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ANTIMICROBIAL ACTIVITY OF DIFFERENT LEAF EXTRACTS OF *CALOTROPISPROCERA* AND *CALOTROPISGIGANTEA* AGAINST *P. AERUGINOSA*

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ABSTRACT

Calotropisgigantea, sometimes known as milk weed, is a xerophyte and also an erect shrub that grows up to 4 metreslong.It is a wasteland weed habitant of Asian nations that comprises Philippines, China, Thailand, Srilanka, Indonesia, Malaysia, and India. Purple waxy blooms with 5 pointed petals and a little exquisite 'crown' emerging from the middle to keep the stamens adorn the plant. The plant's leaves are round and light green, with a milky stem. It has been reported as traditional medicinal plant in Homeopathic, Unani and Ayurveda medication system for the treatment of different ailments. This study is conducted to assess the antimicrobial activity of *Calotropis* against *P. aeruginosa*.

INTRODUCTION

Antimicrobial agents are compounds that may either suppress or eliminate infections while causing negligible or little harm to the host cells (Vaidya*et al*., 2007). 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 now used to denote artificial antimicrobials like sulphonamides. Antibiotics were discovered, developed, and used clinically in the nineteen century, resulting in a significant reduction in bacterial infection death. Antibiotics are one of the most regularly prescribed medications. For examples, one or more than one rounds of antibiotic treatment are given to thirty percent of hospitalized patients. Antiviral medicines are a kind of medicine that is used to treat viral infections.

Antivirals, like antibiotics, are used to treat certain viruses. Because they are largely innocuous to the host, they may be utilized to treat infections.

Antifungals are applied to cure fungal diseases like candidiasis, ringworm, and athlete's foot, as well as dangerous systemic infections like cryptococcalmenigitis. Antifungals function by taking advantage of distinctions between mammalian and fungal cells to eliminate the fungus without harming the host.

Antiparasitics are a kind of drug used to cure parasitic infections caused by round worms, trematodes, cestodes, infectious amoebas, and protozoa. Antimicrobial research has become a worldwide problem, owing to the widespread application of infectious illnesses, which has resulted in microbial resistance to medications applied to cure common infectious disorders (Rao *et al.*, 2011; Faizi*et al.*, 1995). Since pathogenic microbes' resistance to conventional antibiotics has attained unhealthy levels in growing countries, and data suggests further

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increment (Okeke*et al*., 2001), these drug-resistant micro-organism are more infective with a high death rate and have become a big hurdle in the healthcare and pharmaceutical industries. Researchers are looking forward to the discovery of alternative and innovative medications to combat microorganism drug resistance. Animals, algae and plants contain a variety of natural therapeutic substances that may be used to cure a variety of contagious ailments (Kubmurawa*et al*., 2007).

Asclepiadaceae is one of the families whose plants show antimicrobial activities. The family includes 130 genera with about 2000 species mainly located in tropical and subtropical regions. They are a collection of perennial herbs, twining shrubs, lianas, and twets, but they also comprise a large no. of leafless stem succulents, each of which belongs to the Gentianales order. This family name arises from the Asclepias genus (milkweed), therefore the family is also known by the name milk weed family (Iwu*et al*., 1999).Various plants of this family show antimicrobial activities for example *Calotropisprocera*, *Calotropisgigantea, Leptidenia, Gymnemasylvestre*etc.

MATERIALS AND METHODS

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

To some extent, *Calotropisprocera* 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.

Calotropisgigantea, 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

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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:-

Anumber 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).

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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

The goal of this research was to assess the antimicrobial characteristics of*Calotropisproceraand Calotropisgigantea*of family Asclepiadaceae against various pathogens. The different leaf fractions of *Calotropisprocera* and *Calotropisgigantea* at different concentration (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml) were formed in different solvents viz. distilled water, acetone, methyl alcohol, , and ethylacetate were studied for the antimicrobial properties. The plant's antimicrobial activity studied alongside with two strains of bacteria:-
P. aeruginosa and *S. aureus* and the antifungal activity was studied against *R.stolonifer*. Different culture media were used for performing experiment and the maintenance of the strains. Nutrient broth media and NAM (Nutrient agar media) were used for antibacterial study and PDA (Potato Dextrose agar) media for antifungal study.
Standard Disc diffusion method was utilized for the antimicrobial properties of the test plant were checked by (Mukherjee *et al*., 1995).

