COMPARATIVE ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS, PROXIMATE COMPOSITION AND ENERGY VALUE OF *Hibiscus sabdariffa* L. LEAF AND PETAL EXTRACTS

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ABSTRACT
This study evaluates the phytochemical constituents, proximate composition, and energy values of methanolic, aqueous and ethanolic extracts from *Hibiscus sabdariffa* L. leaves and petals. Qualitative phytochemical analysis showed the presence of bioactive compounds such as steroids, alkaloids, terpenoids, and flavonoids, with petal extracts showing significantly higher amounts of bioactive compounds across all solvents. The quantitative analysis indicated that aqueous extracts had the highest concentration of flavonoids at 267 mg/100 g, while ethanolic petal extracts had the highest terpenoid content at 7.5mg/100g. The proximate analysis demonstrated that the leaves possessed a greater energy value of 280.36 ± 1.20 kcal/100 g and a carbohydrate content of 45.56 ± 0.89%. In contrast, the petals had a higher protein content of 11.42 ± 0.38% and crude fibre content of 37.49 ± 0.64%. These findings underscore the nutritional and medicinal benefits of *H. sabdariffa*. The leaves can provide dietary energy, whereas the petals may have potential applications in antioxidant therapies and functional foods. Future research should focus on their pharmacological applications and potential industrial uses.

Keywords: *Hibiscus sabdariffa*, phytochemical analysis, proximate composition, energy value, nutrition

1.0 INTRODUCTION

Hibiscus sabdariffa L., generally identified as Roselle, is a medicinal and food plant extensively cultivated in tropical and subtropical areas which belongs to the Malvaceae plant family and is known for its rich phytochemical composition, which contributes to numerous health benefits and applications in traditional medicine (Maganha, 2010; Osman et al., 2011). The calyces of Roselle are popularly used to make a non-alcoholic beverage known as "zobo" in Nigeria and some parts of West Africa. This drink is refreshing and a good source of essential vitamins and minerals (Babalola, 2000; Singh et al., 2017).

Phytochemicals such as alkaloids, flavonoids, and terpenoids are bioactive compounds that significantly impact plant pharmacological activities. Roselle is recognised for having anti-inflammatory, antimicrobial, and antioxidant properties, primarily due to these compounds (Okereke et al., 2015; Puro et al., 2016). Its medicinal applications include managing hypertension, aiding digestion, and reducing cholesterol levels, making it a potential therapeutic agent in modern medicine (Duke et al., 2003; Azza et al., 2011).

Nutritionally, *H. sabdariffa* is recognised for its high carbohydrate, fibre, and protein content, contributing to its role as a dietary staple in many regions. Extensive studies on the plant's proximate composition have revealed its potential as a source of essential nutrients and energy (Ansari et al., 2013; Cid-Ortega & Guerrero-Beltrá, 2015). However, variations in the nutritional and phytochemical profiles of different parts of the plant, including the leaves and petals of the plant, are not well documented, despite their potential significance for dietary and medicinal use (Nnam & Onyeke, 2003; Adanlawo & Ajibade, 2006).

This study aims to address this knowledge gap by comparing the phytochemical constituents, proximate composition, and energy values of the leaves and petals of *H. sabdariffa*. By exploring the unique properties of these plant parts, this research seeks to highlight their potential contributions to human nutrition and health, providing insights for their application to certain industries dealing with food and pharmaceuticals.

2.0 MATERIALS AND METHODS

2.1 Plant collection and preparation

Fresh leaves and petals of *H. sabdariffa* were collected from the botanical garden of the Federal Polytechnic Ilaro, Ogun State, Nigeria. The collected plant materials were washed to remove dirt and debris, dried at 60°C, and then pulverised into powder using a grinder. It was then stored in airtight containers under dry conditions for analysis. (Maganha, 2010; Ansari et al., 2013).

2.1.1 Extract Preparation

The extract was prepared following the method described by Harborne (1998). Dried leaves and petals of *H. sabdariffa* were extracted using aqueous, ethanol, and methanol solvents. Specifically, 15 grams of the dried powder of leaves and petals were suspended separately in 500 mL of each solvent. After soaking the mixture for 72 hours, it was then filtered with Whatman No. 42 filter paper and the resulting extract was preserved in a glass bottle and stored in a refrigerator for further analysis.

