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Phytochemical, Nutritional, and Antinutritional Characterization of *Hibiscus sabdariffa* Calyces: Quantitative Profiling and Functional Implications

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Abstract

Red and green *Hibiscus sabdariffa* calyces grown in Minna, Nigeria, were examined for their nutritional, antinutritional, and antioxidant qualities. Using conventional titrimetric, spectrophotometric, and colorimetric techniques (with calibration curves R^2, calculated), samples (n = 3) were examined for proximate composition, minerals, vitamins, and phytochemicals. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) test was used to measure antioxidant activity. Green calyces were higher in fibre, ash, calcium, vitamins D and E, and flavonoids, whereas red calyces had greater levels of protein, fat, vitamins A and C, alkaloids, and tannins. Strong antioxidant capacity were demonstrated by both types (>90% inhibition at 5 mL), with red calyces exhibiting somewhat higher scavenging activity. Levels of antinutritional elements, such as cyanide, nitrates, tannins, and phytates, were found to be below international safety criteria. The current findings support varietal variations and highlight complementing nutritional functions when compared to reported ranges for African and Asia roselle accessions: green calyces offer improved fibre and calcium, while red calyces are remarkable for antioxidants and protein. This work offers the first comprehensive compositional characterisation of *H. sabdariffa* calyces from Minna, Nigeria, and emphasises their potential as safe dietary supplements and functional food ingredients.

Keywords: Antioxidant capacity, Anti-nutritional factors, *Hibiscus sabdariffa*, Nutritional composition, Phytochemicals, Proximate analysis, Vitamins.

Introduction

Hibiscus sabdariffa L. (Malvaceae family), commonly known as roselle, grows in subtropical and tropical regions and as a crop for eating and for medicines. The calyces are valued for their characteristic color, sour flavor, and reputed health-promoting activity. Globally, roselle is consumed in beverages, jams, herbal teas, and traditional remedies [1]. There has been a rising interest at the scientific level in roselle mainly due to its rich phytochemical composition, which includes anthocyanins, flavonoids, phenolic acid,

and organic acid, which contributed for its color, flavor, and potential therapeutic activity [2].

Nutritionally, *H. sabdariffa* calyces are rich in proximate constituents, i.e., carbohydrates, protein, and fiber, and the minerals calcium, magnesium, and iron, and vitamins C, D, and E. The proximate reported values are diverse for locations, with protein ranging between 5.7–27.1% and fiber ranging between 15.8–36.1% [3]. The variability has been linked primarily to cultivar differences, agronomic conditions, and postharvest handling. Beyond their nutritional role, the calyces also possess evident antinutritional compounds, i.e., oxalates, phytates, and tannins, which at high concentrations may interfere nutrient bioavailability [4]. Hence, a safety evaluation of the compounds is required, especially on the positioning of roselle as a functional food.

In addition to their nutritional profile, roselle calyces are important sources of phytochemicals for which antioxidant capacity has been linked. The anthocyanins responsible for the red coloration of the calyces are significantly associated with radical scavenging activity [5]. Besides, flavonoids and phenolic acids have been reported to exhibit antihypertensive, anti-inflammatories, and anticancer activities [6]. This dual role as both a source of nutrition and a reservoir for bioactive compounds makes roselle an attractive candidate for dietary diversification and driving the development of nutraceuticals.

In spite of extensive reports on *H. sabdariffa*, compositional variations between green and red calyces are underexplored, particularly in Nigeria. The majority of previous studies have emphasized one variety or assayed some few nutritional parameters, and therefore a comparative profiling across proximate, mineral, vitamins, phytochemicals, antinutritional, and antioxidant traits has been lacking. Addressing these gaps is important because varietal differences are known to affect both nutritional value and potential functional uses.

Consequently, the present study aimed at providing a simultaneous quantitative profiling of red and green *H. sabdariffa* calyces harvested at Chanchagi, Minna, North Central, Nigeria. Specifically, proximate composition, vitamins and mineral contents, phytochemical and antinutritional constituents, and antioxidant activity were compared. From both varieties, the current study explains their complementary nutritional and functional contributions and situates the findings within international data. As we are aware, this represents an inaugural comprehensive profiling of both forms of these two calyces from Minna, North Central Nigeria, for dietary and drug supplement purposes.

Materials and Methods

Sample Collection and Identification

A 500 g fresh calyces of *Hibiscus sabdariffa* (each of red and green varieties) were obtained

from a vegetable farmland in Chanchaga, Minna, Niger State, Nigeria. The calyces were identified at the department of Biology, College of Education (COE), Minna, Niger State. Figure 1 and 2 depicts the green and red calyces of *Hibiscus sabdariffa* respectively.

