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Pharmacognostical Investigation On Leaves Of Citrus Limetta Risso

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Abstract

The Citrus species are inherent source of valuable essential oil which possess various pharmacological actions. *Citrus limetta* Risso belongs to Rutaceae family. It is commonly known as Mosambi (Sweet Lime) rich in vitamin C and also acts as Antioxidant, Anticancer, Antibacterial, Immune boosters, Antiviral agent. The current study is aimed to explore the Pharmacognostical parameters and perform proximate analysis including Ash values, Loss on drying, Extractive values, Crude fibre content, Foaming index for the leaves that might enable the detection of adulterants from the genuine raw materials and further can be included as standard reference in various Pharmacopoeias.

Keywords: Citrus limetta Risso, Pharmacognostical studies, Proximate analysis

Introduction

The universal role of plants in the treatment of diseases is exemplified by their employment in all system of medicines and various plants have become of interest to the world at large. There is a good deal of diversity of citrus species that possess various pharmacological actions. These aromatic plants comprises the third largest fruit industry after banana & mango and occupies about 75% of the land under fruits. They are mainly farmed, distributed in the Indo-Malaysian region, South-East Asia and China but cultivated throughout the tropical and temperate regions. One of such plants is *Citrus limetta* Risso. It is commonly known as Mosambi (Sweet lime) that is rich in vitamin C. The essential oil from the leaves and fruits consists of various phytoconstituents including D-limonene, α-pinene, β-pinene, β-Myrcene, Neral, Citronellal, Camphene, etc.[1] These constituents are reported to possess Antioxidant, Anticancer, Antibacterial and Antiviral properties and also serve as Immune boosters. It is probably of hybrid origin and is commonly grown in central and northern parts of India as it is lasting and yields heavily. These are small to medium round-topped tree of less vigor and growth habit; fruits small, rounded or depressed, circular furrow in the center of which is prominent fleshy papilla or nipple. The leaves, flowers, fruits, peels contain volatile oil, ethno medically

the leaf extracts are used to treat hypertension.[2,3] The Pharmacognostical evaluation of the leaves of the selected plant remains unidentified, hence this work will demonstrate the complete Pharmacognostical details and proximate analysis of *Citrus limetta* leaves.

Materials and Methods

Collection of specimens

The plant specimens for the proposed study were collected in Odathurai village, Erode district Tamil Nadu in the month of December 2019. Care was taken to select healthy plants and normal organs. The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin -5ml+ Acetic acid-5ml +70% Ethyl alcohol-90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary Butyl alcohol. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60ºC) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Figure 1 - Citrus limetta fruits along withdifferent sized leaves

Figure 2 - Whole plant of Citrus limetta

Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 µm. Dewaxing of the sections was done by customary procedure [4]. The sections were stained with toluidine blue [5]. Since toluidine blue is a polychromatic stain, the staining results were remarkably good and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. Wherever necessary sections were also stained with safranin and fast-green and IKI (for Starch)

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid were prepared [6]. Glycerine mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with sodium hydroxide and mounted in glycerine medium after staining. Different cell components were studied and measured.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon labphoto 2 microscopic unit. For normal observations bright field was used. For the study of crystals, starch grains and liginified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive

terms of the anatomical features are as given in the standard anatomy books [7,8]

Proximate Analysis

Proximate analysis of powdered plant material of *Citrus limetta* was carried out using reported methods with reference to standard procedures [8-13]

Following determinations were done: Total ash, Acid insoluble ash, Water soluble ash, Sulphated ash, Loss on Drying, Extractive value, Alcohol soluble extractives, Water soluble extractives, Crude fibre content, and Foaming index.

Results and Discussion

Pharmacognostical studies

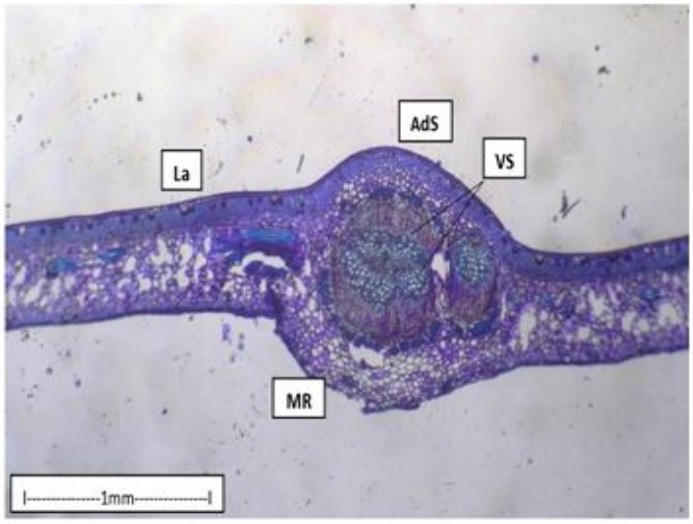

Figure 3 - T.S of leaf through midrib.

