INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

PHARMACOGNOSTIC INVESTIGATION OF PLANT OF *BREYNIA RETUSA (DENNST).* (FAMILY - EUPHORBIACEAE)

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ARTICLE INFO

Article history
Received 07/09/2017
Available online
09/12/2017

Keywords
Breynia Retusa (Dennst),
Macroscopical,
Microscopical,
Physico-Chemical Analysis.

ABSTRACT

Pharmacognostic standardization including physico-chemical evaluation is meant for identification, authentication, and detection of adulteration and also compilation of quality control standards of crude drugs. Standardization of herbal drugs has traditionally been based on appearance and today microscopic evaluation is indispensable in the initial identification of herbs, as well as in identifying small fragments of crude or powdered herbs and detection of foreign matter and adulterants. Therefore the present study is to investigate the macroscopical, microscopical, and physico-chemical characteristics of the plant of *Breynia Retusa (Dennst)*. (Family - Euphorbiaceae). The results obtained from the study may play a major role in setting particular standards for the plant of *Breynia Retusa (Dennst)*. This might broaden its pharmacognostic, pharmacological, botanical and economical importance.

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*Please cite this article in press as **Y. Bhagyasri** et al. Pharmacognostic Investigation of Plant of Breynia Retusa (Dennst). (Family - Euphorbiaceae). Indo American Journal of Pharmaceutical Research.2017:7(11).*

INTRODUCTION

Simple pharmacognostic techniques used in standardization of plant material include evaluation of the morphological, anatomical and physico-chemical characteristics (1, 2). Physico-chemical parameters include Ash values which determine the quality and purity of a crude drug, especially in the powdered form. Hence ash determination furnishes a basis for judging the identity and quality of the drug that gives information to its adulteration with inorganic matter. Extractive values are useful for determination of the nature of the chemical constituents present.

Breynia retusa belongs to family Euphorbiaceae. Euphorbia is the largest genus in the family Euphorbiaceae and one of the sixth largest genera of flowering plants in the world, consisting of about 2000 species. Out of 81 species of Euphorbia occurring in India, about 40 species have been ethanobotanically studied. (3, 4)Many plants of this family have been used in traditional Chinese medicine for more than 2000 years as anti-tumor drugs. Ethnobotanical studies have revealed the folklore medicinal claim of *Breynia retusa* spp. (5) the plant has been proved to possess herbicide potential against Parthenium hysterophorus. (6) An herbal drug consisting of extracts of *Breynia retusa* has been used as a galactogogue. The juice of the stem is used in conjunctivitis and leaves as poultice to hasten suppuration. (7) Hence the present study attempted to explore the macroscopic, microscopic & physic-chemical, parameters of *Breynia retusa* leaf and stem with future scope to work on this species.

MATERIALS AND METHODS

Collection and Authentication of the plant material

The leaves and stem of *Breynia retusa* were collected from Tirupati forest region, India in the month of March 2016 and it was identified and authenticated. The taxonomical identification and authentication of the plant was done by Dr. K. Madhava Chetty,Assistant professor, Department of Botany, Sri Venkateswara university, Tirupati, india. The vocher specimens (March-2016/2141) were preserved in laboratory, Department of Pharmacognosy, Gland institute of pharmaceutical sciences for further reference.

Physico-Chemical Parameters (9, 10)

The physico-chemical evaluation of a crude drug involves the determination of identity, purity and quality. Purity depends upon the absence of foreign matter whether organic or inorganic, while quality refers essentially to the concentration of the active constituents in the drug that makes it valuable to medicine. The following standardization parameters were evaluated to obtain the qualitative information about the purity and quality of the parts of *Breynia retusa (Dennst)*. (Family - Euphorbiaceae).

a) Determination of foreign matter

Foreign matter in herbal drugs consists of either parts of the medicinal plant or it may be any organism, part or product of an organism. It may also include mineral admixtures not adhering to the medicinal plant materials e.g. soil, stones, dust etc. The specified quantity of plant material was spread on a thin layer of paper. By visual inspection or by using a magnifying lens (5X or 10X), the foreign matters were picked out and the percentage was recorded.

b) Determination of moisture content

An accurately weighed quantity of about 2 g of powdered drug was taken in a tared glass petridish. The powder was distributed evenly. The petridish was kept open in vacuum oven and the sample was dried at a temperature between 100 to 105ºC for 3 h until a constant weight was recorded. Then it was cooled in a desiccator to room temperature, weighed and recorded. % Loss on drying was calculated using the following formula.

