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To study plant *Calotropis gigantea* extract for anti-inflammatory activity

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Abstract

The leaves of *Calotropis gigantea* have a long history of use in treating various conditions, including edema, cancer, wounds, recurrent fevers, paralysis, and joint pain. Additionally, they serve as an antispasmodic and anti-asthmatic, and are applied externally for ailments such as leprosy, eczema, boils, ulcers, scabies, and hemorrhoids. While there are indications of positive anti-inflammatory effects, research has yet to conclusively demonstrate these properties. This study aims to assess the anti-inflammatory potential of an extract from *Calotropis gigantea* leaves using an in vitro method. The extraction process involved sequentially using petroleum ether (60–80°C), chloroform, ethyl acetate, n-butanol, ethanol, and distilled water. The resulting extract was then evaluated for its anti-inflammatory efficacy through a slightly modified albumin denaturation inhibition assay. For comparison, etoricoxib was used as the standard reference drug at a dosage of 100 mg/kg. The ethanolic extract of *Calotropis gigantea* leaves exhibited a denaturation Inhibition percentage of 85.71%, comparable to that of etoricoxib, suggesting that the extract has significant anti-inflammatory properties.

Keywords: *Calotropis gigantea*; Antispasmodic; Antiasthmatic; Antiinflammatory Activity

1. Introduction

Calotropis gigantea, a member of the Asclepiadaceae family, is widely distributed across India, including the Andaman Islands, and can grow up to 900 meters in elevation. It is commonly found in dry waste areas referred to as “mudar.” The roots have a whitish-grey exterior, and a transverse section of the mature root reveals a cork zone. This zone consists of thin-walled, polyhedral to roughly cubic cells arranged in rows of thirty to fifty, with small cubical crystals present in the inner row. The phellogen is distinctive. The cortex Is relatively narrow and composed of several layers of thin-walled cubic, rectangular, or oblong cells, predominantly filled with starch grains. The phloem consists of broad radial bands of thin-walled cells interspersed with very thin strips of medullary rays, along with laticiferous cells and calcium oxalate crystals. The cambium is also unique. The leaves are smooth on the upper surface and cottony underneath, exhibiting a glaucous green color. They are lanceolate to oblong in shape, with an acute apex that is rarely rounded and a cordate base, measuring between 6 to 20 cm in length and 3 to 8 cm in width when fresh. The petioles range from 0.3 to 2 cm in length. The juvenile stems and branches are covered in soft, loosely applied white pubescence that can appear waxy or sometimes powdery. The woody stem features a golden-white bark. The fruits are turgid, measuring 7 to 10 cm in length, and can be found either in pairs or solitary.

2. Material and method

2.1. Profile

Calotropis gigantea, commonly referred to as the “Rui Tree,” is a perennial shrub or small tree that can reach heights of up to 5.4 meters. It is characterized by its tall, heavily branched structure and the presence of milky latex.

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2.2. Collection of Plant Parts

Fresh leaves and flowers of *Calotropis gigantea* were collected from the Sasaram Rohtas garden in Bihar. This plant belongs to the Asclepiadaceae family. The extraction of *Calotropis gigantea* is conducted in the pharmacognosy laboratory at Ganpati Institute of Pharmacy (GIP) in Bilaspur.

2.3. Drying & Size Reduction

The leaves of *Calotropis gigantea* are dried and then subjected to size reduction for further processing.

