

Phytochemical composition and *in-vitro* antioxidant activity of *Hibiscus sabdariffa*

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ABSTRACT

Hibiscus sabdariffa calyxes has long been used in traditional food and medicine, including cold or hot drinks, jellies, puddings, and as flavoring agents. This study evaluated the phytochemicals composition and in vitro antioxidant activities of *Hibiscus sabdariffa* calyxes in aqueous extract form. The analysis qualitatively and quantitatively identified several phytochemicals, revealing 4634.41 ± 10.75 mg of total phenolics, 329.99 ± 4.57 mg of alkaloids, 179.38 ± 0.83 mg of flavonoids, 14.42 ± 0.23 mg of tannins, 1.69 ± 0.00 mg of glycosides, 3.45 ± 0.00 mg of steroids, 173.48 ± 0.13 mg of reducing sugars, and 137.70 ± 2.77 mg of terpenoids per 100 grams of extract. The antioxidant activity was measured through several assays. The DPPH radical scavenging assay resulted in an EC₅₀ of 34.89 µg//ml, compared with the standard at 29.9 µg//ml. Lipid peroxidation inhibition showed an EC₅₀ of 8.53 µg//ml, compared to 2.73 µg//ml for the standard. Nitric oxide scavenging had an EC₅₀ of 3.217 µg//ml, while the standard was shown to have a value of 0.895 µg//ml. The ferric reducing antioxidant power (FRAP) assay demonstrated the extract’s ability to reduce Fe³⁺ to Fe²⁺. Additionally, the extract reduced molybdenum (vi) to molybdenum (v). This study on *Hibiscus sabdariffa* calyxes confirms that the aqueous extract from *Hibiscus sabdariffa* calyxes is rich in phenolics, contributing to its antioxidant properties and supporting its traditional medicinal uses.

Keywords: *Hibiscus sabdariffa*, antioxidant, phytochemicals, calyxes.

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INTRODUCTION & AIM

- ❑ Plant-derived natural products are gaining increasing attention for their pharmacological and bioactive compounds, which can scavenge free radicals and restore biochemical balance (Mumtaz et al., 2020).
- ❑ The calyxes of *Hibiscus sabdariffa*, used in preparation of a nonalcoholic drink known as ‘zobo’ in Nigeria, have been reported to contain antioxidants that play a role in the treatment of atherosclerosis, cardiovascular diseases, hypertension, hyperlipidemia, cancer, and other inflammatory diseases of the liver and kidneys (Kumoye & Remi-esan, 2024).
- ❑ This study aimed to evaluate the phytochemical constituents and the *in-vitro* antioxidant activities of *Hibiscus sabdariffa* calyxes from Nsukka

METHOD

Extraction of the Plant Sample

A quantity of the air-dried *Hibiscus sabdariffa* calyxes was measured using a weighing balance, washed and boiled in 250ml of water for 30 minutes, and was decanted and filtered using Whatman No. 1 filter paper. The resulting filtrate was centrifuged to get a clearer filtrate and used. This process was repeated daily to get a fresh aqueous extract for each experiment in order to mitigate error due to microbial activities on the leftover sample.

Qualitative phytochemical analysis of *Hibiscus sabdariffa* calyxes

The qualitative phytochemical analysis of *Hibiscus sabdariffa* calyxes aqueous extract was determined by the methods of Trease and Evans (1989) and Harbourne (1978). A known quantity, 1 g of test sample, was placed in a test tube each to assay for phytochemicals such as reducing sugar, tannins, total phenol, steroid, flavonoid, glycoside, alkaloids, and terpenoids.

Quantitative phytochemical analysis of *Hibiscus sabdariffa* calyxes

The quantitative phytochemical analysis of *Hibiscus sabdariffa* calyxes aqueous extract was determined by the methods of Trease and Evans (1989) and Harbourne (1978).

Determination of *in-vitro* antioxidant properties

The quantitative determination of antioxidant properties of *Hibiscus sabdariffa* aqueous extract was carried out using these methods: Gyamfi et al. (1999), Banerjee et al. (2005), Benzie et al. (1996), Greengayam (1997), and Jayaprakasha et al. (2002).

