



STUDIES ON PHARMACOGNOSTICAL SPECIFICATIONS OF *AZIMA TETRACANTHA* LAM.

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ABSTRACT

In ethnomedicinal practices the traditional healers use the leaves and root barks of *Azima tetraacantha* in the treatment of various ailments. Scientific parameters are not yet available to identify the true plant material and to ensure its quality. Therefore the present work has been undertaken to establish the necessary pharmacognostic standards for evaluating the plant material. In our studies, anatomical studies on the root bark, stems and leaves of *Azima tetraacantha* Lam. have been carried out. Various parameters like morphology and microscopy of the root bark, stems and leaves and also powder analysis, fluorescence characteristics and physico-chemical constants of root bark were studied and the salient diagnostic features are documented. Obvious morphological features of different organs of this plant and the microscopic characteristics were found in the tissue structures of the root bark, stem, leaf and many diagnostic elements were found to be useful evidences in the identification of this medicinal plant.

KEY WORDS: *Azima tetraacantha*, ethnomedicine, microscopy, pharmacognostical parameters.

INTRODUCTION

In recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance (Reddy *et al.*, 1999, Venkatesh *et al.*, 2004). Despite the modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. According to the World Health Organization

(WHO, 1998), the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken.

Azima tetraacantha Lam. (Family: Salvadoraceae) locally known as “*Mulsangu*”, is a rambling spinous shrub flowering throughout the year found in Peninsular India, West Bengal, Orissa, African Countries and extends through Arabia to tropical Asia. The juice of the leaves is said to relieve the cough phthisis and asthma. In western India juice of the leaves is applied as eardrops against

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earache and crushed leaves are placed on painful teeth. In India and Sri Lanka the root, root bark and leaves are administered with food as a remedy for rheumatism (Chopra, 1956, Kritiker and basu, 1987; Hebbar *et al.*, 2004). The plant is considered as a powerful diuretic and is also used to treat rheumatism, dropsy, dyspepsia, chronic diarrhoea and as a stimulant tonic for women after confinement (Nadkarni, 1976).

According to an ethnomedicinal survey carried out by Hebbar *et al.*, the rural population of Dharwad district in Karnataka, India—used this plant to treat tooth ache in oral health care. This plant is also used as a food and for various herbal medicines in Africa, India and Madagascar (Bennet *et al.*, 2004). Locally, the traditional healers from Tirunelveli district of Tamilnadu are using root bark (paste with butter milk) of this plant as potent remedy for jaundice (Personal information). This plant has been reported to possess different biological activities like anti-inflammatory, wound-healing, diuretic and analgesic activities (Ismail *et al.*, 1997, Jaswanth *et al.*, 2001, Nandgude *et al.*, 2007,).

Most of the cases of accidental herbal medicine misuse start with wrong identification of a medicinal plant prescribed. Many of the traditional systems have records where one common vernacular is supplied in place of two or more entirely different species. However, no scientific parameters are available to identify the true plant material and to ensure its quality. Therefore the present work has been undertaken to establish the various pharmacognostical parameters, which could serve as a valuable source of information and provide suitable standards for the future identification of this plant.

MATERIALS AND METHODS

Plant materials

Fresh plant was collected from Wastelands of Kadyanallur, Tirunelveli (District), Tamilnadu, India. The plant specimen was authenticated by Dr.V.Chelladurai, Research Officer Botany, Survey of Medicinal Plants Unit, Tirunelveli. A voucher specimen (Voucher No. ATC27/08/2005) has been deposited at the herbarium unit of the Department of Botany, Siddha Medical College, Palayamkottai, Tirunelveli (District), Tamilnadu, India. All the reagents used were of analytical grade obtained from Sigma Chemical Co, St. Louis, USA or Fine Chemicals Ltd., Mumbai, India.