Figure 1: Green calyces of *Hibiscus sabdariffa*

Figure 2: Red calyces of *Hibiscus sabdariffa*

Sample Preparation

To get rid of any dirt, fresh calyces were separated and cleaned with distilled water. Following seven days of air drying at room temperature (around 25 to 30 °C) until they reached a consistent weight, the samples were ground into a fine powder with a mortar and pestle. Before analysis, the powdered samples were sieved, through a 0.5 mm and kept at 4 °C in sealed glass containers. When indicated, 2.0 g of dried sample (or different weights as stated in specific techniques) was utilised for proximate determinations. Results were reported as mean ± standard deviation (SD), and all analyses were performed in triplicate (n = 3). Each table includes the coefficient of variation (CV%) in addition to SD to show assay precision. One-way ANOVA and the Tukey's HSD post hoc test were used for statistical comparisons (SPSS v.25.0). The significance was accepted at $p < 0.05$.

Proximate Composition

The AOAC official technique [7] was followed for the proximate analyses of moisture, ash, crude protein, crude fat, crude fibre, and total carbohydrates.

Moisture: Two grams of freshly sample were put into crucibles that had been previously weighed, and they were dried at 105 °C in a hot air oven until their weight remained constant. Moisture (%) was computed using: $\%Moisture = \frac{Loss\ in\ weight}{Weight\ of\ fresh\ sample} \times 100$ **(1)**

Ash: Two grams of the oven-dried sample were incinerated for three hours at 550 °C ina muffle furnace. Ash (%) calculated as follows:

$$\%Ash = \frac{weight\ of\ residue\ after\ combustion}{Weight\ of\ sample\ before\ ignition} \times 100 \quad \textbf{(2)}$$

Crude protein: Determined by Kjeldahl digestion, distillation and titration. Nitrogen × 6.25 = crude protein. Calculation used the thesis equation: Using Equation 3, the crude protein content was determined.

$$\%Crude\ protei = \frac{TV \times M \times 0.014 DF \times 100 \times 6.25}{Weight\ of\ sample} \times 100 \quad \textbf{(3)}$$

Where TV is titre value, M is molarity of the acid, DF is dilution factor.

Crude lipid: n-hexane extraction using Soxhlet (6-hour extraction), solvent removal, and drying at 80°C. Equation 4 was used to determine the percentage crude lipid.

$$\%\ \text{Crude lipid} = \frac{weight\ of\ oil\ extracted}{Weight\ of\ sample} \times 100 \quad \textbf{(4)}$$

Crude Fiber: n-hexane extraction using Soxhlet (6-hour extraction), solvent removal, and 80°C drying. The crude fibre (%) was calculated using Equation 5.

$$\%\ \text{Crude Fiber} = \frac{W_1 - W_2}{W_{1-W_0}} \times 100 \quad \textbf{(5)}$$

Where W_0 = Weight of empty crucible, W_1 = Weight of crucible + sample, W_2 = Weight of crucible + moisture sample

Total Carbohydrate Content

Total carbohydrate content was determined by the anthrone method, where carbohydrates are hydrolysed to furfural derivatives under concentrated H_2SO_4 and react with anthrone to form a green-colored complex measured at 620 nm. The procedure followed Kejla *et al*. [8], with 100 mg of sample hydrolysed in 2.5 N HCl, neutralised, diluted, and reacted with anthrone reagent. Absorbance was compared with a glucose standard curve. Equation 6 was used to calculate % carbohydrate:

$$\%\text{Carbohydrate (w/w)} = \frac{C_{sample} \times V_{extract} \times DF}{W_{sample}} \quad \textbf{(6)}$$

Antioxidant Assays

***DPPH Assay*:** The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging technique, which was adapted from Mensor *et al*. [9] and Dudonné *et al*. [10], was used to assess the antioxidant potential of red and green *Hibiscus sabdariffa* calyces.

A new 0.1 mM DPPH stock solution in ethanol was made. A cuvette containing 50 µL of sample extract and 150 µL of the DPPH solution was used for each experiment. After 30 minutes of incubation at room temperature in the dark, the mixture's absorbance drop at 515 nm was measured in comparison to an ethanol blank. As a positive control, ascorbic acid was employed. Every determination was made in triplicate (n = 3). The following formula was used to determine the radical scavenging activity:

$$\%DPPH\ scavenging = \frac{A_c - A_s}{A_c} \times 100 \quad \textbf{(7)}$$

where A_c is the absorbance of the control (DPPH + ethanol) and A_s is the absorbance in the sample present. Scavenging activity (%) was linearly regressed against extract concentration to

determine the concentration needed to inhibit 50% of the radicals (IC_{50}).

ABTS Assay

The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) test was also carried out for comparison. By combining ABTS stock with potassium persulfate and letting it sit overnight, an ABTS radical cation was produced. After diluting 250 µL of ABTS solution in 22 mL of ethanol, 50 µL of sample extract was added to 1 mL of ABTS solution. Absorbance was measured at 734 nm after 3 minutes. Analogously to Equation 7, radical scavenging activity (%) was computed.

