

**MICROSCOPICAL OBSERVATIONS ON *CISSUS VITIGINEA* L.**G. Penchala Pratap^{1*}, G. Sudarsanam², G. P. Prasad³, J. Sekhar¹¹Research Scholar, ²Professor in the Dept. of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India.³Research Officer (Ayu.), National Institute of Indian Medical Heritage (NIIMH), Hyderabad, Telangana, India.

Received on: 22/07/2014

Revised on: 18/08/2014

Accepted on: 28/08/2014

ABSTRACT

The present study discloses detailed investigation on Macro and Microscopical characters of the leaves and roots of *Cissus vitiginea* L. Yanadhi tribes of Nellore district, have been using the root powder and leaf paste of *C. vitiginea* as a single drugs remedy to heal the wounds and to get immediate relief from swellings and sprains respectively. The plant belongs to Vitaceae family. In dried form identification of leaves and roots is hard and at present authentic pharmacognostical data is not available. With this view, leaves and roots of *C. vitiginea* were subjected to various pharmacognostical studies like, powder microscopy, sectioning, maceration and florasense studies to identify authentically. In the microscopical studies, different cell structures like trichomes, elongated fibers, raphide sacs, acicular crystals and different arrangements like, raphide sacs in the parenchymatous region reddish tannin content in the phloem region were noticed.

KEYWORDS: Ethnobotanical, Macro and Microscopical studies, pharmacognostical studies, & *C. vitiginea*.

INTRODUCTION

Yanadi, chenchu and nakkalas are the 3 primitive inhabiting tribal groups of Nellore district from immemorial days. Yanadi tribes of Rapur forest division in Nellore District have been claiming that the external application of root powder of *C. vitiginea* heals the wounds and the external application of leaf paste mixed with a little amount of CaCO₃ on affected area relieves swellings and sprains. The same efficacy is already reported in past literatures. (Bharath Kumar 2008 & Balasubramanian 1997). Locally the plant is called as Adavi Drksha, as per Encyclopedia on Indian Medicinal Plants (FRLHT) Sanskrit name of the plant is Amlavetasah. Identification of leaves and roots in dried form is very difficult. The major difficult that is encountering today in all traditional systems is the usage of several different botanical species under the same drug name. These are claimed as having same therapeutic efficacy and used by the physicians as the

same drug. Today such drugs are termed as controversial drugs. In such cases pharmacognostical studies only help to identify the genuine drug which prevent adulteration of drugs by using standardization and microscopical methods (G.Penchala pratap et al. 2012). With all these views present work "Pharmacognosy of leaves and roots of *Cissus vitiginea* L." was conducted.

The work was carried out from Department of Botany, Sri Venkateswara University, Tirupathi, Andhra Pradesh.

Taxonomy of the plant

Woody straggling or Climbing shrubs. Branchlets densely pubescent, tendrils simple, stout. Leaves simple, broadly cordate, lobed, pubescent, dentate, acuminate. Flowers pale yellow, in dichotomous cymes. Berried ovoid, purple when ripe. (Fig:2&3) (Gamble, J.S 1967)

MATERIALS AND METHODS

Fresh leaves and roots of *C. vitiginea* L. were collected from the Yanadi tribes of Nellore district. Identification and confirmation were done by Department of Botany Sri Venkateswara University. The voucher herbarium specimen was processed followed by standard procedure present in Jain and Rao 1977. Microscopical studies of leaf using fresh plant material were carried out with standard procedures like Johansen, 1940; Wallis, 1985, Khandelwal et al. 1996. and Kokate 1991. During these studies T.S of the leaf, Powder microscopical studies and Maceration were done to observe peculiar characters of the leaf. Fluorescence analysis was carried out according to methods of Kokoski et al. 1958 and Indian Pharmacopoeia 2007. Fluorescence characters of powdered drug play a vital role in the determination of quality and purity of the drug material.

Author conducted an Ethnobotanical survey in Nellore district from 25-06-2011 to 25-07-2011.

Macroscopical characters of the leaf

Leaves simple, broadly cordate, pubescent, 5-7X 3-8cm., lobed or 5 angular, thick coriaceous. Base cordate, apex acute-acuminate, margin dentate. Petiole 1-9 cm. (Fig:2&3)

Microscopical Characters of the leaf

T.S of the leaf dorsiventral in structure shows both upper and lower epidermis covered by thick cuticle, some of the epidermal cells of both upper and lower epidermis shows elongated to short multicellular trichomes (Fig:4,5&6). Towards the upper epidermis, cells are rectangular and followed by 2 to 3 layered collenchymatous layer (Fig: 5&6) and 2-3 layered parenchymatous layer (Fig: 5&6). In the centre vascular bundle is represented by xylem and phloem in two separate bundles towards upper and lower region with protoxylem and metaxylem, phloem represented by thin polygonal cells with abundant reddish tannin contents as dots like structure (Fig: 4,6&7). Near the phloem region, parenchymatous region prominent raphide sacs are present (Fig: 8&9). Towards the lower region, collenchymatous cells are 2-

3 layered and parenchymatous cells are 6-8 layered and raphide sacs are represented in parenchymatous region (Fig:5,8,9&21). T.S of the leaf through laminar region shows both upper and lower epidermis, covered by thick cuticle. Palisade layer is 2 layered and some of the palisade cells show prominent raphide sacs and also reddish tannin content. Spongy tissue is 2-3 layered loosely arranged (Fig: 11). In the lower epidermis of ranunculaceous type of stomata are present. Both upper and lower epidermis shows elongated multicellular trichomes.