Statistical Analysis

The data obtained were analyzed using the Statistical Product and Service Solutions (SPSS version 23.0) and expressed as mean ± SEM values. The statistical significance of the difference in mean values was determined using a one-way analysis of variance (ANOVA) with Duncan’s multiple comparison test for post-hoc analysis. A probability value of p < 0.05 was used as the criterion for statistical significance.

RESULTS & DISCUSSION

Qualitative and quantitative phytochemical constituents of aqueous extract of *Hibiscus sabdariffa* calyx.

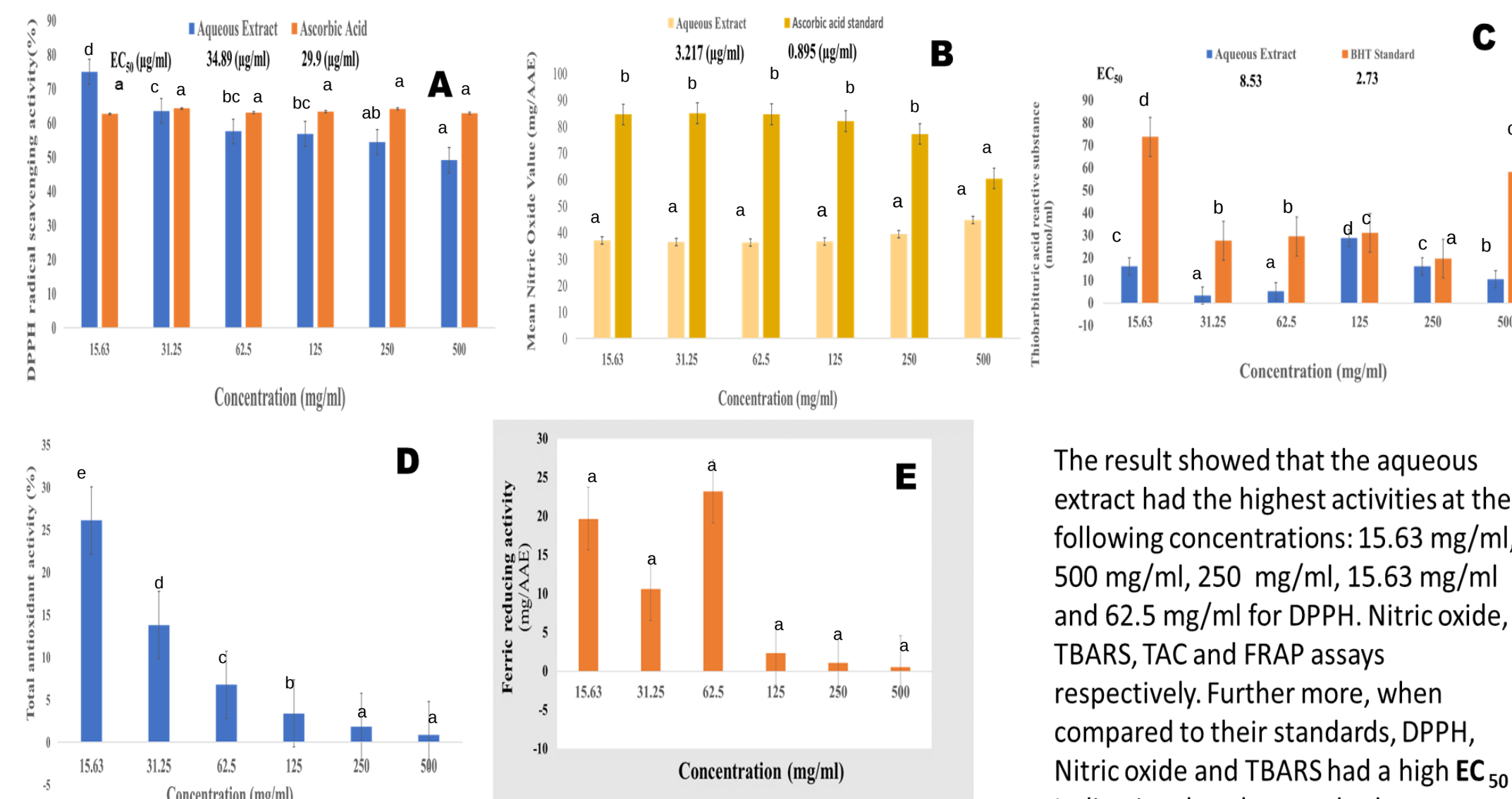
Phytochemical Constituents	Bioavailability	Phytochemical Constituents	Mean (mg/100g) ± SEM	Wavelength (nm)
Reducing Sugar	+++	Alkaloids	329.99 ± 4.57 ^d	565
Glycoside	+++	Flavonoids	179.38 ± 0.83 ^e	490
Alkaloid	+	Steroid	3.45 ± 0.00 ^a	550
Steroid	+++	Glycoside	1.69 ± 0.00 ^a	490
Terpenoid	+++	Reducing sugar	173.48 ± 0.13 ^c	420
Flavonoids	+++	Total phenol	4634 ± 10.75 ^e	765
Tannin	++	Tannin	14.42 ± 0.23 ^a	735
Phenol	+++	Terpenoid	137 ± 2.77 ^b	700
Saponin	ND			

