

# Antimicrobial Activity of *Calotropis gigantea* against *Staphylococcus aureus*: Eco-Friendly Management

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## ABSTRACT

*Calotropis gigantea* (Madaar) Linn. is a potent medicinal herb that has active compounds in the form of alkaloids, glycosides, lactones, and steroids. All these active compounds have immune-modulatory and physiological roles of different types; the plant is also reported as effective in treating skin, digestive, respiratory, circulatory, and neurological disorders and was used to treat fevers, elephantiasis, nausea, vomiting, and diarrhea. In this study, the phytochemical and the zone of inhibition was measured in *C. gigantea* L. It proves that *C. gigantea* L. is more effective against *Staphylococcus aureus*. Aqueous extract of *C. gigantea* L. was extracted by the aqueous method. In the qualitative phytochemical analysis presence of various secondary metabolites were found as alkaloids, flavonoids, tannins, and saponin. In the quantitative analysis, carbohydrate was found in *C. gigantea* L. about concentration is found (470 µg/mL). Antimicrobial activity was also quite good against *S. aureus*. The study demonstrates that the *C. gigantea* L. contains the presence different of bioactive compounds indicated a potent antimicrobial activity of *C. gigantea* L. against *S. aureus* so that we called as eco-friendly management.

**Keywords:** Antibacterial activity, Eco-friendly management, Eco-management.

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## INTRODUCTION

The *C. gigantea* Linn. commonly known as *Madar* or *Yercum*, belongs to the family Asclepiadaceae. Leaves of this plant were investigated for its morphological, microscopical, and phytochemical constituents to check the authenticity of the plant. *C. gigantea* L. R. Br. (Asclepiadaceae) has been known to the traditional systems of medicine and plant known as *Madar* in the *Unani* medicinal system (Rastogi and Mehrotra, 1999; Yelne *et al.*, 2000). Widely it is used medicinally to treat boils, infected wounds, and other skin problems in people. *C. gigantea* L. is regarded as a useful medicinal plant and used in folk medicine. This plant is popularly known because it produces a large quantity of latex. Medicinal plants have no doubt remained the major sources of traditional medicine worldwide (Yogi *et al.*, 2016). A scrutiny of the literature revealed some notable pharmacological activities of the plant, such as analgesic, hepato-protective, anti-diarrhoeal, anti-diabetic, antimalarial, antinociceptive, anti-inflammatory, anthelmintic, anticonvulsant, antimicrobial, anticancer, antifertility, and antioxidant activity. The present review is an attempt to highlight the various ethano-botanical and traditional uses as well as phytochemical and pharmacological reports on *C. procera* (Murti *et al.*, 2010; Muzammal, 2014; Yogi *et al.*, 2016).

Herbal medicines have been used from the earliest times to the present day. Ethnopharmacology is as old as man himself. Herbal medicines exhibit a remarkable therapeutic diversity. *C. gigantea* L. is an *Ayurvedic* plant which is used in several traditional medicines to treat a variety of diseases. The extracts from different parts of the plant have significant therapeutic value. The whole plant, when dried, exhibits good tonic, antihelmintic, and expectorant activities (Quazi *et al.*, 2013; Yogi *et al.*, 2016). The roots also have similar activities and also act as an effective laxative. Traditionally, the powdered root is used to treat bronchitis, asthma, leprosy, eczema, elephantiasis, while the latex is used to, treat vertigo, baldness, hair loss,

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toothache, intermittent fevers, rheumatoid/joint swellings, and paralysis, The leaves are used to treat joint pain, and reduce swelling. Besides its *Ayurvedic* use, *C. gigantea* L. is also used as homeopathic medicine. In ancient *Ayurvedic* medicine, the plant *C. gigantea* L. was known as "Raktaarka." The pungent latex extracted from the leaves and flowers of *C. gigantea* is processed and used in the commercial preparation of eye tonics (Quazi *et al.*, 2013).

*C. gigantea* L. is a widely used traditional medicinal plant to treat various ailments. It is an erect, perennial shrub luxuriantly thriving in wastelands. Plants are the richest sources of bioactive organic chemicals on earth. They are the storehouse of secondary metabolites such as alkaloids, terpenoids, steroids, and flavonoids, etc. The traditional medicine involves the use of different plant extracts or bioactive chemicals. The results suggest that the phytochemical properties of the stem, leaves, and flowers for curing various ailments (Shrivastava *et al.*, 2013).