Diagnostic Characters

1. Presence of abundant raphide sacs in the parenchymatous region of midrib as well as in the palisade cells.
2. Presence of reddish content (tannin) in the phloem region prominently.
3. Presence of multicellular trichomes in midrib region and laminar region.
4. Presence of ranunculaceous type of stomata in the lower epidermis.
5. Presence of mucilage in the epidermal cells of the midrib and laminar region.

Powder Microscopical Studies

Leaf powder Green in colour, coarse to touch, smell agreeable, tastes slightly sweetish, and mucilaginous, when treated with chloral hydrate solution and water following fragments of different tissues were observed under the microscope.

- 1) Fragments of elongated, multicellular trichomes. (Fig:14)
- 2) Fragments and elongated Acicular crystals. (Fig:17&19)
- 3) Fragments of thinwalled parenchymatous cells. (Fig:16)
- 4) Abundant raphide bundles. (Fig:15)
- 5) Fragments of Abundant multicellular trichomes in groups. (Fig:18)
- 6) Fragments of thinwalled parenchymatous cells with abundant mucilaginous cells. This is a characteristic character of the drug powder. (Fig:20)

Diagnostic characters

- 1) Presence of simple multicellular trichomes abundantly.
- 2) Presence of abundant acicular crystals.
- 3) Presence of abundant Raphide bundles.
- 4) Presence of abundant mucilage cells.

**Table 1: Behavior of the drug (Leaf) powder with different Chemical reagents.
(Kokoski et al,1958, Anonymous, 1959. and Chase et al,1949)**

S.No	Treatment	Colour observations		
		Ordinary light	U.V Light	
1.	Powder +Distilled water	Light Green and mucilaginous	255nm	365nm
2.	Powder+5%Aqueous FeCl ₃	Black	Black	Brownish Black
3.	Powder+Glacial acetic acid	No change	No change	No change
4.	Powder+5% HNO ₃	No change	No change	No change
5.	Powder+N/10 Iodine Solution	Blue colour	Dark Blue	Blue
6.	Powder+Con HCL	Light Green	Dark Green	Green
7.	Powder+ConH ₂ SO ₄	Black	Black	Brownish Black
8.	Powder+Ammonia solution	Light buff		
9.	Powder+5%Aqueous NaOH	No change	No change	No change
10.	Powder+5% Aqueous KOH Solution.	Brown	Brown	Light brown

Macroscopical characters roots

Roots measures 30 cm. to 50 cm. long and 10-15 cm. width. Juvenile roots have smooth and soft surface, light brown coloured, easily breakable by hand but mature roots are hard and not easily breakable by hand, fracture fibrous, smell agreeable, taste slightly sweet and mucilaginous. (Fig: 1&2)

Microscopical characters roots: T.S of the root is circular in outline shows outermost Phellum, Phellogen and Phelloderm with prominent Stellar region (Fig:22). Phellum is many layered cells, (Fig: 23&24) thin walled, rectangular, small, phellogen 3to 5 layered, cells broad. Phelloderm many layered cells are thin walled, parenchymatous, tangentially elongated filled with simple starch grains and abundant reddish tannin content (Fig: 23,25&26). Cortex shows prominent raphide sacs (Fig: 30&31). Pholem well developed, cells thin walled, small, Cambium prominent (Fig: 28&29). Xylem vessels were well developed with tracheids. Medullary rays are uni to biseriate and filled with simple starch grains (Fig: 23, 27, 28 & 29). Pith absent and central region is occupied by secondary xylem.

Diagnostic characters:

1. Presence of abundant reddish tannin content in cortex region
2. Presence of raphide sacs in cortex region.

3. Presence of simple, rounded to oval starch grains in cortex region and also in medullary ray region.

4. Presence of well developed xylem with tracheids.

Maceration studies

The root is cut into 1 cm² slices and subjected to a solution, consist Chromic acid and HNO₃ in 3:1 ratio. This treatment is given for 5-6 hours. By this treatment root is converted in to fine powder form. Now this powder is subjected to microscopical studies. During these study vessels, xylem parenchyma and xylem fibers are observed. Vessel elements are wider and short, measures 250-500 μm long and 25-30 μm wide without short tails and with simple, oblique Perforation plates. (Fig. 33). In root, only narrow fibers are observed, they are 650-950μm long and 5 -7μm wide (Fig. 35& 37). A series of xylem parenchyma cells are observed (Fig. 36).

