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Antibacterial potential of *Thespesia populnea* (Linn.) Sol. ex Corr. leaves and its corresponding callus against drug resistant isolates

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Effect of methanolic extracts of leaves of *Thespesia populnea* (Linn.) Sol. ex Corr. and its corresponding callus were studied against drug resistant strains of *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae* and *Salmonella typhii*. The antibiotic sensitivity pattern for all the clinical isolates was studied by Bauer-Kirby method and all the clinical isolates were found to be resistant to more than one antibiotic. Both, the leaf of the plant and its corresponding callus showed great potential as source of antibacterial agents.

Keywords: Antibacterial, Callus, Drug resistance, Methanolic extracts, *Thespesia populnea*.

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Introduction

In the indigenous health care delivery system, numerous plant species and natural products derived from plants are used to treat diseases of infectious origin. Considerable research on pharmacognosy, chemistry, pharmacology and clinical therapeutics has been carried out on Ayurvedic medicinal plants and many drugs have been entered in the international pharmacopoeia through the study of ethno-pharmacology[1].

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[2].

The medicinal plant, *Thespesia populnea* (Linn.) Sol. ex Corr. (Family — Malvaceae) widely used by the traditional medicinal practitioners for the treatment of infectious diseases, is put to systematic scientific investigation in the present study. It is commonly known as *Gardhabhanda* and *Parisa* in Sanskrit. It is a fast growing small tree and generally reaches a height of 9 m with a spread of 3.6 m. The shiny green leaves are generally oval or triangular in shape and range in size from 5.1 to 30.5 cm. The Hibiscus-like flowers (5.1-7.6 cm) are yellow with a maroon center (Plate 1). The leaf extracts are given in cases of diarrhoea, dysentery, leucoderma and other skin diseases, cough, asthma

Plate 1 — *Thespesia populnea*

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and other respiratory diseases[3,4]. Considering the high costs of the synthetic drugs and their various side effects, the search for alternative products from plants used in traditional system of medicine is justified.

Materials and Methods

Plant collection and processing

Leaves of T. *populnea* were collected from the urban fringe area of Nagpur city. A voucher specimen (ND 2318) is deposited in the Department of Botany; RTM Nagpur University, Nagpur.

The leaves were washed under running tap water and air dried under shade. After 15 days the dried leaves were macerated in a mixer grinder to yield a fine powder which was sieved to yield particle size of 50-150 mm. This dried powder (50 g) was extracted in a Soxhlet apparatus using 100 mL of petroleum ether (60-80°C), chloroform (61°C), methanol (78.5°C) and water (80°C)[(Ref. 5)]. The extracts obtained were dried and stored in sealed tubes at 4°C. The methanolic extracts were found to be more potent than the other solvent counterparts and hence used in this study[6].

Clinical isolates and control

Clinical isolates were obtained from Department of Microbiology, Jawaharlal Nehru Medical College and Hospital, Sawangi, District-Wardha (M.S.) and Department of Pathology, MGIMS, Sewagram, District-Wardha. Thirteen strains of *Escherichia coli,* ten strains of *Staphylococcus aureus,* nine strains of *Klebsiella pneumoniae* and six strains of *Salmonella typhii* were tested against standard antibiotics to get the antibiotic sensitivity pattern. Standard antibiotic discs were purchased from HiMedia, Mumbai. The bacterial cultures were maintained on Nutrient Agar (Himedia, Mumbai) at 4°C and subcultured every two weeks. *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *K. Pneumoniae* ATCC 10921 and *S. typhii* ATCC 19430 were procured from National Centre for Cell Sciences, Pune.

Inoculum preparation

Stock cultures of clinical isolates were maintained at 4°C on nutrient agar slants. A working bacterial inoculum was prepared by inoculating a loop full of the clinical isolate into a 3 ml sterile nutrient broth tube and incubated at 37°C for 24 hours. The turbidity was matched with 0.5 Mc Farland's Nephelometer Standard[7,8]. Dilutions to the tube were done with sterile nutrient broth to get a cell density corresponding to 2×10^6 CFU/ml.

