

Antimicrobial activity of *Thespesia populnea* Soland. ex Correa bark extracts

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Received 8 March 2011; Accepted 18 June 2011

Thespesia populnea Soland. ex Correa, also called as Indian tulip tree, is tropically distributed plant. In present study antimicrobial activity of bark of this plant was investigated by well diffusion method against four bacteria and two fungi (*Escherichia coli*, *Candida albicans*). Petroleum ether extract showed significant activity against all organisms whereas ethanolic and aqueous extracts showed moderate to mild activity. The study scientifically validates use of plant in traditional medicine.

Keywords: *Thespesia populnea*, Indian tulip tree, Mother culture, Griseofluvin, Antibacterial.

IPC code; Int. cl. (2011.01)—A61K 36/00, A61P 31/04.

Introduction

Immune compromised individuals are frequently found suffering from skin infections that are difficult to cure¹. A wide array of natural products from botanicals are traditionally in use over several hundred years as the plant kingdom is gold mine for novel and affordable natural drugs including skin care through novel mechanisms against pathogens. Plants are effective in the treatment of infectious diseases while simultaneously mitigating many side effects associated with synthetic antimicrobials. The plant *Thespesia populnea* Soland. ex Correa, belonging to family Malvaceae and also known as Indian tulip tree, is one among such plants used in indigenous medicine for the treatment of diarrhoea, wounds and cholera. It is also useful in dermatopathy such as scabies psoriasis and leprosy²⁻³. It is reported to possess antioxidant, antibacterial and anti-implantation activities⁴⁻⁸. It is compact quick growing ever green tree with greyish brown fissured bark, flowers yellow with purple base⁹. In present investigation the

antimicrobial activity of bark extracts of *T. populnea* were tested, using standard procedures, against some reference strains of human pathogenic bacteria and fungi to assess the feasibility of applying crude preparations (Plate 1).

Materials and Methods

T. populnea bark was collected in the month of November from the fields of Harapanahalli, Karnataka and authenticated by Prof. K Prabhu, Department of Pharmacognosy. A voucher specimen (No.SCSCP/PG/31/04) has been deposited at S.C.S College of Pharmacy, Harapanahalli, Karnataka.

Preparation of extracts

The shade dried, bark was coarse powdered and packed into Soxhelt column and extracted successively with petroleum ether (60-80°) and ethanol (70%) followed by cold maceration with distilled water. Obtained extracts were concentrated under reduced pressure (temp) and the yield of each extract obtained was, 2.16, 6.70 and 2.03%, respectively. The dried extracts were stored in airtight containers in refrigerator below 10°C. Test solutions of petroleum ether, ethanolic and aqueous extracts were prepared by using DMF and used for the studies.

Micro-organisms

The extracts were tested against four micro-organisms including *Staphylococcus aureus* MTCC87, *Streptococcus pyogenes* (Gram+ve), *Escherichia coli* MTCC46, *Pseudomonas aeruginosa* MTCC442



Plate 1—Bark of *Thespesia populnea*

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(Gram-ve), and fungi *Candida albicans* MTCC 227 and *Aspergillus flavus* MTCC 277. All strains of microorganisms were provided by Institution of Microbial Technology (IMTECH), Chandigarh, India.

Preparation of inoculums

The subculture medium was sterilized by autoclaving at 15 lbs/sq inch. for 15 minutes. Loops full of organisms were transferred from a laboratory maintaining mother culture into a 250 mL conical flask containing sterilized subculture medium. The flask was incubated for 20 h at 37°C. Petri dishes, glass syringe, cork borer (8 mm), conical flask and test tubes were sterilized by autoclaving at 15 lbs/sq. inch. for 15 minutes.

Antibacterial activity

The antibacterial activity was tested by Agar-cup diffusion method. The technique described by Hug and Russel (1984) was adopted for antibacterial activity. Briefly 20 ml of nutrient agar (Hi Media Pvt. Ltd) was poured into the petri dishes and 8 mm well bored in the agar medium, different concentration of three extracts of petroleum ether, 70% ethanolic and aqueous extracts of *T. populnea* bark at the conc. 25 and 50 mg/mL were prepared by using dimethyl formamide (DMF) and used for anti-microbial activity. Commercially available Ceftraixon at the conc. 25 and 50µg/mL was used as positive (Std) control and DMF (Dimethyl formamide) as negative control. Standard drug solutions were prepared in sterile water for injection.

Each conical flask with the medium was cooled to 46°C and inoculated with test organism (20 ml of subculture medium/100 ml of the assay medium). Then 20 ml of inoculated media was distributed into each petri dish. After solidification of the media five bores are made at equal distance by using sterile steel cork borer (8 mm diam.). Different concentrations of standard drugs (25 and 50 µg/ml) and extracts (25 and 50 mg/ml) were introduced into these cups. Dimethyl formamide (DMF) was used as a control.

After introduction of standard drugs and extracts, the plates were placed in a refrigerator at 8-10°C for proper diffusion of drugs into the media. After two hours of cold incubation, the petri plates were transferred to incubator and maintained at 37±2°C for 18-24 h. After the incubation period, the petri plates were observed for zone of inhibition by using vernier scale.

