

Analysis of Comparative Antioxidant and Antimicrobial Activity of Extracts of *Calotropis gigantea*

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Abstract:

The rising prevalence of oxidative stress-related disorders and multidrug-resistant pathogens has intensified global interest in medicinal plants as alternative and complementary therapeutic resources. *Calotropis gigantea* (Apocynaceae), a perennial shrub widely used in Ayurveda, has been traditionally recognized for its broad-spectrum 'pharmacological properties, including antioxidant, antimicrobial, anti-inflammatory, and anticancer activities'. However, systematic comparative studies on different plant parts and propagation sources remain limited.

The current study determines the anti-oxidative and antibacterial activity of *C. gigantea* ethanol extracts. 'The (DPPH) radical scavenging method was used to calculate the antioxidant and antimicrobial properties tested on Gram-positive, Gram-negative, and fungal pathogens via the agar well diffusion method and broth dilution. The results indicated that the leaf extract possessed the highest antioxidant activity with an IC₅₀ of 54.2 µg/mL after the root extract (61.8 µg/mL), but latex and flower extracts showed lower activity. The antimicrobial conditions showed a wide range of activities whereby the greatest zones of inhibition were observed against *S. aureus* (17.3 0.6 mm) and *B. subtilis* (16.8 0.5 mm). These findings were confounded by (MIC) values, which exhibited more sensitivity to Gram-positive organisms than Gram-negative bacteria'. Moderate susceptibility was also observed in the fungal pathogens, indicating antifungal phytoconstituents-terpenoids and flavonoids. Collectively, the results reveal that *C. gigantea* (and more specifically its leaves) is a potentially useful natural source of antioxidant and antibacterial compounds. The complementarity of the uses of plants emphasises the need to combine conservation and biotechnological strategies towards sustainable use of the species. These findings are scientifically relevant to the future use in bioassay-guided isolation of active compounds and their future pharmaceutical use.

Keywords: *Calotropis gigantea*, antioxidant activity, antimicrobial activity, DPPH, MIC, micropropagation.

1. INTRODUCTION

The mounting global health burden caused by the oxidative stress-related diseases and by antimicrobial resistance in the last decades has precipitated the demand to find new natural-derived therapeutic agents. 'Another important issue related to the role of oxidative stress in the etiology of a broad spectrum of chronic and degenerative diseases, including diabetes mellitus, cardiovascular diseases, and neurodegenerative diseases, including Alzheimer's and Parkinson's disease'. Natural antioxidants, especially those of vegetable origin, are essential in neutralizing (ROS), thus limiting the oxidative damage in biomolecules, i.e., DNA, proteins, and lipids. Thus, there is an effort to utilize medicinal plants rich in phenolics, flavonoids, and terpenoids as a possible substitute that is safer and more sustainable than synthetic antioxidants (Pietta, 2000; Prior et al., 2005).

The occurrence of ‘multidrug-resistant’ (MDR) pathogens, including bacteria and fungi like ‘*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida* spp’. has rendered many conventional antibiotics less effective, prompting an urgent need for new antimicrobial agents (Ventola, 2015). Medicinal plants, long valued in traditional healing systems, have therefore regained prominence as reservoirs of structurally diverse phytochemicals with promising antimicrobial properties (Cowan, 1999; Newman & Cragg, 2016).

Within this framework, *Calotropis gigantea* (L.) Dryand., commonly known as Crown Flower or “Madar,” has garnered significant scientific attention. Belonging to the family Apocynaceae, *C. gigantea* is a perennial shrub widely distributed across the Indian subcontinent, Southeast Asia, and parts of Africa, often thriving in arid and wasteland environments. Ethnobotanical records underscore its importance in Ayurveda, Siddha, and folk medicine, where it has been traditionally employed to treat ailments ranging from fever, asthma, and digestive disorders to skin infections, ulcers, rheumatism, and microbial diseases (Singh et al., 2011; Rahman & Wilcock, 1991). The pharmacological potential of *C. gigantea* is attributed to its rich repertoire of ‘secondary metabolites, including alkaloids, flavonoids, terpenoids, cardenolides, glycosides, tannins, and phenolic compounds’ (Yadav et al., 2017; Goyal & Sharma, 2012). Various biological activities assigned to these bioactive compounds cover those that are antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, anticancer, and analgesic.

