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Antibacterial and Antifungal Activities of *Thespesia populnea* Leaf extracts against Human Pathogens

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Abstract: *Thespesia populnea* (L.) Soland. ex Correa, commonly known as 'Portia tree' or 'Indian tulip tree', is widely used in traditional medicines, particularly in the Indian System of Medicine for its astringent, cooling, depurative, haemostatic, anti-inflammatory and antidiarrhoeal properties. The present study examines the antibacterial and antifungal activities of *Thespesia populnea* leaf extracts (hot and cold) using seven solvents (hexane, chloroform, dichloromethane, ethyl acetate, acetone, methanol and water) against human pathogens. Antibacterial and antifungal activities of crude extracts were determined by disc diffusion method. The highest antibacterial activity was exhibited by methanol cold extract against *Staphylococcus epidermidis* (15 mm) and *Bacillus cereus* (14 mm) respectively. Hot hexane extract showed an inhibition zone of 12 mm against *Pseudomonas aeruginosa.* Both the cold and hot extracts of all the seven solvents exhibited inhibition zones against *Candida albicans.*

Keywords: Antibacterial activity, *Candida albicans*, pathogenic microorganism, *Pseudomonas aeruginosa, Thespesia populnea*.

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 and 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 in those countries.[3] Therapeutically active principles are extracted from all parts of the plant body, but the concentration of these components varies from part to part. Normally, parts known to contain the highest concentration of the principles are preferred to therapeutic purposes and it can either be the leaves, stems, barks, roots, bulks, corms, rhizomes, woods, flowers, fruits or the seeds.[4] It is therefore important to consider the commonly considered or preferred part of the target plant by the traditional healers while exploring effective therapeutic agents.

Thespesia populnea (L.) Soland. ex Correa, commonly known as 'Portia tree' or 'Indian tulip tree' of family Malvaceae 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. Fruits are globose brown capsules. The tree yields valuable pink to dark red close-grained wood and also an oil from its seeds. Though the tree is able to grow in a wide range of soil types in the coastal environments, it prefers near neutral soils (pH of 6 - 7.4). *T. populnea* is one of the common trees in the coastal lowlands and midlands of Kerala, which is often cultivated in the home gardens and in other agroforestry systems for its multifarious uses.

The leaves are applied locally for their anti-inflammatory effects in swollen joints.[5] The phytochemical study of bark reveals the presence of gossypol, tannin, acacetin, quercetin, coloring matter, etc. and the leaf extract indicates the presence of lupeol, lupenone, and β-sitosterol.[6] Gossypol was found to be the major component of *T. populnea* responsible for anti-inflammatory and antifertility effects in rats as well as in human beings.[5] The flowers contained kaempferol, kaemperol-7-glucoside and gossypetin. The fruit kernels were reported to contain β-sitosterol, ceryl alcohol and a yellow pigment, thespesin.[7]

The plant is traditionally claimed to possess useful medicinal properties[8,9] such as antifertility, anti-inflammatory, antioxidant, purgative and hepatoprotective[10] activities and its bark, leaves and flowers are useful in cutaneous infections such as scabies, psoriasis, eczema, ring worms, guinea worm,[11] anti-inflammatory for poultice as a folk medicine etc. In addition to these, *T. populnea* has been scientifically proved to possess medicinal properties such as antibacterial, antifertility, and antinociceptive activities,[12] as also in the treatment of Alzheimer's disorder,[13] for its memory enhancing activity,[13] antioxidant and hepatoprotective activity,[14] anti-psoriatic activity,[15] diuretic activity[16] and wound healing activity.[17] The purpose of the present study was to examine the antibacterial and antifungal activity of leaf extracts of *T. populnea* leaf samples collected from Kuttanad wetlands, Kerala State, India.

EXPERIMENTAL

Collection of Plant Material

Healthy leaf samples were collected from Kuttanad wetlands after authenticating the taxonomic identity of the source plant by Dr. T. Shaju, Plant Taxonomist, Division of Plant Systematics and Evolutionary Science, Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Palode, Kerala, and the voucher specimens of the samples (leafy twigs with flowers) were deposited in the Herbarium of Environmental Resources Research Centre (ERRC), Thiruvananthapuram, Kerala. The plant materials were washed and shade dried and pulverized to coarse powder using electric grinder. The powder was then stored in airtight bottles for further studies.

