Antimicrobial Activity of Latex of *Calotropis Gigantea* Against Pathogenic Microorganisms - An *In Vitro* Study

Gaurav Kumar, Loganathan Karthik and Kokati Venkat Bhaskara Rao *

Molecular and Microbiology Research Lab, Environmental Biotechnology Division, School of Bio Science and Technology, VIT University, Vellore-632 014, Tamil Nadu, India

***Corresponding author:**
Dr. Kokati Venkat Bhaskara Rao, Assistant Professor (Senior), Environmental Biotechnology Division, School of Bio Sciences and Technology, VIT University, Vellore-632 014, TN, India
Tel: +91 9894350824, E-mail: kokatibhaskar@yahoo.co.in

Summary

The antimicrobial activity of crude aqueous extract of latex of *Calotropis gigantea* (Apocynaceae) was tested against six pathogenic species of bacteria (*Staphylococcus aureus, Bacillus cereus, Escherichia coli, Micrococcus luteus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) and two species of fungi (*Candida krusei* and *Aspergillus niger*). *In vitro* antimicrobial test was performed by agar well diffusion method on Mueller Hinton agar and Sabouraud Dextrose agar for bacteria and fungi respectively. The extract of the latex of *C. gigantea* showed 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*. Minimum inhibitory concentration test was performed by modified agar well diffusion method. MIC values ranged in between 62.5 to 125 µg/ml. Fourier Transform Infrared (FT-IR) spectroscopic analysis of the latex powder showed the presence of major functional groups in the sample.

Keywords: *Calotropis gigantea*; Latex; Well diffusion method; Antimicrobial activity; Fourier Transform Infrared spectroscopic

Introduction

Herbs and plants have been in use as a source of therapeutic compounds in traditional medicinal system since ancient time. There is a continuous need of the development of new effective antimicrobial drugs because of the emergence of new infectious diseases and drug resistance. [1, 2] Most recently plants got a great attention of scientists for the development of alternative drugs to cure several lethal diseases. [3]

C. gigantea is a widely growing plant native to India, Indonesia, Malaysia, Philippines, Thailand, Sri Lanka and China, commonly known as milk weed or crown flower weed. *C. gigantea* is latex bearing plant and release the latex after a tissue injury. Plant latex is a mixture of alkaloids, tannins, gum, sugars, starch, resins and protein. [4]

Leaves, roots, stem, flowers and latex of *C. gigantea* are used in traditional medicinal system to cure several diseases and medicinal potential of the *C. gigantea* proved scientifically. The flowers of the *C. gigantea* are used in stomachic, bechic, antiasthmatic, analgesic activity. [5] Roots are used for the treatment of lupus, tuberculous leprosy, and syphilitic ulceration. Roots also contain anti-pyretic activity [6](Chitme *et al*., 2005), cytotoxic activity [7], antimicrobial activity [8, 9, 10], CNS activity [11] and pregnancy interceptive properties [12]. Leaves and areal parts of the plant are used in the treatment of external swellings and diarrhoea. [13] Latex is reported to contain purgative properties, procoagulant activity [14] and wound healing activity. [15] *C. gigantea* also uses to cure toothache, earache, sprain, anxiety, pain, epilepsy and mental disorders.

The focus of this investigation was to determine antimicrobial activity of latex of *C. gigantea* against pathogenic microorganisms *in vitro*.

Materials and Methods

Chemicals

Nutrient broth (NB), MHA, SDA, penicillin G disc, polymyxin-B disc, amoxycillin disc, amphotericin-B disc, dimethyl sulfoxide (DMSO) were purchased from Hi-Media, Mumbai, India. Potassium Bromide (KBr-FTTR grade) was purchased from S.D. Fine, Mumbai, India.

Plant material

C. gigantea plant was collected from the wasteland of Vellore district, TN, India (12°54′40″N 79°8′10″E) in the month of December, 2008. Plant was identified in Herbal Garden of VIT University, TN, India, voucher specimen was maintained in our laboratory (Accession number: VIT/SBST/MMRL/CG/10.1.2009/101)

Collection of latex from the plant

The apical part of the plant was collected from the natural population of *C. gigantea*. Fresh latex was collected as an exudate from the apical part of plant. Latex sample was brought to the Molecular Microbiology Research Lab, VIT University, TN, India. The latex sample was dried in hot air oven at 42°C. Dried latex was powdered using mortar and pestle.

