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COMPARATIVE PHARMACOGNOSTIC AND PHYTOCHEMICAL STUDIES OF *OCIMUM TENUIFLORUM* L. AND ITS SUBSTITUTE *VITEX NEGUNDO* L.

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ABSTRACT

India is endowed with a rich wealth of medicinal plants which have been a valuable source of natural products for maintaining human health. A large number of these medicinal plants are used in various formulations for the treatment of several disease caused by microbes. Medicinal plants constitute an effective source of traditional and modern medicines. *Ayurveda* a system of Indian traditional form of alternative medicines. A major aspect of conservation of medicinal plants is their considerable economic and social value. Natural source of medicinal plants are often unable to meet demand for popular herbal products. Populations of many species have limited distribution in their natural habitats, led to genuine or arbitrary substitution by other plants. Unavailability of such medicinal plants has led to arbitrary substitution and adulteration in the raw drug market. Aromatic plants are prime economic importance because of the continuous increased demands for their products by local foreign markets. At present the adulteration and substitution of herbal drugs is the burning problem in herbal industry and it has caused a major advancement in the research on commercial natural products. The article throws light on preliminary pharmocognostic and phytochemical investigation of one pair of *Abhava Pratinidhi Dravya viz. Tulasi* and *Nirgundi*.

KEYWORDS: *O.tenuifloum, V.negundo*, Morphology, Anatomy, Phytochemistry and Fluorescent analysis.

INTRODUCTION

India is endowed with a rich wealth of medicinal plants which have been a valuable source of natural products for maintaining human health. A large number of these medicinal plants are used in various formulations for the treatment of several diseases caused by microbes. World Health Organization (WHO) estimated that 80% of the people worldwide rely on plant based medicines for their primary health care needs[1]. Medicinal plants are now more focused than ever because they have the capability of producing many benefits to society indeed to mankind, especially in the line of medicine and pharmacological[2-3]. The phytochemical constituents and medicinal properties of most of the medicinal plants were recorded in the last few decades by a number of workers [4-5]. A major aspect of conservation of medicinal plants is their considerable economic and social value.[6]

Natural sources of medicinal plants are often unable to meet demand for popular herbal products. Populations of many species have limited distribution in their natural habitats, requiring conservation strategies for protection.[7] or due to highly reduced population size and consequent threatened status of the taxa. This has led to genuine or arbitrary substitution by other plants.[8] Unavailability of such medicinal plants has led to arbitrary substitution and adulteration in the raw drug market.[9] Even for some of the top-traded drugs such as Asoka (*Saraca asoca* Roxb.), there appear to be substitutes in today's market. Ayurvedic texts from the 16th century and later, name several pairs of substitutes (*Abhava Pratinidhi Dravya*) for preferred plants, if unavailable (*Abhava*). For example, for *Plumbago zeylandica* L. (*Chitraka*) of the Plumbaginaceae family, they name *Baliospermum montanum* Willd[10] (*Danthi*), belonging to an entirely different family (Euphorbiaceae). While the concept of substitute use is mentioned as early as Charaka Samhita,[11] Bhavaprakasha Nighantu[12] and Bhaishajya Ratnavalis[13] name plant pairs. Drug unavailability may have pertained to specific regions and not necessarily across the country.

Even a cursory glance at the list of *Abhava Pratinidhi Dravya* mentioned in classical Ayurveda texts excites scientific curiosity concerning the Ayurvedic principles behind selection of substitute drug. In this paper we report preliminary pharmacognostic, phytochemical and pharmacological investigation of one pair of *Abhava Pratinidhi Dravya viz*., *Tulasi* (*Ocimum tenuiflorum L.*; Lamiaceae) [Figure 1] and *Nirgundi* (*Vitexnegundo* L.; Verbenaceae) [Figure 2].

The present study deals with the Comparitive pharmacognostic and phytochemical studies of medicinal plant *Ocimum tenuiflorum.* L and its substitute *Vitex negundo.* L. A popular local quote of the Bengalis in western Himalayan region of India which translated as-a man cannot die of disease in an area where *Vitex negundo* found the plant holds a great promise as a commonly available medicinal plant and it is indeed no surprise that the plants is referred to in the Indian traditional circles as *Sarvaroganivarini*-the remedy for all disease. *Tulasi* known in English as Holy Basil and botanically called *Ocimum sanctum* is described as a sacred and medicinal plant in Ancient literature. *Tulsi* is also called as the 'Elixir of Life'

since it promotes the longevity. It is an important symbol of Hindu religious tradition and is found in most of the Indian homes and worshipped[14-15].

