



Comparative Morphological, Microscopical Study of Ocimum Species: Research Article

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ABSTRACT

Now a days, increasing use of herbal products needs to study the medicinal plants by more appropriate study in relation to maintain its quality standards. In this paper, the literature and the published work on comparative Pharmacognostical study of Ocimum species. Aim of present study is to establish comparative standards for different species of Ocimum, to collect and comparatively evaluate morphological microscopical characters of different species of Ocimum, to comparatively evaluate micro chemical parameters of different species, to develop comparative standards to differentiate species of Ocimum. The T.S of leaf shows the presence of epidermis, parenchymatous cells, collateral vascular bundle, lateral vein, glandular trichomes which are peltate type and sessile. Powder microscopy of the leaf shows the presence of epidermal peelings and multicellular unbranched trichomes. In present study, the authenticated plants were studied for Pharmacognostical Evaluations including study of Macroscopic Evaluations, Microscopic Evaluations. The macroscopic of leaves and steam of each species was studied by observing the organoleptic characters such as colour, size, texture and surface characteristics.

Keywords: *Ocimum americanum*, *Ocimum basilicum*, *Ocimum kilimandscharicum*, *Ocimum gratissimum*, *Ocimum sanctum*.

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INTRODUCTION

Standardization as defined by American Herbal Product association: “Standardization refers to the body of information and control necessary to product material of reasonable consistency. Standardization of drugs means confirmation of its identity and determination of its quality and purity. At present due to advancement in the Morphological knowledge of crude drugs various methods like botanical, chemical and micro chemical to develop literature for comparative study of *Ocimum* species. Plants have been known to relieve various diseases in Ayurveda. Standardization of herbal medicine is process of prescribing a set of standards or inherent characteristic, constant, definitive, qualitative & quantities value that carry an assurance of quality, efficacy, safety & predictability. It is process of developing and agreeing upon chemical standards. The specific standards are workout by experimentation & observation which would lead to process of observing prescribing set of chromatistics exhibiting by particular herbal medicine.(1)

When the active principles chiefly responsible for the desired therapeutic effect are known, the levels of such constituents may be adjusted by blending different lots of raw material, whether whole herb or extract. The highest aim of standardization is obviously to provide a product of consistent high quality which reliably delivers a health benefit. In the vast majority of cases, however, active principles are either totally unknown or are not well defined. As a consequence, the herbal preparation in its entirety is regarded as the medicine. In most cases, therefore, one has to rely on chemical markers, preferably highly characteristic of the plant and, if even not significantly contributory to its therapeutic effect, at least correlated with it.

***Ocimum* species**

Throughout the world, there are so many species of *Ocimum* are available, in the most of the species are used as medicinal plant for the various treatments. So in present thesis is dedicated to comparative study of five local species of *Ocimum* available in Amravati and Akola i:e in Vidarbha region in Maharashtra in India.

Medicinal properties of *Ocimum* are known for thousand years to various civilizations of the world. This medicinal herb is considered as a sacred plant by the Hindus in the India subcontinent. Scientific explorations of traditional belief of medicinal properties of *Ocimum* have got momentum mostly after the middle of the 20th century. (2) Tulsi is a sacred medicinal plant considered as a Holy Hindu Laxmi Goddess. It is member of mint or Labiatae family from India. Medicine is obtained from leaves, seed & stem.

Uses:

cold, influenza, H1N1 (swine flu) hepatitis, bronchitis, stress .cancer, headache, heart disease, malaria, digestive disorder. This is powerful anti-oxidant demonstrated as anti-fungal antibacterial. Act as anti-inflammatory, immunomodulatory, increase metabolism and lowering down stress hormones. This is also a repellent for mosquitos. This has strong healing power in catarra matter and phelgm. This also work as supplements to combat Stress by lowering corticosteroid level by active compounds at therapeutic dosages & standardized for Eugenol, caryophyllene and triterpenoic acid such as ursolic and oleanolic acid .Tulsi has antifertility &anti sperm role by reducing estrogen in female It has strong immunomodulator and Adoptogen to counter life style diseases in cancer hypertension diabetes and stress.(3)

The complex taxonomy of the genus, Determined by interspecific hybridizations and polyploidy, includes 150 species. Whole plant is used as a source of remedy. In India two forms of Tulsi are more common - dark or *Shyama* (Krishna) Tulsi and light or Rama Tulsi. The former possesses greater medicinal value and is commonly used for worship. Various other species are also commonly found in India like *O. canum*, *O. basilicum*, *O. kilimandscharicum*, *O. ammericanum*, *O. camphora* and *O. micranthum* in the past few decades, many of these benefits have been investigated and verified by modern scientific research. *Osmium*'s main Ayurvedic guna (quality) as a healing herb is its ability to enhance the energetic resonance between the body and the environment. "The Elixir of Life", *Ocimum* has been traditionally employed in hundreds of different formulations for the treatment of a wide disorders including those of the mouth and throat, lungs, heart, blood, liver, kidney, and the digestive, metabolic, reproductive and nervous systems. (2)

EXPERIMENTAL WORK***Ocimum ammericanum:***

Plant material was purchased from local Gayatrii nursery, Badnera road Amravati. Identification and authentication was made from Dr. Satish N. Malode, H.O.D Botany department, Government Vidarbha Institute of science & Humanities, Amravati (M.S.)

Ocimum basilicum:

Plant material was purchased from local Gayatrii nursery, Badnera road Amravati. Identification and authentication was made from Dr. Satish N. Malode, H.O.D Botany department, Government Vidarbha Institute of science & Humanities, Amravati (M.S.)

Ocimum kilimandscharicum:

Plant material was purchased from Nagarjun Garden Dr. P.D.K.V. Akola. Identification and authentication was made from Dr. Satish N. Malode, H.O.D Botany department, Government Vidarbha Institute of science & Humanities, Amravati (M.S.)

Ocimum gratissimum:

Plant material was purchased from Nagarjun Garden Dr. P.D.K.V. Akola. Identification and authentication was made from Dr. Satish N. Malode, H.O.D Botany department, Government Vidarbha Institute of science & Humanities, Amravati (M.S.)

Ocimum sanctum:

Plant material was collected from my home garden. Identification and authentication was made from Dr. Satish N. Malode, H.O.D Botany department, Government Vidarbha Institute of science & Humanities, Amravati (M.S.)