Collection of Specimens

The different organs of this plant were cut and removed from the plant and fixed in FAA (Formalin 5ml+ Acetic acid 5ml+70%Ethyl alcohol 90 ml) for histological studies; transverse sections (T.S) of the different organs of the plant material. After 24 hours of fixing, the specimens were dehydrated with graded series of tertiary-butyl alcohol (TBA) as per the schedule given by Sass, 1940. Infiltration of the specimens was carried out by

gradual addition of paraffin wax (melting point 58-68°C) until TBA solution attained supersaturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were section with the help of rotary microtome. The thicknesses of the sections were 10-12 µm. Dewaxing of the sections was performed by customary procedure (Johansen, 1940). The sections were stained with toluidine blue as according to the method prescribed by O'Brien *et al.*, 1964. Wherever necessary, the sections were also stained with saffranin and Fast-green. The microphotographs of the sections were made using Olympus BX 40 microscope attached with Olympus DP12 digital camera.

Physico-chemical constants

Physico-chemical constants such as consistency and organoleptic characters (Pratt and Chase, 1949), fluorescence (Kokashi *et al.*, 1958) and the percentage of total ash, acid-insoluble ash, water-soluble ash and alkalinity of water soluble ash values and loss on drying (LOD) were calculated as per the Indian Pharmacopoeia (Anonymous, 1985).

RESULTS

The External features of the plant;

The plant is armed, straggling spinous shrub; young branches are tetragonous and pubescent; spines axillary, thick and pointed (**Fig. 1a**).

Leaves are elliptic, obovate to lanceolate, acute, spine-tipped and dark green to pale green colored. (Fig.1a). The flowers are small, greenish white (or) yellow colored, unisexual, zygomorphic, axillary clusters. Calyx; campanulate, lobes 4 toothed, unequal, imbricate. Corolla; cream colored, 4 lobed, free. Stamens; 4, free. Pistillode; conical. Ovary; 2 locular, one oval per cell, staminodes four. Fruits; globular, white shiny. Seeds; compressed, circular. Root bark; (Fig. 1b)

Microscopical features of the leaf

Transverse section of the leaf shows dorsiventral nature of the leaf. Following are the important tissues in the midrib regions, lamina, petiole and the (**Fig. 2**).

Midrib: The midrib is flat on the adaxial side and hemispherical on the abaxial side (**Fig-2.1**). The midrib is 400µm in vertical plane and 200 µm in horizontal plane. The epidermal layer of the midrib is thin, rectangular with prominent cuticle; the abaxial epidermis thinner and the cells are spindle shaped (**Fig-2.1**). The ground tissue on the adaxial part consists of a vertical mass of hyalane, compact parenchyma cells. The abaxial midrib has small, compact, thin walled parenchyma cells.

The vascular bundle is single, top shaped and consists of a few parallel, short, radial multiples of vessels and an abaxial arc shaped phloem. There is no distinct bundle sheath; no sclerenchyma elements are seen in the vascular bundle (**Fig-2.1**).

Lamina: The lamina is 230 μm thick. Both adaxial and abaxial sides are smooth and even; no trichomes are evident. The adaxial epidermis is slightly thicker than the abaxial epidermis; it consists of horizontally rectangular cells with distinct cuticle. Beneath the epidermal is a single layer of large, hyaline, rectangular hypodermal layer of cells. The abaxial epidermis consists of narrow, spindle shaped cells; this layer is stomatiferous. The mesophyll tissue is differentiated into adaxial palisade zone, which is 80 μm in height and it consists of two layers of short, less compact thin walled cells. The lower zone is spongy mesophyll which has four or five layers of lobed, loosely arranged cells. The vascular bundles of the lateral veins are embedded in the median part of the mesophyll tissue.

Epidermal tissues (Fig.2.2): The epidermal tissue as seen in paradermal section. It consists of stomata and epidermal cells. The stomata are tetracytic with four subsidiary cells or anisocytic with four unequal subsidiary cells. The guard cells are uniformly elliptical with prominent nuclei. The epidermal cells are polygonal or more predominantly rectangular in surface view. They have prominent nuclei. The anticlinal walls of the epidermal cells are straight and fairly thick.

