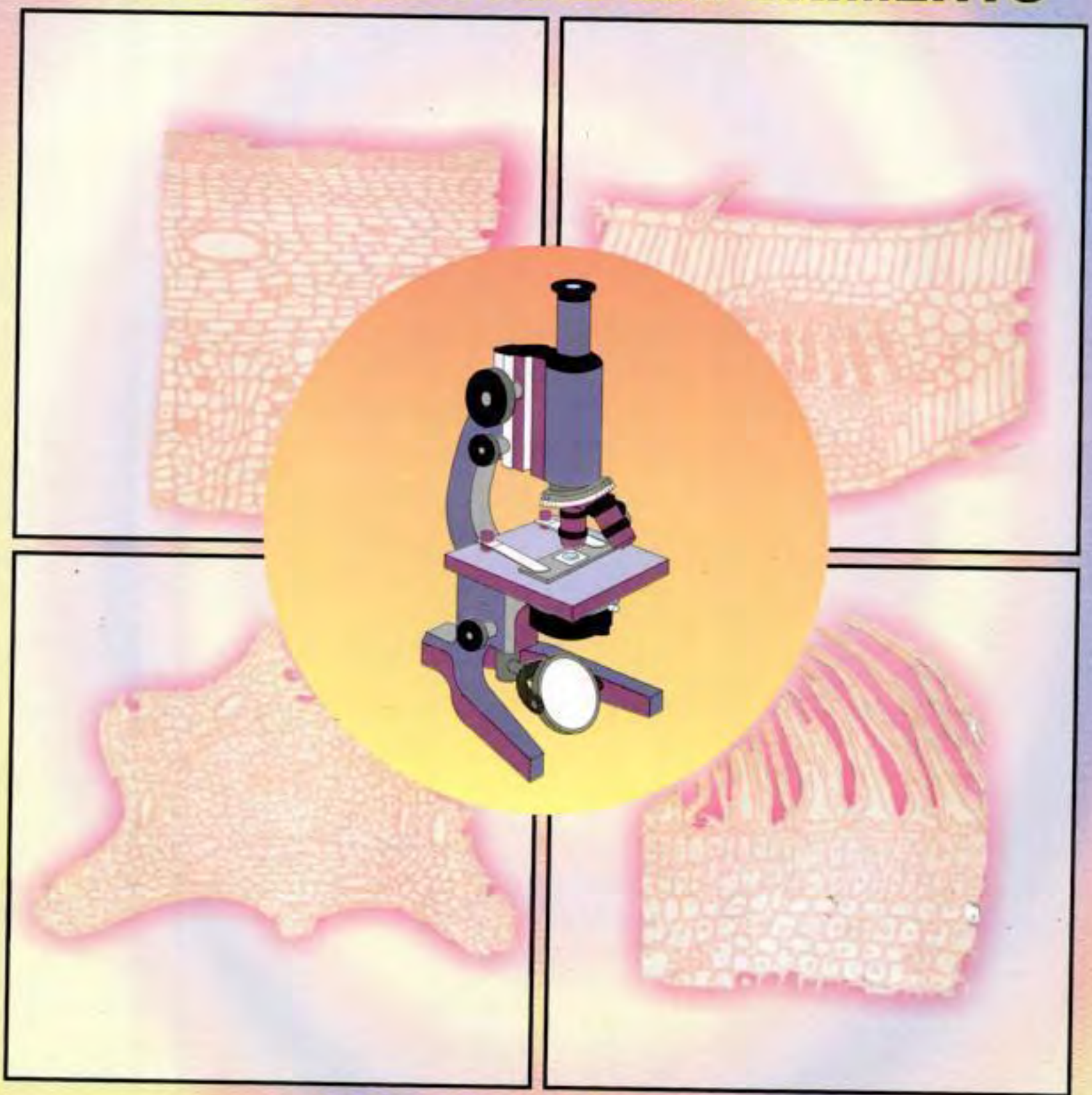
PRACTICAL

PHARMACOGNOSY

TECHNIQUES AND EXPERIMENTS



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Study of crude drugs :

i) **Wild cherry bark**
ii) **Capsicum fruit**
iii) **Andrographis stem (Kalmegh)**
iv) **Asparagus root (Shatavari)**
v) **Eucalyptus leaf**
vi) **Azadiracta leaf (Neem)**
vii) **Podophyllum root**
viii) **Tinospora stem (Gulvel)**
ix) **Withania root (Ashwagandha)**

1 THE MICROSCOPE

A microscope may be defined as an optical instrument, comprising of a lens or a combination of lenses which enables to view magnified images of a minute object .

MICROSCOPE

SIMPLE	**COMPOUND**
One set of lens.	Two sets of lenses-Eyepiece and Objective
Lower magnification.	Higher magnification.

1.1 SIMPLE MICROSCOPE (DISSECTING MICROSCOPE)

It helps to reveal the morphological characteristics of the object.