The major objectives of this study are, the study of morphological, anatomical and powder characters of root, stem and leaves and also the fluorescent analysis and phytochemical constitutes.

Basil is one of the species that used for the commercial seasoning. Herbal medicine has become an important part of our health care systems. There is a great demand for herbal medicine in developed as well as developing countries like India, because of their wide range of biological activities, higher safety of margin than the synthetic drugs and lesser costs[16]. The leaf epidermal anatomy of *Vitexne gundo*, shows the stomatal type, epidermal cell shape, size and trichomes. Micro hairs were noted in *V.negundo*. trichomes are well segmented in vitex.[17] *Tulasi* is a herb that is bestowed with enormous antimicrobial substances and used to treat a variety of illnesses ranging from diabetes, arthritis, bronchitis, skin diseases, etc)[18]. The name *Tulsi* which in Sanskrit means 'The incomparable One', has got two varieties, Krishna *Tulsi* (black) and Rama *Tulsi* (green)[19]. Phytochemistry describes the large number of secondary metabolic compound found in plants. Many of these are known to provide protection against diseases. They also exhibit a number of protective functions to plants and animals. Medicinal plants rich in phytochemicals have been used for centuries in the treatment and prevention of diseases. In traditional systems of medicinal, different parts of Ocimum have been recommended for the treatment of Bronchitis, Malaria, dysentery etc. Morphological and anatomical characters play a vital role in crude drug identification and standardisation. The result of microscopical and powder characters are used for the identification of different species of *Ocimum* which furnishes a basis of judging the authenticity of the plant and also differentiate the drug from its allied species and detect its adulterants. [20]

MATERIALS AND METHODS

Plant materials

1) Ocimum tenuiflorum L.; (Synonym: *Ocimum sanctum*)

Systematic classification: (Gamble 2006)

Kingdom :	Plantae
Subkingdom:	Phanerogamae
Class:	Dicotyledonae
Sub class:	Gamopetalae
Series:	Bicarpellatae
Order:	Lamiales
Family:	Labiatae
Genus:	*Ocimum*
Species:	*Tenuiflorum*

- Vernacular names: *Katturamathulasi, Kattuthrithavu, Kattuthulasi.*
- Parts used: Roots, Stem bark, Leaves.

2) Vitex negundo **L**

Systematic classification : (Gamble 2006)

Kingdom:	Plantae
Subkingdom:	Phanerogamae
Class:	Dicotyledonae
Series:	Bicarpellatae
Order:	Lamiales
Family:	Verbenaceae
Genus:	*Vitex*
Species:	*negundo*

- **Vernacular names:** *Indrani, Karinochi, Nochi, Vellanochi, Vennochi.*
- **Parts used:** Roots, Stem bark, Leaves.

Collection and authentication of samples: Fresh mature samples of plants were collected and the specimens were thoroughly washed in tap water as well as in distilled water. Parts were dried, powdered and stored in air tight containers.

Study of Morphology: The collected samples were subjected to morphological and taxonomical analysis using features such as the shape, size, color and floral characters

Study of Anatomy: The samples of root stem and leaves of selected plants were preserved in formalin acetic acid-alcohol (40% formalin: 5 mL: 50% ethanol: 90 mL; glacial acetic acid: 5 mL). Transverse sections, were taken by using razor blade, stained with toluidine blue 0.05% in benzoate buffer (benzoic acid 0.25 g in 200 mL water pH 4.4) washed with water, observed under a compound microscope and the photographic images were captured using a digital camera fixed with the microscope.

Powder Microscopy: The plants collected were properly washed and dried and made into coarse powder and stored in well-sealed bottle for further analysis. These powders were placed on the grease free microscopic slide along with glycerine and water (1:1). Then it was covered with a clean cover slip and observed under compound microscope at 10X followed by 40X magnification.

Fluorescence analysis: A small quantity of dry plant powder was placed on grease free clean microscopic slide and 1-2 drops of freshly prepared reagent solution was added, mixed by gentle tilting the slide and waited for few minutes. Then the slide was placed inside the UV chamber and The colour observed by application of different reagents were recorded. The reagents used were:

- powder+1NNaOH(aq)
- powder+50%HCl
- powder+50%H2SO4
- powder+ picric acid
- powder+ ethanol
- powder+ methanol
- powder+ petroleum ether
- powder+ ammonia
- powder + ethyl acetate

Preparation of plant material: The freshly collected samples will be washed thoroughly with distilled water and air dried under shade at room temperature for 30-45 days. Upon drying, the samples will be grinded into fine powder mechanically using an electric blender then sieved using a muslin cloth. Finely powdered samples will then store in air tight bottles at ambient temperature until requires.

