Research Article

iMedPub Journals
www.imedpub.com

Journal Nutraceuticals and Food Science

2021
Vol.6 No.3:11

Nutraceutical Properties of *Hibiscus sabdariffa* Stem and Leaf Extract as a Potential Alternative to Management of Clinical Manifestations Associated with Diabetes Mellitus

Ogo Agbor Ogo[1*], Onmata Gloria[2], Inalegwu Bawa[2] and Eru Ukonu Emmanuel[3]

1 Department of Biochemistry, College of Health Sciences, Benue State University, Makurdi, Nigeria
2 Department of Biochemistry, College of Science, University of Agriculture, Makurdi, Nigeria
3 Department of Physiology, College of Health Science, Benue State University Makurdi, Nigeria

***Corresponding author:** Ogo Agbor Ogo

oogo@bsum.edu.ng

Department of Biochemistry, College of Health Sciences, Benue State University, Makurdi, Nigeria

Citation: Ogo OA, Gloria O, Bawa I, Emmanuel EU (2021) Nutraceutical Properties of *Hibiscus sabdariffa* Stem and Leaf Extract as a Potential Alternative to Management of Clinical Manifestations Associated with Diabetes Mellitus. J Nutraceuticals Food Sci Vol.6 No.3:11.

Abstract

The calyxes of roselle (*Hibiscus sabdariffa L.*), popularly known as the zobo plant in Nigeria, are often used in the preparation of drinks for recreational and medicinal purposes. However, the seasonal availability makes utilization of this part of the plant sub-optimal. Thus, evaluating additional parts of the plant would be a useful venture aimed at maximizing the benefits of the plant for both nutritional cum therapeutic uses. Thus this study was carried out to assess the nutritional evaluation as well as therapeutic properties of stem and leaf methanolic extracts of *Hibiscus sabdariffa*. Nutritional properties were determined by carrying out proximate analysis, amino acid profile, and mineral content using standard procedures. Therapeutic properties were assessed by evaluating blood-sugar-lowering and antilipidemic effects of the extracts of roselle stem and leaf in alloxan-induced diabetic Wistar albino rats. Thirty experimental animals weighing between 180 gm and 220 gm were divided into five groups of six rats each and treated 28 days with blood samples taken every 7 days for biochemical assays. Results show that the proximate composition of the leaf showed a higher percentage of carbohydrate (33.73 ± 0.05%) and fat (20.50 ± 0.08%) compared to the stem, however, the latter recorded a higher percentage of protein (20.40 ± 0.03%), fiber (29.40 ± 0.22%) and ash (10.33 ± 0.18%), whereas moisture contents of both organs had similar values of (12.52 ± 0.03) and (12.30 ± 0.22) for leaf and stem respectively. Also, both parts contain a reasonable number of both essential and non-essential amino acids, indicating a wide distribution but varying degrees per 100 gm protein. Similarly, significant amounts of minerals including Fe, Ca, K, Mn, Mg, P, Cu, Se, and Zn in both leaf and stem, with values comparable to daily requirements were recorded. Furthermore, *H. sabdariffa* stem and leaf extracts each have a lowering effect on hallmarks of diabetic conditions including hyperglycemia, hyperlipidemia, and a concomitant increase in HDL level compared to the untreated group. Summarily, this study revealed that both stem and leaf of *H. sabdariffa* have beneficial significant nutritional components comparable to those reported for carlyxes, in addition to therapeutic properties and may serve as a potential source of functional ingredients required for optimal nutrition and health as nutraceuticals.

Keywords: *Hibiscus sabdariffa*; Nutritional Composition; Hyperglycemia; Dyslipidemia; Nutraceutical.

