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COMPARATIVE EVALUATION OF NUTRIENTS AND ANTI-NUTRIENTS CONTENTS OF THREE VARIETIES OF *HIBISCUS SABDARIFFA LINN* CALYCES

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ABSTRACT

The nutrients and anti-nutrients contents of H. sabdariffa linn (roselle) calyces of dark red, red and green varieties sourced from Zuru, Kebbi State, Nigeria, were sun-dried and grounded into powder form and then analysed in triplicate. Nutrients contents were assessed by proximate analyses, minerals by atomic absorption spectrophotometer, flame photometer and spectrophotometer. Vitamin C was analysed spectrophotometry while anti-nutrients (phytic acid, oxalate and nitrate) by titrimetric, saponin by gravimetric and cyanogenic glycoside by spectrophotometry. Dark red calyx was rich in carbohydrates (37.67%) and had the highest crude lipid content (2.36%). The highest levels of ash, moisture, fibre and proteins were observed in the green calyx (9.50%), green calyx (8.83%), red calyx (11.00%) and dark red calyx (9.86%) respectively. Calorific values for all calyces were between 142.48 and 211.12 kcal. The mean minerals values in most abundant were Calcium, magnesium and potassium with levels ranging from 91.04 to 208.46 mg /100g. The highest levels of Calcium, magnesium and potassium were observed in the red calyx (208.46

mg/100g and 203.68 mg/100g) and dark red calyx (133.33 mg/100g) respectively. Sodium, manganese, phosphorus, iron, zinc and copper were present in small amounts. Vitamin C levels ranged from 77.13 to 108.66 mg/100 g, with the highest level in the green calyx (108.66 mg/100g). Oxalate, cyanogenic glycoside and nitrate were present at appreciable levels than phytate and saponin, in green calyx (405.00 mg/100g), dark red calyx (355.88 mg/100g and 51.00 mg/100g) respectively. In conclusion, the results indicated that these calyces are good sources of valuable nutrients with lower anti-nutrients, which is processed and consumed could contribute towards meeting the human nutritional requirement.

KEYWORDS: Nutrients, anti-nutrients, three varieties, roselle, calyces.

1. INTRODUCTION

African vegetables were reported to provide essential nutritional requirements, such as carbohydrates, fats, proteins, vitamins and mineral for human and animals' consumption., and also serve as a source of income and raw material to be utilized by various industries.[1] It was reported that the disease catastrophic associated with nutritional deficiencies, including scurvy, rickets and night blindness, which are commonly found in Africans, can be alleviated to a lesser extent by proper consumption of these vegetables.[2] *Hibiscus sabdariffa linn* commonly called roselle is an annual erect, bushy plant with the cylindrical stem. The plant is commonly found in the North and middle belt regions of Nigeria, probably because of the climate condition.[3] Typically, soil supplied with rich organic manure and essential nutrients favours the survival of this plant.[4] It can grow relatively in high temperature and requires an optimum rainfall of approximately 45-50cm^3.[5] The plant has been recognized and utilized by millions of people from the West African sub-region, particularly among the youths, who considered it as calyces drinks during the social gathering.[5] Furthermore, it also serves as resources of food, medicine, food supplement and other organic compounds.[6]

The nutrients contents of *H. sabdariffa* leaves, seeds and calyces were reported in several studies, possibly due to differences in genetic, environment, ecology and harvesting conditions.[7] The roselle calyces are obtained from its capsules containing in the seeds and are used for the preparation of herbal drinks, beverages, jams and jellies.[8] Additionally, calyces of *H. sabdarifta* were reported to be rich in vitamins, carbohydrate, protein, antioxidants and minerals.[9]

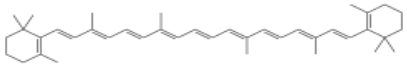

Figure 1: Structure of β-caroteine (antioxidant).

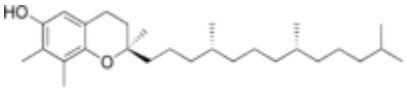

Figure 2: Structure of γ-tocopherol.