DISCUSSION AND CONCLUSION

Cissus vitiginea L. is one of the important plants used in the treatment of healing the wounds, swellings, and sprains by Yanadi tribes of Nellore district. This plant belongs to Vitaceae family and has resemblance with most of the plants of this family. The identification of the plant is difficult. By this study the plant was authentically identified. In this paper the

macro and microscopical characters of the leaf and root along with the florasense studies (Table No-1) are presented. The parameters of present study can be used as a reference for further scientific investigations.

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Cite this article as:

G. Penchala Pratap, G. Sudarsanam, G. P. Prasad, J. Sekhar, Microscopical Observations on *Cissus Vitiginea* L. Int. J. Ayur. Pharma Research. 2014;2(4): 47-58

Source of support: Nil, Conflict of interest: None Declared

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PHOTOGRAPHS



Fig: 1. Yanadi tribe with long roots of *C. Vitiginea*; Local Name: *Adavi Draksha*; Sanskrit Name: *Amlavetasah* Fig: 2 *C. Vitiginea* plant in juvenile stage with soft tuberous root



Fig: 3. *C. Vitiginea* with flowering and fruiting

IMAGES OF MICROSCOPICAL CHARACTERS OF THE LEAF

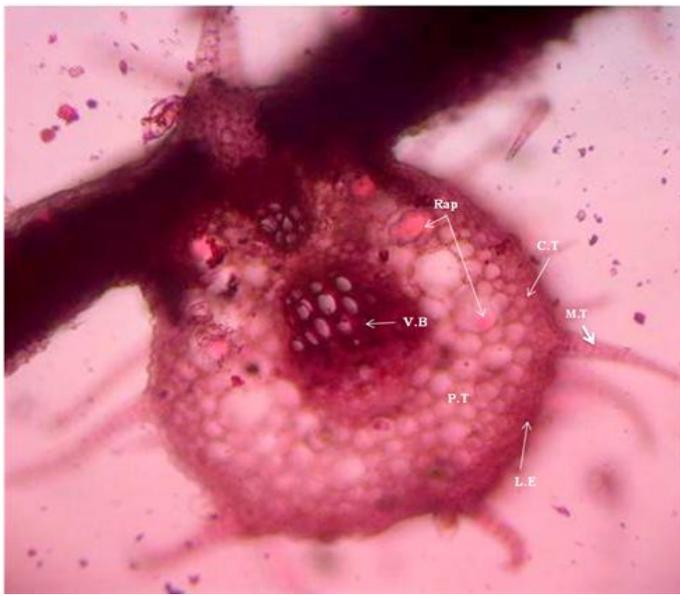


Fig:4.T.s of the leaf 10xX10x: L.E: Lower epidermis; M.T: Multi cellular trichome; Rap: Raphide sacs in Parenchyma tissue; C.T: 2 to 3 layered collenchymatous tissue ; L.E: Lower epidermis; P.T: Parenchyma tissue.

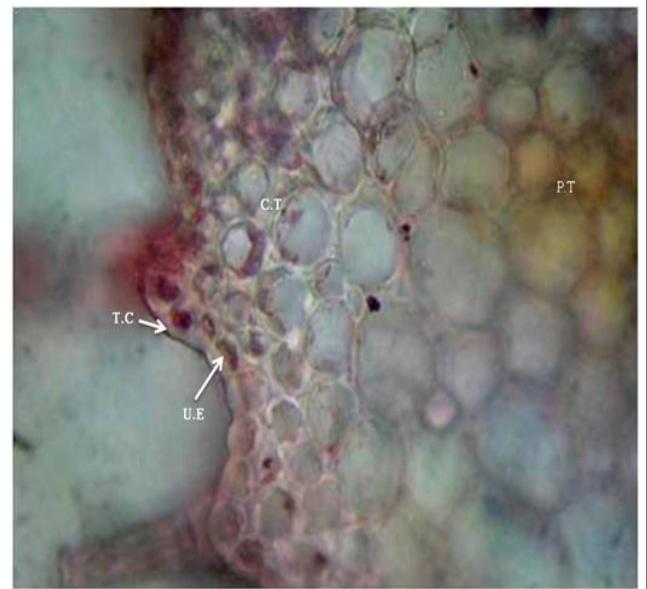


Fig:5. Upper region enlarged10x40x: U.E: Upper epidermis; T.C: Thick cuticle; C.T:2 to 3 layered collenchymatous tissue; P.T: Parenchyma tissue