Preparation of plant extracts: 5gm each of the air-dried finely powdered plant samples were weighed and were

soaked in 50ml each of acetone, methanol, petroleum ether and distilled water contained in separate 100ml sterile conical flasks. The flasks were covered with a sterile cotton plugs followed by wrapping with aluminium foil and shaken at 4 hour intervals for 24hours at room temperature. These crude extracts were then filtered and supernatants were collected, covered, labeled and used for the phytochemical screening.

Phytochemical screening

Test for alkaloids

Meyer's test – To 1ml of each of the sample solution few drops of Meyers reagent was added. The formation of creamish white precipite indicate the presence of alkaloids.

Wagner's test – to a little of the sample solution, Wagner's reagent (iodine in potassium iodide) was added. the formation of reddish brown precipitate indicate the presence of alkaloids.

Hager's test **-** To 1ml of sample solution few drops of Hager's reagent was added, yellow precipitate was formed reacting positively to alkaloids.

FeCl3 test **-** one drop of Fecl3 added to each sample, formation of yellow precipitate was resulting positively for alkaloids.

Test for glycosoides

Kellarkillani test – 1 ml of concentrated sulphuric acid was taken in a test tube. To it add 5ml of plant extract and 2ml of glacial acetic acid with one drop of ferric chloride. A blue coloration indicates the presence of glycosides.

Con. Sulphuric acid test – a reddish coloration on addition of conc. sulphuric acid to the plant extract indicates the presence of glycosides.

Bromine water test - when sample treated with Bromine water. Formation of yellow colour indicates the presence of glycosides.

Test for tannins and phenolic compounds

Ferric chloride test – when few drops of ferric chloride were added to the sample solution, appearance of blackish precipitate occurs.

Alkaline test – on addition of sodium hydroxide to the sample solution, the appearance of yellow to red precipitate indicates the presence of tannins and phenolic compounds.

Test for flavonoids

Ferric chloride test – to a little amount of test sample taken separately, few drops of ferric chloride was added. The formation of blackish red precipitate indicates the presence of flavonoids.

Alkaline reagent test – when sodium hydroxide solution was added to the test samples formation of intense yellow colour, which turns to colour less on addition of few drops of dilute acid indicates the presence of flavonoids.

Zinc hydrochloride reduction test **-** To test the sample solution for the flavonoids added a mixture of zinc dust and concentrated hydrochloric acid solution result in red/pink colour.

*Lead acetate test***-** when aqueous basic lead acetate was added to test sample produces reddish brown precipitate.

Test for carbohydrates

Molisch test - when Alpha naphthol and concentrated H2SO4 were treated with test sample reddish violet ring formed between the junction of two layers was resulted.

Test for quinones

Alcoholic KOH test – when alchoholic KOH was added to the test samples, a red to blue color appears indicating the presence of quinones.

Test for saponins

Foam test - 5ml extracts was shaken vigorously to obtain stable persistent froth. The froth was then mixed with three drops of olive oil and observed for the formation of an emulsion, which indicates the presence of saponins.

RESULTS AND DISCUSSION

Morphological characters

Table 1: Comparative morphological characters

Parameters	***Ocimumtenuiflorum* L**	***Vitexnegundo* L**
Distribution	Throughout the India	Throughout the India
Habitat	Common in waste places, and also found in all plain district	These are commonly found near water bodies, recently distributed land, grassland and mixed open forest
Habit	Strongly scented small annual herb, grows as a small bush 0.5-5 m in height	Large and erect aromatic shrub/small tree, which grown to height 5-9 m
Root	Tap root system	Tap root system
Stem	Aerial, hairy and usually quadrangular, red or purple and aromatic	Quadrangular, densely whitish with tomentosebranch lets. Bark is reddish to brown color.
Leaves	Simple, opposite about 2-4 cm. long and variable in breath, base narrowed, margins toothed, hairy, on both surface, aromatic, exstipulate, petiole in 5mm long. Leaf is ovate and dark green in colour with gland dotted.	Digitate with five lanceolate leaflets, sometimes three. Each leaflet is around 4-10 cm in length with central leaflet being the largest and possessins a stalk. The leaflet in palmately arranged and acute terminal leaflet (16-32cm) with petiolate having 1-2 cm long.
Inflorescencce	Raceme of cyme. Special inflorescence is known as thyrsus. Usually 6-8 inch long.	Commonly racemes

