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ANTI- INFLAMMATORY ACTIVITY OF EQUAL PROPORTION OF DIFFERENT SOLVENT EXTRACT OF *CALOTROPIS GIGANTEA* LEAVES & ROOTS

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ABSTRACT

The present study investigates the anti-inflammatory and anti-arthritic potential of hydroalcoholic extracts derived from the leaves and roots of *Calotropis gigantea*, administered in equal proportions. The extracts were evaluated for percentage yield, phytochemical constituents, and total bioactive content including phenols and flavonoids. Preliminary phytochemical screening revealed the presence of various secondary metabolites such as flavonoids, glycosides, saponins, tannins, and phenolic compounds, which are known to contribute to anti-inflammatory activity. Quantitative analysis indicated a higher phenolic content in leaves and higher flavonoid content in roots. The anti-arthritic activity was assessed using the Freund's adjuvant-induced arthritis model in Wistar rats. Animals treated with the plant extract at doses of 100 mg/kg and 200 mg/kg showed a significant and dose-dependent reduction in paw edema over a 28-day period, compared to the untreated arthritic control group. The effect observed at 200 mg/kg was comparable to the standard drug aspirin. The results suggest that the hydroalcoholic extract of *Calotropis gigantea* exhibits promising anti-inflammatory activity and may serve as a potential natural alternative for managing inflammatory and arthritic conditions.

Keywords: *Calotropis gigantea*, Anti-inflammatory activity, Hydroalcoholic extract, Arthritis, Phytochemical screening, Phenolic content, Flavonoids, Freund's adjuvant model

INTRODUCTION

Inflammation is a protective biological response triggered by infection, injury, or irritation. Although it is essential for tissue repair and immune defense, chronic inflammation can lead to a range of disorders, including arthritis, cardiovascular diseases, and neurodegenerative conditions (Medzhitov *et al.*, 2008). Conventional non-steroidal anti-inflammatory drugs (NSAIDs) are effective but often associated with adverse effects like gastric irritation and renal toxicity (Vane *et al.*, 1998). As a result, there is growing interest in plant-based therapies that offer safer alternatives.

Calotropis gigantea (L.) R. Br., commonly known as "giant milkweed," is a perennial shrub widely used in traditional medicine systems such as Ayurveda and Unani. It is reputed for treating a wide variety of ailments including inflammation, wounds, skin diseases, and fever (Nadkarni *et al.*, 2002). Phytochemical studies have revealed that *C. gigantea* is rich in bioactive compounds like flavonoids, alkaloids, saponins, glycosides, and terpenoids, which contribute to its diverse

pharmacological properties (Gupta *et al.*, 2003; Parotta *et al.*, 2001).

Previous research has shown the anti-inflammatory potential of either leaf or root extracts of *C. gigantea* prepared using various solvents (Basu *et al.*, 2005; Kumar *et al.*, 2011). However, limited studies have evaluated the combined effect of both leaf and root extracts in equal proportions, especially when extracted with solvents of varying polarities. Solvent selection significantly affects phytochemical extraction and, consequently, biological activity.

The present study is therefore designed to assess the anti-inflammatory potential of equal proportions of different solvent extracts (e.g., petroleum ether, chloroform, ethyl acetate, methanol, and aqueous) of *Calotropis gigantea* leaves and roots. This combined approach may reveal enhanced or synergistic therapeutic effects and contribute to the development of safe, effective, and natural anti-inflammatory agents.

MATERIALS AND METHODS

Materials

All chemicals and reagents used, including ethanol, ferric chloride, Folin–Ciocalteu reagent, aluminum chloride, and standard drugs such as aspirin, were of analytical grade and procured from reputed suppliers. Experimental animals (Wistar rats, 150–200 g) were housed under standard laboratory conditions and used for the anti-inflammatory study following ethical approval.

Methods

Collection and preparation

Fresh leaves and roots of *Calotropis gigantea* were collected, thoroughly washed, shade-dried, and coarsely powdered. The powdered plant materials were extracted using a hydroalcoholic solvent system (ethanol: water, 70:30) by maceration for 72 hours. The extracts were filtered and evaporated to dryness using a rotary evaporator.

