



Research Article

Comparative Qualitative and Quantitative Phytochemical Screening of *Hibiscus sabdariffa* (Linn.) and *Allium sativum* (Linn.) Crude Extracts

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ABSTRACT

Medicinal plants remain an important source of bioactive compounds with therapeutic potential, particularly in resource-limited regions where access to orthodox medicine is constrained. This study evaluated the phytochemical composition of *Hibiscus sabdariffa* (Roselle) calyces and *Allium sativum* (Garlic) bulbs from Kaduna, Nigeria, through solvent extraction, qualitative screening, and quantitative phytochemical assays. Ethanolic extraction of *H. sabdariffa* and methanolic extraction of *A. sativum* yielded 17.04% and 4.69% (w/w), respectively, followed by column chromatographic fractionation using hexane/ethyl acetate and ethyl acetate/methanol gradients, yielding seven pooled fractions from roselle (R1-R7) and five from garlic (G1-G5). Qualitative screening confirmed saponins, alkaloids, flavonoids, tannins, cardiac glycosides, and terpenes in both plants, with anthraquinones detected only in roselle. Quantitative analysis showed roselle contained significantly higher alkaloids (876.78 +/- 12.4 vs 629.26 +/- 10.8 mg/g, $p < 0.001$), flavonoids (288.63 +/- 6.2 vs 233.13 +/- 5.7 mg QE/g, $p < 0.001$), polyphenols (6.61 +/- 0.3 vs 4.88 +/- 0.2 mg GAE/g, $p < 0.01$), and tannins (13.88 +/- 0.8 vs 9.42 +/- 0.5 mg TAE/g, $p < 0.01$) than garlic, while saponin content did not differ significantly (52.14 +/- 3.1 vs 49.76 +/- 2.9 mg/g, $p > 0.05$). This is among the first studies to directly compare the phytochemical yield and fractionation profile of Nigerian-grown *H. sabdariffa* and *A. sativum* under standardised conditions, providing a quantitative baseline for future bioactivity-directed isolation of compounds from these widely consumed Nigerian plants.

Keywords: Alkaloids; *Allium sativum*; Extraction; Flavonoids; Fractionation; *Hibiscus sabdariffa*; Phytochemical screening

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INTRODUCTION

Plants synthesize a wide range of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, and terpenoids, many of which possess significant pharmacological activities such as antioxidant, antimicrobial, antihypertensive, and anti-inflammatory properties (Harborne, 1998; Sofowora, 2008). *Hibiscus sabdariffa* (Roselle, Family Malvaceae) and *Allium sativum* (Garlic, Family Amaryllidaceae) are widely consumed in Nigeria, both as food and traditional medicine, and have been extensively studied for their phytochemical and

pharmacological properties (Da-Costa-Rocha *et al.*, 2014; Block, 2010).

Despite the wide use of both plants, comparative studies that systematically extract, fractionate, and quantify their phytochemical constituents under standardized conditions, particularly using Nigerian-grown specimens, remain limited. Establishing an accurate phytochemical profile is a necessary first step before any bioactivity-guided fractionation or pharmacological evaluation can be meaningfully interpreted (Trease and Evans, 2002).

We hypothesised that *H. sabdariffa*, owing to its established anthraquinone content and broader

reported polyphenolic diversity in its calyces, would exhibit greater overall phytochemical richness than *A. sativum*, whose principal bioactive compounds are more narrowly organosulfur-based. Directly comparing these two commonly consumed Nigerian plants under standardised extraction, fractionation, and quantification conditions allows their relative phytochemical potential to be benchmarked against one another, a comparison that is largely absent from the existing literature despite each species being extensively studied in isolation.

This study therefore aimed to extract, fractionate, and comparatively screen the phytochemical constituents of *H. sabdariffa* and *A. sativum* using standard qualitative and quantitative methods.

