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Research Article

PHARMACOGNOSTIC AND PRELIMINARY PHYTOCHEMICAL EVALUATION OF THE LEAVES OF *Dalbergia sissoo* Roxb.

JOSEPHIN NERLING RASHIDA.G *, VENKATARATHNAKUMAR T, AJITHA DHAS ARUNA, GOWRI R, PARAMESHWARI R, SHANTHI M, RAADHIKA K

Department of pharmacognosy, madurai medical college, madurai-20, tamil nadu, india.Email: rashipharm@gmail.com

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ABSTRACT

Dalbergia sissoo Roxb (Fabaceae), known as Indian rosewood, is reported to be useful in many conditions including fever, ulcers, digestive disorders, anti diabetic and skin diseases. Leaves of the plant are used in ayurvedic system of medicine. They require standardization before it enters into the market. The present study was aimed at pharmacognostic and preliminary phytochemical evaluation of the leaves of *Dalbergia sissoo* .The pharmacognostic investigations were carried out in terms of organoleptic, microscopic and physical parameters. The dried leaves powder was subjected to cold maceration using hexane, petroleum ether, chloroform, methanol, ethanol and water. The leaf powder was subjected to a preliminary phytochemical screening to detect the different chemical principle. The phytochemical evaluation revealed the presence of flavonoids, glycosides, tannins, phenols and terpenoids.

Keywords: *Dalbergia sissoo*, Phamacognosy, Phytochemistry.

INTRODUCTION

Dalbergia sissoo Roxb. (Fabaceae), known as Indian Rosewood. It is a medium to large-sized deciduous tree, growing upto 30 m in height under favourable conditions. This plant belongs to the Fabaceae family and widely planted outside its natural range. It has been established in irrigated plantations, along road sides and canals, and around farms and orchards as windbreaks. The sissoo plant is a folk remedy for excoriations, gonorrhea and skin ailments[1]. Ayurveda prescribe the leaf juice for eye ailments, wood and bark are abortifacient, anthelmintic, antipyretic, aphrodiasiac, expectorant and refrigerant. They use the wood and bark for anal disorders, blood diseases, burning sensations and dysentery, dyspepsia, leucoderma and skin ailments[2-4]. *Dalbergia sissoo* Roxb. (Fabaceae) leaves are alternately arranged, compound and oddly pinnate with 3-5 glabrous, leathery leaflets, elliptical to ovate, tapering to a point and native to Pakistan, Oman, Bhutan, India, Nepal has a long history of human consumption[5] . Bioactive richness of the active constituents, potential therapeutic activities and absence of pharmacognostic studies and phytochemical action on leaves of *Dalbergia sissoo* Roxb have promoted us to undertake the present study.

MATERIALS AND METHODS

Chemicals

All the chemicals were of highest available purity and were procured from E. Merck, Mumbai, India, Hi Media Laboratories, Mumbai, India and SD Fine Chemicals, Mumbai, India.

Procurement of plant material

The leaves of *Dalbergia sissoo (D.sissoo)* were collected in an around Madurai and it was authenticated by Dr.Stephan, taxanomist, American college, Madurai, Tamilnadu, India. The present study involves anatomical study as well as preliminary phytochemical standardization of the leaves of *Dalbergia sissoo* Roxb. For anatomical investigation, customary technique of microtome was followed[6]. Physical constant, behaviour of powder with chemical reagents and preliminary tests for extracts were also carried out [7].

Pharmacognostic evaluation

Organoleptic evaluation

In organoleptic evaluation, various sensory parameters of the plant material, such as size, shape, colour, odour, and taste of the leaves were recorded. It includes conclusions drawn from studies resulted due to impressions on organs of senses.

Microscopical investigation

The histological features of the leaf of *Dalbergia sissoo* were determined using the methods of Evans for quantitative study anatomical sections and the microscopy & chemo-microscopy of powdered samples were carried out according to methods outlined by Kay and Evans [6, 8].

Powder analysis

To a little quantity of powder taken onto a microscopic slide, 1-2 drops of o.1% phloroglucinol solution and a drop of concentrated hydrochloric acid were added, mounted in glycerol, covered with a slip and observed under microscope with 10x10 magnification. The characteristic features of the powder were reported below [6].

