



Antibacterial Activity and Phytochemical Analysis of Leaf Extracts of *Calotropis procera*

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Abstract

Phytochemical and antibacterial activities of petroleum ether, chloroform and ethanolic extract obtained from the leaves of *Calotropis procera* were investigated in an attempt to evaluate its medicinal potentials. The phytochemical screening revealed the presence alkaloids, saponins, tannins, flavonoids, amino acids and other phenolic compounds with a very high content in ethanolic extracts. The concentration of the phytochemical constituents were in the order of ethanol > chloroform > petroleum ether. Antibacterial activity was determined against *E. coli*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae* was carried in well diffusion method. The zone of inhibition produced by the crude extracts of petroleum ether, chloroform and ethanol extracts against sensitive bacteria. The results obtained showed that petroleum ether, chloroform and ethanol extracts of *Calotropis procera* Linn, had inhibitory effect on the growth of isolates. The effect exhibited by ethanolic extract of leaves was significantly greater than the other two. The obtained results provide a support for the use of *Calotropis procera* Linn, in traditional medicine and suggest its further advance investigation.

Keywords: Antibacterial Activity; Phytochemical Analysis; *Calotropis procera*

Introduction

World over the medicinal plants are used as a main source of traditional and orthodox medicines. The attention has been made towards developing the new antibiotics that reduce the increasing resistance among the microorganisms [1]. The medicinal plants generally contain number of compounds that may be potential natural antimicrobial agents which may serve as alternative, effective, cheaper and safe antimicrobial agents for the treatment of common microbial infections [2].

Calotropis procera belongs to family Asclepiadaceae. The whole plant has strong odour and found to be in growing in all rural areas. Medicinal properties of this plant are antiseptic, anti-inflammatory, analgesic and useful in eczema and also for thrush and in mycotic enteritis [3]. The whole plant when dried and consumed is a good tonic, anti-helminthic and as an expectorant [4]. The roots, besides being endowed with similar qualities serves as an effective laxative. Traditionally, the dried root is powdered and effectively used to cure bronchitis, asthma, leprosy, eczema and elephantiasis, hepatic and splenic enlargement [5]. In the present we provide new information on the antibacterial activities of leaves of *Calotropis procera* using known microbial pathogens as test organisms.

Materials and Methods

Plant Material: Leaves of *Calotropis procera* were collected from Bhopal city of Madhya Pradesh state in the month of January. The species were identified by senior taxonomists from botany department of M.V.M. College Bhopal (M.P). The leaves collected were room dried and pulverised into powder and powdered part were subject to organic solvent extraction.

Extraction and Preparation of Material for Phytochemical Screening

Continuous hot percolation 'Soxhlation' method was used for extraction. The advantage of this method, compared to previously other methods of extraction, is that large amounts of drug can be extracted with a much smaller of solvent quantity. Powder of leaves of *Calotropis procera* was placed in thimble of Soxhlet apparatus. Extraction was performed at 40°C using petroleum ether as non-polar solvent at first. Extraction was continued for a period of 12 hours. The yield of pet ether extract was 4.20 grams. Exhausted plant material (marc) was dried and afterward was extracted with Chloroform. The extraction was continued for a period of 13 hours at 40°C, the yield was 6.5 grams. Marc obtained after chloroform extraction was subjected to extraction with ethanol. The extraction was continued for a period of 14 hours 30 minutes for complete extraction at the same temperature and the yield was 7 grams. The extracts thus obtained was used for pharmacological screening.

Organisms

The bacterial species used: *E. coli*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae* obtained from Pinnacle Biomedical Research institute Bhopal (M.P).

Antibacterial activity

By plant extracts the growth of inhibition of bacteria was determined by using well diffusion method. The nutrient agar (Muller Hilton) plates were prepared and seeded with the test organisms. In a plate four wells were prepared at equidistance to each other. Extracts of different concentration 62.5 mg/ml, 125 mg/ml, 187.5mg/ml and 250 mg/ml were instilled into the well at volume

of 50 µl in each well. Plates were placed in incubator set to 35°C within 15 minutes. After 1 hour, plates were inverted and again placed in incubator for about 18 - 24 hrs. The plates were examined for evidence of zone of inhibition which appear as a clear area around the holes. The diameter of such zone of inhibition was measured by vernier caliper and the value was recorded and expressed to the nearest millimeter.

Phytochemical screening

The methods described by Trease and Evans [6], was used for Phytochemical screening.

Results and Discussion

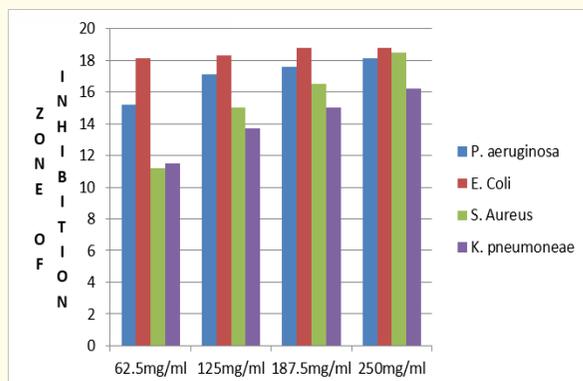
Phytochemical screening of crude extracts was done by applying different tests for different Phytochemicals. The results shows that, Tannins were found in Petroleum ether extract. Alkaloids, Tannins and Amino acids compounds were found in Chloroform extract., Flavonoids, Amino acids, Alkaloids, Saponins, Tannins and phenolic compounds were found in ethanolic extract. The results have shown that the main components i.e., flavonoids, alkaloids, tannins and phenolic compounds were present in ethanolic extract. The presence of these compounds were present in *Calotropis procera* [7]. Majority of the compounds were present in ethanol extract. These compounds are found to be more useful for anti-bacterial activity [8].