MATERIALS AND METHODS

Plant material collection and authentication

Fresh calyces of *Hibiscus sabdariffa* and bulbs of *Allium sativum* were obtained from local markets in Kaduna, Nigeria, and authenticated by a taxonomist at the Department of Biological Sciences, Nigerian Defence Academy, Kaduna. Voucher specimens were deposited in the departmental herbarium under voucher numbers NDA/BIOH/202644 for *H. sabdariffa* and NDA/BIOH/202645 for *A. sativum*.

Sample preparation

Fresh roselle calyces were air-dried under shade for two weeks to preserve thermolabile constituents, then pulverised into fine powder using a laboratory mill and stored in an airtight container at room temperature until extraction. Fresh garlic bulbs were peeled, cleaned, and used directly for extraction without prior drying to preserve volatile organosulfur compounds. Ethanol and methanol were selected as extraction solvents for roselle and garlic, respectively, based on established protocols for maximising recovery of their principal metabolite classes (polyphenolic/anthocyanin pigments in roselle and organosulfur/flavonoid compounds in garlic); the implications of this solvent difference for direct quantitative comparison between the two species are addressed in the Discussion.

Extraction

Dried and pulverised *H. sabdariffa* calyces (200 g) were macerated in ethanol (1000 mL) for 72 hours at room temperature with occasional shaking. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate concentrated using a rotary evaporator at 40°C under reduced pressure to obtain the crude ethanolic extract. Fresh garlic cloves (200 g) were blended with methanol (1000 mL) and allowed to stand for 72 hours at room temperature with

intermittent agitation. The mixture was filtered and concentrated under reduced pressure at 40°C to obtain the crude methanolic extract. Percentage yield (% w/w) was calculated as: % yield = (weight of dried crude extract ÷ weight of starting dried plant material) × 100.

Fractionation by column chromatography

The crude *H. sabdariffa* extract was subjected to column chromatography over silica gel (60–120 mesh), eluted with a hexane/ethyl acetate gradient system of increasing polarity. Eluates were collected in fractions of approximately 50 mL and monitored by thin layer chromatography (TLC) using silica gel GF254 plates visualised under UV light (254 and 366 nm) and by spraying with vanillin-sulphuric acid reagent. TLC plates were developed using a stepwise gradient of hexane:ethyl acetate (increasing polarity from 9:1 to 1:9, v/v) for the roselle column and ethyl acetate:methanol (7:3, v/v) for the garlic column; R_f values of major resolved spots were recorded for each pooled fraction. Fractions exhibiting similar TLC profiles were pooled, yielding seven major pooled fractions (R1–R7). The crude garlic extract was similarly fractionated using an ethyl acetate:methanol (7:3) elution system, yielding five major pooled fractions (G1–G5).

Qualitative phytochemical screening

The crude extracts and pooled fractions were subjected to qualitative phytochemical screening for the presence of saponins, alkaloids, flavonoids, phenols, tannins, cardiac glycosides, terpenoids, and anthraquinones using standard procedures described by Harborne (1998), Trease and Evans (2002), and Sofowora (2008). Tests included the Mayer's and Dragendorff's tests for alkaloids, Shinoda test for flavonoids, ferric chloride test for tannins and phenols, froth test for saponins, Keller-Kiliani test for cardiac glycosides, Salkowski test for terpenoids, and Borntrager's test for anthraquinones.

Quantitative phytochemical analysis

Total alkaloid content was determined gravimetrically following acid-base extraction and precipitation. Total saponin content was determined by the gravimetric method following butanol extraction. Total flavonoid content was determined by the aluminium chloride colorimetric method, with absorbance read at 510 nm and results expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g). Total polyphenol content was determined using the Folin-Ciocalteu colorimetric method, with absorbance read at 760 nm and results expressed as milligrams of gallic acid equivalents per gram (mg GAE/g). Total tannin content was

determined using the Folin-Denis method, with results expressed as milligrams of tannic acid equivalents per gram (mg TAE/g). Calibration curves for the flavonoid, polyphenol, tannin, saponin, and alkaloid assays were constructed using appropriate reference standards (quercetin, gallic acid, tannic acid, diosgenin, and atropine, respectively) over a defined concentration range, and sample concentrations were interpolated from the resulting linear regression equations. All determinations were performed in triplicate.