Flower	Tiny, purple in colour, bisexual, zygomorphic, hypogynous, bracteate and bracteolate. Flowers arranged in whorls and pentamerous.	Sessile or pedunculate cymes forming terminal bract. Small often caduceus. Bluish purple in colour. Flowers are born in panicles 10-20 cm in length
Calyx	5, gamosepalous, persistent, tubular below and lobed above. Imbricate in aestivation.	Tubular, funnel shaped shortly 5 lobed covered with dense hairs.
Corolla	5, gamopetalous, bilabiate, petals often two lipped arranged as 4/1	Small, 2 lipped, tubes usually short and upper lip of 2 and lower lip of 3 lobes. The middle much longest. Covered with dense hairs.
Androecium	4 stamens, epipetalous and didynamous	4, didynamous, divaricate always attached to the tip only
Gynoecium	Bicarpellary, syncarpous, superior and bilocular. Tetra locular by the formation of false septum. Ovary placed on nectariferous disc. Ovule anatropous. One ovule in each locus. Gynobasic style and bifid stigma	2-4 celled, 4 ovules style filiform, stigma bifid
Fruit	Carcerulus	Succulent drupe, black or purple when ripe, round to egg shaped ana about 4mm in diameter.

Anatomical characters

Anatomically these two plants show variations in many characters. Details of anatomical characters of root, stem and petiole are given in Table 2(Fig: 3a, 3b, 4a,4b), Table 3 (5a, and Table 4(Fig 6a and Fig. 6b).

Table 2: Comparative root anatomical characters

Parameters	***Ocimum tenuiflorum (Fig:3a)***	***Vitex negundo (Fiog:3b)***
Epidermis	Ovate shaped	Round in shape
Cork	Presence of cork with 3 rows of thin walled hexagonal shaped cells	Presence of cork with 5 rows of thin walled rectangular shaped cells
Cambium	Continuous layer of cambium is present	Single layer of cambium present
Phloem	Less or absent	Comparatively narrow
Primary xylem	Primary xylem limited in number	Primary xylem limited in number
Secondary xylem	Major portion of root occupied by secondary xylem	Half of the portion of root wood is occupied by secondary xylem
Xylem vessels	Large number of xylem vessels are present but are scattered in distribution. (Fig:4a)	Large number of xylem vessels are present and are compactly arranged
Medullary rays	Medullary rays not distinct	Medullary rays usually biseriate and numerous number (Fig:4b)

Table 3: Comparative stem anatomical characters

Parameters	***Ocimum tenuiflorum (Fig:5a)***	***Vitex negundo* (Fig.5b)**
Similarities		
Shape	T.S of stem is quadrangular	T.S of stem is quadrangular
Epidermis	Uniseriate with cuticle	Uniseriate with cuticle
Cortex	Many layered made up of parechymatous cells	Many layered made up of parechymatous cells
Stele	Endarch	Endarch
Intercellular space	No intercellular space between the cells	No intercellular space between the cells
Pith	Large and made up of parenchymatous cells	Large and made up of parenchymatous cells
Differences		
Cambium	Cambium ring is not continuous	Cambial ring is continuous
Xylem vessels	Comparatively less in number	Xylem vessels are numerous number
Trichomes	Glandular trichomes are arising from the epidermis	Simple trichomes are arising from the epidermis

Table 4: Comparative petiole anatomical characters

Parameters	***Ocimum tenuiflorum (Fig:6a)***	***Vitex negundo (Fig:6b)***
Similarities		
Cortex	Many layered made up of parechymatous cells	Many layered made up of parechymatous cells
Stele	Endarch, Phloem towards periphery and xylem towards pith	Endarch, Phloem towards periphery and xylem towards pith
Phloem and xylem	Equal	Equal
Pith	Large and made up of parechymatous cells	Large and made up of parechymatous cells
Differences		
Shape	Oval shaped	'U' shaped
Epidermis	Uniseriate with simple lengthy trichome	Uniseriate with neumerous short trichomes
Number of stele	Distelic condition present	Polystelic condition present

Powder Microscopy

Table 5: Comparative powder characters

Parameters	***Ocimum tenuiflorum* (Fig:7a,8a &8b)**	***Vitex negundo* (7b,8c & 8d)**
Powder colour	Military green	Dark green
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 amoeboid in outline. Stomata are also seen in the powder	The peeling displays the surface features of the epidermal cells and stomatal morphology. The epidermal cells are small, polyhedral and thick walled. The anticlinal walls are slightly wavy. The stomata are densely distributed
Stomata	Diacytic	Anamocytic
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 non glandular trichomes are multi cellular, unbranched and uniseriate. The trichomes may be two to five celled. The cells are vertically elongated and thick walled. The trichomes are gradually tapering into pointed tip.
Glandular trichome	Capitate type with 4 cells	Glandular trichomes are not seen

Flourescent Analysis (Fig: 9)

The powdered drug with different chemical reagents showed different colours when seen with naked eye and under visible light. The different colour observed shows the presence of different type of phytoconstituents (Table 4). Powder with reagents, when observed under UV light showed dark blue colour.

