

Qualitative and Quantitative Phytochemical Profiling of *Hibiscus sabdariffa* L. Seeds and Implications for Nutraceutical and Therapeutic Applications

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ABSTRACT

Hibiscus sabdariffa L. (Malvaceae), commonly known as roselle, is a widely cultivated multipurpose plant of considerable ethnopharmacological relevance across tropical and subtropical regions of Africa, Asia, and the Americas. Despite sustained global interest in the phytochemistry of its calyces and leaves, systematic characterisation of the seed fraction remains comparatively underexplored. This study reports a parallel qualitative and quantitative phytochemical analysis of methanolic extracts of *H. sabdariffa* seeds sourced from the Onigambari Research Station, Ibadan, Nigeria. Qualitative screening conducted according to Harborne (1973) and Evans (2009) detected seven of nine secondary metabolite classes tested. Alkaloids, terpenoids, and steroids were recorded as abundantly present (++), while flavonoids, saponins, cardiac glycosides, and anthraquinones were present (+). Tannins and free phenols were absent (-). Gravimetric quantification yielded alkaloids at $1.55 \pm 0.002\%$ (w/w), terpenoids at $1.30 \pm 0.001\%$ (w/w), and saponins at $0.45 \pm 0.002\%$ (w/w). Spectrophotometric analyses, performed in triplicate against validated calibration curves ($R^2 \geq 0.99$), gave: total tannin content (TTC) of $1.05 \pm 0.007\%$ (w/w) gallic acid equivalents (GAE); total flavonoid content (TFC) of $0.28 \pm 0.249\%$ (w/w) quercetin equivalents (QE); and total phenolic content (TPC) of $0.83 \pm 0.001\%$ (w/w) GAE. The detected phytochemical assemblage is consistent with reported antioxidant, anti-inflammatory, antimicrobial, and cardioprotective activities of the species. These findings provide an empirical basis for the seed's continued evaluation as a nutraceutical ingredient and herbal medicine resource.

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INTRODUCTION

Hibiscus sabdariffa L. (family Malvaceae), commonly referred to as roselle, sorrel, or karkadé, is an annual or perennial herb distributed throughout tropical and subtropical Africa, Asia, and the Americas (Ali et al., 2005; Da-Costa-Rocha et al., 2014). The plant occupies a dual role in human society: as a food crop consumed as hot and cold beverages, jams, and dried condiments and as a traditional medicinal herb credited with antihypertensive, diuretic, antimicrobial, anti-inflammatory, and antioxidant activities (Ali et al., 2005; Hopkins et al., 2013; Da-Costa-Rocha et al., 2014; Serban et al., 2015). These properties have been attributed to a diverse assemblage of secondary metabolites distributed across the calyx, leaves, stem, roots, and seeds, including anthocyanins (notably delphinidin-3-O-sambubioside and cyanidin-3-O-sambubioside), phenolic acids (protocatechuic acid, hibiscus acid), flavonoids (quercetin, kaempferol, rutin), saponins, alkaloids, and sterols (Tsai et al., 2002; Da-Costa-Rocha et al., 2014; Ojokoh et al., 2019; Montalvo-González et al., 2022; Onyeukwu & Dibia, 2023). Growing clinical and epidemiological evidence supports the use of calyx-derived preparations for management of mild-to-moderate hypertension and hyperlipidaemia, reinforcing scientific interest in the bioactive potential of the plant (Serban et al., 2015; Umeoguaju & Ephraim-Emmanuel, 2021). Phytochemical screening encompassing both qualitative detection and quantitative measurement of secondary

metabolite classes constitutes an indispensable preliminary step in natural product drug discovery and functional food development (Hamrita et al., 2022; Nogueira da Cruz & Khiatah, 2022). Qualitative assays (colorimetric precipitation and spot tests) enable rapid, low-cost identification of compound categories, while quantitative methods (gravimetric precipitation and spectrophotometric calibration against certified standards) deliver the concentration estimates required for pharmacological standardisation, dosage modelling, and regulatory dossier preparation (Hamrita et al., 2022; Nogueira da Cruz & Khiatah, 2022). The complementary use of both approaches is therefore critical, particularly when qualitative results are used to prioritise classes for downstream chromatographic and bioactivity-guided fractionation.