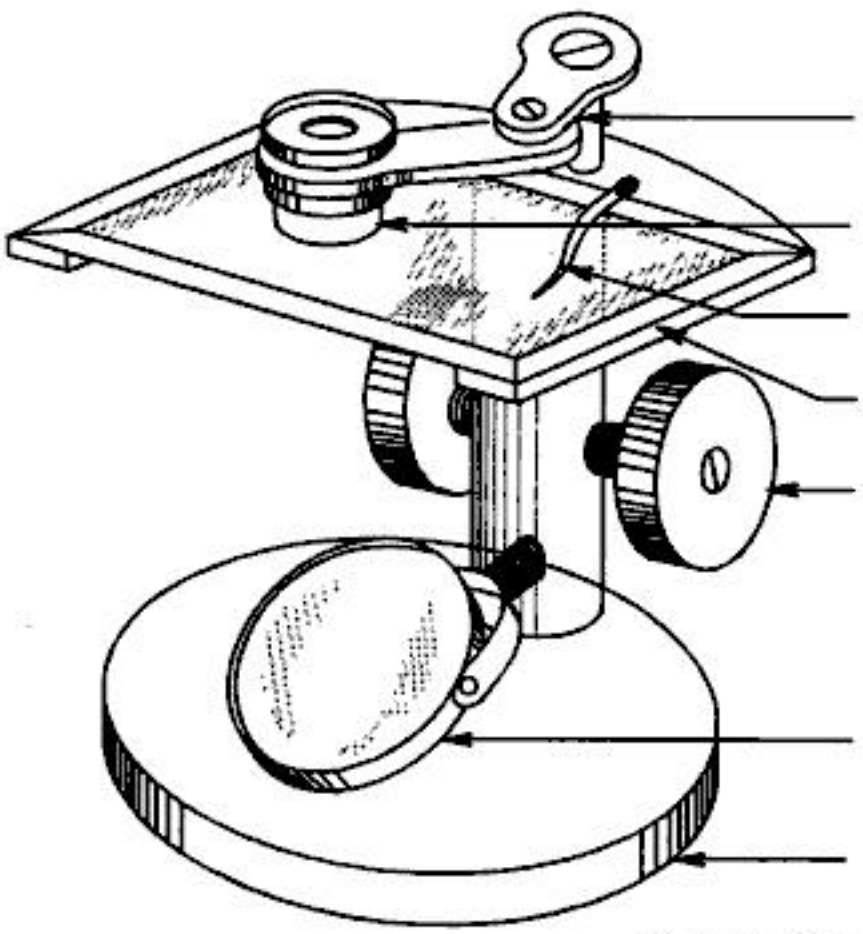


Fig. 1.1 : Simple Microscope

1.2 COMPOUND MICROSCOPE

The compound microscope essentially consists of three major systems.

(I) Support system :

It comprises of base, stage and body tube.

(II) Illumination system :

It throws light on the object for proper viewing. It comprises of light source or mirror, iris diaphragm and condenser . The light source may be a plain or concave mirror or electrically illuminated by a tungsten filament lamp or a halogen lamp. Mirror and electric light source are generally interchangeable.

(III) Magnification system :

This includes a set of lenses aligned in such a manner so that a magnified real image can be viewed. The objective is a set of lenses placed near the object. It partially magnifies the object, which can be observed through the EYEPIECE in a more magnified form.