Extraction of plant materials by maceration method

The powdered leaves (45 gm) and roots (53 gm) of *Calotropis gigantea* were accurately weighed and then packed into an extraction bottle. The defatted plant materials underwent extraction using a hydroalcoholic solvent (ethanol: water; 75:25 v/v). The resulting liquid extracts were collected in a tarred conical flask. Subsequently, the solvent was removed through distillation, with the final traces of solvent being eliminated under vacuum conditions. The extracts obtained using the solvent were then weighed until a constant weight was achieved. The percentage weight/weight (w/w) basis of the extracts was calculated to quantify the concentration of the plant constituents in the final extract (Mukherjee; 2007).

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 (Khandelwal; 2005). The % yield was calculated by using formula:

$$\% \text{ yield} = [(\text{weight of dried extract}) / (\text{weight of dried plant sample})] \times 100$$

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 (Kokate; 1994).

Quantitative estimation of phenols and flavonoids

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 (Mishra *et al.*, 2017).

Estimation of total flavonoids content

Preparation of standard solution 10mg quercetin was weighed and made up to 10ml with Methanol in a 10ml volumetric flask. From the above solution (1mg/ml), 1ml was pippeted out and made up to 10ml with Methanol to get 100µg/ml Quercetin standard solution (stock solution). From the stock solution, solutions of concentration 5, 10, 15, 20 and 25 µg/ml were prepared. 3 ml of each standard and test was mixed with 1 ml of 2% Aluminium chloride solution. The solutions were mixed well and the absorbance was measured against the blank at 420nm using UV-Visible spectrophotometer. A standard graph was plotted using various concentrations of Quercetin and their corresponding absorbance (Mishra *et al.*, 2017).

***In vivo* anti-arthritis activity**

Animals

Albino Wistar rats of either sex (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*. Animas 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 rat 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.

Acute oral toxicity study

Acute oral toxicity was conducted according to the method of Organisation for Economic Co-operation and Development (OECD) (OECD; 2002). Hydroalcoholic extract of leaves and root of *Calotropis gigantea* (5, 50, 300, and 2000 mg/kg) was 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-arthritic effect. Freund's adjuvant induced arthritis in rats: Animals were divided into five groups containing six animals each. Arthritic

syndrome was induced by subcutaneous injection of 0.1ml of complete Freund's adjuvant (10mg of heat killed mycobacterium tuberculosis per ml of paraffin oil) into the planter surface of the left hind paw (Jaijesh *et al.,* 2009).

Group I Served as normal and received 2% gum acacia

Group II Served as arthritis control-untreated received 2% gum acacia.

Group III Received Aspirin (200 mg/kg p.o) served as reference standard

Group IV Received extract of hydroalcoholic extract of leaves and root of *Calotropis gigantea* of doses of 100mg/kg p.o.

Group V Received extract of hydroalcoholic extract of leaves and root of *Calotropis gigantea* of doses of 200mg/kg p.o.

The drug treatment was started from 14th day of adjuvant induction and terminated on 28th day. The changes in paw volume was measured weekly by using Plethysmograph. At the end of experiment histopathology was done to check the inflammation.

Statistical analysis

The values were expressed as mean ± SEM (n=6). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Tukey's test and $P<0.05$, $P<0.01$, and $P<0.001$ were considered to be statistically significant.

RESULTS AND DISCUSSION

The current study aimed to evaluate the anti-inflammatory potential of hydroalcoholic extracts of *Calotropis gigantea* leaves and roots, along with their phytochemical composition and bioactive content. The extraction yielded 6.2% w/w for leaves and 7.5% w/w for roots, indicating a moderate yield and good solubility of active constituents in the hydroalcoholic solvent system.

