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Pharmacognostic evaluation and HPTLC study of leaf part of the medicinal shrub- *Justicia adhatoda* L.

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Abstracts: In the modern era, the exploration of medicinal plants as an herbal drug has gained international attention and interest in handling various kinds of ailments. Conventionally, *Justicia adhatoda* L. is extensively used in the treatment of bronchitis, gonorrhea, asthma, leprosy, heart troubles, cough, loss of memory, tuberculosis, leucoderma, jaundice, diabetes, mouth troubles and wound healing, etc. The current study has been carried out to delineate the pharmacognostic stature of *Justicia adhatoda* L. leaf by macroscopy, microscopy, and Thin Layer Chromatography (TLC) fingerprint essay for validation and description. Macroscopic, microscopic, and powder study analysis was performed by standard methods as established in the Ayurveda Pharmacopoeia of India. The methanol extract of the leaf powder was performed for chromatographical evaluations. Results expressed from the macroscopic and microscopy investigation divulge certain paramount characters in the form of the rectangular lamina, reniform petiole, and oval shape rachis having single-layered of epidermis embedded with blunt covering trichomes and sessile glandular trichomes. The study also exhibited centrally placed cup-shaped, half-circle shaped and circular shape vascular bundle in the midrib, petiole, and rachis region of the leaf. The presence of starch grains, oil globules, and prismatic calcium oxalate crystal in the parenchyma region of the leaf is also a noted feature of the plant. Thin Layer Chromatography fingerprints showed various spots at 366 nm & after derivatization. Findings of the powder microscopic study disclosed the existence of fragments of collenchymatous cells, trichomes, pitted, spiral vessels thickening, and fibers. The data evaluated from the current study will aid to authenticate and proper identification of the crude drug sample.

Keywords*:* HPTLC, *Justicia adhatoda*, leaf, microscopy, pharmacognostic study, powder study

INTRODUCTION

Since the inception of human civilization, plants act as a reservoir of natural resources and are the vital component of medicine. There are attention and international interest for the plant-based products in the field of medicine, health, pharmaceutics, cosmetics, and agriculture. Medicinal plants

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have curative, healing, and protective activities due to the existence of various complex chemical constituents present in the form of secondary metabolites.

The plant *Justicia adhatoda* L. (*Adhatoda zeylanica* Medik, *A. vasica* Nees.) popularly known as vasaka, belongs to the family Acanthaceae is commonly found in India. The plant is a vital platform for the polyphenolic compounds and flavonoids that displayed active anti-oxidant properties which aid in the elimination, safeguard, and remedy of several oxidative stress-related problems. The plant is very well explored in the Indian subcontinent (Assam, Bangladesh, India, Nepal, and Sri Lanka), Laos, and Myanmar. In India, it is widely distributed from plains to 4000 ft, elevation, in sub-Himalayan tracts. The plant is diffuse shrub having a height of 1.5 to 2m. The leaves are ovate or elliptic-lanceolate, acuminate, up to 20 cm long, minutely puberulous when young, glabrous when mature, entire, dark green above, paler beneath, base tapering; with reticulate venation between; petioles 1-2.5 cm. long. Flower spikes or panicles, white or cream-white in color, and having irregular zygomorphic, bisexual, and hypogynous patterns [1]. The plant is very well explored in traditional systems; Ayurveda, Unani, and Siddha for their therapeutic activities. Different parts of this plant have been used as the herbal remedy against, chronic bronchitis, whooping-cough, dysentery, colds, diarrhea, jaundice, and painful inflammatory swellings [2-4]. The leaves of *A. vasica* contains many secondary metabolites and phytochemicals such, vasicine, vasicinone, vasicine acetate, 2-acetyl benzyl amine, vasicinolone, vasicol, vasicoline, vasicolinone, and adhatodine [4-6] responsible for its biological properties [7, 8]. The leaves have been reported to possess antibacterial [9, 10], wound healing [11], anti-tubercular [12], immunomodulatory [13], antitussive [14] properties. Vasicine is reported to have bronchodilatory, respiratory stimulant, and uterine stimulant effects [15]. Vasicine acetate showed anti-mycobacterial activity [16].

