

ANTIBACTERIAL ACTIVITY OF AQUEOUS EXTRACT OF *Calotropis gigantea* LEAVES – AN *IN VITRO* STUDY**Gaurav Kumar, Loganathan Karthik, Kokati Venkata Bhaskara Rao ***

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*Email: kokatibhaskar@yahoo.co.in**ABSTRACT**

Calotropis gigantea is a common wasteland weed and known for various medicinal properties. The aim of the present study was to screen leaves of *Calotropis gigantea* for the antimicrobial activity against clinical isolates of bacteria. The aqueous extract of the *C. gigantea* was studied for its antagonistic activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Micrococcus luteus* and *Klebsiella pneumoniae*. *In vitro* antimicrobial activity was performed by well diffusion method in MH agar. The extract showed significant effect on the tested organisms. The extract showed maximum zone of inhibition against *E. coli* (17.6±1.15), whereas, lowest against *K. pneumoniae* (12.6±1.52). Crude extract showed maximum relative percentage inhibition against *B. cereus* (188.52 %) and lowest relative percentage inhibition against *M. luteus* (24.92 %). Minimum Inhibitory Concentration (MIC) was measured by modified agar well diffusion method. Extract showed 50, 25, 6.25, 3.1, 1.5 and 12.5 mg/ml MIC values for *S. aureus*, *K. pneumoniae*, *B. subtilis*, *P. aeruginosa*, *M. luteus* and *E. coli*, respectively.

Keywords: Antibacterial; *Calotropis gigantea*; Well diffusion method; antagonistic activity.**INTRODUCTION**

In current scenario of medical and pharmaceutical advancement, microbes involve in the change of their metabolism and genetic structure to acquire resistant against the drugs used in the treatment of common infectious disease^{1,2}. These drug resistant candidates are more pathogenic with high mortality rate and become a great challenge in the pharmaceutical and healthcare industry. To overcome microbial drug resistant, scientists are looking forward for the development of alternative and novel drugs. Natural sources such as plants, algae and animals provides an array of natural medicinal compounds for the treatment of various infectious diseases.

Plants are exploited as medicinal source since ancient age. The traditional and folk medicinal system uses the plant products for the treatment of various infectious diseases. In recent times, 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^{3,4,5}. Plants are reported to have antimicrobial, anticancer, antiinflammatory, antidiabetic, hemolytic, antioxidant, larvicidal properties etc.

Calotropis gigantea is a wasteland weed better known as milkweed, habitat of Asian countries that includes, India, Indonesia, Malaysia, Philippines, Thailand, Sri Lanka and China. Tribal people were using this plant parts to cure several illnesses such as toothache, earache, sprain, anxiety, pain, epilepsy, diarrhoea and mental disorders. *C. gigantea* is scientifically reported for its anti-*Candida* activity, cytotoxic activity, antipyretic activity and wound healing activity^{6,7,8,9}. Current study was focused to investigate the antibacterial activity of the crude leaves extract of *C. gigantea* against clinical isolates of bacteria

MATERIALS AND METHODS**Plant material**

C. gigantea plant was collected from the natural population growing in the wasteland of Vellore, TN, India, during December 2008. The plant sample was brought to the Molecular and Microbiology Research Laboratory, VIT University. Plant was identified in Herbal Garden of VIT University, Vellore, TN, India and voucher specimen was maintained in our laboratory (Accession number: VIT/SBST/MMRL/CG/10.1.2009/101).

Processing of the plant

Plant leaves were collected and washed properly with distilled water. The leaves were shade dried at room temperature. Dried leaves were uniformly grinded using mechanical grinder. The leaves powder was extracted in distilled water. Ten gram of plant powder was soaked in 100 ml of distilled water in a conical flask and loaded on an orbit shaker at a speed of 120 rpm for 24 hours. The mixture was filtered using Whatman filter paper number 1. The filtrate was concentrated using rotary evaporator and dried using lyophilizer. Dried extract was collected in an air tight container and stored at 4°C.

The extracted powder was dissolved in sterilized distilled water to make 1000 µg/ml solution. This mixture was used to perform antibacterial assay.

Test microorganism

The following six clinical isolates of bacteria were used for the study: *S. aureus*, *K. pneumoniae*, *B. cereus*, *P. aeruginosa*, *M. luteus* and *E. coli*. All these cultures were maintained on nutrient agar plates at 4°C.



