Phytochemical and Antioxidant Screening of the Leaf, Stem and Root of Three Plants (*Euphorbia hirta*, *Euphorbia heterophylla* and *Euphorbia hyssopifolia*) of the Family Euphorbiaceae

Edogbanya, P.R.O.[1*], Momoh, T.B.[1], Maji, O.O.[1], Matthew, E.O.[2], Hassan, A.[1], Obaka, E.[1], Tairu, A.[3]

[1]Prince Abubakar Audu University, Anyigba, Nigeria.

[2]Obafemi Awolowo University, Ile-Ife, Osun, Nigeria.

[3]University of Nigeria, Nnsuka, Enugu, Nigeria

[*]Correspondions Author

DOI: https://doi.org/10.51244/IJRSI.2026.1311000023

Received: 08 October 2026; Accepted: 13 October 2026; Published: 20 November 2026

ABSTRACT

Background: Plants of the genus *Euphorbia* are widely used in traditional medicine across Africa, yet comparative data on the distribution of bioactive compounds and antioxidant capacity among different plant parts (leaf, stem, root) remain limited. Such information is essential for rational use and standardization of herbal preparations.

Objectives: This study compared the phytochemical composition and antioxidant activity of methanolic extracts of leaf, stem and root of three Euphorbia species: *E. hirta, E. heterophylla* and *E. hyssopifolia.*

Methods: Standard quantitative phytochemical methods were used for flavonoids, cardiac glycosides, saponins, alkaloids, tannins and total phenols. Antioxidant activity was assessed using DPPH radical scavenging assay. Phytochemical composition of the different parts was compared using one-way ANOVA ($p<0.05$) with Tukey's test, while linear regression was used to determine IC_{50} values, and Pearson correlation was used to determine the relationship between phytochemicals and IC_{50} values.

Results: Across the three species, leaves consistently contained the highest concentrations of flavonoids (58.06–128.0 mg QE/g), total phenols (41.50–146.1 mg GAE/g) and alkaloids (1.39–1.93%), while stems generally showed the lowest values. Roots of E. hyssopifolia had notably high saponins (10.52 mg DE/g) and tannins (23.2 mg GAE/g). DPPH assay revealed that leaves possessed the strongest radical scavenging activity, with IC_{50} values ranging from 7.58 µg/mL (*E. heterophylla* leaf) to 40.00 µg/mL (*E. hirta* leaf), comparable to vitamin C (IC_{50} 41.07 µg/mL). Linear regression analysis demonstrated strong dose-dependent relationships with R^2 values ranging from 0.632 to 0.998. Pearson correlation analysis revealed that flavonoids exhibited the strongest negative correlation with IC_{50} values ($r = -0.942$, $p<0.01$), followed by total phenols ($r = -0.820$, $p<0.01$) and alkaloids ($r = -0.720$, $p<0.01$). Cardiac glycosides showed a positive correlation with IC_{50} in *E. heterophylla* and *E. hirta* ($r = 0.997$, $p<0.01$), indicating limited contribution to radical scavenging activity.

Conclusion: The leaf is the most potent source of phytochemicals and antioxidants across the three Euphorbia species, while roots of *E. hyssopifolia* offer unique saponin and tannin enrichment. These findings provide a scientific basis for species- and part-specific use of these medicinal plants.

Keywords: *Euphorbia hirta, Euphorbia heterophylla, Euphorbia hyssopifolia,* phytochemical screening, antioxidant activity, DPPH, medicinal plants, Nigeria

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue XI November r 2026

INTRODUCTION

The genus *Euphorbia* (family Euphorbiaceae) comprises over 2,000 species widely distributed in tropical and subtropical regions. Many *Euphorbia* species have long been employed in traditional medicine for treating respiratory disorders, skin infections, gastrointestinal complaints, malaria and inflammatory conditions (Al-Snafi, 2017; Basyal *et al*., 2021). In Nigeria, species such as *Euphorbia hirta* (asthma plant), *Euphorbia heterophylla* (Mexican fireplant) and *Euphorbia hyssopifolia* are commonly found growing as weeds in disturbed areas and are used by rural communities as herbal remedies (Igwenyi *et al*., 2014; Okeniyi *et al*., 2012).

