World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

ANTIBACTERIAL PROPERTIES OF DIFFERENT PARTS OF CALOTROPIS GIGANTEA: AN IN-VIVO STUDY

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Article Received on 02/03/2021 **Article Revised on 22/03/2021** **Article Accepted on 12/04/2021**

ABSTRACT

C gigantea, a noncultivable weed found abundantly in Africa and Asia, is commonly known by the names "crown flower," "giant milkweed," and "shallow wort" and is known for many medicinal properties. The aim of the present study was to investigate antimicrobial and antifungal activities of aqueous extracts of *Calotropis gigantea* against clinical isolates of bacteria and fungi. *In vitro* antimicrobial and antifungal activity was performed by cup well diffusion method. The extract showed significant effect on the tested organisms. The extract showed maximum zone of inhibition against *E. coli* (18.1±1.16) and lowest activity against *K. pneumoniae* (11.4±1.44). latex of C. gigantea showed maximum relative percentage inhibition against *B. cereus* (178.2 %) followed by *E. coli* (171.2), *P. aeruginosa* (102.4), *K. pneumoniae* (79.5), *S. aureus* (46.04) and *M. luteus* (23.7 %) respectively. Minimum Inhibitory Concentration (MIC) was measured by cup and plate method and the aqueous extract exhibited good antibacterial and antifungal.

KEYWORDS: Antimicrobial; *Calotropis gigantea*; cup and plate method; minimum inhibitory concentration.

1. INTRODUCTION

A medicinal plant is any plant that can be used for therapeutic purpose or which is a precursor for synthesis of useful medicinal drugs. The increased occurrence of severe opportunistic bacterial and fungal infections is an important problem. Therefore, there is an increased need for discovery of new classes of natural products that may be effective against bacteria and/or fungi. Herbal extracts reminds us of the continuous effort to find new compounds that are effective against multi resistant bacteria. Most of the plants have antimicrobial, anticancer, anti-inflammatory, antidiabetic, haemolytic, antioxidant, larvicidal properties, etc. About 20%of all the available antibiotics present in the market are either obtained naturally or semi-synthetically. The Plant Calotropis gigantea belongs to Apocynaceae family and is an medicinal plant with high medicinal values. Its cultivation is native to Cambodia, but it is also found in many parts of Asian subcontinent such as Malaysia, Indonesia, Philippines, Nepal, Thailand, China, India, Pakistan and some parts of Africa.

The genus Calotropis (family Apocynaceae, subfamily Asclepiadaceae) contains six species.[1] C gigantea, a noncultivable weed found abundantly in Africa and Asia, is commonly known by the names "crown flower," "giant milkweed," and "shallow wort." In India, it is commonly called "Madar" in the Hindi dialect. It grows in waste lands and can be identified by its thick oblong leaves and odorless purplish flowers (Figure 1).

Figure 1: Plant and flower of Calotropis Gigantea.

Milky white acrid juice exudates out once the leaves or stalks of the plant are incised or broken. The phytoconstituents of C gigantea that are derived from various parts of the plant include numerous glycosides, alkaloids, flavones, tannins, and so forth such as calotroxin, uscharin, uschridin, and proceroside.[2] Additionally, numerous cardenolides, flavonoids, terpenes, pregnanes, and a non-protein aminoacid have been isolated.[1] Most of the well-studied plant components isolated from the sap, root bark, and/or leaves are cytotoxins. The plant yields a durable fiber that is used in rural households to make bowstring, ropes, carpets, and sewing threads. Latex of the plant has been used in treating a variety of ailments in folklore medicine for thousands of years. Remedies based on traditional systems of medicine such as Ayurveda are often imported into Western countries and their use remains popular without any evidence base. Before the advent of penicillin, the milky sap was thought to be effective against syphilis. Hence, the plant was referred to as "vegetable mercury." Calotropis is reportedly used in treating fever, indigestion, diarrhea, cold, cough, asthma, rheumatism, leprosy, leukoderma, and others in Ayurveda, Chinese,and homeopathic medicines.[1-4] Constituents of the sap have recently been studied for anti-inflammatory and anti-tumor properties and for its effectiveness against certain cancers.[1,3]

Though the antimicrobial activity of this plant against certain microbes was investigated already, the information about antimicrobial activity of flower and complete comparative antimicrobial analysis of different parts of this plant is poorly reported. So, the aim of this study was to evaluate the antibacterial activities of different parts of *Calotropis gigantea.*

2. MATERIALS AND METHODS

The fresh plants of C. gigantea was collected from roadside of Coimbatore. The plant specimens are further identified by Prof. Ravikumar Ex-reader in Botany, Govt. College of Arts and Science, Coimbatore.

