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Research Article

Comparative Evaluation of In-Vitro Antioxidant Activity and Phytochemical Profiling of Hydroethanolic Root Extracts of *Calotropis gigantea* and *Bauhinia variegata*

Arshad Ali[1]*, S.S. Sisodia[1]

[1]Department of Pharmacology, Bhupal Nobles' Institute of Pharmaceutical Sciences, Bhupal Nobles' University, Udaipur, Rajasthan, India.

Corresponding Author*: Arshad Ali, Department of Pharmacology, Bhupal Nobles' Institute of Pharmaceutical Sciences, Bhupal Nobles' University, Udaipur, Rajasthan, India.

Email ID: aliarshad055@gmail.com

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ABSTRACT

Background and Objective: Oxidative stress is a primary contributor to carcinogenesis, making antioxidant-rich medicinal plants highly relevant to cancer chemoprevention. The present study was designed to evaluate and compare the phytochemical composition and in-vitro antioxidant potential of hydroethanolic root extracts of *Calotropis gigantea* (CG) and *Bauhinia variegata* (BV).

Materials and Methods: Both plants were authenticated at the Botanical Survey of India, Jodhpur. Hydroethanolic extracts (70:30 ethanol:water) were prepared by cold maceration. Quantitative phytochemical analysis was performed for total phenolic content (TPC), total flavonoid content (TFC), total tannin content (TTC), saponin index, and terpenoid content. Antioxidant activity was evaluated by five complementary assays: DPPH radical scavenging, ABTS radical cation decolorization, ferric reducing antioxidant power (FRAP), hydrogen peroxide (H_2O_2) scavenging, and reducing power assay. Ascorbic acid and Trolox served as reference standards. IC_{50} values were calculated by linear regression.

Results: BV extract yielded a higher extractive value (14.92% w/w) and contained significantly greater TPC (89.36 ± 2.14 mg GAE/g) and TFC (68.93 ± 1.62 mg RE/g) compared to CG (TPC: 72.48 ± 1.92; TFC: 54.21 ± 1.47 mg RE/g). CG showed higher terpenoid content (63.84 ± 1.88 mg LE/g) and saponin index (280). BV demonstrated superior antioxidant activity across all assays with DPPH IC_{50} = 48.2 µg/mL and ABTS IC_{50} = 43.9 µg/mL, compared to CG (DPPH IC_{50} = 64.8; ABTS IC_{50} = 58.6 µg/mL). A significant positive correlation was established between TPC/TFC and radical scavenging activity ($p < 0.01$).

Conclusion: BV exhibits superior antioxidant activity attributable to its flavonoid-dominant phytochemical profile, whereas CG possesses a terpenoid- and cardenolide-rich composition. Both species offer promising chemopreventive potential through complementary redox-modulatory mechanisms.

KEYWORDS: *Calotropis gigantea, Bauhinia variegata, antioxidant activity, DPPH, ABTS, FRAP, total phenolic content, total flavonoid content, oxidative stress, cancer chemoprevention.*

Introduction

Cancer remains one of the leading causes of mortality worldwide, with the Global Cancer Observatory (GLOBOCAN 2022) reporting approximately 19.3 million new cases and nearly 10 million deaths annually. A fundamental mechanism underlying carcinogenesis is oxidative stress, wherein an imbalance between reactive oxygen species (ROS) generation and endogenous antioxidant defenses results in irreversible DNA damage, lipid peroxidation, and protein oxidation. These events initiate and propagate mutagenic alterations in oncogenes and tumor suppressor genes, ultimately driving malignant transformation[1,2].

Endogenous antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) constitute the primary cellular defense. However, dietary and phytochemical antioxidants serve as critical exogenous supplements, particularly flavonoids and polyphenols, which scavenge free radicals, chelate transition metals, and activate the Nrf2–Keap1 pathway to induce endogenous antioxidant gene expression[3,4]. The U.S. National Cancer Institute (NCI) estimates that over 60% of approved anticancer agents originate from natural products or their derivatives[5].