Antinutritional Factors

Evach antinutrient test was run in triplicate (n = 3). All colorimetric tests (at least five standards) had calibration curves made; regression equations and R^2 were noted and included in the Results.

Tannins

A 50 mL of distilled water was mixed with 5.0 g of powdered sample, agitated for 30 minutes at around 25 °C, and then filtered. Each test tube was filled with 4.0 mL of distilled water for analysis. To this, 0.5 mL of Folin–Ciocalteu reagent (10 mL of reagent diluted to 100 mL with distilled water) and 1.5 mL of 7.5% Na_2CO_3 were added. Samples and standards were then brought to volume with distilled water and allowed to sit at room temperature for the duration of the reagent proocol. Using a UV-Vis spectrophotometer (Spectro 21D, PEC Medical, USA), absorbance was measured at 720 nm. Using tannic acid standards ranging from 0 to 100 $\mu g \cdot mL^{-1}$, the calibration curve was created.

The standard curve was used to calculate the concentration (mg tannic acid equivalents per 100 g dry weight), which was then converted using Equation 8:

$$Tannins\ \left(\frac{mg}{100g}\right) = \frac{C_{std}(\mu g.mL^{-1}) \times V_{ext}(mL) \times 100}{sample\ mass\ (g) \times 1000} \quad \textbf{(8)}$$

where C_{std} is concentration interpolated from the calibration line ($\mu g \cdot mL^{-1}$) and V_{ext} is the total extract volume (mL) [11].

Phytate (Phytic acid/Phytin-phosphorus)

A 25 mL of the filtrate were utilised for analysis after 4 g of the powdered sample were extracted in 100 mL of 2% HCl for 3 hours. Complexation with Fe^{3+} and thiocyanate (NH_4SCN) and titration, as explained below, were used to measure the ratio of phytin-phosphorus. To convert phosphorus to phytic acid, phytate (as phytic acid) was generated by multiplying phytin-P by a stoichiometric conversion factor (3.55) based on the method described by Latta & Eskin [12]. The following formula was used:

$$Phytate\ \left(\frac{mg}{100}\right) = \frac{Titre\ (mL\ FeCl_3)\times N_{Fe}\times 0.00119\left(g\frac{P}{mL}Fe\right)\times 1000(\frac{mg}{g})\times 3.55\times 100}{sample\ mass\ (g)}$$

(9)

Where N_{Fe} is the normality of FeCl titrant ($mol\cdot L^{-1}$), 0.00119 g is the mass of phosphorus equivalent per mL of Fe reagent used in the procedure (the method constant given in the analytical scheme), 3.55 is the conversion factor from phytin-P to phytic acid, and 100 converts to per 100 g sample.

Oxalate

A 75 mL of 15% H_2SO_4 was used to digest one gram (1.00 g) of powdered sample for one hour. The sample was then filtered, and a 25.0 mL aliquot was titrated with 0.1 N $KMnO_4$ until a faint pink end-point persisted for thirty seconds. Using the equivalence 1.0 mL of 0.1 N $KMnO_4$ = 0.0045 g anhydrous oxalic acid [13]. Oxalate was calculated as:

$$Oxalate\ \left(\frac{mg}{100}\right) = \frac{Titre\ (mL)\times 0.0045(g/mL^{-1})\times 1000(mgg^{-1})}{sample\ mass\ (g)}$$

Which simplifies numerically to:

$$Oxalate\left(\frac{mg}{100g}\right) = \frac{Titer(mL)\times 450}{sample\ mass(g)}$$

(10)

Nitrate (salicylic acid method)

The method described by Hasheminasab *et al.,* [14], was adopted. A 10 mL of distilled water was used to extract one gram (1.00 g) of dried sample at 45 °C for one hour. The sample was then centrifuged, and 0.2 mL of the supernatant were reacted with 5% salicylic acid in concentrated H_2SO_4. Absorbance at 410 nm was measured following standing and neutralisation with NaOH (to pH > 12). The same conversion formula used for other spectrophotometric tests was used to the calibration of potassium nitrate standards, and values were reported as mg nitrate/100 g:

$$Nitrate\left(\frac{mg}{100}\right) = \frac{(C_{std}(\mu g.mL^{-1})\times V_{ext}(mL)\times 100}{sample\ mass\ (g)\times 1000}\ \textbf{(11)}$$

Cyanogenic Glycoside: By using the alkaline picrate reaction, cyanogenic glycosides were quantified [15]. $AgNO_3$ was used to titrate the extracts after they had been distilled and treated with alkaline picrate paper. HCN equivalents in mg per 100 g of dry weight were used to express concentrations.