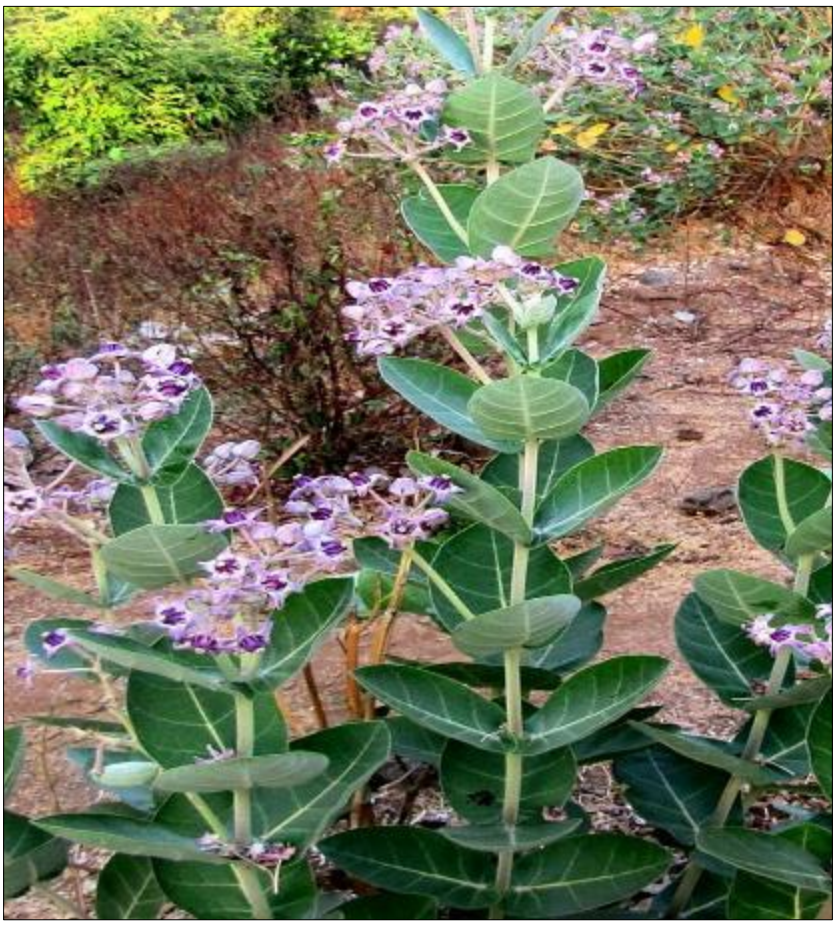

Figure 1 Plant *Calotropis gigantea*

3. Method

3.1. Preparation of extract

For this experiment, fresh leaves of *Calotropis gigantea* were collected and dried in the shade. The dried leaves were then ground into a coarse powder using a mechanical grinder and sieved through a No. 40 mesh to yield approximately 1 kg of powder with the desired particle size.

This 1 kg of powdered material was extracted using a series of solvents: petroleum ether (60-80°C), chloroform, ethyl acetate, n-butanol, ethanol, and distilled water. The extraction process was deemed complete when the solvent in the thimble turned transparent. After each extraction, the extracts were concentrated at low temperatures, and the solvents were distilled off.

The percentage yield of the extracts obtained from distilled water, petroleum ether (60-80°C), chloroform, ethyl acetate, n-butanol, and ethanol were recorded. Additionally, a preliminary phytochemical analysis was performed on the crude extracts to identify various phytoconstituents.