Antifungal activity

The cup plate technique described by Hugo and Russel (1984) was adopted for anti-fungal activity also. The subculture medium was sterilized by autoclaving at 15 lbs/sq.inch for 15 minutes. Loops full of organisms were transferred from a laboratory maintained mother culture into a 250 mL conical flask containing sterilized subculture medium. The flask was incubated for 48 h at 37°C. Standard drug (Griseofulvin 25 and 50 µg/mL) solutions were prepared in sterile water for injection.

Preparation of petri plates

Each conical flask with the medium was cooled to 46°C and inoculated with test organism (20 mL of subculture medium/100 mL of the assay medium). Then 20 ml of inoculated media was distributed into each petri plate. After solidification of the media five bores were made at equal distance by using sterile steel cork borer (8 mm diam). Different concentrations of standard drugs (25 and 50 µg/ml) and extracts (25 and 50 mg/ml) were introduced into these cups; DMF was used as a blank.

After introduction of standard drugs and extracts, the plates were placed in a refrigerator at 8-10°C for proper diffusion of drugs into the media. After two hours of cold incubation, the petri plates were transferred to incubator and maintained at 37±2°C for 48 h. After the incubation period, the petri plates were observed for zone of inhibition.

Results and Discussion

The zone of inhibition obtained with different concentrations of *T. populnea* bark extracts are shown in Table 1. Petroleum ether extract at 25 mg/mL and 50 mg/ml of concentration were found to be having significant antibacterial activity against *S. aureus* and *S. pyogenes* and *E. coli* and *P. aeruginosa*. Ethanolic extract was found to be having moderate and aqueous extract having mild antibacterial activity against both bacteria at concentration of 25 mg/ml and 50 mg/ml (Plate 2 a & b).

The results indicate petroleum ether extract at 25 mg/ml and 50 mg/ml of concentration was found to be having significant anti-fungal activity against *C. albicans* and *A. flavus*, whereas ethanolic and aqueous extracts were showed better and less potent activity against fungal pathogens (Table 1).

Many studies have proved that naphthoquinones have analgesic, anti-inflammatory and antipyretic effects in rat models and also inhibitory effect

Table 1—Effect of *Thespesia populnea* bark extracts on selected strains of micro-organisms

Strain	DMF (Control)	Mean zone of inhibition (mm)							
		Petroleum ether extract (mg/ml)		Ethanol extract (mg/ml)		Aqueous extract (mg/ml)		Standard (mg/ml)	
		25	50	25	50	25	50	25	50
<i>Staphylococcus aureus</i> (MTCC 87)	–	19	22	13	17	10	14	28	34
<i>Streptococcus pyogenes</i>	–	18	25	12	16	9	13	25	30
<i>Escherichia coli</i> (MTCC 46)	–	13	17	12	15	10	12	23	28
<i>Pseudomonas aeruginosa</i> (MTCC 442)	–	18	24	11	15	11	14	24	31
<i>Candida albicans</i> (MTCC 227)	–	16	18	12	16	9	13	24	30
<i>Aspergillus flavus</i> (MTCC 277)	-	15	16	11	14	10	12	25	29

Standard drugs used: Ceftriaxone for Gram positive and negative bacteria; Griseofulvin for Fungi

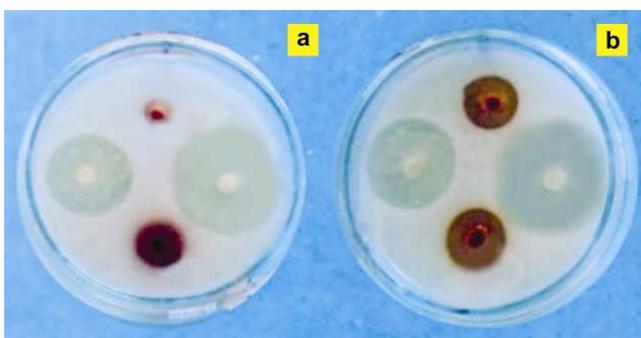


Plate 2—Zone of inhibition, A. Ethanol extract; B. Petroleum ether extract against bacteria

against *Proteus* and *S. aureus*¹⁰. In addition to that naphthoquinones are effective against common urinary pathogens¹¹ and fungal infection¹². Plant may be responsible for its antibacterial and anti-fungal activities due to the presence of naphthoquinones. Anti-fungal activity against *C. albicans* and *Aspergillus flavus*, suggesting that the extracts of *T. populnea* bark contains naphthoquinone¹³.

Conclusion

It may be concluded that the petroleum ether extract of *T. populnea* possesses considerable anti-microbial activity against the tested bacterial and fungal organisms as compared to ethanolic and aqueous extracts. However, the results of extracts were found less potent to the reference standard drug for microbial infections and it has validated its applications in traditional medicines. There is a need to pursue the characterization of active principles to optimize the observed activities.

Acknowledgements

We are thankful to TMEA Society Harapanahalli, for providing necessary facilities for this study in S.C.S College of pharmacy.

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