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Assessment of Antimicrobial Property of Different Leaf Extracts of *Calotropis procera* and *Calotropis gigantea* against *Staphylococcus aureus*

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ABSTRACT
Antimicrobial agents are compounds that may either suppress or eliminate infections while causing negligible or little harm to the host cells. Antimicrobials applied on non-living substances are known as cleaning agents. Antibiotics, antiviral, antifungal, and other antimicrobials are the most common types. In most cases, antibiotics are applied to cure bacterial infections. Antibiotic poisoning in humans and other animals is typically thought to be minimal. Formerly, the word antibiotic exclusively pertained to compositions generated from live organisms, but it is now used to denote artificial antimicrobials like sulphonamides. Hence the present study is designed to assess antimicrobial activity of *Calotropis* against bacteria *Staphylococcus aureus.*

INTRODUCTION
Therapeutic plants are gifts from nature that may be used to treat a wide range of ailments in humans (Barbour *et al*., 2008). Because of the richness of plants on the planet's surface, researchers are looking at diverse extracts derived from ancient plants as possible sources of novel antibacterial agents (Bennett *et al*., 2003). Plants have been effectively utilised as efficient medicinal tools for treating ailments and different other health risks by the human race since the beginning of civilization on Earth.
Joubert and Pasteur found that one kind of bacterium could block the development of the other bacterium, which began the history of antimicrobials. They didn't realize at the time that the cause one bacteria couldn't grow was because another was manufacturing antibiotics. Antimicrobials such as penicillin and tetracycline were discovered, paving the door for improved health for millions of people all over the globe. There was no genuine therapy for pneumonia, or strep throat, gonorrhoea, until penicillin became an effective pharmaceutical cure in the beginning of 1940s. Clients with infected wounds were often forced to have an injured limb amputated or risk infection-related mortality. Almost all of these illnesses may now be readily treated with a minimal course of antibiotics (Kone *et al.*, 2004).

Plants have long been utilised by traditional practitioners to stop or treat contagious illness. Most of these plants have been professionally studied for antibacterial action, and several plant compounds have been demonstrated to prevent the development of harmful germs. Plants produce compounds that are beneficial to the health of people and other animals. Most of these molecules are secondary metabolites, and at least 12,000 of them have been identified, accounting for less than ten percent of the overall (Mohanty *et al.*, 2006).

In India, there are around forty seven thousands plant species, with seven thousands five hundred of them having therapeutic significance. Medicines obtained by plants, on the other hand, are made from just eight hundred plant species (Agarwal, 2005). Scientists have observed that several sections of plants, such as the flowers, leaves, root, bark, and stem, contain antibacterial properties. When used alone or in conjunction with conventional antibacterial medications, natural compounds derived from plants were discovered to have potential antimicrobial properties (Williams, 1998)

MATERIALS AND METHODS

The current investigation on antimicrobial activities of *Calotropis procera* and *Calotropis gigantea* (Asclepiadaceae) growing at different sites of Agra city during 2010-2011 is studied.

To some extent, *Calotropis procera* is salt resistant, drought resilient and seeds are dispersed by animals and wind. It spreads rapidly as a weed along deteriorated roadside ditches, lagoon margins, and pastured native grasslands. It prefers and is frequently abundant in regions of abandoned agriculture, particularly sandy soils in low-rainfall locations; this is thought to be a sign of over-cultivation. It is a shrub with soft wood, a single or several stems, and an occasional tree reaching a height of 6m. While lesions, overall portions of the plant emit a white milky latex.

Calotropis gigantea, the gigantic milkweed, is endemic to the Old World tropics but has spread extensively over the New World tropics, comprising the Caribbean and across the continent from Mexico to Brazil. South China, Sri Lanka, the Malay Islands, Singapore, and India, are all home to this species. It may be found in arid coastal environments, on beaches, and up to 600 feet in elevation. It spreads and becomes prevalent in highly grazed pastures because it is unpleasant to sheep and cattle. It grows in a broad range of soils, sometimes in places where few other plants can.

Collection and Storage of Plants

The plant material from the two *Calotropis* species was carefully harvested and stored in plastic bags that were then sealed to protect it from dust. The specimens were transported to the laboratory and kept in a refrigerator. After properly washing the stored specimens with tap water, they were sterilised with distilled water. Following cleaning, the leaves were dried in the shade and crushed into powder form.