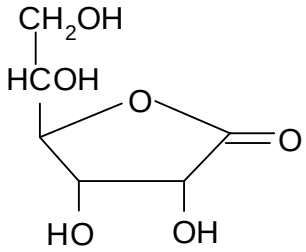

Figure 3: Structure of ascorbic acid.

Though reported to be nutritious, but also contained anti-nutritional factors, that are naturally found, such as phytic acid, saponin, oxalic acid, tannin, nitrate, which alter the absorptions of vitamins, minerals and other nutrients.[5]

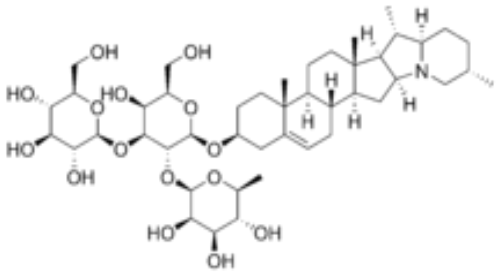

Figure 4: Structure of Saponin.

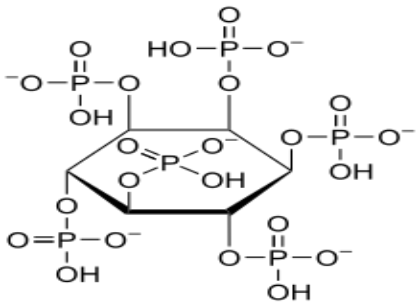

Figure 5: Structure of phytate.

However, some of these anti-nutrients compounds have benefits as opposed to most harmful effects, as some are reported to be an anti-cancer agent.[4]

This study was aimed to investigate the nutrients and anti-nutrients contents of three forms of *H. sabdarifta* calyces (the red, dark red and green) with a view to provide data for dietary planning.

Plate 1: Green roselle.

Plate 2: Red roselle.

Plate 3: Dark red roselle.

2. MATERIAL AND METHODS

2.1 Sample Collection and Preparation

Six hundred grams (200g of each) matured sun-dried *H. sabdariffa* (dark red, red and green) calyces were used for this study. They were obtained in fresh form from the nine (9) local farmers, including Gwammawa, Rikoto and Zanga site, in Zuru Local Government Area of Kebbi State, Nigeria, in October 2019. The sample compounds were characterized at Botany unit, Usmanu Danfodiyo University, Sokoto, with the voucher number giving as (UDUH/ANS/0219). Dried loose roselle calyces of the red, dark red and green varieties were cleaned and then 100 grams of each was weighed and grounded to fine powder that passed sieve of 1 mm diameter.[10]

2.2 Methods

2.2.1 Proximate Analyses

The proximate analyses of Roselle calyces were analyzed according to AOAC.[10] The moisture content was obtained through drying in an oven (SM 9053, uniscope, England) at 105°C and ash were analyzed by weighing the incinerated residue obtained in a furnace (Lenton Furnace SN 4422, England) at 600°C for 3 hours. The total crude protein was also analyzed by the Kjeldahl method (kjeltec 2100, Foss, Sweden) while the crude protein was

calculated as % N_2 (Nitrogen) x6.25. The total lipid was determined by Soxhlet method and an available carbohydrate was estimated by anthrone reagent method.[11]

2.2.2 Macro and Micro Minerals Analyses

The powdered samples (2.0 g) of each was put in a crucible and placed into a muffle furnace and ashed at 600°C for three hours. After cooling Ten millilitres of 5.0N HCl was then added to the ash to digest the minerals. The digest was filtered and transferred to 50 cm^3 volumetric flask, and the elements were determined by Atomic Absorption Spectrophotometer (A A 6300 Shimadzu Model, England). The flame photometer (Model 400, Corning U.K.) was applied for potassium (K) and sodium (Na) determination while phosphorous was determined by the vanodo-molybdate method using spectrophotometer (optima sp- 300 model) at 660 nm according to the method described by AOAC.[10]

2.2.3 Ascorbic Acid Analyses

The ascorbic acid (vitamin C) was analyzed according to Rutkowski and Grzegorczyk method.[12]

2.2.4 Anti-nutrients Analyses

Anti-nutritional factors, *viz.* phytate, nitrate, cyanogenic glycoside, saponin and oxalate were analyzed. The phytic acid, oxalate and nitrate content were determined by titration method adapted by.[13] The saponin was obtained by gravimetric method and cyanogenic glycoside was determined by colourimetric method following the AOAC method.[10]

3. Statistical Analyses

The data generated were expressed as Mean ± standard deviation of triplicate determinations using analyses of variance (ANOVA) with smart view statistical package.[14] A p-value of < 0.05 was considered as statistically significant among the mean values.

