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Biological evaluation of antimicrobial activity of *Calotropis procera* against a range of bacteria

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Abstract
Plants are reported to have anticancer, antimicrobial, anti-diabetic, anti-inflammatory and antioxidant properties. In the present study, methanolic extract of leaves of *Calotropis procera* was used to check the antibacterial activity against *Proteus mirabilis, Pseudomonas aeruginosa, Bacillus cereus, Escherichia coli, Klebsiella pneumonia, Salmonella typhi* and *Enterococcus faecalis* using the disc diffusion method. Different concentrations of extract were made and applied on filter discs. Bacteria were cultured on nutrient agar and discs having different concentrations were applied to petri plates, incubated for 24 hours at 37 °C and after 24 hours, result were recorded in the form of zones of inhibition. Results showed that leaves extract were more effective against *Proteus mirabilis, Pseudomonas aeruginosa and Bacillus cereus*. There was no antibacterial activity of extract against *Escherichia coli, Klebsiella pneumonia, Salmonella typhi, and Enterococcus faecalis*. It has been concluded that leaves extract of *Calotropis procera* may be used as a treatment for the infection caused by *Proteus mirabilis, Pseudomonas aeruginosa* and *Bacillus cereus*. Also, different chemicals present and crude extract should be purified and individual chemical should be used against various bacteria to sort out the chemical(s) having antibacterial activity.

Keywords: *Calotropis procera*, antibacterial activity, crude extract, Disc diffusion method

Introduction
Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, mainly based on their use in traditional medicine (Mahesh & Satish, 2008) [7]. WHO defined medicinal plant as a plant which in one or more of its parts, contains substances that can be used for therapeutic purposes or which are substrates for the synthesis of useful drugs (Zahoor *et al.*) [12]. This plant based traditional medicine system continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care (Murugan & Mohan, 2011) [9]. Studies by various researchers have proved that plants are one of the major sources for drug discovery and development (G. Kumar, Karthik, & Rao, 2010) [5].

Many antibacterial agents have been discovered and it include antibacterial agents, such as the penicillins (from *Penicillium* species), cephalosporins (from *Cephalosporium acremonium*), aminoglycosides, tetracyclines and polyketides (all from *Streptomyces* species); immunosuppressive agents, such as the Cyclosporins and rapamycin (from *Streptomyces* species); cholesterol lowering agents, such as mevastatin (compactin) and lovastatin (from *Penicillium* species); as well as anthelmintics and antiparasitic drugs, such as the ivermectins (from *Strepto myces* species) (Buss, Cox, & Waigh, 1995) [2]. The development of bacterial resistance agent of currently available antibiotics has presented the need to search for new antibacterial drug (Zafar *et al.* 2016) [11].

Calotropis procera (Ait.) R. Br, a plant of Asclepiadaceae family, is originally from Africa, India and Persia, and is known popularly as jealousy, jealousy cotton, silk, flower-silk, milk or queimadeira (Sharma, Kharb, & Kaur, 2011) [10]. They are commonly known as milk weeds because of the latex they produce. *Calotropis* species are considered common weeds in some parts of the world (D. Kumar, 2015) [4]. *Calotropis procera* has a considerable reputation as potent medicine in the treatment of Leucoderma, leprosy, ulcers, tumors, and piles, diseases of the spleen, liver and abdomen. The plant has been investigated phytochemically for cardenolides, anthocyanin, hydrocarbon and triterpenoids and has been reported to show a number of pharmacological activities such as cardio tonic, hepatoprotective, antimicrobial and anticancer as well as infertility (Ahmed, Rana, & Dixit, 2004) [1].

In this study the antibacterial activity of Calotropis procera (Ait.) R. Br extract has been determined by using disc diffusion method.

Experimental

Plant simple collection and preparation

The *Calotropis procera* plant was collected from the botanical garden of the University of Malakand. The leaves of *Calotropis procera* (Ait.) R. Br (2 kg) were extracted with methanol. The metabolic extract 115mg was obtained by evaporating the solvent through rotary evaporator. Water bath at 65 °C for 24 hrs. Was used for the excess water removal from the extract.

