Antioxidant Potential of Calotropis gigantea (L.) Leaf Extract: A FRAP and CUPRAC Based Evaluation

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***Abstract*—The present study was undertaken to evaluate the in-vitro antioxidant potential of the leaf extract of Calotropis gigantea (L.) Dryand. (Apocynaceae), a widely distributed medicinal shrub traditionally used to treat inflammation, skin disorders and wounds, using ferric- and cupric-ion reducing assays. Shade-dried, powdered leaves of C. gigantea were extracted successively by maceration, Soxhlet extraction and magnetic stirrer-assisted extraction using an ethanol-water solvent system. The reducing/antioxidant capacity of the extract was determined by the Ferric Reducing Antioxidant Power (FRAP) assay, measured at 593 nm against a ferrous sulphate standard, and the Cupric ion Reducing Antioxidant Capacity (CUPRAC) assay, measured at 450 nm against a quercetin dihydrate standard. The Soxhlet and magnetic stirrer extractions yielded 1.54% and 3.17% w/w extract, respectively. In the FRAP assay, sample absorbance ranged from 0.250 to 0.757, corresponding to a reducing capacity of 25.6-77.5 µg/ml ferrous sulphate equivalent (mean 53.54 ± 19.67 µg/ml; R^2 = 0.919). In the CUPRAC assay, absorbance ranged from 1.13 to 3.94, corresponding to 33.3-116.2 µg/ml quercetin equivalent (mean 62.00 ± 33.09 µg/ml; R^2 = 0.842). Antioxidant capacity increased proportionally with absorbance in both assays. The leaf extract of Calotropis gigantea exhibited appreciable ferric- and cupric-reducing antioxidant capacity, supporting its traditional medicinal use and its candidacy as a natural antioxidant source for nutraceutical and pharmaceutical applications, warranting further evaluation by complementary radical-scavenging and in-vivo assays.**

***Index Terms*—Calotropis gigantea, Antioxidant activity, FRAP assay, CUPRAC assay, Leaf extract, Phytochemical screening, Free radical scavenging**

I. INTRODUCTION

Oxidative stress arises from an imbalance between the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and the capacity of the endogenous antioxidant defence system to neutralise them [1], [2]. Free radicals and ROS, though essential at physiological concentrations for cell signalling, immune defence and gene regulation, become damaging when produced in excess, attacking lipids, proteins, carbohydrates and nucleic acids [1]. This oxidative damage is implicated in the pathogenesis of cardiovascular disorders, diabetes mellitus, cancer, neurodegenerative disease, inflammatory conditions and ageing [2], [3]. Antioxidants counter this damage by donating electrons or hydrogen atoms to free radicals, thereby stabilising them and interrupting the oxidative chain reaction [3].

A variety of in-vitro assays are used to evaluate antioxidant activity, each interrogating a different mechanism of action; these include the DPPH radical scavenging assay, the ABTS radical cation decolourisation assay, the Ferric Reducing Antioxidant Power (FRAP) assay, the Cupric ion Reducing Antioxidant Capacity (CUPRAC) assay, and the phosphomolybdenum total antioxidant capacity assay [3]. The FRAP assay is based on the reduction of a colourless ferric-tripyridyltriazine (Fe3+-TPTZ) complex to its intensely blue ferrous form, read at 593 nm [14]. The CUPRAC assay complements FRAP by measuring the reduction of cupric (Cu2+) to cuprous (Cu+) ions in the presence of neocuproine, forming a chromogen read at 450 nm; it operates closer to physiological pH and correlates well with total phenolic content [15].

Calotropis gigantea (L.) Dryand. (family Apocynaceae), commonly known as giant milkweed or crown flower, is a hardy perennial shrub distributed across the tropical and subtropical wastelands, roadsides and coastal areas of Asia, Africa and Australia [6], [7]. Its various parts have long been used in Ayurveda, Siddha and Unani medicine for inflammation, pain, wounds, respiratory ailments and skin disorders [6], [12]. Phytochemical studies have identified cardiac glycosides, flavonoids, alkaloids, terpenoids, steroids, saponins, tannins and phenolic compounds in its leaves, associated with antimicrobial, anti-inflammatory, antioxidant and wound-healing activity [3], [12], [20]. The present study was designed to prepare an ethanol-water leaf extract of C. gigantea and evaluate its in-vitro antioxidant (reducing) potential using the FRAP and CUPRAC assays.