Physical evaluation

In physical evaluation, ash values viz., total ash, acid insoluble ash, water soluble ash, and extractive values viz., alcohol, hexane, petroleum ether, chloroform and water extractive values were determined[8-13]. 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 content of drug. The determinations were performed in triplicate and the results are expressed as mean± SD. The percentage w/w values were calculated with reference to the air-dried drug.

Determination of total ash value

Two gram of leaf powder of *D.sissoo* was taken in a tarred silica crucible and incinerated at a temperature not exceeding 450^{0}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

The total ash obtained from 2g powder was boiled for 5 min 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 2 g of leaf powder was boiled for 5 min with 25 ml of water and the insoluble matter was collected on the 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 alcohol soluble extractive value

Accurately weighed powder (5g) of leaf was taken and macerated with 100 ml of 95% alcohol for 24 hours. The content was frequently shaken during the first 6 hours and allowed to remain for 18 hours. The extract was filtered and 25 ml of the filtrate was evaporated and it was dried at 105^{0}C to a constant weight [12].

Determination of water soluble extractive value

Water soluble extractive value was determined using the procedure described for alcohol soluble extractive; instead of alcohol chloroform water was used as solvent.

Determination of hexane, petroleum ether and chloroform soluble extractive value

The procedure adopted under alcohol soluble extractive was followed using Hexane, petroleum ether and chloroform as a solvent instead of alcohol.

Preliminary phytochemical screening

The leaf powder was subjected to cold maceration using petroleum ether, chloroform, alcohol and water for 8 hours and the extract were evaporated to dryness. The dried extracts were weighed, and the percentage yield was calculated. The extracts were used for preliminary phytochemical screening with a battery of chemical tests viz., molisch's, fehling's benedicts and barfoed's test for carbohydrates; biuret and millon's tests for proteins; ninhydrin's test for amino acids; salkowski and libermann-burchard's reactions for steroids; borntrager's test for anthraquinone glycosides; foam test for saponin glycosides; shinoda and alkaline tests for flavonoids glycosides; dragendorff's, mayer's, hager's and wagner's tests for alkaloids; and ferric chloride, lead acetate tests for tannins and phenols [14].

Foaming index

1 gm coarse powder of *Dalbergia sissoo* was weighed accurately and transferred to a 500 ml conical flask containing 100 ml of water. It continued at moderate boiling for 30 minutes. Allowed to cool and filtered in to a 100 ml volumetric flask and volume was diluted by adding sufficient amount of water. The decoction was poured in to 10 stoppered test tubes in successive portion of 1 ml, 2 ml, 3 ml up to 10 ml and the volume of liquid in each test tube was adjusted to 10 ml with water. The tubes were stoppered and they were shaken in a lengthwise motion for 15 sec., two shake per sec. and they were allowed to stand for 15 minutes and the height of foam was measured [12].

Fluorescence analysis

The leaf powder and the extracts were subjected to fluorescence analysis with various reagents. The organic molecules absorb light usually over a specific range of wave length and many of them reemit such radiations. So if the powder is treated with different chemical reagents and seen in the UV chamber, different colours will be produced. Therefore it can be used for the identification of the drug. The fluorescence characteristic of the drug powder with different chemical reagent was studied by observing under UV Light at 254nm and 365 nm [13].

RESULTS AND DISCUSSION

Pharmacognostic evaluation

Macroscopy characteristics

Leaves alternate, bifarious, imparipinnate, leaf rhachis zigzag; petioles terete, very downy when young; stipules lanceolate, caducous. Leaflets 3 to 5, firm 3.8 to 6.3 by 3.5 to 4 cm, the terminal the largest and the smallest; distant, alternate, suborbicular, conspicuously and abruptly acuminate, puberulous when young, soon glabrescent, base narrowed or cuneate; petiolules 3 to 6 mm long.

Microscopical characteristics

T.S of leaf

Petiole

Transverse section of petiole is circular in outline (Fig 1.A). The outer most epidermis is made up of single layer of cells. Most of the cells elongate to form uniseriatetrichome. Epidermal cells are papillose. The cortex is broad and composed of round, closely arranged parenchyma cells (Fig 1.B and Fig 1.C). In the centre 'U' shaped with strongly incurved ends and approximately circular, leaving a small gap on the adaxial side, large, collateral vascular bundle is seen. Vascular bundle is surrounded by sclerenchyma fibres (Fig 1 D).