4. RESULTS

The results of the analysis for proximate, minerals, vitamin C and anti-nutritive contents of the calyces of three varieties of *H. sabdariffa linn* (roselle) were summarized in Tables 1, 2 and 3 respectively**.**

4.1 Proximate contents

The results of the mean proximate content of the three varieties of *H. sabdariffa* calyces are presented in Table 1. The varieties had mean protein content ranged from 7.70 to 9.86%,

fibre from 9.33 to 11.0%, available carbohydrate from 22.67 to 37.67%, lipid from 2.07 to 2.36%, ash from 550 to 9.50% and moisture from 8.00 to 8.83%. There were significant differences ($p<0.05$) in the mean values of protein, fibre and available carbohydrate among the three varieties.

4.2 Minerals contents

The results of the mean minerals values of the three varieties of *H. sabdariffa* calyces are presented in Table 2 in mg/100g. The varieties had mean Na content ranged from 3.63 to 4.20, K from 117.00 to 133.33, Ca from 91.04 to 208.46, Mg from 199.49 to 203.68, P from 4.10 to 4.30, Fe from 2.06 to 2.27, Zn from 1. 75 to 3.56 and Mn from 3.42 to 5.31. There were significant differences ($p<0.05$) in the mean values of Na, K, P, Zn and Mn among the three varieties. The difference was observed between dark red and red for Na, dark red and green as well as red and green for K. Similarly, the difference was observed between dark red and green as well as dark red and red for Zn and Mn. For P, the difference was observed between the red and green.

4.3 Anti-Nutrients and Ascorbic acid Contents

The results of the mean anti-nutrients contents of the three varieties of *H. sabdariffa* calyces are presented in Table 3 in mg/100g. The varieties had mean Cyanogenic glycoside content ranged from 147.44 to 355.88, nitrate from 27.50 to 51.00, oxalate from 180.00 to 405.00, phytate from 1.41 to 2.28, a saponin from 7.33 to 7.67 and ascorbic acid from 77.13 to 108.66. No significant differences ($p<0.05$) in the mean values of phytate, saponin and ascorbic acid among the three varieties.

Table 1: Proximate Content of the three varieties of *H. sabdariffa linn* calyces in percentage (%) dry basis.

	Calyx types		
Parameter	**Dark red**	**Red**	**Green**
Moisture	8.33 ± 2.47^{a}	8.00 ± 1.32^{a}	8.83 ± 2.93^{a}
Ash	6.83 ± 2.02^{a}	5.50 ± 0.50^{a}	9.50 ± 2.65^{b}
Crude Lipid	2.36 ± 0.31^{a}	2.33 ± 0.29^{a}	2.07 ± 0.40^{a}
Crude Fibre	9.33 ± 0.29^{a}	11.00 ± 0.87^{b}	10.33 ± 0.58^{ab}
Crude Protein	9.86 ± 0.20^{a}	7.70 ± 0.18^{b}	9.63 ± 0.76^{a}
Available Carbohydrate	37.67 ± 0.58^{a}	22.67 ± 0.58^{b}	33.67 ± 1.53^{c}
Caloricfic Value (kcal/100g)	211.12 ± 3.01^{a}	142.48 ± 3.12^{b}	191.71 ± 6.05^{c}

Data reported as Mean ± SD of triplicate determinations. Means followed by different letters (a–c) in the same row are significantly different from each other ($p < 0.05$).

Table 3.2: Macro and Micro minerals Content of the three varieties of *H. sabdariffa linn* calyces in mg/100g dry basis.

