

RESEARCH ARTICLE

COMPARATIVE PHARMACOGNOSTICAL CHARACTERIZATION OF SELECTED SPECIES OF OCIMUM

*Amit Joshi¹, A.K. Pathak¹, Mukul Tailang²¹Department of Pharmacy, Barkatullah University, Bhopal (M.P.) India²Department of Pharmacy, Jiwaji University, Gwalior (M.P.) India*Corresponding Author's E-mail: amit_joshi_4580@yahoo.co.in, Contact No.:- +91-9425094311

ABSTRACT

In traditional systems of medicine, different parts of *Ocimum* have been recommended for the treatment of bronchitis, bronchial asthma, malaria, diarrhoea, dysentery, skin diseases, arthritis, chronic fever and insect bite. Morphological and anatomical characters play a vital role in crude drug identification and standardization. In the present study, comparative Pharmacognostical evaluation was performed for the dried leaves powder of selected species of *Ocimum* such as *O. gratissimum*, *O. sanctum* and *O. canum*. The result from macroscopical, microscopical and powder characters are used for the identification of *O. gratissimum*, *O. sanctum* and *O. canum*. The results throw immense light on the botanical identity of various species of *Ocimum* which furnish a basis of judging the authenticity of the plant and also to differentiate the drug from its allied species and detect adulterants.

Keywords: *Ocimum gratissimum*, *Ocimum sanctum*, *Ocimum canum*, Comparative, Pharmacognostical characterization.

INTRODUCTION

The *Ocimum* (Basil) comprises some of the most popular herbs in the world. It belongs to the family Lamiaceae, sub family ocimoideae and includes more than 150 different species and varieties distributed in the tropical regions of Asia, Africa, Central and South Africa considered as one of the largest genera of the Lamiaceae family. The name Tulsi is derived from 'Sanskrit', which means "matchless one"¹⁻³

Among the plants known for medicinal value, the plants of genus *Ocimum* are very important for their therapeutic potentials. Because of its popularity basil is often referred to as King of herbs, being widely utilized due to its economic, nutritional, industrial and medicinal properties. *Ocimum sanctum* L. (Tulsi), *Ocimum gratissimum* (Ram Tulsi), *Ocimum canum* (DulalTulsi), are examples of known important species of genus *Ocimum* which grow in different parts of the world and are known to have medicinal properties.⁴⁻⁶

Pharmacognosy is basically divided into conventional and modern pharmacognosy. Conventional pharmacognostical study is based on macroscopic, microscopic and quantitative microscopy. Macroscopic characters include shape, size, colour and texture of the drug in crude or powdered form. Microscopic characters include the anatomical details of drug producing plant as seen in transverse and longitudinal sections, maceration study and the size measurement of various type of cells. Quantitative microscopy includes the vein islet number, palisade ratio, stomatal number and stomatal indices and so restricted to leaf drug only. The modern pharmacognosy utilizes characteristics of analytical, phytochemical and certain physical constant values over the traditional science of taxonomy in plant systematics. Most of the botanical, chemical, physical and microbial techniques employed in pharmacognosy are applicable to the analysis of drugs and therefore, used by public

analysts, forensic scientists and quality control chemists associated with industries. In this research work we have carried out the macroscopical, microscopical and powder characterization for the identification of *O. gratissimum*, *O. sanctum* and *O. canum*.⁷⁻¹⁰

Materials and Methods

Collection and Authentication of Plant Material

There are three varieties of *Ocimum* namely, *Ocimum gratissimum* (Rama Tulsi), *Ocimum sanctum* (Krishna Tulsi) and *Ocimum canum* (Vana Tulsi). The leaves of the above plants were collected from local area of Bhopal Madhya Pradesh India and authenticated at Department of Pharmacy, Barkatullah University, Bhopal, Madhya Pradesh.

Pharmacognostical study

All leave samples were subjected to morphological and microscopical examination to study the variations among them.

Morphological Evaluation^{3,6-8,10}

Morphological study of the plant was carried out as per the reported method for its Organoleptic characters such as colour, odour, taste, shape, size etc were observed and evaluated botanically. Organoleptic evaluations can be done by means of organs of special sense which includes the above parameters and thereby define some specific characteristics of the material which can be considered as a first step towards establishment of identity and degree of purity.

Colour: the untreated samples was properly examined under diffused sunlight or artificial light source with wavelengths similar to that of daylight.

Shape and size: the length, breadth and thickness of the drugs are of great importance while evaluating crude

drugs. A graduated ruler with basic unit in millimeter is adequate for the measurement. Bark and leaves were measured by aligning ten of them on a sheet of a calibrated paper approx. 1mm apart between the lines and the result was divided by 10. Average length, breadth and thickness were determined.

Odour and taste: the odour and taste of crude drugs were extremely sensitive criteria based on individual's perception. Therefore the description of this feature may sometimes cause some differences of opinion. The sample was crushed in a mortar by applying pressure by pestle and the strength of the odour like weak, distinct, strong was first noted and then the odour sensation like rancid, aromatic etc was determined.

Surface characteristics, texture and fracture: the texture was best examined by taking a small quantity of material and rubbing it between the thumb and forefingers, it was usually described as smooth, rough and gritty. The physical evaluation of the bark and leaves by palpitation (touch) of the material determines the softness or hardness. The study of morphology of bark and leaves was done by taking ten samples and was observed for various qualitative and quantitative macroscopical characters.

Microscopical evaluation¹⁻³

The following Microscopical parameters were observed such as Arrangement of tissues in a transverse section, type of epidermal cells, stone cells, testa and endosperm, Presence and type of crystalline structures eg. Calcium oxalate, starch etc, Presence of oil globules, aleurone grains and trichomes.

All determination was carried out by using Almicro compound microscope (10x, 40x) attached with a camera.

Powder microscopy^{3,6,10}: The dried Leaves of plant *Ocimum gratissimum*, *Ocimum sanctum* and *Ocimum canum*, were powdered and sieved to obtained fine powder and then these were taken up for powder microscopy evaluation as follows:-

1. A small quantity of powder was kept on a slide and after mounting on glycerin, 10 min were provided as spread out time. Finally, it was observed for powder microscopical characters.
2. Another small quantity of powder was stained with phloroglucinol and HCl, ruthenium red, safranin, sudan red III, iodine and acetic acid respectively. Mounted with glycerin on microscopical slide and observed for powder microscopic characters.

