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INVITRO ANTIMICROBIAL ACTIVITIES AND PHYTOCHEMICAL ANALYSIS OF CRUDE LEAF EXTRACTS OF *THESPESIA POPULNEA* (L)

***Neethu S. Kumar and Neethu Simon**

Department and Research Centre of Botany, Mahatma Gandhi College, Thiruvananthapuram, Kerala, India

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ABSTRACT

Plants have been one of the important sources of medicines since the beginning of human civilization. *Thespesia populnea* is a large tree belonging to the family Malvaceae found in tropical regions and coastal forests of India. Various parts of this tree 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, scabies, urinary tract infections, diabetes, cholera, cough, asthma, guneaworm infections and also for various skin diseases including psoriasis. The barks and flowers possess astringent, hepatoprotective and antioxidant activity. A comparative antimicrobial activity of dried leaf extracts of *T.populnea* (L) were evaluated against two gram negative bacterial strains namely Escherichia coli and Pseudomonas *aeroginosa* and two clinical fungal pathogens namely Candida albicans and *Aspergillus niger* by agar cup method. Qualitative phytochemical screening was carried out using the crude leaf extracts in three different solvents such as water, alcohol and chloroform. Phytochemical analysis of the extracts revealed the presence of glycosides, alkaloids, oils, saponins and flavanoids. The leaf extracts of T.*populnea* (L) was found to have high antibacterial activity than anti fungal activity. The results suggest that the leaves are a rich source of valuable primary and secondary metabolites exhibiting the antimicrobial activity.

Key words: *Thespesia populnea* , Phytochemical analysis, Antimicrobial, Agar cup method.

INTRODUCTION

Since ancient times, people have been exploring the nature particularly plants in search of new drugs which has resulted in the use of large number of medicinal plants with curative properties to treat various diseases (Verpoorte *et al*., 1998). According to WHO survey, 80% populations living in the developing countries rely exclusively on traditional medicine for their primary health care needs of which most involve the use of plant extracts (Sandhya *et al*., 2006). The studies of plants continue principally for the discovery of novel secondary metabolites or phytochemicals which are the non essential nutrients derived from plants exhibiting a number of protective functions for human consumers. *Thespesia populnea* is a large tree belonging to the family Malvaceae found in tropical regions and coastal forests of India. Various parts of this tree are found to possess useful medicinal properties. The leaves are applied locally in swollen joints for their anti inflammatory effects and also for hepatitis, jaundice, ulcers, wounds, scabies, urinary tract infections, diabetes, cholera, cough, asthma, gunea worm infections, various skin diseases including psoriasis and so on. The fruits of the plant are used in Ayurveda for the control of diabetes (Sathyanarayana *et al.,* 2004).

Corresponding author: Neethu S. Kumar,
Department and Research Centre of Botany, Mahatma Gandhi College , Thiruvananthapuram, Kerala, India

The barks and flowers possess astringent, hepatoprotective and antioxidant activity (Ilavavarasan *et al.,* 2003). Phytochemical screening is a method which exposes or reveals certain components or properties readily available in plants for bio-activity or ethno-medical applications. Plant based antimicrobials has enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials (Iwu *et al*., 1999). Thus it is anticipated that phytochemicals with adequate antibacterial efficiency can be used for the treatment of bacterial infections (Balandrin *et al*., 1985). Antioxidants and antimicrobial properties of various extracts from many plants have recently been of great interest in both research and in food industry, because of their possible use as natural additives to replace synthetic antioxidants and antimicrobials with natural ones (Deba *et al*., 2008). Thus medicinal plants play an important role in the development of newer drugs because of their effectiveness, less side effects and relatively low cost when compared with synthetic drugs (Raj *et al*., 2011). The present study aims in exploring the phytochemical constituents, antibacterial and antifungal properties of the crude leaf extracts of *Thespesia poulnea* (L).

MATERIALS AND METHODS

Collection and extraction of plant materials

The fully matured fresh leaves of *T. populnea*(L) were collected from Kattakada area in Thiruvananthapuram district,

kerala. The leaves were washed thoroughly, shade dried and finely powered. The dried powdered leaves were extracted with three different solvents such as water, acetone and chloroform.

