Qualitative phytochemical screening and antimicrobial studies of *Calotropis gigantea* Linn latex.

Sarkar S* 1, Sen M 2, Bhattacharya P, Ghosh A.

1Pharmacognosy lab, 2Microbiology & Biotechnology lab, Bengal College of Pharmaceutical Sciences and Research, B.R.B. Basu Sarani, Bidhannagar, Durgapur-713212, W.B, India


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**ABSTRACT**

**Background:** *Calotropis* genera comprise of two species, with 90% inhabiting southern Asian country and are most endemic to the India, Indonesia, Malaysia, Thailand, and Srilanka, China. **Objective:** The present project attempts to study the antimicrobial activity of the latex of *Calotropis gigantea* L. **Material and Methods:** The latex of *Calotropis gigantea* L. is subjected to various extractions using ethanol and distilled water. The extracts were subjected to phytochemicals were screening and antimicrobial study was performed against different bacterial species i.e. *E. coli*, *S. aureus*, *S. dysenteriae*, *B. subtilis* using disc diffusion method. **Result:** The aqueous extracts and ethanolic extracts show presence of alkaloid, tannin, flavonoid, saponin and cardiac glycosides. The aqueous extract was inactive against all the samples, i.e. *Escherichia coli*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Bacillus subtilis* and does not show any significant antibacterial properties. The ethanol extract was active only against *Staphylococcus aureus*, *Shigella dysenteriae* and does not show any significant antibacterial properties on the remaining ones. **Conclusion:** Thus it may be suggested that ethanolic latex extracts of *Calotropis gigantea* L. may be used to treat infections caused by *Staphylococcus aureus*, *Shigella dysenteriae*.

**Keywords:** *Calotropis gigantea*; phytochemical screening; Antimicrobial activity; ethanolic extract; zone of inhibition.

**INTRODUCTION:**

The prevalence of invasive, opportunistic bacterial infections is increasing at an alarming rate. This trend has attributed to the increasing use of cytotoxic drugs. The microbes cause a broad spectrum of infections ranging from systemic and potentially fatal diseases to localized cutaneous, subcutaneous or mucosal infections. Based on the knowledge & folklore claims that, plants develop their own defense against microbes they appear as an interesting source for antimalarial compounds. Plant-produced compounds are of interest as sources of safer or more effective substitutes for synthetically produced antimicrobial agents. Since ancient times, plants have been a variable source of drugs, hence research work on medicinal plants is intensified and information on these plants is exchanged. This thought will go a long way in the scientific exploration of medicinal plants for the benefit of man and is likely to decrease the dependence on synthetic drugs with high side-effects.

Medicinal plants are the ‘back bone’ of traditional remedy Plants have been a rich source of drugs because they produce wide array of bioactive molecules, most of which probably evolved as chemical defense against predation or infection. Herbs are widely exploited in the traditional medicine and their curative potentials are well documented. Antibacterial active principle isolated from higher plants is appeared to be one of the important alternative approaches to contain antibiotic resistance and the management of disease. It is believed that plant based drugs cause less or no side effect when compared with synthetic antibiotics.

There are estimated around 2500 effective plant based formulations which are available in the medicinal traditional medicinal texts, India. The Charak Samhita records the use over 340 drugs of vegetable source. Herbal medicine is a major component in all indigenous people’s tradition and a common element in Ayurvedic, Homeopathic medicine. Herbal medicines are preferred to synthetic drugs.

*Calotropis gigantea* L. is a traditional medicinal plant it belongs to the family of *Asclepiadaceous* habitat of Asian countries that includes India, Indonesia, Malaysia, Thailand, Srilanka and China. It is commonly known as milkweed or swallows worth. The plant grows up to 2–4.3 meters long. It has oral, light green leaves and milky stem. The leaves are very much succulent in nature. *Calotropis gigantea* L. is medicinally used to treat disease such as fevers, rheumatism, indigestion, cough, cold, eczema, asthma elephantiasis, nausea, vomiting, leprosy, diarrhoea. *Calotropis gigantea* L. is scientifically reported for its cytotoxic, antipyretic and wound healing activity. The whole plant of *Calotropis gigantea* L. have many medicinal uses;
milky sap is used to stop bleeding, and for treating boils, scabies, burns, bruises, cuts, sores and wounds. The present communication attempts to study the antimicrobial activity of *Calotropis gigantea* L. because the antimicrobial property was more effective against bacteria that are investigated against a few clinically isolated as well as standard microbial cultures of bacteria using disc diffusion method.

