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Phytopharmacognostical Study, Development & Assessment of Topical Herbal Ointment from Leaves of *Calotropis gigantea L.*

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Abstract

Joint pain is a common problem that affects people all over the world and calls for efficient treatment solutions. The present study investigated the creation of a topical formulation for the treatment of joint pain that uses an extract from the leaves of Calotropis gigantea. C. gigantea is sometimes referred to as huge milk weed and aak leaves. It is a member of the Apocynaceae family. This versatile plant has been valued for its medicinal properties for millennia. It is one of those plants that has access to the best natural resources and traditional wisdom. The anti-inflammatory qualities of C. gigantea leaves aid to reduce joint ache suffering. It mostly consists of mudarine, cardenolides, flavonoids, and alkaloids. The present study included the extraction procedure, formulation optimization, and effectiveness assessment of the prepared topical medication. When developing the formulation, a number of factors were taken into account, including stability, excipients, and extract concentration. Furthermore, investigations conducted in vitro looked into the anti-inflammatory qualities of C. gigantea leaves. The results showed encouraging trends, suggesting that the topical formulation that was created has the potential to be a secure and effective treatment for joint discomfort. The present study highlighted the value of herbal-based therapies in contemporary healthcare practices and advanced the investigation of natural treatments for joint diseases.

Keywords: *Calotropis gigantea* L., joint pain, anti-inflammatory, topical formulation, herbal ointment

INTRODUCTION

Inflammation is the body's natural reaction to damaging stimuli, such as infections, damaged cells, or irritants. A number of intricate biological processes involving blood vessels, the immune system, and signaling molecules define it. The goal of inflammation is to start tissue repair by eliminating the original source of cell damage. Redness, swelling, heat, and discomfort at the location of the injury or infection are typical symptoms. Acute inflammation is a helpful and protective reaction, but persistent inflammation can lead to a number of illnesses and ailments [1, 2].

Calotropis gigantea L. is a species of flowering plant in the Apocynaceae family, commonly referred to as gigantic milkweed or crown flower. It is extensively dispersed in nations such as India, Thailand, and Australia and is native to tropical parts of Asia and Africa. It is a big shrub or small tree that reaches a height of 4–6 m. It bears clusters of lovely, star-shaped flowers with purple or white petals, and it has thick, waxy leaves. Because of its

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therapeutic qualities, several sections of the *C. gigantea* L plant have been employed in traditional medicine. The plant's latex has been used as an antibacterial and to heal wounds and skin conditions.

Furthermore, the plant's extracts have been investigated for possible pharmacological effects, such as those that are anti-inflammatory, antibacterial, and anti-cancer. According to *Ayurveda*, dried whole plant is a good expectorant, anthelmintic, depurative, and tonic. Traditional practitioners use the leaf extract to treat rheumatic and inflammatory painful disorders. Common illnesses including fever, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting, and diarrhea are also treated with *C.gigantea* [3, 4].

MATERIALS AND METHODS

Plant Material

In December 2023, leaves of *C. gigantea* were taken from Nadiad, in the Kheda district of Gujarat, India. The leaves were verified by a botanist from J & J College of Science, Nadiad, Gujarat, India. For future reference, a leaf sample herbarium specimen was submitted to the Department of Pharmacognosy at the Faculty of Pharmacy, Dharmsinh Desai University, Nadiad Gujarat, India.

Chemicals and Instruments Required

Polyethylene glycol (400), polyethylene glycol (4000), methyl paraben, eucalyptus oil, leaves extract, pH meter of control dynamics and UV-visible spectrophotometer.

Collection of Plant Material

C.gigantea leaves were sun-dried, ground into a 40 mesh powder, and then kept in airtight receptacles. The macroscopic characteristics were examined in accordance with the protocol outlined in the World Health Organization (WHO) recommendations for techniques of quality control for medicinal plant materials.

Extraction from Calotropis gigantea Leaves

Approximately 1 kg of plant leaves were gathered, properly cleaned with distilled water, and allowed to dry in the shade for a period of 15 days. The dried leaves were crushed into a rough powder. About 500 ml of methanol was infused with 100 g of powder for a full day in order to macerate it. After that, the macerate was put into a round-bottom flask and extracted over the course of 3 h using a Soxhlet apparatus. To obtain the *C.gigantea* crude extract, the extract was evaporated over a water bath [5].

Formulation of Ointment [6–9]

Table 1 shows the ingredients used in the formulation of *C. gigantea* leaves extract.

Table 1. Formulation of herbal ointment.

S.N.	Name of ingredients	Quantity to be taken (50 g)
1.	Polyethylene glycol (400)	60%
2.	Polyethylene glycol (4000)	40%
3.	Leaves extract	3.2 g
4.	Methyl paraben	0.2%
5.	Eucalyptus oil	0.2%

Method of Making Herbal Ointment

First, the foundation for the ointment was made by precisely weighing polyethylene glycol (400) and polyethylene glycol (4000). Both were carefully mixed together until they congealed after being heated to 650°C in a water bath. In order to create a smooth paste for the herbal ointment, precisely weighed leaf extract was combined with the ointment base using the levigation method [10].