Chemicals

The solvents used for the extraction process were of analytical grade, procured from the SD Fine Chemicals, Mumbai, India. Ciprofloxacin (5 µg/ml) discs, Amphotericin B (10µg/ml), Mueller Hinton agar and Nutrient agar medium were obtained from Hi-Media Laboratories, Mumbai, India. All other chemicals were of analytical grade and procured locally.

Preparation of Extracts

Thirty grams of leaf powder was subjected to hot and cold extraction using 250 ml of solvents in the increasing order of polarity (hexane, chloroform, dichloromethane, ethyl acetate, acetone, methanol and water). The final filtrate of each extract concentrated using a rotary vacuum evaporator (IKA, RV 10 digital, Germany) was collected, evaporated to dryness and stored at 4ºC for further studies. The percent extractive of hot and cold extracts of all the seven solvents was calculated using the formula.

$$\text{Percent extractive} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant material}} \times 100$$

Pathogenic Bacterial Strains

Ten bacterial strains were used in the present study. Out of the ten bacterial cultures investigated, four gram positive bacteria were *Bacillus subtilis* (MTCC 619), *Bacillus cereus* (MTCC 430), *Staphylococcus simulans* (MTCC 3610) and *Staphylococcus epidermidis* (MTCC 3615) while the six gram negative ones were *Escherichia coli* (MTCC 729), *Pseudomonas aeruginosa* (MTCC 4676), *Salmonella typhi* (MTCC 3216), *Vibrio cholerae* (MTCC 3904), *Klebsiella pneumoniae* (MTCC 432), and *Proteus mirabilis* (MTCC 425).

Pathogenic Fungal Strains

Fungal strains, *Candida albicans* (MTCC 183), *Trichophyton rubrum* (MTCC 296), *Cryptococcus gastricus* (MTCC 1715), *Aspergillus tubingensis* (MTCC 2425), *Aspergillus flavus* (MTCC 873) and *Aspergillus fumigatus* (MTCC 343) were used in the study. All these strains were procured from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India. The cultures were stored at 4ºC and sub cultured frequently in suitable agar slants.

Growth Medium

Nutrient agar, nutrient broth, Mueller Hinton agar, tryptone soya broth and Luria Bertani broth were used for culturing bacteria. Potato dextrose agar (PDA), yeast extract peptone dextrose (YEPD) agar and Czapek dox broth were used to maintain fungal cultures.

Assay of Antibacterial Activity

Antibacterial assay was carried out by disc diffusion method and it was evaluated by measuring the diameter of the inhibition zone.[18] Different solvent extracts of the plant were tested against selected bacterial strains. The test bacterial cultures were evenly spread over Mueller Hinton agar plates using a sterile cotton swab. The sterile discs (6 mm in diameter) were impregnated with extract solution and placed in the inoculated agar. All plates were then incubated at 37°C for 24 hours and the zones of inhibition were subsequently measured in mm. Ciprofloxacin (5 µg/ml) was used as a positive control and respective solvents served as negative controls. The experiment was done in triplicates and the mean values were taken. The extracts which had shown high antibacterial activity were further subjected to disc diffusion assay at varying concentrations (5, 10, 25, 50, 100, 200, 300, 400, 500 mg/ml) to check for the maximum activity at a definite concentration.

Antifungal Assay

Antifungal activity was measured using disc diffusion method.[19] The test fungal cultures were evenly spread over respective agar plates using a sterile cotton swab. Subsequently, sterile paper discs (6 mm diameter) impregnated with suitable extract was placed on the agar. The activity was measured after 72 h of incubation at 27°C. Amphotericin B (10µg/ml) was used as a positive control and respective solvents as negative controls. All tests were done in triplicate and the mean values were presented.

RESULTS AND DISCUSSION

The percentage extractives of *T. populnea* leaves extracted in the seven different solvents are given in Table 1. The highest percentage extractive value was obtained in hot hexane extract with a value of 5.3% and the lowest was found in hot dichloromethane extract with a value of 0.43%.

