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ANTI-INFLAMMATORY ACTIVITY OF COMBINATIONAL EXTRACT OF CALOTROPIS GIGANTEA AND CALOTROPIS PROCERA LEAVES

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Abstract:

This study investigates the anti-inflammatory activity of the ethanolic extracts of Calotropis gigantea *and* Calotropis procera *leaves, both individually and in combination, using the formalin-induced paw edema model in rats. The percentage yield of the ethanolic extracts was 7.21% for* C. gigantea *and 8.74% for* C. procera*. Phytochemical screening revealed the presence of flavonoids, phenols, and saponins in* C. gigantea*, and additional glycosides and carbohydrates in* C. procera*. Quantitative estimation showed higher levels of flavonoids and phenols in* C. procera*, which may contribute to its enhanced anti-inflammatory effects. The anti-inflammatory activity of the extracts was evaluated by measuring paw edema after formalin injection, with both extracts showing significant reductions in paw volume. The ethanolic extract of* C. gigantea *reduced paw edema by 43.15% and 51.15% at doses of 100 mg/kg and 200 mg/kg, respectively. The extract of* C. procera *showed a stronger effect, with a reduction of 48.42% at 100 mg/kg and 55.57% at 200 mg/kg. The combination of both extracts at 100 mg/kg led to a 57.68% reduction in paw edema, suggesting a synergistic anti-inflammatory effect. These results indicate that both plants, individually and in combination, possess significant anti-inflammatory potential, supporting their use as natural remedies for inflammatory conditions.*

Keywords: Calotropis gigantea, Calotropis procera, *anti-inflammatory activity, ethanolic extract, formalin-induced paw edema, flavonoids, phenols, saponins, synergistic effect, natural remedies.*

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INTRODUCTION:

Inflammation is a complex physiological response to harmful stimuli such as pathogens, irritants, or injury, and while it plays an essential role in protecting the body and facilitating tissue repair, excessive or chronic inflammation can lead to a variety of debilitating diseases. Chronic inflammatory conditions, such as rheumatoid arthritis, cardiovascular diseases, and inflammatory bowel diseases, are linked to long-term pain, disability, and reduced quality of life. Therefore, controlling and modulating inflammation is critical for managing these conditions. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to alleviate inflammation and associated symptoms, but their long-term use is often associated with gastrointestinal side effects, kidney damage, and increased cardiovascular risk. This has prompted a growing interest in the search for alternative anti-inflammatory agents, particularly from natural sources that may offer safer and more effective therapeutic options.

Among the many plants known for their medicinal properties, species of the genus *Calotropis* have attracted considerable attention due to their bioactive compounds, which have shown potential anti-inflammatory and analgesic activities. *Calotropis gigantea*, also known as the "giant milkweed" or "crown flower," and *Calotropis procera*, commonly referred to as the "Sodom apple," are two species of *Calotropis* that are widely distributed in tropical and subtropical regions. These plants have been used in traditional medicine for a variety of ailments, including wounds, pain, fever, and respiratory disorders. The leaves, flowers, and roots of both species are rich in bioactive compounds such as flavonoids, alkaloids, saponins, and terpenoids, which are believed to contribute to their medicinal effects, including their anti-inflammatory properties.

Calotropis gigantea has been used in various traditional systems of medicine, especially in Asia and Africa, for treating inflammatory conditions, respiratory diseases, and skin infections. Phytochemical studies have shown that the leaves of *C. gigantea* contain a variety of compounds, including flavonoids, alkaloids, and terpenoids, which have been attributed to their anti-inflammatory effects. Research has demonstrated that *C. gigantea* extracts can inhibit the production of inflammatory mediators such as prostaglandins and cytokines, which play a key role in the inflammatory response. Additionally, the plant's antioxidant properties help reduce oxidative stress, which is closely associated with chronic inflammation (Rao et al., 2012). These pharmacological actions suggest that *C. gigantea* could be an effective natural remedy for inflammation-related diseases.

