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A PHARMACOGNOSTIC DISCOURSE ON *IMPATIENS WALLERIANA*

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Keywords:

Impatiens walleriana, Balsaminaceae, Pharmacognostic study, Folk medicine

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ABSTRACT: Pharmacognostic study is important in laying down diagnostic parameters for standardization and authentication of plant materials in their fresh, dried or powdered state. Pharmacognostic study serves as a tool to identify adulterants and prevent substitution. Authentic plant material serves as raw material for herbal drug industry and local markets where people prefer folk medicine. *Impatiens walleriana* (Family – Balsaminaceae) is a folk medicine. Pharmacognostic studies on *Impatiens walleriana* species are not reported and our findings shall contribute to proper authentication of the plant material and also resolve the complexity associated with species having same common names as other species in the same genera. In the present study various parameters organoleptic, chemomicroscopic, macroscopic and microscopic characteristics have been studied in addition to fluorescence, physical and photochemical analysis. This study therefore generates resourceful data helping to identify *Impatiens walleriana* and guide researchers to explore therapeutic worthiness of this plant.

INTRODUCTION: In the recent times interest in herbal drugs has been increasing rapidly based upon the idea that herbal medicines are supposedly safer and cost effective [1]. Various drugs in current therapy are the ones developed or obtained from plant products [2]. They have drawbacks like quality issues and adulteration. Every plant has unique properties in terms of its botany, chemical constituents and medicinal property. In traditional medicine, plants are used for healing various diseases mainly based on belief passed on from generation to generation. Drawback of folklore medicines are, there are no firm quality control parameters for standardization and hence liable for adulteration, substitution [1].

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Their efficacy is hence doubted. Therefore it is essential to study pharmacognostic characters of each medicinal plant to identify unadulterated plant samples. Therapeutic efficacy of medicinal plants relates to quality and quantity of chemical constituents. Abuse of herbals begins with inaccurate identification and evaluation [3]. The most common misconception is one common vernacular name is given to two or more similar species [4]. Such issues can be figured out by pharmacognostic studies of medicinal plants. It is essential and beneficial to lay down pharmacognostic description of medicinal plants that are used as drugs.

Apart from taxonomic identification, Pharmacognostic study includes powder evaluation. This is required because once the plant is dried and made into powder it loses its morphological character and is easily prone to adulteration [1]. Pharmacognostic studies help in authentication of the medicinal plants and make sure reproducible quality of herbals [3]. On the basis of pharmacognostic study similar herbal medicines which claim to be the

same can be compared for the purpose of authenticity, efficacy, genuiness, purity, reproducibility and overall quality [5]. The more productive the natural drug is, its demand and chances of unavailability increase. This also gives scope for adulteration with poor quality material [4].

The abuse of herbal medicines can also start with wrong recognition. Such problems can be resolved by pharmacognostic specifications of medicinal plants. The pharmacognostic studies of medicinal plants have become imperative for various reasons. As per WHO norms every drug has to undergo botanical standardization in the form of macroscopic and microscopic characterization as they are important constitutes of pharmacognostic determination [4]. This primary step helps the researcher to affirm phytodrugs. The botanical standardization is based on the fact that certain characters are constant for a plant. Plants grown in the wild are the starting materials for local communities and herbal industries [6].

Impatiens genera has close to 1200 species with new species discovery further contributing to the number. Genus *Impatiens* is found in South China, South-East Asia, India and Africa. Several of its species are used in alternative medicine. *Impatiens walleriana* belonging to the family Balsaminaceae is part of folk medicine and is used in Africa. It is also used in China, Indonesia and is listed in Hawaiian ethnopharmacopoeia [2]. Pharmacognostic study detailing microscopic, macroscopic, chemomicroscopic characters and fluorescence analysis of *Impatiens walleriana* species is not reported, hence the present study.

MATERIALS AND METHODS:

Plant Collection and Authentication: The fresh plants of *I. walleriana* were collected from a local garden in Belagavi from July to August 2024. Authentication was carried out at RMRC (Regional Medical Research Centre, Belagavi) and specimen is deposited in RMRC herbarium with accession number RMRC-1341.

FIG. 1: ***I. WALLERIANA***

METHODS:

Macroscopic Analysis: The leaves and fruits of *I. walleriana* were exposed to organoleptic assessment. All samples were washed then leaves, fruit, root, stem and flowers were investigated for their morphological characteristics.

Morphological parameters such as color, smell, size, shape, and taste (organoleptic studies) were determined with help of sensory organs. Presence of petiole, leaf venation were studied and various measurements were taken such as node length, node number, leaf margin, venation, texture, spur length, petal number, petiole length and seed size.

Microscopic Analysis [8, 9]: The transverse sections of leaves, root, stem and petiole of *I. walleriana* were examined with help of a simple microscope. Standard histological examinations were also performed on the crude powdered plant material. Thin transverse sections of leaf, root, stem and petiole of *I. walleriana* were dissected with a sharp edge and kept in phloroglucinol and HCl for 1-2 min. The thin transverse sections of leaf, root, stem, seed and petiole of *I. walleriana* were transferred onto a clean and dry glass slide with the help of a brush, then 2-3 drops of glycerin was placed on it and covered with a cover slip. Then observation was carried out using simple microscope for

phloem, xylem, collenchyma cells, parenchyma cells, palisade layer, cortex etc. Quantitative leaf microscopy was carried out to determine stomatal index, stomata number, palisade ratio, vein islet and vein termination number. Powder of stem, leaf and root was examined for xylem, phloem, lignified vessel, vascular bundles, fibers, stone cells, stomata and trichome. The macroscopic and microscopic analysis images were taken by iphone 12 pro (2x zoom).

