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PHYTOCHEMICAL COMPOSITION, ANTIOXIDANT CAPACITY AND ANTIMICROBIAL ACTIVITY OF *Hibiscus sabdariffa* L. (ROSELLE SEED) EXTRACT

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ABSTRACT

Hibiscus sabdariffa L. (Roselle) seeds are emerging as a potent source of bioactive compounds with both antioxidant and antimicrobial properties. This study evaluated the phytochemical composition, antioxidant capacity and antimicrobial activity of Roselle seed extracts. Seeds were cleaned, shade-dried, ground, and extracted using 70% ethanol and distilled water. Phytochemical screening revealed the presence of saponins and terpenes**,** while tannins, flavonoids, and alkaloids were absent. Antioxidant activity, assessed using the DPPH radical scavenging assay, showed a dose-dependent increase**,** with maximum inhibition of 78.34% at 200 µg/mL. The strong radical scavenging effect is attributed to saponins and terpenes, highlighting their role as primary antioxidants even in the absence of flavonoids. Antimicrobial testing using agar well diffusion and MIC determination demonstrated significant activity against *Staphylococcus aureus* and *Candida albicans* (MIC 50 mg/µL), moderate inhibition of *Pseudomonas aeruginosa* (MIC 100 mg/µL), and resistance of *Escherichia coli*, consistent with the known defense mechanisms of Gram-negative bacteria. These effects are linked to the membrane-disruptive and bioactive properties of saponins and terpenes. The results confirmed that Roselle seed extracts possessed dual antioxidant and antimicrobial activities, supporting their traditional medicinal uses and potential application as natural preservatives or therapeutic agents. Further research is recommended to isolate and characterize the active compounds, explore their synergistic effects with conventional drugs, and evaluate their efficacy in in vivo models. Overall, Roselle seeds offer a promising, natural source of bioactive compounds for pharmaceutical and nutraceutical development.

Key words: *Hibiscus sabdariffa,* Roselle seed, Antioxidant activity, Antimicrobial activity, Saponins

1.0 INTRODUCTION

Plants are rich sources of bioactive compounds with diverse health benefits, including antioxidant and antimicrobial properties. Among these, *Hibiscus sabdariffa* L. (Roselle) is a widely cultivated medicinal plant known for its edible calyces, seeds, and leaves, which are traditionally used in beverages, jams, and herbal remedies (Ali *et al.,* 2020). While the calyces are commonly consumed for their anthocyanin content, Roselle seeds have recently gained attention due to their rich content of polyphenols, flavonoids, and essential fatty acids, which contribute to their bioactivity (Olayemi *et al.,* 2021). Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) and antioxidants, is implicated in various chronic diseases including cardiovascular disorders, cancer, and neurodegeneration. Natural antioxidants from plant sources can scavenge free radicals and protect biomolecules from oxidative damage (Chen *et al.,* 2021). Several studies have demonstrated that *H. sabdariffa* seed extracts possess significant free radical scavenging activity, indicating their potential as natural antioxidants in food and pharmaceutical applications (Ahmed *et al.,* 2022).

In addition to antioxidant activity, Roselle seed extracts exhibit antimicrobial properties against a range of pathogenic microorganisms, including *Escherichia coli, Staphylococcus* aureus, and *Candida albicans* (Akinmoladun *et al.,* 2019; Ogunmoyela *et al.,* 2022). These activities are largely attributed to secondary metabolites such as phenolic acids, flavonoids, and tannins, which disrupt microbial cell walls and inhibit growth. The dual antioxidant and antimicrobial effects of *H. sabdariffa* seeds suggest their potential for development as natural preservatives or therapeutic agents.

Despite growing interest, studies specifically evaluating the antioxidant and antimicrobial potential of Roselle seed

extracts remain limited, highlighting the need for further research to quantify their bioactive properties and explore practical applications in food and pharmaceutical industries.