It is a highly valued Indian medicinal plant and used in the treatment of respiratory diseases like asthma, cough, bronchitis, and tuberculosis [17, 18]. The flowers, leaves, and roots have antispasmodic properties [19]. Moreover, *A. vasica* possesses several biological activities including anti-inflammatory, antispasmodic, antibleeding, anti-diabetic, and anti-jaundice effects [20]. The leaves are also used to treat malarial fever, chronic fever, intrinsic hemorrhage, cough, asthma, leprosy, skin diseases, and piles [21]. Authenticity, purity, and assay are the three major attributes for standardization and quality control [22]. Hence keeping this view in mind, the present study was conducted to standardize the *J. adhatoda* L. leaf through pharmacognostic, macroscopic, and microscopic studies.

MATERIAL AND METHODS

Collection and preparation of plant material: The fresh raw drug sample *of J. adhatoda* L. leaf was collected from Kamla Nehru Nagar Ghaziabad (U.P) India during February 2020. Drug authentication was done based on macroscopic, microscopic, and powder features of the leaf. The API, HPI, and UPI standards were used for pharmacognostic authentication.

Method of preparation of sample: J. adhatoda: leaves were washed gently with normal tap water to discard the adhered dust particles and then with distilled water. Thin and fine section of the leaf was taken manually with the aid of potato pith, and then stained and mounted following usual micro-techniques [14] and individual photographs at lower and higher magnification were taken with the help of Dewinter microscope attached with Dewinter microscope digital camera (DIGI510), New Delhi, India. For Powder studies, the leaves were shade dried at normal temperature followed by the oven for 60ºc for 5 hrs. After the proper drying of the leaf sample, it was ensured that the drug sample

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is not contaminated with any type of pathogenic microorganisms and then it was processed for the study.

Thin Layer Chromatography (TLC) analysis: 2 g of the sample was soaked in 20 ml of ethanol overnight, boiled for 10 minutes, concentrated, and made up to 10 ml volumetric flask. 10 µl of the extract was manually applied on E. Merck aluminum plate precoated with Silica gel 60F254 of 0.25 mm as a band and develop the plate up to 8 cm from the base in a CAMAG twin trough chamber. Detection of the spots was carried out by UV lamp and anisaldehyde-sulphuric acid reagent. Rf values were then measured.

$$Rf = \frac{Distance\ traveled\ by\ the\ compound}{Distance\ traveled\ by\ the\ solvent\ front}$$

RESULTS

Macroscopic evaluation: Leaves are large, lance-shaped, opposite, exstipulate, green adaxial surface, paler abaxial surface, base tapered with reticulate venation. The odor characteristic and taste is bitter.

Microscopic observation:

Midrib: TS shows a narrow conical projection on the upper side of the midrib. Single layered of epidermis covered with a thin and smooth cuticular layer. The epidermis on the upper and lower surface is covered with covering unbranched trichomes. Four to five layers of collenchymatous cells present in the upper and lower side. Parenchymatous cells followed ground tissue with oil globules and starch grains. Centre consists of 20-24 rows of xylem elements present in the cup-shaped form.

Lamina: TS shows curvy rectangular shape lamina. Single layered of epidermis present on the upper and lower side covered with a smooth cuticular layer. Simple bi-cellular covering trichome and glandular trichomes embedded with the epidermal layer. Mesophyll cells are differentiated into the upper layers of palisade cells with the prismatic crystal of calcium oxalate, oil globules, and on lower side 4-5 layer of spongy parenchyma cells with starch granules and oil globules. Parenchymatous ground tissue and pitted thickening present in the center**.**

Petiole: TS of petiole has reniform or kidney shape and covered with the thick cuticular layer. Upper and the lower surface of petiole consist of the rectangular thick-walled epidermis. This epidermal layer is embedded with blunt covering trichome and glandular trichomes. 6-7 layers of collenchymatous cells followed by parenchymatous ground tissue are present having starch granules and oil globules. Half circle shape vascular bundle with 36-40 radial rows of xylem elements occupies the central position. Patches of xylem elements are also inserted on the upper side of parenchymatous cells. Pericyclic fibers are present on the outer side of phloem parenchyma cells.

Rachis: TS of rachis shows an oval shape. The outermost covering is single-layered epidermis having bi-cellular covering unbranched trichome and glandular trichome and covered with a smooth layer of cuticle. Below this 6-7 layer of collenchymatous cells followed with parenchymatous ground tissue filled with starch granules. Vascular bundles are oval in shape. 50-60 rows of xylem elements are present in the center having the circular shape. Phloem parenchyma cells are surrounded with xylem elements and pericyclic fiber and collenchymatous cells are filled with scattered oil globules.