Among the plant organs of *H. sabdariffa*, the seed fraction has attracted comparatively less scientific attention relative to the calyx, despite constituting a nutritionally significant co-product of roselle processing. The seeds are reported to contain 25–33% crude protein, 20–26% oil (rich in linoleic and oleic acids), substantial dietary fibre, and macro- and micro-mineral elements (Chukwu et al., 2019; Mohamed et al., 2024). Phytochemical investigations of the seed in Sudanese and Nigerian accessions have confirmed the presence of alkaloids, saponins, and flavonoids, and have demonstrated in vitro antioxidant and antibacterial activities in seed extracts (Hagr & Adam, 2020; Mohamed et al., 2024).

However, comprehensive dual-method profiling integrating gravimetric and spectrophotometric quantification under internally consistent, validated analytical conditions from West African germplasm is absent from the published literature. Such data are necessary to establish reference phytochemical baselines for Nigerian accessions, support extract standardisation, and guide the rational development of seed-based nutraceutical formulations.

Considerable methodological heterogeneity characterises published phytochemical data for *H. sabdariffa* seeds, with wide variation in reported constituent concentrations attributable to differences in extraction solvent polarity, solid-to-liquid ratios, maceration duration, and analytical platform (Harborne, 1973; Hamrita et al., 2022; Nogueira da Cruz & Khiatah, 2022). This variability complicates cross-study comparisons and underscores the need for fully described, reproducible analytical protocols. Accordingly, the present study aimed to (i) determine the qualitative phytochemical profile of methanolic seed extracts of *H. sabdariffa* using standard colorimetric and precipitation assays; (ii) quantify alkaloids, terpenoids, and saponins by validated gravimetric methods; (iii) determine total tannin, flavonoid, and phenolic contents by spectrophotometric calibration against certified reference standards; and (iv) contextualise the findings within the broader pharmacological and nutraceutical literature on the species.

MATERIALS AND METHODS

Plant Material and Authentication

Seeds of *Hibiscus sabdariffa* L. were manually harvested from mature, fully dehiscent capsules in August 2024 at the Onigambari Research Station, Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria (latitude 7°30'N, longitude 4°00'E; altitude approximately 227 m above sea level).

Sample Preparation

Seeds were rinsed with three successive changes of distilled water to remove surface impurities, then spread in a single layer on clean nylon-mesh drying trays and air-dried at ambient temperature (25–30 °C) until constant mass was attained (approximately 72 h). Dried seeds were pulverised to a fine powder using a stainless-steel laboratory blender (Binatone model BLG-450), and the powder was sieved through a 0.5-mm mesh (British Standard sieve No. 36) to obtain a uniform particle size. The powdered material was stored in airtight amber glass vials at 4 °C until use.

Preparation of Methanolic Extract

Extraction was performed by cold maceration. One gram (1.0 g, accurately weighed) of powdered seed was macerated in 20 mL of 80% (v/v) methanol (Sigma-Aldrich, analytical grade) in a stoppered 100-mL Erlenmeyer flask at room temperature (25 °C) for 48 h with intermittent hand-shaking at 8-h intervals. The slurry was filtered through Whatman No. 1 filter paper (pore size 11 µm). The filtrate was transferred to a pre-weighed evaporating dish and concentrated to dryness on a thermostatically controlled water bath at 40 °C. The percentage extraction yield (% w/w) was calculated as: % yield = (mass of dried extract / mass of starting powder) × 100. Dried extracts were stored at –20 °C in sealed amber vials until analysis.

Qualitative Phytochemical Screening

Qualitative screening of the methanolic seed extract was performed in accordance with the standard procedures

described by Harborne (1973) and Evans (2009). The following nine secondary metabolite classes were tested:

Alkaloids: Dragendorff's reagent (potassium bismuth iodide), Mayer's reagent (potassium mercuric iodide), and Wagner's reagent (iodine–potassium iodide) tests; red or brown precipitate taken as positive.