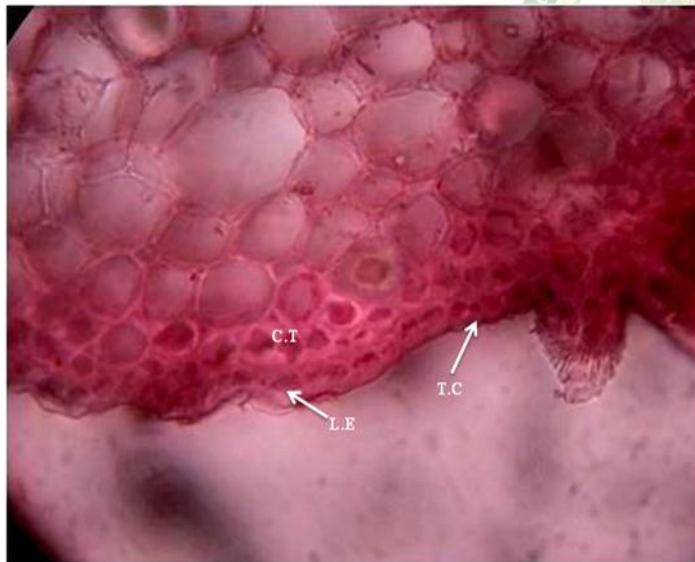


Fig:6 Lower region enlarged10x40x: L.E: Lower epidermis; T.C: Thick cuticle; C.T:2 to 3 layered collenchymatous tissue.



Fig:7 Vascular bundle enlarged with Phloem and Tannin contents 10xX40x

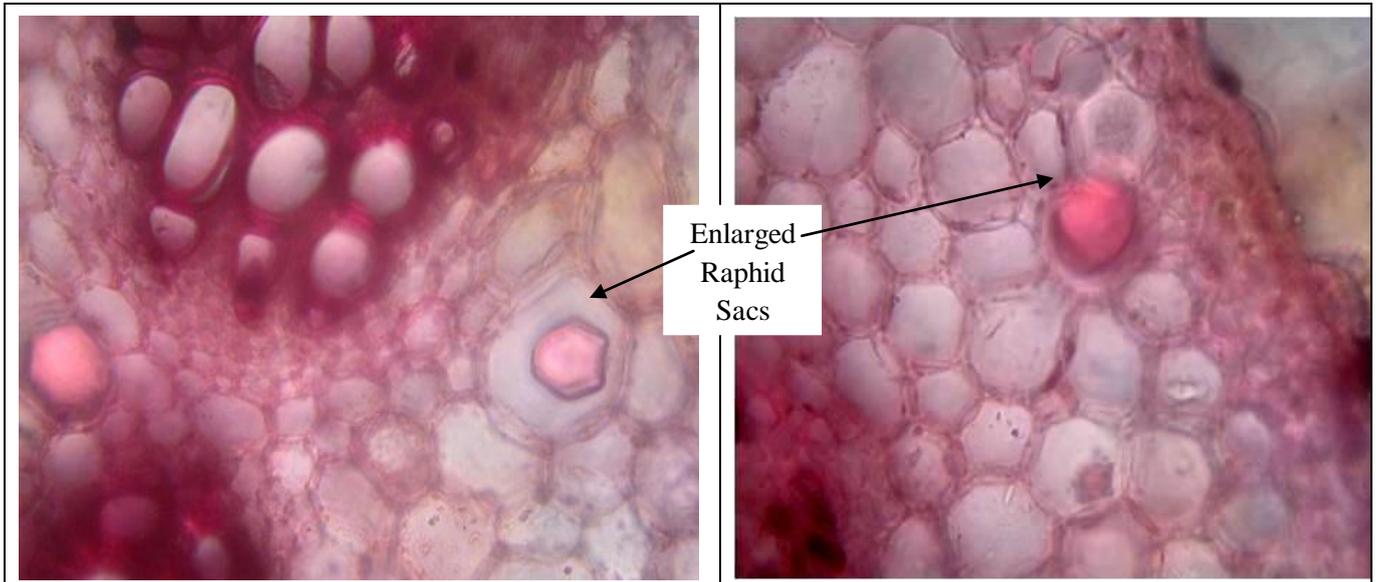


Fig:8 Parenchymatous region with prominent raphide sacs near to the phloem region.

