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PHARMACOGNOSTIC CHARACTERIZATION OF STEM AND ROOT OF *ADHATODA ZEYLANICA* MEDICUS

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ABSTRACT

Adhatoda zeylanica Medicus is a well-known plant drug in Ayurvedic, Unani-Tibbi, Siddha, Allopathic, Homeopathic, Naturopathic & Home Remedies. The present communication deals with the pharmacognostic evaluation on the stem and root of *Adhatoda zeylanica* Medicus (Acanthaceae). Macroscopic and microscopic examinations of the different organ, observations, and differential microchemical test has been carried out for the authentication of the samples. Physiochemical values such as the percentage of total ash, acid insoluble ash, acid soluble ash, extractive values like petroleum ether-soluble extractives, alcohol-soluble extractives and water-soluble extractives were calculated as well as histochemical tests and colour reactions with different chemicals were performed.

INTRODUCTION:

Botanical Name : *Adhatoda zeylanica* Medicus

Family : Acanthaceae
Sunskrit Name : Amalaka, Atursta, Sinhika, Vasaka
Marathi Name : Adulsa, Adusa, Vasuka.
Hindi Name : Adalso, Arusho, Vasaka.
English Name : Mulabar nut.

Botanical Description: *Adhatoda zeylanica* Medicus also known as *A. vasica* Nees., *Justicia adhatoda* Linn. It is a dense shrub 1.2-2.4 m. sometime arborescent, 6m. high with many long opposite ascending branches; stem with yellowish bark, terete, glabrous. Leaves 10-20 by 9-8 cm., elliptic-lanceolate, acuminate, minutely puberulous when young, glabrous when mature, entire, dark green above, paler beneath, base tapering; main nerves 10-12 pairs with reticulate venation between; petioles 1-2.5 cm. long. Flowers in short dense axillary pedunculate spikes 2-8 cm. long, towards the end of the branches; peduncles 3-10 cm., stout, shorter than the leaves; bracts reaching 1-2 by 0.5-1.2 cm, elliptic subacute, glabrous or nearly so, 5-7 nerved, closely reticulately veined; bracteoles 1.5-2 by 0.3-0.4 mm., oblong-lanceolate, acute, with ciliolate margins, 1-nerved, reticulately veined. Calyx rather less than 1.3 cm. long, glabrous or slightly pubescent, divided to within 2 mm. of the base; sepals imbricate, oblong-lanceolate, acute, 3-nerved, reticulately veined. Corolla white, with a few irregular pinkish coloured bars in the throat, 2.5-3 cm. long, pubescent outside; tube 1-2 cm. long, the lower half cylindric, 4mm. diam., the upper half much laterally inflated; upper lip 2 by 1.3 cm. long, ovate-oblong, curved, obtuse, notched; lower lip as long as the upper, the lobes 1.3 cm. deep, ablong, rounded, the middle lobe the broadest.

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Filaments hairy at the base, long, stout, curved; lower anther-cells minutely apiculate (not white-apurred) at the base. Ovary pubescent; lower part of style pubescent, Capsules 1.5-2 by 0.6-0.8 cm. clavate, subacute, shortly and bluntly pointed, pubescent; solid stalk flattened, 1 cm. long. Seeds 5-6 mm. long, orbicular-oblong, tubercular- verrucose, glabrous.

Medicinal uses: The plant is pungent, bitter, acrid, Cooling; causes "vata", useful in bronchitis, leprosy, blood impurities, heart troubles, thirst, asthma, fever, vomiting, loss of memory, leucoderma, jaundice, tumours, diseases of the mouth. The root facilitates the expulsion of the foetus; useful in strangury and leucorrhoea with blood discharges (Ayurveda).

The root is diuretic; useful in bronchitis, asthma, bilious vomiting, sore eyes, fevers and gonorrhoea. The leaves are emmenagogue; useful in gonorrhoea. The flowers improve the circulation of the blood; lessen strangury and jaundice. The fruit is useful in bronchitis (Yunani).

Traditional uses: *A. zeylanica* has been most frequently used for the treatment of respiratory complaints and for cough, asthma and colds [1]. It is used as an expectorant, bronchodilator and to liquefy sputum [2-6]. *A. zeylanica* as an expectorant and antispasmodic agent was described and an alkaloid with a bitter taste was identified and named vasicine [2]. Jain *et al.,* observed that the Neterhat people in Bihar used a decoction of the leaves to stimulate and heal before and after delivery [7]. Nath *et al.,* observed the use of *A. zeylanica* as an abortifacient in the Gora village of Lucknow, Uttar Pradesh. They found that 70% of the pregnant women use the leaves of *A. zeylanica* to induce abortion [8]. The macerated roots of the Vasak (*A. zeylanica*) are applied on the pubic region and the vagina to help parturition [9]. It is also described in the Gazetteer of Bombay State [10] and by Kirtikar and Basu, that 'root facilitates the expulsion of foetus' [3]. Pushpangadan *et al.* stated the use of the whole plant of *A. zeylanica* for treating impotence and sexual disorders [11]. The use of *A. zeylanica* leaves for checking postpartum haemorrhage was also mentioned.