**MATERIAL AND METHODS**

**Plant material**

*Calotropis gigantea* plants were randomly and aseptically collected from the medicinal plant garden of Bengal College of Pharmaceutical Sciences and Research, Durgapur-12. The plant was first identified using standard keys and descriptions. It’s botanical identity was further confirmed and authenticated at the herbarium section of the Botanical Survey of India, Howrah-711103, West Bengal, India. Voucher specimen SS/BSPSR/01 were preserved and stored at the herbarium for future reference.

**Plant material and latex collection:**

Here, the latex of *C. gigantea* plant was obtained as exudates by hand plucking of fresh leaves of actively growing plant using aseptic techniques. The fresh latex of *C. gigantea* was collected aseptically from the aerial parts of the healthy plants into two different clean and sterile test tubes. After collection, the containers were cotton-plugged and stored at a temperature of 4°C until required for use. The collections were made in the mornings on the days of each analysis.

**Crude extract preparation**

This was carried out according to the method of percolation. The latex and solvent is taken into two test-tubes containing distilled water and ethanol in each of them to yield a dilution rate of 1:8 (latex: solvent) in both the cases respectively. Each was allowed to stand for two weeks with constant shaking at regular interval at room temperature. The percolates were then filtered and the solvents (ethanol and water) were evaporated to obtain ethanolic and aqueous extracts of the latex. These served as stock solutions which were stored in a refrigerator at 4°C until needed for analysis.

**Phytochemical analysis**

**Test for alkaloids**

*Dragendorff’s test:* A reddish brown precipitate was obtained while treating the extract with Dragendorff’s reagent (Potassium bismuth iodide solution) indicating presence of alkaloids.

*Mayer’s test:* A cream colour precipitate was obtained while treating the extract with Mayer’s reagent (Potassium mercuric iodide solution) indicating presence of alkaloids.

*Wagner’s test:* A reddish brown precipitate was obtained while treating the extract with Wagner’s reagent (Iodine potassium iodide solution) indicating presence of alkaloids.

**Test for tannins**

Treatment with ferric chloride solution gave mild green colour in presence of condensed tannins.

**Test for flavonoids**

*Shinoda test:* To the test few magnesium turnings and concentrated hydrochloric acid drop-wise were added; effervescence occurs after few minutes indicating absence of flavonoids.

*Zinc hydrochloride test:* To the test a mixture of zinc dust and concentrated hydrochloric acid were added, that gave effervescence with a muddy colour after a few minutes indicating absence of flavonoids.

**Test for steroids and triterpenoids**

*Salwoski test:* The extract was treated with few drops of concentrated sulphuric acid, which leads to the formation of milky colour with cloudy appearance indicates absence of triterpenoids.

**Test for saponin glycosides**

*Froth formation test:* 2ml solution of drug in water in a test tube was taken, and shaken frequently; a stable froth is formed in presence of saponin glycosides.

**Test for cardiac glycosides**

*Baljet’s test:* The test solution was treated with picric acid, that gave orange colour indicating presence of cardiac glycosides.

**Test for pentoses**

The extract was treated with same amount of hydrochloric acid and a small amount of phloroglucinol, and heated that gave pale yellow colour in absence of pentose sugar.

**Test for mucilage**

The extract was treated with ruthenium red solution, that gave a cloudy appearance, that indicates the absence of mucilage.

**Test for protein**

Test solution was heated that did not produce any coagulation which indicates the absence of proteins.

**Test for starch**

To the extract addition of weak aqueous iodine solution gave a pale greenish yellow colour indicating absence of starch.