Collection of bacterial sample

The bacterial samples of *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Bacillus cereus* and *Proteus mirabilis* were collected from microbiology laboratory, Department of Pharmacy, University of Malakand.

Media sterilization and culture of bacterial sample

The media were prepared and sterilized in an autoclave at 121 °C for 20 minutes. From each bacterial specie, enough amount of bacterium was mounted into the sterilized media in flask. The whole process was carried out in the positive pressure under laminar flow. The media were allowed for incubation of the bacterial species at 37 °C for 24 hours for increasing the bacterial population.

Preparations of dilutions from plant extract

The leaves extract of *Calotropis procera* was dried in a water bath, and 10 grams of the dried extract were dissolved in 10 ml of DMSO and further dilutions were made as 10µg, 9µg, 5µg, 4.5 µg, 4µg, 2.5µg, 2µg, 2.75µg, 1.25µg, 1µg, 0.5µg, 0.025µg and 0.005µg.

Antibacterial assay

Nutrient agar media was used for antibacterial assay. Agar media was prepared and sterilized in autoclave at 121 °C for 20 minutes. Nutrient agar media was transferred to each sterilized petri dish in laminar airflow. The media was allowed to cool and solidify. Then through cotton swab method, bacterial culture was inoculated.

After inoculation of bacterial sample, and uniform distribution of bacteria on the surface of the media, already prepared discs having different extract concentration were impregnated on surface of media through forcep.

After putting discs, petri dishes were transferred to incubator for incubation. Incubation was done for 24 hours at 37 °C, and after 24 hours, results were recorded in the form of measuring zones of inhibition.

Statistical analysis

Data was statistically analyzed, mean and standard deviation of the zones were determined using online software Graph pad prism, Demo version 5 (www.graph pad.com).

Results

The crude extract of *Calotropis procera* was used against various bacteria using disc agar diffusion method. Result of antibacterial activity of extract against bacteria have been shown as follows.

The table no 1 shows different concentrations of plant extract which have been used against different bacteria.

It has been concluded that plant extract has different effect at different concentration against a specific bacteria at different concentration.

Table 1: Zones of inhibition of different concentrations of leaves extract against various bacteria

Bacteria	Concentration												
	10 µg	9 µg	5 µg	4.5 µg	4 µg	2.75 µg	2.5 µg	2 µg	1.25 µg	1 µg	0.5 µg	0.025 µg	0.005 µg
	Zone of inhibition												
Pr. Mirabelus	17±2	0	0	12±2	17±2	15±2	22±3	0	13±2	15±2	0	0	16±2
P. aeruginosa	19±2	20±2	22±2	13±2	12±2	19±2	14±3	0	0	12±2	0	19±2	16±2
B. cereus	16±2	14±2	22±2	0	15±2	10±2	18±3	16±2	11±2	18±2	0	15±2	16±2
E. coli	0	0	0	0	0	0	0	0	0	0	0	0	0
K. pneumonia	0	0	0	0	0	0	0	0	0	0	0	0	0
S. typhi	0	0	0	0	0	0	0	0	0	0	0	0	0
E. faecalis	0	0	0	0	0	0	0	0	0	0	0	0	0

M = Mean, SD = Standard deviation

From the table, it is clear that there is no antibacterial activity of *Calotropis procera* extract against *Escherichia coli*, *klebsiella pneumonia*, *Salmonella typhi*, and *Enterococcus faecalis* at various concentrations. It has been concluded that these bacterial infections may not be treated with the extract at these doses.

