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PHARMACOGNOSTICAL AND PHYTOCHEMICAL STUDIES ON STEM BARK OF *AILANTHUS EXCELSA* ROXB. (SIMAROUBACEAE)

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ABSTRACT

Ailanthus excelsa, Roxb (Family-Simaroubaceae) is a large deciduous tree, 60-80 inch in height and 6-8 inch in girth with rough and light grey bark. It has a large panel of indication and it would be more suitable to evaluate the bark since it has been used in diarrhoea, dysentery, cholera, astringent, febrifuge, anthelmintic and liver tonic. The present paper highlights the exomorphology, histomorphology, physicochemical evaluation and preliminary phytochemical studies of the bark. For the better understanding of structural details Scanning Electron Microscopy (SEM) also employed. These observation will enable to standardize the botanical identify of the drug in its crude form. Data evolved in this investigation could be used in laying down pharmacopoeial standards for the drug studied, as standardization of herbal medicines is absolutely essential and is need of the hour.

KEY WORDS

Ailanthus excelsa, Simaroubaceae, Exomorphology, Histomorphology, Scanning Electron Microscope.

INTRODUCTION

Ailanthus excelsa, Roxb, (Family: Simaroubaceae) is a large, deciduous tree indigenous to central and Southern India and Sri Lanka[1]. It is known as tree of heaven or tree of Gods, Maharuk in Hindi, Mattipongilyam in Malayalam, Peru or Perumaruthu in Tamil[2]. Bark juice is a tonic to women after delivery; bark powder is useful in asthma, bronchitis, fever, weakness and its decoction in cholera [3-5]. Five quassinoids were isolated and derivitised namely Glaucurubin, a C_{20} quassinoid and three unnamed quassinoids (AX1, AY2) from the bark of *A. excelsa*[6-7]. Three quassinioids 1, 2 and 3, 4 dihydro excelsin were isolated from the stem bark along with five quassinoids, excelsin, glaucarubine, ailanthinone, glaucarubinone and glaucarubolone[8]. When the plant is subjected into a qualitative standardization, botanical identity is a pre-requisite. In the present study, the pharamacognostic parameters of the stem bark

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of *A.excelsa* is studied such as exomorphological, histomorphological, powder microscopy, quantitative microscopy and physical standards like ash values, extractive values, bitterness value, forming index, and loss on drying. Scanning electron microscopy is also employed to obtain the best possible structural details to assist in the solution of taxonomic problem, to avoid misleading of diagnostic features by oversimplified description and to locate presence of phytoconstituents which are not visible under light microscope.

MATERIALS AND METHODS

Plant material

The stem bark of *A.excelsa* was collected from Azhagar kovil Hills, Madurai, Tamil Nadu, India. It was identified and authenticated by Prof.S.Stephen, Department of Botany, The American College, Madurai and a voucher specimen (PCG/AE/015/2008) has been deposited in the herbarium of Department of Pharmacognosy, Madurai Medical College, Tamil Nadu, India.

Preparation of specimen

The barks were cut and removed from the plant and fixed in FAA (Formalin 5 ml + Acetic acid 5 ml+ 70% Ethanol 90 ml). After 24 hours of fixing, the specimens were dehydrated with graded series of tertiary butyl alcohol. Infiltration of the specimen was carried by gradual addition of paraffin wax (melting point 58-60°C) until tertiary butyl alcohol solution attained super saturation. The specimens were cased into paraffin blocks[9].

Sectioning

The paraffin embedded specimen was sectioned with the help of rotary microtome. The thickness of the section was 10-12µm. After dewaxing, the sections were stained with toluidine blue. Since toluidine blue is a polychromatic stain, the staining results were remarkably good and some phytochemical reactions were also obtained[10]. 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 iodine for starch[11-12].

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Labphot- 2 microscope units. For normal observations bright filed was used. For the study of crystals, starch grains and lignified cells polarized light were employed. Since these structures have birefringent property, under polarized light they appear bright against dark back ground[13].

