

Pharmacognostical studies on *Zanthoxylum armatum* leaves

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Abstract: The present study deals with the Pharmacognostical investigations on the leaves of *Zanthoxylum armatum* along with fluorescence characteristics, ash and extractive values with an aim to identify and differentiate it from its possible adulterants and / or substitutes.

Keywords: *Zanthoxylum armatum*, Pharmacognosy, Fluorescence analysis, Powder characteristics.

Introduction:

Zanthoxylum armatum DC (Rutaceae) is commonly known as “darmar” or “Nepali Dhaniya” in Hindi. It is an armed scandent or erect shrub or a small tree, 6M tall or more with dense foliage, found in the hot valleys of the Himalayas from Jammu to Bhutan at altitudes of 1,000-2100 M and in Eastern Ghats in Orissa and Andhra Pradesh at 1,200 M. The bark, fruits and seeds are extensively used in indigenous system of medicine as a carminative, stomachic and anthelmintic. The stem has exhibited hypoglycemic activity in the preliminary trials. The fruits and seeds are employed as an aromatic tonic in fever and dyspepsia. A number of alkaloids have been reported from its stem-bark, wood and roots viz. berberine, dictamnine, magnoflorine, xanthoplanine, sikimianine, dictamnine and γ fagarine. The stem bark and leaves also contains an essential oil and resins^{1,2}. The antioxidant potential of *Zanthoxylum armatum* leaves has already been reported by us³. In view of the importance of this plant, the systematic pharmacognostical investigation of its leaves, hitherto not reported, has been now undertaken.

Material and Methods:

Fresh sample of the leaf of *Zanthoxylum armatum* was collected from Jawaharlal Nehru Krishi Vishwa Vidyalaya, Krishi Nagar, Jabalpur, M. P. It was identified and authenticated by taxonomic division, National Herbarium of Cultivated Plants, National Bureau of Plant Genetic and Resources, New Delhi and the specimen voucher no. NHCP/NBPGR/2007/100/2225 dated 22/08/2007 was preserved in the department for future references.

Microtome sections were taken, stained and mounted as usual and the cell content and cell wall structure were studied according to the method described by Sass (1940)⁴, Johnson (1940)⁵ and O'Brian et al (1964)⁶. Photograph of different magnifications were taken

with Nikon Labphot 2 microscopic unit.

Powder characteristics

Preliminary examination, behavior of powder with different chemical reagents and microscopical examination was carried out (Kay, 1938)⁷.

Fluorescence Analysis:

Fluorescence characteristics of powdered material were recorded under ultraviolet light as per the method mention by Kokaski (1958)⁸.

Physicochemical parameters

The various physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, moisture content (Loss on drying), water content, Foreign organic matter, extractive values (Petroleum ether, chloroform, alcohol and water) have been studied as per WHO guidelines (WHO 2004)⁹.

Results and Discussion:

Macroscopical studies:

Leaves imparipinnate, 10-23 cm long, the rachis with a foliaceous green wing up to 4 mm broad, often-bearing straight pink prickles up to 15 mm long. Leaflets 5-11, lanceolate, more or less serrate and each serrature with a pellucid gland, sparsely pellucid, punctuate, acute or acuminate, sessile, glabrous, dark glossy green above, pale beneath; the terminal 6-11 by 1.5-2.8 cm lateral smaller (Plate-1).

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Microscopical Studies:**Midrib:**

In transactional view, the leaf appears dorsiventral with thick, broad elliptical midrib and thus wing like lamina (**Plate 2, Fig. 1.1**). The midrib is biconvex in sectional view with more or less slightly curved adaxial and abaxial sides, the later showing more convexity than the former. It is 1.2 mm thick along median vertical plane, about 2.7 mm in horizontal plane and comprises of heterogeneous ground tissue within which lie two rows of vascular bundles (**Plate 2, Fig. 1.2**), three on the adaxial side and five on the abaxial side. The epidermis is thin and consists of small thick walled cells covered externally with a thin cuticle. Along the adaxial side, beneath the epidermis there exists a broad wide discontinuous band of chlorenchyma about 50 μm comprising of 5-7 layers of cells, which appears to be a lateral extension of the mesophyll tissue of the lamina. Along the abaxial side, above the epidermis is a continuous band of 4 or 5 layers of collenchyma cells, about 70 μm wide. The remaining ground tissue consists of fairly large, thin walled, compact angular or circular parenchymatous cells. Along the median horizontal region, some of the cells are dilated forming a transverse region of larger cells (**Plate 3, Fig. 1.1**).