*C. gigantea* L., commonly known as *Madar* or *Yercum*, belongs to the family Asclepiadaceae. Leaves of this plant were investigated for its morphological, microscopical, and phytochemical constituents to check the authenticity of the

plant. A study to evaluate *in vitro* antibacterial activity of methanol extract of the leaves against gram-negative bacteria such as *Salmonella typhi*, *Pseudomonas fluorescens*, *P. aeruginosa*, and *Escherichia coli* was carried out. The pathogens were tested by disc diffusion assay method, and minimum inhibitory concentration was evaluated. An attempt has been made to compare the activity of extract with standard ciprofloxacin. The antibacterial activity, therefore, shows clearly that the resistance to the pathogens may be minimized by these natural products of the plant origins (Sethi *et al.*, 2014).

In this study, the antimicrobial activity of leaves obtained from *C. gigantea* L. has been assessed against selected pathogenic bacterial strain.

## MATERIALS AND METHODS

### Sample Collection

The sample is collected from Jiyamau, Hazratgunj, and Telibag areas in Lucknow. The samples were stored at room temperature (37°C) until further use. Drying of leaves of *C. gigantea* L. was done at room temperature for 3 to 4 days.

### Preparation of Aqueous Extract

The plant part leaves were maceration by the help of mortar and pestle to make it into powdered form, and about 5 grams of powder was extracted with 0.8% saline water. The extract was kept for one hour, and after that, it was filtered and stored in airtight containers. The extract was preserved in the refrigerator at approximately 4°C, stored in airtight containers.

### Phytochemical Test

#### Saponin

A 1 mL sample was dissolved in 5 mL distilled water. It was shaken well, and froth formation took place. The stability of froth confirms the presence of saponin in the sample.

#### Tannin

A 1 mL sample was dissolved in 1 mL 5% FeCl<sub>3</sub>. The appearance of dark blue, black, or dark green confirms the presence of tannin in the sample.

#### Flavonoid

A 1 mL sample was dissolved in 2 mL 1% NaOH. The presence of yellow color indicates the presence of flavonoids in the sample.

#### Alkaloid

A 1 mL iodine was dissolved in 1 mL sample. The appearance of reddish-brown precipitate confirms the presence of alkaloids in the sample.

### Salicylic Acid DNS Method for Carbohydrate Estimation

Take ten clean, dry test tubes, pipette, and standard sugar solution in the range of 0 to 3 mL in different test tubes and make up the volume of all test tubes to 3 mL with distilled water concentrations ranging from 0 to 750 mg. Add 1 mL DNS reagent to all the test tubes and mix plug the test tube with cotton or marble and keep the test tube in a boiling water bath for

5 minutes. Take the tubes and cool them to room temperature. Read extinction at 540 nm against the blank. Please note that all the tubes must be cooled to room temperature before reading since the absorbance is sensitive to temperature. Prepare standard curves of the sugars provided and use them to estimate the concentration of the unknowns provided.

### Studied Activity

Antimicrobial activity by the disc diffusion method and determination of minimum inhibitory concentration (MIC).

### Screening of Antifungal Activities

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. Similarly to the procedure used in the disk diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a well with a diameter of 6–8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100 µL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Then agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested.

The strain (*S. aureus*) from the plate was inoculated in the nutrient broth, and then inoculum was left for 1–2 days at 30°C in the incubator, after the growth of bacteria in the broth, it is used to perform the agar well diffusion method with the given plant samples. NAM media were prepared for three plates of 15 mL, then subjected it to autoclave. Immediately after autoclaving, it was poured into a Petri plate. Agar media was allowed to cool and solidify at room temperature. The glass spreader and plates were put in UV light. And after that 10 µL of the sample was put in plates and spread with the spreader evenly on the surface of the plate. Then it was dried for 4–5 minutes. Then the well was punched out of four wells, antibiotic was added in one well, and rest three well was added with the extracts. The plate was kept in an incubator for 24–48 hours.