Positive and negative control

Amoxycillin (10 µg/disc) was used as positive control for *B. cereus* and *K. pneumoniae*, Penicillin G disc (10 µg/disc) *S. aureus* and *M. luteus* and Polymyxin-B (10 µg/disc) for *E. coli* and *P. aeruginosa*. Sterilized distilled water was used as negative control.

Antibacterial assay

Antimicrobial activity of the crude extracts was determined by the agar well diffusion method¹⁰. All test organisms were inoculated in Mueller Hinton broth (pH 7.4.) for 8 hours. The concentration of the suspensions was adjusted to 0.5 (optical density) by using a spectrophotometer. Isolates were seeded on Mueller Hinton agar plates by using sterilized cotton swabs. Agar surface was bored by using sterilized gel borer to make wells (7 mm diameter). 100 µl of the test extract and 100 µl of sterilized distilled water (negative control) were poured in to separate wells. The standard antibiotic disc was placed on the agar surface as positive control. Plates were incubated at 37°C for 48 hours. Triplicate plates were maintained for each organism.

Determination of relative percentage inhibition

The relative percentage inhibition of the test extract with respect to positive control was calculated by using the following formula^{6,11}.

Relative percentage inhibition of the test extract =

$$\frac{100 \times (x-y)}{(z-y)}$$

Where,

x: total area of inhibition of the test extract

y: total area of inhibition of the solvent

z: total area of inhibition of the standard drug

The total area of the inhibition was calculated by using area = πr^2 ; where, r = radius of zone of inhibition.

Determination of minimum inhibitory concentration (MIC)

MIC of the leaf extract was performed by modified agar well diffusion method. Two fold serial dilution of the stock solution was prepared in sterilized distilled water to make a concentration range from 0.1-100 mg/ml^{12,13}.

The concentration of test cultures was adjusted to 0.5 McFarland standards. The bacterial suspensions were seeded on MHA plates using a sterilized cotton swab. In each of these plates four wells were cut out using a standard cork borer (7 mm). Using a micropipette, 100 µl of each dilution was added in to wells. All the plates were incubated at 37°C for 24 hours.

Antimicrobial activity of the leaf extract was evaluated by measuring the zone of inhibition. Experiment was carried out in triplicates for each test organism.

Statistical analysis

The values of antimicrobial activity of the aqueous leaves extract of *C. gigantea* were expressed as mean ± standard deviation (n= 3) for each sample.

RESULTS AND DISCUSSION

Medicinal plants are being probed as an alternate source to get therapeutic compounds based on their medicinal properties. *C. gigantea* is easily available in most of the agricultural and non agricultural fields and the usage of this plant for medicinal purpose was reported by several researchers. The aqueous extract of *C. gigantea* leaves exhibited the antibacterial activity against six clinical isolates of bacteria (Tables 1 and Figure 1) and the results were expressed as mean ± standard deviation (n=3). Extract showed maximum antibacterial activity against *E. coli* (17.6±1.15) and lowest activity against *K. pneumoniae* (12.6±1.52).

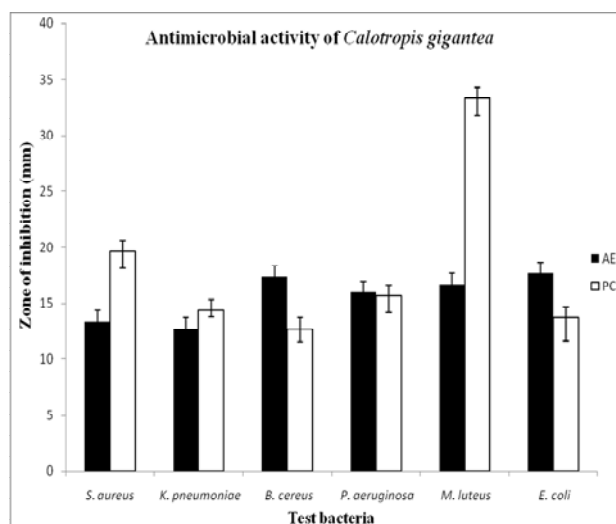
Table 1: Antimicrobial activity of *Calotropis gigantea*

Test organisms	Inhibition zone diameter (mm)		
	AE	PC	NC
<i>Staphylococcus aureus</i>	13.3±1.15	19.6±1.52	0
<i>Klebsiella pneumoniae</i>	12.6±1.52	14.3±0.57	0
<i>Bacillus cereus</i>	17.3±1.52	12.6±1.15	0
<i>Pseudomonas aeruginosa</i>	16.0±1.73	15.6±1.52	0
<i>Micrococcus luteus</i>	16.6±1.52	33.3±1.52	0
<i>Escherichia coli</i>	17.6±1.15	13.6±2.08	0

AE: aqueous extract, PC: positive control, NC: negative control. Values are expressed as mean ± standard deviation of the three replicates.