There are eight prominent and collateral vascular bundles, three on the adaxial side and five on the abaxial side, juxtaposed and alternate with each other, measuring 170X200 μm . The xylem elements are located in the centre and towards the outer part of which is phloem. Each of the vascular bundles has a massive hemispherical mass of bundle sheath (**Plate 3, Fig. 1.2**).

Lamina:

The lamina shows a dorsiventral arrangement of the mesophyll (**Plate 4, Fig. 1.1**). The upper epidermis is composed of cubical to tangentially elongated type of cells covered externally with a thick cuticle. The cells of the lower epidermis are comparatively smaller but tangentially elongated. In the surface, view the cells of the upper and lower epidermis shows wavy and sinuous walls (**Plate 4, Fig. 1.2**).

Stomata occur on the lower surface only and are rubiaceous type, the stomata being surrounded by

2 to 3 subsidiary cells two of which are parallel to guard cells. The guard cells are 20X15 μm in size. Hair are absent on both surfaces. The stomatal number of the leaf is 275 - 300 and stomatal index is 16 - 18.

Below the upper epidermis is found a two layered palisade tissue, the cells of which are broad and squarish with straight anticlinal walls. They are compactly arranged and bear a large number of chloroplast. Both the palisade layers extend right up to the margin of the leaf, which is thin and blunt. Below the palisade tissue is the spongy tissue which consists of 6-8 layers of irregularly arranged polyhedral type of parenchyma through which the vein transverse in various direction. The cells of the spongy mesophyll in general contain cluster of calcium oxalate crystals. They are about 15-22 μm in diameters. The vascular bundles are surrounded by bundle sheath consisting of 8-10 cells. The cells of the bundle sheath are slightly circular than the surrounding mesophyll parenchyma (**Plate 3, Fig. 1.3**).

Venation pattern:

The lateral primary and tertiary veins are fairly prominent. The vein islets are prominent distinct, wide, polyhedral and randomly oriented. The boundaries of the islet are thick. The vein terminations are often forked repeatedly forming dendroid outline (**Plate 5, Fig. 1.1, 1.2**). Generally, only one vein termination is seen in an islet.

Powder microscopic observation:

Coarse powder of the leaf exhibits small fragments of the epidermis with abundant rubiaceous stomata and thick masses of mesophyll cells. The epidermal cells are small, polygonal with wavy anticlinal walls (**Plate 6, Fig. 1.1**). Clusters of calcium oxalate crystals are sporadically seen in the powder within the mesophyll tissue (**Plate 6, Fig. 1.2**).

Fluorescence Analysis

Fluorescence characteristics of plant under ultraviolet light are recorded in Table I.

Physicochemical parameters

The various physicochemical parameters are recorded in Table II.

Table I Fluorescence characteristics of the leaf of *Zanthoxylum armatum*
Under ultra- violet light

Treatment	Fluorescence
Powder as such	Greenish
Powder mounted in nitrocellulose	Buff green
Powder treated with 1N NaOH in methanol	Bright Yellow with Green Fluorescence
Powder treated with 1N NaOH in methanol	Bright green
Dried and mounted in nitrocellulose	Fluorescence
Powder treated with 1N HCl	Yellow
Powder treated with 1N HCl, dried and mounted in nitrocellulose	Grayish brown
Powder treated with HNO ₃ (1:1)	Blackish green
Powder treated with H ₂ SO ₄ (1:1)	Fluorescent green

Table II Physico-chemical parameters of the leaf of *Zanthoxylum armatum*

S.No.	PARAMETERS	OBSERVATIONS (Mean ^a ± SEM)
1.	Total ash	8.22% w/w
2.	Acid insoluble ash	6.2% w/w
3.	Water soluble ash	2.40% w/w
5.	Moisture content	8.97%
6.	Water content	12.87%
7.	Foreign organic matter (FOM)	2.25% w/w
8.	Water soluble extractive	13.655%
9.	Alcohol soluble extractive	12.262%
10.	Chloroform soluble extractive	4.890%
11.	Petroleum ether soluble extractive	4.142%

^a Mean value of three readings.