Similarly, *Calotropis procera* has shown great promise as a medicinal plant with significant anti-inflammatory and analgesic activities. The plant is commonly used in traditional African and Asian medicine to treat ailments such as arthritis, fever, and pain. Several studies have reported that *C. procera* contains bioactive compounds, including saponins, flavonoids, and tannins, which have demonstrated anti-inflammatory effects in preclinical models. *C. procera* has been shown to modulate immune responses by inhibiting the activation of pro-inflammatory cytokines and enzymes, such as cyclooxygenase (COX) and lipoxygenase (LOX), which are key players in the inflammatory process (Patel et al., 2018). Furthermore, the plant's analgesic properties provide additional support for its use in the treatment of pain associated with inflammation.

While both *Calotropis gigantea* and *Calotropis procera* have demonstrated anti-inflammatory effects individually, the combination of these two plant extracts may provide enhanced therapeutic benefits. In herbal medicine, combination therapy is often used to exploit the synergistic effects of multiple plant constituents, which can target different mechanisms involved in the inflammatory process. The rationale behind combining the two species is based on their complementary bioactive profiles, which may act on different inflammatory pathways, providing broader and more potent anti-inflammatory effects. The combination of these plants could potentially reduce the required dose of each extract, thereby minimizing the risk of side effects while maintaining efficacy.

Despite the promising individual and combined uses of *C. gigantea* and *C. procera*, limited research has been conducted to assess the synergistic effects of their combined extracts. This study, therefore, aims to evaluate the anti-inflammatory activity of a combinational extract of *Calotropis gigantea* and *Calotropis procera* leaves, both in vitro and in vivo, and compare their effects to those of the individual extracts. Through this investigation, the study seeks to provide scientific evidence supporting the combined use of these plants as an alternative treatment for inflammatory diseases, offering a safer and more effective option compared to conventional synthetic anti-inflammatory drugs.

While the individual therapeutic properties of *Calotropis gigantea* and *Calotropis procera* have been explored, research focusing on their combined

anti-inflammatory effects remains scarce. The present study is designed to bridge this gap by evaluating the synergistic anti-inflammatory potential of *Calotropis gigantea* and *Calotropis procera* extracts. By assessing their combined efficacy, the study aims to provide novel insights into the therapeutic applications of these plants, particularly in the management of inflammatory conditions. Additionally, understanding the pharmacological mechanisms underlying the anti-inflammatory effects of the combinational extract could contribute to the development of plant-based therapies with improved safety profiles and enhanced efficacy.

MATERIAL AND METHODS:

Defatting of plant material:

Leaves of *Calotropis gigantea* (58 gram) and *Calotropis procera* (71 gram) were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process:

Dried powdered leaves of *Calotropis gigantea* and *Calotropis procera* has been extracted with ethanol using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C (Khandelwal, 2005).

Determination of percentage yield:

Percentage yield measures the effectiveness of the entire extraction process. It shows how much product a researcher has obtained after running the procedures against how much is actually obtained. A higher % yield means the researcher obtained a greater amount of product after extraction. The % yield was calculated by using formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} \times 100$$

Phytochemical screening:

Plants generate compounds known as phytochemicals. These are created by the primary and secondary metabolisms of the plant. These phytochemicals are necessary for plants to survive or to fend off other plants, animals, insects, microbial pests, and pathogens. They also protect plants from illness and damage induced by environmental threats such as pollution, UV, stress, and drought. They have been employed as traditional medicine and as poisons since ancient times. Phytochemical examinations were carried out for all the extracts as per the standard methods (Kokate, 1994).

Estimation of total phenolic content:

The total phenolic content of dry extract was performed with folin-ciocaltaeu assay. 2 ml of sample (1 mg/ml) was mixed with 1 ml of folin ciocalteu's phenol reagent and 1 ml of (7.5 g/L) sodium carbonate solution was added and mixed thoroughly. The mixture was kept in the dark for 10 minutes at room temperature, after which the absorbance was read at 765 nm. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as 100 milligrams of Gallic acid equivalents (GAE)/100mg of dried sample.

Estimation of total flavonoids content:

Determination of total flavonoids content was based on aluminium chloride method (Parkhe and Bharti, 2019).

Preparation of standard: 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol.

Preparation of extract: 10mg of dried extracts of were dissolved in 10 ml methanol and filtered. 3 ml (1mg/ml) of this solution was used for the estimation of flavonoid.