Herbarium preparation; -

A herbarium is a historical record of documenting of plant information. A herbarium is an institution housing a collection of preserved plants that have been gathered and classified over long period of time. Importance 1) conservation of biodiversity. 2) Provide material for Comparative botanical study.3) It helps to identify variation within the species. 4) Any herbarium is bank of rare and endangered species. 5) Herbarium provide sample for the identification of plant that may be significant in criminal investigation. Steps in herbarium preparation:

- Collection
- Identification of plant specimen
- Pressing and drying plant specimen
- Mounting herbarium specimen s
- Herbarium specimen labels(4)

Pharmacognostical Evaluations

Macroscopic Evaluations:

Macroscopy of whole plant was studied by observing the organoleptic characters such as color, odor, size, shape, taste and special features including touch and texture etc. 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 .(5)

Microscopic Evaluations:

Microscopic evaluation is indispensable in the initial identification of herbs as well as identifying small fragment of crud or powdered herbs, and in detection of adulterants. This study helps in identifying characteristics tissue features. Every plant possess a characteristics tissue structure which can be demonstrated through study of tissue arrangement, cell walls and configurations, when properly maintained in stains, reagents and media. Thus it determines the size, shape, and relative structure of different cell (5) Microscopical parameters observed were,

- Arrangement of tissues in a transverse section.
- Type of epidermal cells, stone cells, testa and endosperm.
- Presence and type of crystalline structures e.g. Calcium oxalate, starch etc.
- Presence of oil globules, aleurone grains and trichomes.

All determination was carried out by using Light microscope (Olympus, model no. U-APT, Cx31RTSF). (10x, 40 xs)

Procedure for taking transverse sections:

Here, fresh leaves and stems was used for sectioning part of plant. Stems were sectioned vertically holding in finger with the help of sharp razor blade. In case of leaves, potato cubes was used for taking proper sections of the leaves. Using sharp blade transverse section were taken and transferred to watch glass containing water with the help of brush. Thin uniform section was treated with different reagents. (5).

Powder microscopy of whole dried plant:

The powder of respective plant was used for powder microscopy study (5). Place one or two drop of water, glycerol/ethanol on a glass slide. The tip of needle was moisten with water and dip into the powder. The small quantity of material that adheres to the needle tip was transferred into the drop of fluid on the slide. Stirred thoroughly, and cover glass was applied. Cover glass was press with the help of Handel of the needle. And excess of fluid was removed from the margin of the cover glass with strip of filter paper. Treat a powder sample with following reagents:

Phloroglucinol and hydrochloric acid –

It establish lignification's in cells and tissues. To the material on slide one or two drops of solution if Phloroglucinol was added mixed, the solvent was allowed to evaporate almost completely. One or two drops of hydrochloric acid were added and the material was covered with cover glass. T. S. was examined immediately: science the colour gradually fades. Heavy lignification's give deep red colour and slight lignification's a pink pale colour. (5) **Iodine solution:** It was used to conform starch grains content. (5) **Sudan red solution:** It was used to

determine the oil globules if present in the section. The structure seen under microscope were studied and captured with the help of camera. (5)

RESULTS AND DISCUSSION

Total five species of *Ocimum* i.e. *Ocimum sanctum* Linn, *Ocimum americanum* Linn, *Ocimum basilicum* Linn, *Ocimum gratissimum* Linn, *Ocimum kilimandscharicumguerke* were collected from different locations and identified as well as authenticated from Dr. Satish N. Malode, H.O.D Botany department, Government Vidarbha Institute of Science and Humanities, Amravati (M.S.) Authenticated species further evaluated comparatively for their morphological, microscopical, physiochemical, and phytochemical parameters and also evaluated for their antioxidant and antimicrobial activity by in vitro methods.

Macroscopy of leaves and stem as well as powder of whole plant studied in detail. Microscopical examination of leaf *O. americanum* shows isobilateral lamina covered with cuticle; glandular trichomes with multicellular head and multi cellular warty covering trichomes. Mid rib with arc shaped vascular bundle consisting of xylem and phloem. three to four layers of collenchymatous tissue present on upper side of vascular bundle whereas stem part shows cork, vascular bundle containing xylem & phloem, spongy tissue, collenchymatous cells, pith. Powder microscopy shows reticulate xylem vessel, stone cell, epidermal cell, collenchymas, cork cell.

Ocimum basilicum leaf shows the presence of multicellular curved trichomes, Upper epidermis, collenchymatous cells, upper palisied cell, vascular bundle containing xylem & phloem, spongy tissue, lower epidermis, stem section shows unicellular covering trichomes, epidermal cell, collenchymatous cells, vascular bundle containing xylem & phloem, spongy tissue, pith, spongy parenchyma. Powder microscopy shows presence of medullary rays lamellar collenchymas, Cork cell, Epidermal cell, Fibers, Stone cell. One species contain trichomes on the stem such as *Ocimum gratissimum*

Ocimum gratissimum showed Single layered epidermis, multicellular covering trichomes and glandular trichomes, collenchymatous cells, Vascular bundle consisting of xylem and phloem, Palisade cells and spongy tissue A transverse section of stem showed Shape of section was rectangular Compressed bark cells followed by single layered epidermis Multicellular covering trichomes and glandular trichomes, Collenchymatous cells, Vascular bundle contains xylem and phloem, Spongy tissue was present at center. Powder microscopy glandular trichomes with multicellular head, Thin walled fiber with pointed end, multicellular covering trichome,















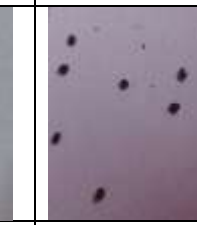
Diacytic stomata, Wavy walled epidermal cells, Collenchymatous cell with intercellular spaces, Vessels. This species can be identified as highly multicellular trichome containing species.

Ocimum. kilimandscharicum leaf part shows multicellular covering trichomes, multicellular curved trichomes, upper epidermis upper palisade cell, vascular bundle containing xylem & phloem, Collenchymatous cells, and stem part shows cork, Collenchymatous cells, vascular bundle containing xylem & phloem, multicellular curved trichomes, pith and powder microscopy shows Storage parenchyma, Reticulate xylem vessel, multi cellular curved trichomes, Stone cell.

