Pharmacognostical Studies of *Azima Tetracantha* Lam. (Salvadoraceae)

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ABSTRACT

The present study deals with evaluation of pharmacognostical characters of an important medicinal plant, *Azima tetracantha* Lam. The pharmacognostical studies were carried out such as organoleptic, macroscopic, microscopic and fluorescence analysis. Microscopic studies include transverse and longitudinal sections, powder microscopy and quantitative microscopy such as stomatal number, stomatal index, vein islet number and veinlet termination number. Various pharmacognostical characters observed in this study help in the identification and standardization of *Azima tetracantha.*

Keywords: *Azima tetracantha*, Microscopy, Organoleptic, Fluorescence analysis, Identification.

INTRODUCTION

Over the last few decades, there has been a growing interest in drugs of plant origin in contrast to the synthetics that are regarded as unsafe to human and environment.[1] *Azima tetracantha* Lam., is a unique folk medicinal plant known as Mulsangu in Tamil, belonging to the family "Salvadoraceae", *Azima tetracantha* is a perennial shrub growing upto 3m height in hot, dry riverine scrub, particularly on alluvial or saline soil. It occurs naturally in central, eastern and southern Africa as well as in the Indian Ocean Islands and extends through Arabia to tropical Asia. It has various medicinal properties like stimulant, antispasmodic, anti-rheumatism, diuretic, anti-inflammatory, anti-microbial, hypoglycemic, antioxidant and hypolipidemic activity. It is also used in the treatment of cancer, dyspepsia and chronic diarrhoea.[2,3] Microscopy is an important tool for authentication of crude drugs and study of powdered drugs.[4] It is important to interpret morphological and anatomical descriptions of crude drugs as well as characteristic features of drugs and adulterants of commercial significance.[5] Establishment of the pharmacognostic, morphological and microscopical characters of leaves and stem of the plant will assist in standardization, which can guarantee quality, purity and identification of samples.

MATERIALS AND METHODS

Procurement of plant materials: Fresh leaves and stem of *Azima tetracantha* were collected from Vellore district, Tamilnadu, India. Identification of the plant was done by Dr. G.V.S Murthy, Scientist 'F', Botanical Survey of India, under reference number BSI/SRC/5/23/2016/Tech./178.

Organoleptic evaluation[6]: Organoleptic evaluation can be done by means of sense organs, which provide the simplest as well as quickest means to establish the identity and purity to ensure quality of particular drug. Various sensory parameters of the plant material (such as colour, odour, size, shape, and taste) were studied by organoleptic evaluation.

Macroscopic evaluation[7]: Various macroscopic characters of fresh leaves and stem of *Azima*

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tetracantha were recorded such as type of leaf base, presence or absence of petiole and characters of lamina. Lamina consists of characteristic features such as composition, incision, shape, venation, margin, apex, base, surface and texture. The stem is morphologically studied for its size, shape, surface, fracture and configuration.[6]

Microscopic evaluation: In microscopic evaluation, studies were conducted on both grounds qualitatively and quantitatively.

Qualitative microscopy: In this study, transverse and longitudinal sections of leaf and stem were studied under photomicrograph. Staining reagents (such as phloroglucinol-HCl and methyl orange) were used as per standard procedures.[8-11] The various identifying characters were studied with or without staining and recorded.

Powder microscopy: The dried leaves and stem were powdered and studied under microscope. Different staining reagents (such as iodine for detection of starch grains and phloroglucinol for detection of lignified components) were used. A little quantity of leaves and stem powder were taken onto a microscopic slide, 1–2 drops of 0.1% w/v phloroglucinol solution and a drop of concentrated hydrochloric acid were added and covered with a cover slip. The slide preparation was mounted in glycerol and examined under microscope. The presence of starch grain and calcium oxalate crystal was detected by the formation of blue colour on addition of 2–3 drops of 0.01 M iodine solution.[12] The characteristic structures and cell components were observed and their photographs were taken using photomicrography.