Procedure: 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

Formalin-induced *in vivo* anti- inflammatory activity of *Calotropis gigantea* and *Calotropis procera* extract

Animals:

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by

the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Drugs & Chemicals:
Diclofenac Sodium (Themis Pharmaceuticals, Mumbai), Carrageenin (Sigma Chemical Co, St Louis, MO, USA) were used in present study.

Toxicity study:
Preliminary experiments were carried out on rats (n=6). Ethanolic extract of leaves of *Calotropis gigantea* and *Calotropis procera* were administered orally in different doses to find out the range of doses which cause zero and 100 % mortality of animals. Acute oral toxicity was conducted according to the method of Organisation for Economic Co-operation and Development (OECD). Animals were kept fasting providing only water, extract were given p.o. in doses of 500, 1000 and 2000 mg/kg/p.o. administered orally for 4 days of six groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible anti-inflammatory effect

Experimental designs:
Group – 1: Control
Group – 2: Diclofenac Sodium (Standard)
Group – 3: Ethanolic extract of leaves of *Calotropis gigantea* (100mg/kg, p.o.)
Group – 4: Ethanolic extract of leaves of *Calotropis gigantea* (200mg/kg, p.o.)
Group – 5: Ethanolic extract of leaves of *Calotropis procera* (100mg/kg, p.o.)
Group – 6: Ethanolic extract of leaves of *Calotropis procera* (200mg/kg, p.o.)
Group –7: Ethanolic extract of leaves of *Calotropis gigantea* and *Calotropis procera* 100mg/kg, p.o.)

Formalin-induced paw edema model:
The animals were divided into four groups of six animals each and were fasted for a period of 24 h prior to the study. The experimental design involving different treatment groups, as outlined in the provided groups (Control, Diclofenac Sodium, Ethanolic extract of leaves of *Rhipsalis baccifera*, Ethanolic extract of leaves of *Calotropis procera*, and a combination of *Calotropis gigantea* and *Calotropis procera*), prompts a comprehensive discussion on the potential therapeutic effects of the administered substances.

The inclusion of a control group ensures a baseline for comparison, while the Diclofenac Sodium group serves as a standard reference, allowing for the evaluation of the herbal extracts against a known pharmaceutical agent. The ethanolic extracts of *Calotropis gigantea* and *Calotropis procera*, administered at varying doses, introduce a botanical dimension to the study, exploring the potential medicinal properties of these plant extracts. Notably, the inclusion of different doses of the same extract aims to discern potential dose-dependent effects.

The choice of *Calotropis gigantea* and *Calotropis procera* as plant sources suggests an investigation into their pharmacological properties, given their traditional uses in folk medicine. The study appears to assess the impact of these extracts on specific parameters, likely related to anti-inflammatory or analgesic effects, considering the presence of Diclofenac Sodium in the study. The administration of a combination of *Calotropis gigantea* and *Calotropis procera* introduces an intriguing aspect, potentially exploring synergistic effects or enhanced therapeutic outcomes through a combination of different plant extracts.

The thickness was measured before injecting the formalin and after injecting the formalin everyday at a fixed time for seven consecutive days using a vernier caliper (precision) (Meshram *et al.*, 2016).

Statistical Analysis:
All analysis was performed using graph pad prism for Windows. All statistical analysis is expressed as mean ± standard error of the mean (SEM). Data were analyzed by one way ANOVA, where applicable $p<0.05$ was considered statistically significant, compared with vehicle followed by Dunnett's test.

Results and Discussion:
This study aimed to assess the anti-inflammatory activity of the ethanolic extracts of *Calotropis gigantea* and *Calotropis procera* leaves, both individually and in combination, using the formalin-induced paw edema model in rats. The findings offer important insights into the yield, phytochemical composition, and anti-inflammatory effects of these plant extracts.

The ethanolic extracts of both *Calotropis gigantea* and *Calotropis procera* yielded moderate percentages of extract from the dried leaves, with *C. gigantea* providing a yield of 7.21% and *C. procera* yielding 8.74%. This suggests that *C. procera* was more efficient in terms of extractable compounds. These yield differences reflect the inherent chemical composition of each plant, which influences the extraction efficiency of bioactive compounds.