Calotropis gigantea (L.) W.T. Aiton (family Apocynaceae), commonly known as "Giant Milkweed" or "Aak" in traditional Indian medicine, is a perennial shrub widely distributed across tropical Asia. Classified as Arka in Ayurveda, it has been historically employed for the treatment of skin disorders, ulcers, tumors, and inflammatory conditions. Phytochemically, C. gigantea is characterized by cardenolide glycosides (calotropin, calactin, uscharin), flavonoids (kaempferol, quercetin, isorhamnetin), terpenoids (lupeol, β-amyrin), and proteolytic enzymes (calotropain)[6].

Bauhinia variegata (L.) Benth. (family Fabaceae), referred to as Kanchanar in Ayurveda and a principal ingredient in the classical formulation Kanchanar Guggulu, is a deciduous tree native to the Indian subcontinent. Traditionally employed for glandular swellings (Granthi), tumors (Arbuda), and metabolic disorders, its bark and leaves are rich in flavonoids (kaempferol, quercetin, rutin, apigenin), phenolic acids (gallic acid, ferulic acid), and triterpenoids (lupeol, β-sitosterol, oleanolic acid), imparting potent antioxidant and hepatoprotective properties[7-9].

Despite individual reports on both species, a systematic comparative evaluation of their antioxidant activity and phytochemical profile using multiple complementary methods has not been conducted. The present study addresses this gap by quantifying major phytochemical constituents and assessing antioxidant activity through five standardized assays, establishing a mechanistic correlation between phytochemical composition and free radical scavenging capacity.

Materials and Methods

Plant Collection and Authentication

The roots of C. gigantea and B. variegata were collected from naturally occurring populations in Jaipur district, Rajasthan, India, during the winter season when phytochemical concentrations are optimal. Plant specimens were authenticated at the Botanical Survey of India (BSI), Arid Zone Regional Centre, Jodhpur, by Shri Sriman Lal Meena (Scientist-E), under Certificate No. 1/2012/Tech./2024-25 (Pl. Id.)/88, dated 05/02/2025. Authenticated voucher specimens were deposited for future reference.

Preparation of Hydroethanolic Extracts

Collected roots were washed, shade-dried at 25–30°C for 10–12 days, and coarsely powdered (40-mesh sieve). Cold maceration was performed using a hydroethanolic solvent system (ethanol:water = 70:30 v/v) for 7 days in stoppered glass containers. The filtrate was concentrated under reduced pressure using a rotary vacuum evaporator (40–45°C), and the dried extracts were stored at 2–8°C until use. Percentage extractive yield was calculated with respect to dried plant material weight[10].

Quantitative Phytochemical Estimation

Total Phenolic Content (TPC) was determined by the Folin–Ciocalteu method using gallic acid as standard, measuring absorbance at 765 nm and expressed as mg Gallic Acid Equivalent per gram of extract (mg GAE/g)[11]. Total Flavonoid Content (TFC) was measured by the aluminium chloride colorimetric method with rutin as standard, reading absorbance at 415 nm and expressing results as mg Rutin Equivalent per gram (mg RE/g)[12]. Total Tannin Content (TTC) was estimated using the Folin–Denis method with tannic acid as standard at 760 nm, expressed as mg Tannic Acid Equivalent per gram (mg TAE/g). The Saponin Index was determined by the foam height method. Total Terpenoid Content was estimated using the vanillin–perchloric acid colorimetric method at 548 nm, expressed as mg Linalool Equivalent per gram (mg LE/g). All estimations were performed in triplicate (n = 3) and results expressed as Mean ± SEM.

In-Vitro Antioxidant Assays

DPPH Radical Scavenging Assay: A 0.1 mM methanolic DPPH solution was mixed with varying concentrations of plant extracts (20–100 µg/mL) and incubated in the dark for 30 minutes. Absorbance was recorded at 517 nm and percentage scavenging calculated relative to the control. Ascorbic acid served as the standard[13].

ABTS Radical Cation Decolorization Assay: $ABTS^{\bullet+}$ radical was generated by reacting ABTS (7 mM) with potassium persulfate (2.45 mM) for 12–16 hours in darkness. The radical solution was diluted to an absorbance of 0.70 ± 0.02 at 734 nm, and percentage inhibition was measured against Trolox as standard[14].