II. MATERIALS AND METHODS

A. Collection and Authentication of Plant Material

Fresh leaves of Calotropis gigantea (L.) Dryand. were collected in july 2026 from Adagapadi (PIN 636803), Tamil Nadu, India, and were identified by the Department of Pharmacognosy of the institute.

B. Preparation of the Extract

The collected leaves were washed under running water, shade-dried at room temperature for 2-3 weeks, and pulverised to a fine powder using a mechanical grinder. The powder was passed through sieve no. 80 and stored in an airtight container. Approximately 25 g of the dried leaf powder was used for successive extraction.

C. Extraction of Plant Material

Maceration, Soxhlet extraction and magnetic stirrer-assisted extraction were used to isolate bioactive phytoconstituents. In the Soxhlet method, dried leaf powder packed in a cellulose thimble was continuously extracted with an ethanol-water solvent system (approx. 75:20 v/v, 300 ml ethanol: 100 ml water) for 12 h. In the magnetic stirrer method, powdered leaf material was stirred in the same solvent system at 50-80°C for 3 h. Both extracts were filtered through Whatman No. 1 filter paper and concentrated on a heating mantle at 30-50°C. Percentage yield was 1.54% w/w (Soxhlet) and 3.17% w/w (magnetic stirrer); dry extract weights were 1.47 g and 3.02 g, respectively (maceration yielded 2.52 g). The concentrated extract was black-green, gummy, and possessed a pungent odour.

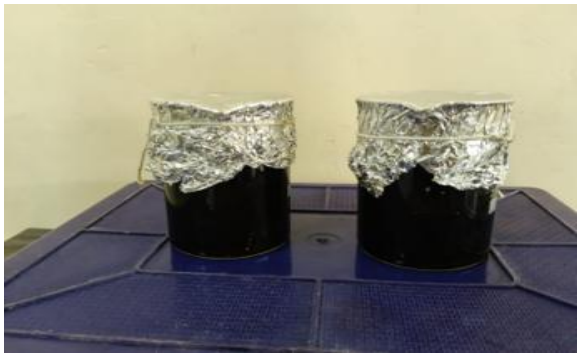

Fig no 1: Concentrated ethanol-water leaf extracts of Calotropis gigantea

D. Chemicals and Reagents

Acetate buffer, TPTZ, ferric chloride, ferrous sulphate (FRAP standard), copper (II) chloride, neocuproine, ammonium acetate buffer and quercetin dihydrate (CUPRAC standard) were of analytical grade. Absorbance was recorded on a UV-Visible spectrophotometer using matched quartz cuvettes.

E. FRAP Assay

Antioxidant capacity was determined by the method of Benzie and Strain [14]. The FRAP reagent (acetate buffer pH 3.6, TPTZ solution and ferric chloride solution) was mixed; 1 ml of extract was added to 3 ml of reagent, incubated at 37°C for 30 min, and absorbance was read at 593 nm against a blank. Results were interpolated on a ferrous sulphate calibration curve and expressed as ferrous sulphate equivalents (µg/ml).

F. CUPRAC Assay

The CUPRAC assay was performed per Apak et al. [15]. Copper (II) chloride, neocuproine and ammonium acetate buffer (pH 7.0) were mixed with the extract and incubated at room temperature for 30 min; the yellow-orange Cu+-neocuproine chelate was measured at 450 nm. Results were interpolated on a quercetin dihydrate calibration curve and expressed as quercetin equivalents (µg/ml).

G. Statistical Analysis

Results are expressed as mean ± SD. Linear regression was used to derive the calibration equation and R^2 for each standard curve, and sample values were interpolated accordingly.

III. RESULTS

A. FRAP Assay

The ferrous sulphate calibration curve gave y = 0.00262x + 0.428 (R^2 = 0.919) at 593 nm. The extract showed a concentration-dependent increase in absorbance, corresponding to 25.6-77.5 µg/ml FSE (mean 53.54 ± 19.67 µg/ml; CV 36.74%), with the highest value for sample S5 (Table 1, Fig no 2).

