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Pharmacognostic and phytochemical studies of *Thespesia populnea* Linn

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ABSTRACT

The present study deals with the pharmacognostic and phytochemical studies of leaves of Thespesia Populnea Linn. The objective of the present work comprise of collection, identification, microscopical and phytochemical evaluation of leaves of Thespesia Populnea Linn. The preliminary phytochemical studies indicates the presence of alkaloids, flavonoids, carbohydrates, phytosterols, tannins, saponins, proteins and aminoacids, terpenes, phenols, gums and mucilage's. The sections were taken and cellular structures were studied. The T.S of leaf shows the presence of epidermis, vascular bundle, glandular trichomes which are peltate type, cleared leaf showing vein-islet, vein-termination and mucilage masses, Peeling of the lamina showing abaxial epidermis- with stomata .Powder microscopy of the leaf shows the presence of crystals in the peltate scale, crystals in the ground parenchyma and phloem cells.

Keywords: *Thespesia Populnea*, Malvaceae, Alkaloids, Flavanoids.

INTRODUCTION

Thespesia Populnea Linn commonly called as Hibiscus populnea belongs to the Family: Malvaceae. *Thespesia populnea* is an evergreen tree. The Leaves are alternate, simple, with petioles of length 5-10cm long. The flowers Hibiscus like single at upper leaf axils, corolla yellow with a red center. The Fruits are Globose. The Seeds are Black, hairy. The main chemical constituents are Kaempferol, Quercetin and its glycosides, herbacetin and its glucoside, populneol, populnin, populnetin, rutin, gossipetin, gossypol, lupeol, sesquiterpenoidal quinones viz *;* thespeson, thespone, mansonones C,D,E and F, amino acids and carbohydrates. The main uses of *Thespesia Populnea* are cutaneous infections, skin and liver diseases. Fruit juices are used on rheumatism sprains, scabies, swellings, insect bites and warts. Pulps of fresh fruits were applied for relief of migrane.Unripe fruit juice was used to cure piles. Decoction of bark was given to treat diarrhoea and arthritis. Root, fruit and leaf used in psoriasis, scabies and other cutaneous diseases. Bark was used for the treatment of hemorrhoids and chronic dysentery. Leaf used as an anti-inflammatory[1,2].

EXPERIMENTAL SECTION

The plant selected for the present study is *Thespesia Populnea* Linn belongs to the family Malvaceae was collected and authontificated by the botanist Dr.P.Jayaraman, M.Sc., Ph.D. Director Plant Anatomy Research center (PARC), Tambaram, Chennai.

Microscopical study of the leaves [3-13]

Collection of specimen

The plant specimens for the proposed study were taken to select healthy plants and normal organs. The required samples of leaves were cut and removed from the plant and fixed in FAA (formalin -5ml+acid acid 5ml +70%ethyl alcohol 90ml). After 24hrs of fixing, the specimens were dehydrated with graded series of tertiary butyl alcohol as per the schedule given by sass, 1940 .infiltrations of the specimens was carried by gradual addition of paraffin wax (mp58-60) until TBA solution attained super saturation .The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thick nests of the section were 10-12 micro meter. Dewaxing of the section was by customary procedure (Johnson, 1940). The section was stained with Toluidine blue as per the method published by O'Brien et al (1964).since Toluidine blue is a polychromatic stain the staining results were remarkably good, and some phytochemical reactions were also obtained. The dye rendered pink color to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. wherever necessary sections were also stained with safranin and fast green and I+KI(for starch).

For studying the stomata morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5%NaoH (OR) epidermal pealing by partial maceration employing Jeffery's maceration fluid (Sass, 1940) were prepared. Glycerin mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with NaoH and mounted in glycerin medium after staining. Different cell components were studied and measured.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic units. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells polarized light microscope was employed. Since these structures have birefrigent property, under polarized, UV light they appear bright against dark back ground. Magnifications of the figures are indicated by the scale bars. Descriptive terms of anatomical features are as given in the standard anatomy books (Esau, 1964).

Identification of the plant constituents by phytochemical tests: [14, 15]

Ethanolic extract is subjected to various preliminary phytochemical analysis to test for the presence or absence of various phytoconstituents by the following tests.