Chemomicroscopic Examination [10]: *I. walleriana* plant transverse sections were treated with phloroglucinol, hydrochloric acid and iodine to determine presence and absence of trichromes, calcium oxalate crystals, starch grains, fats, oils and inulin.

Fluorescence Analysis [11]: A small quantity of fine powder of leaves, stem and root sample were dried separately, placed on a grease free microscopic glass slide and 1-2 drops of reagent solutions were mixed by gently tilting the slide followed by waiting for 1-2 minutes.

The slide was then placed inside the UV chamber and viewed in day light, short (254 nm) and long (365nm) ultraviolet radiations. The colors were observed and recorded.

Physicochemical Values Determination [12, 13]:

a) Total Ash: Empty silica crucible was weighed and 2gm of *I. walleriana* powdered crude drug was added, then kept in the Muffle furnace at a temperature of about 500°C - 600°C until carbon-free ash was formed. It was cooled, weighed, and the percentage of total ash was calculated.

b) Acid-insoluble Ash: Obtained ash was boiled with 25ml of (70 g/l) hydrochloric acid for 5min and filtered. Ash-less filter paper was washed with hot water. Both ashless filter paper and residue were transferred into a crucible, kept in the Muffle furnace at a temperature of about 600°C until a constant weight was obtained and the percentage of acid-insoluble ash was calculated.

c) Water-soluble Ash: Obtained ash was boiled with 25ml of water for 5min and the ashless filter paper was washed with hot water. Both ashless filter paper and residue were transferred into a crucible, kept in the Muffle furnace at a temperature of about 600°C until a constant weight was obtained. Percentage of water-soluble ash was calculated.

d) Sulphated Ash: 2g of ash was weighed using a previously heated and weighed porcelain dish. The sample was heated until it got completely charred, then allowed to cool. Remaining residue was moistened with 1ml of concentrated sulphuric acid and heated again until all carbonaceous material was eliminated.

This was continued until no fumes were produced. Finally it was placed in a furnace at 550-650°C, cooled in a desiccator and weighed. Then percentage of residue was calculated.

Loss on Drying: Petri plate was weighed empty and 2gm of *I. walleriana* powdered crude drug was added. It was then kept in the oven at temperature 105°C for 2 hrs. After cooling it was weighed and the percentage of loss on drying was calculated.

RESULTS:

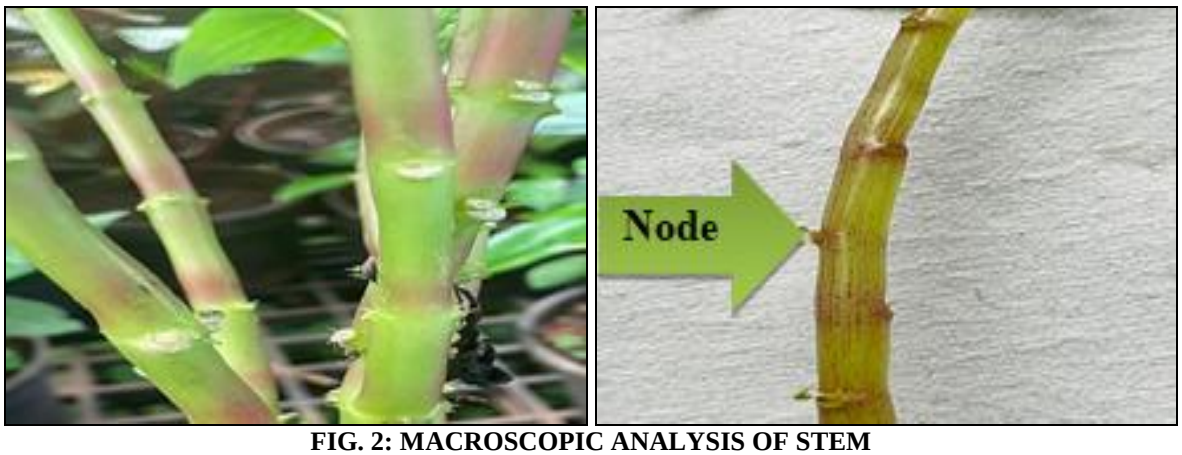


FIG. 2: MACROSCOPIC ANALYSIS OF STEM

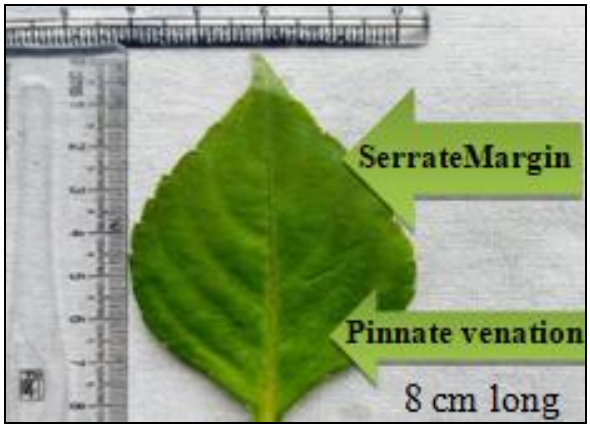
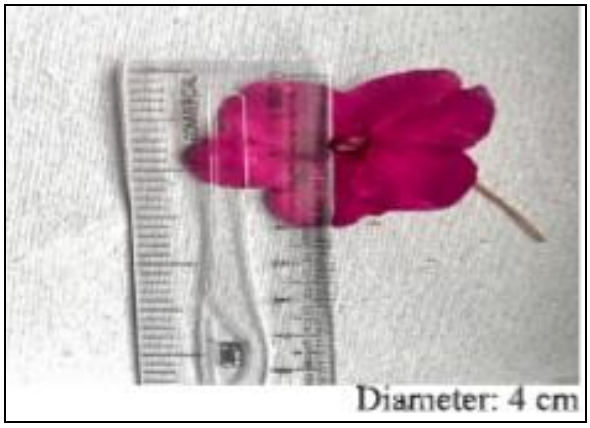


