Available online at http://www.ijcrls.com

International Journal of Current Research in Life Sciences
Vol. 07, No. 06, pp.2347-2350, June, 2018

ISSN: 2319-9490

RESEARCH ARTICLE

IN VITRO ASSESSMENT OF ANTIBACTERIAL ACTIVITY OF *CALOTROPIS GIGANTEA*

***Priyanka Sharma and Gajanand Modi**

Department of Biotechnology, IASE University, Sardarshahr, Rajasthan

Received 07th April, 2018; Accepted 29th May, 2018; Published 30th June, 2018

ABSTRACT

The aim of the present study was to evaluate aqueous, ethanolic and ethyl acetate extracts of *plant Calotropis gigantea* traditionally used in Indian folklore medicine for the treatment of various bacterial and fungal infections were investigated for in vitro antibacterial activity against pathogens namely *Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae* by disc diffusion method compared with standard antibiotic antibacterial activity. The results revealed that, among the different types of tested extract, ethyl acetate extract of leaves showed 16.9±1.06mm, 19.4±0.54mm, and 21.5±0.85mm maximum zone of inhibition *against E.coli, K. pneumoniae* and *S.aureus* respectively. Minimum Inhibitory Concentration (MIC) was measured by broth dilution method. Results revealed that MIC value was recorded for aqueous extract at 2.75 mg/ml against *S.aureus* and *K. pneumoniae* respectively, whereas for ethyl acetate extract MIC was observed at 2.25mg/ml for E.coli and 2 mg/ml for both *S.aureus* and *K. pneumoniae*. In case of ethanol MIC value was recorded at 2.25mg/ml for S.aureus and 2.5 mg/ml for both E.coli and K. pneumoniae. The results provided justification for the use of plants in folk medicine to treat various infectious diseases.

Key words: *Calotropis Gigantea,* leaf Extract, Minimum Inhibitory Concentration (MIC), Zone of Inhibition.

Citation: Priyanka Sharma and Gajanand Modi, 2018. "In vitro assessment of Antibacterial activity of *Calotropis gigantea*" *International Journal of Current Research in Life Sciences*, 7, (06), 2347-2350.

INTRODUCTION

The genus *Calotropis* R. Br (Asclepiadaceae) comprises four species, but in Indian Subcontinent including Bangladesh, it is mainly represented by two popular species viz., *Calotropis procera* and *Calotropis gigantea* (Wang *et al.*, 2008).*C.gigantea* has glabrous or hoary, lactiferous shrubs or small trees, commonly known as THE SWALLOW-WORT or MILKWEED. Currently 25% of prescribed drugs worldwide are derived from plant sources in spite of the great progress and advancement of organic synthesis (Habib and karim, 2016). So, plants are being extensively explored for harboring medicinal properties. Studies by various researchers have proved that plants are one of the major sources for drug discovery and development (De Pasquale, 1984; Gordon and David,2005; Rates, 2001). The major phytochemicals of *C. gigantea* are flavonol, glycoside, uscharidin, calotropin, frugoside, calotroposides A to G. Other constituents are α-amyrin, β-amyrin, taraxas-terol, β-sitosterol, α- and β-amyrinmethylbutazone, gigantursenylacetate A and B (Mishra *et al.,*2016). It grows in the tropical region and is most abundant in Bangladesh, India, Burma, and Pakistan and in the sub-Himalayan tract (Pathak and Argal, 2006).

Corresponding author: **Priyanka Sharma,**
Department of Biotechnology, IASE University, Sardarshahr, Rajasthan.

According to Ayurveda dried whole plant is asthma, expectorant, depurative, and anthelmintic and leaves are useful in the treatment of paralysis, arthralegia, leprosy, anthelmintic ,anti-*Candida* activity, wound healing activity (Agharkar, 1991; Warrier *et al.,* 1994; Chitme *et al*., 2005; Saratha *et al*., 2009; Mayee *et al.*, 2011; Rahman *et al.*, 2013). The various uses of this plant are biogas production, substitute for petroleum products, cleansing of water, energy plantation, fibers, fodder, latex or rubber, substitute for paper etc. There is a continuous need of the development of new effective antimicrobial drugs because of the emergence of new infectious diseases and drug resistance (Richard, 1998; Raghunath, 2008). The selection of this plant for the present study was based on its medicinal properties and use in traditional medicinal system. Present investigation was carried out to study the antibacterial activity on various extracts of *C. gigantea* leaves.

