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Antimicrobial Activity of Ethanolic Crude Extracts of *Thespesia populnea* Flowers

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: To analyze the antimicrobial activity of ethanolic crude extract of *Thespesia populnea* Flowers.
Study Design: Antimicrobial activity of *Thespesia populnea* was carried out using disc diffusion method against crude extract.
Place and Duration of Study: PG & Research Department of Chemistry, Periyar E.V.R. College (Autonomous), Trichy, Tamilnadu, India. During the month of August and September 2014.
Methodology: Four bacterial strains such as *S. typhi*, *E. coli*, *E. faecalis*, *B. cereus* and two fungal strains such as *C. lunata*, and *C. albicans* were identified by using disc diffusion method. The anti bacterial activity of ethanolic extract is almost comparable with standard Chloramphenicol, which is

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used as an antibacterial agent. The anti fungal activity of ethanolic crude extract is almost comparable with standard Fluconazole, which is used as an antifungal agent.

Results: The disc diffusion method result showed the zone of inhibition for 10 mg/ml as 0 mm, 0 mm, 0 mm and 0 mm, for 20 mg/ml as 9 mm,0 mm , 0 mm and 7 mm, for 30 mg/ml showing 21 mm, 18 mm, 20 mm and 16 mm and for 40 mg/ml as 27 mm, 26 mm, 25 mm and 25 mm, against *S. typhi, E. coli*, *E. faecalis* and *B. cereus* respectively when compared with standard drug Chloramphenicol showing 22 mm, 19 mm, 23 mm and 25 mm zone of inhibition respectively. Then it is evident from the data presented in Table II that the sample possesses antifungal activity. The disc diffusion method result showed the zone of inhibition for 10 mg/ml as 0 mm and 0 mm, for 20 mg/ml as 9 mm and 0 mm, for 30 mg/ml as 19 mm and 18 mm and for 40 mg/ml as 23 mm and 26 mm against *C. lunata*, and *C. albicans* respectively when compared with standard drug Fluconazole showing 25 mm and 20 mm of inhibition respectively.

Conclusion: This study justifies the antimicrobial activity of ethanolic crude extract of *Thespesia populnea* Flowers. Further detailed analysis of this sample is required to identify the presence of bioactive compounds responsible for antibacterial and antifungal activities. Studies are highly needed for future drug development.

Keywords: Thespesia populnea; antibacterial activity; antifungal activity; diffusion method; chloramphenicol; fluconazole.

1. INTRODUCTION

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. [1,2]. Screening of medicinal plants for therapeutically active bio-molecules including those with antimicrobial properties has gained an unprecedented importance in the recent years. World Health Organization (WHO) has recently shown genuine interest in promoting the development and utilization of indigenous medicinal plant resources in the developing countries so as to extend safe and effective healthcare to maximum number of population on those countries [3]. Emerging antibiotic resistant infections are one of the most serious problems the medical professionals face today. Due to the immense cost of discovery and regulatory uncertainties, large pharmaceutical companies are hesitant to commit to antibiotic discovery programs. The result is tens of millions of unwarranted deaths per year. Recently, considerable attention has been paid to utilize eco-friendly plant based products for prevention and cure of different human diseases since they are safe and effective. Studies are conducted to shed light on the antibacterial activity of some indigenous medicinal plants. Nonetheless, the investigations have primarily been restricted to screening only. In order to promote herbal drugs there has to be an evaluation of therapeutic potentials of drugs [4]. The present Study aims to screen the antibacterial & antifungal activities of *Thespesia populnea*, by using modern scientific approaches and innovative scientific tools.

1.1 Plant Description

T. populnea (Malvaceae) is most commonly known as 'portia tree' or 'poovarasu' or 'Indian tulip tree'. It is a small to medium sized tree with a pantropical distribution, normally found along the coastal stretches. The tree grows to a height of 15 m. Its leaves are simple and heart-shaped, with a distinct tip. Flowers are bisexual, solitary or in cymes, showy and yellow. The tree yields valuable pink to dark red close-grained wood and also an oil from its seeds. The tree is able to grow in a wide range of soil types in the coastal environments. The leaves are applied locally for their anti-inflammatory effects in swollen joints [5]. The plant is traditionally claimed to possess useful medicinal properties [6,7] such as anti-inflammatory, antioxidant, purgative and hepatoprotective [8] activities. In addition to this *T. populnea* flowers has been scientifically proved to possess medicinal properties such as antibacterial, antifertility, antinociceptive activities, antioxidant and hepatoprotective activity [9,10,11,12,13,14].