Ocimum sanctum leaf microscopy shows Unicellular covering trichomes, Glandular trichomes Upper epidermis, Upper palisade cell, Vascular bundle containing xylem & phloem, collenchymatous cells, Parenchyma cell, Epidermal cell. Stem shows cork, collenchymatous cells, pith, and medullar rays. Powder microscopy shows, glandular trichomes, fiber *multi-cellular* curved trichomes, sclerenchymatous fiber, and stomata cell cork cell bicuspid epidermal cells.

Table 1: Comparative table of morphology of *Ocimum* species

Parameter	<i>Ocimum americanum</i>	<i>Ocimum basilicum</i>	<i>Ocimum gratissimum</i>	<i>Ocimum kilimandscharicum</i>	<i>Ocimum. sanctum</i>
Colour	Green to yellow green	Green or some time purple	Light green	Pale green	Green to purple
Odour	Aromatic	Faint	Aromatic	Aromatic	Aromatic
Taste	Characteristic, mint like flavour	Characteristic	Oily and sharp, tingling taste like cloves, pungent.	Aromatic camphor like	Warm & pungent, aromatic and sharp.
Height	30-60 cm	60-80 cm	1 to 1.5 m	15 to 30 cm	20-60 cm
Herb	Branched herb, branches are sub-quadrangular striate; light puff colored stem	Erect, strongly aromatic, nearly glabrous branching herb, covered with soft spreading hairs	Stem and branches are green	Perennial aromatic evergreen under shrub Stems are brownish green,	Much branched, stems and branches usually purplish, sub-quadrangular, woody, Covered with soft spreading hairs

Plant					
Leaves					
Venation	Pinnate	Pinnate	Pinnate	Pinnate	Pinnate
Margin	Serrate	Lobed	Entire	Serrate	Entire
Fruit					
Shape	Small; nutlets are pitted,	Ellipsoid nutlets, And pitted.	Elongated, round at one end and flattened at the other	Ovoid, smooth or minutely tuberculate,	Caeruleus
Colour	Black	Black	Black	Black	Brownish Black

Microscopical evaluations

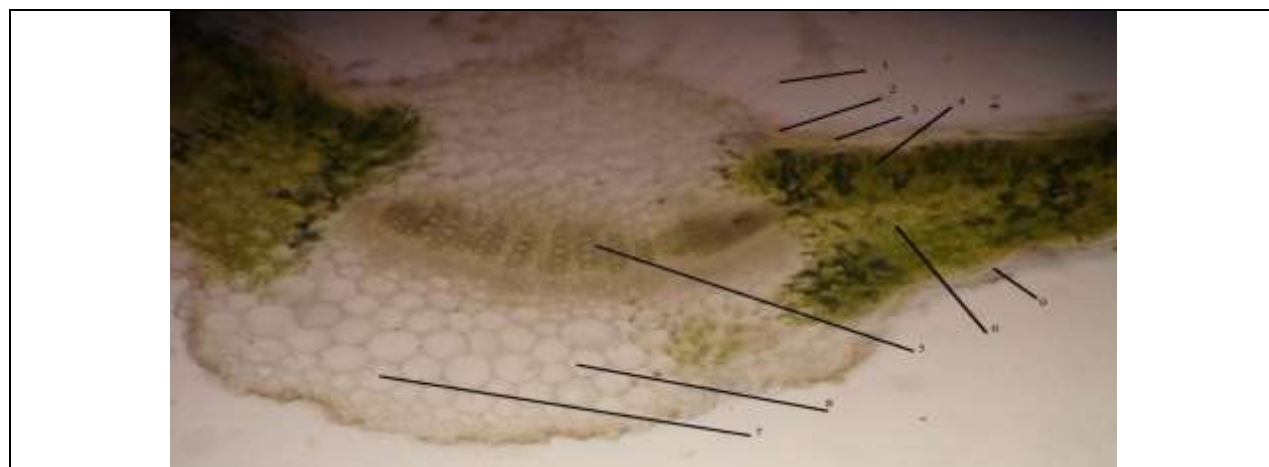


Figure 1: Microscopy leaf of *O. americanum* where 1-Multicellular covering trichomes, 2- Glandular trichomes, 3- Upper epidermis, 4- Upper palisade cell, 5-Vascular bundle containing xylem & phloem, 6- Collenchymatous cells, 7- Oil globules, 8-Spongy tissue, 9-

Lower epidermis

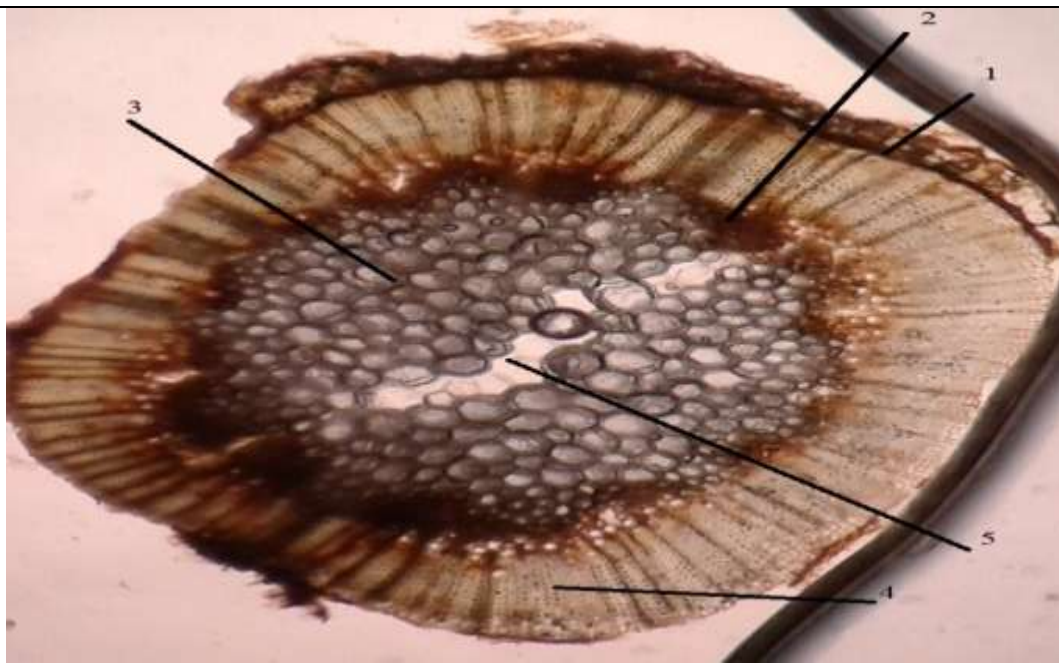


Figure 2: Microscopy of stem of *O. americanum* where 1-Cork, 2- Vascular bundle containing xylem & phloem, 3-Spongy tissue, 4-Collenchymatous cells, 5-pith.