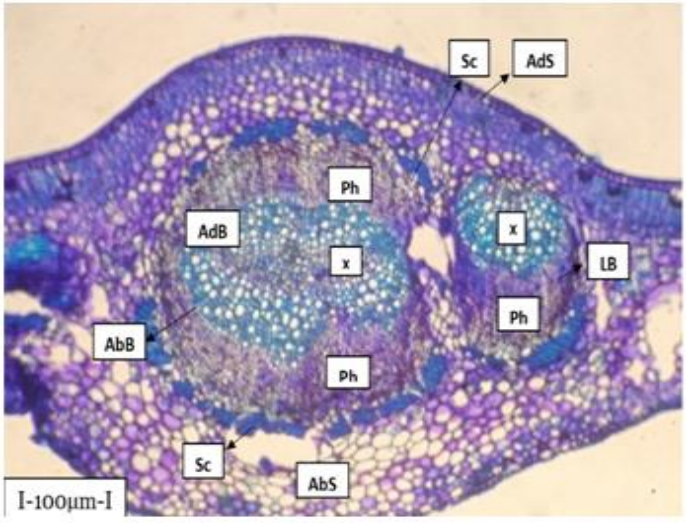

Figure 4 - T.S of midrib enlarged.

[AbB- Abaxial Bundle; AdB- Adaxial Bundle; AbS- Abaxial Side; Ads- Adaxial Side; LB- Lateral Bundle; La- Lamina; MR- Midrib; Ph- Phloem; Sc- Sclerenchyma; VS- Vascular Strand; X- Xylem