Various biological activities assigned to these bioactive compounds cover those that are antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, anticancer, and analgesic. With long-term ethnomedicinal application, there are still quite a few systematic studies on the comparative efficacy of varying plant parts (leaves, latex, flowers, and roots) (Liguori et al., 2018). Research on individual extracts has been ample, but there is little work in terms of overall studies where phytochemical, antioxidant, and antimicrobial analyses have been combined. These comparative evaluations are essential, as due to the variances of phytochemical distribution across tissues and their possible impact on bioactivity and therapeutic potential, special care must be taken (Kumar et al., 2016; Mishra et al., 2013).

Limited natural spread is another difficulty for the extended use of *C. gigantea*. Its seed viability and little vegetative regeneration place restraints on the accessibility of the species to pharmacological use. The recent developments in micropropagation methods have brought a hopeful alternative, because they allow for preserving germplasm and producing large quantities of uniform plant material (Kumar & Gupta, 2020). In vitro propagation systems are not only beneficial in conservation but can also alter secondary metabolism accumulation, which may have an effect on antioxidant and antimicrobial activity of resulting extracts (Murthy et al., 2014; Sharma et al., 2018). This study will determine the ethnopharmacological usefulness of *C. gigantea*, (i) validate the ethnopharmacological significance of *C. gigantea* by integrating phytochemical screening with functional assays (ii) determine the parts of the plant that possess the highest amount of bioactive potential and (iii) show the relevance of micropropagation as an environmentally-friendly method of maintaining an unending supply of bioactive compounds.

The findings are expected to not only contribute to the growing body of evidence supporting the therapeutic potential of *C. gigantea* but also provide valuable insights for its application in natural drug discovery, nutraceutical development, and plant-based pharmacology in the context of the dual global challenges of oxidative stress and antimicrobial resistance.

2. MATERIALS AND METHODS

2.1 Plant Material Collection

Fresh and healthy parts of *Calotropis gigantea* (leaves, roots, latex, and flowers) were collected from mature plants growing in natural habitats. The plant was taxonomically authenticated by a botanist, and voucher specimens were deposited in the departmental herbarium for reference.

2.2 Preparation of Plant Extracts

Plant parts (leaves, roots, and flowers) were thoroughly washed under the running tap water, rinsed with distilled water, dried at room temperature under a shade for 10–15 days, and powdered finely using a sterile grinder. Latex was collected aseptically in a sterile vessel. The powdered material (50 g each) was subjected to extraction with 250 mL of ethanol using Soxhlet apparatus for 6–8 hours until exhaustion. Whatman No. 1 filter papers were used to filter the extracts and concentrate them under reduced pressure with the help of a rotary evaporator at 40–45 °C. Extraction of the concentrated materials was done in airtight containers at 40°C until later time of utilization.

2.3 Antioxidant Activity Assay

DPPH solution preparation: DPPH solution was prepared at 0.1 mM in methanol. Assay procedure: Extracts at different concentrations (25–200 µg/mL) were prepared in ethanol. ‘3 mL of DPPH solution was prepared, and 1 mL aliquot of each extract was combined and incubated in the dark at room temperature. Absorbance was measured at 517 nm with UV-Vis Spectrophotometer’.

Calculation of % inhibition: % Inhibition = $\frac{A_c - A_s}{A_c} \times 100\%$

‘Inhibition = $\frac{A_c - A_s}{A_c} \times 100$, where A_c = absorbance of control (DPPH solution without extract), and A_s = absorbance of sample. The IC₅₀ value (concentration required to scavenge 50% of DPPH radicals) was calculated from the dose–response curve.’

2.4 Antimicrobial Activity

2.4.1 Microorganisms Tested

The extracts were tested against the following ‘Gram-positive bacteria: *Staphylococcus aureus* and *Bacillus subtilis*. Gram-negative bacteria: Example: *Escherichia coli*, *Pseudomonas aeruginosa*. Molds: *Candida albicans*, *Aspergillus niger*’. Cultures of pure cultures were taken and placed into the Microbiology Laboratory culture collection and grown in nutrient agar.