Petiole (Fig.2.3): The basal and upper parts of the petiole differ in cross-sectional outline; but the ground tissue and the vascular bundle remain similar. The basal and upper part of the petiole is circular in outline in measuring 1.15 μm in diameter. The surface is smooth and even. The petiole has thin, continuous epidermis made up of thick walled elliptical epidermal cells. The ground tissue has homogenous, parenchymatous, compact thin walled cells. The vascular strand is single, collateral and deeply arc shaped. It consists of closely arranged parallel, radial files of xylem elements and a thin continuous arc of phloem on the abaxial sides of the xylem band. A butting arc of phloem occurs in a discontinuous row of sclerenchyma patches.

The distal (upper) part of the petiole is 1mm in horizontal plane and 850 μm in vertical plane. It is semicircular in outline; the adaxial side is flat with short, thick lateral wings. The abaxial part is semicircular and even (Fig 2.3). It consists of thin epidermis, compact, homogenous, thin walled parenchymatous ground tissue and single, collateral arc shaped vascular bundle, supported by abaxial small masses of sclerenchyma cells.

Microscopic features of the stem (Fig 3.1): Young stem, measuring 1.5mm thick was studied. It shows initial stage of secondary growth. It is somewhat four-angled in cross sectioned view. The outline is smooth and even. The stem consists of a distinct continuous epidermis, cortex, vascular cylinder and pith.

Epidermis is thin and the epidermal cells are squarish or rectangular, coated with thick cuticle. Stomata are frequently seen in the epidermis.

Cortex is 150 μm wide. It consists of chlorenchyma and parenchyma cells which are compact and homogenous (Fig 3.1 and 3.2).

Pith is wide, homogenous and parenchymatous. The pith cells are angular and thick walled; they vary in size and cells of different sizes intermixed (Fig 3.2)

Vascular cylinder is in the form of the outline of the stem. It consists of about 29 discrete vascular bundles, separated from each other by narrow medullary rays. The rays are 3-5 cells wide; as they reach the periphery, the cells dilate tangentially into rectangular cells (Fig 3.2). The vascular bundles are radially elongated; a thick, semicircular mass of sclerenchymatous bundle-cap occurs on the cortex part of the phloem of each bundle. Primary xylem occurs on the inner part of the vascular bundle. Secondary xylem consists of outer cluster of wide vessels and inner narrow, compact band of thick walled fibres and a few wide vessels.

Microscopical features of the root bark

The root-bark has wide, well developed superficial Periderm wide pseudocortex and wide secondary phloem (Fig 4.1).

Periderm (Fig 4.1); It is superficial in position; the Periderm surface has no deep fissures. It consists of homogeneous phellan cells; phelloderm is not evident. The phloem zone is 200 μm wide. The cells are fairly thick walled; the cells are tabular in shaped, arranged in regular radial rows; the cell walls are suberised.

Inner to the Periderm is a wide parenchymatous zone; the cells are rectangular and are arranged tangential files. These cells are derivatives of the dilated phloem rays simulating the cortex and this zone is sometimes called pseudocortex (Fig 4.1).

Secondary phloem constitutes the major portion of the bark. The phloem consists of two zones; i) Collapsed phloem, and, ii) Non-collapsed phloem

i) **Collapsed phloem** is wider than the non collapsed phloem. It includes wide dilated phloem rays and conical bands consisting of enclosed sieve elements and tangential bands of sclerenchyma cells. The dilated rays have transverse bands of this walled rectangular cell. The collapsed phloem is wider in the inner part and gradually becomes tapering into thin tails towards the periphery. Within the collapsed phloem curves are seen dark, thin rectangular lines alternating with tangential bands of fibers. The dark lines represent the crushed sieve elements of the phloem.

ii) **Non-collapsed phloem;** The intact phloem in narrow semicircular segments, innermost to the collapsed phloem and just outer to the secondary xylem.

