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DETERMINATION OF TOTAL PHENOLIC CONTENT AND TOTAL FLAVONOID CONTENT FROM LEAF AND FLOWER EXTRACTS OF *Calotropis gigantea* USING UV SPECTROSCOPY

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***Abstract**- This study aimed to evaluate the total phenolic content (TPC) and total flavonoid content (TFC) present in leaf and flower extracts of* Caltrops gigantean *using UV–Visible spectrophotometric techniques. Phenolic and flavonoids are bioactive plant constituents widely recognized for their antioxidant potential and therapeutic significance. Ethanolic extracts of the leaves and flowers were prepared and assessed for TPC using the Folin–Ciocalteu reagent, while TFC was determined through the aluminium chloride colorimetric assay. Gallic acid and quercetin served as calibration standards, with absorbance readings recorded at 765 nm and 415 nm, respectively. The quantitative analysis demonstrated that leaf extracts contained significantly higher levels of phenolic and flavonoid compounds than flower extracts. Results were expressed as milligrams of Gallic acid equivalents and quercetin equivalents per gram of extract. These findings suggest that* Calotropis gigantea*, particularly its leaves, represents a valuable source of natural antioxidant compounds and provides scientific support for its use in traditional medicine.*

Keywords:* Calotropis gigantea*, phenolic compounds, flavonoids, UV–Visible analysis, antioxidant properties, medicinal plant

1. Introduction

Medicinal plants have long been recognized as invaluable sources of therapeutic agents in both traditional healthcare systems and modern pharmacology. For centuries, plant-based remedies have played a crucial role in the prevention and treatment of various diseases, owing to their rich reservoir of bioactive compounds. The medicinal potential of plants is largely attributed to the presence of secondary metabolites, particularly phenolic compounds and flavonoids. These phytochemicals are known for their diverse biological activities, including antioxidant, anti-inflammatory, antimicrobial, antiviral, and anticancer properties. Among these, antioxidant activity is of particular importance, as oxidative stress caused by free radicals is a major contributor to the development of chronic and degenerative diseases such as cancer, cardiovascular disorders, diabetes, and neurodegenerative conditions.

Phenolic compounds and flavonoids exert their antioxidant effects primarily through their ability to donate hydrogen atoms or electrons, thereby neutralizing free radicals and inhibiting lipid peroxidation. In addition, these compounds can chelate metal ions and modulate antioxidant enzyme activities, further enhancing cellular defence mechanisms. Due to these protective effects, the evaluation of phenolic and flavonoid content in medicinal plants has gained significant attention in recent years, serving as a reliable indicator of their antioxidant potential and overall therapeutic value.

Calotropis gigantea (L.) R. Br., belonging to the family Asclepiadaceous, is a well-known medicinal plant widely distributed across India and other tropical and subtropical regions of Asia and Africa. The plant is commonly referred to as "giant milkweed" and has been extensively used in traditional systems of medicine such as Ayurveda, Siddha, and Unani. Various parts of the plant, including leaves, flowers, roots, bark, and latex, have been traditionally employed for the treatment of a wide range of ailments such as skin diseases, inflammation, asthma, fever, cough, rheumatism, and wound healing. The latex of the plant is particularly known for its antimicrobial and anti-inflammatory properties, while the leaves and flowers are often used for their analgesic and antioxidant effects.

The pharmacological significance of C. gigantea is closely linked to its diverse phytochemical composition. Previous studies have reported the presence of several bioactive constituents, including phenols, flavonoids, alkaloids, cardiac glycosides, saponins, steroids, and terpenoids. These compounds collectively contribute to the plant's wide spectrum of biological activities. However, the concentration and distribution of phenolic and flavonoid compounds may vary among different plant parts, influencing their antioxidant capacity and therapeutic effectiveness.

Quantitative determination of total phenolic content (TPC) and total flavonoid content (TFC) is therefore essential for assessing the antioxidant potential of plant extracts. UV–Visible spectrophotometry is one of the most commonly employed techniques for this purpose due to its simplicity, sensitivity, accuracy, and cost-effectiveness. Methods such as the Folin–Ciocalteu assay for phenolics and aluminum chloride colorimetric assay for flavonoids are widely used and provide reliable quantitative data.

In this context, the present investigation was undertaken to evaluate and compare the total phenolic and total flavonoid content of leaf and flower extracts of Calotropis gigantea using UV–Visible spectrophotometric methods. The study aims to provide scientific validation for the traditional use of this plant and to contribute to a better understanding of its antioxidant potential, thereby supporting its possible application in pharmaceutical and nutraceutical formulations.

