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Comparative Pharmacognostical and Pharmacological Evaluation of Conventional and Dry-Heat-Assisted 50% Ethanolic Extracts of *Calotropis Gigantea* Whole Plant"

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Abstract: The present study was designed to conduct a comparative pharmacognostical and pharmacological evaluation of conventional and dry-heat-assisted 50% ethanolic extracts of the whole plant of *Calotropis gigantean***.** The whole plant material of *Calotropis gigantea* was collected, authenticated botanically, cleaned, dried, and subjected to appropriate pharmacognostical evaluation The powdered whole plant was subsequently extracted using 50% ethanol by a conventional extraction procedure and by a dry-heat-assisted extraction method under controlled experimental conditions. Preliminary phytochemical investigation was carried out to identify the major classes of secondary metabolites present in both extracts. The extracts were evaluated for the presence of phytoconstituents such as alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, glycosides, saponins, steroids, and other relevant constituents. DPPH activity expressed convention extract demonstrated a scavenging activity of 74.4±2.3%, whereas the dry heat extract showed an inhibition value of 72.28±3.46%. OECD 423 followed single oral administration of 2000 mg/kg of animals shown no toxic symptoms. Carrageenan-induced edema paw volume was subsequently observed, with values convention extract & dry heat extract decreasing to 1.6 ± 0.13 &1.0 ± 0.615 ml at the second hour, 1.5 ± 0.89 & 1.8 ± 0.122 ml at the third hour, 1.4 ± 0.62 & 1.6 ± 0.13 ml at the fourth hour, and remaining at 1.4 ± 0.843 & 1.4 ± 0.843 ml at the sixth hour. The findings are expected to provide useful information regarding the influence of processing and extraction methodology on the pharmacologically potential of the plant. The study may also contribute to the standardization and further scientific investigation of *C. gigantea* as a potential source of bioactive phytoconstituents. Further studies involving detailed phytochemical characterization, isolation of active constituents, mechanistic investigations, and comprehensive safety evaluation are warranted to establish the therapeutic potential of the more active extract.

Key Words: *Calotropis gigantean*, 50% ethanol, DPPH activity, OECD 423, Carrageenan-induced edema, toxic symptoms and bioactive phytoconstituents

INTRODUCTION

Medicinal plants have played an important role in traditional healthcare systems for centuries and continue to serve as valuable sources of biologically active compounds. The therapeutic potential of plants is largely associated with their diverse secondary metabolites, including flavonoids, phenolic compounds, alkaloids, terpenoids, steroids, glycosides, tannins, and other specialized constituents. The increasing interest in natural products has encouraged systematic scientific investigation of medicinal plants to establish their identity, quality, chemical composition, biological activities, and safety. Pharmacognostical and pharmacological investigations are therefore important components of the scientific evaluation and standardization of medicinal plant materials. Among the medicinal plants of considerable traditional importance, *Calotropis gigantea* is a well-known species belonging to the family Apocynaceae. It is commonly distributed throughout tropical and subtropical regions and is frequently found in dry, wasteland, roadside, and open areas. The plant is a perennial shrub characterized by a well-developed root system, thick branches, opposite leaves, milky latex, and distinctive flowers. Different parts of *C. gigantea*, including the roots, leaves, flowers, bark, latex, and whole plant, have been traditionally used for various medicinal purposes. Its extensive traditional utilization has generated considerable interest in the scientific investigation of its pharmacognostical characteristics and pharmacological properties. Proper identification and authentication of medicinal plant materials are essential for ensuring reproducibility and consistency in herbal research. Plant materials can vary considerably in their morphological, anatomical, physicochemical, and phytochemical characteristics depending on geographical origin, environmental conditions, stage of development, season of collection, drying conditions, and methods of processing. Pharmacognostical evaluation provides important information regarding the macroscopic and microscopic characteristics of a plant and assists in distinguishing the genuine drug from adulterants and closely related species. Evaluation of physicochemical parameters such as moisture content, ash values, and extractive values further contributes to the establishment of quality-control standards for medicinal plant materials. The biological activity of a medicinal plant is strongly influenced by the method used to extract its chemical constituents. Extraction is an important preliminary step in phytochemical and pharmacological investigations because the nature and concentration of the constituents obtained depend on several factors, including solvent polarity, temperature, extraction time, particle size, solvent-to-drug ratio, and extraction technique. Ethanol and hydroethanolic mixtures are widely used for the extraction of plant materials because they can dissolve a broad range of polar and moderately non-polar phytoconstituents. In particular, 50% ethanol provides a hydroalcoholic medium capable of extracting diverse classes of constituents that may not be efficiently recovered using either water or absolute ethanol alone. Conventional extraction techniques are commonly employed for the preparation of plant extracts because of their simplicity and practical applicability. However, conventional methods may require prolonged extraction periods and may not always provide optimal recovery of desired constituents. Extended exposure to extraction conditions can also influence the stability of certain thermolabile compounds. Consequently, researchers have increasingly explored alternative processing and extraction approaches that may improve the recovery or availability of biologically active constituents while maintaining acceptable extract quality. Dry-heat-assisted extraction represents one such approach in which controlled thermal treatment is incorporated into the preparation of the plant material before or during the extraction process, depending on the experimental design. Heat treatment can influence plant tissues by modifying cellular structures, reducing moisture, facilitating the release of intracellular constituents, and potentially altering the extractability of certain phytochemicals. In some cases, controlled heating may enhance the availability of compounds that are otherwise less accessible in untreated plant material. However, excessive temperature or prolonged exposure may cause degradation or transformation of heat-sensitive constituents. Therefore, appropriate control of the heating conditions is essential when investigating the influence of dry heat on plant extracts. A comparative evaluation of conventional and dry-heat-assisted extraction is therefore scientifically relevant because the two approaches may produce extracts with differences in yield, physicochemical characteristics, phytochemical composition, and biological activity. Such differences cannot be assumed solely from the extraction method and need to be established experimentally. Comparison of the resulting extracts can provide information regarding whether dry-heat treatment produces a meaningful change in the extraction characteristics or pharmacological potential of *C. gigantea*. Phytochemical investigation

