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Phytochemical Composition, Anti-nutrient Properties and Antioxidant Potentials of Raw *Hibiscus sabdariffa* Seeds

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Authors' contributions

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

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ABSTRACT

Aims: This study was aimed at ascertaining the phytochemical, anti-nutrient and antioxidant potentials of raw seeds of *Hibiscus sabdariffa* (HS).
Place and Duration of Study: Rivers and Anambra states, Nigeria, between January and April, 2017.
Methodology: Dried seeds of *Hibiscus sabdariffa* were gotten from Mangu Local Government Area of Plateau state, Nigeria. They were properly cleaned, sorted and ground into powder for analyses. Phytochemical constituents and anti-nutrients were quantified using a BUCK M910 Gas chromatography equipped with an on-column automatic flame ionization detector (GC-FID) under standard chromatographic conditions. Antioxidant vitamins A, C and E were determined using standard spectrophotometric methods, while *in vitro* enzymatic antioxidants were determined using standard protocols.
Results: The phytochemical screening revealed the presence of the following in their order of abundance: ribalidine>epicatechin>oxalate>catechin>saponin>sapogenin>kaempferol>tannin>

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lunamarine>rutin>anthocyanin>phytate>spartein. The anti-nutrient levels in the seeds of HS were: alkaloids (47.00 µg/ml; 39.20%), saponins (14.62 µg/ml; 12.19%), oxalates (14.06 µg/ml; 11.72%), tannin (4.79 µg/ml; 3.99%) and phytates (0.55 µg/ml; 0.46%). Antioxidant vitamins detected were vitamin A (2.76 ± 0.26 mg/kg), vitamin C (2.60 ± 0.07 mg/kg) and vitamin E (5.06 ± 0.30 mg/kg). Some *in vitro* enzymatic antioxidants were Catalase (26.71 ± 3.68 µmol/ml), Peroxidase (13.29 ± 1.72 µmol/ml), Glutathione reductase (24.43 ± 0.78 µmol/ml) and Superoxide Dismutase (0.88 ± 0.05 unit enzyme).

Conclusion: Seeds of HS contain several phytochemicals which exist in great amounts, some of which may act as anti-nutrients that interfere with food absorption. Furthermore, HS seeds possess some antioxidant potentials which can be exploited for therapeutic purposes.

Keywords: Phytochemicals; anti-nutrients; antioxidants; Hibiscus sabdariffa seeds; toxicity.

1. INTRODUCTION

Hibiscus sabdariffa (HS) Linn. is a shrub that belongs to the Malvaceae family and believed to be of native to East Africa, Asia (India to Malaysia) or Tropical Africa. The plant is broadly cultivated in tropics like The Caribbean, Central America, India, Africa, Brazil, Australia, Hawaii, Florida and Philippines as a home patio nursery crop. They are generally regarded to have medicinal value and contain high amount of protein, dietary fiber, lipids, and minerals [1-4]. The dietary compositions of roselle seeds just as their functional properties are seldom examined contrasted with the calyces. Roselle seeds can be exploited as an alternate protein source to alleviate protein-energy-malnutrition. In addition, consumption of Roselle seeds might have a cardio-protective effect. They also have the potential to act as antioxidants, lower the level of cholesterol and prevent the risk of atherosclerosis [5]. Furthermore, raw roselle seeds are toxic with respect to transaminases and creatinine and the relative weight of liver and kidney of rats [6]. It was likewise discovered that the seed contained anti-nutritional components which lead to undesirable physiological disorders in non-ruminants like gossypol, tannins [7] and phytic acid [8].

Phytochemicals are non-nutritive plant chemicals that possess disease protective and preventive properties. They are non-essential nutrients – implying that they are not required to sustain life of humans. However, these chemicals produced abundantly by plants, have been shown to have various chemotherapeutic and chemopreventive effects against many diseases [9]. Anti-nutritional factors are natural agents in food that constrains the bioavailability of nutrients, and to get the best from our food these compounds need to be expelled amid food processing [10]. Most phytochemicals have the ability to protect our cells from oxidative damage and limit the risk of developing certain types of diseases as a result of their antioxidant activity. Examples: allyl sulfides (onions, leeks, garlic), carotenoids (fruits, carrots), flavonoids (fruits, vegetables), polyphenols (tea, grapes) [11]. Antioxidants are compounds that scavenge free radicals and bind to them in order to make them less toxic to the cell. Antioxidants can donate electrons and thus, can inactive free radicals and converting them to less harmful compound like water [12]. Antioxidant phytochemicals can be found in numerous foods and therapeutic plants, and assume an imperative role in the aversion and treatment of incessant ailments brought about by oxidative stress. They often possess strong antioxidant and free radical scavenging abilities, as well as anti-inflammatory action, which are likewise the premise of other bioactivities and health benefits, for example, anticancer, anti-aging, and defensive activity for cardiovascular diseases, diabetes mellitus, obesity and neurodegenerative maladies diseases [13]. This study was aimed at ascertaining the phytochemical components, anti-nutrients and antioxidant potentials of raw seeds of *Hibiscus sabdariffa* (HS).