Figure legends:



PLATE 1: *Zanthoxylum armatum* Flowering branch and Fruits

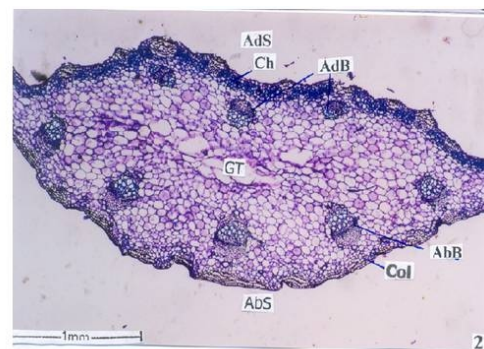
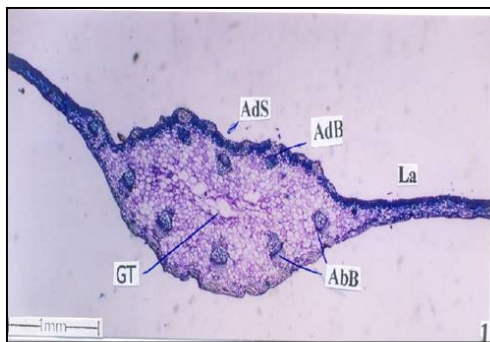


PLATE 2: T.S. of Midrib.

Figure: 1.1: T.S of leaf through midrib with lamina 2X2.5

Figure: 1.2: T.S. of midrib enlarged 4X2.5

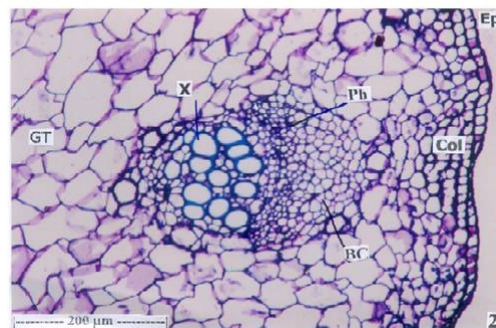
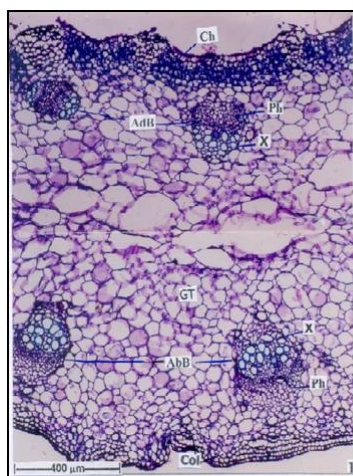