$$\% \text{ Loss on drying} = \frac{\text{Loss in weight of the sample}}{\text{Weight of the sample}} \times 100$$

c) Determination of total ash values

Weighed accurately, 3 g of the powdered material in a silica crucible which was previously ignited and weighed. The powdered material was spread as a fine even layer at the bottom of the crucible. The crucible was incinerated until a red hot material was obtained, not exceeding 450°C temperature and free from carbon. The crucible was cooled and weighed. The percentage of the total ash was calculated with reference to the air dried powdered sample.

$$\% \text{ Total ash value} = \frac{\text{Wt. of total ash}}{\text{Wt. of crude drug taken}} \times 100$$

Determination of acid insoluble ash value

The obtained total ash was boiled with 25 ml of 2N Hcl for 5 min. The insoluble ash was collected on ash less filter and washed with hot water. The insoluble ash was transferred into pre-weighed silica crucible, ignited, cooled and weighed. The procedure was repeated till the constant weight was obtained. The percentage of acid insoluble ash was calculated with reference to the air dried drugs.

$$\% \text{ Acid insoluble ash value} = \frac{\text{Wt. of acid insoluble ash}}{\text{Wt. of total ash}} \times 100$$

Determination of water soluble ash value

The total ash obtained was boiled with 25 ml of chloroform water I.P. For five min. The insoluble matter was collected on a ash less filter paper & and washed with hot water. The insoluble ash was transferred into pre-weighed silica crucible, ignited for 15 min at a temperature not exceeding 450°C, cooled and weighed. The weight of the insoluble matter was subtracted from the weight of total ash. The percentage of water soluble ash was calculated with reference to the air-dried sample drug.

$$\% \text{ Water soluble ash value} = \frac{\text{Wt. of total ash - Wt. of water insoluble ash}}{\text{Wt. of crude drug taken}} \times 100$$

d) Determination of alcohol soluble extractive value

About 5 g of air dried coarse powdered drug was weighed and macerated with 100ml of 90%alcohol in a closed flask for 24 hrs, shaking frequently during the first 6 hrs & allowed to stand for 18 hrs .Thereafter it was filtered rapidly taking precautions against loss of the solvent. 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed dish, dried at 105°C & weighed. The percentage w/w of the alcohol soluble extractive was calculated with reference to the air-dried drug.

% Alcohol soluble extractive value = 80 x Wt. of residue

Determination of water soluble extractive value

About 5 g of air dried coarse powdered drug was weighed and macerated with 100ml of chloroform water I.P. in a closed flask for 24 hrs, shaking frequently during the first 6 hrs & allowed to stand for 18 hrs .Thereafter it was filtered rapidly taking precautions against loss of the solvent. 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed dish, dried at 105°C & weighed. The percentage w/w of the water soluble extractive was calculated with reference to the air-dried drug.

% Water soluble extractive value = 80 x Wt. of residue

MACROSCOPICAL STUDIES

Macroscopical features of the parts of *Breynia retusa (Dennst)*. (Family - Euphorbiaceae). Was studied directly in the field and photographed under original environment. The organoleptic features of powder prepared from parts (leaves and stems) were evaluated.

MICROSCOPIC STUDIES

Transverse Section of the Specimen

Collection of specimens

The plant specimens for the proposed study were collected from tirumala forest, tirupathi. Care was taken to select healthy plants and normal organs. The required samples of different organs were cut and removed from the plant and fixed in FAA (formalin-5ml+ acetic acid-5ml+ 70% ethyl alcohol-90ml). After 24hrs of fixing, the specimens were given by Sass, 1940. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60^0 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 sections was 10-12 µm. De-waxing of the sections was done by customary procedure (Johansen, 1940). The sections were stained with Toluidine blue as per method published by O'Brian *et al* (1964). 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). For studying stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of the leaf with 5 % sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid (Sass, 1940) were employed. Glycerine mounted temporary preparations were made for macerated materials. Powdered materials of different parts were cleared with NaoH and mounted in glycerine medium after staining. Different cell component were studied and measured.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Labphot 2 microscopic unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was used as they appear bright against dark background. Magnifications of the figures are indicated by the scalebars. Descriptive terms of the anatomical features are as given in the standard anatomy books (Esau, 1960, 1964).

