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ANTIMICROBIAL ACTIVITY OF LEAVES AND LATEX EXTRACT OF THE HERBAL PLANT *CALOTROPIS GIGANTEA* (ERUKKU IN TAMIL)

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ABSTRACT

The leaf and latex extract were obtained by the organic solvents methanol and ethyl acetate. The obtained extracts were analyzed for the presence of phytochemicals and it was found to contain Aminoacids, Anthraquinones, Flavanoids, Phenolic compounds. In addition there were four clinically important bacterial species (*Bacillus subtilis, Micrococcus luteus, Pseudomonas aeruginosa and Serratia mascersans*) and six plant fungal pathogens (*Aspergillus niger, Aspergillus flavus, Alternaria sp, Fusarium sp, Penicillium sp, Rhizopus sp*) were evaluated for antibacterial and antifungal activity respectively. The results obtained from this study inferred that the leaf and latex extracts of *Calotrpis gigantea* was effectively inhibited (concentration ranges from 1mg/ml - 8mg/ml) the growth of test organism.

Keywords: Organic solvent, Leaf and Latex Extract, Phyto-chemical Analysis, Antimicrobial Activity

INTRODUCTION

India is rich in its biodiversity. A number of plants have been documented for their medicinal potential, which is in use by the traditional healers, herbal folklorists and in Indian system of medicine namely Siddha, Ayurveda and Unani. There are about 45,000 species in India with concentrated Hot Spot is the region of

Eastern Himalayas, Western Ghats and Andaman and Nicobar Islands. The officially documented plants with medicinal potential are 3000 but traditional practitioners use more than 6000 plants. India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world **[1]**. Plants have the capacity to produce a large number of organic chemical called as phytochemicals. The accumulation of phytochemical in the plant cell cultures had been studied for more than thirty years and the generated knowledge had helped in realization of using cell culture for the production of desired phytochemicals **[2]**. *Calotropis gigantea* commonly known as Mudar Yercum belongs to the family *Asclepiadaeae* a shrub about 6 M high is widely distributed in Eastern and southern parts of India, Ceylon, Eastern Asia and other parts of tropics. In India the genus is represented by two species. Viz., *Calotrpis gigantea* and *Calotropis procera*. The plants produces white or violet coloured flower in bunches, much branched, tall, erect, large and perennial with latex throughout. The antibacterial activity of leaf extract of *Calotropis gigantea* **[3]** and antifungal activity of *Calotropis procera* the related species was reported **[4]**. This study aimed to find out the phytochemical content and its antimicrobial activity of leaf and latex extracts of *Calotropis gigantea*.

MATERIALS AND METHODS

Plant Material

The leaves and latex part of the above plant is used for the present study. The healthy and mature leaves were collected from Kattankulathur, Chennai, Tamilnadu, India. The plants and the parts screened together with their families and vernacular names and the taxonomic identities of these plants were confirmed by Dr.M.Krishnan, Registered Indian Medical Practitioner (RIMP Siddha) Guduvancherry, Chennai.

Processing

The collected leaves were washed thoroughly with sterile distilled water to remove the debris. The leaves were air dried until all the moisture content in the leaves is lost. The completely dried leaves were blended into powder using an electric blender (Moulinex) and stored at 4ºC until further use.

The latex oozing out from the plant was collected aseptically through giving V

shaped incision on the branches of the plant. The aseptically collected latex was transformed into a sterile centrifuge tube and centrifuged using a bench centrifuge at 1500 rpm for 5 minutes. After centrifugation the supernatant was discarded and the pellet was collected in sterile container. The pellets were evaporated to dryness using Rota-evaporator at 100ºc and stored at 4ºc in an airtight container or is best preserved by adding little chloroform until use.

Extraction

The extraction was carried out using the organic solvent namely: Methanol and Ethyl acetate. 10gm of dried leaf powder and latex were accurately weighed and dissolved in 100 ml of appropriate solvents in an air tight cork bottle and labeled accordingly. The suspended solutions were kept in rotary shaker for 24 hour and the supernatant was concentrated by drying. Dried extract was used for phytochemical and bioassays and stored at 4ºC until use **[5]**.

Phytochemical Analysis

The extracts were analysed for the presence of Aminoacids, Anthroquinones, Flavonoids, Phenolic compounds, Saponins, Steroids, Tannins and Triterpenes were tested **[4]**.