TLS view of the bark (Fig 5.1); The phloem tissue of the bark has wide and light phloem rays; they are nonstoried. The rays are of oak-type. The individual rays are homocellular; the cells are small, polygonal compact and thin walled. The rays are 2mm in height and 350-400µm wide. Ray frequency is 6/mm.

Sieve elements or sieve tube members are more or less in horizontal tiers (**Fig 5.1**). They are narrow, straight and simple oblique sieve plate. The tube members are 250µm in length.

RLS view of the bark (Fig 5.2); In RLS view, the rays appear as wide horizontal panel. The cells are in regular parallel files. They are brick-like and hexagonal. All the cells are procumbent-type; upright cells are not evident.

Powder microscopy of stem-bark (Fig 6)

i). Fibers are few, lignified well developed sclerenchymatous fibers from the vascular bundle region, thin, and isolated fibers measure 200 - 600 microns in length and 10 - 20 microns in breadth.

ii). Numerous anomocytic or ranunculaceous stomata opening thereby that the cells surrounding the stomatal

pores are irregularly arranged and cannot be differentiated from other epidermal cells.

iii). Fragments of mesophyll tissue containing vascular strands are seen good many in number.

iv). Fragments of leaf showing dorsiventral structure.

Physico-chemical constants of stem bark powder

The powder of the stem bark was analyzed for various physico-chemical constants and loss on drying (LOD).

Determination of consistency and organoleptic characters

The stem bark powder was tested with various solvents and chemicals to determine consistency and organoleptic characters are given in **Table 1 and 2**.

Ash values

Total ash, water-soluble ash, alkalinity of water soluble ash and acid-insoluble ash values of the stem bark powder was done and the results are tabulated in **Table 3**.

Fluorescence analysis of stem bark powder

The powder of stem bark is examined in daylight, short (at 254nm) and long UV (at 365nm) to detect the fluorescent compounds and the observations are given in **Table 4**.

Table 1: Organoleptic characters of *A.tetracantha* stem bark powder

1. **Colour** : Pale brownish yellow
2. **Appearance**: Coarse powder
3. **Odour**: No characteristic odour
4. **Taste**: No characteristic taste

Table 2: Determination of consistency of stem bark powder

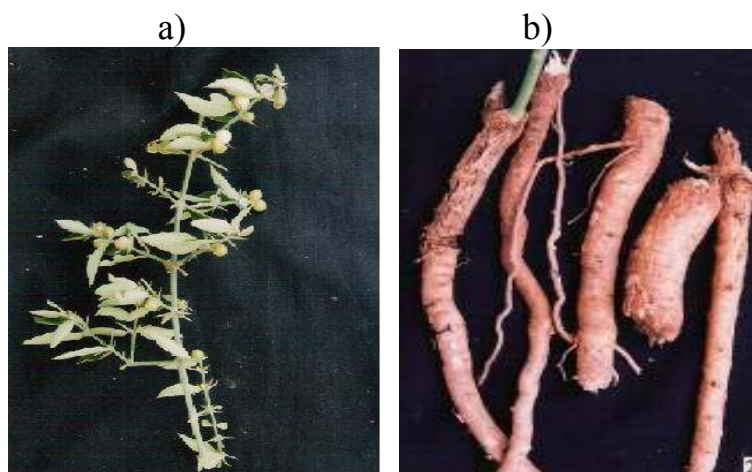
Treatment	Observation
Powder treated with water	Non-sticky
Powder shaken with water	Honey comb like froth
Powder treated with 5% aqueous sodium hydroxide	Pale yellow
Powder treated with 60% aqueous sulphuric acid	Reddish brown
Powder pressed between filter paper for 24 hours	No oil stain

