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Antimicrobial properties and HPTLC fingerprint profile of ethnomedicinal plant *Calotropis procera L.* (Rui) from Poladpur taluka of Raigad district

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Abstract
Calotropis procera L. (Rui) is an important ethnomedicinally plant from the family Asclepideaceae found in this region. There are two species of *Calotropis,* namely, *Calotropis procera L.* and *Calotropis gigantia* L., which are found in Poladpur. The screening of leaf extracts in water, alcohol, and ethyl acetate for antimicrobial properties exhibited antibacterial as well as antifungal activities. *Calotropis procera* leaf extract was subjected to extensive HPTLC analysis for a comprehensive HPTLC profile. Sonication was carried out using ethanol extract. Rf value, peaks in the densitogram, and chemical variation were evident in the results of the densitometric analysis. Therefore, HPTLC fingerprint analysis shows phytochemical variations present in selected leaf extract. The HPTLC fingerprint analysis profile of the plant will be helpful for drug identification, checking adulteration, and also act as a biomarker for the plant in the pharmaceutical industry.

Keywords: asclepiadaceae, *Calotropis procera L*, antimicrobial activities, HPTLC

Introduction
Since antiquity, people have been turning to medicinal plants as a source of drugs and other human compounds. About 805 medications have been derived from plants in India, which has a long history of employing herbs and plants for medicinal purposes. In India, there are 2,500 plant species with documented medicinal value. Flora and fauna abound in the Raigad area in the Kankan region, which is well-known for its wide variety. There is a forest tucked away in the mountains around the region. In the Sahyadri mountains possesses a lot of natural resources and information about medicinal plants have been passed down through the years (More and Baig, 2013) [13].
Different parts of the plant *C. procera* possess a varied number of pharmacological properties, such as fruits, flowers, roots, leaves, and latex. Leprosy, ulcers, tumours, haemorrhoids, and rheumatism have been treated with this plant in traditional Indian medicine. (Warrier *et al*., 1994) [25]. Many of the relevant pharmacological activities of Calotropis procera are related to or to its latex. In chemical analyses of two crude extracts of latex of *C. procera*, various compounds have been identified, such as active cardenolides, proteolytic enzymes, alkalides, and carbohydrates (Seiber *et al*., 1982) [19], apart from steroids, terpenes, and organic carbonates (Gallegos-Olea *et al*., 2002) [5]. Cysteine proteases (Dubey; Jagannadham, 2003; Singh *et al*., 2010) [2] and uma osmotin (Freitas *et al*., 2011) are the most recently identified non-latex of C. procera, indicating the presence of a protein with papain inhibitor activity (Ramos *et al*., 2010) [18]. Attempts to examine the antibacterial and antifungal properties of *Calotropis procera L.* leaves against selected bacteria and fungi were carried out. The leaves were extracted in water, ethanol, and ethyl acetate following methods given in material and methods, and accordingly, the activity was assessed by the agar well method with these dilutions.

Material and Methods
Collection of plant material
Calotropis procera L. leaves were gathered from Poladpur district Raigad and brought to laboratory for further analysis. The *Calotropis procera* L. leaves cleaned gently using tap water to remove dust and pollutants deposited. The leaves were dried in shade and this plant material was grounded to powder in a grinder.

Microbial culture and maintenance
Culture of the used E.coli, Staphylococcus aureus, Candida albicans, and Trichophyton rubrum were obtained from Department of Microbiology, Yeshwant College, Nanded. Results are presented as the mean of three experiments and three replicates.
The microbial cultures were preserved on the medium given in the table 1. Sub-culturing was done every fortnightly and were incubated at room temperature, while storing of the cultures was in refrigerator.

Table 1

Sr. no	Organism	Culture Medium
1	*Escherichia coli*	Nutrient Agar
2	*Staphylococcus aureus*	Nutrient Agar
3	*Candida albicans*	Sabouraud Agar

Extraction of plant material
A Soxhlet Extractor (Borosil) was used to extract approximately 10 gm of *Calotropis procera* powder in 70% ethanol for six hours. Whatmann filter was used to filter the ethanol extracts and the solution The extracts were dried after evaporation. It was then utilised for additional phytochemical and HPTLC analysis with the filtered extracts Win CATS Planar Chromatography Manager.