RESU LTS AND DISCUSSION

Table-1: Physico-chemical parameters of. *Breynia Retusa (Dennst)*.

S.NO	PARAMETERS	VALUES	
		LEAF	STEM
1	Foreign matter	0.13 gm	0.11 gm
2	Moisture content	1%	0.9%
3	Alcohol-soluble extractive value	0.54 gm	0.58 gm
4	Water soluble extractive value	0.23 gm	0.27 gm
5	Total ash value	0.86	0.89
6	Water soluble ash value	0.05	0.03
7	Acid insoluble ash value	0.01	0.01

Table-2: Macroscopical Features of *Breynia retusa (Dennst)*.

Characteristics	Observations		Powdered plant material	
	Leaves	Stem	Leaf	Stem
Colour	Bright green-coloured	Light-brown-coloured.	Pale green	Light-brown-coloured.
Odour	Characteristics.	Characteristics.	Characteristic	Characteristics.
Taste	Tasteless	Sweet	Slightly Sweet	Tasteless
Shape	Blade Is Ovate	Longitudinally fissured.	_	_

Figure 1: Whole plant of *Breynia retusa (Dennst)*.

Figure2. Leaf of *Breynia retusa (Dennst)*.

Figure3. Stem of *Breynia retusa (Dennst)*.

Macroscopical Studies

It is assumed that macroscopical evaluation of any plant drug is considered to be the primary step for establishing its quality control profile. Proper authentication of a drug depends almost entirely on macroscopical characters. The macroscopical description of a crude drug includes size, shape, nature of outer and inner surfaces, type of fracture and organoleptic characteristics like colour, odour, taste, consistency, etc. The macroscopical feature of the fresh whole plant parts of *Breynia retusa* Willd. As well as the powder of dried of plant was studied and the results were shown in table 1. The photographs of both plant parts are shown in Figures 1 –3.

Microscopical studies of *Breynia retusa* (Dennst)

Microscopical study of an organized drug either in entire or powdered form is one of the important aspect of its histological evaluation. The arrangement of tissues in transverse and longitudinal section and types of cells and cell contents are revealed by suitable histological study of a crude drug with the aid of microscope. Microscopical evaluation of a crude drug also involves linear measurements, study of surface preparations of leaves, quantitative microscopical aspects and chemo-microscopy. Certain microscopical characteristics like stomata, trichomes, calcium oxalate crystals, starch grains, stone cells, palisade ratio, vein islet number, vein termination number, etc., are important anatomical characteristics of organized drugs. The microscopical studies of *Breynia retusa* Willd. Was carried out and it shown following features.

LEAF:

The leaf is mesomorphic and dorsiventral. It constists of a prominent midrib and uniformily thin and smooth lamina (fig-4.1). The midrib is planoconvex with flash adaxial side and broadly conical abaxial part (fig-4.2). The midrib is 500um thick and 550um wide. The adaxial epidermis consists of small, squarish thin walled cells. The abaxial epidermis has small, leaf thick walled cells. Beneath the adaxial epidermis are three layers of small collenchyma cells (fig-4.2)

The palisade layer is horizontally transcurrent beneath the adaxial epidermis. The ground parenchyma tissue in the abaxial part of the midrib consists of angular, thick walled compact cells.

The vascular bundle of the midrib is single, collateral and flat. It is located in adaxial part of the midrib. The vascular bundle consists of about 11 vertical lines of xylem vessels. The vessels are circular and elliptical, thick walled and linginified. The metaxylem elements are 30um wide. Phloem occurs in thin layer beneath the xylem unit. The phloem elements are thin walled and dorssely stained. Beneath vascular bundle is a thick are of sclenchyma cells (fig-4.3).

Lamina: (fig-5.1)

The lamina is 110um thick. The adaxial epidermis consists of squarish or rectangular thick walled cells with prominent cuticle. The elder leaves have narrowed the epidermis cells. The abaxial epidermis has thin cells which are cylindrical or circular with outer tanglutical walls being slightly raised. The palisade cell is thin, narrow and cylindrical and arranged in wide gaps in single horizontal layer. The spongy parenchyma tissue is many layered; the cells are small and interconnected to form wide air-spaces. (Fig-5.1)

Leaf –margin: (fig-5.2).

