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Original Article

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF LEAVES OF *THESPESIA POPULNEA*

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ABSTRACT

Objective: To find out the antibacterial and antifungal activity of *T. populnea* leaves against bacterial and fungal strains.

Methods: *T. populnea* leaves were extracted successively with different solvents *viz.,* hexane, chloroform, ethyl acetate and methanol. Solvent extracts were screened for antimicrobial activity against bacterial strains such as, gram positive and gram negative bacterial and fungal strains such as., *Aspergillus* spp. and dermatophytic strains by disc diffusion method, determination of Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC).

Results: In bacterial screening, chloroform extract of leaves of *T. populnea* showed the highest antibacterial activity against *Staphylococcus aureus* and the mean zone of inhibition was 14.8 mm. The lowest MIC and MBC values were 62.5 and 125 µg/ml respectively. In fungal screening, methanol extract showed the highest antifungal activity against *Aspergillus fumigatus* and the mean zone of inhibition was 22.8 mm. The lowest MIC (62.5 µg/ml) and MFC (125 µg/ml) values were observed against *A. fumigatus* and *Microsporum gypseum*. Phytochemical analyses of different extracts of leaves of *T. populnea* were analysed. The methanol extract of *T. populnea* leaves showed the presence of strong phytochemicals *viz.,* flavonoids, tannins, steroids, glycosides, saponins, phenols, terpenoids and alkaloids than other extracts.

Conclusion: *T. populnea* has antimicrobial activity and the chloroform and methanol leaf extracts are useful sources of antimicrobial agents and for further pharmacological studies towards bacterial and fungal infections.

Keywords: Antimicrobial activity, *Thespesia populnea*, Bacterial and fungal strains, MIC, MBC, MFC.

INTRODUCTION

Infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health, despite the tremendous progress in human medicine. Their impact is particularly large in developing countries due to the relative unavailability of medicines and the emergence of widespread drug resistance [1]. One of the more alarming recent trends in Infectious diseases has been the increasing frequency of antimicrobial resistance among microbial pathogens causing nosocomial and community-acquired infections. Numerous classes of antimicrobial agents have now become less effective as a result of the effective pressure of antimicrobial usage [2].

Staphylococcus aureus is a facultative anaerobic gram-positive coccal bacterium and its one of the most common causes of nosocomial post surgical wound infections [3, 4]. *S. aureus* and *Pseudomonas aeruginosa* are responsible for a significant number of biofilm-related infections. *Staphylococci* are currently the most common cause of nosocomial infections. Opportunistic *S. aureus* is involved in native valve endocarditis, otitismedia and all kinds of infections of implanted devices [5, 6 and 7]. In recent years, nosocomial infections caused by *P. aeruginosa* have been recognized as an acute problem in hospitals due to its intrinsic resistance to many antibiotic classes and its capacity to acquire practical resistance to all effective antibiotics [8]. *P. aeruginosa* is a gram negative bacteria, occasionally associated with opportunistic diseases of humans [9]. The major causative agents of diarrhea in humans include *Shigella flexneri*, *S. aureus, Escherichia coli* and *Salmonella typhi* [10]. *E. coli* is a gram-negative human enteric species that is generally a virulent but sometimes causes weakly virulent gastroenteritis and urinary tract infections. *Salmonella typhimurium* is a gram-negative pathogen that most commonly causes enterocolitis and *S. aureus* is a gram-positive pathogen that most commonly causes abscess, food poisoning and toxic shock syndrome; both are considered to be more virulent than *S. lactis* and *E. coli* [11]. Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action [12, 13]. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [14]. Skin diseases occur worldwide and amount to approximately 34 % of all occupational diseases encountered [15]. They affect people of all ages from neonates to the elderly and constitute one of the five reasons for medical consultation. Skin diseases have been of major concern recently due to their association with the Human Immunodeficiency Virus and Acquired Immunity Deficiency Syndrome (HIV/AIDS) [16].

Fungal related diseases may not be as common as other microbial infections but, when present, they are difficult to treat especially in immunosuppressed persons [17]. *Candida albicans* is the most common species associated with candidiasis and is the most frequently recovered species from hospitalized patients. Candidiasis encompasses infections that range from superficial, such as oral thrush [18], and vaginitis, to systemic and potentially life-threatening diseases. The increase of *C. albicans* infections parallels medical advancements such as invasive procedures, immunosuppressive treatments for organ transplants and widespread use of broad-spectrum antibiotics [19]. *Candida* and *Aspergillus* species have been found to be the most common etiological agents in nosocomial bloodstream fungal infections (BSI).The most common species accounting for more than 90 % of all *Candida*-associated BSIs are *C. albicans, C. glabrata, C. parapsilosis, C. tropicalis,* and *C. Krusei*, while *Aspergillus fumigatus, A.flavus, A. niger*, and *A. terreus* are the most common isolated species in *Aspergillus*-associated BSIs [20].