Antibacterial and antifungal properties of plant extract

The assessment of antimicrobial properties of the plant extracts was done by well-diffusion method given by diameter of inhibition zone in mm for *E.coli, Staphylococcus aureus, Candida albicans and Trichophyton rubrum*.

The use of overnight Nutrient Broth culture or Sabouraud agar culture was used to test bacteria or fungus where the inoculum for the bioassay contained 1X106 CFU. The media was seeded with 1ml of the spores/cells and the plates were filled with their respective mediums in Petri dishes and allowed to solidify. A sterilised cork borer was used to make wells of 5 mm in diameter in the solidified seeded medium in the plate. The filling of wells was done using 0.5 mL of extract. Bacterial plates were incubated at 37oC for 24 hours, whereas fungal plates were incubated at 25oC for 72 hours. Plant extracts were tested for their properties to inhibit microbial growth and were compared with the standard concentration of antibiotics, streptomycin, bought from Hi-media in Mumbai. In 1000 ml of sterile distilled water, 100 mg of each antibiotic was dissolved individually, and 0.5 ml was used to fill the wells. 0.5 ml of sterile distilled water was used to fill the wells with 1 gm of amphotericin B obtained from Hi-media, Mumbai. (Mahadevan and Sridhar, 1982).

HPTLC analysis

Preliminary phytochemical analysis of *Calotropis procera L*. is done following method suggested by Sita Kumari and Nirmal Babu Rao (2015) [21] and HPTLC profiling of herbals used in preparation of *Calotropis procera L*. is done by using CAMAG HPTLC System with WIN CATS software.

Morphology of plant

Erect shrub, leaves ovate, obovate, umbellate cyme flower, corolla lobe truncate with a recurved spur at the base, The follicle is boat-shaped, recurved, and cottony pubescent; the seed is ovoid, compressed, and silky white. The quest for eco-friendly prototypes to substitute chemically synthesised drugs is fast growing. the plants used in traditional medicine has been explored a lot. A number of human ailments have been successfully treated with the use of C. procera comprising of anti-diarrheal and anti-diarrheal properties. These ailments also include colds and fevers, leprosy and other skin disorders, asthma, rheumatism, and eczema (Al-Rowaily *et al*., 2020) [1]. In Saudi Arabia, the decoction of aerial plant parts were used to treat fever, joint pain, muscular spasms, and constipation (Mossa *et al*., 1991) [14]. The plant was also used to treat neuropsychiatric disorders in Burkina Faso (Kinda *et al*., 2020) [9]. The medicinal attributes of *C. procera* L. can be credited to secondary metabolites and cardio tonic substances present in the plant (Hagaggi and Mohamed, 2020) [7]. The extracts of plant parts of *C. procera* L. exhibited strong antipyretic, analgesic, antidepressant, and neuromuscular blocking activity (Garabadu *et al*., 2019) [6] and latex contained anticancer activity (Tenzin *et al*., 2006) [23].

Table 2: Rf value of the peak formed of *Calotropis procera*. L. leaf extract

Peak	start Rf	Start height	Max Rf	Max height	Max%	End Rf	End height	Area	Area %	Assigned substance
1	0.09	0.2	0.13	17.8	3.25	0.15	0.0	432.4	2.66	unknown
2	0.19	0.0	0.22	30.1	5.49	0.25	2.7	542.0	3.34	unknown
3	0.28	0.3	0.32	21.6	3.94	0.34	3.2	424.8	2.62	unknown
4	0.35	3.5	0.38	37.6	6.80	0.40	6.7	739.7	4.55	unknown
5	0.40	6.9	0.45	236.7	43.11	0.50	0.1	5114.0	31.48	unknown
6	0.59	0.0	0.63	13.6	2.47	0.66	3.3	342.3	2.11	unknown
7	0.66	3.4	0.72	46.0	8.38	0.77	0.2	1560.0	9.60	unknown
8	0.77	0.3	0.80	11.2	2.03	0.83	0.0	236.3	1.45	unknown
9	0.86	1.4	0.96	134.7	24. 53	1.00	3.3	6852.6	42.19	unknown

