

Phytochemical Evaluation and Anti-Inflammatory Activity of Ethanolic Extract of *Calotropis procera* Leaves

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ABSTRACT

Background: Medicinal plants have been used for centuries to cure various diseases. There is a huge potential to investigate the medicinal impacts of different parts of plants. Roots, stem, leaves and fruits of *Calotropis procera* are known for their biological activities. *Calotropis procera* plant shows multiple pharmacological activities like anti-cancer, anti-microbial, antioxidant, antimalarial, hepatoprotective and anti-diabetic activities.

Objectives: The objective of the current research was ethanolic extraction of *Calotropis procera* leaves and to study phytochemistry and anti-inflammatory activity.

Methodology: In this study, we used the extract of *Calotropis procera* leaves for detection of phytochemicals and anti-inflammatory activity *in vitro* by hypotonicity induced hemolysis on 2% HRBC suspension, using UV-Vis spectrophotometer.

Results: Phytochemicals like alkaloids, terpenoids, and flavonoids were present in large amount while tannins, saponins, steroids and cardiac glycosides were in small amount, whereas phlobatannins and anthraquinone were not detected. The potential of the ethanolic extract of *Calotropis procera* leaves was compared with Diclofenac sodium (100µl/ml, 200µl/ml). The leaves extract of *Calotropis procera* (100, 200, 300, 400, 500µl/ml each) showed significant anti-inflammatory activity by hypotonicity induced hemolysis on 2% HRBC suspension.

Conclusion: The *Calotropis procera* leaves have the potential to cure inflammatory diseases and can be used as anti-inflammatory medicine and analgesic.

Keywords

Anti-inflammatory activity, *Calotropis procera* leaves, phytochemical evaluation, antioxidant, HRBC suspension, UV-Vis spectrophotometer

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INTRODUCTION

Anciently, men used herbal medicines for curing various diseases. Pharmacology expands rapidly with the advancement of scientific knowledge. These plant-based medicines are safe and easily available¹. Traditional systems such as Hikmat, Ayurveda, Unani, Siddha and homoeopathy suggested 95% different medicinal plants in

the treatments². World Health Organization (WHO) claimed that 60-80% population of this world use ancient medicinal remedies for common diseases based on plants³. *Calotropis procera* is a medicinal plant of the family Asclepiadaceae. It is widely found in areas of Africa and Asia⁴. *Calotropis procera* name in other languages⁵ is

shown in Table 1. The botanical name and other taxonomical data⁶ of *Calotropis procera* is shown in Table 2.

Calotropis procera is found in Pakistan, India, Afghanistan, Nepal, Iran, Algeria, Kenya, Nigeria, Niger, Oman, U.A.E, Saudi Arabia, Yemen, Vietnam and Zimbabwe^{6,7}. In Pakistan, *Calotropis procera* grows almost in all parts of Pakistan as a shrub in plain, sandy, and alkaline lands⁸. Morphologically, it is a much-branched, erect small tree-like structure of about 5.4m height with milky latex throughout. The bark is soft and corky. Leaves are subsessile, opposite, oblong, thick, and green colored. Flowers are umbellate, cymes and tomentose on young⁹.

Table 1. Vernacular Names of *Calotropis procera*.

S.No.	Language	Name
01	Hindi	Madar
02	English	Crown flower
03	Bengali	Akanda
04	Sanskrit	Adityapuspikar
05	Punjabi	Aak, Ak

Table 2. Classification of *Calotropis procera*.

S.No.	Kingdom	Plantae
01	Division	Magnoliophyta
02	Class	Magnoliopsida
03	Subclass	Asteridae
04	Order	Gentianales
05	Family	Asclepiadaceae
06	Subfamily	Caesalpinioideae
07	Genus	Calotropis
08	Species	Procera

Traditionally, different parts of the *Calotropis procera* have been used for ailments and are still utilized for the said purpose. For instance, latex has been used as a wound-healing agent and abortifacient in folk medicines¹⁰. Roots are used for eczema, leprosy, elephantiasis, asthma, cough and rheumatism¹¹. Flowers use as anti-dandruff, also used in cholera and dysentery¹². Leaves are used against swelling, joints pain, sores, skin diseases, rheumatic joints, snake bite, scabies, veterinary medicine, boils, anti-lice and scorpion stings^{13,14,15}.

Pharmacologically, *Calotropis procera* is used for many activities for e.g. dry latex shows anti-diabetic activity¹⁶, and *Calotropis procera* flowers are used for potent hepatoprotective agent against induced hepatic injuries¹⁷. *Calotropis procera* roots show anti-tumor activity¹⁸, while leaves extract show anti-microbial¹⁹ as well as antioxidant activity²⁰. Likewise, the ethanolic extract of *Calotropis procera* leaves significantly exhibited the anti-malarial activity^{21,22}.