Received: March 04, 2021; **Accepted:** March 15, 2021; **Published:** March 22, 2021

Introduction

Roselle (*Hibiscus sabdariffa L*) is a bushy annual flower plant grown in many parts of the world including Nigeria, where it is popularly known as the "zobo" plant. It is an important vegetable of the Malvaceae family together with Okra. It is suggested that all parts of roselle may be important sources of food and raw materials for foreign exchange [1]. However, the most common

commercially important part of the plant is the fleshy calyx (sepals) surrounding the fruit (capsules). The whole plant can be used as a beverage, or the dried calyces can be soaked in water to prepare a colorful refreshing drink or may be boiled in water and taken as a hot drink [2]. It has been reported to be medicinal having diuretic, cholerectic, febrifugal, and hypotensive properties; decreasing the viscosity of the blood and stimulating intestinal peristalsis as reported by Alarcon-Alonso et al.[3]. Numerous research findings have reported that roselle calyx extract can also lower blood glucose levels in experimental animal models of Diabetic Mellitus by increasing the activity of catalase and glutathione enzymes. In histological experiments, roselle caused osmotic diuresis in the proximal renal tubule of diabetic animals as shown Jiménez-Ferrer et al. [4]. This medicinal importance of roselle calyxes is in addition to the nutritional value being the reason behind the drink made from it. Unfortunately, the calyxes are seasonal and not available all year round for these benefits, making it imperative to utilize other parts of the plant that may have comparable benefits. Furthermore, evidence-based information on the leaf is scanty whereas that for the stem is unavailable. Consequently, this study was designed to bridge this gap in knowledge by investigating the nutritional composition as well as the therapeutic potential of the leaf and stem extract of *H. sabdariffa* by evaluating the effects on blood sugar and lipids in alloxan-induced diabetic model rats.

Materials and Methods

Collection and preparation of plant materials and extraction

Fresh roselle (*Hibiscus Sabdariffa*) leaves and stem was collected and identified at the Department of Botany, University of Agriculture, with voucher number FUAM/BOT/00513 and a sample deposited at the university herbarium. The fresh leaves and stem were cleaned (sorted), washed under running water, drained, and air-dried in the shade for 7 days before extraction. The extracts were made by soaking 100 gm of the pulverized leaf and stem in 1000 ml of methanol and allowed to stand 48 hours. The extract was evaporated to complete dryness with a rotary evaporator for 24 hours, while sticky concentrate of the samples was reclaimed and stored in the refrigerator for further use.

Proximate analysis

The proximate composition was determined according to the standard methods described by the Association of Analytical Chemists [5]. Moisture content was determined by oven-drying the samples at 105℃ until a constant weight was obtained. Ash content was obtained by taking the difference in mass before and after incineration of oven-dried samples in a muffle furnance at >500℃. Total carbon and nitrogen contents (expressed as % dry mass) were determined by combustion on the freeze-dried samples that were previously pulverized using an elemental analyzer (Thermo Scientific Flash EA 1112). Crude protein of each sample was estimated by multiplying the total nitrogen content, N by 6.25 factor, whereas, total carbohydrate was calculated by different methods thus: Total carbohydrate=100% - (%crude fiber+ %protein+ %fat+ %ash+ %moisture).

Determination of amino acid profile

The Amino Acid profile of samples was determined using the previously adopted method by Ogo et al. [6,7]. The *H. sabdariffa* leaves and stem were dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator, and loaded into the Applied Biosystems PTH Amino Acid Analyzer model (120 APTH). About 4 gm of the sample was put in an extraction thimble and extracted for 15 hours in the soxhlet extraction apparatus. 1 gm of the defatted sample was weighed into a glass ampoule and 7 ml of 6N HCl was added and oxygen expelled by passing nitrogen into the ampoule to avoid possible oxidation of sulfur-containing amino acids including methionine and cysteine. The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105℃ for 22 h. The ampoule was allowed to cool before being broken open at the tip and the content filtered to remove the humins. The filtrate was then evaporated to dryness using a rotary evaporator and the residue dissolved with 5 ml acetate buffer of pH 2 before being cooled. About 60 µL of the preparation mixture was dispensed into the cartridge of the amino acid analyzer (model 120 APTH), which is designed to separate and analyze free acidic, neutral, and basic acids of the hydrolysate. The peak area which corresponds to the concentration of each amino acid was calculated from the integrator attached to the analyzer thus:

$$Concentration\ of\ amino\ acid\left(g/100\ gm\ protein\right)=NH\times width\times\frac{NH}{2}\times Sstd\times C$$

