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Pharmacognostical Study of *Justicia adhatoda* Linn. Leaf

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ABSTRACT

Justicia adhatoda Linn. Synonym *Adhatoda vasica* Nees. (Acanthaceae) a plant species which is commonly used in preparation of indigenous medicine. The macroscopy, microscopy, quantitative analysis, extractive values in ethanol and water, phytochemical screening and TLC of the leaf were investigated. The leaf is simple, entire, wavy, ovate, lanceolate, apex acuminate, 6 to 14 cm long, 3 to 5.5 cm broad, and midrib prominent at the lower surface. Transverse section of leaf TS passing through the midrib is highly convex on the lower side, centrally depressed on the upper side, containing a centrally located arc of meristemes and few other secondary meristemes on its either side, underneath the upper epidermis lies two layer of palisade cells containing oil globules and elongated warty cystoliths extending from the epidermis to the lower cells of palisade, 5-10 rows of collenchymatous tissue found on both side of midrib. Midrib contains 3-4 conjoint, collateral meristemes. There are some small glandular trichomes found on lower epidermis. Leaf powder was dark green in colour, bitter in taste. Alkaloids, Glycosides, Steroidal/triterpenes and Resin were present in both extracts while saponin was present only in aqueous extract. TLC confirmed the chemical composition present in leaf. The pharmacognostic profile of *Justicia adhatoda* Linn. Leaf is helpful in sample identification, quality and purity standards.

Keywords: *Justicia adhatoda* Linn., Leaf, Phytochemical testing, TLC.

1. Introduction

Justicia adhatoda Linn. belongs to family Acanthaceae, is a Diffuse shrubs. Leaves ovate or elliptical- lanceolate, acuminate, up to 20 cm long. Flowers are white, streaked with pink or purple. Spikes pedunculate clustered towards branch end. Bract large, leafy, ovate, glabrous, 6 - 7 nerved, bracteoles 1- nerved. Corolla tube short upper tip galeate, subentire, lower spreading and 3- lobed. Stamens 2 inserted near the mouth of corolla tube; filaments hairy near base. The fruit is a legume, 30-60 cm (12-23 in) long and 1.5-2.5 cm (0.5-1 in) broad, with a pungent odour. Seeds are Sub-orbicular, rugose. It is found throughout India ascending to 4000 ft, elevation, in sub- Himalayan tracts and commonly in the plains. It is 1.5-2 meter tall shrub. Its leaves are ovate or elliptical- lanceolate, acuminate, up to 20 cm long. *Justicia adhatoda* Linn. is mentioned as vasa in Ayurvedic classics and has been used in various dosage forms to treat asthma, cough, wound ulcer, fever and hiccough. The leaves are bitter in taste. The leaves extract has been reported to contain Polysaccharide, Proteins, uronic acids, Saponins, tannins, triterpenes, alkaloids, and flavonoids, essential oils having antitussive, wound healing and antimicrobial activity^[1, 2, 3].

The leaves have been reported to contain alkaloids, vasicinone, vasicinol, adhatodine, adhatonine, adhavasine, anisotine and peganine as major constituents^[4]. The leaves has been reported to possess antibacterial^[5], woundhealing^[6], antitubercular^[7], immunomodulator^[8], antitussive^[9] properties. Authenticity, purity and assay are the three major attributes for standardization and quality control^[10]. Hence, in this work we just made an attempt for the standardization of *Justicia adhatoda* Linn. by carrying out its pharmacognostic evaluation.

2. Materials and Methods

2.1. Plant material

The leaves of *Justicia adhatoda* Linn. was collected from the ayurvedic garden of Department of Dravyaguna, Institute of Medical Sciences, Banaras Hindu University, Varanasi, in the month of Oct- Nov.

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2.2. Macroscopic and microscopic analysis

Leaf was studied macroscopically for important identification points, i.e. odour, taste and texture and for microscopical studies; a transverse section was prepared and stained [11]. Microscopy of powder was investigated according to method of Kokate (2010) [12].

2.3. Physiochemical analysis

Physiochemical studies such as moisture content, total ash, foreign matter, acid insoluble ash, sulphated ash were determined according to WHO guidelines on quality control method for medicinal plants [13].

2.4. Phytochemical screening

Phytochemical screening was carried out by using the standard methods.

2.5. Thin layer chromatography (TLC)



Fig 1: Macroscopic Study of *Justicia adhatoda* Linn.

