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RESEARCH ARTICLE

IN VITROANTIBACTERIAL ACTIVITIES ASSESSMENT OF *CALOTROPIS PROCERA* LEAF EXTRACT

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ABSTRACT

The present study was conducted to determine antimicrobial activity of *Calotropis procera* against *Escherichia coli* and *Proteus vulgaris.* In plant parts leaves were chosen for this activity. The leaf extracts were prepared in three solvents: aqueous, petroleum ether and ethyl acetate. Concentration of plant extract 0.25-3mg/ml was used in study. The maximum zone of inhibition (20.3±0.93mm) was recorded in case of aqueous leaf extract of *C. procera* against *Proteus vulgaris.* Results revealed that MIC value was recorded for aqueous extract at 2.50 mg/ml and 2.75 mg/ml against *E.coli* and *P.vulgaris* respectively, whereas for ethyl acetate extract MIC was observed at 2.50mg/ml and 2.75 mg/ml for *E.coli* and *P. vulgaris* respectively. In case of petroleum ether MIC value was recorded at 2.50mg/ml and 2.75mg/ml for *E.coli* and *P.vulgaris* respectively.

Key words:Calotropis Procera, Antimicrobial Activity, Leaf Extract, Inhibition Zone.

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INTRODUCTION

*Calotropisprocera*R. Br. (Asclepiadaceae) is widely available in Mansa, Punjab, India. The leaves, flowers, latex, roots and bark of *C.procera* generally grow up to 2.5 - 4 meter high, having ethno-medicinal properties (Verma *et. al*., 2010). This plant is used for treatment of several infectious diseases including purulent wound infections, purgative, antihelmintic, digestive, stomachic, emetic, expectorant, sedative, an antidote for snake poisoning, ulcers, tumors, leprosy, asthma, boils, eczema, piles, diseases of liver and spleen disorders, larvicidal activity against mosquitoes (Kirtikar and Basu, 1935; Nadkarni and Nadkarni, 1960; Markouk *et. al.*, 2000; Sammer, 2010). It isused for making gun powder, the latex is used in treating vertigo, baldness, hair fall, tooth aches, intermittent fevers, rheumatoid/joints swellings, paralysis and for the treatment of ring worms (Vohra, 2004; Kuta, 2008). The whole plant when dried and consumed is good tonic, antihelmintic and expectorant (Agharkar, 1991; Warrier *et. al*., 1994). Due to these medicinal properties *C. procera* was selected for further research studies. Present report will provide new information on the antibacterial activities of *C. procera* against following pathogens: *Escherichia coli* and *Proteus vulgaris* as test organism.

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MATERIALS AND METHODS

Sample Collection and Preparation

The *Calotropis procera* leaf samples were collected from hedges of agriculture fields, Mansa, Punjab. The plant was identified on the basis of botanical identity and standard description. Further the leaf samples were properly washed and shade dried for 5-8 days. The dried samples were grinded by mixer and converted into the powdered form.

Extraction and Preparation of Material

Leaf extract was obtained by using following solvents: water, ethyl acetate, petroleum ether. The dried leaf powder was subjected to extraction in soxhlet extractor with these solvents for 70 hours. The collected extract is evaporated to dryness by rotator vacuum evaporator. This dry leaf extracts were stored at 4°C.

Antibacterial Screening

Test organisms

The microorganisms used for antibacterial activity, was*E.coli* (MTCC 1692) and *P. vulgaris*(MTCC 744) obtained from Imtech Chandigarh.

Table 1. Inhibition zone (mm/ml) of *Calotropis procera* leaf extracted against *Escherichia coli* and *Proteus vulgaris* using three solvents

Micro-organisms	Aqueous Extract	Petroleum Ether Extract	Ethyl Acetate Extract
Escherichia coli	8.2±0.87	10.3±0.94	12.5±1.06
Proteus vulgaris	20.3±0.93	15.4±0.79	14.2±0.49

Table 2.MIC results of *Calotropis procera* Aqueous, petroleum ether, ethyl acetate leaf extract against *Escherichia coli* and *Proteus vulgaris*