The margial part of the lamina is slightly currerd down. It is 120um thick. The epidermal layer of the leaf- margin includes small circular, highly thick walled cells. The mesophyll tissue is not differentiated into palisade and spongy parenchyma, undifferentiated compact tissue occurs at the margial part

Crystal distribution

Calcium oxalate crystals are common in mesophyll cells of the midrib (fig-6.1) and in the phloem parenchyma cells of the midrib (fig-6.2). The crystals are druses they are spherical bodies with spiny surface. The crystal are random is distribution. Only one occurs in a cell. The crystal containing cells are normal cells and not modified. The maximum size of the crystal is 10um.

STEM:

Thin stem (fig-7.1, 2) .The thin stem is circular in outline measuring 2.1 mm in diameter. The stem consists of epidermis, cortical zone, and vascular cylindrical and contral pith (fig-4.1).

The epidermal layer consists of rectangular thick walled cells with prominent cuticle. Inner of the epidermis occurs a wide zone of about seven layers of cortical parenchyma. Some of the cortical cells possess chloroplasts (fig.7.2).

Along the inner boundary of the cortex occurs a thin wavy continuous layer of sclenchyma cells. The sclenchyma layer is one or two layered. The cells are small angular and lignified. (Fig-7.2)

The secondary phloem some is 100um thick. It includes small thick walled angular dorsely stained sieve elements and phloem parenchyma cells.

Secondary xylem cylinder is a hollow cylinder of 700um thickness. It consists of vessels, fibers and rays. Most of the vessels are in long radial multiplas and a few vessels are solitary xylem fiberes are narrow, thick walled lignified cells. Xylem rays are thin, straight lines. The ray cells are radially along, thick walled and lignified (fig-7.2).

Thick stem:

Thick stem consists of well developed periderm at certain regions, periderm is lacking in other regious (fig-8.1).the cortex is chlorenchymatous. The sclerotic layer lying inner of the cortex is up to 4 layers thick. The periderm originates from the phallogen layer, which has produced phallem outside and phalloderm in side. The phellem cells are two layered, wide and radially oblong. The cell walls are thick (fig-8.2).

The phelloderm is single layered and the cells are horizontally elongated and rectangular in shape.

Secondary phloem (fig-9.1)

The secondary phloem consists of rectangular thick walled darkly stained cells called sieve elements. The sieve elements are in regular vertical compact lines. Phloem parenchyma cells are also sens in this tissue.

Secondary xylem:

Secondary xylem includes wide, circular lightly thick walled vessels which are is long radial multiples (fig-9.2). The vessels are 40um in diameter. Xylem fibers are wide radially oblong very thick walled lignified cells. Their cell lumen is wide, they are 15um in diameter. The xylem rays are single cell thick, they are less straight and their cells are squarish thick walled and lignified.

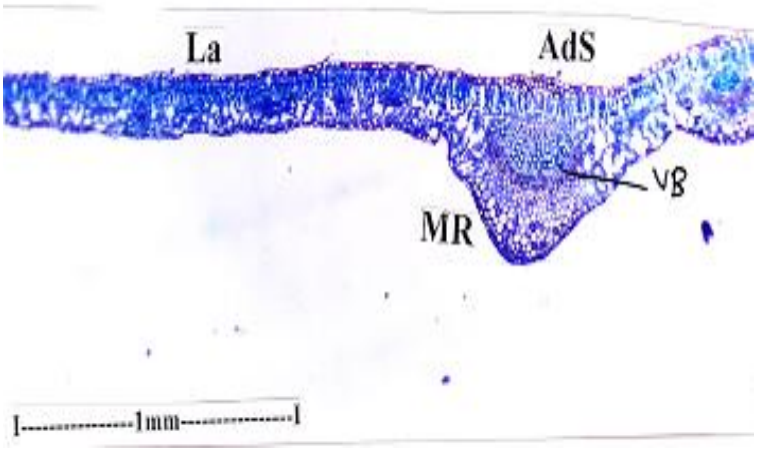


Figure -4.1: TS of Leaf through Midrib.