Where,

$$C=\frac{Dilution\times 16}{SampleWt\left(gm\right)\times N\%\times vol.\text{loaded}}\div NH\times W\left(nleu\right)$$

S_{std}=$NE_{std\times}$ Mol. Weight × µ AA_{std}

Tryptophan chemically decomposes during acid hydrolysis, therefore, it was prepared separately by using thioglycolic acid with 6 N hydrochloric acid to preserve it. The tryptophan in the sample was hydrolyzed with 4.2 M sodium hydroxide before further determination.

Determination of mineral composition

One gram (1 gm) of each sample was digested by adding 15 ml of tri-acid mixture (HNO_3, H_2SO_4, and $HClO_4$) in 5:1:1 ratio and heated to 80℃ until a transparent solution was obtained. After cooling, the digested sample was filtered using Whatman No. 42 filter paper and the filtrate was made up to 50 mL with distilled water. The samples were then analyzed using atomic absorption spectrophotometer (Perkin-Elmer 8650). Ten minerals including iron (Fe), calcium (Ca), potassium (K), manganese (Mn), magnesium (Mg), phosphorus (P), selenium (Se), copper (Cu), zinc (Zn), and sodium (Na) were analyzed.

Experimental animals and study design

Thirty (30) male albino rats weighing 220±25 gm acquired from the animal house of College of Health Sciences, Benue State University, were maintained at standard conditions at the Department of Biochemistry, the University of Agriculture Makurdi with 12 hours light/12 hours dark cycle and allowed to acclimatize for a week. They were also allowed access to standard feeds (Chikum Feeds Ltd., Lagos) and water ad libitum. Approved institutional procedures for animal care and treatment were strictly observed. The animals were grouped into 5 containing 6 rats each and described as follows:

Group I: NC- Normal Control

Group II: NC+Alloxan: induced with alloxan but not treated

Group III: Diabetic+glibenclamide: induced with alloxan; treated with clamide (5 mg/kg).

Group IV: Diabetic+HS Stem: induced with alloxan; treated with HS Stem extract (200 mg/kg)

Group V: Diabetic+HS leaf: induced with alloxan; treated with HS leaf extract (200 mg/kg)

Induction, treatment and preparation of blood samples for biochemical assays

The animals in group II-V were induced through intraperitoneal injection with a single dose of alloxan monohydrate (100 mg/kg body weight) dissolved in normal saline. Rats were confirmed diabetic with measurement of blood sugar level after 72 hours before commencement of treatment. Blood samples were collected after a 4-week treatment course by intra-cardiac puncture into plain sterilized tubes and centrifuged at 300 rpm for 15 minutes. The serum samples obtained were stored at -4°C for biochemical analysis using test assay kits according to the manufacturer's guidelines.

Statistical analysis

Data were expressed as mean ± standard deviation and analyzed by the Analysis of Variance (ANOVA) using statistical package for social sciences version 20 (SPSS Inc., Chicago, USA). The difference between the extracts and the albino rats groups was compared using Duncan's Post Hoc Test. A p-value less than 0.05 were considered significant.

Results

Proximate composition of *Hibiscus sabdariffa* leaf and stem

Results of the proximate composition of *Hibiscus sabdariffa* leaf and stem are represented in **Figure 1.** The result shows that total crude protein and dietary fiber were significantly higher in the stem than in the leaves on one hand, while the total fat and carbohydrates were higher in the leaves than the stem.

Amino acid profile of *Hibiscus sabdariffa* Leaf and stem

The results of amino acid profile of *Hibiscus sabdariffa* leaves and stem are presented in **Table 1**. Showing the various amino acids and, calculated, total essential amino-acid, total conditional amino acids, total non-essential amino acids, and the total amino acid content. The result shows that glutamic acid has the highest amount while tryptophan was recorded the least in both samples studied.