provides an important link between the traditional medicinal use of a plant and its biological activity. *Calotropis gigantea* has been reported to contain several groups of secondary metabolites, including flavonoids, phenolic constituents, terpenoids, steroids, glycosides, and other characteristic compounds. The distribution and concentration of these constituents may vary among different plant parts and may also be influenced by extraction conditions. Since the present investigation uses the whole plant, the resulting extract may contain a broader spectrum of constituents derived from different tissues and organs. Comparative phytochemical evaluation of conventionally prepared and dry-heat-assisted extracts can therefore provide useful information regarding the influence of processing on the chemical profile. Pharmacological evaluation is another essential component of medicinal plant research. Traditional claims regarding the therapeutic value of *C. gigantea* require experimental investigation to determine their biological relevance. Various pharmacological properties have been investigated for different parts of the plant, and these activities have been associated with the presence of diverse bioactive constituents. The biological effects of an extract, however, depend not only on the plant species but also on the plant part, extraction solvent, extraction technique, dose, and experimental model employed. Therefore, comparison of extracts prepared using different extraction approaches can provide a better understanding of how processing influences biological efficacy. The selection of 50% ethanol as the extraction solvent in the present investigation is particularly relevant because a hydroethanolic solvent system can facilitate the extraction of compounds with a wide range of polarities. The use of the same solvent concentration for both conventional and dry-heat-assisted extraction allows the extraction method to remain the principal variable under comparison. This approach can help determine whether controlled dry-heat treatment influences the quality and pharmacological performance of the resulting extract independently of major changes in solvent composition. The present study is therefore designed to undertake a comparative pharmacognostical and pharmacological evaluation of conventional and dry-heat-assisted 50% ethanolic extracts of the whole plant of *Calotropis gigantea*. Pharmacognostical evaluation is intended to establish the identity and quality characteristics of the plant material, while comparative extract evaluation is aimed at determining differences associated with the two extraction approaches. Pharmacological investigation is intended to assess the biological potential of the prepared extracts using appropriate experimental models. The findings may provide useful information regarding the influence of dry-heat-assisted processing on the extractive characteristics and biological activity of *C. gigantea*. A systematic comparison of these two extraction approaches may also contribute to the development of a more reproducible method for preparing *C. gigantea* extracts for further research. If dry-heat-assisted processing demonstrates improved extract characteristics or biological activity under the defined experimental conditions, it may provide a useful basis for further optimization of the extraction process. Conversely, if conventional extraction produces superior results, the findings would support the continued use of the conventional approach. In either case, comparative investigation is important for establishing an evidence-based understanding of the relationship between processing conditions and the biological properties of medicinal plant extracts. Thus, the present study seeks to bridge traditional knowledge and experimental investigation by evaluating *Calotropis gigantea* through pharmacognostical and pharmacological approaches. The comparison of conventional and dry-heat-assisted 50% ethanolic extracts is expected to provide valuable information on the effect of extraction methodology on the quality and biological potential of the whole plant. The study may serve as a foundation for further phytochemical characterization, isolation of active constituents, mechanistic investigations, standardization, and safety assessment of *Calotropis gigantea* extracts.

MATERIALS AND METHODS

1. Plant collection and authentication

Fresh and healthy whole plants of *Calotropis gigantea* were collected from a suitable local area in Tamil Nadu, India, during June 2018. The collected plant material was carefully examined to ensure that it was free from visible disease, insect infestation, and mechanical damage. The plant material was submitted to the Department of Botany, University of Coimbatore, for botanical identification and authentication. The identity of the plant was confirmed by a qualified botanist based on its characteristic morphological features

2. Conventional Extract prepared by Cold Maceration Process

The dried leaves of *Calotropis gigantea* were pulverized into a coarse powder using a mechanical grinder and stored in airtight containers until further extraction. Approximately 500 g of the powdered plant material was subjected to successive solvent extraction by the cold maceration method using solvents of increasing polarity. Initially, the powdered material was immersed in petroleum ether in a clean, dry glass container at a plant material-to-solvent ratio of approximately 1:2 (w/v)**.** The mixture was maintained at room temperature for 14 days and shaken intermittently several times a day to facilitate the extraction of non-polar constituents. After completion of the extraction period, the mixture was filtered through muslin cloth to remove the plant residue. The resulting filtrate was concentrated under reduced pressure using a rotary vacuum evaporator and subsequently dried to obtain the petroleum ether extract. The dried extract was weighed, and the percentage yield was calculated. The extract was then transferred into an airtight, amber-colored container and stored at 4°C until further analysis. The percentage yield of the extract was calculated using the following formula:

Percentage Yield (%) = (Weight of Dried Extract / Weight of Air-Dried Powdered Plant Material) × 100

3. Dry Heat Extraction

A measured quantity of the dry-heat-treated powdered plant material was placed in a Soxhlet extraction thimble and loaded into the Soxhlet apparatus. 50% ethanol was prepared using ethanol and purified water in the required proportion and used as the extraction solvent. The solvent was transferred into the round-bottom flask connected to the Soxhlet apparatus, and the system was assembled carefully. The extraction was carried out under controlled heating conditions, allowing the 50% ethanol to undergo repeated evaporation, condensation, and percolation through the powdered plant material. The extraction was continued until the solvent in the siphon became substantially clear, indicating satisfactory exhaustion of the extractable constituents. The extraction temperature was maintained appropriately to avoid unnecessary degradation of heat-sensitive phytoconstituents. After completion of the extraction, the solvent containing the extracted constituents was collected and concentrated under reduced pressure using a rotary vacuum evaporator.