2.4.2 Agar Well Diffusion Method

Using the agar well diffusion method, the antimicrobial potential of ethanol extracts was determined (Perez et al., 1990). Nutrient agar plates were set up and inoculated with the test organisms (10⁶ CFU/mL). Aseptic-punched wells (6 mm diameter) were added with plant extracts (100, 100, and 200 µg/mL) in 100 µL volume. Plates were incubated at 37 °C and 24 h (bacteria) and 28 °C 48–72 h (fungi). ‘Zones of inhibition (ZOI)’ were estimated with the length measured in millimeters (mm).

2.4.3 Minimum Inhibitory Concentration (MIC)

‘MIC values were determined by the broth dilution method. Extracts were tested at serial concentrations (12.5–200 µg/mL) in broth. The lowest concentration without visible turbidity after incubation was recorded as MIC’.

2.4.4 Standard Controls

‘**Positive controls:** Ciprofloxacin (for bacteria) and Fluconazole (for fungi), **Negative controls:** Solvent (ethanol) without extracts’.

2.5 Statistical Analysis

‘All experiments were performed in triplicate (n=3). Results were expressed as mean ± standard deviation (SD). IC₅₀ values were calculated using regression analysis, while antimicrobial data were analyzed using one-way ANOVA followed by post hoc Tukey’s test. Statistical significance was considered at $p < 0.05$ ’.

3. RESULTS

3.1 Antioxidant Activity

‘The antioxidant potential of ethanol extracts of *Calotropis gigantea* was assessed using the DPPH radical scavenging assay’. The IC₅₀ values are presented in Table 1.

Table 1. Antioxidant activity (IC₅₀ values) of ethanol extracts of *Calotropis gigantea*

Plant Part (Extract)	IC ₅₀ (µg/mL)	Relative Activity
Leaf Extract	54.2	Strongest
Root Extract	61.8	Moderate
Latex Extract	Higher than 61.8 (weaker)	Low activity
Flower Extract	Higher than 61.8 (weaker)	Low activity

These findings correlate well with the earlier phytochemical analysis, which reported the highest concentrations of phenolic and flavonoid compounds in leaves, moderate levels in roots, and comparatively lower levels in latex and flowers. Since phenolics and flavonoids are well-known contributors to antioxidant activity, their abundance in leaves explains the higher radical scavenging potential. ‘The data revealed that the leaf extract exhibited the strongest radical scavenging activity, with an IC₅₀ value of 54.2 µg/mL, followed by the root extract (61.8 µg/mL)’. In contrast, latex and flower extracts showed significantly higher IC₅₀ values (>61.8µg/mL), indicating much weaker antioxidant potential.

3.2 Antimicrobial Activity

Ethanol extracts of *C. gigantea* were evaluated in vitro against six clinically significant microorganisms for their antimicrobial activity. Table 2 summarizes the results of the agar well diffusion assay (zone of inhibition), and Table 3 tabulates the minimum inhibitory concentrations (MICs) obtained by the broth dilution method.

Table 2. Zone of Inhibition (mm) of *C. gigantea* Extracts Against Test Organisms

Organism	Leaf Extract	Root Extract	Latex Extract	Standard Drug
<i>Staphylococcus aureus</i>	17.3 ± 0.6	15.2 ± 0.4	12.1 ± 0.5	22.4 ± 0.3
<i>Bacillus subtilis</i>	16.8 ± 0.5	14.5 ± 0.6	11.3 ± 0.6	23.0 ± 0.4
<i>Escherichia coli</i>	14.6 ± 0.4	12.3 ± 0.7	10.4 ± 0.5	20.5 ± 0.5
<i>Pseudomonas aeruginosa</i>	13.9 ± 0.3	11.7 ± 0.5	9.6 ± 0.3	21.0 ± 0.4
<i>Candida albicans</i>	15.1 ± 0.6	13.4 ± 0.5	11.2 ± 0.4	19.8 ± 0.6
<i>Aspergillus niger</i>	14.3 ± 0.5	12.1 ± 0.4	10.7 ± 0.4	18.5 ± 0.3

The leaf extract showed the greatest antimicrobial activity, producing the largest inhibition zones across all test organisms. ‘The strongest effect was against *Staphylococcus aureus* (17.3 mm) and *Bacillus subtilis* (16.8 mm), both Gram-positive bacteria. Moderate activity was observed against ‘*E. coli* and *P. aeruginosa*’ (Gram-negative bacteria), with inhibition zones of 14.6 mm and 13.9 mm, respectively’. Fungal strains (*C. albicans* and *A. niger*) also showed susceptibility, though inhibition zones were smaller compared to standard antifungal agents.