Table 1: Amino acid profile of *H.sabdariffa* leaf and stem.

Amino Acid	Leaf (gm/100 gm protein)	Stem (gm/100 gm protein)
Leucine	8.06 ± 0.00	5.84 ± 0.01
Lysine	4.14 ± 0.00	5.14 ± 0.00
Isoleucine	4.26 ± 0.01	3.93 ± 0.01
Phenylalanine	4.26 ± 0.01	4.97 ± 0.01
Tryptophan	0.97 ± 0.00	1.05 ± 0.00
Valine	4.62 ± 0.01	4.85 ± 0.00
Methionine	1.02 ± 0.00	0.91 ± 0.00
Proline	3.86 ± 0.00	4.06 ± 0.01
Arginine	5.08 ± 0.00	6.19 ± 0.00
Tyrosine	2.75 ± 0.00	2.92 ± 0.00
Histidine	2.24 ± 0.01	2.36 ± 0.01
Cysteine	0.97 ± 0.01	1.21 ± 0.01
Alanine	3.87 ± 0.00	4.10 ± 0.01
Glutamic acid	10.30 ± 0.00	11.20 ± 0.01
Glycine	4.01 ± 0.01	3.92 ± 0.01
Threonine	2.89 ± 0.01	3.00 ± 0.00
Serine	2.54 ± 0.01	2.30 ± 0.00
Aspartic acid	8.22 ± 0.00	9.37 ± 0.00
TEAA	32.46	32.05
TCAA	19.24	20.57
TNEAA	41.6	45.27
TAA	93.3	98.89

Values are mean ± SD, for two independent determinations

TEAA: Total Essential Amino Acid

TCAA: Total Conditional Amino Acid

TNEAA: Total Non-Essential Amino Acid

TAA: Total Amino Acid

Mineral contents of *Hibiscus sabdariffa* leaves and stems

The concentration of minerals in *H. sabdariffa* leaf and stem recorded in 1 Kg of sample is presented in **Figure 2.**

Effect of *H. sabdariffa* leaf and stem extract treatment on glucose level of alloxan-induced diabetic rats

The result of glucose levels of animals following treatment with extracts of *H. sabdariffa* leaf and stem is presented in **Table 2.** It revealed that both leaf and stem extracts had a

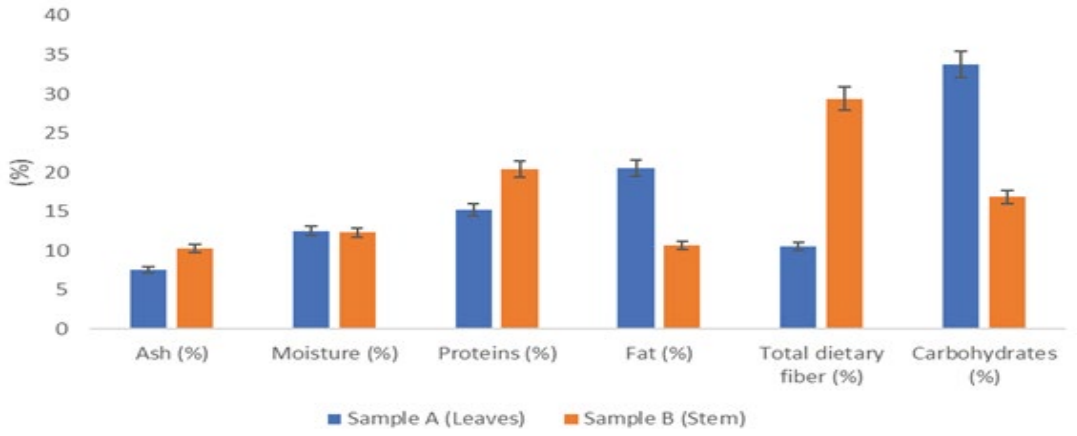

Figure 1 Proximate composition of H. sabdariffa stem and leaf.

