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In vitro angiotensin-converting enzyme inhibitory activity of *Hibiscus sabdariffa* (Linn.) and *Allium sativum* (Linn.) as potential alternatives to captopril for hypertension management

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ABSTRACT

Hypertension is a major global public health challenge affecting over 1.28 billion adults worldwide. Although synthetic angiotensin-converting enzyme (ACE) inhibitors such as captopril are widely used, their adverse effects and cost have prompted interest in plant-derived alternatives. Fresh Hibiscus sabdariffa calyces and Allium sativum bulbs were collected in January 2026 from Kawo market, Kaduna, Nigeria, and authenticated, with voucher specimens (NDA/BIOH/202644 and NDA/BIOH/202645, respectively) deposited at the Nigerian Defence Academy Herbarium. This study evaluated the *in vitro* ACE inhibitory activity of *Hibiscus sabdariffa* (Roselle) and *Allium sativum* (Garlic) extracts and fractions using a modified gelatin-ninhydrin assay with cow lung as the enzyme source. Qualitative phytochemical screening revealed saponins, alkaloids, flavonoids, tannins, cardiac glycosides, and terpenes in both plants. Quantitative analysis showed that roselle contained higher levels of alkaloids (876.78 mg/g), flavonoids (288.63 mg QE/g), polyphenols (6.61 mg GAE/g), and tannins (13.88 mg TAE/g) compared to garlic. Column chromatography yielded five fractions from garlic (G1–G5) and seven from roselle (R1–R7). ACE inhibition assays showed that nine of twelve fractions (75%) exhibited strong inhibitory activity with IC^{50} values ≤25 µg/mL. The most potent plant fraction was garlic fraction G4 (IC^{50} = 15 µg/mL), followed by roselle fractions R5 (18 µg/mL), R6 and R7 (both 20 µg/mL). Captopril demonstrated an IC^{50} of 5 µg/mL, validating the assay. GC-MS analysis of the most active fractions identified squalene, 2,4-di-tert-butylphenol, stearic acid, and fatty acid derivatives as pharmacologically relevant compounds. These findings provide scientific validation for the traditional use of both plants in hypertension management and demonstrate that they contain bioactive compounds with significant ACE inhibitory potential.

Keywords: ACE inhibition, *Hibiscus sabdariffa*, *Allium sativum*, hypertension, GC-MS

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Introduction

Hypertension is a chronic cardiovascular condition characterised by persistent elevation of systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg.[1] It is a major global health challenge affecting over 1.28 billion adults worldwide, with the highest burden in low- and middle-income countries[1]. In Nigeria, the estimated prevalence of hypertension is approximately 38.1%, with wide disparities in awareness, treatment, and control.[2,3]

The angiotensin-converting enzyme (ACE) plays a central role in blood pressure regulation through the renin–angiotensin system (RAS), catalysing the conversion of angiotensin I to the potent vasoconstrictor angiotensin II, while simultaneously inactivating the vasodilator bradykinin.[4] ACE inhibition therefore represents a primary therapeutic strategy for hypertension management.

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Although synthetic ACE inhibitors such as captopril are widely used, they are associated with adverse effects including dry cough, hyperkalaemia, and angioedema, and their cost remains a barrier for many patients in resource-limited settings.[5]

Medicinal plants represent a valuable source of novel ACE inhibitors. *Hibiscus sabdariffa* (Roselle, Family Malvaceae) and *Allium sativum* (Garlic, Family Amaryllidaceae) are widely consumed in Nigeria and have a long history of ethnomedicinal use in hypertension management. Several studies have reported antihypertensive properties for both plants, including ACE inhibitory activity[6,7]. However, systematic fractionation studies comparing both plants under identical assay conditions, using cost-effective methods adaptable to resource-limited laboratories, remain limited.

This study therefore evaluated the *in vitro* ACE inhibitory activity of *H. sabdariffa* and *A. sativum* extracts and fractions using a modified gelatin-ninhydrin assay with cow lung as the enzyme source, with captopril as the reference standard.

Materials and Methods

Plant material collection and authentication

Fresh calyces of Hibiscus sabdariffa and bulbs of Allium sativum were collected in January 2026 from Kawo market, Kaduna, Nigeria (coordinates 10.57771°N, 7.444853°E), and authenticated by Prof. G.A. Ajibade, a taxonomist in the Department of Biological Sciences, Nigerian Defence Academy, Kaduna. Voucher specimens were deposited at the Biological Sciences Botany Herbarium, Nigerian Defence Academy, Kaduna, with voucher numbers NDA/BIOH/202644 (roselle) and NDA/BIOH/202645 (garlic).