1. Test for alkaloids:

To the extract dilute hydrochloric acid will be added and filtered. The filtrate will be treated with various alkaloid reagents

a) Mayer's test:
The filtrate will be treated with Mayer's reagent: appearance of cream colour indicates the presence of alkaloids.

b) Dragendroff's test:
The filtrate will be treated with Dragendroffs reagent: appearance of reddish brown precipitate indicates the presence of alkaloids.

c) Hager's test:
The filtrate when treated with Hager's reagent, appearance of yellow colour precipitate indicates the presence of alkaloids.

2) Test for carbohydrates and reducing sugar
The small quantities of the filtrate will be dissolved in 4ml of distilled water and filtered. The filtrate will be subjected to

a) Molisch's test:
A small portion of the filtrate will be treated with Molisch's reagent and sulphuric acid. Formation of a violet ring indicates the presence of carbohydrates.

b) Fehling's test:
The extract will be treated with Fehling's reagent A and B. The appearance of reddish brown colour precipitate indicates the presence of reducing sugar.

c) Benedict's test:
The extract will be treated with Benedict's reagent; appearance of reddish orange colour precipitate indicates the presence of reducing sugar.

d) Barfoed's test:
The extract will be treated with barfoed's reagent and heated. Appearance of reddish orange colour precipitate indicates the presence of non reducing sugars.

3) Test for steroids:
Liebermann bur chard's test:
The extract will be treated with 3ml of acetic anhydride, few drops of glacial acetic acid followed by a drop of concentrated sulphuric acid. Appearance of bluish green colour indicates the presence of steroids.

4) Test for proteins:
a) Biuret test:
The extract will be treated with copper sulphate solution, followed by addition of sodium hydroxide solution; appearance of violet colour indicates the presence of proteins.

b) Millon's test:
The extract will be treated with Millon's reagent; appearance of pink colour indicates the presence of proteins.

5) Test for tannins:
The extract will be treated with 10% lead acetate solution; appearance of white precipitate indicates the presence of tannins.

6) Test for phenolic compounds:

a) The extract will be treated with neutral ferric chloride solution; appearance of violet colour indicates the presence of phenolic compounds.

b) The extract will be treated with 10% sodium chloride solution; appearance of cream colour indicates the presence of phenolic compounds.

7) Test for flavonoids:

a) 5ml of extract will be hydrolyzed with 10%sulphuric acid and cooled. Then, it will be extracting with diethyl ether and divided in to three portions in three separate test tubes. 1ml of diluted sodium carbonate, 1ml of 0.1N sodium hydroxide, and 1ml of strong ammonia solution will be added to the first, second and third test tubes respectively. In each test tube. Development of yellow colour demonstrated the presence of flavonoids.

b) Shinoda's test:

The extract will be dissolved in alcohol, to which few magnesium turnings will beaded followed by concentrated HCL drop wise and heated, and appearance of magenta colour shows the presence of flavonoids.

8. Test for gums and mucilage:

The extract was treated with 25 ml of absolute alcohol, and filtered. The filtrate will examine for its swelling properties.

9. Test for glycosides

When a pinch the extract was treated with glacial acetic acid and few drops of ferric chloride solution, followed by the addition of conc. Sulphuric acid, formation of ring at the junction of two liquids indicates the presence of glycosides.

10. Test for saponins

Foam test

About 1 ml of the extract was diluted to 20 ml of with distilled water and shaken well in a test tube. The formation of foam in the upper part of test tube indicates the presence of saponins.

11. Test for Triterpenoids

The substance was warmed with tin and thionyl chloride. Pink colour indicates the presence of triterpenoids.

RESULTS

Anatomy of the leaf

The leaf has prominent and thick abaxial midrib and lateral veins. The lamina is thin with wide shallow glandular pits (Fig: 1, 2 and 3) the midrib is 350 µm thick and 400µm wide. It consists of a short, semi-circular adaxial part and wide, thick abaxial .The epidermis is thin and continuous comprising small, thick walled squarish cells.