RESULT AND DISCUSSION

Pharmacognostical Studies

Morphological Evaluation

The dried leaves powder of selected species of *Ocimum* such as *O. gratissimum*, *O. sanctum* and *O. canum* were subjected to morphological study. The photograph of the sample leaves are shown in Fig.No.1, 2 & 3. Morphology of all the species was compared and tabulated in Table No.1

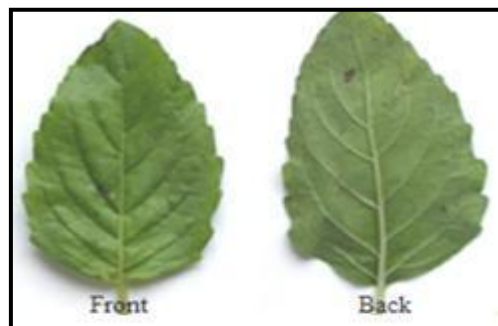


Fig.1 Leaf of *Ocimum gratissimum* L



Fig.2 Leaf of *Ocimum Sanctum* L

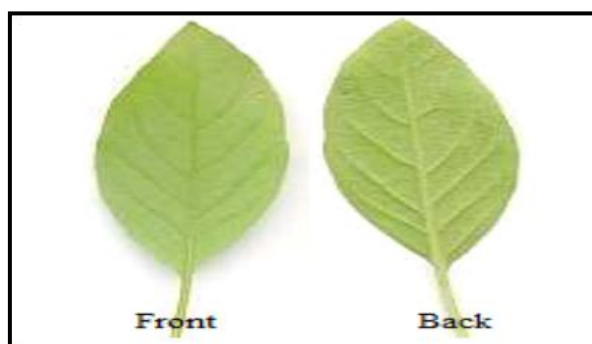


Fig.3 Leaf of *Ocimum canum* L

Table 1 Morphological features of the leaves of the three species used as *Ocimum*

Parameters	Species of <i>Ocimum</i>		
	<i>Ocimum gratissimum</i>	<i>Ocimum Sanctum</i>	<i>Ocimum canum</i>
Colour	Green	Green to purple	Green
Odour	Aromatic smell	Aromatic smell	Aromatic smell
Taste	Pungent taste	Warm pungent taste	Sharp pungent taste
Size and Shape	2.5-5 cm long and 1.6-3.2 cm broad, oval, pointed and sharp	2.5-5 cm long and 1.5-3.2 cm broad, elliptical, oblong	2.5-6 cm long, 1-2.5 scm broad Lanceolate to oblong-lanceolate, scattered

Microscopical evaluation

Microscopy of Leaf of *Ocimum gratissimum* L.

The leaf consists of thick and wide midrib and their Lamina. The Midrib is slightly raised on the adaxial side bearing dense non glandular trichomes. The abaxial part is wider and thick. The midrib 1 mm thick and 1.5 mm wide. The epidermal layer of the midrib is thin and the cells are small and thick walled. 2 or 3 layers of ground tissue inner to the epidermis are smaller in size and slightly thick walled remaining ground tissue includes larger, thin walled, angular, compact parenchyma cells (Fig 4 and 5). The vascular systems consists of a broadly bowl shaped vascular strand. It is collateral, comprising upper band of xylem and lower zone of phloem. The xylem arc consists of several, parallel, lines of xylem elements with wide gaps in between the lines. The xylem elements are angular, wide and thick walled. They are 35 μ m in diameter fig (7). In the adaxial part of the xylem arc there are 2 thin plates of phloem elements (Fig 6). Small spindle shaped calcium oxalate crystals are densely distributed in the parenchyma cells lying in the upper zone of the xylem band (Fig 8).

On the lower edge of the xylem strands occurs a thick isolated mass of phloem. The phloem elements are wide, angular and thick walled (Fig 9).

The lateral vein is also prominent with flat adaxial part where cluster of non-glandular trichomes are situated. The abaxial part of the lateral vein is conical having parenchymatous ground tissue. The vascular strand is single and wedge shaped. It consist of a few rows of thick walled angular xylem elements and prominent hemispherical cap of phloem elements (Fig 10).

1. Lamina

The lamina is dorsiventral. It consists of thick adaxial and abaxial epidermal layers and the cells are being thin walled with thin cuticle. The epidermal cells are rectangular in shape. The adaxial epidermal cells are 10 μ m thick, the abaxial epidermal cells are more stretched and are 20 μ m thick (Fig 11). The mesophyll tissue includes dense and compact cylindrical palisade cells which are 30 μ m in height. The spongy parenchyma is 5 or 6 layered which are small, lobed and loosely arranged (Fig 11).

2. Trichomes:

Glandular trichomes are more abundant on the lamina. They are located within wide and deep epidermal pits. It is sessile having a wide cup shaped glandular head. It consists of several radiating secretory cells forming a circle. The secretory head is 20 μ m in height and 60 μ m in diameter (Fig 12).