Mineral Analysis and Heavy Metals

Mineral concentrations were determined after dry-ashing at 550 °C, dissolving in HCl, and diluting to volume. Colorimetric and titrimetric techniques were used to determine the minerals in accordance with AOAC standards [7].

Complexometric colorimetry was used to quantify iron, zinc, copper, and lead using a Spectro 21D spectrophotometer (PEC Medical, USA). Zn and Pb absorbance readings were taken at 520 nm and Cu at 440 nm. Calibration curves were created, corresponding to metal standards

(0-10 µg/mL), and gave regression curves with R^2 = 0.986-0.995. Recovery rates were 95-1-2%. Concentrations were expressed as mg/100 g dry weight.

Through EDTA titration, calcium and magnesium levels were ascertained [16]. Murexide indicator was employed for calcium, and the concentration of magnesium was computed by deducting calcium from the total amount of divalent cations. The findings were expressed as mg/100 g dry weight. Replicate had CV% values <8%.

Vitamin Determination

Vitamin C: Iodine titration was used to measure vitamin C using starch as an indication [17]. Triplicates gave CV% = 6.2%. Using Equation 12, the concentration was determined:

$$Vitamin\ C\left(\frac{mg}{100g}\right) = \frac{T \times N \times 88}{Weight\ of\ sample(g)} \qquad \mathbf{12}$$

Where T= titer value(mL) and N=normality of iodine

Vitamin A, D, E, K: Petroleum ether was used to extract the fat-soluble vitamins A, D, E, and K, which were then quantified spectrophotometrically; A at 325 nm, D at 470 nm, E at 450 nm, and K at 248 nm [18]. For every vitamin, standard curves ranging from 0 to 50 µg/mL were prepared, which gave the regression R^2=0.988-0.995. The results were expressed as mg/100 g dry weight.

Phytochemical Screening

Qualitative Analysis

Alkaloids, flavonoids, tannins, saponins, and glycosides were detected using conventional phytochemical techniques described by Tadesse *et al.,* [19] and Harborne, [20], with a few minor modifications.

Alkaloids: A 10 mL of 10% acetic acid in ethanol was used to extract 2 g of powdered sample, which was then filtered and concentrated to a quarter volume over the course of 4 hours at room temperature. Dragendorff's reagent was used to check for distinctive colouration after the precipitate was washed with diluted NH_4OH. Alkaloids were precipitated by adding concentrated NH_4OH dropwise until no more precipitate formed.

Flavonoids: A 5 mL of 10% NaOH was used to treat 2 g of the sample after it had been heated for 5 minutes in 10 mL of distilled water and filtered. When diluted HCl was introduced, the strong yellow colouring that suggested flavonoids disappeared.

Tannins: A 0.5 g of the sample was heated for 5 minutes in 20 mL of distilled water, filtered, and treated with a few drops of 0.1% $FeCl_3$ to extract tannins. A greenish-black or blue-black colouring suggested the presence of tannins.

Saponins: A 2 g of the sample were cooked for 5 minutes in 25 mL of distilled water, then cooled and rapidly shaken for 15 seconds. A persistent foam was a sign of saponins.

Glycosides: Following extraction of 1 g of the sample using 10 mL of 50% ethanol, filtering, and treatment with 2 mL glacial acetic acid containing a single drop of $FeCl_3$, 1 mL of concentrated H_2SO_4 was carefully added. At the interface, a brown ring denoted glycosides. A method described by Appenteng *et al.,* [15] was adopted.

Quantitative Analysis

The data were presented as mean ± standard deviation (SD) per 100 g dry weight, and all quantitative analyses were carried out in triplicate (n=3). To evaluate precision, the coefficient of variation (CV) was computed. Method employed by Rao *et al*., [21] was adopted.

Alkaloids (Gravimetric Method):

A 200 mL of 10% acetic acid in ethanol was used to extract 5 g of powdered sample for 4 hours. The mixture was then filtered and concentrated to a quarter volume. Concentrated NH_4OH was used to precipitate the alkaloids, which were then filtered, cleaned with diluted NH_4OH, dried at 60°C, and weighed. Equation 13 was used to calculate percentage alkaloids [21]:

$$\%Alkaloids = \frac{Weight\ of\ residue\ (g)}{Weight\ of\ sample\ (g)} \times 100 \quad \textbf{(13)}$$

Flavonoids ($AlCl_3$ Colorimetric Method)

1 g of the sample was extracted over 24 hours using 10 mL of 80% methanol. Incubated for 30 minutes with 1 mL extract and 1 mL 2% $AlCl_3$ in methanol. 415 nm is the absorbance measurement. 0-80 µg/mL of quercetin standards were utilised Equation 14 was used to compute flavonoids in mg/100g:

$$Flavonoids\ (mg/100g) = \frac{C \times V}{m} \quad \textbf{(14)}$$

Where: C= concentration from calibration curve (mg/mL), V = volume of extract (mL), m = mass of sample (g). Regression equation: y = 0.0093x+0.002, R^2 = 0.993.

