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Assessment of pharmacognostic and phytochemical standards of *Thespesia populnea* (L.) root

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ABSTRACT

Objective: To Assessment of pharmacognostic and phytochemical parameters of root of *Thespesia populnea* (L.) soland. **Methods:** Macroscopical, miscroscopical, physico-chemical evaluation, florescence analysis, behavior of root powder and preliminary phytochemical analysis, quantitative estimation of phytoconstituents were determined of various extracts of *T. populnea*. **Results:** microscopic study shows the general characteristic of root with presence of periderm, cortex, xylem and phloem region, abundant starch grains. Physico-chemical investigation shows the total ash, acid insoluble ash, water soluble ash and sulphated ash values were 10.59±0.02 % w/w, 1.02±0.03 % w/w, 2.72±0.03 % w/w and 8.56±0.02 % w/w respectively. However, the aqueous soluble, alcohol soluble extractives and moisture content were found to be 14.23±0.46 % w/w, 10.54±0.23 % w/w and 4.60±0.01% w/w respectively. The preliminary phytochemical assessment revealed the presence of glycosides, steroid/triterpenoids, flavonoids, tannins, phenolic compounds saponins, carbohydrates and proteins. Total phenolic and flavonoids content of root was 0.271% and 0.344% respectively. Behavior of root powder with various chemicals confirmed the presence of phytoconstituents. **Conclusions:** The pharmacognostic and phytochemical assessment of *T. populnea* may helpful towards founding for quality, purity and sample identification and standardization.

1. Introduction

Thespesia populnea (L.) Soland ex Correa (family: Malvaceae) is a large avenue tree found in the tropical regions and coastal forests in India. The bark, leaves, flowers and fruits are useful in cutaneous infections such as scabies, psoriasis, eczema, ringworm and guinea worm [1]. A decoction of the bark is commonly used for the treatment of skin and liver diseases. Oil of bark mixed with vegetable oil is useful in urethritis and gonorrhea [2]. The bark, roots and fruits were used in dysentery, cholera and hemorrhoids; bark is employed as a poultice for wounds. In the indigenous system of medicine, the paste of fruits, leaves and roots are applied externally for various skin diseases. The leaves are applied locally for their anti-inflammatory effects in swollen joints [3]. The alcoholic seed extract was also evaluated for hypoglycemic and anti-hyperglycemic [4], astringent, hepatoprotective [5] and antioxidant activity in rats [6]. The seed extract and its fractions were evaluated for analgesic and antipyretic activity in mice [7]. A polyherbal formulation containing *T. populnea* as one of the ingredient was shown useful remedy for Alzheimer's disease [8]. Four naturally occurring quinines, viz. thespone, mansonone-D, mansonone-H, and thespesone, have also been extracted from heartwood of *T. populnea* [9]. Much work has been done and reported on various plant parts especially on bark and leaves.

As literature survey and scientific data revealed that a large number of indigenous drugs have already been investigated as regards their botany and chemistry is concerned, however a systematic standardization including pharmacognostical and physico-chemical study is still lacking. The present investigation of *Thespesia populnea* (L.) is therefore taken up to evaluate certain botanical and chemical standards which would help in crude drug identification as well as in checking adulteration, if any. Further the study will greatly help in quality assurance of finished product of herbal drugs.

2. Material and methods

2.1 Plant material

The plant material was obtained from Nasik district (M.S.) and authenticated by Dr. D. A. Patil, reader and the

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authorized plant identifier of Department of Botany, SSVPS College, North Maharashtra University, Dhule (M.S) India; a specimen is preserved in the college herbarium (KBHSS/PCG/2009/12).

2.2 Macroscopic and microscopic examination

Macroscopic studies were done using simple microscope. The color, shape, size, taste and odour of root were determined. Microscopic study was carried out by preparing of thin hand section (longitudinal and transverse) of root. The sections were cleared with chloral hydrate and stained with concentrated hydrochloric acid – phloroglucinol (1:1). Powdered drug was separately treated with concentrated hydrochloric acid – phloroglucinol, iodine solution, 60% sulphuric acid for identification of lignified elements, starch grains, calcium oxalate crystals in the powdered root by reported methods [10, 11].