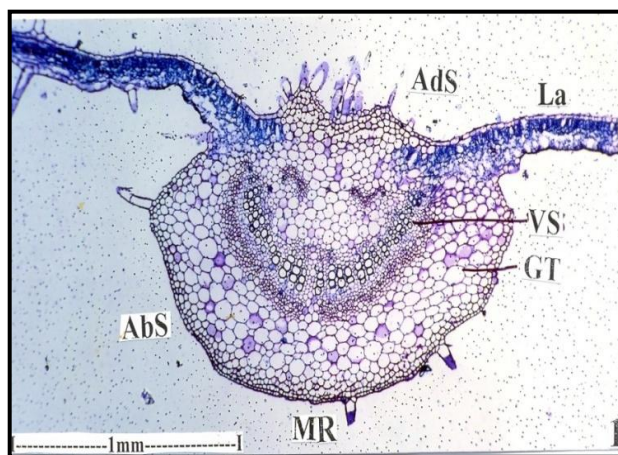


Fig. 4 T S of Leaf through Midrib with Lamina

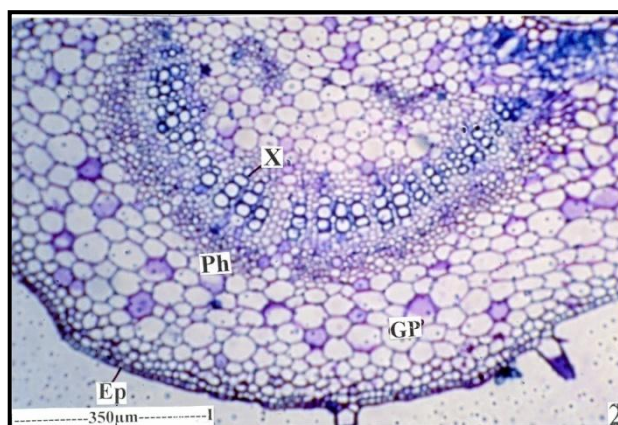


Fig. 5 Midrib Vascular Bundle Enlarged

(AbS – Abaxial side, AdS – Adaxial side, Ep – Epidermis, GP – Ground parenchyma, GT – Ground tissue, La – Lamina, MR – Midrib, Ph – Phloem, VS – Vascular strand, X – X - Xylem)

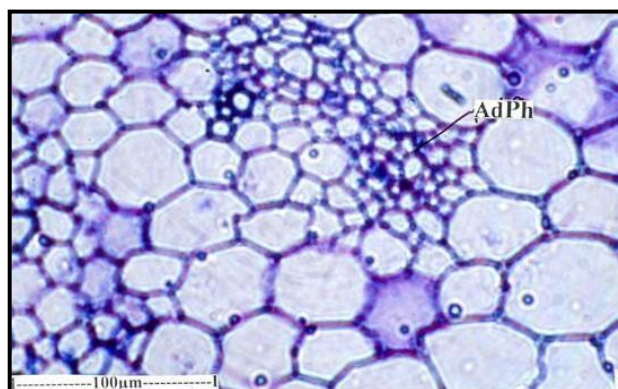


Fig 6 Adaxial (accessory) Vascular bundle of the midrib enlarged

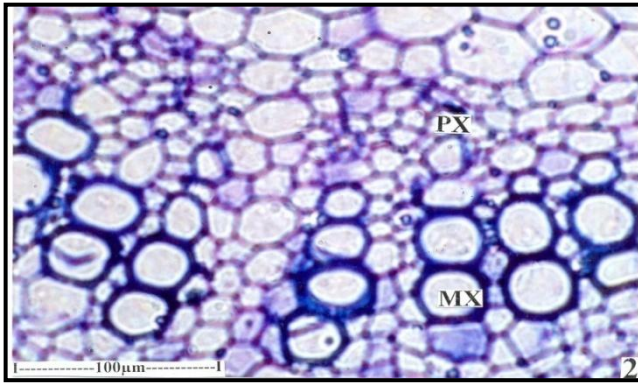


Fig 7 Median Bundle of the midrib – Magnified

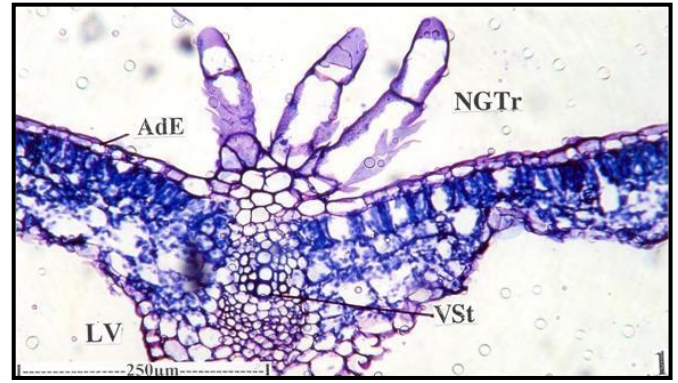


Fig 10 T S of leaf through lateral vein showing non glandular Trichomes on the adaxial epidermis

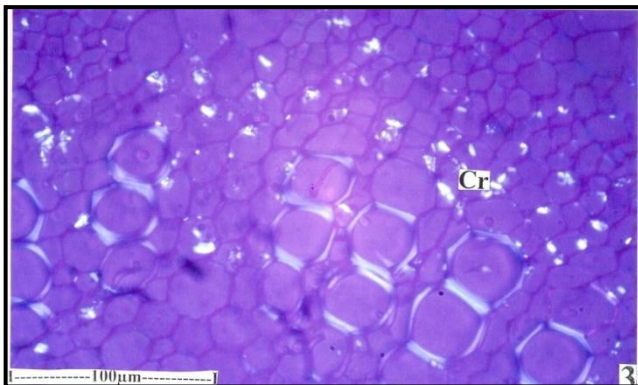


Fig 8 Crystal distribution in the phloem cells (as seen with polarized light Microscope)

(AdPh – Adaxial phloem, Cr – Crystals, MX – Meta xylem, PX – Proto xylem)

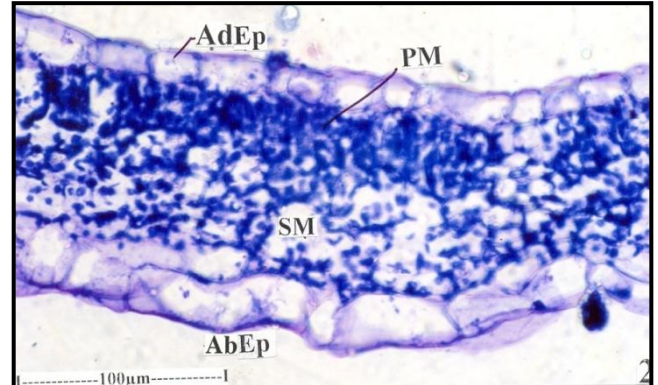


Fig 11 T S of Lamina

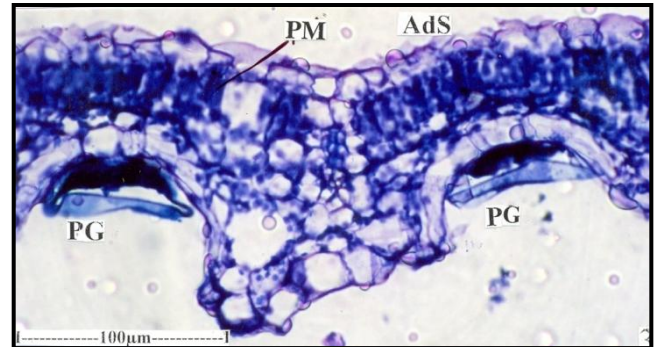


Fig 12 T S of Lamina showing two peltate glandular trichomes on the abaxial epidermis cells