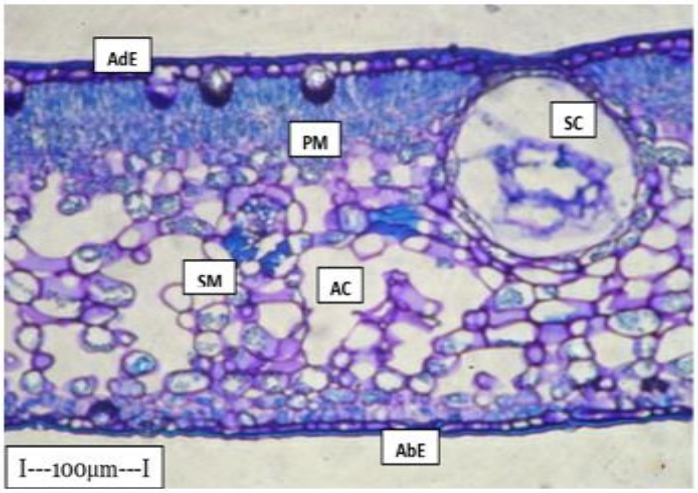

Figure 5 - T.S of Lamina

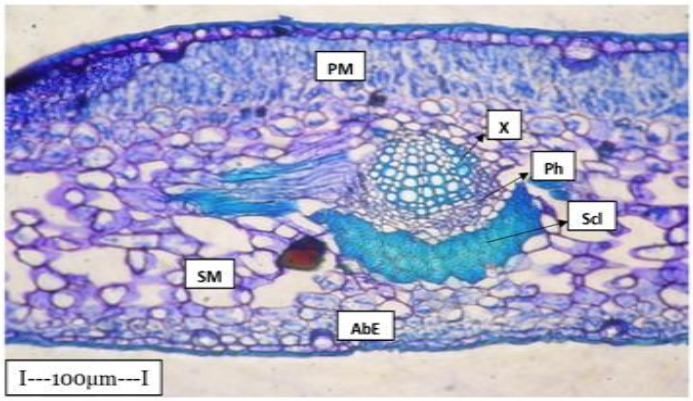

Figure 6 - T.S of Lamina with lateral vein.[AbE- Abaxial

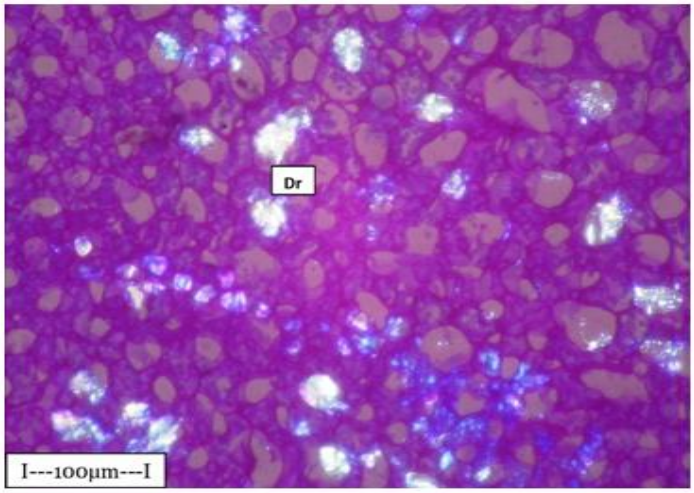

Figure 7 - Distribution of calcium oxalate crystals. [Cr-Crystal; Dr- Druse]

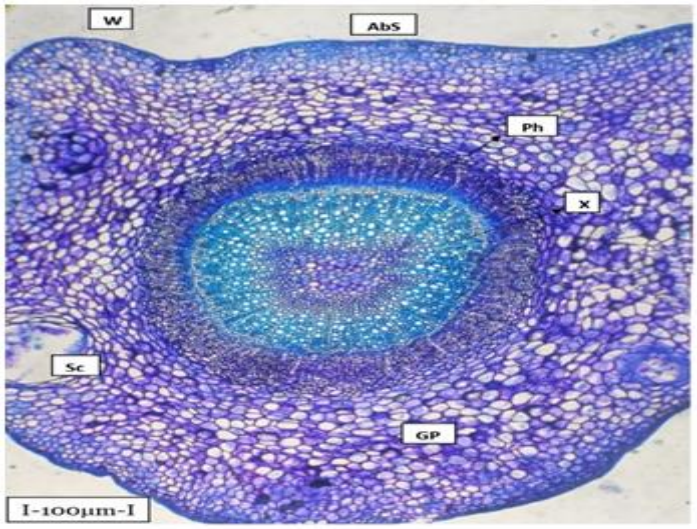

Figure 8 - T.S of petiole entire view

[GP- Ground Parenchyma; W-Wing; Pa- Parenchyma

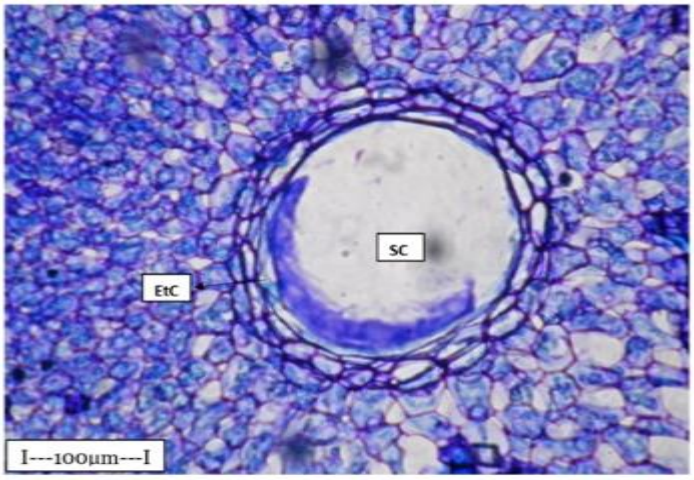

Figure 9 - Secretory Cavities in surface view.

[Cr- Crystal; Etc- Epithelial cells; SC- Secretory Cell]

