

**NOTE****Antimicrobial Activity of *Calotropis procera* Seeds**

V.H. BHASKAR\* and S.S. AJAY

Department of Pharmaceutical Chemistry, Vinayak Missions University, Salem-636 001, India

Fax:/Tel: (91)(427)3987000; E-mail: ajay\_singh\_20042004@yahoo.com

The present study is related to evaluate antimicrobial activities of chloroform and methanol extracts of seeds of *Calotropis procera* located in the forest area of Ghaziabad, India. Chloroforms extract of *Calotropis procera* seeds exhibited better antimicrobial activity. On the other hand, the extracts obtained *Calotropis procera* seeds tested have been evaluated for their possible *in vitro* antibacterial activities based on paper disc method.

**Key Words:** *Calotropis procera* seeds, Asclepiadaceae, Antimicrobial activity.

*Calotropis procera* is large shrubs belong to the family Asclepiadaceae. The plant grows mainly in semi-arid tropical areas. It grows best in dry sandy and alkaline soils and warm climate. It is distributed in India and Africa. It is grown well as Rubbiss heap, waste<sup>1</sup>. The plant is indigenously known as Alarka. In Sanskrit, Alarka, in Hindi Akha, English Name milkweed. The various medicinal properties have been attributed to the plant *Calotropis procera*, in a folk medicine as antiinflammatory activity and anticancerous property. It is also used for the treatment of skin disease, enlargement of abdominal viscera, intestinal worm, cough, ulcer and as anti bacterial in traditional medicine *i.e.* (latex of *Calotropis procera*) reported for the anti bacterial activity. Phytochemically the plant is reported to contain glycoside, alkaloid, flavonoids<sup>2</sup> and reducing antiinflammatory activity<sup>3</sup>.

Plant samples of *Calotropis procera* seeds were collected from different locations in forest area of Ghaziabad, India. The taxonomic identification of plant materials was confirmed by raw material herbarium and museum, National Institute of Science communication and Information Resources. They were assigned voucher specimen/ Ref. NISCAIR/RHMD/CONSULT/2008-09/996/27/ and were deposited in Herbarium Department. The collected plant materials were dried in the shade and the seeds of plant were separated from the stem and ground in a grinder with a 2 mm in diameter mesh<sup>4</sup>.

Methanol and chloroform were obtained from Sigma (St. Lonis, MO). All other solvents were of analytical grade and obtained from Merek (Darmstadt, Germany).

**Preparation of extracts:** The dried and powdered seeds (500 g) were extracted with 1 L of chloroform and methanol using soxhlet extractor (ISOPAD, Heideberg, Germany) for 72 h at a temperature not exceeding the boiling point of the solvent<sup>5,6</sup>, respectively. The extract was filtered using Whatman filter paper No. 1 and then concentrated *in vacuo* at 40 °C using a rotary evaporator (BUCHI Labortechnic AG., Flawil, Switzerland). The residues obtained were stored in a freezer (Nauire, polymouth, USA) at -80 °C until further tests.

#### Antimicrobial activity<sup>6</sup>

**Microbial strain:** Chloroform and methanol extracts were tested individually against a range of 7 microorganisms. Microorganisms were provided by Department of Microbiology, Santosh Medical College, Ghaziabad, India, The identify of the microorganisms used in this study was confirmed by the microbial identification system in Department of Biotechnology, C.P.S. Mohuda, India.

**Bacterial cultures:** The test organisms<sup>7</sup> used in this study were as follow: *Staphylococcus aureus* MTCC 737, *Bacillus subtilis* MTCC, *Escherichia coli* MTCC 118, *Mycobacterium smegmatis* MTCC 106, *Aeromonas hydrophila* ATCC 79, *Morganella morganii* ATCC 102, *Proteus vulgaris* MTCC 201.