Scanning Electron Microscopy (SEM)

Scanning Electron Microscope forms a three dimensional image on a cathode ray tube by moving a beam of focused electrons across an object and reading both the electrons scattered by the object and the secondary electrons produced by it. The electromagnetic lenses are used in this microscope and focusing is

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done by varying the current and the image is projected in photographic plate on screen giving easily comprehensive , quasi three dimensional representation of the objects examined leading to the better understanding of the ultra structure of plant cells. In addition, it also reveals the spatial relations, unsuspected details and previously undesirable characters. In other words the micrograph obtained by SEM, shows the best possible structural details of the specimens[14-15]. Sample for SEM analysis were mounted on the specimen stub using fevicol adhesive. Small sample was mounted directly on scotsch double adhesive tape. Samples were coated with gold to a thickness of 100 A^0 using Hitachi Vacuum evaporator. Coated samples were analyzed in a Hitachi Scanning Electron Microscope model S-450 operated at 15 kV and photographed.

As a part of quantitative microscopy length and width of the phloem fibers, the total ash, water-soluble ash, acid insoluble ash, bitterness value, foaming index, extractive values for various solvents and loss on drying were determined. The dried powdered materials were also subjected to identification tests for the detection of various phytoconstituents [11,16].

RESULTS AND DISCUSSION

Exo-Morphology

The bark exhibit grayish brown (outer surface), pale yellow (inner surface) color with longitudinal striations on inner side. Fresh bark emits disagreeable odors and it was intensively bitter. It breaks with irregular fracture and powdery mass.

Histo-Morphology

The bark is 1.6 cm thick and is differentiated into outer zone of periderm or outer bark and inner zone of secondary phloem or inner bark.

Outer Bark

The outer bark or periderm is 600 µm to 1mm thick. The outer part is an excess and many deep, narrow irregular tissues are seen with periderm. The periderm consists of broad phellem and narrow zone of phelloderm which is not prominent. The cells of the phellem are tabular zone in shape and thin walled with suberinised cell walls. Along the periphery of the bark, a narrow, concave band of phellem is formed enclosing a small island of phloem tissues. This portion of the periderm is called '*shell-bark*'. The *shell-bark* has outer phellem and inner concave shaped phloem with sclerenchyma elevation in between (fig.1).

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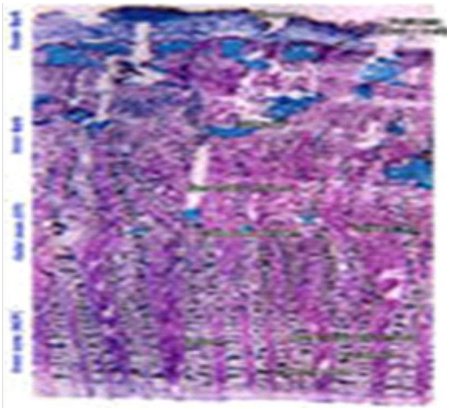

Fig 1.*Tranverse section of stem bark of A.excelsa*

Inner Bark

It consists of two zones, the outer zone, beneath the periderm is called collapsed phloem. The collapsed phloem is widest zone and consists of dilated rays, crushed sieve elements and tangentially distributed irregular sclerenchyma masses. The dilated rays are narrow in the inner zone and become wider in tangential plane comprising of horizontal bonds of rectangular cells. They are spindle shaped in the middle and become funnel shaped along the outer part. The ray cells are thin walled. In between the dilated rays are thin, dark, wavy streaks, these structures are crushed and obliterated sieve element known as crushed sieve elements. Few collapsed phloem also has prominent tangential blocks of sclerenchyma elements which occur more, less in tangential lines traversed by the dilated rays. The sclerenchyma blocks consist of both fibers and sclereids. The parenchyma cells in the collapsed phloem are wider and intact. They serve as storage cells of many ergastic substances (fig.2).