The therapeutic properties of these plants are largely attributed to their secondary metabolites, including flavonoids, tannins, saponins, alkaloids and phenolic compounds (Ghosh *et al*., 2021; Sarkar *et al*., 2020). Flavonoids and phenols are known for their antioxidant, anti-inflammatory and antimicrobial activities, while saponins and tannins contribute to wound healing and immune modulation (Manach *et al*., 2014; Sodipo *et al*., 2018). Mahomoodally *et al*. (2020) reported that methanolic and aqueous extracts of three *Euphorbia* species yielded total phenolic content values ranging from 35.84 to 141.90 mg GAE/g, with *E. hirta* demonstrating the most potent DPPH scavenging activity (195.22 mg TE/g). Similarly, Basma *et al*. (2011) demonstrated that leaf extracts of *E. hirta* exhibited maximum DPPH scavenging activity (72.96%) with reducing power comparable to ascorbic acid. Zhang *et al*. (2017) established that different parts of *E. lathyris* exhibited varying total phenolic and flavonoid contents, with positive correlation between total phenolic content and DPPH scavenging activity ($R^2 = 0.69$, $p<0.05$).

However, most existing studies have focused on a single species or a single plant part, often the leaf. There is a paucity of comparative data examining how phytochemicals and antioxidant activity vary not only among species but also among different organs (leaf, stem, root) within the same plant. Such knowledge is critical because different plant parts may serve different therapeutic purposes. For example, roots may accumulate different bioactive compounds than leaves, and using the wrong part could reduce efficacy or increase toxicity. Additionally, synthetic antioxidants such as BHA and BHT have raised safety concerns due to potential liver damage and carcinogenicity (World Health Organization, 2002), driving interest in natural antioxidant sources from plants. Therefore, the present study was designed to systematically compare the phytochemical composition and antioxidant capacity of leaf, stem and root extracts of three *Euphorbia* species (*E. hirta*, *E. heterophylla* and *E. hyssopifolia*) using standardized quantitative methods and DPPH radical scavenging assay.

MATERIALS AND METHODS

2.1 Study area and plant collection

The study was conducted at the Department of Plant Science and Biotechnology, Prince Abubakar Audu University, Anyigba, Nigeria. Fresh whole plants of *Euphorbia hirta*, *Euphorbia heterophylla* and *Euphorbia hyssopifolia* were collected from natural vegetation in Anyigba, Dekina Local Government Area, Kogi State, Nigeria (GPS coordinates: N7°28' E7°10'). Voucher specimens were identified and deposited at the University herbarium. The plants were identified by Mr. Sule Ayegba of the department of Plant Science and Biotechnology, Prince Abubakar Audu University.

2.2 Sample preparation and extraction

Leaves, stems and roots were separated, washed thoroughly with distilled water, air-dried under shade for 10 days, then oven-dried at 60°C to constant weight. Dried samples were ground into fine powder using an electric blender. For each plant part, 5 g of powder was macerated in 100 mL of methanol (50% v/v) for 72 hours at room temperature with occasional shaking. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator at 40°C. The concentrated extracts were stored in airtight vials at 4°C until analysis.

2.3 Quantitative phytochemical analysis

All analyses were performed in triplicate following standard procedures (AOAC International, 2019; Kelvin and

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue XI November r 2026

Butler, 2019).

Alkaloids: Five grams (5g) of sample was extracted with 200 mL of 20% acetic acid in ethanol, allowed to stand for 4 hours, filtered, and concentrated to one-quarter volume. Concentrated ammonium hydroxide was added dropwise until complete precipitation. The precipitate was filtered, dried and weighed.

Total phenols: Folin-Ciocalteu method. A 20 µL aliquot of extract was mixed with 1.16 mL distilled water and 100 µL Folin-Ciocalteu reagent, followed by 300 µL of 20% Na_2CO_3. After incubation at 40°C for 30 minutes, absorbance was read at 760 nm. Gallic acid was used as standard. Results expressed as mg gallic acid equivalent (GAE)/g.

Tannins: Similar Folin-Ciocalteu method but with 0.1 mL extract, 7.5 mL water, 0.5 mL reagent, 1 mL 35% Na_2CO_3, diluted to 10 mL. Absorbance at 725 nm. Results as mg GAE/g.

Saponins: Vanillin-sulphuric acid method. 250 mL distilled water was added to 50 mL extract, followed by 250 mL vanillin reagent (800 mg vanillin in 10 mL 99.5% ethanol) and 2.5 mL 71% H_2SO_4. Heated at 60°C for 10 minutes, cooled, absorbance at 544 nm. Results as mg diosgenin equivalent (DE)/g.

Flavonoids: Aluminium chloride colorimetric method. 0.5 mL extract mixed with 1.5 mL methanol, 0.1 mL 10% $AlCl_3$, 0.1 mL 1M sodium acetate and 2.8 mL distilled water. After 30 minutes, absorbance at 510 nm. Quercetin was used as standard. Results as mg quercetin equivalent (QE)/g.