Method for Anti-Inflammatory Activity

Ultraviolet (UV) visible spectrophotometer was used to measure anti-inflammatory activity. About 20 mg extract was weighed and dissolved in 20 ml water.

20 mg/20 ml = 1 mg/ml =1000 µg/1000 µl

Three test tubes were taken and 1 ml (200 µg/ml), 1.5 ml (300 µg/ml) and 2 ml (400 µg/ml) solutions were taken from the stock solution and the volume was made up to 5 ml. Two test tubes for each concentration or one extract were made. From this concentration, 200 µg/ml, 300 µg/ml and 400 µg/ml test tube, 2 ml in different test tubes were taken and 2 ml BSA solution and 2 ml normal saline was added to make a solution. For control 4 ml of normal saline and 2 ml of BSA solution was taken. After that, all the test tubes were heated on water bath at 60 °C–70 °C for 3–5 min. After the test tube was cooled, UV spectroscopy was used to measure the absorbance at 660 nm.

Evaluation Parameters [11–15]

Different physicochemical characteristics that were investigated to describe the topical herbal ointment from *C. gigantea* leaves are described below:

Color and Smell

Color and scent were among the physical characteristics that were analyzed visually. It was discovered that the ointment had a distinct smell and was pale yellow in hue.

Consistency

A visual inspection was conducted to assess the texture and uniformity of each ointment batch and the consistency of the ointment was observed. It is silky and devoid of any avarice in the goods.

pH

The pH of the prepared herbal ointment was measured with a digital pH monitor. The ointment solution was prepared using 100 ml of distilled water and allowed to stand for 2 h. The pH was measured three times, and the average value was calculated to provide the answer. The Systronics pH meter model was utilized for the calculation of pH.

Spreadability

About 1 g of the sample was placed between two slides that had been uniformly thinned by applying a 100 g weight for 5 min in order to assess the spreadability. The spreadability was better the faster the two slides could be separated. The spreadability was calculated using the following formula:

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

Where, S= spreadability
M = weight placed on upper slide
L = length of glass slide
T = time taken to separate the slides.

Loss on Drying

The formulation was placed in a petriplate set over a water bath and dried at 105℃ to determine the loss on drying (LOD). Following formula was used to compute the LOD:

$$\text{LOD (\%w/w)} = \frac{W2-W3}{W2-W1} \times 100$$

Where, W2 – W3 = weight of the sample
W2 – W1= weight lost

Solubility

The procedure by which a solute dissolves in a solvent to form a uniform system is called solubility. Achieving the appropriate medication concentration in systemic circulation for the intended pharmacological reaction depends critically on solubility.

Washability

Actions to be taken prior to the test: Pick a surface that works well, such as fabric or skin. Make sure the surface is cleaned to ensure that nothing remains that could interfere with the test. After measuring a consistent amount, apply the ointment to the test surface in a controlled manner. Ensure that the entire surface has an even coating of the substance.

Establishing Time: Leave the cream on the skin for a predetermined period of time.

Cleaning: After the prescribed setting time has elapsed, try rinsing the ointment off with water and a little soap.

Effect Without Irritation

The produced herbal ointment was applied to human skin, and the result was monitored. To conduct the test, a small amount of sample was applied to the hand, and the results such as redness, erythema, inflammation, etc. were monitored for a few minutes. Consequently, no such impact was noticed, indicating that it does not irritate skin [11–15].

RESULTS AND DISCUSSION

Physicochemical Evaluation of Formulated Ointment

Based on the results of chemical tests conducted, the sample being analyzed was likely to contain alkaloids, carbohydrates, saponins, steroids and triterpenoids, glycosides. flavonoids, and tannins. However, proteins, amino acids, fats and oils were not present in the sample. The ointment's physical attributes, such as color, smell, and condition were assessed. The produced ointment had cream color, a distinct smell, a semisolid texture, and a silky feel. It verified the consistent distribution of extract in the ointment by look and touch (Table 2).

Table 2. Physicochemical evaluation of formulated ointment.

Physicochemical parameters	Observation
Colour	Light yellow
Odor	Characteristic
Texture	Smooth
pH	6
Spreadability	133.63 (g.cm/s)
Loss on drying	14%
Solubility	Soluble in ether, chloroform, and water
Washability	Good
Non-irritancy	Non-irritant

The herbal pharmaceutical ointment of *C.gigantea L.* has been found to pass all of the mentioned parameters, indicating that it has promising potential as a medicinal product. The appearance of the ointment was smooth and consistent, indicating that it was well-mixed and homogeneous. The after-feel of the ointment was also found to be suitable, with no sticky or greasy residue upon application. The pH of the ointment was within the desired range, indicating that it was stable and not likely to cause irritation upon application. The ointment was also found to be easy to remove and did not cause any irritation during use, making it suitable for human use. Overall, the *C.gigantea L.* herbal pharmaceutical ointment has demonstrated excellent potential for use in a wide range of medicinal applications. Its

properties are well-suited for topical application and it has passed a range of stringent tests, confirming its safety and efficacy. With further research and development, this ointment could be used to treat a range of conditions, bringing relief, and improving quality of life for many people.