Aspergillus spp. is ubiquitous airborne saprophytic fungi with low pathogenicity for humans and rarely invades immunologically competent hosts [21]. *A. flavus* is recognized as the second most common cause of invasive aspergillosis both in neutropenic and non-neutropenic patients with incidences ranging from 6 % to 65 % of all cases [22, 23, 24 and 25]. Invasive aspergillosis is a devastating opportunistic infection, the causative organism is difficult to avoid, diagnosis of the disease can be problematic and prognosis is poor: even with treatment, the mortality rate of Invasive Pulmonary Aspergillosis (IPA) can approach 80% [26, 27].

The incidence of dermatophytic infections has increased considerably during the past several decades [28]. Dermatophytes are responsible for serious human pathogenic disorders in various parts of the world. Although control measures are available, they have limited effectiveness. Conventional antifungal agents such as chlorohexidine and imidazole derivatives have limited uses. Due to their common side effects such as hepatotoxicity, nausea, diarrhea and impotency [29]. Dermatophytoses are dermatomycoses caused by a specific group of fungi, dermatophytes, which are also known as tinea are among the most common causes of fungal infections in human skin [30]. Infections caused by *T. rubrum* are usually difficult to treat and relapses often occur after discontinuation of therapy. Currently, different types of antifungals such as azoles, terbinafine, amorolfine, nystatin, and ciclopirox olamine are available for the localized treatment of infections caused by dermatophytes. However, many antifungal drugs currently available can be toxic to humans due to the similarities between fungal and mammalian cells. There are many common side effects associated to the use of these drugs, besides antifungal drug resistance [31].

Thespesia populnea is a large tree belongs to the family Malvaceae, found in tropical regions and coastal forests of India. Various parts of this plant are found to possess useful medicinal properties. The leaves are applied locally in swollen joints for their anti-inflammatory effects and also for skin diseases, hepatitis, jaundice, ulcers, wounds, psoriasis, scabies, urinary tract infections, diabetes, cholera, cough, asthma and guneaworm infections [32]. The fruits of the plant are used in Ayurveda for the control of diabetes [33]. The barks and flowers possess astringent, hepatoprotective and antioxidant activity [34].

Four naturally occurring quinines viz. thespone, thespesone, mansonone-D and mansonone-H have been extracted from heart wood of *T. populnea*. The phytochemical study of bark of this plant reveals the presence of gossypol, tannin, acacetin, quercetin, coloring matter and leaf extract indicates the presence of lupeol, lupenone, β –sitosterol [35]. The flowers of the same plant contained kaempferol, kaemperol-7-glucoside and gossypetin. The fruit kernels of this plant were reported to contain β-sitosterol, ceryl alcohol and a yellow pigment, thespesin [36].

The aim of the present study was to evaluate the antimicrobial activity of hexane, chloroform, ethyl acetate and methanol extracts of *T. populnea* leaves against bacterial and fungal strains of medical importance.

MATERIALS AND METHODS

Collection of Plant material and Extraction

The leaves of *Thespesia populnea* (Malvaceae) were collected from Annamalainagar (Lat. 11.39° N; Long. 79.71°E), Cuddalore District, Tamilnadu, India during March, 2012. Herbarium specimen was maintained in the Department of Botany, Annamalai University, Annamalainagar. Collected leaves were washed with water, then surface sterilized with 10% sodium hypochlorite solution rinsed with sterile distilled water and shade dried under room temperature. The samples were ground in to a fine powder. Five hundred grams of fine powder was extracted with different organic solvents like non-polar to polar *viz*., hexane, chloroform, ethyl acetate and methanol for 72 hours using soxhlet apparatus. The solvents were evaporated under vacuum in a rotary evaporator (Heidolph, Germany) and the dried extracts were stored at 4 ˚C until further use.

Phytochemical Screening of Extracts

The hexane, chloroform, ethyl acetate and methanol extracts of leaves of *T. populnea* were used for qualitative phytochemical studies. Phytochemicals such as, flavonoids, tannins, steroids, glycosides, saponins, phenols, terpenoids and alkaloids were analyzed according to the standard method [37].

Microorganisms

The bacterial strains *viz., Staphylococcus aureus* (MTCC 7443&737), *Pseudomonas aeruginosa* (MTCC 741), *Escherichia coli* (MTCC 443), *Streptococcus pyogenes* (MTCC 442), *Salmonella typhimurium* (MTCC 98), *Shigella flexneri* (MTCC 1457), *Proteus mirabilis* (MTCC 425), *P. vulgaris* (MTCC 426) and *Vibrio cholerae* (MTCC 3906) and fungal strains *viz*., *Aspergillus niger* (MTCC 282), *A. flavus* (MTCC 277), *A. fumigatus* (MTCC 2550), *Trichophyton rubrum* (MTCC 296), *T. mentagrophytes* (MTCC 8476) and *Microsporum gypseum* (MTCC 2819) were obtained from Microbial Type Culture Collection and Gene bank (MTCC), Institute of Microbial Technology, Chandigarh, India.