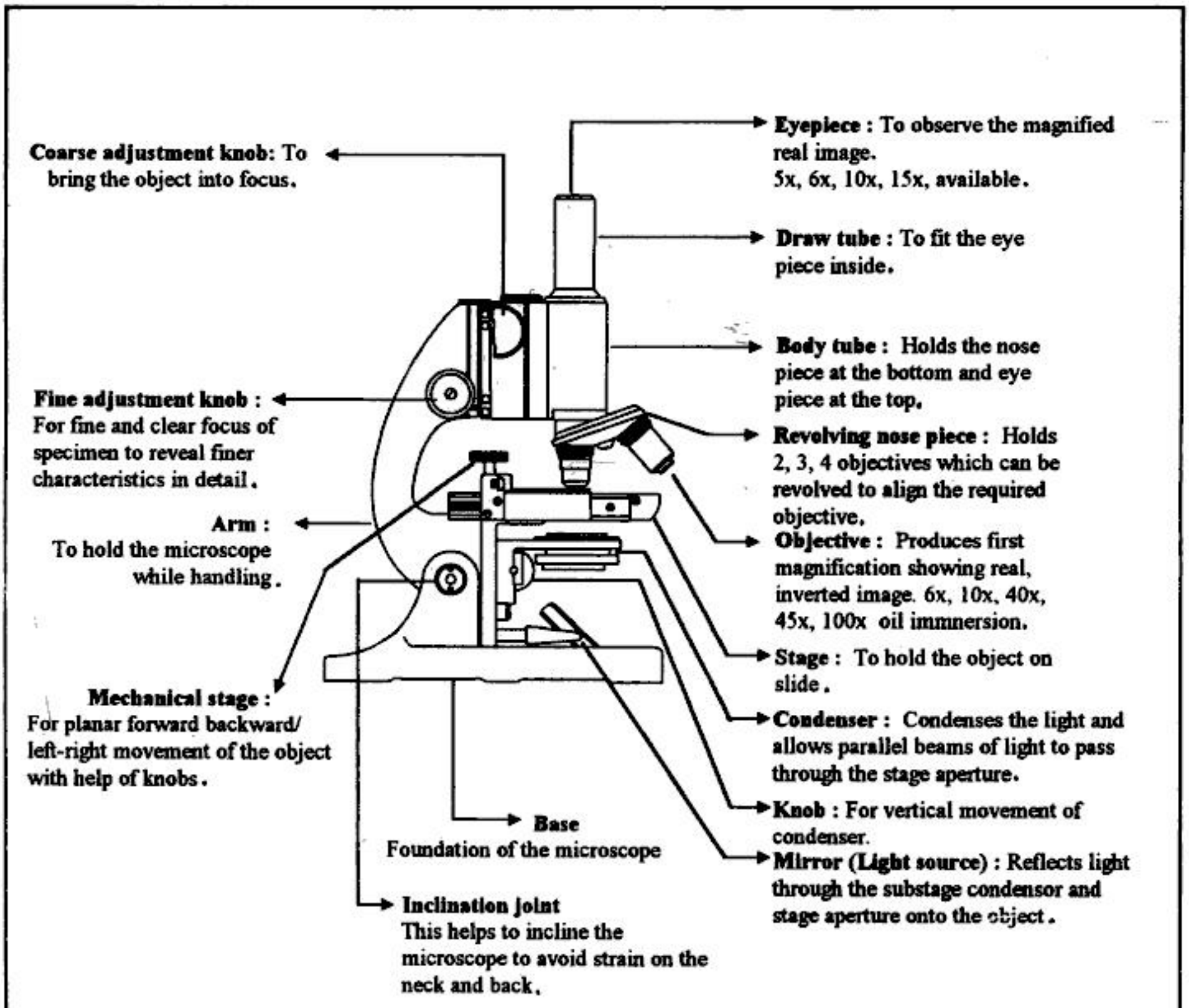


Fig. 1.2 : Compound Microscope

1.3 KNOW YOUR MICROSCOPE

1.3.1 DIFFERENT ILLUMINATION SYSTEMS USED IN MICROSCOPES

(A) Plain Mirror

Use ***Plain Mirror*** when a fixed source of light is used.

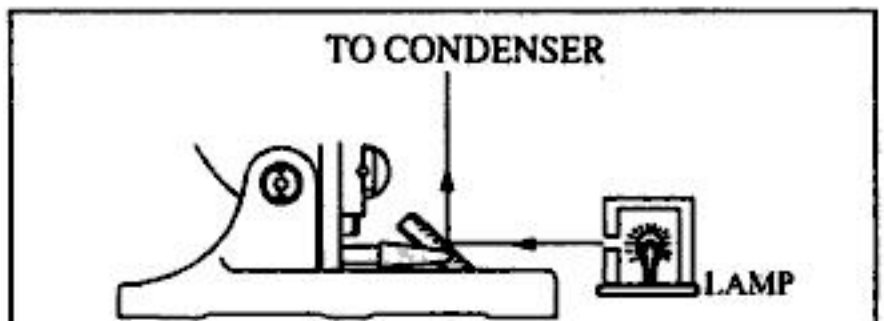


Fig. 1.3 A

(B) Concave Mirror

When skylight is used, ***Concave Mirror*** helps to converge the beam onto the condenser.

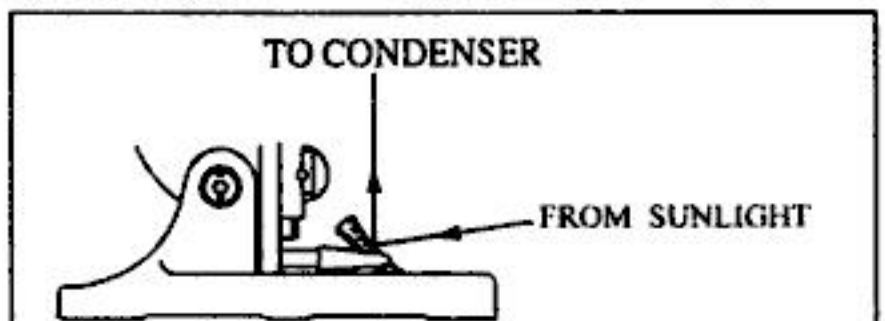


Fig. 1.3 B

(C) Substage Lamp Interchangeable with mirror

Where there is no electricity or battery, mirror can be used.