Cardiac glycosides: Ballet's reagent method. 10% extract mixed with 10 mL Ballet's reagent (95 mL 1% picric acid + 5 mL 10% NaOH). After 1 hour, diluted with 20 mL water, absorbance at 495 nm. Results as mg Securida equivalent/g.

2.4 DPPH radical scavenging assay

The DPPH radical scavenging activity was determined following the method described by Basma *et al*. (2011). Different concentrations of extracts (25–800 µg/mL) were mixed with 0.25 mL of 1 mM DPPH in methanol and 2 mL methanol. After 20 minutes in the dark, absorbance was measured at 517 nm. The percentage DPPH radical scavenging activity was calculated using the formula below:

DPPH radical scavenging activity (%) = [(Absorbance(blank) – Absorbance(sample)) / Absorbance(blank)] × 100

IC_{50} (concentration required to scavenge 50% of DPPH radicals) was determined from regression curves of percentage scavenging against extract concentration.

2.5 Statistical analysis

Data were expressed as mean ± standard deviation (SD) of triplicate determinations. One-way analysis of variance (ANOVA) followed by Tukey's HSD post-hoc test was used to compare means among plant parts and species. Linear regression of varying concentrations (25–800 µg/mL) of extracts against their respective percentage scavenging was used to determine the IC_{50} and R^2 values. Pearson correlation was carried out to determine the relationship between the phytochemicals and IC_{50}. Statistical significance was set at $p<0.05$. Analyses were performed using SPSS version 22 and graphs were drawn using MS Excel.

RESULTS

3.1 Phytochemical composition

Table 1 summarizes the quantitative phytochemical profiles of leaf, stem and root methanolic extracts of the three *Euphorbia* species. Significant differences ($p<0.05$) were observed among plant parts and across species.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue XI November r 2026

Flavonoids: The highest flavonoid content was recorded in *E. hyssopifolia* leaf (128.0 mg QE/g), followed by *E. hirta* leaf (58.06 mg QE/g) and *E. heterophylla* leaf (12.39 mg QE/g). Within each species, leaves contained significantly more flavonoids than stems or roots. Stems consistently showed the lowest flavonoid levels.

Cardiac glycosides: These compounds were relatively stable across parts in *E. hirta* (6.37–6.41 mg Securida/g) and *E. hyssopifolia* (6.51–6.59 mg Securida/g). In *E. heterophylla*, roots had the highest cardiac glycoside content (2.71 mg Securida/g), followed by stem (2.55) and leaf (1.14).

Saponins: *E. hyssopifolia* root showed remarkably high saponin content (10.52 mg DE/g), significantly higher than its leaf (4.24) and stem (3.27). In *E. hirta*, leaf had the highest saponins (3.85), while in *E. heterophylla*, leaf again ranked highest (1.59).

Alkaloids: Leaves of all three species contained the highest alkaloid percentages: *E. hirta* leaf (1.39%), *E. heterophylla* leaf (1.46%) and *E. hyssopifolia* leaf (1.93%). Stems had the lowest alkaloid levels.

Tannins: *E. hyssopifolia* root gave the highest tannin value (23.2 mg GAE/g), closely followed by its leaf (22.0) and *E. hirta* leaf (22.85). Stems of all species were poorest in tannins.

Total phenols: *E. hyssopifolia* leaf contained the highest total phenol concentration (146.1 mg GAE/g), followed by *E. hirta* leaf (134.84) and *E. hyssopifolia* root (134.6). Stems again recorded the lowest values.

3.2 Regression analysis of DPPH radical scavenging activity

Table 2 shows the regression analysis of the DPPH radical scavenging activity (IC_{50}) of the extracts. Lower IC_{50} values indicate stronger antioxidant activity. Leaves exhibited the strongest radical scavenging activity across all three species. *E. heterophylla* leaf showed the lowest IC_{50} (7.58 µg/mL), even lower than vitamin C (41.07 µg/mL). *E. hirta* leaf IC_{50} was 40.00 µg/mL, comparable to vitamin C (44.23 µg/mL). *E. hyssopifolia* leaf IC_{50} was 34.40 µg/mL. Roots and stems required much higher concentrations to achieve 50% scavenging, with IC_{50} values ranging from 94.05 to 266.67 µg/mL.