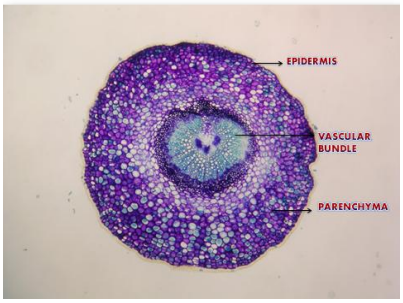

Fig1.A: T.S. of petiole

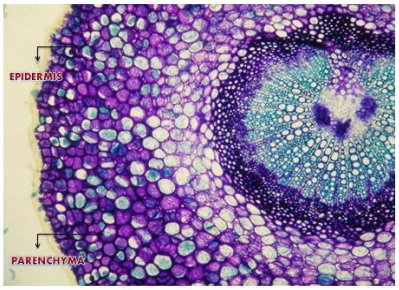

Fig 1.B: T.S. of petiole – A portion enlarged

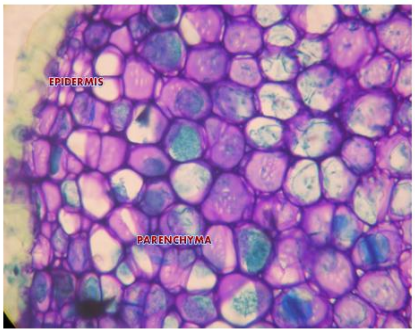

Fig 1.C: T.S. of petiole showing cortical cells

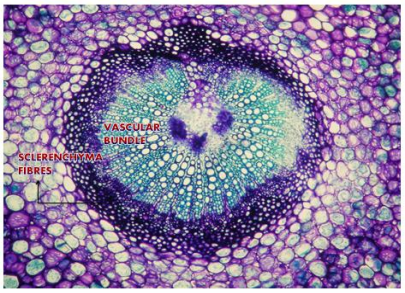

Fig 1.D: T.S. of petiole - Vascular bundle enlarged

Leaflet

Midrib

Transverse section of midrib shows a flat surface on the adaxial side and convexity on the abaxial side (Fig. 1 E, F). The epidermis is made up of single layer of rectangular transversely elongated cells (Fig. 1 E, F). Abaxial epidermis is papillose and inner walls are gelatinized. The hypodermal region of adaxial and abaxial epidermis is composed of 2 to 4 rows of collenchyma cells.

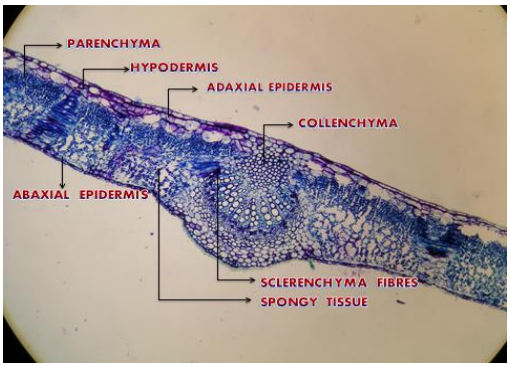

Fig 1.E: T.S. of leaf

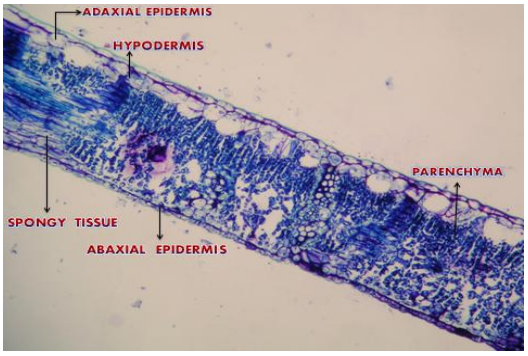

Fig 1.F: T.S. lamina

A large arc shaped collateral vascular bundle is situated in the centre. Sclerenchyma fibres are present on the adaxial and abaxial side of the vascular bundles.

