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PHYTOCHEMICAL SCREENING IN n-HEXANE/ETHYL ACETATE EXTRACT OF *THESPESIA POPULNEA* (L.) STEM BARK

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ABSTRACT

The present study was planned to identify the chemical compounds present in the n-hexane-ethyl acetate (80:20 v/v) extract of *Thespesia populnea* (L.) stem bark by using GC-MS technique and its activities were compared using phytochemical and ethnobotanical databases. The chemical compounds were investigated using gas chromatography-mass spectrometry, and the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. A total of 24 compounds were identified as hydrocarbons, carbohydrates, fatty acid, fatty acid ester, alcoholic compounds, alkaloids, terpenoids, ketones, alkenes etc. These different active phytochemicals have been found to possess a wide range of pharmacological activities.

Key words: *Thespesia populnea* (L.) stem bark, n-Hexane-ethyl acetate solvent, Phytochemical analysis.

INTRODUCTION

Natural products have provided biologically active compounds for many years and many of today's medicines are either obtained directly from natural sources or were developed from a lead compound originally obtained from a natural source. *T. populnea* (L.) Linn. (Fam. Malvaceae), a fast growing, medium-sized evergreen tree, distributed throughout coastal forests of India is also largely grown as a roadside tree. The plant *Thespesia populnea* traditionally claimed to be useful in the treatment of cutaneous affections such as scabies, psoriasis, ringworm, guinea worm, eczema and herpetic diseases. *T. populnea* ground up bark is used to treat skin diseases (India), dysentery and hemorrhoids (Mauritius). Oil prepared by boiling the ground bark in coconut oil is applied externally in psoriasis and scabies. The plant contains glycosides such as quercetin, gossypol, β-sitosterol[1] and sesquiterpene. Thespesenone and dehydrooxoperezinone-6-methyl ether were isolated

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from the red hard wood of *Thespesia populnea*[2]. Alanine, arginine, methionine and tryptophan were isolated from seed of *Thespesia populnea*[3]. It also contains lupenone and lupeol[4]. The ethanolic extract of *Thespesia populnea* bark has been reported to show antiinflammatory and analgesic activity[5]. Aqueous and methanolic extracts of the *Thespesia populnea* showed antioxidant activity against carbon tetrachloride induced liver injury in rats[6]. *Thespesia populnea* is also recommended for antifertility activity, wound healing[7], antifungal and hepatoprotective activity. Metal oxides play a very important role in many areas of chemistry, physics and material science. The metals are able to form a large diversity of oxide compounds. In technological applications, oxides are used in the fabrication of microelectronic circuits, sensors, piezoelectric devices and fuel cells, coatings for the passivation of surfaces against corrosion and as catalysts[8-23]. Rao et al.[24-47] have reported their work on different oxide materials in their earlier studies. The present research is an attempt to investigate more phytochemical compounds in stem bark of *Thespesia populnea* with n-hexane: ethyl acetate (80:20 v/v).

EXPERIMENTAL

Materials and methods

The *Thespesia populnea* (L.) stem bark (Fig. 1) used for the present study was collected from Autonagar, Vijayawada, India (80°40'17.55"E/16°29'33.4"N). All Chemicals used in the entire study were AR grade and obtained from SD fine chemicals, India, Pvt. Ltd. The plant material was authenticated at the Botanical Survey of India, Howrah and West Bengal, India.

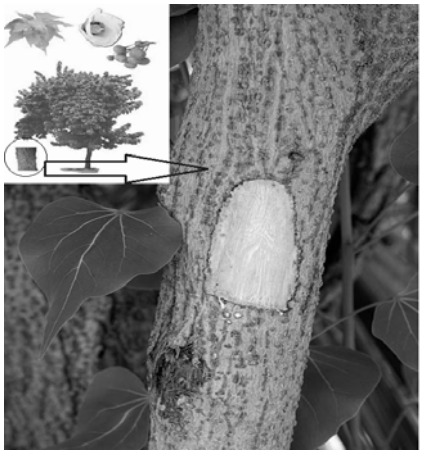


Fig. 1: Stem bark of *T. Populnea* (L.) (Inset total plant, flower, leaf) Source (https://www.pinterest.com/pin/383439355750005690/)

