Phytochemical Investigation and Assessment of Antibacterial Activity of Calotropis procera Leaf

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ABSTRACT

The aim of the present study was to find out antimicrobial property of calotropis procera (C. procera), family Asclepiadaceae. The plant is referred to number of names such as Giant swallow Wort, Milk Weed, Sodom apple and also local names like Madar, Akanda, Arks, Aak. In ayurvedic all parts of the tree used in different healing procedures for different diseases. The plant is also known for its toxic properties that include iridocyclites, dermatitis and acts like a poison and produces lethal effects. The potentials of C. procera leaves in water treatment and its ability to reduce total viable count have also been reported. The phytochemical screening indicated the presence of alkaloid, tannin, flavonoid, steroid in the extracts. The presence of alkaloids calotropin, calotaxein and uskerin has been reported and are stimulant to heart. The antibacterial effect of Methanol and aqueous extracts of leaf of C. procera on different bacteria namely, Escherichia coli, Staphylococcus aureus, Staphylococcus epidermid, Proteus vulgaris, Pseudomonas aeruginose, Bacillus subtilis, Micrococcus luteus and determined using agar well diffusion method. Agar well diffusion method allows better diffusion of the extracts into the medium thus enhancing contact with the organisms. Antibacterial activity was expressed as the zone of inhibition (mm) produced by the plant extract compared with the streptomycin (positive controls). The Methanolic and Aqueous extract shows antibacterial activity on the Escherichia coli, Staphylococcus aureus, Proteus vulgaris. The result of this study justifies the use of water extracts of C. procera in ethnomedicine for the treatment of infectious diseases caused by susceptible bacterial species. The aqueous extract shows good antibacterial activity than methanolic Extract.

Keywords: Antibacterial, Calotropis procera, Leaf Extract, Phytochemical screening.

INTRODUCTION

Calotropis Procera (C. procera) is one of the species of family Asclepiadaceae, native to tropical and subtropical Asia and Africa, in dry sandy and alkaline soils in waste land and grows abundantly as a Weed, Nigeria and Bangladesh.¹ The plant is referred to number of names such as Giant swallow Wort, Milk Weed, Sodom apple and also local names like Madar, Akanda, Arks, Aak.² It is a shrub which grows up to 5.5 m high, occasionally branchless to a height of 2.5 m. Leaf is large ovate, opposite, sessile, up to 30 cm long and 16 cm wide shown in figure¹.³ C. procera is used as a folk medicine in traditional system for the various diseases like skin disease, analgesic, antimicrobial, antioxidant, purgative, tumor , paralysis .⁴ It is either used alone or with other herbs to treat common diseases such as fevers, rheumatism, indigestion, cold, eczema and diarrhea.⁵ The methanolic extract of leaves of C. procera was subjected to the antioxidant & antibacterial activity.⁶ The plant is also known for its toxic properties that include iridocyclites, dermatitis and acts like a poison and produces lethal effects. The whole plant when dried and consumed is a good tonic, antiemetic and as an expectorant.⁷ Leaf extracts, chopped leaves and latex of C. procera have shown great promise as a nematicide in vitro and in vivo.⁸ The potentials of C. procera leaves in water treatment and its ability to reduce total viable count have also been reported.⁹ The dry leaves in northern Nigeria are used as a remedy for asthma, cough, etc.¹⁰ Plants have provided a good source of emetine, quinine, berberine, tannins, terpenoids, alkaloids and flavonoids.¹¹ The presence of alkaloids calotropin, calotaxein and uskerin has been reported and are stimulant to heart.¹²

Figure 1: Calotropis Procera

MATERIALS AND METHODS

Collection of plant materials

The experiment was conducted in the year 2013 in the college. Leaves were collected from the C. procera in the Herbal Garden. The species was identified and authenticated at the Herbarium Department of Biological Sciences, M.S. University, Vadodara, Gujarat. It was ensured that the plant was healthy and uninfected. The leaves were washed under running tap water to eliminate dust and other foreign particles and to cleanse the leaves thoroughly and dried.
Source of Microorganism

The Strains of organisms used were Escherichia Coli (MTCC No:40), staphylococcus aureus (MTCC No:87), staphylococcus Epidermidis (MTCC No:9041), Proteus vulgaris (MTCC No:742), Pseudomonas aeruginose (MTCC No:424), Bacillus subtilus (MTCC No:441), Micrococcus luteus (MTCC No:106). The organism were obtained from MTCC Chandigadh and maintain according to specification. Subculturing was done at every 15 days interval.

Preparation of Methanolic, ethanolic and aqueous leaf extract

Methanolic Extract

The leaf powder was dissolved in 100 ml of methanol. The suspended solution was left to stand for 2 to 5 days. The extract filtered using sterile Whatman no. 1 filter paper. The extract was stored at 4°C.13

Aqueous Extract

The leaf powder was dissolved in 100 ml of water. The suspended solution was left to stand for 2 to 5 days.