**Microorganisms**

Clinical isolates of bacteria were used for the study. The studies include *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Shigella dysenteriae*. The isolates were obtained from the microbiology department under the Department of Pharmaceutical technology of Jadavpur University, Kolkata. The isolates were maintained on freshly prepared nutrient broth and kept in refrigerator at 4°C until required for use.

**Preparation of extract concentrations**

This was carried out using standard method. Stock solution of the ethanolic extract was prepared by weighing 10 mg and it was dissolved in 1 ml distilled water. This gave an exact concentration of 10mg/ml (stock solution). Two dilutions were prepared from the stock solution i.e. 1mg/ml, 0.1mg/ml. The same procedure was repeated using distilled water for the aqueous extract.
Preparation of sensitivity discs
The sensitivity discs were prepared using sterile Whatman’s No. 1 filter paper. The discs (6.0 ±1.0 mm in diameter each) were prepared by punching the filter paper appropriately. Ten discs were dispensed into each concentration (impregnation by means of sterile forceps). Water was used as control.

Sensitivity testing
Disc diffusion method was employed. The freshly prepared nutrient agar plates were kept in incubator for about 10 minutes to remove surface moisture. The paper discs were sterilized by exposing both sides to UV radiation. The plates were aseptically inoculated uniformly with the test organism by streaking method. With the aid of sterile forceps, impregnated paper discs containing the latex extract of C. gigantea at different concentration were arranged radially and pressed firmly onto the inoculated agar surface to ensure even contact. Each disc was sufficiently spaced out and kept at least 15 mm from the edge of the plate to prevent over-lapping zones. The plates were incubated aerobically at 37°C for 48 hours. Diameters of the zone of inhibition were measured using millimeter ruler, recorded and interpreted.

RESULTS
The data for phytochemical screening of latex of Calotropis in aqueous and ethanol are shown in Table 1 and data for antimicrobial for crude extract in aqueous and ethanol are shown in Table 2 and Table 3 respectively. The phytochemicals are present alkaloid, tannin, flavonoid, saponin, cardiac glycosides. The aqueous extract shows no antimicrobial activity and but the ethanolic extract shows antimicrobial activity against two strains S. aureus and S. dysenteriae.

Table 1: Phytochemical analysis of aqueous and ethanolic latex extracts of Calotropis gigantea.

<table>
<thead>
<tr>
<th>Phytochemical constituents</th>
<th>Aqueous extract</th>
<th>Ethanol extract</th>
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<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tanins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroids and triterpenoids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac glycoside</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pentose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mucilage</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Protein</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Starch</td>
<td>-</td>
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</tr>
</tbody>
</table>

Table 2: Antibacterial activity of aqueous extract of latex of Calotropis gigantea Linn.

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Diameter of zone of inhibition (mm)</th>
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<tbody>
<tr>
<td></td>
<td>10</td>
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<tr>
<td>Escherichia coli</td>
<td>N.Z</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1.73</td>
</tr>
<tr>
<td>Shigella dysenteriae</td>
<td>1.1</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>N.Z</td>
</tr>
</tbody>
</table>

DISCUSSION
The extracts were studied shown antimicrobial activities against S. aureus and S. dysenteriae. Ethanolic extracts inhibited the growth of above two strains. The inhibition zone analysis of the organisms tested suggests that the ethanol extracts of Calotropis gigantea were most active. The S. aureus was inhibited with the maximum zone of inhibition of 1.73 cm.

CONCLUSION
Thus, from the phytochemical analysis of aqueous and ethanolic latex extracts of Calotropis gigantea it can be concluded that alkaloids, condensed tannins, saponin glycosides and cardiac glycosides are present in the latex extracts of the plant and from the antibacterial activity of aqueous extract of latex of Calotropis gigantea Linn it is clear that the aqueous extracts of the plant don’t have any antimicrobial effect on the microbes chosen i.e. Escherichia coli, Staphylococcus aureus, Shigella dysenteriae, Bacillus subtilis. While the antibacterial activity of ethanolic extract of latex of Calotropis gigantea Linn. Shows activity against only Staphylococcus aureus, Shigella dysenteriae while Escherichia coli, Bacillus subtilis shows no activity.

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REFERENCES


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