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RESEARCH

NUTRITIONAL AND NON-NUTRITIONAL SCREENING OF *AZANZA GARCKEANA* FRUIT METHANOL EXTRACT

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Abstract: This study investigates the nutraceutical properties of *A. Garckeana* fruit extract. Standard procedure of the nutritional and non-nutritional composition of the extract was evaluated. The results showed that, the extract elicited a considerable percent of moisture, ash, fibre content, fat, nitrogen, protein, and carbohydrate contents. The fruit extract had stearic acid (36.5442 ug/ml) with the highest peak and Docosahexaenoic acid (0.0728 ug/ml) with the least peak. Also, amino acids had glutamate (14.67031 g/16gofN) as the highest peak and Docosahexaenoic acid (g/16gofN) as the least peak. Zinc content (0.893 ppm) is more abundant, ascorbic acid (vitamin C) (69.47 mg/kg) at an appreciable amount. The phytochemicals present include; alkaloids, flavonoids, tannins, saponins, phenol, glycosides, and oxalate, with saponins (72.82 %) the highest quantity and oxalate (10 mg/100g) the least quantity.2, 2-Diphenyl-1-1-picrylhydrazyl (DPPH), ferric reducing antioxidant capacity, and total antioxidant capacity of the extract had scavenging effect when compared with the standards.In conclusion, this study validated the nutraceutical uses of *A. garckeana*fruit extract.

Keywords*:* Nutritional, Non-nutritional, *Azanza garckeana*, Methanol Extract

INTRODUCTION

Currently, it is estimated that approximately 80% of the population in developing countries depend on alternative medicine derived from plants and animals as primary sources of health care. In Nigeria, plants are used in alternative medicine as a remedy, usually prepared in extracts or use as an infusion for the management and treatment of different illnesses or diseases such as; gastrointestinal, respiratory, skin, urinary, liver disorders, and many more. Medicinal plants show an imperative function in drug development (Raskin *et al*., 2002, Burke *et al*., 2017, Simon and West 2006, Zhao and Yang 2018).

Azanza garckeana (snot apple) fruit tree is an underutilized indigenous fruit tree (IFT) that is well distributed in the warm woodlands of Southern Africa (Maroyi and Cheikhiyoussef 2017). *Thespesia garckeana* (commonly known as *A. garckeana*) fruit tree belongs to the *Malvaceae* family and its domestication is important to support nutrition, health, and income generation in most communities in sub-Saharan Africa. *A. garckeana* plant is rich in nutritional, phytochemicals, potential medicinal use, cultivation, agronomy, and productivity of the whole plant. The fruit has not been fully exploited including the existing potential for a wider application in food processing and other technological applications. The fruit has the potential to be utilized and used in the production of new food products (Van Wyk 2011). The bark, fruits, leaves, roots, and stems of *A. garckeana* are reported to possess diverse medicinal properties and are used to treat or manage various diseases and ailments (Mshelia *et al*. 2016, Nkafamiya *et al*. 2016, Dikko *et al*. 2016).

There are previous studies that showed some nutritional perspectives attached to various plants (Embrapa 2006,Garzón and Wrolstad 2009,Mlcek and Rop 2011,Niizu and Rodriguez-Amaya 2005). Different medicinal plants as well can be utilized as food with their medicinal profit, examine their dietetic significance (Faokunla *et al*. 2017). Some species can certainly display anti-nutritional components such as protein blockers, tannins, nitrates, calcium oxalates, and others (De Jesus *et al*. 2011, Van Velzen *et al*. 2008).

MATERIALS AND METHODS

Collection and preparation of Plant material

The fruit of *Azanza garckeana* was purchased from Tula village in Michika local government area Adamawa state, Nigeria. It was identified and authenticated by Dr. Timothy Odaro in the Herbarium Unit of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences,

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University of Benin, Edo State, Nigeria with the voucher number UBH-A317.

The fruits were rinsed in clean water, open into pierces, air dried for 21 days and oven dried at 45^{o}c for 24 hours. The dried fruits were powdered using the British milling machine. The weight of the pulverized fruit was taken. The pulverized fruit weighed 1500 g and was extracted using the maceration of 4800 ml of methanol for 72 hours. The percentage yield of the extract was calculated: weight of extract/weight of sample x 100/1 = 56.64 %.

Estimation of Vitamin

All procedures were carried out in the dark to avoid the interference of light. 1g of sample was mixed with 1.0 ml of saponification mixture and refluxed for 20 minutes at 60^{0}C in the dark. The tubes were cooled and 20 ml of water was added and mixed well. The vitamins were extracted twice with 10ml of (40 ^{0}C - 60^{0}C) petroleum ether. The two samples were pooled and washed thoroughly with water (Akindahunsi and Salawu 2005,Glew *et al*. 2005,Vadivel and Janardhanan 2005, Sulieman *et al*. 2012, Saka and Msonthi 1994, Nwaogu *et al*. 2000).