Table 6: Colour of Powders observed under visible light using different reagent

REAGENT		***Ocimum tenuiflorum***			***Vitex negundo***		
		Root	**Stem**	**Leaf**	**Root**	**Stem**	**Leaf**
1	1N NaOH(aq)	Light brown	Brown	Dark brown	Light yellow	Light brown	Dark brown
2	Dil. HCl	Light red	Light brown	Black	Light red	Black	Light brown
3	Dil.H2SO4	Colourless	Light brown	Black	Colourless	Green	Black
4	Picric acid	Yellow	Yellow	Black	Yellow	Black	Green
5	Ethanol	Light red	Light brown	Black	Light brown	Black	Black
6	Methanol	Black	Light brown	Black	Light brown	Light brown	Black
7	Petroleum ether	Black	Black	Black	Brown	Black	Black
8	Ammonia	Light yellow	Light yellow	Black	Brown	Light red	Light green
9	Ethyl acetate	Black	Black	Black	Black	Black	Black

Qualitative Phytochemical Screening

The qualitative phytochemical screening of the root, stem and leaf powder of both plants showed the presence of carbohydrates, saponines, alkaloids etc..The detailed results of the tests are recorded in the Table 7

Table 7: Qualitative phytochemical screening of plants under different solvents

Solvent	**Distilled water**						**Acetone**						**Petroleum ether**						**Methanol**					
Phytoconstituent	**R1**	**R2**	**S1**	**S2**	**L1**	**L2**	**R1**	**R2**	**S1**	**S2**	**L1**	**L2**	**R1**	**R2**	**S1**	**S2**	**L1**	**L2**	**R1**	**R2**	**S1**	**S2**	**L1**	**L2**
Flavonoids	+	+	_	+	+	+	+	+	+	_	_	+	_	_	_	_	_	_	+	+	_	+	+	_
Quinones	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
Saponins	+	+	+	+	+	+	+	_	_	_	_	_	+	_	_	_	_	_	+	_	_	_	_	_
Alkaloids	+	+	+	+	+	_	_	_	+	_	_	+	+	_	+	_	+	_	+	+	+	_	+	+
Glycosides	+	_	_	+	+	+	+	+	+	-	+	+	+	+	-	-	_	_	+	+	+	+	+	+
Tannin	+	+	_	_	+	+	_	_	+	+	_	_	_	_	_	_	_	_	+	+	_	_	+	_
Phenols	_	_	_	_	+	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
Carbohydrates	_	_	_	_	_	_	_	_	_	_	_	_	+	+	+	+	+	+	_	_	_	_	_	_

*R1 – root extract of *O. tenuiflorum**S1 – Stem extract of *O. tenuiflorum* *L1 – Leaf extract of *O. tenuiflorum*
*R2 – root extract of *V. negundo**S2 – Stem extract of *V. negundo**L2 – Leaf extract of *V. negundo*

In the Ayurvedic raw drug markets, *Tulasi* is substituted in total or in part by *Nirgundi* which is relatively very cheap and easily available as it is a tree. The present study aims at comparing the pharmacognostical, and phytochemical studies of *Ocimum tenuiflorum* L with *Vitex negundo* L. Morphologically both plants are dissimilar and also under different families. Thus there is no chance for its use as substitute. Anatomically they showed few similarities and many differences. Fluorescence analysis showed same result for both the plants in UV light but colour under visible light showed slight variations. Hence crude drugs are often assessed qualitatively in this way and it is an important parameter for pharmacognostic evaluation of crude drugs. This study can help in authentication and identification of the plant material. Such information can act as reference information for correct identification of a particular plant. It can act as a tool to detect adulterants. Phytochemical analysis of the plant extracts helps to reveal the presence of different chemical components present in the parts. Both the plants are medicinally important. The medicinal properties of the plants are generally believed to be due to the presence of different chemical components present in it. The comparative phytochemical studies of root, stem and leaves of both the plants revealed the presence of carbohydrates, flavanoids, tannins, saponins, alkaloids glycosides and phenol. The previous studies on the leaf extract of *V. negundo* have revealed the presence of important classes of phytoconstituents like alkaloids, flavonoids and carbohydrates[21]. Earlier studies on the leaf sample extract of *O. tenuiflorum* showed the presence of phytochemical constitutes such as tannins, saponins, flavonoids, steroids, terpenoids and cardiac glycosides present study support earlier findings.