The leaves were toxic to 'all forms of lower life' and have insecticidal effect [2, 10, 12, 13]. It was used in intermittent, typhus fevers, febrifuge and diphtheria [14]. It was also used for stomach catarrh with constipation, rheumatism, gout, and urinary stone [15]. Decoction of leaves is used for fever [16]. Ash of leaves, smoke from leaf and wood are used for cough and asthma [17]. Leaves and seeds are used for asthma, cough, colds dysentery and diarrhea [18]. Externally warmed leaves used for rheumatic pains and dislocation of joint [19]. Yellow leaves are used for cough [20]. Decoction and ash of leaves is used for bronchial complaints such as cough, asthma, tuberculosis and antipyretic [21, 22]. Leaf juice orally is used for coughs, fever, malarial fever [23]. Plant juice is used for cough, asthma and bronchitis [24]. Leaf powder is used for cough and jaundice [25]. Leaf powder boiled in sesame oil is used to stop bleeding, ear aches, and pus from ears [26]. Root decoction is used for gonorrhoea [27]. Water extract of leaf is used to relieve acidity [28].

With this background, *A. vasica* is a well-known plant drug in Ayurvedic, Unani-Tibbi, Siddha, Allopathic, Homeopathic, Naturopathic & Home Remedies. It becomes extremely important to make an effort towards standardization of the plant material to be used as medicine. These studies help in identification and authentication of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy. Simple pharmacognostic techniques used in standardization of plant material include its morphological, anatomical and biochemical characteristics.

MATERIALS AND METHODS: The fresh Parts of *A. zeylanica* were collected from Botanical garden of KSPMS's, Homoeopathic Medical College, MIDC, Latur (India). The Stem and roots were washed and used for the present study. The macroscopic observations were noted down. For microscopic studies, the epidermal peels and cross sections of the stem and roots were prepared and stained. Quantitative microscopy was carried out for stem and root Vessel.

The histochemical tests and colour reactions with different chemicals were performed by the standard methods [29, 30]. For study of vessels, fragments of plant organs, especially stem at nodal region and root, were macerated using nitric acid (10%) and potassium dichromate (10%) solution in equal proportions. The vessel elements were stained with aqueous saffranin

(1%), dehydrated and mounted in DPX. Some vessel members were also examined in glycerine. The line and cellular sketches of the figures were drawn using a Camera Lucida. The range of length and diameter of vessel elements was determined by the measurement of 20-25 vessel elements.

Transections of leaf, petiole, node, stem and root were taken by free hand. Fresh and preserved materials were used. Sections were stained in safranin (1 %), light green (1 %) and mounted in DPX after the customary dehydration. Some hand sections were also examined in glycerine. Microphotographs of stem and root sections were taken by using Jenaval and Mirax Laborec Cameras affixed to microscope. Physio-chemical values such as the percentage of total ash, acid insoluble ash, acid soluble ash, extractive values like petroleum ether-soluble extractives, alcohol-soluble extractives and water-soluble extractives were calculated according to the methods described in the Indian pharmacopoeia [31, 32]. Phytochemical studies such as quantitative examination were done on the dried powdered material.

RESULTS AND DISCUSSIONS: The first step towards ensuring quality of starting material is authentication. Thus, in recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance [33, 34]. Despite the modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. According to the World Health Organization, 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. The result of this study as follows:

T. S. Stem: A transverse section shows the presence of both glandular (**Fig. 1C, D**) and non-glandular trichomes (**Fig.1E, F**); the former being present in small depressions of the epidermis. Following the epidermis is a band of collenchyma of 8-10 layers of cells and a wide zone of parenchyma consisting of 12-15 layers of cell. The stem possesses a siphonostele which encloses a wide central pith. Starch grains, calcium oxalate crystals of acicular and prismatic types and cystoliths are found abundantly in the region of the stem (**Fig. 1A**).

T. S. of Root: In sectional view it is circular. Epidermis has small cells compactly arranged. Periderm formation is noted. Cork is many layered made up of rectangular cells compactly arranged in radial rows. Below it a few layered phelloderm. Ground tissue has phloem fibres, stone cells, oxalate crystals and starch grains. Secondary growth is more. Xylem developed in large amount. Medullary rays of 2-3 rows of cells (**Fig. 1B**).