(AbEp – Abaxial epidermis, AdEp – Adaxial epidermis, AdS – Adaxial side, LV – Lateral vein, NGTr – Non-glandular trichomes, PG – Peltate gland, PM – Palisade mesophyll, SM – Spongy mesophyll, VSt – Vascular strand)

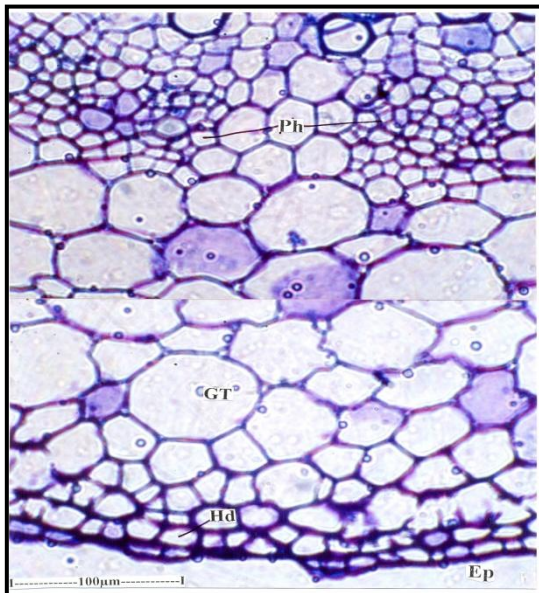


Fig 9 T S of Midrib showing Abaxial Phloem cells and ground tissue parenchyma cells

(Hd – Hypodermal cells, GT – Ground tissue, Ph - Phloem)

Powder Microscopy of Leaf of *Ocimum gratissimum* L.

The powder microscopy of leaf was done and data mentioned below

1. Epidermal trichomes:

Unicellular, unbranched, covering – type or non – glandular type having thick lignified walls with echinate cuticle, 450 – 600 mm long and 30 mm thick.

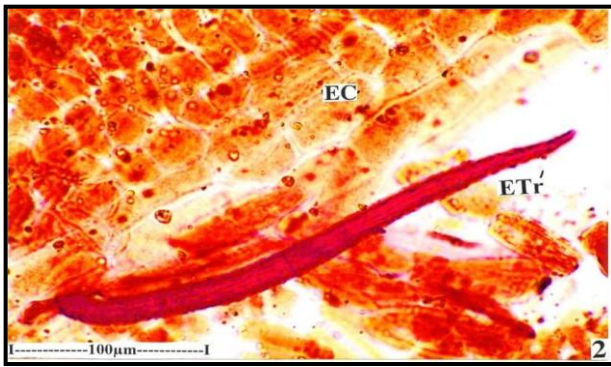


Fig 13: Fragment of epidermal cells with trichomes enlarged
(EC – Epidermal cells ETr – Epidermal trichomes)

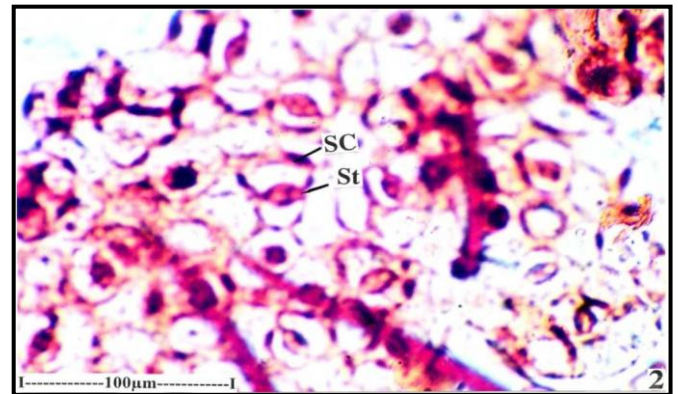


Fig 16: Fragments of abaxial epidermis with stomata
SC – Subsidiary cells, St – Stoma

2. Calcium oxalate crystal:

Sphaero crystals, diffuse in distribution and occur in the mesophyll tissue and may be the associated with veins

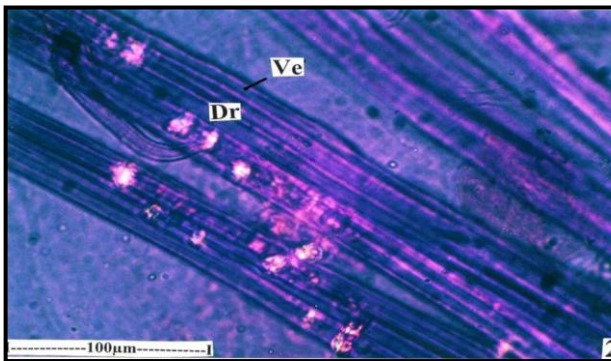


Fig 14: Druses in the leaf powder
(Dr – Druses Ve Vein)

3. Broken xylem elements

Broken xylem elements of the veins are seen scattered in the powder. The xylem elements have spiral, annual and scalariform lateral wall thickenings.



Fig 15: Trichomes & xylem elements in the leaf powder
(Tr – Trichomes XE – Xylem elements)

4. Epidermal peelings:

Fragments of epidermal layer with stomata are seen into powder. The stomata are paracytic type. The epidermal cells have straight, thin walls.

Microscopy of Leaf of *Ocimum Sanctum*

The leaf consists of a thick plano convex midrib. The adaxial part of the midrib is slightly raise band bears non glandular Trichomes. The basal part is wide and thick and semicircular in outline (Fig 26.1) The midrib is 550 um thick and 700 um wide. The adaxial part consist of epidermis and a thick mass of collenchymas cells. The basal part has thin epidermal layer of small, squarish thick walled cells. The ground tissue is homogenous and parenchymatous with thin walled compact cells. The vascular strand is single bowl shaped and prominent. It is 400 um wide, 150um thick. It consists of several long narrow parallel lines of Xylem elements which are narrow, angular and thick walled. Along the lower end of the strand occur more or less continuous layer of phloem elements (Fig 26.2).The xylem elements are 20 um wide along the adaxial part of the vascular strand occurs small nest of Phloem elements.