This implies that ‘Gram-negative cells are more resistant to *C. gigantea* extracts than Gram-positive species’ probably by virtue of the difference in the composition of the cell wall in these two organisms.

Table 3. Minimum Inhibitory Concentration (MIC) (mg/mL) of *C. gigantea* Extracts Against Test Organisms

Organism	Leaf Extract	Root Extract	Latex Extract	Standard Drug
<i>Staphylococcus aureus</i>	50	75	100	12.5
<i>Bacillus subtilis</i>	50	75	100	12.5
<i>Escherichia coli</i>	75	100	125	25
<i>Pseudomonas aeruginosa</i>	100	125	150	25
<i>Candida albicans</i>	75	100	125	25
<i>Aspergillus niger</i>	75	100	125	50

MIC results corroborate the ZOI data. The lowest MIC values were recorded for ‘Gram-positive bacteria (*S. aureus* and *B. subtilis*)’ at 50 mg/mL, indicating the strongest antimicrobial action. Gram-negative bacteria required higher concentrations (75–150 mg/mL) for inhibition, reflecting their relative resistance. Similarly, fungal pathogens displayed moderate sensitivity with MIC values ranging from 75 to 125 mg/mL.

Taken together, the antimicrobial assays confirm that leaves are the most potent plant part in terms of antimicrobial efficacy, followed by roots, with latex showing the weakest activity.

- **Leaves** are the richest source of antioxidant compounds and exhibit the strongest radical scavenging and antimicrobial activity.
- **Gram-positive bacteria** were more sensitive than Gram-negative strains, consistent with structural differences in bacterial cell envelopes.
- **Fungi** (*C. albicans*, *A. niger*) showed moderate susceptibility, suggesting potential antifungal activity.
- Standard drugs (Ciprofloxacin, Fluconazole) showed higher potency overall, but the activity of *C. gigantea* extracts remains significant and warrants further investigation.

4. DISCUSSION

The results of this research demonstrate that the leaves of *Calotropis gigantea* represent the richest source of antioxidants among the plant parts tested. ‘The DPPH radical scavenging assay revealed an IC₅₀ value of 54.2 µg/mL, which is markedly lower than that of the roots (61.8 µg/mL), latex (>70 µg/mL), and flowers (>80 µg/mL)’. These findings are consistent with the phytochemical screening, which showed the highest concentration of phenolic and flavonoid compounds in leaves, moderate levels in roots, and much lower levels in latex and flowers. As polyphenols and flavonoids are known to be good contributors to antioxidant activity through their ability to donate hydrogen and radical-scavenging capacity (Rice-Evans et al., 1997), their high concentration in leaves contributes to the high radical scavenging power (Pietta, 2000).

The antioxidant activity indicated in this work is consistent with the previous research, comparatively. As an example, the study by (Yadav et al., 2017) of the extracts of leaves of *C. gigantea* showed significant DPPH scavenging activity, and, in this case, it is explained by the presence of flavonoids, including quercetin alkaloid derivatives. Similarly, ethanolic extracts of the *C. gigantea* leaf showed a high scavenging of free radicals when compared to root and stem extracts (Rao and Patel, 2013). Our results support these observations and offer quantitative data (IC₅₀ values) to indicate that the leaf extract is the antioxidant fraction with the strongest effect. These implications are significant, bearing in mind that oxidative stress is the basis of a broad range of disorders such as cardiovascular disorders, neurodegeneration, and cancer (Valko et al., 2007). As an antimicrobial antibiotic, the antibacterial, anti-yeast, and antifungal activities of *C. gigantea* ethanol extracts were found to be broad-spectrum and more pronounced on Gram-positive bacterial species than Gram-negative. The zone of inhibition (ZOI)

results showed maximum activities on *S.aureus* (17.3mm) and *Bacillus subtilis* (16.8mm), moderate activities on *E.coli* (14.6mm) and *Pseudomonas aeruginosa* (13.9mm), and no activities on *Candida albicans* (0mm). These observations were also supported by the 'minimum inhibitory concentration (MIC) the lowest minimum inhibitory concentration values were obtained (50 mg/mL) against Gram-positive strains and intermediate MICs against Gram-negative bacteria'.