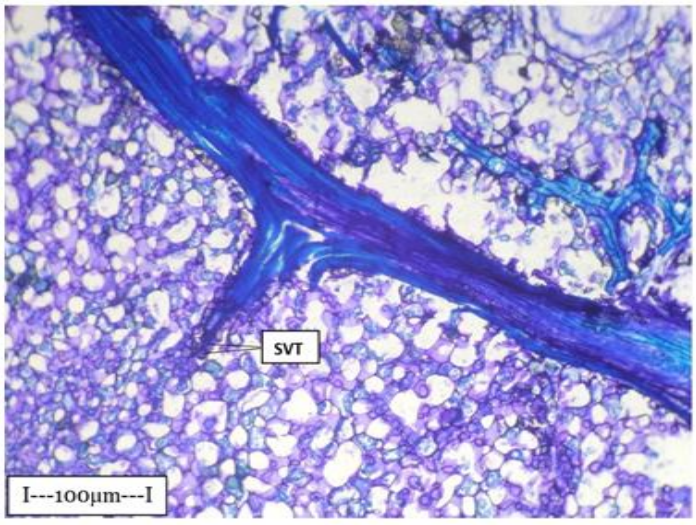

Figure10 - Simple vein termination

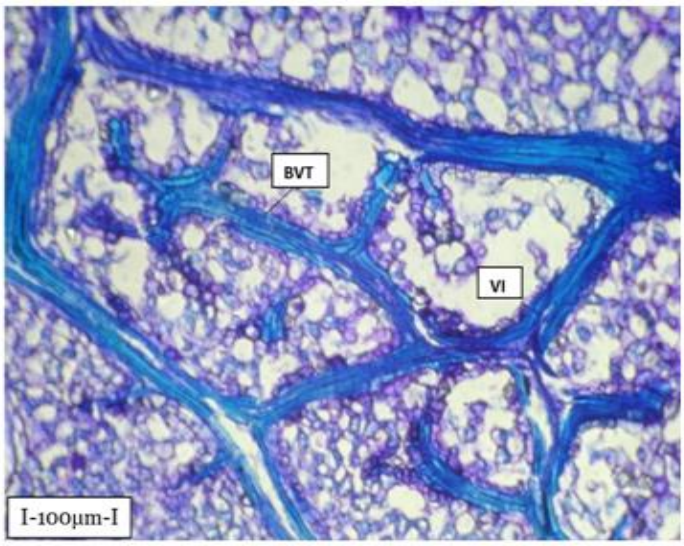

Figure 11 - Branched vein termination

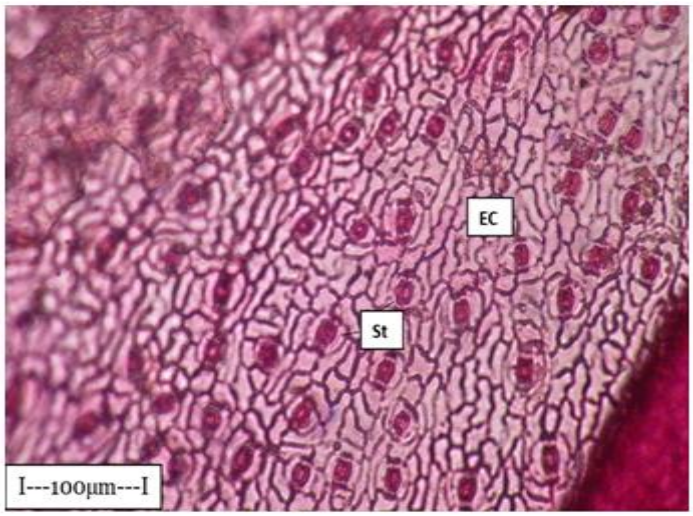

Figure 12 - Abaxial epidermis in paradermal section

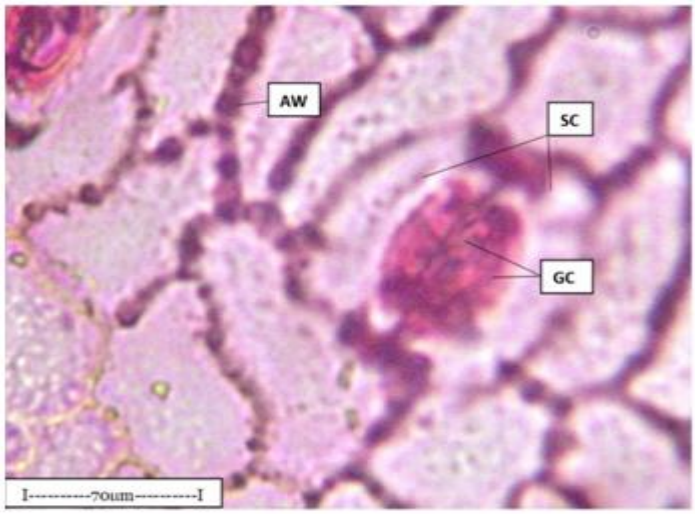

Figure 13 - Paracytic subsidiary cells.