Media

Nutrient Agar (M001), Agar Agar Type I (RM666), Mueller Hinton Agar No. 2 (M1084) and Nutrient broth (M002) were procured from Hi-Media, Mumbai. The preparation of media was done strictly according to the manufacturer's instructions.

Antibiotic discs

Commercially available standard antibiotic discs were obtained from Hi-Media, Mumbai. The abbreviations and strength of the antibiotics are given in brackets; the antibiotic discs used were: Amoxycillin SD 076 (Am-30 µg), Ampicillin SD 002 (A-10 µg), Chloramphenicol SD 006 (C-30 µg), Erythromycin SD 013 (E-15 µg), Penicillin-G SD 028 (P-10 µg), Kanamycin SD 017 (K-30 µg), Tetracyclin SD 037 (T-30 µg), Cephalexin SD 048 (Cp-30 µg), Ciprofloxacin SD 060 (Cf-5 µg), Co-trimoxazole SD 010 (Co-25 µg), Gatifloxacin SD 737 (Gf- 5µg), Norfloxacin SD 057 (Nx-10 µg), Ofloxacin SD 087 (Of- 5 µg), Pe-floxacin SD 070 (Pf-5 µg), Sparfloxacin SD 162(Sc-5 µg) and Streptomycin SD 031 (S-10 µg).

Antibiotic sensitivity test

The antibiotic sensitivity of the clinical isolates was studied by Bauer-Kirby disc diffusion method[9]. A sterile non-toxic cotton swab was dipped into the inoculum tube and rotated firmly against the upper inside wall of the tube to express excess fluid. This swab was now used to streak the entire agar surface of the plate three times turning the plate 60° between each streaking. Five antibiotic discs were placed aseptically on each plate with enough spacing. All the plates were incubated at 37°C for 18-24 hours. After incubation, plates were examined for zone of inhibition. Zones were measured and recorded as sensitive, resistant or intermediate referring the zone size interpretive chart[10].

Antibacterial susceptibility test

A suspension (0.1 ml) of the test organisms from the 18 h cultures was thoroughly mixed with 20 ml of sterile Mueller Hinton Agar maintained at 45-50°C. The seeded M.H. Agar is poured in presterilized petri plates and set aside. After solidification, the seeded agar was punched with a flamed (sterile) 10 mm cork borer in order to obtain a well of 10 mm diam in the center of the petri plate. Methanolic plant extract (100 µl) was loaded into the well accurately with a micropipette (with presterilized tips) to obtain concentration of 20, 40, 60, 80 and 100%. The petri plates were delicately handled and kept in refrigerator

for 30 min and then at room temperature for 30 min which facilitated diffusion of the plant extract. The petri plates were then incubated at 37°C for 24 h[11]. The zone of inhibition was measured in mm with HiAntibiotic ZoneScale (PW096), HiMedia, Mumbai.

Callus induction

The plant species being lost at an alarming rate has focused attention on alternatives to whole plants for production of natural products which are of either direct or indirect potential benefit to mankind. Plant tissue and cell culture techniques are valuable for producing commercially important plant products. The secondary metabolites accumulate in specialized cells in the plant. Most of these have a complex structure involving many chiral centers which determine their biological activity. In recent years, interest in screening higher plants for novel biologically active products has been renewed so as to combat ailments which have defied the synthetics and antibiotics.

With the progress in tissue culture research and ever increasing demand of natural products, attention is towards the production of secondary metabolites through culturing of tissues. Plant cells generally produce small amounts of useful secondary compounds which when cultured may produce the secondary metabolites at a higher concentration by changing the physical factors like, culture media, temperature, *p*H, photoperiod, osmotic pressure, etc. In cultivated plants, the secondary metabolites often accumulate in the roots, stems, barks and leaves of the tree. Extraction of these compounds involves destruction of the entire tree. The need to conserve and cultivate the desired plant species for the production of high value compounds is, therefore, imperative. Plant tissue and cell culture techniques are advantageous because they produce desirable compounds. In this study, the callus of T. *populnea* was established to study the antibacterial activity.