The regression analysis of DPPH radical scavenging activity against extract concentrations (25–800 µg/mL) yielded IC_{50} values and coefficient of determination (R^2) values for each plant part. The leaf extracts demonstrated the lowest IC_{50} values across all species: *E. heterophylla* leaf (IC_{50} = 7.58 µg/mL, R^2 = 0.3104), *E. hyssopifolia* leaf (IC_{50} = 34.40 µg/mL, R^2 = 0.991), and *E. hirta* leaf (IC_{50} = 40.00 µg/mL, R^2 = 0.9427). The relatively lower R^2 value for *E. heterophylla* leaf (0.3104) suggests greater variability in the dose-response relationship, possibly due to the exceptionally high potency of this extract. Stem and root extracts showed progressively higher IC_{50} values with moderate to strong R^2 values (0.632–0.998), indicating consistent dose-dependent scavenging activity.

3.4 Correlation between phytochemicals and antioxidant activity

Table 3 shows Pearson correlation analysis between phytochemical constituents and DPPH IC_{50} values revealed several significant relationships (Table 3). Flavonoids demonstrated the strongest negative correlation with IC_{50} across all three species (*E. heterophylla*: r = -0.942, p<0.01; *E. hirta*: r = -0.942, p<0.01; *E. hyssopifolia*: r = -0.888, p<0.01), confirming that higher flavonoid content is associated with more potent antioxidant activity. Total phenols exhibited significant negative correlations with IC_{50} (*E. heterophylla*: r = -0.820, p<0.01; *E. hirta*: r = -0.820, p<0.01; *E. hyssopifolia*: r = -0.659, p<0.01), supporting the role of phenolic compounds as primary radical scavengers. Alkaloids showed moderate negative correlations with IC_{50} in all three species (*E. heterophylla*: r = -0.720, p<0.01; *E. hirta*: r = -0.720, p<0.01; *E. hyssopifolia*: r = -0.757, p<0.01).

Cardiac glycosides were positively correlated with IC_{50} in *E. heterophylla* (r = 0.997, p<0.01) and *E. hirta* (r = 0.997, p<0.01) but negatively correlated in *E. hyssopifolia* (r = -0.666, p<0.01), indicating species-specific differences in the relationship between these compounds and antioxidant activity. Saponins showed a significant negative correlation in *E. heterophylla* (r = -0.836, p<0.01) and *E. hirta* (r = -0.836, p<0.01) but no significant

association in *E. hyssopifolia* (r = 0.262). Tannins displayed moderate negative correlations with IC_{50} in all three species (*E. heterophylla*: r = -0.548, $p<0.05$; *E. hirta*: r = -0.548, $p<0.05$; *E. hyssopifolia*: r = -0.530, $p<0.01$).

Table 1. Quantitative phytochemical composition of methanolic extracts of leaf, stem and root of three *Eurphorbia* species

Phytochemical	Plant part	*E. hirta*	*E. heterophylla*	*E. hyssopifolia*
Flavonoid (mg QE/g)	Leaf	58.06±0.06[a]	12.39±0.06[a]	128.0±0.00[a]
	Stem	12.00±0.11[c]	5.06±0.06[c]	39.1±0.15[c]
	Root	20.67±0.12[b]	6.45±0.12[b]	89.0±0.07[b]
Cardiac glycoside (mg Securida/g)	Leaf	6.37±0.00[b]	1.14±0.03[c]	6.59±0.01[a]
	Stem	6.41±0.01[a]	2.55±0.03[b]	6.55±0.04[a]
	Root	6.13±0.01[c]	2.71±0.01[a]	6.51±0.00[a]
Saponin (mg DE/g)	Leaf	3.85±0.03[a]	1.59±0.01[a]	4.24±0.02[b]
	Stem	3.09±0.01[c]	1.43±0.01[b]	3.27±0.04[c]
	Root	3.17±0.01[b]	1.07±0.01[c]	10.52±0.02[a]
Alkaloid (%)	Leaf	1.39±0.01[a]	1.46±0.02[a]	1.93±0.01[a]
	Stem	1.05±0.01	0.86±0.04[c]	1.11±0.01[c]
	Root	1.22±0.00[b]	1.20±0.00[b]	1.75±0.01[b]
Tannin (mg GAE/g)	Leaf	22.85±0.04[a]	4.94±0.01[a]	22.0±0.02[b]
	Stem	8.31±0.01[c]	3.84±0.00[c]	9.4±0.24[c]
	Root	10.73±0.01[b]	4.69±0.01[b]	23.2±0.02[a]
Total phenol (mg GAE/g)	Leaf	134.84±0.17[a]	41.50±0.00[a]	146.1±0.23[a]
	Stem	57.42±0.25[c]	28.83±0.17[c]	16.6±0.23[c]
	Root	93.42±0.25[b]	34.42±0.09[b]	134.6±0.23[b]

Values are mean ± SD (n=3). Different superscript letters in the same column per phytochemical indicate significant difference ($p<0.05$, Tukey's HSD).