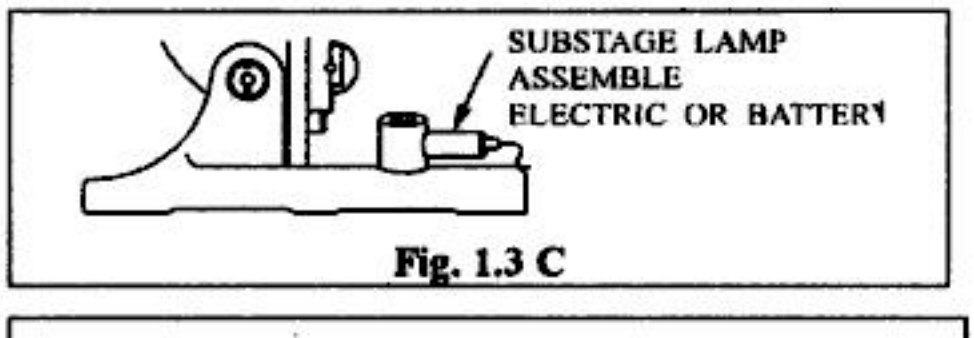


Fig. 1.3 C

(D) Built-in substage lamp (Tungsten-Filament or Halogen Lamp) with intensity adjustment

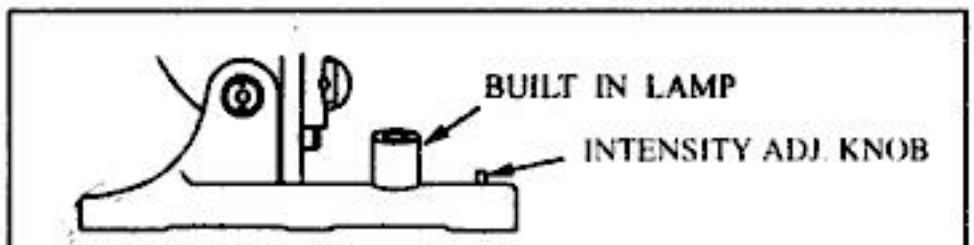


Fig. 1.3 D

1.3.2 LIGHT ADJUSTMENT IN A MICROSCOPE

While viewing an object, sometimes, the object has to be brightly illuminated; on other occasions, less light is needed.

(I) The light rays on the object can be altered in 2 ways by means of CONDENSER :

(A) Condenser can be moved upwards with the knob so as to make the object more brighter.

(B) Condenser can be moved downwards to make the objects less brighter.

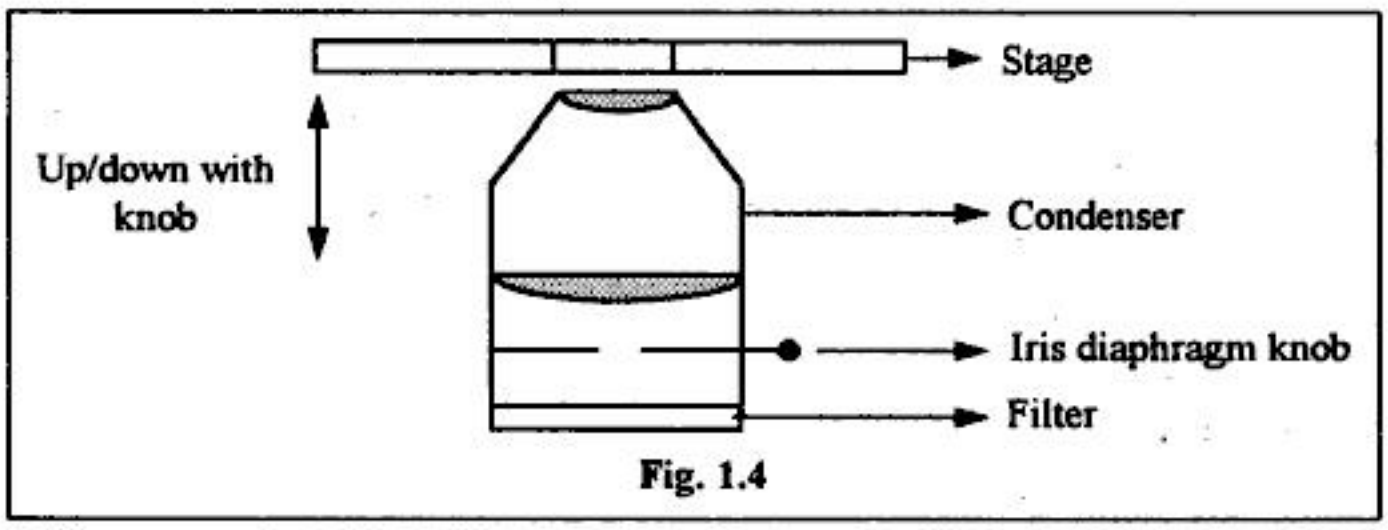
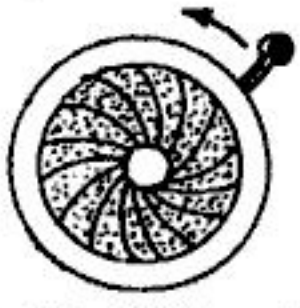