MATERIAL AND METHODS

Plant collection and identification

Calotropis gigantea was collected from wasteland of Mansa District, Punjab, India in the month of November 2017.The plant was identified by Department of Biotechnology, IASE Deemed University, Sardarshahr, Rajasthan.

The samples were properly washed, 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 extraction was done by using following solvents: aqueous, ethanol and ethyl acetate. The dried powder of leaves was subjected to extraction in soxhlet extractor with these solvents for 70 hours. The collected extract is evaporated to dryness by rotatory vacuum evaporator. This dry leaf extracts were stored at 4 ˚C.

Determination of Antimicrobial activity

Test organisms: The leaves extract of *C. gigantea* was tested against pathogenic bacteria (MTCC 7443) *Staphylococcus aureus,*(MTCC 1692) *Escherichia coli* and(MTCC 7407) *Klebsiella pneumoniae*.The microorganisms used for antibacterial activity assay were obtained from IMTECH Chandigarh.

Positive and negative control: Penicillin and tetracycline were used as positive control (PC) for bacterial strains. Solvents were used as negative control (NC).

Antibacterial activity: The antibacterial activity was carried out by disc diffusion method (Kirby Bauer, 1959)**.** Bacterial cultures (adjusted to 1×10^6 CFU/ml using spectrophotometer) were used to lawn nutrient agar plates evenly using asterile 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 plant extracts were placed on the nutrient agar surface.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).

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 a concentration of 1×10^6 CFU/ml. Extract of about 0.5 ml (0.25-3 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 a colorimeter. The lowest concentration of the compound that inhibits the growth of the organism was determined as the MIC.

RESULTS AND DISCUSSION

This plant is known for antimicrobial, anti-diarrhoeal, antipyretic, wound healing and CNS activity etc. In this study leaf extract of *C.gigantea* with various solventswere tested against pathogenic species of bacteria. The antimicrobial activities of the various leaf extract of *C. gigantea* against bacterial strains with respect to positive and negative control are listed in Table 1. Table 1 showed that ethyl acetate was the best solvent for extracting antimicrobial substance as compared to aqueous and ethanol.

The widest zone of inhibition (21.5±0.85) was demonstrated by ethyl acetate extract of *C. gigantea* leaves extract while value dropped to (18.9±0.33) and (15.8±0.15) mm for ethanol and aqueous extract respectively, when tested against the same organism (Table 1).My results are ,however, not in agreement with Sukanya *et al*., (2009) who found *C. gigantea* to be ineffective or showed poor inhibitionon tested human (*Escherichia coli* and *Staphylococcus aureus*) and phytopathogenic bacteria (*Xanthomonas vesicatoria* and *Ralstonia solanacearum*)**.** Kumar *et al,* (2010), reported significantly high inhibitory effect on *S. aureus, B. cereus* and *E. coli*, moderate effect on *C. krusei,* whereas, no effect on *M. luteus, K. pneumonia* , *P. aeruginosa* and *A. niger*. Antibacterial activity of different solvent extracts of *C. gigantea* showed varying degrees of antibacterial activity against all microorganisms tested (Subhramaninanand Saratha, 2010).

Table 1. Antimicrobial activity of leaf extract of *Calotropis gigantea* on tested organisms

Micro-organisms Name	Positive Control		Aqueous Extract	Ethanol Extract	Ethyl Acetate Extract
	Tetracycline (in mm)	Penicillin (in mm)			
Escherichia coli	5.6±0.37	7.5±0.96	12.4±0.74	14.5±0.65	16.9±1.06
Klebsiella pneumonia	7.0±0.46	8.3±0.38	10.5±0.67	13.3±0.76	19.4±0.54
Staphylococcus aureus	26.8±0.78	30.4±0.63	15.8±0.15	18.9±0.33	21.5±0.85

Table 2. MIC results of *Calotropis gigantea* aqueous leaf extract against three bacterial strains