Phytochemical screening of the extracts revealed the presence of important primary metabolites like carbohydrates and proteins in both leaves and roots, while amino acids were found only in the leaf extract. The extracts also tested positive for key secondary metabolites such as glycosides, saponins, flavonoids, tannins, phenols, and diterpenes. These constituents are known to possess significant pharmacological actions including anti-inflammatory and antioxidant properties.

Quantitative estimation showed that the leaf extract had a higher total phenolic content, while the root extract showed a higher total flavonoid content. These findings suggest that both parts of the plant are rich in bioactive compounds that may contribute to the observed pharmacological effects.

The anti-arthritic activity was evaluated using the Freund's adjuvant-induced arthritis model in rats. The arthritis control group exhibited a steady and significant increase in paw volume over 28 days, confirming the development of inflammation and joint swelling. In contrast, animals treated with aspirin showed a significant reduction in paw volume, indicating effective suppression of inflammation.

Treatment with the hydroalcoholic extract of *Calotropis gigantea* at both 100 mg/kg and 200 mg/kg doses resulted in a dose-dependent decrease in paw swelling. The group receiving the higher dose showed a significant reduction in paw volume, closely approaching the effect seen with aspirin, especially by Day 28. This

suggests a promising anti-inflammatory effect of the plant extract when administered orally. The observed activity can be attributed to the synergistic action of flavonoids, phenolic compounds, saponins, and glycosides, which are known to modulate inflammatory pathways and reduce oxidative stress. The combination of leaves and roots in equal proportion appears to enhance this activity, potentially offering a broader range of bioactive compounds.

Table 1: % Yield of hydroalcoholic extract of *Calotropis gigantea*

Extract	Colour	Consistency	Yield (% w/w)
Calotropis gigantea			
Leaves	Dark green	Solid	6.2%
Root	Brown	Solid	7.5%

Table 2: Preliminary qualitative phytochemical tests for *Calotropis gigantea* leaves extract

Phytoconstituents	*Calotropis gigantea* leaves extract
i) Primary Metabolites	
Carbohydrates	(+)
Amino acids	(+)
Proteins	(+)
Fats and oils	(-)
ii) Secondary Metabolites	
Steroids	(-)
Triterpenoids	(-)
Volatile oils	(-)
Diterpenes	(+)
Glycosides	(+)
Saponins	(+)
Flavonoids	(+)
Tannins & Phenol	(+)
Alkaloids	(-)
HE = Hydroalcoholic extract; '+' = Present; '-' = Absent	

Table 3: Preliminary qualitative phytochemical tests for *Calotropis gigantea* root extract

Phytoconstituents	*Calotropis gigantea* root extract
i) Primary Metabolites	
Carbohydrates	(+)
Amino acids	(-)
Proteins	(+)
Fats and oils	(-)
ii) Secondary metabolites	
Steroids	(-)
Triterpenoids	(-)

Volatile oils	(-)
Diterpenes	(-)
Glycosides	(+)
Saponins	(+)
Flavonoids	(+)
Tannins & Phenol	(+)
Alkaloids	(-)
HE = Hydroalcoholic extract; '+' = Present; '-' = Absent	

Table 4: Total bioactive constituents content of *Calotropis gigantea*

S. No.	Extract	Total phenol content	Total flavonoid content
		mg/ 100mg	
1.	Leaves extract	0.568	0.622
2.	Root extract	0.452	0.702

Table 5: Anti-arthritis activity of hydroalcoholic extract of leaves and root of *Calotropis gigantea* against Freund's adjuvant induced arthritis in rats

Paw volume (mL)					
Group		**Day 7**	**Day 14**	**Day 21**	**Day 28**
Group I	Normal	0.23±0.60	0.25±0.50	0.25±0.50	0.25±0.40
Group II	Arthritis control-untreated	0.75±0.20	0.90±0.35	0.95±0.30	0.96±0.40
Group III	Aspirin (200 mg/kg p.o)	0.65±0.20	0.60±0.40**	0.50±0.55***	0.45±0.60***
Group IV	Hydroalcoholic extract of Leaves and Root of *Calotropis gigantea*100mg/kg p.o	0.68±0.40	0.65±0.20*	0.62±0.50*	0.60±0.20*
Group V	Hydroalcoholic extract of Leaves and Root of *Calotropis gigantean* 200mg/kg p.o	0.65±0.30**	0.62±0.40**	0.57±0.40***	0.52±0.40***

