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# Formulation And Development Of Herbal Ointment By Using Active Compounds Of Calotropis Gigantea For Anti-Inflammatory Activity

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**Abstract**

The leaves of Calotropis gigantea have long been used to treat edema, cancer, wounds, recurrent fevers, paralysis, and hurting joints. As an antispasmodic and anti-asthmatic, it is also applied externally for leprosy, eczema, boils, ulcers, scabies, and piles. Positive anti-inflammatory effects seem to be present. However, research has not demonstrated that it has anti-inflammatory qualities. Therefore, the objective of this study is to evaluate, via an in vitro method, the anti-inflammatory qualities of an extract derived from Calotropis gigantea leaves. Using petroleum ether (60–800), chloroform, ethyl acetate, n-butanol, ethanol, and distilled water, the leaves of Calotropis gigantea were extracted gradually. After that, the extract was tested utilizing a slightly modified inhibition of albumin denaturation approach for in vitro anti-inflammatory efficacy. We employed 100 mg/kg of etoricoxib as the usual reference drug. Calotropis gigantea leaf ethanolic extract produced a denaturation inhibition percentage of 85.71%, which was comparable to that of etoricoxib, indicating that the extract possesses potent anti-inflammatory qualities.

**Keyword:** *Calotropis gigantea*, antispasmodic, antiasthmatic, antiinflammatory activity.

## Introduction

The entire plant, Calotropis gigantean (Asclepiadaeceae), is distributed throughout India, including the Andaman Islands, reaching heights of 900 meters. Also present in areas of dry waste called in English as "mudar." The color of the roots is 1whitish grey on the outside. The cork zone is seen in the adult root's transverse section. Composed of thin-walled, polyhedral to roughly cubic cells arranged in rows of thirty to fifty. The inner row contains cubical crystals that are quite tiny. Phellogen is unique. The cortex is made up of a few rows of thin-walled cubic, rectangular, or oblong cells, the majority of which are packed full of starch grains. The cortex is rather narrow. Phloem is a zone that is made up of several wide radial bands of thin-walled cells that are crossed by extremely thin strips of medullary rays. There are laticiferous cells and a calcium oxalate crystal. Cambium is unique. Leaves are smooth above and cottony underneath, glaucous green, lanceolate, oblong, apex acute, rarely rounded, base cordite, and measuring 6–20 cm long by 3–8 cm broad when fresh. Petioles, 0.3–2 cm in length. Soft, loosely applied pubescence that is white, waxy, or sometimes powdery covers the juvenile stem and branches. With golden white bark, the stem is woody. Fruits are in length from 7 to 10 cm, are paired or solitary, turgid, and recovered. The seeds are 2.5–3.2 cm

long, broadly oval, flattened brown in color, with a pointed end and a white tuft of silky hair. Lilac, pale pink, purple, and occasionally light greenish flowers that are yellow or white in color, inodorous, and borne non axillary pedunculate corymbs have spreading reflexed corolla lobes. Flowers virtually all year round, however in central India, they bloom most frequently from November to March.

## Material and Methods

### Profile

C. gigantea, often known as the "Rui Tree," is a perennial shrub or small tree that grows to a height of 5.4 m and is covered in milky latex. It is tall and heavily branched.

### Collection of Plant Parts

From the Sasaram Rohtas garden in Bihar, we obtained fresh leaves and flowers of Calotropis gigantea figure no., a member of the Asclepiadaeceae family. We extract the Calotropis gigantea in pharmacognosy at the GIP (Ganpati Institute of Pharmacy) laboratory, which is located in BILASPUR.

### Drying & Size reduction

Calotropis gigantea leaves were harvested fresh, shade-dried in typical weather conditions, and then coarsely powdered to reduce size.

**Figure 1: Plant of calotropis gigantean**

**Figure 2: Drying of calotropis gigantea**

## Methods

### Preparation of extract:

The Calotropis gigantea for this experiment, new leaves were collected and dried in the shade. Using a mechanical grinder, the dried material was ground into a coarse powder and then sieved No. 40 to obtain about 1 kg of powder with the required particle size. One kilogram of powdered material was extracted using the following techniques: petroleum ether (60-800), chloroform, ethyl acetate, n-butanol, ethanol, and distilled water. The solvent in the thimble was allowed to turn transparent, indicating that the extraction procedure was complete. Following each extraction, the extract was concentrated at a low temperature and the solvent was distilled off. The following represents the percentage yield of distilled water extract, petroleum ether (60–800), chloroform, ethyl acetate, n-butanol, and ethanol.A preliminary phytochemical analysis was conducted on the obtained crude extract to examine different phytoconstituents.