Table 3: Fluorescence characteristics of *A.tetracantha* stem bark powder

Treatment	Day light	UV light	
		254nm	365nm
Powder	Pale-brownish yellow	Pale green	Brown
Powder + 1N NaOH (aqueous)	Pale yellow	Pale yellow	Black
Powder + 1N NaOH (alcoholic)	Orange	Yellowish green	Black
Powder + 1N Hydrochloric acid	Pale yellow	Black	Black
Powder + 50% Sulphuric acid	Reddish brown	Dark brown	Black
Powder + 50% Nitric acid	Orange	Yellowish green	Black
Powder + Picric acid	Yellow	Green	Black
Powder + Acetic acid	Brown	No visible colour	Black
Powder + Ferric chloride	Orange	Green	Black
Powder + Con. Nitric acid	Brown	Green	Black
Powder + Nitric acid + Ammonia	Reddish orange with precipitate	Green	Black

Table 4: Ash values of *A.tetracantha* stem bark powder

1. Total ash value :	21.625 %
2. Water-soluble ash value:	13.945 %
3. Alkalinity of water soluble ash value:	1.73 ml
4. Acid-insoluble ash value:	0.665 %



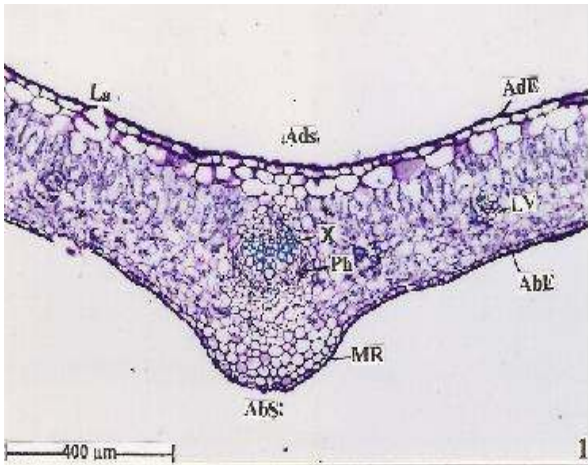


Figure 1: a) A twig of *Azima tetraacantha* b) Roots

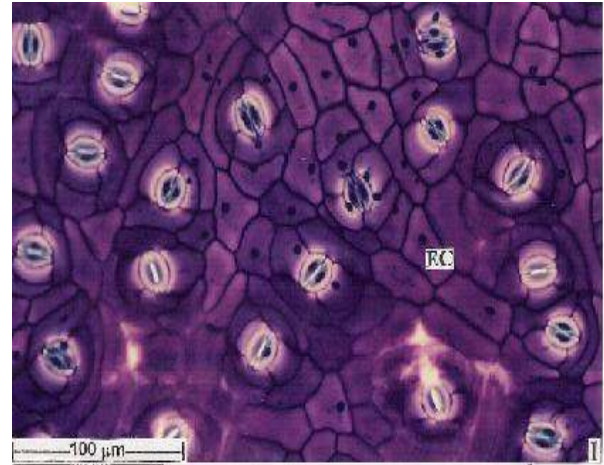


Fig 2.1: T.S of leaf through midrib: La, Lamina; Ads, Adaxial side; AdE, Adaxial epidermis; X, Xylem; Ph, Phloem; LV, Lateral vein; AbE, Abaxial epidermis; MR, Midrib; AbS, Abaxial side.