Values expressed as mean ± SEM (n=6) *P<0.05, **P<0.01, *** P<0.001 as compared to arthritis Control

CONCLUSION

The findings of the present study demonstrate that the hydroalcoholic extracts of *Calotropis gigantea* leaves and roots, when administered in equal proportions, possess significant anti-inflammatory and anti-arthritic properties. The presence of phytoconstituents such as flavonoids, tannins, and phenolic compounds likely contributes to these therapeutic effects. The extract showed a marked, dose-dependent reduction in paw edema in the Freund's adjuvant-induced arthritis model, with the 200 mg/kg dose yielding effects comparable to the standard drug aspirin. These results support the traditional use of *Calotropis gigantea* in inflammatory disorders and highlight its potential as a natural and effective alternative for the treatment of arthritis and related

inflammatory conditions. Further studies focusing on the isolation of active compounds and elucidation of their mechanisms are warranted.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

REFERENCES

- Medzhitov, R., & Janeway, C. A. (2008). Origin and physiological roles of inflammation. *Nature, 454*(7203), 428–435. https://doi.org/10.1038/nature07201
- Vane, J. R., & Botting, R. M. (1998). Mechanism of action of nonsteroidal anti-inflammatory drugs. *The American Journal of Medicine*, *104*(3A), 2S–8S. https://doi.org/10.1016/S0002-9343(97)00203-9
- Nadkarni, K. M. (2002). *Indian materia medica* (Vol. 1–2). Mumbai: Popular Prakashan.
- Gupta, M., Mazumder, U. K., Gomathi, P., & Selvan, V. T. (2003). Antiinflammatory evaluation of leaves of *Calotropis gigantea*. *Fitoterapia*, *74*(7–8), 745–749. https://doi.org/10.1016/S0367-326X(03)00107-0
- Parrotta, J. A. (2001). *Healing plants of peninsular India*. Wallingford, UK: CABI Publishing. https://doi.org/10.1079/9780851995050.0000
- Basu, A., & Chaudhuri, A. (2005). Antibacterial activity of *Calotropis gigantea* leaves extract. *Indian Journal of Pharmaceutical Sciences*, *67*(1), 150–151.
- Kumar, S., Dewan, S., Sangameswaran, B., & Kumar, V. (2011). Phytochemical and anti-inflammatory investigation of root extracts of *Calotropis gigantea*. *Journal of Applied Pharmaceutical Science*, *1*(7), 123–126.
- Mukherjee, P. K. (2007). *Quality control of herbal drugs* (2nd ed., pp. 2–14). New Delhi: Business Horizons.
- Khandelwal, K. R. (2005). *Practical pharmacognosy: Techniques and experiments* (23rd ed., pp. 15, 29, 56, 149). Pune: Nirali Prakashan.
- Kokate, C. K. (1994). *Practical pharmacognosy* (4th ed., pp. 112, 120). New Delhi: Vallabh Prakashan.
- Mishra, A. G., Singh, R., Meha, P., & Parkhe, G. (2017). Determination of total phenolic, flavonoid content, antioxidant and antimicrobial activity of *Gloriosa superba* seed extract. *Asian Journal of Pharmaceutical Education and Research*, *6*(2), 12–17.
- OECD. (2002). *Acute oral toxicity: Acute oral toxic class method guideline 423*. In *Eleventh addendum to the OECD guidelines for the testing of chemicals*. Paris: Organisation for Economic Co-operation and Development.
- Jaijesh, P., Srinivasan, K. K., Bhagath, K. P., Sreejith, G., Raju, S. K., & Sareesh, N. N., et al. (2009). Anti-arthritic potential of the plant *Justicia gendarussa* Burm F. *Journal of Clinics*, *64*, 357–362.