4. Preliminary Phytochemical Screening Procedures

1. Detection of Alkaloids

Preparation of extract solution

A suitable quantity of each extract was dissolved in dilute hydrochloric acid, warmed gently, cooled, and filtered. The filtrate was used for alkaloid detection.

A. Mayer’s Test

The prepared filtrate was treated with a few drops of Mayer’s reagent. The formation of a cream-coloured precipitate indicated the presence of alkaloids.

B. Dragendorff’s Test

A small quantity of the filtrate was mixed with Dragendorff’s reagent. The appearance of an orange or reddish-brown precipitate confirmed the presence of alkaloids.

C. Wagner’s Test

The filtrate was treated with Wagner’s reagent. Development of a reddish-brown precipitate indicated the presence of alkaloids.

D. Hager’s Test

A few drops of Hager’s reagent were added to the filtrate. Formation of a yellow precipitate suggested the presence of alkaloids.

2. Detection of Carbohydrates

A. Molisch's Test

The extract solution was treated with a few drops of Molisch's reagent. Concentrated sulphuric acid was carefully added along the wall of the test tube to form a separate layer. Development of a violet ring at the junction indicated the presence of carbohydrates.

B. Fehling's Test

The extract solution was mixed with equal volumes of Fehling's solution A and Fehling's solution B and heated. Formation of a brick-red coloured precipitate indicated the presence of reducing sugars.

C. Barfoed's Test

The extract solution was mixed with Barfoed's reagent and heated in a boiling water bath. Formation of a red precipitate indicated the presence of monosaccharides.

3. Detection of Proteins and Free Amino Acids

A. Ninhydrin Test

The extract solution was treated with ninhydrin reagent and heated gently. Development of a blue or violet colour indicated the presence of free amino acids.

B. Biuret Test

The extract solution was treated with sodium hydroxide solution followed by dilute copper sulphate solution. Formation of a violet colour indicated the presence of proteins.

C. Xanthoprotein Test

The extract solution was treated with concentrated nitric acid and heated gently. After cooling, ammonia solution was added. Development of yellow to orange colour indicated the presence of aromatic amino acids.

4. Detection of Tannins and Phenolic Compounds

A. Ferric Chloride Test

The extract solution was treated with ferric chloride solution. Development of blue-black, green, or dark green colour indicated the presence of tannins and phenolic compounds.

B. Lead Acetate Test

The extract solution was treated with lead acetate solution. Formation of a white precipitate indicated the presence of tannins.

C. Gelatin Test

The extract solution was mixed with gelatin solution containing sodium chloride. Formation of a white precipitate indicated the presence of tannins.

5. Detection of Phytosterols

A. Liebermann–Burchard Test

The extract was dissolved in chloroform and treated with acetic anhydride followed by careful addition of concentrated sulphuric acid. Development of a greenish-blue colour indicated the presence of phytosterols.

B. Salkowski Test

The extract was dissolved in chloroform and concentrated sulphuric acid was added carefully. Formation of a reddish-brown colour at the interface indicated the presence of phytosterols.6. Detection of Flavonoids

A. Shinoda Test

The extract solution was treated with magnesium turnings followed by concentrated hydrochloric acid. Development of pink, red, or orange colour indicated the presence of flavonoids.

7. Detection of Saponins

A. Foam Test

The extract solution was diluted with distilled water and shaken vigorously. Formation of persistent froth indicated the presence of saponins.

8. Detection of Glycosides

A. Keller–Killiani Test

The extract solution was treated with glacial acetic acid containing ferric chloride solution, followed by careful addition of concentrated sulphuric acid. Formation of a brown ring at the junction indicated the presence of glycosides.

9. Detection of Terpenoids

A. Salkowski Test

The extract was dissolved in chloroform and concentrated sulphuric acid was added carefully along the side of the test tube. Development of a reddish-brown colour at the interface indicated the presence of terpenoids.

5. Pharmacognostical

1. Ash Values

A. Total Ash

About **2–3 g** of each dried extract (petroleum ether, ethyl acetate, ethanol, and aqueous extracts) was accurately weighed and placed in a previously ignited and weighed silica crucible. The sample was incinerated gradually until all carbonaceous matter was removed and a constant weight of ash was obtained. The crucible was cooled in a desiccator and weighed.

The percentage of total ash was calculated based on the weight of the original sample.

Total ash (%) = (Weight of total ash / Weight of sample) × 100

B. Acid-Insoluble Ash

The total ash obtained from each extract was boiled with dilute hydrochloric acid. The insoluble residue was collected by filtration using ashless filter paper and washed with hot water until free from acid. The residue was transferred into a crucible, ignited until constant weight was obtained, cooled in a desiccator, and weighed.

The percentage of acid-insoluble ash was calculated.

Acid-insoluble ash (%) = (Weight of acid-insoluble residue / Weight of sample) × 100

C. Water-Soluble Ash

The total ash obtained from each extract was boiled with distilled water. The insoluble portion was collected by filtration, washed with hot water, and ignited to constant weight. The weight of insoluble ash was subtracted from the total ash value to determine the water-soluble ash content.