3 Preparation of the Extract

3.1 *Ethanolic Extract*

The leaves were separated from the whole plant and is washed thoroughly. The leaves are cut into small pieces and are allowed to shade dry at room temperature for 15 days. The dried leaves are finely powdered and 100 gm powder was taken for extraction in absolute ethanol by Soxhlet apparatus (Borosil Glass Work Limited, Worli, Mumbai, India) at 40 _C for 18–20 h. The extract was filtered through Whatmen no.1 filter paper and concentrated at room temperature. The extracts are stored at 4 _C till further analysis.

3.2 Methanolic Extract

The leaves were separated from the whole plant and is washed thoroughly. The leaves are cut into small pieces and are allowed for shade dry at room temperature for 15 days. The dried leaves are finely powdered and 100 gm of the powder is taken for extraction in methanol by Soxhlet apparatus (Borosil Glass Work Limited, Worli, Mumbai, India) at 40 _C for 18–20 h. The extract was filtered through Whatmen no.1 filter paper and concentrated at room temperature. The extracts are stored at 4 _C till further analysis.

3.3 Chloroform Extract

The leaves were separated from the whole plant and is washed thoroughly. The leaves are cut into small pieces and are allowed for shade dry at room temperature for 15 days. The dried leaves are finely powdered and 100 gm of the powder is taken for extraction in chloroform by Soxhlet apparatus (Borosil Glass Work Limited, Worli, Mumbai, India) at 40 _C for 18–20 h. The extract was filtered through Whatmen no.1 filter paper and concentrated at room temperature. The extracts are stored at 4 _C till further analysis.

3.4 Test Microorganism and Growth Media

Bacteria strains *B. cereus, E. coli, P. aeruginosa, K. pneumoniae, S. aureus* and *M. luteus a fungal* strain of Rhizopus nigricans were chosen based on their clinical and pharmacological importance. The bacterial strains obtained from the Department of Microbiology, RVS College of Arts and Science, Coimbatore, were used for evaluating antimicrobial activity. The bacterial and fungal stock cultures were incubated for 24 h at 37 _C on nutrient agar and potato dextrose agar (PDA) medium (Hi-Media), respectively, following refrigeration storage at 4 _C. The bacterial strains were grown in Mueller–Hinton agar (MHA) plates at 37 _C (the bacteria were

grown in the nutrient broth at 37 _C and maintained on nutrient agar slants at 4 _C), whereas the fungal strains were grown in Sabouraud dextrose agar and PDA media, respectively, at 28 _C. The stock cultures were maintained at 4 _C.

4. Antimicrobial Test

The antimicrobial activities of ethanolic extracts of C. gigantea were determined by filter paper disc diffusion method.

4.1 Disc Diffusion Method

A sterile filter disc (diameter 4 mm, Whatman paper No. 3) was placed in Petri dishes (diameter 90 mm) filled with Mueller–Hinton agar and seeded with 0.3 ml of the test organism. The disc was impregnated with test concentrations (5, 25, 50, 100 mg/ml) of the compounds investigated dissolved in DMSO. The zones of growth inhibition around the discs were measured after 24 h of incubation at 37 _C. Each microorganism was tested in triplicate and the solvent (DMSO) was used as a control, while streptomycin was used as a positive control for antibacterial activity and nystatin for antifungal activity.

4.2 Minimum Inhibitory Concentration

Different concentrations extract of C. gigantea were prepared to obtain 2.5, 5.0, 7.5 mg/ml. Three drops of overnight broth culture of the test organisms were inoculated into the dilutions and incubated at 37 _C for 24 h. The lowest concentration of the extracts that inhibited the growth of the test organisms was recorded as the minimum inhibitory concentration (MIC).

5. 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 the results were expressed as mean ± standard deviation. Extract showed maximum antibacterial activity against *E. coli* (18.1±1.16) and lowest activity against *K. pneumoniae* (11.4±1.44).

Table 1: Antimicrobial activity of *Calotropis gigantean.*

Test Organisms	Inhibition Zone Diameter (mm)		
	Aqueous Extract	Positive Control	Negative Control
Staphylococcus aureus	13.3±1.15	19.6±1.52	0
Klebsiella pneumoniae	11.4±1.44	12.2±0.37	0
Bacillus cereus	17.3±1.52	12.6±1.15	0
Pseudomonas aeruginosa	16.0±1.73	15.6±1.52	0
Micrococcus luteus	16.6±1.52	33.3±1.52	0
Escherichia coli	18.1±1.16	11.4±1.88	0

The results of antimicrobial activity of crude extract was compared with the positive control (Standard drugs) for evaluating their relative percentage inhibition (Table 2), while the aqueous extract exhibits maximum relative percentage inhibition against *B. cereus* (178.2 %) followed by *E. coli* (171.2), *P. aeruginosa* (102.4), *K. pneumoniae* (79.5), *S. aureus* (46.04) and *M. luteus* (23.7 %) respectively.