Screening for Antibacterial Activity

The extracts were screened against the four bacterial species viz., *Bacillus subtilis, Micrococcus sp, Pseudomonas aeruginosa and Serratia mascersans* by disk diffusion method. Sterile 6mm disk was obtained from Hi Media, impregnated with 5mg/ml of four extracts and air dried under laminar airflow at room temperature for 8 hours. The bacterial culture was adjusted to 0.5 McFarland standards and swab inoculated on Mueller Hinton Agar (MHA) plates. The inoculated plates were kept at room temperature for 30 minutes. The impregnated disks were placed on MHA plates along with positive control, and incubated at 37^{o}c for 18 hours. The positive controls used in this experiment were Ciprofloxin, Tetracycline and Polymixin(10µg/disc) **[6].**

MIC Determination

The MIC determination was performed for the four extracts viz., leaf & latex extracts of methanol and leaf &latex extract of Ethyl acetate by disc diffusion method. Discs of 1, 2, 4 and 8 mg/ml were prepared. The inoculated plates were incubated at 35-37ºC for 18 hrs. The MIC

for all the test organisms were read by naked eye and tabulated **[6].**

Antifungal Activity

The extracts were screened against six fungal species viz., *Aspergillus niger*, *Aspergillus flavus*, *Alternaria sp*, *Fusarium sp*, *Penicillium sp*, *Rhizopus sp.* for antifungal activity by agar block method. The concentration used for screening was5mg/ml. The concentrations used for MIC determination were 1, 2, 4 and 8 mg/ml. The leaf and latex extracts were added to freshly prepared SDA medium before solidification at the above concentration and mixed uniformly and transferred to sterilized Petri plates. The plates were allowed to solidify at room temperature. After solidifying a small piece of agar cube was cut and removed from the centre of the agar plate and was replaced by same volume of agar cube cut from a lawn culture plate of the test organism. The inoculated plates were incubated at room temperature for 48 hours. After 48 hours of incubation, the plates were observed for growth **[7]**.

RESULTS

The extracts of both leaf and latex were obtained using two organic solvents: methanol and ethyl acetate and were subjected to phytochemical analysis antibacterial and antifungal activity.

Phytochemical Analysis

The phytochemical analysis showed that the latex extracts of methanol and ethyl acetate contains significant quantities of Aminoacid, Anthroquinones, Flavonoids, and Tannins **(Table 1)**. Saponins found significantly in all the four extract. Amino acid found significantly in the methanol leaf extract. Phenolic compounds found significant quantities only in methanol extract of leaf. Triterpenes was not found in all the four extracts.

Antibacterial Activity

All the four extracts at highest concentrations (8mg/ml) showed sensitivity. The latex methanol extracts of four concentrations were not inhibited the growth of *Pseudomonas aeruginosa*. Ethyl acetate extracts of 1 and 2mg/ml concentration did not inhibit the growth of *P.aeruginosa* and *S.marcescenes*. Similar concentrations of latex ethyl acetate extract not showed sensitivity to *P. aeruginosa*. The highest zone of inhibition was observed with latex ethyl acetate extract at 8mg/ml for

S.marcescenes whereas the lowest zone was observed in *B. subtilis* at 1mg/ml.

Antifungal Activity

All the concentrations of four extracts inhibited the growth of all the six fungal Species tested **(Table 2)**.

Table 1: Phyto-chemical Analysis of *Calotropis gigantea* Extract

COMPONENTS	METHANOL EXTRACT		ETHYL ACETATE EXTRACT	
	LEAF	LATEX	LEAF	LATEX
AMINO ACIDS	++	++	-	++
ANTHRAQUINONES	-	++	+	++
FLAVONOIDS	-	++	-	++
PHENOLIC COMPOUNDS	++	-	-	-
SAPONINS	++	++	++	++
STEROIDS	++	++	+	+
TANNINS	++	++	-	++
TRITERPENES	-	-	-	-

++ = Significantly Present; + = Present; - = Absent

Table 2: Table Antibacterial Activity of Methanolic Leaf Extract of *Calotropis gigantea*

EXTRACTS		CONCEN-TRATION (mg/ml)	TEST BACTERIA (ZONE OF INHIBITION IN mm)			
			1	2	3	4
LEAF	METHANOL	1	10	6	NZ	12
		2	11	8	NZ	15
		4	12	10	6	17
		8	14	13	8	19
	ETHYL ACETATE	1	8	6	NZ	NZ
		2	10	8	NZ	NZ
		4	12	10	6	8
		8	13	12	10	10
LATEX	METHANOL	1	NZ	NZ	NZ	6
		2	7	8	NZ	8
		4	9	9	NZ	10
		8	11	10	NZ	12
	ETHYL ACETATE	1	6	NZ	NZ	13
		2	7	NZ	9	15
		4	8	7	12	17
		8	9	9	14	23