Water-soluble ash (%) = Total ash (%) − Water-insoluble ash (%)

D. Sulphated Ash

A known quantity of each extract was placed in a previously weighed silica crucible and moistened with concentrated sulphuric acid. The sample was gently heated until fumes ceased and then ignited at a controlled temperature until constant weight was achieved. The crucible was cooled in a desiccator and weighed.

The percentage of sulphated ash was calculated as:

Sulphated ash (%) = (Weight of sulphated ash / Weight of sample) × 100

2. Loss on Drying (Moisture Content)

The sample was dried in a hot air oven at 105°C until a constant weight was obtained. The dish was cooled in a desiccator and weighed.

The percentage loss on drying was calculated using:

Loss on drying (%) = (Loss in weight / Weight of sample) × 100

3. Crude Fibre Content

The insoluble residue was collected by filtration, washed thoroughly with hot water, dried, weighed, and then incinerated to determine the ash content. The crude fibre content was calculated by subtracting the weight of ash from the weight of the dried residue.

Crude fibre (%) = (Weight of residue − Weight of ash / Weight of sample) × 100

6. DPPH activity

A 0.1 mM DPPH solution was prepared by accurately weighing 3.94 mg of DPPH and dissolving it in 100 mL of methanol. The conventional and dry-heat-assisted 50% ethanolic extracts of *Calotropis gigantea* were separately dissolved in a suitable solvent to prepare a stock solution of 1 mg/mL. From the stock solution, different concentrations such as 25, 50, 100, 200, and 400 µg/mL were prepared for the assay. Ascorbic acid was prepared similarly at corresponding concentrations and used as the reference standard. For each concentration, 1.0 mL of the test extract solution was mixed with 1.0 mL of freshly prepared 0.1 mM DPPH solution in a test tube. The final reaction volume was therefore 2.0 mL. The same procedure was followed for the ascorbic acid standard. A control solution was prepared by mixing 1.0 mL of methanol with 1.0 mL of DPPH solution, without adding the plant extract. The reaction mixtures were thoroughly mixed and incubated in the dark at room temperature for approximately 30 minutes. After incubation, the absorbance was measured at 517 nm using a UV–Visible spectrophotometer.

7. Animals

Healthy adult Wistar albino rats weighing approximately 180–250 g were used for the experimental study. The animals were acclimatized to the laboratory environment for at least 7 days before commencement of the experiment. They were maintained under standard laboratory conditions with controlled temperature, humidity, and light–dark cycles. The animals were provided with a standard pellet diet and had free access to drinking water throughout the experimental period, except where fasting was required according to the experimental protocol.After acclimatization, the animals were randomly divided into ten groups, with an appropriate number of animals in each group. The experimental groups were treated as follows:

Group:I: Control 0.5% CMC

Group:II: 2mg/Kg Diclofenac Positive Control

Group:III: 200 mg/Kg Conventional

Group:IV: 400 mg/Kg Conventional

Group:V: 200 mg/Kg Dry heat

Group:VI: 400 mg/Kg Dry heat

8. OECD 423

The extracts were administered orally as a single dose of 2000 mg/kg body weight using an appropriate dosing vehicle. A suitable control group received the vehicle alone under identical experimental conditions. Animals were observed continuously during the initial period following administration for any immediate signs of toxicity or behavioral changes. Thereafter, the animals were observed periodically for changes in general behavior, locomotor activity, respiration, food and water intake, posture, skin, fur, eyes, and other visible signs of toxicity. Particular attention was given to the occurrence of tremors, convulsions, salivation, lethargy, diarrhea, abnormal respiration, or mortality. The animals were monitored for 14 days, with body weight and general health status recorded at appropriate intervals. No mortality or observable toxic signs were recorded at the administered dose of 2000 mg/kg, indicating that both extracts were well tolerated under the conditions.

9. Carrgeenan induced paw oedema in rats

Acute paw edema was induced by subplantar administration of a freshly prepared carrageenan suspension into the hind paw of each rat. Paw volume was measured immediately before carrageenan administration to obtain the baseline value and subsequently at predetermined time intervals using a suitable paw-volume measuring instrument. The increase in paw volume was calculated by comparing each post-carrageenan measurement with the corresponding baseline value. The percentage inhibition of paw edema produced by each extracts were calculated in comparison with the control group. The percentage inhibition of edema was calculated using the following formula: Percentage inhibition of edema (%) = [(Vc − Vt) / Vc] × 100

RESULTS AND DISCUSSION

1 . Pharmacognostical

The extractive value was higher for the conventional extract (15.4% w/w) than for the dry-heat extract (13.7% w/w), indicating greater extractable matter in the conventional preparation.

The total ash value of the conventional extract was 6.3%, whereas the dry-heat extract showed a comparatively lower value of 5.34%. The lower ash value of the dry-heat extract may indicate a relatively reduced proportion of inorganic residues compared with the conventional extract.

