

## Phytochemical Constituents, and Antioxidant Activities of Sokoto-Grown *Hibiscus Sabdariffa* (Zobo)

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### ABSTRACT

Oxidative stress is a harmful physiological condition caused by an imbalance between free radical production and the body's antioxidant defense system. It contributes to the development of many chronic diseases, including diabetes, cardiovascular disorders, neurodegenerative diseases, and cancer. *Hibiscus sabdariffa* Linn., locally known as "zobo" in Nigeria, is a medicinal plant traditionally consumed as a beverage and used for various therapeutic purposes. This study evaluated the phytochemical composition and antioxidant potential of *Hibiscus sabdariffa* leaf extracts. Dried red and black zobo leaves were collected from Tsohuwar Kasuwa market in Sokoto and authenticated at the Laboratory, Biochemistry Department, Usmanu Danfodiyo University, Sokoto. The samples were soaked in distilled water for 24 hours, filtered, and stored. Phytochemical screening was conducted using standard qualitative and quantitative methods, while antioxidant activity was assessed using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The phytochemical results revealed the presence of flavonoids, tannins, saponins, glycosides, cardiac glycosides, steroids, balsams, anthraquinones, and volatile oils. Quantitatively, black zobo exhibited higher flavonoid ( $2.26 \pm 0.01\%$ ) and glycoside ( $3.82 \pm 0.02\%$ ) contents, while red zobo contained more amount of saponins ( $2.78 \pm 0.01\%$ ). Antioxidant activity showed that black zobo had a higher radical scavenging activity ( $73.86 \pm 0.37\%$ ) compared to red zobo ( $66.42 \pm 0.85\%$ ) at 0.2 mg/mL. A decline in activity was observed at higher concentrations. The results suggest that *Hibiscus sabdariffa* leaves, particularly the black variant, are rich in phytochemicals with significant antioxidant activity and could serve as a potential natural remedy for oxidative stress-related conditions.

**Keywords:** *Hibiscus sabdariffa*, Phytochemicals, Antioxidant activity, DPPH assay

### 1.0 INTRODUCTION

Plants are a rich source of bioactive compounds called phytochemicals, which play a key role in promoting health by influencing both nutritional and therapeutic functions (Hu et al., 2023). These secondary metabolites, such as flavonoids, alkaloids, tannins, saponins, and phenolic compounds, are responsible for many of the physiological and medicinal properties attributed to plants. Historically, people have used phytochemicals in traditional medicine in various forms, including infusions, decoctions, and extracts, to treat numerous ailments (Kaushik et al., 2021). Their potential for developing pharmacologically active agents remains a major focus of modern biomedical research. One of the most important biological effects of phytochemicals is their contribution to antioxidant defense (Myhrstad & Wolk, 2023). Antioxidants are molecules that protect cells from damage by neutralizing free radicals, unstable molecules produced during normal cellular processes, especially during oxidative phosphorylation in the mitochondria. This oxidation process is vital for life, supporting key functions such as cellular respiration,

immune responses, and signal transduction (Kozlov et al., 2024).

However, excessive production of reactive oxygen species (ROS), combined with weakened antioxidant defenses, leads to a harmful condition called oxidative stress. This imbalance disrupts redox homeostasis, causing oxidative damage to lipids, proteins, and nucleic acids, ultimately impairing cellular function and integrity (Kowalczyk et al., 2021; Siddika et al., 2024). The biological consequences of oxidative stress are significant, with studies linking it to the development of several chronic diseases, including diabetes mellitus, cardiovascular diseases such as atherosclerosis and hypertension, neurodegenerative disorders like Alzheimer's and Parkinson's diseases, various types of cancer, and the aging process itself (Sharifi-Rad et al., 2020).