Aqueous/Petroleum ether/ Ethyl acetate Extract (mg/ml)	*E.coli*/*P.vulgaris* suspension (ml)	Nutrient Broth (ml)	Absorbance (at 600 nm)					
			Aqueous extract		Petroleum ether		Ethyl acetate	
			E.coli	*P.vulgaris*	*E.coli*	*P.vulgaris*	*E.coli*	*P.vulgaris*
0	0.5	4.5	2.99	2.88	2.99	2.88	2.99	2.88
0.25	0.5	4.25	2.50	2.75	2.85	2.80	2.61	2.65
0.5	0.5	4	2.31	2.43	2.52	2.45	2.22	2.33
0.75	0.5	3.75	2.10	2.21	2.29	2.16	1.93	2.07
1	0.5	3.5	1.89	2.02	2.06	1.83	1.65	1.77
1.25	0.5	3.25	1.63	1.79	1.84	1.47	1.28	1.46
1.5	0.5	3	1.41	1.58	1.34	1.24	0.99	1.18
1.75	0.5	2.75	1.18	1.39	1.04	1.07	0.62	0.84
2	0.5	2.5	0.90	1.20	0.74	0.83	0.40	0.63
2.25	0.5	2.25	0.51	0.90	0.51	.64	.17	0.41
2.5	0.5	2	0.28	0.43	0.20	.37	0.0	0.20
2.75	0.5	1.75	0.0	0.15	0.0	0.14	0.0	0.09
3	0.5	1.5	0.0	0.0	0.0	0.0	0.0	0.0

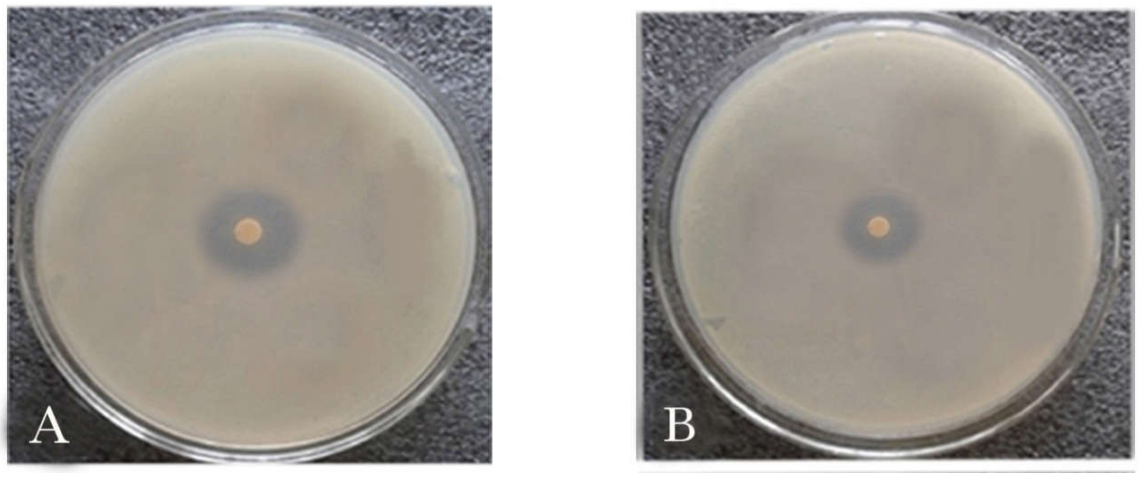

Figure 1. Antibacterial activity of aqueous extract of leaves of *Calotropis procera* against A. E*scherichia coli* B. *Proteus vulgaris*

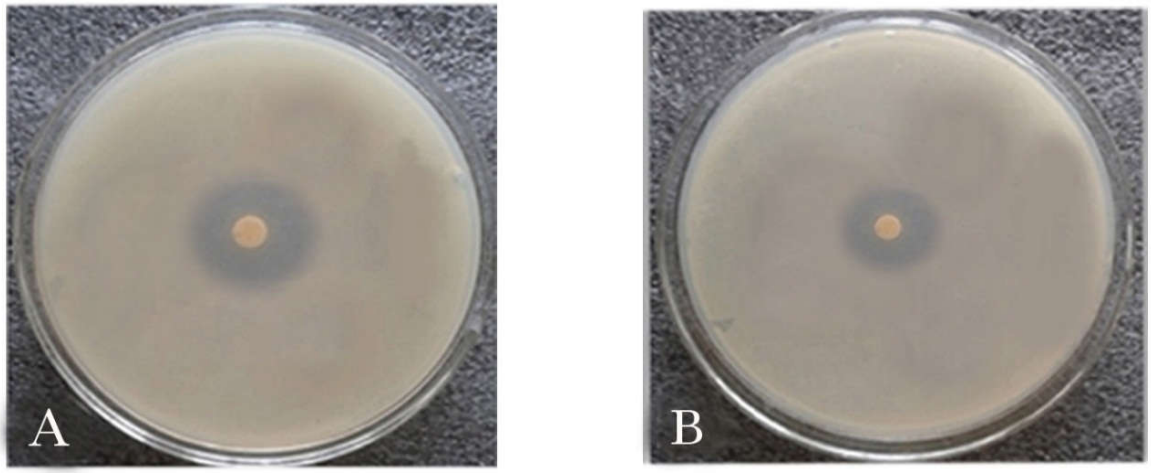

Figure 2. Antibacterial activity of petroleum ether extract of leaves of *Calotropis procera* against A. E*scherichia coli* and B. *Proteus vulgaris*