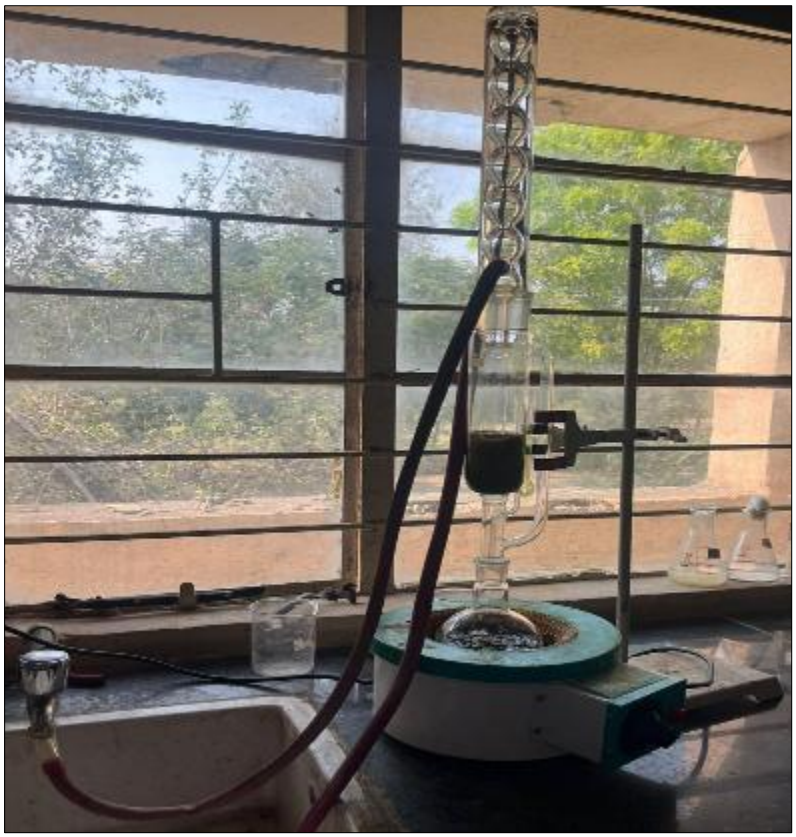

Figure 2 Soxhlet extraction

3.2. Preparation of herbal ointment

- Ointment Base: To prepare the ointment base, hard paraffin was first weighed and finely crushed. The crushed paraffin was then placed in an evaporating dish over a water bath set to 65°C. Once the hard paraffin melted, additional ingredients added and vigorously stirred to ensure thorough melting and uniform mixing. The ointment base was then allowed to cool.
- Herbal Ointment Preparation:

For the herbal ointment, precisely weighed extracts of *Calotropis gigantea* leaves were incorporated into the ointment base using a levigation technique. The exact weight of the leaf extract was combined with the ointment base, resulting in a smooth paste that weighed two to three times that of the base. After achieving a homogeneous mixture, more base.

Table 1 Formulation design of ointment base

Sr no	Ingredients	Quantity
1	Beeswax	1gm
2	Antioxidant (Vit E)	0.15gm
3	Preservative (methyl paraben)	0.04gm
4	Emulsifier	0.7gm
5	Petroleum jelly	21gm

Table 2 Formulation of herbal ointment

Sr no.	Ointment base	Methanolic extract
F1	10	2gm
F2	10	4gm
F3	10	6gm

Figure 3 Formulation

3.3. Evaluation of ointment

3.3.1. Organoleptic Properties

The physical characteristics of the prepared ointment, including color and odor, were evaluated visually. It was observed that the ointment has a smooth and consistent texture.

3.3.2. Ointment's pH

The pH of the herbal ointment was measured using a digital pH meter. To prepare the solution, 50 milliliters of distilled water were heated for a few minutes in a 100-milliliter dry beaker, then allowed to cool for two hours. The pH was measured using the pH meter, with three readings taken to calculate an average pH value.

3.3.3. Spreadability

Spreadability was assessed by timing how long it took for two glass slides to separate when a specific force was applied between them. A sample of the ointment was placed between the two slides, and weight was applied to ensure a uniform thickness. The time taken to separate the slides was recorded. Spreadability (S) was calculated using the following formula:

[S = \frac {L \times M} {T}]

Where:

- (L) = length of the glass slide,
- (M) = weight applied to the upper slide,
- (S) = spreadability,
- (T) = time taken to separate the slides.

3.4. Extrudability

- The mixture was put into a container including a tube that may collapse. Check to see if the preparation is
- Consistent. The force required to drive material out of the tube is known as extrudability.
- The extrudability was calculated using the formula below.
- Extrudability is defined as applied weight (gram) / area (cm2) for extruding ointment from a tube.

3.4.1. Diffusion study

- The diffusion study required the preparation of nutrient agar media. A medium with a hole in the middle held
- The ointment. The knot that the ointment had assisted in diffusing was observed after 60 minutes.
- Solubility
- The preparation mildly soluble in distilled water, insoluble in water, and miscible in ethanol, chloroform, and
- Ether.

3.4.2. Washability

- After applying the formulation to the skin, the ease and thoroughness of the water washing were assessed.
- Stability study
- The herbal ointment underwent a one-month physical stability test at a variety of temperatures, including 20 c,
- 250 c, and 300 c. It was discovered that the herbal ointment was physically at several temperatures—20, 250,
- And 300 degrees Celsius.

3.4.3. Drug content

- Each formulation (1g) was added to a 50 ml volumetric flask, filled to the top with the methanol, and
- Thoroughly shaken to ensure the active ingredients were completely dissolved. After passing the solution
- Through Whatman filter paper, 0.1 ml of it was pipetted out and diluted with methanol to make 10 ml. A
- Standard curve with a cutoff point of 450 nm was used to perform the spectrophotometric measurement of the
- Active omponent content.