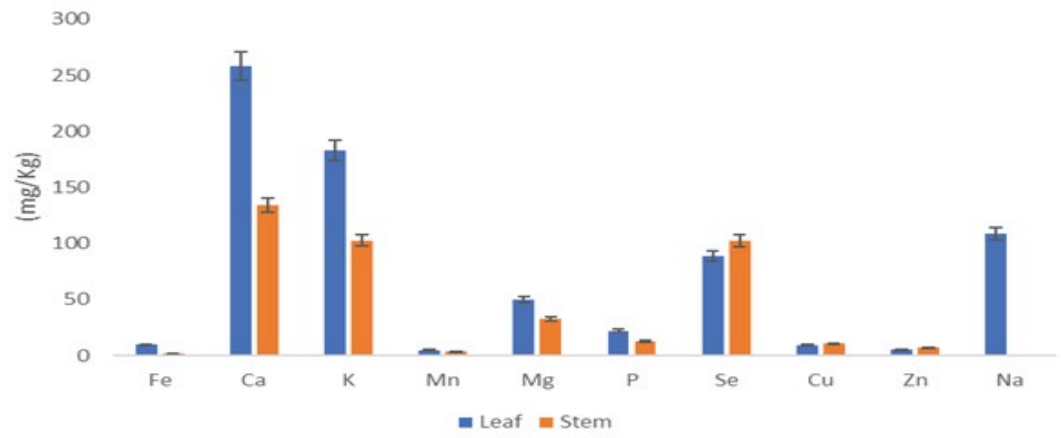

Figure 2 Mineral content in *H. sabdariffa* leaf and stem.

Table 2: Effect of *H. sabdariffa* leaf and stem extract treatment on glucose level of alloxan-induced Diabetic rats.

Treatment	Blood Glucose Level (mg/dL)					
	Normal	Diabetic (After 72 hours)	Week I	Week II	Week III	Week IV
NC	70.0 ± 2.6^{a}	108.8 ± 1.8^{a}	105.0 ± 3.7^{a}	97.0 ± 3.3^{a}	89.0±2.9^{a}	78.5 ± 2.0^{a}
NC+Alloxan	77.3 ± 3.4^{a}	262.0 ± 2.8^{b}	332 ± 3.1^{c}	338 ± 2.3^{c}	296 ± 3.0^{d}	287 ± 3.2^{d}
Diabetic+Glibenclamide	95.0 ± 3.6^{b}	326.8 ± 2.9^{c}	313.4 ± 2.6^{b}	242.3 ± 3.1^{b}	168.3 ± 2.7^{b}	158.8 ± 3.5^{b}
Diabetic+HS Stem	92.3 ± 3.0^{b}	266.5 ± 2.4^{b}	256.5 ± 1.8^{c}	233.0 ± 1.9^{b}	177 ± 2.8^{c}	149.3 ± 2.5^{b}
Diabetic+HS Leaf	92.5 ± 3.3^{b}	315.0 ± 3.0^{c}	296.3 ± 3.4^{c}	220.0 ± 2.4^{b}	185.5 ± 3.2^{c}	143.5 ± 2.9^{b}

significant lowering effect on increased sugar level in a manner comparable to the effect of the standard drug, Glibenclamide.

Values are presented as mean ± SD for 2 determinations. Values with different superscripts across the row are statistically significant at p<0.05.

Effect of *H. sabdariffa* leaf and stem extract treatment on lipid profile of alloxan-induced diabetic rats

The result of serum lipid profile following treatment with extracts of *H. sabdariffa* leaf and stem is presented in **Table 3.** It shows that both leaf and stem extracts had a significant lowering

Table 3: Effect of *H. sabdariffa* leaf and stem extract treatment on serum lipid profile of alloxan-induced diabetic rats.

