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## Antimicrobial activity of *Cascabela thevetia* (Flowers)

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**Abstract**

A large number of medicinal plants are claimed to be useful in treating skin diseases in all traditional system of medicine. The present study was carried out to investigate the antimicrobial effect of the compound isolated from the ethylacetate fraction of flowers of *Cascabela thevetia*. This compound was shown to possess antimicrobial activity against bacteria and fungi. Four bacterial strains *Salmonella typhi*, *Escherichia coli*, *Enterococcus faecalis*, *Bacillus cereus* and two fungal strains *Curvularia lunata* and *Candida albicans* were tested by using disc diffusion method. The anti-bacterial activity of the compound isolated from ethyl acetate fraction is almost comparable with standard *Chloramphenicol*. The antifungal activity is almost comparable with standard *Fluconazole*.

**Keywords:** *Cascabela thevetia*, antibacterial activity, antifungal activity, diffusion method, chloramphenicol, fluconazole

**Introduction**

Medicinal plants and their extracts are used in traditional treatments of various diseases [1-5]. Plants are a potential source of antimicrobial compounds and several researchers throughout the world are investigating the antimicrobial activity of medicinal plants, which are utilized in the traditional or alternative healthcare systems [6, 7]. The antimicrobial activities of plant extract can be determined by various methods such as disc diffusion, agar well diffusion and twofold serial dilution techniques [6]. The agar well diffusion technique for screening of the antimicrobial activity of medicinal plant is normally considered [8].

**Plant Description**

*Cascabela thevetia* (L.) belongs to the family Apocynaceae and commonly known as the Mexican Oleander is a native plant of Mexico and Central America and a close relative to *Nerium oleander* [9]. It is an evergreen tropical shrub or small tree that bears yellow, trumpet like flowers and its fruit are deep green or black in colour encasing a large seed that bears resemblance to a Chinese plant "be-still tree" [10, 11]. The leaves of *Cascabela thevetia* are used to control toothache; the barks of the plant have shown decongestant activity [12].

**Materials and Methods****Collection of Flowers**

Fresh flowers of *Cascabela thevetia* were collected from S. Pudur, Sivagangai (Dt), Tamil Nadu, India, during the month of February and identified by Dr. S. John Britto, Director, The Rapinat Herbarium and Centre for Molecular Systematics (Authentication No. SS005 dated: 03/06/2016). St. Joseph's College (Campus), Tiruchirappalli, Tamil Nadu, India.

**Extraction and fractionation**

Fresh flowers (3 kg) of *Cascabela thevetia* were extracted with 90% ethanol (5x500ml). The combined alcoholic extract was concentrated in vacuo and the aqueous extract was successively fractionated with petroleum ether (60-80 °C) (6x250ml), Peroxide free diethyl ether (4x250ml) and ethyl acetate (8x250ml). Petroleum ether fraction and diethyl ether fraction did not yield any isolable material. Ethyl acetate fraction on concentration yielded a dry powder which was dissolved in DMSO to get various concentrations and were used for further study.

**Antimicrobial procedure****Screening of antibacterial activity****Bacteria tested**

Four bacterial strains were used throughout this investigation. All the bacterial cultures were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology,

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Chandigarh, India. The young bacterial broth cultures were prepared before the screening procedure.

#### Preparation of inoculums

Stock cultures were maintained at 4 °C on slopes of nutrient agar. Active cultures of experiment were prepared by transferring a loop full of cells from the stock cultures to test tube of Muller-Hinton Broth (MHB) that were incubated without agitation for 24 hrs at 37 °C. The cultures were diluted with fresh Muller-Hinton Broth to achieve optical densities corresponding to  $2.0 \times 10^6$  colony forming units (CFU/ml).

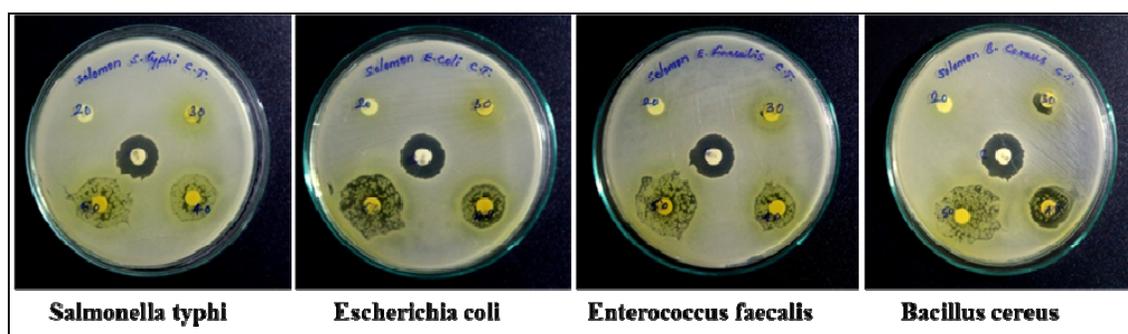
#### Antibacterial susceptibility test

The disc diffusion method (Bauer *et al.*, 1966) was used to screen the antibacterial activity. In-vitro antibacterial activity was screened by using Muller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1% inoculum suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes. The compound