To mitigate oxidative stress, the body depends on a comprehensive antioxidant defense system made up of enzymatic antioxidants, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase

(GPx), as well as non-enzymatic antioxidants like vitamins C and E, beta-carotene, and polyphenols (Jena et al., 2023). The interest in dietary and plant-based antioxidants has increased significantly due to their relative safety, availability, and therapeutic potential. One plant of notable pharmacological and nutritional interest is *Hibiscus sabdariffa* Linn., locally known in Nigeria as “zobo.” It belongs to the Malvaceae family and is widely cultivated in tropical and subtropical regions. The plant’s calyces are commonly used to make a traditional beverage popular across West Africa, especially in northern Nigeria. Besides its cultural importance, *H. sabdariffa* is valued for its strong antioxidant, anti-inflammatory, antihypertensive, and antimicrobial properties, which are linked to its rich phytochemical content, especially anthocyanins, flavonoids, and phenolic acids (Anas, 2024; Ghaly et al., 2025). Additionally, nutritional studies have shown that *H. sabdariffa* calyces are a significant source of essential minerals like iron, calcium, magnesium, potassium, and manganese, which are crucial for physiological functions including enzyme activity, oxygen transport, and bone growth (Ogunsola et al., 2023). Clinical and preclinical research has indicated that regular intake of *H. sabdariffa* extracts or drinks can help lower blood pressure and cholesterol levels, supporting cardiovascular health (Montalvo-González et al., 2022; Sridevi et al., 2024). Given the rising prevalence of diseases related to oxidative stress and the need for affordable, plant-based treatments in developing regions, exploring the antioxidant potential of *Hibiscus sabdariffa* offers promising public health and clinical benefits. This study examines the phytochemical and antioxidant properties of *H. sabdariffa*, emphasizing its importance as a functional

food and a natural remedy for oxidative stress-related conditions.

## 2.0 MATERIALS AND METHODS

### 2.1 Sample Collection

Dried red and black *Hibiscus sabdariffa* leaves were purchased from the Tsohuwar Kasuwa market in Sokoto and authenticated at the Laboratory, Biochemistry Department, Usmanu Danfodiyo University, Sokoto. Samples were labeled A and B.

### 2.2 Sample Preparation and Extraction

Five (5) grams of each sample (red and black variants) were weighed using an analytical balance. The leaves were then placed separately into two clean, labeled conical flasks (labeled A and B). Each flask received 50 mL of distilled water, which served as the extraction solvent. The samples were left to soak (macerate) at room temperature for 24 hours to allow phytochemicals to diffuse from the plant matrix into the aqueous medium. After 24 hours, the mixtures were filtered to separate the solid plant residue from the liquid extract. After filtration, the aqueous extracts were stored for analysis (Sofowora, 1993).

### 2.3 Phytochemical Screening

#### 2.3.1 Qualitative Analysis

Standard procedures were used to test for the presence of flavonoids, tannins, saponins, alkaloids, glycosides, cardiac glycosides, steroids, balsams, anthraquinones, volatile oils, and saponin glycosides. Colorimetric reactions or precipitate formation were used as indicators for the presence of phytochemicals (Harbone and Evans, 1998) as described below.

**Table 1: Qualitative Phytochemical Analysis of *Hibiscus sabdariffa* Leaf Extracts**

Phytochemical	Test Procedure	Indicator of Presence
Flavonoids	Extract + a few drops of 10% NaOH	Intense yellow color
Tannins	Extract + 2–3 drops of 5% FeCl <sub>3</sub> , shaken	Greenish precipitate
Saponins	Extract shaken vigorously with equal volume of distilled water	Persistent frothing
Glycosides	Extract + H <sub>2</sub> SO <sub>4</sub> , heated → cooled → neutralized with NaOH → Fehling’s solution added and heated	Brick-red precipitate
Alkaloids	Extract + 10% HCl → Mayer’s reagent added	Yellow precipitate
Cardiac Glycosides	Extract + FeCl <sub>3</sub> → layered with conc. H <sub>2</sub> SO <sub>4</sub>	Reddish-brown ring at the interface
Steroids	Extract in chloroform → layered with conc. H <sub>2</sub> SO <sub>4</sub>	Reddish-brown ring at the junction
Balsams	Extract + 90% ethanol → alcoholic FeCl <sub>3</sub> added	Dark green color
Anthraquinones	Extract + benzene → 10% ammonia solution added	Pink/violet/red color in ammoniacal layer
Volatile Oils	Extract + dilute HCl	White precipitate
Saponin Glycosides	Extract + Fehling’s solution A & B	Bluish-green precipitate