Leaf is dorsiventral in structure. Adaxial epidermal cells are larger than the abaxial epidermal cells. Hypodermis on the upper side is made up of large rectangular parenchyma cells (Fig. 1. G).The palisade tissue is made up of 2 rows of columnar closely packed cells. The spongy tissue is composed of 5 to 7 rows of loosely arranged round parenchyma cells. A small crystalline grains or prisms or rod shaped crystals are seen in the mesophyll tissue. The stomatal index for abaxial epidermis is 17 to 21 (Fig. 1.H); palisade ratio 3 to 4; vein islet number 18 to 22 (Fig. 1.J). The smaller veins of the leaf are vertically transcurrent.

Fig 1.G: Adaxial foliar epidermis

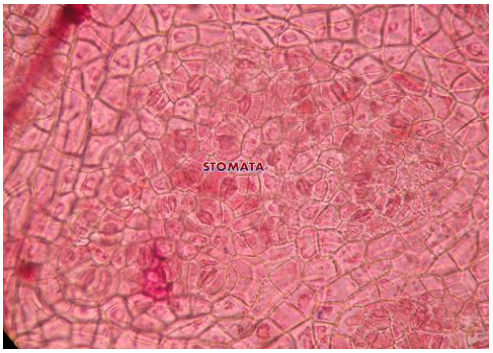

Fig 1.H: Stomata

Fig 1 j: Vein islets

Epidermis in surface view

The adaxial foliar epidermis is made up of polygonal parenchyma cells with straight wall and devoid of stomata. Uniseriate trichomes are noticed (Fig. 1. L).

Fig 1 L: Trichome - Enlarged

The abaxial foliar epidermal cells are also polygonal in shape with straight walls but smaller in size. It is perforated by rubiaceous stomata or stomata surrounded by a rosette of cells (Fig. 1. I).

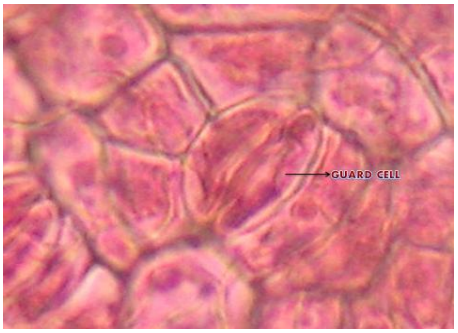

Fig 1 I: Stomata enlarged

Trichome

Trichomes are numerous, simple, uniseriate with a short basal cell accompanied by an elongated terminal cells with blunt tip (Fig. 1.K, L).

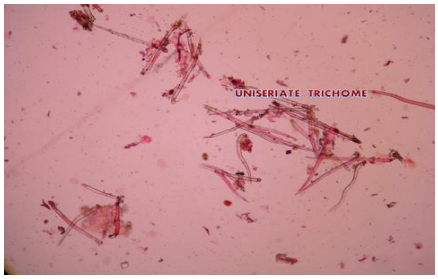

Fig 1 K: Trichome

Organoleptic characters

In organoleptic evaluation, appropriate parameters like taste, odour, size, shape, colour of the leaf and leaf powder were studied.

Nature : Coarse
Colour : Greenish yellow
Odour : Characteristic
Taste : Bitter followed by astringent taste.

Powder characters

Powder characters such as epidermal cells with rubiaceous stomata, Uniseriatetrichomes, polygonal parenchyma cells, sclerenchyma fibres, vascular bundles, lignified xylem fibres were noticed.

Quantitative microscopical data pertaining to stomatal frequency, palisade ratios and venation features are given in Table 1.

Table 1: Quantitative analytical microscopical parameters of the leaf of *Dalbergia sissoo*

S. No.	Parameters	Values *obtained
1	Stomatal number in upper epidermis	31.17 ± 0.27
2	Stomatal number in lower epidermis	16.84 ± 1.32
3	Stomatal index in upper epidermis	21.8 ± 0.78
4	Stomatal index in lower epidermis	24.3 ± 0.96
5	Vein islet number	18.5 ± 0.94
6	Vein termination number	5.7 ± 1.79
7	Palisade ratio in upper epidermis	3.40 ± 0.29

*** mean of 6 readings ± SEM**

Physical evaluation

Ash values

Total ash, acid-insoluble ash, sulphated ash, water soluble ash of leaf powder were done as per the reported methods mentioned in standard books and result are tabulated in Table 2a.