Table 1. Phytochemical analysis *Thespesia populnea* (L) leaf extracts

Phytochemicals	Glycosides	Phytosterols	Alkaloids	Oils	Saponins	Phenols	Flavanoids
Water	-	+	-	+	+	+	-
Acetone	-	+	+	+	+	+	-
Chloroform	-	-	+	+	+	-	-

+: Present - : Absent

For aqueous extraction, ten grams of the powdered leaves were mixed with 100ml distilled water, boiled for about two hours and filtered. Whereas acetone and chloroform extracts were prepared by mixing ten grams of powdered leaf samples with 100ml of each solvent separately in mechanical shaker for 48 hours at room temperature. Extracts were then filtered, concentrated, dried and were stored in the refrigerator at 4°C for future use.

Phytochemical analysis

The prepared plant extracts were analysed for the presence of alkaloids, glycosides, saponins, proteins, aminoacids, fixed oils, phenolic compounds, tannins, flavonoids, gum and mucilages etc. (Harborne et al., 1998)

Preparation of plant extract for antimicrobial screening

For antimicrobial screening the concentrated, dried and powdered ethanol leaf extract was dissolved in 10 % dimethyl sulfoxide (DMSO) and were stored at 4^0 C for further use.

Test Organisms

Antibacterial activity was carried out against two selected gram negative pathogens (such as *Escherichia coli and Pseudomonas aeroginosa*) whereas antifungal against two clinical fungal isolates such as *Candida albicans* and *Aspergillus niger*. The strains used for the present study were obtained from Biogenix Research centre, Valiyavila, Thiruvananthapuram. In order to access the biological significance and ability of the plant part, the minimal inhibitory activity was determined by Agar cup method.

Antibacterial activity

Petri plates containing 20ml of Muller Hinton medium were seeded each with 24hr old culture of bacterial strains such as *E.coli and P. aeroginosa* . Wells of approximately 10mm diameter were bored using a well cutter and 25 µl , 50 µl and 100µl of the extracts were added to the wells from a stock concentration of 0.1g/1ml. The plates were then incubated at 37°C for 24 hours. Antibacterial activity was assayed by measuring the diameter of the inhibition zone in millimeters formed around the wells.[10]Gentamycin (standard antibacterial agent, concentration: 20mg / ml) was used as a positive control.

Antifungal activity

Antifungal activity was also determined by Agar cup method. Potato Dextrose agar plates were prepared and overnight grown isolates of fungi such as *Candida albicans* and *Aspergillus niger* were swabbed. Wells of approximately 10mm diameter were bored using a well cutter and extracts of 25 µl, 50 µl and 100 µl concentrations were added and the zones of inhibition were measured after overnight incubation which were then compared with that of standard antibiotics. Clotrimazole was used as a positive control.

RESULTS AND DISCUSSION

Phytochemical analysis

Table 1 represent the various phytochemical constituents present in the leaf extracts of *T. populnea* (L). The phytochemical studies of all the three extracts conclude that acetone extracts of leaf samples had more positive result. The aqueous extract of *Thespesia populnea* had shown the presence of glycosides, phytosterols, oils, saponins and phenols (Table1). Primarily phenolic compounds are of great importance as cellular support material because polymeric phenols form the integral part of the cell wall structure (Guptha *et al.,* 2010). The leaf extract of acetone showed the presence of phytosterols, alkaloids, oils, saponins etc.The chloroform extract also had shown the presence of alkaloids, oils, saponins and phenols. All the three extracts were positive for Glycosides, oils, sapponins and phenols. Bioactive polyphenols have attracted special attention because they can protect the human body from the oxidative stress which may lead to many diseases including cancer, cardiovascular problems and ageing (Robards *et al.,* 1999).

