



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 epidermidis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *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 diarrhoea.⁵ The methanolic extract of leaves of *C.Procra* 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, antihelmintic 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.Procra* 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 aeruginosa* (MTCC No:424), *Bacillus subtilis* (MTCC No:441), *Micrococcus luteus* (MTCC No:106). The organisms were obtained from MTCC Chandigarh and maintained 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 Whatman 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 described.¹⁴

Alkaloids

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

Hager's Test: In 1ml of Extract added 0.5ml of Hager reagent.

Dragendorff's Test: In 1ml of Extract added 0.5ml of Dragendorff 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 Ethanolic, 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.¹⁸ Similarly, *Staphylococcus aureus* is least susceptible in this study and same observation was reported.¹⁹ 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).²⁰ 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

Solvents used for Extraction	Alkaloid	Flavanoid	Steroid	Tannin	Glycoside	Cardiac glycoside
Methanol	+	-	+	+	-	-
Water	+	+	+	+	+	-

KEY: + (Present) , - (Absent)

Table 2: Antibacterial Activity of Various Leaf Extract on Microorganism

Microorganism	Zone of inhibition (mm)		
	Control streptomycin	Methanolic extract	Aqueous extract
<i>Escherichia coli</i>	5mm	4mm	3mm
<i>Staphylococcus aureus</i>	5mm	3mm	5mm
<i>Proteus vulgaris</i>	6mm	-	2mm
<i>Pseudomonas aeruginosa</i>	5mm	-	-
<i>Bacillus subtilus</i>	6mm	-	-
<i>Staphylococcs epidermidid</i>	6mm	-	-
<i>Micrococcus luteus</i>	5mm	-	-

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

- Perwez A, Mohammad A, Phytochemical investigation of *Calotropis procera* in roots, Indian Journal of chemistry, 48B, 2008, 443-446.
- Irvine FR, Woody plants of Ghana, Oxford University Press, 1961, 48-50.
- Aliu BS, Common ethnomedicinal plant of the semi arid region of west Africa their description and phytochemicals, Triumph publishing company limited kano, Nigeria, 2006, 193-196.
- Vohra R, *Calotropis* the medicinal weed, Online medicinal book store, India, 2004.
- Kew F, The useful plants of west tropical Africa, Royal botanical gardens publications United States of America, 1985, 219-224.
- Gaurav K, Karthik L, Kokati Venkata Bhaskara R, Antibacterial activity of aqueous extracts of *Calotropis gigantea* leaves – An invitro study, International journal of pharmaceutical Sciences Review and Research, 2010, 141-144.
- Agharkar SP, Medicinal plant of Bombay Presidency, Scientific Publication, 1991, 48-49.
- Khirstova P, Tissot M, Soda–Anthroquinone pulping of *HibiscusSabdarriffa* (Karkadeh) and *Calotropis procera* from Sudan, Bioresource Technology, 53, 1995, 677-672.
- Shittu BO, Popoola TOS, Taiwo O, Potentials of *Calotropis procera* leaves for Wastewater treatment, Proceedings of the International Conference on Science and Natiional Development held at University of Agriculture, Abeokuta, 2004, 97-101.
- Oke OA, Afolayan AJ, Oyedeji AO, Validation Of Antibacterial Potetials Of *Anacardium Occidentale* *Calotropis Procera* Commy used agaist Cough Symoptoms, Journal Of Research and Review in science, 3, 2004, 56-60.
- Marjorie MC, Plant Products as Antimicrobial agents, Clinical Microbiology Review, 1991, 562-582.
- Ashwari k, Productivity of *Calotropis procera* and its use in renewable energy science Blogging, Retrieved on 5/01/2010 from <http://www.scientificblogging.com>, 2009.
- Kareem SO, Akpan I, Ojo OP, Antimicrobial Activities of *Calotropis procera* on Selected Pathogenic Microorganisms, African Journal of Biomedical Research, 11, 2008, 105-110.
- Khandelwal KR, Pratical Pharmacognosy, Nirali prakashan, 12th edition, 25.1-25.6.



15. Kenner D, Requena Y, Botanical medicine: A European Professional Perfective, Paradigm Publication Brookline, Massachusetts, 1996, 12.
16. Cheesbrough M, Discript Laboratory Praticce in Tropical Countries, Part 1, Cambridge University Press, London, 2001, 157-206.
17. Hassan SW, Umar RA, Ebbo AA, Matazu IK, Phytochemical, Antibacterial and toxicity Studies of Parkinsonia aculeate L. (sFabaceae), Nigerian gernal of Biochemistry and Molecular Biology, 20(2), 2005, 89-97.
18. Omeka CA, JO Osuoha, Antibacterial Potency of Grapefruit Seed extract on five Selected Pathogens, Nigerian Journal Of Microbiology, 14(2), 2000, 39-42.
19. Alierio AA, Aliero BL, Buhari U, Preliminary Phytochemical and Antibacterial Screening of Scadoxus multiflorus, International Journal of Pure and applied Sciences, 2(4), 2008, 13.
20. Hussain t, Muhammad A, Sarzamin K, Hamid S, Muhammad Subhan Q, In vitro Screening Of Menthol Plant extract, For their Antibacterial activity, Pak.J.BOT., 43(1), 531538, 2011.

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