

Research Article

Pharmacognostical and Phytochemical Studies of The Leaves of *Albizia Odoratissima* (L.F) Benth

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ABSTRACT

Various traditional systems of medicine enlightened the importance of the leaves of *Albizia odoratissima* (L.F) benth (Mimosoideae) to have a great medicinal value. The present study was aimed at pharmacognostic and preliminary phytochemical evaluations of *A.odoratissima* leaves. The pharmacognostic investigations were carried out in terms of organoleptic, macroscopy, microscopic and physical parameters. The dried leaves powder was subjected to successive Soxhlet extraction using petroleum ether, chloroform, ethyl acetate, methanol, ethanol and water. These solvent extracts were subjected to a preliminary phytochemical screening to detect the different chemical principles present viz., carbohydrates, proteins, amino acids, steroids, glycosides, alkaloids, flavonoids, tannins and phenolic compounds, fixed oils. The phytochemical evaluation revealed the presence of carbohydrates, glycosides, flavonoids, steroids, tannins and phenolic compounds, fixed oils. These findings will be useful towards establishing pharmacognostic standards on identification, purity, quality and classification of the plant, which is gaining relevance in plant drug research.

KEY WORDS: *Albizia odoratissima* (L.F) benth leaves, organoleptic, macroscopy, microscopy, physiochemical, phytochemical.

INTRODUCTION

Pharmacognostical study is the preliminary step in the standardization of crude drugs. The detailed pharmacognostical evaluation gives valuable information regarding the morphology, microscopical and physical characteristics of the crude drugs. Pharmacognostic studies have been done on many important drugs, and the resulting observations have been incorporated in various pharmacopoeias [1]. There are a number of crude drugs where the plant source has not yet been scientifically identified. Hence pharmacognostic study gives the scientific information regarding the purity and quality of the plant drugs [2]. *Albizia Odoratissima* (L.F) benth belongs to family Mimosoideae is a large sized tree distributed throughout India, Srilanka and Nepal. A large tree growing up to 20 m height. The plant contains *Albizia adianthi folia*, *Albizia amora*, *Albizia lebek*, *Albizia rhizonse*, *adionthifoliosides* A&B, *gummiferaosides*, flavonoids, glycosides. All part of the plant having anxiety, depression. The flower head having oxytocic, digestive, sedative, insomnia, anthelmintic, diuretic. The stem part having analgesic, stimulant, swelling, injuries, abscesses, diuretics, anthelmintic and mostly used for diabetes [3-4]. Pharmacognostic and

preliminary phytochemical studies have not been reported for the leaves part of this plant. Therefore the main aim of the present study Pharmacognostical and preliminary phytochemical investigation such as organoleptic, morphologic, microscopic was including leaf constants, measurement and other applicable physico - chemical parameters of leaves part of *Albizia odoratissima* (L.f) Benth which could be used to prepare a monograph for the proper identification of the plant.

MATERIALS AND METHODS

Collection of plant material

Table 1 Vein-islet and Veinlet Termination Number of *Albizia odoratissima* (L.F) benth leaves

| S.no | Species | Range of vein-islet number | Range of veinlet termination number |
|------|--|----------------------------|-------------------------------------|
| 1 | <i>Albizia odoratissima</i> (L.f) benth leaves | 6-12 | 12-20 |

