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Antipathogenic Efficacy and Hemolytic Activity of *Calotropis procera* Leaves

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Abstract: The work delt with the growth-inhibitory impact of methanolic extract of *Calotropis procera* leaves on five pathogenic bacteria, analysed by agar diffusion and broth dilution methods. By agar diffusion method, the extract was found very effective against *B. subtilis* (MIC= 2.5 mg), while moderate activity was noted against *P. mirabilis* (MIC= 5 mg). Zone of inhibition was not found for *P. aeruginosa, S. aureus* and *S. typhi* for the concentrations screened. MIC was found 9 and 8 mg for *S. aureus* and *S. typhi* respectively by broth dilution method. The haemolytic activity exhibited by the crude extract showed time and concentration dependence. The present findings suggested that *Calotropis procera* is a potential source of antimicrobial compounds.

Key words: *Calotropis procera* · MIC · Antibacterial · Hemolytic Activity

INTRODUCTION

Infectious diseases pose serious problems to health and they are the main cause of morbidity and mortality worldwide [1]. Recent trends show the failure of chemotherapeutics due to emergence of multiple drug resistance, re-emergence of infections and changing pattern of susceptibility [2-4]. This calls for the discovery of new antimicrobial compounds.

Plant-derived medicines have been a part of traditional health care in most parts of the world for thousands of years [5] and continue to be almost exclusive source of drugs for a major part of the world even today [6]. Hence researchers are turning their interests towards the field of ethno-botany which provides immense prospects for the new drug leads pertaining to the unequalled availability of chemical diversity [7-14].

India harbors rich diversity of medicinal plant species, which are known to produce several bioactive metabolites and are being used in the folk medicine system [15, 16]. One such plant is *Calotropis procera* (Asclepiadaceae). It is a perennial shrub commonly found in the tropical parts of the world, including Asia, Africa and Arabian Peninsula. It has also been reported in Australia, Mexico, South-Central America and in few Caribbean and Pacific islands [17]. In traditional and folk medicine systems, *C. procera* has been used to treat a variety of ailments like leprosy, fever, menorrhagia, malaria, headache and rheumatism [18, 19] in the Sudani, Unani, Arabic and Indian traditional medicine systems [20]. It has also been used as *in vitro* and *in vivo* nematicide and as an antidote to snake bite [21, 22]. There is however, an insufficiency of information regarding the antibacterial efficacy of the leaf extract of the plant. Also, information regarding the haemolytic activity of *C. procera* leaf is lacking.

With this background, the present study was undertaken to assess the antibacterial efficacy of the methanolic leaf extract of *Calotropis procera*, along with study of haemolytic activity of the aqueous extract.

MATERIALS AND METHODS

Collection of Plant Material: The fresh mature leaves of *C. procera* were collected from Ranchi district of Jharkhand state (India) during February' 2013. Samples were washed with deionized water and disinfected with 0.1% $HgCl_2$ solution for five minutes, chopped into small pieces and shade dried. Upon drying, the sample was powered in an electric grinder, sieved with fine mesh and stored in air-tight container for further use.

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Preparation of Extract: 50 g of the powder was subjected to extraction by soxhlet using methanol and distilled water separately. The extract obtained was filtered, concentrated after dryness in rotary flash evaporator maintained at 45°C, percentage yield of each extract was calculated and the dried extract was stored in air tight containers at room temperature for further studies.

Antibacterial Assay: The antibacterial activity of the extracts was determined using five Gram positive and Gram negative bacteria: *Bacillus subtilis, Staphylococcus aureus*, *Proteus mirabilis Pseudomonas aeruginosa* and *Salmonella typhi* by agar diffusion and broth dilution methods. In agar diffusion method, following Threlfall *et al.* [23], the agar plates were prepared and wells were made in the plate. Each plate was inoculated with 18 h old cultures (100 µl) of the selected bacteria and spread evenly on the plate. After 20 min, the wells were filled with different concentrations of sample extracts. The control wells were filled with Gentamycin along with solvent. All the plates were incubated at 37°C for 24 h and the diameter of inhibition zones were noted.

Growth inhibition by methanolic extract was measured (in percentage) by broth dilution method, as proposed by Walker [24]. The tubes containing the culture media were prepared, autoclaved and respective concentrations of the samples were added. Each tube was inoculated with 18 h old cultures (100 µl). A control tube with inoculums and without any sample was prepared along with a sterile media tube as blank. All the tubes were incubated at 37°C on a shaker with 140 rpm for 24 h. The inhibition % was measured at 660 nm.

