



## GC-MS ANALYSIS, PHYTOCHEMICAL STUDIES AND ANTIMICROBIAL COMPOUNDS FROM BARK OF *THESPESIA POPULNEA* (L) SOLAND EX CORREA

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### ABSTRACT

The phytochemical analysis and antimicrobial compounds by GC-MS analysis from bark of *Thespesia populnea* were studied. The confirmation of antimicrobial activity of bark extract was done by agar well diffusion method. Active compounds were extracted by ethanol solvent extraction method. The presence of active compounds were confirmed by UV-Spectrophotometer, FT-IR and GC-MS analysis and it was confirmed that the presence of Methyl salicylate, Hexadecanoic acid- ethyl ether and Oleic acid. The results of this study suggested that the method adopted for extracted active metabolites and can be fruitfully employed for obtaining novel antibiotic compounds to treat various bacterial and fungal diseases.

**KEYWORDS:** *Thespesia populnea*, GC-MS, FT-IR, antimicrobial, and phytochemical .



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## INTRODUCTION

Most Immuno compromised persons are frequently affected from skin diseases that are very difficult to cure. The plants were effective in the treatment of infectious diseases while simultaneously mitigating side effects associated with synthetic antimicrobials<sup>1</sup>. *Thespesia populnea* is under the family Malvaceae and also known as Indian tulip tree. The bark possess astringent, hepato-protective and antioxidant activity<sup>2</sup>. The trees well grows under the full sunlight and tolerates drought climatic conditions. The tree has valuable as coastal windbreak because it is it highly resistant to wind. It propagates easily and grows rapidly in all places. Therapeutically active principles are extracted from all parts of the plant body, but the concentration of these components varies from part to part. Normally the 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, woods, flowers, fruits or the seeds<sup>3</sup>. Many medicinal plants possesses diverse active principles and are useful as curative in various human and animal diseases. The continuing use of herbs in medicine reveals that the functional value and its necessity in the future. In modern medicine, the importance of medicinal plants was increasing with pharmaceutical and cosmetic industries and progressively uses more plant sources of rural or untainted areas<sup>4</sup>. Four naturally occurring quinines are thespone, thespesone, mansonone-D and mansonone-H have been extracted from heart wood of *Thespesia. populnea*. The phytochemical study of bark of this plant reveals that the presence of gossypol, tannin, acacetin, quercetin, coloring matter. A compound oil of bark and capsules were useful in urethritis and gonorrhoea. The astringent bark, root and fruits are stated to be used in dysentery, cholera, hemorrhoids and the mashed bark is employed as a hot fomentation for wounds<sup>5</sup>. The aim of the present study was to evaluate the presence of compounds in bark extracts and antimicrobial activity of methanolic bark extracts of *Thespesia populnea* against bacterial and fungal strains of medical importance.

## MATERIALS AND METHODS

### Processing of bark sample

*Thespesia populnea* bark were collected from Sathyabama university garden, Chennai situated in the state of Tamil Nadu. The healthy plant materials like bark were washed with running tap water followed with 0.1% of sodium hypochlorite solution simultaneously and sterile distilled water. After washing the healthy plant parts are shade dried for two weeks and pulverized to coarse powder using electric mixer grinder.

The powder was then sieved and stored in airtight bottles for further studies<sup>6</sup>.

### Bark extraction

The dried bark powder was subjected to extraction using ethanol as a solvent. Powders with solvent kept in a soxhlet extractor for 72hrs. The aqueous extract was prepared by maceration with distilled water. The extracts were stored in airtight containers in refrigerator below 10°C for further use<sup>7</sup>.

### Phytochemical analysis of bark extracts

The ethanolic bark extracts of *Thespesia populnea* were used for qualitative phytochemical analysis. Phytochemicals such as carbohydrates, proteins, flavonoids, tannins, phytosterols, glycosides, saponins, phenols, terpenoids and alkaloids were analyzed according to the standard method<sup>8</sup>.

### Antimicrobial activity

Antibacterial and antifungal activity was carried out by agar well diffusion method and it was evaluated by measuring the Zone of inhibition. Ethanolic bark extract was tested against selected bacterial and fungal strains. The test cultures were evenly spread over on agar plates using a sterile cotton swab. The sterile wells were filled with 50µg/ml bark extract. Bacterial test plates were incubated at 37°C for 24hrs and fungal plates were incubated at 28°C for 72hrs and the zones of inhibition were subsequently measured in mm. Ciprofloxacin (5µg/ml) and Amphotericin B (10µg/ml) was used as a positive control and respective solvents served as negative control<sup>9</sup>.

### Identification of active compounds

The presence of active compounds were identified by using UV-spectrophotometer in the range from 200nm to 400nm. FT- IR spectrometer analysis for identification of functional groups of compounds in the spectral range of 4000 – 50 cm<sup>-1</sup>. Gas chromatography-mass spectrometry (GC-MS) method used to identify chemical compounds present in the bark extract<sup>10</sup>.