Acid-insoluble ash was 3.10% in the conventional extract and decreased markedly to 1.23% in the dry-heat extract. The lower acid-insoluble ash value of the dry-heat extract suggests a comparatively lower level of acid-insoluble inorganic matter. Loss on drying was very low and comparable between the conventional (0.99%) and dry-heat (0.97%) extracts, indicating low residual moisture under the test conditions. The crude fiber content was 11.2% in the conventional extract and 11.4% in the dry-heat extract, showing only a minor difference between the two preparations. Overall, the conventional extract exhibited a higher extractive value, whereas the dry-heat extract showed lower total ash and acid-insoluble ash values with nearly comparable moisture and crude fiber contents. Alkaloids were detected in all three samples, as confirmed by Mayer’s, Dragendorff’s, Wagner’s, and Hager’s tests, indicating that these constituents were retained after both extraction procedures.Carbohydrates were consistently detected in the raw, conventional, and dry-heat samples based on positive Molisch’s, Fehling’s, and Barfoed’s tests.Proteins and amino acid-related constituents were detected in all samples; however, the Ninhydrin test was positive only in the raw material and negative in both extracts, suggesting a possible reduction or absence of detectable free amino acids after extraction. Biuret and Xanthoproteic tests were positive in all three samples, supporting the presence of protein-related constituents. Tannins and phenolic compounds were consistently detected by ferric chloride, lead acetate, and gelatin tests in the raw material and both extracts. Phytosterols were detected in the raw and dry-heat extracts but were not detected in the conventional extract by either Liebermann–Burchard or Salkowski tests, indicating a difference in the detectable phytochemical profile between the extraction methods. Flavonoids and saponins were present in the raw material as well as in both conventional and dry-heat extracts, suggesting that these constituents were extractable and remained detectable following processing. Glycosides were detected in the raw material but were not detected in either the conventional or dry-heat extract, whereas terpenoids were detected in the raw and conventional samples but not in the dry-heat extract.

2. Pharmacological

A. DPPH assay

At 10 µg/mL, ascorbic acid showed the highest inhibition (69 ± 2.97%), followed by the conventional extract (61 ± 0.9%) and dry-heat extract (45.33 ± 3.45%). At 20 µg/mL, the inhibition increased to 74 ± 1.93% for ascorbic acid, 62.4 ± 0.2% for the conventional extract, and 53.34 ± 1.2% for the dry-heat extract. At 40 µg/mL, the conventional extract showed 64.2 ± 0.6% inhibition, whereas the dry-heat extract increased to 60.45 ± 1.34%, demonstrating an improvement with increasing concentration. At 80 µg/mL, the conventional and dry-heat extracts exhibited closely comparable activity, with inhibition values of 67.7 ± 0.6% and 67.77 ± 1.36%, respectively. At the highest tested concentration of 160 µg/mL, the conventional extract produced 74.4 ± 2.3% inhibition, while the dry-heat extract showed 72.28 ± 3.46% inhibition.

Ascorbic acid exhibited the highest radical-scavenging activity throughout the tested concentration range, reaching 87 ± 0.35% at 160 µg/mL, confirming its stronger antioxidant activity than both plant extracts. The progressive increase in DPPH inhibition with increasing extract concentration suggests that both extracts contain antioxidant constituents capable of donating hydrogen atoms or electrons to neutralize DPPH radicals. Although the conventional extract showed slightly higher activity at most concentrations, the dry-heat extract approached the activity of the conventional extract at 80 and 160 µg/mL, indicating that dry-heat treatment did not eliminate the antioxidant potential of the extract.

B. Acute Oral Toxicity

The acute oral toxicity study was conducted at a dose of 2000 mg/kg body weight, and the animals were observed for a period of 15 days. No mortality was recorded throughout the observation period, indicating that the administered dose was well tolerated under the conditions of the study. No transient signs of toxicity were observed, and 100% of the animals remained active during the experimental period. No convulsions or remarkable alterations in locomotor activity were observed following administration of the test extract. The animals did not show any noticeable abnormality in their general behavioral pattern during the observation period. Feeding behavior remained apparently normal, with no marked reduction in food consumption reported.

No significant abnormalities in grooming behavior were observed during the study. Urination and fecal output remained apparently normal, with no notable changes recorded. Gross examination of the liver, kidney, stomach, and heart did not reveal any obvious macroscopic abnormalities based on the observations/photographs provided. Overall, the absence of mortality and observable signs of toxicity at 2000 mg/kg suggests that the extract was well tolerated at this dose under the experimental conditions; however, this finding should be reported together with the complete OECD guideline

C. Carrageenan-induced paw oedema

The carrageenan-induced paw oedema model demonstrated a clear increase in paw volume in the control group, from 0.97 ± 0.68 mL at 0 min to 3.40 ± 0.126 mL at 6 h, confirming the development of acute inflammation. The positive control showed a progressive reduction in paw volume after the initial inflammatory response, reaching 1.40 ± 0.843 mL at 6 h, indicating a marked anti-inflammatory response. The conventional extract at 200 mg/kg produced paw volumes of 1.50 ± 0.71, 2.00 ± 0.15, 2.15 ± 0.20, 2.40 ± 0.149, and 3.00 ± 0.1838 mL at 1, 2, 3, 4, and 6 h, respectively. The conventional extract at 400 mg/kg produced a comparatively lower paw volume, reaching 1.60 ± 0.1612 mL at 6 h, suggesting greater inhibition of carrageenan-induced oedema than the 200 mg/kg dose. The dry-heat extract at 200 mg/kg showed a progressive increase in paw volume and reached 3.2667 ± 0.0843 mL at 6 h, indicating relatively weak inhibition of oedema at this dose. In contrast, the dry-heat extract at 400 mg/kg produced a marked reduction in paw volume after carrageenan administration, with a value of 1.40 ± 0.856 mL at 6 h. The 400 mg/kg dry-heat extract therefore showed a response comparable to the positive control at the 6-hour observation point, suggesting considerable anti-inflammatory potential under the experimental conditions. The conventional 400 mg/kg extract also demonstrated appreciable activity, although its 6-hour paw volume (1.60 ± 0.1612 mL) was slightly higher than that observed with the dry-heat 400 mg/kg extract. The difference between the 200 and 400 mg/kg doses indicates that the anti-inflammatory response was more pronounced at the higher dose, particularly for the dry-heat-assisted extract. Overall, the results suggest that dry-heat-assisted 50% ethanolic extract at 400 mg/kg exhibited the most promising anti-inflammatory activity, followed by the conventional extract at 400 mg/kg;