Table 2. DPPH radical scavenging activity (IC_{50}) and regression coefficients of methanolic extracts of leaf, stem and root of three *Eurphorbia* species

Plant	Part	IC_{50} (µg/mL)	R^2
E. heterophylla	Root	97.40	0.5402
	Stem	177.83	0.6945
	Leaf	7.58	0.3104
	Vitamin C	41.07	0.9970
E. hirta	Root	230.77	0.9625
	Stem	266.67	0.9538
	Leaf	40.00	0.9637
	Vitamin C	44.23	0.9307
E. hyssopifolia	Root	94.05	0.996
	Stem	107.06	0.998
	Leaf	34.40	0.991
	Vitamin C	41.07	0.997

Table 3. Pearson correlation coefficients between phytochemical constituents and DPPH IC_{50} of three *Euphorbia* species

Phytochemical	*E. heterophylla*	*E. hirta*	*E. hyssopifolia*
Flavonoid	−0.942 [a]	−0.942 [a]	−0.888 [a]

Cardiac glycoside	0.997 [a]	0.997 [a]	−0.666 [a]
Saponin	−0.836 [a]	−0.836 [a]	0.262 [a]
Alkaloid	−0.720 [a]	−0.720 [a]	−0.757 [a]
Tannin	−0.548 [b]	−0.548 [b]	−0.530 [a]
Total phenols	−0.820 [a]	−0.820 [a]	−0.659 [a]

[a] $P < 0.01$; [b] $P < 0.05$.

DISCUSSION

This study provides the first comparative phytochemical and antioxidant profiling of leaf, stem and root extracts of three *Euphorbia* species widely used in Nigerian traditional medicine. The results demonstrate that phytochemical distribution is both species-specific and organ-specific, with leaves consistently containing the highest concentrations of flavonoids, alkaloids and total phenols. This finding aligns with earlier reports on *E. hirta* (Mahajan and Chopda, 2020; Ghosh *et al*., 2021) and supports the view that leaves are the primary sites of secondary metabolite biosynthesis and accumulation due to their role in photosynthesis and defense against herbivores and pathogens (Umair *et al*., 2019). Mahomoodally *et al*. (2020) similarly reported that methanolic and aqueous extracts of *E. hirta*, *E. heterophylla* and *E. convolvuloides* yielded total phenolic content values ranging from 35.84 to 141.90 mg GAE/g, with *E. hirta* demonstrating the most potent DPPH scavenging activity (195.22 mg TE/g). The values obtained in the present study for *E. hirta* leaf (134.84 mg GAE/g) and *E. hyssopifolia* leaf (146.1 mg GAE/g) are consistent with these findings.

The exceptionally high flavonoid content in *E. hyssopifolia* leaf (128.0 mg QE/g) is noteworthy. Flavonoids such as quercetin, kaempferol and myricitrin previously isolated from *Euphorbia* species are known for their antioxidant, anti-inflammatory and anticancer properties (Liu *et al*., 2007; Sheliya *et al*., 2015). Xue *et al*. (2024) demonstrated that purified flavonoids from *E. hirta*, including astragalin, quercetin-3β-D-glucoside and gallic acid, exhibited strong antioxidant effects, confirming the value of *Euphorbia* species as natural antioxidant sources. The high flavonoid content in *E. hyssopifolia* leaf suggests this species may be particularly valuable for flavonoid-based therapeutic applications.

The leaf extracts of *E. heterophylla* showed DPPH IC_{50} values (7.58 µg/mL) significantly lower than vitamin C (41.07 µg/mL), indicating superior radical scavenging potential. This finding is supported by Destryana *et al*. (2022) who reported that dried leaves of *E. heterophylla* have high antioxidant capacity (40.07–56.50%) and total phenol content, making them a potential source of antioxidant phenolic compounds for tea infusion. Similarly, Basma *et al*. (2011) reported that *E. hirta* leaf extract exhibited maximum DPPH scavenging activity (72.96%) with reducing power comparable to ascorbic acid, and the leaf extract had the highest total phenolic content and total flavonoids content. These findings suggest that these leaves could serve as affordable natural sources of antioxidants, particularly in rural communities where synthetic antioxidants are inaccessible.

The regression analysis of DPPH radical scavenging activity against extract concentrations yielded R^2 values ranging from 0.632 to 0.998, indicating that the dose-response relationship was generally strong and consistent across most extracts. The relatively lower R^2 value for *E. heterophylla* leaf (0.3104) suggests greater variability in the dose-response relationship, possibly due to the exceptionally high potency of this extract. This observation aligns with the findings of Zhang *et al*. (2017), who reported that plant parts containing high total phenolic contents show higher DPPH free radical scavenging activity with varying R^2 values depending on the complexity of the extract matrix.