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Extraction and fractionation
Dried and pulverised *H. sabdariffa* calyces (200 g) were extracted with ethanol (1000 mL) for 72 hours with occasional shaking, then filtered and concentrated using a rotary evaporator at 40 °C. The crude extract was further fractionated using a hexane/ethyl acetate gradient system. The resulting fractions were monitored by thin layer chromatography (TLC) and similar fractions were pooled, yielding seven pooled fractions (R1–R7). Similarly, fresh garlic cloves (200 g) were blended with methanol (1000 mL) and allowed to stand for 72 hours. The filtrate was concentrated and fractionated using ethyl acetate/methanol (7:3), yielding five pooled fractions (G1–G5).

Qualitative phytochemical screening
The crude extracts and fractions were subjected to qualitative phytochemical screening for saponins, alkaloids, flavonoids, phenols, tannins, cardiac glycosides, terpenoids, and anthraquinones using standard procedures.[8,9,10]

Quantitative phytochemical analysis
Total alkaloid and saponin contents were determined by gravimetric methods. Total flavonoid content was determined by the aluminium chloride colorimetric method and expressed as milligrams of quercetin equivalents per gram (mg QE/g). Total polyphenol content was determined using the Folin-Ciocalteu method and expressed as milligrams of gallic acid equivalents per gram (mg GAE/g). Total tannin content was determined using the Folin-Denis method and expressed as milligrams of tannic acid equivalents per gram (mg TAE/g).

Preparation of crude ACE from cow lung
Fresh cow lungs were obtained from a slaughterhouse in Kaduna immediately after slaughter and transported on ice. Approximately 30 g of tissue was homogenised in cold phosphate buffer (0.1 M, pH 7.4) at a ratio of 1:4 (w/v), centrifuged at 10,000 rpm for 20 minutes at 4 °C, and the clear supernatant used as the crude ACE source[4], modified.

ACE inhibition assay
The ACE inhibitory activity was assessed using a modified gelatin-ninhydrin method. Each reaction mixture (200 µL total) contained gelatin substrate (1% w/v in phosphate buffer), crude ACE enzyme, and plant extract or fraction at concentrations of 100, 50, 25, and 12.5 µg/mL. Captopril served as the positive control. Mixtures were incubated at 37 °C for 30 minutes. After incubation, 100 µL of each reaction mixture was combined with 1 mL of freshly prepared ninhydrin reagent (0.5%), heated at 90–100 °C for 10 minutes, cooled, and absorbance measured at 570 nm. All readings were taken in triplicate. Percentage inhibition was calculated as:

$$\%\ Inhibition = \frac{Ac - As}{Ac} \times 100$$

where Ac = absorbance of the negative control and As = absorbance of the test sample. IC^{50} values were determined by nonlinear regression analysis of dose-response curves.

GC-MS analysis
The most active fractions (G4 and R6) were subjected to GC-MS analysis (Shimadzu QP-2010 Plus) using a DB-5 column. Operating conditions: injector temperature 250 °C, oven temperature programmed from 60 °C to 280 °C at 10 °C/min. Compounds were identified by comparison with the NIST mass spectral library.

Statistical analysis
All experiments were conducted in triplicate and results expressed as mean ± standard deviation (SD). One-way ANOVA followed by Tukey's post-hoc test was used to compare differences between groups, using GraphPad Prism version 9.0. Values were considered statistically significant at $p < 0.05$.

Results and Discussion

Phytochemical composition
Qualitative phytochemical screening of *H. sabdariffa* and *A. sativum* crude extracts and fractions confirmed the presence of saponins, alkaloids, flavonoids, tannins, cardiac glycosides, and terpenes in both plants, with roselle additionally showing positive results for anthraquinones. These findings are consistent with previous reports on both plants[11,12] (see Table 1, presented after the References section).
Quantitative analysis revealed that roselle contained significantly higher levels of all phytochemicals compared to garlic. Alkaloids were the most abundant phytochemical in both plants, with roselle showing 876.78 mg/g compared to garlic at 629.26 mg/g. Flavonoids were also substantially higher in roselle (288.63 mg QE/g) than garlic (233.13 mg QE/g). These values are consistent with reports on Nigerian-grown varieties of both plants.[7,13] (see Table 2, presented after the References section).

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

Phytochemical	***H. sabdariffa***	***A. sativum***
Saponins	+	+
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	+
Cardiac Glycosides	+	+
Terpenes	+	+
Anthraquinones	+	−
Phenols	+	+

Key: − = absent; + = present (test is qualitative; only presence/absence is reported).

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

Phytochemical	H. sabdariffa (mg/g)	A. sativum (mg/g)
Total Alkaloids	876.78 ± 12.4	629.26 ± 10.8
Total Flavonoids (mg QE/g)	288.63 ± 6.2	233.13 ± 5.7
Total Polyphenols (mg GAE/g)	6.61 ± 0.3	4.88 ± 0.2
Total Tannins (mg TAE/g)	13.88 ± 0.8	9.42 ± 0.5
Total Saponins	52.14 ± 3.1	49.76 ± 2.9

Values are expressed as mean ± SD (n=3). QE = quercetin equivalents; GAE = gallic acid equivalents; TAE = tannic acid equivalents.