Table 1: Ideals of Extracts

S No	Constraints	G.V % w/w
1	**Extractive Ideals**	
a.	Convention extract	15.4
b.	Dry heat extract	13.7
2	**Ash Values**	
a.	Convention extract	6.3

b.	Dry heat extract	5.34
3.	**Acid insoluble Ash**	3.54
a.	Convention extract	3.10
b.	Dry heat extract	1.23
4.	**Loss on Drying**	
a.	Convention extract	0.99
b.	Dry heat extract	0.97
5.	**Crude fiber content**	11.2
a.	Convention extract	11.2
b.	Dry heat extract	11.4

Table 2: Preliminary Phytochemical screening

S. No	TEST	Raw	Convetion	Dry Heat
(1)	Alkaloids	+	+	+
A	Mayer's test	+	+	+
B	Dragendroff's test	+	+	+
C	Wagner's test	+	+	+
D	Hager's test	+	+	+
(2)	Carbohydrates	+	+	+
A	Molisch's test	+	+	+
B	Fehling's test	+	+	+
C	Barfoed's test	+	+	+
(3)	Proteins and Free Amino acids	+	+	+
A	Ninhydrin test	+	-	-
B	Biuret test	+	+	+
C	Xantho Protein test	+	+	+
(4)	Tannins and Phenolic Compounds	+	+	+
A	Ferric chloride test	+	+	+
B	Lead acetate test	+	+	+
C	Gelatin test	+	+	+
(5)	Phytosterols	+	-	+
A	Libermann Burchard test	+	-	+
B	Salkowski test	+	-	+
(6)	Flavanoids	+		+
A	Shinoda test	+	+	+
(7)	Saponins	+	+	+
(8)	Glycosides	+	-	-
9	Terpenoids	+	+	-

Table 3: DPPH Assay

Conc	Ascorbic acid	Convention	Dry Heat
10	69 ± 2.97	61. ± 0.9	45.33 ± 3.45
20	74 ± 1.93	62.4 ± 0.2	53.34 ± 1.2
40	80. ± 0.48	64.2 ± 0.6	60.45 ± 1.34
80	83. ± 1.62	67.7 ± 0.6	67.77 ± 1.36
160	87. ± 0.35	74.4 ± 2.3	72.28 ± 3.46

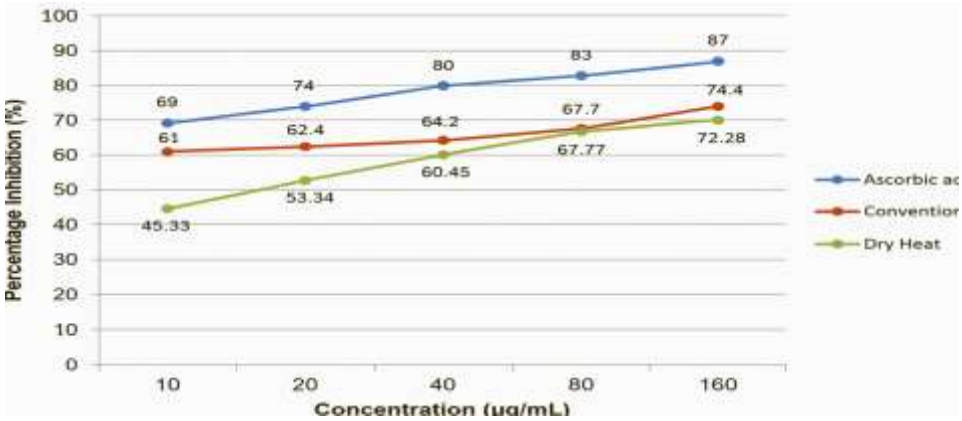

Fig 1: DPPH activity excerpts

Table 4: Poisonousness Study: 15 days observation report 2000 mg/ kg

S. No	Parameters	Inference
1.	Mortality	No
2.	Measurement Transience	0
3.	Booming Measurement	100
4.	Paroxysm	Not establish assemblies
5.	Propulsion	Inattentive
6	Snuffling	Extraordinary surveillance establish the eccentric nonetheless remaining supplementary existences vague
7.	Nurturing	Extraordinary comment were establish eccentric but remaining supplementary days preoccupied
8.	Tutoring	Extraordinary comment Retro establish character residual additional existences inattentive
9.	Mane defeat	Nope
10.	Leftover urination	Nullity
11.	Leftover Feces riddance	Vague