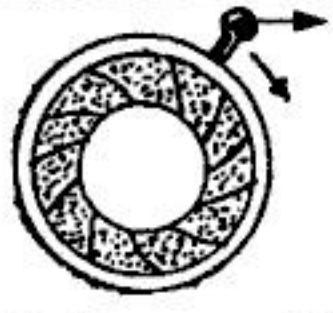
Fig. 1.4

(II) Light illuminating the object may be adjusted with IRIS DIAPHRAGM. The diaphragm may be opened or closed to increase or diminish the light falling on the condenser hence making the object more or less bright.

IRIS DIAPHRAGM (looking up from underneath)



Nearly shut, little light is getting through this hole into the condenser



Nearly open, much light is getting through this big hole into the condenser

Fig. 1.5

1.3.3 FACTS AND FIGURES ABOUT (YOUR) MICROSCOPE

(A) Magnifying Power (M.P.) :

M.P. – Magnification of objective × Magnification of eye piece. e.g. if you are observing an object on a slide using a 10x objective and 5x eyepiece then MP = 10 × 5 = 50
Thus, the object viewed is magnified 50 times.

(B) Resolving Power of objective (R.P.) :

Resolving power of an objective is defined as the ability to separate distinctly two small elements of an object which are situated a short distance apart. R.P. can be measured by Numerical Aperture (N.A) of an objective Greater the N.A., greater is the resolving power.

(C) Working distance :

The distance between the object and the objective is known as working distance

The working distance decreases with increasing magnification. This means higher the power of objective, lesser is the working distance.

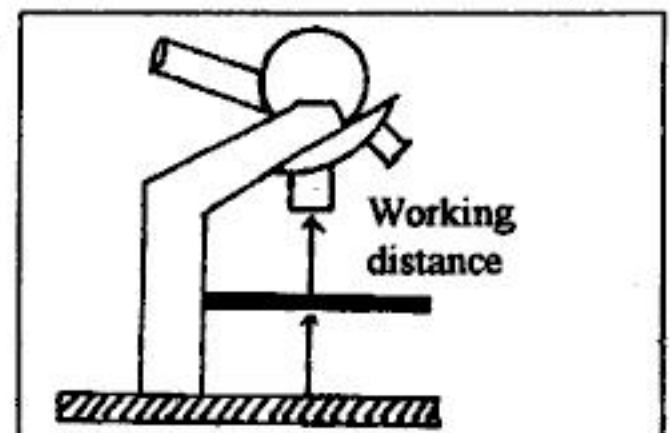


Fig. 1.6

(D) Focusing :

Focusing an object while viewing through an eye piece means, adjustment of working distance. This is done, with the help of coarse adjustment and fine adjustment knob. Coarse adjustment knob is rotated to bring the object in field of view and the fine adjustment knob is rotated to get a sharp image.

(E) Field of view :

The area of the object which one can view through the eye piece is the field of view. The field of view narrows as magnification increases.

(F) Objectives : Different objectives used in microscopy.

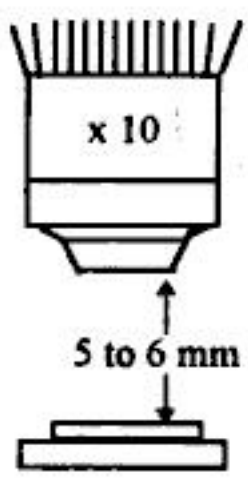
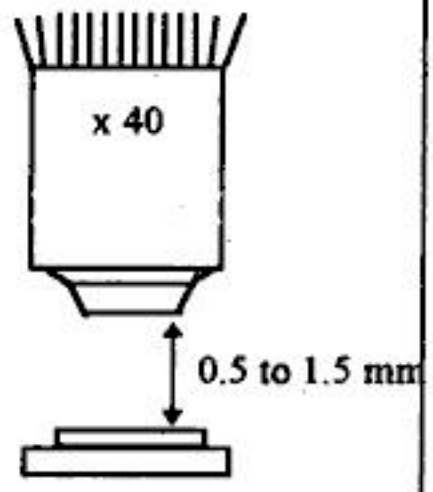
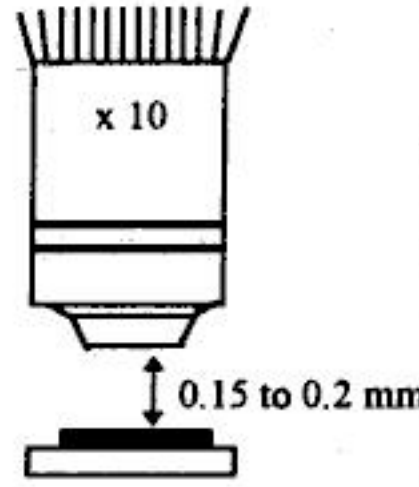
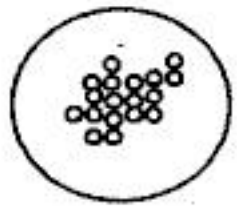
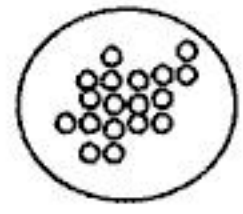
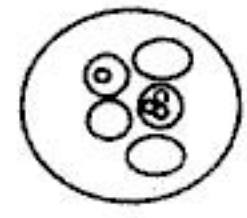
(i) 4x – Also known as scanner, which is very low power and is used mainly, to bring a particular part of the, object in the field of view.