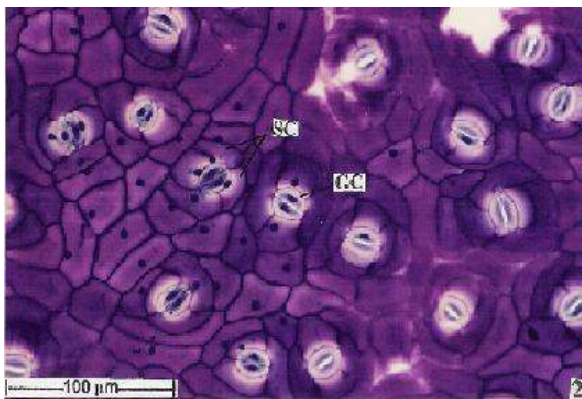


Fig 2.2: Stomata: EC, Epidermal cell; SC, Subsidiary cell; GC, Guard cell

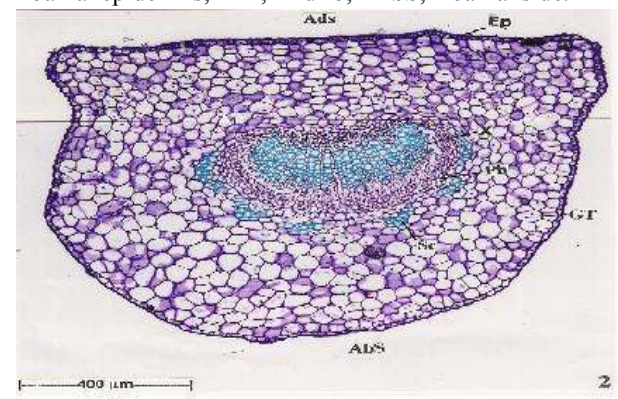


Fig 2.3: T.S of petiole (Upper region): Ads, Adaxial side; Ep, Epidermis; AbS, Abaxial side; GT, Ground tissue; X, Xylem; Ph, Phloem; Sc, Schlerenchyma.



Fig 3.1: T.S of Stem (A portion enlarged): Co, Cortex; St, Stomata; Cu, Cuticle; Ep, Epidermis; Sc, Schlerenchyma; SPh, Secondary phloem; SX, Secondary xylem; MR, Medullary rays; PX, Primary xylem

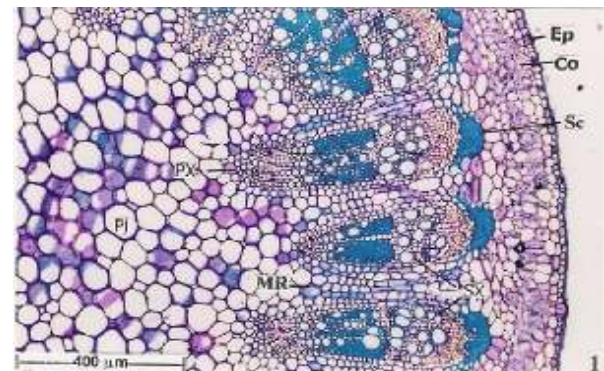


Fig 3.2: T.S of Young stem: Ep, Epidermis; Co, Cortex; Sc, Schlerenchyma; PX, Primary xylem; MR, Medullary rays; SX, Secondary xylem; Pi, Pith

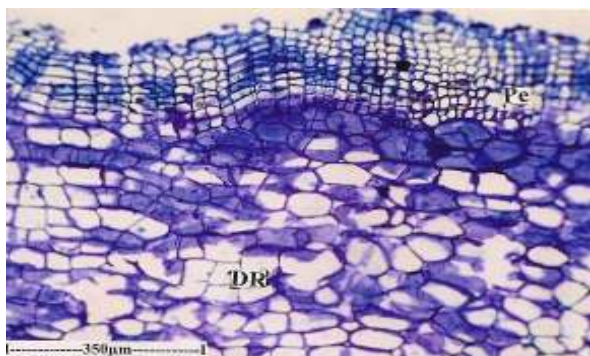


Fig 4.1: T.S of root bark showing Periderm and pseudo cortex: **DR**, Dilated rays; **Pe**, Periderm

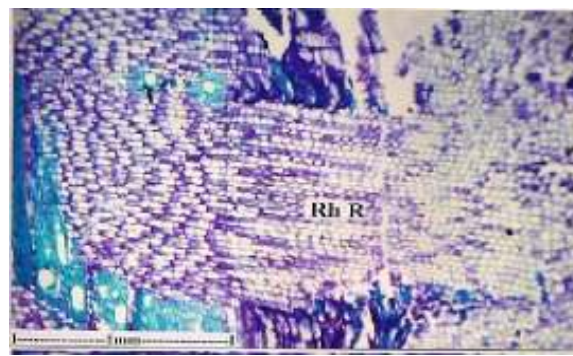


Fig.5.2: RLS of secondary Phloem: **PhR**, Phloem ray.