Five (5) g of sample was dissolved in 20 ml of anhydrous glacial acetic acid and warmed slightly.5ml of acetic anhydride was added and mixed.2 -3 drops of crystal violet solution were added as an indicator. Titrate with 0.1 M perchloric acid to a greenish-blue colour (Nkafamiya *et al*. 2016).

Five (5) g of sample was dissolved in a mixture of 5ml of anhydrous glacial acetic acid and 6ml of 0.1 M mercury II acetate solution. 2 drops of crystal violet were added as an indicator. Titrate with 0.1 M perchloric acid to a green colour endpoint (Sulieman *et al*. 2012, Nkafamiya *et al*. 2016, De Jesus *et al*. 2011, Akindahunsi and Salawu 2005, Vadivel and Janardhanan 2005).

Determination of Elemental Analysis

Heavy metal analysis was conducted using Agilent FS240AA Atomic Absorption Spectrophometer according to the method of APHA 1995 (American Public Health Association) (Abdel-Muti 2002, Djama *et al*. 2012).A series of standard metal solutions in the optimum concentration range are prepared, the reference solutions were prepared daily by diluting the single stock element solutions with water containing 1.5 mL concentrated nitric acid/litre. A calibration blank was prepared using all the reagents except for the metal stock solutions. A calibration curve for each metal was prepared by plotting the absorbance of standards versus their concentrations (Costa *et al*. 2008).

Antioxidant Assay

The fruit samples (20 µl) were added to 0.5 ml of 0.1 mM methanol solution of DPPH and 0.48 ml of methanol. The mixture was allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol, without the leaf samples, served as the positive control while butylatedhydroxytoluene (BHT) served as reference (Dikko *et al*. 2006).

Ferric Reducing Antioxidant Property of the extract will be determined as described by Bowler *et al*. (1992). 0.25 ml of the extracts will be mixed with 0.25 ml of 200 Mm Sodium phosphate buffer pH 6.6 and 0.25 ml of 1% Potassium ferrocyanide (Lako*et al*.2007).

The Total Antioxidant Capacity (TAC) of extract in different extracting solvents (absolute ethanol, 70%, and 50% ethanol) will be determined by the phosphomolybdate method according to Mustapha *et al*. (2011), Zhang *et al*. (2015). The blank will be incubated under the same conditions as the test samples. Ascorbic acid will be used as a standard reference compound to compare the activities of the extract.

Amino acid

The procedure used was a modification of the method of Hassan and Umar (2006). A standard solution (5, 10, 15, or 20 µl) or 50 µl of sample solution was pipetted into a 10- × 5-mm tube and dried in vacuum at 65°C. To the residue, 30 µl of methanol-water-Phenylisothiocianate (2:2:1 [v/v]) was added and then removed in a vacuum at 65°C. Next, 30 µl of the derivatizing reagent methanol-water-Phenylisothiocianate (7:1:1:1 [v/v]) was added, and the tube was agitated and left to stand at room temperature for 20 min. Finally, the solvents were removed under a nitrogen stream, and the tube was sealed and stored at 4°C, pending analysis. Before injection, 150 µL of diluent consisting of 5 mM sodium phosphate with 5% acetonitrile was added to each tube (Isong *et al*. 1999).Chromatography was carried out at a constant temperature of 30°C using a gradient elute ion as follows. Eluent A was an aqueous buffer prepared by adding 0.5 mL/L Triethylamine to 0.14 M sodium acetate and titrating it to pH 6.20 with glacial acetic acid; eluent B was acetonitrile-water (60:40 [v/v]) (Osagie 1998).

Fatty acid profile

Dissolve 1 ml of the filtered residue of the extract in 50 ml of chloroform and transferred to a 100 ml volumetric flask and dilute to the mark. Evaporate most of the chloroform at room temperature next, add 1 ml of the reagent {20 vol% benzene and 55 vol.% methanol}. Seal it, and heat it in a 40^{0}C water bath for 30 minutes Hassan and Umar (2006).

Data Analysis

Results were analysed with Graph pad prism version 6. Data were presented as Mean ± S.E.M, and statistical significance was calculated using one-way ANOVA, followed by Dunnett's test where $P<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Qualitative and Quantitative Phytochemical Screening

Table 1 showed the qualitative phytochemical screening results of *Azanza garckeana* fruit methanol extract indicated the presences of alkaloids, flavonoids, tannins, saponnins, phenols, glycosides and oxalate. Saponnins, tannins, phenol and glycosides are more abundant.

Phytochemical screening of *Azanza garckeana* fruit methanol extract showed that alkaloids, flavonoids, tannins, saponnin, phenols, glycoside, and oxalate were present in the plant fruit extract as shown in Table 1. A study by Rajurkar and Kunda (2012) also screened for the phytochemicals and metal constituents in *Adiantumcapillus*. The result of their study exhibited that *Adiantum capillu*s is present with alkaloids; saponnin, flavonoids, and terpenoids in the leaf extract, when compared with *A. garckeana* fruit methanol extract of this study, showed the presence of similar phytochemicals in the plant (Leal *et al*.2005). From this study, *A. garckeana* had been proven to contain latent biological properties due to the phytochemicals present. This report is in line with the findings of Sabandar *et al*. (2013), Faokunla *et al*. (2017).