Mineral Element	Calyx types		
	Dark red	Red	Green
Sodium (Na)	4.20±0.17[a]	3.73±0.12[b]	3.63±0.57[ab]
Potassium (K)	133.33±2.89[a]	131.67±2.89[a]	117.00±2.65[b]
Calcium(Ca)	91.04±21.56[a]	208.46±30.16[b]	129.85±39.74[ac]
Magnesium (Mg)	201.20+4.48[a]	203.68±7.85[a]	199.49±2.64[a]
Phosphorus (P)	4.17±0.10[a]	4.10±0.08[b]	4.30±0.10[c]
Iron (Fe)	2.27±0.72[a]	2.06±1.04[a]	2.13±0.32[a]
Copper (Cu)	0.36±0.21[a]	0.17±0.10[a]	0.20±0.06[a]
Zinc (Zn)	3.56±0.37[a]	1.75±0.50[b]	2.30±0.47 [b]
Manganese (Mn)	5.31±0.68[a]	3.42±0.50[b]	4.09±0.12[b]

Data reported as Mean ± SD of triplicate determinations. Means followed by different letters (a–c) in the same row are significantly different from each other ($p < 0.05$).

Table 3: Anti-Nutrients and Ascorbic acid Content of the three varieties of *H. sabdariffa linn* calyces in (mg/100g) dry basis.

Parameter		Calyx types	
	Dark red	Red	Green
Cyanogenic glycoside	355.88±61.64[a]	147.44±7.52[b]	156.11±32.52[b]
Nitrate	51.00±3.46[a]	27.50±1.73[b]	32.33±6.51[b]
Oxalate	195.00±51.96[a]	180.00±45.00[a]	405.00±45.0[b]
Phytate	2.28±0.25[a]	2.11±0.25[a]	1.41±0.49 [a]
Saponin	7.33±1.53[a]	7.33±1.53[a]	7.67±4.04[a]
Ascorbic acid	77.13±27.73[a]	91.99±21.56[a]	108.66±13.69[a]

Data reported as Mean ± SD of triplicate determinations. Means followed by different letters (a–c) in the same row are significantly different from each other ($p < 0.05$).

5. DISCUSSION

5.1 Proximate Content of *H. sabdariffa* calyces

The values obtained from ash content were within the range (6.5-6.8%) previously reported by[3] on red, green and dark red roselle calyces, but lower when compared with the values reported by[15] 11.24±0.02% and[16] 10.60% in red and 9.50% in green. The crude lipid observed high values than the values reported by[16] 0.16% in red and 0.12% in green calyces and[15] 0.46±0.03% in the red calyx. The fibre contents were found to concord the results reported by[3] 9.8% in dark red, 8.5% in red and 11.2% in green and[15] 11.17±0.02% in red calyx, but lower compared with the results reported by[16] 13.2% in red and 12% in white. The crude protein values agreed with the values reported by[15] 7.5±0.02% in red calyx and[3] 9.8% in dark red, 8.5% in red and 11.2% in green, which agreed with the literature. The crude

protein content indicates that the roselle calyces could be an important source for protein supplements. The available carbohydrate contents shows inconsistent values when compared with the reported by[17] 12.3%,[7] 11.2%,[19] 15% and[16] 57.16% in red and 61.55% in green, this may be as a result of method of analysis used or soil condition.

5.2 Macro and Micro Minerals Content of *H. sabdariffa* calyces

The values of sodium concentration were low compared with the values reported by[4] 96.66mg/100g in red and 48.19mg/100g in green calyces,[3] reported 9.5mg/100g in green, 5.5 mg/100g in red and 6.5mg/100g in dark red. Na plays an important role in reducing health risk such as osmotic regulation of the body fluids.[19] The potassium content obtained was higher compared with the values reported by[4] 49.35mg/100g in the red calyx and 49.59mg/100g in green and[15] 20.60±0.02mg/100g in the red calyx.[3] Reported very high values of 1850 mg/100g in the green calyx, 2060 mg/100g in the red calyx and 2320 mg/100g in the dark red calyx. The presence of a low amount of sodium to a high amount of potassium in the results indicates the use of roselle calyces as antihypertensive. The calcium contents were in lower concentration compared to what[3] reported as 1209 mg/100g in the green calyx, 1583 mg/100g in the red calyx and 1602 mg/100g in the dark red calyx. The lower values were also reported by[4] 12.65mg/100g in red and 21.58mg/100g in the green calyx. Calcium intakes, especially from regular use of calcium supplements may be associated with increased risk of kidney stone.[20] The magnesium content obtained was in high concentration compared with the values reported by[4], 38.65mg/100g in red calyx and 47.54mg/100g in green, but, lower compared to the results of[15] 315.21±1.0mg/100g in red calyx and[3] 235mg/100g in green, 316mg/100g in red and 340mg/100g in dark red. Magnesium is an essential element in most reactions involving phosphate transfer such as structural stability of nucleic acid[19], while lack of it causes health problems such as diarrhea.[21] The phosphorus contents obtained were lower than the results of[15] 36.22±1.0 mg/100g in red and[4] 36.60 mg/100g in red and 15.05 mg/100g in green. Phosphorus is an essential element in body development.[20, 22]