(ii) 10 x – Low power objective; with the help of this one identifies the part to be observed in high power. This does not reveal much details.

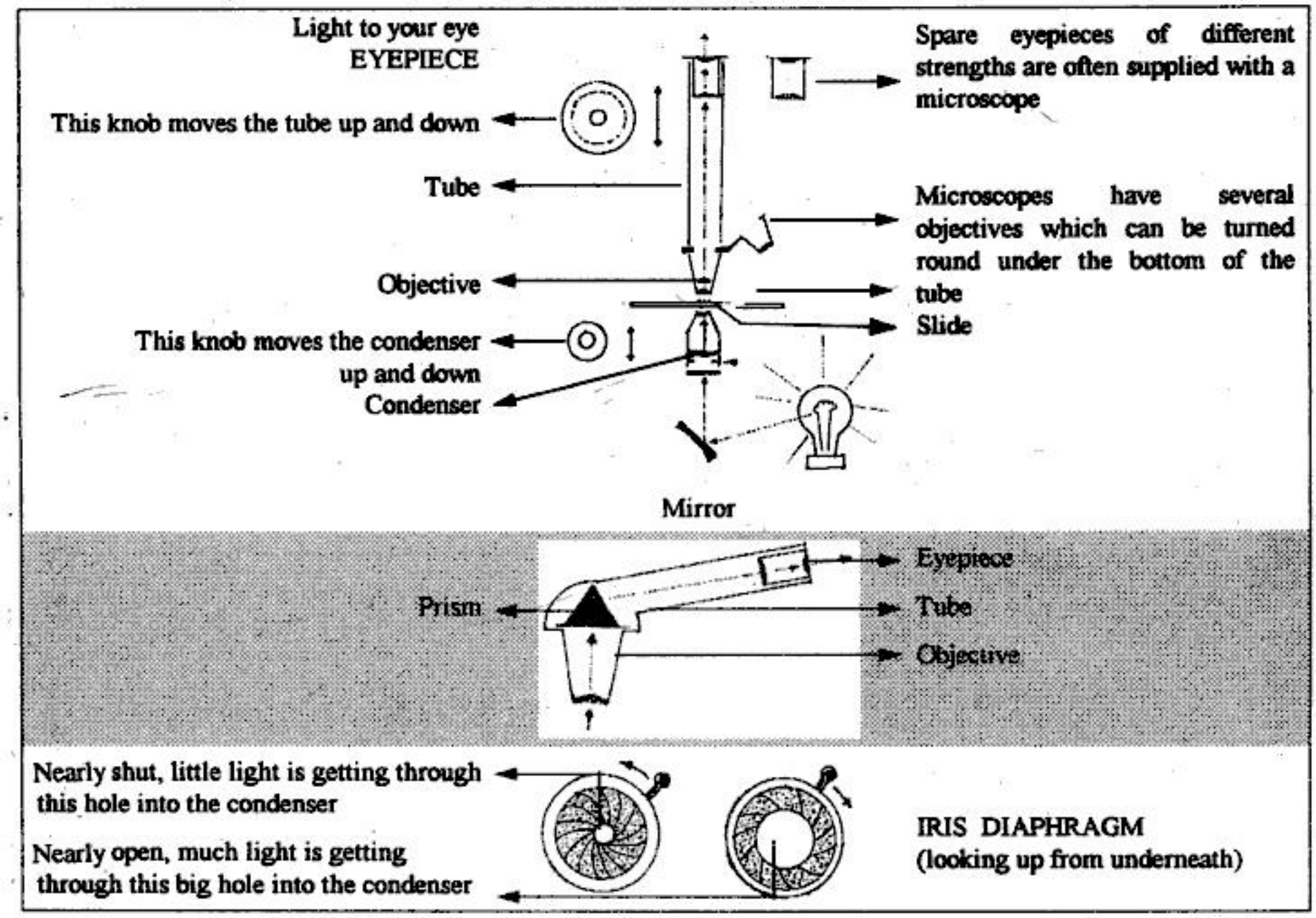
(iii) 40 x – This is a high power objective to reveal finer details of the object. This is spring loaded, which means that a spring is fitted between the front and back lenses of the objective. This is to protect the front lens, as the working distance is low in high magnification and the lens may touch the slide while focussing. The spring does not allow pressure on the front lens when it touches the slide.