Fig:9 Raphid Sacs in the Lower region enlarged

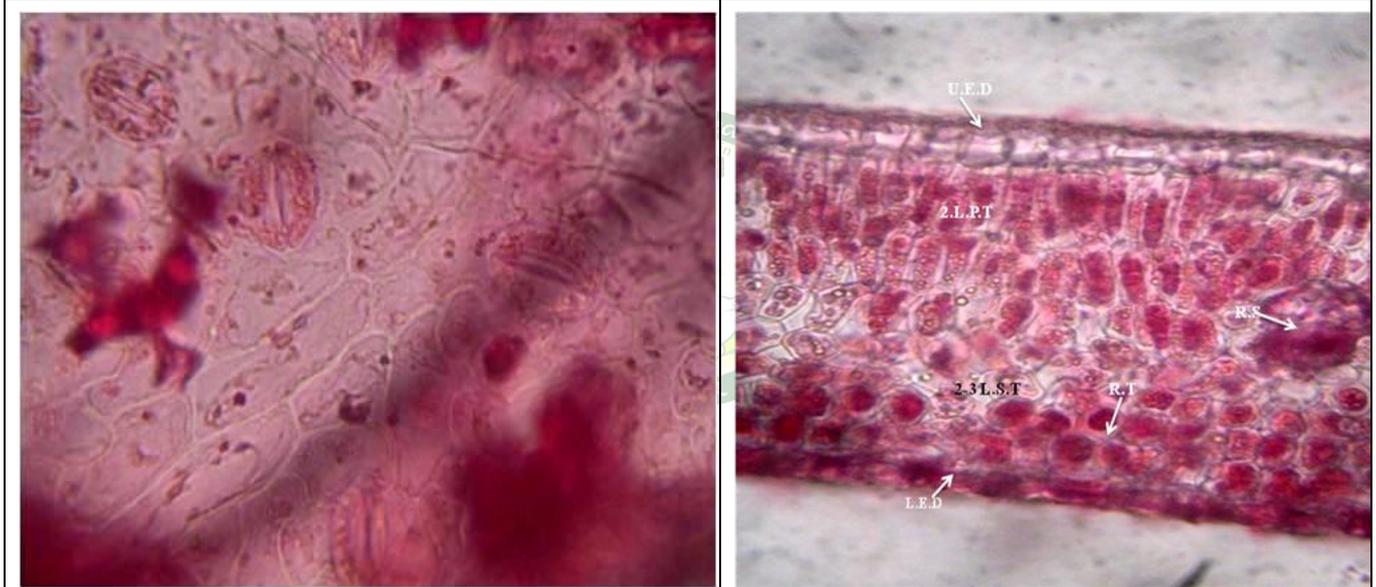


Fig:10 Ranunculaceae type of stomata in the lower epidermis

Fig:11Stomata 10x40x: U.E.D: Upper epidermis; L.E.D: Lower epidermis; 2L.P.T: 2 layered Palisade tissue; R.S: prominent raphide sacs in palisade cells; 2-3L.S.T: 2-3 layered loosely arranged Spongy tissue .

Images of Powder microscopy images:



Fig:12 Leaf powder

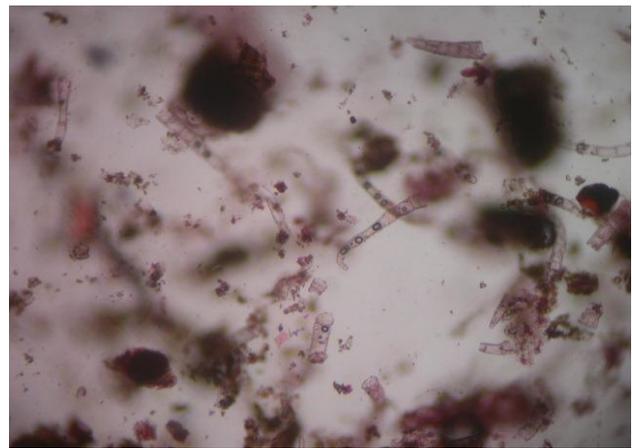


Fig:13 Different fragments of tissues 10x X10x

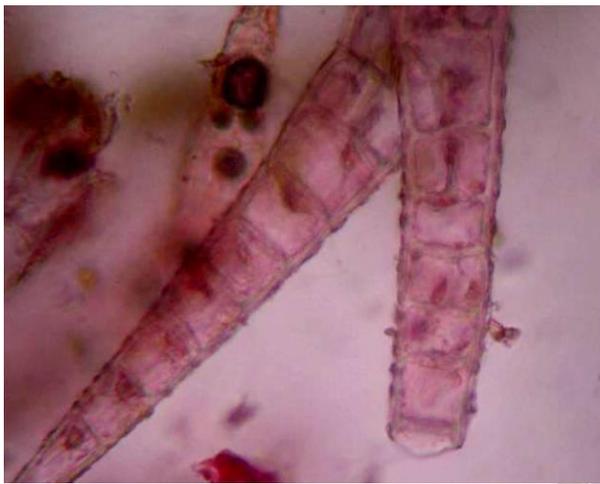


Fig:14 Multicellular trichomes 10xX40x



Fig:15 Raphide bundles 10x X10x



Fig:16 Parenchymatous cells 10x X40x



Fig:17 Acicular crystals 10x X40x

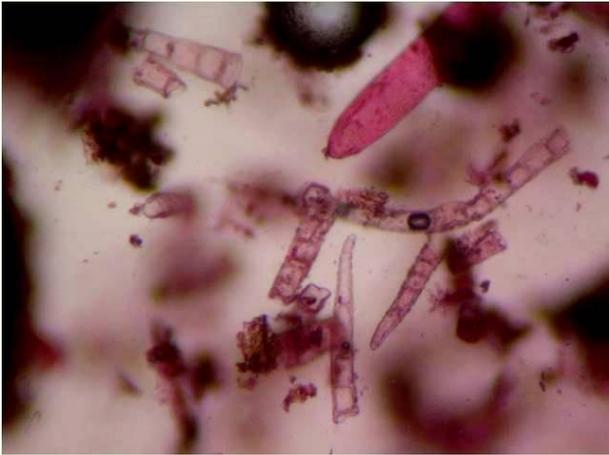


Fig:18 Abundant trichomes and raphide bundles. 10x X10x

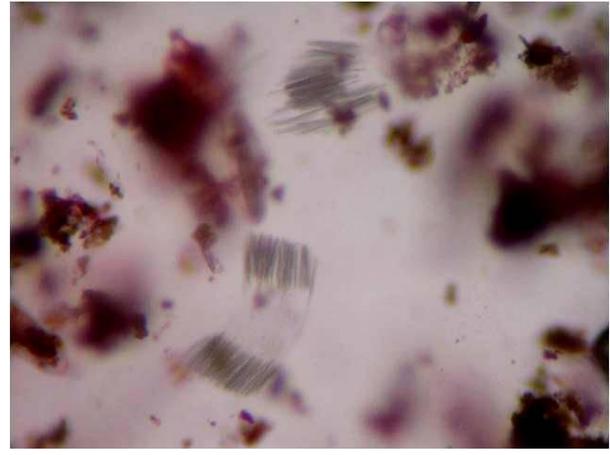


Fig:19 Abundant acicular crystals 10x X10x



Fig:20 Mucilage cells and trichomes 10x X10x

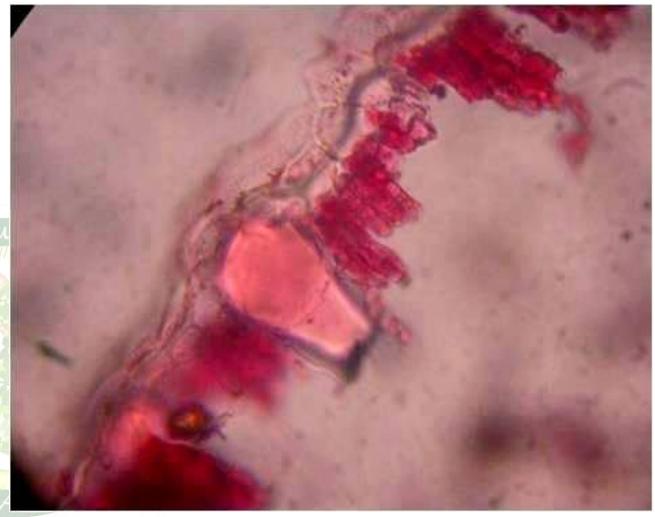


Fig:21 Palisade region with raphide sacs 10x40x

Images of Transverse section cutting of the Root:



Fig:22 T.s of the root .10xX4x with circular out line.