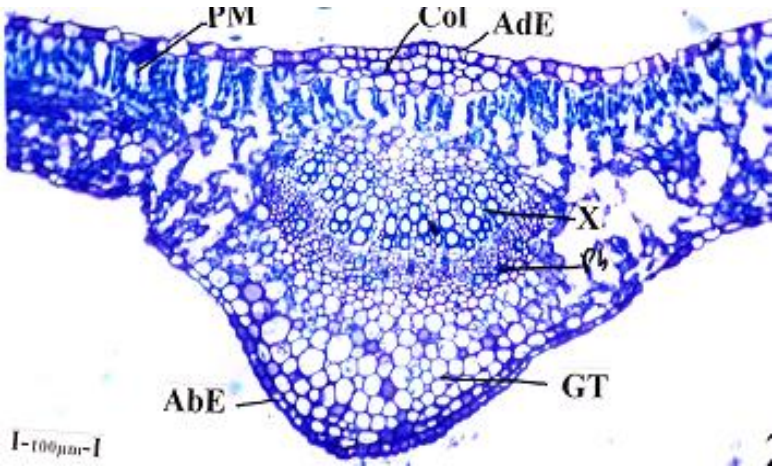


Figure -4.2: TS of Midrib.

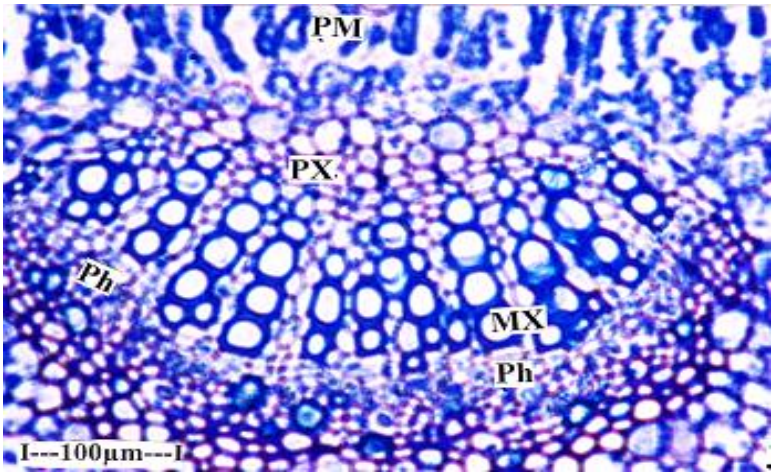


Figure -4.3: Vascular tissue of the midrib.

(ABE. Abaxial Epidermis; ADE: Adaxial Epidermis; ADS: Adaxial Side; Col: Collenchyma; GT: Ground tissue; La: Lamina; MR: Midrib; MX: Metaxylem; Ph: Phloem; PM: Palisade Mesophyll; PX: Proto Xylem; VB: Vascular Bundle; X: xylem).

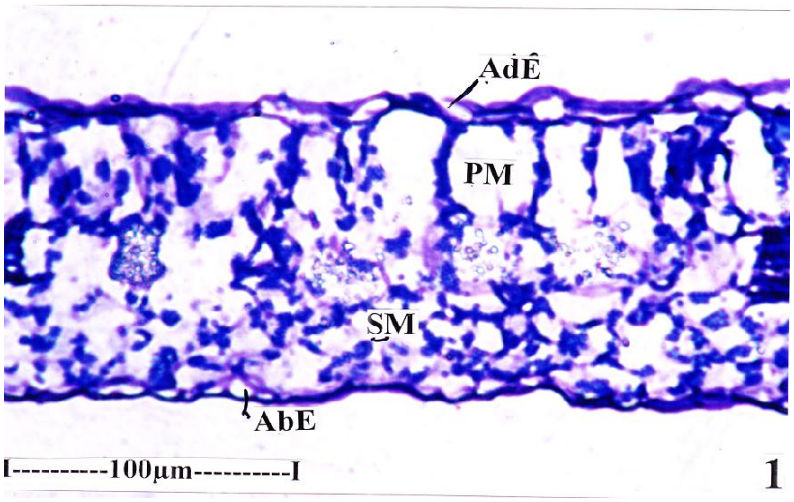


Figure -5.1: TS of Lamina;