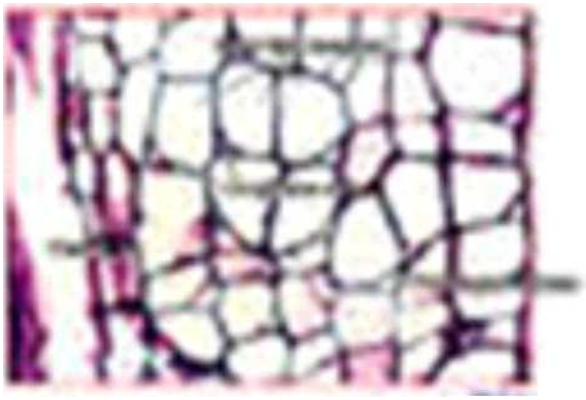

Fig 2.*Collapsed Phloem*

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Non-collapsed phloem

It is narrow zone consisting of intact sieve elements, narrow rays and small phloem parenchyma cells. The sclerenchyma cells are few or absent. The sieve elements and parenchyma cells occur in radial parallel rows in between the phloem rays. The sieve tube members are polyhedral in T.S view, they are few, and they are either in clusters or in radial rows. Their walls are thick. The companion cells occur along the lateral sieve tube. The sieve plate has wide sieve-pores (fig.3).

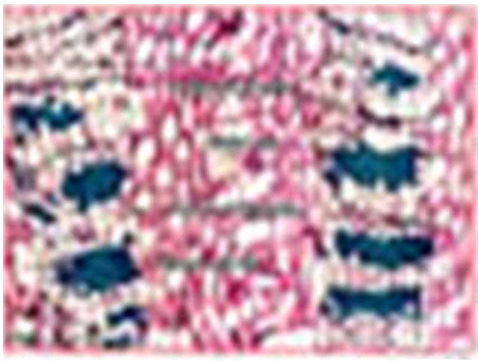

Fig 3.*Non-Collapsed phloem*

Powder microscopic observation

The powder of the bark when viewed under the microscope, it shows the presence of prismatic crystals and rosettes, cuboidal sclereids called levachy sclereids or stone cell and elongated figure like sclereids and long narrow thick walled fibers (fig.4).

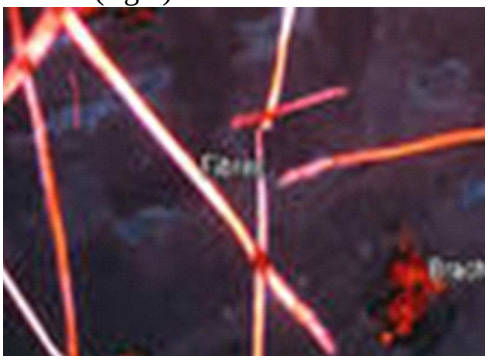

Fig 4.*Powder microscopical characters of A.excelsa*

Quantitative microscopy

The length and width of the phloem fibers, ash values, and extractive values in different solvents, bitterness value, foaming index and loss on drying were given in table no. 1, 2 & 3.

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Table 1.
Data showing different ash values of Ailanthus excelsa,Roxb.

S. No	Type of ash	% of ash values
1.	Total ash	8.15
2.	Water soluble ash	5.56
3.	Acid insoluble ash	3.05
4.	Sulphated ash	6.12

Table 2.
Data showing different extractive values of Ailanthus excelsa,Roxb.

S. No	Solvents used	% of extractive values
1.	Petroleum ether (60-80^{o}C)	2.56
2.	Chloroform	1.34
3.	Ethyl acetate	2.68
4.	Methanol	4.52
5.	Ethanol	7.38
6.	Water	15.32

Table 3.
Data showing quantitative parameters of Ailanthus excelsa,Roxb.

S. No	Parameters	Result
1.	Length of the phloem fiber	250-712.5 µm
2.	Width of the phloem fiber	12.5-37.5 µm
3.	Loss on drying	6.10%w/w
4.	Bitterness value	160 units/gram
5.	Foaming index	Less than 100

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Qualitative chemical test

The qualitative phytochemical tests reveals the presence of phytosterols, carbohydrates, proteins, saponins, terpenoids and mucilages and it was found that absence of fixed oils, alkaloids, glycosides, tannins, flavonoids and volatile oils in the stem bark of *A.excelsa.*

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