2.3 Physical constant study

The Physicochemical parameters of the powdered drug such as total ash, water-soluble ash, acid-insoluble ash and sulphated ash were determined. Alcohol and water soluble extractives values were determined to find out amount of water and alcohol soluble components. The moisture content was detected by loss on drying method [12].

2.4 Fluorescence analysis

Powdered material was analyzed under visible light, short ultra- violet light, long ultra- violet after treatment with various organic/ inorganic reagents like NaOH, HCl, HNO_3 and H_2SO_4 was also carried out for the powder [13].

2.5 Behaviour of root powder

Behaviors of root powder of *T. populnea* with different chemical reagent were performed to detect the occurrence of phytoconstituents along with color changes under ordinary day light by standard method [14].

2.6 Preparation of extracts

The collected root was sun dried for 5 days and pulverized into a dry powder. The powder was subjected to extraction in soxhlet extractor using petroleum ether, ethyl acetate, methanol and water for 72h. The extracts were filter and each filtrate was evaporated by distillation under reduced pressure using rotary vacuum evaporator at 30^0C and stored [15].

2.7 Phytochemical screening

The obtained extracts were dried and weighed. The presence of various phytoconstituents viz. steroids and terpenoids (Leibermann Burchard test), alkaloids (Dragendroffs test), tannins and phenolics (Ferric chloride test), flavonoids (Shinoda test), Sugars (Fehling solution test), amino acids (Ninhydrin test), etc. was detected by usual methods prescribed in standard texts [16].

2.8 Quantitative estimation of phytoconstituents

2.8.1 Estimation of total phenolic content

The phenolic content was determined as per the method described in Khadbadi [17]. The Folin- Ciocalteu reagent (FCR) also called as the Gallic acid equivalent method (GAE), was preferred for the estimation.

2.8.2 Estimation of total flavonoids content

An aliquot (l ml) of standard solution of rutin (20-100 μg/ml) was added to volumetric flask containing 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and the volume was made up to 10 ml with distilled water. Same dilution was prepared with the test solution. Mixed well and take absorbance at 358 nm. The total flavonoids content was determined from calibration curve [17]

3. Results

3.1 Macroscopic examination

The root of *T. populnea* have reddish brown colour. Outer bark of root consisting of reddish brown colour with yellowish white wood, Root have characteristic odour, acrid in taste.

3.2 Microscopic examination

Root is mainly divided in to periderm, cortex, phloem and xylem.

Periderm: periderm shows the presence of tangentially elongated cork (phellum), which is non- lignified and suberinized cork, followed by phellogen which is non living, cellulosic rectangular cell. Phelloderm is the last layer which is cellulosic, live and is square in shape this is also known as primary cortex. All these collective layers form the periderm. (Fig 1)

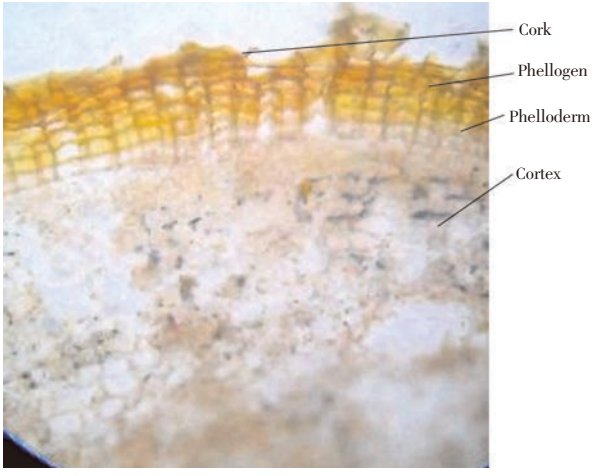

Fig 1: Transverse section of *T. populnea* root showing the cortex and periderm which consist of cork, phellogen and phelloderm.

Cortex: it is made of polygonal cellulosic loosely arranged parenchymatous cells. These are made for storage of food material. The cortex shows the abundant amount of bluish starch grains when stained with iodine solution. (Fig 2)

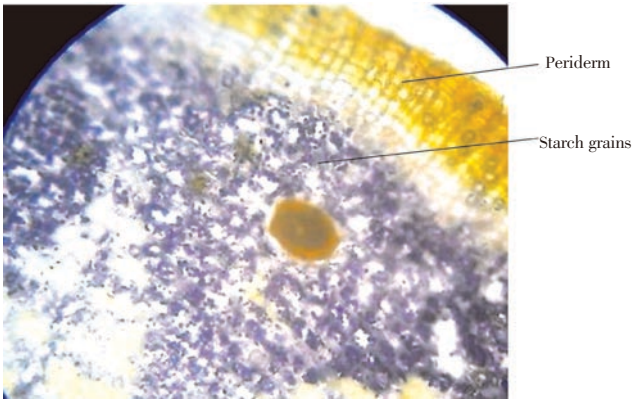

Fig 2: Transverse section of *T. populnea* root showing the presence of starch grains (blue colored) when stained with iodine solution.