2. MATERIALS AND METHODS

2.1 Materials

Dried *Hibiscus sabdariffa* seeds were collected from Mangu Local Government Area, Plateau State, Nigeria (9°23'25.87" N 9°10'46.85" E). They were properly cleaned by removing all dirt and sorting out damaged seeds. The cleaned dried seeds were put in a container and stored properly for further use. A portion of the raw seeds was pulverized into a fine powder with an electric blender and stored in a lid-tight container for further analyses in the laboratory.

2.2 Phytochemical Analysis Using Gas Chromatography with Flame-Ionization Detector (GC-FID)

2.2.1 Extraction of phytochemicals

Exactly 1 g of sample was weighed and transferred in a test tube and 15 ml ethanol and 10 ml of 50%m/v potassium hydroxide were added. The test tube was allowed to react in a water bath at 60°C for 60 mins. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel. The tube was washed successfully with 20 ml of ethanol, 10 ml of cold water, 10 ml of hot water and 3 ml of hexane, which was all transferred to the funnel. This extracts were combined and washed three times with 10 ml of 10%v/v ethanol aqueous solution. The solution was dried with anhydrous sodium sulfate and the solvent was evaporated. The sample was solubilized in 1000 ul of pyridine of which 200 ul was transferred to a vial for analysis.

2.2.2 Quantification by GC-FID

The analysis of the sample was performed on a BUCK M910 Gas chromatography equipped with a flame ionization detector (FID). A RESTEK 15 meter MXT-1 column (15 m x 250 µm x 0.15 µm) was used. The injector temperature was 280°C with splitless injection of 2 µl of sample and a linear velocity of 30 cms^{-1}, Helium 5.0 Psi was the carrier gas with a flow rate of 40 $mlmin^{-1}$. The oven operated initially at 200°C, it was heated to 330°C at a rate of 3°C min^{-1} and was kept at this temperature for 5 minutes. The detector operated at a temperature of 320°C. Phytochemical concentration was determined by the ratio between the area and mass of internal standard and the area of the peaks of the identified phytochemicals. The concentrations of the different phytochemicals were expressed in µg/ml.

2.3 Determination of Antioxidants

Vitamins A, C and E were determined using spectrophotometric methods of Kirk and Sawyer [14]. Catalase, Superoxide Dismutase (SOD), Glutathione Peroxidase and Reductase were assayed according to the methods of Luck [15], Kakker et al. [16], Reddy et al. [17] and David and Richard [18] respectively.

3. RESULTS AND DISCUSSION

The result of the phytochemical screening of raw seeds of *Hibiscus sabdariffa* is presented in Table 1. Flavonoids, oxalate, tannin, phytate, polyphenols, alkaloids and saponins were found to be present, while there is absence of terpenes, steroids, glycosides and phlobotannins. From the qualitative analysis, the most abundant phytochemicals are flavonoids and alkaloids (+++), followed by saponins and polyphenols (++) and then oxalate, tannin and phytate (+). These phytochemicals have been shown to have good antioxidant and physiological activities. Saponins and polyphenols were also present in appreciable amounts.

Table 1. Qualitative phytochemical screening of raw seeds of *Hibiscus sabdariffa*

Phytochemicals	Concentration
Flavonoids	+++
Terpenes	-
Oxalate	+
Tannin	+
Phytate	+
Steroids	-
Glycosides	-
Polyphenols	++
Alkaloids	+++
Phlobatannins	-
Saponins	++

Key: Absent (-), Low (+), High (++), Very High (+++)