Equipments Used

The detail of the equipments used in the study is given in table-1

S.No.	Equipments	Company
1	Autoclave, Hot air oven	Scientific equipment work
2	Electronic analytical balance	Sartorius
3	Laminar air flow	Zenith
4	Incubator	Toshiba
5	Deep freeze and refrigerator	Sonyo
6	Sterile Cotton swab tube	HI-Media
7	Inoculating loop and Needle	HI-Media

8	Micropipette	Tarson, Hirschmann, Laborgerate
9	Soxhlet extractor, Rotary evaporator	Heidolph
10	Glass wares	Borosil

Preparation of Extracts

a) **Aqueous Extract:** For the aqueous extract, leaf powder was individually homogenised in a pestle and mortar with sterile distilled water at a 1:8 w/v ratio and filtered through muslin cloth. The resulting filtrate was further strained using Whattman No. 1 filter paper. At room temperature, the extraction was performed.

b) **Organic Extract**: Organic extract was produced using the Soxhlet technique. A 0.5mm whatmann filter paper was used to create a thimble. A total of around 100 g of powder material was packed evenly into a thimble and put through a Soxhlet extractor. Soxhlet apparatus is combination of extractor, condenser and round bottom flask. For extraction of compounds the round bottom flask is heated on the heating mentle and evaporated solvent goes to siphon tube of an extractor. Here it is cooled by the water moving in the condenser and then solvent come back to Round bottom flask with compounds of *Calotropis* plant (leaf). It was exhausted extracted with solvent for about 48 hours or 22 cycles, or until the solvent in the extractor's syphon tube became colourless. Following that, the extracts were filtered using filter paper and the solvent was evaporated from the extracts using a Rotary evaporator to get the syrupy consistency. To eliminate any traces of solvent, the residue was dried over anhydrous sodium sulphate. The extract was then stored at 4°C in the refrigerator to determine antimicrobial property.

MEDIA USED FOR THE MAINTENANCE OF TEST ORGANISM:-

A number of conventional culture media were used for isolation and culturing bacterial and fungal strains in artificial conditions. The details of various media used are listed in Table below Details of antibiotic disc used for susceptibility test (Table-2)

S.No.	Antibiotics	Symbol
1	Cephalothin	Ch
2	Clindomycin	Cd
3	Co-Trimoxazole	Co
4	Erythromycin	E
5	Gentamycin	G
6	Ofloxacin	Of
7	Penicillin-G	P
8	Vaneomycin	Va

PROCEDURE FOR TESTING ANTIMICROBIAL PROPERTIES

Disc diffusion method

To determine the presence of an antimicrobial substance, antimicrobial susceptibility tests using the standard disc diffusion method were performed (Nadkarni and Nadkarni, 1976). After dissolving the plant extract in a suitable solvent, solutions of different concentrations of plant extracts (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, and 3.125mg/ml) were prepared via serial dilution. Empty 6 mm diameter sterile discs were impregnated with 25µl of each serial dilution of the extract solution. These impregnated discs were then incubated for 15 minutes at various concentrations of extract (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg, 6.25mg, and 3.12mg/disc). On the other side, specific colonies from the pure culture

were aseptically packed and blended (emulsified) in nutritional broth (7µl/ml broth). The entire area of the nutrient agar plate was infected with this broth using a culture moistened cotton swab. After inoculation, wait 5-6 minutes to enable the liquid culture to seep into the agar surface. Herbal extracts containing discs were put on the infected surface of an agar plate using sterile forceps. The plates were incubated at thirty seven degree celcius for twenty four hours and the inhibitory zone was measured in millimetres.

RESULTS AND DISCUSSION

Table-3: Zones of inhibition of *Calotropis gigantea* and *Calotropis Procera* leaf extracts in Methanol, Distilled water, Acetone and Ethyl acetate against *Staphylococcus aureus*.

S.No.	Solvent	Concentration (mg/ml)	*Calotropis procera*	*Calotropis gigantea*
			Zone of inhibition in mm	
1	Methanol	200	16.0±4.58	19.33±4.16
		100	12.67±2.52	18.0±3.0
		50	10.33±2.52	18.0±2.65
		25	06.67±0.58	16.67±2.52
		12.5	06.67±0.58	14.33±2.08
		6.25	06.33±0.57	07.66±2.08
		3.125	06.33±0.57	06.33±0.57
		Drug	22	20
2	Distilled water	200	-	12.33±2.52
		100	-	-
		50	-	-
		25	-	-
		12.5	-	-
		6.25	-	-
		3.125	-	-
		Drug	11	12
3	Acetone	200	19.0±4.36	14.67±4.16
		100	16.67±3.51	11.33±3.05
		50	12.67±3.46	07.33±0.55
		25	08.33±3.21	06.67±0.58
		12.5	06.67±0.58	06.67±0.58
		6.25	06.33±0.57	06.33±0.57
		3.125	06.33±0.57	06.33±0.57
		Drug	11	20
4	Ethyl acetate	200	14.0±1.0	13.33±0.58
		100	13.0±1.0	10.0±3.0
		50	11.67±0.57	09.0±2.64
		25	07.0±1.0	07.0±1.0
		12.5	06.67±0.58	07.33±2.30
		6.25	06.33±0.57	06.33±0.57
		3.125	06.33±0.57	06.33±0.57
		Drug	11	18