Table-3: Zones of inhibition of *Calotropisgigantea* and *CalotropisProcera* leaf extracts and in Methanol, Distilled water, Acetone and Ethyl acetate against *Pseudomonasaeruginosa*

S.No.	**Solvent**	**Concentration (mg/ml)**	***Calotropisprocera***	***Calotropisgigantea***
			Zone of inhibition in mm	
1	Methanol	200	11.66±0.57	11.33±2.51
		100	9.67±0.58	10.0±2.64
		50	8.33±0.58	09.33±2.08
		25	7.33±0.58	8.67±3.05
		12.5	6.67±0.58	07.66±2.88
		6.25	6.33±0.57	06.67±1.15
		3.125	6.67±1.15	6.33±0.57
		Drug	**15**	**16**
2	Distilled water	200	11.33±2.30	14.33±0.58
		100	8.66±2.78	13.67±0.58
		50	8.33±3.21	11.33±2.89
		25	6.67±0.58	6.67±1.15
		12.5	6.33±0.57	06.37±0.58
		6.25	6.33±0.57	06.33±o.57

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		3.125	6.33±0.57	06.33±o.57
		Drug	**16**	**11**
3	Acetone	200	20.33±3.21	09.33±1.15
		100	15±3.60	07.67±0.58
		50	14±4.36	06.67±0.58
		25	9.66±4.04	06.33±0.57
		12.5	08±3.64	06.33±0.57
		6.25	07.33±1.52	06.33±0.57
		3.125	06.33±0.57	06.33±0.57
		Drug	**17**	**20**
4	Ethyl acetate	200	16.33±1.53	10.33±3.51
		100	10.66±1.52	07.67±2.08
		50	09±1.0	08.66±1.52
		25	08±0.57	07.33±2.30
		12.5	07±1.0	07.0±0.50
		6.25	06.33±0.57	06.33±0.57
		3.125	06.33±0.57	06.33±0.57
		Drug	**16**	**18**

The studies of antifungal and antibacterial properties of various fractions of the test plants against various pathogens are described separately under the following headings.

The study of antibacterial properties was performed by disc diffusion method for *Calotropisgigantea and Calotropisprocera's* various leaf extracts of family Asclepiadaceae.

Table-3 shows the zones of inhibition of leaf extracts of *Calotropisgigantea and Calotropisprocera*in methanol, distilled water, acetone and ethylacetate against *P. aeruginosa* at various concentrations (Fig. 1-3).

The methanolic leaf extracts of *Calotropisprocera* and *Calotropisgigantea* exhibited moderate activity against *P. aeruginosa* with 11.66, 9.67, 8.33, 7.33, 6.67, 6.33 and 6.67mm and 11.33**,** 10.0**,** 09.33**,** 8.67**,** 07.66**,** 06.67 and6.33(mm) zone of inhibition respectively at 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml concentration. The maximum inhibition of 11.66±0.57mm and 11.33±2.51mm at a concentration of 200mg/ml was found for *Calotropisprocera*and *Calotropisgigantea* respectively (Table-3).

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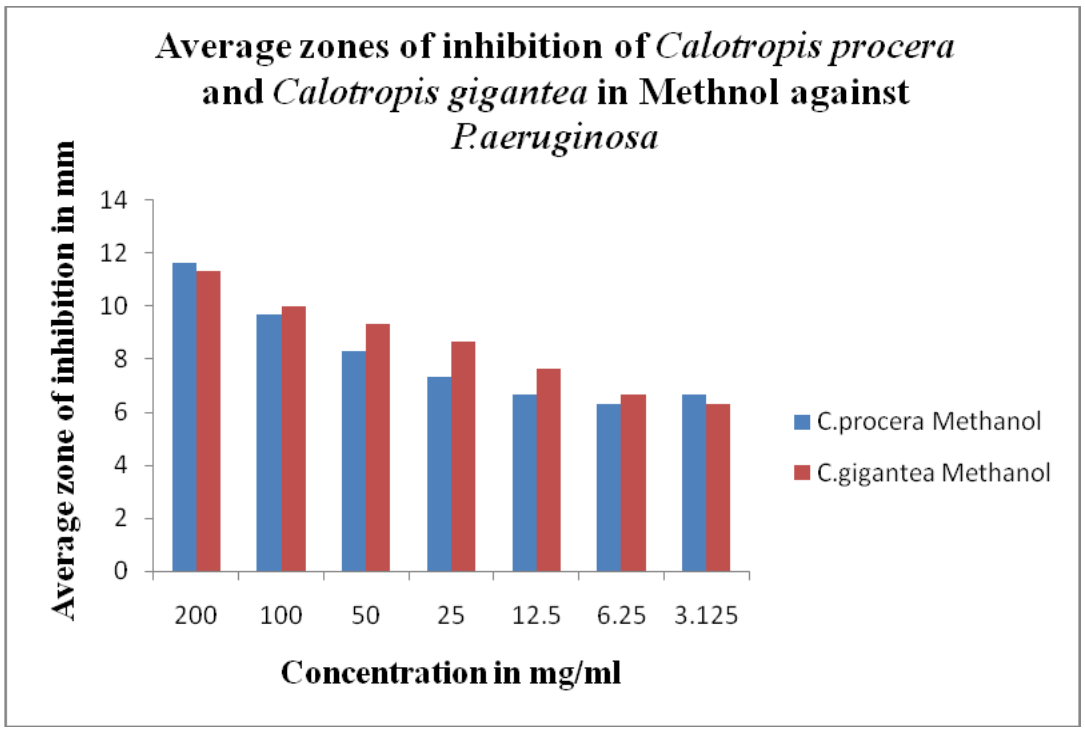

