Research Article

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GC-MS analysis, antioxidant and antibacterial activities of *Thespesia populnea* Linn. Leaf – *in vitro* study

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ABSTRACT

Thespesia Populnea (*T. populnea*) Linn has been used conventionally to treat various diseases like rheumatism, diabetes and also as an anti-inflammatory agent. In this study, ethanolic extract of *T. populnea* leaves was screened for chemical constituents (Chemical methods and GC-MS analysis) and pharmacological properties such as antioxidant capacity (DPPH and ABTS models) and antibacterial property (Agar-well diffusion and micro broth dilution methods) by *in vitro* assays. The leaf extract showed significant antioxidant and antibacterial activities. Preliminary phytochemical screening discloses the presence of bioactive constituents including flavanoids phenols and alkaloids. Results of GC-MS analysis showed 19 different phytocompounds. The present results suggest that the ethnopharmacological role of *T. populnea* could be attributed from active principles.

Key words: *Thespesia populnea*,GC-MS, DPPH, ABTS, MIC

INTRODUCTION

It is well known fact that traditional systems of medicine always played important role in meeting the global health care needs. Plants are used in different systems of medicine like Ayurvedha, Siddha, Unani and even Allopathy. India is one of the richest floristic regions of the world known for its ancient heritage regarding medicinal plant drugs since time of Rig-Veda. A large number of plant products have long been utilized as a source of therapeutic agent worldwide [1, 2]. Plants produce certain chemicals which are naturally toxic to bacteria [3] and many plants have been investigated for the development of novel drugs with therapeutic properties [4]. Recently, herbal medicines have been used to treat many diseases including several infections.

Thespesia populnea

Thespesia populnea Linn commonly called as *Hibiscus populnea* belongs to the family: Malvaceae. *T. populnea* is an evergreen tree. Different parts of this plant such as bark, root, fruit and leaf are also used in psoriasis, scabies, hemorrhoids, chronic dysentery, cutaneous and anti-inflammatory diseases [5]. The main medicinal uses are cutaneous infections, skin and liver diseases. Fruit juices are used on rheumatism sprains, scabies, swellings, insect bites and warts. Pulps of fresh fruits are applied for relief of migrane. Unripe fruit juice is used to cure piles. Decoction of bark is given to treat diarrhoea and arthritis [6]. In line with this, the present study was designed to explore the chemical constituents, antioxidant and antibacterial effects of *T. populnea* leaves using established *in vitro* methods.

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MATERIALS AND METHODS

Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), 2, 2'- azino-bis (3-ethylbenzothiozoline-6-sulphonic acid) diammonium salt (ABTS) were purchased from Sigma-Aldrich Co. Muller Hinton media (Agar and broth) were procured from Hi-Media. All other chemicals and solvents were of analytical grade and purchased from Merck and S.D. Fine Chemicals.

Plant material and extraction

The plant material *T. populnea* was collected locally. Authentication of the plant material was made by Prof. Dr. Jayaraman, Director, Plant Anatomy Research Centre, National Institute of Herbal Science, Chennai, Tamilnadu, India. About 100 gm of shade dried leaves were ground to powder and exhaustively extracted with 600 ml ethanol using soxhlet apparatus and extract was concentrated under reduced pressure and then stored in an air tight container for further study.

Phytochemical studies

Preliminary phytochemical screening

Preliminary phytochemical screening of *T. populnea* leaf extract was

performed using standard chemical methods [7, 8]

GC-MS analysis

T. populnea extract was analyzed by GC-MS (GC Clarus 500 Perkin Elmer) equipped with Elite-1 column (100% dimethyl poly siloxane), 30 × 0.25 mm x 1 µm (df) and mass detector: Turbomass gold Perkin Elmer. The temperature program was set as follows, 110°C for 2 min hold rose at 10°C/min up to 200°C and rose at 5°C/min up to 280°C with 9 min hold. Injector temperature was set at 250°C. The mass range was scanned from 45 to 450 (m/z). The control of the GC-MS system and data peak processing was controlled by means of Turbomass software version 5.2. Compound identification was verified based on the relative retention time and mass fragmentation pattern spectra with those of standards and the NIST Version (2005) LIB database of the GC-MS system [9].