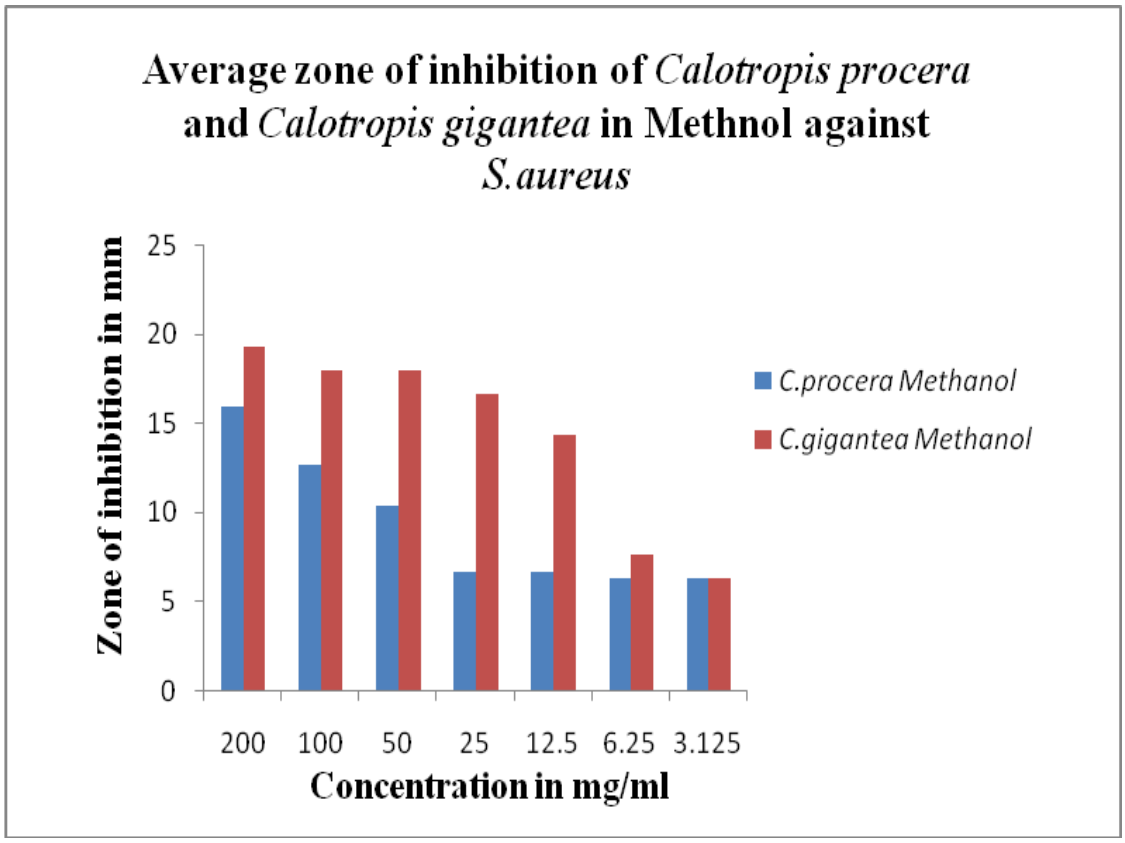

Fig. 1: Zones of inhibition of *Calotropis gigantea* and *Calotropis procera* leaf extracts in methanol alongside *S. aureus.*