2. MATERIALS AND METHODS

2.1 Collection of Flowers

Fresh flowers of *Thespesia populnea* were collected from O. Koothur Village, Ariyalur

district, Tamil Nadu, India, during the month of August and identified by Head, PG & Research Department of Botany, St. Joseph's College (Autonomous) Trichy. Tamilnadu, India. (Authentication no: SS001-30/03/2015)

2.2 Flower Extraction

2 kg of fresh flowers were soaked with 90% ethanol at room temperature (25ºC-30ºC). After 72 hrs the ethanolic extract was filtered. This extract was distilled and finally the crude was obtained. This ethanolic crude to get required concentrations and were used for screening antimicrobial activities.

2.3 Antimicrobial Procedure

2.3.1 Screening of antibacterial activity

2.3.1.1 Bacteria tested

Four bacterial strains such as *S. typhi, E. coli, E. faecalis* and *B. cereus* were used throughout this investigation. All the bacterial cultures were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The young bacterial broth cultures were prepared before the screening procedure.

2.3.1.2 Preparation of inoculums

Stock cultures were maintained at 4ºC 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 was incubated without agitation for 24 hrs at 37ºC. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities corresponding to $2.0x10^6$ colony forming units (CFU/ml).

2.3.1.3 Antibacterial susceptibility test

The disc diffusion method 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 extracts of concentration 10 mg/ml, 20 mg/ml, 30 mg/ml, 40 mg/ml were loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the extract was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37ºC for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. Standard antibiotic Chloramphenicol of concentration 1mg/ml was used as positive control.

2.3.2 Screening of antifungal activity

2.3.2.1 Culture media

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

2.3.2.2 Inoculum

The fungal strains were inoculated separately in Sabouraud's dextrose broth for 6 h and the suspensions were checked to provide approximately 105 CFU/ml.

2.3.3 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 Sabourauds dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with plant extracts. Standard antibiotic (Fluconazole, concentration 1 mg/ml) was used as positive control and fungal plates were incubated at 37ºC for 72 h. The diameters of zone of inhibition observed were measured.

3. RESULTS AND DISCUSSION

In the present study, ethanolic crude extract of *T. populnea* flowers exhibited significant antimicrobial activity when compared with standard drug. It is evident from the data presented in Table 1 and Figs. 1 and 2 that the sample possesses antibacterial activity. The disc diffusion method result showed the zone of inhibition for 10 mg/ml as 0 mm, 0 mm, 0 mm and 0 mm, for 20 mg/ml as 9 mm,0 mm, 0 mm and 7 mm, for 30 mg/ml showing 21 mm, 18 mm, 20 mm and 16 mm and for 40 mg/ml as 27 mm, 26 mm, 25 mm and 25 mm, against S. typhi, *E. coli, E. faecalis* and *B. cereus* respectively when compared with standard drug chloramphenicol showing 22 mm, 19 mm, 23 mm and 25 mm

zone of inhibition respectively. Then it is evident from the data presented in Table 2 and Figs. 3 and 4 that the sample possesses antifungal activity. The disc diffusion method result showed the zone of inhibition for 10 mg/ml as 0 mm and 0 mm, for 20 mg/ml as 9 mm and 0 mm, for 30 mg/ml as 19 mm and 18 mm and for 40 mg/ml as 23 mm and 26 mm against *C. lunata*, and *C. albicans* respectively when compared with standard drug Fluconazole showing 25 mm and 20 mm of inhibition respectively. The above result shows that the activity of ethanolic crude extracts of *T. populnea flowers* shows significant antibacterial and antifungal activities and also the possession of antimicrobial activities against a number of microorganisms.

Table 1. Antibacterial activity of *Thespesia populnea* ethanolic crude extract in different strains

S. no.	Name of organisms	Zone of inhibition (mm)				
		Standard (chloramphenicol)	Sample concentration (mg/ml)			
			10	20	30	40
1.	*S. typhi*	22	0	9	21	27
2.	*E. coli*	19	0	0	18	26
3.	*E. faecalis*	23	0	0	20	25
4.	*B. cereus*	25	0	7	16	25

Table 2. Anti fungal activity of *Thespesia populnea* flowers of ethanolic crude extract in different strains

S. no.	Name of organisms	Zone of inhibition (mm)				
		Standard (fluconazole)	Sample concentration (mg/ml)			
			10	20	30	40
1.	*C. lunata*	25	0	9	19	23
2.	*C. albicans*	20	0	0	18	26