The amounts of the phytochemicals are embodied in Table 2. Flavonoids possess antioxidant, anti-inflammatory, hypocholesterolemic, hepatoprotective and anti-microbial potentials [19]. The flavonoids found in the HS seeds (anthocyanin, rutin and kaempferol) have all been shown to possess such antioxidant properties [20]. The alkaloids – Ribalidine (35.44%), Lunamarine (3.75%) and Spartein (0.02%) were the highest occurring phytochemicals. Alkaloids have been shown to possess therapeutic potentials like anti-malarial [21], analgesic, and antioxidant properties [22] and anti-plasmodial properties [23]. The Saponins (6.61%) and sapogenins (5.59%) were also present in a reasonable amount. Saponins exhibit cholesterol lowering (by binding with bile salts and cholesterol in the intestinal tract and preventing its re-absorption), wound healing and hemolytic properties [24] while sapogenins are useful in neutralization of viruses such as the inhibition of the replication of HIV-1 virus possibly through the inhibition of its protease activity [25]. Saponins have also been shown to possess antitumor and anti-mutagenic activities and can lower the risk of human cancers, by preventing cancer cells from growing [26,27]. Saponins possess anti-inflammatory properties [28], anti-

cancer and anti-platelet aggregation activity [29]. Few studies have shown that saponins can cause apoptosis of leukemia cells by inducing mitotic arrest. Other roles of Saponins include: immune booster, reduction of bone loss and antioxidant effects. The foaming characteristics of saponins and in addition to its biological activities may be exploited in cosmetic, food and pharmaceutical industries.

Polyphenols present in the HS seeds are Catechin (5.14%) and Epicatechin (13.31%). Catechin is useful in treatment of heart diseases and improvement of blood pressure [30]. Catechin influences the molecular mechanisms associated with angiogenesis, degradation of extracellular matrix, the regulation of cell death, and multidrug resistance in malignant growths and related disorders [31]. Also, catechin and epicatechin can be effective in preventing coffee berry disease by the appressorial melanization of Colletotrichum kahawae [32]. The antioxidant and pro-oxidative activities of catechins can possibly influence malignancy signaling, contingent upon the bioavailability of catechins and context of the cellular condition [33]. Epicatechin and catechin flavonoids may confer protection against neurotoxic oxidative stress caused by the HIV-Tat protein [34]. Furthermore, Catechin has been shown to possess hypotensive and hypocholesterolemic potential [35,36].

The HS seeds were shown to contain 3.99% tannins. Tannins possess possible anti-carcinogenic effect [37], antimicrobial and antioxidant properties [38,39]. Tannins have been used for many years in folk medicine to treat gastric problems. The mechanism of action that explains why tannins improve gastric symptoms is based on their ability to chelate metals, antioxidant activity, and their complexation power with other molecules [40].

Anti-nutrients have the ability to control the nutritional and food qualities [41]. The anti-nutrients found in the HS seeds (Table 3) were: alkaloids (47.00 µg/ml; 39.20%), saponins (14.62 µg/ml; 12.19%), oxalates (14.06 µg/ml; 11.72%), tannin (4.79 µg/ml; 3.99%) and phytates (0.55 µg/ml; 0.46%).

Alkaloids were found in very high amount in HS seeds (47.00 µg/ml) and alkaloids have been shown to interfere with the body's ability to regulate the enzyme acetylcholine, responsible for conducting nerve impulses, leading to symptoms such as sweating, vomiting, diarrhoea and bronchospasm [42]. Alkaloids in high amounts also show toxicity in human [43,44].

Table 2. Quantitative phytochemical analysis of *Hibiscus sabdariffa* seeds

Parameters	Concentration (µg/ml)	Percentage composition (%)
Spartein	0.02	0.02
Anthocyanin	0.57	0.48
Oxalate	14.06	11.72
Tannin	4.79	3.99
Rutin	2.77	2.31
Lunamarin	4.50	3.75
Saponin	7.92	6.61
Sapogenin	6.70	5.59
Ribalidine	42.49	35.44
Kaempferol	6.16	0.46
Catechin	13.42	5.14
Phytate	0.56	11.19
Epicatechin	15.96	13.31

Table 3. Anti-nutrient composition of raw *Hibiscus sabdariffa* seeds

Parameters	Concentration (µg/ml)	Percentage composition (%)
Alkaloids	47.00	39.20
Saponins	14.62	12.19
Oxalates	14.06	11.72
Tannin	4.79	3.99
Phytates	0.55	0.46

Saponins (12.19%) were present in appreciable amount in the sample. Saponins have been implicated in some serious animal and human health conditions. Hypocholesterolemia is a disease caused when saponin binds with cholesterol in order to reduce its assimilation [45].

The oxalate level present in the HS seeds is (11.72%). Oxalates bind to calcium to form calcium oxalate crystals which are deposited as urinary stones that are associated with the renal tubule blockages and prevent its absorption in the human body [46]. According to Noonan and Savage [47], high oxalate levels in foods cause selective reabsorption or impaired absorption of some minerals like calcium and iron.