### Preparation of aqueous extract

The Calotropis gigantea flowers were powdered and their crude aqueous extract was prepared according to standard protocol. Simply said, 100 g of the powdered flowers and 500 mL of distilled water were put in a 1-liter flask, and the mixture was allowed to boil for 1.5 hours. Once the "brew" cooled to 40 ◦C, it was filtered through Whatman No. 1 filter paper. After filtering, the extract was stored at 4 °C until required. After that, a vacuum rotary evaporator was used to concentrate it.

### Preparation of methanolic extract

Methanol was used to fully extract powdered Calotropis gigantea flowers using a Soxhlet technique Crude methanolic extract, which had been evaporated and dried, was stored at 4 °C until it was required.

### Preparation of test sample

To prepare the extract for in-vitro testing, it was dissolved in dimethyl formamide (DMF) and diluted with phosphate buffer (0.2 M, pH 7.4), resulting in a final concentration of 2.5%.

**Figure 3: Methanolic Extract**

**Percentage Yield**

The ideal quantity of product, or theoretical yield, is obtained by solving a stoichiometry problem. By calculating the quantity of product produced, we may ascertain the true yield. By dividing the actual yield by the theoretical number, we can calculate the percentage yield.

**Percentage yield = (*Actualyield/Theoreticalyield*) ×100**

The amount of product actually made compared with the maximum calculated yield is called the **percentage yield.**

**Phytochemical Screening Methods**

Several qualitative tests were performed in order to determine the phytochemical components that were present in the hydroalcoholic extracts. Multiple tests were conducted to determine the content of carbohydrates, flavonoids, steroids, glycosides, alkaloids, saponins, and other compounds.

**Test for alkaloid**

**Mayer's test**

1 milliliter of extract was mixed with a few drops of Mayer's reagent. The precipitate turns yellowish brown, signifying the presence of alkaloids.

**Dragendroff's reagent**

An orange-red precipitate that forms when 1 milliliter of Dragendorf's reagent is added to 2 milliliters of extract suggests the presence of alkaloids.

**Test for Glycoside**

**Keller killani test**

Ten milliliters of aqueous plant extract, one milliliter of concentrated H2SO4, and one drop of 2% Fecl3 combination were combined with four milliliters of glacial acetic acid solution. Between the layers, a brown ring developed that revealed the cardiac glycoside molecule.

**Test for Carbohydrates**

**Molisch's test**

Fill a dry test tube with 2 milliliters of the plant extract solution. Add two to three drops of the test reagent for Molisch. As you carefully pour 2 ml of concentrated H2SO4 along the test tube's sides, tilt the test tube. At the intersection of the two liquids—Molisch's test reagent and the dehydrated carbohydrate solution a purple or violet colored ring is visible.

**Fehling test**

To dissolve the test sample, add it to the test tube with water and shake. Drops of concentrated H2SO4 should be added gradually down the test tube's walls. The presence of carbohydrates is indicated by the creation of a purple ring.

**Test for Flavonoids**

**Ammonia test**

A small amount of the extract's aqueous filtrate was mixed with five milliliters of diluted ammonia. H2SO4 concentrate (1 ml) was added. A yellow tint that goes away when you stand up suggests that there are flavonoids present.

**Test for Saponin**

5 ml of extract was placed in a test tube along with a drop of Na2CO3 solution. It was given a good shake and then allowed to rest for five minutes. The production of foam signifies the existence of saponins.

**Test for Terpenoids:**

**Salkowski test**

To create a layer, salkowski test extract (5 ml) was combined with 2 ml of chloroform and 3 ml of carefully added concentrated H2SO4. The interface developed a reddish brown tint, indicating the presence of terpenoids.

**Test for Phenolic compound:**

**Ferric chloride test**

Three drops of the sample are placed in a tube, and the tube is agitated after around 20 drops of a yellow solution, 5% FeCl3 solution, are added. An bright tint ranging from purple to reddish brown to green is seen as a sign of a positive test.

**Preparation of Herbal ointment**

a) Ointment Base: The ointment is made using firstly weighed and finely crushed hard paraffin. After that, it is put over a water bath at 650C in an evaporating dish. Once the hard paraffin had melted, the additional ingredients were added and vigorously stirred to help with melting and uniform mixing. The ointment base was then allowed to cool.

b) Herbal Ointment by Ointment Base: To manufacture the herbal ointment, precisely weighed extracts of Calotropis gigantea leaves were blended with the ointment base using a levigation technique, an exact weight extract of Calotropis gigantea leaves was combined with the ointment base to create the herbal ointment. As a result, a smooth paste that weighed two or three times the base was produced. More base was gradually added to the ointment once it was homogeneous before it was put in the appropriate container