Ferric Reducing Antioxidant Power (FRAP) Assay: FRAP reagent (acetate buffer + TPTZ + $FeCl_3$ in 10:1:1 ratio) was incubated with extract at 37°C for 30 minutes. Absorbance at 593 nm was measured and antioxidant capacity expressed as µM Fe^{2+} equivalents using an $FeSO_4$ calibration curve[15].

Hydrogen Peroxide (H_2O_2) Scavenging Assay: Extract (0.6 mL) was mixed with 40 mM H_2O_2 in phosphate buffer (pH 7.4) and incubated for 10 minutes. Residual H_2O_2 was measured at 230 nm and percentage scavenging calculated[16].

Reducing Power Assay: The extract was mixed with phosphate buffer (pH 6.6) and potassium ferricyanide (1%), incubated at 50°C for 20 minutes, followed by addition of TCA and $FeCl_3$. Prussian blue formation was measured at 700 nm. Higher absorbance indicated greater reducing power[17].

For DPPH, ABTS, and H_2O_2 assays, IC_{50} values were determined from percentage inhibition versus concentration plots using linear regression analysis. All assays were conducted in triplicate (n = 3), and results expressed as Mean ± SEM. Statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparison test ($p < 0.05$ and $p < 0.01$).

Results

Percentage Yield of Extracts

The hydroethanolic maceration yielded 12.24% w/w for C. gigantea and 14.92% w/w for B. variegata, demonstrating higher extractive efficiency for BV. The greater yield of BV is consistent with its higher polyphenolic content, as polar phytochemicals including flavonoids and phenolic acids are efficiently extracted by hydroethanolic solvents.

Qualitative and Quantitative Phytochemical Analysis

Preliminary qualitative screening confirmed the presence of flavonoids, phenolics, tannins, alkaloids, saponins, glycosides, steroids, triterpenoids, and carbohydrates in both extracts, though with differential intensities. CG showed strongly positive reactions for alkaloids (+++), saponins (+++), and triterpenoids (+++), while BV exhibited stronger responses for flavonoids (+++), phenolics (+++), and tannins (+++). Quantitative data are summarized in Table 1.

DPPH Radical Scavenging Activity

Both extracts showed concentration-dependent DPPH scavenging. BV was consistently more potent than CG across all concentrations (20–100 µg/mL). IC_{50} values: BV = 48.2 µg/mL; CG = 64.8 µg/mL; ascorbic acid = 21.6 µg/mL. At 100 µg/mL, BV achieved 84.61 ± 1.36% vs. CG at 73.92 ± 1.48% inhibition ($p<0.01$).

Table 1: Quantitative Phytochemical Composition of Hydroethanolic Root Extracts

Phytochemical Parameter	*C. gigantea* (CG)	*B. variegata* (BV)
Extractive Yield (% w/w)	12.24	14.92
Total Phenolic Content (mg GAE/g)	72.48 ± 1.92	89.36 ± 2.14**
Total Flavonoid Content (mg RE/g)	54.21 ± 1.47	68.93 ± 1.62**
Total Tannin Content (mg TAE/g)	41.68 ± 1.29	57.14 ± 1.35**
Saponin Index	280 ± 0.12	220 ± 0.10*
Total Terpenoid Content (mg LE/g)	63.84 ± 1.88*	48.72 ± 1.51

*Values expressed as Mean ± SEM (n = 3). *p < 0.05; **p < 0.01 vs. CG. GAE: Gallic Acid Equivalent; RE: Rutin Equivalent; TAE: Tannic Acid Equivalent; LE: Linalool Equivalent.*