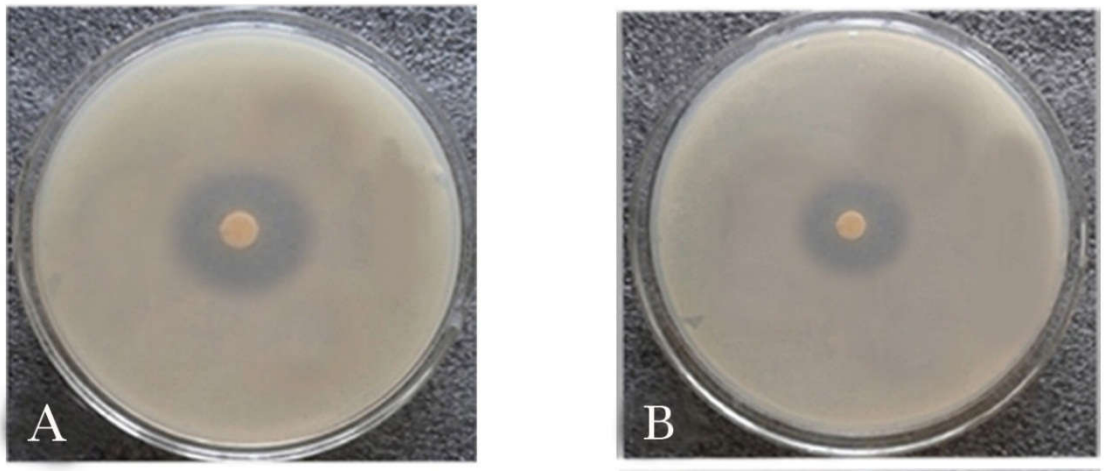

Figure 3. Antibacterial activity of ethyl acetate extract of leaves of *Calotropis procera* against A. *Escherichia coli* and B. *Proteus vulgaris*

Antibacterial activity

The antibacterial activity was carried out by disc diffusion method. Bacterial cultures (adjusted to 1×10^6 CFU/ml using spectrophotometer) were inoculated on nutrient agar plates evenly using sterile swab. The plates were dried for 15 min and sterile discs (5 mm in diameter, Whatman No.1) impregnated with 10 µl (1 mg/ml) of the leaf extracts. 10 µl of the respective solvent served as the negative control. The plates were then incubated at 37°C for 18-24 h. After overnight incubation the plates were examined for the zone of inhibition (Omenka and Osuoha, 2000). All tests were carried out in triplicate. The zone of inhibition was measured using a transparent meter ruler. The calculation was done by using SPSS software.

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) was carried out by broth dilution method. The test organisms were grown in nutrient broth medium to the concentration of 1×10^6 CFU/ml. Extract of about 0.5 ml (0.25-2 mg/ml) was mixed with 4 ml of nutrient broth inoculated with 0.5 ml of bacterial suspension. The tubes containing 4.5 ml of broth and 0.5 ml of bacterial suspension served as bacterial control and 5 ml of un-inoculated broth served as blank. The tubes were incubated at 37°C for 18 h. Inhibition of bacterial growth was determined by measuring the absorbance at 600 nm in Spectrophotometer.

RESULTSANDDISCUSSION

The antibacterial activity and Minimum inhibitory concentration (MIC) were evaluated by using aqueous, ethyl acetate and petroleum ether leaf extracts against *Escherichia coli* and *proteus vulgaris* (Table 1 & 2).The widest zone of inhibition (20.3±0.93 mm) was recorded for *P.vulgaris*using aqueous leaf extract of *C. procera* while value dropped to 15.4±0.79mm and 14.2±0.49 mm for petroleum ether and ethyl acetate leaf extract respectively (Table 1, Figure 1, 2 &3). Results also revealed that for aqueous extract the MIC value was recorded at 2.50 mg/ml and 2.75 mg/ml against *E.coli* and *P.vulgaris* respectively. In ethyl acetate leaf extract MIC was observed at 2.50mg/ml and 2.75 mg/ml for *E.coli* and *P. vulgaris* respectively.