Solvent extract

Fresh plant barks were collected and air-dried in shade at room temperature. The dried barks were powdered by using a kitchen blender. The dried stem bark material (100 g) of *Thespesia populnea* was pulverized using pestle and mortar and 15 g of the ground sample was placed inside the Soxhlet extractor with 90 cm^3 of the n-hexane: ethyl acetate (80:20 v/v) as the extracting solvent. The temperature of the heating mantle was adjusted to cover the range of 40-60^oC to keep the solvent volatile enough and to get semisolid sticky residue (5 g).

Column chromatography

n-Hexane: ethyl acetate (80:20 v/v) extract of the plant material (10 g) was subjected to column chromatography using silica gel (80 -120 #) as adsorbent and eluted with the mixture of n-hexane: water (80:20 v/v) in gradient manner. n-Hexane: chloromethane (90: 10 v/v) fraction yielded dark brown color semi-solid.

GC-MS analysis

GC-MS analysis of the extract was performed using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a Elite-I, fused silica capillary column (30 mm x 0.25 mm 1D x 1 μMdf, composed of 100 % dimethyl polysiloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999 %) was used as the carrier gas at constant flow rate 1 mL/min and an injection volume of 2 μL was employed (split ratio of 10:1); injector temperature 250°C; and ion-source temperature 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 min.

Identification of components

Identification was based on the molecular structure, molecular mass and calculated fragments. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name, molecular weight and structure of the components of the test materials were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The spectrum of the unknown component was compared with the spectrum of the component stored in the NIST library version (2005), software, Turbomas 5.2. This is done in order to determine, whether this plant species contains any individual compound or group of compounds, which may substantiate its current commercial and traditional use as an herbal medicine?

Table 1: List of phytoconstituents in n-hexane:ethyl acetate extract of T. Populnea (L.) stem bark

S. No.	RT	Constituents (molecular formula/Molecular weight)	M.W.	Molecular structure	Peak area (%)	Medicinal value#
1	2.75	Octanoic acid	144	$C_8H_{16}O_2$	2.02	Straight chain fatty acid
2	3.78	1,14-Tetradecanediol	230	$C_{14}H_{30}O_2$	2.21	Antimicrobial
3	4.15	Dodecanoic acid, methyester (CAS)	214	$C_{13}H_{26}O_2$	6.21	Antieczemeic
4	6.07	2-Methoxy-4-formylphenol	152	$C_8H_8O_3$	3.2	Antioxidants
5	6.77	n-Dodecanoic acid (Lauric acid)	200	$C_{12}H_{24}O_2$	1.05	Saturated medium-chain fatty acid
6	8.01	Cedran-diol, 8S,14-	238	$C_{15}H_{26}O_2$	1.03	Antimicrobial, antiinflammatory
7	9.50	2-Pentanone, 1-(2,4,6-trihydroxyphenyl)	210	$C_{11}H_{14}O_4$	1.14	-
8	10.53	Tetradecanoic acid; Myristic acid	228	$C_{14}H_{28}O_2$	24.35	Myristic acid is a saturated long-chain fatty acid
9	11.10	9, 12-Octadecadienoic acid (Z,Z)-(Synonyms: Linoleic acid)	280	$C_{18}H_{32}O_2$	0.51	Antiinflammatory, insectifuge hypocholesterolemic, cancer preventive, nematicide, hepatoprotective, insectifuge,
10	11.93	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	295	$C_{20}H_{40}O$	0.8	Antimicrobial
11	13.17	Hexadecanoic acid	256	$C_{16}H_{32}O_2$	1.84	Antioxidant, hypocholesterolemic nematicide, pesticide, lubricant, antiandrogenic, flavor, hemolytic
12	14.29	3-O-Methyl-d-glucose	194	$C_7H_{14}O_6$	2.6	Preservative
13	14.71	5-Naphthalen-1-yl-2H-pyrazol-3-ol	210	$C_{13}H_{10}N_2O$	1.94	-
14	15.31	Tetradecanoic acid	228	$C_{14}H_{28}O_2$	0.84	Antioxidant