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

Test Organisms	Relative Percentage Inhibition (%)
Staphylococcus aureus	***47.2***
Klebsiella pneumoniae	***79.5***
Bacillus cereus	***178.2***
Pseudomonas aeruginosa	***102.4***
Micrococcus luteus	***23.7***
Escherichia coli	***171.2***

Using cup plate method the test bacteria (E.coli & staphylococcus aureus) are used to inoculate on the nutrient agar plates with the help of sterile cotton swabs to develop the culture. Then well punched in agar plates. Then the extracts are poured into the well. The plates were incubated at 37°C for 24hours and examined for clear zones of inhibition. Calotropis gigantea latex extract was used for the test of antibacterial activity. The bacteria E.coli & staphylococcus aureus are inoculated in the nutrient broth culture and incubated at 37°C for 48 hours can be used. The zone of inhibition is shown in figure 1.

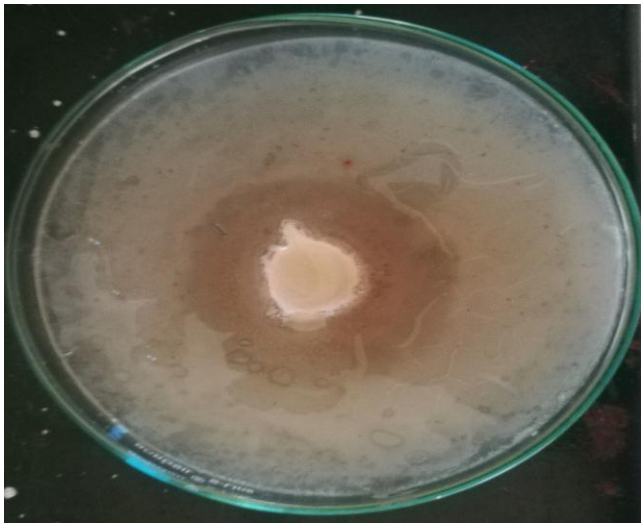

Figure 1: Antibacterial activity of Calotropis gigantea extract on E.coli & staphylococcus aureus.

Calotropis gigantea latex was used for the test of antifungal property. Fungicidal property of a substance refers to its effect of that substance to kill fungi. These substances which can kill the fungi are of great importance to many fields of medicinal and agricultural areas. The fungi Rhizopus nigricans are inoculated in the nutrient broth culture & and place in the aseptic area(room temperature).

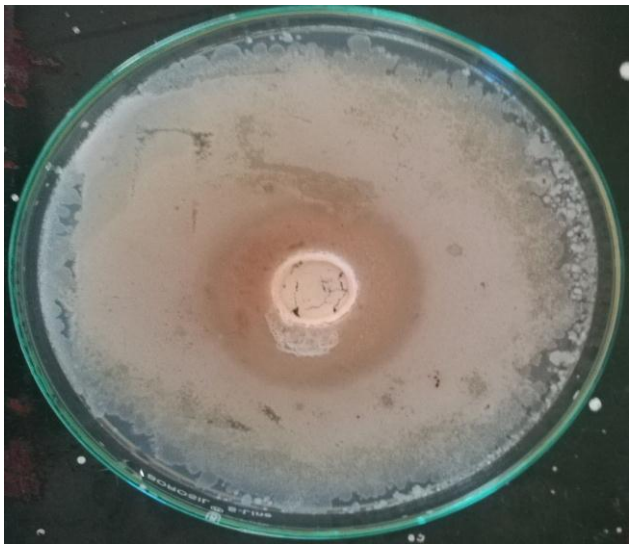

Figure 2: Antifungal activity of Calotropis gigantea extract on Rhizopus nigricans.

6. 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.[5-9] 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). 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* (178.2 %) followed by *E. coli* (171.2), *P. aeruginosa* (102.4), *K. pneumoniae* (79.5), *S. aureus* (46.04) and *M. luteus* (23.7 %) respectively.

7. CONCLUSION

The encouraging results indicate that the extracts of various parts of calotropis gigantea might be exploited as natural drug for the treatment of several infectious diseases caused by these organisms. Our results showed that in present work that latex extracts obtained different parts of the plant of calotropis gigantea using centrifuge are rich sources of potent phytochemicals especially the latex extract and has inhibitory effects on the experimental microbes. These bioactive complex

phytochemicals can be used for the development of potent drugs, medicines or antimicrobial agents that can be used for various purposes for human welfare upon further extensive & systematic studies. Antibacterial & Antifungal property has been found in the latex of the species.

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