Preparation of aqueous extract

Ten grams of latex powder was extracted with 100 ml of distilled water for two days at room temperature. Extract was filtered by using four layers of muslin cloth. Extract was concentrated in rotavapor and dried by using lyophilizer. The extracted powder was dissolved in DMSO as 100 mg/ml (10% w/v) solution.

Determination of antimicrobial activity

Test microorganisms

The aqueous extract of the latex of *C. gigantea* was tested against pathogenic bacteria *S. aureus, B. cereus, E. coli, M. luteus, K. pneumoniae* and *P. aeruginosa* and fungi *C. krusei* and *A. niger*. All the cultures were isolated from the clinical samples. All the test organisms were inoculated in to nutrient broth and incubated at 37°C for 8 hours.

Positive and negative control

Penicillin G disc (10 µg/disc) was used as positive control (PC) for *S. aureus* and *M. luteus*, Polymyxin-B (10 µg/disc) for *E. coli* and *P. aeruginosa*, Amoxycillin (10 µg/disc) for *B. cereus* and *K. pneumoniae* and Amphotericin-B (20 µg/disc) for fungal cultures. DMSO was used as negative control (NC).

Antimicrobial assay

Antimicrobial activity of the crude latex extract was determined by agar well diffusion method. [16] The concentration of the microbial suspensions was adjusted to 0.5 McFarland standards. The bacterial suspensions were seeded on MHA plates and fungal suspensions on SDA (in triplicates). In each of these plates two wells were cut out using a sterilize cork borer. Using a micropipette, 100 µl of crud extract and negative control was added in to different wells. A positive control antibiotic disc was placed in the plate. Bacterial plates were incubated for 24 hours at 37°C and fungal plates for 72 hours at room temperature. Antimicrobial activity was evaluated by measuring the zone of inhibition.

Determination of relative percentage inhibition

The relative percentage inhibition of the crude latex extract with respect to positive control was calculated by using the following formula [17]

$$\textbf{Relative percentage inhibition of the test extract} = \frac{\mathbf{100 \times (\mathit{a}\text{-}\mathit{b})}}{\mathbf{(\mathit{c}\text{-}\mathit{b})}}$$

Where,

a: total area of inhibition of the test extract

b: total area of inhibition of the solvent

c: 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)

The MIC of the crude extract was determined by modified agar well diffusion method. [18, 19] The extract was dissolved in DMSO to obtain a concentration range of 62.5, 125, 250, 500, 1000, 5000 and 10000 µg/ml. The bacterial suspensions were seeded on MHA plates and fungal suspension on SDA plates (in triplicates).

In each of these plates four wells were cut out using a cork borer. Using a micropipette, 100 µl of each dilution was added in to wells. Bacteria plates were incubated at 37ºC for 24 hours and fungal plates were incubated at room temperature for 48 to 72 hours. The minimum concentration of each extract showing a clear zone of inhibition was considered to be MIC.

FT-IR analysis of the plant sample

The dried latex of *C. gigantea* was ground into fine powder using mortar and pestle. Two milligrams of the sample were mixed with 200 mg KBr (FT-IR grade) and pressed into a pellet. The sample pellet was placed into the sample holder and FT-IR spectra were recorded in the range 4000-450 cm^{-1} in FT-IR spectroscopy (AVATAR 300 FT-IR, Thermo Nicolet, USA). [20]

Statistical analysis

The results of the antimicrobial activity of latex extract *C. gigantea* are expressed as mean ± standard deviation of the response of 3 replicates determinations per sample. Level of significance was assessed by the Student *t* test at P□0.05. Results were analyzed statically by using Microsoft Excel 2007 (Roselle, IL, USA).

Results and Discussion

The selection of this plant for the present study was based on its medicinal properties and use in traditional medicinal system. This plant is known for antimicrobial, anti-diarrhoeal, antipyretic, wound healing and CNS activity etc. In this study crude aqueous extract of latex of *Calotropis gigantea* was tested against pathogenic species of bacteria and fungi.

The antimicrobial activities of the aqueous extract of *C gigantea* latex with respect to positive and negative control are listed in Table 1. All values were expressed as mean ± standard deviation of three replicates. The results were statistically analyzed by student *t* test at P□0.05 confidence limit, plant showed significantly higher inhibition against *S. aureus, B. cereus, E. coli,* significantly low inhibition against *C. krusei*, whereas, no activity was reported against *M. luteus, K. pneumoniae*, *P. aeruginosa* and *A. niger*. The inhibitory effect showed by the latex extract was find higher than the standard antimicrobial drugs used against *S. aureus, B. cereus, E. coli*, whereas, slightly lower for *C. krusei*. Earlier, anti-Candida activity of leaves of *C. gigantea* was reported against clinical isolate of *Candida albicans, C. parapsilosis, C. tropicalis* and *C. krusei*. [8] *C. gigantea* was reported to possess antibacterial activity against *S. aureus*, *E. coli, B. cereus, P. aeruginosa, M. luteus and K. pneumoniae*. [9] Antifungal activity of *C. gigantea* was reported against plant pathogenic fungi *Fusarium mangiferae*. [21]