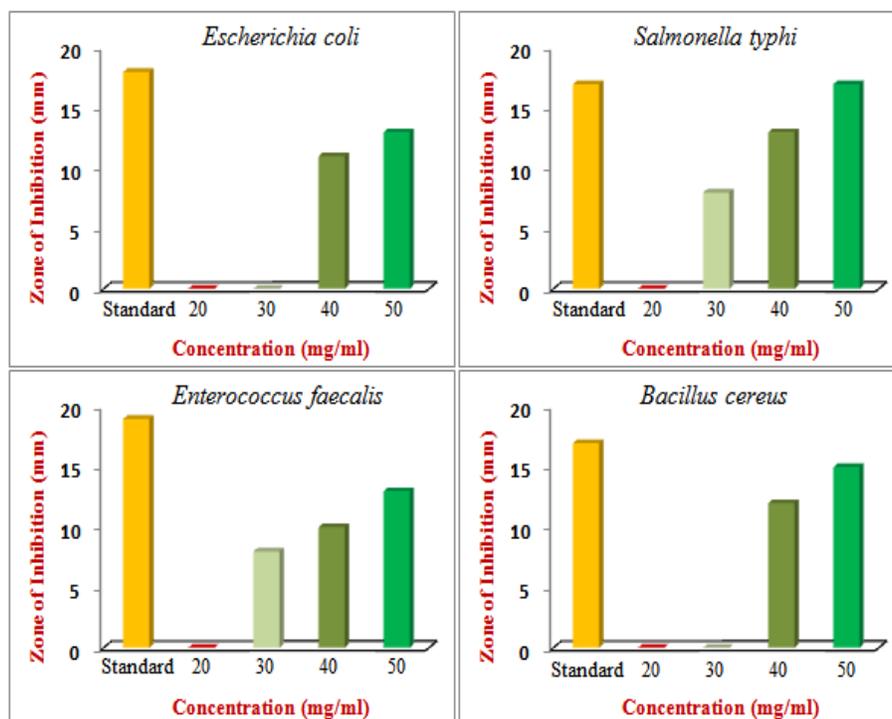
of concentration 20 mg/ml, 30 mg/ml, 40 mg/ml, 50 mg/ml were loaded on 6 mm sterile disc. The loaded disc were placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37 °C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with a transparent ruler in millimeter. Standard antibiotic chloramphenicol of concentration 1mg/ml was used as positive control [13].

**Table I:** Antibacterial activity of the compound isolated from the ethyl acetate fraction of flowers of *Cascabela Thevetia*

S. No	Organisms	Zone of inhibition(mm)				
		Standard (Chloramphenicol)	Sample Concentration (mg/ml)			
			20	30	40	50
1	<i>Salmonella typhi</i>	17	00	08	13	17
2	<i>Escherichia coli</i>	18	00	00	11	13
3	<i>Enterococcus faecalis</i>	19	00	08	10	13
4	<i>Bacillus cereus</i>	17	00	00	12	15



**Fig I:** Antibacterial activity of the compound isolated from the ethyl acetate fraction of flowers of *Cascabela Thevetia*



**Graph I:** Graphical representation of anti-bacterial activity of the compound isolated from the ethyl acetate fraction of flowers of *Cascabela thevetia* (Standard: Chloramphenicol, concentration 1 mg/ml)

### Screening of antifungal activity

#### Culture Media

The media used for antifungal test was Sabouraud's dextrose agar/broth of Hi media Pvt. Bombay, India.

#### Inoculum

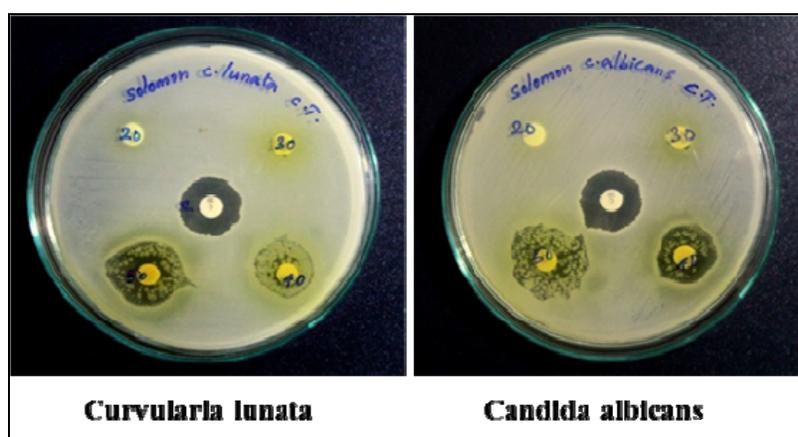
The fungal strains were inoculated separately in Sabouraud's dextrose broth for 6 hrs and the suspensions were checked to provide approximately 10<sup>5</sup> CFU/ml.