Phloem region: The phloem is sandwiched between the two medullary rays. The medullary rays are cellulosic parenchymatous, they also shows the presence of starch grains. The medullary arrays are bi-serrate to multi-serrate. Phloem is well developed shows the presence of phloem fiber which are always found as group and are lignified. The phloem also shows the presence of sieve tube, companion cell and phloem parenchyma the starch is present abundantly in phloem parenchyma. (Fig 3)

The xylem region: In the xylem region similar to phloem the xylems are also guarded by bi-serrate lignified medullary rays. Xylem tissues consist of xylem vessels, xylem parenchyma, xylem tracheids and xylem fibers. Xylem vessels are larger, lignified found as solitary or in pairs; these are mainly responsible for conduction of water. Xylem fibers and xylem tracheids are present in groups. Xylem parenchyma is also lignified and stores some amount of starch. (Fig 3)

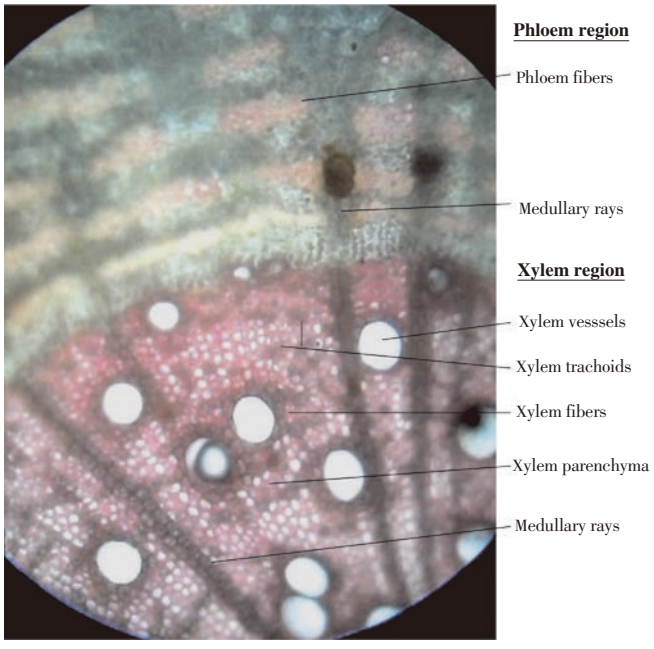

Fig 3: Transverse section of *T. populnea* root showing medullary rays, phloem and ylem region.

3.3Powder characteristic

Powder microscopy of the root of *T. populnea* showed the presence of xylem tissue, phloem fibers, medullary rays, epidermal cell, cortex parenchymatous cell, and starch grains as we seen with transverse section study. (Fig 4)