Statistical analysis

All quantitative determinations were performed in triplicate (n = 3), and results are expressed as mean ± standard deviation (SD). Differences in phytochemical content between *H. sabdariffa* and *A. sativum* were assessed using Welch's two-sample t-test, which does not assume equal variances between groups, with statistical significance set at p < 0.05. Data were analysed using GraphPad Prism version 9.0.

RESULTS

Extraction yield

Ethanol extraction of *H. sabdariffa* calyces (200 g dried starting material) yielded a dark reddish-brown crude extract of 34.08 g, corresponding to a percentage yield of 17.04% (w/w). Methanolic extraction of *A. sativum* bulbs (200 g starting material) yielded a pale-yellow crude extract with a characteristic pungent odour, weighing 9.37 g, corresponding to a percentage yield of 4.69% (w/w). The higher extraction yield obtained for roselle indicates a greater proportion of solvent-extractable constituents in the calyx tissue relative to garlic bulb tissue under the conditions used. The fractionation process yielded seven pooled fractions from roselle

(R1–R7) and five pooled fractions from garlic (G1–G5), reflecting the broader polarity range of constituents present in the roselle extract.

Qualitative phytochemical composition

Qualitative screening of both crude extracts and their respective fractions confirmed the presence of saponins, alkaloids, flavonoids, tannins, cardiac glycosides, and terpenes in both plant species. Anthraquinones were detected only in roselle, consistent with its classification as an anthraquinone-bearing plant species. These results are broadly consistent with previous phytochemical reports on both plants (Da-Costa-Rocha *et al.*, 2014; Block, 2010; Okhale *et al.*, 2016).

Quantitative phytochemical content

Quantitative analysis revealed that roselle contained significantly higher concentrations of alkaloids, flavonoids, polyphenols, and tannins compared to garlic (Table 2). Alkaloids were the most abundant phytochemical class detected in both plants, with roselle recording 876.78 ± 12.4 mg/g compared to 629.26 ± 10.8 mg/g in garlic (Welch's t-test, t = 26.07, p < 0.001). Flavonoid content followed a similar trend, with roselle recording 288.63 ± 6.2 mg QE/g compared to 233.13 ± 5.7 mg QE/g in garlic (t = 11.41, p < 0.001). Polyphenol content was 6.61 ± 0.3 mg GAE/g in roselle versus 4.88 ± 0.2 mg GAE/g in garlic (t = 8.31, p < 0.01), and tannin content was 13.88 ± 0.8 mg TAE/g in roselle versus 9.42 ± 0.5 mg TAE/g in garlic (t = 8.19, p < 0.01). Saponin content, by contrast, did not differ significantly between the two species (52.14 ± 3.1 mg/g in roselle versus 49.76 ± 2.9 mg/g in garlic; t = 0.97, p = 0.39), indicating that this phytochemical class is present at comparable levels in both plants despite their taxonomic and morphological differences.

Table 1: Qualitative phytochemical composition of *H. sabdariffa* and *A. sativum* crude extracts

| Phytochemical | <i>H. sabdariffa</i> | <i>A. sativum</i> |
|--------------------|----------------------|-------------------|
| Saponins | + | + |
| Alkaloids | ++ | + |
| Flavonoids | +++ | ++ |
| Tannins | ++ | + |
| Cardiac Glycosides | + | + |
| Terpenes | + | + |
| Anthraquinones | + | - |
| Phenols | ++ | + |

Key: - = absent; + = present (weak); ++ = present (moderate); +++ = present (strong)

Table 2: Quantitative phytochemical content (mg/g dry weight) of *H. sabdariffa* and *A. sativum*

| Phytochemical | <i>H. sabdariffa</i> (mg/g) | <i>A. sativum</i> (mg/g) | p-value |
|------------------------------|-----------------------------|--------------------------|-----------|
| Total Alkaloids | 876.78 ± 12.4 | 629.26 ± 10.8 | < 0.001 |
| Total Flavonoids (mg QE/g) | 288.63 ± 6.2 | 233.13 ± 5.7 | < 0.001 |
| Total Polyphenols (mg GAE/g) | 6.61 ± 0.3 | 4.88 ± 0.2 | < 0.01 |
| Total Tannins (mg TAE/g) | 13.88 ± 0.8 | 9.42 ± 0.5 | < 0.01 |
| Total Saponins | 52.14 ± 3.1 | 49.76 ± 2.9 | 0.39 (ns) |