Flavonoids: Ammonia/H₂SO₄, aluminium chloride (1% AlCl₃ in methanol), and ethyl acetate/ammonia tests; yellow colour with AlCl₃ and yellow fluorescence under UV taken as positive.

Saponins: Persistent frothing (Frothing–Haemolysis) test; stable foam persisting for ≥ 10 min after vigorous shaking of a 2% aqueous extract taken as positive.

Tannins: Ferric chloride test (1% FeCl₃ in water); blue-black or greenish-black coloration taken as positive.

Anthraquinones: Bornträger's test (Soda extract acidified and extracted with ether, followed by addition of dilute ammonia); pink or red coloration of the ammoniacal layer taken as positive.

Terpenoids: Salkowski test (extract dissolved in chloroform, then concentrated H₂SO₄ added carefully); reddish-brown coloration at the interface taken as positive.

Cardiac glycosides: Keller–Kiliani test (glacial acetic acid + FeCl₃, then H₂SO₄ underlay); reddish-brown ring at the junction and blue-green colour in the upper layer taken as positive.

Free phenols: Folin–Ciocalteu colour reaction in the absence of the sodium carbonate step; a blue colour taken as positive.

Steroids: Liebermann–Burchard test (extract dissolved in chloroform + acetic anhydride, then H₂SO₄ added); green-blue colour taken as positive.

Results were categorised as absent (–), present (+), or abundantly present (++), based on the relative intensity of colour development or volume of precipitate formed compared with known positive controls. All tests were conducted in triplicate to confirm consistency.

Quantitative Phytochemical Analysis

Alkaloids (Gravimetric Method)

Alkaloid content was determined by the acid–base gravimetric precipitation method of Harborne (1973). Briefly, 1.0 g of powdered seed was stirred with 20 mL of 10% (v/v) acetic acid in ethanol for 4 h, filtered through Whatman No. 1 paper, and the filtrate concentrated to approximately 5 mL on a water bath. Alkaloids were precipitated by dropwise addition of concentrated ammonium hydroxide solution to pH ≥ 11, the precipitate collected on pre-weighed Whatman No. 42 paper (ashless), dried at 60 °C to constant mass, cooled in a desiccator, and weighed. The analysis was carried out in duplicate and the mean reported. Percentage yield (%w/w) = (mass of precipitate / mass of sample) × 100.

Terpenoids (Gravimetric Method)

Terpenoid content was determined by a modified hexane-partition gravimetric method based on Harborne (1973). One gram of powdered seed was extracted with 20 mL of absolute ethanol with magnetic stirring for 1 h, filtered, and the filtrate transferred to a 60-mL separating funnel. An equal volume of n-hexane (analytical grade) was added, the mixture shaken vigorously, and the two phases allowed to separate. The hexane layer (upper phase, enriched in terpenoids and steroids) was collected, transferred to a pre-weighed porcelain crucible, and evaporated at 40 °C to constant mass. The analysis was carried out in duplicate and the mean reported. Percentage yield was calculated as above.

Saponins (Gravimetric Method)

Saponin content was determined by the gravimetric method of Obadoni and Ochuko (2001). One gram of powdered seed was extracted with 20 mL of 20% (v/v) aqueous ethanol at 55 °C for 4 h with continuous magnetic stirring, filtered, and the extraction repeated twice; the combined filtrates were pooled. Non-saponin lipophilic impurities were removed by washing the pooled filtrate three times against 10 mL n-diethyl ether. The clarified aqueous phase was transferred to a pre-weighed porcelain crucible, evaporated to dryness at 60 °C, and weighed after equilibration in a desiccator. The analysis was carried out in duplicate and the mean reported.

All gravimetric determinations were performed in duplicate (n = 2); mean values are reported. Results are expressed as mean $\pm$ standard deviation (SD) in % (w/w) dry weight basis.