Quantitative microscopy[9,13]
Determination of stomatal number and stomatal index: Stomatal number is the average number of stomata per sq mm of epidermis of the leaf. The percentage proportion of the ultimate divisions of the epidermis of a leaf which can be converted into stomata is termed as stomatal index. Stomatal index can be calculated by using following equation:

$$S. I = S / E + S \times 100$$

Where, I = stomatal index, S = number of stomata per mm^2 and E = number of ordinary epidermal cells per mm^2.

A piece of leaf was cleaned and the upper and lower epidermis was peeled out separately by means of forceps. It was kept on slide and mounted in glycerin water. Camera lucida was attached and drawing board was placed for drawing the cells. A square of 1 mm by means of stage micrometer was drawn on it. The slide with cleared leaf was placed on the stage and the epidermal cells and stomata were traced. The number of stomata and the number of epidermal cells in each field were counted. The numbers of stomata were counted as stomatal number and the stomatal index using the above formula was calculated separately for upper and lower surface.

Determination of vein-islet and vein termination number: Vein islet is the minute area of photosynthetic tissue encircled by the ultimate division of the conducting strands. Vein termination number is the number of veinlet terminations per sq mm of leaf surface.

A piece of the leaf was cleared by boiling in chloral hydrate solution and camera lucida and drawings board were arranged and 1 mm line was drawn with help of stage mm. A square was constructed on this line in the centre of the field. The slide was placed on the stage. The veins included within the square were traced off, completing the outline of those islets which overlap two adjustment side of the square. The average number of vein islet from the four adjoining square, to get the value for one square mm was calculated. The number of veinlet termination present within the square was counted and the average number of veinlet termination number from the four adjoining square to get the value for 1 sq mm was found known as vein termination number

Physicochemical analysis[14,15]**:** Physicochemical constants such as ash values, extractive values, loss on drying, volatile oil content, swelling index, foaming index and foreign organic matter were observed

Fluorescence analysis[16]**:** Fluorescence analysis of powder of leaves was done by standard procedure. In this analysis the powder were treated with various acidic and basic solvents and were then observed in UV/ visible chamber under visible, short wave and long wave regions simultaneously.

RESULTS

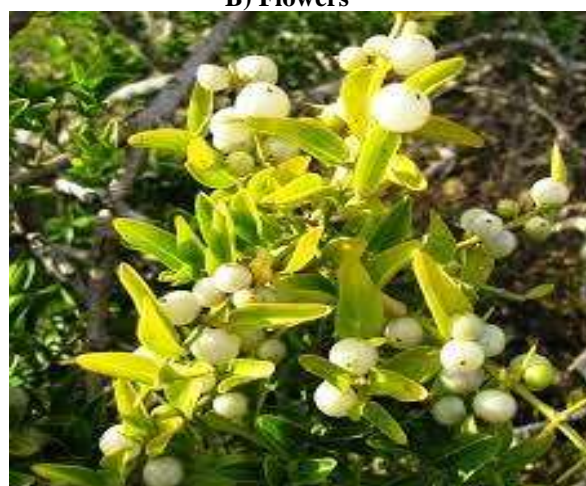**Macroscopical characters**

A) Leaf

B) Flowers

C) Stem with thorns

D) Fruits

E) Length of leaf

F) Breadth of Leaf

Fig 1: Macroscopy of *Azima tetracantha* Lam.
Morphology of *Azima tetracantha* A) Leaf, B) Stem with thorns, C) Flower, D) Fruits, E) Length of Leaf and F) Breadth of Leaf

Organoleptic evaluation

Table 1: Organoleptic evaluation of *Azima tetracantha* Lam.

S. No	Particulars	Observations
1.	Colour	Dark green to pale green
2.	Odour	Characteristic odour
3.	Taste	Tasteless
5.	Margin	Simple and entire
6.	Apex	Mucronate, sharp-tipped
7.	Base	Acute base
9.	Shape	Aristate with a spine on tip
10.	Vein	Cross venulate
11.	Stipules	Absent or rudimentary
12.	Leaflets	Pinnate