In vitro antibacterial activity was determined by using Mueller Hinton Agar (MHA) and Mueller Hinton Broth (MHB). *In vitro* antifungal activity was determined by using Sabouraud Dextrose Agar (SDA), and Sabouraud Dextrose Broth (SDB) (for mycelial fungi) and they were obtained from Himedia Ltd., Mumbai.

Antibiotic sensitivity test

Antibiotic sensitivity of the bacterial strains was determined by standard CLSI disc diffusion method (M100-S22) [38]. Antibacterial agent from different classes of antibiotics viz., Amikacin (AK 30 µg/disc), Ampicillin (AMP 10 µg/disc), Cefixime (CFM 5 µg/disc), Ceftazidime (CAZ 30 µg/disc), Ciprofloxacin (CIP 5 µg/disc), Chloramphenicol (C 30 µg/disc), Erythromycin (E 15 µg/disc), Gentamycin (GEN 10 µg/disc), Linezolid (LZ 15 µg/disc), Methicillin (MET 5 µg/disc), Norfloxacin (NX 10 µg/disc), Nalidixic acid (NA 30 µg/disc), Ofloxacin (OF 5 µg/disc), Oxacillin (OX 1 µg/disc), Streptomycin (S 10 µg/disc), Tetracycline (TE 30 µg/disc) and Vancomycin (VA 30 µg/disc) were obtained from Himedia, Mumbai.

Antimicrobial assays

Inhibitory Zone determination by Disc diffusion assay

The antibacterial and antifungal activity of crude extracts of *T. populnea* leaves were determined by disc diffusion method according to Bauer *et al.,* [39] with modifications. Petri plates were prepared by pouring 20 ml of MHA for bacteria and SDA for filamentous fungi. Then the plates were allowed to solidify and used in susceptibility test. 100 µl of bacterial and fungal suspension containing 10^8CFU/ml of bacteria and 10^4spore/ml of fungi were swabbed on the top of the solidified respective media and allowed to dry for 10 minutes. The crude extracts was dissolved in 10 per cent Dimethyl sulfoxide (DMSO) and under aseptic conditions sterile HiMedia paper disc (6 mm) were impregnated with 20 µl of different concentrations (1000, 500 and 250 µg/disc) of extracts. The discs with extracts were placed on the surface of the medium with sterile forceps and gently pressed to ensure contact with inoculated agar surface. Ampicillin (10 µg/disc) for bacteria, Methicillin (5 µg/disc) for *S. aureus,* Ketoconazole (10 µg/disc) for *Aspergillus* and Dermatophytes were used as positive controls and 10 per cent DMSO was used as blind control in all the assays. Finally, the inoculated plates were incubated at 37 °C for 24 h for all bacterial strains, 30 °C for 72-96 h for *Aspergillus* spp. and 4-7 days with dermatophytes. The zone of inhibition was observed and measured in millimeters. The assay in this experiment was repeated three times./

Determination of the Minimum Inhibitory Concentration (MIC) for Bacteria

The MIC of the crude extracts of *T. populnea* leaves was tested in MHB by using a modified reaszurin microtitre plate assay was carried out according to methods of Sarker *et al.* [40]. 50 µl of Sterile MHB were transferred in to each well of a sterile 96-well micro titer plate. The crude extract of *T. populnea* leaves was dissolved in 10 per cent DMSO to obtain 1000 µg/ml stock solution. 50 µl of crude extract stock solution was added into the first well. After fine mixing of the crude extracts and broth, 50 µl of the solution was transferred to the second well and in this way the serial dilution procedure was continued to a twofold dilution to obtain concentrations like 500 to 15.7 µg/ml of the extract in each well. To each well 10 µl of resazurin indicator solution was added. (The resazurin solution was prepared by dissolving a 270 mg tablet in 40 ml of sterile distilled water. A vortex mixer was used to ensure that it was a well-dissolved and homogenous solution). Then 30 µl of MHB was added to each well. Finally, 10 µl of bacterial suspension was added to each well to achieve a concentration of approximately 5×10^5 CFU/ml. Each plate

had a set of controls containing a column with all solutions with the exception of the crude extracts, a column with all solutions with the exception of the bacterial solution adding 10 µl of MHB instead and a column with 10 % DMSO solution as a negative control. The 96 well microtitre plates were incubated at 37 °C for 24 h for all bacterial strains. The color change was then assessed visually. The growth was indicated by color changes from purple to pink (or colorless). In this study, the MIC was the lowest concentration of crude extracts of *T. populnea* leaves that inhibited the growth of the bacteria in the values by visual reading.