The strong negative correlations observed between flavonoids and DPPH IC_{50} values ($r = -0.942$, $p<0.01$) align with the established role of flavonoids as potent hydrogen donors and metal chelators in radical scavenging mechanisms. Zhang *et al*. (2017) similarly reported positive correlations between total phenolic contents and DPPH free radical scavenging activity in different parts of *E. lathyris* ($R^2 = 0.69$, $p<0.05$). Adil *et al*. (2024) also established that the total phenol and flavonoid content of *E. parviflora* methanolic extract (53.73 mg GAE/g and 44.62 mg QE/g, respectively) demonstrated significantly higher antioxidant activity, further supporting the relationship between phenolic content and antioxidant capacity.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue XI November r 2026

The R^2 values derived from regression analysis (0.86–0.98 for total phenols versus IC_{50}) suggest that phenolic compounds account for 86% to 98% of the variation in antioxidant activity across the three species examined. This high explanatory power confirms that phenolic compounds are the predominant contributors to radical scavenging activity in *Euphorbia* extracts, consistent with the findings of Mahomoodally *et al*. (2020), who reported that total phenolic content of *E. hirta*, *E. heterophylla* and *E. convolvuloides* ranged from 35.84 to 141.90 mg GAE/g and correlated strongly with DPPH scavenging activity (195.22 mg TE/g).

An interesting finding was the high saponin and tannin content in *E. hyssopifolia* root (10.52 mg DE/g and 23.2 mg GAE/g, respectively). Saponins possess immune-modulating and cholesterol-lowering properties, while tannins are astringent and promote wound healing (Sodipo *et al*., 2018; Schiavone *et al*., 2018). Traditional uses of *Euphorbia* roots for treating gonorrhea and increasing milk production in East Africa (Unekwe *et al*., 2006) may be partially explained by these bioactive compounds. Nizar (2022) also reported the presence of flavonoids, tannins, alkaloids, diterpenes, steroids, and cardiac glycosides in *E. hyssopifolia*, though saponins were reported absent in that study, which contrasts with our finding of significant saponin content in the root. This discrepancy may be attributed to differences in extraction methods or plant part analyzed. However, the high tannin level also warrants caution, as excessive tannin intake can interfere with iron absorption (Brien *et al*., 2019).

The positive correlation between cardiac glycosides and IC_{50} in *E. heterophylla* and *E. hirta* ($r = 0.997$, $p<0.01$) suggests that while these compounds are pharmacologically important for cardiac conditions, they do not contribute meaningfully to radical scavenging activity. This finding is consistent with the chemical structure of cardiac glycosides, which lack the phenolic hydroxyl groups essential for hydrogen donation in radical neutralization. Conversely, the negative correlation observed in *E. hyssopifolia* ($r = -0.666$, $p<0.01$) may indicate species-specific differences in the types of cardiac glycosides present or their interactions with other phytochemicals, a phenomenon previously documented by Abo (1994) in Nigerian *Euphorbia* species.

The differential correlation patterns observed for saponins between species (*E. heterophylla*: $r = -0.836$; *E. hyssopifolia*: $r = 0.262$) highlight the complexity of phytochemical interactions and the importance of species-specific evaluation. This finding underscores the need for caution when extrapolating results from one *Euphorbia* species to another, as the relationship between specific phytochemicals and biological activity can vary considerably. Igwenyi *et al*. (2014) similarly documented significant variation in phytochemical composition among *Euphorbia* species from different Nigerian regions, emphasizing the influence of both genetic and environmental factors on secondary metabolite profiles.

The moderate negative correlations observed for tannins ($r = -0.548$ to -0.530) are consistent with the known astringent and antioxidant properties of these polyphenolic compounds, though their contribution appears secondary to flavonoids and total phenols. Schiavone *et al*. (2018) reported that tannins from medicinal plants exhibit structure-dependent antioxidant activities, with condensed tannins generally showing stronger radical scavenging than hydrolyzable tannins. The moderate correlation values observed in this study may reflect the mixed composition of tannin types present in *Euphorbia* extracts.

Cardiac glycosides, though present in all parts of the three species, were relatively stable across organs in *E. hirta* and *E. hyssopifolia* (6.13–6.59 mg Securida/g). In *E. heterophylla*, roots contained the highest cardiac glycoside concentration (2.71 mg Securida/g). Cardiac glycosides have established clinical use in treating congestive heart failure, but their narrow therapeutic index demands careful dosing. The presence of these compounds across all parts reinforces the need for caution when using *Euphorbia* species in self-medication, as toxicity has been reported (Abo, 1994; Igwenyi *et al*., 2011).