PLATE 3: Structure of the midrib

Figure: 1.1: Adaxial and abaxial vascular bundles of the midrib 10X2.5

Figure: 1.2: One adaxial vascular bundle enlarged 10X5

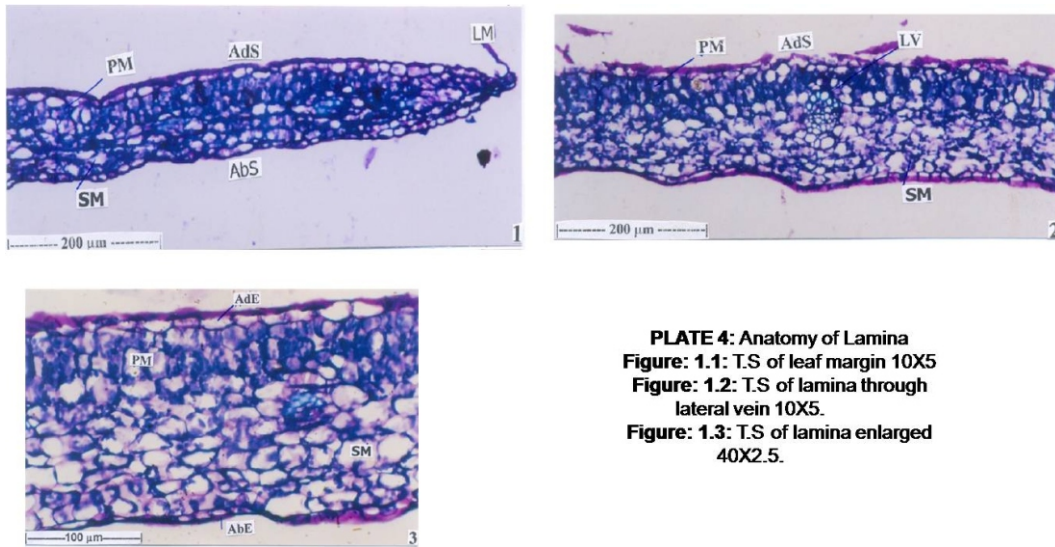


PLATE 4: Anatomy of Lamina
Figure: 1.1: T.S of leaf margin 10X5
Figure: 1.2: T.S of lamina through lateral vein 10X5.
Figure: 1.3: T.S of lamina enlarged 40X2.5.

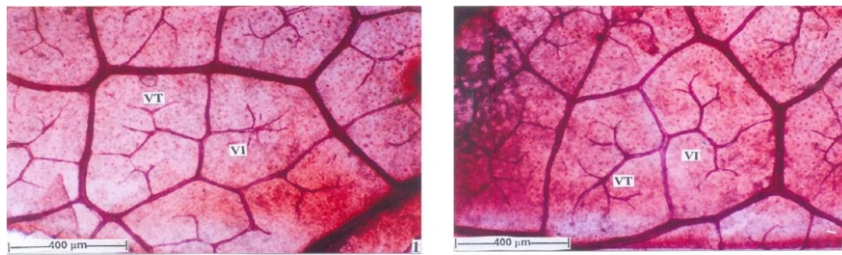


PLATE 5: Venation Pattern

Figure: 1.1: Paradermal section of leaf showing vein-islet and vein termination 10X2.5

Figure: 1.2: Paradermal section of leaf showing vein-islet and vein termination Enlarged 10X5

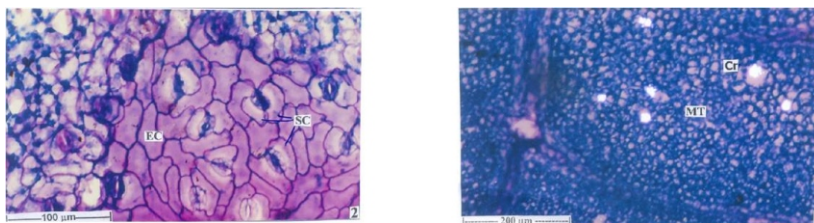


PLATE 6: Powder microscopy of the leaf

Figure: 1.1: Abaxial epidermis with stomata under high magnification 40X2.5

Figure: 1.2: Crystals in the mesophyll tissues (Under polarized light microscope) 10X5

Conclusion:

From the forgoing observations it is seen that the leaf of *Zanthoxylum armatum* appears dorsiventral with thick, broad elliptical midrib and thus wing like lamina. There are eight prominent and collateral vascular bundles, three on the adaxial side and five on the abaxial side, juxtaposed and alternate with each other. The xylem elements are located in the centre and towards the outer part of which is phloem. The lamina shows a dorsiventral arrangement of the mesophyll. In the surface, view the cells of the upper and lower epidermis shows wavy and sinuous walls. Stomata occur on the lower surface only and are rubiaceous type. Hair are absent on both surfaces. Below the upper epidermis is found a two layered palisade tissue, the cells of which are broad and squarish with straight anticlinal walls. The cells of the spongy mesophyll in general contain cluster of calcium oxalate crystals.

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Abbreviations used

AbB- Abaxial bundle, **Abs**- Abaxial side, **AdB**- Adaxial bundle, **Ads**- Adaxial side, **Ch**- Chlorenchyma, **Col**-Collenchyma, **GT**- Ground tissue, **La**-Lamina, **BC**-Bundle cap, **Ch**- Chlorenchyma, **Col**-Collenchyma, **Ep**-Epidermis, **Ph**-Phloem, **GT**- Ground tissue, **X**-Xylem. **AbE**- Abaxial epidermis; **AdE**- Adaxial epidermis; **LM**-Leaf margin; **LV**-Lateral vein; **PM**-Palisade mesophyll; **SM**- Spongy mesophyll; **VI**- Vein-islets; **VI**- Vein termination, **EC**- Epidermal cell; **SC**- Subsidiary cells; **St**- Stomata, **MT**- Mesophyll tissue; **Cr**- Crystals.