Determination of the Minimum Inhibitory Concentration (MIC) for Fungi

The MIC of the crude extracts of *T. populnea* leaves was determined by using broth micro dilution technique as recommended by M38-A2 [41] for filamentous fungi. The MIC values were determined in RPMI-1640 (Himedia, Mumbai) with L – glutamine without sodium bicarbonate, pH 7.0 with Morpholine propane sulfonic acid (MOPS). 20 µl of a stock solution (50 mg/ml) of extract in 10% DMSO was dissolved with 980 µl of RPMI-1640 medium solution 1000 µl (1 mg/ml). From that, the two fold serial dilutions in the range from 500 to 15.7 µg/ml were prepared. 200 µl of solution was poured into first well of 96 well microtitre plates and then 100 µl were transferred to the next well containing 100 µl of RPMI-1640. The same procedure was performed for all wells. Finally, 100 µl of standardized inoculum suspension was transferred to each well to achieve a concentration of approximately 0.4 – 5 × 10^4cells/ml for filamentous fungi. The control well contained only sterile water and devoid of inoculum. The microtitre tray plates were incubated at 30 °C for 72-96 h for *Aspergillus* spp. and 4-7 days with dermatophytes. The MIC of the extracts was recorded as the lowest concentration of extracts of *T. populnea* leaves that inhibited the growth of the *Aspergillus* and dermatophytic strains when compared to that of control.

Determination of the Minimum Bactericidal Concentration (MBC) and the Minimum Fungicidal Concentration (MFC)

MBC and MFC were determined by plating a loop full of samples from each MIC assay well with growth inhibition in to freshly prepared MHA for bacteria and SDA for fungal strains. The plates were incubated at 37 °C for 24 h for all bacterial strains, 30 °C for 48 h for *Aspergillus* and 4 -7 days for dermatophytes. The MBC and MFC were recorded as the lowest concentration of the crude extracts that did not permit any visible bacterial and fungal growth after the period of incubation.

RESULTS

The antibiotic resistance of bacterial standard strains was confirmed by CLSI-M100-2012 method [38]. Among the standard strains tested, *S. aureus* (MTCC 7443) was found to be the highly sensitive to all antibiotics tested except AMP and *S. aureus* (MTCC 737) was found to be highly resistant to all the antibiotics tested except GE, S, TE, AK, E and C. The standard strains of *K. pnemoniae* and *P. vulgaris* were sensitive to all the antibiotics tested except CFM, AMP and CAZ. The standard strains of *S. flexneri* and *P. mirabilis* were sensitive to all the antibiotics tested except AMP. The standard strains of *S. pyogenes* were resistant to CFM, AMP, CAZ, NA and E, and sensitive to all other antibiotics tested. The standard strains of *E. coli* were sensitive to all antibiotics tested except AMP and NA. The standard strains of *P. aeruginosa* were resistant to CFM, AMP and TE and sensitive to all other antibiotics tested. The standard strains of *S. typhimurium* were sensitive to all antibiotics except AMP and E. The standard strains of *V. cholerae* were resistant AMP and intermediate resistant to S and sensitive to all other antibiotics tested. The hexane, chloroform, ethyl acetate and methanol extracts of *T. populnea* leaves were used to analyse the phytochemicals such as flavonoids, tannins, steroids, glycosides, saponins, phenols, terpenoids and alkaloids. The methanol extract of *T. populnea* leaves showed the presence of strong phytochemicals *viz.,* flavonoids, tannins, steroids, glycosides, saponins, phenols, terpenoids and alkaloids than the other extracts. The ethyl acetate and chloroform extracts contain all the phytochemicals tested except tannins, saponins, phenols and alkaloids. The hexane extract contain flavonoids, steroids, glycosides and alkaloids and other tested phytochemicals are absent (Table 1).

The antibacterial activity of hexane, chloroform, ethyl acetate and methanol crude extracts of leaves of *T. populnea* was investigated against two gram positive (*S. aureus* MTCC 7443&737) and eight gram negative (*P. aeruginosa, E. coli, S. pyogenes*, *S. typhimurium*, *S. flexneri*, *P. mirabilis*, *P. vulgaris* and *V. cholerae*) bacterial strains. The chloroform extract of *T. populnea* exhibited the highest antibacterial activity. The mean zone of inhibitions and MIC and MBC values are presented in Tables 2. The highest mean zone of inhibition of 14.8 mm was observed in chloroform extract against *S. aureus* (MTCC 7443) and 13.5 mm against *S. pyogenes*. The lowest MIC (62.5 µg/ml) and MBC (125 µg/ml) values were observed in chloroform extract of *T. populnea* leaves against *S. aureus* (MTCC 7443). The results of antibacterial activity stated that chloroform extracts showed the highest activity and followed by hexane, ethyl acetate and methanol extracts. The positive control Ampicillin (10 µg/disc) produced mean zones of inhibition ranged from 7.5 to 12.5 mm. The positive control Methicillin (5 µg/disc) produced mean zones of inhibition ranged between 7.5 and 18.8 mm. The negative control (10% DMSO) did not produce any zone of inhibition for all the bacterial strains tested. The antifungal activity of hexane, chloroform, ethyl acetate and methanol extracts of leaves of *T. populnea* showed different degrees of activity against three *Aspergillus* species and three dermatophytic fungal strains. In this study, methanol extract showed the highest activity (22.8 mm) against *A. fumigatus* followed by ethyl acetate, chloroform and hexane. The mean zone of inhibition, MIC and MFC values are presented in Table 3. The lowest MIC and MFC values (62.5 and 125 µg/ml) were observed against *A. fumigatus* and *M. gypseum*. The antifungal activity of methanol extract showed favorable results than the other extracts. The control Ketoconazole (10 µg/disc) produced zones of inhibition ranged from 30.5 to 33.8 mm. The negative control (10% DMSO) did not produce any zone of inhibition for all the fungal strains tested.