The leaves were taken from a 1-2 year old *T. populnea* plant and wrapped in perforated aluminum foil and then incubated in dark at 26 ± 1°C for 24 h on a Murashige and Skoog (MS) medium supplemented with 2.0ppm 2-*iso*-pentenyladenine (2-iP) and 0.5ppm NAA. Callus was cultured at 3000 lux light intensity provided for 16 h per day followed by a dark period of 8 h (Plate 2). Two month old callus derived form the leaf explant was weighed and dried by wrapping them in perforated aluminum foils and keeping at 40°C for 72 h in an oven. The dried weight was recorded. This dried callus was ground into fine powder and methanol soxhletion yielded the extract which was cooled to room temperature. This was filtered through Whatmann No. 1 filter paper and the filtrate was utilized in this study at various concentrations of 20, 40, 60, 80 and 100 mg/ml.

Results and Discussion

The antibiotic sensitivity to the control microorganisms is given in Table 1. *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *K. pneumoniae* ATCC 10921 and *S. typhii* ATCC 19430 were used as controls.

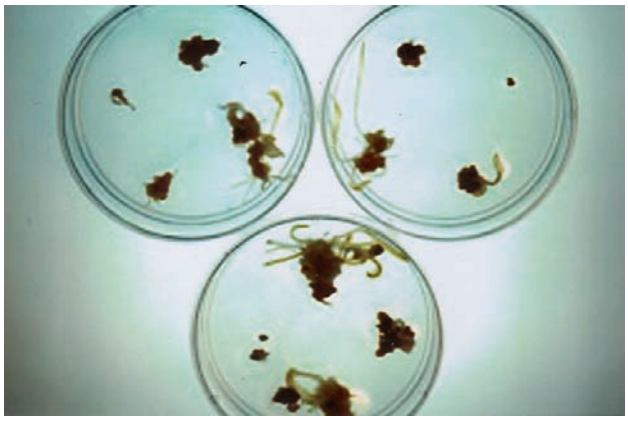

Plate 2 — Callus culture from leaves of *Thespesia populnea*

Table 1 — Antibiotic sensitivity of control microorganisms (Zone of inhibition in mm diameter)

S. No.	Control micro-organisms	Antibiotics														
		A	Am	C	E	P	K	T	Cp	Cf	Co	Gf	Nx	Of	Pf	Sc
1	*Escherichia coli* ATCC 25922	18	22	24	19	17	19	22	15	33	24	29	32	32	30	32
2	*Staphylococcus aureus* ATCC 25923	32	33	26	27	22	26	27	29	28	29	19	19	26	26	32
3	*Klebsiella peumoniae* ATCC10921	20	22	25	22	25	22	22	16	32	20	26	28	26	22	30
4	*Salmonella typhii* ATCC 19430	18	20	21	20	18	17	20	14	22	18	20	20	30	30	30

A=Ampicillin, T=Tetracycline, Nx=Norfloxacin, Am=Amoxycillin, Cp=Cephalexin, Of=Oflaxacin, C=Chloramphenicol, Cf=Ciprofloxacin, Pf=Pefloxacin, E=Erythromycin, Co= Co-trimoxazole, Sc=Sparfloxacin, P=Penicillin-G, Gf=Gatifloxacin, S=Streptomycin, K=Kanamycin

The wet and dry weight of the two month old leaf callus of *T. populnea* was 0.26 g and 0.15 g, respectively. The effect of MeOH leaf and callus extract at 20, 40, 60, 80 and 100mg/ml concentration is given in Table 2. The callus extract showed a higher antibacterial activity as compared to leaf extracts at all the concentrations studied against all the four control microorganisms.