The cleared leaf showing vein-islet, vein-termination and mucilage masses (Fig: 4 and 5)
The leaf shows peeling of the lamina showing abaxial epidermis- with stomata (Fig: 6 and 7)

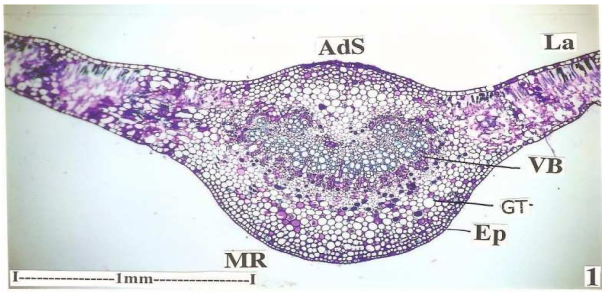

Fig.1: T,S of leaf through midrib with lamina

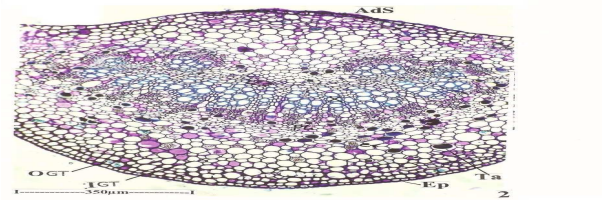

Fig. 2: T.S of midrib enlarged

(Ads- Adaxial side; Col-Collenchyma; Ep-Epidermis; GP-Ground parenchyma; La- lamina; Lv- Lateral vein; MR-midrib; Vs-vascular strand).

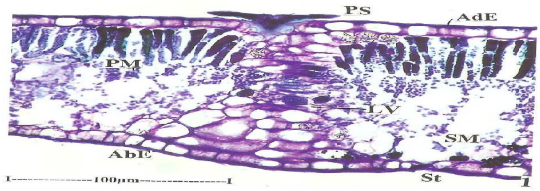

Fig.3: T.S of lamina showing peltate scale

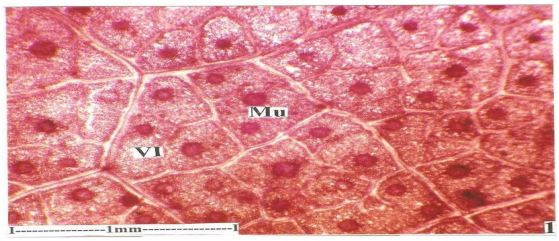

Fig.4: Cleared leaf showing vein-islet, vein-termination and mucilage masses

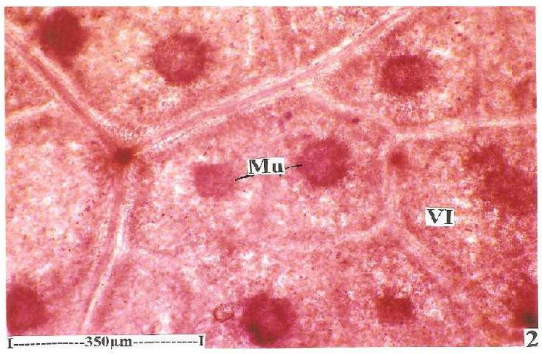

Fig.5: Cleared leaf showing vein-islet, vein-termination and mucilage masses – enlarged.

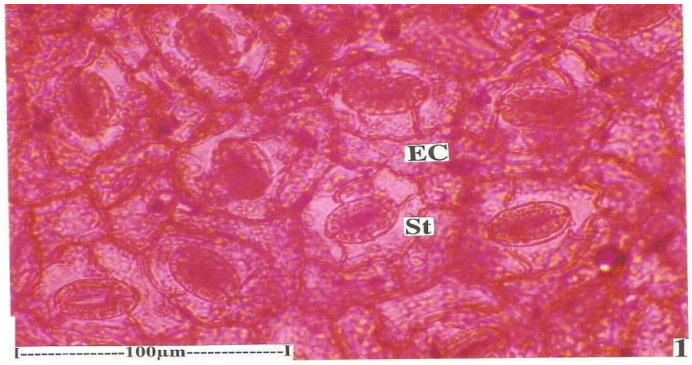

Fig.6: Peeling of the lamina showing abaxial epidermis- with stomata

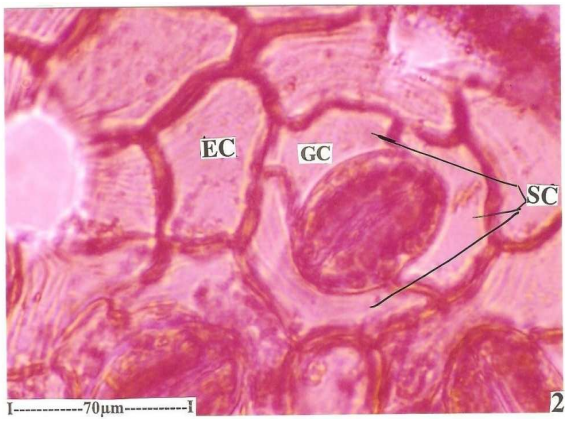

Fig.7: A Stoma with epidermal cells – enlarged

Powder microscopy of the leaf: The leaf powder when examined under the microscope exhibit the following inclusions