2.2 Phytochemical analysis

2.2.1 Qualitative Phytochemical Analysis

This analysis used established standard methods (Harborne, 1998; Okereke et al., 2015; Singh et al., 2017).

To test for alkaloids, 0.2 grams of the samples were boiled in 5 mL of 2% hydrochloric acid in a steam bath, and the mixture was subsequently filtered. Two drops of picric acid and Mayer's reagent were added to each of the four test tubes containing 1 mL of the filtrate. Two milligrams of the extracts were boiled in 100 mL of ethyl acetate in boiling water for three minutes to perform the flavonoid test. After the mixtures were filtered, 4 mL of the filtrate was combined with 1 mL of a 1% aluminium chloride solution for the aluminium chloride test. Flavonoids were present because a light-yellow precipitate formed. One gram of each extract was refluxed with 9 mL of ethanol, filtered, and then concentrated to 2.5 mL in a boiling water bath for terpenoids and steroids. Five millilitres of distilled water was added, and the mixture was left to settle for an hour before the waxy substance was filtered off. The filtrate was extracted using 2.5 mL of chloroform and a separating funnel. In the presence of steroids, a reddish-brown border formed when 0.5 mL of the extract was combined with 1 mL of concentrated sulphuric acid for the steroid test. The presence of terpenoids was confirmed by the formation of a grey hue after a further 0.5 mL of the extract was dried out and heated with 3 mL of concentrated sulphuric acid for 10 minutes.

2.2.2 Quantitative Phytochemical Analysis

This analysis was conducted using the established standard gravimetric method described by Harborne 1998 and modified.

2.2.2.1 Determination of Alkaloids

Five grams of dust were dissolved in 100 mL of 10% acetic acid. After a good shake, the mixture was allowed to stand for four hours (Harborne, 1998). Following this time frame, the Whatman No. 42 filter paper was used to filter the mixture. Next, a heated plate with a magnetic stirrer was used to evaporate the filtrate to

a quarter of its initial volume. To precipitate the alkaloids, concentrated ammonium hydroxide was added to the solution dropwise. After another filtering of the solution, 1% ammonium hydroxide was used to wash the precipitate. After 30 minutes of drying at 60°C in an oven, the filter paper holding the precipitate was let to cool for 10 minutes before being weighed. The weight difference was used to calculate the alkaloids' weight, which was then converted using the proper procedure to a percentage of the original sample.

$$\% \text{ Alkaloid} = \frac{W_3 - W_2}{W_1} \times 100 \quad \text{Eqn. 1}$$

Where,

W_1 = Sample weight

W_2 = Beaker weight

W_3 = Beaker weight + extract

2.2.2.2 Determination of Flavonoids

Five grams of the samples were heated for thirty minutes in 100 millilitres of a 2M hydrogen chloride solution (Harborne, 1998). The mixture was allowed to cool after boiling, and then the Whatman No. 42 filter paper was used to filter it. Ethyl acetate was added to the filtrate drop by drop until an excess was achieved. After that, the precipitated flavonoid was recovered by filtering it through filter paper that had been previously weighed, drying it in an oven at 80°C, cooling it in a desiccator, and then reweighing it. A percentage of the original sample weight was used to represent the flavonoid weight difference. The proper formula was used to get the flavonoid proportion.

$$\% \text{ Flavonoid} = \frac{W_2 - W_1}{W_1} \times 100 \quad \text{Eqn. 2}$$

Where,

W = the sample weight that is being examined.

W_1 = Empty crucible weight.

W_2 = Filter paperweight plus flavonoid precipitate.

2.2.2.3 Determination of Terpenoids

Ninety millilitres of solvent were used to soak 10 grams of dried plant extract (Indumathi et al., 2014). Following filtration, a separating funnel was used to filter the extract once again after it had been combined with 10 millilitres of petroleum ether. Measurements were taken when the extract had completely dried.

$$\% \text{ Terpenoid} = \frac{W_i - W_f}{W_i} \times 100 \quad Eqn. 3$$

Where,

W_i = dried plant extracts

W_f = extracts after drying.