Spreadability plays a critical role in maximizing the ointment's efficacy and uptake. A product with a low spreadability reading can be applied evenly and easily over the intended area without requiring a lot of force or friction. This test demonstrated the formulation's strong spreadability qualities.

Table 3. Results of absorbance of test solution.

S.N.	Concentration (µg/ml)	%inhibition of BSA	Average % inhibition. of BSA
1	200	0.007	32.35%
2	300	0.008	52.94%
3	400	0.006	64.70%

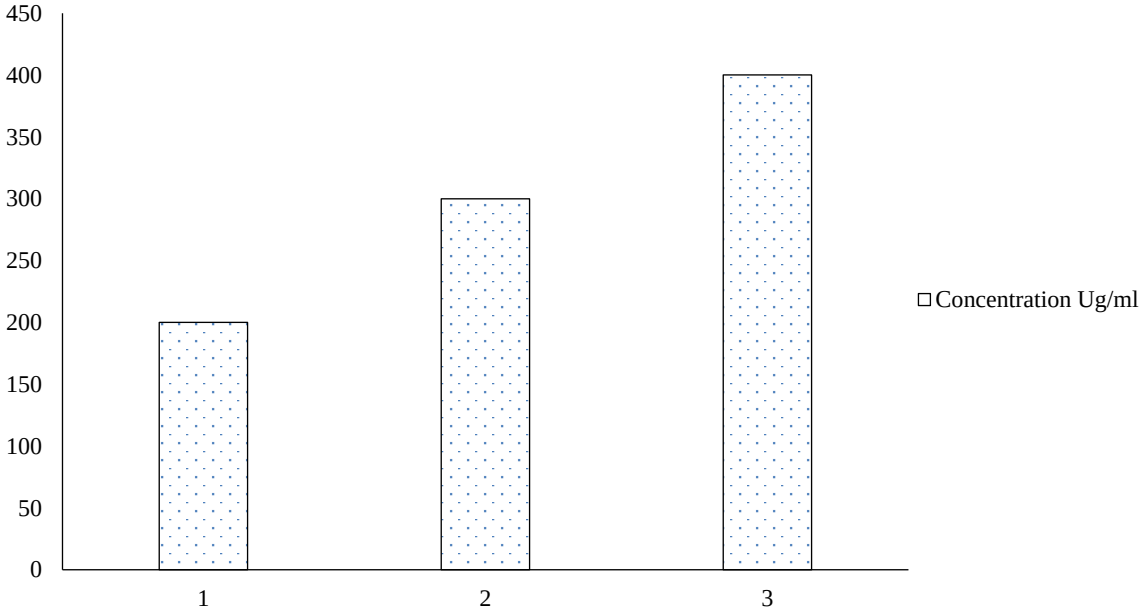

Figure 1. Percentage inhibition versus concentration of test solution.

Table 3 shows the result of absorbance of *C.gigantea* L. leaves extract of different concentration with their percentage inhibition. The highest % inhibition of 64.70% was shown by 400 µg/ml solution, which is found to be good for the anti-inflammatory activity. Figure 1 shows the graph of percentage inhibition versus concentration where 400 µg/ml showed the highest %inhibition.

The goal of the present study was to formulate and assess the herbal ointment. In order to produce a good yield of extract and ensure that the chemical contents and their activity were unharmed, the herbal extracts were prepared using a straightforward maceration and Soxhlet extraction procedure. In order to achieve homogeneous mixing of the herbal extract with the ointment base, the levigation method was utilized to manufacture the ointment, which was found to be stable throughout storage. After studying the physicochemical characteristics, favorable findings were obtained for spreadability, washability, solubility, drying loss, and other factors.

CONCLUSION

To sum up, *C.gigantea L*. is a useful medicinal plant having a variety of therapeutic uses. Numerous phytoconstituents that contribute to the plant's medicinal qualities were found in the phytopharmacognostical analysis of the plant. The anti-inflammatory activity of a topical ointment formulated and evaluated with *C.gigantea* L. extract demonstrated encouraging outcomes. In vitro and

in vivo models demonstrated that the ointment was safe and effective, with an efficacy that was on par with anti-inflammatory medications that are sold commercially. The best ointment formulation for stability and a longer shelf life under various storage circumstances was also determined by the present study. The creation of a natural remedy such as *C.gigantea* L. for joint pain has the potential to be a safe and effective topical treatment for anti-inflammatory conditions. The product's consistency and efficacy were guaranteed for a considerable amount of time due to the formulation's good stability and extended shelf life. Additionally, the formulation showed good spreadability, smoothness to touch, good characteristics, lack of irritancy, ease of absorption, ease of washing, etc. Every criterion was below the standard deviation. The research not only showed that *C. gigantea* L. can effectively treat inflammation, but it also emphasized the value of phytopharmacognostical investigations in the creation of herbal products.

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Conflict of Interest Statement

The authors declared no conflict of interest.

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