E. coli causes urinary tract infections, diarrhoea, pyogenic infections and septicemia[12-15]. Clinical isolates of *E. coli* showed resistance to commonly used antibiotics like Amoxycillin, Penicillin, Cephalexin, Streptomycin, etc.; the resistance pattern is shown in Table 3. These isolates when treated with 100 mg/ml MeOH leaf extract of *T. populnea* and its corresponding callus show a zone of inhibition of 16 mm and 14 mm diam, respectively which clearly indicates that the methanolic leaf extract has more antibacterial activity than its corresponding callus extract (Table 3).

S. aureus causes localized suppurative lesions in human beings. Its ability to develop resistance to Penicillin and other antibiotics enhances its importance as a human pathogen, especially in the hospital environment[16]. In this study, the clinical isolates of *S. aureus* show resistance against most commonly used antibiotics such as Amoxycillin, Co-trimoxazole, Cephalexin, Ampicillin, Kanamycin, Penicillin, etc. Interestingly, the MeOH leaf extracts show activity against multi-drug resistant S. *aureus* strains and show a zone of inhibition even at low concentration of 20 mg/ml, worth mentioning is the activity shown by both leaf and callus extracts at all the concentrations (Table 4).

K. pneumoniae causes pneumonia, urinary infection, and other pyogenic infections[17]. The clinical isolates of *K. pneumoniae* show resistance to more than one antibiotic as clearly seen from the resistance pattern in Table 5. *T. populnea* leaf MeOH extract is effective at >60 mg/ml while the corresponding callus extract is effective at >80 mg/ml concentration for most of the clinical isolates tested.

The genus *Salmonella* consists of bacilli that infect human beings, leading to enteric fever, gastroenteritis, and septicemia. Enteric fever is endemic in all parts of India. Mortality in neonates is very high unless early treatment is started with antibiotics to which the infecting strain is sensitive[18,19]. The clinical isolates obtained in this study show a common resistance pattern for Amoxycillin, Ampicillin, Pencillin and Cephalexin. The *T. populnea* MeOH callus extract showed great potential as an antibacterial agent as compared to the MeOH leaf extracts (Table 6).

Table 2 — Effect of methanolic extracts of *Thespesia populnea* leaf and its corresponding calli on control microorganisms (Zone of inhibition in mm diameter)

S. No.	Control Micro-organisms	100 µl of MeOH leaf extract (conc. mg/ml)					100µl of MeOH callus extract (conc. mg/ml)					
		20	40	60	80	100	20	40	60	80	100	20
1	*Escherichia coli* ATCC 25922	14	16	18	20	22	16	18	20	24	26	16
2	*Staphylococcus aureus* ATCC 25923	14	16	20	22	24	14	16	20	22	24	14
3	*Klebsiella peumoniae* ATCC10921	10	14	16	18	20	2	14	16	18	24	2
4	*Salmonella typhii* ATCC 19430	14	16	18	20	22	10	12	16	20	22	10

Table 3 — Effect of *T. populnea* extracts on multi drug resistant strains of *Escherichia coli* (Zone of inhibition in mm diameter)

S. No.	Clinical isolates	100 µl of MeOH leaf extract (conc. mg/ml)					100µl of MeOH callus extract (conc. mg/ml)					Antibiotics resistance pattern
		20	40	60	80	100	20	40	60	80	100	
1	*Escherichia coli* 1 (EC1)	11	13	13	15	16	-	-	-	12	14	E,P,K,Co,S,Cp,Gf
2	*Escherichia coli* 2 (EC2)	11	12	12	15	16	-	-	11	11	14	Am,E,P,Co,S
3	*Escherichia coli* 3 (EC3)	12	12	13	14	15	-	-	-	12	13	Am,A,E,P,T,Gf,Nx,S
4	*Escherichia coli* 4 (EC4)	-	-	13	14	17	-	-	-	12	13	E,P,Cp,Nx,S
5	*Escherichia coli* 5 (EC5)	11	12	12	14	16	-	-	11	12	14	Am,C,P,K,S,G
6	*Escherichia coli* 6 (EC6)	12	13	13	14	17	-	-	11	11	14	Am,E,P,K,T,S
7	*Escherichia coli* 7 (EC7)	12	13	12	15	16	-	-	11	11	14	Am,E,P,K,Co
8	*Escherichia coli* 8 (EC8)	13	13	13	14	15	-	-	11	11	14	Am,E,P,K,Cp
9	*Escherichia coli* 9 (EC9)	-	11	13	15	15	-	-	-	-	14	Am,A,P,Co,Nx
10	*Escherichia coli* 10 (EC10)	-	12	13	15	16	-	-	11	12	14	E,P,K,Nx,S,Gf
11	*Escherichia coli* 11 (EC11)	11	12	12	15	16	-	-	-	12	13	Am,A,E,P,Of
12	*Escherichia coli* 12 (EC12)	-	-	12	15	16	-	-	-	11	14	E,P,Cp,Co,Of
13	*Escherichia coli* 13 (EC13)	-	-	13	14	16	-	-	11	12	14	Am,A,E,P,K,K,Cp,Of