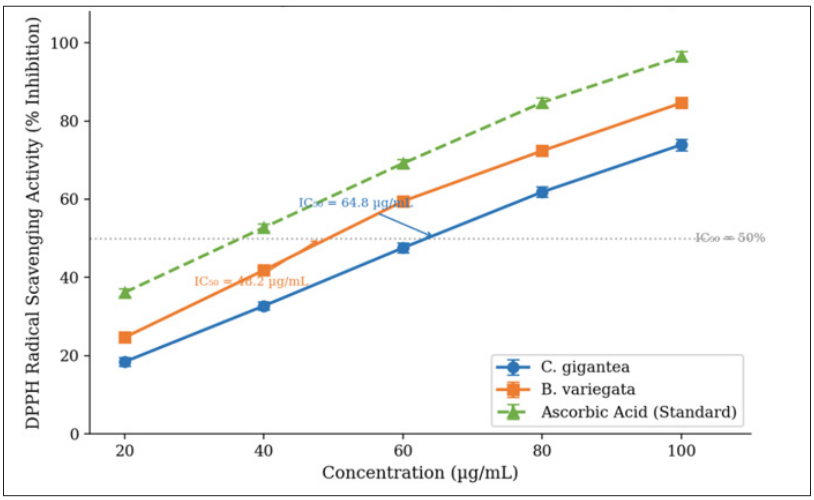

Figure 1: DPPH radical scavenging activity of hydroethanolic root extracts of C. gigantea and B. variegata compared with ascorbic acid. Mean ± SEM (n=3). IC_{50} values calculated by linear regression from percentage inhibition vs. concentration plots

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ABTS Radical Cation Scavenging Activity

BV showed greater ABTS radical scavenging activity than CG at all concentrations. At 100 µg/mL, BV inhibited 88.52 ± 1.35% vs. CG 78.46 ± 1.41% ($p<0.01$). IC_{50}: BV = 43.9 µg/mL; CG = 58.6 µg/mL; Trolox = 19.4 µg/mL. Both extracts showed statistically significant activity from 40 µg/mL onwards.

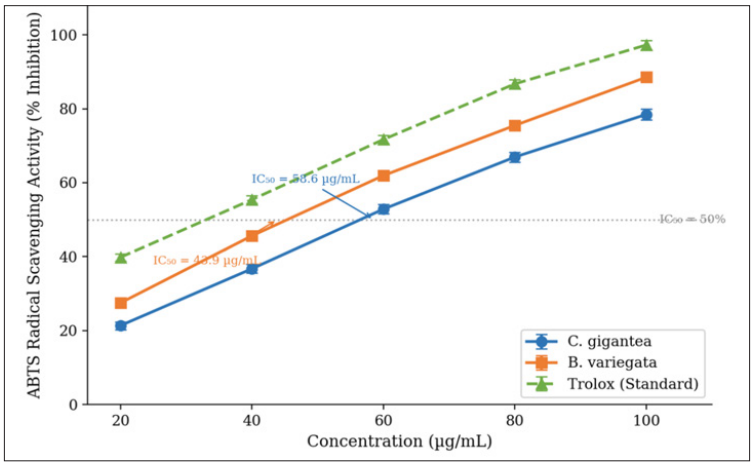

Figure 2: ABTS radical cation scavenging activity of hydroethanolic root extracts of C. gigantea and B. variegata compared with Trolox. Mean ± SEM (n=3)

Table 2: IC_{50} Values (µg/mL) for In-Vitro Antioxidant Assays

Antioxidant Assay	*C. gigantea*	*B. variegata*	Standard
DPPH Radical Scavenging	64.8	48.2	21.6 (Ascorbic acid)
ABTS Radical Scavenging	58.6	43.9	19.4 (Trolox)
H_2O_2 Scavenging	66.3	51.7	24.8 (Ascorbic acid)

Lower IC_{50} values indicate higher antioxidant potency. FRAP and reducing power assays are expressed as concentration-dependent absorbance values (non-IC_{50}). All values represent mean of triplicate determinations.

FRAP, H_2O_2 Scavenging, and Reducing Power Assays

In the FRAP assay, BV extract demonstrated higher ferric reducing capacity at all concentrations (254.3 ± 7.1 to 812.5 ± 11.2 µM Fe^{2+} equivalents) compared to CG (218.6 ± 6.4 to 718.9 ± 10.6 µM Fe^{2+} equivalents). The H_2O_2 scavenging assay yielded IC_{50} values of 51.7 µg/mL (BV) and 66.3 µg/mL (CG), compared to ascorbic acid (24.8 µg/mL). The reducing power assay further confirmed the superior electron-donating capacity of BV, with absorbance values consistently higher than CG at each concentration ($p < 0.05$ to $p < 0.01$). Table 2 summarizes all IC_{50} values.