Cont…

S. No.	RT	Constituents (molecular formula/Molecular weight)	M.W.	Molecular structure	Peak area (%)	Medicinal value#
15	16.06	14-Cyclopropyltetradecanoic acid	394	$C_{26}H_{50}O_2$	0.94	Antioxidant
16	17.24	Ledene oxide-(I)	220	$C_{15}H_{24}O$	42.24	Anti-tumor, analgesic antibacterial, anti-inflammatory sedative, fungicide
17	17.59	Ethyl isoallocholate	436	$C_{26}H_{44}O_5$	2.34	Antibacterial, antioxidant, anti-tumer, cancer preventiv
18	17.82	Hexanoic acid pentadecyl ester	326	$C_{21}H_{42}O_2$	0.84	Antioxidant
19	18.67	Oleic acid	282	$C_{18}H_{34}O_2$	1.05	Antioxidant
20	20.09	1,2-Benzenedicarboxylic acid, Diisooctyl ester	390	$C_{24}H_{38}O_4$	0.94	Antifouling Antimicrobial
21	21.41	Squalene	410	$C_{30}H_{50}$	1.09	Antibacterial, antioxidant, pesticide, antitumor, cancer preventive, immunostimulant, chemo preventive, lipoxygenase-inhibitor
22	22.52	Lupeol	426	$C_{30}H_{50}O$	0.4	Antimalarial, antioxidant, antiflue antihyperglycemic, antitumor antiviral, pesticide, cytotoxic antiinflammatory
23	23.80	1H-Perimidine, 2,3-dihydro-2-(2,4,5-trimethoxyphenyl)-	336	$C_{20}H_{20}N_2O_3$	0.22	Antimicrobial antioxidant antiinflammatory
24	27.88	7,8-Dimethoxy-13-carbomethoxy-15-(3,4,5-trimethoxybenzoxy) 13, 14-didehydroalloberban	567	$C_{31}H_{37}NO_9$	0.2	Antimicrobial

#**Source: Dr. Duke's phytochemical and ethnobotanical databases [Online database]

Further it helps to determine the most appropriate methods of extracting these compounds. These results will consequently be discussed in the light of their putative biological or therapeutic relevance. The name, molecular weight and structure of the components of the test materials were ascertained (Table 1 & Fig. 2).

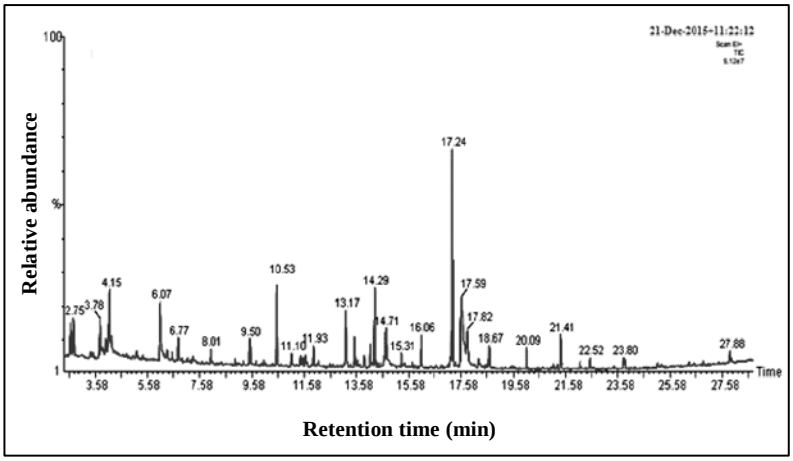


Fig. 2: GC-MS chromatogram of *T. populnea* (L.) stem bark

Preliminary phytochemical screening

Initially, the extract was subjected to qualitative analysis for various phytochemical constituents including alkaloids, carbohydrates, steroids, proteins, phenols, tannins, flavonoids, glycosides, saponins, terpenes, etc.

Qualitative analysis of plant extract

Different qualitative chemical tests were performed for establishing the chemical profile of the extract. The following tests were performed to detect various phytoconstituents present in extracts[48].