Hemolytic Activity: Hemolytic activity of the aqueous leaf extract was determined using goat blood. An erythrocyte suspension was prepared by adding 5% (by volume) of sodium citrate (36.5 g/l) to fresh blood and centrifuged at 3000 rpm for 5 min to separate the erythrocytes. 2% erythrocyte suspension was prepared by adding 49 mL phosphate buffer (pH 7.4) to 1 mL packed erythrocytes. Serial dilution of plant extracts were prepared using phosphate buffer. 1 ml of citrated blood was mixed with equal volume of diluted plant extracts and the volume was adjusted to 5 mL by phosphate buffer. The mixture was allowed to stand for 6 hrs at room temperature. Hemolysis was monitored spectrophotometrically at 540 nm, depicted by an increase in the optical density of the solution due to the release of hemoglobin through time [25, 26].

RESULTS AND DISCUSSION

The results were depicted in Table 1 and Figures 1-6.

Antibacterial Efficacy: Table-1 shows the zone of inhibition obtained by methanolic leaf extract of *C. procera* against the selected Gram positive and Gram negative bacteria. Minimum inhibitory concentration (MIC) values represent the efficacy of the extract against the bacteria. The lower the MIC, more effective is the extract against that bacterium.

It is apparent from the table that the extract showed considerable activity against *B. subtilis* (MIC = 2.5 mg), while moderate activity was shown against *P. mirabilis* (MIC = 5 mg). MIC values were not obtained for the extract against *S. aureus, P. aeruginosa* and *S. typhi* for the concentrations screened.

The MIC values obtained by the broth dilution method were 2.5, 9, 5, 10 and 8 mg for *B. subtilis, S. aureus, P. mirabilis, P. aeruginosa* and *S. typhi* respectively and Figures 1-5 depict the growth inhibitory kinetics of the five above stated bacteria respectively. By broth dilution method too, the methanolic leaf extract of *C. procera* was found most effective against *B. subtilis* among the studied bacteria, while moderate activity was noted against *P. mirabilis*. At higher concentrations, the extract also inhibited *S. aureus, P. aeruginosa* and *S. typhi.*

Table 1: Zone of inhibition obtained for methanolic extract of *Calotropis procera* leaves against the selected bacteria by Agar diffusion method (values in mm)

S. No.	Conc. of extract (mg)	0.13	0.36	0.612	1.25	2.5	5	MIC (mg)
1.	*B. subtilis*	0	0	0	0	3	10	2.5
2.	*S. aureus*	0	0	0	0	0	0	NF
3.	*P. mirabilis*	0	0	0	0	0	4	5
4.	*P. aeruginosa*	0	0	0	0	0	0	NF
5.	*S. typhi*	0	0	0	0	0	0	NF

NF- MIC was not found with the concentrations screened

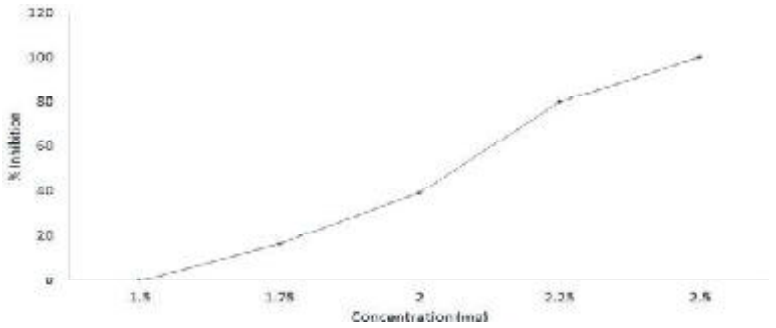

Fig. 1: Growth inhibitory efficacy of methanolic leaf extract of *Calotropis procera* on *B. subtilis*

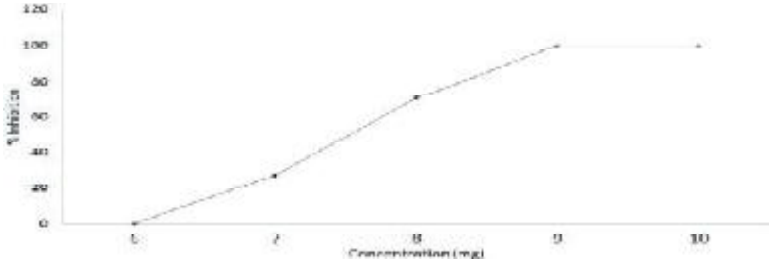

Fig. 2: Growth inhibitory efficacy of methanolic leaf extract of *Calotropis procera* on *S. aureus*