The presence of tannins is implicated for astringency in the taste of HS seeds, coupled with the obstruction of protein absorption through tannin precipitation [48]. Protein digestibility is affected by tannins due to the formation of complexes [49] and according Oakenfull and Sidhu [50], high consumption of tannins could be very injurious to the body. Tannins chelate iron and zinc limiting their absorption and furthermore interfere with digestion by displaying anti-trypsin and anti-amylase activity [51].

The phytate composition (0.55%) of the HS seeds is lower contrasted with other anti-nutrients. Phytate influences the intestinal uptake of minerals by forming stable complexes with dietary minerals, thus inducing the inadequacy of the mineral [52]. Phytate binds with various minerals such as magnesium, calcium, zinc and iron and thus cause increase in the mineral deficiency in digestive tract of animals [53]. However, given the low amount of phytate in the sample, the tendency of causing selective or impaired mineral reabsorption is highly reduced.

3.1 Antioxidant Property

The antioxidant vitamins contents shown in Table 4 are vitamin A (2.60 ± 0.07 mg/kg), vitamin C (2.76 ± 0.26 mg/kg) and vitamin E (5.06 mg/kg). These vitamins (A, C and E) are nutritive antioxidants which confer additional therapeutic functions to the seeds of *Hibiscus sabdariffa* by scavenging free radicals generated in the body. They do this by binding to them the free radicals in order to make them less toxic to the cell [54]. Vitamin C provides first line of defence against oxidative stress [55]. Antioxidants can donate electrons and thus, can inactive free radicals and convert them to less harmful compound like water [12]. Free radicals cause damage to organic molecules like proteins, nucleic acids and lipids. This ultimately results to cellular injury and consequently plays a role in the pathogenesis of certain diseases [12,56]. Hence, raw HS seeds may possess ameliorative potentials if augmented with other anti-oxidant rich plants against diseases linked with oxidative stress.

Table 4. Antioxidant vitamins of raw *Hibiscus sabdariffa seeds*

Parameter	Concentration (mg/kg)
Vitamin A	2.60 ± 0.07
Vitamin C	2.76 ± 0.26
Vitamin E	5.06 ± 0.30

Values are mean ± SD of triplicate determinations

The levels of enzymatic antioxidants such as CAT, SOD, Glutathione reductase (GR) and Peroxidase are shown in Table 5. The CAT level of HS seed was found to be (26.71±3.68 umol/ml). CAT seems, by all accounts, to be best protection against hydrogen peroxide radical. It is available in peroxisomes of about every single oxygen consuming cell and serves to shield the cell from the dangerous impacts of hydrogen peroxide by catalyzing its decay without the formation of free radicals [57].

Glutathione reductase level was found to be (24.43±0.78 mmol/ml). Glutathione antioxidant systems play a fundamental role in cellular defence against free radical and their oxidant species. Glutathione reacts with superoxide radical, peroxy radical and singlet oxygen followed by the formation of oxidized glutathione and other disulphides [58]. Rajan and Pushpa [59] also opined that seeds of *Syzygium cumini* and *Momordica charantia* have proven to be potent source of antioxidants in eradicating the free radicals.

Table 5. In vitro enzymatic antioxidants of raw *Hibiscus sabdariffa seeds*

Parameter	Concentration
Catalase mmol/ml	26.71 ± 3.68
SOD unit enzyme	0.88 ± 0.05
Peroxidase mmol/ml	13.29 ± 1.72
Glutathione reductase mmol/ml	24.43 ± 0.78

Values are Mean ± SD of triplicate determinations

4. CONCLUSION

Seeds of HS contain several phytochemicals which exist in great amounts. These phytochemicals are biologically important in several metabolic activities and normal functioning of the body. However, some of them may act as anti-nutrients that interfere with food absorption, digestibility and availability of useful nutrients. Furthermore, HS seeds contain certain biologically important antioxidants that can be exploited for nutritive and therapeutic purposes.