#### 2.3.2 Quantitative Analysis

The leaves of *Hibiscus sabdariffa* were subjected to quantitative phytochemical analysis for saponin, flavonoid, and glycosides

**Table 2: Quantitative Phytochemical Analysis of *Hibiscus sabdariffa* Leaf Extracts**

Phytochemical	Method Summary	Percentage Yield (% w/w) Formula
<b>Flavonoids</b>	Boiled with dilute HCl → filtration → ethyl acetate treatment → drying and weighing of residue	$\% \text{ Flavonoid} = \frac{W_3 - W_1}{W_2} \times 100$ Where, W <sub>1</sub> - weight of empty filter paper W <sub>2</sub> - weight of sample W <sub>3</sub> - weight of filter paper after drying
<b>Saponins</b>	Extracted with 50% ethanol → precipitation with acetone → collection and weighing of precipitate	$\% \text{ Saponin} = \frac{W_3 - W_1}{W_2} \times 100$ Where, W <sub>3</sub> - weight of filter paper + saponin W <sub>1</sub> - weight of empty filter paper W <sub>2</sub> - weight of sample
<b>Glycosides</b>	Alcoholic extraction → reaction with sodium phosphate and Baljet's reagent → spectrophotometric reading at 495 nm	$\% \text{ Glycoside} = \frac{\text{absorbance of sample}}{17 \text{ (constant)}} \times 100$

#### 2.4 Antioxidant Activity (DPPH) Assay

The antioxidant capacity was determined using the DPPH free radical scavenging activity (Blois, 1958). A 1 mM DPPH solution was prepared in 95% methanol. Varying concentrations of plant extracts were reacted with DPPH, and absorbance was measured at 517 nm. The radical scavenging activity (RSA) was calculated as:

$$\text{RSA (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

### 3.0 RESULTS

#### 3.1 Phytochemical Screening

##### 3.1.1 Qualitative and Quantitative Phytochemical Screening

Phytochemical analysis of *Hibiscus sabdariffa* leaves showed the presence of flavonoids, glycosides, saponins, tannins, and other secondary metabolites. Black zobo contained higher levels of flavonoids (2.26%) and glycosides (3.82%), while red zobo had more amount of saponins (2.78%). Alkaloids were not detected in either variety (Table 3).

**Table 3: Phytochemical screening tests of *Hibiscus Sabdariffa* Leaves Extract.**

Tests	Black Zobo	Red Zobo
<b>Flavonoids</b>	+	+
<b>Tannins</b>	+	+
<b>Saponins</b>	+	+
<b>Glycosides</b>	+	+
<b>Alkaloids</b>	ND	ND
<b>Cardiac glycosides</b>	+	+
<b>Steroids</b>	+	+
<b>Saponin glycosides</b>	ND	+
<b>Balsams</b>	+	+
<b>Anthraquinones</b>	+	+
<b>Volatiles oils</b>	ND	+
<b>Flavonoids (%)</b>	2.2633 ± 0.0104	0.8883 ± 0.0126
<b>Glycosides (%)</b>	3.8200 ± 0.0173	3.2100 ± 0.0100
<b>Saponin (%)</b>	1.4400 ± 0.0100	2.7750 ± 0.0150

Key: + = Present, ND= Not Detected

### 3.2 Antioxidant Activities

The antioxidant activity of *Hibiscus Sabdariffa* leaf extracts was evaluated using the DPPH radical scavenging assay. Black zobo exhibited consistently higher radical scavenging activity (RSA) across all concentrations, peaking at  $73.86 \pm 0.37\%$  at 0.2 mg/mL. Red zobo followed with a maximum RSA of  $66.42 \pm 0.85\%$  at the same concentration. A gradual decline in RSA was observed with increasing extract concentration in both variants, indicating concentration-dependent activity (Table 4).