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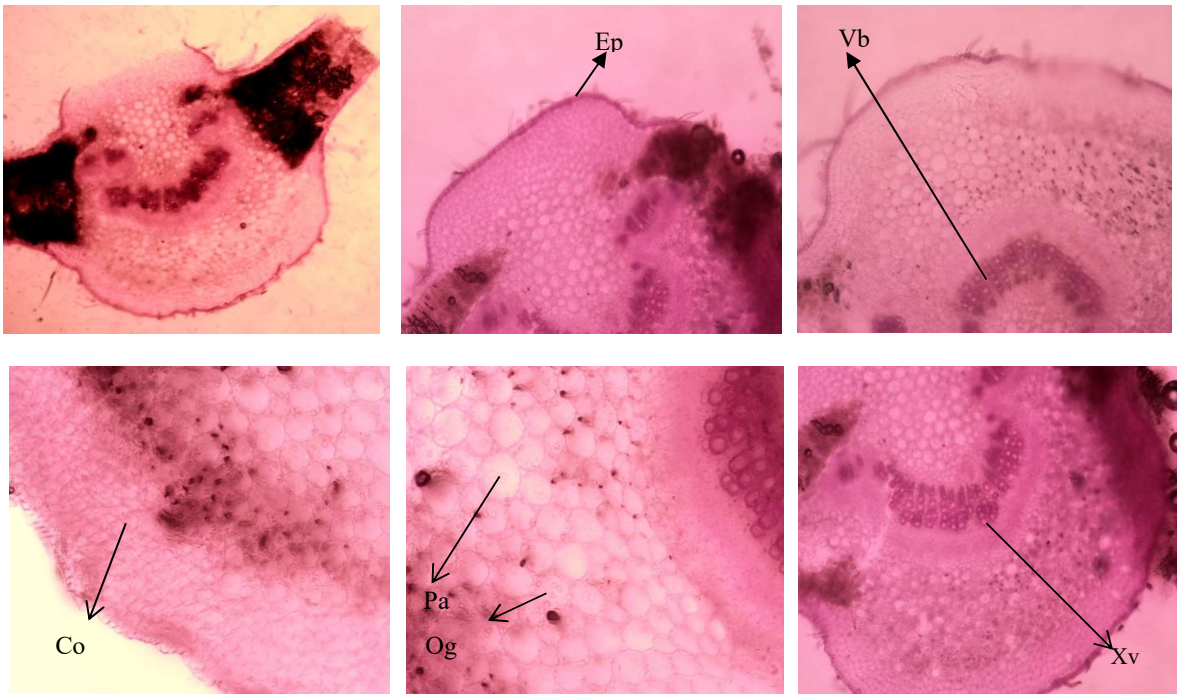

Figure 1-8: Section of T.S of leaf midrib of *Justicia adhatoda* L.