Epidermal Trichomes are densely distributed both on the adaxial and abaxial surfaces of the lamina. There are two types of Trichomes

(i) **Glandular Trichomes:** these are secretory structures bearing aromatic compounds. Two types of glandular Trichomes are seen

(a) **Peltate type of trichome:** These are bowl shaped Trichomes with single short and wide stalk cell and cup shaped body. These peltate Trichomes are usually situated in shallow cavities of the Lamina (fig 27.1 and 27.2),the body of the trichome is multicellular with radiating cells of darkly strained cell contents. The glands ore 25 um in height and 60 um in diameters.

(b) **Capitate type of trichome:** These are multicellular Trichomes with thick, wide stalk cell and multicellular spherical body. The body consists of mostly 4 cells and possesses dense aroma compounds (fig 28.1 and 28.2).These glands are 40 um in height and body is 35 um thick.

(ii) **Non-Glandular Trichomes:** These Trichomes are multicellular, uniserrate and unbranched.They have broad basal part and gradually tapering pointed terminal part. The trichome is 150 um long and 20 um thick at the base.

Lamina: The lamina is bifacial with distinction into adaxial side and abaxial side. An adaxial epidermis

consists of fairly wide, spindle shaped cells with prominent cuticle.

The cells are 20 μm thick. The abaxial epidermis is comparatively thin, rectangular in shape and the cuticle is prominent. The mesophyll tissue consists of an adaxial band of single row of cylindrical, compact palisade cells which are 70 μm in height. The lower part of the lamina includes 4 or 5 layer of lobed loosely arranged spongy mesophyll tissue.