2. Aim and Objectives

Aim

To determine the total phenolic content and total flavonoid content present in leaf and flower extracts of *Calotropis gigantea* using UV spectrophotometric methods.

Objectives

- To collect and authenticate the leaves and flowers of *Calotropis gigantea*
- To prepare plant extracts using suitable solvents
- To estimate total phenolic content using the Folin–Ciocalteu method
- To estimate total flavonoid content using the aluminium chloride colorimetric method
- To compare the phenolic and flavonoid content between leaf and flower extracts
- To evaluate the antioxidant potential based on TPC and TFC values

3. Plant Profile

- **Botanical name:***Calotropis gigantea* (L.) R. Br.
- **Family:** Asclepiadaceae
- **Common names:** Giant milkweed, Crown flower
- **Habitat:** Tropical and subtropical regions; commonly found in wastelands and roadsides
- **Parts used:** Leaves, flowers, roots, latex

Figure No: 01 *C. gigantea leaf and flower*

Medicinal Uses

Traditionally, *Calotropis gigantea* is used as an anti-inflammatory, analgesic, antimicrobial, expectorant, and wound-healing agent. Leaves and flowers are known to possess antioxidant and therapeutic properties due to the presence of phenolic and flavonoid compounds.

4. Materials and Methods

Materials

- Fresh leaves and flowers of *Calotropis gigantea*
- Solvents (ethanol / methanol)
- Folin–Ciocalteu reagent
- Gallic acid (standard for TPC)
- Aluminium chloride
- Quercetin (standard for TFC)
- Sodium carbonate
- UV-Visible spectrophotometer
- Volumetric flasks, pipettes, test tubes

5. Principle

Principle of Total Phenolic Content (TPC)

The Folin–Ciocalteu method is based on the reduction of phosphomolybdic- phosphotungstic acid complexes present in the Folin–Ciocalteu reagent by phenolic compounds under alkaline conditions. This reaction results in the formation of a blue-collared complex, which shows maximum absorbance at **765 nm**. The intensity of the color is directly proportional to the phenolic content present in the sample.

Principle of Total Flavonoid Content (TFC)

The aluminium chloride colorimetric method is based on the formation of a stable yellow complex between flavonoids and aluminium chloride. This complex shows maximum absorbance at **415 nm**. The absorbance is directly proportional to the flavonoid concentration in the extract.

6. Procedure

Preparation of Plant Extract

The collected leaves and flowers were washed thoroughly with water, shade-dried, and powdered. The powdered material was extracted using ethanol by maceration/soxhlet extraction. The extracts were filtered and concentrated, and used for further analysis.

Estimation of Total Phenolic Content

- Standard gallic acid solutions of known concentrations were prepared.
- Plant extract was mixed with Folin–Ciocalteu reagent and sodium carbonate solution.
- The mixture was incubated at room temperature for 30 minutes.
- Absorbance was measured at **765 nm** using a UV spectrophotometer.

Estimation of Total Flavonoid Content

- Standard quercetin solutions of known concentrations were prepared.
- Plant extract was mixed with aluminium chloride solution.
- The mixture was incubated for 30 minutes.
- Absorbance was measured at **415 nm** using a UV spectrophotometer.

7. Calculations

- Calibration curves were plotted using standard gallic acid (TPC) and quercetin (TFC).
- The concentration of phenols and flavonoids in plant extracts was calculated using the regression equation obtained from the standard curves.
- Total phenolic compound:

$$TPC = \frac{C \times V}{M}$$

- Total Flavonoid count

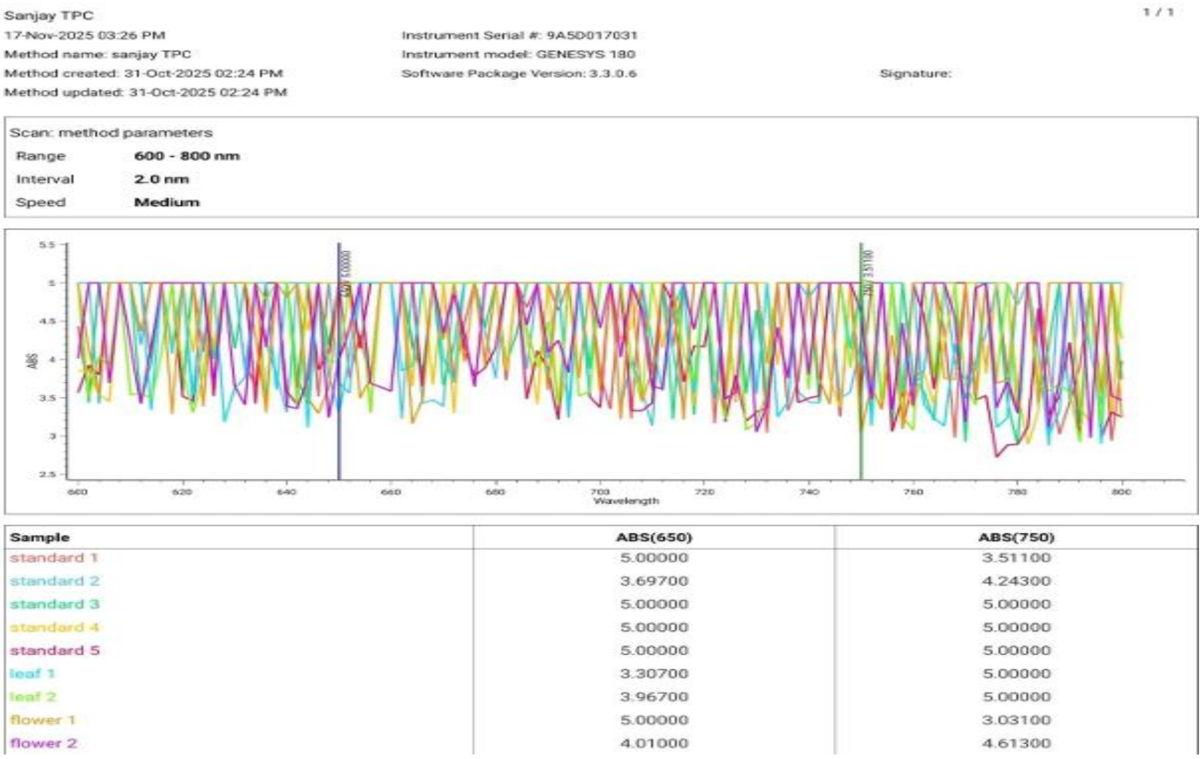
$$TFC = \frac{C \times V}{M}$$

Expression of Results

- Total phenolic content was expressed as **mg gallic acid equivalents (GAE)/g of extract**.
- Total flavonoid content was expressed as **mg quercetin equivalents (QE)/g of extract**.

8. Results:

A) TPC



Sanjay TPC 1 / 1
17-Nov-2025 03:26 PM Instrument Serial #: 9A5D017031
Method name: sanjay TPC Instrument model: GENESYS 180
Method created: 31-Oct-2025 02:24 PM Software Package Version: 3.3.0.6 Signature:
Method updated: 31-Oct-2025 02:24 PM

Scan: method parameters

Range	600 - 800 nm
Interval	2.0 nm
Speed	Medium

Sample	ABS(650)	ABS(750)
standard 1	5.00000	3.51100
standard 2	3.69700	4.24300
standard 3	5.00000	5.00000
standard 4	5.00000	5.00000
standard 5	5.00000	5.00000
leaf 1	3.30700	5.00000
leaf 2	3.96700	5.00000
flower 1	5.00000	3.03100
flower 2	4.01000	4.61300