Detection of alkaloids

50 mg of solvent free extract was stirred with few mL of dilute hydrochloric acid and filtered. To a few milliliter of filtrate, a drop or two of Mayer's reagent (1.36 g of mercuric chloride and 5 g of potassium iodide in 100 mL distilled water) was added along the sides of the test tube. The formation of white or creamy precipitate indicates the test as positive.

Detection of carbohydrates and glycosides

100 mg of the extract was dissolved in 5 mL of water and the filtrate was collected. To 2 mL of filtrate, two drops of alcoholic α-naphthol solution was added, the mixture was subjected to vigorous shaking and 1 mL of concentrated sulphuric acid was added slowly along the sides of the test tube and allowed to stand. A violet ring indicates the presence of carbohydrates.

Detection of saponins

50 mg of extract was diluted with distilled water and made up to 20 mL. Then the suspension was shaken in a graduated cylinder for 15 min. The formation of 2 cm layer foam indicates the presence of saponins.

Detection of phenolic compounds

50 mg of extract was dissolved in 5 mL of distilled water and few drops of neutral 5% (w/v) ferric chloride solution were added. Formation of deep blue or black colour indicates the presence of phenolic compounds.

Detection of tannins

50 mg of extract was dissolved in water and, heated on a water bath for 1 hr followed by treating with 10% (w/v) ferric chloride. Formation of blue or dark greenish grey colour indicates the presence of tannins.

Detection of terpenoids (Salkowski test)

0.2 g of the extract of the plant sample was mixed with 2 mL of chloroform followed by the addition of concentrated H_2SO_4 (3 mL). A reddish brown coloration in the interface indicates positive results for the presence of terpenoids.

Detection of flavanoids

The extract was treated with sulphuric acid and observed for the formation of orange colour, which indicates the presence of terpenoids.

Detection of sterols

1 mL of extract was treated with chloroform and acetic anhydride followed by adding few drops of H_2SO_4. The formation of dark pink or red colour indicates the presence of sterols.

Detection of anthraquinones

About 50 mg of methanolic extract was heated with 10% (w/v) ferric chloride solution and 1 mL of concentrated hydrochloric acid. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia. The formation of pink or deep red coloration of aqueous layer indicates the presence of anthraquinones.

RESULTS AND DISCUSSION

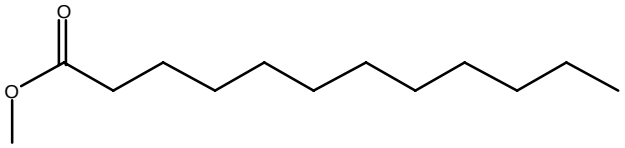
GC-MS analysis

Traditional medicine also known as indigenous or folk medicine comprises medical knowledge systems that developed over generations within various societies before the era of modern medicine. Traditional medicines are prepared from a single plant or combination of more than one plants. Phytochemical constituents are responsible for medicinal activity of plant species. Hence, in the present study, preliminary phytochemical screening of *T. populnea* (L.) stem bark a medicinal plant, was carried out. Qualitative phytochemical analysis of this plant confirms the presence of various secondary metabolites like alkaloids, glycosides, tannins, saponin, flavonoids, steroid, triterpenes and phenols.

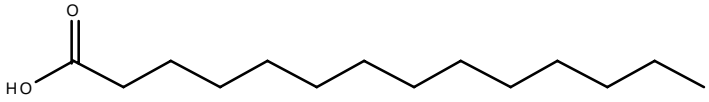
The phytochemical screening of *Thespesia populnea* stem bark extract revealed the existence of flavonoids, phenols, steroids, saponins and tannins etc., (Table 3). The strong antibacterial and antifungal activity was due to the presence of tannins. The flavonoids, phenols, steroids and saponins are found to be used in synthetic drugs as starting materials. Alkaloids are significantly used as anesthetics, stimulants, analgesics and antibacterial. GC-MS chromatogram of the n-hexane: ethyl acetate stem bark extract of *T. populnea* (L.) (Fig. 2) showed 24 peaks indicating the presence of 24 phytochemical constituents. On comparison of the mass spectra of the constituents with the NIST library[49], the 24 phytoconstituents were characterized and identified and listed in Table 1. The various phytochemicals, which contributes to the medicinal activity of the plant and similar possible structure are presented in Table 2. The major compounds present in the bark were methyl dodecanoate (6.21), tetradecanoic acid (24.35%), ledene oxide (I) (42.24%), 3-O-methyl-d-glucose (2.6%), ethyl isoallocholate (2.34%) etc. Other major and minor compounds were also present (Table 1). The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. In addition to this, the results of the GC-MS profile can be used as pharmacognostical tool for the identification of the plant compounds with different chemical structures. The presence of various bioactive compounds confirms the application of *Thespesia populnea* stem bark for various ailments by traditional