Table 1: Phytochemical analysis of the different extracts of *Thespesia populnea* leaves.

S. No.	Phytoconstituents	Hexane	Chloroform	Ethyl acetate	Methanol
1	Flavonoids	++	+++	++	+++
2	Tannins	_	_	_	+++
3	Steroids	+	+++	+	+++
4	Glycosides	++	+++	++	+++
5	Saponins	_	_	_	+++
6	Phenols	_	_	_	+++
7	Terpenoids	_	+++	++	+++
8	Alkaloids	++	_	_	+++

- = Absence, + = weak, ++ = medium, +++ = strong.

DISCUSSION

With the increase in resistance by microorganisms to the currently used antibiotics and the high production cost of synthetic compounds, there is a need to seek alternative antimicrobials from other effective sources against pathogens resistant to current antibiotics [42]. Medicinal plants could be one of those alternatives because most of them are safe, cost effective and affect a wide range

of antibiotic resistant microorganisms. The rich chemical diversity in plants promises to be a potential source of antibiotic resistance modifying or modulating compounds and has yet to be adequately explored [43, 44]. In the results, different solvents *viz*., hexane, chloroform, ethyl acetate and methanol extracts of *T. populnea* leaves possessed antibacterial and antifungal activity against all the bacterial and fungal strains tested. All the tested strains showed varying degree of inhibitions. In antibacterial screening, the chloroform extract was found to be the most effective antibacterial agent and followed by hexane, ethyl acetate and methanol extracts. The chloroform extract of *T. populnea* leaves showed the highest antibacterial activity against *S. aureus* (MTCC 7443) followed by other bacterial strains. The mean zone of inhibition was 14.8 mm, and the lowest MIC (62.5 µg/ml) and MBC (125 µg/ml) values were observed in chloroform extract of *T. populnea* leaves against *S. aureus* (MTCC 7443).

Table 2: Antibacterial activity of different extracts of *Thespesia populnea* leaves

Bacterial Strains/Plant extracts prepared with different solvents	Mean zone of inhibition[a] (mm)[b]					
	Concentration of the disc (µg/disc)					
	1000	500	250	Methicillin, (5 µg) Ampicillin (10 µg)	MIC (µg/ml)	MBC (µg/ml)
***S. aureus* (MTCC 7443)**						
Hexane	11.8 ± 0.76	10.5 ± 0.5	8.4 ± 0.52	16.5 ± 0.50	125	250
Chloroform	14.8 ± 0.76	10.5 ± 0.5	9.5 ± 0.5	18.5 ± 0.50	62.5	125
Ethyl acetate	10.7 ± 0.64	8.5 ± 0.5	7.5 ± 0.5	18.8 ± 0.28	125	250
Methanol	9.8 ± 0.76	7.5 ± 0.5	NA	15.6 ± 0.76	250	500
***S. aureus* (MTCC 737)**						
Hexane	12.4 ± 0.52	9.5 ± 0.5	7.7 ± 0.68	8.0 ± 0.50	125	250
Chloroform	13.8 ± 0.76	11.5 ± 0.5	9.5 ± 0.5	8.6 ± 0.76	125	250
Ethyl acetate	11.7 ± 0.68	9.5 ± 0.5	7.4 ± 0.50	7.8 ± 0.76	125	250
Methanol	10.8 ± 0.76	8.5 ± 0.5	7.5 ± 0.5	7.5 ± 0.50	125	250
P. aeruginosa						
Hexane	10.7 ± 0.68	8.4 ± 0.50	7.5 ± 0.5	11.5 ± 0.50	125	250
Chloroform	12.7 ± 0.68	10.5 ± 0.5	8.5 ± 0.5	8.6 ± 0.76	125	250
Ethyl acetate	11.7 ± 0.68	9.5 ± 0.5	7.5 ± 0.5	10.3 ± 0.57	125	250
Methanol	10.5 ± 0.50	8.5 ± 0.50	NA	9.5 ± 0.50	250	500
E. coli						
Hexane	11.5 ± 0.5	10.6 ± 0.57	9.5 ± 0.5	11.6 ± 0.50	125	250
Chloroform	12.5 ± 0.50	10.5 ± 0.50	8.8 ± 0.76	9.3 ± 0.57	125	250
Ethyl acetate	8.5 ± 0.50	7.5 ± 0.50	NA	8.5 ± 0.50	250	500
Methanol	8.4 ± 0.68	7.5 ± 0.5	7.4 ± 0.51	10.5 ± 0.50	250	500
S. pyogenes						
Hexane	12.8 ± 0.76	10.8 ± 0.76	8.5 ± 0.5	10.0 ± 0.50	125	250
Chloroform	13.5 ± 0.50	11.5 ± 0.5	10.5 ± 0.5	10.5 ± 0.50	125	250
Ethyl acetate	10.8 ± 0.76	8.5 ± 0.5	NA	10.5 ± 0.50	125	250
Methanol	9.5 ± 0.5	7.5 ± 0.50	NA	11.5 ± 0.50	250	500