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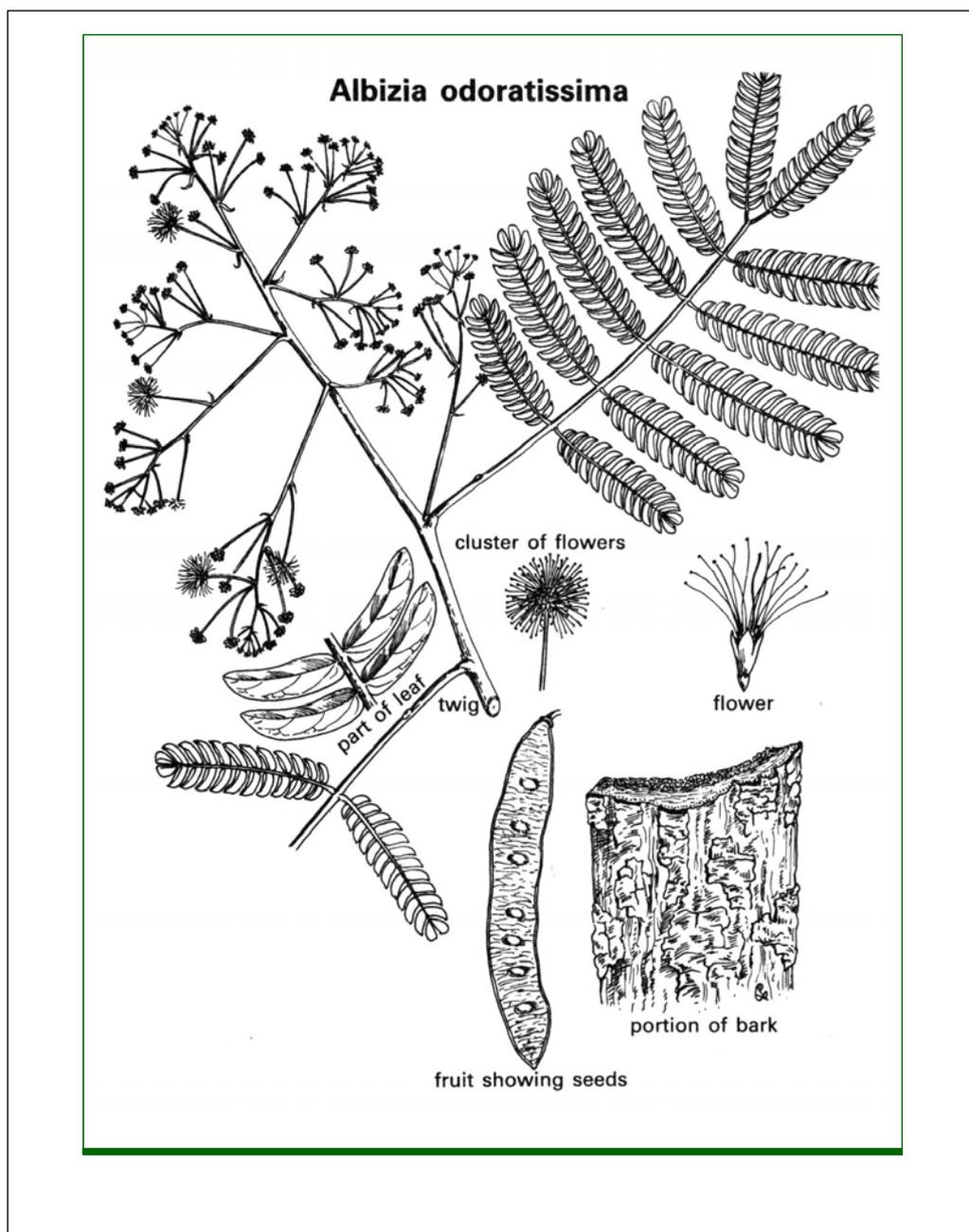


Figure 1 Morphological features of *Albizia odoratissima* (L.f) Benth

The species for the proposed study that is *Albizia odoratissima* (L.f) Benth. were collected from Kolli Hills, Namakkal district, Tamilnadu and authenticated as *Albizia odoratissima* (L.f) Benth by Prof.P.Jayaraman, Ph.D., Director, National Institute of Herbal Science, Chennai-45, Pharmacognostical studies

Macroscopical studies

Organoleptic Characters

In organoleptic evaluation, appropriate parameters like taste, odor, size, shape and color of the roots and root powder were studied [3-4].

Morphological Characters

Morphological investigations of the plant leaves were studied [3-4].

Microscopical studies

Table 2 Stomatal number and Stomatal Index of *Albizia odoratissima* (L.F) Benth leaves

| S. no | Species | Range of Stomatal index | Range of Stomatal number |
|-------|--|-------------------------|--------------------------|
| 1 | <i>Albizia odoratissima</i> (L.f) Benth leaves | 8-16 | 1-3 |

Care was taken to select healthy plant and normal organ. The required samples of different organs were cut and removed from the plant and fixed in FAA

Table 3 Physico-chemical parameters of *Albizia odoratissima* (L.f) benth leaves

| S.no. | Parameters | <i>Albizia odoratissima</i> (L.f) benth (%w/w) |
|-------|----------------------------|--|
| 1 | Total ash | 4.56 |
| 2 | Acid-insoluble ash | 0.39 |
| 3 | Water soluble ash | 6.9 |
| 4 | Sulphated ash | 11.9 |
| 5 | Alcohol soluble extractive | 20.14 |
| 6 | Water soluble extractive | 22.15 |

(Formalin-5ml + Acetic acid-5ml+ 70% Ethyl alcohol-90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary-butyl alcohol as per the schedule [5]. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60°C) 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 thickness of the section was 10-12 µm. dewaxing of the sections was by customary procedure [6]. The sections were stained with toluidine blue as per the methods [7]. Since toluidine blue is a polychromatic stain. The staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour 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 IKI (for starch). Glycerin mounted temporary preparation were made for macerated/cleared materials. Powdered materials of different parts were cleared with NaOH and mounted in glycerine medium after staining. Different cell component were studied and measured [8-9].