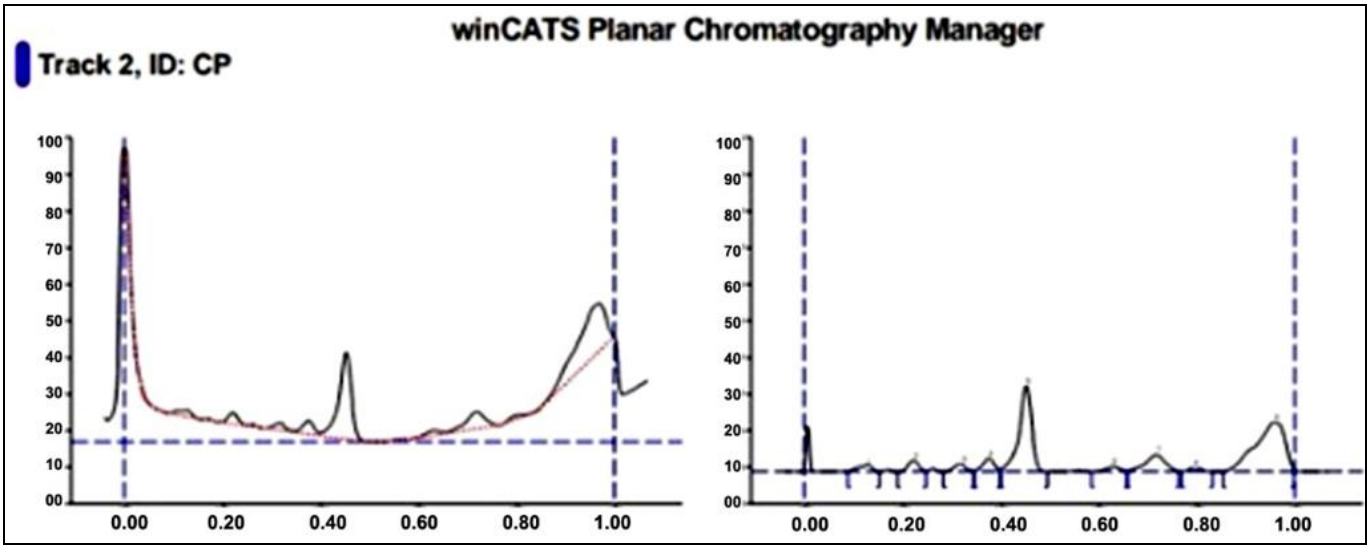

Fig 1: Chromatogram of *Calotropis procera* L. leaf extract

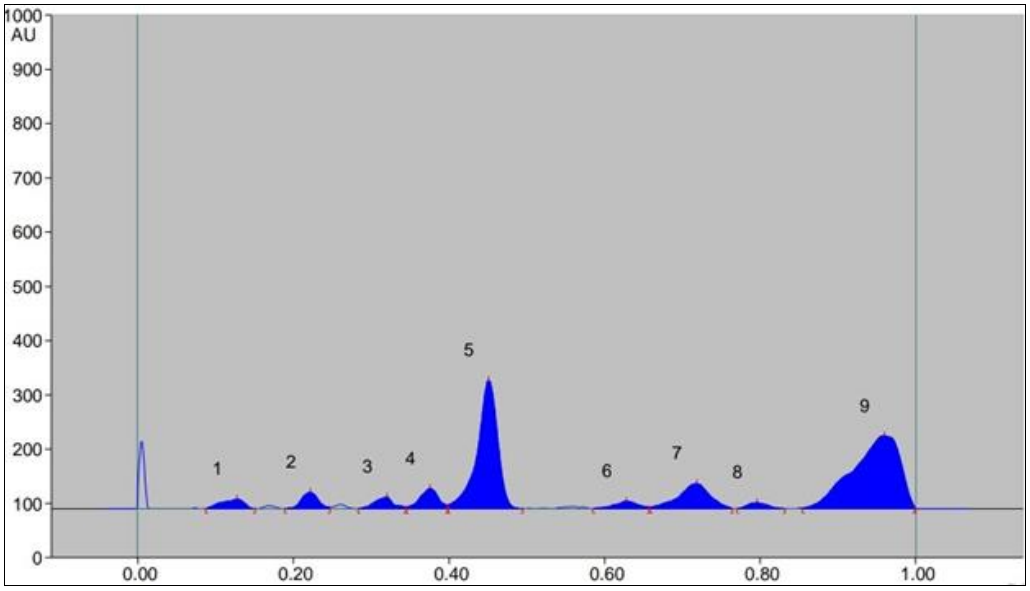

Fig 2: HPTLC Chromatogram of *Calotropis procera*. L. leaf extract.