Values are expressed as mean ± SD (n=3). P-values from Welch's two-sample t-test comparing *H. sabdariffa* and *A. sativum*; ns = not significant (p > 0.05). QE = quercetin equivalents; GAE = gallic acid equivalents; TAE = tannic acid equivalents

DISCUSSION

The significantly higher alkaloid, flavonoid, polyphenol, and tannin content recorded in roselle relative to garlic may partly reflect the greater overall extraction yield obtained for roselle (17.04% vs 4.69%), suggesting that a larger proportion of extractable secondary metabolites, rather than solely their innate concentration in fresh tissue, contributed to the observed differences. It may also reflect genuine physiological differences between calyx tissue, which accumulates anthocyanins, flavonoids, and organic acids as part of roselle's characteristic pigmentation, and bulb tissue, whose secondary metabolism in garlic is comparatively specialised towards organosulfur compound biosynthesis (Block, 2010; Tudu *et al.*, 2024).

These findings are broadly consistent with previous phytochemical reports on both plants grown in Nigeria (Iwalokun *et al.*, 2011; Okhale *et al.*, 2016) and with recent reviews highlighting the phenolic richness of roselle calyces (Edo *et al.*, 2023) and the phytochemical diversity of garlic (El-Saadony *et al.*, 2024; Tudu *et al.*, 2024). However, the pharmacological relevance of these differences should be interpreted with caution, as this study did not include direct bioactivity assays (e.g., antioxidant, antimicrobial, or antihypertensive assays); the higher phytochemical concentrations recorded for roselle do not necessarily translate into proportionally greater biological activity, since garlic's principal pharmacological effects are attributed largely to organosulfur compounds not captured by the alkaloid, flavonoid, polyphenol, and tannin assays used here (Block, 2010).

The lack of a statistically significant difference in saponin content between the two species suggests that, unlike the other phytochemical classes assessed, saponin biosynthesis may be comparably active in both plant tissues regardless of extraction yield or solvent system used. It is also worth noting that the use of different extraction solvents for the two species (ethanol for roselle, methanol for garlic),

while consistent with established protocols for each plant, introduces a methodological difference that should be considered when interpreting the magnitude, though not necessarily the direction, of the differences reported here. Future comparative studies may consider a standardised single-solvent extraction protocol applied uniformly across both species to isolate the effect of plant matrix from that of solvent choice.

The fractionation profile obtained for both plants, with seven pooled fractions from roselle and five from garlic, reflects the relative chemical complexity and polarity range of constituents in each species and provides a useful basis for subsequent bioassay-guided isolation of specific bioactive compounds, particularly given recent advances in the structural characterisation of *Hibiscus* and *Allium* metabolites (Edo *et al.*, 2023; El-Saadony *et al.*, 2024).

CONCLUSION

This study successfully extracted, fractionated, and comparatively profiled the phytochemical constituents of *Hibiscus sabdariffa* and *Allium sativum*. Both plants contained saponins, alkaloids, flavonoids, tannins, cardiac glycosides, and terpenes, with roselle additionally containing anthraquinones and significantly higher quantities of alkaloids, flavonoids, polyphenols, and tannins, while saponin content did not differ significantly between the two species. These findings provide an initial phytochemical baseline for both plants grown in Kaduna, Nigeria. Future studies should incorporate bioactivity-guided fractionation, *in vitro* and *in vivo* pharmacological assays (particularly antioxidant, antimicrobial, and antihypertensive assays), and structural characterisation (e.g., GC-MS, NMR) of the isolated fractions to establish the therapeutic relevance of the phytochemical differences reported here, alongside a standardised single-solvent extraction protocol to enable more direct comparison between the two species.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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