Phytochemicals	Petroleum ether extract (leaves)	Chloroform extract (leaves)	Ethanol extract (leaves)
Alkaloids	-	+	+
Tannins	+	+	+
Amino acids	-	+	+
Flavonoids	-	-	+
Saponins	-	-	+
Carbohydrates	-	+	-
Glycosides	+	-	-

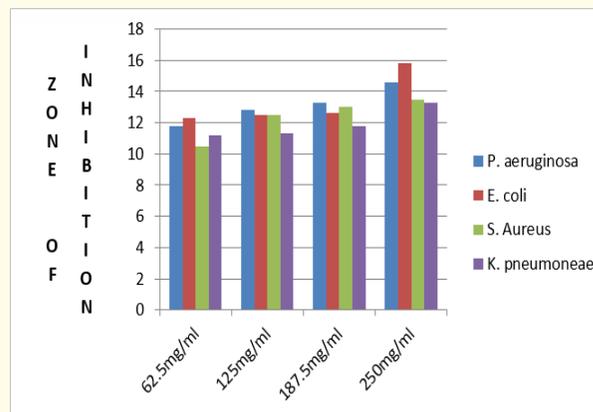
Table: Phytochemical constituents of *Calotropis procera*.

The results of antibacterial activity measured in terms of diameter of zone of inhibition, the antibacterial activity of *Calotropis procera* on the test organisms using petroleum ether, chloroform and ethanolic extracts of leaves of *Calotropis procera* exhibited antibacterial activity against Gram positive and Gram negative bacteria. i.e. *E. coli*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae*. It was found that antibacterial activity of petroleum ether, chloroform and chloroform against these pathogens at selected concentrations 62.5 mg/ml, 125 mg/ml, 187.5 mg/ml and 250 mg/ml. Zone of inhibition was found to be maximum for ethanolic than petroleum ether and chloroform also reported by [9].

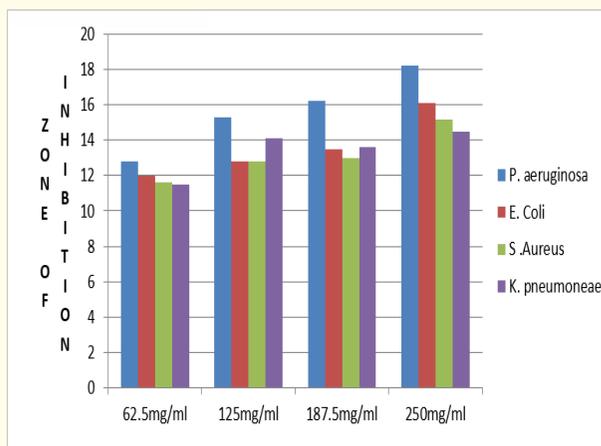
The results obtained in the present study shown that the Petroleum ether, Chloroform and ethanol extracts extract of *Calotropis procera* and its purified isolated bioactive compound from ethanolic extracts (Rutin disaccharide) displayed significant antibacterial activity.



Graph 1: Antibacterial activity of Ethanolic extract of *Calotropis procera*.



Graph 2: Antibacterial activity of Chloroform extract of *Calotropis procera*.



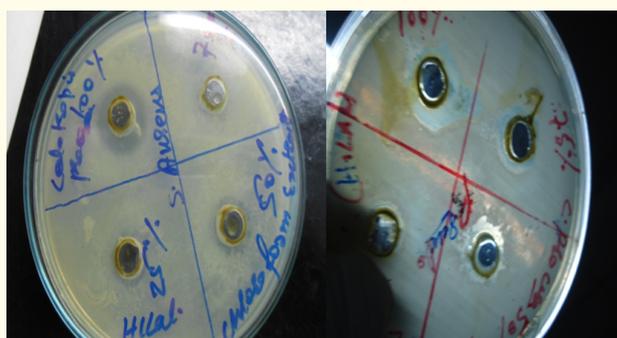
Graph 3: Antibacterial activity of pet. Ether extract of *Calotropis procera*.



Zone of Inhibition against *S. aureus* Zone of Inhibition against *E. coli*.



Zone of Inhibition against *Pseudomonas* Zone of Inhibition against *Klebsiella*



Zone of Inhibition against *S. aureus* Zone of Inhibition against *Pseudomonas*

Conclusions and Recommendations

Based on the pharmacological results of the present study, it could be said that the plant extracts contain chemical constituents of pharmacological significance. The presence of these chemical constituents in this plant is an indication that the plant, if properly screened using additional solvents, could yield drugs of pharmaceutical significance. Further research is therefore recommended to isolate, purify and characterise these chemical constituents with a view to supplementing conventional drug development especially in developing countries.

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