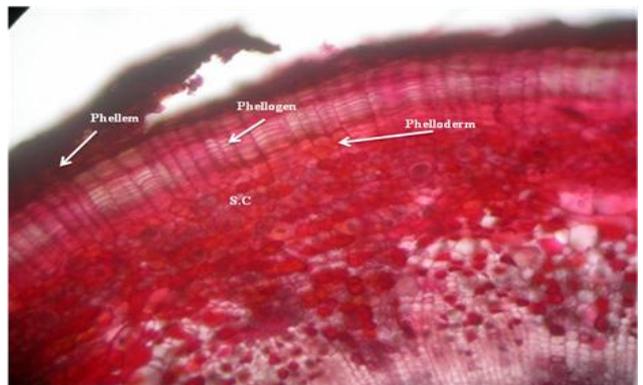
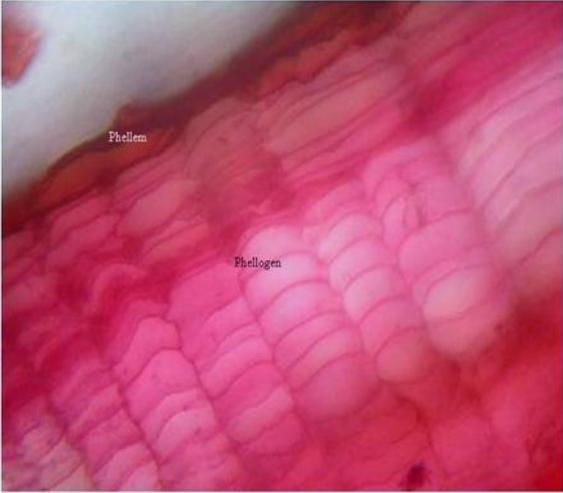
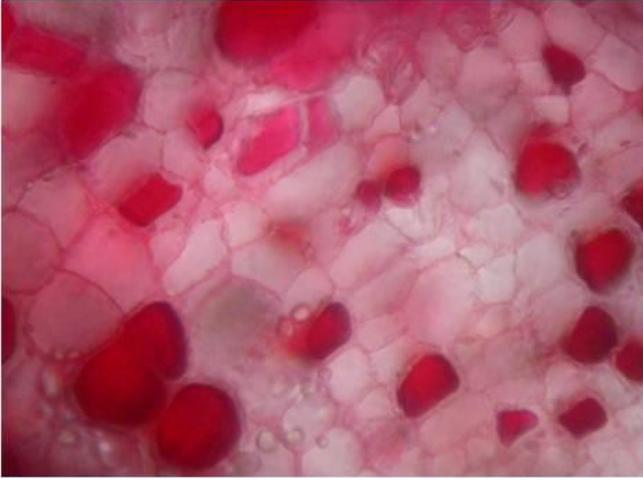
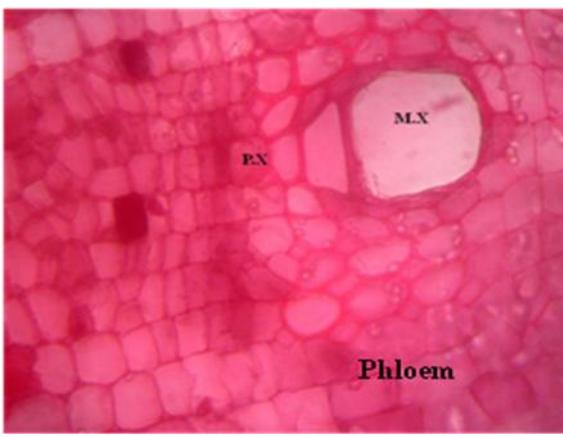
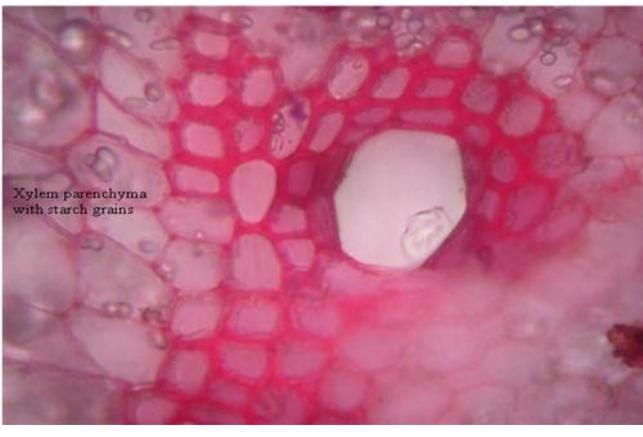
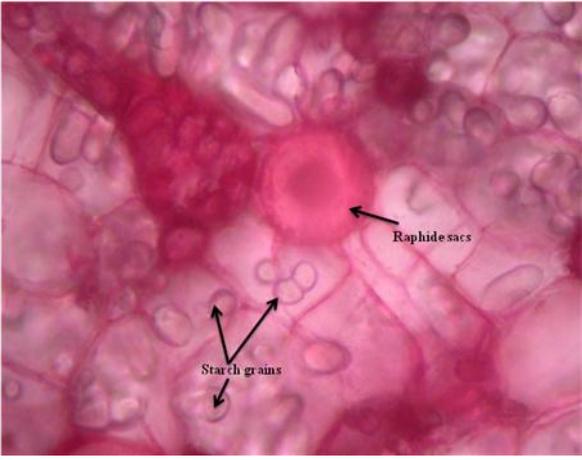
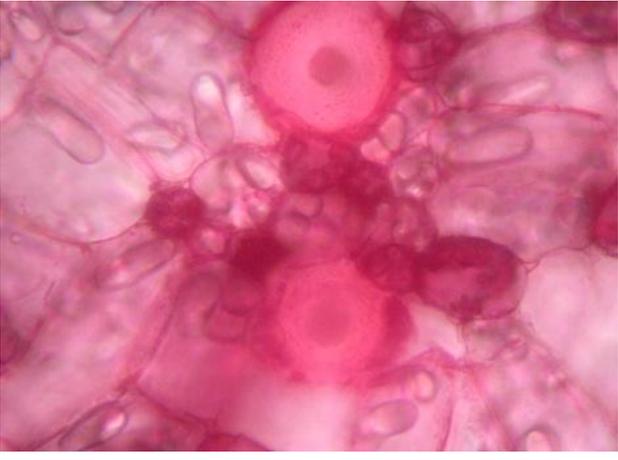
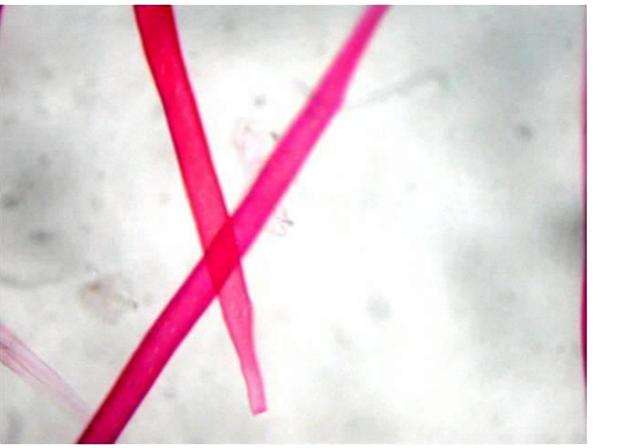
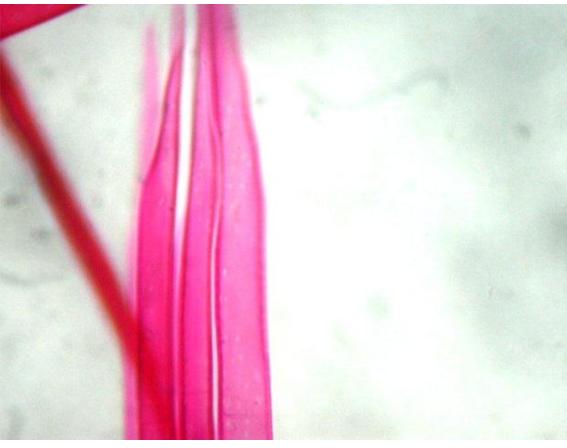