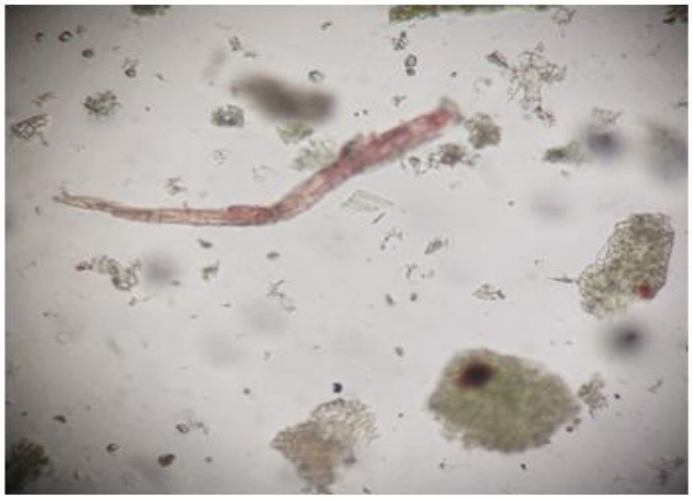

Figure 14 - Fragments of tissues along with trichomes

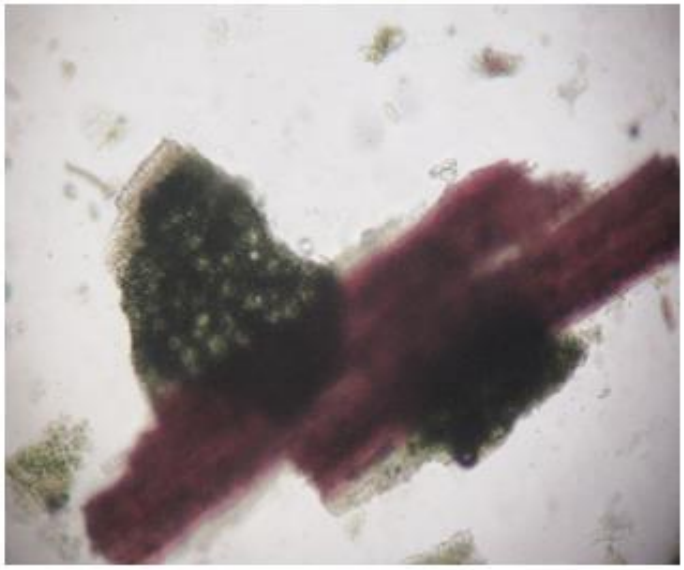

Figure 15 - Group of fibres

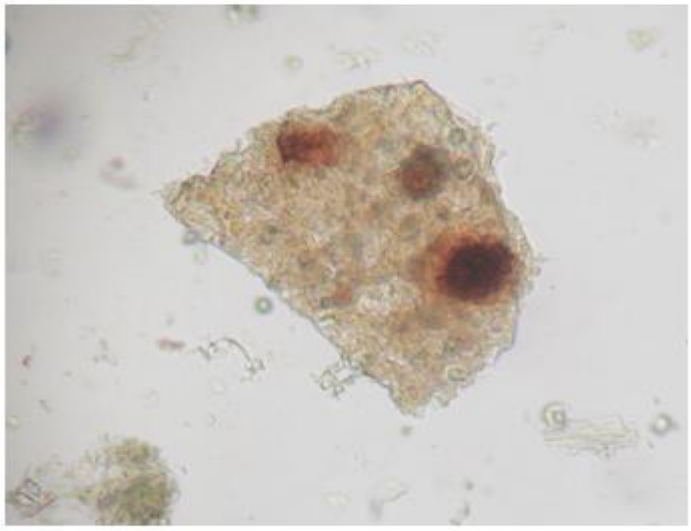

Figure 16 - Secretory cells

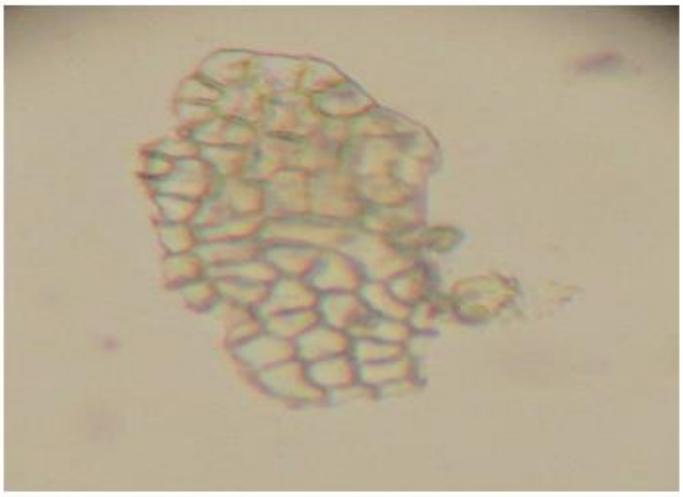

Figure 17 - Epidermal cells

In cross sectional view, the leaf possess a thick biconvex midrib and thick smooth lamina. The midrib is 150µm thick and the lamina is 350µm thick. The midrib has multi stranded vascular system. The vascular system of the midrib consists of two bundles of vascular segments on the abaxial side and single segment of adaxial bundle. There is a circular, thick vascular bundle on the lateral side. The vascular bundles are collateral with xylem tissue on the upper side and phloem tissue on the lower side. In the adaxial bundle, phloem is on the upper side and xylem is on the lower side (Figure 3 & 4).

Lamina is distinctly dorsi-ventral with differentiation of adaxial side and abaxial side. The epidermal cells of the lamina have small fairly thick walled cells with thick prominent cuticle. Some of the adaxial epidermal cells are dilated and possess small calcium oxalate crystals. The mesophyll tissue includes two horizontal layers of cylindrical palisade cells. The remaining portion of the lamina has reticulate spongy parenchyma cells which are prominent and large. Air chambers are formed by reticulate partitions of the spongy mesophyll tissue and are very wide, circular secretory cavities located either towards adaxial side or abaxial side of the lamina. The secretory cavities have two or more layers of epithelial cells encircling the inner surface. (Figure 5)

Prominent collateral lateral veins are seen in the middle part of the lamina. The vascular bundle of the vein consists of conical xylem strand comprising long parallel four or five lines of the thick walled xylem elements. Phloem occurs in the form of arc beneath the xylem strand. The protoxylem elements are directed towards the upper side of the lamina. (Figure 6)

Calcium oxalate crystals are abundant in the mesophyll tissues of the leaf. The crystals are mostly druses. Rarely prismatic crystals are also seen. The crystals are located individually in the mesophyll tissue. The crystals possess brief ringent property. (Figure 7)