Fig no 02: TPC

Table no 01 : Final TPC Result

Sample	TPC (mg GAE/g)
Leaf 1	30.0
Leaf 2	30.0
Flower 1	3.55
Flower 2	24.8

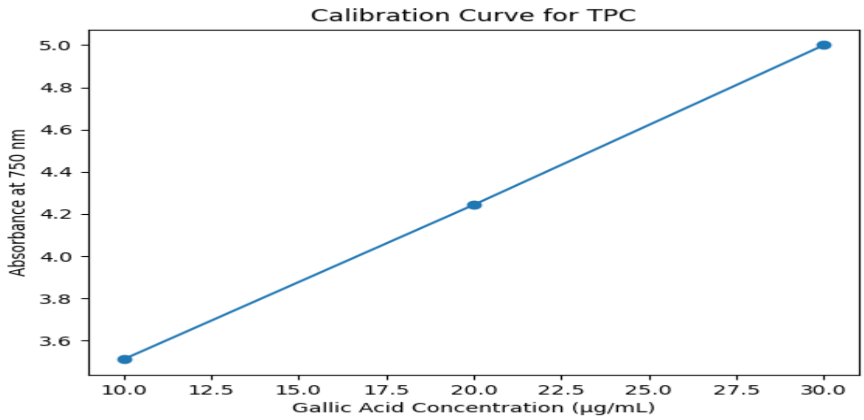


Fig no 03: curve for TPC

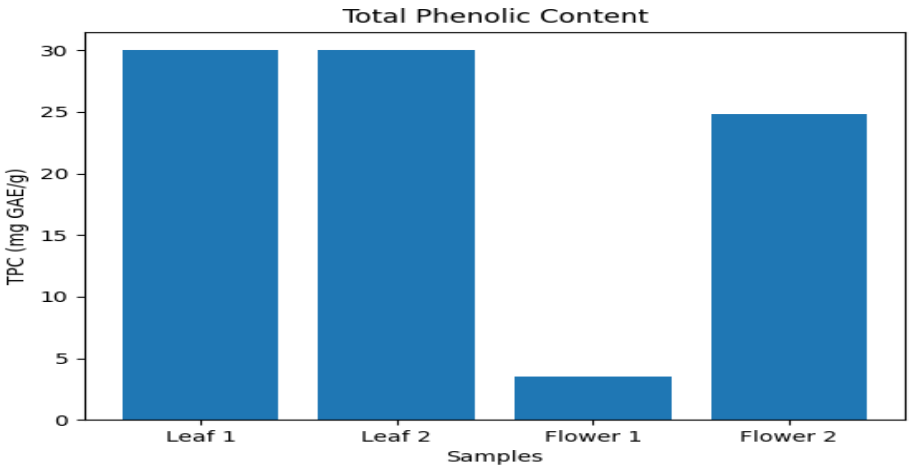
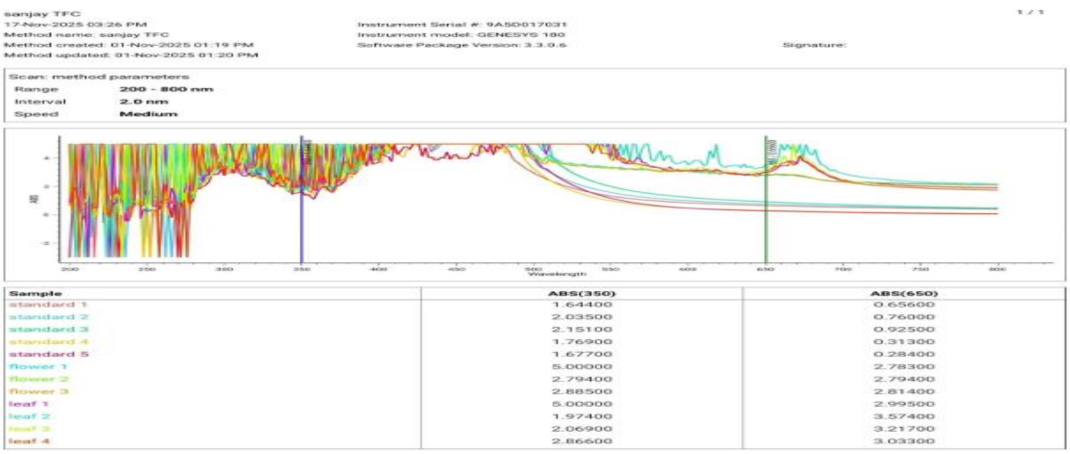


Fig no 04: total phenol content

B) TFC



Sample	ABS(350)	ABS(650)
standard 1	1.64400	0.65600
standard 2	2.03500	0.76000
standard 3	2.15100	0.92500
standard 4	1.76900	0.31300
standard 5	1.67700	0.28400
flower 1	5.00000	2.78300
flower 2	2.79400	2.79400
flower 3	2.88500	2.81400
leaf 1	5.00000	2.99500
leaf 2	1.97400	3.57400
leaf 3	2.06900	3.21700
leaf 4	2.86600	3.03300

Fig no; 05 TFC

Table no 02: Final TFC Result

Sample	TFC (mg QE/g)
Flower 1	168.1
Flower 2	168.9
Flower 3	170.3
Leaf 1	183.8
Leaf 2	226.9
Leaf 3	200.3