### Determination of antifungal activity

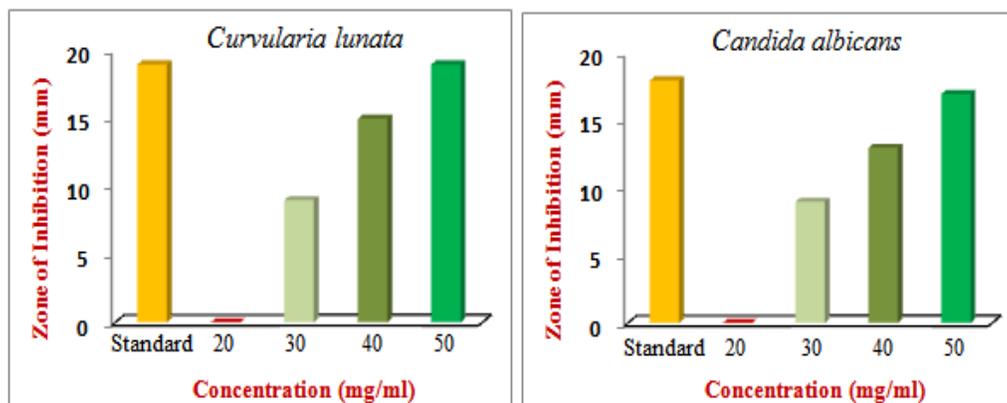
The agar well diffusion method (Perez, 1993) was modified. Sabouraud's dextrose agar (SDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in Sabouraud's dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with sample solution and solvent blanks (hydro alcohol, and hexane). Standard antibiotic (Fluconazole, concentration 1 mg/ml) was used as positive control and fungal plates were incubated at 37 °C for 72 hrs. The diameters of zone of inhibition observed were measured.

**Table II:** Antifungal activity of the compound isolated from the ethyl acetate fraction of flowers of *Cascabela Thevetia*

S. No	Organisms	Zone of inhibition(mm)				
		Standard (Fluconazole)	Sample Concentration (mg/ml)			
			20	30	40	50
1	<i>Curvularia lunata</i>	19	00	09	15	19
2	<i>Candida albicans</i>	18	00	09	13	17



**Fig II:** Antifungal activity of the compound isolated from the ethyl acetate fraction of flowers of *Cascabela Thevetia*



**Graph II:** Graphical representation of anti-fungal activity of the compound isolated from the ethyl acetate fraction of flowers of *Cascabela thevetia* (Standard: Fluconazole, concentration 1 mg/ml)

### Results and Discussion

In the present study, *Cascabela thevetia* flowers were screened for antimicrobial activity and compared with standard drug. It is evident from the data presented in Table I that the compound isolated from the ethyl acetate fraction of *Cascabela thevetia* flowers possesses antibacterial activity. The disc diffusion method result showed the zone of inhibition for 20 mg/ml as 0 mm, 0 mm, 0 mm and 0 mm, for 30 mg/ml as 0 mm, 8 mm, 0 mm and 8 mm, for 40 mg/ml showing 13 mm, 11 mm, 10 mm and 12 mm, for 50 mg/ml as

17 mm, 13 mm, 13 mm and 15 mm, for the test sample against *Salmonella typhi*, *Escherichia coli*, *Enterococcus faecalis* and *Bacillus cereus* respectively when compared with standard drug *Chloramphenicol* showing 17 mm, 18 mm, 19 mm and 17 mm zone of inhibition respectively.

Then it is evident from the data presented in Table II that the test sample possesses antifungal activity. The disc diffusion method result showed the zone of inhibition for 20 mg/ml as 0 mm and 0 mm, for 30 mg/ml as 9 mm and 9 mm, for 40

mg/ml as 15 mm and 13 mm, for 50 mg/ml as 19 mm and 17 mm for the test solution against *Curvularia lunata*, and *Candida albicans* respectively when compared with standard drug *Fluconazole* showing 19 mm and 17 mm of inhibition respectively.

### Conclusion

Present study indicates that the compound isolated from the ethyl acetate fraction of flowers of *Cascabela thevetia* is having good pharmacological action. Antimicrobial activities are aggravated by increasing the quantity of this compound, and hence this compound could be used as an alternative for antibiotics.

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