Evaluation of antioxidant activity

DPPH radical scavenging assay

The free radical scavenging activity of the extract was measured by decrease in the absorbance of ethanolic solution of DPPH [10]. Different concentration of extract (50-250 µg/ml) and positive control ascorbic acid (250 µg/ml) was added separately to 2 ml of freshly prepared DPPH. The measurement was performed in triplicates. After incubation for about 30 min at room temperature in dark, the absorbance was measured at 520 nm using spectrophotometer (RAYLEIGH). Radical scavenging activity (%) was calculated using the following formula:-

$$\text{DPPH radical scavenging (\%)} = \frac{\text{OD of Control} - \text{OD of Sample}}{\text{OD of Control}} \times 100$$

ABTS radical cation-scavenging activity

The antioxidant activity of the extract was determined by the improved $ABTS^{\cdot+}$ radical cation scavenging ability as described by Baltrusaityte *et al* (2007) [11] with minor modifications. $ABTS^{+}$ radical cation was produced by mixing 7 mM 2,2'- azino-bis (3-ethylbenzothiozoline-6-sulphonic acid) diammonium salt (ABTS) and 2.45 mM potassium persulfate ($K_2S_2O_8$), incubated at room temperature in dark. To determine the ABTS radical scavenging activity, 3.9 ml of $ABTS^{+}$ solution was mixed thoroughly with 0.1 ml of different concentration (50-250 µg/ml) of extract. The reaction mixture was allowed to stand at room temperature for 6 min and the absorbance was immediately measured at 734 nm. Appropriate blank and standard ascorbic acid were also maintained. The experiment was performed in triplicate and the percentage decrease in absorbance was calculated by the following formula:

$$\text{\% Inhibition} = \frac{\text{OD of Control} - \text{OD of Sample}}{\text{OD of Control}} \times 100$$

Determination of antimicrobial activity

Test microbial strains

Test bacterial strains such as *Bacillus subtilis* (*B. subtilis*) *Escherichia coli* (*E.coli*), *Klebsiella pneumonia* (*K. pneumonia*), *Staphylococcus aureus* (*S. aureus*), and *Salmonella typhimurium* (*S. typhimurium*) were obtained from Post Graduate and Research department of Microbiology, M.G.R College, Hosur, India. Test organisms were subcultured periodically and maintained on their respective growth media for further study.

Agar-well diffusion assay

The modified Collins *et al*. (1995)[12] agar-well diffusion method was employed to determine the antimicrobial activity of the leaf extract against the bacterial strains mentioned above. Approximately, 10 ml of sterile Muller Hinton agar was poured in to sterile culture plates and allowed to set and wells of about 8 mm in diameter were punched on the plates. About 50-250 µg/ml of the extract was dispensed in to the wells and the plates were incubated at 37 ^{0}C for 24 h.

Micro broth dilution assay

Minimum inhibitory concentration (MIC) was determined by micro broth dilution method [13] using 96-well micro titre plates. Total volume of the assay system in each well was kept 200 µL. Muller-Hinton broth was used as growth medium. Inoculum density of the test organisms was adjusted to that of 0.5 McFarland standards. Broth was dispensed into wells of microtitre plate followed by addition of test extract and inoculum. Test extract (reconstituted in DMSO) was serially diluted into each of the wells. A DMSO control was included in the assay. Gentamicin served as a positive control. Plates were incubated at 37ºC for 16-20 h, before being read at 655 nm in a plate reader. MIC was recorded as the lowest concentration at which no growth was observed.

Statistical analysis

Results are expressed as the mean ± S.D. of three independent experiments (n=3). Student's *t*-test was used for statistical analysis; *P* values > 0.05 were considered to be significant. IC_{50} was calculated by linear regression analysis using Graph pad prism statistical software.

RESULTS AND DISCUSSION

Phytochemical studies

Preliminary phytochemical screening of the leaf extract of *T. populnea* revealed the presence of bioactive constituents such as alkaloids, terpenoids, tannins, steroids, flavonoids, saponins, cardiac glycosides and phenols (table 1). Previous reports suggest that the presence of these bioactive constituents in plant preparations could contribute to the antioxidant, antibacterial, antifungal and antiviral properties [14, 15, 16].