## RESULTS AND DISCUSSION

### Phytochemical analysis

The plant bark part were collected and surface sterilized successfully. After the hot soxhlet extraction of the bark extract was investigated for the preliminary phytochemical study. It was revealed that ethanolic bark extract of *Thespesia populnea* contains carbohydrates, proteins, flavonoides, saponins, phenols, terpenoids and alkaloids. Phytosterols, tannins and glycosides were absent in the extract shown in Table 1.

**Table 1**  
**Phytochemical characterization of Bark extracts**

S.No	Phytoconstituents	Ethanol
1	Carbohydrates	+
2	Proteins	+
3	Flavonoids	+
4	Tannins	-
5	Phytosterols	-
6	Glycosides	-
7	Saponins	+

8	phenols	+
9	Terpenoids	+
10	Alkaloids	+

- = Absence , + = presence

### Antimicrobial activity

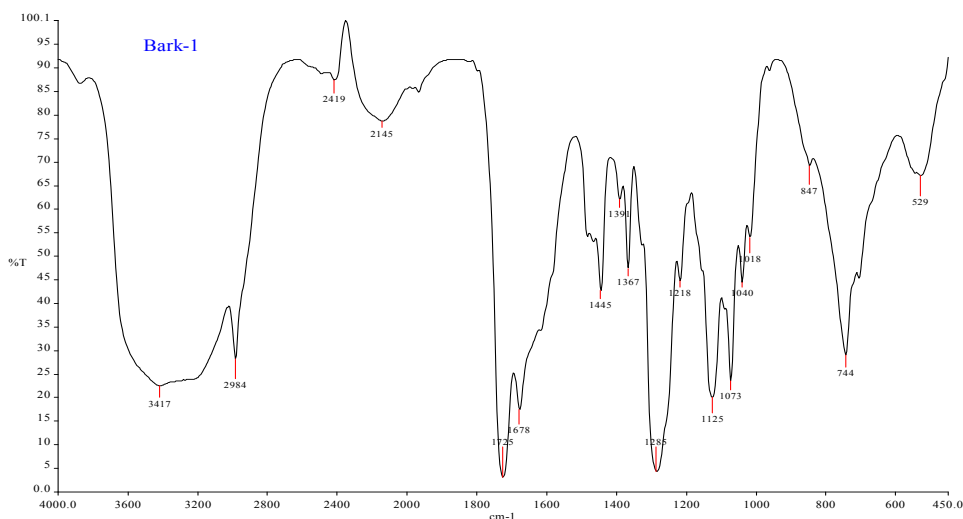
The zone of inhibition obtained with ethanolic bark extracts of *Thespesia populnea* shown in Table 2. The diameter of the zone of inhibition for each strain was recorded as per <sup>11</sup>. Ethanolic bark extracts were showed significant activity against *Escherichia coli* for bacteria and *Aspergillus flavus* for fungal strains.

**Table 2**  
Antimicrobial activity of bark extract (50µg/ml) of *Thespesia populnea*  
Mean zone of inhibition (mm)

S.No	Test Organisms	Ethanolic bark extract	Ciprofloxacin (5µg/ml)
1	<i>Streptococcus pyogenes</i>	13.5±0.50	16.8±0.76
2	<i>Escherichia coli</i>	14.5±0.50	19.5±0.50
3	<i>Staphylococcus aureus</i>	12.5±0.50	15.5±0.50
4	<i>Pseudomonas aeruginosa</i>	9.5±0.50	11.5±0.50
<b>Amphotericin B (10µg/ml)</b>			
5	<i>Aspergillus niger</i>	15.8±0.76	19.5±0.50
6	<i>Aspergillus flavus</i>	16.5±0.50	20.5±0.50