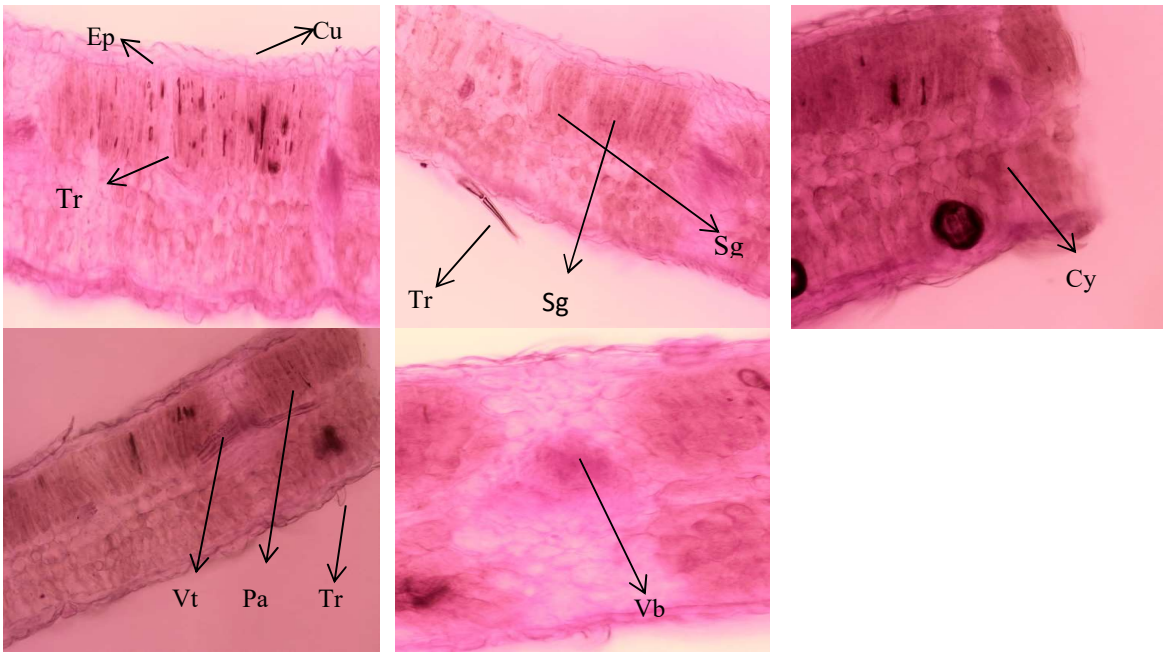

Figure 9-13: Section of T.S of leaf lamina of *Justicia adhatoda* L.

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Powder Microscopy: Powder microscopy of the leaf sample manifest light green in color, characteristic odor, and pungent test. Microscopy shows covering trichomes and oil globules. The study also exhibited a fragment of collenchymatous cells, pitted, spiral vessels thickening, and fibers.