Therefore, the present study is to find how much the leaves of *Calotropis procera* depicts the anti-inflammatory activity.

MATERIALS AND METHODS

Collection of Plant Material

The mature leaves of *Calotropis procera* were collected from the roadside in the area of sabzazar scheme Multan road, district Lahore, Punjab. All the leaves were washed well to eliminate all the dust, then dried in shade for 10 days. After that, the leaves were crushed with a grinder to make pieces as small as possible.

Preparation of Plant Extract

The known amount (50g) of dried leaves of *Calotropis procera* were subjected to Soxhlet apparatus to get extract using ethanol (250ml) as a solvent. After two days, the obtained extract was evaporated by putting it in the oven and finally, the crude green colored semi-solid extract was obtained.

Phytochemical Evaluation of Plant Extract

Qualitative evaluation of phytochemicals in ethanolic extract of *Calotropis procera* leaves was done by dissolving a small amount of extract in ethanol²³, and used further for the following detections:

1. Tannins

Leaves extract (1ml) was mixed with FeCl₃ (5ml) solution, giving a dark green color which confirmed the presence of tannins²⁴.

2. Flavonoids

Approx. 2ml of leaves' extract was mixed with 1ml of NaOH solution giving yellow color at first, which disappeared besides of solution of acid²³, confirming the presence of flavonoids in the leaf extract.

3. Alkaloids**Hager's test:**

A small amount (1-2ml) of leaves' extract was mixed with Hager's reagent and observed for yellow coloration²³.

4. Cardiac Glycosides:

Approx. 2ml mixture of leaves' extract was mixed with 5ml of water, and 2ml of glacial acetic acid containing one drop of FeCl₃ solution. After that, 1ml of conc. H₂SO₄ was added resulting in the formation of a brownish ring, thus indicating deoxy sugar characteristics of cardenoloids²⁵.

5. Saponins**Froth test:**

Approx. 1ml of leaves' extract was shaken with 5-10ml of water resulting in the stable froth, thus indicating the presence of saponins²⁵.

6. Phlobatannins

A small amount of extract was boiled with 1% HCl, causing deposition of a red precipitate, hence, confirmed the presence of phlobatannins²⁵.

7. Steroids**Salkowski Test**

Leaves extract (1ml) was mixed with 2ml of chloroform and 2ml of conc. H₂SO₄, forming red coloration in the chloroform layer and greenish-yellow in an acid layer, hence, confirmed the presence of steroids in the extracts²³.

8. Terpenoids

A small amount of leaves' extract was mixed with 2ml of chloroform and 3ml of conc. H₂SO₄ carefully to make a layer of reddish-brown coloration, verifying the presence of terpenoids²⁵.

9. Anthraquinone:

To check the presence of anthraquinones, leaves extract was boiled with 10ml of dil. H₂SO₄ and filtered while hot. To the filtrate, 5ml of chloroform was added further. The mixture was shaken and the chloroform layer was transferred into another test tube containing 1ml of dil. ammonia. The appearance of violet colour confirmed the presence of anthraquinone²⁵.

Preparation of Extract Solution For *In Vitro* Activity

An equal volume of extract and distilled water were shaken vigorously and left overnight. The next day, the solution was filtered to be used for further activities^{26,27}.

Anti-Inflammatory Activity (*In Vitro* Study)**Human Red Blood Cell Suspension (HRBC) Preparation**

Blood (5ml) from healthy human volunteers, free from NSAID, was drawn in a tube containing heparin and centrifuged at 3000rpm for 10min. Plasma was discarded, and residual blood cells and RBCs were washed three times with normal saline. Following this, 2% v/v suspension was prepared with normal saline for further use^{28,29}.

Hypotonicity Induced Hemolysis

In each tube, 0.5ml of 2% HRBC suspension and 0.5ml normal saline (NaCl 0.03%) was mixed. Later on, variable concentrations of leaf extract were added (i.e. 100-500µl/ml) in each tube. Control was prepared without extract. Furthermore, Diclofenac sodium injection (100, 200µl/ml) was added as a standard drug and tubes were incubated at 37°C for 30min followed by centrifugation at 3000rpm for 10min. The supernatant was decanted, and hemoglobin content was estimated at 540nm spectrophotometrically^{30,31}. The % protection was calculated by using the formulas:

$$\text{Hemolysis \%} = (\text{Abs of test sample} / \text{Abs of control}) \times 100$$

$$\text{Protection \%} = 100 - (\% \text{ hemolysis})$$

RESULTS**Phytochemical evaluation**

Ethanollic extract of *Calotropis procera* leaves was subjected to check the presence of phytochemicals in it. The phytochemical evaluation depicted that flavonoids, alkaloids, tannins, terpenoid, saponins, steroids and

cardiac glycosides were present but phlobatannins and anthraquinone were absent³² (Table 3).