FIG. 3: MACROSCOPIC ANALYSIS OF LEAF

FIG. 4: MACROSCOPIC ANALYSIS OF ROOT



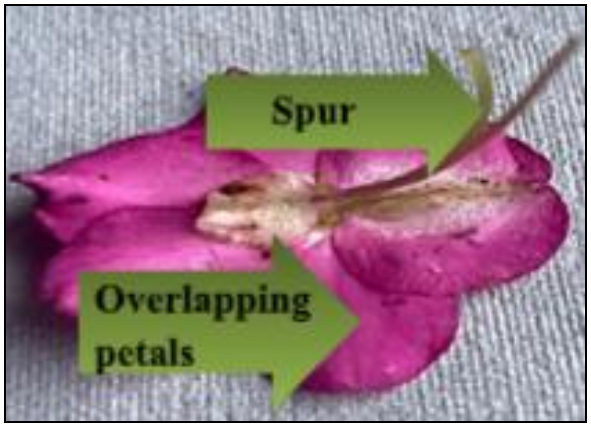


FIG. 5: MACROSCOPIC ANALYSIS OF FLOWER

FIG. 6: MACROSCOPIC ANALYSIS OF SEEDS

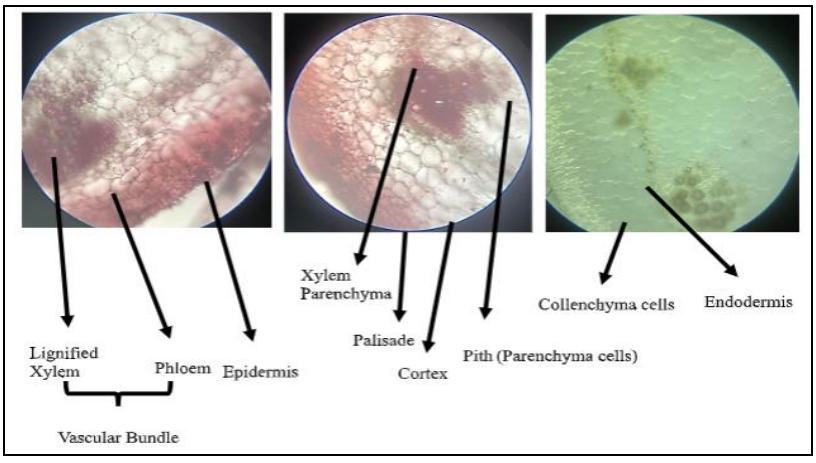


FIG. 7: MICROSCOPIC ANALYSIS OF STEM

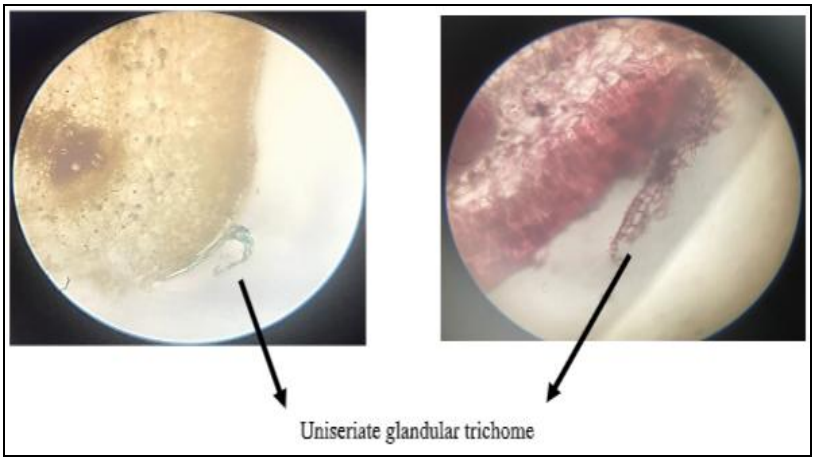


FIG. 8: MICROSCOPIC ANALYSIS TRICHOME

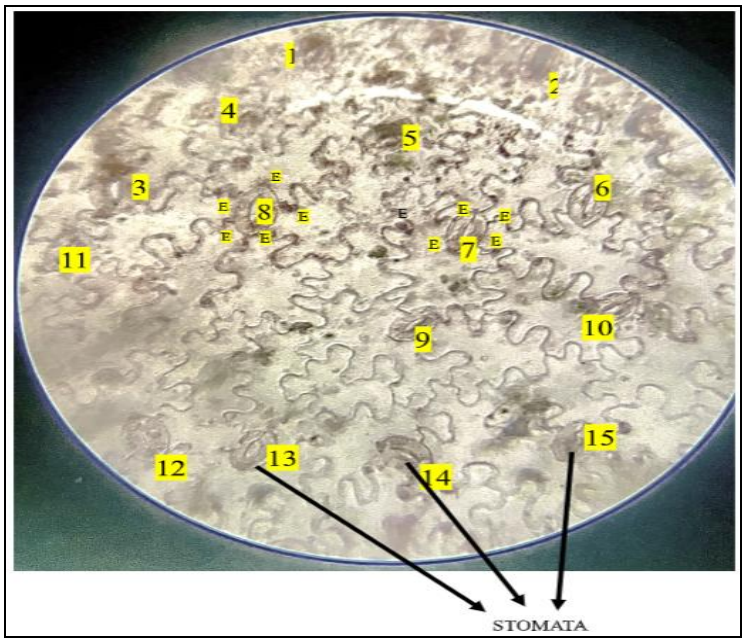


FIG. 9: STOMATAL INDEX OBSERVED IN STEM

Total 15 Anomocytic stomata's & 61 epidermal cells were observed.

Stomatal Index (SI) = Number of stomata per unit area/(Number of stomata+Epidermal cells) × 100