Discussion

The present study provides a systematic comparative analysis of the antioxidant activity and phytochemical composition of two pharmacologically relevant Ayurvedic medicinal plants. The significantly higher extractive yield of B. variegata (14.92% vs. 12.24%) reflects greater availability of polar, hydroethanolic-soluble phytoconstituents, particularly polyphenols. This is corroborated by the quantitative data showing notably higher TPC, TFC, and TTC in BV compared to CG.

The superior antioxidant activity of BV across all five assays is mechanistically attributable to its polyphenol-rich composition. Flavonoids such as quercetin, kaempferol, and rutin possess ortho-dihydroxyl (catechol) configurations in their B-ring that facilitate efficient electron or hydrogen atom donation to neutralize free radicals, a mechanism underpinning both DPPH and ABTS scavenging[18]. Moreover, the 3-hydroxyl-4-carbonyl structural feature in the C-ring enables metal chelation, thereby inhibiting iron-catalyzed Fenton reactions responsible for the highly reactive hydroxyl radical (•OH)[4]. These dual mechanisms explain the correlation observed between TPC/TFC and radical scavenging potency in BV.

The FRAP and reducing power assays measure the capacity to reduce ferric ions (Fe^{3+} to Fe^{2+}), reflecting the electron-donating capacity of antioxidants. The consistently higher FRAP values in BV confirm that its phenolic constituents function as effective electron donors through single-electron transfer (SET) mechanisms. The H_2O_2 scavenging activity, relevant to prevention of hydroxyl radical generation via the Fenton and Haber–Weiss reactions, was also significantly higher in BV, indicating a stronger protective role against ROS-mediated cellular damage.

Conversely, C. gigantea exhibited a terpenoid- and cardenolide-dominant profile (TPC: 72.48 mg GAE/g; terpenoid: 63.84 mg LE/g; saponin index: 280). While its

antioxidant activity was moderate compared to BV, the presence of calotropin, calactin, and uscharin (cardenolide glycosides) imparts unique cytotoxic properties through Na^+/K^+-ATPase inhibition, mitochondrial dysfunction, and caspase activation, mechanisms distinct from conventional radical scavenging. Flavonoids such as kaempferol and isorhamnetin contribute to its moderate antioxidant capacity through Nrf2–Keap1 pathway activation, upregulating endogenous antioxidant enzyme expression[3].

Taken together, both plants demonstrate clinically relevant antioxidant potential through complementary mechanisms. BV functions primarily as a potent redox-modulator through polyphenol-mediated free radical scavenging and metal chelation, suitable for antioxidant-based chemopreventive strategies. CG, while a moderate antioxidant, contributes to redox balance through terpenoid-mediated NF-κB suppression and COX-2 inhibition, demonstrating a multi-targeted anti-inflammatory antioxidant profile. The phytochemical-antioxidant correlation established in this study provides a scientific basis for the traditional ethnomedicinal claims of both species in oxidative stress-related disorders.

Conclusion

The present investigation establishes that both C. gigantea and B. variegata hydroethanolic root extracts possess significant in-vitro antioxidant activity through multiple mechanistic pathways. B. variegata, enriched in total phenolics, flavonoids, and tannins, demonstrates superior radical scavenging, ferric reducing, and hydrogen peroxide scavenging activities, consistent with its polyphenol-driven redox modulation. C. gigantea, characterized by higher terpenoid and saponin content with moderate antioxidant activity, offers complementary anti-inflammatory antioxidant mechanisms through its cardenolide and terpenoid constituents. The significant positive correlation between phytochemical composition and antioxidant potency confirms the mechanistic basis for the observed activities. These findings support the development of standardized phytopharmaceutical preparations from both species for chemopreventive and adjunctive therapeutic applications in oxidative stress-related disorders, including cancer.

Conflict of Interest

None

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