3.2. Microscopic characteristics

Transverse section passing through the midrib is highly convex on the lower side, centrally depressed on the upper side, containing a centrally located arc of meristeles and few other secondary meristeles on its either side. Upper and lower epidermis are composed of rectangular to squarish arranged cells covered with thin cuticle, cellwall of upper epidermis is more wavy than lower

Silica gel and distilled water were used to prepare a slurry coating materials and plates were coated by using the spreading device, with a layer about 0.30 mm thick coated plates were then dried and activated in oven for 30 min. a pencil line is drawn near the bottom and a small drop of Test solution (hydro alcoholic extract of leaves) and Standard solution containing vasicine were placed separately on it; and the spot were placed to become dry. Plates were placed in chromatographic chamber containing the Ethyl acetate: Methanol: Ammonia (8:2:0.2) and R_f value were recorded.

3. Result

3.1. Macroscopic characteristics

The leaf is simple, entire, wavy, ovate, lanceolate, apex acuminate, 6 to 14 cm long, 3 to 5.5 cm broad, and midrib prominent at the lower surface, slightly grooved on the upper surface, lateral veins 6-10 pairs running parallel to each other. Leaves having some odour, test slightly bitter. (Figure 1)

epidermis, underneath the upper epidermis lies two layer of palisade cells containing oil globules and elongated warty cystoliths extending from the epidermis to the lower cells of palisade, 5-10 rows of collenchymatous tissue found on both side of midrib. Midrib contains 3-4 conjoint, collateral meristeles. There is some small glandular trichomes found on lower epidermis (Figure 2).

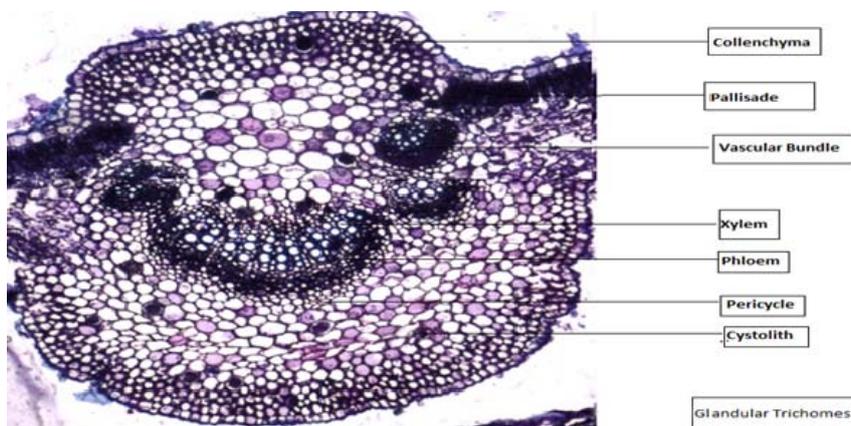


Fig 2: Microscopic characteristics of *Justicia adhatoda* Linn., T.S. of leaf through midrib showing ground tissue of Collenchyma cell with abundant cystolith and Glandular trichomes.

3.3. Powder characteristics

The fine powder of dry leaves of *Justicia adhatoda* Linn. was first treated with chloral hydrate and then stained with Phloroglucinol and HCl in ratio of 1:1, the treated powder was then mounted on

slide with glycerine and observed under microscope, On observation there were diacytic stomata covered with epidermal cell, lignified spiral xylem vessels and trichomes were found (Fig 3).

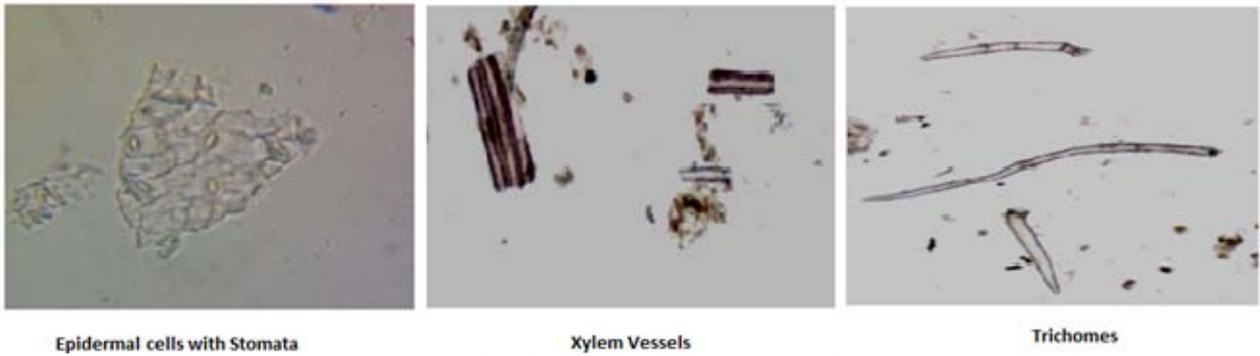


Fig 3. Powder characteristics of *Justicia adhatoda* Linn., Having epidermal cells with stomata, xylem vessels and trichomes

3.4. Physicochemical analysis

Physicochemical studies such as moisture content, total ash, foreign matter, acid insoluble ash and sulphated ash were presented in table 1.