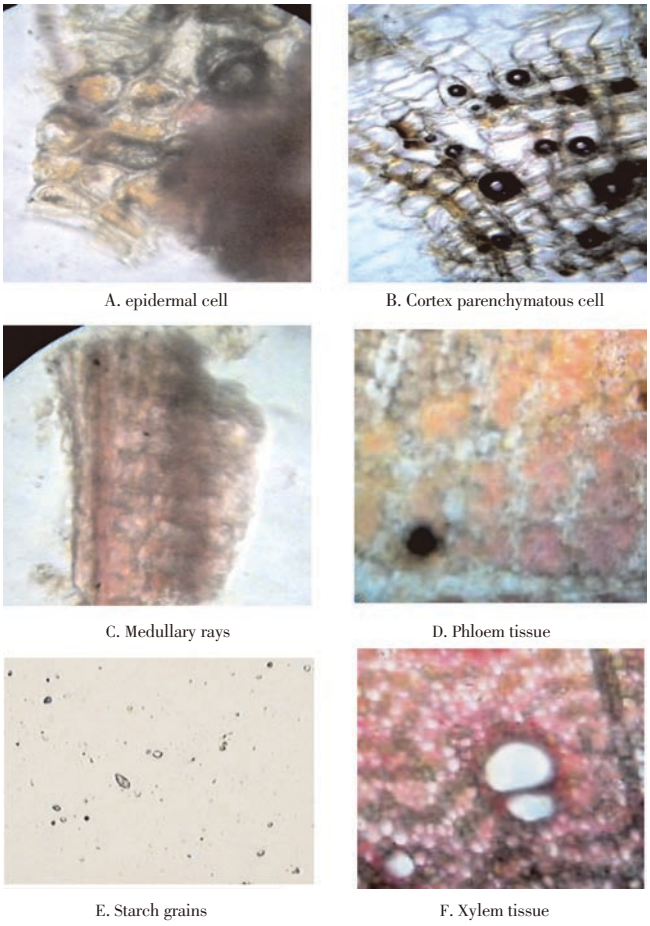

Fig 4 (A-F): Powder characteristic of *T. populnea* root.

3.4 Physical constant study

In physical constant study, the ash values, extractive values, moisture content of root were determined. The total ash, acid insoluble ash, water soluble ash and sulphated ash values were 10.59±0.02 % w/w, 1.02±0.03 % w/w, 2.72±0.03 % w/w and 8.56±0.02 % w/w respectively. However, the alcohol soluble and water soluble extractive values were found to be 10.54±0.23 % w/w and 14.23±0.46 % w/w respectively. The moisture content of root powder was 4.60±0.01 % w/w. (Table 1)

Table 1

Physical constants of *T. populnea* root

Physical parameter	Result (% w/w)
Total ash	10.59 ±0.02
Acid insoluble ash	1.02 ±0.03
Water soluble ash	2.72 ±0.03
Sulphated ash	8.56 ±0.02
Moisture content	4.60 ±0.01
Alcohol soluble extractive	10.54 ±0.23
Water soluble extractive	14.23 ±0.46

Table 2

Florescence analysis of powdered root of *T. populnea*

Treatment	Colour observed		
	Day light	UV light (Short, 254 nm)	UV light (long, 366 nm)
Powder as such	Dark brown	Dark brown	Black
1N NaOH in methanol	Brown	Light greenish brown	Blue
1N HCl	Brown	Light brown	Dark brown
1N NaOH in water	Brown	Dark orange	Dark blue
Powder + HNO3	Brown	Dark brown	Dark brown
Powder + H2SO4	Brown	Black	Dark brown

3.5 Fluorescence analysis

Fluorescence characteristic of powdered root of *T. populnea* were observed for resolution of doubtful specimen. The root powder of *T. populnea* was observed in visible, short and long ultra- violet light. (Table 2)

3.6 Behavior of root powder

Behavior of root powder of *T. populnea* with different chemical reagent showed presence of steroids, starch, tannins, flavonoids and saponins (Table 3)

Table 3

Behavior of *T. populnea* root powder with different chemical reagents

Reagents	Colour/ ppt	Constituents
Picric acid	No ppt	Alkaloids absent
Conc. H_2SO_4	Reddish brown	Steroids/ triterpenoids present
Aq. $FeCl_3$	Bluish black ppt	Tannins present
Iodine solution	Blue colour	Starch present
5% Aq. KOH	No change	Antroquinone glycosides absent
Mayer's reagent	No ppt	Alkaloids absent
Spot test	No stains observed	Fixed oils absent
Aq. AgNO3	Precipitation	Proteins present
Aq. NaoH	Yellow colour	Flavonoids present
Mg – HCl	Magenta colour	Flavonoids present
Dragendroff's reagent	No ppt	Alkaloids absent
Aq. Lead acetate	White change	Tannins present
Liberman Burcherd's test	Reddish green	Steroids and tannins are present

3.7 Phytochemical investigation and extractive values

Preliminary phytochemical analysis revealed the presence of flavonoids, terpenoids, tannins, saponins, steroids, carbohydrates, phenolic compounds, carbohydrates and proteins (Table 4). The extractive values of petroleum ether, ethyl acetate, methanol and aqueous extract were found to be 2.08 w/w (yellowish brown), 3.28 w/w (brown), 11.29 w/w (reddish brown) and 15.64 (dark brown) w/w respectively.