Table 1. Preliminary qualitative and quantitative phytochemical screening of *Azanzagarckeana* fruit

Phyto-constituents	Qualitative	Quantitative
Alkaloids	+	2.80 ± 0.02
Flavonoids	+	7.00 ± 0.14
Tannins	++	25.00 ± 1.41
Saponnins	+++	72.82 ±2.06
Phenols	++	15.00 ± 0.51
Glycosides	++	20.00 ± 0.95
Oxalate	+	10.00 ± 0.49

Keys: Present (+), highly present (++) and very present (+++)

Table 1 showed the quantitative (mg/kg) phytochemicals screening results of *A. garckeana* fruit methanol extract indicated the quantity of total phenol (15 mg/100 g), total flavonoid (7%), total alkaloid (2.80%), tannins content (25 mg/100 g), total saponnins (72.82 %), glycosides (20 mg/100 g) and oxalate (10 mg/100 g). saponnins (72.82 %) as the highest quantity and oxalate (10 mg/100 g) with least quantity.

A. garckeana extract showed diverse quantities of the phytoconstituents of which saponnins and tannins content were the highest in *A. garckeana* fruit extract at (72.82 % and 25 mg/100g) followed by glycosides and phenols at (20.0 and 15.0 mg/100g). This is similar to the work of Saini (2015), which showed similar results in an equivalent trend, with the amount of alkaloids and flavonoids of the fruit extract with the lowest quantity (2.80 and 7.0 %). A study conducted by Rajurkar and Kunda (2012), estimated that the soxhlet extraction of *Adiantum capillus* veneris on preliminary phytochemicals exhibited alkaloids at 16 mg/kg, flavonoids at 17 mg/kg, and phenolic at 13 mg/kg as major constituents, this report is in line with those reported by Faokunla *et al*. (2017) on *J. Gossypiifolia* for its traditional uses, phytochemistry, pharmacology and toxicology profile.

Proximate analysis

The dried weight percentage composition showed the quantity of proximate analysis such asmoisture content in 3.24 %, ash content 4.41 %, crude fibre 9.88 %, crude fat 2.27 %, crude, crude nitrogen 1.34%, protein 8.40 %, and carbohydrate content 70.46 % were present in *Azanza garckeana* fruit methanol extract. Carbohydrate content was more abundant (70.46 %) and nitrogen (1.34%) least abundant (Table 2).

The moisture content exhibited is present as found in *A. garckeana* fruit extract. The ash content is known as the residue left after the whole moisture is isolated also with organic materials included to the protein, carbohydrates, fat, and vitamins, which was being cremated at a temperature of approximately 50^0C (Onwuka 2005), the ash content evaluated the mineral constituents in unique plant materials. The ash content unyielding in the fruit of *A. garckeana*showed ash content (4.41 %) as shown in Table 2. The rate of the ash content is less than this present study when compared with 1.80 % *Ipomoea batatas* leaf, reported by Asibey-Berko and Tayie (1999) and 5% *Tribulus terrestris* leaf reported by Nwaogu *et al*. (2000), by the reduction in several leafy vegetables usually ingested in Nigeria includes *Talinum trangulare* at (20%) showed in the findings of Akindahunsi and Salawu (2005).

The results got from crude fibre attained from the fruit methanol extract of *A. garckeana* with 9.88%. This is in line with Akpabio and Ikpe (2013) work. Fibres found in diet could be required for simple absorption and efficient waste removal (Okwu 2006,Faokunla*et al*. 2017,Vadivel and Janardhanan 2005). Crude fatsubstances in *A. garckeana* fruit extract elicited the fat content as 2.27. The result obtained is greater than Uzama *et al*. (2012) values reported from the work on spinach leaves at (0.3%), *Amaranthus hybridus* leaves at (1.60 %), and *Securinega virosa* at (4.70 %). This study also indicated the crude nitrogen and protein gotten from *A. garckeana* fruit extract, which amounted to 1.34 and 8.40 % respectively. This is comparable to the

report of Isong *et al*. (1999), Hassan and Umar (2006),Faokunla *et al*. (2017) that worked on Balsam apple and *Momordica foecide* leaves amounted to 11.29 % and 4.6 % of crude protein.Nosiri*et al*.(2011) recorded that carbohydrate substance in the seed of *Irvingiagabonensis* at 15.71 to 55.00%, which is useful in modern and traditional medicine and implicated in the management of numerous diseases. The presence of carbohydrate content found in *A. garckeana* fruit extract at 70.46 % as shown in Table2.