The extract filtered using sterile Whatmatman no. 1 filter paper. The extract was stored at 4°C.13

Phytochemical Analysis

Qualitative tests for alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins, Terpenoids, Proteins and Anthraquinone were performed according to the procedure describe.14

Alkaloids

Wagner’s Test: In 1ml of Extract added 0.5ml of wagner reagent.

Hagger’s Test: In 1ml of Extract added 0.5ml of hagger reagent.

Dragondroff’s Test: In 1ml of Extract added 0.5ml of dragondroff reagent.

Baljet Test: In 1ml of Extract added 1ml of sodium picrate.

Flavonoids

3ml of each extract was added to 10ml of distilled water the solution was shaken. 1ml of 10%NaOH solution was added to the mixture.

Steroids

Salkowski Test: 2 ml extract, add 2ml chloroform and 2ml concentrated sulphuric acid, shake well and chloroform layer appear red and acid layer shows greenish yellow fluorescence.

Tannins

1ml extracts and add few drop of lead acetate.

Glycoside

25ml of dilute sulphuric acid was added to 5ml extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10%NaOH, then 5ml of Fehling solution added.

Cardiac glycoside

Legal Test: In 1ml Extract added 1ml sodium, 1ml pyridine and sodium hydroxide added.

Determination of Antimicrobial Activity

The antimicrobial activities of aqueous and Ethanol, Ethanolic extracts were determined by agar well diffusion methods as described.

Agar well diffusion method

The culture plates seeded with test organisms were allowed to solidify and punched with a sterile cork borer (6.0 mm diameter) to make open wells. The wells were filled with 0.05 ml of the extract. The plates were incubated at 37°C for 24 hours. The zones of inhibition were measured and recorded.13

RESULTS AND DISCUSSION

Results

The present study reveals that C.Procera plant shows the presence of phytochemical constituents like alkaloids, flavonoids, carbohydrates, glycosides, proteins, saponins, tannins, terpenoids and anthraquinones in different solvent extracts as shown in Table 1.

The potential sensitivity of the extract was obtained against all the microorganisms tested and the zone of inhibition was recorded and presented below in the tabulation drawn (Table 2).

Discussion

The results of phytochemical screening of water, methanol and ethanol leaf extracts of C.procera revealed the presence of alkaloids, flavonoids, saponins, tannin and glycosides Table 1. The concentrations of the various classes of secondary metabolite vary amongst the extracts evaluated. The presence of these components in this species is an indication that it may have some medicinal potential. This is due to the fact that each of the components identified has one therapeutic usage or another. For instance, plants rich in saponins have immune boosting and anti inflammatory properties.15 Similarly, tannins have been reported to have antibacterial potential due to their basic character that allows them to react with proteins to form stable water soluble compounds thereby killing bacteria by directly damaging its cell membrane.16 The antibacterial activities of alkaloids and flavonoids have been reported by a number of authors.17 The antibacterial activity of C. procera water, methanol extracts obtained from the leaves on the test organisms exhibited antibacterial activity Table 2. Generally, E.coli, S.aureus, P.vulgaris were observed to be the most susceptible organisms.
Agar well diffusion method allows better diffusion of the extracts into the medium thus enhancing contact with the organisms. Similarly, *Staphylococcus aureus* is least susceptible in this study and same observation was reported. The methanol extracts of *C. procera* did not show appreciable activity against *p.vulgaris*. While methanolic and aqueous extract did not show zone of inhibition against the *p.aeuginosa*, *B.subtilus*, *M. luteus*. Antibacterial activity was expressed as the zone of inhibition (mm) produced by the plant extract compared with the streptomycin (positive controls). The result of this study justifies the use of water extracts of *C. procera* in ethnomedicine for the treatment of infectious diseases caused by susceptible bacterial species.

### Table 1: Phytochemical constituent Present in various Extracts

<table>
<thead>
<tr>
<th>Solvents used for Extraction</th>
<th>Alkaloid</th>
<th>Flavanoid</th>
<th>Steroid</th>
<th>Tannin</th>
<th>Glycoside</th>
<th>Cardiac glycoside</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Water</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

KEY: + (Present) , - (Absent)

### Table 2: Antibacterial Activity of Various Leaf Extract on Microorganism

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control streptomycin</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>5mm</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>5mm</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>6mm</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>5mm</td>
</tr>
<tr>
<td><em>Bacillus subtilus</em></td>
<td>6mm</td>
</tr>
<tr>
<td><em>Staphylococcs epidermidid</em></td>
<td>6mm</td>
</tr>
<tr>
<td><em>Micrococcus luteus</em></td>
<td>5mm</td>
</tr>
</tbody>
</table>

KEY: - No Inhibition

### CONCLUSION

These findings suggest a new pathway in elucidating a potent antibacterial agent from *C. procera*. Present study indicates that the plant contains antibacterial compound that can be further developed as phytomedicine for the therapy of infection. Such screening of various natural organic compounds and identification of active agents is the need of the hour because successful prediction of lead molecule at the onset of drug discovery will pay off later in drug development. Lastly, to conclude the extracts were found to inhibit the growth of Gram positive bacteria as well as the Gram-negative bacteria.

### REFERENCES


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