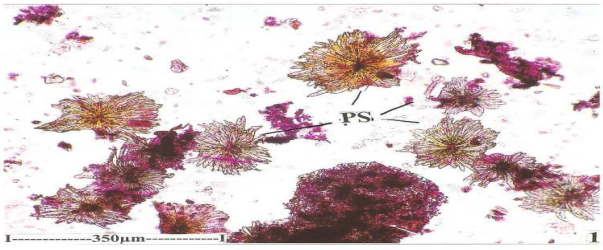

Fig.8: Leaf powder showing peltate scales

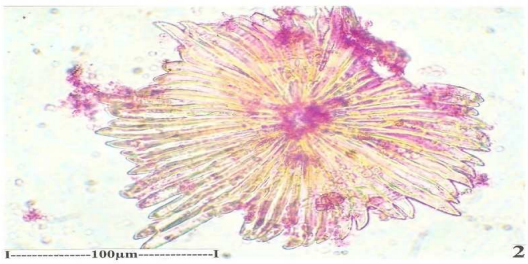

Fig.9: A peltate scale magnified

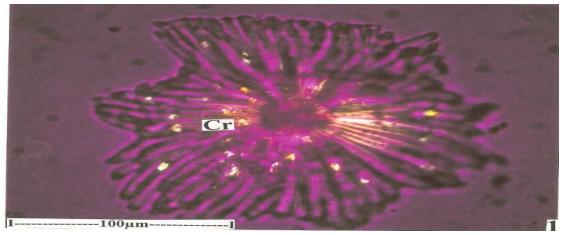

Fig.10: Crystals in the peltate scale

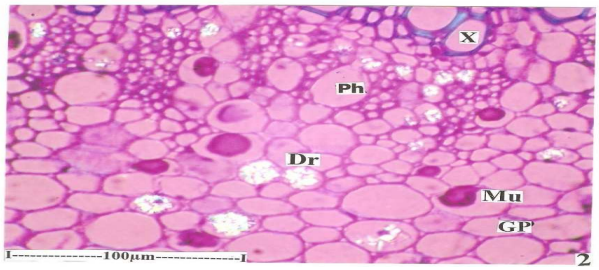

Fig.11: Crystals in the ground parenchyma and phloem cells

Preliminary Phytochemical Screening of Ethanol Extract of Leaves of *Thespesia populnea* Linn

S.No	Test	Alcoholic Extract
1	ALKALOIDS	+
2	CARBOHYDRATES	+
3	GLYCOSIDES	-
4	PHYTOSTEROLS	+
5	VOLATILE OILS	-
6	SAPONINS	+
7	TANNINS	+
8	PROTEINS AND AMINO ACIDS	+
9	GUMS AND MUCILAGE	+
10	FLAVANOIDS	+
11	TERPENES	+
12	PHENOLS	+

(-)=Absent (+) =Present

DISCUSSION

Pharmacognostic studies

The plant *Thespesia populnea* was pharmacognostically identified by studying its microscopical characters with main focus on its leaf. The T.S of leaf shows the presence of epidermis, vascular bundle, glandular trichomes which are peltate type, cleared leaf showing vein-islet, vein-termination and mucilage masses, Peeling of the lamina showing abaxial epidermis- with stomata .Powder microscopy of the leaf shows the presence of crystals in the peltate scale, crystals in the ground parenchyma and phloem cells.

CONCLUSION

The pharmacognostic studies include the microscopic characters and powder analysis for the leaves of *Thespesia populnea* was performed. It shows the presence of epidermis,

parenchymatous cells, vascular bundle, lateral vein, glandular trichomes. The preliminary phytochemical studies for different extracts of *Thespesia populnea* show the presence of alkaloids, flavonoids, carbohydrates, phytosterols, tannins, saponins, proteins and aminoacids, terpenes, phenols, gums and mucilage's.

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