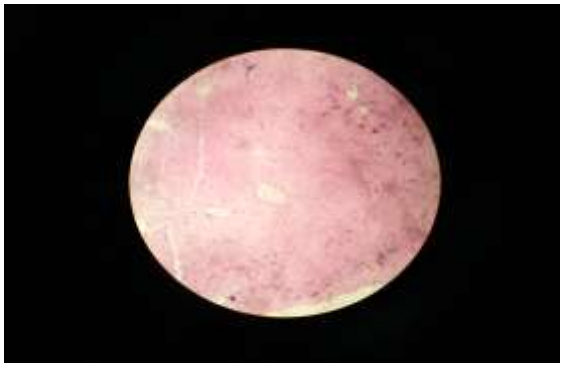

Fig 2: Liver

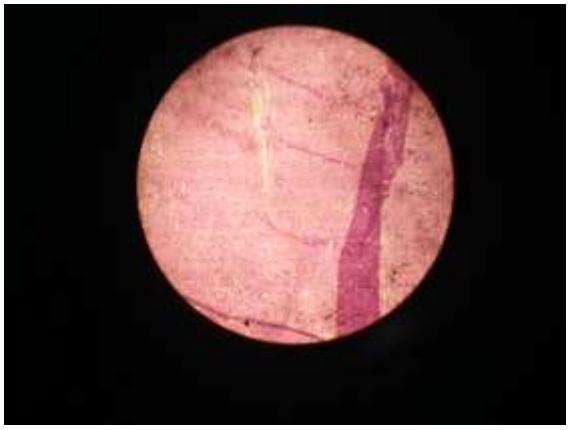

Fig 3: Kidney

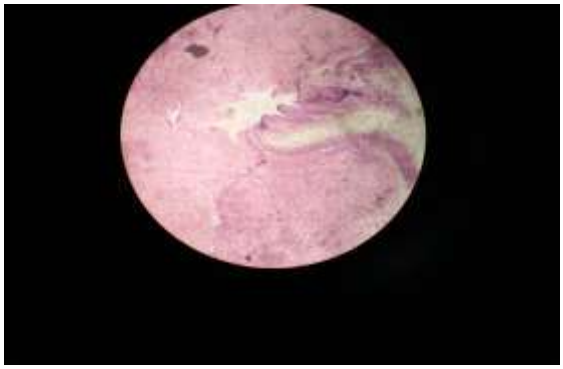

Fig 4: Stomach

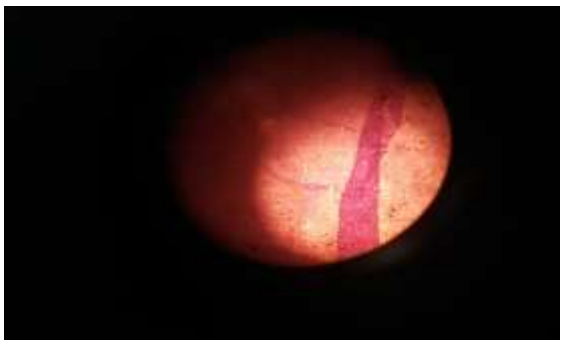

Fig 5: Heart

Table 5: Antiinflammatory Activity of different Extract using Carrageenan induced Paw oedema

Treatment	Dose Mg/Kg	Mean Paw Volume (Ml)					
		0 min	1 hr	2hr	3 hr	4 hr	6 hr
Control	0.5 % CMC	0.97 ± 0.68	1.5 ± 0.69	2.2 ± 0.67	2.6 ± 0.12	2.7 ± 0.13	3.4 ± 0.126
Positive Control	10	1.0 ± 0.615	1.8 ± 0.122	1.6 ± 0.13	1.5 ± 0.89	1.4 ± 0.62	1.4 ± 0.843
Convention	200	0.9 ± 0.447	1.5 ± 0.71	2 ± 0.15	2.15 ± 0.2	2.4 ± 0.149	3.0 ± 0.1838
Convention	400	1.0 ± 0.667	1.2 ± 0.1125	1.3 ± 0.1461	1.7 ± 0.1544	1.6 ± 0.0843	1.6 ± 0.1612
Dry Heat	200 mg/kg	0.9 ± 0.0447	1.4667 ± 0.0422	2.0 ± 0.1862	2.333 ± 0.0842	2.533± 0.1606	3.2667 ± 0.0843
Dry Heat	400	1.0 ± 0.76	0.9 ± 0.47	1.5 ± 0.61	2.0 ± 0.12	1.6 ±0.183	1.4 ± 0.856

SUMMARY AND CONCLUSION

The present study demonstrates that *Calotropis gigantea* whole plant possesses considerable pharmacognostical and pharmacological potential and that the method of extraction can influence the characteristics and biological performance of the resulting extract. The conventional 50% ethanolic extraction produced a higher extractive value and showed slightly greater DPPH radical-scavenging activity than the dry-heat-assisted extract. In contrast, dry-heat-assisted extraction produced lower total ash and acid-insoluble ash values and showed a favorable qualitative phytochemical profile. Both extracts demonstrated appreciable antioxidant activity, as evidenced by their concentration-dependent DPPH radical-scavenging effects. The conventional extract exhibited slightly higher activity The absence of mortality and obvious clinical signs at 2000 mg/kg suggests good acute tolerability under the conditions of the toxicity study. The most notable pharmacological finding was the anti-inflammatory activity observed in the carrageenan-induced paw oedema model. The dry-heat-assisted 50% ethanolic extract at 400 mg/kg produced comparable to that of the positive control while the conventional extract at 400 mg/kg also showed substantial inhibition of oedema. These observations suggest that dry-heat-assisted processing may enhance or preserve constituents contributing to the anti-inflammatory activity of *C. gigantea*. Therefore, among the tested preparations, the dry-heat-assisted 50% ethanolic extract at 400 mg/kg appears particularly promising for anti-inflammatory activity, whereas the conventional extract demonstrated advantages in extractive yield and DPPH radical-scavenging activity.Further investigation of *C. gigantea*. However, statistical analysis, quantitative phytochemical estimation, chromatographic fingerprinting, isolation of active constituents, mechanistic studies, and detailed safety evaluation are required to establish the pharmacological significance and reproducibility of these findings.