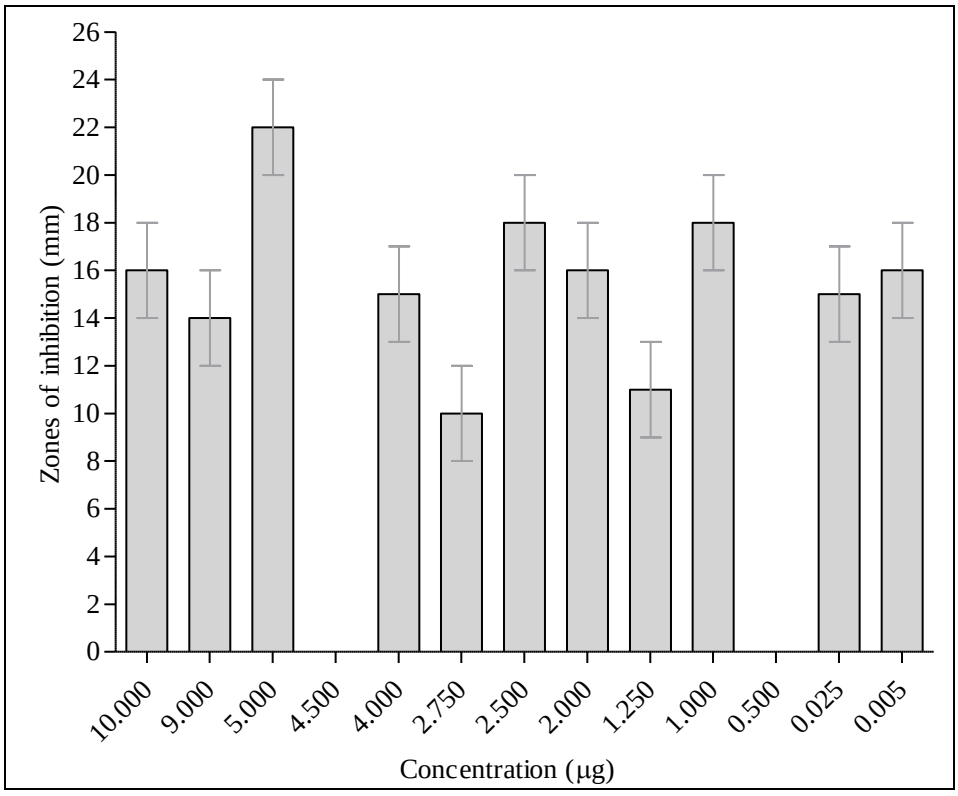

Fig 1: Antimicrobial activity of *Calotropis procera* against *Bacillus cereus*

Different disc were prepared having concentrations of 10, 9, 5, 4.5, 4, 2.75, 2.5, 2, 1.25, 1, 0.5, 0.025 and 0.005µg /10µl and applied against *Bacillus cereus* to determine the antibacterial activity of *Calotropis procera.*

It has been concluded that leaves extract showed different antibacterial activity at different concentration. There was no any effect on bacterial growth at 4.5 and 0.5µg concentration. Maximum effect was achieved when 5 µg of leaves extracts was used against *Bacillus cereus* with a zone of 22 ± 2mm, while least activity was obtained at 2.75 µg with a zone of 10 ± 2mm. when the dose was increased to 9 µg and 10 µg, there was no any significant increase in zone of inhibition. It has been concluded that leaves of *Calotropis procera* may be used at the dose which may achieve a concentration of 5 µg/ml in blood to control *Bacillus cereus* infection *in vivo*.