Table 1: Physicochemical characteristics of *Justicia adhatoda* leaf

Parameters	Value
Foreign matter	.20%
Total ash	20%
Acid insoluble ash	.82%
Water soluble ash	4.5%
Alcohol soluble extractive	6.8%
Water soluble extractive	18.45%

4. Phytochemical Screening

Alkaloids, Glycosides, Steroidal/triterpenes, Phenolic & tannins, Resins are present in both aqueous and alcoholic extracts but saponin present only in aqueous extract (Table 2).

4.1 TLC

Test (Hydro alcoholic) extracts contain the fluorescence component. Alkaloid was confirmed on TLC plate and produced orange color after spraying with Dragendorff, s reagent of ninhydrin an orange color appeared in test extract due to presence of Vasicine (Figure 4, table 3).

Table 2: Phytochemical screening of *Justicia adhatoda* leaf

Chemical constituents	Chemical tests	Aqueous Extract	Alcoholic Extract
Alkaloids	Dragendroff Test	+	+
Flavonoids	Shinoda's test	-	-
Triterpenoids	Liebermann Burchard test	+	+++
Proteins	Biuret's test	-	-
	Moillon's test	-	-
Resins		+	+
Saponins	Foam test	+	-
Steroid	Liebermann Burchard test	+	+++
Tannins	Ferric chloride test	+	++
	Iodine test	+	+
Glycoside	Keller-kiliani test	++	+

+: Present; - : Negative

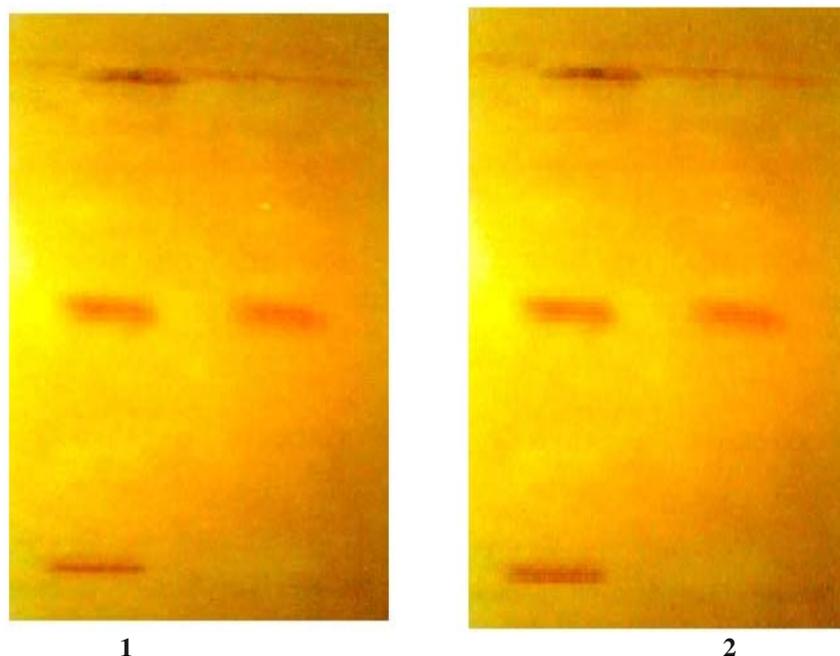


Fig 4: T.L.C. of *Justicia adhatoda* Linn. (1) Test solution (2) Vasicine Standard

Table 3: T.L.C Results of *Justicia adhatoda* leaf

UV 254 nm	Derivatized (Dragendorff's reagent)
R _f value	R _f value Colour of the band
0.17	
0.31	
0.49 (Vasicine)	0.49 Orange (Vasicine)
0.74	
0.97	

5. Discussion

As we know there are many species and varieties are in market of a single genus. So it is necessary to identify the documented variety and species. The efficacy of herbal drugs and herb mineral drugs is also affected because of this adulteration and substitution. For solving this problem of adulteration and substitution, it is necessary to standardized plant drugs. WHO gives guideline for that is Authentication, Identify Foreign matter, organoleptic evaluation, and Macroscopic and Microscopic examination of taken plant part, Ash value, Extractive value, T.L.C, H.P.T.L.C. The preliminary phytochemical analysis will reveal the chemical nature of the drug and explore the source of pharmacologically active chemical compounds^[14]. These parameters are much authenticated for identification of a particular part of a particular plant species for increasing efficacy of Ayurvedic formulations.

The present work was undertaken with a view to lay down standard of *Justicia adhatoda* Linn., leaf which could be useful for authenticity of the leaf. The pharmacognostic parameters could be useful in the identification and standardization of a crude drug.

6. Conflict of interest statement

We declare that we have no conflict of interest.

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