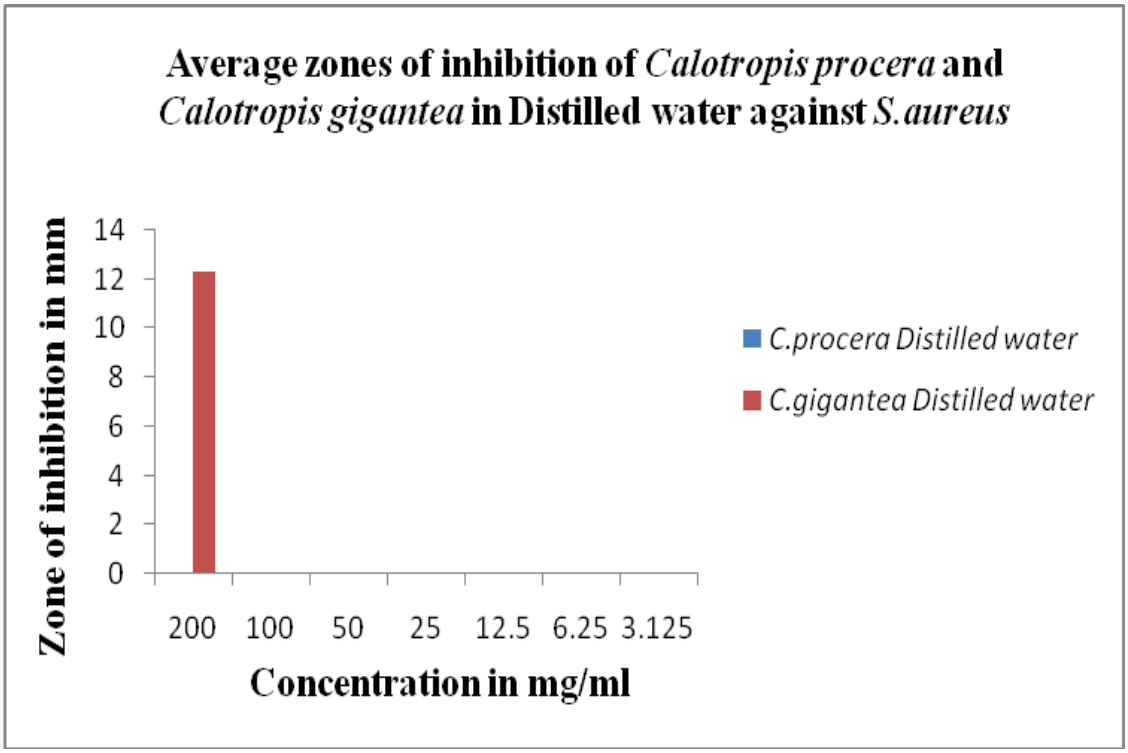

Fig. 2: Zones of inhibition of aqueous leaf extracts of *Calotropis procera* and *Calotropis gigantea* in against *S. aureus.*

The aqueous leaf extract of *Calotropis procera* does not show any activity against *S. aureus* at all, while the aqueous leaf extracts of *Calotropis gigantea* showed antibacterial activity only at

200mg/ml concentration (Fig. 2) (Table-3). From the table-3, it is clear that methanol is better solvent for the extraction of leaves of *Calotropis gigantea* than *calotropis procera*.
An excellent activity was shown by the acetonic leaf extract of *Calotropis procera* against *S. aureus*. The zones of inhibition were found to be 19.0, 16.67, 12.67, 08.33, 06.67, 06.33 and 06.33 (mm) at 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.12mg/ml concentration individually with a maximum of 19.0±4.36 mm at 200 mg/ml while the acetonic leaf extract of *Calotropis gigantea* showed potential activity at various concentrations against *S. aureus*. The zones recorded at 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml concentrations were found to be 14.67, 11.33, 07.33, 06.67, 06.67, 06.33 and 06.33(mm) correspondingly with a maximum zone of inhibition of 14.67±4.16mm at a concentration of 200mg/ml (Fig. 3), (Table-3).