***1. Bacillus subtilis* ; 2. *Micrococcus luteus*; 3. *Pseudomonas aeruginosa*; 4. *Serratia marcescens*; NZ – No zone**

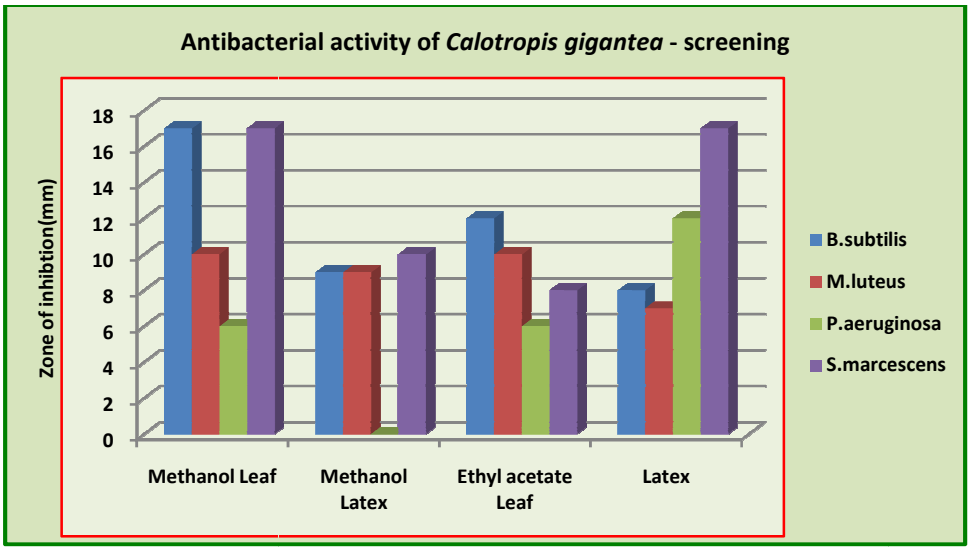

Figure 1: Screening for Antibacterial Activity of *Calotrpis gigantea* Extracts to Four Test Bacterial Species

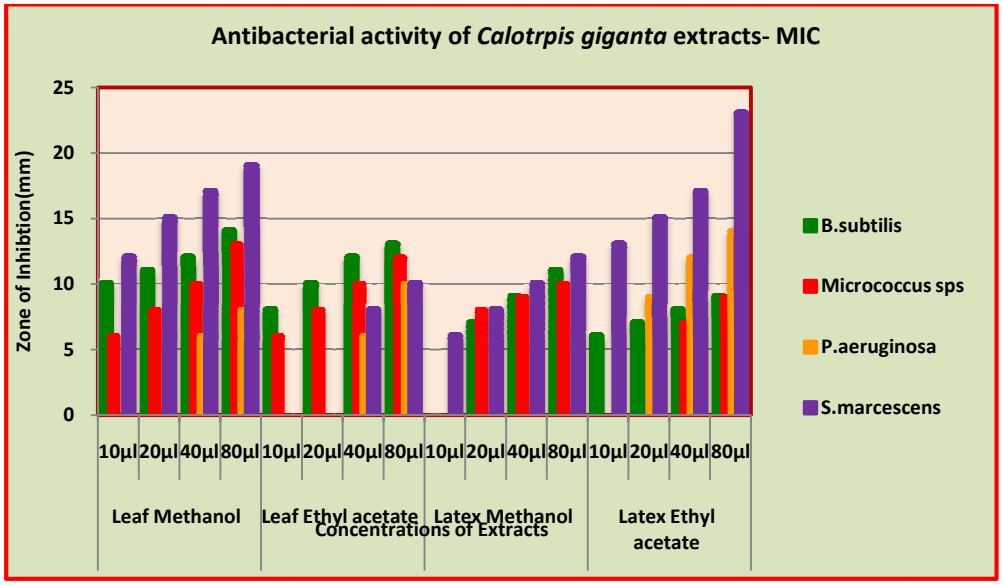

Figure 2: Minimum Inhibitory Concentration (MIC) Determination of *Calotrpis gigantea* Extracts to Four Test Bacterial Species