Photomicrograph

Microscopic descriptions of tissue are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon labphoto 2 microscopic units. For normal observation bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard anatomy book [10-11].

Determination of Vein-islet Number and Veinlet Termination Number

TABLE 4 Qualitative phytochemical analysis of extracts of *Albizia odoratissima* (L.f) benth leaves

| Test of extract | Petroleum ether extract | Chloroform extract | Ethyl acetate extract | Ethanol extract |
|------------------------------|-------------------------|--------------------|-----------------------|-----------------|
| Carbohydrates | - | + | - | + |
| Proteins & amino acids | - | - | - | - |
| Glycosides | + | + | + | + |
| Alkaloids | - | - | - | - |
| Phytosteroids | + | + | + | + |
| Favonoids | + | + | + | + |
| Saponin | - | - | - | - |
| Tannins & phenolic compounds | + | + | + | + |
| Fixed oils & fats | + | + | - | - |
| Gums & mucilages | - | - | - | - |

(+) = indicates presence,

(-) = indicates absence,

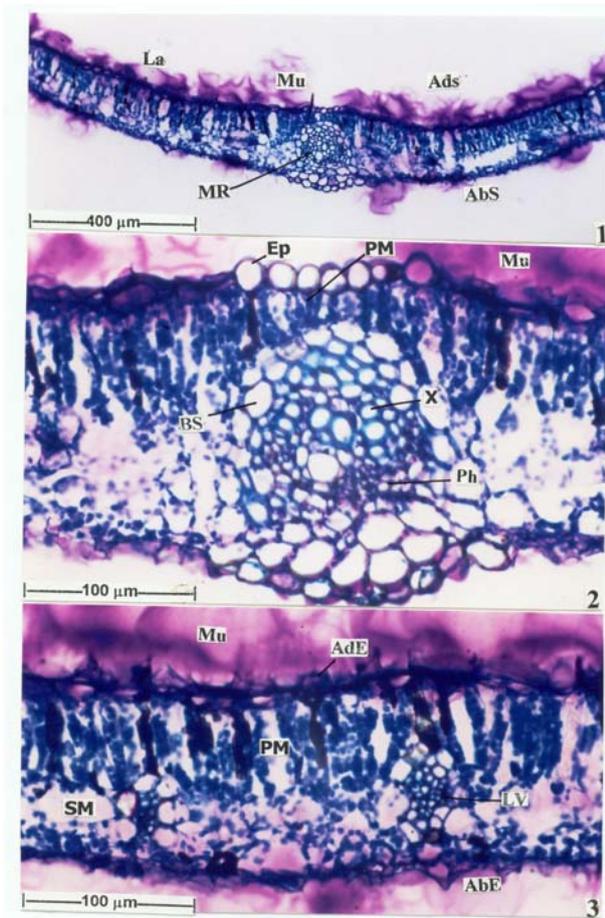


Figure 2 Transverse sections of leaves
Anatomy of the leaf

- 2.1 Ts of leaf through midrib with laminar
- 2.2 Ts of midrib enlarged
- 2.3 Ts of lamina enlarged

[AbE- Abaxial epidermis; Abs- abaxial side; Ads – adaxial side; Bs- bundle sheath extension; La- lamina; Lv- lateral vein; Mu- mucilage; MR- midrib; Ph- phloem; Pm- palisade mesophyll; SM- spongy mesophyll; X- xylem].