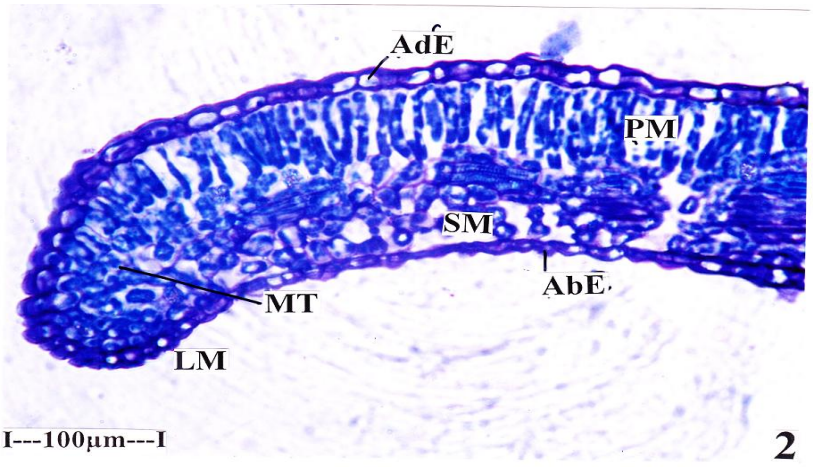
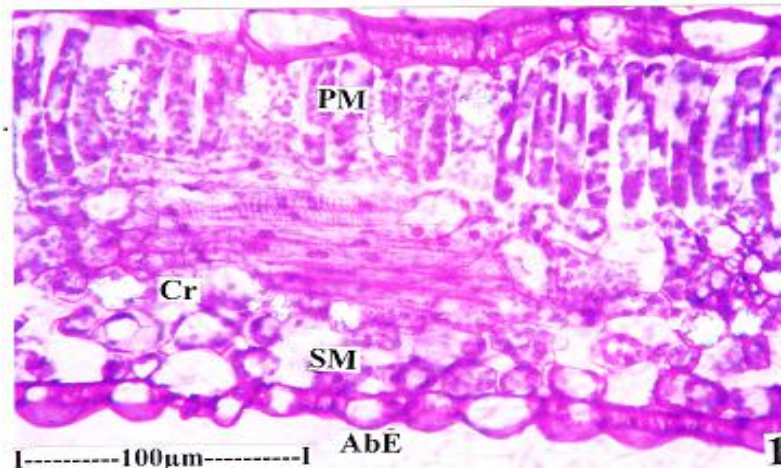
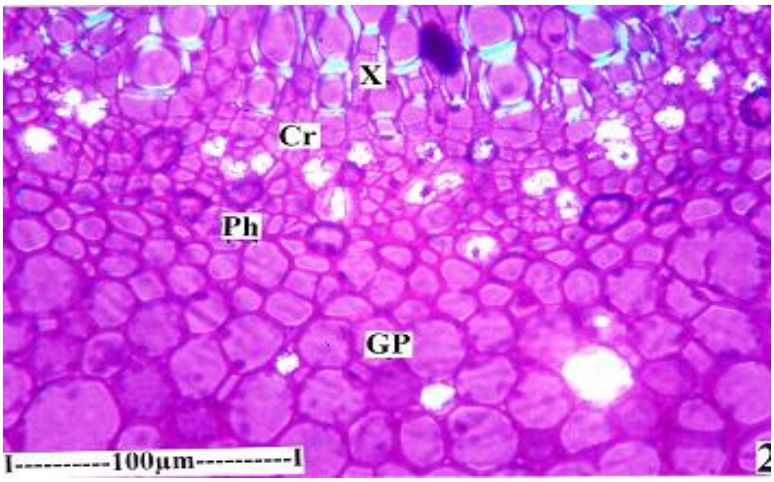


Figure -5.2: TS of leaf margin.

(ABE: Abaxial Epidermis; ADE: Adaxial Epidermis; LM: Leaf Margin; MT: Mesophyll Tissue; PM: Palisade Mesophyll; SM: Spongy Mesophyll;)



Druses in the leaf mesophyll cells.



Calcium oxalate druses in the phloem parenchyma of the petiole (as secondary polarized light).

Figure -6.1: Calcium Oxalate.

(ABE: Abaxial Epidermis; CR: Crystal's; GP: Ground Parenchyma; Ph: Phloem; Pm: Palisade Mesophyll; Sm: Spongy Mesophyll)