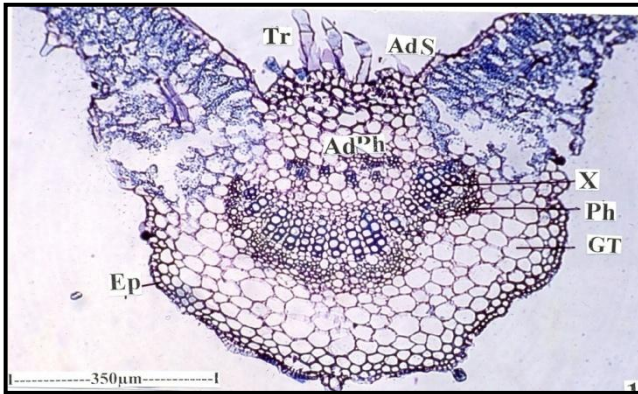


Fig 17 T S of leaf through midrib with Lamina

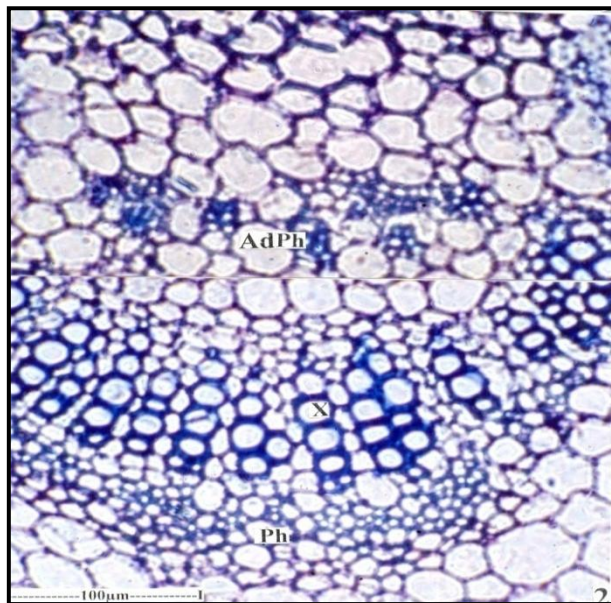


Fig 18 Midrib Vascular Bundle Enlarged

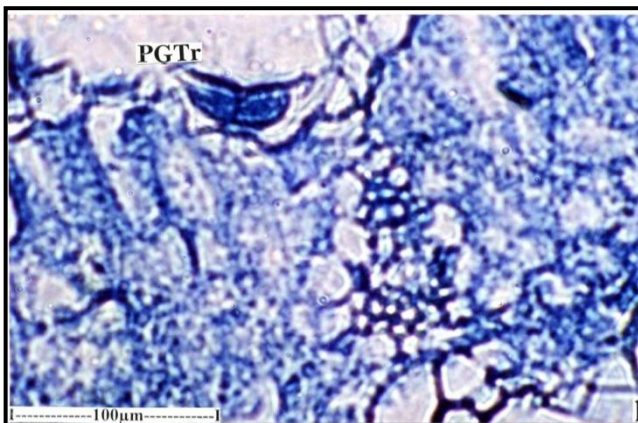


Fig 19 T S Lamina through Lateral Vein Adaxial Glandular Trichome

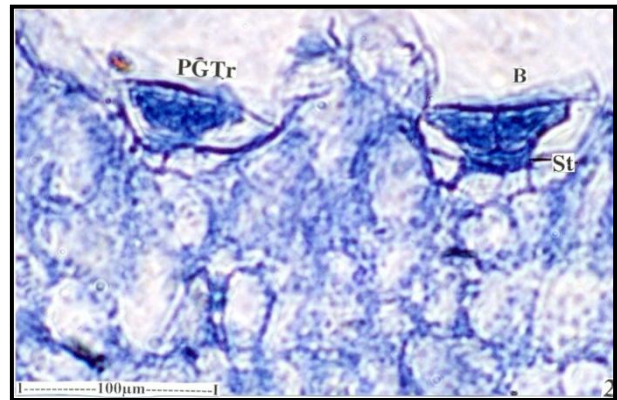


Fig 20 Two Peltate Glandular Trichome enlarged

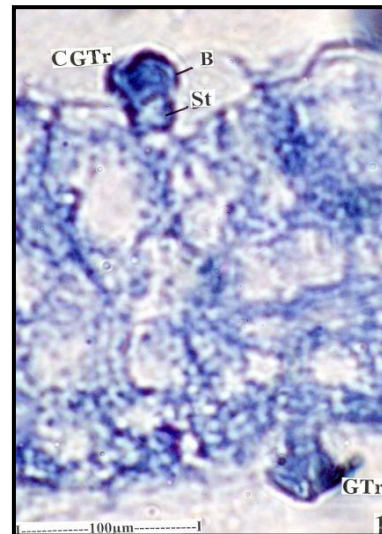


Fig 21 Lamina showing capitate Glandular Trichomes on the adaxial side

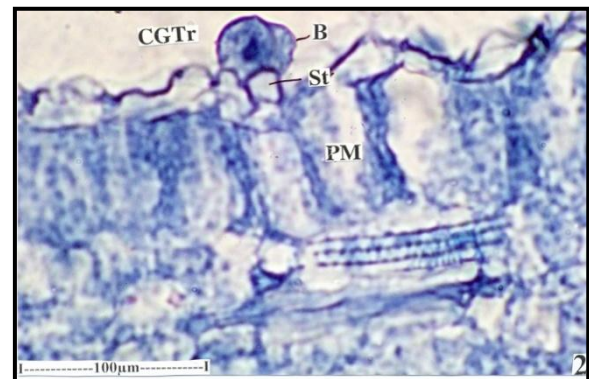


Fig 22 Lamina showing Capitate Glandular Trichome on abaxial side

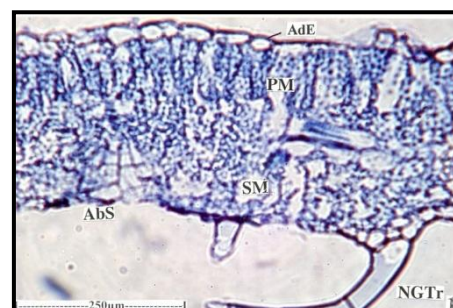


Fig 23 T S Lamina

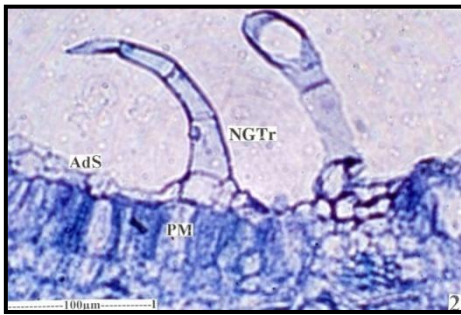


Fig 24 T S of Lamina Showing Non Glandular Trichome

Powder Microscopy of Leaf of *Ocimum Sanctum*



Fig 25 Powder microscopy showing stomata



Fig 26 Glandular Trichomes



Fig 27 Covering Trichomes

Microscopy of Leaf of *Ocimum canum*

The leaf has prominent and thick abaxial midrib and lateral veins. The lamina is thin with wide shallow glandular pits the midrib is 350 µm thick and 400µm wide. It consists of a short, semi-circular adaxial part and wide, thick abaxial. The epidermis is thin and continuous comprising small, thick walled squarish cells. The

ground tissue abaxial part is collenchymatous and adaxial part is parenchymatous. The cells are angular, thin walled and compact.

The vascular system includes a single, horizontally, oblong, collateral vascular bundle.

The bundle is 70µm thick and 180µm wide. The vascular bundle consists of about 10 short, parallel line of xylem elements which are angular and thick walled. The metaxylem elements are 20µm in diameter. Phloem elements occur in small clusters of three to five elements along the lower end of xylem segment

1. **Lateral vein:** the lateral vein is slightly concave on the adaxial side and prominently semi-circular on the abaxial side. It is about 200µm in thickness. The abaxial part consists of thick walled epidermal layer and two layers of wide, compact, parenchyma cells. The vascular strand of the lateral vein comprises two short lines of xylem elements and small group of phloem elements.

2. **Leaf margin:** the marginal part of the lamina is semi-circular and the epidermal cells are dilated and angular in out line. The cuticle is very thick. The mesophyll tissue consists of palisade and spongy parenchyma similar to middle part of the lamina.

3. **Lamina:** lamina is thin and uneven in surface due to the presence of shallow pit. The abaxial epidermis is broad with thick walled, rectangular cells. The cells are 20µm in thickness. The abaxial epidermis is thin with narrow, rectangular. The mesophyll tissue consists of a single upper layer of narrow, loosely arranged vertical cylinders of palisade cells and two or three layers lobed spongy parenchyma cells. Both on the lower and upper epidermal layer there are wide, shallow pits in which sessile, peltate glandular trichomes are situated.

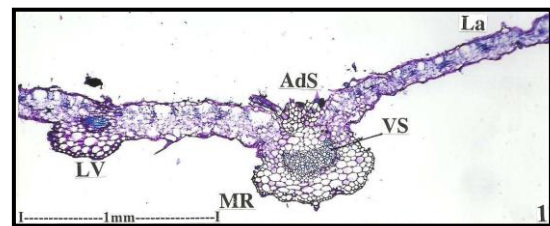


Fig.28 T.S of leaf through midrib with lamina

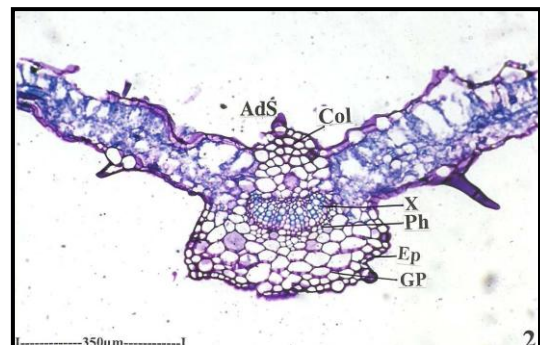


Fig.29 T.S of midrib with lamina magnified
(AdS- Adaxial side; Col-Collenchyma; Ep-Epidermis; GP- Ground parenchyma; La- lamina; Lv- Lateral vein; MRmidrib; Ph-phloem; Vs-vascular strand; x- xylem).