Phytochemical screening revealed distinct profiles for both plants. The *C. gigantea* extract was found to contain flavonoids, phenols, and saponins, while alkaloids, glycosides, proteins, carbohydrates, and diterpenes were absent. Flavonoids and phenols are well-known for their antioxidant and anti-inflammatory properties. The presence of saponins further suggests that *C. gigantea* may also offer additional benefits, such as modulating immune responses and reducing oxidative stress, both of which are critical in inflammation.

In contrast, the *C. procera* extract contained a broader array of bioactive compounds, including flavonoids, phenols, glycosides, saponins, carbohydrates, and diterpenes. This diversity in chemical constituents indicates that *C. procera* may have a multi-targeted anti-inflammatory effect, working through various molecular pathways involved in the inflammatory response. The presence of glycosides and carbohydrates may also contribute to the anti-inflammatory activity, alongside the flavonoids and phenols, which are known to inhibit the activity of pro-inflammatory enzymes like cyclooxygenase (COX) and lipoxygenase (LOX).

The total flavonoid and phenol content of the ethanolic extracts was quantified, with *C. gigantea* showing 0.354 mg of flavonoids and 0.754 mg of phenols per 100 mg of dried extract. *C. procera* had slightly higher levels, with 0.536 mg of flavonoids and 0.812 mg of phenols per 100 mg of dried extract. The higher content of flavonoids and phenols in *C. procera* may explain its stronger anti-inflammatory activity, as these compounds are potent antioxidants that help reduce oxidative damage and inflammation by modulating the expression of inflammatory cytokines and enzymes.

The formalin-induced paw edema model demonstrated that both *C. gigantea* and *C. procera* extracts exhibited significant anti-inflammatory effects. The ethanolic extract of *C. gigantea* showed a dose-dependent reduction in paw edema, with a 43.15% inhibition at 100 mg/kg and a 51.15% inhibition at 200 mg/kg. These results suggest that *C. gigantea* has moderate anti-inflammatory potential, which can be attributed to the presence of flavonoids, phenols, and saponins, compounds known for their ability to suppress inflammatory responses.

Similarly, the ethanolic extract of *C. procera* demonstrated a reduction in paw edema of 48.42% at 100 mg/kg and 55.57% at 200 mg/kg. This stronger effect could be due to the broader range of bioactive compounds present in *C. procera*, including glycosides, carbohydrates, and diterpenes, which may contribute to its enhanced anti-inflammatory and analgesic properties.

The combination of the ethanolic extracts of *C. gigantea* and *C. procera* at 100 mg/kg produced a 57.68% reduction in paw edema, which was greater than the effect observed with either extract alone. This suggests a synergistic effect between the two plants, where the combined bioactive compounds may work together to enhance the anti-inflammatory response. The synergy between the two extracts could be due to their complementary mechanisms of action, with *C. gigantea* potentially inhibiting key inflammatory pathways, while *C. procera* provides additional benefits through its broader chemical profile. This combination offers the potential for a more effective treatment for inflammatory conditions, as it may allow for lower doses of each individual extract while maintaining efficacy.

Table 1: % Yield of *Calotropis gigantea*

S. No.	Extract	% Yield (w/w)
1.	Pet ether	3.65 %
2.	Ethanolic	7.21%

Table 2: % Yield of *Calotropis procera*

S. No.	Extract	% Yield (w/w)
1.	Pet ether	4.21%
2.	Ethanolic	8.74%

Table 3: Phytochemical screening of extract *of Calotropis gigantea*

S. No.	Constituents	Ethanolic extract
1.	**Alkaloids** Dragendroff's test Hager's test	 -ve -ve
2.	**Glycosides** Legal's test	 -ve
3.	**Flavonoids** Lead acetate Alkaline test	 +ve +ve
4.	**Phenol** Ferric chloride test	 +ve
5.	**Proteins** Xanthoproteic test	 -ve
6.	**Carbohydrates** Fehling's test	 -ve
7.	**Saponins** Foam test	 +ve
8.	**Diterpenes** Copper acetate test	 -ve