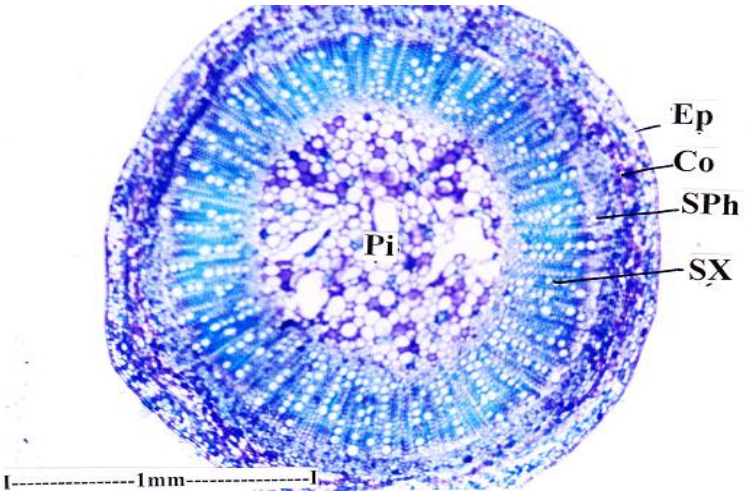


Figure -7.1: TS of Thin Stem Entire View.

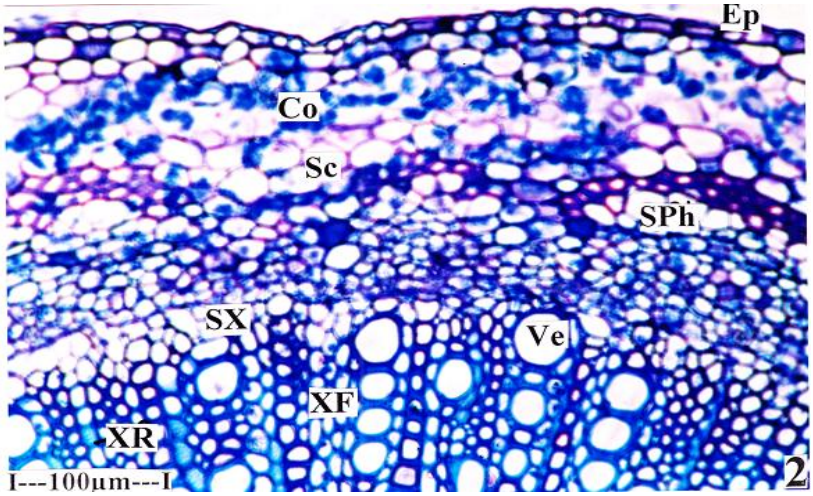


Figure -7.2: TS of thin stem a sector Enlarged.

(Co: Cortex; Ep: Epidermis; Se: Sclenchyma; Sph: Secondary Phloem; Sx: Secondary Xylem; Ve: Vessel; Xf: Xylem Fibres; Xr: Xylem Ray ;)

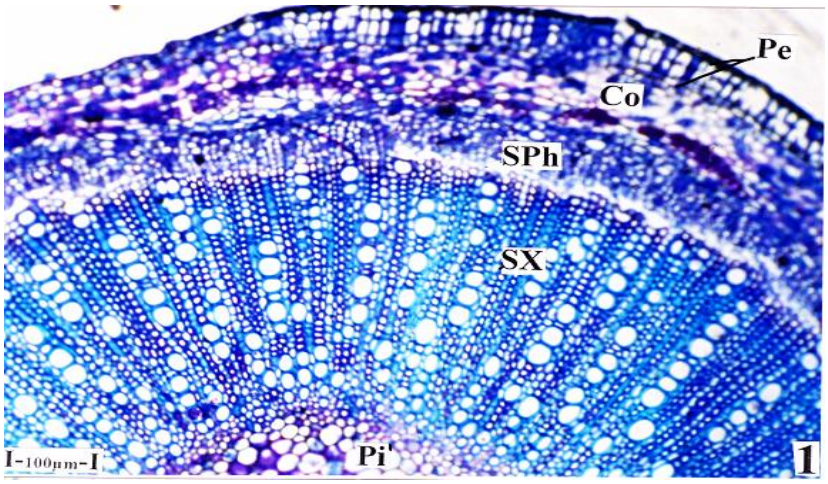


Figure -8.1: TS of Thick Stem.