Boil a few leaves in chloral hydrate solution in a test-tube placed in a boiling water-bath. If the leaves are difficult to clear in this manner, soak them in water, treat successively with chlorinated soda for bleaching, 10% hydrochloric acid for the removal of calcium oxalate and finally chloral hydrate solution was added. Mount the preparation in glycerin water. Set up the camera lucida and divide the paper into squares of 1sq.mm by means of the stage micrometer. Replace the stage micrometer by the clear leaf preparation and trace the veins in four continuous squares either in a square of 2mmx2mm or a rectangle of 1mmx4mm. Trace the vein-islets and veinlet termination by looking through the microscope when a super imposed image of the leaf portion and paper is seen at same time. Count the number of veinislets and veinlet termination with in the square or rectangle and also be

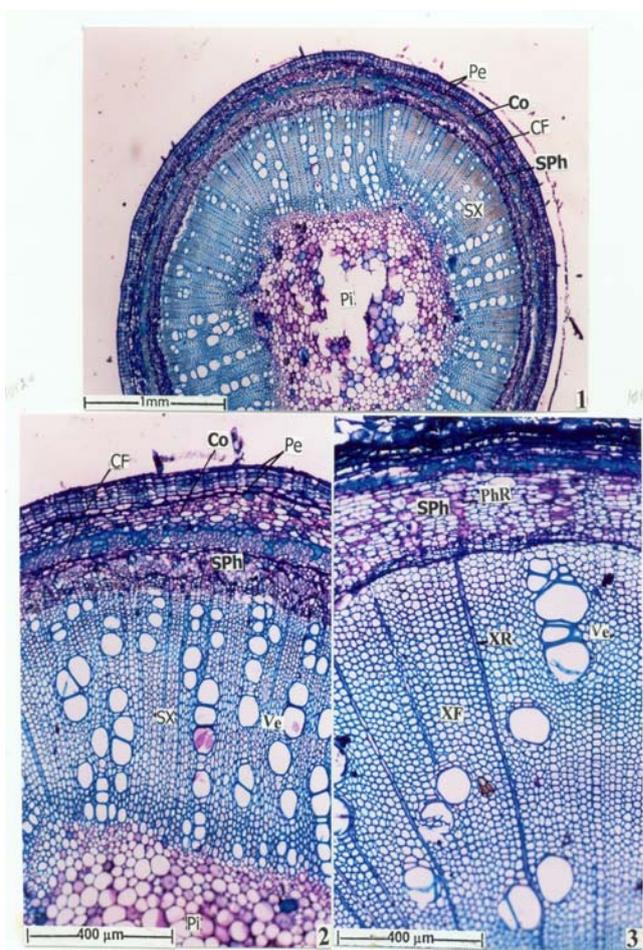


Figure 3 Transverse section of the stem

Anatomy of the stem

- 3.1 Ts of stem- ground plan
- 3.2 Ts of young stem –a sector
- 3.3 Ts of old stem – a sector

[CF- cortical fibres; Co- cortex; Pe- periderm ; Pi- pith; Phr- phloem ray; SPh – secondary phloem; SX- secondary xylem; Ve- vessels; XF- xylem fibres; XR- xylem ray].

taking into consideration incomplete vein-islets on any two adjacent sides of the square or rectangle. Divide the total number of vein-islets and vein let termination numbers in four adjoining squares by 4 in order to get the value for one sq.mm. Take at least ten sets of such counts. Record the observations in the form of range and also indicate the mean value [12].

Determination of Stomatal Number and Stomatal Index

Clear the fragments of leaf from the middle of lamina by boiling with chloral hydrate solution or alternatively with chlorinated soda. Peel out upper and lower epidermis, separately by means of forceps. Prepare the mounts of low and upper epidermis separately is

glycerin water. Draw a square of known dimension by means of a stage micrometer and camera Lucida on a drawing paper. Replace the stage micrometer