## RESULTS AND DISCUSSION

A plant with as diverse a role as *C. gigantea* L. is a versatile resource for all forms of life. There are reports as already discussed that the plant extracts have active compounds in the form of alkaloids, glycosides, lactones, and steroids. All these active compounds have immune-modulatory and physiological roles of different types, thereby demonstrating the diverse versatility of the plant. Studies need to be conducted with aspects of how the active compounds actually interact with the living systems and affect the structure-function relationship. The plant is also reported as effective in treating skin, digestive,

**Table 1:** Phytochemical analysis of *C. gigantea* L. leaves

Tests	<i>C. gigantea</i> L. (+/-)
Flavonoid	++
Alkaloid	+++
Saponin	++
Tanin	+



**Fig. 1:** Madar flower leaves



**Fig. 2:** Sample in saline water

respiratory, circulatory, and neurological disorders and was used to treat fevers, elephantiasis, nausea, vomiting, and diarrhea.

The zone of inhibition was measured in *C. gigantea* L. It proves that leaves are more effective against *S. aureus*. Aqueous extract of *C. gigantea* L. was extracted by the aqueous method. In the qualitative phytochemical analysis presence of various



**Fig. 3:** Antibacterial activity against *S. aureus*

**Table 2:** Antimicrobial activity of essential oil of *C. gigantea* L.

Sample used ( $\mu\text{L}$ )	Zone of inhibition (mm)
50	$3 \pm 0.12$
75	$5 \pm 0.14$
100	$7 \pm 0.10$
Positive control	$8 \pm 0.14$

\*Values are mean of three replicates, with  $\pm$  standard error

secondary metabolites was found in aqueous extract of *C. gigantea* L. were alkaloids, flavonoids, tannins, and saponin. In the quantitative analysis, carbohydrate was found in *C. gigantea* L. about concentration is found ( $470 \mu\text{g/mL}$ ) (Table 1; Figs 1 and 2). Antimicrobial activity was also quite good, in *C. gigantea* L. against *S. Aureus* (Table 2; Fig. 3). The study demonstrates that the *C. gigantea* L. contains the presence of different bioactive compounds.

Comparative study of plant extracts crude and aqueous, methanolic, and ethanolic with antibiotics, provide evidence that *C. gigantea* L. extracts have the similar antibacterial activity as these antibiotics against test pathogens, i.e., *Salmonella typhi* and *E. coli* (Anonymous, 1956). The analysis of the antimicrobial activity of aqueous, methanolic, and ethanolic extract of leaves and flower of *C. gigantea* L. was carried out in disc method and also determined MIC value at through optical density using a spectrophotometer. The zone of inhibition produced by extracts was examined and compared it with the zone produced by antibiotics. The effect exhibited by the ethanolic extract of leaves and flowers was significantly greater than the aqueous and methanolic extract of leaves and flowers. Crude extracts, i.e., latex, leaves, fruit, and flower crude extracts, among them, flower crude extracts, show a similar zone of inhibition to test antibiotics. While in MIC value, we made different concentrations of extracts and antibiotics, i.e., for crude we made the concentration of crude juice and for the aqueous, methanolic and ethanolic dimethyl sulfoxide, and same antibiotics concentration. Aqueous leaves extracts show MIC against *E. coli* while against *Salmonella* it shows MIC. We also determine phytochemical analysis for the presence of different compounds in crude extracts. The obtained results provide support for the use of *C. gigantea* L., in traditional medicine and suggest its further advance investigation (Mainasara *et al.*, 2011).

Phytochemical and antibacterial activities of water, methanol, and ethanol extracts obtained from the fruit and bark of *C. gigantea* L. were investigated in an attempt to evaluate its medicinal potentials. The phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, and cardiac glycosides with a very high content in water extracts. The concentration of the phytochemical constituents was in the order of water > methanol > ethanol. Antibacterial activity was also determined against *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*, and *Streptococcus pyogenes*. Water extracts showed inhibition against the tested organisms. Methanol and ethanol extracts did not show an appreciable activity, respectively. The result of this study validates the use of water extract of this species in ethnomedicine and could provide a lead in the isolation of antibacterial agents from water extracts of *C. gigantea* L.

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