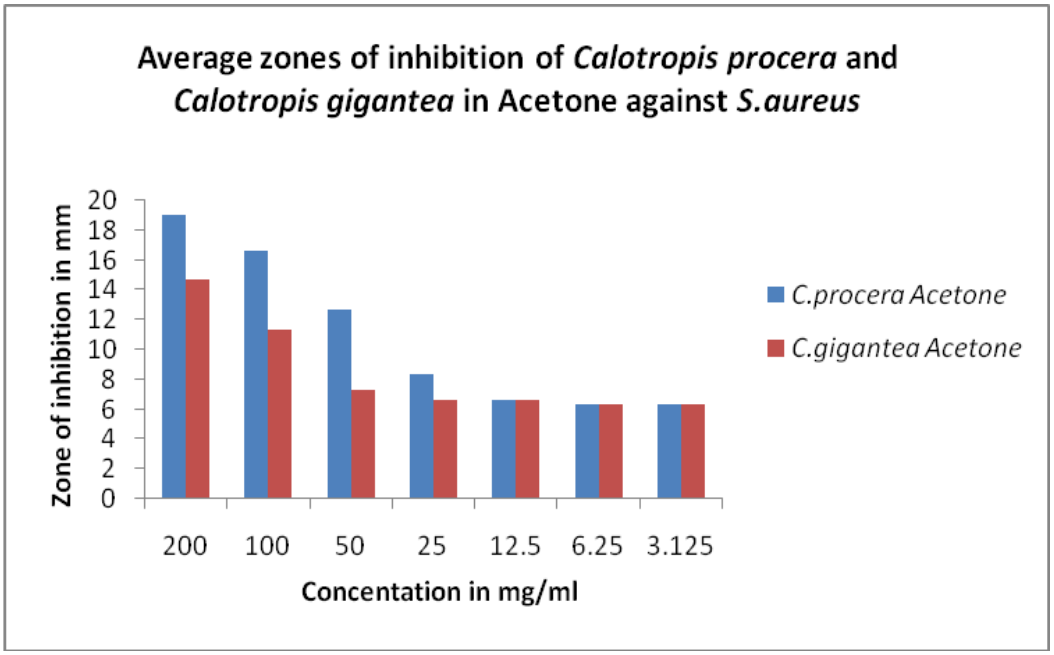

Fig. 3: Zones of inhibition of *Calotropis gigantea* and *Calotropis procera* leaf extracts in acetone alongside *S. aureus.*

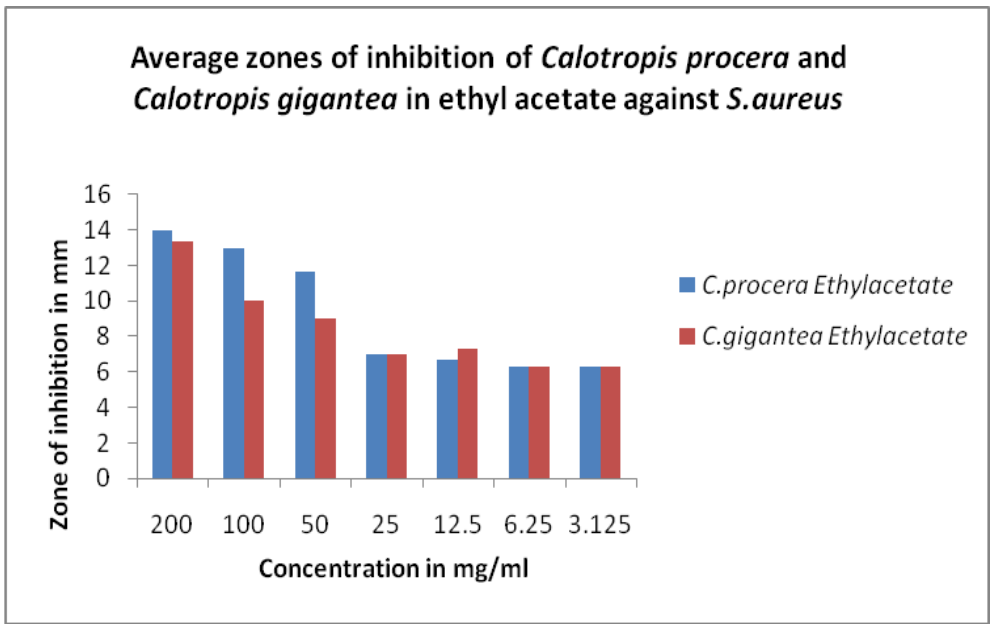

Fig. 4: Zones of inhibition of *Calotropis gigantea* and *Calotropis procera* leaf extracts in ethyl acetate against *S. aureus.*

The leaf extract of *Calotropis procera* and *Calotropis gigantea* in ethylacetate exhibited potential activity with 14.0, 13.0, 11.67, 07.0, 06.67, 06.33 and 06.33(mm) and 13.33, 10.0, 09.0, 07.0, 07.33, 06.33 and 06.33 (mm) at 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml respectively (Table-3). The maximum zone of inhibition for *Calotropis procera* and *Calotropis gigantea* was recorded to be 14.0±1.0 and 13.33±0.58 mm respectively (Fig.34). The study's findings indicated that the leaf extract of *Calotropis procera* in acetone and ethylacetate showed better antibacterial activity against *S. aureus* than the corresponding extracts of *Calotropis gigantea* (Table-3, Fig 3 and 4).

REFERENCES

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a) **Aqueous Extract:** For the aqueous extract, leaf powder was individually homogenised in a pestle and mortar with sterile distilled water at a 1:8 w/v ratio and filtered through muslin cloth. The resulting filtrate was further strained using Whattman No. 1 filter paper. At room temperature, the extraction was performed.

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