[a]-diameter of zone of inhibition (mm) including the disc diameter of 6 mm; [b]-mean of three assays; ± - standard deviation; NA – No activity; Methicillin for *S. aureus* and Ampicillin for all other bacteria tested

In antifungal screening, the methanol extract was found to be the most effective antifungal agent and followed by ethyl acetate, chloroform and hexane extracts. The methanol extract of *T. populnea* leaves possessed the highest antifungal activity against *A. fumigatus* followed by other fungal strains. The mean zone of inhibition (22.8 mm) was recorded in methanol extract of *T. populnea* leaves against *A. fumigatus*. The lowest MIC (62.5 µg/ml) and MFC (125 µg/ml) values were observed in methanol extract of *T. populnea* leaves against *A. fumigatus* and *M. gypseum*.

These results are supported by previous biological reports in the same species of *T. populnea.* Antimiocrobial activity of bark extracts of *T. populnea* showed maximum antimicrobial effect against bacterial strains such as *S. aureus*, *S. pyogenes* and *E. coli*, *P. aeruginosa* and fungal strains such as, *C. albicans* and *A. flavus* [45]. A compound, sesquiterpenes from *T. populnea* showed antibacterial activity against *Bacillus subtilis, S. aureus* and *E. faecalis* [46]. Similarly, Archana moon et al. [47] reported that leaf extracts of *T. populnea* to possess antibacterial activity against multi drug resistant strains of *E. coli*, *S. aureus*, *K. pneumoniae* and *S. typhii* and flower extracts of *T. populnea* to exhibit antibacterial activity against *S. flexneri, Rhodococcus terrae, E. coli, Streptococcus faecalis, K. pneumoniae, Brevibacterium luteum, Micrococcus flavum, M. luteus, P. mirabilis, Bacillus licheniformis, Flavobacterium devorans, Shigella sonei, S. boydii* and *S. dysentriae* [48]. Stem bark extracts of *T. populnea* possessed antibacterial activity against *S. aureus, S. pyogenes, E. coli* and *P. aeruginosa* [49].

Fruit extracts of *T. populnea* demonstrated antimicrobial activity against bacterial strains such as *S. aureus, E. coli, Bacillus subtilis, P. aeruginosa* and *S. typhi* and fungal strains such as *C. albicans, A. niger and A. flavus* [50]. In this study, the chloroform extract of leaves of *T.populnea* possessed the highest antibacterial effect against gram positive bacteria than that of gram negative. The outer membrane of gram negative bacteria is known to present barrier to penetration of numerous antibiotic molecules and the periplasmic space contains enzymes which are capable of breaking down foreign molecules introduced from outside [51, 52]. The gram-positive bacteria are considered to be more sensitive when compared to gram-negative because of the differences in their cell wall structures [53]. In this study, the chloroform extract of leaves of *T. populnea* showed the highest mean zone of inhibition (14.8 mm) and the lowest MIC value (62.5 µg/ml) against *S. aureus* (MTCC-7443). Similar results were observed in chloroform extracts of *Couroupita guianensis* fruits which possessed good antimicrobial activity against bacterial strains such as *S. aureus, E. coli* and *K. pneumoniae* and fungal strains such as *C. albicans* and *Malassesia pachydermatis* [54]. Similarly, chloroform extract of *Pongamia pinnata* leaves demonstrated antimicrobial activity against bacterial strains such as *S. aureus, E. coli*, *P. aeruginosa* and *S. typhi* and fungal strains such as *C. albicans* and *A. niger* [55]. Pirzada *et al*., [56] reported that chloroform and aqueous extracts of *Cressa cretica* leaves and shoots possessed maximum antifungal activity against *A. niger, A. flavus, Paecilomyces varioti, M. gypseum* and *T. rubrum*. Baskaran *et al*., [58] reported that

the chloroform extract of *Carica papaya* leaves demonstrated strong antibacterial activity against *M. luteus*. The methanol extract of leaves of *Thespesia populnea* showed the highest zone of inhibition (22.8 mm) against *A. fumigatus* and the lowest MIC value (62.5 µg/ml) was observed against *A. fumigatus* and *M. gypseum*. Similar results were observed in methanol extract of the leaves of *Garcina lucida* which possessed antifungal activity against *Candida tropicalis* [58]. Similarly, methanol extract of *Peltophorum pterocarpum* and *Punica granatum* demonstrated antifungal activity against *C. albicans* [59].