2.3 Proximate analysis

This involves assessing the nutritional quality and organic composition of the plant extracts (Okeke et al., 2020: Kehinde and Augustine, 2022)

2.3.1 Determination of Moisture Content

This process made use of the gravimetric method described by the AOAC (2005). Initially, 5 grams of the sample was put into a moisture container which had been previously weighed. After three hours, this sample was dried in an oven set to 105°C. The container was taken out after drying and left to cool in a desiccator before being weighed once again. The process of drying, cooling, and weighing was repeated every hour until there were no more weight variations, signifying that a stable weight had been achieved. A percentage of the initial weight of the sample under analysis was used to compute the weight of the moisture lost:

$$\text{Moisture Content (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \quad \text{Eqn. 4}$$

Where W_1 is the weight of the empty moisture can.

W_2 = Weight of can + Sample weight before drying

W_3 = Weight of can + Sample weight after drying to constant weight

2.3.2 Determination of Protein

The Kjeldahl method was used to determine total nitrogen, which was then multiplied by 6.25 to calculate protein concentration. A 0.5-gram sample was mixed with 10 mL of concentrated sulfuric acid and heated until a clear solution formed, then diluted to 100 mL. Ten millilitres of the digest was combined with an equal volume of 45% sodium hydroxide for distillation. After distillation, the mixture was titrated with 0.02 N EDTA, using a mixed indicator in 10 mL of 40% boric acid. The nitrogen content was calculated using a formula to find protein content, with procedures also performed on a reagent blank.

$$N^2(\%) = \frac{100}{W} \times \frac{N \times 14}{1000} \times \frac{Vt}{Va} \times T.B \quad \text{Eqn. 5}$$

Where W is the sample's weight (0.5 g).

N = Titrant normality (0.02 N H_2SO_4)

Vt = 100 mL of total digest volume

Va is the digested volume (10 mL).

T is the titre value of the sample.

B = No titre value

2.3.3 Determination of Total Ash Content

The furnace incinerator approach was used to perform the gravimetric method for calculating the amount of ash (AOAC, 2005). A ceramic crucible that had been previously weighed was filled with 5 grams of the sample. After that, the crucible was heated for around three hours at 550°C in a muffle furnace. Following this time, the crucible was cautiously taken out of the furnace, given time to cool in a desiccator, and then weighed again. A percentage of the weight of the sample under analysis was used to represent the difference between the starting and final weights.

$$\text{Ash (\%)} = \frac{W_2 - W_1}{\text{Weight of sample}} X\ 100 \quad \text{Eqn. 6}$$

Where W_1 is the empty crucible's weight (g).

W_2 = Crucible weight + Ash

2.3.4 Determination of Crude Fibre

James (1995) method for determining crude fibre involves refluxing a 5 g processed sample in 150 mL of a 1.25% H_2SO_4 solution for 30 minutes, followed by thorough rinsing. The sample is then treated with 150 mL of a 1.25% NaOH solution for an additional 30 minutes, washed again, and dried in an oven at 105°C until stable. Finally, the sample is burned in a muffle furnace to leave only ash, allowing for the calculation of crude fibre weight as a percentage of the initial sample weight.

$$\text{Crude fiber (\%)} = \frac{W_2 - W_3}{\text{Weight of sample}} \times 100 \quad \text{Eqn. 7}$$

W_3 is the weight of the crucible plus the sample following washing, boiling, and drying.

W_2 = crucible weight plus ash sample

2.3.5 Determination of Crude Fat

The gravimetric method, as described by Kirk and Sawyer (1980), was used to extract oil from 5 grams of the sample wrapped in Whatman filter paper. The sample was placed in a Soxhlet reflux flask connected to a flask containing 200 mL of petroleum ether. After four hours, the defatted sample was removed, and the solvent was recovered. The remaining solvent was evaporated by heating the flask at 60°C for 30 minutes. Finally, the flask was cooled and weighed, allowing for the calculation of the oil extract's percentage based on the initial sample weight.

$$\text{Fat (\%)} = \frac{W_2 - W_1}{\text{Weight of sample}} \times 100 \quad \text{Eqn. 8}$$

Where W_1 is the empty extraction flask's weight (g).

W_2 is the flask weight plus the oil (fat) extract.