Treatment	Lipid profile (mg/dL)			
	T-CHL	TAG	HDL-C	LDL-C
NC	136.4 ± 3.4^{b}	107.6 ± 2.5^{c}	87.7 ± 3.2^{a}	93.1 ± 2.6^{b}
NC+Alloxan)	192.7 ± 1.8^{c}	135.6 ± 3.1^{d}	64.6 ± 2.1^{b}	137.6 ± 2.0^{c}
Diabetic+Glibenclamide	139.5 ± 2.4^{b}	107.6 ± 2.5^{c}	78.1 ± 2.5^{a}	113.0 ± 1.9^{b}
Diabetic+*HS*Stem	123.9 ± 3.0^{a}	90.6 ± 2.1^{b}	72.0 ± 3.3^{c}	81.8 ± 2.4^{a}
Diabetic+*HS*Leaf	126.4 ± 3.3^{a}	71.5 ± 2.3^{a}	71.3 ± 3.1^{c}	80.6 ± 3.3^{a}

effect on increased total cholesterol, triglycerides, and low-density lipoprotein levels while showing a significantly increased effect on high-density lipoprotein levels compared to alloxan-induced control rats.

Values are presented as mean ± SD for 2 determinations. Values with different superscripts across the row are statistically significant at $p<0.05$.

Discussion

The nutritional composition as well as the therapeutic potential of roselle stem and leaf extracts on hallmarks of diabetes including hyperglycemic and dyslipidemia induced by aloxan in Wistar albino rats were investigated in this study. The results for proximate composition show that the ash, protein, and fiber contents of *H. sabdariffa* stem are higher than that of the leaves. On the contrary, the leaves recorded higher contents of moisture, crude fat, and total carbohydrate, which are similar to the results obtained by Ayodeji et al. [8] who analyzed the proximate composition of *H.sabdariffa* leaves along with other leaves, and those of Amin et al.[9]that determined the nutritional composition of *H. sabdariffa* seeds. The range of protein content in the stem recorded in this study was comparable to values obtained in other studies carried out by Getsoet al. [10], Adebayo-Tayo and Samuel [11] and Shagalet al. [12] but significantly different from those obtained in nutritional assessment of Indian roselle seeds by Bako et al. [13] and Mabrouk et al. [14] for analysis of the nutritional composition of roselle leaves, calyxes, and seeds respectively. Although variations in values exist in some previous measurements and the present study owing to differences in geography and season as well as species used, the results indicate that this flowering plant appears to be a complete reservoir of both macro and micronutrients. Thus, supplementation in children's food with products made from these roselle parts could help upgrade their nutritional requirements necessary to mitigate disturbing incidences of malnutrition that appear to be on the increase, particularly in Sub-Saharan Africa.

Owing to the significant amount of protein detected in the samples from proximate analysis, further analysis was carried out to determine the amino acids that may be present in the plant parts studied. About seventeen essential and non-essential amino acids were detected in both the leaf and stem-like previous findings of the amino acid composition of HS seeds by Amin et al. [15]. Interestingly, the stem recorded high values in the amounts of essential amino acids such as leucine, isoleucine, valine, lysine, and phenylalanine comparable to those found in the leaf. Total Amino Acids (TAA) found in the stem was higher than those found in the leaves, which is similarly higher than those found by Adubiaro et al. [16]. This result is promising as it could give additional nutritional benefits when food is consistently supplemented with products from these plant parts for improved nutrient enrichment as used in biscuit supplementation by Walaaet al. [17]. The nutritional value of food item is also determined by its mineral contents, thus the study investigated the elemental composition of roselle leaf and stem and found higher concentrations of Fe, Ca, K, P, and Na in leaf sample, while the stem recorded a higher concentration for Se, Cu, and Zn. These results align with the findings of Anhwange et al. [18]. Who found significant amounts of P, Ca, and Mg in roselle. Minerals in foods are essential for many critical cellular and metabolic processes either as components of regulatory proteins or as cofactors of enzymes that catalyze reactions for the sustenance of an organism. In addition to providing nutritional benefits, the leaf and stem of HS can also be used for therapeutic purposes as the significant composition of sodium and potassium may be adequate in replenishing lost electrolytes during diarrhoeal episodes.