Table 1: Preliminary phytochemical screening of *T. populnea*

Phytochemicals	*T. populnea*
Alkaloids	+
Tannins	+
Cardiac glycosides	+
Saponins	+
Flavonoids	+
Terpenoids	+
Steroids	+
Phenols	+

+ Present

In the present GC-MS analysis of *T. populnea* leaf extract (Figure 1), 19 different phytochemicals were identified (Table 2) that include Copaene, α-Murolene, δ-Cadinene, Phytol, Linoleic acid, and trans-squalene. The other compounds present in the extract belong to fatty acid and fatty acid esters. The chromatogram (Figure 1) shows 19 prominent peaks in the retention time (RT) range 2.07 - 31.04. The peak at 19.72 (RT) has the peak area 38.50 % represents linoleic acid (MW 280) followed by the second less prominent peak at 16.82 (RT) with peak area 21.23 corresponds to n- hexadecanoic acid (MW 256). The third less significant peak (RT 31.04) with the peak area 9.83 is characteristic of trans-squalene (MW 410). The fourth less prominent peak (RT 19.10) with the peak area 9.83 denotes phytol (MW 296). The other peaks at their retention times with respective peak area % are given in table 2.

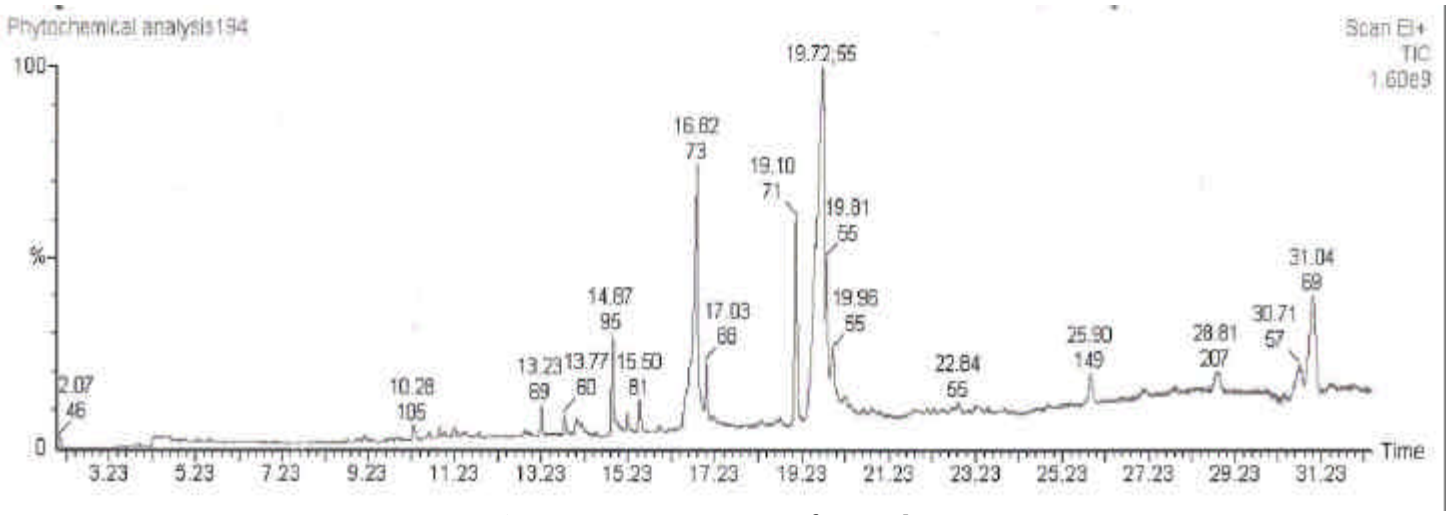

Figure 1: GC-MS spectrum of *T. populnea*

Table 2: Phytocompounds identified in *T. populnea* by GC-MS analysis

S. No	RT	Name of the Compound	Molecular Formula	MW	Peak area %
1	2.07	1,3-Propanediol, 2-methyl-2-nitro-, dinitrate	$C_4H_7N_3O_8$	227	0.47
2	3.87	Benzene, (l-methylethyl)-	C_9H_{12}	120	0.17
3	9.15	Copaene	$C_{15}H_{24}$	204	0.28
4	9.73	5,9-Undecadien-2-one, 6,1O-dimethyl-, (E)- (Synonyms: trans-Geranylacetone)	$C_{13}H_{22}O$	194	0.15
5	10.28	1H-Cycloprop[e]azulene, decahydro-l, 1.7-trimethyl-4-methylene-, [1aR-(l*aa4aß,7a,7aß,7ba)* (Synonyms: L-Alloaromadendrene)	$C_{15}H_{24}$	204	0.56
6	10.63	Naphthalene, 1,2Aa,5,6,8a-hexahydro-4.7-dimethyl-l-(methylethyl)-, (1a.*4aa,8aa)*- (Synonyms:a-Muurolene)	$C_{15}H_{24}$	204	0.21
7	10.88	Naphthalene, 1,2,3,5,6,8a-hexahydro-4, 7-dimethyl-l-(methylethyl)-, (1S-cis)- (Synonyms: d-Cadinene)	$C_{15}H_{24}$	204	0.31
8	11.21	Dodecanoic acid	$C_{12}H_{24}O_2$	200	0.32
9	13.23	2,6,1O-Dodecatrien-l-ol, 3.7, ll-trimethyl-, (E,E)- (Synonyms: (E,E)-Farnesol)	$C_{15}H_{26}O$	222	0.80
10	13.77	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.63
11	14.87	3,7,11,15-Tetramethyl-2-hexadecen-l-ol	$C_{20}H_{40}O$	296	3.76
12	16.82	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	21.23
13	17.03	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	2.89
14	19.10	Phytol	$C_{20}H_{40}O$	296	7.20
15	19.72	9, 12-Octadecadienoic acid (Z,Z)- (Synonyms: Linoleic acid)	$C_{18}H_{32}O_2$	280	38.50
16	19.81	Ethyl oleate	$C_{20}H_{38}O_2$	310	5.21
17	19.96	Octadecanoic acid	$C_{18}H_{36}O_2$	284	3.93
18	25.90	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	3.54