Table 3. Phytochemical Analysis of *Calotropis procera* Leaves.

S.No.	Phytochemicals	Result
01	Tannins	+
02	Alkaloid	++
03	Terpenoid	++
04	Flavonoid	++
05	Saponins	+
06	Phlobatannins	—
07	Anthraquinone	—
08	Steroids	+
09	Cardiac Glycosides	+

++ = high amount, + = less amount, — = Absent

Anti-inflammatory activity

In vitro activity of ethanolic extract of *Calotropis procera* leaves was checked against 2% HRBC suspension. Results in Table 4 indicated that the concentrations of extract (100, 200µl/ml) did not show significant membrane-stabilizing effect whereas, the concentration ranges of (300-500µl/ml) presented significant stabilization with maximum protection around 73.11% at 500µl/ml, whereas, standard diclofenac sodium showed 67.24% stabilization at 200µl/ml.

Table 4 & Fig. 1 shows % protection of ethanolic extract *Calotropis procera* leaves against hypotonicity induced hemolysis on 2% HRBC suspension.

Table 4. Percentage Protection of *Calotropis procera* Leaves on 2% HRBC Suspension by Hypotonicity Induced Hemolysis.

S.No.	Treatment(s)	Concentrations (µl/ml)	Absorbance (A)	Heamolysis (%)	Protection (%)
01	Control	—	1.056 ± 0.18	—	—
02	ECPL	100	0.668 ± 0.02	63.25	36.75
03	ECPL	200	0.577 ± 0.03	54.64	45.36
04	ECPL	300	0.429 ± 0.03	40.62	59.38
05	ECPL	400	0.343 ± 0.01	32.48	67.52
06	ECPL	500	0.284 ± 0.01	26.89	73.11
07	DS	100	0.561 ± 0.01	48.39	51.61
08	DS	200	0.346 ± 0.01	32.76	67.24

Each value is shown in mean ± SD, Here ECPL= Extract of *Calotropis procera* leaves, DS= Diclofenac sodium

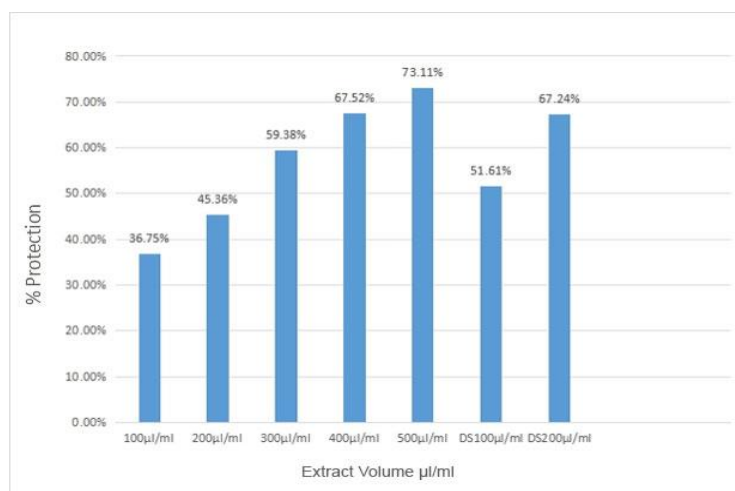


Figure 1. % protection of *Calotropis procera* against 2% HRBC suspension.

DISCUSSION

Medicinal plants contain many phytochemicals that are responsible for the treatment of different diseases. Anciently, men used medicinal plants due to their therapeutic potential, which facilitated them to cure diseases. Nature has given us medicinal plants which have medicinal values³³.

In the current study, there were many phytochemicals detected from ethanolic extract of *Calotropis procera* leaves by general identification methods, which showed therapeutic potential.

For checking the therapeutic potential of ethanolic extract of *Calotropis procera* leaves, we used *in vitro* anti-inflammatory activity by HRBC erythrocyte membrane stabilization method. The principle behind this method is that when Red Blood Cells (RBCs) get exposure to substances such as hypotonic medium, heat etc., lysis of membrane take place^{34,35}. These erythrocyte membranes are analogous to the lysosomal membranes and their stabilization implies that the extract may well stabilize the lysosomal membrane.