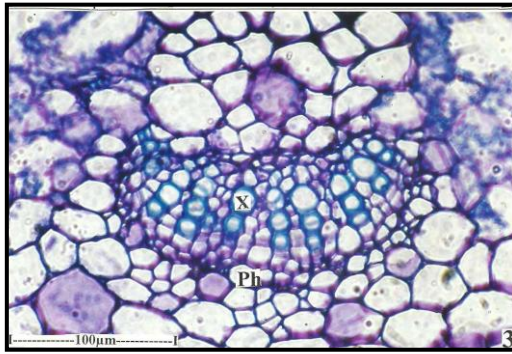


Fig. 30 T.S of midrib vascular bundle magnified
(Ads- Adaxial side; Col-Collenchyma; Ep-Epidermis; GP-Ground parenchyma; La- lamina; Lv- Lateral vein; MRmidrib; Ph-phloem; Vs-vascular strand ; x- xylem).

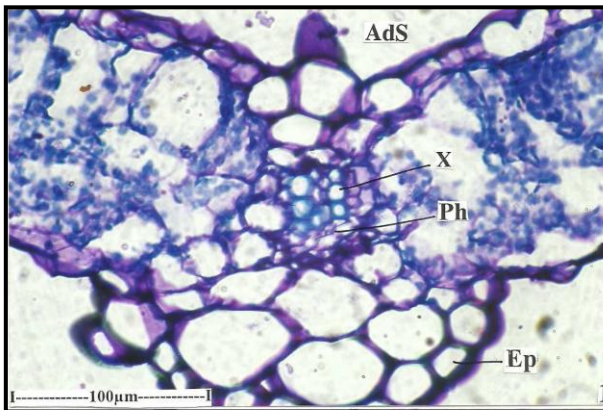


Fig. 31: T S of lamina through lateral vein

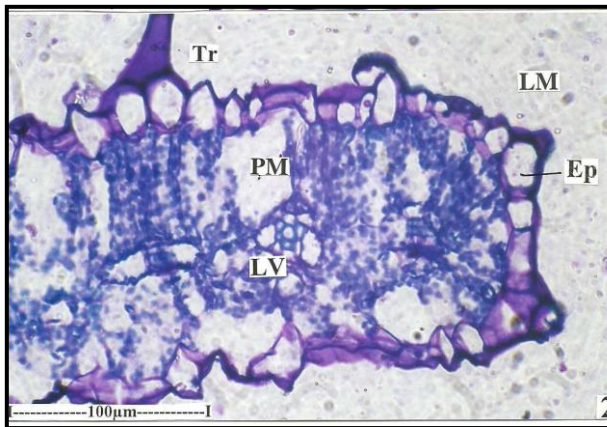


Fig. 32 T.S. of leaf margin

(Ads- adaxial side; EP-Epidermis; LM-Leaf margin; Lv- Lateral vein; Ph-Phloem; PM-Palisade mesophyll; Tr-trichome; X- Xylem).

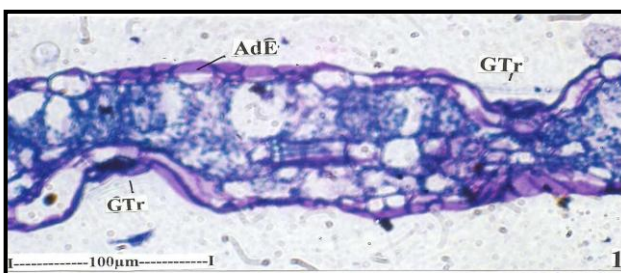


Fig. 33 T.S. of lamina showing glandular trichomes on the abaxial and adaxial side

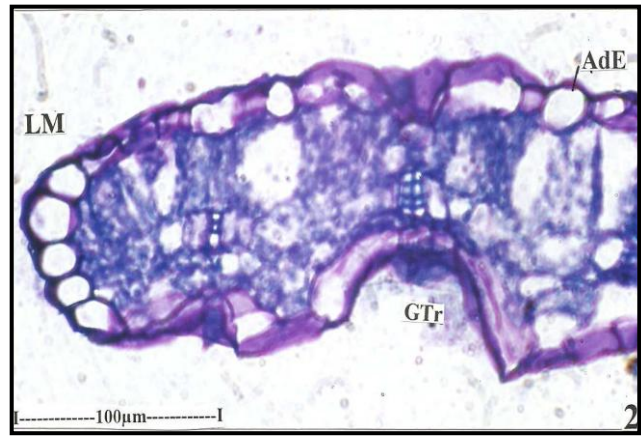


Fig. 34 T.S. of lamina with glandular trichome on the abaxial epidermis

(AbE-Abaxial epidermis; AdE-Adaxial epidermis; GTr-Glandular trichome; LM- Leaf margin; LV-Lateral vein; Tr-Trichome; PM-Palisade mesophyll; SM-Spongy mesophyll)

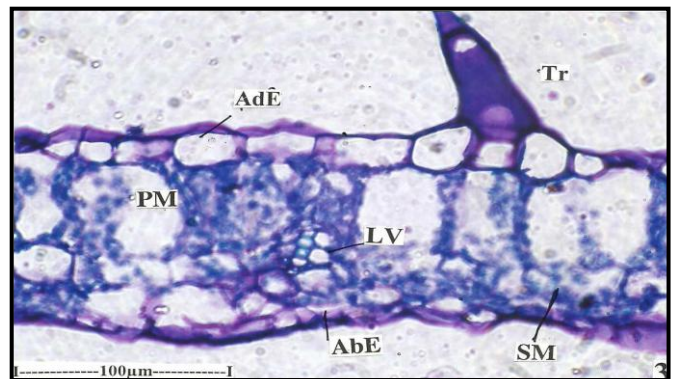


Fig. 35 T.S. of lamina with showing non-glandular trichome on the adaxial epidermis

(AbE-Abaxial epidermis; AdE-Adaxial epidermis; GTr-Glandular trichome; LM- Leaf margin; LV-Lateral vein; Tr-Trichome; PM-Palisade mesophyll; SM-Spongy mesophyll)

Powder microscopy of the leaf: The leaf powder when examined under the microscope exhibit the following inclusions.

Epidermal peeling: thin pieces of epidermal peelings are visible in the powder. In surface view the epidermal cells have thin, highly, wavy anticlinal walls; the epidermal cells appear amoeboidal in outline. Stomata are also seen in the powder. Stomata are “diacytic” type.

Covering or non-glandular trichomes:

Multicellular, uniseriate, unbranched trichomes are frequently seen in the powder. They are three to five cells. The basal cell is wider, and the upper cells are gradually narrow and tapering in to pointed tip. The basal cell is 40µm thick and the terminal cells are up to 10µm thick. The surface of the cell wall have minute cuticular echinate out growths.