Table 2. Proximate analysis of *Azanzagarckeana*fruit

Proximate Analysis	*Azanzagarckeana* Fruit extract (%)
Moisture content	3.24 ± 0.09
Ash content	4.41 ± 0.10
Fibre	9.88 ± 0.31
Fat	2.27 ± 0.07
Nitrogen	1.34 ± 0.05
Protein	8.40 ± 0.25
Carbohydrate content	70.46 ± 1.73

Values was expressed as Mean ± SEM

Fatty acids

Table 3 elicited the Fatty acids and their various indices present in *A. garckeana*fruit methanol extract such asLinolenic acid, Lauric acid, Docosahexaenoic acid, Palmitic acid,Eicosapentaenoic acid, Eicosadienoic acid, Oleic acid, Stearic acid, Eicosatetraenoic acid and Linoleic acid were present in the extract with Stearic acid (36.5442 ug/ml) with the highest peak and Docosahexaenoic acid (0.0728 ug/ml) had the least peak level.The values of fatty acids of *A. Garckeana* extract showed diverse fatty acids constituents of which stearic acid and Eicosapentaenoic acid were the highest peaks in *A. garckeana*fruit extractat (36.5442 and 11.5447 ug/ml) followed by Linolenic acid and Palmitic acid at (10.0426 and 9.4340 ug/g) with Eicosadienoic acid and Docosahexaenoic acid (0.1142 and 0.0728 ug/ml) as the least peak values. This is similar to the work of Uzama *et al*. (2012), which showed similar results as shown in Table 3. This is a tie to the several biological properties elicited by *the A. garckeana* plant.

Table 3. Fatty acids and their various indices present in *Azanzagarckeana*fruit

Peak number	Name of compound	Molecular formular	Retention	Area	Height	Values
1	Linolenic acid	C18:3	0.473	5578.4404	438.214	10.0426 ug/ml
2	Lauric acid	C12	11.893	4026.5014	316.642	3.8459 ug/ml
3	Docosahexaenoic acid	C22:6	13.943	56.5842	4.959	0.0728 ug/ml
4	Palmitic acid	C16	15.223	13892.1697	1076.189	9.4340 ug/g
5	Eicosapentaenoic acid	C20:5	17.183	9016.3813	704.561	11.5447 ug/ml
6	Eicosadienoic acid	C20:2	25.323	66.5530	5.135	0.1142 ug/ml
7	Oleic acid	C18:1	27.270	5845.8952	459.211	5.4406 ug/ml
8	Stearic acid	C18	34.333	22150.7922	1653.921	36.5442 ug/ml
9	Eicosatetraenoic acid	C20:4	38.290	6621.7925	519.750	4.5847 ug/ml
10	Linoleic acid	C18:2	40.760	4906.8424	385.723	7.4810 ug/ml

Amino acids

Table 5 revealed the Amino acids and their various indices found in *A. garckeana* fruit methanol extract, which include; Glycine, Alanine, Serine, Proline, Valine, Threonine, Isoleucine, Leucine, Aspartate, Lysine, Methionine, Glutamate, Phenylalanine, Histidine, Arginine, Tyrosine, Tryptophan, and Cysteine were present in the extract with Glutamate (14.67031 g/16gofN) with the highest peak and Docosahexaenoic acid (Tryptophan g/16gofN) had the least peak level.

The values of amino acids of *A. garckeana* extract showed diverse amino acids constituents of which Glutamate and Aspartate were the highest peaks in *A. garckeana* fruit extract at (14.67031 and 12.07126 g/16gofN) followed by Leucine and Lysine at (7.20846 and 6.11876 g/16gofN) with Cysteine and Tryptophan (1.29790 and 1.15379 g/16gofN) as the least peak values. This is similar to the work of Isong *et al*. (1999); Hassan and Umar (2006),Faokunla *et al*. (2017), which showed similar results as shown in Table 4. Hence, it displayed numerous biological properties.

Table 4. Amino acids and their various indices present in *Azanzagarckeana* fruit

Peak number	Name of Amino acid	Ret Time (min)	Area (pA*s)	Ams/area	Amount (g/16gofN)
1	Glycine	9.343	20.76649	1.70938e-1	3.54978
2	Alanine	10.850	26.35212	1.51221e-1	3.98499
3	Serine	12.267	9.00644	5.37347e-1	4.83959
4	Proline	13.659	13.70012	3.16268e-1	4.33290
5	Valine	14.923	35.63976	1.35705e-1	4.83649
6	Threonine	16.190	7.04605	5.23875e-1	3.69125
7	Isoleucine	17.090	85.97766	4.81067e-2	4.13610
8	Leucine	18.049	131.61325	5.47700e-2	7.20846
9	Aspartate	19.515	123.37109	9.78451e-2	12.07126
10	Lysine	20.591	29.20164	2.09535e-1	6.11876
11	Methionine	21.675	53.99881	2.40415e-2	1.29821
12	Glutamate	22.596	97.64228	1.50246e-1	14.67031
13	Phenylalanine	23.227	40.09413	1.26745e-1	5.08174
14	Histidine	24.998	25.73695	9.00975e-1	2.31883
15	Arginine	24.998	26.19519	2.05038e-1	5.37102
16	Tyrosine	25.616	13.97092	2.42628e-1	3.38974
17	Tryptophan	26.012	7.28376	1.58405e-1	1.15379
18	Cysteine	26.555	4.60651	2.76944e-1	1.29790