Table 1: FRAP values of C. gigantea leaf extract (mean ± SD 53.54 ± 19.67 µg/ml)

Sample	Abs. (593 nm)	FRAP (µg/ml FSE)
S1	0.250	25.6
S2	0.433	44.4
S3	0.554	56.7
S4	0.620	63.5
S5	0.757	77.5

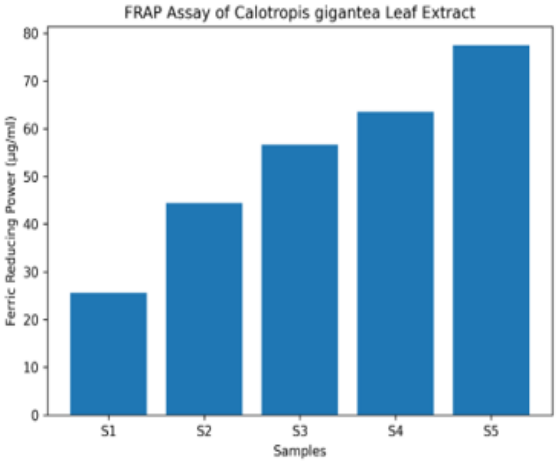

Fig no 2: FRAP assay of Calotropis gigantea leaf extract

B. CUPRAC Assay

The quercetin dihydrate calibration curve gave y = 0.00426x + 1.779 (R^2 = 0.842) at 450 nm. Cupric-reducing capacity ranged from 33.3 to 116.2 µg/ml QE (mean 62.00 ± 33.09 µg/ml; CV 53.37%), with the highest value for sample S1 (Table 2, Fig no 3).

Table 2: CUPRAC values of C. gigantea leaf extract (mean ± SD 62.00 ± 33.09 µg/ml)

Sample	Abs. (450 nm)	CUPRAC (µg/ml QE)
S1	3.939	116.2
S2	2.363	69.7
S3	1.471	43.4
S4	1.131	33.3
S5	1.609	47.4

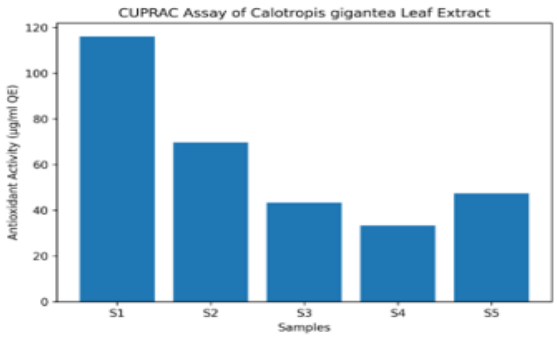

Fig no 3: CUPRAC assay of Calotropis gigantea leaf extract

IV. DISCUSSION

Both assays demonstrated concentration-dependent reducing capacity, consistent with earlier reports of significant antioxidant activity in Calotropis gigantea extracts attributed to phenolic and flavonoid content [16], [20]. The higher mean and greater variability in CUPRAC (62.00 ± 33.09 µg/ml QE, CV 53.37%) relative to FRAP (53.54 ± 19.67 µg/ml FSE, CV 36.74%) reflects the differing chemistries of the two methods: CUPRAC operates near physiological pH and detects a broader range of hydrophilic and lipophilic reductants, whereas FRAP operates under strongly acidic conditions with a fixed 30 min window [14], [15].

The stronger linear fit for FRAP (R^2 = 0.919) versus CUPRAC (R^2 = 0.842) may reflect greater matrix sensitivity of the CUPRAC chromogen to co-extracted pigments and resinous latex constituents [15]. Nonetheless, the concordant increasing trend across both assays strengthens confidence in the extract's overall reducing-power profile, corroborating earlier phytochemical screening of C. gigantea leaves identifying flavonoids, phenolics, tannins, alkaloids and cardiac glycosides as key contributors [3], [12], [20]. As FRAP and CUPRAC are purely chemical, non-radical, electron-transfer assays, the present results indicate electron-donating capacity rather than a comprehensive measure of overall antioxidant efficacy.

V. CONCLUSION

The leaf extract of Calotropis gigantea possesses appreciable ferric- and cupric-ion reducing (antioxidant) capacity, as shown by concentration-

dependent activity in both the FRAP and CUPRAC assays, attributable to its phenolic and flavonoid phytoconstituents. These findings support the traditional ethnomedicinal use of C. gigantea leaves and indicate its potential as a natural antioxidant source for nutraceutical and pharmaceutical formulations. Further studies incorporating DPPH/ABTS radical-scavenging assays, quantitative phenolic/flavonoid estimation, and in-vivo evaluation are recommended.

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DECLARATIONS

Conflict of Interest: The authors declare that they have no conflict of interest.
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Use of Generative AI: No artificial intelligence (AI) or AI-assisted tools were used in the design, conduct, or data analysis of this study.

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