Microscopic evaluation
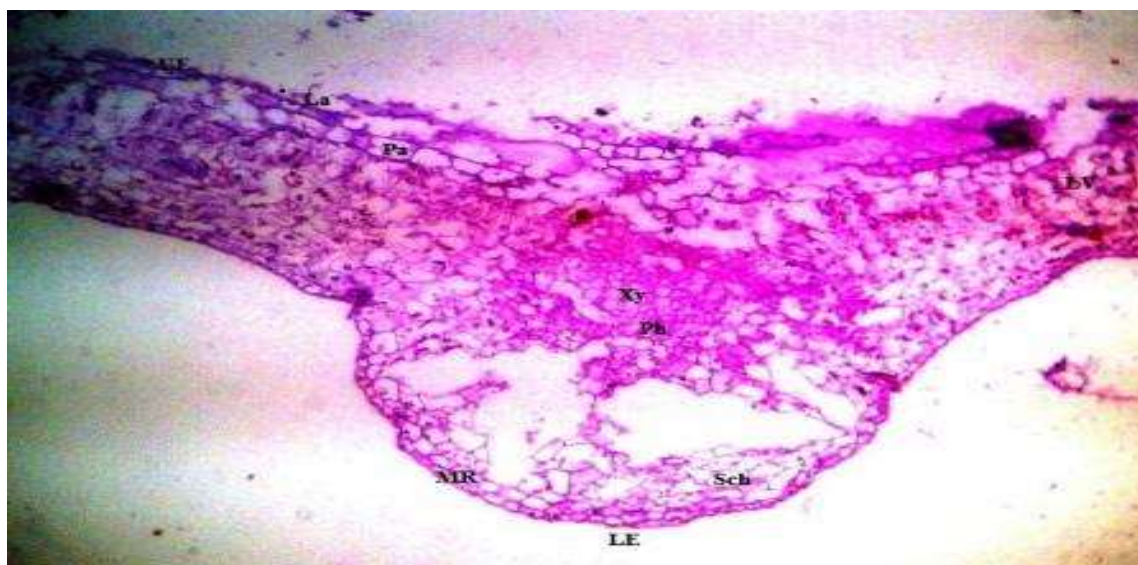
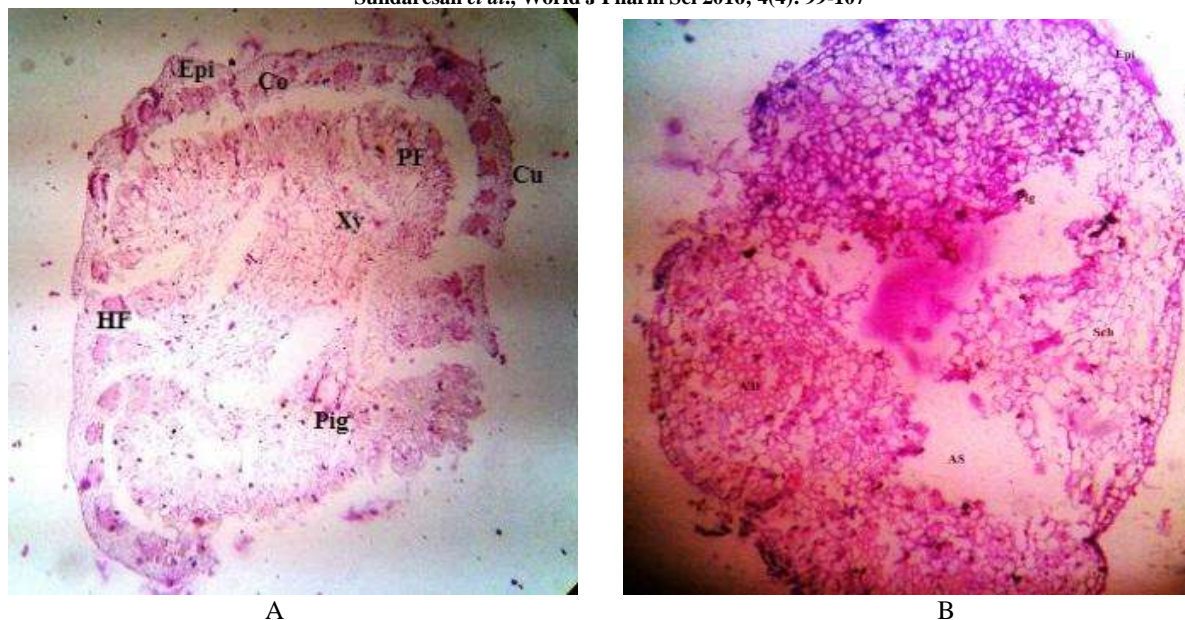**Fig 2: Transverse section *Azima tetracantha* Leaf**