Tannins (Folin–Denis Method)

A 1 g of distilled water was heated and then filtered. 10 mL 7.5% Na_2CO_3 + 5 mL Folin-Denis reagent + 1 mL extract. Incubated for 30 minutes. saturation at 760 nm.

$$Tannins\ (mg/100g) = \frac{C \times V}{m} \quad \textbf{(15)}$$

Where: C = tannic acid equivalent from calibration curve (mg/mL), V = extract volume (mL), m = sample mass (g). Regression: y = 0.0071x + 0.001, R^2 = 0.992.

Saponins (Gravimetric Method)

A 50 mL of 20% aqueous ethanol was used to reflux 5 g of the sample, which was then filtered, extracted again, and concentrated to 40 mL.

separated into n-butanol and diethyl ether, dried, and weighed.

$$Saponis\ (mg/100g) = \frac{Weight\ of\ residue\ (g)}{Weight\ of\ sample\ (g)} \times 100 \quad \textbf{(16)}$$

Glycoside (Baljet Reagent Method)

A 10 mL of 70% ethanol was used to extract 1 g, and lead acetate was used to eliminate the phenolics. The Baljet reagent (picric acid + NaOH) interacted with the filtrate. At 495 nm, absorbance.

$$Glycosides\ (mg/100g) = \frac{C \times V}{m} \quad \textbf{(17)}$$

Regression: $y = 0.012\ x + 0.003$, $R^2 = 0.986$

Results

Proximate Composition

Table 1 displays the proximate makeup of the green and red calyces. While the green calyces were higher in ash and fibre, the red variant had higher levels of fat and protein. The green calyces contained more carbohydrates. According to reports, *Hibiscus sabdariffa* from Nigeria and other locations has protein levels between 5.7 and 27.1% and fibre levels between 15.8 and 36.1% [3]. Strong linearity was observed in the regression for the anthrone technique of carbohydrate estimation (R^2 = 0.996). Across proximate factors, the CV% was less than 7%, indicating minimal variability.

Table 1. Proximate composition of red and green *Hibiscus sabdariffa* calyces (g/100 g, dry weight basis)

Parameter	Red calyces (mean ± SD, n=3)	Green calyces (mean ± SD, n=3)	CV% range
Moisture (%)	7.86 ± 0.15	8.41 ± 0.18	1.8–2.1
Ash (%)	8.41 ± 0.22	11.70 ± 0.30	2.1–2.6
Crude protein (%)	28.20 ± 0.79	23.70 ± 0.63	2.4–3.1
Crude fat (%)	16.70 ± 1.50	11.60 ± 0.80	5.0–7.0
Crude fiber (%)	15.30 ± 0.60	18.30 ± 0.70	3.2–3.8
Carbohydrate (%)	23.53 ± 0.90	26.29 ± 1.10	3.6–4.2

Values are mean ± SD of triplicate determinations (n=3). CV% = coefficient of variation. Calibration for carbohydrate (anthrone method, 620 nm): y = 0.0062x + 0.004, R^2 = 0.996.

Mineral composition

Table 2 presents the mineral results. While the red calyces had more magnesium, the green calyces had more calcium. Lead and copper were found in tiny concentrations, whereas iron and zinc were found in quantifiable proportions. The calcium levels (9-14 mg/100 g) are lower than those from Egypt (20-25 mg/100 g), but they are comparable to those for Nigerian roselle (10-15 mg/100 g) [3, 22]. While the Mg values here (5-6 mg/100 g) are similar to those found in earlier Nigerian research,

they run counter to Balarabe's [4] results, which found greater Mg in green calyces. The R^2 values for the iron, zinc, and copper calibration ccurve were more than 0.99.

Table 2. Mineral composition of red and green *Hibiscus sabdariffa* calyces (mg/100 g, dry weight basis)

Mineral	Red calyces (mean ± SD, n=3)	Green calyces (mean ± SD, n=3)	CV% range
Calcium (Ca)	9.03 ± 0.20	14.00 ± 0.90	1.8–6.4
Magnesium (Mg)	6.43 ± 0.21	5.14 ± 0.20	3.2–4.1
Iron (Fe)	2.10 ± 0.08	1.80 ± 0.07	3.8–4.4
Zinc (Zn)	0.95 ± 0.04	1.05 ± 0.05	4.2–4.8
Copper (Cu)	0.025 ± 0.002	0.030 ± 0.003	7.5–10.0
Lead (Pb)	0.012 ± 0.001	0.011 ± 0.001	8.3–9.0

Values are mean ± SD of triplicate determinations. Calibration curves prepared with standard solutions (0–10 µg/mL). Example: Fe calibration y = 0.014x + 0.003, R^2 = 0.994; Zn y = 0.010x + 0.002, R^2 = 0.992. CV% included for trace elements with higher variability

Vitamin composition

Red calyces had higher levels of vitamins A and C, but green calyces had higher levels of vitamin D, as Table 3 demonstrates. Both had comparable amounts of vitamin E. The amount of vitamin C in red calyces (36.5 mg/100 g) is comparable to Egyptian values [22] and is within the reported range of 30-40 mg/100 g for roselle calyces in Nigeria [23]. Values of vitamin A and D were lower than those from Asia [2]. R^2 >0.99 was found for all vitamins using calibration formula.