Total Tannin Content (TTC) — Spectrophotometric Method

TTC was determined by the Folin–Denis colorimetric method (Harborne, 1973) using gallic acid ($\geq 97.5\%$ purity, Sigma-Aldrich) as the reference standard. Working extract solutions at concentrations of 200, 400, 600, 800, and 1000 $\mu\text{g/mL}$ were prepared by serial dilution of the stock methanolic extract in 80% methanol. Each dilution (1.0 mL) was added to 0.5 mL of Folin–Denis reagent (prepared freshly from sodium tungstate, phosphomolybdic acid, and phosphoric acid), followed by 1.0 mL of 35% (w/v) sodium carbonate solution; the mixture was made up to 10.0 mL with distilled water, mixed thoroughly, and incubated at 25 °C for 30 min in the dark. Absorbance was measured at 760 nm against a reagent blank (Jenway 6305 UV–Vis spectrophotometer; Bibby Scientific, UK). A calibration curve was constructed using gallic acid standards at 32, 63, 125, 250, 500, and 1000 $\mu\text{g/mL}$; linearity was confirmed by least-squares regression ($R^2 \geq 0.99$). TTC was expressed as % (w/w) gallic acid equivalents (GAE). All measurements were performed in triplicate (n = 3).

Total Flavonoid Content (TFC) — Spectrophotometric Method

TFC was determined by the aluminium chloride (AlCl_3) colorimetric method (Evans, 2009; Harborne, 1973) using quercetin ($\geq 95\%$ purity, Sigma-Aldrich) as the reference standard. Aliquots (1.0 mL) of each extract dilution (200–1000 $\mu\text{g/mL}$) were combined with 4.0 mL distilled water, 0.30 mL of 5% (w/v) NaNO_2 solution, and 0.30 mL of 10% (w/v) AlCl_3 solution; after 5 min, 2.0 mL of 1 M NaOH was added, and the total volume adjusted to 10 mL with distilled water. Absorbance was measured at 430 nm against a reagent blank. A quercetin calibration curve was prepared at 20, 40,

60, 80, and 100 $\mu\text{g/mL}$ ($R^2 \geq 0.99$). TFC was expressed as % (w/w) quercetin equivalents (QE). All measurements were performed in triplicate (n = 3).

Total Phenolic Content (TPC) — Spectrophotometric Method

TPC was determined by the Folin–Ciocalteu method (Evans, 2009; Harborne, 1973) using gallic acid as the reference standard. Aliquots (0.10 mL) of each extract dilution (200–1000 $\mu\text{g/mL}$) were combined with 0.50 mL of Folin–Ciocalteu reagent (diluted 1:10 v/v with distilled water) and mixed. After 5 min, 1.0 mL of 20% (w/v) sodium carbonate solution was added, and the total volume adjusted to 10 mL with distilled water; the mixture was incubated at 25 °C for 30 min in the dark. Absorbance was measured at 760 nm against a reagent blank. A gallic acid calibration curve was constructed at 80, 120, 160, and 200 $\mu\text{g/mL}$ ($R^2 \geq 0.99$). TPC was expressed as % (w/w) gallic acid equivalents (GAE). All measurements were performed in triplicate (n = 3).

Statistical Analysis

All values are expressed as mean $\pm$ standard deviation (SD). Gravimetric data represent the mean of duplicate extractions (n = 2); spectrophotometric data represent the mean of triplicate readings (n = 3). Calibration curve linearity was evaluated by the coefficient of determination (R^2) derived from least-squares linear regression in Microsoft Excel 2019. Normality of spectrophotometric datasets was assessed by the Shapiro–Wilk test (IBM SPSS Statistics v.26; IBM Corp., Armonk, NY, USA). A significance threshold of $p < 0.05$ was applied throughout.

RESULTS AND DISCUSSION**Qualitative Phytochemical Screening**

Qualitative phytochemical screening of the methanolic seed extract of *H. sabdariffa* confirmed the presence of seven of the nine secondary metabolite classes tested (Table 1). Alkaloids, tested by three independent precipitation reagents (Dragendorff's, Mayer's, and Wagner's), were all strongly positive (++) across all three reagent systems, indicating abundant alkaloid content in the seed matrix. Terpenoids (Salkowski test) and steroids (Liebermann–Burchard test) were likewise recorded as abundantly present (++) . Saponins, flavonoids, cardiac glycosides, and anthraquinones were detected at moderate (+) levels. Tannins (FeCl_3 test) and free phenols (Folin–Ciocalteu reaction) were absent (–) from the seed extract. Results were reproducible across all three replicate tests.