A=Ampicillin, T=Tetracycline, Nx=Norfloxacin, Am=Amoxycillin, Cp=Cephalexin, Of=Oflaxacin, C=Chloramphenicol, Cf=Ciprofloxacin, Pf=Pefloxacin, E=Erythromycin, Co= Co-trimoxazole, Sc=Sparfloxacin, P=Penicillin-G, Gf=Gatifloxacin, S=Streptomycin, K=Kanamycin

Table 4 — Effect of *T. populnea* extracts on multi drug resistant strains of *Staphylococcus aureus* (Zone of inhibition in mm diameter)

S. No.	Clinical isolates	100µl of MeOH leaf extract (conc. mg/ml)					100µl of MeOH callus extract (conc. mg/ml)					Antibiotics resistance pattern
		20	40	60	80	100	20	40	60	80	100	
1	*Staphylococcus aureus* 1 (SA1)	18	19	21	23	25	13	18	20	21	22	Am,A,P,K,T,Cp,Co
2	*Staphylococcus aureus* 2 (SA2)	18	18	20	22	25	14	18	19	22	27	Am,A,C,P,K,Cp,Co,Nx,Sc
3	*Staphylococcus aureus* 3 (SA3)	16	19	20	23	24	14	14	21	21	29	Am,A,P,K,Cp,Co
4	*Staphylococcus aureus* 4 (SA4)	17	19	21	22	24	13	16	20	20	28	Am,C,P,K,Cp,Cf,Co,Nx
5	*Staphylococcus aureus* 5 (SA5)	16	18	20	23	25	13	18	20	22	28	Am,A,E,P,K,Cp,Co
6	*Staphylococcus aureus* 6 (SA6)	18	19	21	22	25	14	14	19	20	25	Am,A,P,K,Cp,Co
7	*Staphylococcus aureus* 7 (SA7)	17	18	21	23	24	13	15	18	21	26	Am,A,P,K,Cp,Co,Sc
8	*Staphylococcus aureus* 8 (SA8)	18	19	20	23	25	13	13	20	20	24	Am,A,P,K,T,Cp,Co
9	*Staphylococcus aureus* 9 (SA9)	17	18	20	22	25	14	14	16	19	27	Am,A,P,K,Cp,Cf,Co,Sc
10	*Staphylococcus aureus* 10 (SA10)	18	19	20	23	24	14	16	17	20	26	A,C,P,K,T,Cp,Co

A=Ampicillin, T=Tetracycline, Nx=Norfloxacin, Am=Amoxycillin, Cp=Cephalexin, Of=Oflaxacin, C=Chloramphenicol, Cf=Ciprofloxacin, Pf=Pefloxacin, E=Erythromycin, Co= Co-trimoxazole, Sc=Sparfloxacin, P=Penicillin-G, Gf=Gatifloxacin, S=Streptomycin, K=Kanamycin

Table 5 — Effect of *T. populnea* extracts on multi drug resistant strains of *Klebsiella pneumoniae* (Zone of inhibition in mm diameter)