Fig. 1: Zones of inhibition of *Calotropisgigantea and Calotropisprocera*leaf extracts in methanol against *P. aeruginosa*.

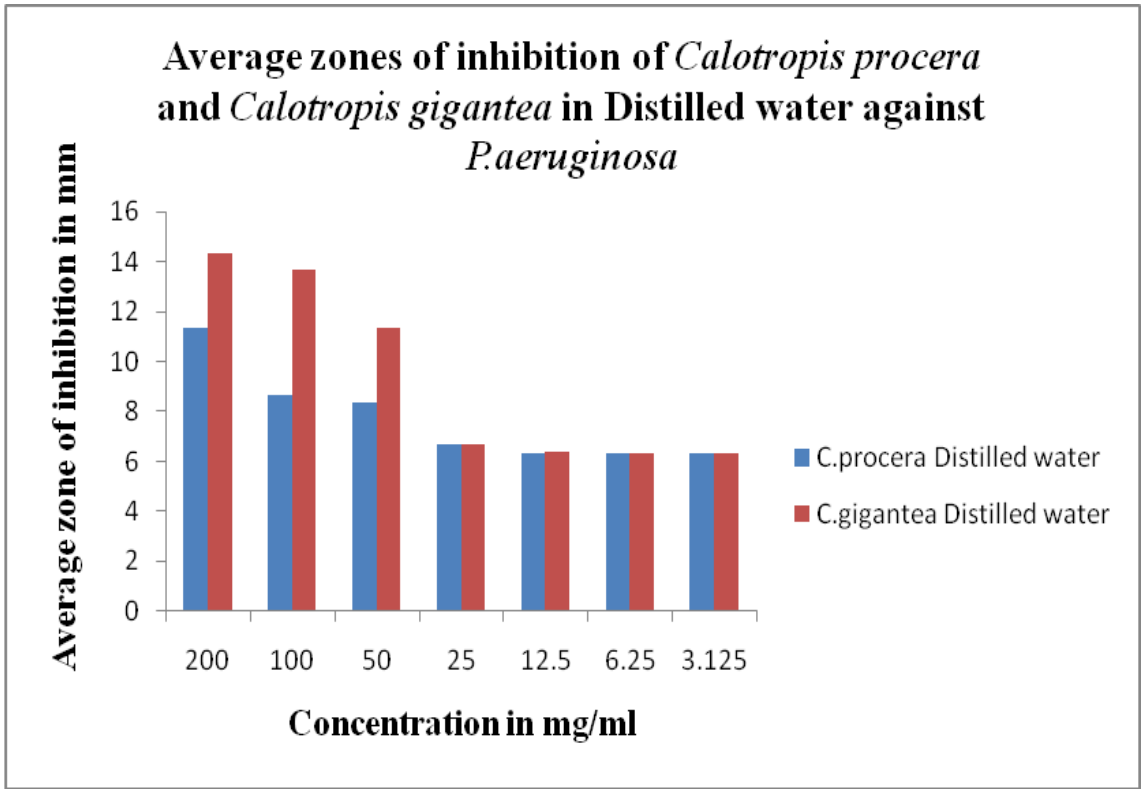

Fig.2: Zones of inhibition of aqueous leaf extracts of *Calotropisgigantea* and *Calotropisprocera*against *P. aeruginosa*.