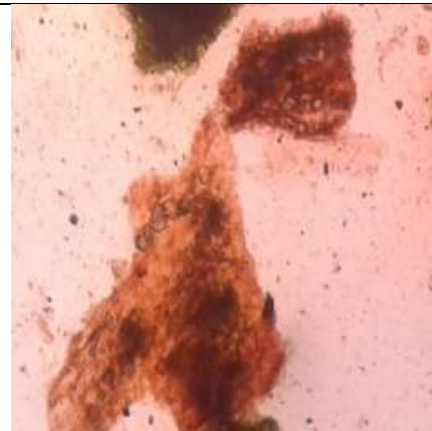
1



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Figure 3: Powder microscopy *Ocimum americanum* where 1-Cork cell, 2-Stone cell, 3-epidermal cell, 4-Collenchymas, 5-Reticulate Xylem vessel

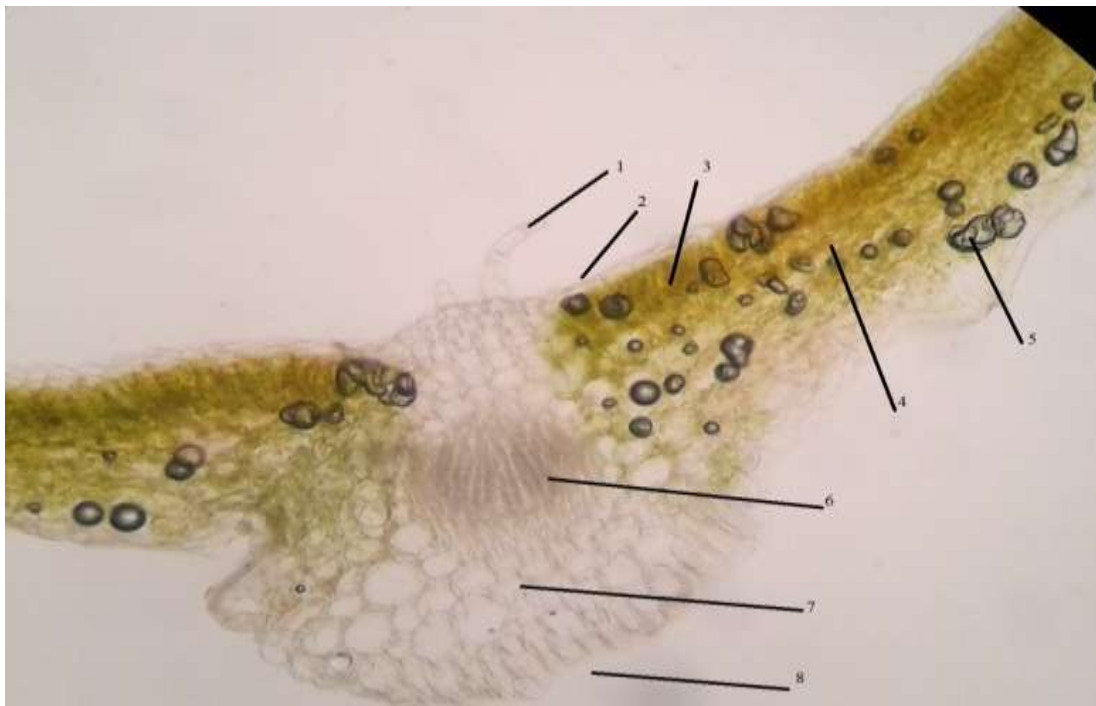


Figure 4: Microscopy leaf of *O. basilicum* where 1-Multicellule curved trichomes, 2- Upper epidermis, 3-Collenchymatous cells, 4- Upper palsied cell, 5-oil globules, 6- Vascular bundle containing xylem & phloem, 7- Spongy tissue 8- Lower epidermis

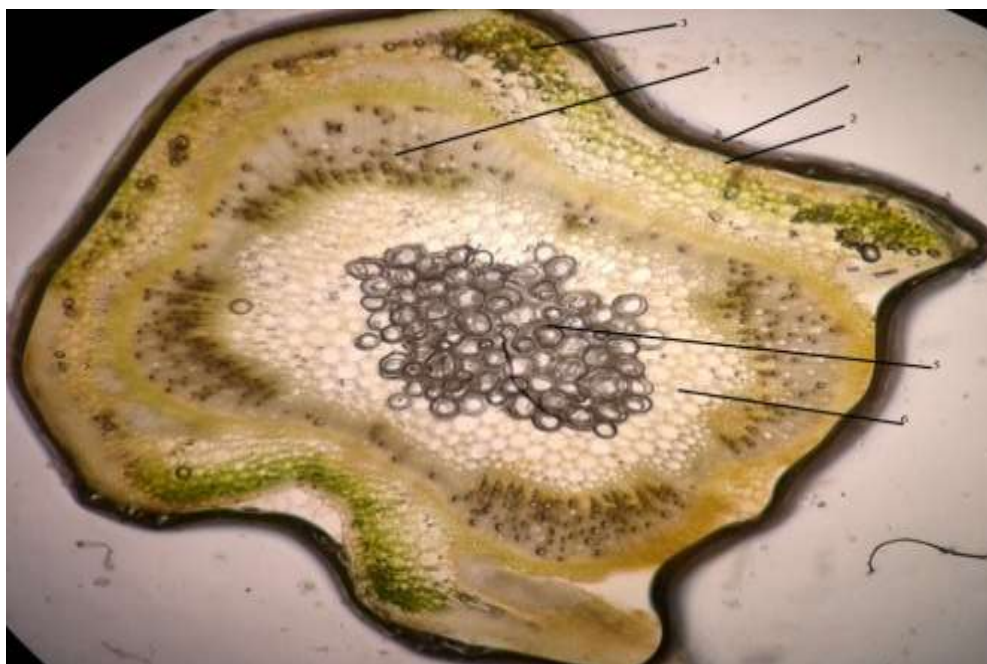


Figure 5: Microscopy stem of *O. basilicum* 1-Unicellular covering trichome, 2- Epidermal cell, 3- Collenchymatous cells, 4-Vascular bundle containing xylem & phloem, 4-Spongy