(SI) = 15/(15 + 61) × 100 = 19.7%

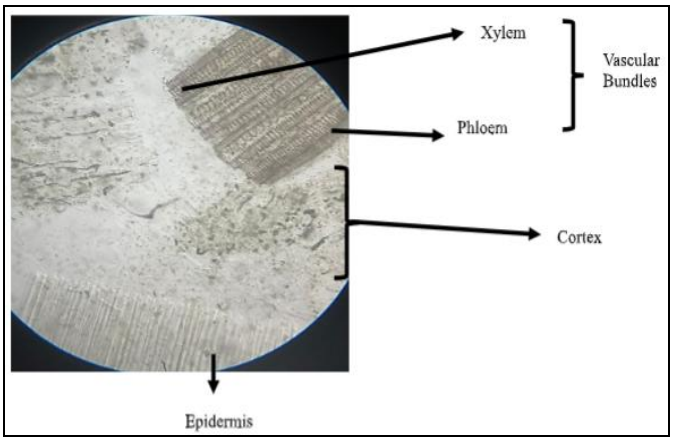


FIG. 10: T.S OF PETIOLE

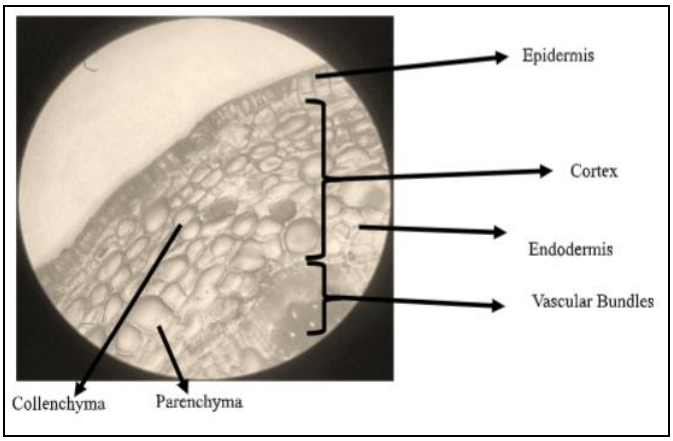


FIG. 11: T.S OF ROOT

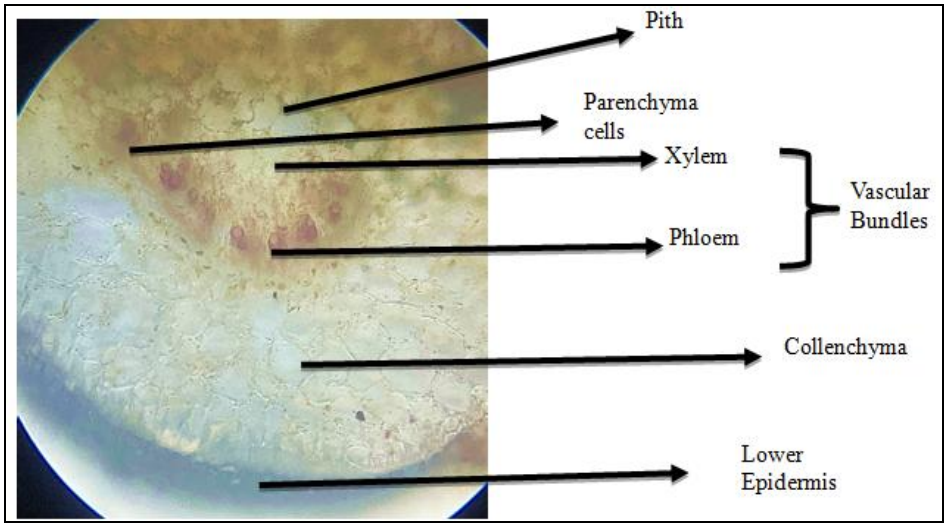


FIG. 12: T.S OF LEAF

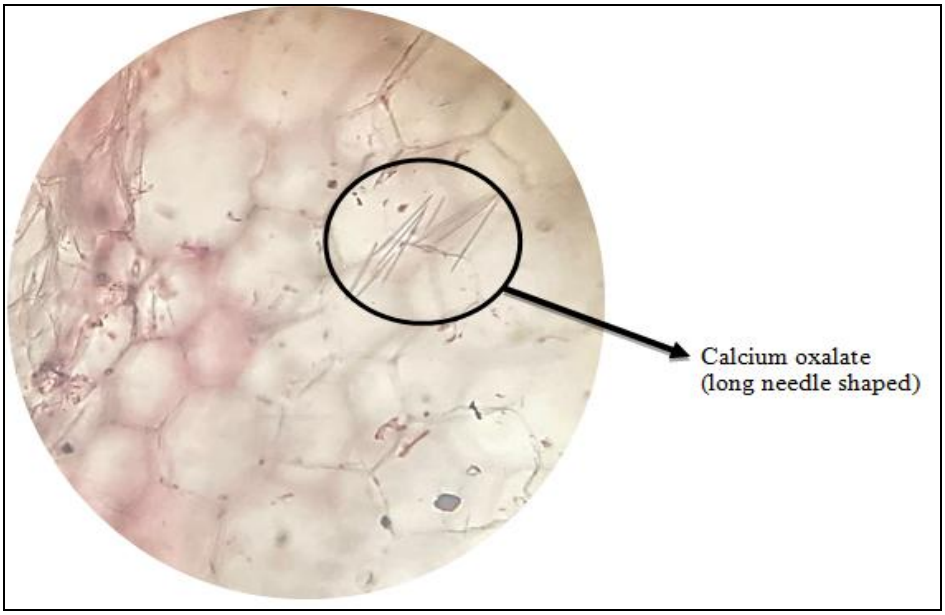


FIG. 13: CHEMOMICROSCOPIC EXAMINATION OF CALCIUM OXALATE IN STEM

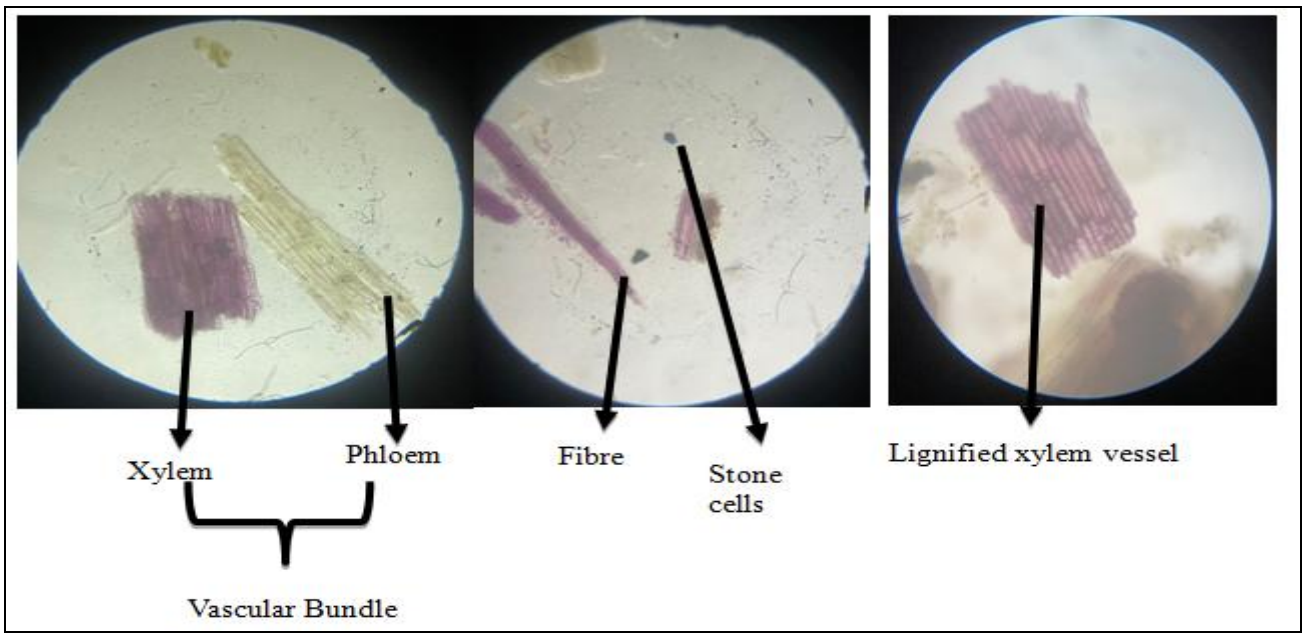


FIG. 14: POWDER MICROSCOPY OF STEM

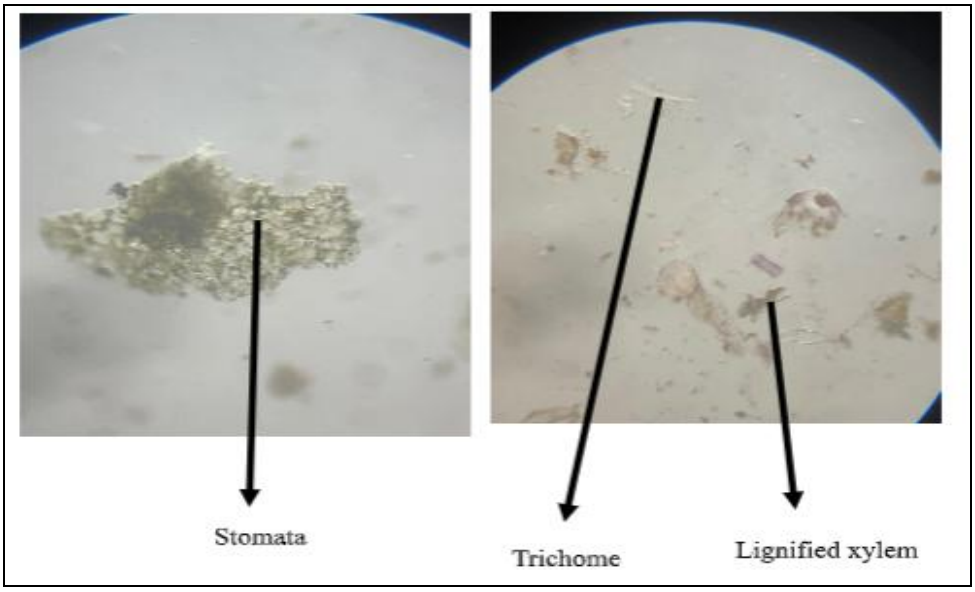


FIG. 15: POWDER MICROSCOPY OF LEAF

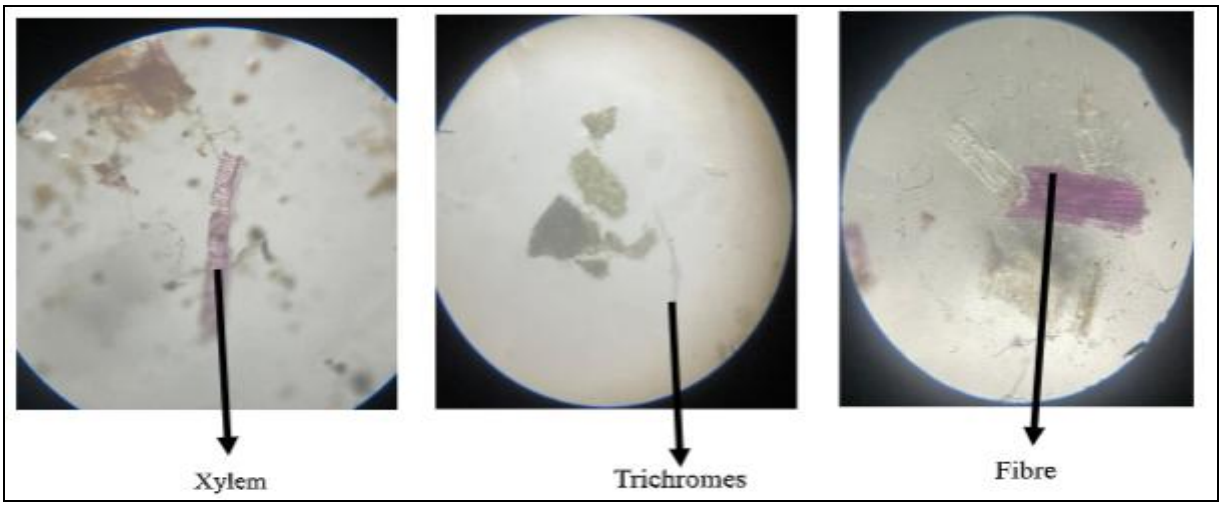


FIG. 16: POWDER MICROSCOPY OF ROOT

TABLE 1: FLUORESCENCE ANALYSIS OF STEM

Sl. no.	Chemicals	Visible Light	Short UV (254nm)	Long UV (365nm)
1	KOH	Yellowish green	Green	Light green
2	1N NaOH(aq*)	Golden	Black	Light green
3	Ammonia	Light green	Black	Light green
4	Ethyl acetate	Yellow	Black	Pink