In case of petroleum ether leaf extract the MIC value was recorded at 2.50mg/ml and 2.75mg/ml for *E.coli* and *P.vulgaris* respectively. Similar type of antimicrobial activity was recorded using ethanol leaf extract of *C.procera* against three pathogen, *Escherichia coli,Staphylococcus* sp.and *Streptococcus* sp.at department of microbiology, University of Agriculture, Abeokuta, Nigeria, where result revealed that highest antimicrobial activity was recorded at concentration 2.5 mg/ml for *E.coli* (Kareem *et. al.*, 2008). SimilarlyShittu *et. al.*(2004)at college of natural science,recorded stronger antibacterial activity of *C.procera*leaf extract than roots. Methanol, ethanol and water extracts of *C. procera* showed significant antibacterial activity againstboth the Gram positive and Gram negative bacterial strain (Yesmin *et. al.*, 2008). Study conducted by Kawo *et. al*., (2009) on weak antibacterial properties of ethanolic leaf extracts and latex of *C. procera* against *E. coli*, *S. aureus*, *Salmonella* sp. and *Pseudomonas* sp. was recorded by using paper-disc diffusion and broth dilution techniques. The results revealed that ethanol was the best extractive solvent for antibacterial activity. Similarly some researchers reported methanol extraction yielded higher antimicrobial activity than hexane and ethyl acetate (Manilal *et. al*., 2009; Rangaiah *et. al*., 2010). Salem *et. al*. (2014) also reported the antibacterial activity properties of *C.procera* aqueous and ethanol leaf extracts on different strain ofbacteria. Hence, the remarkable bactericidal effects of *C. procera* leaves extracts suggested that these extracts can be a useful source for the development of novel antibacterial formulations.

REFERENCES

Agharkar S. P. 1991. Medicinal plant of Bombay presidency.Scientific publication, India.48-49.

Kareem S.O, Akpan I and Ojo O.P.2008. Antimicrobial activities of *Calotropis procera* on selected pathogenic microorganisms. *Afr. J.Biomed.Res*. 11:105- 110.

Kawo A.H., Mustafa A., Abdullahi B.A., Rogo L.D., Gaiya Z.A. and Kumurya A.S. 2009.Phytochemical properties and antibacterial activities of the leaf and latex extracts of *Caltropis procera* (Ait).*Bayero J. Pure Appl. Sci*. 2:34-40.

Kirtikar K.R. and Basu B.D.1935.Indian Medicinal Plants, M/S Bishen Singh Mahendra Pal Singh, Dehradun, 2nd Edition.1606-1607.

Kuta, F.A.2008. Antifungal effect of *Calotropis procera* stem of*Epidermophyton flocosum.African Journal of Biotechnology.*7 (13); 2116-2118.

Manilal A., Sujith S., Selvin J., Shakir C and Kiran G.S. (2009).Antibacterial activity of *Falkenbergia hillebrandii* (Born) from the Indian coast against human pathogens.*Phyton*. 78:161-166.

Markouk M., Bekkouche K., Larshini, M., Bousaid M., Lazrek H.M. and Jana M. 2000. Evaluation of some Moroccan medical plant extracts for larvicidal activity. *J. Ethnopharmacol*., 73(1-2): 293-297.

Nadkarni, A.K. and Nadkarni, K.M. 1960. Indian MateriaMedica, Popular Book Depot, Bombay. 69-71.

Omenka C. A. and OsuohaJ. O. 2000. Antimicrobial potency of Grapefruit seed extraction five selected pathogens. *Nigerian Journal of Microbiology*.14 (2): 39-42.

Rangaiah S.G., Lakshmi P. and Manjula E. 2010.Antimicrobial activity of seaweeds *Gracillaria, Padina* and *Sargassum sps*.on clinical and phytopathogens. *Int. J. Chem. Anal. Sci.* 1(6):114-117.

Salem W.M., Sayed W.F., Haridy M. and Hassan N. H. 2014. Antibacterial activity of Calotropis procera and Ficussycomorus extracts on some pathogenicmicroorganisms (Hurgada, Egypt). *A fr J. biotech.* 13(32):3271-3280.

Sameer H. Q. M. 2010. DNA-RAPD Fingerprinting and Cytogenetic Screening of Genotoxic and Antigenotoxic Effects of Aqueous Extracts of *Costus Speciosus* (Koen.). *JKAU: Sci*. 22; 133-152.

Shittu B. 2004. Potential of *Calotropis procera* leaves for wastewater treatment. College of Natural Sciences Proceedings 97-108.

Verma, R., Satsangi, G.P. and Shrivastava, J.N. 2010. Ethno-medicinal profile of different plant parts of *Calotropis procera* (Ait.) R. Br. *Ethno botanical Leaflets*, 14: 721–742

Vohra R. 2004. *Calotropis* the medicinal weed. Online medicinal book store, India

Warrier P.K., Nambiar V. P.K. and Mankutty C.1994. Indian medicinal plants, orient longman: Chennai, India. 341-345

Yesmin M.N., Uddin, N.S., Sanzida M. and Muhammad A. A. 2008. Antioxidant and Antibacterial Activities of *Calotropis procera. American-Eurasian Journal of Agric and Environmental Science*. 4 (5): 550-553.

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