The present work revealed that the ethanolic extract of *Calotropis procera* leaves has potential against inflammation of the erythrocyte membrane. The activity was increased by increasing the extract dose. 100µl/ml and 200µl/ml showed non-significant efficacy such as 36.75% and 45.36%, respectively. The extract dose 300µl/ml and 400µl/ml showed significant efficacy such as 59.38% and 67.52%, respectively. The extract dose 500µl/ml showed greater efficacy of 73.11% than the standard Diclofenac sodium 100µl/ml and 200µl/ml which showed 51.61% and 67.24%, respectively. The results were also indicated graphically in the figure 1.

CONCLUSION

It is concluded that the ethanolic extract of *Calotropis procera* leaves have the potential to cure inflammatory diseases and can be used as anti-inflammatory and analgesic medicine.

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LIST OF ABBREVIATION

DS	Diclofenac Sodium
ECPL	Extract of <i>Calotropis procera</i> Leaves
HRBCs	Human Red Blood Cells
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs

REFERENCES

1. Modak M, Dixit P, Londhe J, Ghaskadbi S, Devasagayam TP. Recent advances in Indian herbal drug research guest editor: Thomas Paul Asir Devasagayam Indian herbs and herbal drugs used for the treatment of diabetes. J Clin Biochem Nutr. 2007; 40(3):163-73.
2. Satyavati, G.V., Gupta AK, Tandon N. Med Plants of India, ICMR. 1987. P. 16. 2.
3. Zhang Q, Sharan A, Espinosa SA, Gallego-Perez D, Weeks J. The path toward integration of traditional and complementary medicine into health systems globally: The World Health Organization report on the implementation of the 2014–2023 strategy. J Altern Complement Med. 2019; 25(9):869-71.
4. Mossa JS, Tariq M, Mohsin A, Ageel AM, Al-Yahya MA, Al-Said MS, Rafatullah S. Pharmacological studies on aerial parts of *Calotropis procera*. Am J Chinese Med. 1991; 19(04):223-31.
5. Sholapur HN, Patil BM. Effect of *Moringa oleifera* bark extracts on dexamethasone-induced insulin resistance in rats. Drug Res. 2013; 63(10):527-31.
6. Yogi B, Gupta SK, Mishra A. *Calotropis procera* (Madar): A medicinal plant of various therapeutic uses—A review. Bull Env Pharmacol Life Sci. 2016; 5(7):74-81.
7. Chatterjee A, Parkashi S. The treatise of Indian medicinal plants, volume IV. New Delhi: Publication and Information Directorate CSIR. 1995.
8. Kabir H, Naz A, Khan NA. Unani medicinal plants in Hamdard University campus, India. Hamdard Medicus (Pakistan). 2003; 1:34-40.
9. Verma R, Satsangi GP, Shrivastava JN. Ethnomedicinal profile of different plant parts of *Calotropis procera* (Alt.) R Br Ethnobotanical Leaflets. 2010; 14:721-42.
10. Bhaskar VH, Ajay SS. Evaluation of antihyperglycemic activity of extracts of *Calotropis procera* (Ait.) R. Br on streptozotocin induced diabetic rats. Glob J Pharmacol. 2009; 3(2):95-8.
11. Sen SK, Behra LM. Ethnomedicinal plants used in touch therapy at Bargarh district of Orissa. Ethnobot. 2007; 19(1&2):100-04.
12. Anis M, Sharma MP, Muhammad I, Herral. Ethnomedicine for the Gwalier forest division in Madhya Pradesh, India. Pharm Biol. 2007; 38(4): 241-53.