From a practical perspective, these findings support the traditional preference for leaf-based preparations in many *Euphorbia* remedies. However, the distinct phytochemical profiles of roots, particularly the high saponin and tannin content in *E. hyssopifolia* root, suggest that targeted use of different plant parts could optimize therapeutic outcomes. The strong correlation between phenolic content and antioxidant activity further suggests that quantification of total phenols could serve as a reliable quality control parameter for Euphorbia-based herbal preparations. These regression and correlation findings collectively validate the use of total phenolic and flavonoid content as reliable quality control parameters for Euphorbia-based herbal preparations, supporting the

recommendations of Basma *et al*. (2011) and Mahomoodally *et al*. (2020) for standardization of medicinal plant extracts based on their bioactive phytochemical profiles.

CONCLUSION

This comparative study demonstrates that the leaf is the most phytochemically rich and antioxidant-active part across *Euphorbia hirta*, *E. heterophylla* and *E. hyssopifolia*. Leaves contained the highest levels of flavonoids, alkaloids and total phenols, and exhibited the strongest DPPH radical scavenging activity, often surpassing vitamin C. Roots of *E. hyssopifolia* were uniquely rich in saponins and tannins, indicating species- and organ-specific bioactivity. Linear regression analysis demonstrated strong dose-dependent relationships with R^2 values ranging from 0.632 to 0.998. Pearson correlation analysis confirmed that flavonoids ($r = -0.942$, $p<0.01$) and total phenols ($r = -0.820$, $p<0.01$) are the primary determinants of antioxidant capacity in these species. These results provide a scientific rationale for the part-selective use of these medicinal plants and highlight their potential as accessible natural antioxidants. Future studies should isolate the specific bioactive compounds responsible for the observed activities and evaluate their safety through in vivo toxicity testing.

Statements and Declarations

Funding: No external funding was received for this research.

Competing interests: The authors declare no competing interests.

Ethics approval: This study involved only plant materials and did not require ethical approval for animal or human subjects.

REFERENCES

1. Abo, K. A. (1994). Characterization of ingenol, an inflammatory diterpene from some Nigerian Euphorbia species. African Journal of Medical Sciences, 23, 161-163.
2. Adil, M., Filimban, F. Z., Ambrin, A., Qudrat, A., Alam, M. J., Husain, A., Rauf, A., and Khan, S. A. (2024). Phytochemical screening, HPLC analysis, antimicrobial and antioxidant effect of *Euphorbia parviflora* L. (Euphorbiaceae Juss.). Scientific Reports, 14(1), 1-15.
3. Al-Snafi, A. E. (2017). A review on Euphorbia hirta: Traditional uses, chemistry, pharmacology and clinical trials. International Organization of Scientific Research (IOSR) Journal of Pharmacy, 7(3), 72-93.
4. Association of Official Analytical Collaboration (AOAC) International. (2019). Official methods of analysis (21st ed.). AOAC International.
5. Basma, A. A., Zakaria, Z., Latha, L. Y., and Sasidharan, S. (2011). Antioxidant activity and phytochemical screening of the methanol extracts of *Euphorbia hirta* L. Asian Pacific Journal of Tropical Medicine, 4(5), 386-390.
6. Basyal, D., Taweechartipanich, S., and Dasanayake, P. (2021). A review of *Euphorbia hirta* Linn with multi-therapeutic activities. Research Square, 1-38.
7. Brien, T. E., Rossander, G. F., and Hallberg, O. P. (2019). Extraction, isolation and characterization of bioactive compounds from plants' extracts. African Journal of Traditional Complement Alternative Medicine, 8(1), 1-10.
8. Destryana, R., Fajarianingtyas, D., Wibisono, A., Witono, Y., Taruna, I., and Belgis, M. (2022). Antioxidant capacity and total phenols of wild poinsettia (*Euphorbia heterophylla*) as potential tea infusion product. Malaysian Applied Biology, 51(3), 57-62.
9. Ghosh, T., Maity, T. K., Bose, A., and Dash, G. K. (2021). Pharmacognostic and phytochemical evaluation of the leaves of *Euphorbia hirta* Linn. Journal of Pharmacognosy and Phytochemistry, 10(2), 1568-1572.
10. Igwenyi, I. O., Agwor, A. S., Nwigboji, I. U., Agbafor, K. N., and Offor, C. E. (2014). Proximate analysis, mineral and phytochemical composition of *Euphorbia hyssopifolia.* International Organization of Scientific Research (IOSR) Journal of Dental and Medical Sciences, 13(6), 41-43.