The results of relative percentage inhibition are reported in Table 2. The latex extract showed the maximum relative percentage inhibition against *E. coli* (381.1%), whereas, no relative percentage inhibition against *M. luteus, K. pneumoniae* and *P. aeruginosa* and *A. niger* (0%).

The results of the MIC for the latex extract are mentioned in Table 2. All values were expressed as mean of three replicates. MIC values for *S. aureus* and *E. coli* was find 62.5 µg/ml, whereas, 125 µg/ml for *B. cereus* and *C. krusei.*

The results for FTIR analysis for the functional groups present in the latex of *C. gigantea* are listed in Table 3 and Figure 1. The FT-IR spectrum showed Weak absorption bands at 3629.48 cm^{-1} is representative for O-H stretching vibration for amino acids. Strong absorption bands at 3472.99 and 3432.32 cm^{-1} are representative for O-H and N-H stretching vibrations, characteristic of amino acids. The very strong absorption band at 2923.99 is representative for Aliphatic – CH_3 and CH_2 Stretching of chlorophyll. Very strong absorption band at 1730.70 and 1634.55 cm^{-1} are representative for C=0 stretching vibration for acids and Secondary amides respectively. Strong absorption band at 1320.51 and 1378.77 cm^{-1} are representative for C=H deformations of –CH2 or –CH3 groups for lignin in aliphatics. Strong absorption band at 1243.52 cm^{-1} is representative for ester carbonyl group, phenol. Strong absorption band at 1104.21 cm^{-1} is rrepresentative for C-H deformation, C-O, C-C stretching for carbohydrates. Strong absorption band at 1018.28 cm^{-1} represent C-O Stretching of polysaccharides, Si-O asymmetric stretch for starch and silicate impurities. Absorption band at 778.87 and 676.24 cm^{-1} represent CH out of plane bending for carbohydrate (Ramamurthy and Kannan, 2007).

The results of the current study conclude that aqueous extract of latex of *C. gigantea* possess significant amount of antimicrobial activity against a wide range of microorganisms.

Table 1: Antimicrobial activity of latex extract of *Calotropis gigantea* on tested organisms

Test organisms	Zone of inhibition (mm)		
	Latex	**PC**	**NC**
Staphylococcus aureus	30±1	17.6±0.5	-
Bacillus cereus	16±1	11.3±0.5	-
Escherichia coli	24.6±0.5	12.6±0.5	-
Micrococcus luteus	-	34.6±0.5	-
Pseudomonas aeruginosa	-	15±1	-
Klebsiella pneumoniae	-	14±1	-
Candida krusei	13.6±0.5	15.3±0.5	-
Aspergillus niger	-	17.3±0.5	-

All the values are mean ± standard deviation (n=3)
PC- positive control; NC- negative control
Zone of inhibition not include well diameter