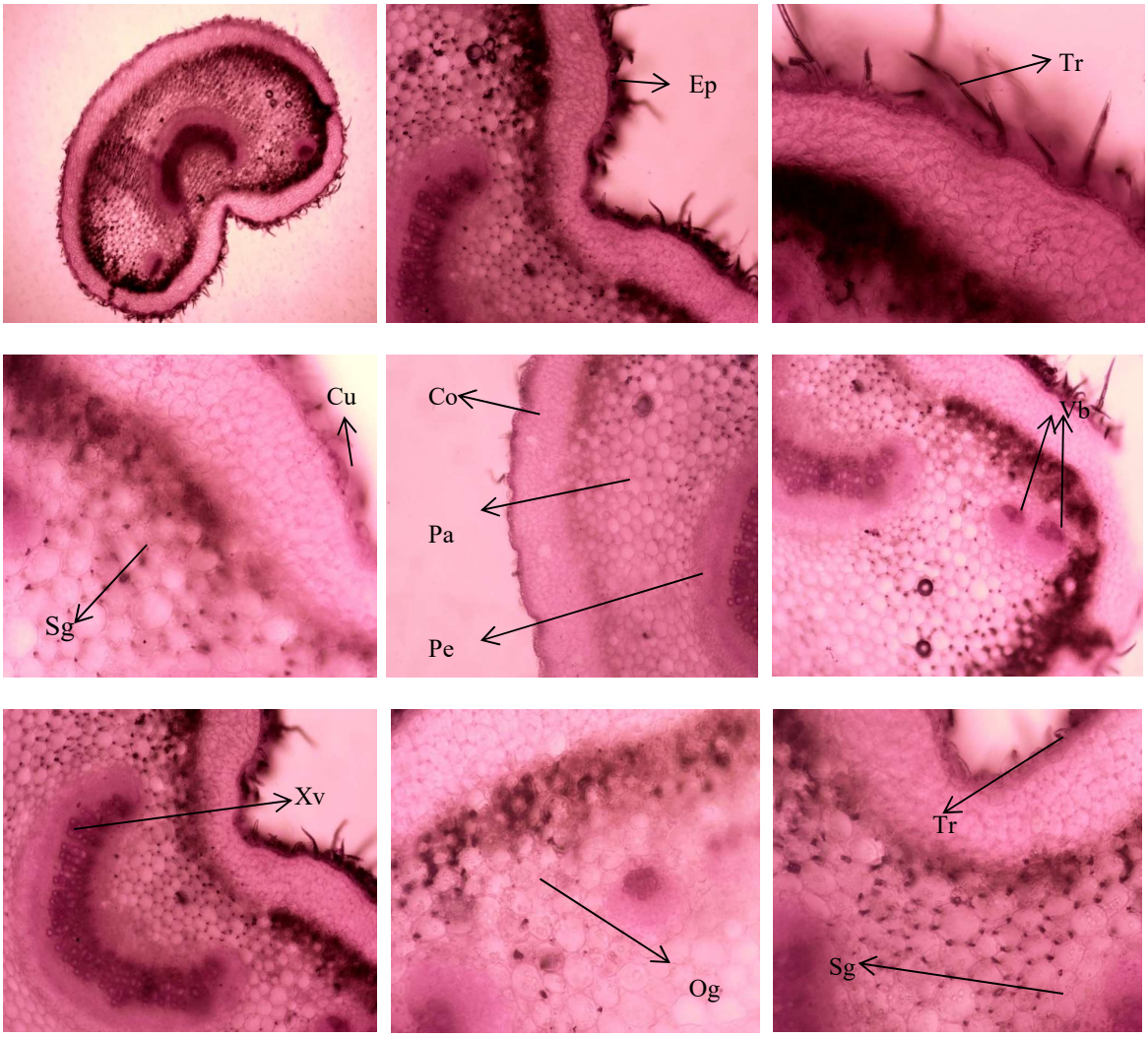

Figure 14- 22: Section of T.S of leaf petiole of *Justicia adhatoda* L.

Thin Layer Chromatography (TLC) profile: TLC- Thin Layer Chromatography fingerprinting is a common analytical tool and is a very linear, precise, accurate method for plant authentication and identification. It is mainly used to characterize herbal medicine. The HPTLC method is widely used for the phytoconstituents analysis of plants and quantification of important phytochemicals of the herbal medicines For the TLC profiling of ethanol extract *Justicia adhatoda* leaf, several solvent systems viz; ethyl acetate: methanol: ammonia (8:2:0.2), ethyl acetate: methanol: ammonia (9:1:0.2), toluene: *n*-butanol: formic acid (8:1.5:0.5) & toluene: n-butanol: formic acid (9:0.5:0.5) were tried to get better results. Finally, the best separation was achieved in the solvent system; ethyl acetate:

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methanol: ammonia (9:1:0.2). After the development of the TLC plate, it was air-dried and scanned at a wavelength of 366 (Figure 1). Thereafter, the plate was dipped in anisaldehyde-sulphuric acid reagent and heated in an air circulated oven at 105°C till the colorful spots appeared and photos were documented under white light (Fig. 43). After derivatization TLC fingerprint of *Justicia adhatoda* leaf showed 10 bands at Rf: 0.04, 0.13, 0.19, 0.28, 0.53, 0.59, 0.65, 0.68, 0.81, and 0.84 (Figure 43).