Table 1: Percent extractive of *T. populnea* leaf extracts

Plants	Extraction Process	Hexane (%)	Chloroform (%)	Dichloromethane (%)	Ethyl acetate (%)	Acetone (%)	Ethanol (%)	Distilled Water (%)
Thespesia populnea (leaf)	Hot	5.3	2.96	0.43	0.66	0.5	2.2	1.85
	Cold	2.0	3.36	1.58	0.64	0.82	3.38	2.02

The extracts showed varying degrees of growth inhibition patterns against clinically important pathogens. The effects of different hot and cold solvent extracts of *T. populnea* leaf against different pathogens are given in Tables 2 and 3. Hot extract of hexane and chloroform showed the highest zone of inhibition of 12 mm each against *P. aeruginosa* and *B. subtilis* and the control drug ciprofloxacin showed an inhibition zone of 30 mm and 26 mm respectively. In case of hot extracts, except water, all the extracts showed activity against *P. aeruginosa.* Hexane, chloroform and dichloromethane extracts alone showed activity against *B. subtilis*, and except methanolic extract no other extracts showed activity against *E. coli.*

Udayakumar and Hazeena Begum[20] reported that *T. populnea* leaf extracts were found to be effective against *E. coli* with an inhibition zone of 10 mm and *S. typhi* with 11 mm zone. In the present study, we have observed similar results for *E. coli* and there was no activity against *S. typhi*. According to Bauer's method[18] the microbicidal activity is classified into resistant if the zone of inhibition in millimeter is less than 7, intermediate if it is 7-9 mm, and if the inhibition zone is 10 mm or more, it is sensitive.

In the case of cold extracts, the methanol extract alone showed highest activity against *S. epidermidis* (15 mm) and *B. cereus* (14 mm) whereas the control drug ciprofloxacin showed an inhibition zone of 29 mm and 27 mm respectively. The chloroform, dichloromethane and ethyl acetate extracts showed intermediate activity against *S. epidermidis.* Except, methanol and chloroform extracts, all other extracts did not show any activity against *E. coli.* The hexane, dichloromethane, ethyl acetate and acetone extract showed intermediate activity against *P. aeruginosa* (Table 3). The seven cold and hot extracts tested did not show any activity against *S. typhi, V. cholerae* and *S. simulans.* In the present study, it was found that the cold leaf extract showed good antibacterial activity.

Table 2: Antibacterial activity of different hot leaf extract of *T. populnea*

Extraction solvents	Diameter of zone of inhibition (mm)									
	E. coli	*K. pneumoniae*	*P. mirabilis*	*S. epidermidis*	*B. cereus*	*P. aeruginosa*	*B. subtilis*	*S. typhi*	*V. cholerae*	*S. simulans*
Hexane	-	-	8±1	-	-	12±1	10±2	-	-	-
Chloroform	-	-	-	-	-	10±1	12±0.81	-	-	-
Dichloromethane	-	-	-	-	-	10±0	10±1	-	-	-
Ethyl Acetate	-	8±0	-	-	-	10±0	-	-	-	-
Acetone	-	-	-	-	-	10±1	-	-	-	-
Methanol	11±0	7±0	-	12±1	-	10±2	-	-	-	-
Water	-	-	-	-	-	-	-	-	-	-
Ciprofloxacin (5 µg/ml)	32±1.15	28±1.52	31±0.57	29±1	27±1	30±0	26±0	30±1.52	31±0	25±0.57

"-" No activity

Table 3: Antibacterial activity of different cold leaf extract of *T. populnea*

Extraction solvents	Diameter of zone of inhibition (mm)									
	E. coli	*K. pneumoniae*	*P. mirabilis*	*S. epidermidis*	*B. cereus*	*P. aeruginosa*	*B. subtilis*	*S. typhi*	*V. cholerae*	*S. simulans*
Hexane	-	-	-	7±0	-	9±0	-	-	-	-
Chloroform	8±1	-	9	10	-	-	-	-	-	-
Dichloromethane	-	-	-	10±0.81	-	10±0	9±2	-	-	-
Ethyl Acetate	-	7±0.8	-	10±0	-	11±1	-	-	-	-
Acetone	-	-	-	-	-	10±1	-	-	-	-
Methanol	11±1	-	-	15±1	14±1	-	-	-	-	-
Water	-	-	-	-	-	-	-	-	-	-
Ciprofloxacin (5 µg/ml)	32±1.15	28±1.52	31±0.57	29±1	27±1	30±0	26±0	30±1.52	31±0	25±0.57