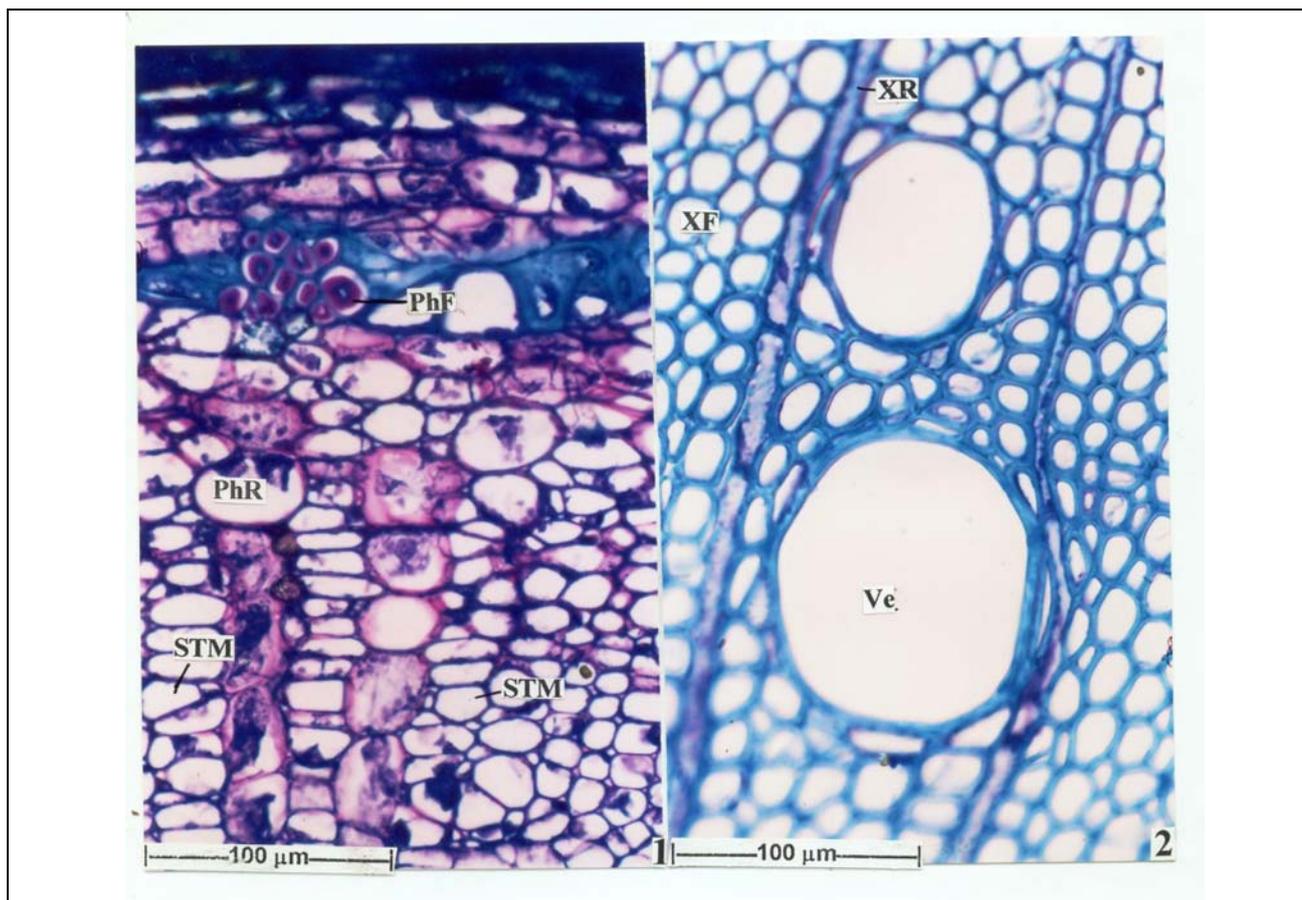


Figure 4 Transverse sections of secondary phloem and secondary xylem – enlarged
Anatomy of secondary phloem and secondary xylem – enlarged.

4.1 Ts of secondary phloem

4.2 Ts of secondary xylem

[PhF- phloem fibres; PhR- phloem ray; STM- sieve tube member; Ve- vessel; XF- xylem fibres ; XR- xylem ray].

by the clear leaf preparation, focus under the same

magnification and trace the epidermal cells and stomata by looking through the microscope. When a super imposed image of the leaf is seen at the same time, count the number of epidermal cells and stomata (the two guard cells and ostiole being considered as unit) within the square. a cell being counted if at least half of its area lies within the square provided two adjacent sides are considered for purpose of circulation. Examine successive adjacent fields until about 400 cells are counted and calculate the stomatal number *i.e.* number of stomata per sq.mm of leaf preparation. Calculate the Stomatal Index using the formula

$$SI = \frac{S \times 100}{E + S}$$

Determine the values for each surface where the leaf bears stomata on both surfaces [12].

Physico-chemical standards

In the physico-chemical evaluation, ash values viz., total ash, acid insoluble ash and water soluble ash, sulphated ash, and extractive values viz., alcohol soluble extractive value, water soluble extractive and ether soluble extractive values, and loss on drying were

determined [13-14]. The ash values represent the inorganic salts present in the drug. Extracts obtained by exhausting crude drugs are indicative of approximate measures of certain chemical compounds they contain, the diversity in chemical nature and properties of contents of drug. The percentage w/w values were calculated with reference to the air-dried drug.