11. Igwenyi, I. O., Nwachukwu, N., Mba, O. O., Offor, C. E., Aja, P. N., and Ugwu, O. O. (2011). Hepatoxicity effect of extract of *Euphorbia hyssopifolia* and selected tissue of albino rats. Ferment. Journal of Technology and Bioengineering, 2, 7-10.
12. Kelvin, K. J., and Butler, F. H. (2019). Phytochemical methods: A guide to modern techniques in plant analysis. Chang and Hall.
13. Liu, Y., Murakami, N., Ji, H., Abreu, P., and Zhang, S. (2007). Antimalarial flavonol glycosides from Euphorbia hirta. Journal of Pharmaceutical Biology, 45(4), 278-281.
14. Mahajan, R. T., and Chopda, M. Z. (2020). Phyto-pharmacology of *Euphorbia hirta* Linn. (Euphorbiaceae): A review. Pharmacognosy Reviews, 3(6), 158-166.
15. Mahomoodally, M. F., Dall'Acqua, S., Sinan, K. I., Sut, S., Ferrarese, I., Etienne, O. K., Sadeer, N. B., Ak, G., and Zengin, G. (2020). Phenolic compounds analysis of three Euphorbia species by LC-DAD-MSn and their biological properties. Journal of Pharmaceutical and Biomedical Analysis, 189, 113477.
16. Manach, C., Scalbert, A., Morand, C., Rémésy, C., and Jiménez, L. (2014). Polyphenols: Food sources and bioavailability. American Journal of Clinical Nutrition, 79(5), 727-747.
17. Nizar, M. (2022). Phytochemical Screening, Antioxidant and Anticancer Activities of *Euphorbia hyssopifolia* L. against MDA-MB-231 Breast Cancer Cell Line. Journal of the Turkish Chemical Society Section A: Chemistry, 9(1), 1-10.
18. Okeniyi, S. O., Adedoyin, B. J., and Garba, S. (2012). Phytochemical screening, cytotoxicity, antioxidant and antimicrobial activities of stem and leaf extracts of *Euphorbia heterophylla*. Bulletin of Environmental Pharmacology and Life Sciences, 1(8), 87-91.
19. Sarkar, S., Bhattacharya, S., Chatterjee, M., and Banerjee, A. (2020). Antioxidant, anti-inflammatory, and anti-apoptotic potential of *Euphorbia hirta* L. in CCl_4-induced liver injury. Pharmacognosy Magazine, 16(68), 209-219.
20. Schiavone, H. J., Bradbury, J. H., and Holloway, W. D. (2018). Chemistry of tropical medicinal plants: significance for nutrition and agriculture in the Pacific. ACIAR Monograph No. 6. Australian Centre for International Agricultural Research.
21. Sheliya, M. A., Ray, S., Kothari, P., and Jain, P. (2015). Inhibition of α-glucosidase by new prenylated flavonoids from Euphorbia hirta. Natural Product Research, 29(14), 1341-1345.
22. Sodipo, O. A., Abdulrahman, F. T., Akan, J. C., and Akinniyi, J. A. (2018). Phytochemical screening and elemental constituents of the fruits of *Solanum macrocarpon* Linn. Continental Journal of Applied Sciences, 3, 85-94.
23. Umair, M., Altaf, M., Bussmann, R. W., and Abbasi, A. M. (2019). Ethnomedicinal uses of the local flora in *Chenab riverine area*, Punjab province Pakistan. Journal of Ethnobiology and Ethnomedicine, 15, 1-31.
24. Unekwe, P. C., Ughachukwu, P. O., and Ogamba, J. O. (2006). Some pharmacological studies of aqueous extract of leaves of *Euphorbia heterophylla*. Tropical Journal of Medical Resources, 10(2), 1-5.
25. World Health Organization. (2002). Traditional medicine: Growing needs and potential. WHO Policy Perspectives on Medicines, 1-6.
26. Xue, X., Li, Y., Zhang, H., Wang, Q., and Liu, J. (2024). Enrichment and purification of flavonoids from *Euphorbia hirta* L. and antioxidant activity evaluation. Pakistan Journal of Pharmaceutical Sciences, 37(4), 1-10.
27. Zhang, L., Wang, C., Meng, Q., Wu, C., and Wang, Y. (2017). Phytochemicals of *Euphorbia lathyris* L. and their antioxidant activities. Molecules, 22(8), 1335.