2.3.6 Determination of Carbohydrates

This determination was made using the James (1995) approach. A total of 45 mL of each sample extract was diluted to 450 mL using distilled water. One mL of each diluted filtrate was pipetted into a different test tube. Additionally, one test tube was filled with 1 millilitre of distilled water as a blank, and another test tube was filled with 1 mL of glucose as a standard. Five millilitres of newly made 0.10% Anthrone reagent were added to each test tube, and the mixture was properly mixed by shaking the tube gently. After being labelled, each tube was put on a test tube rack and immersed in a water bath set to 30°C for 12 minutes. The tubes were then taken out and let to cool to room temperature. A spectrophotometer was used to measure the absorbance of the samples and standard at 630 nm, with the blank serving as a reference. For about two hours, the green colour formed remained constant which signifies the presence of glucose. The following approach was used to determine the total amount of accessible carbs as a percentage of glucose:

$$\text{Glucose (\%)} = \frac{25A_1}{X \times A_2} \times 100 \quad \text{Eqn. 9}$$

Where X is the sample weight (g).

A_1 is the diluted sample's absorbance.

A_2 is the diluted standard's absorbance.

2.4 Energy Value Determination

The Atwater physiological fuel parameters were used to determine the energy value of the *H. sabdariffa* leaves and petals based on their proximate composition. The following estimates were made for the energy contributions of fat, protein, and carbohydrates:

(4 × Protein) + (4 × Carbohydrate) + (9 × Fat) = Energy (kcal/100g) Eqn. 10

2.5 Statistical Analysis

All values are reported as means ± standard deviation (SD). The differences between the extracts of leaves and petals from *H. sabdariffa* were assessed using a one-way Analysis of Variance (ANOVA), followed by Duncan's Multiple Range Test to determine significant differences among the groups. A statistical significance threshold was set at $p < 0.05$. All analyses were conducted using SPSS (Version 25). The energy value of the extracts was calculated based on the proximate composition data, following established guidelines (AOAC, 2005; McDonald et al., 2014).

3.0 RESULTS

3.1 Qualitative Phytochemical Composition of *H. sabdariffa* L. Leaf and Petal Extracts

The results as seen in Table 1 revealed the presence of alkaloids, flavonoids, terpenoids, and steroids in varying concentrations across different solvent extractions. Alkaloids and terpenoids were consistently found in all extracts, while flavonoids and steroids were observed in fewer extracts.

3.2 Quantitative Phytochemical Composition of *H. sabdariffa* L. Leaf and Petal Extracts

The quantitative analysis of alkaloids, flavonoids, and terpenoids in both leaf and petal extracts reveals that the petal extracts consistently have a higher phytochemical content compared to the leaf extracts (as shown in Table 2). Notably, the ethanolic petal extract contains the highest amount of terpenoids at 7.5 mg per 100 g, while the aqueous petal extract follows with 6.3 mg per 100 g.

3.3 Proximate Composition and Energy Value of *H. sabdariffa* L. Leaf and Petal Extracts

The proximate composition of extracts from the leaves and petals of *H. sabdariffa* is outlined in Table 3. The results reveal variations in moisture, ash, protein, fibre, fat, and carbohydrate content between the two extracts. Specifically, the petal extract has higher levels of fibre and protein, while the leaf extract contains increased carbohydrate levels. Additionally, the calculated energy values highlight the nutritional potential of the leaves as a source of dietary energy.

Table 1: Qualitative Phytochemical Composition of *H. sabdariffa* L. Leaf and Petal Extract

Sample	Leaf			Petal		
Extracts	Aqueous	Ethanol	Methanol	Aqueous	Ethanol	Methanol
Alkaloids	-	-	-	+	+	+
Flavonoids	+	-	-	+	-	-
Terpenoids	-	-	-	+	+	+
Steroids	-	-	-	-	+	+

Keys: (+): presence; (-): absence

Sample	Leaf			Petal		
Extracts	Aqueous	Ethanol	Methanol	Aqueous	Ethanol	Methanol
Alkaloids (mg/100 g)	-	-	-	0.0162 ± 0.0023a	0.0205 ± 0.0023a	-
Flavonoids (mg/100 g)	1.545±0.005a	-	-	2.614±0.005a	-	-
Terpenoids (mg/100 g)	-	-	-	39.69 ± 0.0023a	6.25 ± 0.0023a	13.08±0.0023a

Table 2: Quantitative Phytochemical Composition of *H. sabdariffa* L. Leaf and Petal Extract

Results are reported in mean ± SD. Mean values with the same letter in a column are not significantly different (P>.05)

Table 3: Proximate Composition and Energy Value of *H. sabdariffa* L. Leaf and Petal Extract