T. S. of Node: The leaves are opposite and decussate. The vascular cylinder in the subnodal region is compact and somewhat rectangular. It breaks in nodal region and diverges off one median curved arc shaped foliar trace on the opposite sides leaving behind a gap. The two traces for axillary bud are given out which unit to form a ring like structure. The median trace remains unbranched and enters in to the petiole. The node is unilacunar one traced (**Fig. 1G**).

Vessel Element: Vessel elements are observed in the stem and roots. Their length, width, perforation, plates, end-wall and lateral wall thickening are recorded which are described below.

Vessels elements in Roots (**Fig. 1H, I; Table 1**):
Length of vessel element – 66.6 μm. to 177.6 μm.
Average length – 122.1μm.
Diameter of vessels elements - 22.2 μm to 31.08 μm.
Average diameter – 26.64 μm.
Shape - Tubular, cylindrical, spindle like, fusifrom.
Lateral wall thickening – Simple pitted.
Pits arrangement – Alternate.
Perforation plate – Simple.
Shape of perforation plate – Circular, oval.
Position of plate – Oblique, lateral, transverse.
Tail - Short and blunt.

Vessels elements in Stem (**Fig. 1 J, K, L; Table 1**):
Length of the vessels elements – 85.74 to 314.38 μm.
Average length – 200.06 μm.
Diameter of vessels elements – 14.29 μm to 28.58 μm.
Average diameter – 21.43 μm.
Shape – Spindle shaped, tubular, column like and cylindrical.
Lateral thickening – Reticulate and simple pitted.
Pits arrangements – Alternate.
Perforation plate – Lateral, transverse.
Tail - Short and blunt.

Histochemistry: On the epidermis of stem glandular and non-glandular trichomes are observed. The cortical parenchymatous cells of stem show the presence of starch, protein, alkaloids, tannin, while the pith parenchymatous cells of stem shows presence of tannin and fat. Root shows the presence of starch, protein, fats, saponin and glycoside in the cells of cortex.