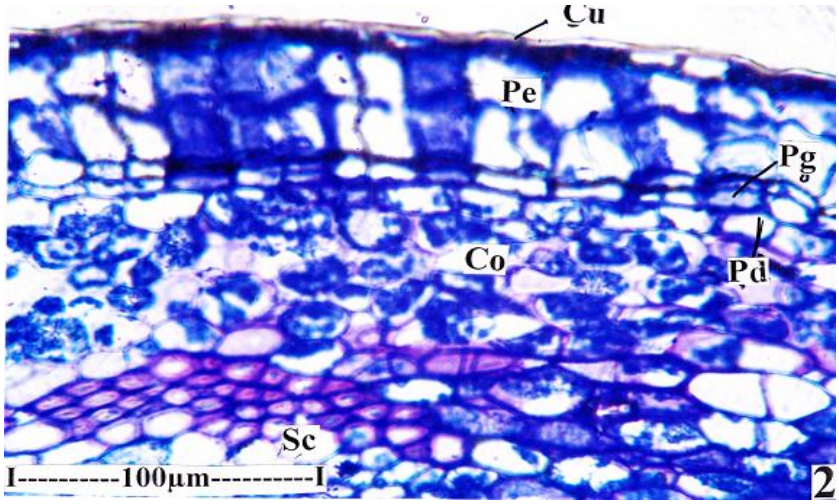


Figure -8.2: TS of Thick Stem Outer Portion.

(Co: Cortex; Cu: Cuticle; Pe: periderm (phellem), Pg: Phallogen; Pd: Phelloderm; Pi: Pith; Sc: Selenchyma; Sph: Secondary Phloem; Sx: Seconsary Xylem)

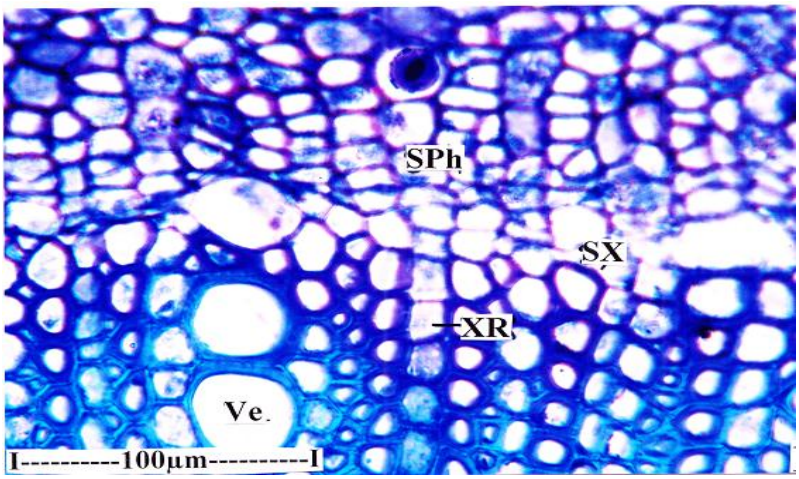


Figure -9.1: TS of Old Stem: Secondary Phloem.

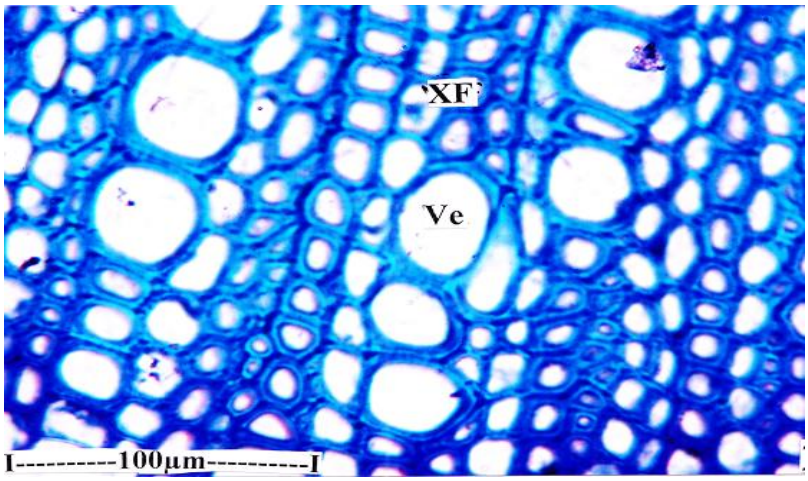


Figure -9.2: TS of Old Stem: Secondary Xylem.

(Sph: Secondary Phloem; Sx: Secondary Xylem; Ve: Vessels; Xf: Xylem Fiberes; Xr: Xylem Ray)

CONCLUSION

The results obtained from present study may play a major role in setting particular standards for the plant of *Breynia Retusa (Dennst)*. This might broaden its pharmacognostic, pharmacological, botanical and economical importance. These parameters may also prove beneficial in identification of the plant. Thus, with the help of these standards we identify the adulteration of the plant of *Breynia retusa (Dennst)*. This will be of great use for the future workers in selecting the correct herbal specimen.

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