(iv) 100x – Oil immersion lens – This also is a spring loaded objective, requiring very low working distance and will give an image only when the object is immersed in CEDAR WOOD OIL. This oil is used as it has high refractivity and allows very high resolving power.

COMPARISON OF OBJECTIVES

TYPE OF OBJECTIVE	LOW POWER	HIGH POWER	OIL IMMERSION
MAGNIFICATION	10 X	40 X	100 X
COLOUR CODE FOR IDENTIFICATION	GREEN	YELLOW	RED
ILLUMINATION REQUIRED	MINIMUM	MORE THAN LOW POWER	MAXIMUM
FIELD OF VIEW	WIDE	NARROWER THAN LOW POWER	NARROWER THAN HIGH POWER
WORKING DISTANCE			
RESOLVING POWER AND NA	 LOW NA = 0.3	 HIGH NA = 0.65	 VERY HIGH NA = 1.3
MAGNIFYING POWER (M.P.) USING X10 EYE–PIECE	10 × 10 = 100	10 × 40 = 400	10 × 100 = 1000

Proper knowledge of handling a microscope will thus, enable a pharmacognosist to get the best possible results with even the simplest of microscopes, accompanied with good quality of optics.



1.4 RECENT ADVANCES IN MICROSCOPES

Since the invention of microscope, it has undergone many changes in size, shape, material and uses.

The commonly used student microscope with one eyepiece is the **Monocular microscope**, with a vertical body tube and draw tube. (Fig. 1.6 A).

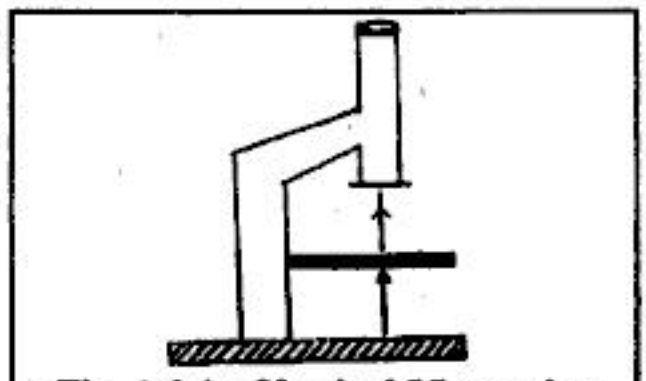


Fig. 1.6 A : Vertical Monocular

To avoid fatigue to neck and back, this microscope was built with an **angular or inclined draw tube** (Fig. 1.6 B).

This inclined draw tube was made fully rotatable through 360°, so as to enable more than one person to view the object without changing seats but by only rotating the draw tube head.