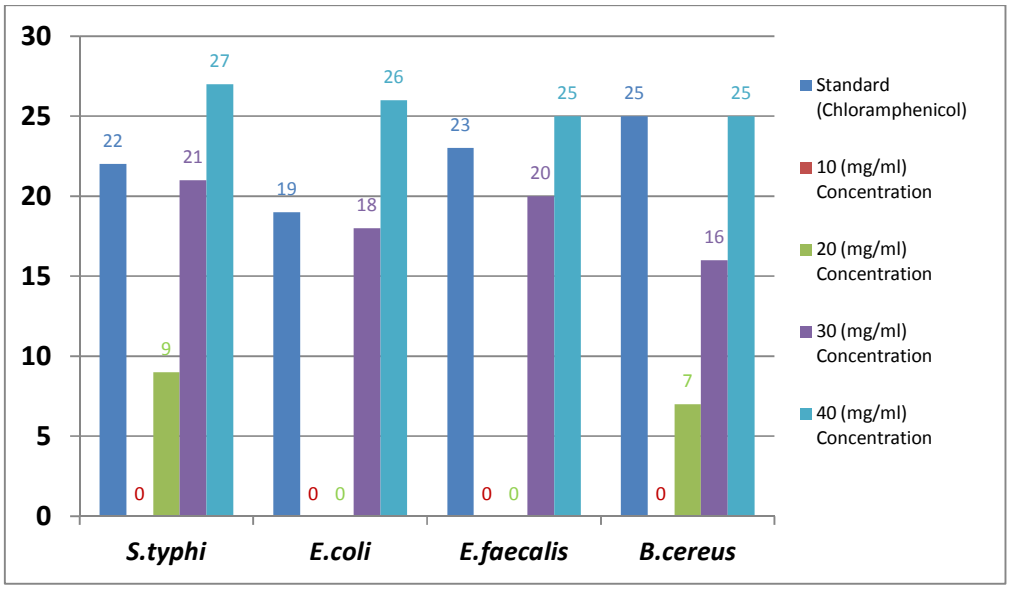


Fig. 1. Graphical representation of anti bacterial activity of ethanolic crude extract of flowers of *Thespesia populnea*. (Standard: Chloramphenicol, concentration 1 mg/ml)

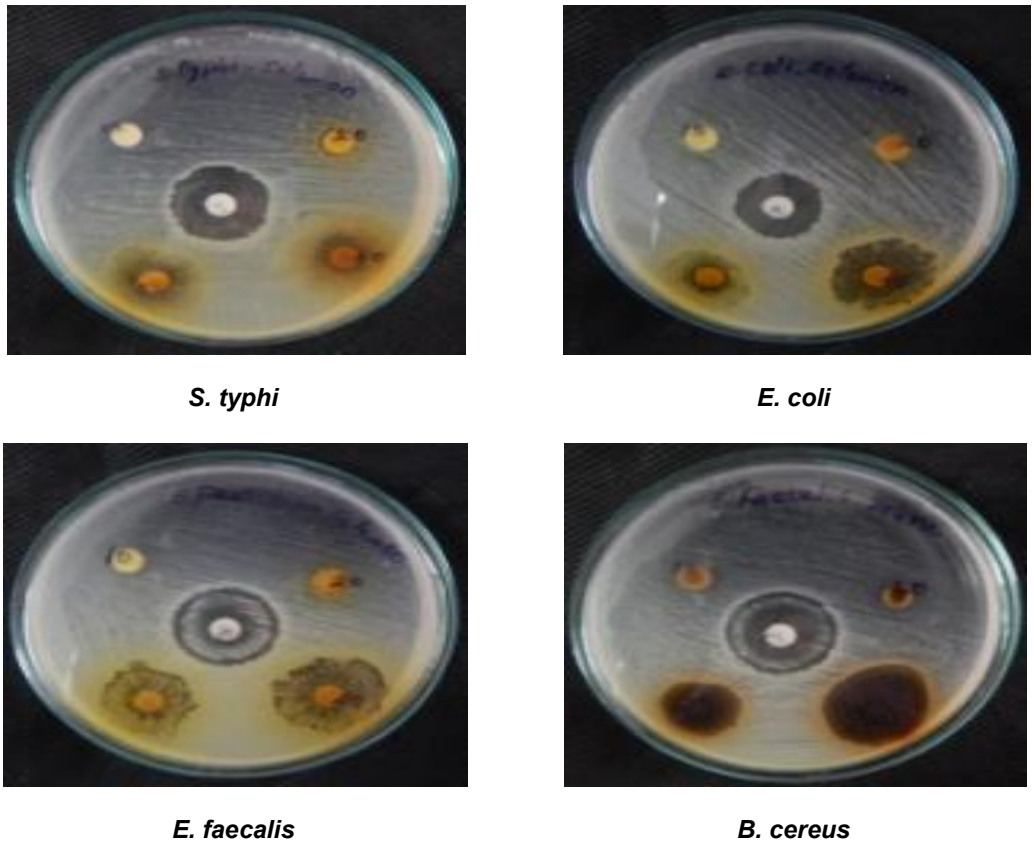


Fig. 2. Inhibition of bacterial growth by ethanolic crude extract of *Thespesia populnea* flowers by disc diffusion method