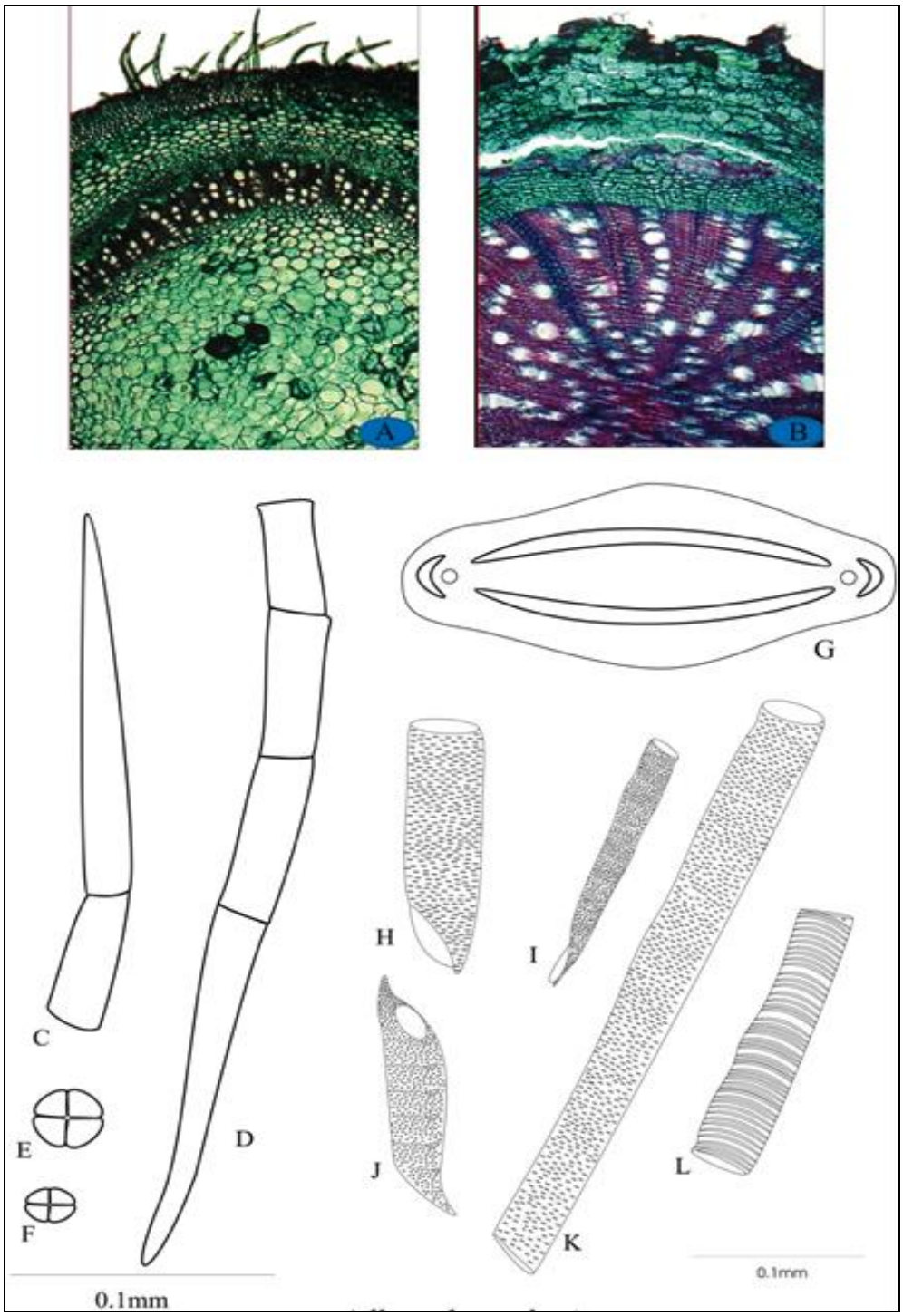

FIGURE 1: *ADHATODA ZEYLANICA*; A- T. S. STEM; B- T. S. OF ROOT; C, D- NON GLANDULAR TRICHOME; F, G- GLANDULAR TRICHOME; G- T.S. STEM AT NODE; H, I- ROOT VESSELS; J, K, L- STEM VESSELS

Fluorescence analysis: For fluorescence analysis, the powders stems and root of *A. zeylanica* were treated various **chemical reagents to give different colours** (**Table 2**). This may be helpful to identify the purity of the drug. The results of various types of ash may provide a basis to identify the quality and purity of the drug.

The present study on micro morphological features, other physical values and chemical parameters will helpful to identify the correct species of the plant when there is possibility of adulteration. In ordinary light powder shows different colour with different chemicals are summarized in **Table 3**.

Physical Constants: Results of moisture contents, ash analysis and extractive values of the dried stem and root have been presented in **Table 4**.

TABLE 1: VESSEL ELEMENTS IN *ADHATODA ZEYLANICA*

Name of species	Length of vessel members (µm)			Diameter of vessel members (µm)		
	Minimum length	Maximum length	Average length	Minimum diameter	Maximum diameter	Average diameter
Stem	85.74	314.38	200.06	14.29	28.58	21.43
Root	66.6	177.6	122.1	22.2	31.08	26.64

TABLE 2: FLUORESCENT ANALYSIS OF EXTRACTS FROM ORGANIC SOLVENTS (IN UV-360 nm)

Sr. no.	Name of plant	Ethanol	Ethyl Acetate	Distilled water	Ether
1	Stem	Dark green	Dark green	Dark green	Light green
2	Root	Light greeen	Dark green	Yellowishgreen	Pale green

TABLE 3: EFFECT OF CHEMICALS ON POWDER OF *ADHATODA ZEYLANICA*

Sr. no.	Reagent	Stem	Root
1	Powder	Yellowish brown	Yellowish brown
2	Powder + Iodine	orange	reddish orange
3	Powder + 5% Ferric chloride	Yellowish black	Tar black
4	Powder + 1N NaOH	Yellowish orange	Yellowish orange
5	Powder + Acetic acid	wine	Dark wine
6	Extract + Acetic acid + 50% H_2SO_4	Light yellow	Light brown
7	Powder + 50% H_2SO_4	Greenish brown	brown
8	Powder + Conc. HCl	Brown	Brown
9	Powder + Ammonia	Light Yellow	Light Yellow
10	Extract + 4% NaOH + $CuSO_4$	Yellowish green	green
11	Extract + 40% NaOH + 1% Lead acetate	Yellowish green	Dark orange
12	Powder + 50% HNO_3 + Ammonia	Light yellow	Yellow

TABLE 4: PHYSICAL EVALUATION (%) OF *A. ZEYLANICA*

Sr. No.	Parameter	Value (%)	
		Stem	Root
1.	**Moisture content**	6.5	6.3
2.	**Extractive values**		
	a) Petroleum Ether	7.5	5.2
	b) Alcohol	22.5	17.7
	c) Water	15.0	13.0
3.	**Ash values**		
	a) Total ash	6.4	6.6
	b) Acid insoluble Ash	0.5	0.6
	c) Acid soluble Ash	5.9	6.0

CONCLUSION: The present study shows the major pharmacongonstic characters in the selected parts of species *A. zeylanica*. The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent [35]. In the present investigation we observed the high extracti ve values in ethanol compared to other solvents. The pharmacognostical characters reported in this work can serve as a valuable source of information and provide suitable diagnostic tool for the standardization as well as identification of adulterants in future investigation or application.

It will also be immense using carrying out further research and revalidation of its use. The microscopic features could help in laying down micro morphological standards as per WHO guidelines for authentication of the original drug.

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