Fig 3: Transverse section of *Azima tetracantha* A) Stem, B) Thorn

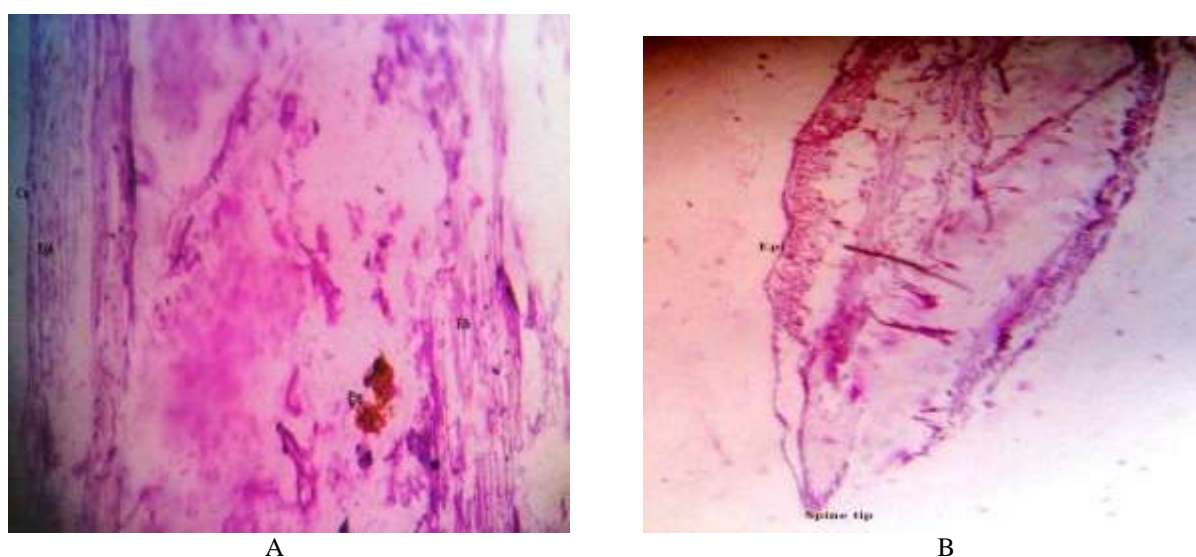**Fig 4: Longitudinal section of *Azima tetracantha* Leaf**

Fig 5: Longitudinal section of *Azima tetracantha* A) Stem, B) Thorn
UE- Upper epidermis, La- Lamina, Pa- Palisade cells, Xy- Xylem, Ph- Phloem, MR- Midrib, LE- Lower epidermis, Sch- Sclerenchyma, LV- Lateral vein, AS- Airsac, VB- Vascular bundle, Pig- Pigments, HF- Hypodermal fibres, PF- Pericyclic fibres, Cu- Cuticle, Co- Cortex

Powder microscopy

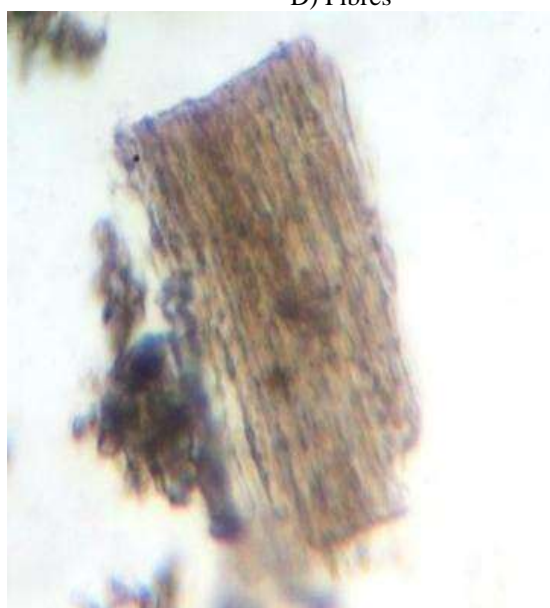Parenchyma
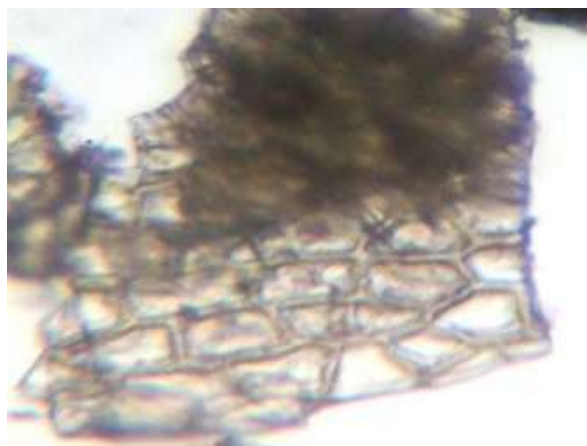
B) Sclerenchyma