tissue, 5-Pith, 6- spongy parenchyma

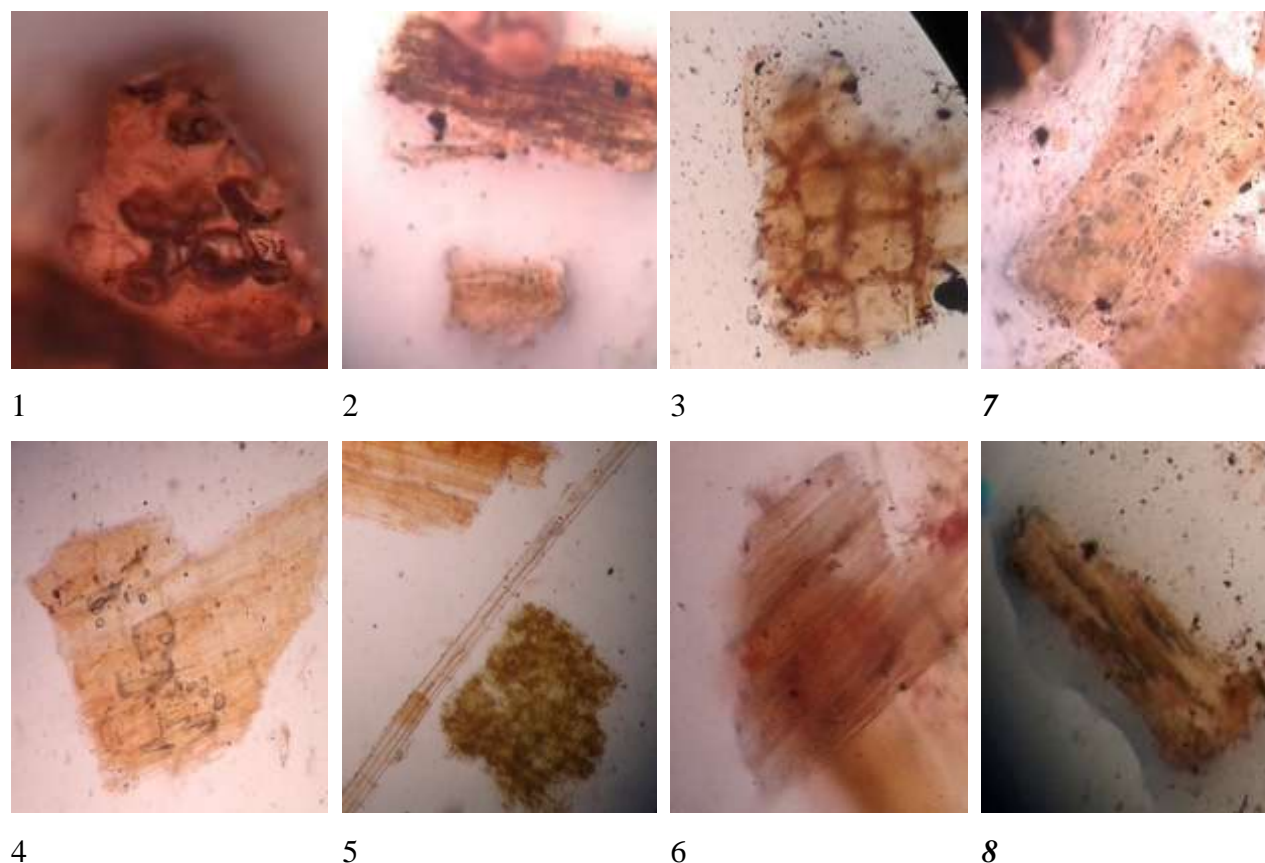


Figure 6: Powder microscopy of *O. basilicum* where 1-Stone cell, 2-Lamellar collenchymas, 3-Cork cell, 4-Epidermal cell, 5-Fibers, 6-Medullary rays, 7-Wood element



Figure 7: microscopy leaf of *O. gratissimum* where 1-Multicellular covering trichomes, 2-Glandular trichomes, 3- Upper epidermis, 4- Upper palisied cell, 5-Collenchymatous cells, 6- Vascular bundle containing xylem & phloem, 7- oil glands

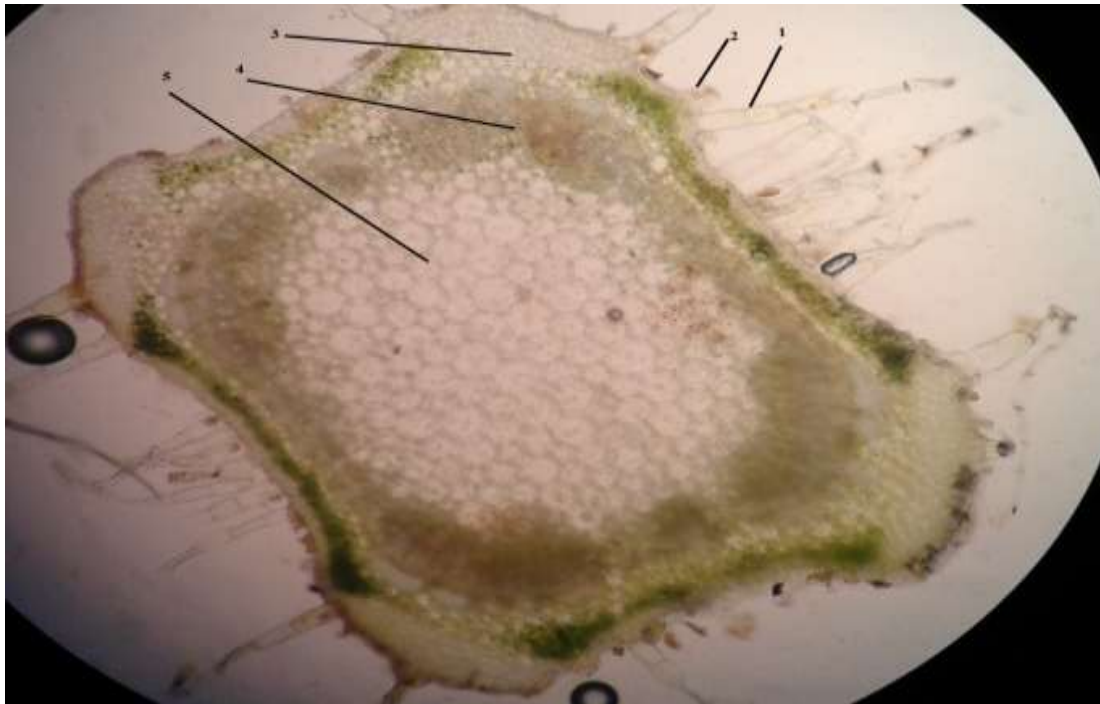


Figure 8:Microscopy stem of *O. gratissimum* Where 1-Multicellular covering trichome, 2- Glandular trichome, 3-Collenchymatous cells, 4-Vascular bundle containing xylem & phloem, 5-Spongy tissue