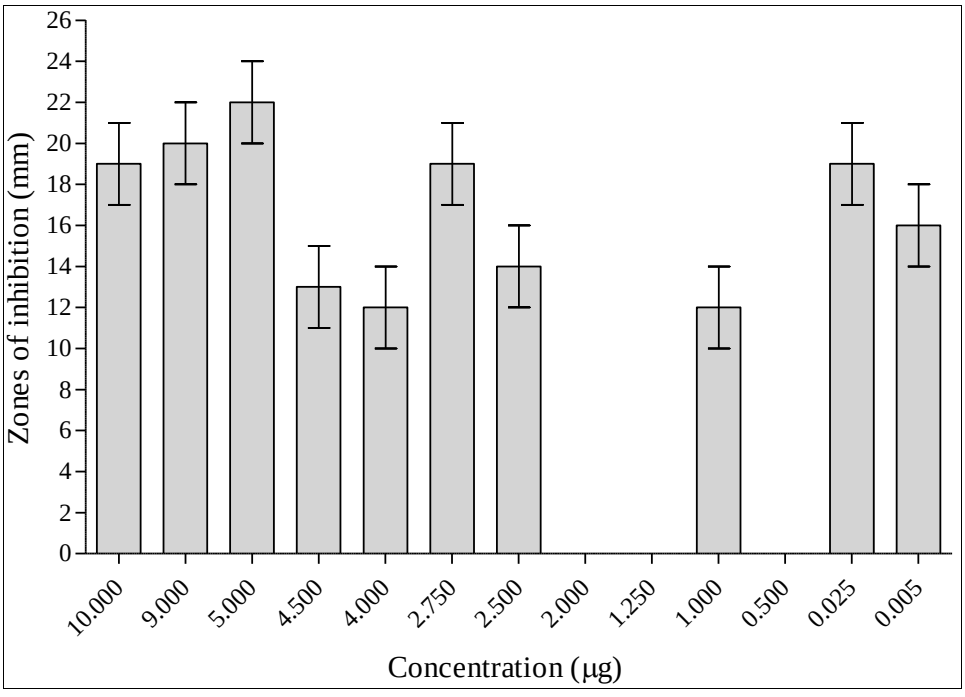

Fig 2: Antimicrobial activity of *Calotropis procera* against *Pseudomonas aeruginosa*

Different disc were prepared having concentrations of 10, 9, 5, 4.5, 4, 2.75, 2.5, 2, 1.25, 1, 0.5, 0.025 and 0.005µg /10µl and applied against *Pseudomonas aeruginosa* to determine the antibacterial activity of *calotropis procera.*

It has been concluded that leaves extract showed different antibacterial activity at different concentration. There was no any effect on bacterial growth at 2, 1.25 and 0.5µg concentration.

Maximum effect was achieved when 5 µg of leaves extracts was used against *Pseudomonas aeruginosa* with a zone of 22 ± 2mm, while least activity was obtained at 1, 4µg, with a zone of 12± 2, 12±2mm. when the dose was increased to 9 µg and 10 µg, there was no any significant increase in zone of inhibition. It has been concluded that leaves of *Calitropis procera* may be used at the dose which may achieve a concentration of 5 µg/ ml in blood to control *Pseudomonas aeruginosa* infection *in vivo*.

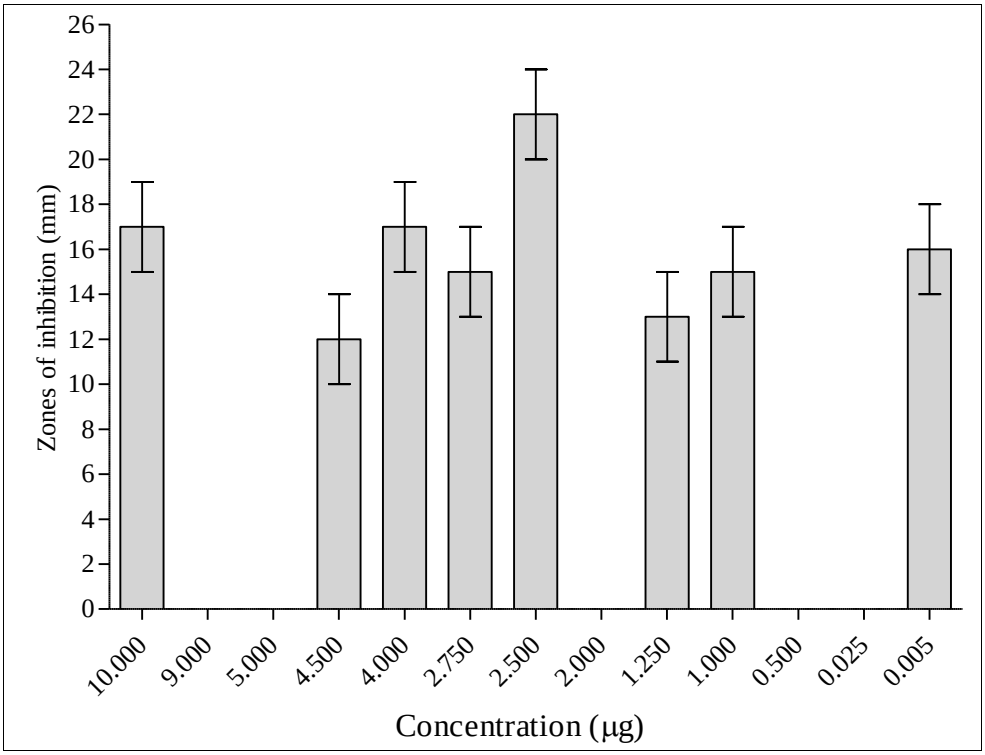

Fig 3: Antimicrobial activity of *Calotropis procera* against *Proteus mirabilis*