Table 2. Zone diameter of inhibition of ethanol leaf extract of *Thespesia populnea* (L)

Test organisms	Zone of inhibition in mm			Positive Control
	Concentration of leaf extracts			
	25	50	100	
E.Coli	-	-	15	20
P.aeroginosa	-	-	11	20

Table 3. Zone diameter of inhibition of ethanol leaf extract of *Thespesia populnea* (L)

Test organisms	Zone of inhibition in mm			Positive Control
	Concentration of leaf extracts			
	25	50	100	
C. albicans	-	-	-	25
A. niger	-	-	-	25

Antibacterial activity

Antibacterial activity of *T. populnea* (L) (leaf ethanol extract with DMSO) was assayed invitro by agar cup method against clinical isolates of *E.coli* and *P.aeroginosa.* The given table shows the microbial growth inhibition of ethanolic leaf extracts of *T.populnea* (L). Among the varying concentration

of leaf extracts, higher concentration exhibited maximum antibacterial activity against the two clinical isolates. Table 2 shows the zone of inhibition formed by the extracts against the bacterial strains on Muller Hinton agar. The sequence of antibacterial activity of leaf extract against *E.coli* exhibited no activity in 25µl and 50µl but produced a 15 mm zone of inhibition in 100µl concentration respectively (Table 2). Similarly the plant extract had shown no activity in both 25and 50 µl but showed a 11mm zone of inhibition at 100µl concentration (Table 2). Thus antibacterial activity was expressed at varying degrees with the increase in concentration. Higher concentration of the leaf extract shows highest antibacterial activity. The result obtained might be considered sufficient for further studies for isolation and identification of active principle and for the evaluation of possible antimicrobial activity of other extracts from other parts of *T. populnea* (L).

Antifungal activity

In order to access the biological significance and ability of the plant extract, antifungal activity of *T. populnea* (L) (leaf ethanol extract with DMSO) was assayed invitro by agar cup method against two clinical fungal isolates viz. *Candida albicans* and *Aspergillus niger*. The given table shows antifungal activity of the plant species. The sequence of antifungal activity of leaf extract against *C. albicans* and *A.niger* exhibited no activity in all the three (25µl, 50µl and in 100µl) concentrations respectively (Table 3). The present study reveals that the ethanol leaf extracts of *T. populnea* (L) were more active against the clinical bacterial pathogens viz. *E.coli* and *P.aeroginosa*. Anti fungal activity was not found when compared to bacterial activity. In literature it has been reported that the antibacterial activity is due to the presence of different chemical agents in the leaf extract including essential oils, Phenolics, flavanoids, terpenoids and other components which are classified as active antimicrobial compounds. The results of the study supports to a certain degree, the use of traditional medicinal plants in human and animal disease therapy and reinforce the concept of ethno botanical approach in screening plants as potential sources of bioactive substances. The aqueous extract generally exhibits a high degree of antibacterial activity which seems to confirm the traditional therapeutic claims of this plant.

Summary and conclusion

Medicinal plants were the potent source of human health due to the presence of active phytochemical compounds that are responsible for its various pharmacological activities. On the basis of the results obtained, the present work conclude that the leaves of *T. Populnea* (L) are rich in phytochemical constituents even though the phytochemical screening of the leaf extracts of samples had shown variation in their phytochemical constituents with the presence and or absence of some components. Most components were present in aqueous extracts of leaves. The presence of various secondary metabolites such as glycosides, phytosterols, alkaloids, oils, sapponins, phenols and flavanoids were believed to exhibit the antibiotic properties of *T. populnea* (L) leaves and confirmed their antimicrobial efficacy against selected pathogens. The present work highlights the possible use of *T. Populnea* (L) leaf extracts as a source of antioxidants and as antibacterial agents that can be used to prevent enteric diseases. The study reveals that the results of extraction yield, total phenol and flavonoid compounds and bioactivity tests varied depending upon the type of solvent being used. Hence it can be concluded that the leaves of (*T. Populnea* L) would direct to the establishment of some compounds that could be used to invent new and more potent anti microbial drugs of natural origin. Therefore future research should be addressed on the application of using *T. Populnea* (L) leaves as natural remedied and to protect against infectious diseases.

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