Determination of total ash value

Three gram of root powder of *Anacyclus pyrethrum* Dc was taken in a tared silica crucible and incinerated at a temperature not exceeding 450 °C until free from carbon. The resultant ash was cooled and weighed. The percentage of ash was calculated with reference to the air-dried drug.

Acid insoluble ash value

The total ash obtained from 3g of root powder was boiled for 5 minutes with 25 ml of dilute hydrochloric acid and the insoluble matter was collected on an ashless filter paper. It was washed with hot water,

ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

Water Soluble Ash Value

The total ash obtained from 3g of root powder was boiled for 5 minutes with 25 ml of water and the insoluble matter was collected on an ashless filter paper.

It was washed with hot water, ignited and weighed. The percentage of water-soluble ash was calculated with reference to the air-dried drug.

Determination of sulphated Ash Value

The total ash obtained from 3g of root powder was moistened with 1 ml of concentrated sulphuric acid, heated gently until the white fumes were no longer evolved, ignited and weighed. The percentage of sulphated ash was calculated with reference to the air-dried drug.

Determination of Alcohol Soluble Extractive Value

Accurately weighed powder (5 g) of roots was taken and macerated with 100 ml of 95% alcohol for 24 h. The contents were frequently shaken during the first 6 h and allowed to remain for 18 h. After 24 h, the extract was filtered and 25 ml of the filtrate was evaporated. The extract was dried at 105°C to a constant weight.

Determination of Water Soluble Extractive Value

Water soluble extractive value was determined using the procedure described for alcohol soluble extractive, except that chloroform water was used for maceration.

Determination of ether Soluble Extractive Value

Ether soluble extractive value was determined using the procedure described for alcohol soluble extractive, except that chloroform water was used for maceration.

Loss on Drying

Weigh accurately about 1.5 gm of the powdered drug in a tared porcelain dish, which was previously dried at 105°C in hot air oven to constant weight and then weighed. From the difference in weight, the percentage loss on drying with reference to the air-dried substance was calculated.

Preliminary phytochemical studies

Preparation of extracts of *A. Odoratissima* (L.F) benth

The powder was extracted with hexane to remove lipids. It was then filtered and the filtrate was discarded. The residue was successively extracted with petroleum ether, chloroform, ethyl acetate, ethanol, using cold percolation method [15-16].

Preliminary phytochemical screening

One gram of the petroleum ether, chloroform, ethyl acetate, ethanol extracts of *A. Odoratissima* (L.F) benth leaves were dissolved in 100 ml of its own mother solvents to obtain a stock of concentration 1% (v/v). The extracts thus obtained were subjected to preliminary phytochemical screening following the standard procedure [12, 17].

Screening Procedure

1. Tests for Carbohydrates

a) Molish's test

To 2-3 ml aqueous extract, added few drops of α -naphthol solution in alcohol, shaken and added concentrated H₂SO₄ from sides of the test tube was observed for violet ring at the junction of two liquids.

b) Fehling's test

1 ml Fehling's A and 1ml Fehling's B solutions was mixed and boiled for one minute. Added equal

volume of test solution. Heated in boiling water bath for 5-10 min was observed for a yellow, then brick red precipitate.

c) Benedict's test

Equal volume of Benedict's reagent and test solution in test tube were mixed. Heated in boiling water bath for 5 min. Solution may appear green, yellow or red depending on amount of reducing sugar present in test solution.

2. Tests for Proteins and Amino acids

a) Biuret test

To 3 ml test solution added 4% NaOH and few drops of 1% CuSO₄ solution observed for violet or pink colour.

b) Million's test

Mixed 3 ml test solution with 5 ml Million's reagent, white precipitate. Precipitate warmed turns brick red or precipitate dissolves giving red color was observed.

c) Xanthoprotein test

Mixed 3ml test solution with 1 ml concentrated H₂SO₄ observed for white precipitate.

d) Ninhydrin test

3 ml test solution and 3 drops 5% Ninhydrin solution were heated in boiling water bath for 10 min. observed for purple or bluish color.