The micro-nutrient elements are of great biochemical interest and exhibit nutritional and clinical importance. The concentrations of the iron content obtained agreed with the values reported by[4] 3.22mg/100g in red and 3.37mg/100g in the green calyx. But, lower than the results reported by[15] 37.80±1.0 in the red calyx and[3] 32.8mg/100g in green, 37.8mg/100g in red and 34.6mg/100g in dark red calyces. Adequate iron in a diet is very important and will

help to decrease the incidence of anaemia in pregnant women, nursing mothers and infants.[23] The copper contents obtained were lower compared with the value reported in red calyx by[15] 4.32±0.03mg/100g in red, although, it appeared absent in[3] report. Lack of copper in the body is associated with many health conditions, including the cardiovascular disorders.[24] The zinc content values obtained in the samples were lower than the values reported by[4] 12.12mg/100g for red and 16.28mg/100g in the green calyx. Zinc plays a very vital role during normal body development, being an integral element during protein and nucleic acid productions.[24] The manganese values obtained agreed with the work of[4] 2.39mg/100g in the red calyx and 5.61mg/100g in green and[15] reported 2.39±0.03mg/100g in the red calyx.

5.3 Anti-Nutritive Content of *H. sabdariffa* calysces

It was observed that hydrocyanic acid value obtained in the dark red calyx was elevated compared to the red and green calyces as shown in Table 3. These values were higher compared with the values reported by[25] 132mg/100g and[4] 0.16mg/100g in the red calyx and 0.13mg/100g in green. The nitrate contents obtained were above the permissible daily intake of 3.7 mg/kg body weight.[26] The oxalate content obtained were lower than the values reported by[4] 6150mg/100g in red calyx and 6050mg/100g in green. The phytate content in the green calyx was lower than in the dark red and red calyx (Table 3). The phytate values were lower compared to the values reported by[27] 174.81mg/100g and[4] 0.32% (320mg/100g) in the red calyx and 1.13% (1130mg/100g in green. The saponins content observed were decreased compared to the values reported by[27] 75.00mg/100g and[26] 0.96% (960mg/100g). Saponin acts to lower the body cholesterol during reabsorption.[20]

5.4 Vitamin C Content of *H. sabdariffa* calyces

The values of vitamin C concentration were down compared with the values reported by[27] 139.51mg/100g,[18] 280-360mg/100g and[15] 140.13±3.0mg/100g in red calyx, but higher compared with the report of[4] 16.67mg/100g in red calyx and 12.50mg/100g in green. The allowable vitamin C intake is 60 mg/day for a person.[28] Based on the results obtained, roselle calyces can supplement the daily vitamin C requirement.

6. CONCLUSION

This study has shown that the roselle calyces sourced from Zuru will contribute important nutrients to many diets. Therefore, *H, sabdariffa* calyces (dark red, red and green) are good sources of proteins, fibre, carbohydrates, vitamins c and minerals. A significant amount of

cyanogenic glycoside and oxalates are contained in the calyces of dark red and green respectively, it can, therefore, be concluded that the calyces should be processed and used as food supplement in low protein and minerals diets.

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8. Authors' contributions: G A, wrote the manuscript GA, YY and HAS, designed the project topic, performed data analysis and interpretations while IKK, HSI and SG performed reagents preparations and samples analysis.

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