**Table 1: Formulation Design of ointment base**

| s.no. | Chemicals | Quantity |
|---|---|---|
| 1 | Wool fat | 0.48g ±5 |
| 2 | Cetostearyl alcohol | 0.49g ±5 |
| 3 | Hard paraffin | 0.44g ±5 |
| 4 | Yellow soft paraffin | 8.4g ±5 |

**Table 2: Formulation of Herbal Ointment**

| Sr.No | OINTMENT BASE | METHANOLIC EXTRACT (QTY) |
|---|---|---|
| **F1** | 10 gm | 2ml |
| **F2** | 10gm | 4ml |
| **F3** | 10gm | 6ml |
| **F4** | 10 gm | 8ml |


**Figure 4: Loaded Calotropis gigantea Ointment**

**Evaluation of ointment**

**Organoleptic Properties**

The physical characteristics, such as color and smell, were assessed visually.It is noted that the prepared ointment is smooth and consistent.

**Ointment's pH**

A digital pH meter was used to measure the pH of the produced herbal ointment. 50 milliliters of distilled water were heated for a few minutes in 100 milliliters of dry beaker, and then the mixture was allowed to cool for two hours to create the ointment. To find pH 10, a pH meter was utilized. Three measurements of the pH of the solution were made, and an average was calculated.

**The ability to spread**

Timing the amount of time it takes for two slides to separate from the ointment when a specific force is applied between them allows one to measure spreadability. The additional sample was placed between the two glass slides, and some weight was applied to compress the uniform thickness of the glass slides. It was noted how long it took to split the two slides. Spreadability was calculated with the help of the subsequent formula.

$$S=(L\times M)/T$$

where L is the glass slide's length,

M is the weight tide to the upper slide,

S is its spreadability,

T is the amount of time it takes to separate the slides.

**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.

**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.

**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.

**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 component content.

**Anti-inflammatory Activity**

A slightly modified version of the prevention of albumin denaturation technique was utilized to screen Calotropis gigantea flower extract at 200 mg/kg for anti-inflammatory activity. The standard drug and test chemicals were dissolved in the least amount of DMF and then diluted with phosphate buffer (0.2 M, pH 7.4). The final DMF concentration was less than 2.5 percent in all solutions. A 1 ml test solution containing different medication dosages was mixed with 1 ml of 1M albumin solution in phosphate buffer, and the mixture was incubated in a water bath for 15 minutes at 27 ºC + 1 ºC. After cooling, the turbidity was measured at 650 nm. The control group, to which no medicine was administered, was used to calculate the percentage of denaturation inhibition.

## Result & discussion

### Percentage yield

Calotropis gigantea yield percentage is shown in Table 3 below.

**Table 3: Results of Percentage yield of Extracts of Whole plant**

| Sr.no. | Extract | Weight | Percentage yeild |
|---|---|---|---|
| 1 | Petroleum-ether | 29.5gm | 0.99% |
| 2 | Chloroform | 14.8ml | 1.48% |
| 3 | Ethyl acetate | 09.6ml | 1.26% |
| 4 | n-Butanol | 4.7ml | 1.24% |
| 5 | Ethanol | 19.7ml | 2.45% |
| 6 | Distilled water | 64.7ml | 11.8% |

### Phytochemical screening

Several qualitative tests were performed in order to determine the phytochemical components that were present in the hydroalcoholic extracts. Numerous tests were conducted to check for the presence of carbohydrates, flavonoids, steroids, glycosides, alkaloids, saponins, and other compounds. The results of the phytochemical screening are displayed in table 4:

**Table 4: Phytochemical Screening of Calotropis gigantean**

| Sr.no. | Class of Compounds | Test Performed | Result |
|---|---|---|---|
| 1 | Alkaloid | Tests by Drangendroff and Mayer | + |
| 2 | Carbohydrates | Fehling test and Molish test | + |
| 3 | Glycosides | Test Keller Killiani | + |
| 4 | Flavonoids | Test for ammonia | + |
| 5 | Saponins | With Na2CO3-infused water | + |
| 6 | Terpenoids | Salkowski examination | + |
| 7 | Steroids | Burchard-Libmann test | + |
| 8 | Phenolic compounds | Test for ferric chloride | + |
| 9 | Polyuronoids | Test for haemotoxylin | - |
| 10 | Peroxides | Test for Potassium Iodide | - |

### Assessment of the ointment

### Properties of Organoleptic Systems

The smooth consistency, scent, and color of the produced ointment were evaluated by visually assessing its golden hue.

**pH of Ointment**

Using a digital pH meter, the pH of the prepared herbal ointment was examined, and the results showed that the pH was between 6.5 and 7.