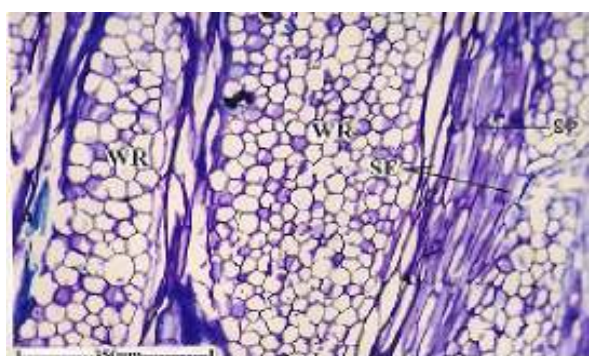


Fig.5.1: T.S of secondary Phloem showing a portion of the ray and sieve tubes enlarged: **SE**, Sieve elements; **SP**, Sieve plate; **WR**, Wide ray

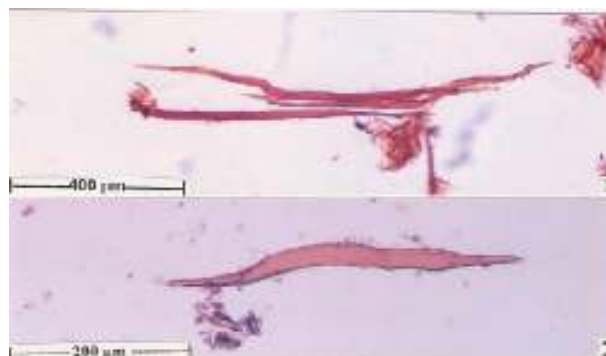


Fig 6: Diagnostic features for the powder microscopy of the stem bark of *A.tetracantha*:

DISCUSSION AND CONCLUSION

In ethnomedicinal practices the traditional healers use *A.tetracantha* in treatment of various ailments, especially in jaundice. Microscopic evaluation is an indispensable tool for identification of medicinal herbs and is one of the essential parameter in modern monograph. In this regard the important microscopic features of the various parts of the plant have been documented such as T.S of the leaf showed the absence of trichomes and presence of anisocytic stomata. Midribs showed the presence of hyalane mass in the adaxial part and spindle shaped thinner epidermal cells in the abaxial part, absence of bundle sheath and sclerenchyma around the top shaped vascular bundle and the thick lamina showed the presence of four or five layers of lobed, loosely arranged spongy mesophyll which has vascular bundles in which lateral veins are embedded in the median part of the mesophyll tissue are the distinct features of the leaf microscopy.

Presence of distinct continuous squarish or rectangular epidermis, cortex packed with compact and homogenous chlorenchyma and parenchyma, vascular cylinder which

comprises 29 discrete vascular bundles separated from each other by 3-5 celled narrow medullary rays and thick walled pith cells of different sizes intermixed are the characteristic features observed in the microscopy of the stem. T.S of root bark showed wide, well developed superficial Periderm, wide pseudocortex and wide secondary phloem.

Studies on qualitative microscopical features and physico-chemical constants of powder can serve as a valuable source of information and provide suitable standards to determine the quality of this plant material in future investigations or applications.

In conclusion, the present study on pharmacognostical characters of different parts of *Azima tetracantha* Lam. will be providing useful information for the future identification of this plant.

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