Glandular trichome:

Glandular trichomes are also frequently seen in the powder. They are peltate type and sessile. The glands are embedded in the epidermal pits. The gland has short,

horizontally oblong basal cell and semi-circular body comprising four to eight secretory cells. The glandular trichome is 80µm wide.

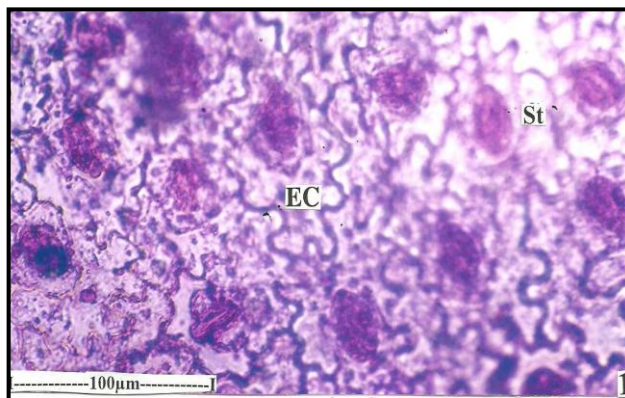


Fig. 36 Fragment of abaxial epidermis with stomata

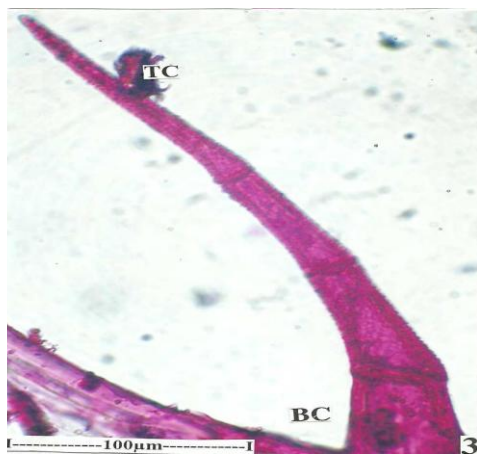


Fig. 38 Non-glandular trichome in the leaf powder magnified

(BC- Basal cell of trichome; EG-Epidermal cell; St-Stomata; TC-Tip cell of trichome; Tr-Trichome)

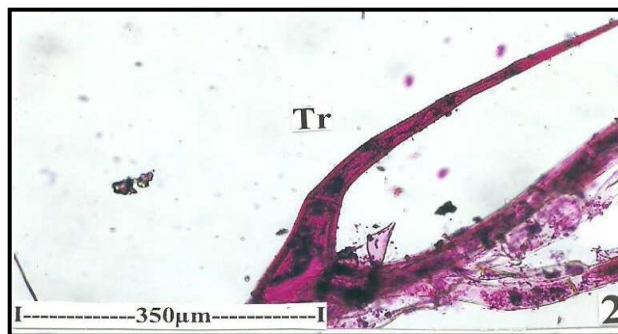


Fig. 37 Non-glandular trichome in the leaf powder (BC- Basal cell of trichome; EG-Epidermal cell; St-Stomata; TC-Tip cell of trichome; Tr-Trichome)



Fig. 39 A detected glandular trichome in surface view

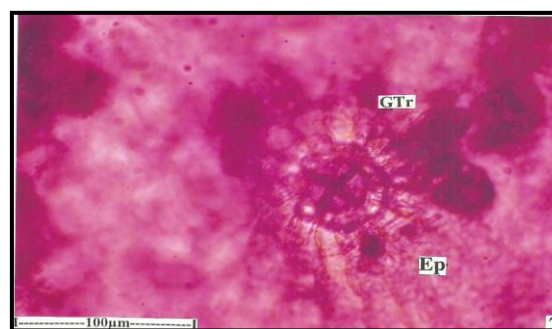


Fig. 40 A gland attached on the epidermal pit (BC-Basal cell; Ep- Epidermis; GTr-Glandular trichome; Sc-Secretory cell)

REFERENCES

- Nadkarni AK, Nadkarni KM, Indian Materia Medica, Popular Prakashan Pvt. Ltd., Bombay, 1976.
- Kirtikar KR, Basu BD. In: Ocimum sanctum in Indian Medicinal Plants, LB Basu, Allahabad, 1965.
- Trease, G. and Evans W. C. Textbook of Pharmacognosy. 12th ed., Balliere, Tindall, London, 1983; 343 – 383.
- Asolkar LV, Kakkar KK, Chakre OJ. Glossary of Indian medicinal plants with active principles (part 1), CSIR New Delhi, 2005; 327-328.
- Atal CK, Kapur BM. Cultivation and utilization of Aromatic plants, Reprint edition, 1989; 1- 2, 90-92, 95, 641.
- Gupta SK, Prakash J, Srivastav S. Validation of claim of Tulsi, Ocimum sanctum Linn as a medicinal plant, J Expt. Biol., 2002;40; 765-773.
- Ghosh GR. Tulasi (N.O. Labiatae, Genus-Ocimum) .New Approaches to Medicine and Health (NAMA); 3: 1995; 23–29.
- Simpson BB and Conner OM. Economic Botany plants in our world. McGraw Hill Book company Hamburg. 1986; 640.
- Simon JE Quinn J and Murray RG. Basil A source of essential oils, in new crops, Timber press Portland. 1990; 484-489.
- Khosla MK. Study of interrelationship, phylogeny and evaluatory tendencies in genus Ocimum. Ind J Gene plnt breed. 1995;55; 71-83.
- Atal CK and Kapoor BM. Cultivation and utilization of medicinal plants (Eds. PID CSIR); 1989.
- Sen P. Therapeutic potentials of Tulsi: from experience to facts. Drugs News & Views. 1993;1(2); 15–21.
- Nagarajun S, Jain HC and Aulakh GS. Indigenous plants used in the control of Diabetes. In: Cultivation and utilization of medicinal plants. Editors: Atal CK and Kapoor BM (Published by PID CSJR), 1989; 584.
- Miller R and Miller S. Tulsi green and Herbs. India's Holy Basil. <http://www.omorganic.com/Tulsi> 23J03;1-6.