S. No.	Clinical isolates	100µl of MeOH leaf extract (conc. mg/ml)					100µl of MeOH callus extract (conc. mg/ml)					Antibiotics resistance pattern
		20	40	60	80	100	20	40	60	80	100	
1	*Klebsiella pneumoniae*1 (KA1)	-	-	15	16	19	-	-	-	16	18	Am,C,Cp,S
2	*Klebsiella pneumoniae*2 (KA2)	11	12	14	17	18	11	11	14	16	17	E,T,Co,S
3	*Klebsiella pneumoniae*3 (KA3)	13	14	15	15	16	13	14	15	16	18	Am,E,T,Cp,S
4	*Klebsiella pneumoniae*4 (KA4)	-	13	15	17	18	-	12	14	15	19	A,T,Cp,Co,S
5	*Klebsiella pneumoniae*5 (KA5)	12	13	16	16	19	12	13	14	17	18	Am,Cp,Co,S
6	*Klebsiella pneumoniae*6 (KA6)	-	-	16	17	19	11	13	15	16	17	A,E,T,Cp,S
7	*Klebsiella pneumoniae*7 (KA7)	14	15	14	16	17	12	14	16	16	18	Am,T,Cp,Co,S
8	*Klebsiella pneumoniae*8 (KA8)	11	13	15	16	18	-	11	12	14	15	A,T,Cp,Co,S
9	*Klebsiella pneumoniae*9 (KA9)	13	14	15	16	18	13	14	14	15	15	A,E,Cp,Co,S

A=Ampicillin, T=Tetracycline, Nx=Norfloxacin, Am=Amoxycillin, Cp=Cephalexin, Of=Oflaxacin, C=Chloramphenicol, Cf=Ciprofloxacin, Pf=Pefloxacin, E=Erythromycin, Co= Co-trimoxazole, Sc=Sparfloxacin, P=Penicillin-G, Gf=Gatifloxacin, S=Streptomycin, K=Kanamycin

Table 6 — Effect of *T. populnea* extracts on multi drug resistant strains of *Salmonella typhii* (Zone of inhibition in mm diameter)

S. No.	Clinical isolates	100µl of MeOH leaf extract (conc. mg/ml)					100µl of MeOH callus extract (conc. mg/ml)					Antibiotics resistance pattern
		20	40	60	80	100	20	40	60	80	100	
1	*Salmonella typhii 1* (ST1)	-	-	18	20	23	-	17	20	24	27	Am,A,C,E,P,Cp,S,Of
2	*Salmonella typhii 2* (ST2)	11	13	17	21	24	13	15	18	23	27	Am,A,P,S,T,Cp,T,Of
3	*Salmonella typhii 3* (ST3)	14	15	18	22	24	12	16	19	25	27	Am,A,P,S,T,Cp,Of
4	*Salmonella typhii 4* (ST4)	13	16	19	20	23	-	-	13	22	25	Am,A,P,S,Cp,Of
5	*Salmonella typhii 5* (ST5)	12	14	15	18	21	11	16	19	23	26	Am,A,P,S,E,Cp,Of
6	*Salmonella typhii 6* (ST6)	11	13	14	16	19	13	18	21	25	28	Am,A,P,S,K,Cp,T

A=Ampicillin, T=Tetracycline, Nx=Norfloxacin, Am=Amoxycillin, Cp=Cephalexin, Of=Oflaxacin, C=Chloramphenicol, Cf=Ciprofloxacin, Pf=Pefloxacin, E=Erythromycin, Co= Co-trimoxazole, Sc=Sparfloxacin, P=Penicillin-G, Gf=Gatifloxacin, S=Streptomycin, K=Kanamycin

Conclusion

From the results of antibacterial screening of MeOH leaf extracts and its corresponding callus extract used in this study, it is clear that *T. populnea* exhibits significant antibacterial activity. Further research in this study focuses on the isolation of bioactive phytochemicals and also inducing the callus to produce higher concentrations of bioactive phytochemicals which are responsible for the antibacterial activity to combat the multi drug resistance shown by the human pathogenic bacteria.

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