Trichomes

D) Fibres

1.   Stomata

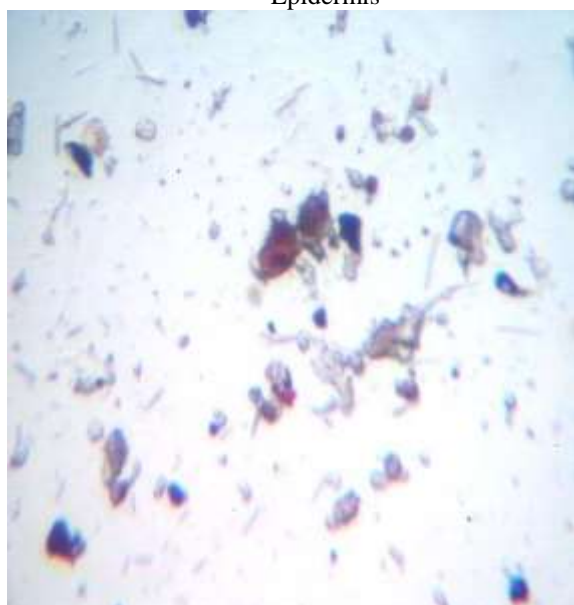E) Crystal sheath

Xylem vessels
Fig 6: Powder microscopy of *Azima tetracantha* Leaf powder


Epidermis

B) Fibres

Pigments

D) Xylem vessels

Fig 7: Powder microscopy of *Azima tetracantha* Stem powder

Quantitative microscopy

Table 2: Quantitative microscopy of *Azima tetracantha* Leaf

S. No	Parameters	Values
1.	Stomatal number- Lower epidermis	23-27
2.	Stomatal index- Lowder epidermis	19-20
3.	Vein islet number- Lowder epidermis	9-15
4.	Veinlet termination number- Lowder epidermis	15-19
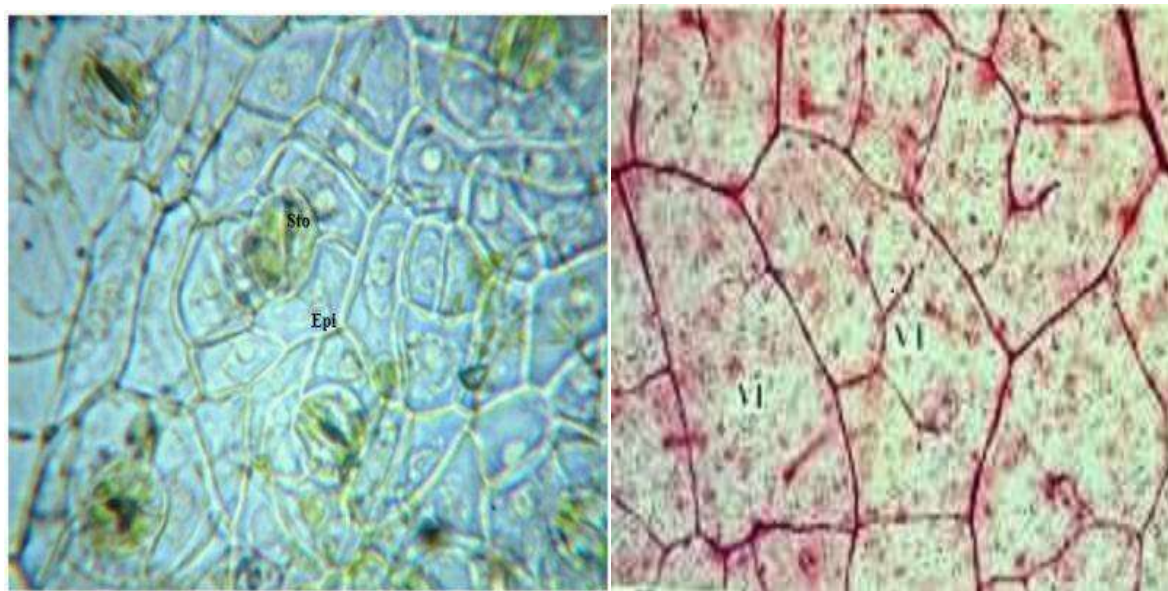
Fig 8: Quantitative microscopy of *Azima tetracantha* Leaf