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Calotropisprocera's aqueous leaf extracts displayed significant activity against *P. aeruginosa*. The average zones of inhibition at 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.12mg/ml were found to be 11.33, 08.66, 8.33, 6.67, 6.33, 6.33 and 6.33(mm) respectively with a maximum inhibition of 11.33±2.30mm at 200mg/ml whereas *Calotropisgigantea*'s aqueous leaf extracts displayed significant activity alongside *P. aeruginosa* with 14.33, 13.67, 11.33, 6.67, 06.37, 06.33 and 06.33(mm) zone of inhibition at 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.12mg/ml concentrations correspondingly. The maximum inhibition 14.33±0.58 mm was found at a concentration of 200mg/ml (Table-3 and Fig. 2). It is evident from the Fig.2that aqueous extracts of *Calotropisgigantea*shows wider zones of inhibition than *Calotropisprocera.*

The leaf extract of *Calotropisprocera* in acetone displayed an excellent activity against *P. aeruginosa*. The acetonic leaf extract of *Calotropisprocera* exhibited 20.33, 15.0, 14.0, 9.66, 08.0, 07.33, and 06.33(mm) zone of inhibition at 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.12mg/ml inhibitory concentration correspondingly. The zones of inhibition decreased with decreasing concentration of the leaf extract and a maximum inhibition of 20.33 ± 3.21 mm was recorded at a concentration of 200mg/ml (Fig. 3), (Table-3). The same trend was found in case of leaf extract of *Calotropisprocera* in ethyl acetate with 16.33, 10.66, 09.0, 08.0, 07.0, 06.33 and 06.33 (mm) zone of inhibition at 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.12mg/ml concentration respectively with a maximum inhibition of 16.33±1.53mm at an inhibitory concentration of 200mg/ml (Table-3), while leaf extracts of *Calotropisgigantea* in acetone and ethylacetate at various concentrations showed moderate activity against *P. aeruginosa*. The zones of inhibition of leaf extract of *Calotropisgigantea* were found to be 09.33, 07.67, 06.67, 06.33, 06.33, 06.33 and 06.33(mm) in acetone and 10.33, 07.67, 08.66, 07.33, 07.0, 06.33 and 06.33 (mm) in ethyl acetate at 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.12mg/ml concentration respectively.

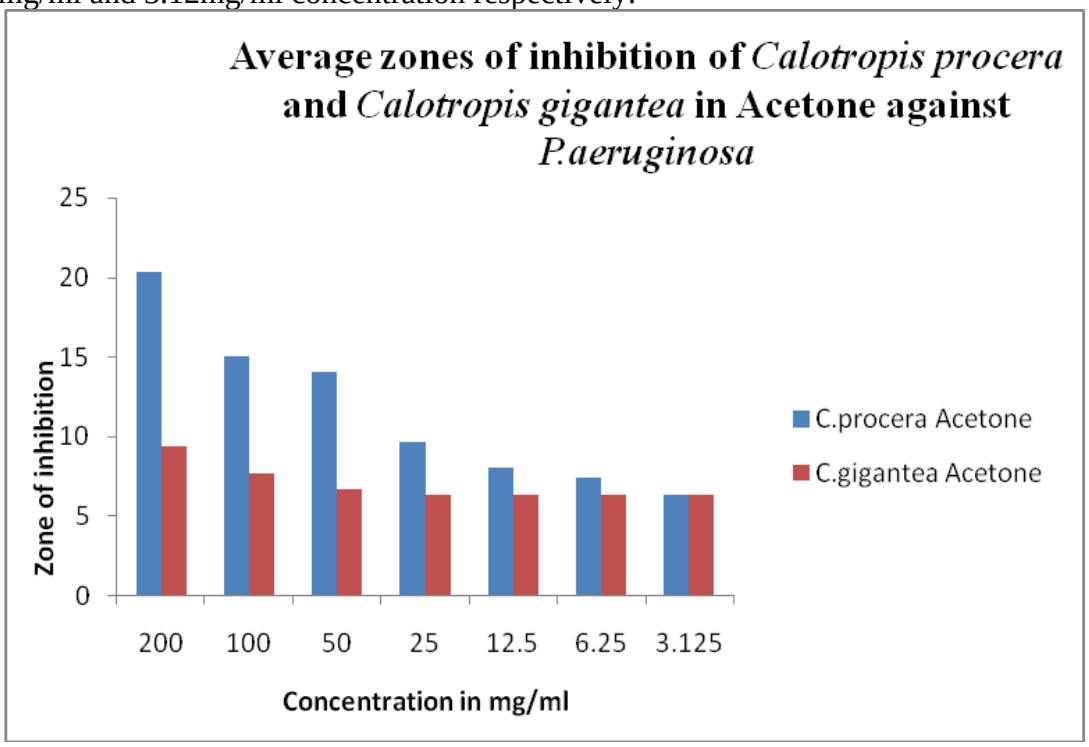

Fig. 3: Zones of inhibition of leaf extracts of *Calotropisgigantea*and *Calotropisprocera* in acetone against *P. aeruginosa*.