### Identification of active compounds

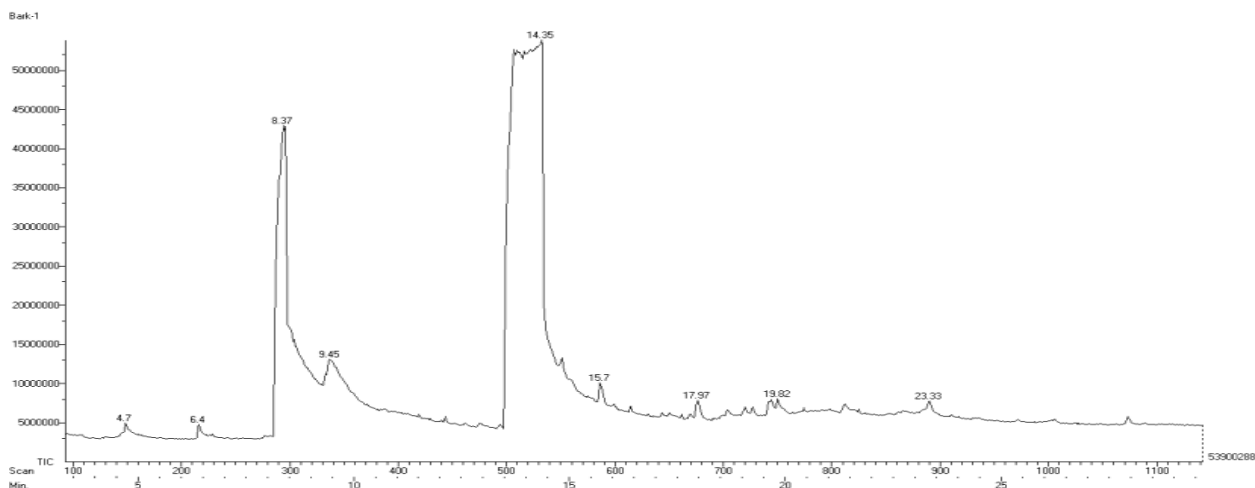
The ethanolic bark extracts were showed peak at 342 nm in UV-Visible spectrophotometer. The functional groups present in the ethanolic extracts of bark were identified by FT-IR (Table 3 and Fig 1 ). The mass spectrum was recorded using Agilent GC-MS-5975C Mass spectrometer under the current (MA) 100 and the temperature at 70°C. The compound was quantified using Gas Chromatograph (Shimadzu QP 2010) equipped with a VF-5 ms column (diameter 0.25 mm, length 30m, film thickness 0.25µm) mass spectrometer (ion source 2000C; EI -70 eV), programmed at temperature 40 - 650°C with a rate of 4°C/min. Injector flow rate was 200°C; carrier gas was He 99.9995% purity, column flow rate 1.51ml/min, injection mode-split. The compounds present in the ethanolic bark extract of *Thespesia populnea* were identified by GC-MS analysis. The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) and peak area in percentage are presented in Table 4. The result revealed that the presence of 9 major compounds (Fig 2). Similar observation was reported <sup>12</sup> in which the phyto-constituent rich ethanolic extract of *Maranta arundinacea*. L subjected to GC-MS analysis revealed the presence of 49 compounds.. The results of the GC-MS analysis provide 9 major peaks determining the presence of phytochemical compounds with different therapeutic activities.



**Figure 1**  
FT-IR analysis of Bark extracts

**Table 3**  
FT-IR report of Bark extracts

Bond	Functional groups	Frequency range/ cm <sup>-1</sup>
N-H	Amines, Amides	3417
O-H	Carboxylic acids, Alkanes and Alkyls	2984
C-H	Ketones , Aldehydes, Esters.	1725
C-H	Amides , Ketones	1678
C=O	Alkenes, esters.	1285
C=O	Alkenes, alcohols, ethers.	1125
C=O	Alkyl halides, alcohols, ethers.	1073



**Figure 2**  
Gas chromatography – mass spectrometry analysis of Bark extracts

**Table 4**  
Phytochemicals present in the Bark of *Thespesia populnea* using GC – MS

Peak No	R.Time	Name of the compound	Molecular formula	Mol.Wt.	Area%	Activity
1	4.7	Oxime methoxy phenyl	C <sub>8</sub> H <sub>9</sub> NO <sub>2</sub>	151	0.70	Antimicrobial
2	6.4	2-carbomyl-3-methylbutanoic acid	C <sub>9</sub> H <sub>17</sub> NO <sub>3</sub>	187	0.68	Antibacterial
3	8.37	Methyl salicylate	C <sub>8</sub> H <sub>8</sub> O <sub>3</sub>	152	4.25	Antimicrobial
4	9.45	1,4-benzene dicarboxylic acid,diethyl ether	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>	222	1.30	Antimicrobial
5	14.35	Hexadecanoic acid, ethyl ether	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284	5.50	Antibacterial and antifungal
6	15.7	Oleic acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282	1.05	Antibacterial <sup>13</sup>
7	17.97	Androst 5,7-dine-3-ol-17-one	C <sub>19</sub> H <sub>26</sub> O <sub>2</sub>	286	0.95	Anticandidal
8	19.82	Benzoic acid,2-hydroxy,ethyl ether (ethyl salicylate)	C <sub>9</sub> H <sub>10</sub> O <sub>3</sub>	166	0.90	Antimicrobial, antioxidants <sup>14</sup>
9	23.33	18,19secoyohimban 19-oic acid 16,17,20,21-tetrahydro-16-(hydroxymethyl),methyl ester,(15a,16e)	C <sub>28</sub> H <sub>30</sub> O <sub>10</sub>	526	0.83	Antibacterial and antifungal <sup>15</sup>

## CONCLUSION

The results of this study revealed that ethanolic bark extracts of *Thespesia populnea* exhibited strong antimicrobial activity against bacterial and fungal strains. The clinical effect of this plant part will be helpful to pharmaceutical research to prepare antibiotics for treating various microbial infectious diseases.

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## CONFLICT OF INTEREST

Conflict of interest declared none.

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