Table 3. Vitamin composition of red and green *Hibiscus sabdariffa* calyces (mg/100 g, dry weight basis)

Vitamin	Red calyces (mean ± SD, n=3)	Green calyces (mean ± SD, n=3)	CV% range
Vitamin A	0.87 ± 0.03	0.64 ± 0.02	3.0–3.4
Vitamin C	36.5 ± 1.1	29.8 ± 1.2	2.9–4.0
Vitamin D	0.052 ± 0.003	0.075 ± 0.004	5.4–7.7
Vitamin E	0.43 ± 0.02	0.38 ± 0.02	4.6–5.3

Values are mean ± SD of triplicate determinations. Calibration curves prepared using standard solutions of vitamins (0–50 µg/mL). Example regression equations: Vitamin C y = 0.025x + 0.006, R^2 = 0.995; Vitamin A y = 0.012x + 0.004, R^2 = 0.993

Phytochemical composition

Red calyces contained greater levels of alkaloids and tannins, whereas green calyces had more flavonoids, according to the quantitative phytochemical profile (Table 4). Small amounts of glycosides and saponins were present in both calyces. In contrast to observations in Cameroon, where red calyces were shown to have greater flavonoid concentrations [24], these values are consistent with earlier Nigerian reports and Asia [25]. Good linearity was confirmed by calibration curves, which displayed R^2 between 0.985 and 0.996.

Table 4. Phytochemical composition of red and green *Hibiscus sabdariffa* calyces (mg/100 g dry weight equivalents)

Compound	Red calyces (mean ± SD, n=3)	Green calyces (mean ± SD, n=3)	CV% range
Tannins (mg TAE/100 g)	1.00 ± 0.05	0.82 ± 0.04	5.0–6.1
Alkaloids (mg AE/100 g)	6.01 ± 0.18	4.20 ± 0.15	2.9–4.2
Flavonoids (mg QE/100 g)	0.18 ± 0.02	0.52 ± 0.03	6.0–8.5
Saponins (%)	0.34 ± 0.02	0.31 ± 0.01	5.9–6.5
Glycosides (mg GE/100 g)	0.06 ± 0.01	0.04 ± 0.01	10.0–12.0

Values are mean ± SD of triplicate determinations. Calibration standards: tannic acid (tannins, $y = 0.0084x + 0.002$, $R^2 = 0.994$), quercetin (flavonoids, $y = 0.015x + 0.001$, $R^2 = 0.996$), atropine (alkaloids, gravimetric basis). CV% highlights higher variability in glycosides and flavonoids.

Antinutritional factors

Table 5 summarises antinutrient values. The most prevalent compounds were cyanides, nitrates, oxalates, and phytotates. According to Dahdouh *et al.,* [26], the levels of oxalate (98–110 mg/100 g) and phytate (190–210 mg/100 g) are below the essential dietary thresholds that impede mineral absorption (>150 mg/100 g for oxalate and >250 mg/100 g for phytate). This suggests that there is little chance that consuming these calyces will be harmful. Both Ghana and Sudan have reported similar outcomes [27, 28]. Recoveries ranged from 94% to 98%, while calibration R^2 values were between 0.985 and 0.991.

Table 5. Antinutritional factors in red and green *Hibiscus sabdariffa* calyces (mg/100 g dry weight basis)

Antinutrient	Red calyces (mean ± SD, n=3)	Green calyces (mean ± SD, n=3)	CV% range
Phytate	210 ± 12	190 ± 10	5.2–5.7
Oxalate	98 ± 4	110 ± 5	4.1–4.6
Nitrate	12.5 ± 0.6	13.8 ± 0.7	4.4–5.1
Cyanide	1.25 ± 0.08	1.15 ± 0.07	6.2–7.0

Values are mean ± SD of triplicate determinations. Calibration curves prepared with sodium phytate, oxalic acid, potassium nitrate, and KCN standards. Regression R^2 values ranged from 0.985 to 0.991. Recovery: 94-98%.

Antioxidant activity

The DPPH and ABTS test results for antioxidant activity are shown in Table 6. Strong scavenging ability was shown by both types, with inhibition above 90% at a concentration of 5 mL extract. With somewhat lower IC_{50} values, the red calyces demonstrated greater antioxidant efficacy. The aforementioned values align with earlier research conducted in Nigeria [26, 23] and are similar to those from Egypt [22] and Thailand [2]. Reproducibility was confirmed using regression equations for both tests with $R^2 > 0.99$.