DISCLAIMER

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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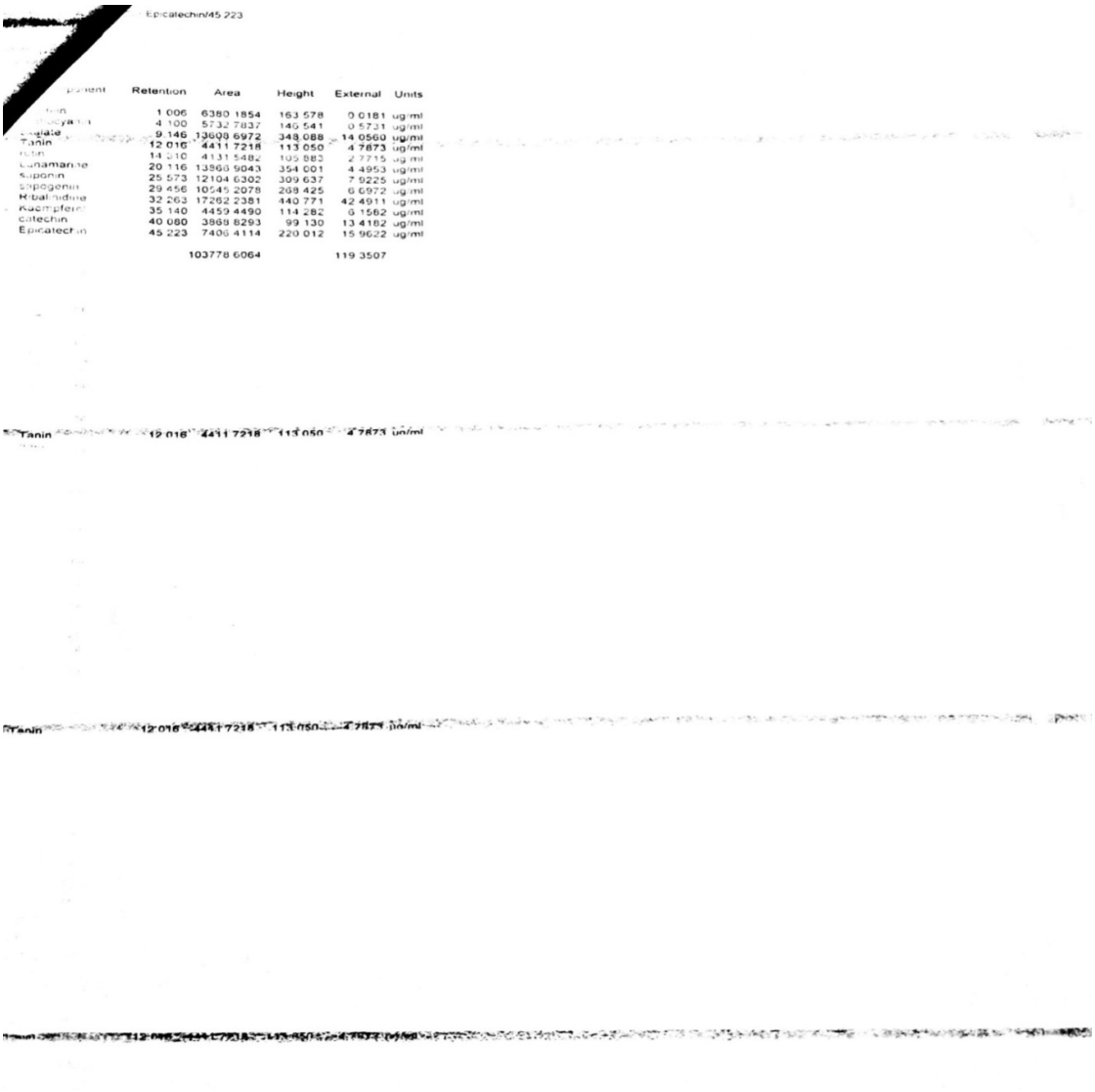
APPENDIX

Chromatogram of Phytochemical Analysis of Raw *Hibiscus sabdariffa* Seeds



Epicatechin/45.223

Retention	Area	Height	External	Units
1.006	6380.1854	163.578	0.0181	ug/ml
4.100	5732.7837	146.541	0.5731	ug/ml
9.146	13609.6972	348.088	14.0560	ug/ml
12.016	4411.7218	113.050	4.7873	ug/ml
14.310	4131.5482	105.883	2.7715	ug/ml
20.116	13563.5043	354.001	4.4953	ug/ml
25.573	12104.6302	309.637	7.5225	ug/ml
29.456	10545.2078	268.425	6.6972	ug/ml
32.263	17262.2381	440.771	42.4911	ug/ml
35.140	4459.4490	114.282	6.1562	ug/ml
40.080	3869.8293	99.130	13.4182	ug/ml
45.223	7406.4114	220.012	15.9622	ug/ml
	103778.6064		119.3507	

Tanin 12.016 4411.7218 113.050 4.7873 ug/ml

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