CONCLUSION

Due to the non- availability of the required original drug in specific regions, arbitrary substitution is on the increase in more recent times due to market-driven demands. One such drug complex where substitution (both genuine and arbitrary) is prevalent to a greater extent is the *Tulasi-Nirgundi* complex studied here. There appears to be no doubt about the botanical correlation as per [22-23] of Ayurvedic *Tulasi* and *Nirgundi* with *Ocimum tenuiflorum* and *Vitex negundo*, respectively. Pharmacognostical studies and phytochemical screening can serve as a basis for proper identification, collection and investigation of the plant. These parameters, which are being reported, could be useful in identification of the genuine and substitute source plants and also in the preparation of the herbal monograph for its proper identification. The present study helps to distinguish *Ocimum tenuiflorum* L with *Vitex negundo* L conveniently and coming to a conclusion that the genuine plant *Tulasi* is equated with *Nirgundi* used as the substitute. For further confirmation pharmacological studies are to be carried out It is also likely that different chemicals present in these taxa may cause the same therapeutic actions. Hence, the commonalities and their chemical basis need to be explored at greater depth before making any definite conclusion on this aspect. The high degree of phytochemical similarity in all two candidates is probably the reason for the high degree of similarity in their therapeutic activities as recorded by many earlier investigators. Whatever differences are there in phytochemical parameters may account for the more specific therapeutic actions exhibited by each one of the taxa. These also need to be ascertained by more detailed individual chemical based therapeutic action studies.