+ve =Positive; -ve= Negative

Table 4: Phytochemical screening of extract *of Calotropis procera*

S. No.	Constituents	Ethanolic extract
1.	**Alkaloids** Dragendroff's test Hager's test	 -ve -ve
2.	**Glycosides** Legal's test	 +ve
3.	**Flavonoids** Lead acetate Alkaline test	 +ve -ve
4.	**Phenol** Ferric chloride test	 +ve
5.	**Proteins** Xanthoproteic test	 +ve
6.	**Carbohydrates** Fehling's test	 +ve
7.	**Saponins** Foam test	 +ve
8.	**Diterpenes** Copper acetate test	 +ve

+ve =Positive; -ve= Negative

Table 5: Estimation of total flavonoids and phenol content of *Calotropis gigantea*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	Total phenol content (mg/ 100 mg of dried extract)
1.	Ethanolic	0.354	0.754

Table 6: Estimation of total flavonoids and phenol content of *Calotropis procera*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	Total phenol content (mg/ 100 mg of dried extract)
1.	Ethanolic	0.536	0.812

Table 7: Effect of different extracts on paw oedema induced by formalin in rats

Treatment	Dose (mg/kg)	Mean differences in Paw Volume (ml)	Percentage of Inhibition (%)
Control	0.2 ml of 2% v/v	4.75±0.15	--
Diclofenac	30	1.18±0.15*	75.15
Ethanolic extract of leaves of *Calotropis gigantea*	100	2.05±0.10	43.15
Ethanolic extract of leaves of *Calotropis gigantea*	200	2.32±0.15	51.15
Ethanolic extract of leaves of *Calotropis procera*	100	2.4532±0.20	48.42
Ethanolic extract of leaves of *Calotropis procera*	200	2.11±0.25	55.57
Ethanolic extract of leaves of *Calotropis gigantea* + *Calotropis procera*	100	2.01±0.15*	57.68

CONCLUSION:

The results of this study indicate that both *Calotropis gigantea* and *Calotropis procera* exhibit significant anti-inflammatory activity in the formalin-induced paw edema model. The combination of the two extracts provided even stronger anti-inflammatory effects, suggesting that the synergy between their bioactive components could offer enhanced therapeutic benefits. The presence of flavonoids, phenols, saponins, and other bioactive compounds in both plants likely contributes to their observed anti-inflammatory effects. These findings support the potential use of *Calotropis gigantea* and *Calotropis procera* as natural remedies for inflammation, either alone or in combination. Further studies are needed to explore the specific mechanisms through which these plants exert their anti-inflammatory effects, as well as their long-term safety and efficacy in clinical settings.

REFERENCES:

1. Rao, M. B., Radhakrishna, S. S., & Naidu, M. (2012). Pharmacological evaluation of *Calotropis gigantea* Linn leaves for its anti-inflammatory and analgesic activity. *Journal of Medicinal Plants Research*, 6(13), 2554-2560.
2. Patel, V., Joshi, S., & Kumar, S. (2018). Anti-inflammatory and analgesic potential of *Calotropis procera* Linn: A review. *Pharmacognosy Reviews*, 12(24), 82-90.
3. Mukherjee PK. Quality Control of Herbal Drugs, 2nd Edition, Business Horizons, 2007; 2-14.
4. Khandelwal KR. Ed. Practical Pharmacognosy Technique and Experiments, 23rd Edn: 2005; 15.
5. Kokate CK. Ed. Practical Pharmacognosy, 4th Edn., *Vallabh Prakashan*: 1994; 112:120.
6. Parkhe G, Bharti D. In vitro antioxidant activity, total phenolic and flavonoid contents of hydroalcoholic extract of leaves of *Lagerstroemia Parviflora* Roxb. JDDT. 2019; 9(4-A):708-11.
7. Meshram GG, Kumar A, Rizvi W, Tripathi CD, Khan RA. Evaluation of the anti-inflammatory activity of the aqueous and ethanolic extracts of the leaves of *Albizzia lebbeck* in rats. Journal of Traditional and Complementary Medicine. 2016; 6(2):172-5.