Component	Leaf (%)	Petal (%)
Moisture Content	11.19 ± 0.34a	9.87 ± 0.25b
Ash Content	8.43 ± 0.29a	7.92 ± 0.19b
Protein Content	10.25 ± 0.44b	11.42 ± 0.38a
Crude Fiber Content	21.34 ± 0.55b	37.49 ± 0.64a
Crude Fat Content	3.22 ± 0.18a	2.89 ± 0.11b
Carbohydrate Content	45.56 ± 0.89a	30.41 ± 0.77b
Energy Value (kcal)	280.36 ± 1.20a	241.22 ± 1.05b

Values are expressed as mean ± standard deviation; letters (a, b) indicate statistical significance using Duncan's test.

4.0 DISCUSSION

The phytochemical analysis of *H. sabdariffa* reveals the rich presence of bioactive compounds with significant variations between the leaf and petal extracts. Alkaloids and terpenoids were consistently identified in all tested solvents, with higher concentrations in petal extracts. These findings corroborate previous studies highlighting the therapeutic potential of *H. sabdariffa* petals as a rich source of alkaloids and terpenoids (Akindahunsi & Olaleye, 2004; Mungole & Chaturvedi, 2011).

The findings from this study reaffirm the established nutritional and medicinal significance of *H. sabdariffa* while providing new insights into the effects of its phytochemical richness. The higher concentrations of flavonoids, terpenoids, and alkaloids in the petals align with prior research, such as the work by Okeke et al. (2015), which highlighted the superior bioactive compound profile in *H. sabdariffa* petals compared to other plant parts. These compounds are well-documented for their therapeutic roles, including antioxidant, anti-inflammatory, and antimicrobial activities (Osman et al., 2021; Kehinde & Augustine, 2022).

Oxidative stress is a prevalent mechanism in chronic diseases, including diabetes, neurological disorders, and cardiovascular issues. Flavonoids, which are strong antioxidants found in the petals, lessen this stress. According to recent research, flavonoids in *H. sabdariffa* can lower blood pressure, enhance endothelial function, and improve lipid profiles (Okeke et al., 2020; Sobowale et al., 2023). Because of these qualities, the petals are a useful component of functional foods and drinks that support metabolic and cardiovascular health.

The terpenoids found abundantly in the petals contribute to antimicrobial and anti-inflammatory effects. Previous studies supported their role in modulating immune responses and fighting bacterial and fungal infections (Akinjogunla et al., 2020). Additionally, the alkaloid content in *H. sabdariffa* enhances its therapeutic significance, as these compounds are known for their pain-relieving, and antimicrobial properties, thereby expanding their potential applications in pharmaceutical formulations.

The study's findings highlight the dual functionality of the plant parts from a nutritional standpoint. According to previous studies, the leaves have a high carbohydrate and energy content, making them ideal for energy-rich diets. On the other hand, petals, higher in protein and fibre are more appropriate for weight control and digestive health. It was also stated that fibre content, in particular, is crucial for bowel health, cholesterol management, and lowering the risk of obesity (Osman et al., 2021; Sobowale et al., 2023).

The observed nutritional differences may stem from the physiological roles of leaves and petals within the plant. Leaves play a crucial role in photosynthesis and carbohydrate storage, while the petals are richer in secondary metabolites and structural components such as fibre (Okereke et al., 2015). The higher fibre content in petals may also contribute to their medicinal uses, including cholesterol regulation and improved bowel health (McKay & Blumberg, 2010; Okeke et al., 2020).

5.0 CONCLUSION

This study highlights the nutritional and medicinal benefits of *H. sabdariffa* leaves and petals through their phytochemical constituents and proximate compositions. The petal extracts contained higher concentrations of bioactive compounds, such as alkaloids, terpenoids, and flavonoids, confirming their antioxidative and therapeutic properties.

Conversely, the leaves exhibited higher carbohydrate content and energy value, highlighting their role as a dietary energy source. The proximate analysis further revealed significant differences in macronutrient distribution: petals are a richer source of fibre and protein, while leaves are more carbohydrate-dense.

These insights are consistent with recent advancements in understanding the plant's pharmacological and nutritional roles, emphasising its significance in the food, health, and pharmaceutical industries. Future research should focus on exploring the bioavailability of its bioactive compounds and optimizing its industrial applications for sustainable use.

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