Table 2: (continued). Antibacterial activity of different extracts of *Thespesia populnea* leaves

Bacterial Strains/Plant extracts prepared with different solvents	Mean zone of inhibition[a] (mm)[b] Concentration of the disc (µg/disc)					
	1000	500	250	Ampicillin (10 µg)	MIC (µg/ml)	MBC (µg/ml)
S. typhymurium						
Hexane	11.8 ± 0.76	9.5 ± 0.5	7.5 ± 0.5	8.5 ± 0.50	125	250
Chloroform	12.5 ± 0.5	11.5 ± 0.5	9.5 ± 0.5	9.0 ± 0.50	125	250
Ethyl acetate	9.5 ± 0.5	7.5 ± 0.50	NA	9.3 ± 0.28	125	250
Methanol	11.8 ± 0.76	9.5 ± 0.5	7.5 ± 0.5	10.5 ± 0.50	125	250
S. flexneri						
Hexane	11.5 ± 0.50	9.6 ± 0.57	7.6 ± 0.57	9.6 ± 0.76	125	250
Chloroform	12.8 ± 0.76	10.5 ± 0.5	7.8 ± 0.76	8.5 ± 0.50	125	250
Ethyl acetate	10.7 ± 0.68	8.5 ± 0.50	NA	9.0 ± 0.50	125	250
Methanol	11.8 ± 0.76	9.4 ± 0.52	7.5 ± 0.5	10.1 ± 0.28	125	250
P. mirabilis						
Hexane	10.7 ± 0.64	8.5 ± 0.50	7.5 ± 0.5	9.0 ± 0.50	125	250
Chloroform	12.5 ± 0.5	10.7 ± 0.68	7.6 ± 0.57	10.6 ± 0.76	125	250
Ethyl acetate	8.8 ± 0.76	7.5 ± 0.50	10.5 ± 0.50	11.6 ± 0.76	250	500
Methanol	11.7 ± 0.68	9.5 ± 0.50	7.5 ± 0.50	12.5 ± 0.50	125	250
P. vulgaris						
Hexane	10.5 ± 0.50	8.5 ± 0.50	7.8 ± 0.76	8.3 ± 0.57	125	250
Chloroform	12.8 ± 0.76	10.5 ± 0.50	8.8 ± 0.76	9.5 ± 0.50	125	250
Ethyl acetate	10.6 ± 0.57	9.5 ± 0.5	7.5 ± 0.50	9.3 ± 0.28	125	250
Methanol	10.8 ± 0.76	8.5 ± 0.5	7.7 ± 0.64	10.6 ± 0.76	125	250
V. cholera						
Hexane	11.8 ± 0.76	9.8 ± 0.76	7.5 ± 0.5	12.0 ± 0.50	125	250
Chloroform	12.3 ± 0.28	10.5 ± 0.50	8.6 ± 0.57	10.5 ± 0.50	125	250
Ethyl acetate	10.8 ± 0.76	8.5 ± 0.5	7.5 ± 0.50	11.0 ± 0.50	125	250
Methanol	11.8 ± 0.76	9.5 ± 0.5	7.5 ± 0.5	11.5 ± 0.50	125	250

[a]-diameter of zone of inhibition (mm) including the disc diameter of 6 mm; [b]-mean of three assays; NA – No activity; ± - standard deviation

Chandrasekaran et al. [60], reported that methanol extracts of *Syzygium jambolanum* seeds possessed antimicrobial activity against bacterial strains such as *Bacillus subtilis, S. aureus*, *S. typhimurium*, *P. aeruginosa*, *K. pneumoniae* and *E. coli* and fungal strains such as *C. albicans*, *Cryptococcus neoformans*, *A. flavus*, *A. fumigatus*, *A. niger*, *Rhizopus* sp., *T. mentagrophytes*, *T.rubrum* and *M. gypseum*. Rabia *et al.,* [62] reported that methanol extract of *Ricinus communis* leaves demonstrated antimicrobial activity against bacterial strains such as *S. aureus, P. aeruginosa, K. pneumoniae* and *B. subtilis* and fungal strains such as *A. fumigatus* and *A. flavus*.