19	31.04	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23- hexamethyl-. [all-E}- (Synonyms: *trans*-Squalene)	$C_{30}H_{50}$	410	9.83

Antioxidant activity

Free radicals are molecular sharks that damage molecules in cell membranes, mitochondria, DNA and are very unstable; tend to rob electrons from the molecules in the immediate surroundings in order to replace their own losses [17]. Reactive oxygen species (ROS) includes not only the oxygen radicals but also some non-radical derivatives of oxygen; these include hydrogen peroxide, hypochlorous acid and ozone [18]. Numerous disorders are reported as ROS mediated [19, 20]. The role of ROS in stress induced diseases and involvement in the process of ageing has been well established [21, 22]. Screening of compounds which scavenge the free radicals, could lead to promising compounds. Most of the antioxidants used in therapy are derived from natural sources. About 28 % drugs approved by the FDA between 1981 and 2002 are either natural products or chemicals derived from them [23, 24]. Many synthetic antioxidant components have shown toxic and/or mutagenic effects. Hence attention has been given to naturally occurring antioxidants. Therefore, identification of effective antioxidants and free radical scavengers from plant origin is an ideal strategy for new drug development. Hence, present study was designed to explore the antioxidant potential and free radical scavenging activity of *T. populnea* leaves by DPPH and ABTS methods.

DPPH radical scavenging activity

The interaction of *T. populnea* extract with DPPH radicals is presented in table 3. The leaf extract exhibited a significant concentration dependent (50-250 µg/ml) DPPH radical scavenging activity with an IC_{50} value of 142.9 µg/ml. The scavenging effect of test extract was lesser when compared to that of reference compound ascorbic acid (IC_{50} 65.54 µg/ml).

Table 3: DPPH scavenging activity of *T. populnea*

Test compound	Concentration (µg/ml)	DPPH Inhibition (%)	IC_{50} (µg/ml)
T. populnea	50	27.7 ± 5.65	142.9
	100	41.4 ± 3.17	
	150	51.4 ± 4.31	
	200	62.2 ± 3.11	
	250	75.5 ± 6.31	
Ascorbic acid	250	93.33 ± 1.09	65.54

ABTS cation scavenging activity

The ABTS cation scavenging values of ethanolic extract are presented in table. 4; results are expressed as the ratio percentage of sample absorbance decrease and the absorbance of ABTS solution in the absence of extract at 517 nm. From the analysis of table 4, it can be inferred that the scavenging effects of *T. populnea* extract on ABTS radicals increased (13.19 to 69.81 %) with the concentration increase (50 to 250 µg/ml). The scavenging effects of the extract (IC_{50} 182.37 µg/ml) were lower than ascorbic acid (74.6 µg/ml).