**Assay procedure (paper disc method):** The peptone solution was poured on the solidified agar medium<sup>8,9</sup>. The contact time was 3-5 min. Excess peptone solution was removed by inverting the plate or with the help of micro pipette (per sterilized in oven of 3 h at 110 °C in order to avoid contamination sterile Whatman filter paper disc (6 mm diameter) were thoroughly moistened with different concentrations of chloroform and methanol extract and chloroamphenicol disc moistened with strile DMF was used as control<sup>9</sup>. The paper discs were previously sterilized in autoclave by moist heat sterilization. The disc were placed on the plate with the help of fine pointed previous sterilized forceps. Then the plates were incubated at  $37 \pm 1$  °C. All work was done under aseptic condition<sup>10</sup>. The assay plates were kept at 5-10 °C for 24 h. After incubation the bacterial activity was determined by zone of inhibition method where diameter was measured by using millimeter scale<sup>11</sup>.

TABLE-1  
ANTIBACTERIAL ACTIVITY OF CHLOROFORM  
EXTRACT OF *Calotropis procera* SEEDS

Test microorganism	CC (100 µg/mL)	Concentration of extract			Control DMF
		1 mg/mL	5 mg/mL	10 mg/mL	
<i>Staphylococcus aureus</i>	20.65±0.48	15.35±0.16	18.64±0.54	23.16±0.43	7.35±0.12
<i>Bacillus subtilis</i>	20.23±0.66	13.10±0.11	17.49±0.75	22.41±0.13	7.35±0.12
<i>Escherichia coli</i>	15.56±0.35	12.32±0.31	14.28±0.61	20.03±0.15	7.35±0.12
<i>Mycobacterium smegmatis</i>	16.23±0.41	10.21±0.10	12.11±0.61	19.13±0.10	7.35±0.12
<i>Aeromonas hydrophila</i>	15.17±0.20	12.21±0.32	14.70±0.21	18.21±0.14	7.35±0.12
<i>Morganella morganii</i>	18.73±0.31	11.30±0.12	15.71±0.60	20.41±0.38	7.35±0.12
<i>Proteus vulgaris</i>	18.90±0.32	14.15±0.61	16.52±0.40	21.01±0.16	7.35±0.12

CC = Concentration of chloramphenicol.

In this screening, the different gram-positive and gram-negative bacteria used are *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *M. morganii*, *P. vulgaris*, *M. smegmatis* and *A. hydrophila* out of all the extract. The chloroform extract was most effective as compared to methanol extract. All extract are having good antibacterial activity, but chloroform extract is having the best activity (Tables 1 and 2). The activity may be due to the presence of flavonoids<sup>2</sup>.

TABLE-2  
ANTIBACTERIAL ACTIVITY OF METHANOL  
EXTRACT OF *Calotropis procera* SEEDS

Test microorganism	CC (100 µg/mL)	Concentration of extract			Control DMF
		1 mg/mL	5 mg/mL	10 mg/mL	
<i>Staphylococcus aureus</i>	20.65±0.48	12.35±0.55	15.46±0.51	18.12±0.54	7.35±0.12
<i>Bacillus subtilis</i>	20.23±0.66	11.24±0.62	14.86±0.61	17.40±0.25	7.35±0.12
<i>Escherichia coli</i>	15.56±0.35	10.22±0.32	11.63±0.52	13.45±0.41	7.35±0.12
<i>Mycobacterium smegmatis</i>	14.16±0.18	09.15±0.13	11.50±0.44	13.21±0.16	7.35±0.12
<i>Aeromonas hydrophila</i>	14.20±0.16	10.13±0.21	12.61±0.26	16.11±0.11	7.35±0.12
<i>Morganella morganii</i>	17.19±0.15	10.20±0.11	12.01±0.19	14.11±0.30	7.35±0.12
<i>Proteus vulgaris</i>	16.25±0.41	11.41±0.51	13.20±0.14	14.21±0.16	7.35±0.12

CC= Concentration of chloramphenicol.

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