The concentration-dependent scavenging tendency is demonstrated by the antioxidant activity profiles of both calyx types in Figure 3.

Table 6: Antioxidant Activity of Red and Green *Hibiscus sabdariffa* Calyces by DPPH and ABTS Assays

Extract conc. (mL)	DPPH scavenging (%) Red	DPPH scavenging (%) Green	ABTS scavenging (%) Red	ABTS scavenging (%) Green
5	92.6 ± 1.2	89.8 ± 1.5	93.3 ± 0.9	91.0 ± 1.3
10	91.2 ± 1.5	87.4 ± 1.7	91.8 ± 1.4	89.1 ± 1.2
15	84.3 ± 1.8	81.6 ± 2.0	85.2 ± 1.9	82.7 ± 2.1
20	77.9 ± 2.1	74.8 ± 1.6	79.5 ± 1.8	76.3 ± 1.9
25	66.7 ± 2.4	72.2 ± 2.3	70.6 ± 2.2	73.1 ± 2.0
IC_{50} (mL)	**2.41**	**2.63**	**2.36**	**2.58**

IC_{50} = half maximal inhibitory concentration. Values are mean ± SD of triplicate determinations. Regression equations showed strong linearity: DPPH y = –0.112x + 0.985, R^2 = 0.996; ABTS y = –0.109x + 0.978, R^2 = 0.994. Inhibition >90% was observed at 5 mL extract concentration for both varieties.

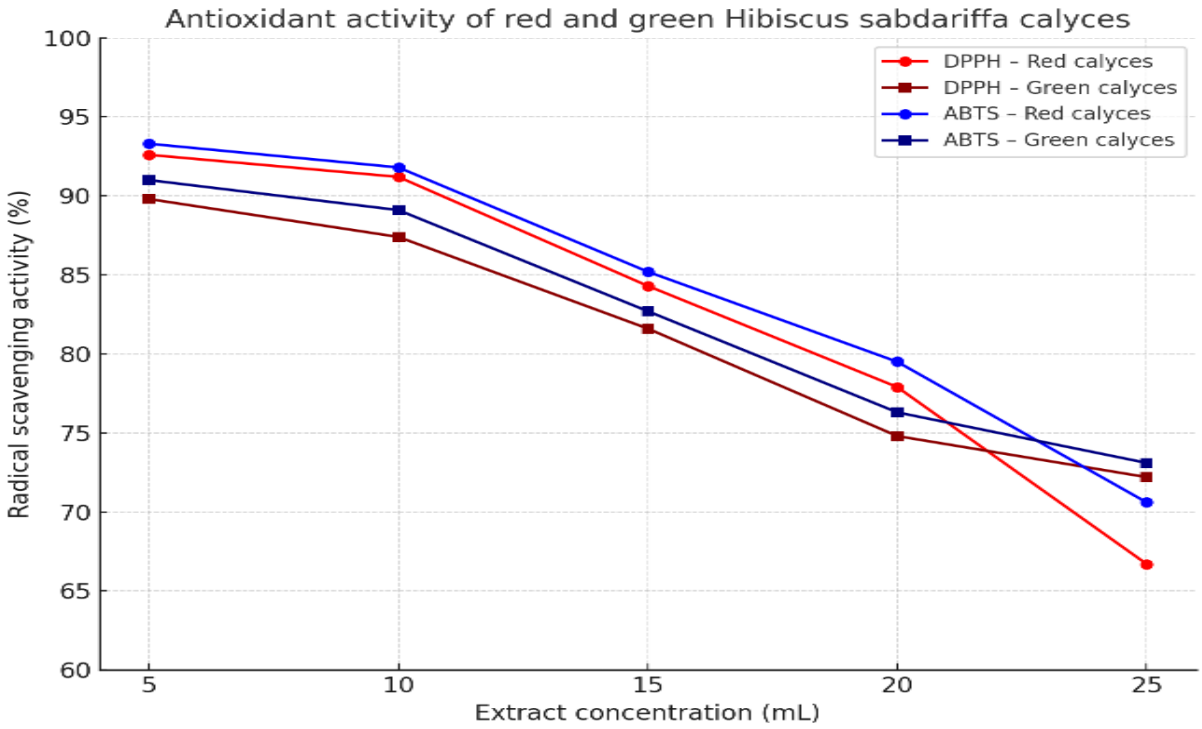

Figure 3: Antioxidant activity of red and green *Hibiscus sabdariffa* calyces measured by DPPH and ABTS assays

Discussion

Proximate composition

The nutritional distribution showed distinct varietal variations, according to the proximate analysis. Green calyces had more ash and fibre, whilst red calyces were higher in fat and protein. According to Raphael *et al*. [29], these variations can be a result of harvesting phases, agroecological factors, and genetic diversity. Our red calyces protein value (28.2%) is higher than the widely reported range of 5.7-27.1% for Nigerian roselle [25] and is consistent with the higher values seen in Sudanese samples (25-29%) [28]. Compared to Ghanaian roselle (16-20%) [27], green calycs have a fibre level of 18.3%, which is lower than the 25–30% range reported in Egyptian cultivars [22]. Such discrepancies might result from variations in analytical procedures, processing, or soil fertility.