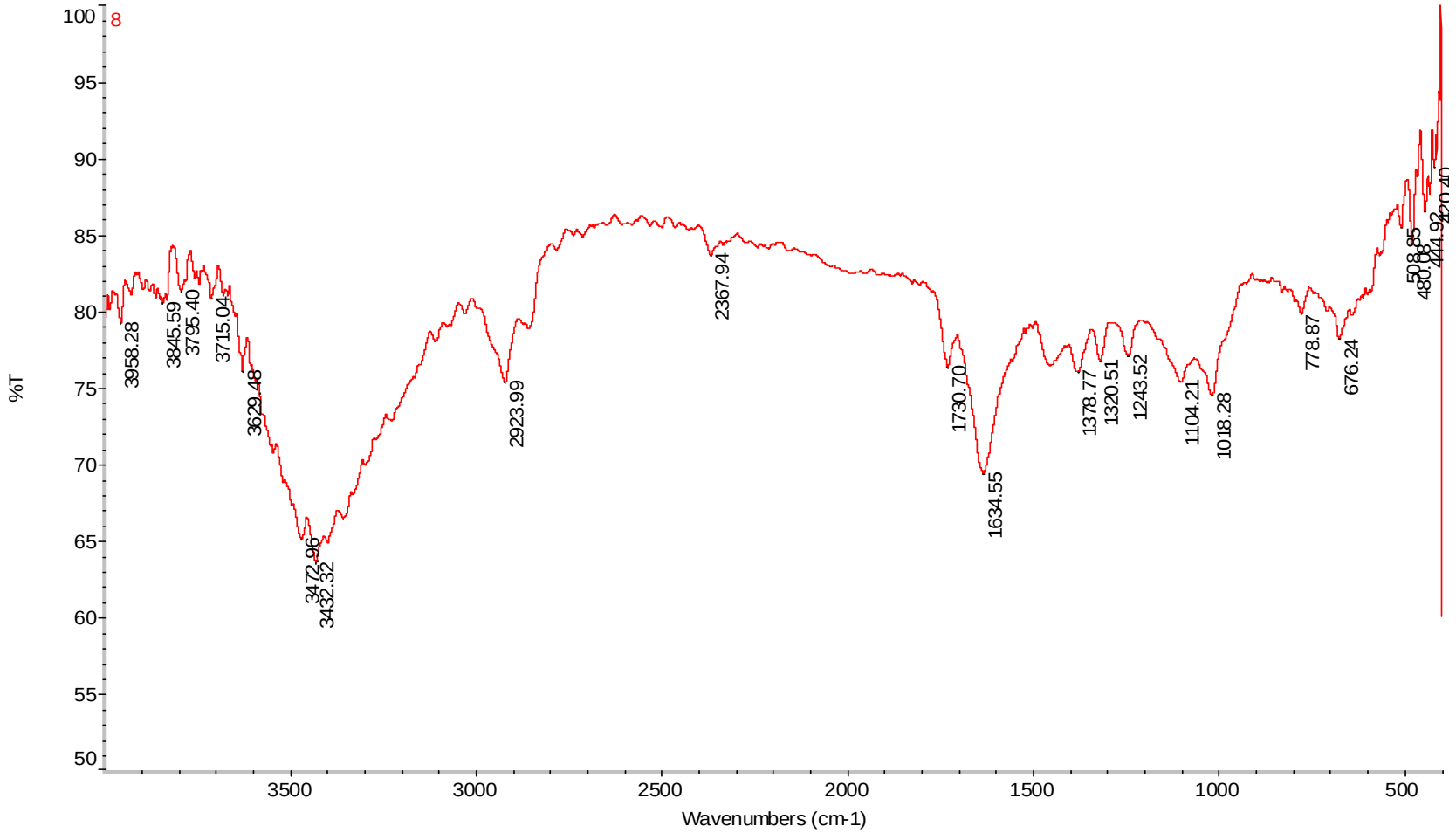
Table 2: Relative percentage inhibition of latex extract of *Calotropis gigantea* on tested organisms

Test organisms	Relative percentage inhibition (%)	MIC (In µg/ml)
Staphylococcus aureus	290.5	62.5
Bacillus cereus	200.4	125
Escherichia coli	381.1	62.5
Micrococcus luteus	-	-
Pseudomonas aeruginosa	-	-
Klebsiella pneumoniae	-	-
Candida krusei	79.0	125
Aspergillus niger	-	-

Table 3: Assignment of FT-IR absorption bands in the spectra of plant samples (*Calotropis gigantea*)

S. No	Absorption frequency (cm^{-1})	Tentative assignment
1.	676.24	CH out of plane bending (carbohydrate)
2.	778.87	CH out of plane bending (carbohydrate)
3.	1018.28	C-O Stretching of polysaccharides, Si-O asymmetric stretch (Starch and silicate impurities)
4.	1104.21	C-H deformation, C-O, C-C stretching (carbohydrates)
5.	1243.52	Ester carbonyl group, phenol
6.	1320.51	C=H deformations of –CH2 or –CH3 groups (lignin) in aliphatics
7.	1378.77	C=H deformations of –CH2 or –CH3 groups (lignin) in aliphatics
8.	1634.55	C=0 Carbonyl Stretching (Secondary amides)
9.	1730.70	C=0 stretching vibration (acids)
10.	2923.99	Aliphatic – CH3 and CH2 Stretching (chlorophyll)
11.	3432.32	N-H Stretching Vibration (amino acids)
12.	3472.99	N-H Stretching Vibration (amino acids)
13.	3629.48	O-H Stretching Vibration (amino acids)

Figure 1: FTIR spectrum of the latex of *Calotropis gigantea*



Acknowledgement

Authors wish to thank management of VIT University, Vellore, TN, India, for providing necessary facilities and support for the completion of this work.

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