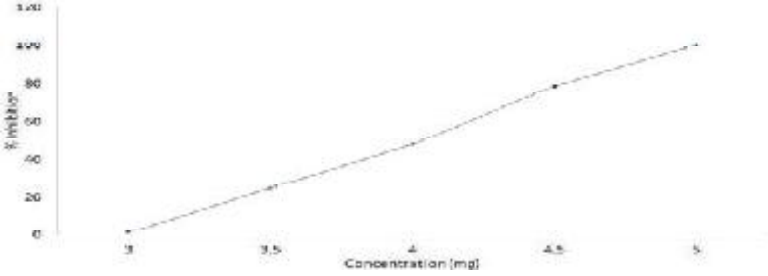

Fig. 3: Growth inhibitory efficacy of methanolic leaf extract of *Calotropis procera* on *P. mirabilis*

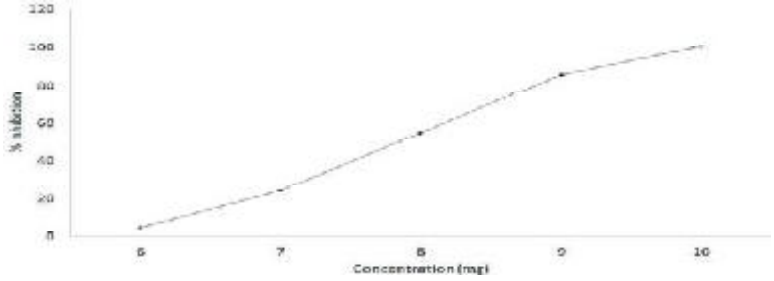

Fig. 4: Growth inhibitory efficacy of methanolic leaf extract of *Calotropis procera* on *P. aeruginosa*

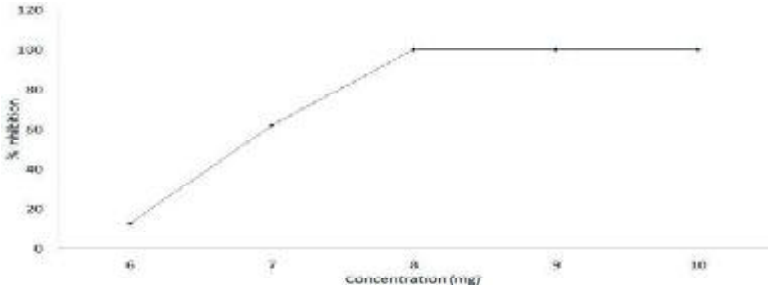

Fig. 5: Growth inhibitory efficacy of methanolic leaf extract of *Calotropis procera* on *S. typhi*

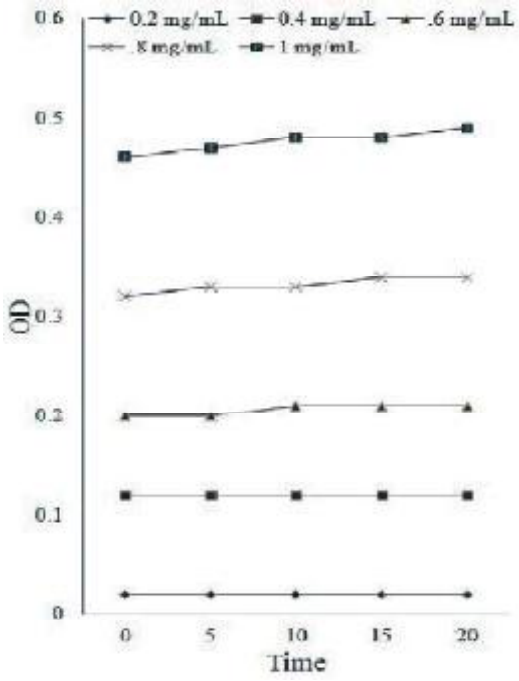

Fig. 6: Hemolytic activity of aqueous leaf extract of *C. procera*

The antibacterial activity of the plants is an attribute to the secondary metabolites produced by them for protection against microbial infections, insect pests and herbivores [7]. Substantial activity of various plant extracts has been reported against these bacteria by several workers [27]. By comparing those reports with the present results we may consider the use of crude methanolic leaf extract of *C. procera* against the studied infective pathogens.

Hemolytic activity of the leaf extract was studied and the results are graphically represented in figure 6 as gradual increase in OD value with time and concentration of extract. The hemolytic activity is due to the saponins contained in the plant parts [28]. These saponins are known to increase the membrane permeability [29] and hence are considered as potential adjuvants. However, because of the same property, they cause hemolysis [28]. This hemolytic activity of a compound depends upon several factors, like the membrane composition, saponin side chain, temperature and time of incubation [29]. The present results showed concentration-dependent increase in the hemolytic activity and a slight increase in the same with time of incubation. The hemolytic activity of few plant extracts have been reported [26, 28-30]. By comparing the results, we may say that the hemolytic activity of *C. procera* is not very pronounced and hence the use of leaf extract is safe.

With the present findings, we may qualify the use of crude extracts of *Calotropis procera* as a potent and safe antibacterial agent.

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