S. No.	Aqueous Extract (mg/ml)	*Escherichia coli* / *Klebsiella pneumoniae* / *Staphylococcus aureus* suspension (ml)	Nutrient Broth (ml)	Absorbance((at 600 nm)		
				Escherichia coli	*Klebsiella pneumoniae*	*Staphylococcus aureus*
1	0	0.5	4.5	2.99	3.05	3.02
2	0.25	0.5	4.25	2.88	2.93	2.76
3	0.5	0.5	4	2.62	2.72	2.51
4	0.75	0.5	3.75	2.43	2.51	2.37
5	1	0.5	3.5	2.11	2.28	2.14
6	1.25	0.5	3.25	1.88	1.96	1.9
7	1.5	0.5	3	1.72	1.73	1.59
8	1.75	0.5	2.75	1.62	1.54	1.32
9	2	0.5	2.5	1.47	1.3	1.1
10	2.25	0.5	2.25	1.28	0.96	0.83
11	2.5	0.5	2	1.02	0.64	0.54
12	2.75	0.5	1.75	0.79	0.32	0.26
13	3	0.5	1.5	0.61	0.0	0.0

Table 4. MIC results of *Calotropis gigantea* ethyl acetate leaf extract against three bacterial strains.

S. No.	Ethyl acetate Extract (mg/ml)	*Escherichia coli* / *Klebsiella pneumoniae* / *Staphylococcus aureus* suspension (ml)	Nutrient Broth (ml)	Absorbance (at 600 nm)		
				Escherichia coli	*Klebsiella pneumoniae*	*Staphylococcus aureus*
1	0	0.5	4.5	2.99	3.05	3.02
2	0.25	0.5	4.25	2.83	2.63	2.47
3	0.5	0.5	4	2.64	2.42	2.26
4	0.75	0.5	3.75	2.39	2.21	1.92
5	1	0.5	3.5	2.12	2.02	1.53
6	1.25	0.5	3.25	1.93	1.85	1.21
7	1.5	0.5	3	1.54	1.37	0.95
8	1.75	0.5	2.75	1.29	0.84	0.62
9	2	0.5	2.5	0.82	0.45	0.31
10	2.25	0.5	2.25	0.36	0.0	0.0
11	2.5	0.5	2	0.0	0.0	0.0
12	2.75	0.5	1.75	0.0	0.0	0.0
13	3	0.5	1.5	0.0	0.0	0.0

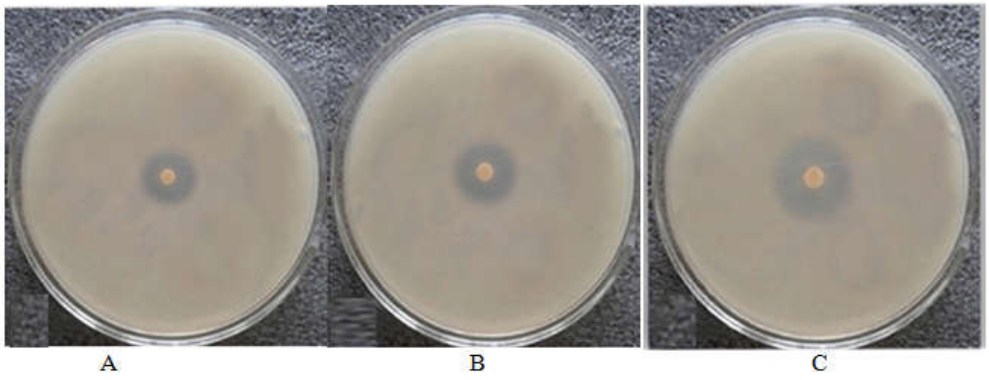

Figure 1. Images A, B and C is revealing the Antimicrobial activity of aqueous extracts on *E. coli, S.aureus* and *K. pneumoniae* respectively