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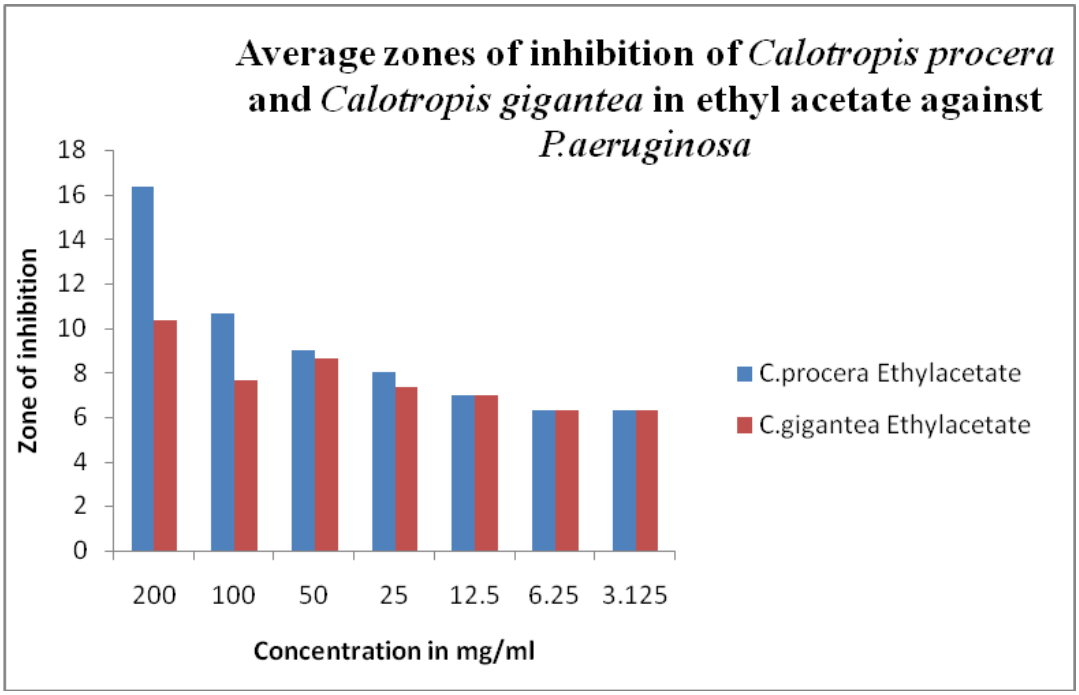

Fig. 4: Zones of inhibition of leaf extracts of *Calotropisgigantea*and *Calotropisprocera*in ethyl acetate against *P. aeruginosa*.

The maximum inhibition of *Calotropisgigantea*in acetone and ethyl acetate was found to be 09.33± 1.15 and 10.33 ±3.15 mm respectively against *P. aeruginosa (*Table -3). The data in Table-3 shows that leaf extracts of *Calotropisprocera* in acetone and ethylacetate was more effective than leaf extracts of *Calotropisprocera* in the said solvents against *P. aeruginosa*.
The data in the Table 8 shows the inhibition zones of *Calotropisgigantea* and *Calotropisprocera* and in methanol, distilled water, acetone and ethylacetate against *S. aureus*. From table-3 it is clear that*Calotropisprocera's*methanolic leaf extract has potential activity alongside with *S. aureus*. The extract exhibited 16.0, 12.67, 10.33, 06.67, 06.67, 06.33 and 06.33 (mm) zone of inhibition at 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.12mg/ml concentration correspondingly. The maximum inhibition of 16.0±4.58 mm was found at 200mg/ml concentration. The methanolic extracts of *Calotropisgigantea*also showed excellent activity with a maximum zone of inhibition of 19.33±4.16 mm at a concentration of 200mg/ml. The zone of inhibition decreased with decreasing concentration of the leaf extract.

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