2.0 MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Makurdi, the capital of Benue State, Nigeria, located within the Southern Guinea Savannah ecological zone. Geographically, Makurdi lies approximately at latitude 7°44′N and longitude 8°32′E, along the banks of the River Benue, which significantly influences the local microclimate and agricultural activities in the area. The location is characterized by a mix of urban and peri-urban settings, supporting active trade in agricultural commodities, including medicinal plants and seeds. The climate of Benue State is typically tropical sub-humid, marked by distinct wet and dry seasons. The rainy season generally occurs from April to October, while the dry season spans November to March. Average annual rainfall ranges between 1,200 and 1,600 mm, with peak rainfall usually recorded between July and September. Temperatures are relatively high throughout the year, ranging from about 24 °C to 35 °C, with higher values recorded during the dry season. Relative humidity is high during the rainy season and drops significantly during the harmattan period. These climatic conditions support the growth and availability of diverse plant species used for medicinal and nutritional purposes. Ecologically, the area is dominated by savannah vegetation interspersed with scattered trees, shrubs, and grasses, making it suitable for the growth of *Hibiscus sabdariffa*. The presence of the River Benue also contributes to fertile alluvial soils, which support agricultural productivity and the availability of plant-based raw materials used in traditional and scientific research.

2.2 Sample Collection

Roselle (Hibiscus sabdariffa L.) seeds were obtained from Modern Market, Makurdi, Benue State. The seeds were sorted to remove impurities, washed, and shade-dried at ambient temperature to preserve bioactive compounds. The dried seeds were milled into fine powder using a sterile electric grinder and stored in airtight containers at room temperature prior to analysis.

2.3 Sample Identification

The plant material was authenticated based on morphological characteristics and taxonomic descriptors. Identification was confirmed using standard botanical references, and the specimen was verified as Hibiscus sabdariffa L. (family Malvaceae) in accordance with established plant taxonomy guidelines (POWO, 2024).

2.4 Sample Preparation

Approximately 50 g of powdered Roselle seeds was extracted using 70% ethanol and distilled water at a solvent-to-sample ratio of 5:1 (v/w). The mixtures were macerated for 72 hours at room temperature with intermittent shaking. Filtration was carried out using Whatman No. 1 filter paper. The ethanol extract was concentrated using a rotary evaporator at 40 °C, while the aqueous extract was evaporated using a water bath. Extracts were stored at 4 °C until further use (Azwanida, 2019).

2.5 Experimental Design

A completely randomized design (CRD) was employed. Qualitative phytochemical analysis was carried out on the extract to detect the presence of alkaloids, flavonoids, tannins, saponins, and terpenoids using standard phytochemical procedures of Khan *et al.* (2021). The antioxidant potential of the extract was evaluated using the DPPH free radical scavenging assay. Serial concentrations of the extract were prepared and reacted with DPPH solution, followed by incubation in the dark. Absorbance was measured at 517 nm, and percentage inhibition was calculated using standard modifications of the Blois method (Munteanu and Apetrei, 2021). Antimicrobial activity was assessed using the agar well diffusion method against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*. The inoculated plates were incubated at 37 °C for 24 hours, after which zones of inhibition were measured. Minimum inhibitory concentration (MIC) values were determined using standard broth dilution techniques in accordance with CLSI guidelines (CLSI, 2022).

2.6 Data Collection

Data collection involved both qualitative and quantitative assessments of the Roselle seed extracts. Phytochemical data were obtained by observing characteristic colour changes or precipitate formation during standard screening tests for alkaloids, flavonoids, tannins, saponins, and terpenoids. Antioxidant data were collected spectrophotometrically by measuring absorbance changes at 517 nm after reaction of different extract concentrations with DPPH solution, and percentage inhibition values were computed from the readings. For antimicrobial assessment, data were collected by measuring the zones of inhibition (in millimetres) around wells on inoculated agar plates after incubation, while minimum inhibitory concentration (MIC) values were determined by identifying the lowest extract concentration that prevented visible microbial growth in broth dilution assays.