**Table 4: Antioxidant activities (DPPH) Assay for Local Zobo “*Hibiscus Sabdariffa*” Leaves**

Conc. (mg/ml)	Black Zobo		Red Zobo	
	ABS (517 nm)	RSA (%)	ABS (517 nm)	RSA (%)
0.2	$0.1077 \pm 0.0015$	$73.86 \pm 0.3721^a$	$0.1383 \pm 0.0035$	$66.42 \pm 0.8528^e$
0.4	$0.1503 \pm 0.0031$	$63.50 \pm 0.7441^b$	$0.2173 \pm 0.0015$	$47.24 \pm 0.3721^f$
0.6	$0.1697 \pm 0.0021$	$58.81 \pm 0.5052^b$	$0.2750 \pm 0.0017$	$33.24 \pm 0.4215^g$
0.8	$0.2027 \pm 0.0031$	$50.80 \pm 0.7387^c$	$0.2863 \pm 0.0015$	$30.49 \pm 0.3721^h$
1.0	$0.2610 \pm 0.0030$	$36.64 \pm 0.7250^d$	$0.3160 \pm 0.0020$	$23.29 \pm 0.4850^i$

The values are presented as mean  $\pm$  SD of triplicate determinations. Different superscript letters in RSA values denote a statistically significant difference ( $p < 0.05$ ).

### 4.0 DISCUSSION

The phytochemical analysis of *Hibiscus sabdariffa* (zobo) leaves identified various bioactive compounds, including flavonoids, glycosides, saponins, tannins, steroids, anthraquinones, and alkaloids. These results align with previous studies by Teye *et al.* (2019), who highlighted *H. sabdariffa*'s rich phytochemical profile contributing to antioxidant, anti-inflammatory, and antihypertensive effects. The qualitative data further support the medicinal importance of zobo leaves in healthcare. Quantitative analyses showed that black zobo had higher flavonoid content ( $2.26 \pm 0.01\%$ ) than red zobo ( $0.89 \pm 0.01\%$ ), indicating varietal differences in secondary metabolites. Similar observations by Onyeukwu *et al.* (2023) suggest these differences may be due to genetic and environmental factors. Glycosides also varied slightly, with black zobo ( $3.82 \pm 0.02\%$ ) higher than red zobo ( $3.21 \pm 0.01\%$ ), while saponins were more abundant in red zobo ( $2.78 \pm 0.01\%$ ) than black zobo ( $1.44 \pm 0.01\%$ ). These complementary phytochemical profiles support their traditional use in health promotion.

Antioxidant activity assessed via the DPPH activity assay revealed significant free radical scavenging in both samples, with black zobo consistently showing higher activity. At 0.2 mg/mL, black zobo achieved a radical scavenging activity (RSA) of  $73.86 \pm 0.37\%$ , compared to  $66.66.42 \pm 0.85\%$  in red zobo. These results align with prior research by ElMajdoub *et al.* (2021) and M'be *et al.* (2023), which emphasized the role of delphinidin-3-sambubioside and cyanidin-3-sambubioside in antioxidant activity. Notably, higher concentrations (1.0 mg/mL) showed a decline in RSA,  $36.64 \pm 0.73\%$  for black zobo and  $23.29 \pm 0.49\%$  for red zobo, indicating potential pro-oxidant effects at elevated levels, as seen in similar DPPH studies (Omar *et al.*, 2021).

While many reports emphasize anthocyanins as primary antioxidants in hibiscus calyces, this study reveals that the leaves also possess notable antioxidant capacity, especially due to flavonoids and glycosides. This suggests that underutilized leaves could be beneficial sources of phytochemicals for nutraceuticals or functional foods, consistent with the findings of Salem *et al.* (2021) and Baskota *et al.* (2025), who identified flavonoids and phenolic acids as key contributors to *H. sabdariffa*'s antioxidant activity. Overall, the rich phytochemical content and robust antioxidant properties of *H. sabdariffa* leaves, especially in the black variety, highlight their potential role in health enhancement and disease prevention. These results provide biochemical support for the traditional use of zobo leaves and establish a scientific basis for their broader application in food and pharmaceutical industries.

### 5.0 CONCLUSION

*Hibiscus Sabdariffa* leaf extract exhibited significant antioxidant activity and was rich in phytochemicals such as flavonoids, glycosides, and saponins. Therefore, further isolation and structural elucidation of the bioactive compounds responsible for its antioxidant effects may serve as potential precursors for the development of natural antioxidant agents or complementary therapies for oxidative stress-related diseases.

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