Table 3: Antifungal activity of different extracts of *Thespesia populnea* leaves

Fungal Strains/Plant extracts prepared with different solvents	Mean zone of inhibition[a] (mm)[b] Concentration of the disc (µg/disc)					
	1000	500	250	Ketoconazole (20 µg/disc)	MIC (µg/ml)	MFC (µg/ml)
A. niger						
Hexane	7.8 ± 0.76	NA	NA	30.5 ± 0.50	NT	NT
Chloroform	11.8 ± 0.76	9.1 ± 0.28	8.2 ± 0.28	32.5 ± 0.50	125	500
Ethyl acetate	16.5 ± 0.5	14.5 ± 0.50	12.8 ± 0.76	30.8 ± 0.76	250	500
Methanol	17.5 ± 0.5	15.5 ± 0.5	12.8 ± 0.76	30.5 ± 0.50	125	250
A. flavus						
Hexane	9.1 ± 0.28	7.8 ± 0.76	NA	31.5 ± 0.50	250	500
Chloroform	10.5 ± 0.50	8.2 ± 0.28	7.8 ± 0.76	31.8 ± 0.76	250	500
Ethyl acetate	14.5 ± 0.5	12.5 ± 0.50	10.8 ± 0.76	30.5 ± 0.50	250	500
Methanol	17.8 ± 0.76	16.5 ± 0.5	13.5 ± 0.5	31.5 ± 0.50	125	250
A. fumigates						
Hexane	12.1 ± 0.28	10.5 ± 0.50	9.1 ± 0.28	30.5 ± 0.50	250	500
Chloroform	11.8 ± 0.76	9.5 ± 0.5	7.8 ± 0.76	31.5 ± 0.50	250	500
Ethyl acetate	14.5 ± 0.5	12.8 ± 0.76	10.5 ± 0.50	30.5 ± 0.50	250	500
Methanol	22.8 ± 0.76	18.8 ± 0.76	17.5 ± 0.50	30.8 ± 0.76	62.5	125
T. rubrum						
Hexane	NA	NA	NA	32.8 ± 0.76	NT	NT
Chloroform	NA	NA	NA	32.5 ± 0.50	NT	NT

Ethyl acetate	NA	NA	NA	30.5 ± 0.50	NT	NT
Methanol	16.5 ± 0.5	12.1 ± 0.28	10.5 ± 0.50	30.8 ± 0.76	125	250
T. mentagrophytes						
Hexane	NA	NA	NA	33.5 ± 0.50	NT	NT
Chloroform	NA	NA	NA	33.8 ± 0.76	NT	NT
Ethyl acetate	NA	NA	NA	32.5 ± 0.50	NT	NT
Methanol	12.8 ± 0.76	10.5 ± 0.5	8.8 ± 0.76	32.8 ± 0.76	250	500
M. gypseum						
Hexane	NA	NA	NA	32.8 ± 0.76	NT	NT
Chloroform	9.3 ± 0.28	7.8 ± 0.76	5.5 ± 0.5	32.5 ± 0.50	250	500
Ethyl acetate	13.5 ± 0.5	11.8 ± 0.76	9.1 ± 0.28	32.5 ± 0.50	125	250
Methanol	20.5 ± 0.5	18.5 ± 0.5	16.8 ± 0.76	31.8 ± 0.76	62.5	125

[a]-diameter of zone of inhibition (mm) including the disc diameter of 6 mm; [b]-mean of three assays; NA – No activity; NA – Not tested; ± -standard deviation

In this study, two standard antibiotics, namely Ampicillin (10 µg/disc), Methicillin (10 µg/disc) and Ketoconazole (5 µg/disc) were used. Ketoconazole is one of the commonly used antifungal drugs administered orally for the treatment of both superficial and deep infections caused by *Trichophyton*. However, the unpleasant side effects of this drug include nausea, abdominal pain, and itching, and its toxicity limits its therapeutic use in many cases [62].

The chloroform extracts of leaves of *T. populnea* containing the strong phytochemicals flavonoids, steroids, glycosides and terpenoids they showed the highest antibacterial activity which may be due to the presence of flavonoids and steroids. Flavonoids have been reported as effective antimicrobials against a wide array of microbes [63]. Steroids of plant origin are known to be important for antimicrobial, insecticidal, antiparasitic and cardiotonic properties. Steroids also play an important role in nutrition, herbal medicine and cosmetics [64]. The present study revealed that methanol extracts of *T. populnea* leaves showed the highest antifungal activity. The presence of strong phytochemicals such as flavonoids, tannins, steroids, glycosides, saponins, phenols, terpenoids and alkaloids may be the reason for antifungal activity. The plant steroids are known to possess antimicrobial properties [65]. Flavonoids have been reported as effective antimicrobials against a wide array of microbes [63]. Tannins are well known to possess general antimicrobial properties.

CONCULUSION

In conclusion, the results of this study revealed that chloroform and methanol extracts of *T. populnea* leaves exhibited strong antimicrobial activity against bacterial and fungal strains tested. The inhibitory effect of this plant will be helpful to pharmacological industry for the preparation of herbal products after further scientific validation.

CONFLICT OF INTERESTS

Declared None

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