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

Naveed Aslam Dogar1,*, M. Hamza Shahid1, Hafiz Usama Shaukat1, M. Abubakar Khan1, Farooq Saleem1,2

1Department of Chemistry, Government College of Science, Lahore, Pakistan
2Faculty of Pharmacy, University of Lahore, Pakistan

**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

**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 languages is
shown in Table 1. The botanical name and other taxonomical data 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. In Pakistan, *Calotropis procera* grows almost in all parts of Pakistan as a shrub in plain, sandy, and alkaline lands. 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 young.

Table 1. Vernacular Names of *Calotropis procera*.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Language</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Hindi</td>
<td>Madar</td>
</tr>
<tr>
<td>02</td>
<td>English</td>
<td>Crown flower</td>
</tr>
<tr>
<td>03</td>
<td>Bengali</td>
<td>Akanda</td>
</tr>
<tr>
<td>04</td>
<td>Sanskrit</td>
<td>Adityapuspikar</td>
</tr>
<tr>
<td>05</td>
<td>Punjabi</td>
<td>Aak, Ak</td>
</tr>
</tbody>
</table>

Table 2. Classification of *Calotropis procera*.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Kingdom</th>
<th>Plantae</th>
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<tbody>
<tr>
<td>01</td>
<td>Division</td>
<td>Magnoliophyta</td>
</tr>
<tr>
<td>02</td>
<td>Class</td>
<td>Magnoliopsida</td>
</tr>
<tr>
<td>03</td>
<td>Subclass</td>
<td>Asteridae</td>
</tr>
<tr>
<td>04</td>
<td>Order</td>
<td>Gentianales</td>
</tr>
<tr>
<td>05</td>
<td>Family</td>
<td>Asclepiadaceae</td>
</tr>
<tr>
<td>06</td>
<td>Subfamily</td>
<td>Caesalpinioideae</td>
</tr>
<tr>
<td>07</td>
<td>Genus</td>
<td>Calotropis</td>
</tr>
<tr>
<td>08</td>
<td>Species</td>
<td>Procera</td>
</tr>
</tbody>
</table>

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 medicines. Roots are used for eczema, leprosy, elephantiasis, asthma, cough and rheumatism. Flowers use as anti-dandruff, also used in cholera and dysentery. Leaves are used against swelling, joints pain, sores, skin diseases, rheumatic joints, snake bite, scabies, veterinary medicine, boils, anti-lice and scorpion stings.

Pharmacologically, *Calotropis procera* is used for many activities e.g. dry latex shows anti-diabetic activity, and *Calotropis procera* flowers are used for potent hepatoprotective agent against induced hepatic injuries. *Calotropis procera* roots show anti-tumor activity, while leaves extract show anti-microbial as well as antioxidant activity. Likewise, the ethanolic extract of *Calotropis procera* leaves significantly exhibited the anti-malarial activity.

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 sabzazzar 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 ethanol, 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 coloration²⁳.

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 anthraquinine²⁵.

**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 activities²⁶,²⁷.

**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 use²⁸,²⁹.

**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 spectrophotometrically³⁰,³¹. The % protection was calculated by using the formulas:

\[
\text{Hemolysis \%} = \left(\frac{\text{Abs of test sample}}{\text{Abs of control}}\right) \times 100
\]

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

**RESULTS**

**Phytochemical evaluation**
Ethanolic 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 absent (Table 3).

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

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Phytochemicals</th>
<th>Result</th>
</tr>
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<tbody>
<tr>
<td>01</td>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>02</td>
<td>Alkaloid</td>
<td>++</td>
</tr>
<tr>
<td>03</td>
<td>Terpenoid</td>
<td>++</td>
</tr>
<tr>
<td>04</td>
<td>Flavonoid</td>
<td>++</td>
</tr>
<tr>
<td>05</td>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>06</td>
<td>Phlobatannins</td>
<td>-</td>
</tr>
<tr>
<td>07</td>
<td>Anthraquinone</td>
<td>-</td>
</tr>
<tr>
<td>08</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>09</td>
<td>Cardiac Glycosides</td>
<td>+</td>
</tr>
</tbody>
</table>

++ = 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.

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

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 values.

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. 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.

ACKNOWLEDGEMENTS

I am very thankful to my supervisor Mr Naveed Aslam Dogar and Mr. Farooq Saleem for their guidance and support in this research work.

LIST OF ABBREVIATION

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>DS</td>
<td>Diclofenac Sodium</td>
</tr>
<tr>
<td>ECPL</td>
<td>Extract of Calotropis procera Leaves</td>
</tr>
<tr>
<td>HRBCs</td>
<td>Human Red Blood Cells</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>Non-Steroidal Anti-Inflammatory Drugs</td>
</tr>
</tbody>
</table>

REFERENCES