2.7 Data Analysis

Data obtained were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was used to determine significant differences among means. Statistical significance was accepted at $p < 0.05$. Results were presented in tables and figures.

3.0 RESULTS

3.1 DPPH Radical Scavenging Activity of the *Hibiscus sabdariffa* Seed Extract

Table 1 and Figure 1 showed that the percentage inhibition increased as the concentration of the extract increased. At 25 µg/mL, the inhibition was 40.55%, while the highest inhibition of 78.34% was recorded at 200 µg/mL. The

absorbance values decreased gradually from 1.400 to 0.510 with increasing concentration. This indicates a concentration-dependent antioxidant activity of the extract.

Table 1: DPPH Radical Scavenging Activity of the *Hibiscus sabdariffa* Seed Extract

Concentration (µg/ml)	Absorbance (A517)	% Inhibition
25	1.400	40.55%
50	1.000	57.54%
100	0.905	61.58%
200	0.510	78.34%
Control	2.355	—

Values represent the mean antioxidant activity of the extract at different concentrations using the DPPH radical scavenging assay.

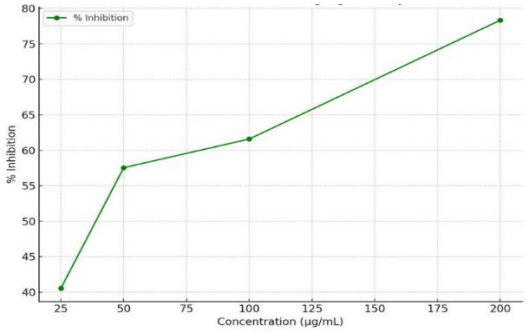

Figure 1: Graph showing the relationship between extract concentration and percentage DPPH radical inhibition

3.2 Phytochemical Constituents of *Hibiscus sabdariffa* Seed Extract

The Phytochemical screening of *Hibiscus sabdariffa* Seed Extract revealed the presence of important bioactive compounds. As shown in Table 2, saponins and terpenes were present in the extract, whereas tannins, flavonoids, and alkaloids were absent. The result indicates that the extract contained only two detectable phytochemical constituents. Saponins and terpenes were strongly present as indicated by (++). The absence of other phytochemicals suggests variation in the chemical composition of the extract.

Table 2: Phytochemical Constituents of *Hibiscus sabdariffa* Seed Extract

Phytochemical	Result
Tannins	Absent (–)
Saponins	Present (++)
Flavonoids	Absent (–)
Alkaloids	Absent (–)
Terpenes	Present (++)

KEY: ++ = Presence of phytochemicals; = Absence of Phytochemicals

3.3 Antibacterial Activity of *Hibiscus sabdariffa* Seed Extract

The antibacterial activity of *hibiscus sabdariffa* seed extract are shown in Table 3. The extract exhibited antimicrobial activity at 50 mg and 100 mg concentrations. *Candida albicans* recorded the highest zone of inhibition of 20 mm at 100 mg concentration, followed by *Staphylococcus aureus* and *Pseudomonas aeruginosa*. No inhibition was observed at 25 mg concentration for all organisms tested. *Escherichia coli* showed no sensitivity to the extract at all concentrations.

Table 3: Antibacterial Activity of *Hibiscus sabdariffa* Seed Extract

Zones of inhibition were measured in millimetres (mm) after incubation of the test organisms with different extract concentrations.

Concentration	*E. coli*	*Pseudomonas*	*Staphylococcus*	*Candida albicans*
100 mg	0 mm	15 mm	16 mm	20 mm
50 mg	0 mm	8 mm	10 mm	13 mm
25 mg	0 mm	0 mm	0 mm	0 mm
Control (Std.)	30 mm	28 mm	35 mm	—

3.4 Minimum Inhibitory Concentration (MIC) of *Hibiscus sabdariffa* Seed Extract

Table 4 presents the minimum inhibitory concentration (MIC) of *Hibiscus sabdariffa* seed extract. This showed that *Staphylococcus aureus* and *Candida albicans* had MIC values of 50 mg/µl, indicating inhibition at lower concentrations. *Pseudomonas aeruginosa* required a higher concentration of 100 mg/µl for inhibition. *Escherichia coli* showed no inhibition throughout the test. The result indicates differences in the sensitivity of the test organisms to the extract.