Experiments were designed to assess the antibacterial and antifungal properties of the leaves of *Caltropis procera* against selected pathogenic bacteria and fungal dermatophytes. The extraction of the leaves was done in distilled water as described in the material and general methods. The appropriate dilutions were made to assess the activity of the extract by the agar well method.

Table 3: Effect of *Calotropis procera* aqueous leaf extract on growth of test bacteria and fungi.

Sr. No.	*Dilution*	*Zone of Inhibition(in mm)*			
		E.coli	*S.aureus*	*C.albicans*	*T.rubrum*
	Water Extract				
1	1	10	9	11	13
2	0.5	8	8	10	11
3	0.25	7	7	9	10
	Ethanol Extract				
1	1	11	10	13	20
2	0.5	9	8	11	15
3	0.25	8	7	9	13
	Ethyl Acetate Extract				
1	1	17	15	19	25
2	0.5	15	13	17	22
3	0.25	13	12	15	18
4	Stm	17	19	-	-
5	Amp. B	-	-	18	16

The leaf extract assay for the zone of inhibition of *Calotropis procera* varied with the dilutions. The maximum zone of inhibition was recorded in 1 dilution against *E. coli*, exhibiting 10 mm and *T. rubrum* with 13 mm, while the minimum was recorded in both bacteria and *C. albicans* with 9 mm in 0.25 dilution (Table no. 2).

As shown in table 3, the zone of inhibition of the leaf extract of *Calotropis procera* varied with the dilutions. The maximum zone of inhibition was recorded in 1 dilution against *E. coli*, exhibiting 11 mm and *T. Rubrum* with 20 mm, while the minimum was recorded in *S. aureus*, i.e., 7 mm in 0.25 dilution.

It is clearly evident from table 3 that the zones of inhibition of leaf extract of *Calotropis procera* varied with the dilutions. The maximum zone of inhibition was recorded in 1 dilution against *E. coli*, exhibiting 17 mm and 25 mm with *T. rubrum*, while the minimum was recorded in *S. aureus* with 12 mm in 0.25 dilution.

Different peaks in high resolution were obtained in the HPTLC analysis. The leaf extract *of Calotropis procera* L. was run along with the standard and it was perceived to validate the presence of phytochemical compounds from the chromatogram after derivatization. The result of the selected plant extract is given in table 1. Ethanol is used as a solvent. Rf values and different wavelengths were obtained. Table 1 shows the picture plate at UV254nm. The graphic representation shows different peaks of polyvalent phytoconstituents. The Rf value starts from 0.15 to 1.00, in which the highest concentration of phytoconstituents was found to be 24.53, and the maximum percentage of stars from 3.22 to 24.53 %, and the maximum height from 17.8 to 134.7. The secondary metabolites glycoside and alkaloid are hazardous to human health, when they are used with any intention, such as to kill the person or blind the person.

The presence of proteins related to defence against pathogens (P R Proteins), such as glucanases and chitinases (Jekel *et al*., 1991; Van Loon; Van Sttrien, 1999) [8, 24], proteinases (Freitas *et al*., 2007; Konno *et al*., 2004; Moussaoui *et al*., 2001) [16, 10, 15] and protein inhibitors (Sritanyarat *et al*., 2006), strengthens the hypothesis that this secretion plays a role in plant defence against or attack by insects and pathogens. Ramos *et al*. (2007) [16] hypothesised that a protease inhibitor was involved in the observed negative effects (Ramos *et al*., 2009) [16].

Conclusion

the HPTLC fingerprint profile of *Calotropis procera* is useful in determining the quality of crude pharmaceuticals is. The separation of secondary metabolites including alkaloids, flavonoids, tannins, saponins, and cardiac glycosides, which are bioactive compounds used to study plant biochemistry and physiology, is also facilitated by this technique.

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