3.5. Extrudability

To assess extrudability, the ointment mixture was placed in a collapsible tube. The consistency of the preparation was evaluated, and the force required to expel the material from the tube was measured. Extrudability was calculated using the following formula:

3.6. Diffusion Study

For the diffusion study, nutrient agar media was prepared with a hole in the center to hold the ointment. After 60 minutes, the area here the ointment had diffused was observed and recorded.

3.7. Solubility

The ointment was found to be mildly soluble in distilled water, insoluble in water, and miscible in ethanol, chloroform, and ether.

3.8. Washability

The ease and thoroughness of washing the formulation off the skin with water were evaluated after application.

3.9. Stability Study

The herbal ointment underwent a one-month physical stability test at various temperatures: 20°C, 25°C, and 30°C. It was observed that the ointment remained physically stable at all tested temperatures.

3.10. Drug Content

To determine the drug content, 1 gram of each formulation was added to a 50 ml volumetric flask and filled to the mark with methanol. The mixture was thoroughly shaken to ensure complete dissolution of the active ingredients. The solution was then filtered through Whatman filter paper. A 0.1 ml aliquot of the filtered solution was pipetted out and diluted with methanol to a final volume of 10 ml. The active component content was measured using spectrophotometry at a wavelength of 450 nm, referencing a standard curve for quantification.

3.10.1. Anti-inflammatory activity

A modified version of the albumin denaturation prevention method was employed to evaluate the anti-inflammatory activity of *Calotropis gigantea* flower extract at a dose of 200 mg/kg. Both the standard drug and test compounds were initially dissolved in minimal amounts of DMF and then diluted with 0.2 M phosphate buffer (pH 7.4), ensuring the final DMF concentration remained below 2.5% in all samples. Each test sample (1 ml) containing varying drug concentrations was mixed with 1 ml of 1 M albumin solution in phosphate buffer. The mixtures were incubated in a water bath at 27 ± 1 °C for 15 minutes. After cooling, turbidity was measured at 650 nm. A control sample without any drug was used to determine the percentage inhibition of protein denaturation.

3.10.2. pH of the Ointment

The pH of the prepared herbal ointment was measured using a digital pH meter and was found to range between 6.5 and 7. This indicates that the formulation is close to neutral, making it suitable for topical application without causing skin irritation.

3.10.3. Spreadability

Spreadability was assessed by measuring the time required for two glass slides to separate under a specific applied force. The spreadability of the F4 formulation was determined to be 6.9 seconds, indicating satisfactory consistency and ease of application.

3.10.4. Solubility

The herbal ointment was found to be slightly soluble in distilled water and soluble in ethanol, chloroform, and ether. This solubility profile suggests compatibility with both polar and non-polar solvents, which may aid in the absorption and stability of the active ingredients.

3.10.5. Washability

The F4 preparation exhibited good washability, as evidenced by the ease and volume of removal with water after application. This characteristic is favorable for ensuring user comfort and product cleanliness post-application.

Table 3 Physicochemical evaluation of herbal ointment.

Physicochemical parameters	F1	F2	F3
Colour	Yellow	Yellow	Yellow
Odour	Characteristics	Characteristics	Characteristics
Consistency	Seamless	Slightly smoothing	Good smoothing
PH	6.7	6.8	6.6
Washability	Slightly less	Average	Good
Spreadiability	6.5 sec	6.6 sec	64 sec

4. Conclusion

The flower of *Calotropis gigantea* may be a valuable component in herbal ointments, as demonstrated by its remarkable anti-inflammatory properties. According to estimates by the World Health Organization, over 80% of people in underdeveloped nations rely on herbal remedies as their primary source of healthcare. The use of organic compounds, particularly plant-derived substances, has gained popularity in both traditional and modern medicine due to their safety and effectiveness for human use. A comprehensive review of existing literature reveals that *Calotropis gigantea* and Calotropis procera are commonly used to treat a variety of ailments by Ayurvedic practitioners, traditional healers, and people from diverse cultural backgrounds. Current research focuses extensively on the Calotropis species, based on the belief that its therapeutic potential is far greater than currently recognized. Notably, the F4 preparation exhibits strong anti-inflammatory activity and fulfills the criteria for an effective anti-inflammatory agent.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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