The petiole exhibits dorsi-ventral symmetry. It consists of convex adaxial side with two thick and wide lateral veins. The petiole consists of distinct continuous epidermal layer, homogenous ground tissue and prominent centrally placed circular vascular cylinder. (Figure 8)

There are wide circular secretory glands in the powder. The glands have short wide one-celled stalk. The glands are 350µm in diameter. (Figure 9)

The lamina contains dense reticulate system with thick lateral veins and vein islets. The veinlet are distinct and they are bound by well developed thick vein boundaries. Within the vein islets there are two types of vein termination. There are well branched vein termination within the vein islets. The other types of vein termination is simple and unbranched vein termination (Figure 10 & 11).

It consists of paracytic stomata.The guard cells are rectangular with central stomatal pore. The stoma is narrowly elliptical measuring 10 x 30µm in size. The stoma has two subsidiary cells parallel to the long axis of the guard cells. (Figure 12 & 13)

Powder microscopy of the crude drug shows presence of fragments of tissues including group of fibers, trichomes, epidermal cells, secretory cells etc. (Figure 14, 15, 16, 17)

Proximate Analysis

Table1. Data showing the Physico -chemical characters of leaves of *Citrus limetta* Risso

S. No	Physicochemical Standards	Values in Percentage (w/w)
1.	Total Ash	12.25
2.	Acid Insoluble Ash	8.97
3.	Water Soluble Ash	16
4.	Sulphated Ash	16
5.	Loss on Drying	11
6.	Water Soluble Extractive	25.6
7.	Alcohol Soluble Extractive	8
8.	Crude Fiber Content	26
9.	Foaming Index	<80

The physiochemical properties of the crude drug have been analyzed and the values are tabulated (Table 1).

Conclusion

The standardization of crude drug is a key factor that will establish the proper identification and guaranteeing the botanical quality. This study might act as solid basis for the identification, collection, authentication of genuine raw materials of *Citrus limetta* Risso. In future these microscopic data and proximate analysis can be included as standard reference in Pharmacopoeias and also for the detection of adulterants and substituents in the crude drug that might aid to maintain the quality and efficacy of the drug.

Acknowledgement

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