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2



3



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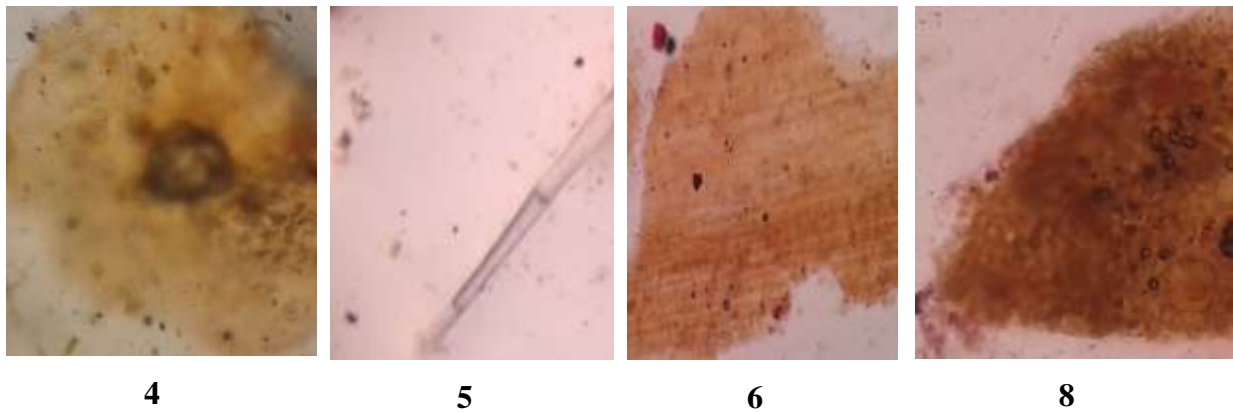


Figure 9: Powder microscopy of *O. gratissimum* where 1-Reticulate xylem vessel, 2-Fiber, 3- Cortical cell, 4-Stone cell, 5-Multicellular trichome, 6-Epidermal cell, 7- Vessels, 8-Oil glands



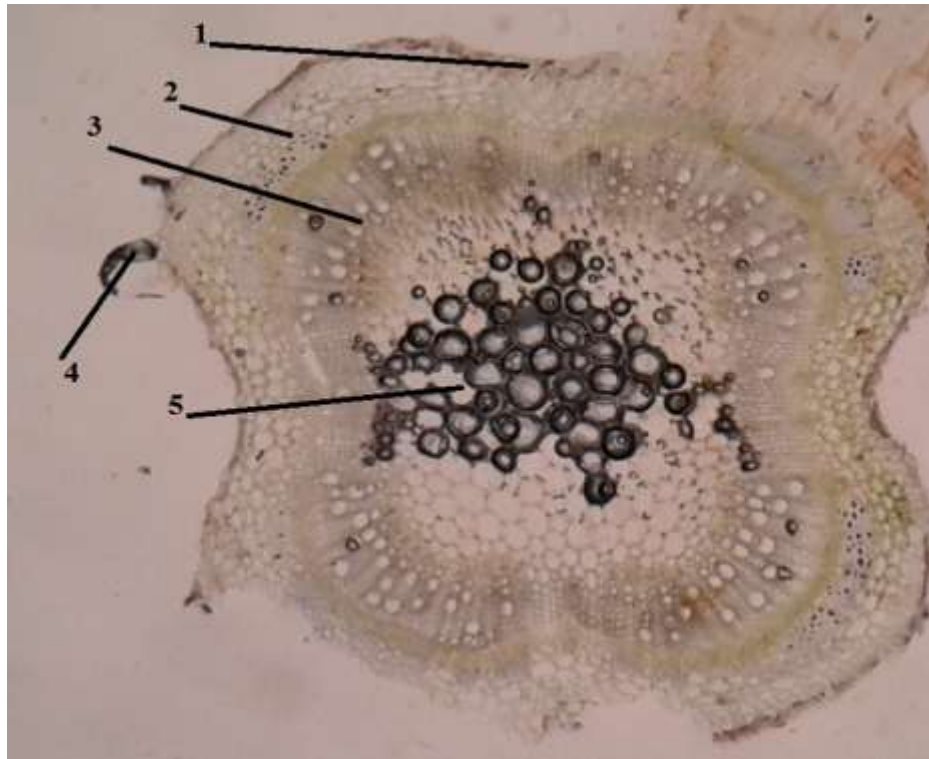
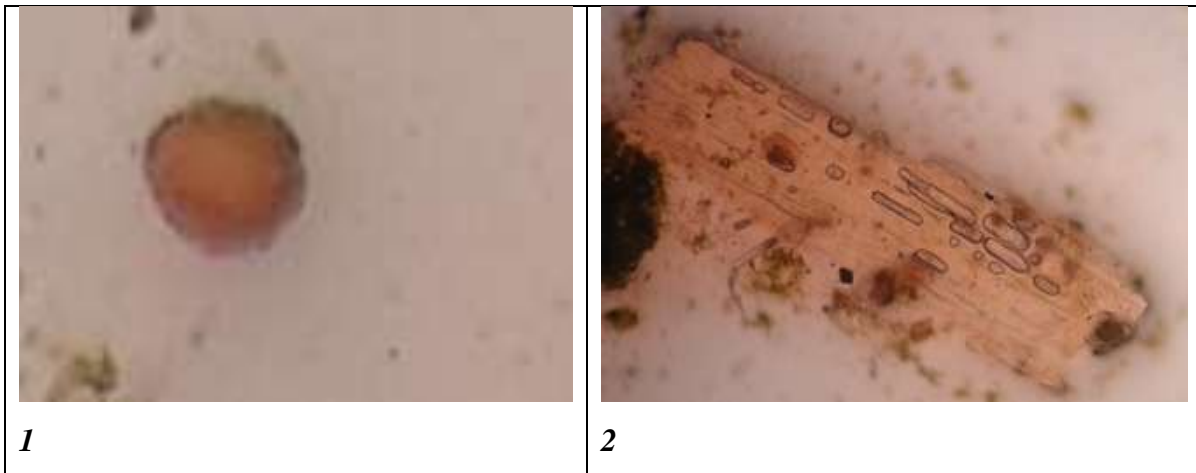