Minerals composition

Table 5 showed the minerals composition which includes; potassium, chromium, copper, selenium, calcium, magnesium, iron, sodium, and zinc were present in *A. garckeana* fruit methanol extract at various concentrations with Zinc (0.893 ppm) more abundant and chromium (0.00) least abundant. Kapha disparity is also called atherosclerosis and was triggered in the mechanism of heart disorders (Singh *et al*. 2003). This present study showed that the fruit extract of *A. garckeana* elicited a slight increase in potassium content at (0.194 ppm). This is compared to the work of Uzama *et al*. (2012) on *Securinegavirosa* leaves at (3.67 mg/g), the leaves of *Indigoferaastragelina* at (14.55 mg/g), and *Mucunasloanei* at (43.21 mg/g). Faokunla *et al*. (2017) suggested that the harmless potassium limit be set at 0.01-0.1 mg/kg, hence the result of this present study is in line with the set limit.

The presence of chromium was absent from the evaluation carried out on *A. garckeana* fruit extract, while copper quantity was not significant (0.024 ppm), showing the low level of toxicity contaminant of heavy metals in the plant. Selenium is a vital mineral required for crucial biological activities such as it enhances the growth of nails and hair and carrying out other endogenous activities. The required quantity of selenium present in the fruit extract of *A. garckeana* had a significant amount needed for daily consumption (0.142 ppm).

This present study showed the level of calcium present in the fruit of *A. garckeana* at (0.0029 ppm) (Table 5). Owing to the important roles calcium interplays in the muscle, heart, bones, and teeth function as early stated by Dosunmu (1997); Turan *et al*. (2003); Faokunla *et al*. (2017) that an increased quantity is needed for several biological functions. Associated with the elemental constituent resolute in *Vitexdoniana* sweet (black plum) stem bark reported by Mustapha *et al*. (2011), calcium is present at 2.423 mg/kg. World Health Organization (WHO) (2006) place a safe limit of 3.6-80 mg/kg.

Thefruit of the plant (*A. garckeana*) showed the presence of magnesium at 0.142 ppm, compared with the report of Nwaogu *et al*. (2000) and Faokunla *et al*. (2017) that worked on *Amaranthushybridus* leaves at (23.18 mg/100g) and *Cassia siamea* (400 mg/100 g). The absence of magnesium is bring about asymmetrical irritability of convulsions and muscle while excess Mg involving in the disproportion of the central nervous system. Thereby, it is suggested the safety limits for magnesium should not exceed 0.1- 0.2 mg/kg as recommended by WHO (2006).

Metal elements like Iron are a vital mineral component that enhanced sturdy bones and blood synthesis (Obiajunwa *et al*. 2002; Khan *et al*. 2011). World Health Organization (2006) also recommended a harmless limit situate iron by World Health Organization is 0.5-07 mg/kg Deficiency of iron brings about osteomalacia and blood deformed illness earlier indicated in the report of Michael (2007). The fruit of *A. garckeana* is a good source of iron as indicated by the presence of 0.054 ppm as shown in Table 5, which aids in the correction of blood deformity disease and bone marrow and kidney repair.

The fruit of *Azanza garckeana* is a restrained basis for sodium at (0.134 ppm) which perhaps aids in lowering blood pressure, amino acid, glucose, and energy production to convey into the body cells. The quantity is elusively smaller than the quantity present in *Berberis asiatica* stem bark (20.89 mg/g) and root

(36.0 mg/kg) as reported by Swati *et al*. (2012), 20.31 mg/kg present in *Securinega virosa* leaf by Uzama *et al*. (2012) and 0.02 mg/g present in *Bryophylum pinnatum* and *Aspilia Africana* reported by Okwu and Josiah (2006). WHO (2006) suggested that the protective sodium limit is positioned at 0.4-0.5 mg/kg.

Zinc is an essential trace element in diet functioning as a vital segment of recurrent enzymes like superoxide dismutase (Powell 2000,Ozturk*et al*., 2003, Uzama *et al*., 2012, Faokunla *et al*., 2017); regulate tissue improvement and therapeutic; and enhances insulin expansion with spermatogenesis (Wong *et al*., 2001,Serfor-Armah *et al*., 2002) and the edict harmless zinc limits of 0.15-20 mg/kg recommended by Faokunla *et al*. (2017). Zinc content in the fruit of *A. garckeana*is 0.893 ppm, is closely related to *Mucuna sloanei* content (0.25 mg/kg) and the leaves of *I. Astragelina* (0.11 mg/kg), but reduces when compared with 6.85 mg/kg in the leaves of *Cassia siamea*. Zinc is comparatively harmless reported by Faokunla *et al*. (2017).