Therapeutic effects of roselle leaf and stem extracts were investigated using experimental animal models that were induced to develop diabetes manifested by increases in blood sugar levels as well as a disproportional lipid profile. Rats that developed these features exhibited an increase in weight and body mass index (results not included), which was attenuated by treatment with extracts from roselle stem and leaf in a manner that agrees with the findings documented by Carvajal-Zarrabal et al. [19]. The sugar lowering effect of roselle stem and leaf extracts was tested weekly on alloxan-induced rats and was found to be effective in comparison with the effect of the standard drug used. The antidiabetic effect of roselle calyx extract has been previously reported but the present study informs the use of other parts in the management of increased blood sugar levels associated with diabetic conditions. Other studies such as Mardiah et al. [20], Zainalabidin et al. [21], and Nana Su et al. [22] reported sugar-lowering effects of HS on experimental animals, thus increasing evidence in support of its use as a robust agent for alternative management of sugar surges that accompany Diabetes Mellitus. Diabetes Mellitus is also characterized by dyslipidemia as having been severally reported. Our results significantly attenuated

changes in lipid cocktail including cholesterol, TAG, and LDL, while increasing HDL in treated rats to untreated diabetic rats. The results show a fashion consistent with reports of Ochani et al. [23], FarombiandIge [24], and Adewoye et al. [25], showing consistency in the ability of extracts of HS in regulating abnormal lipid levels associated with Diabetes Mellitus and could be an effective alternative treatment.

Conclusion

This study document for the first time among other findings information about the nutritional quality of *H. sabdariffa* stem and leaf similar to the composition of more widely analyzed calyxes and could serve additional nutritional benefits to consumers *via* food supplementation. Further studies would explore the antioxidant potential of this part with the view to optimizing its benefits in nutrition. Also, this study shows that the methanolic extracts of *H. sabdariffa* stem and leaf have comparable effects on altered glucose and lipid levels associated with diabetes and could be used for alternative management, thus supporting the nutraceutical properties of *H. sabdariffa*.