3. Tests for Glycosides

a) Hydrolysis of extract

A minimum quantity of the extracts is hydrolyzed with hydrochloric acid for few minutes on water bath and the hydrolysate is subjected to the following tests.

i) Legal's test

To the hydrolysate 1 ml of the pyridine and few drops of sodium nitroprusside solution added, then it is made alkaline with sodium hydroxide solution. Color change shows the presence of glycosides.

ii) Borntrager's test

Hydrolysate is treated with chloroform and the chloroform layer is separated. To this, equal quantity of dilute ammonia solution is added. Color changes in the ammonical layer shows the presence of glycoside.

b) Baljet's test

A test solution observed for yellow to orange color with sodium picrate.

4. Tests for Alkaloids

a) Mayer's test

To the 1 ml of extract, add 1 ml of Mayer's reagent (potassium mercuric iodide solution). Whitish yellow or cream colored precipitate indicates the presence of alkaloids.

b) Dragendroff's test

To 1 ml of the extract, add 1 ml of Dragendroff's reagent (potassium bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

c) Hager's test

To 1 ml of the extract, add 1 ml of Hager's reagent (saturated aqueous solution of picric acid). A yellow colored precipitate indicates the presence of alkaloids.

d) Wagner's test

To 1 ml of the extract, add 1 ml of Wagner's reagent (iodine in potassium iodide solution). Formation of reddish brown precipitate indicates the presence of alkaloids.

5. Tests for Phyto Steroids

Small quantity of extract is dissolved in 5 ml of chloroform separately. The above obtained chloroform solutions are subjected to Salkowski and Liebermann-Burchard tests.

a) Salkowski test

To the 1 ml of above prepared chloroform solution few drops of concentrated sulphuric acid is added. Formation of brown ring indicates the presence of phytosterols.

b) Liebermann-Burchard test

The above prepared chloroform solutions are treated with few drops of concentrated sulphuric acid followed by 1 ml of acetic anhydride solution. A bluish green color solution shows the presence of phytosterols.

6. Tests for Flavonoids

a) Shinoda test

To dried powder or extract added 5 ml 95% ethanol, few drops concentrated HCl and 0.5 g magnesium turnings. Pink color was observed.

b) Ferric Chloride test

Test solution with few drops of ferric chloride solution shows intense green color.

c) Alkaline reagent test

Test solution when treated with sodium hydroxide solution shows increase in the intensity of yellow color, which becomes colorless on addition of few drops of dilute acid.

d) Lead Acetate solution test

Test solution with few drops of lead acetate solution (10%) gives yellow precipitates.

7. Tests for Saponins

a) Foam test

The drug extract or dry powder was shake vigorously with water. Persistent foam was observed.

b) Haemolytic test

Added test solution to one drop of blood placed on glass slide. Haemolytic zone whether appeared was observed.

8. Tests for Tannins and Phenolic Compounds

To 2-3 ml of extract, add few drops of following reagents:

- 5% FeCl₃ solution: deep blue-black color.
- Lead acetate solution: white precipitate.
- Gelatin solution: white precipitate
- Bromine water: decoloration of bromine water.
- Acetic acid solution: red color solution
- Dilute iodine solution: transient red color.
- Dilute HNO₃: reddish to yellow color.

9. Test for Fixed Oils and Fats

a) Spot test

Small quantity of the extract is placed between two filter papers. Oil stain produced with any extract shows the presence of fixed oils and fats in the extracts.

b) Saponification test

Few drops of 0.5N alcoholic potassium hydroxide are added to the extract with few drops of phenolphthalein solution. Later the mixture is heated on water bath for 1-2 hours soap formation indicates the presence of fixed oils and fats in the extracts.

10. Test for Gums and Mucilages

a) Ruthenium red test

Small quantities of extract are diluted with water and added with ruthenium red solution. A pink color production shows the presence of gums and mucilage's.

RESULT

Macroscopical studies

Organoleptic characters

In organoleptic evaluation, appropriate parameters like taste, odor, size, shape and color of the roots and root powder were studied.

| | |
|---------|---------------------------------|
| Colour | : Dark green |
| Odour | : Slightly aromatic |
| Taste | : Characteristically astringent |
| Texture | : Rough |
| Shape | : Oblong elliptic |

Morphological characters

A medium sized unarmed tree about 20 m in height with dark coloured young shoots and grey, rough, irregularly cracked bark with dark patches; leaves abruptly 2-pinnate; main rachis downy, 15-30cm. long, with a large sessile gland on the petiole a little above its base and also at the bases of 1-2 of the upper pinnae; pinnae usually 4 pairs, rather distant, 7.5-15cm. long, their rachises pubescent. Leaflets 8-15 pairs, sessile, 2-2.8 by 1-1.3 cm., obliquely oblong, rounded at the apex, reticulately veined, the midrib about 1/3 the breadth of the blade from the upper edge, dark green and slightly pubescent above, glaucous and pubescent beneath, base very obliquely rounded (Fig 1).