Table 1: Qualitative Phytochemical Profile of Methanolic Seed Extract of *Hibiscus sabdariffa* L

Phytochemical Class	Reagent / Test System	Result
Alkaloids	Dragendorff's / Mayer's / Wagner's	++ (all three)
Terpenoids	Salkowski ($\text{CHCl}_3 + \text{H}_2\text{SO}_4$ conc.)	++
Steroids	Liebermann–Burchard ($\text{CHCl}_3 + \text{Ac}_2\text{O} + \text{H}_2\text{SO}_4$)	++
Flavonoids	AlCl_3 ; $\text{NH}_3/\text{H}_2\text{SO}_4$; EtOAc/NH_3	+
Saponins	Persistent frothing (Frothing–Haemolysis)	+
Cardiac glycosides	Keller–Kiliani	+
Anthraquinones	Bornträger's	+
Tannins	1% FeCl_3 aqueous	–
Free phenols	Folin–Ciocalteu (without Na_2CO_3 alkalisation)	–

Quantitative Phytochemical Analysis**Gravimetric Quantification of Alkaloids, Terpenoids, and Saponins**

Results of gravimetric quantification are presented in Table 2. Alkaloids constituted the highest-yielding fraction at $1.55 \pm 0.002\%$ (w/w), followed by terpenoids at $1.30 \pm 0.001\%$

(w/w) and Tannin at 1.05 ± 0.007 . The relative ranking of these three classes is congruent with the qualitative data, wherein alkaloids and terpenoids were most abundant (++). The precision of duplicate extractions was high, as reflected by the small standard deviations.

Table 2: Gravimetric Quantification of Alkaloids, Terpenoids, and Saponins in Methanolic Seed Extract of *Hibiscus sabdariffa* L.

Phytochemical	<i>Hibiscus sabdariffa</i> L. (% w/w)
Alkaloids	1.55 ± 0.002
Terpenoids	1.30 ± 0.001
Saponins	0.45 ± 0.002
Flavonoids	0.28 ± 0.249
Tannins	1.05 ± 0.007
Phenols	0.83 ± 0.001

Discussion

The present study provides the first systematically documented parallel qualitative and quantitative phytochemical characterisation of methanolic seed extracts of *H. sabdariffa* from the Onigambari accession of the Forestry Research Institute of Nigeria, employing gravimetric and spectrophotometric methods against certified reference standards. The results confirm a rich and pharmacologically diverse secondary metabolite profile in the seed, encompassing alkaloids, terpenoids, steroids, flavonoids, saponins, cardiac glycosides, and anthraquinones. This finding corroborates previous qualitative reports from Sudanese and Nigerian accessions (Hagr & Adam, 2020; Mohamed et al., 2024) and extends the phytochemical knowledge base for West African germplasm with precise gravimetric and spectrophotometric quantification.

The abundant detection of alkaloids (++) supported by a gravimetric yield of $1.55 \pm 0.002\%$ (w/w) is consistent with prior reports of nitrogen-containing secondary metabolites in *Hibiscus* spp. (Ojokoh et al., 2019; Hagr & Adam, 2020). Alkaloids in members of Malvaceae have been broadly associated with analgesic, antispasmodic, antibacterial, and anti-inflammatory activities (Ojokoh et al., 2019; Onyeukwu & Dibia, 2023). The considerably higher alkaloid value reported by Chukwu et al. (2019) for Nigerian *H. sabdariffa* seeds (39.20%) is explicable by the methodological difference: GC-FID-based quantification encompasses a broader population of volatile nitrogen-containing compounds, including amino acid degradation products and volatiles that are not precipitated by the acid–base gravimetric method. This methodological non-equivalence is a recurrent source of apparent discordance in phytochemical literature and underscores the recommendation of Hamrita et al. (2022) and Nogueira da Cruz and Khiatah (2022) that quantitative data be reported alongside full methodological descriptions to permit meaningful comparison.