References

1. Nnebue OM, Ogoke IJ, Obilo OP, Agu CM, Ihejirika GO, et al. (2014). Estimation of planting dates for roselle (*Hibiscus sabdariffa l.*) in the humid tropical environment of Owerri, Southeastern Nigeria. Afe J Ol 14:168-178.
2. Salami SO, Afolayan AJ (2010) Suitability of Roselle-*Hibiscus sabdariffa L*. as raw materials for soft drink production. J Food Qual 4: 1-9.
3. Alarcón AJ, Zamilpa A, Aguilar FA, Herrera RM, Tortoriello J, et al. (2012). Pharmacological characterization of the diuretic effect of *Hibiscus sabdariffa* Linn (Malvaceae) extract. J Ethnopharmacol 139: 751-756.
4. Jiménez FE, Alarcón AJ, Aguilar RA, Zamilpa A, Jiménez FCI, et al.(2012). Diuretic effect of compounds from Hibiscus sabdariffa by modulation of the aldosterone activity. PlantaMed 78: 1893-1898.
5. AOAC (2010). Official methods of analysis of the association of official analytical chemists, 18th Ed Washington D.C.
6. Ogo AO, Kparev M, Amali EOO, Efiong EE, Obochi GO, et al. (2019). Comparative nutritional assessment of products made from fresh and dried tiger nut sold in Makurdi Metropolis. NAPAS 1: 137-143.
7. Ogo AO, Amali EO, Efiong EE, Gbaa A, Enenche D et al. (2019). Optimising soymilk protein nutriture through selection of appropriate processing technique. NAPAS 1: 61-67.
8. Ayodeji PO, Aniebet TA, Onoja US, Olanrewaju OI (2018). Proximate, mineral and vitamin composition of Sorghum Bicolor, *Carica Papaya* and *Hibiscus Sabdariffa* leaves. IOSR-J Env Sci Toxico Food Technol 12: 85-89.
9. Amin I, Emmy HKI, Halimatul SM (2008). Roselle seed: Nutritional composition and protein quality and health benefit. Food 2: 1-16.
10. Getso MM, Sallau MS, Abechi SE, Uba S (2018). The influence of mineral fertilizers in nutrients and supplementation of qualitative calyx production of roselle (*Hibiscus Sabdariffa*). Bayero J Pure Appl Sci 11: 198-204.
11. Adebayo TBC, Samuel UA (2009). Microbial quality and proximate composition of dried *Hibiscus Sabdariffa* Calyxes in Uyo, Eastern Nigeria. Malaysian J Microbiol 5: 13-18.
12. Kubmarawa MH, Shagal AM, Magomya DA,Kitan (2013). Proximate composition of *Hibiscus sabdariffa* and Amaranthuscaudatus. J Med Biomed Sci. 4: 37-41.
13. Bako RK, Chavan UD (2016). Physical properties and nutritional potentials of Indian Roselle (*Hibiscus Sabdariffa L*) Seeds. Int J Curr Res. 8: 38644-38648.
14. Mabrouk OM, Ayat MM, Waffa AA, Abou Samaha (2016). Physical properties, microstructure and proximate chemical composition of Roselle (*Hibiscus sabdariffa*) seeds and kernels. Alex J Fd Sci Technol. 13: 19-32.
15. Hainida KI, Amin HN, Norman MS (2008). Nutritional and amino acid contents of differently treated Roselle (*Hibiscus sabdariffa* L.) seeds. Food Chem 111: 906-911.
16. Adubiaro HO, Ogunbusola EM, Olaleye AA (2017). The amino acid composition of smooth loofah (luffa cylindrical l.), roselle (*Hibiscus Sabdariffa*) and sesame (*SesamumIndicum*) seeds. FUW Trend Sci Technol J 2: 85-88.
17. Walaa ME, El-Kalyoubi MH, Mostafa MM, Salwa A Farghal (2019) Nutritional evaluation of Roselle (*Hibiscus sabdariffa* l.) And its application in biscuit supplementation. Arab Univ J Agric Sci 27: 2563-2571.
18. Anhwange BA, Ajibola VO, Okibe FG (2006). Nutritive value and antinutritional factors in *Hibiscus sabdariffa*. *J Fish* Int, 1:73-76.
19. Carvajal Zarrabal O, Hayward Jones PM, Orta Flores Z, Nolasco HipólitoC, Barradas Dermitz DM, et al. (2009). Effect of *Hibiscus sabdariffa* L. Dried calyx ethanol extract on fat absorption-excretion, and body weight Implication in Rats. J Biomed Biotechnol 2009: 1-5.
20. Mardiah F, Rungkat Z, Endang P, Rizal SD (2014). The effect of roselle extract (*Hibiscus sabdariffa* Linn) on Blood Glucose Level and Total Antioxidant Level on Diabetic Rat Induced by Streptozotocin. IOSR J Pharma 4: 8-16.
21. Zainalabidin S, Budin SB, Anuar NNM, Yusoff NA,Yusof NLM (2018). *Hibiscus sabdariffa* Linn. Improves the aortic damage in diabetic rats by acting as antioxidant. J. Applied Pharm Sci. 8: 108-114.
22. Nana Su, Jinglei Li, Liuqing Yang, GuohuaHou, Ming Ye (2018). Hypoglycemic and hypolipidemic effects of fermented milks with added roselle (*Hibiscus sabdariffa L.*) extract. J Functi Food 43: 234-241.
23. Ochani PC, D'Mello P (2009). Antioxidant and antihyperlipidemic activity of *Hibiscus sabdariffa* Linn. Leaves and calyces extracts in rats. Indian J ExpBiol 47: 276-282.
24. Farombi EO, Ige OO (2007) Hypolipidemic and antioxidant effects of ethanolic extract from dried calyx of *Hibiscus sabdariffa* in alloxan-induced diabetic rats. Fundam Clin Pharmacol 21: 601-609.
25. Adewoye EO, Ige AO. (2016). Lipid profile and electrolyte composition in diabetic rats treated with leaf extract of musa sapientum, J Diet Suppl. 13: 106-117.