Physicochemical analysis

Table 3: Physicochemical constants of *Azima tetracantha* Leaf and Stem

S. No	Parameters	Leaves	Stem
1.	Total ash	7.45%	21.039%
2.	Acid insoluble ash	1.06%	0.493%
3.	Water soluble ash	6.98%	13.985%
4.	Sulphated ash	15.35%	18.84%
5.	Loss on drying	15.27%	11.95%
6.	Water soluble extractive value	1.593%	1.394%
7.	Ethanol soluble extractive value	1.197%	0.399%
8.	Foreign organic matter	3.6%	No
9.	Volatile oil content	1.5%v/w	No
10.	Swelling Index	No	No
11.	Foaming Index	<100	<100

Flourescence analysis

Table 3: Flourescence analysis of *Azima tetracantha* Lam. leaves

Treatment	Day light	UV light	
		254nm	**365nm**
Powder	Pale-brownish green	Pale green	Brown
Powder + 1N NaOH (aqueous)	Yellowish green	Pale yellow	White
Powder + 1N NaOH (alcoholic)	Pale green	Brownish green	Brownish green
Powder + 1N Hydrochloric acid	Pale green	Brown	Black
Powder + 50% Sulphuric acid	Pale green	Bluish green	Pale green
Powder + 50% Nitric acid	Green	Yellowish green	Black
Powder + Picric acid	Dark yellow	Brown	Pale brown
Powder + Acetic acid	Pale green	Brownish green	Pale green
Powder + Ferric chloride	Orange	Brown	Pale brown
Powder +Con. Nitric acid	Brown	Brown	Black
Powder + Nitric acid + Ammonia	Green	Green	Black

DISCUSSION

Macroscopic study showed that leaf shape- blade elliptical-oblong to ovate-oblong or orbicular, base-pinnately veined with one pair of lateral veins and leaf margin was simple and entire with characteristic odour. Young stem measuring 1.5mm thick. Its outline is smooth and even. The characteristic microscopic features of leaves were observed the presence of epidermis, stomata, lamina and stem consists of a distinct continuous epidermis, cortex, vascular cylinder and pith. The characteristic microscopy of leaf powder showed the presence of parenchyma, sclerenchyma, trichomes, fibers, stomata, crystal sheath and xylem vessels. The characteristic microscopy of stem powder showed the presence of epidermis, fibres, pigments and xylem vessels. Quantitative microscopy showed the presence of stomata in the lower epidermis. There was no fluorescence compound in the plant drug.

CONCLUSION

Standardization is an important tool for herbal drugs in order to establish their identity, purity, safety and quality. In order to standardize a drug, various macroscopic, microscopic, fluorescence analysis are done. Morphological and microscopical studies of the leaf will enable to identify the crude drug. The quantitative determination of some pharmacognostical parameters is useful for setting standards for crude drugs. Stomatal number, stomatal index, vein islet and vein termination value determination are equally important in the evaluation of crude drugs. These values help in the evaluation of purity of drugs. In conclusion, the parameters which are reported here can be considered as distinctive enough to identify and decide the authenticity of this drug in herbal industry/ trade and this can be included as microscopic standards in Indian Herbal Pharmacopeia.

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