3.8 Quantitative estimation of phytoconstituents

3.8.1 Estimation of total phenolic content

Total Phenolic content of methanoilc extracts of *T. populnea* root was measured by Folin- Ciocalteu reagent method. The total phenolic content of root was found to be 0.271%. (Table 5)

Table 4

Preliminary phytochemical analysis of *T. populnea* root extract

Constituents	Petroleum ether extract	Ethyl acetate extract	Methanol extract	Aqueous extract
Alkaloids	–	–	–	–
Glycoside	–	–	+	+
Tannins	–	–	+	+
Saponin glycosides	–	–	+	+
Steroids	+	+	+	+
Flavonoids	–	+	+	+
Carbohydrates	–	+	+	+
Proteins	–	–	–	+

+: present, – : absent

Table 5

Absorbane of gallic acid, rutin and root extract of *T. populnea* for determination of total phenolic and flavonoids content.

Concentration (μg/ ml)	Absorbance	
	Toal phenolic content (Gallic acid)	Total flavonoids content (Rutin)
20	0.0746 ±0.0003	0.1963 ±0.0002
40	0.1534 ±0.0003	0.3876 ±0.0002
60	0.2486 ±0.0003	0.5873 ±0.0001
80	0.3168 ±0.0034	0.7721 ±0.0002
100	0.3787 ±0.0002	0.9392 ±0.0001
400 (root extract)	0.0866 ±0.0001	0.2734 ±0.0006

Values are expressed in mean ±S.D. (n=3)

3.8.2 Estimation of total flavonoids content

Total flavonoids content of root extract was determined by aluminium chloride colorimetric method, by standard curve of rutin at various concentrations; and it was found to be 0.344%. (Table 5)

4. Discussion

Assessment of standards is crucial measure for detection of sample quality, purity, authentication and also anthology of quality control of crude drug. Microscopy is one of the simple and cheap methods to start with establishing the correct identity of the source materials. First time, in this study the pharmacognostic and phytochemical evaluation was carried out for the root of *Thespesia populnea*. The present study may enable to identify the crude drug.

The ash of any organic material is composed of their non – volatile inorganic components. Controlled incineration of crude drugs results in an ash residue consisting of an inorganic material (metallic salts & silica). This value varies

within fairly wide limits and is therefore an important parameter for the purpose of evaluation of crude drug [18]. The determination of ash is useful for detecting low grade products, exhausted drugs and excess of sandy or earth matter; it is more especially applicable to powdered drug. On incineration, crude drugs normally leave an ash usually consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium [19]. The crude drugs owe their biological activity mainly due to active chemical constituents. These constituents may be in different polar, semipolar or nonpolar solvents. Total soluble constituents of the drug in any particular solvent or mixture of solvents may be called as extractive value [20]. The extracts obtained by exhausting crude drugs are indicative of approximate measures of their chemicals constitute. Taking into consideration the diversity in chemical nature and properties of contents of drugs, various solvents are used for determination of extractives. The solvent used for extraction is in a position to dissolve appreciable quantities of substances desired [21]. The percentage of active chemical constituents in crude drugs is mentioned on air-dried basis. Hence the moisture content of a drug should be determined and should also be controlled. The moisture content of a drug should be minimized in order to prevent decomposition of crude drugs either due to chemical change or microbial contamination. Florescence analysis and behavior of powdered drug with various chemicals/ reagents are very rapid methods to identify the doubtful specimens. Incase of lacking of physico- chemical evaluation, such methods are very important to check the adulteration.

All crude drugs are standardized for its active constituents. An extract is referring to a concentrated, well dried preparation of active constituents of medicinal crude drug. The concept of standardized extracts defiantly provides scientific validation of crude drug. Here, preliminary phytochemical analysis of the root extract confirmed the presence of flavonoids, terpenoids, tannins, saponins, steroids, carbohydrates, phenolic compounds, carbohydrates and proteins. Additionally, the methanolic extract of root was also examined to identify the total phenolic and total flavonoids content by Folin- Ciocalteu reagent method and aluminium chloride colorimetric method respectively.

In conclusion, present study was undertaken to assessment of pharmacognostic and phytochemical parameters of root of *T. populnea* providing information could be useful of the detection of sample quality, purity, authentication and also anthology of quality control of crude drug.

Conflict of interest statement

We declare that we have no conflict of interest.

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