The high terpenoid ($1.30 \pm 0.001\%$ w/w) and abundant steroid (++) contents are consistent with the lipid-rich character of *H. sabdariffa* seeds, whose oil fraction is documented to contain triterpenoids, phytosterols (β -sitosterol, stigmasterol, campesterol), and polyterpene hydrocarbons as minor lipid components (Da-Costa-Rocha et al., 2014; Montalvo-González et al., 2022). Phytosterols are well characterised as competitive inhibitors of intestinal cholesterol absorption and as modulators of NF- κ B-mediated inflammatory signalling (Awad & Fink, 2000; Plat & Mensink, 2005). Triterpenoids, additionally, have demonstrated hepatoprotective activity in rodent models, potentially via induction of antioxidant

enzyme systems (superoxide dismutase, catalase) (Montalvo-González et al., 2022). The seed's terpenoid and sterol content thus provides a mechanistic basis for anti-inflammatory and lipid-lowering activities reported for *H. sabdariffa* preparations in vivo.

The detection of anthraquinones (+) in the seed extract is noteworthy. Anthraquinone glycosides are well-established laxative agents acting on colonic epithelium via stimulation of prostaglandin-mediated secretion and inhibition of water reabsorption (van Gorkom et al., 1999; Evans, 2009). Their presence in the seed, in conjunction with the dietary fibre content documented in *H. sabdariffa* seeds (Chukwu et al., 2019; Mohamed et al., 2024), suggests potential utility in formulations targeting constipation and gastrointestinal motility. The detection of cardiac glycosides (+) by the Keller–Kiliani test warrants toxicological attention: these compounds inhibit sarcolemmal Na^+/K^+ -ATPase and at supratherapeutic concentrations cause arrhythmia and cardiac toxicity (Evans, 2009). Structural characterisation by HPLC-MS/MS is therefore essential before seed extracts are considered for therapeutic application, in order to identify the specific glycoside(s) and determine safe exposure margins.

The spectrophotometric TTC value of $1.05 \pm 0.007\%$ (w/w) GAE, despite the negative FeCl_3 qualitative result, illustrates a key methodological principle: the visual detection threshold of colorimetric precipitation assays is considerably higher than the detection limit of calibrated spectrophotometric methods. Hamrita et al. (2022) formally demonstrated this discordance and established that Folin–Denis and FeCl_3 assays have non-identical polyphenol specificities; the former responds to a broader range of hydroxylated phenolics including ellagitannins and gallotannins at sub-visual concentrations, while FeCl_3 precipitation requires higher concentrations of hydrolysable or condensed tannins for a visible colour change. The tannin concentration detected spectrophotometrically in the present study therefore likely reflects low but real tannin accumulation in the seed, consistent with the general observation that tannins are less concentrated in reproductive tissues than in vegetative organs of *H. sabdariffa* (Da-Costa-Rocha et al., 2014; Onyeukwu & Dibia, 2023).

Flavonoids in *H. sabdariffa*, including quercetin, kaempferol, and their glycosides, are potent inhibitors of xanthine oxidase, lipoxygenase, and cyclooxygenase-2 at the molecular level, underpinning the species' documented anti-inflammatory activity (Middleton et al., 2000; Montalvo-González et al., 2022; Tsai et al., 2002). The comparatively low TFC in seeds relative to calyx extracts is consistent with the anthocyanin-

dominated flavonoid profile of the calyx, a tissue with markedly stronger polyphenol sink activity (Da-Costa-Rocha et al., 2014; Tsai et al., 2002).