Zone of inhibition not include the diameter of the well.

Figure 1: Antimicrobial activity of *Calotropis gigantea*



AE: aqueous extract, PC: positive control
Values are expressed as mean ± standard deviation of the three replicates

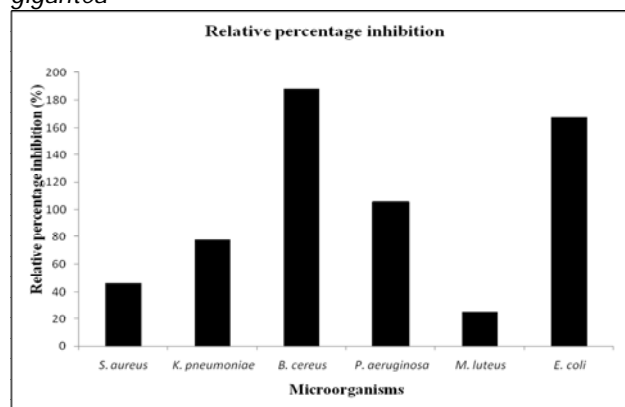


The results of antimicrobial activity of crude extract was compared with the positive control (Standard drugs) for evaluating their relative percentage inhibition (Table 2 and Figure 2), while the aqueous extract exhibits maximum relative percentage inhibition against *B. cereus* (188.52 %) followed by *E. coli* (167.47), *P. aeruginosa* (105.19), *K. pneumoniae* (77.63), *S. aureus* (46.04) and *M. luteus* (24.92 %) respectively.

Table 2: Relative percentage inhibition of *Calotropis gigantea* compare to standard antibiotics

Test organisms	Relative percentage inhibition (%)
<i>Staphylococcus aureus</i>	46.04
<i>Klebsiella pneumoniae</i>	77.63
<i>Bacillus cereus</i>	188.52
<i>Pseudomonas aeruginosa</i>	105.19
<i>Micrococcus luteus</i>	24.92
<i>Escherichia coli</i>	167.47

Figure 2: Relative percentage inhibition of *Calotropis gigantea*



Results of MIC are reported in Table 3. The crude extract showed 50, 25, 12.5, 6.25, 3.1 and 1.5 mg/ml MIC values for *S. aureus*, *K. pneumoniae*, *E. coli*, *B. subtilis*, *P. aeruginosa* and *M. luteus* respectively.

Table 3: MIC values of methanol and aqueous extracts of *Calotropis gigantea* on test organisms

MIC in mg/ml					
<i>S. aureus</i>	<i>K. pneumonia</i>	<i>B. cereus</i>	<i>P. aeruginosa</i>	<i>M. luteus</i>	<i>E. coli</i>
50	25	6.25	3.1	1.5	12.5

Earlier studies on the antimicrobial activity of *C. gigantea* root bark extracts revealed its antibacterial potential against *Sarcina lutea*, *B. megaterium*, *B. subtilis*, *Shigella sonnei*, *E. coli* and *P. aeruginosa*¹⁴. This is in agreement with the present study.

The antifungal activity of *C. gigantea* was also reported in some studies and it provides an important option for the biological control of *Fusarium mangiferae*, a plant pathogenic fungus that causes serious threat in mango cultivation in various countries¹⁵.

Anti-*Candida* activity of the *C. gigantea* leave extracts was reported against clinical isolates of *C. albicans*, *C. tropicalis*, *C. krusei* and *C. parapsilosis*. Aqueous extract of *Calotropis gigantea* showed high inhibitory activity followed by methanol extract, where as ethanol and petroleum ether extracts showed low activity⁶.

Previous studies report the presence of phytochemicals like cardenolides, flavonoids, terpenes, pregnanes, nonprotein amino acid and cardiac glycoside as major constituents in *C. gigantea* may acknowledge the medicinal property of this plant^{7,16}.

We conclude that *C. gigantea* represents a rich source of valuable medicinal compounds and leaves of *C. gigantea* contain high antibacterial property and can be further be explored for the isolation of its bioactive compound.

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