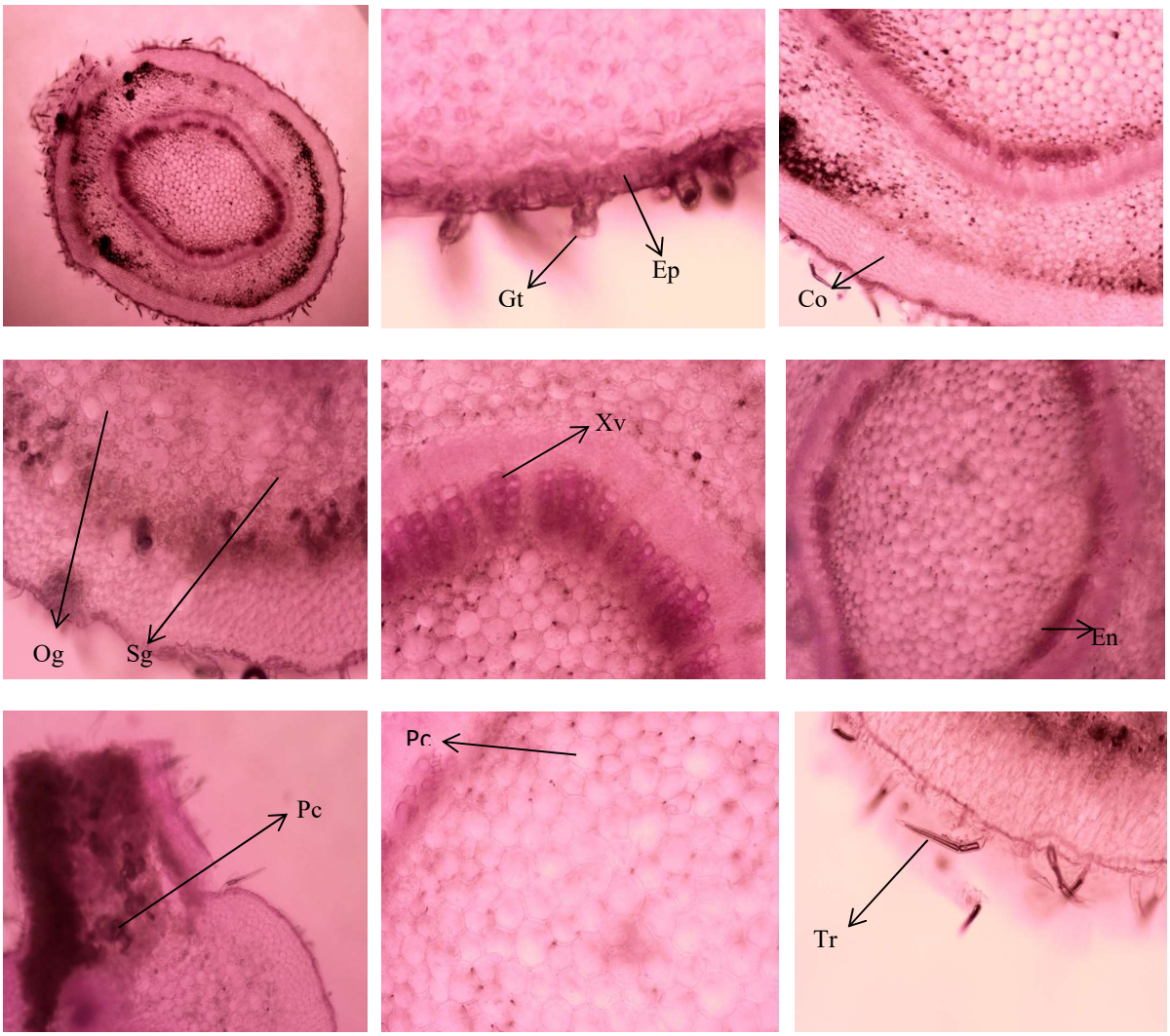

Figure 23- 32: Section of T.S of leaf rachis of *Justicia adhatoda* L.

DISCUSSION

The presence of the 4-5 layers of collenchymatous cells in the midrib region and 6-7 layers of collenchymatous cells in the petiole and rachis region is one of the unique features of the leaf. The presence of oil globules in the parenchymatous region of the midrib, petiole, and rachis is also one of the important features for the identification of the plant. Additionally, some other important

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characters present in the figures 1, 2 and 3, 6 represents 20- 24 rows of the centrally placed cup-shaped vascular bundle and figures 16, 17 and 23 displayed half-circle shaped vascular bundle with