Table 3: Antifungal Activity of Leaf and Latex Extracts of *Calotropis gigantea*

EXTRACTS		CONCENTRATION (mg/ml)	TEST FUNGI					
			1	2	3	4	5	6
LEAF	METHANOL	1	+	+	+	+	+	+
		2	+	+	+	+	+	+
		4	+	+	+	+	+	+
		8	+	+	+	+	+	+
	ETHYL ACETATE	1	+	+	+	+	+	+
		2	+	+	+	+	+	+
		4	+	+	+	+	+	+
		8	+	+	+	+	+	+
LATEX	METHANOL	1	+	+	+	+	+	+
		2	+	+	+	+	+	+
		4	+	+	+	+	+	+
		8	+	+	+	+	+	+
	ETHYL ACETATE	1	+	+	+	+	+	+
		2	+	+	+	+	+	+
		4	+	+	+	+	+	+
		8	+	+	+	+	+	+

1. *Aspergillus niger*; 2. *Aspergillus flavus;* 3. *Alternaria sp.*; 4. *Fusarium sp.*; 5. *Penicillium sp.;*

6. *Rhizopus sp.*; + = Growth Inhibited; - = Growth Commences

DISCUSSION

The phyto-chemical properties and antifungal activity of *Calotropis procera* was evaluated. The phyto-chemical analysis revealed the presence of alkaloids, flavonoids, tannins, steroids, triterpenoids, saponins and saponin glycosides in leaves and root extract and only with flavonoids, triterpinoids and saponins in the stem bark extracts **[4]**. In this study, the phytochemical analysis revealed the presence of amino acids, anthraquinones, flavonoids, phenolic compounds, saponins, steroids, tannins and triterpenes in leaves and latex extracts. These biologically active compounds in the plant extract could be known to possess the antimicrobial property **[8, 9, 10]**.

The aqueous, ethanol and chloroform extract were obtained from the leaves and latex of the *Calotropis procera* and tested against different bacterial pathogens **[11].** Ethanol latex extract provided wider zone of inhibition (9 mm) where as chloroform and water extracts provided lower zone (8.5 mm & 6 mm respectively) against same organism. The zone of inhibition of

ethanol latex extract against *E. coli* was 14.4 mm. The minimum inhibitory (MIC) values of the extract showed that the highest activity was recorded against *E. coli* (MIC 2.5 mg/ml) in ethanol extract of *C. procera* latex and the lowest was observed against *Pseudomonas aeruginosa* and *Streptococcus pyogenes* (20 mg/ml) in aqueous extract of the latex.

In this study during screening the widest zone of inhibition was obtained to methanol leaf extract (5 mg/ml) against *Bacillus subtilis* (17mm) and *Serratia marcescens* (19 mm) and ethyl acetate extracts *Bacillus subtilis* (12 mm). The zone of inhibition for methanol latex extract against *Serratia marcescens* (10 mm) and for ethyl acetate extracts *Pseudomonas aeruginosa* (12 mm) and *Serratia marcescens* (17 mm). The minimum inhibitory concentration (MIC) values of the extract showed that the highest activity was recorded against *Serratia marscens*(1mg/ml) in ethyl acetate latex extract of *C.gigantea* and the lowest activity was observed against *Pseudomonas aeruginosa* (MIC- 8 mg/ml). This value was lower than the MIC value of latex extract of *C. procera* against *P. aeruginosa* **[11].**

The antifungal activity of ethanol and chloroform extracts of both leaf and latex of *C. procera* were tested against four fungal species. The widest zone of inhibition of 8.5mm was obtained against *Aspergillus niger* by the ethanol latex extracts **[11]**. The aqueous and organic solvents of leaf, root and stem barks extracts were screened for antifungal activity against *Aspergillus niger*, *Trichophyton rubrum* and *Microsporum gypseum* by agar dilution method **[4]**. The roots fraction of hexane and petroleum ether extracts showed significant growth inhibitions of *Microsporum gypseum* and *Aspergillus niger* (MIC 2 and 4 mg/ml). In this research the antifungal activity against six plant fungal pathogens such as *Aspergillus niger*, *Aspergillus flavus*, *Fusarium sps*, *Alternaria sps* *Penicillium sps* and *Rhizopus sps*. On testing for antifungal activity, all the four extract (MIC 1mg/ml) showed inhibition against the selected test organism *Aspergillus niger, Aspergillus flavus, Fusarium sp, Alternaria sp, Penicillum sp* and *Rhizopus sp*. The MIC value was lower than the value of **[11]**.

CONCLUSION

The results obtained from our study showed an effective inhibition against the test organism which justify the traditional use of the plant for infectious diseases.

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