"-" No activity

Different concentrations of cold methanolic extracts of *T. populnea* leaves were tested against *S. epidermidis* and *B. cereus.* The concentration 300 and 400 mg/ml were found to be sensitive against *S. epidermidis* and intermediate for *B. cereus* (Table 4). Moon *et al*[21] reported the antibacterial activity of methanolic extract of *T. populnea* leaves and its corresponding callus were studied against *E. coli*, *Staphylococcus aureus*, *K. pneumoniae* and *S. typhi* and found that the leaf extract and its corresponding callus showed great potential as source of antibacterial agents. Shekshavali and Hugar[22] had reported antimicrobial activity of bark of ethanolic extract of *T. populnea* by well diffusion method against four bacterial strains (*Staphylococcus aureus* MTCC 87, *E. coli* MTCC 46, *Streptococcus pyogenes, P. aeruginosa*) and two fungal strains, *Candida albicans* and *A. flavus.* Pet ether extract showed significant activity against all organisms, where as ethanolic and aqueous extracts showed moderate to mild activity.

The antifungal assay of seven cold and hot leaf extracts showed that the plant was only active against *C. albicans* (Table 5 and 6). The highest activity was shown by hot acetone extract (12 mm) whereas the control drug amphotericin B (10 µg/ml) showed an inhibition zone of 24 mm. The seven extract tested had no effect on the growth of all the other fungal pathogens tested. Kumar *et al*[23] also reported antifungal activity against *Candida albicans* (MTCC 10231) by the leaf extract of *T. populnea*. Shekshavali and Hugar[22] also reported the presence of antifungal property of *T. populnea* bark extracts against *Candida albicans* and *A. flavus.* In the present study none of the leaf extracts showed antifungal against *A. flavus* and other pathogenic fungi tested.

Table 4: Antibacterial analysis of different concentration of *T. populnea* leaf extract

Concentration (mg/ml)	Zone of inhibition (mm) against cold methanolic extract	
	S. epidermidis	*B. cereus*
5	-	-
10	-	-
25	-	-
50	-	-
100	-	-
200	-	-
300	10±0	7±2
400	11±0	9±1

"-" No activity

Table 5: Antifungal activity of different hot extracts of *T. populnea*

Extraction solvents	Diameter of zone of inhibition (mm)					
	C. albicans	*T. rubrum*	*C. gastricus*	*A. tubingensis*	*A. flavus*	*A. fumigatus*
Hexane	7±0.81	-	-	-	-	-
Chloroform	8±2	-	-	-	-	-
Dichloromethane	7±1	-	-	-	-	-
Ethyl Acetate	10±0	-	-	-	-	-
Acetone	12±1	-	-	-	-	-
Methanol	6±1	-	-	-	-	-
Water	8±0.8	-	-	-	-	-
Amphotericin B (10 µg/ml)	24±1.73	27±1	23±1.15	28±0.1	26±0	31±0.8

"-" No activity

Table 6: Antifungal activity of different cold extracts of *T. populnea*

Extraction solvents	Diameter of zone of inhibition (mm)					
	C. albicans	*T. rubrum*	*C. gastricus*	*A. tubingensis*	*A. flavus*	*A. fumigatus*
Hexane	8±1	-	-	-	-	-
Chloroform	8±1	-	-	-	-	-
Dichloromethane	8±2	-	-	-	-	-
Ethyl Acetate	7±0	-	-	-	-	-
Acetone	6±0	-	-	-	-	-
Methanol	8±1	-	-	-	-	-
Water	7±1	-	-	-	-	-
Amphotericin B (10 µg/ml)	24±1.73	27±1	23±1.15	28±0.1	26±0	31±0.8

"-" No activity

CONCLUSION

The *T. populnea* extracts, extracted using different solvents from the leaf samples collected from Kuttanad wetlands were sensitive to *E. coli, S. epidermidis, B. cereus, P. aeruginosa* and *B. subtilis* whereas the other extracts showed intermediate activity against other bacterial pathogens. Intermediate antifungal activity was observed against *C. albicans* for all the solvent extracts except for hot ethyl acetate and acetone extracts which were found sensitive to *C. albicans.*

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