5	50% HCl	Golden	Black	Light green
6	Acetic acid	Dark green	Light green	Black
7	Petroleum ether	Yellow	Green	Dark green
8	50% H_2SO_4	Yellow	Green	Black
9	50% HNO_3	Yellowish brown	Light green	Black
10	1N NaOH(alc**)	Golden	Green	Brown
11	$FeCl_3$	Yellowish green	Green	Brown
12	KOH(alc**)	Golden	Dark green	Light green

(aq *) = aqueous, (alc **) = alcoholic

TABLE 2: FLUORESCENCE ANALYSIS OF LEAVES

Sl. no.	Chemicals	Visible Light	Short UV (254nm)	Long UV (365nm)
1	KOH	Yellowish green	Green	Light green
2	1N NaOH(aq*)	Light green	Light green	Black
3	Ammonia	Light green	Black	Black
4	Ethyl acetate	Green	Light green	Pink

5	50% HCl	Golden	Dark green	Black
6	Acetic acid	Green	Light green	Black
7	Petroleum ether	Green	Light green	Creamy
8	50% H_2SO_4	Yellowish green	Green	Black
9	50% HNO_3	Brown	Dark green	Black
10	1N NaOH(alc**)	Golden	Green	Black
11	$FeCl_3$	Yellowish green	Green	Black
12	KOH(alc**)	Golden	Dark green	Light green

TABLE 3: FLUORESCENCE ANALYSIS OF ROOT

Sl. no.	Chemicals	Visible Light	Short UV (254nm)	Long UV (365nm)
1	KOH	Brown	Dark green	Light green
2	1N NaOH(aq*)	Brown	Green	Green
3	Ammonia	Brown	Light green	Light green
4	Ethyl acetate	Brown	Dark green	Black
5	50% HCl	Brown	Light green	Black
6	Acetic acid	Brown	Green	Creamy
7	Petroleum ether	Golden	Light green	Black
8	50% H_2SO_4	Brown	Black	Black
9	50% HNO_3	Brown	Light green	Black
10	1N NaOH(alc**)	Golden	Dark green	Creamy
11	$FeCl_3$	Yellow	Light green	Black
12	KOH(alc**)	Dark brown	Dark green	Light green

TABLE 4: PARAMETERS FOR LEAF

Parameters	Value
Stomatal Index	19.7%
Vein Islet	20
Vein Termination	27
Palisade ratio	4.5

Macroscopic Analysis of Stem:

Type: Dicot stem, stems are sappy, succulent, tender, herbaceous.