Fig:23 Cork and cortex enlarged .10xX10x: Phellem; Phellogen; Phelloderm; S/C: Secondary cortex

 <p>Phellem Phellogen</p>	
<p>Fig:24 Cork enlarged .10xX40x</p>	<p>Fig:25 Cortex enlarged showing abundant tannin content .10xX40x</p>
	
<p>Fig:26 Cortex and stelar region enlarged. With abundant tannin contents .10xX10x</p>	<p>Fig:27 Xylem elements enlarged</p>
 <p>PX MX Phloem</p>	 <p>Xylem parenchyma with starch grains</p>
<p>Fig:28 Xylem and phloem enlarged .10xX40x: M.X:Meta xylem elements; P.X: Proto xylem elements.</p>	<p>Fig:29 Xylem enlarged and Xylem parenchyma with starch grains 10xX40x</p>

 <p>A micrograph showing starch grains and raphide sacs in the cortex region. The starch grains are small, rounded, and have a distinct central core. The raphide sacs are larger, elongated, and contain needle-shaped crystals. Labels with arrows point to a 'Raphide sacs' and 'Starch grains'.</p>	 <p>A micrograph showing enlarged raphide sacs in the cortex region. The sacs are large and contain prominent, needle-shaped crystals.</p>
<p>Fig:30 Starch grain and Raphide sacs in cortex region.10xX40x</p>	<p>Fig:31 Raphide sacs enlarged sacs in cortex region .10xX40x</p>

Images of Maceration studies

 <p>A micrograph showing various macerated elements including vessels, fibers, tracheids, and xylem parenchyma. The elements are fragmented and stained red.</p>	 <p>A micrograph showing xylem vessels with pitted thickenings. The vessels are elongated and have characteristic pitted walls.</p>
<p>Fig:32 Different Macerated elements like vessels, fibers, tracheids, xylem parenchyma . 10x X 10 x</p>	<p>Fig:33 Xylem vessels with pitted thickenings. 10x X 40 x</p>
 <p>A micrograph showing tracheids. The tracheids are elongated, tapered cells with thickened walls.</p>	 <p>A micrograph showing xylem fibers. The fibers are long, thin, and have thickened walls.</p>
<p>Fig:34 Tracheids. 10x X 40 x</p>	<p>Fig:35 Xylem fibers. 10x X 40 x</p>

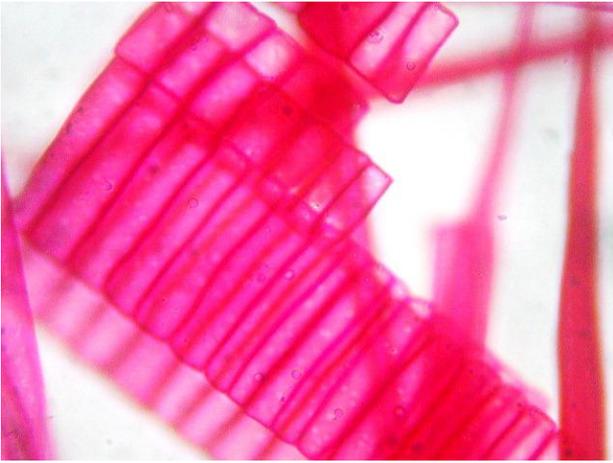


Fig:36 Xylem parenchyma 10x X 40 x

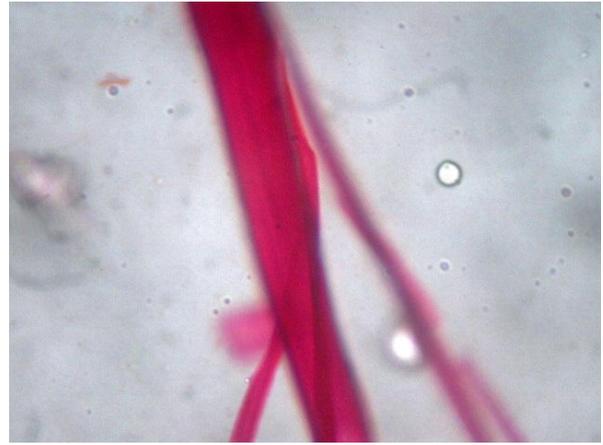


Fig:37 Tracheids and fibers,.10x X 40 x