These conclusions came in line with the findings of other researchers who did not find any exceptions to the high susceptibility of Gram-positive bacteria to plant extracts (Cowan, 1999; Goyal & Sharma, 2012). The basis is that of structural variability in bacterial cell walls: the cell wall of Gram-positive bacteria is relatively simple, consisting of peptidoglycans, and thus phytochemicals like alkaloids, flavonoids, and terpenoids are more apt to enter it. Conversely, the Gram-negative bacteria possess an outer membrane whose major polymer component is lipopolysaccharides; these membranes serve as a permeability barrier, making the effectiveness of the plant-derived antimicrobial agents to the cell form a challenge (Nikaido, 2003).

Interestingly, the extracts also showed antifungal potential against '*Candida albicans* and *Aspergillus niger*, with ZOI values of 15.1 mm and 14.3 mm, respectively'. While this activity was less pronounced compared to bacterial inhibition, it supports the ethnopharmacological use of *C. gigantea* for treating skin infections, ulcers, and fungal disorders (Mehrotra & Kumar, 2007). Similar antifungal effects of *C. gigantea* extracts have been reported by (Kumar et al. 2016), who demonstrated inhibitory action against *Candida* spp., attributing it to bioactive terpenoids and cardenolides.

When benchmarked against standard antibiotics and antifungals, such as ciprofloxacin and fluconazole, the efficacy of *C. gigantea* extracts was comparatively lower. For example, ciprofloxacin produced larger inhibition zones (22–23 mm against bacteria) and fluconazole inhibited fungal growth more effectively than plant extracts. Nevertheless, the significant antimicrobial activity of leaf and root extracts, particularly against Gram-positive bacteria, is notable. These findings suggest that although whole extracts are less potent than standard drugs, they harbor bioactive compounds that merit further bioassay-guided fractionation and purification. Isolated phytoconstituents may demonstrate enhanced potency and specificity, potentially serving as leads for novel antimicrobial agents.

Overall, the present study corroborates earlier findings while providing comparative quantitative data that enrich our understanding of *C. gigantea*'s pharmacological profile. By demonstrating both antioxidant and antimicrobial activities in different plant parts, the study validates its traditional use and underscores its potential for future therapeutic applications.

5. CONCLUSION

The current research reveals the great medical ability of *Calotropis gigantea* as a natural antioxidant and antimicrobial agent. 'Irrespective of the part of the plant, the extract of the leaf showed the highest antioxidant activity, which is indicated by the lowest IC₅₀ value of the DPPH assay'. This was because of its high radical scavenging activity, which is attributed to the high phenolic and flavonoid content found in leaves, and the crucial relevance of this plant organ in pharmacological properties. Root and latex extracts were the best of them with differing levels of antioxidant and antimicrobial activities, with the flower extracts being lowest, showing that bioactive compound distribution is different across different tissues.

C. gigantea extracts produced a wide spectrum inhibitory effect on both bacterial and fungal pathogens, with Gram-positive bacteria exhibiting more susceptibility than Gram-negative ones, such as '*Staphylococcus aureus* and *Bacillus subtilis* v. *Escherichia coli* and *Pseudomonas aeruginosa*'. This relative insensitivity has probably been associated with the multifactorial outer membrane of Gram-negative pathogens, which hinders the entry of phytochemicals. Fungal pathogens such as *Candida*

albicans and *Aspergillus niger* also provided medium sensitivity, implying that the plant also contains antifungal metabolites. In spite of the outputs of the standard antibiotics and antifungal, which were more effective, the activity of extracts of *C. gigantea* was promising to warrant further research to discover plant-based therapeutics. Notably, the results also highlight the importance of micropropagation as a sustainable method of preserving *C. gigantea* and allowing high yields of the compounds with bioactive activities they contain. Since the threat of antimicrobial resistance grows and *C. gigantea* shows promising results, this study offers a fair motive to move it towards bioassay-guided fractionation and compound isolation processes.

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