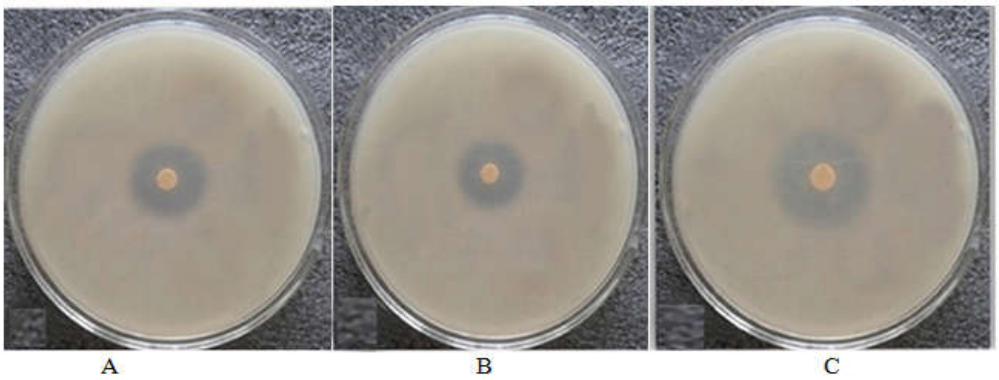

Figure 2. Images A, B and C is revealing the Antimicrobial activity of ethanol extracts on *E. coli, S.aureus* and *K. pneumoniae* respectively

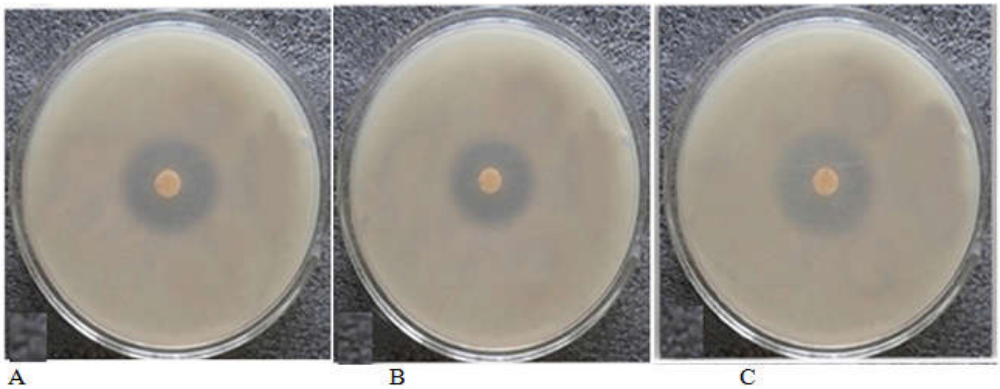

Figure 3. Images A, B and C is revealing the Antimicrobial activity of ethyl acetate extracts on *E. coli, S.aureus* and *K.pneumoniae* respectively

From previous study reported acetone, ethanol and aqueous shade dried leaf extract and ethanol and aqueous shade dried fruits extract of *C. gigantea* showed wide range of antibacterial activity can be used and administered in the ethano medical practice. (Murti and Seshadri, 1945; Ishnava, 2012; Kori and Alawa, 2014). Minimum Inhibitory Concentration (MIC) was measured by broth dilution method.Results revealed that MIC value was recorded for aqueous extract at 2.75 mg/ml against *S.aureus* and *K. pneumoniae* respectively, whereas for ethyl acetate extract MIC was observed at 2.25mg/ml for *E.coli* and 2 mg/ml for both *S.aureus* and *K.Pneumoniae*. In case of ethanol MIC value was recorded at 2.25mg/ml for *S.aureus* and 2.5 mg/ml for both *E.coli* and *K. pneumoniae.* Multi target based approaches of screening of medicinal plant extracts and herbal drugs are expected to yield novel activities (Wang and Wang, 2008; Prabia and Kothari, 2008). The results of the current study conclude that ethyl acetate extract of latex of *C. gigantea* possess significant amount of antimicrobial activity against a wide range of microorganisms. The results obtained from our study showed an effective inhibition against the test organism which justify the traditional use of the plant for infectious diseases.

REFERENCES

Agharkar 1991. Medicinal plants of Bombay presidency. Scientific Publ., India: 48-49.

Bauer AW, Perry DM and Kirby WMM. 1959. Single disc antibiotic sensitivity testing of Staphylococci. *A.M.A. Arch. Intern. Med,* 104:208–216.

Chitme HR, Chandra R and Kaushik S. 2005. Evaluation ofantipyretic activity of *Calotropis gigantea* (Asclepiadaceae) in experimental animals. *Phytotherapy Research,* 19 (5): 454-456.