13. Evans WC. Trease and Evans, Pharmacognosy 2005; 41-7.
14. Ragnathan M, Abay SM. Ethnomedicinal survey of folk drugs used in Bahirdar Zuria district, Northwestern Ethiopia. 2009; 8(2):281-4.
15. Azhar MF, Siddiqui MT, Ishaque M, Tanveer A. Study of ethnobotany and indigenous use of *Calotropis procera* (Ait.) in cholistan desert, Punjab, Pakistan. J Agric Res. 2014; 52(1):117-26.
16. Roy S, Sehgal R, Padhy BM, Kumar VL. Antioxidant and protective effect of latex of *Calotropis procera* against alloxan-induced diabetes in rats. J Ethnopharmacol. 2005; 102(3): 470-3.
17. Setty SR, Quereshi AA, Swamy AV, Patil T, Prakash T, Prabhu K, Gouda AV. Hepatoprotective activity of *Calotropis procera* flowers against paracetamol-induced hepatic injury in rats. Fitoterapia. 2007; 78(7-8):451-4.
18. Van Quaquebeke E, Simon G, André A, Dewelle J, Yazidi ME, Bruyneel F, Tuti J, Nacoulma O, Guissou P, Decaestecker C, Braekman JC. Identification of a Novel Cardenolide (2"-Oxovorusharin) from *Calotropis procera* and the hemisynthesis of novel derivatives displaying potent *in vitro* antitumor activities and high *in vivo* tolerance: Structure- activity relationship analyses. J Med Chem. 2005; 48(3):849-56.
19. Mossa JS, Tariq M, Mohsin A, Ageel AM, Al-Yahya MA, Al-Said MS, Rafatullah S. Pharmacological studies on aerial parts of *Calotropis procera*. Am J Chinese Med. 1991; 19(04):223-31.
20. Yesmin N, Nasir Uddin S, Mubassara S, Ali Ako M. Antioxidant and Antibacterial Activities of *Calotropis procera* Linn AM Eurasian J Agric Environ Sci. 2008; 4(5):550-3.
21. Sharma P, Sharma JD. Evaluation of *in vitro* schizontocidal activity of plant parts of *Calotropis procera*—An ethnobotanical approach. J Ethnopharmacol. 1999; 68(1-3):83-95.
22. Sharma P, Sharma JD. *In vitro* schizontocidal screening of *Calotropis procera*. Fitoterapia. 2000; 71(1):77-9.
23. Ranjit PM, Santhipriya T, Nagasri S. Phytochemical screening and anti-bacterial activities of ethanolic extract of flower against human pathogenic strains. AJPCR 2012; 74(5): 443-50.
24. Gajare SM, Patil MV, Mahajan RT. Phytochemical screening and antimicrobial activity of ethanol extract of *Calotropis procera* root. IJRPP. 2012; 2(3):143-6.
25. Kumar S, Sharma UK, Sharma AK, Pandey AK. Protective efficacy of *Solanum xanthocarpum* root extracts against free radical damage: Phytochemical analysis and antioxidant effect. Cell Mol Biol. 2012; 58(1):171-8.
26. Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Dagainawala HF. Effect of *Fagonia arabica* (Dhamasa) on *in vitro* thrombolysis. BMC Complem Altern M. 2007; 7(1):1-6.
27. Amri O, Zekhnini A, Bouhaimi A, Tahrouch S, Hatimi A. Anti-inflammatory activity of methanolic extract from *Pistacia atlantica* desf. leaves. Pharmacog J. 2018; 10(1):71-76.
28. Sakat S, Juvekar AR, Gambhire MN. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. Int J Pharm Pharm Sci. 2010; 2(1):146-55.
29. Sadique J, Al-Rqobahs WA, Bughaith EI, Gindi AR. The bioactivity of certain medicinal plants on the stabilization of RBC membrane system. Fitoterapia. 1989; 60(6):525-32.
30. Dima J, Raghda L, Jalil GA. Evaluation of hemolytic and anti-hemolytic activity of the aerial parts of *Sonchus oleraceus* extracts. Int J Pharm Sci Nanotechnol. 2017; 10(3):3745-51.
31. Azeem AK, Dilip C, Prasanth SS, Shahima VJ, Sajeev K, Naseera C. Anti-inflammatory activity of the glandular extracts of *Thunus alalunga*. Asian Pac J Trop Med. 2010; 3(10):794-6.
32. Shamim SA, Fatima L. Pharmacological actions and therapeutic uses of Aak (*Calotropis procera*): A Review. J Pharm Innov. 2019; 8(2): 40-7.
33. Heidarian E, Jafari-Dehkordi E, Valipour P, Ghatreh-Samani K, Ashrafi-Eshkaftaki L. Nephroprotective and anti-inflammatory effects of *Pistacia atlantica* leaf hydroethanolic extract against gentamicin-induced nephrotoxicity in rats. J Diet Suppl. 2017; 14(5):489-502.
34. Debnath PC, Das A, Islam A, Islam MA, Hassan MM, Uddin SM. Membrane stabilization—A possible mechanism of action for the anti-inflammatory activity of a Bangladeshi medicinal plant: *Erioglossum rubiginosum* (Bara Harina). Pharmacog J. 2013; 5(3):104-7.
35. Bhatti GR, Qureshi R, Shah SM. Ethnobotany of *Calotropis procera* with special reference to the people of Nara Desert. Scientific Sindh Ann J Res. 1998; 5:13-22.