Branching: Vigorous stem and Sympodial branching (the main axis of growth is terminated by flower and growth is continued by one or more lateral branches. This pattern gives bushy appearance).

Size: 15cm-75cm long and 0.3cm-1cm wide.

Color: Young stems are greenish brown in color and reddish tinged when fully grown.

Texture: Smooth

Shape: Cylindrical and relatively slender.

Node Length: 0.5cm-3cm.

Nodes: Stem has distinct nodes (with 9 to 11 nodes in 30cm plant).

Macroscopic Analysis of Leaf:

Type: Lanceolate (small leaf) to Ovate (fully grown leaf).

Leaf Margin: Dentate

Size: 3cm-12cm long and 2cm-6cm wide

Color: Dark green and variegated when fully grown

Venation: Pinnate

Texture: Glossy, glabrous

Leaflets: Not Present

Taste: Slightly bitter

Macroscopic Analysis of Flower:

Color: Pink

Position: Solitary or in clusters (found on axils of leaves)

Petals: 5 Petals that are free (not fused) and overlapping. Lower petal forming spur (contains nectar which attracts pollinators like bees and butterflies)

Type: Zygomorphic

Size: 2.5cm-5cm in Diameter

Blooming Season: Spring

Taste: Sweet taste

Sepals: 3 Sepals present

Spur Length: upto 4 cm (fully grown flower)

Petiole Length: 4-6 cm

Macroscopic Analysis of Seeds:
Type: Capsule like when mature. It explosively dehisces to disperse the seeds (Ballistic seed dispersal).

Fruit Color: Fruits are green in color

Fruit Size: 1.3cm-2.5cm in length and about 0.5cm-1cm in diameter

Seed Color: Seeds are white in color

Seed Size: 1mm-2mm diameter

Fruit and Seed Taste: Unripe fruit and Immature seeds tasted bitter.

Macroscopic Analysis of Root:

Root System: Fibrous root system, tapering root

Root System Depth: 15cm-20cm

Taste: Unpleasant taste

Color: White to light tan in color

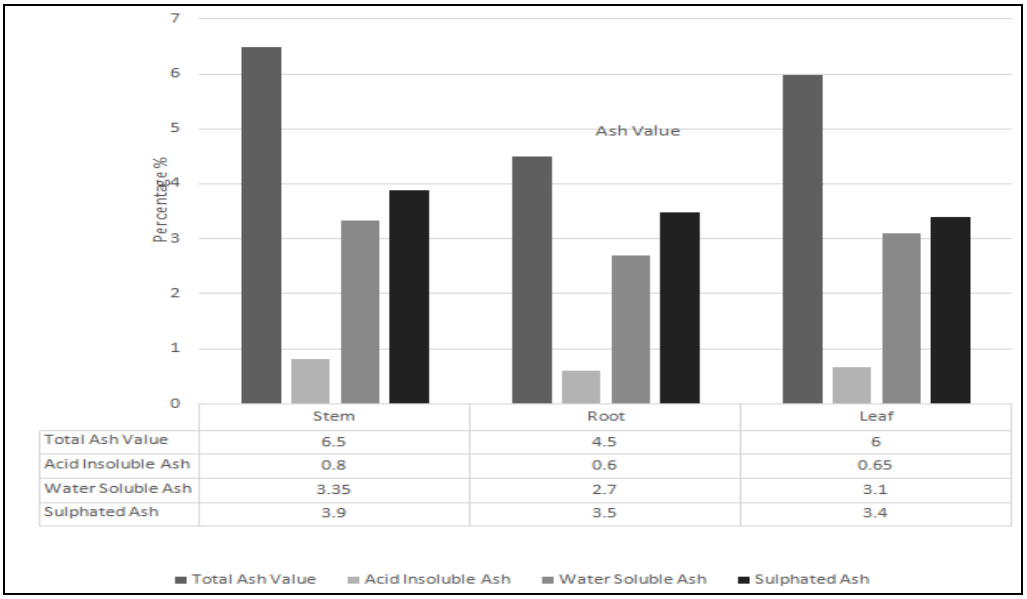
Microscopic Characteristics:
Stem: The T.S of *I. walleriana* stem **Fig. 7** consist of epidermis which is 3 layered surrounded by palisade cells. The cortex region consists of 4-6 layers. The vascular bundles (lignified xylem and phloem) are present around the pith region (parenchyma cells) which is a closed type arrangement in ring form. The vascular bundles are surrounded by xylem parenchyma cells. Uniseriate glandular trichomes are present on the outer surface of epidermis **Fig. 8**. Raphides calcium oxalate and acicular bundles are present in pith region. Trichomes are present on the outer epidermis layer.

Leaf: The T.S of *I. walleriana* leaf **Fig. 9** consists of single layered lower epidermis (as seen in section). Centrally located conjoint collateral vascular bundles (xylem and phloem) are surrounded by parenchyma cells. Anomocytic stomata are present in lower epidermis **Fig. 10.**

Petiole: The T.S of *I. walleriana* petiole **Fig. 10** consist of single layered epidermis where trichomes are absent. The cortex is present around vascular bundles (xylem and phloem).

Root: The T.S of *I. walleriana* root **Fig. 11** consist of single layered epidermis. The cortex region consists of 7-8 layers with collenchyma and parenchyma cells in it. Vascular bundles (xylem and phloem) are surrounded by endodermis.