De Pasquale A. 1984. Pharmacognosy: the oldest modern science.*Journal of Ethnopharmacology*,11(1):1-16.

Gordon MC. and David JN. 2005. Biodiversity: A continuing source of novel drug leads.*Pure Appl Chem*, 77: 7-24.

Habib MR. and Karim MR. 2016. Effect of anhydrosophoradiol-3-acetate of *Calotropis gigantean* (Linn.) flower as antitumoric agent against Ehrlich's ascites carcinoma in mice. *Pharmacol Rep*. 65: 761-767.

Ishnava KB 2012. Antibacterial and phyto-chemical studies on *Calotropis gigantea (L.)R.Br.,*Latex against selected cariogenic bacteria. Saudi. *J. of Bio. Sci,* 19: 87-91.

Kori P and Alawa P 2014. Antimicrobial activity and phytochemical analysis of *Calotropis gigantea* root, latex extracts**.** *IOSR Journal of Pharmacy,* 4 (6):7-11.

Kumar G, Karthik L and Bhaskara Rao KV 2010. Antibacterial activity of aqueous extract of *Calotropis gigantea leaves* – an *in vitro* study. *International Journal of Pharmaceutical Sciences Review and Research*, 4(2):141-144.

Mayee R., Ambrish T. and Kondapure A. 2011. Evaluation of Antiasthmatic Activity of *Calotropis gigantea* Roots.*Asian Journal of Pharmaceutical and Clinical Research,*4(2).

Mishra, M. Gautam, RK. Mathur, R. 2016. Evaluation of antifertility potential of *Calotropis gigantea* linn. in female albino rats. *JPharmaceutical Res Opinion.* 1(3): 92-93.

Murti PBR and Seshadri TR 1945. Chemical composition of *Calotropis gigantea*: Part V. FurtherExamination of the Latex and Root Bark, Proc. *Ind. Acad*, Sci. 21: 143 – 147.

Omenka CA and JO Osuoha 2000. Antimicrobial potency of Grapefruit seed extract on five selected pathogens. *Nigerian Journal of Microbiology*, 14 (2): 39-42.

Pathak AK. and Argal A. 2006. CNS activity of Calotropis gigantea roots, *J.Ethnopharmacol*, 106 (1): 142–145.

Prabia FM and Kothari IL 2008. Natural Product of Radiance, 7, 30

Raghunath 2008 Emerging antibiotic resistance in bacteria with special reference to India. *J Biosci*, 33(4):593-603.

Rahman MS, Moly NN and Hossen MJ 2013. Antimicrobial, cytotoxic and antioxidant activity of the exudates of *Calotropis gigantea. Int. J. of Pharmaceutical Sciences and Res.,*4(2):745-753.

Rates, S.M.K. 2001. Plants as source of drugs*. Toxicon,* 39: 603-613.

Richard 1998. Bacterial evolution and the cost of antibiotic resistance. *Internatl Microbiol*, 1(4):265-270.

Saratha V., Subramanian S. and Sivakumar S. 2009. Evaluation ofwound healing potential of *Calotropis gigantea* latex studied on excision wounds in experimental animals, *Med Chem Res*. 18(8):936-947.

Subhramaninan SP and Saratha V. 2010. Evaluation of Antimicrobial Activity of *Calotropis gigantea* Latex Extract on Selected Pathogenic Bacteria. *Journal of Pharmacy Research*,(13)3.

Sukanya SL, Sudisha J, Hariparasad P, Niranjana SR, Prakash HS and Fathima SK. 2009. Antimicrobial activity of leaf extracts of indian medicinal plants against clinical and phytopathogenic bacteria. *African journal of biotechnology*. 8(23): 6677- 6682.

Wang, Z, Wang, M., Mei, W., Han, Z. and Dai, H. 2008. A New Cytotoxic Pregnanone from *Calotropis gigantea,* Molecules, 13, 3033- 3039

Wang, Z. and Wang, M. 2008. Molecules, 13:3033.

Warrier PK, Nambiar VPK and Mankutty C. 1994. Indian Medicinal Plants. Orient Longman; Chennai, India. 341-345.

****