Total phenolic content ($0.83 \pm 0.001\%$ w/w GAE) reflects the contribution of non-flavonoid polyphenols, potentially protocatechuic acid, hibiscus acid, and hydroxycinnamic derivatives which have been identified by HPLC in calyx extracts of *H. sabdariffa* (Tsai et al., 2002; Da-Costa-Rocha et al., 2014). Given that free phenols were absent by qualitative testing, the TPC reading is attributed to bound or conjugated polyphenolic structures released under the alkaline conditions of the Folin-Ciocalteu assay. The TPC:TFC ratio of approximately 3:1 is consistent with a phenolic pool dominated by non-flavonoid polyphenols, paralleling the polyphenol composition pattern observed in seed fractions of related Malvaceous species (Montalvo-González et al., 2022; Onyeukwu & Dibia, 2023).

Saponins finding consistent with reports for *H. sabdariffa* leaf and calyx fractions, where saponin concentrations are typically modest relative to those in leguminous species (Montalvo-González et al., 2022; Oakenfull, 1981). Nonetheless, triterpenic and steroidal saponins from *Hibiscus* spp. have demonstrated concentration-dependent antiproliferative activity against MCF-7 (breast) and HepG2 (hepatoma) cell lines, antifungal activity, and cholesterol-lowering properties in hyperlipidaemic animal models (Oakenfull, 1981; Montalvo-González et al., 2022). The seed's saponin content, while modest, is therefore pharmacologically relevant and warrants structural characterisation.

CONCLUSION

This study presents a comprehensive, parallel qualitative and quantitative phytochemical characterisation of methanolic seed extracts of *Hibiscus sabdariffa* L. from the Onigambari Research Station, Ibadan, Nigeria. Taken together, the phytochemical assemblage of *H. sabdariffa* seeds provides a scientific basis for the documented antioxidant, anti-inflammatory, antimicrobial, and cardioprotective properties of the species, and supports the seed's further investigation as a nutraceutical ingredient and resource for evidence-based herbal medicine development. Future work should prioritise HPLC and LC-MS/MS profiling, bioactivity-guided fractionation, and evaluation of antioxidant capacity across multiple Nigerian accessions.

COMPETING INTERESTS

The authors declare no competing interests.

REFERENCES

Ali, B. H., Al-Wabel, N. A., & Blunden, G. (2005). Phytochemical, pharmacological and toxicological aspects of *Hibiscus sabdariffa* L.: A review. *Phytotherapy Research*, 19(5), 369–375. <https://doi.org/10.1002/ptr.1628>

Awad, A. B., & Fink, C. S. (2000). Phytosterols as anticancer dietary components: Evidence and mechanism of action. *Journal of Nutrition*, 130(9), 2127–2130. <https://doi.org/10.1093/jn/130.9.2127>

Chukwu, C. N., Ogunka-Nnoka, C. U., & Akaninwor, J. O. (2019). Phytochemical composition, anti-nutrient properties and antioxidant potentials of raw *Hibiscus sabdariffa* seeds. *Archives of Current Research International*, 17(3), 1–10. <https://doi.org/10.9734/acri/2019/v17i330113>

Da-Costa-Rocha, I., Bonnlaender, B., Sievers, H., Pischel, I., & Heinrich, M. (2014). *Hibiscus sabdariffa* L.—a phytochemical and pharmacological review. *Food Chemistry*, 165, 424–443.

<https://doi.org/10.1016/j.foodchem.2014.05.002>

Evans, W. C. (2009). *Trease and Evans' pharmacognosy* (16th ed.). Saunders/Elsevier.

Hagr, T., & Adam, I. (2020). Phytochemical analysis, antibacterial and antioxidant activities of essential oil from *Hibiscus sabdariffa* (L.) seeds (Sudanese Karkadi). *Progress in Chemical and Biochemical Research*, 3(2), 194–201. <https://doi.org/10.33945/SAMI/PCBR.2020.3.2>

Hamrita, B., Ben Gara, A., & Braiek, O. B. (2022). Comparison of qualitative and quantitative phytochemical methods and their implications in standardisation of plant extracts. *Plant Science Today*, 9(1), 1–9. <https://doi.org/10.14719/pst.1569>

Harborne, J. B. (1973). *Phytochemical methods: A guide to modern techniques of plant analysis*. Chapman and Hall.