**The ability to spread**

Timing the amount of time it takes for two slides to separate from the ointment when a specific force is applied between them allows one to measure spreadability. The spreadability was found to be 6.9 seconds. This instance accurately illustrates the F4 preparation's spreadability.

**Solubility**

It was discovered that the herbal ointment was somewhat soluble in distilled water and soluble in ethanol, chloroform, and ether.

**Washability**

Ability to wash We saw increased washability following application of the mixture, both in terms of volume and ease of water washing. In this instance, the F4 preparation displays the appropriate washability result in this preparation.

**Table 5: Physicochemical Evaluation of Herbal Ointment**

| Physicochemical parameters | F1 | F2 | F3 | F4 |
|---|---|---|---|---|
| Colour | yellow | yellow | yellow | yellow |
| Odour | Characteristics | Characteristics | Characteristics | Characteristics |
| Consistency | Seamless | Slightly fluidy | Good smoothing | Good Smoothing |
| pH<br>Washability | 6.7<br>Slightly less | 6.8<br>Average | 6.6<br>Good | 6.9<br>Better |
| Spread-ability (sec.) | 6.5 seconds | 6.6 seconds | 6.4 seconds | 6.4 seconds |
| Phase separation | Slightly phase separation occur | No phase separation | Little phase separation occur | No phase separation |

**Stability study**

The herbal ointment was put through a physical stability test for a month at three different temperatures: 20 °C, 250 °C, and 300 °C. Three different temperatures 20°C, 250°C, and 300°C were used to measure the herbal ointment's physical stability.

**Table 6: Stability study**

| Temp. | Drug Content |
|---|---|
| **$2^0$C** | **85%** |
| **$25^0$C** | **81.5%** |
| **$30^0$C** | **78%** |

**Drug content**

Formulation F4 was determined to contain the most drug (85%), while formulation F1 had the lowest drug concentration (76%).

**Table 7: Drug content**

| Formulation | Drug content |
|---|---|
| F1 | 76% |
| F2 | 81.5% |
| F3 | 78% |
| F4 | 85% |

**Anti-inflammatory properties in vitro**

Results of Calotropis gigantea's anti-inflammatory action are displayed in Table 8. An substantial increase in activity was seen when comparing Calotropis gigantea doses to the control. To be comparable to the effects of 100 mg/kg of etoricoxib, the 200 mg/kg dose of ethanolic extract from Calotropis gigantea demonstrated the most anti-inflammatory potency.Calotropis gigantea exhibited strong anti-inflammatory properties in its methanolic extract, suggesting that its main mechanism of action is to stop the release of inflammatory mediators. More investigation, including animal trials, is necessary to identify and isolate the active ingredient or ingredients that are responsible for its anti-inflammatory impact as well as to define the mechanism(s) underlying its anti-inflammatory action.

**Table 8: In-vitro Anti-inflammatory activity of Calotropis gigantean.**

| Sr.no. | Compounds | Dose(mg/kg) | Absorbance value (Mean ±SE) | Inhibition of denaturation(%) |
|---|---|---|---|---|
| 1 | Control | 10ml/kg | 0.097 ± 0.006 | ---- |
| 2 | Standard (Etoricoxib) | 100mg/kg | 0.181 ± 0.001 | 86.71 |
| 3 | Petroleum ether extract | 250ml/kg | 0.152 ± 0.002 | 53.08 |
| 4 | Chloroform extract | 150ml/kg | 0.144 ± 0.002 | 44.87 |
| 5 | Ethyl acetate extract | 250ml/kg | 0.123 ± 0.005 | 25.53 |
| 6 | n-Butanol | 150ml/kg | 0.166 ± 0.008 | 71.40 |
| 7 | Ethanol | 250ml/kg | 0.174 ± 0.007 | 77.57 |
| 8 | Distilled water | 150ml/kg | 0.159 ± 0.002 | 62.26 |

## Conclusion

The flower may be a valuable component for herbal ointments, as evidenced by the remarkable anti-inflammatory properties of Calotropis gigantea flower extract. More than 80% of people in underdeveloped nations get their primary care from herbal remedies, according to World Health Organization estimates. The utilization of organic molecules, particularly those derived from plants, has grown in popularity recently for both conventional and botanical uses due to their safety and effectiveness when consumed by humans. A comprehensive review of the available data indicates that Calotropis procera and gigantea are widely used as remedies for a variety of ailments by practitioners of Ayurveda, traditional healers, and individuals from diverse ethnic backgrounds. The Calotropis species is the main focus of research due to the belief that it has much more therapeutic potential than is currently recognized.The F4 preparation has strong anti-inflammatory properties and meets the criteria for an anti-inflammatory preparation.

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