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FIGURES

Fig1: Habit of *O.tenuiflorum*

Fig 2: Habit of *V.negundo*

Fig: 3a - *Ocimum tenuiflorum:*T.S. of Root

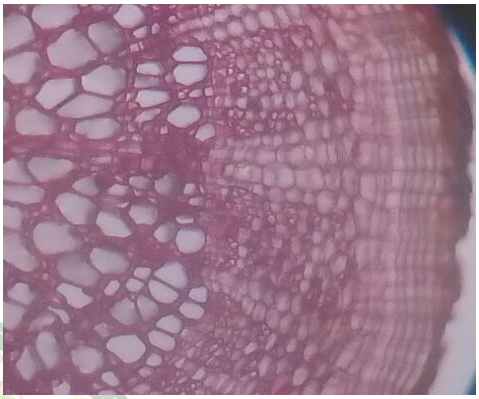


Fig:3b- *Vitex negundo:* T.S of Root

Fig:4a - *Ocimum tenuiflorum:* Stellar region

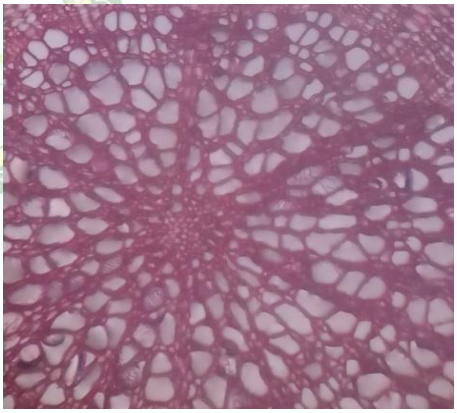


Fig:4b- *Vitex negundo:* Stellar region

Fig: 5a-*Ocimum tenuiflorum*: T.S of Stem

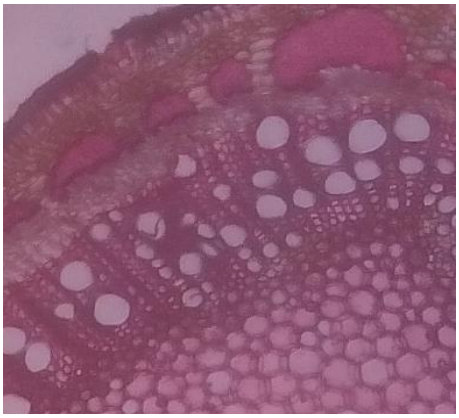


Fig: 5b- *Vitex negundo:*T.S of Stem

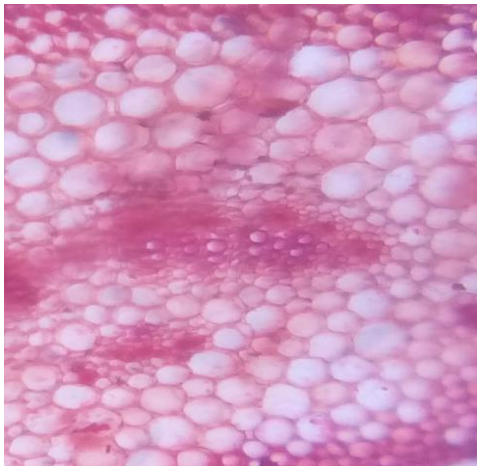


Fig:6a- *Ocimum tenuiflorum:* T.S of Petiole

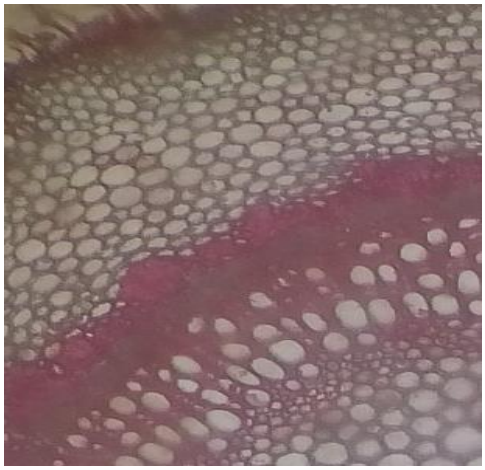


Fig:6b- *Vitex negundo:* T.S of Petiole

Fig: 7a:*O.tenuiflorum:*Epidermis and stomata

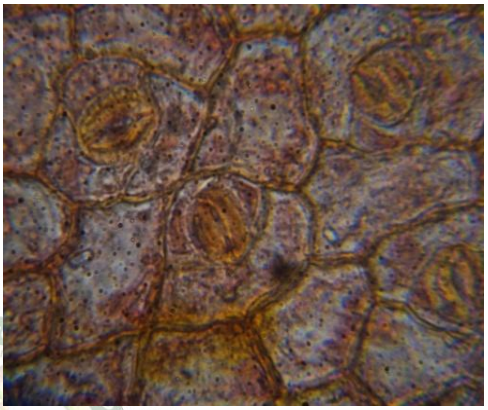


Fig: 7b: V.negundo Epidermis and stomata

Fig:8a - *O.tenuiflorum*: Glandular trichome

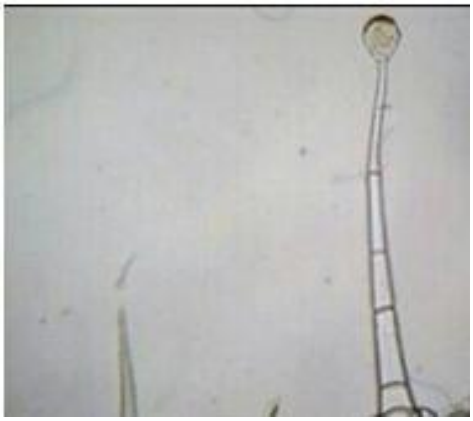


Fig:8b - *O.tenuiflorum*: Capitatetrichome

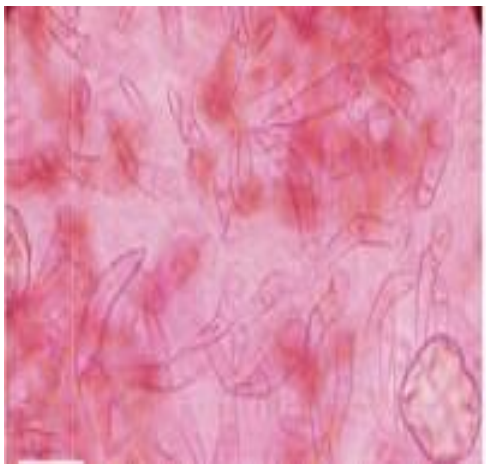


Fig:8a – *V. negundo*: Covering trichome (100X)