Table 1

Table 2

In-vitro antioxidant activities of *Hibiscus sabdariffa* calyxes using DPPH scavenging, TBARS, TAC, FRAP and Nitric oxide Assay

Antioxidant Activity of *Hibiscus sabdariffa* calyxes aqueous extract



The result showed that the aqueous extract had the highest activities at the following concentrations: 15.63 mg/ml, 500 mg/ml, 250 mg/ml, 15.63 mg/ml and 62.5 mg/ml for DPPH. Nitric oxide, TBARS, TAC and FRAP assays respectively. Further more, when compared to their standards, DPPH, Nitric oxide and TBARS had a high EC₅₀ Indicating that the standard poses a better antioxidant activity.

The results presented in Tables 1 and 2 show the qualitative and quantitative phytochemical composition present in the aqueous extract of *Hibiscus sabdariffa* calyxes. From the result, it was established that the highest content of total phenolics was observed (4634.41 ± 10.75). This could be due to some environmental factors such as weather conditions, method of drying, method of extraction, and solvent used. The phenolic acids are associated with the inhibition of cancer, atherosclerosis, as well as age-related degenerative brain disorders (Jeannett et al., 2020). Antioxidant parameters were used in the analysis of the antioxidant activities of the aqueous extracts of the calyxes of *Hibiscus sabdariffa*.

CONCLUSION

- ❑ The results obtained showed that *Hibiscus sabdariffa* possesses abundant phenolics, alkaloids, and flavonoids and exhibited excellent antioxidant activity, thereby suggesting the potency of the aqueous extract of *Hibiscus sabdariffa* calyxes as natural antioxidants. Therefore, *Hibiscus sabdariffa* can be used in the treatment of oxidative stress-related diseases, justifying its use in traditional medicine.

FUTURE WORK / REFERENCES

Further studies can be carried out to evaluate the major functions of the phytochemicals and the mechanisms behind the chemotherapeutic activities and potential effects of *Hibiscus sabdariffa*.

Further studies can also be carried out on *Hibiscus sabdariffa* calyxes from different regions in Nigeria to know which region produces the most effective in terms of phytochemical composition and antioxidant activities.

REFERENCE

❑ Jeannett, A., Diego, A., Nancy, V., Eduardo, M., & Javier, C. (2020). Organic acid from Rosella (*Hibiscus sabdriffa* L.) – A brief review of its pharmacological effects. *Biomedicines*, **8**: 100

❑ Kumoye, D. E. & Remi-Esan, I. A. (2024). Comparative analysis of phytochemical constituents, proximate composition and energy value of *Hibiscus sabdariffa* L. leaf and petal extracts. *Federal Polytechnique Ilaro Journal of Pure and Applied Science* 6(2):12-16.

❑ Mumtaz, S., Ali, S., Shakir, H. A., Tahir, H. M., Mumtaz, S., & Andleeb, S. (2020). Therapeutic role of garlic and vitamins C and E against toxicity induced by lead on various organs. *Environmental Science and Pollution Research*, 27, 8953–8964.