Table 4: $ABTS^{+}$ radical scavenging activity of *T. populnea*

Test compound	Concentration (µg/ml)	DPPH Inhibition (%)	IC_{50} (µg/ml)
T. populnea	50	13.19 ± 2.52	182.37
	100	26.03 ± 5.88	
	150	40.77 ± 3.28	
	200	54.4 ± 2.72	
	250	69.81 ± 0.91	
Ascorbic acid	250	93.47 ± 1.54	74.6

Antibacterial activity

Drug discovery from the medicinal plants has played significant role in the treatment of various diseases and indeed, most new clinical applications of plant's secondary metabolites and their derivatives over the last century. In developing countries, traditional medicine is widely used to treat many of non-infectious and infectious ailments and it is estimated that approximately a total of 80% of the world's population use traditional medicine [25 According to one estimate, 25% of the commonly used medicines contain compounds isolated from plants [26] Medicinal plants are recognized as important sources of novel biomolecules [27, 28] which can theoretically be used in treating multiple life-threatening illness such as malaria [29], diabetes [30], hepatitis B virus [31,32], HIV [33], mycobacterial infection [34], and cancer [35]. Thus, it is important to document the therapeutic uses of plants because such information could help in obtaining maximum benefits from the natural resources and increase the possibility of their safe and efficient use in future for various ailments. In this study, *T. populnea* leaf extract was screened for antibacterial activity by agar well diffusion and micro broth dilution methods. Our finding shows significant results as presented in tables 5 and 6 that ethanol extract of *T. populnea* is found to be bactericidal. *T. populnea* exhibited remarkable antibacterial activity against both gram positive and gram negative bacteria and relatively highest effect was observed against *S. aureus* (33.6 mm) followed by *K. pneumonia* (30 mm) and *E. coli* (20.6 mm). *B. subtilis* and *S. typhimurium* were not susceptible to the extract (table 5). Each bacterial strain has a different intrinsic growth rate and susceptibility to antibiotics and therefore diameter of inhibition zone may vary according to the strains, species and concentra-

Table 5: Antibacterial activity of *T. populnea*

		Zone of growth inhibition (mm)				
Test Compound	Conc. (µg/ml)	*S. aureus*	*E. coli*	*K. pneumoniae*	*B. subtilis*	*S. typhimurium*
T. populnea	50	9.66 ± 0.27	10.33 ±0.33	11.66 ± 0.27	-	-
	100	12.33 ± 0.33	12 ±0.47	20 ± 0.47	-	-
	150	16 ± 0.47	13.66 ±0.27	21.33 ± 0.63	-	-
	200	16.33 ± 0.54	19.66 ±0.72	30.66 ± 0.72	-	-
	250	33.6 ± 0.72	20.66 ±0.7	30 ± 0.47		
Gentamicin	250	40.66 ± 0.27	38 ± 0.94	25 ± 0.90	26.6 ± 0.7	42.6 ± 1.18

tion of antibacterial agents. In terms of MIC values (table 6), *T. populnea* is more potent against *S. aureus* (40 µg/ml) and *K. pneumonia* (43 µg/ml) than *E. coli* (70 µg/ml). Gentamicin used as reference standard was potent against all bacterial strains.

Table 6: MIC of *T. populnea*

Test compound	MIC (µg/ml)				
	S. aureus	*E. coli*	*K. pneumoniae*	*B. subtilis*	*S. typhimurium*
T. populnea	40	70	43	-	-
Gentamicin	36	41	48	61	33

In conclusion, from the results of the present investigation, it could be inferred that *T. populnea* leaf is found to have significant antioxidant and broad spectrum of antibacterial activities. Phytochemical screening and GC-MS study substantiate that *T. populnea* leaves contain pharmacologically active principles. *T. populnea* leaves can be used as source of nutraceuticals.

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