Table 5. Elemental and mineral constituents present in *Azanza garckeana* fruit

Name of Element	eement	*Azanza garckeana* Fruit extract (ppm)
Potassium	K	0.194
Chromium	Cr	0.000
Copper	Cu	0.024
Selenium	Se	0.142
Calcium	Ca	0.0029
Magnesium	Mg	0.142
Iron	Fe	0.054
Sodium	Na	0.134
Zinc	Zn	0.893

Vitamins

Table 6 showed the various vitamins with potent therapeutics implicated in the management of several disease conditions such as; retinol (vitamin A), alpha-tocopherol (vitamin E), ascorbic acid (vitamin C), cholecalciferol (vitamin D), thiamine (vitamin B1) riboflavin (vitamin B2), niacin (vitamin B3), pyridoxine (vitamin B6), cobalamin (vitamin B12) were present in *A. garckeana* fruit methanol extract at various concentrations with ascorbic acid (vitamin C) (69.47 mg/kg) appeared more abundant and thiamine (vitamin B1) and riboflavin (vitamin B2) (0.02 mg/kg) appeared least abundant.

Table 6. Vitamin constituents present in *Azanza garckeana* fruit

Name of Vitamins	Vitamins	*Azanza garckeana* Fruit extract (mg/kg)
Retinol	Vitamin A	5.07
Alpha-tocopherol	Vitamin E	15.46
Ascorbic acid	Vitamin C	69.47
Cholecalciferol	Vitamin D	3.82
Thiamine	Vitamin B_1	0.02
Riboflavin	Vitamin B_2	0.02
Niacin	Vitamin B_3	0.66
Pyridoxine	Vitamin B_6	0.24
Cobalamin	Vitamin B12	4.56

The values of 5.07; 15.46; 69.47 and 3.82 mg/kg of vitamins A, E, C, and D are found in the fruit of *A. garckeana*. Vitamin A, E, C, and D display vital functions in eye functioning, skin enrichment, wound healing and repaired and blood clotting stimulation with the implication of some calcium and proteins for this function (Institute of Medicine, Food and Nutrition Board 2000,Oduola *et al*. 2005). Its definite uses are implicated in antibiotics and blood thinner agents (anticoagulants) including warfarin, which may trigger the deficiency associated with these vitamins.

Azanza garckeana fruit possessed 0.02 mg/kg of vitamin B_2. The function of riboflavin is major as a coenzyme with redox responses (FAD). Also, it aids in erythrocytes and growth formation. The quantity procured (0.02 mg/kg) is virtually in line with a recommended value of 1.3 mg/day dietary allowance (RDA). The absence of adverse effects of drugs has been associated with riboflavin ingestion either as a supplement or as food (Institute of Medicine, Food and Nutrition Board 2000). The fruit extract of *A. garckeana* has a moderate vitamin B_3 quantity (0.66 mg/kg). Just like riboflavin, niacin acts as a

coenzyme to synthesize phosphate form (NADP) or nicotinamide adenine dinucleotide (NAD).

Vitamin B_6 in coincidence with B_3 structures the main mechanism to increase in the nervous system, thus evolving cognitive health, hence preventing any potential danger connected with the advance of deteriorating disorders like Parkinson's, Alzheimer's disease, and Dementia. Vitamin B_3 controls serotonin which is liable for the production of stress hormones thereby deterring the symptoms of stress and anxiety. In the medical investigation, a higher increase in niacin for a stated remedy to raised cholesterol (Altschul *et al*., 1955).

Conversely, hepatic toxicity was reported at an exciting high concentration of nicotinamide and nicotinic acid ingestion ranging from 3 to 9 grams/day for months or annual aging for the treatment of high cholesterol concentration (Capuzzi *et al*., 1998). Deficiency outcome in pellagra and 16 mg/ day for niacin against. An approximation of vitamin B1 (thiamin) present in the fruit of *A. garckeana* was observed at 0.02 mg/kg as shown in Table 7.

Thiamine roles as a coenzyme in thiamine pyrophosphate (TPP) performed in the metabolism of branched-chain amino acids and carbohydrates. Overtly, Mg_2 consistent with TPP contributed to α-ketols development (e.g. between pentose phosphates and hexose) like catalyzed by transketolase and in α-keto acids oxidation (e.g. α-ketoglutarate. branched-chain α-keto acids and pyruvate) via dehydrogenase intricacy. Vitamin B1 regulates kidney stone formation and healthy hair, muscles, skin, and brain.RDA considered for thiamine is 1.2 mg/kg. Deficiency is linked with thiamine-activated failure in muscle role, beriberi, and nerve damage. The quantity of vitamin B_{12} (Cobalamin) in 1000 grams of *A. garckeana* fruit extract was anticipated at 4.56 mg/kg. Vitamin B_{12} helps in preserving a healthy gastrointestinal tract (GIT) and supports probiotics found in GIT. As well, it is determined to control breast, and colorectal cancers, and pancreas development. Folate act in single-carbon transport reactions, needed for red blood cell synthesis, purines, body proteins, and DNA. It's a vital vitamin required throughout the early pregnancy stage to avert babies' abnormalities against spina-bifida.

Antioxidant assay

Figure 1 showed the scavenging power of *A. garckeana* fruit methanol extract against exogenous free radicals. It potentiated a scavenging property against 2, 2-diphenyl-2-picrylhydrazyl radicals at 84.63% when compared with the standard (ascorbic acid) 98.24%.