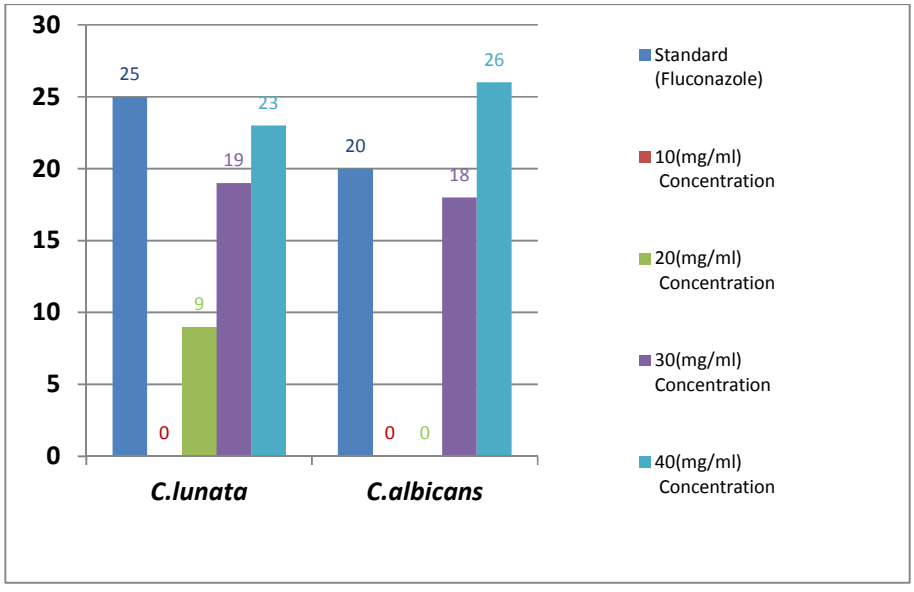
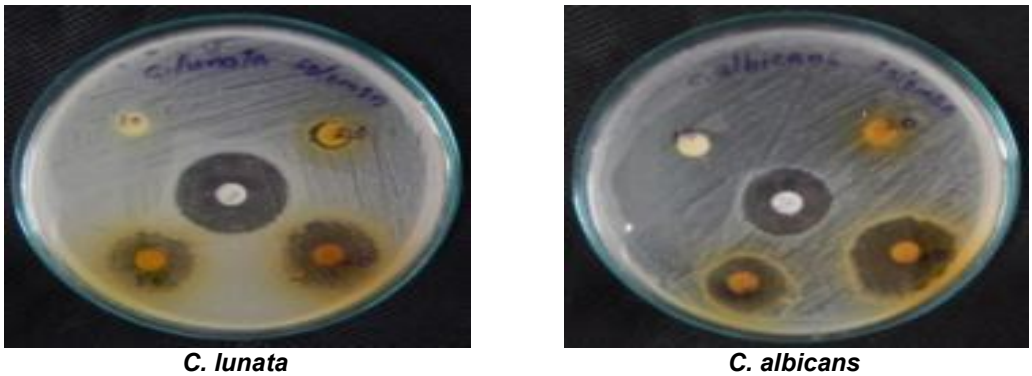


Fig. 3. Graphical representations of anti fungal activity of ethanolic crude extract of *Thespesia populnea* flowers. (Standard: fluconazole, concentration 1 mg/ml)



C. lunata *C. albicans*

Fig. 4. Inhibition of bacterial growth by ethanolic crude extract of *Thespesia populnea* flowers by disc diffusion method

4. CONCLUSION

Based on the result of the above study on the *T. Populnea* we conclude that *T. populnea* shows higher antibacterial and antifungal activity against following micro organisms like *S. typhi, E. coli*, *E. faecalis*, *B. cereus* and *C. lunata*, *C. albicans*. Also it justifies the claimed uses of flowers parts of the *T. populnea* in the traditional system of medicine to treat various infectious disease caused by the microbes. Antimicrobial activities are aggravated by increasing the quantity of this compound, which can be used as an alternative for antibiotics. Therefore, pharmacological test is necessary to isolate and characterize their active compounds. Moreover, extract of the plant should be investigated for better understanding of its safety, efficacy and properties.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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