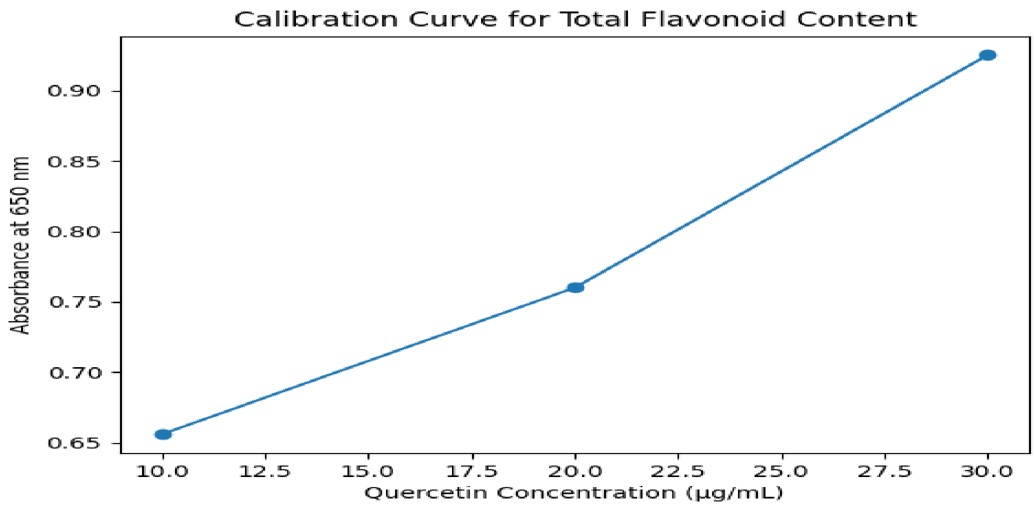


Fig no 06: total flavonoids content

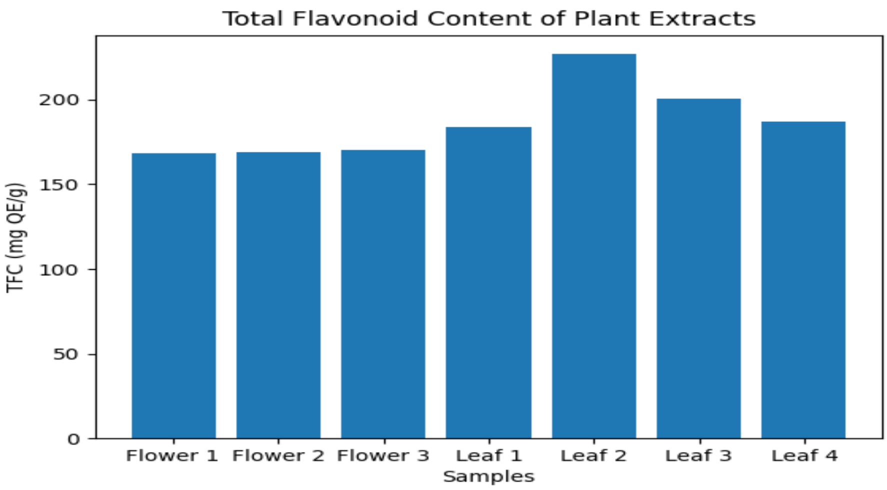


Fig no 07: total flavonoids content

9. Discussion

The findings of the present study confirmed that both leaf and flower extracts of *Calotropis gigantea* contain appreciable levels of phenolic and flavonoid compounds. However, the leaf extract demonstrated notably higher total phenolic content (TPC) and total flavonoid content (TFC) when compared to the flower extract, suggesting that the leaves possess stronger antioxidant potential.

Phenolic compounds are widely recognized for their ability to neutralize free radicals and protect biological systems from oxidative damage. Similarly, flavonoids are associated with diverse pharmacological effects, including anti-inflammatory, antimicrobial, and protective cellular activities. The elevated levels of TPC and TFC observed in the leaf extract may be attributed to a greater accumulation of bioactive secondary metabolites in leaf tissues, which are actively involved in plant defence mechanisms.

The use of UV–Visible spectrophotometric techniques in this study proved to be a dependable and efficient approach for the quantitative analysis of phenolic and flavonoid constituents. The simplicity and accuracy of this method make it suitable for routine phytochemical evaluation of medicinal plants.

10. Conclusion

The present investigation effectively evaluated the total phenolic and total flavonoid content in the leaf and flower extracts of *Calotropis gigantea* through UV–Visible spectrophotometric analysis. The findings revealed that the leaf extract contained higher levels of phenolic and flavonoid compounds compared to the flower extract, indicating superior antioxidant potential. These results provide scientific validation for the traditional medicinal applications of *Calotropis gigantea* and suggest that the plant, especially its leaves, holds promise as a valuable source of natural antioxidant compounds. Further studies are recommended to explore its bioactive constituents and potential pharmaceutical applications.

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