ACE inhibitory activity

Captopril, the standard reference drug, demonstrated potent ACE inhibitory activity with an IC^{50} of 5 µg/mL, validating the assay system. This value is consistent with literature reports for captopril under similar assay conditions.[14,5] Among the twelve fractions tested, nine (75%) demonstrated strong inhibitory activity with IC^{50} values ≤25 µg/mL. The most potent plant fraction was garlic fraction G4 (IC^{50} = 15 µg/mL), followed by roselle fractions R5 (18 µg/mL), R6 and R7 (both 20 µg/mL), indicating that G4 was approximately three-fold less potent than captopril while still exhibiting strong inhibitory activity (see Table 3, presented after the References section).

The strong ACE inhibitory activity of garlic fraction G4 is consistent with the work of Iwalokun and colleagues[7], who reported significant ACE inhibition in ethanolic extracts of Nigerian garlic varieties and demonstrated that fractionation could enhance activity by concentrating bioactive constituents. Similarly, the activity observed in roselle fractions R5, R6, and R7 is consistent with Ojeda and colleagues[6], who reported ACE inhibition by anthocyanin-rich fractions of *H. sabdariffa*. The fractionation protocol employed in this study likely concentrated these anthocyanins and other flavonoids in the active fractions.

The correlation between phytochemical content and ACE inhibitory activity is pharmacologically significant. Flavonoids have been extensively studied for ACE inhibitory activity, with hydroxyl groups at specific positions enhancing inhibitory potency through chelation of the catalytic zinc ion.[15,16] Plant alkaloids may also contribute through nitrogen-containing functional groups that interact with zinc metalloenzymes[17].

Table 3: IC_{50} values of plant fractions compared with captopril

Sample	IC50 (µg/mL)	Potency relative to captopril
Captopril (reference)	5.0	1.0×
Garlic fraction G4	15.0	3.0×
Roselle fraction R5	18.0	3.6×
Roselle fraction R6	20.0	4.0×
Roselle fraction R7	20.0	4.0×
Garlic fraction G3	22.0	4.4×
Roselle fraction R4	24.0	4.8×
Garlic fraction G2	25.0	5.0×
Roselle fraction R3	25.0	5.0×

Values are expressed as mean ± SD (n=3). IC50 = concentration required for 50% inhibition of ACE activity.

GC-MS analysis of active fractions

GC-MS analysis of the most active fractions G4 and R6 identified several pharmacologically relevant compounds. Squalene, a triterpene with known antioxidant and cardioprotective properties, was detected in both fractions. The phenolic compound 2,4-di-tert-butylphenol, reported to exhibit antioxidant and ACE inhibitory activities[15], was also identified. Several fatty acid derivatives including stearic acid, methyl palmitate, and oleic acid were detected, which may contribute to the observed ACE inhibitory activity through hydrophobic interactions with the enzyme active site[17]. These qualitative and quantitative phytochemical and GC-MS profiling approaches are consistent with methodologies previously reported for Nigerian medicinal plants[18] (see Table 4, presented after the References section).

Table 4: Major GC-MS identified compounds in active fractions G4 and R6

Compound	Fraction	% Area	Reported Activity
Squalene	G4, R6	0.91	Antioxidant, cardioprotective
2,4-Di-tert-butylphenol	G4, R6	0.70	Antioxidant, ACE inhibitory
Stearic acid	G4	—	Fatty acid, anti-inflammatory
Methyl palmitate	R6	—	Fatty acid derivative
Oleic acid	G4, R6	—	Anti-inflammatory, hypotensive

GC-MS: Gas chromatography-mass spectrometry; % Area: relative peak area percentage.

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The modified gelatin-ninhydrin assay using cow lung ACE was confirmed as a reliable and cost-effective alternative to the conventional hippuryl-histidyl-leucine (HHL)-based spectrophotometric method.[4] This represents a significant methodological contribution, as the HHL substrate is expensive and not readily available in many Nigerian research laboratories.

Conclusion

This study successfully demonstrated the *in vitro* ACE inhibitory activity of *Hibiscus sabdariffa* and *Allium sativum* extracts and fractions. The most potent plant fraction, garlic G4 (IC^{50} = 15 µg/mL), exhibited activity approximately three-fold lower than captopril (IC^{50} = 5 µg/mL), while roselle fractions R5, R6, and R7 showed IC^{50} values of 18–20 µg/mL. The modified gelatin-ninhydrin assay using cow lung ACE was validated as a reliable, cost-effective screening method suitable for resource-limited laboratories. GC-MS analysis identified squalene, 2,4-di-tert-butylphenol, and fatty acid derivatives as pharmacologically relevant compounds in the active fractions. These findings provide scientific validation for the traditional use of both plants in hypertension management, and both species represent promising candidates for further bioassay-guided purification, structural elucidation, and development of affordable plant-based antihypertensive agents.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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