REFERENCES

1. Aftab A, Ahmad B, Bashir S, Rafique S, Bashir M, Ghani T, et al. Comparative study of microscale and macroscale technique for encapsulation of *Calotropis gigantea* extract in metal-conjugated nanomatrices for invasive ductal carcinoma. Sci Rep. 2023;13:13474.
2. Alafnan A, Sridharagatta S, Saleem H, Khurshid U, Alamri A, Ansari SY, et al. Evaluation of the phytochemical, antioxidant, enzyme inhibition, and wound healing potential of *Calotropis gigantea* (L.) Dryand: a source of a bioactive medicinal product. Front Pharmacol. 2021;12:701369. doi:10.3389/fphar.2021.701369.
3. Azhagu Madhavan S, Vinotha P, Uma V. Phytochemical screening and comparative GC-MS analysis of bioactive compounds present in methanolic leaf and latex extract *Calotropis gigantea* (L). Asian J Adv Med Sci. 2020;2(1):31-43.
4. Dang PH, Tran TH, Le TH, Do TNV, Nguyen HX, Nguyen MTT, et al. A new β-carboline-type alkaloid from the flowers of *Calotropis gigantea* (L.) W.T.Aiton. Nat Prod Res. 2024;38(18).
5. Dwivedi A, Pratap S, Awasthi S, Gautam P, Kadir A. *Calotropis gigantea*: an in-depth review of its therapeutic potential. J Pharmacogn Phytochem. 2024;13(2):715-721. doi:10.22271/phyto.2024.v13.i2e.14915.
6. Jamal M, et al. Phytochemical investigations and pharmacological potential of organic extracts of *Calotropis gigantea* L. leaves. Scientifica. 2025;2025:1669969.
7. Jayalekshmi C, Periakaruppan R, Selvaraj KVS, Al-Dayan N. Spectroscopic analysis of bioactive compounds from latex of *Calotropis gigantea* L. and an evaluation of its biological activities. Analytica. 2024;5(3):384-401. doi:10.3390/analytica5030024.
8. Julius OO, Oluwasusi VO, Ibiyemi MF, Oluwatobi FB. Antibacterial activities and phytochemical screening of crude extracts of *Calotropis gigantea* (giant milk weed). South Asian J Res Microbiol. 2021;9(3):24-31. doi:10.9734/sajrm/2021/v9i330210.

9. Kori P, Nilosey V, Alawa P. Comparative evaluation of phytochemicals, TPC and TFC in aqueous and ethanolic extracts of *Calotropis gigantea* L. leaves and roots. Int J Sci Res. 2018;7(11):859-861. doi:10.21275/ART20192885.

10. Kulkarni VD, Deokate UA, Bhoye L, Lokhande V. Bioactive compounds and pharmacological potential of *Calotropis gigantea*: a comprehensive review. Int J Res Sci Innov. 2026;13(4):306-311. doi:10.51244/IJRSI.2026.1304000028.

11. Mandal S. *Calotropis gigantea*: a brief study on phytochemical and pharmacological profile. Asian J Pharm Res. 2023;13(1):34-40. doi:10.52711/2231-5691.2023.00006.

12. Mandal SC. Evaluation of anti-inflammatory and antinociceptive activity of methanol extract of *Calotropis gigantea* root. Int J Green Pharm. 2017;11(3). doi:10.22377/ijgp.v11i03.1126.

13. Mishra P, Yadav KS, Gautam G. Comparative qualitative and quantitative phytochemical analysis of *Calotropis gigantea* and *Calotropis procera* roots. J Drug Deliv Ther. 2018;8(4):179-184.

14. Patil GP, Dhankani MA, Panwar SP, Dhankani AK. *Calotropis*: a comprehensive review of its phytochemistry, pharmacology and therapeutic potential. Res Rev J Bot. 2025;14(2).

15. Phytochemical analysis, anti-inflammatory, antioxidant activity of *Calotropis gigantea* and its therapeutic applications. J Ethnopharmacol. 2023;303:115963. doi:10.1016/j.jep.2022.115963.

16. Sharma AD, Chhabra R, Rani J, Chauhan A, Kaur I, Kapoor G. Oil/water nanoemulsions developed from essential oil extracted from wildly growing *Calotropis gigantea* (Linn.) Aiton F.: synthesis, characterization, stability and evaluation of anti-cancerous, anti-oxidant, anti-inflammatory and anti-diabetic activities. J Adhes Sci Technol. 2024;35(16):2506-2527. doi:10.1080/09205063.2024.2384801.

17. Sharma RC, Roy AS. A review on comparative studies of phytochemical evaluation and pharmacological activities of *Calotropis* species: *Calotropis procera* and *Calotropis gigantea*. J Phytopharmacol. 2025;14(1):62-69. doi:10.31254/phyto.2025.14109.

18. Shastry VM, Patil VR, Chaudhari RY. Evaluation of acute and chronic anti-inflammatory activity of whole plant of *Calotropis gigantea* in various experimental animal models. Res J Pharm Technol. 2011;4(5):815-818.

19. Tiwari DK, Upmanyu N. Phytochemical and pharmacological activity of wonder shrub: *Calotropis gigantia*. Res J Pharmacogn Phytochem. 2020;12(2):106-110. doi:10.5958/0975-4385.2020.00018.7.

20. Yadav R, Chaurasia S. Evaluation of antioxidant, ROS scavenging activity and metal chelation activity of herbal extracts from *Calotropis gigantea*. J Adv Zool. 2022;43(1).