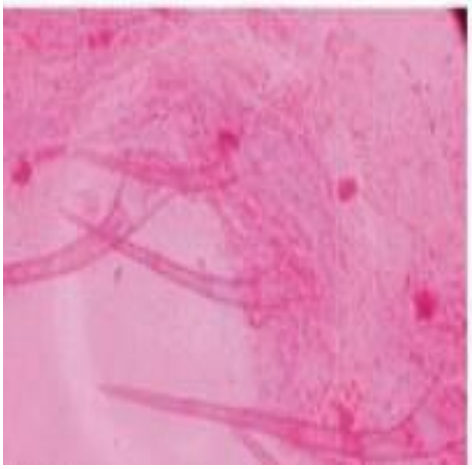
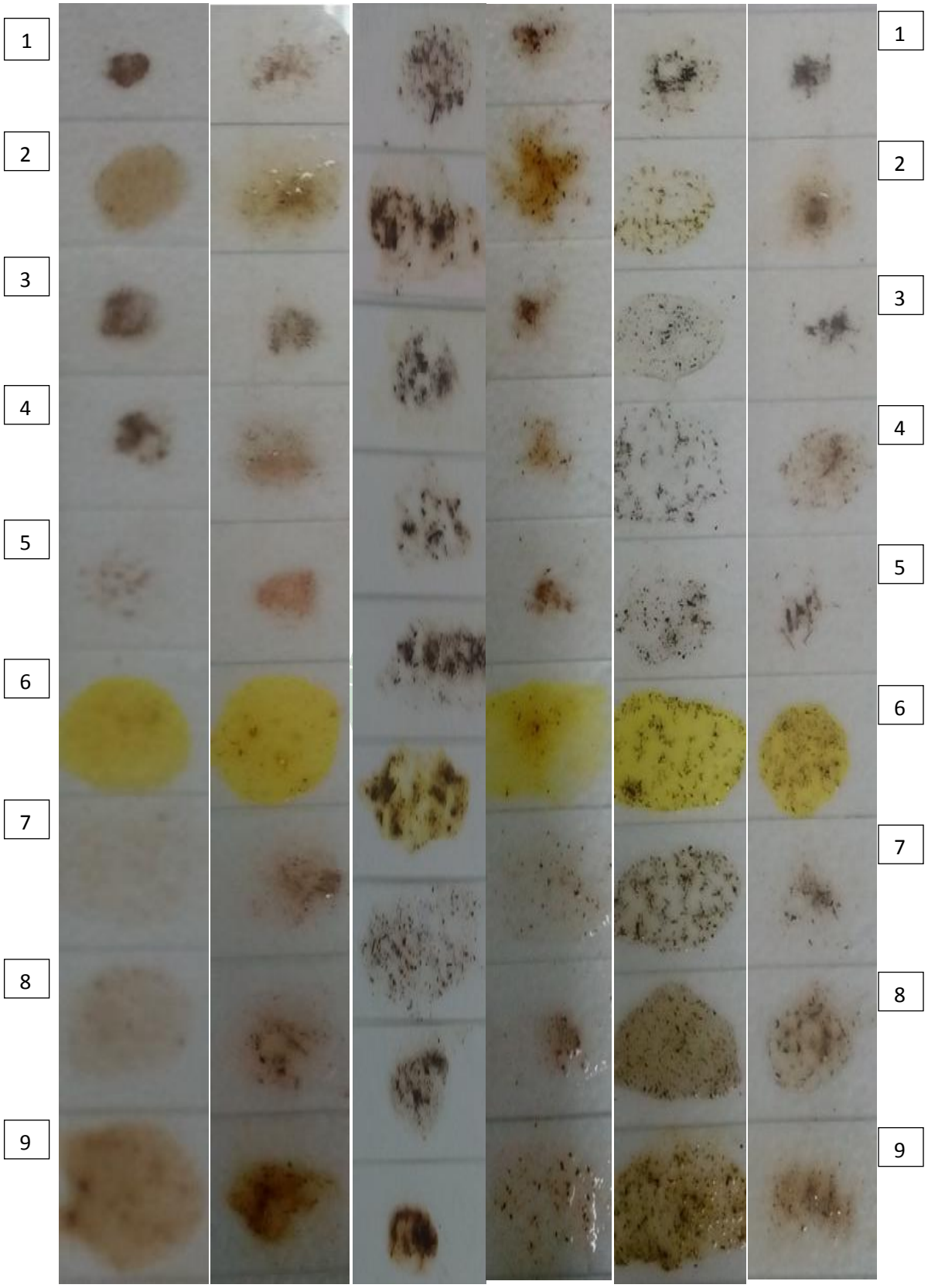


Fig:8a *V. negundo*: Covering trichome (400X)



Root Stem Leaf Root Stem Leaf

O. tenuiflorum V. negundo

Fig: 9- Observation of Powders under visible light using different reagents (1.Ethyl acetate 2.Ammonia solution 3.Petroleum ether 4.Methanol 5.Ethanol 6. Picric acid 7.Dil.H2SO4 8.Dil HCl 9.1N NaOH