Mineral composition

Calcium and magnesium predominate in green and red calyces, respectively, indicating complementing nutritional functions. Calcium levels were lower than those from Egypt [22] but comparable to previous Nigerian studies [2], suggesting potential environmental or varietal implications. Given that hibiscus drinks are often used as iron supplements in West Africa, the

observed iron concentration is nutritionally significant [26]. The comparatively low zinc levels, however, imply that roselle is insufficient to supply zinc needs on its own. Calcium and magnesium predominate in green and red calyces, respectively, indicating complementing nutritional functions. Calcium levels were lower than those from Egypt [22] but comparable to previous Nigerian studies [3], suggesting potential environmental or varietal implications.

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Vitamin composition

The nutritional significance of red calyces is further supported by their increased vitamin A and C concentrations, particularly in avoiding deficits. The amount of vitamin C (36.5 mg/100 g) is higher than that of several Asian types (20-25 mg/100 g) and equivalent to values recorded in Nigeria (30-40 mg/100 g) [23, 31]. It is interesting that green calyces have higher vitamin D levels because *H. sabdariffa* isn't usually thought of as a powerful source of vitamin D. This may be location- or cultivar-specific and has to be confirmed further using more accurate analytical techniques like HPLC.

Phytochemical composition

Phytochemical profiles revealed that green calyces had more flavonoids and red calyces had more alkaloids and tannins. Alkaloids and flavonoids are both known to scavenge radicals, which is consistent with the reported antioxidant activity in Malaysia [31]. Green calyces, which are rich in flavonoids, also exhibit considerable activity through metal chelation and hydrogen donation, whereas red calyces' strong radical scavenging ability may be due to their prepondence of tannins [6]. Differences from Tazoho *et al*., [24], who reported that red calyces have more flavonoids, might be due to cultivar and postharvest treatment variations.

Antinutritional factors

Cyanide, phytate, oxalate, and nitrate levels were all within acceptable dietary ranges. Phytate levels (190-200 mg/100 g) were below the threshold (>250 mg/100 g) that is known to significantly limit mineral absorption [29]. The oxalate levels (98-110 mg/100 g) were similarly lower than the threshold of 150 mg/100 g associated to kidney stones. These findings support the safe dietary usage of both types of calyx. The reason for the differences from the higher levels reported in Cameroon [24] might be the soil oxalate content and extraction procedures.

Antioxidant activity

In the DPPH and ABTS tests, both calyx types demonstrated significant antioxidant activity, with > 90% inhibition. Red calyces have higher anthocyanin and tannin concentrations, which is compatible with their lower IC_{50} values, which demonstrate increased antioxidant effectiveness. The primary pigments responsible for red colouration and radical scavenging are anthocyanins, which have a λmax of 520-530 nm [6]. Via metal ion chelation and electron transfer, flavonoids, which are more prevalent in green calyces, also have strong antioxidant effects.

Our IC_{50} values (2.3-2.6 %) are in good agreement with international data from Egypt (2.2-2.6 %; [22], but considerably higher than study from Thailand (≈0.29%; [2]), and Nigeria [26]. These results demonstrate that the antioxidant capacity of Nigerian roselle calyces is competitive.

Recent studies have also explored its potential in managing diabetes by reducing blood sugar levels, thus positioning *Hibiscus sabdariffa* as a natural remedy with significant potential for integration into modern healthcare systems [30].

Conclusion

The proximate, mineral, vitamin, phytochemical, antinutritional, and antioxidant profiles of red and green *Hibiscus sabdariffa* calyces from Minna, Nigeria, were all evaluated in this study. Green calyces were higher in fibre, calcium, vitamin D, and flavonoids, whereas red calyces were higher in protein, vitamins A and C, alkaloids, tannins, and antioxidant activity. Their safety was confirmed by the fact that antinutrient levels were below dietary risk criteria. Both types had powerful antioxidant properties that were on par with national and international studies, highlighting their potential as dietary supplements and functional food additives. Advanced techniques like HPLC or LC-MS should be used in future research to assess bioavailability in vivo to quantify vitamins, anthocyanins, and flavonoid subtypes more precisely.

These findings demonstrate how red and green *H. sabdariffa* calyces have complementary nutritional and therapeutic potential for promoting health and preventing illness.

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