Figure 11: Microscopy of stem of *O. kilimandscharicum* where 1-Cork, 2-Collenchymatous cells, 3-Vascular bundle containing xylem & phloem, 4-Multicellular curved trichomes, 5-Pith



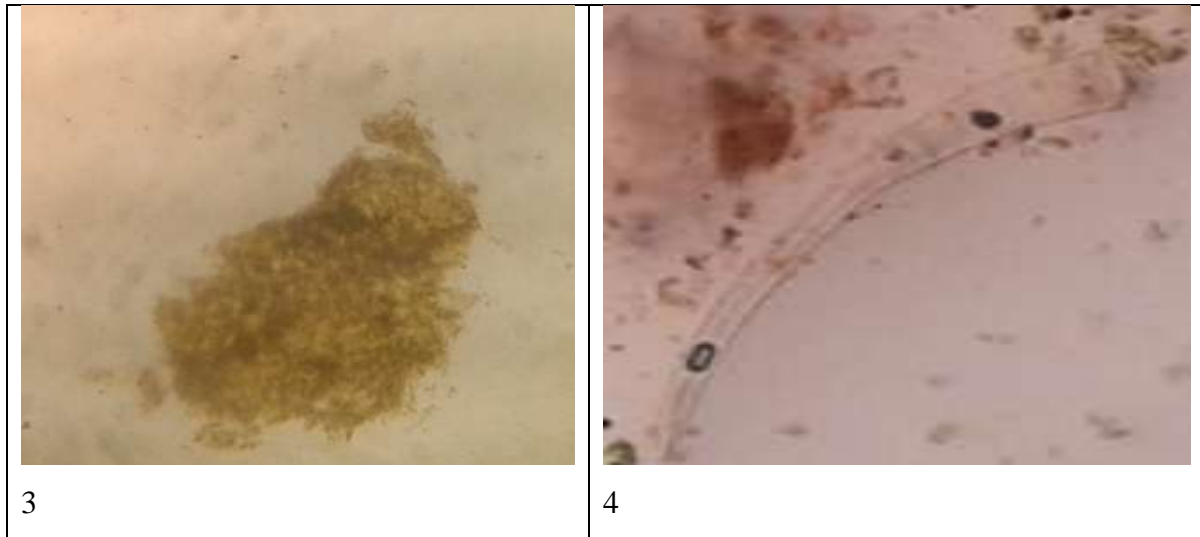
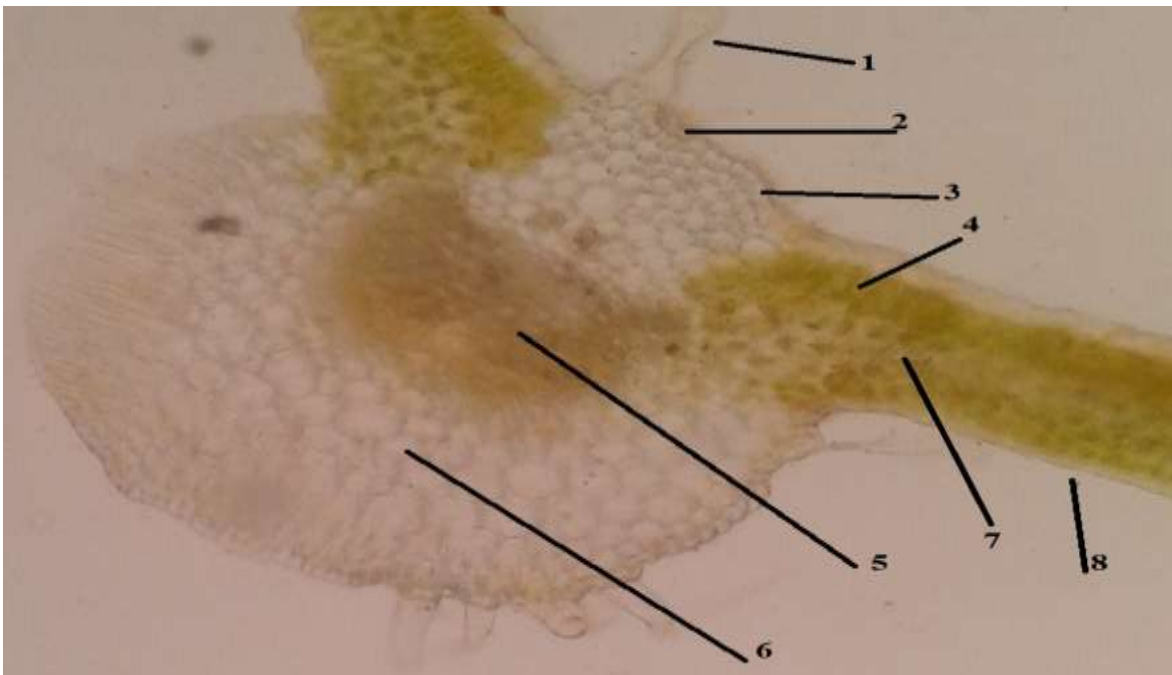


Figure 12: Powder microscopy of *O.kilimandscharicum* where 1- Stone cell, 2-Reticulate xylem vessel, 3-multi cellular curved trichomes, 4-Storage parenchyma,