Foaming index

Foaming index is less than 100.

Preliminary phytochemical screening

The leaf powder was tested for the presence of phytoconstituents using reported methods mentioned in the standards and results are given in Table 3.

Table 2(a): Ash Values of *Dalbergia sissoo* leaf powder

Parameters	Values* expressed as %
Ash values	
Total ash	8.43 ± 0.49
Acid insoluble ash	3.33 ± 0.97
Water soluble ash	4.2±0.90
Water insoluble ash	7.8 ± 0.91

*** mean of 3 readings ± SEM**

Extractive values

Extracts were prepared with various solvents by reported methods mentioned in standard books. Percentages of the extractive values were calculated with reference to air-dried drug. Color and consistency of extract are given in Table 2b.

Table 2(b): Extractive Values of *Dalbergia sissoo*. Leaf

Parameters	Values* expressed as %
Extractive Values	
Hexane extract	7.81 ± 0.91
Petroleum extract	8.86± 0.48
Ether extract	9.82± 0.02
Chloroform extract	4.88± 0.04
Ethanol extract	22.78 ±0.81
70 % Ethanol extract	28.74 ±0.71
Methanol extract	21.47 ± 0.01
Aqueous extract	18.94± 0.74

*** mean of 3 readings ± SEM**

Table 3: Data showing the presence of phytoconstituents present in leaf powder of *Dalbergia sissoo*

Powder + Reagents	Colour / Precipitate	Presence of active principle
Picric acid	Yellow precipitate	Protein present
Conc. sulfuric acid	Reddish brown color	Phyto sterols present
LiebermanBurchard reagent	Reddish brown color	Phyto sterols present
Aqueous ferric chloride	Greenish black color	Tannins present
Iodine solution	Blue color	Starch present
Mayer's reagent	No cream color	Absence of alkaloids
Spot test	No stain	Fixed oils absent
Sulfosalicylic acid	White precipitate	Protein present
Aq. Sodium hydroxide	Yellow color	Flavanoids present
Mg – HCl	Magenta color	Flavanoids present
Aq. Lead acetate	White precipitate	Presence of tannins

The different qualitative chemical tests were performed for establishing the chemicals profile of the extracts. In the present investigation all the extracts of plant was analysed for the presence of carbohydrates, proteins, sterols, phenolic compounds, tannins and flavonoids using standard procedures. The results pertaining to this investigation were presented in Table 3.

Fluorescence analysis of drug powder and extract[15]:

Fluorescence analysis of drug powder and its various extract after treated with acids were studied at day light and U.V. light and the observation are presented in Table 4 &5.

Table 4: Fluorescence Analysis of powder of *Dalbergia sissoo*

Powder +reagent	Day light	UV light(254 nm)	*UV light(366 nm)*
Drug powder	Green	Green	Brown
Drug powder +aqueous 1M sodium hydroxide	Green	Green	Brown
Drug powder + alcoholic 1M sodium hydroxide	Green	Green	Brown
Drug powder + iodine	Red	Brown	Brown
Drug powder + 10% potassium hydroxide	Yellow	Greenish yellow	Brown
Drug powder + 1M hydrochloric acid	Green	Green	Brown
Drug powder + glacial acetic acid	Yellow	Greenish yellow	Brown
Drug powder + 50% sulphuric acid	Green	Green	Brown
Drug powder + 50% nitric acid	Green	Green	Brown
Drug powder + 50% hydrochloric acid	Green	Green	Brown

Table 5: Fluorescence Analysis of extracts of *Dalbergia sissoo*

Extracts	Consistency	Colour in Day Light	Colour under UV Lamp	
			360 nm	**254nm**
Hexane extract	Semisolid	Yellow	Reddish orange	Green
Petroleum extract	Semisolid	Yellow	Orange	Yellow
Ether extract	Semisolid	Greenish brown	Green	Greenish brown
Chloroform extract	Semisolid	Yellowish brown	Orange	Yellowish brown
Ethanol extract	Semisolid	Yellowish green	Orange	Green
Methanol extract	Semisolid	Yellowish green	Orange	Green
Aqueous extract	Semisolid	Brown	Green	Dark green

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