Microscopical studies

T.s of the leaves

The leaflet is thin with less prominent midrib and is adaxially, slightly concave (fig. 2.1). The midrib is 200µm thick and the lamina is 100µm thick the epidermis along the adaxial and abaxial sides have dilated, vertically oblong cells. The palisade is transcurrent along the adaxial part above the vascular bundle (fig. 2.2). Along the abaxial part, these one or two rows of dilated hyaline cells. The Vascular bundle is large, circular and collateral. It consists of a few clustered vessels and a mass of phloem (fig. 2.2).

The lamina has dense mucilaginous epidermal layers. While processing the leaf for sectioning the mucilage oozes out from the epidermal cells in the forms of thick smoke (fig. 2.1-3)

The lateral veins are embedded in the median part of the mesophyll tissues; they do not project above the surface level of the lamina. The vein has a small collateral vascular bundle surrounded by hyaline bundle sheath cells (fig. 2.3)

T.s of the stem

The stem of 3 mm diameter was studied. It is circular even, and smooth in outline (fig. 3.1). It consists

of periderm cortex, cortical fibers and vascular cylinder (fig. 3.2). Periderm is 50µm wide and consists of narrow oblong, thin walled, radial files of phloem cells (fig. 3.1). Cortex is also narrow zone of 50µm width, the cells are parenchymatous, slightly thick walled and compact. Cortical fibers are a continuous cylinder of both scleroids and gelatinous fibers. It forms a border separating the cortex and secondary phloem. Vascular cylinder has a narrow secondary phloem and a wide, dense, hollow cylinder of secondary xylem. Secondary xylem consists of angular, thin wide vessels in radial multiples and thin walled xylem fibers with wide lumen. The pith is wide and parenchymatous. The cells are thin walled and compact. The old stem has wider phloem and xylem cylinder. Phloem consists of slightly dilated rays and tangentially oblong, radial rows of sieve elements and parenchyma cells. It is 100µm wide (Fig: 3.3, 4.1). Secondary xylem has solitary or radial multiples of vessels which are thin walled and angular. Xylem rays are narrow and straight. Xylem fibers are thin walled and narrow lumened. Growth rings are absent. Vessels are 25-40 µm in diameter (fig 4.2)

Quantitative analysis

In this the vein-islet number, veinlet termination number and stomatal number, stomatal index are determined as per standard methods and recorded (tab 1-2).

Physico-chemical standards

The physico-chemical evaluation, ash values viz., total ash, acid insoluble ash and water soluble ash, sulphated ash, and extractive values viz., alcohol soluble extractive value, water soluble extractive and ether soluble extractive values, and loss on drying were calculated and recorded (tab 3).

Preliminary phytochemical studies

Preliminary phytochemical screening to detect the different chemical principles present viz., carbohydrates, proteins, amino acids, steroids, glycosides, alkaloids, flavonoids, tannins and phenolic compounds, fixed oils were recorded (tab 4)

DISCUSSION

Ethnomedically, the leaves and stem bark of this plant were used by local people for in the treatment of various disease conditions without standardization. The standardization of a crude drug is an integral part of establishing its correct identity. Before any crude drug can be included in a herbal pharmacopoeia, pharmacognostic parameters and standards must be established. *Albizia odoratissima* is a plant that has been confused with other species due to their relative similarities. The results of these investigations could, therefore, serve as a basis for proper identification, collection and investigation of the plant. The macro and micro morphological features of the leaf described, distinguishes it from other members of the genera. Leaf surface data, fluorescence analysis and quantitative leaf microscopy are parameters that are unique to the plant and are required in its standardization. Phytochemical investigation of the secondary plant metabolites are known to possess various pharmacological effects and

may be responsible for the various actions of *Albizia odoratissima*.

CONCLUSION

The pharmacognostical and phytochemical evaluation of *Albizia odoratissima* (L.F) benth leaves can provide useful information for identification and authentication of plant. It can also serve as an important source of information to ascertain the identity and to determine the quality and purity of the plant material in future studies.

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