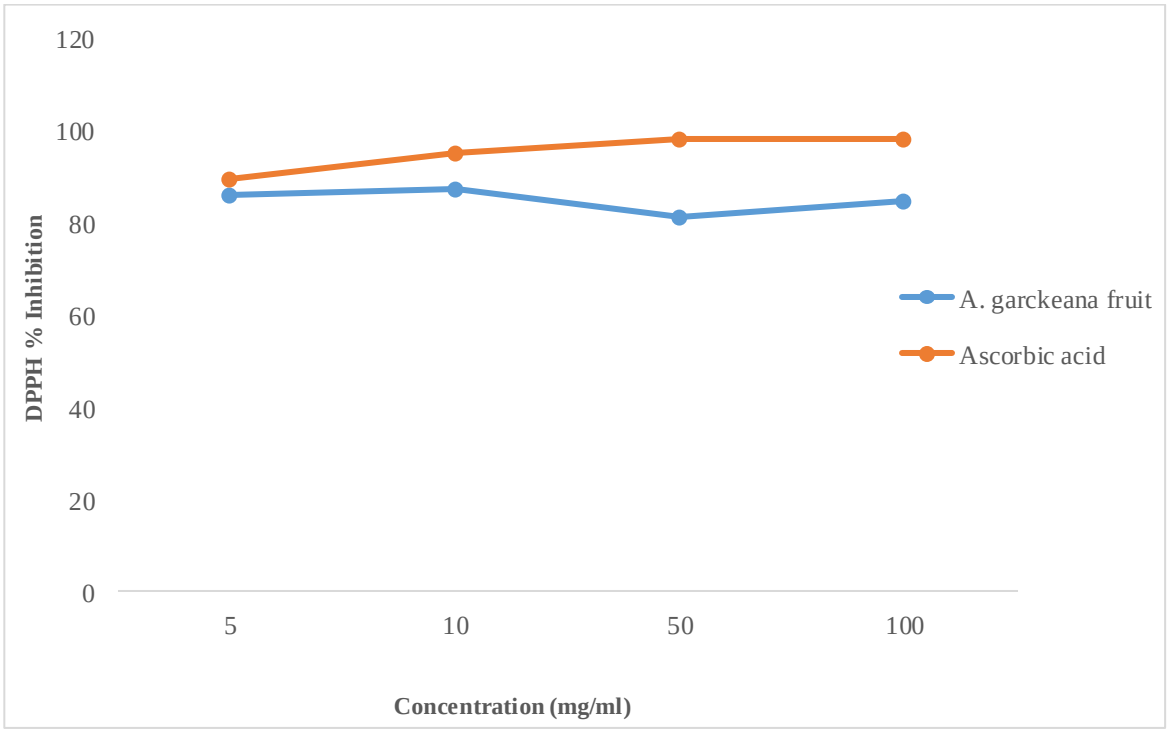

Figure 1. Percentage Inhibitory effect of *Azanza garckeana* fruit extract in 2, 2-diphenyl-2-picrylhydrazyl.

Figure 2 showed the scavenging capacity of *A. garckeana* fruit methanol extract against exogenous (chemical induced) free radicals. It potentiated radical scavenging effect against Ferric Reducing Antioxidant radicals at 75.46 % when compared with the standard (Gallic acid) 99.41 %.

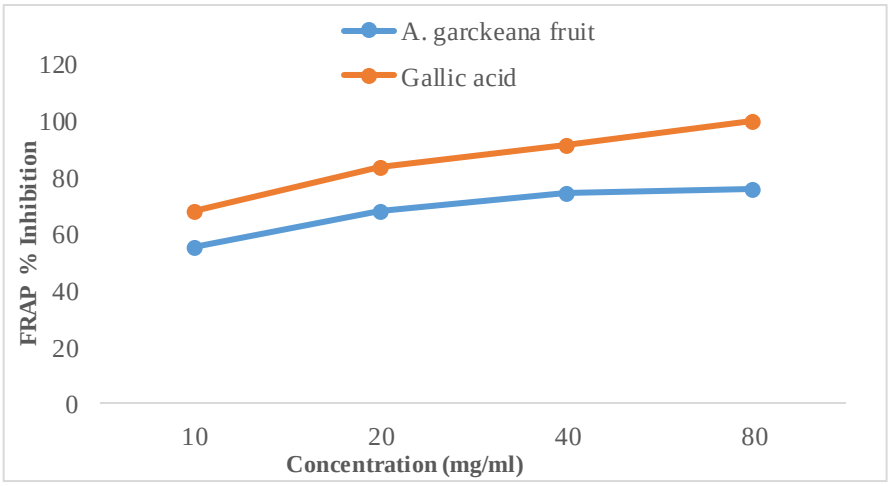

Figure 2. Percentage Inhibitory effect of *Azanza garckeana* fruit extract in Ferric Reducing Antioxidant Property.