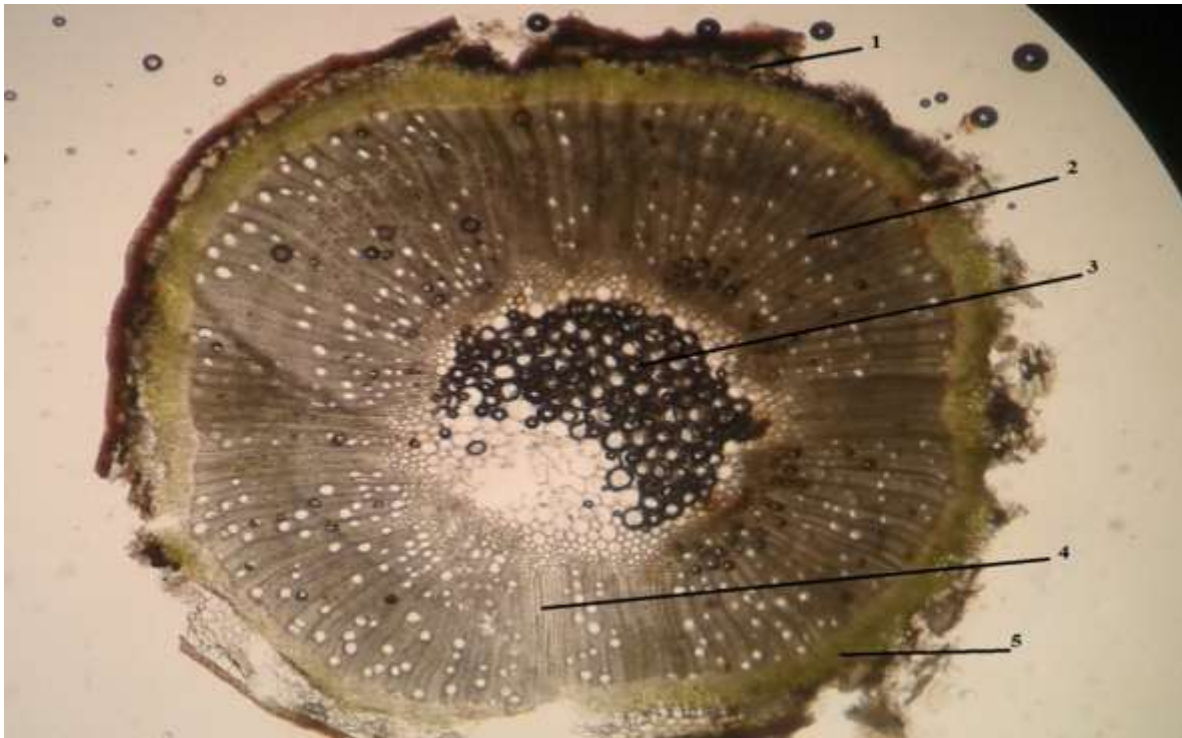


Figure 14: Microscopy stem of *O. sanctum* 1- Cork, 2- Collenchymatous cells, 3- Pith, 4- Medullar rays

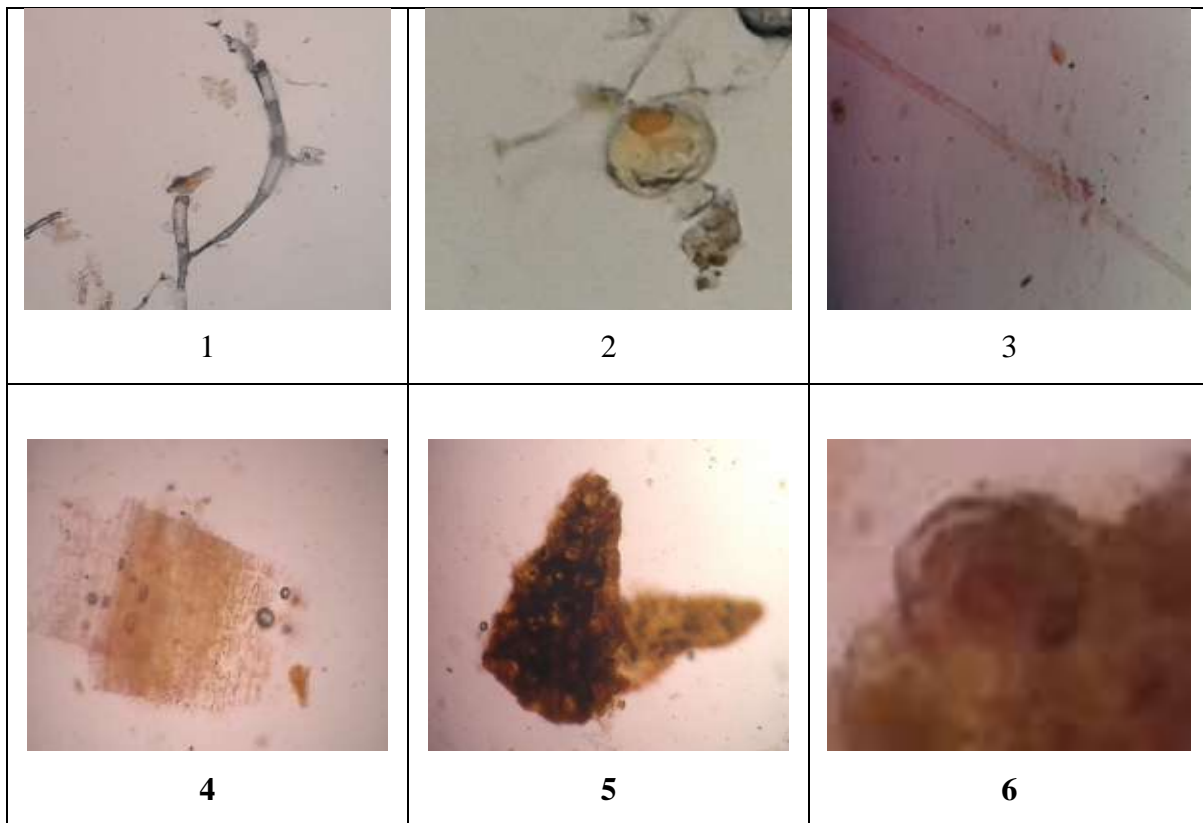


Figure 15: Powder microscopy of *O. sanctum* Where 1- Multicellular curved trichomes,

2-Glandular trichomes, 3-Sclerenchymatous fiber, 4-Epidermal cell, 5-Cork cell 6-Bicuspid stomata

CONCLUSION

As per entitled work “*Comparative Pharmacognostical, evaluation of Ocimum species,*” five species of *Ocimum* are collected and comparatively evaluated for morphological, microscopical characters. Macroscopy of whole plant, microscopy of leaves and stem as well as powder of whole plant studied in detail. Microscopical examination of leaf and powder drug of *O. americanum*, *O. basilicum*, *O. gratissimum*, *O. kilimandscharicum*, *O. sanctum* provided useful information about its correct identity and evaluations. It helps to differentiate species of *Ocimum*.

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