practitioners. However, isolation of individual phytochemical constituents may proceed to find a novel drug.

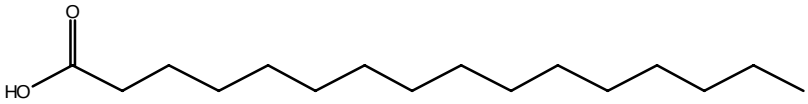
Table 2: Structures of major compounds and elemental composition in n-hexane: ethyl acetate extract of *T. Populnea* (L.) stem bark



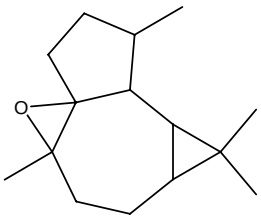
$C_{13}H_{26}O_2$
Exact Mass: 214.19
Mol. Wt.: 214.34
m/e: 214.19 (100.0%), 215.20 (14.4%), 216.20 (1.4%)
C, 72.84; H, 12.23; O, 14.93
Methyl dodecanoate



$C_{14}H_{28}O_2$
Exact Mass: 228.21
Mol. Wt.: 228.37
m/e: 228.21 (100.0%), 229.21 (15.2%), 230.22 (1.1%)
C, 73.63; H, 12.36; O, 14.01
Tetradecanoic acid

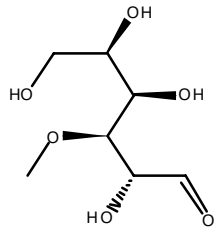


$C_{16}H_{32}O_2$
Exact Mass: 256.24
Mol. Wt.: 256.42
m/e: 256.24 (100.0%), 257.24 (17.4%), 258.25 (1.5%)
C, 74.94; H, 12.58; O, 12.48
Palmitic acid



$C_{15}H_{24}O$
Exact Mass: 220.18
Mol. Wt.: 220.35
m/e: 220.18 (100.0%), 221.19 (16.5%), 222.19 (1.5%)
C, 81.76; H, 10.98; O, 7.26

Ledene oxide-(I)



$C_7H_{14}O_6$
Exact Mass: 194.08
Mol. Wt.: 194.18
m/e: 194.08 (100.0%), 195.08 (7.8%), 196.08 (1.2%)
C, 43.30; H, 7.27; O, 49.44

3-O-Methyl-d-glucose

Table 3: Qualitative analysis of plant extract

1	Alkaloids	+
2	Glycosides	+
3	Tannins	+
4	Saponins	+
5	Flavonoids	+
6	Steroid	+
7	Triterpenes	+
8	Phenol	+
9	Fatty acids	+
10	Anthraquinones	-

CONCLUSION

The medicinal plants appear to be rich in secondary metabolites, widely used in traditional medicine to combat and cure various ailments. The anti-inflammatory, antispasmodic, analgesic and diuretic properties can be attributed to their high alkaloids, phenols, tannins and flavonoids. In conclusion, from the results of the present investigation, it could be inferred that *T. populnea* stem bark is found to have significant medicinal activities. Phytochemical screening and GC-MS study substantiate that *T. populnea* stem bark contains pharmacologically active principles. The phytochemical analysis of the extracts revealed the existence of various constituents including flavonoids, phenols, steroids, saponins and tannins. The active constituent needs to be isolated and should be considered for further *in-vivo* or *in vitro* studies to confirm the tradition.

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