Figure 3 showed the scavenging power of *A. garckeana* fruit methanol extract. It potentiated the scavenging property against Total Antioxidant Capacity at 34.83 % at higher dose when compared with higher conc. of standard (Gallic acid) 0.98 %

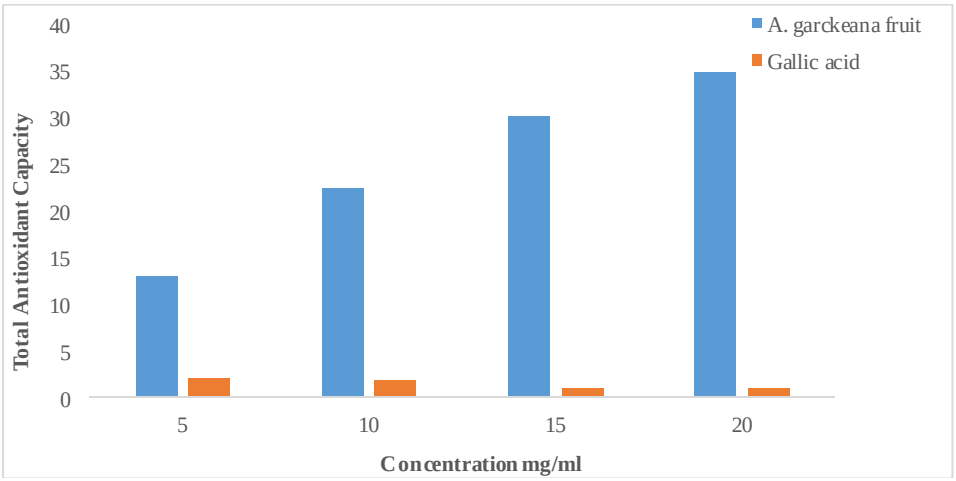

Figure 3. Scavenging effect of *Azanza garckeana* fruit extract against Total Antioxidant Capacity.

The importance of concentration antioxidant and duration of action are considered when determining antioxidant activity (Shukla *et al.*, 2009). The evaluation of 2, 2-diphenyl-2-picrylhydrazyl.antioxidant capacity in this study showed higher antioxidant activity in *A. garckeana* fruit methanol extract at various concentrations (5, 10, 50, and 100 mg/ml) as shown in Figure 3. It has been reported that any fastidious antioxidant activity at higher concentrations (above 50 mg/ml) of the extract is required than the standard ascorbic acid to attain scavenging activity just as reported by Shukla *et al*. (2009); Okoh *et al*. (2016). This phenomenon is observed when measuring the antioxidant activity for scavenging 50 % 2, 2-diphenyl-2-picrylhydrazyl radicals as evaluated in the form of IC_{50} (mg/ml). The free radical scavenging effects measured as percentage inhibition in the case of the stable DPPH were found to be 84.63 mg/ml in fruit methanol extract at 100 mg/ml concentration when compared with 98.24 % ascorbic acid. Standard drug vitamin C showed high inhibition at very low concentration in comparison with the extract. Ascorbic acid is a water-soluble vitamin, which serves as a potent antioxidant in biological or biochemical applications to reduce oxidative stress or damage (Shahwar *et al.*, 2010).

Free radical scavenging activities evaluated as % inhibition of fruit methanol extracts of *Azanza garckeana* at different concentrations (10, 20, 40, and 80 mg/ml), found to scavenge Ferric reducing antioxidant radicals at 75.46 % fruit extract at 100 µg/ml concentrations compared with 99.41% of Gallic acid with the same concentration (Figure 1, 2, 3). Also, an increase in percentage inhibition was commensurate with an increase in the concentration of the plant extract with the assayed mixture during the Ferric Reducing Antioxidant test. Studies from Shahwar *et al*. (2010) and Okoh *et al*. (2016), reported that the selected plants from the family of

Euphorbiaceae such as;*Lauraceae, Malvaceae*, and *Balsaminaceae* had antioxidant activities. The *Azanza garckeana* fruit extract reduced the Ferric Reducing Antioxidant radical to corresponding hydrogen donors. It was noticed that the methanol fruit extract of *A. garckeana* is required in higher concentrations, also Gallic acid (standard) for performing antioxidant activity measured by assessing the concentration required for 50 % scavenging of the Ferric Reducing radical, i.e. IC_{50} (µg/ml). The extract from *A. garckeana* exhibited higher scavenging activity at various concentrations during the Ferric Reducing essay test when compared with the standard. This is by the findings derived from Agati *et al.* (2012).

The free radical scavenging effect from this study was exhibited by the Total Antioxidant Capacity scavenging effect of methanol fruit extract at concentrations of 5, 10, 15, and 20 mg/ml. The radical scavenging property of the plant extract was elicited at 34.83 mg/ml and 0.98 mg/ml of the fruitmethanol extract when compared with Gallic acid. Hence, showed that the fruit of *A. garckeana* extract inhibited Total Antioxidant Capacity induced oxidation. Studies from Shukla *et al.* (2009), Okoh *et al.* 2016) displayed similar protective effects shown in this study.

CONCLUSION

In conclusion, the nutritional and non-nutritional property of *A. garckeana* fruits methanol extract was scientifically validated to ascertain their actual constituents, which could be implicated in the biological efficacy.

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