Table 4: Minimum Inhibitory Concentration (MIC) of *Hibiscus sabdariffa* Seed Extract

Microorganism	MIC Value (mg/µl)
E. coli	No inhibition
Pseudomonas	100 mg/µl
Staphylococcus	50 mg/µl
Candida albicans	50 mg/µl

MIC values represent the minimum concentration of the extract required to inhibit visible microbial growth.

4.0 DISCUSSION

The present study showed that *Hibiscus sabdariffa* seed extract possesses considerable antioxidant and antimicrobial activities. The antioxidant activity increased progressively with concentration, indicating that the extract was capable of scavenging free radicals effectively. The highest inhibition was observed at 200 µg/mL, suggesting strong antioxidant potential at higher concentrations. This activity may be linked to the presence of saponins and terpenes detected during phytochemical screening. Similar findings have been reported for Roselle seed extracts and other medicinal plants containing comparable bioactive compounds as reported by Adekunle *et*

al. (2023). Phytochemical analysis revealed the presence of saponins and terpenes, while flavonoids, tannins, and alkaloids were not detected. The occurrence of antioxidant activity despite the absence of flavonoids suggests that saponins and terpenes contributed significantly to the observed effects. Saponins are known to possess membrane-active and antioxidant properties, whereas terpenes have been associated with antimicrobial and free radical scavenging activities. Similar observations were reported by Ibrahim *et al*. (2022) who noted that non-flavonoid compounds can also contribute substantially to antioxidant activity in plant extracts.

The antimicrobial results demonstrated that the extract was active against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*, but inactive against *Escherichia coli*. The strongest inhibition was recorded against *Candida albicans*, followed by *Staphylococcus aureus*. The resistance shown by *E. coli* may be associated with the protective outer membrane characteristic of Gram-negative bacteria, which often limits the penetration of plant-derived compounds. Previous studies have similarly reported higher susceptibility of Gram-positive bacteria and fungi to plant extracts compared to Gram-negative organisms (Akinyemi *et al.,* 2015; Yusuf *et al.,* 2025). The MIC values obtained in this study further confirmed the antimicrobial potential of the extract. *Staphylococcus aureus* and *Candida albicans* showed greater sensitivity with lower MIC values, while *Pseudomonas aeruginosa* required a higher concentration for inhibition. The differences in susceptibility among the test organisms may be related to variations in cell wall structure and resistance mechanisms. These findings are consistent with reports by Olukoya *et al.* (2024), who observed that the effectiveness of plant extracts depends largely on the nature of the microorganism and the phytochemical constituents present. the study suggests that Roselle seed extract could serve as a natural source of antioxidant and antimicrobial agents. The biological activities observed support the traditional use of the plant in herbal medicine and indicate its possible application in pharmaceutical and food industries. Further studies are recommended to isolate the active compounds and evaluate their effectiveness under in vivo conditions.

5.0 CONCLUSION

This study demonstrated that the Roselle seed extract possesses significant antioxidant and antimicrobial activities, supported by phytochemical screening and MIC determination. DPPH assays showed a dose-dependent antioxidant effect, with a maximum inhibition of 78.34% at 200 µg/mL, primarily due to saponins and terpenes, despite the absence of flavonoids. Phytochemical analysis confirmed only these two bioactive compounds, highlighting that a limited profile can still confer notable biological effects. Antimicrobial tests indicated high efficacy against Staphylococcus aureus and Candida albicans (MIC 50 mg/µL), moderate activity against Pseudomonas aeruginosa (MIC 100 mg/µL), and resistance in E. coli, reflecting known Gram-negative defenses. Overall, the findings validate traditional uses of the plant and suggest its potential for development into natural antioxidant and antimicrobial agents.

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