Hopkins, A. L., Lamm, M. G., Funk, J. L., & Ritenbaugh, C. (2013). *Hibiscus sabdariffa* L. in the treatment of hypertension and hyperlipidemia: A comprehensive review of animal and human studies. *Fitoterapia*, 85, 84–94. <https://doi.org/10.1016/j.fitote.2013.01.003>

Middleton, E., Jr., Kandaswami, C., & Theoharides, T. C. (2000). The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. *Pharmacological Reviews*, 52(4), 673–751.

Mohamed, I. M., Alrasheid, A. A., & Ayoub, S. M. H. (2024). Phytochemical screening, assessment of biological activity and nutritional value of Sudanese *Hibiscus sabdariffa* L. seeds. *Plant Science Today*. <https://doi.org/10.14719/pst.2934>

Montalvo-González, E., Sánchez-González, E. L., Torres-García, G., Velázquez-Estrada, J. L., González-Torres, S., Gutiérrez-Martino, A., Isiordia-Espinoza, M. A., & González-Silva, N. (2022). Biological activities of *Hibiscus sabdariffa* L. (roselle) and its constituents: Review of recent research advances. *Food & Function*, 13(13), 6669–6690. <https://doi.org/10.1039/D2FO00714B>

Nogueira da Cruz, R., & Khiatah, B. (2022). Importance of quantitative versus qualitative phytochemical analysis in drug standardisation. *Journal of Traditional and Complementary Medicine*, 12(1), 1–10. <https://doi.org/10.1016/j.jtcme.2021.06.003>

Oakenfull, D. (1981). Saponins in food—a review. *Food Chemistry*, 6(1), 19.

Obadoni, B. O., & Ochuko, P. O. (2001). Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Sciences*, 8(2), 203–208.

Ojokoh, A. O., Gabriel, M. O., & Lawal, R. T. (2019). A review of chemical constituents and pharmacological properties of *Hibiscus sabdariffa* L. *Journal of Pharmacognosy and Phytochemistry*, 8(3), 2014–2024.

- Onyeukwu, C. C., & Dibie, O. A. (2023). Phytochemical and nutritional evaluation of *Hibiscus sabdariffa* calyces obtained from different agro-ecological zones of Nigeria. *Scientific African*, 19, e01563. <https://doi.org/10.1016/j.sciaf.2023.e01563>
- Plat, J., & Mensink, R. P. (2005). Plant stanol and sterol esters in the control of blood cholesterol levels: Mechanism and safety aspects. *American Journal of Cardiology*, 96(1), 15D–22D. <https://doi.org/10.1016/j.amjcard.2005.06.006>
- Serban, C., Sahebkar, A., Ursoniu, S., Andrica, F., & Banach, M. (2015). Effect of sour tea (*Hibiscus sabdariffa* L.) on arterial hypertension: A systematic review and meta-analysis of randomized controlled trials. *Journal of Hypertension*, 33(6), 1119–1127. <https://doi.org/10.1097/HJH.0000000000000585>
- Tsai, P.-J., McIntosh, J., Pearce, P., Camden, B., & Jordan, B. R. (2002). Anthocyanin and antioxidant capacity in roselle (*Hibiscus sabdariffa* L.) extract. *Food Research International*, 35(4), 351–356. [https://doi.org/10.1016/S0963-9969\(01\)00129-6](https://doi.org/10.1016/S0963-9969(01)00129-6)
- Umeoguaju, F. U., & Ephraim-Emmanuel, B. C. (2021). Pharmacological and nutraceutical potential of *Hibiscus sabdariffa*: Comprehensive review. *Journal of Herbal Medicine*, 28, 100449. <https://doi.org/10.1016/j.hermed.2021.100449>
- van Gorkom, B. A. P., de Vries, E. G. E., Karrenbeld, A., & Kleibeuker, J. H. (1999). Review article: Anthranoid laxatives and their potential carcinogenic effects. *Alimentary Pharmacology & Therapeutics*, 13(4), 443–452. <https://doi.org/10.1046/j.1365-2036.1999.00497.x>



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