Powder Microscopy of Plant: Stem microscopy shows Vascular bundles (lignified xylem and phloem), fibre and stone cells **Fig. 14.** Leaf microscopy show trichomes, lignified xylem and stomata **Fig. 15.** Root microscopy shows xylem, trichomes and fibre **Fig. 16.**



	Stem	Root	Leaf
Total Ash Value	6.5	4.5	6
Acid Insoluble Ash	0.8	0.6	0.65
Water Soluble Ash	3.35	2.7	3.1
Sulphated Ash	3.9	3.5	3.4

FIG. 17: ASH VALUE OF CRUDE POWDER

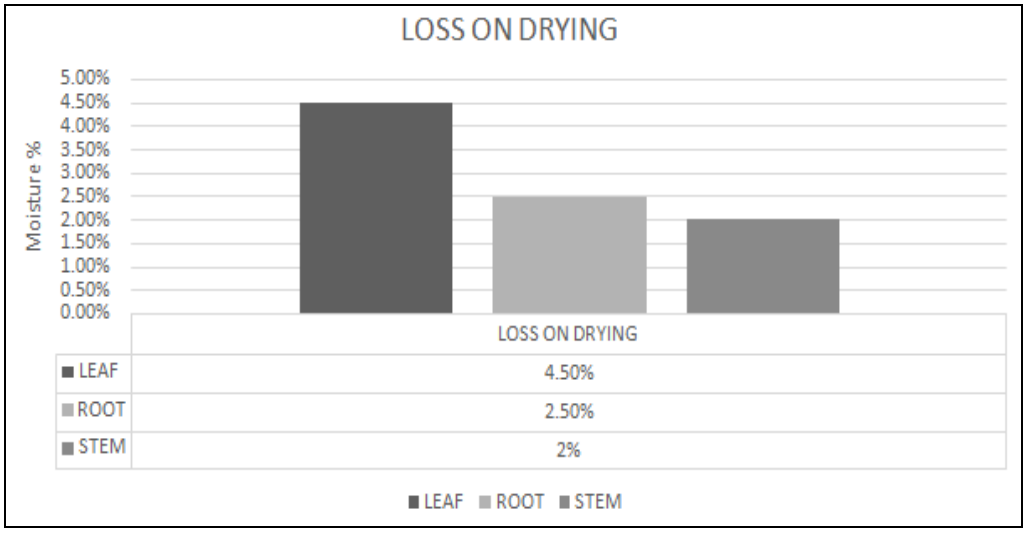


FIG. 18: LOSS ON DRYING

DISCUSSION: Plants growing in the wild are a source of raw material for locals using them and for herbal industry [6]. Safety, efficacy and reproducible quality of herbals is paramount and developing quality control parameters for herbals is therefore essential [14]. Herbals supplied to the market could be in deformed state without proper identification paving way for adulteration or substitution. Pharmacognostic studies help in identifying genuine drugs as the results are plant specific [6]. With pharmacognosy as a tool complete knowledge about herbals can be obtained. Herbals may be fresh or dried plant parts [15]. As per WHO every drug has to undergo botanical standardization mainly pharmacognostic characterization in the form of macroscopic and microscopic studies [4]. All parts of the plant are important as far as pharmacognostic study is concerned [1].

Organoleptic evaluation helps in detecting false or substitute drugs. As macroscopic characteristics have limitation like subjective judging, the study is well supported by microscopic findings using both intact and powdered form of specimen [4]. As per WHO guidelines and botanical standards microscopic detections are diagnostic [5]. Microscopic analysis is a cheaper alternative to identify a particular drug and confirm that raw material [17]. Microscopy also helps to identify and differentiate two herbals that are similar [4]. The distinguishing characters revealed in microscopy get retained even in powder form of drug [3]. Chemomicroscopic examination revealed presence of calcium oxalate crystals but did not reveal presence of any fats, oil, starch grains and inulin. For plant to be used in modern medicine physicochemical standardization is important [1]. Foaming index is not determined in the study as saponins are absent as reported in previous phytochemical studies [18]. Ash value in all indicates the involvement or non-involvement of irrelevant matter. Ash value determines quality and purity of crude drugs. Water soluble ash gives idea about inorganic compounds present in the drug and acid soluble ash indicates presence of earthy materials [4]. Presence of moisture leads to bacterial contamination hence its estimation guides storage and is important for stability of drug during storage [6]. Fluorescence analysis is simple, rapid procedure to detect adulterants [1]. Certain pharmacognostic parameters are specific for a particular drug like the physical parameters being constant for a plant. Study of stomata apart from taxonomic has pharmacognostic value in identification of a plant [6]. Further studies like developing TLC profile and determining extractive values can be taken up to add to the existing data generated through this study. Overall the resourceful data generated hereby will come handy in identifying this plant and will also be of use in the preparation of monograph for this plant.

CONCLUSION: Overall in the present study enough new information has been found and documented with respect to pharmacognostic identification of *Impatiens walleriana*. This study is useful in pharmacognostic standardization of the plant. The parameters laid down are useful in identifying this plant in its crude form and prevent it from adulteration. The information generated

hereby shall serve as one among the references for pharmacopoeial parameters development. Two similar herbals can be identified as different using the resourceful data generated from the study. Further studies to determine extractive values can be carried out to add to the pharmacognostic study. The information provided through this study with help researchers to explore correct species for research in orderto find in scientific way the therapeutic uses of this folk medicine.

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CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

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