Different disc were prepared having concentrations of 10, 9, 5, 4.5, 4, 2.75, 2.5, 2, 1.25, 1, 0.5, 0.025 and 0.005µg /10µl and applied against *Proteus mirabilis* to determine the antibacterial activity of *Calotropis procera.*

It has been concluded that leaves extract showed different antibacterial activity at different concentration. There was no any effect on bacterial growth at 9, 5, 2, 0.5, and 0.025µg concentration.

Maximum effect was achieved when 2.5 µg of leaves extracts was used against *Proteus mirabilis* with a zone of 22 ± 2mm, while least activity was obtained at 4.5 µg with a zone of 12± 2mm. when the dose was increased to 10 µg, there was no any significant increase in zone of inhibition. It has been concluded that leaves of *Calotropis procera* may be used at the dose which may achieve a concentration of 2.5 µg/ ml in blood to control *Proteus mirabilis* infection *in vivo*.

Discussion

The present work investigates the anti-microbial effect of *Calotropis procera* leaves extract against *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Enterococcus faecalis, Klebsiella pneumonia, Bacillus cereus* and *Proteus mirabilis.* There was maximum activity of *Calotropis procera* against *Proteus mirabilis* at concentration of 2.5 µg with a zone of inhibition of 22±2mm. At concentration of 4.5µg, there was minimum activity having zone of inhibition in range of 12±2mm. The same bacterium was tested for the antibacterial activity of *Calotropis procera* flower extract. They used 3.7mg/ml concentration against *Proteus mirabilis* having zone of inhibition of 16.7±0.9mm, which indicates that leaves extract is more effective than flower extract. The leaves extract gives 17±2mm zone of inhibition at concentration of 10µg (Mascolo, Sharma, Jain, & Capasso, 1988) [8].

The maximum activity of *Pseudomonas aeruginosa* was recorded at concentration of 5µg with a zone of inhibition of 22±2mm. Hiren Doshi *et al*., 2011 [3] carried out study on the ethanolic extract of various parts of *Calotropis procera* against same that gave 6mm of inhibition zone at 5µg concentration (Doshi, Satodiya, Thakur, Parabia, & Khan, 2011) [3].

A zone of inhibition of 22±2mm was obtained when leaves extract of *Calotropis procera* was used at concentration of 5µg against *Bacillus cereus*, while 10±2mm zone was observed at 2.75 µg concentration. Kumar *et al*., 2010 found 16±1 mm zone of inhibition at 125µg/ml concentration using *Calotropis Gigantea* (G. Kumar, Karthik, & Bhaskara Rao, 2010) [5].

It has been analyzed that methanolic extract of leaves of *Calotropis procera* may have chemicals that shows antibacterial activity against *Bacillus cereus.* It has also been found that leaves extract have shown antibacterial activity against *Bacillus cereus* determined by mentioned research worker.

Conclusion

In present study, methanolic extract of leaves of *Calotropis procera* (*Spalmai*) was used against various bacteria. No activity was found against *Escherichia coli, Salmonella typhi, Enterococcus faecalis* and *Klebsiella pneumonia*, while there

was highly activity against *Pseudomonas aeruginosa*, *Bacillus cereus and Proteus mirabilis.* The said plant leaves may be used for the treatment of infections caused by these sensitive bacteria.

There is need of further fractionation and isolation of the compounds responsible for antimicrobial activity. The present investigation clearly reveals the antibacterial nature of this plant and suggests that this plant could be exploited in the management of diseases caused by these bacteria in human and animals.

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