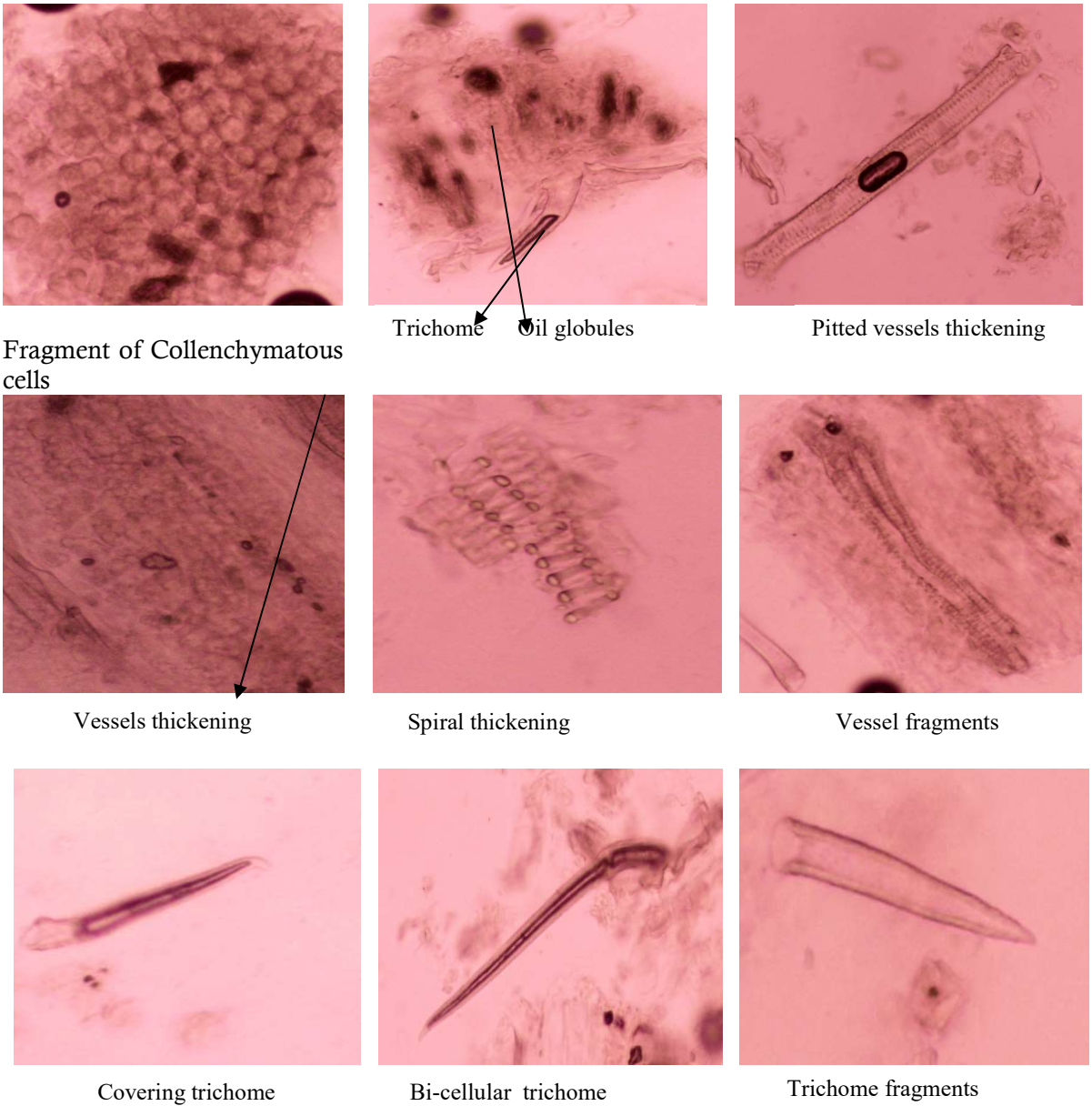

Figure 33- 42: Powder Microscopy of leaf of *Justicia adhatoda* L.

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36-40 radial rows of xylem elements and figure 23 and 28 shows 50-60 rows of xylem elements having a circular shape.

TS also show rosette crystals, oil globules, and abundant trichomes. Powder microscopic figures 35, 36, and 37 show pitted, spiral thickening of vessels and figures 39, 40 shows covering trichome and bi-cellular trichome. TLC fingerprinting reveals the presence of various phytochemicals in *Justicia adhatoda* leaf. TLC profiling of ethanol extract showed that the solvent system: ethyl acetate: methanol: ammonia (9:1:0.2) causes better separation of compounds in the extract. Therefore this solvent system can be used in the future for phytochemical analysis.

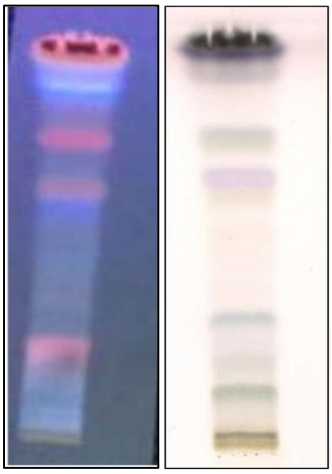

Figure 43. TLC fingerprint of ethanol extract of *Justicia adhatoda* leaf. (a) At 366 nm & (b) After derivatization.

CONCLUSION

The findings of the study manifest the anatomy, microscopy, and TLC analysis of the plant. The authentication and confirmation of crude drug samples by macroscopic, microscopic, powder study and TLC analysis explain the legitimacy and immaculacy and that may serve to assure the standard of the drug. In addition to this, it will assist in monograph preparation and repository for future information about morphoanatomical and pharmacognostic study.

DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

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