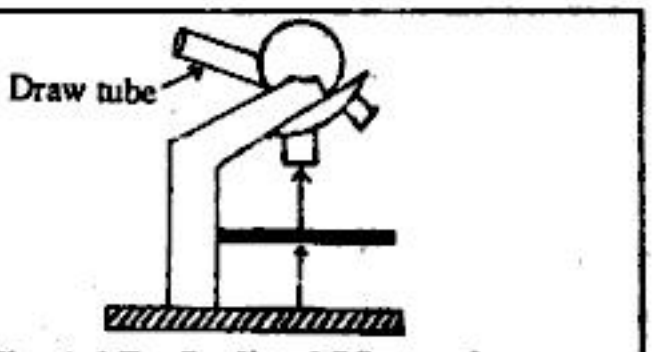


Fig. 1.6 B : Inclined Monocular

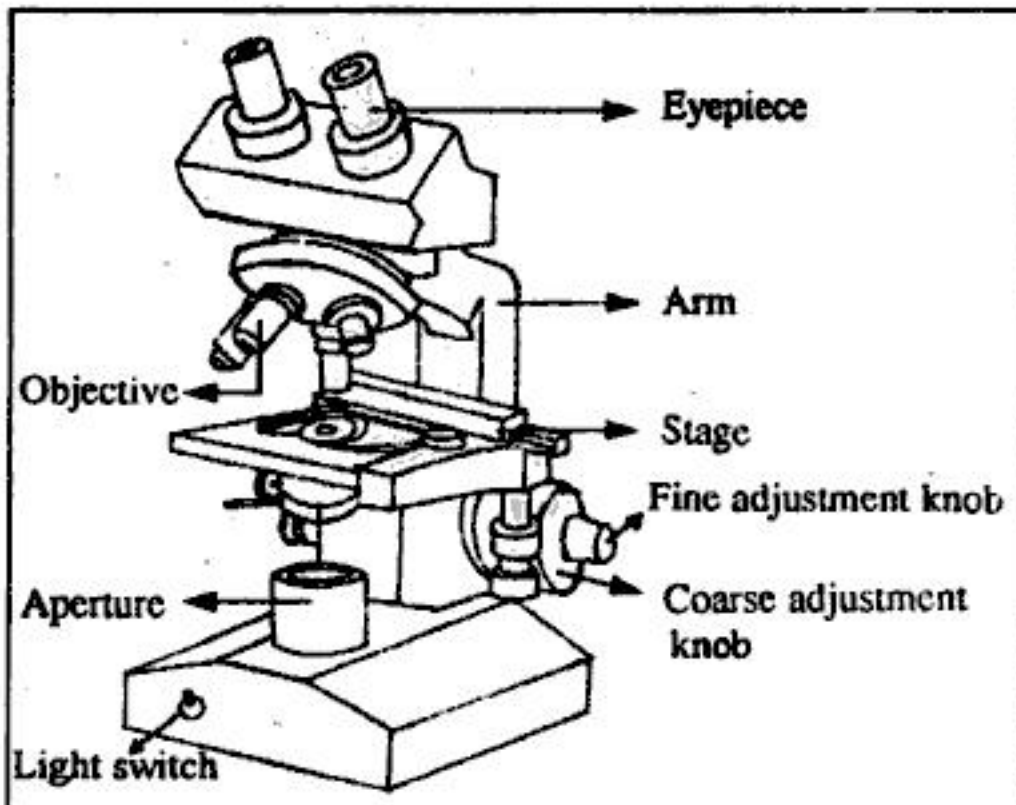


Fig. 1.6 C : Binocular Microscope

Then came the inclined binocular microscope which enabled the viewer to observe with both eyes open hence reducing eye fatigue.

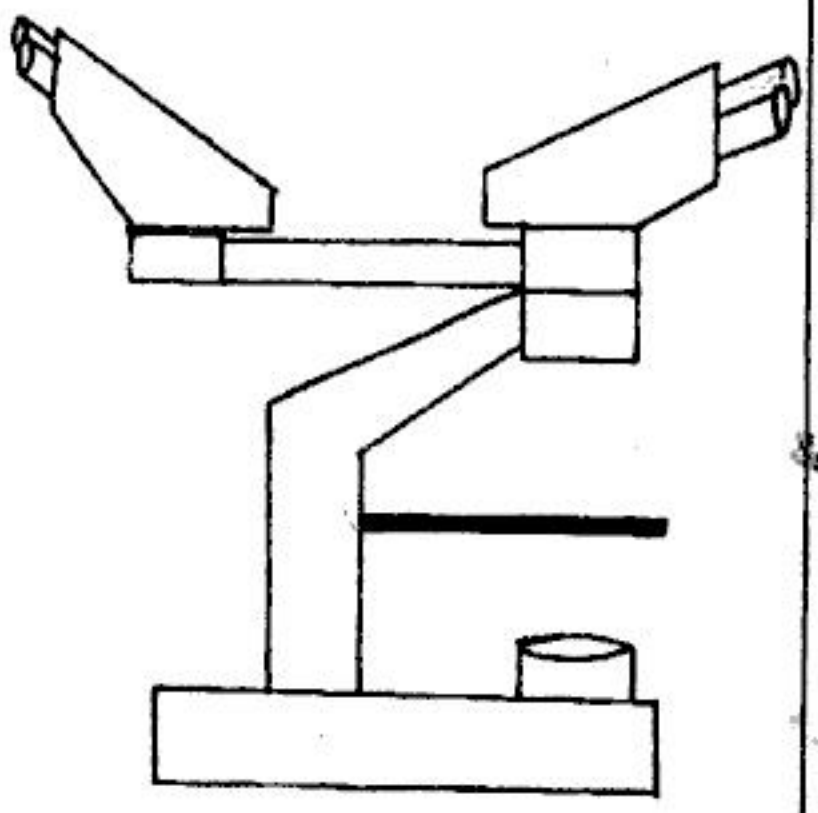


Fig. 1.6 D : Dual Viewing Microscope

Dual viewing microscopes for two persons to observe at a time.

Trinocular Microscopes are available with a binocular head with a vertical draw tube attached, which helps in photomicrographic work. (Fig. 1.6 E)

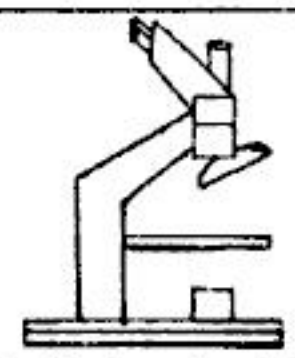


Fig. 1.6 E : Trinocular Microscope

Projection Microscopes are available which project the image of the object on a 6"-8" wide screen, built at the top of the microscope (fig. 1.6 F)

Stereo microscopes are available with zoom facilities with greater flatness and contrast and long working distances ensuring more distinct images.

Advanced photomicrographic equipment is available for taking photagraphs of the slides observed under microscopes.

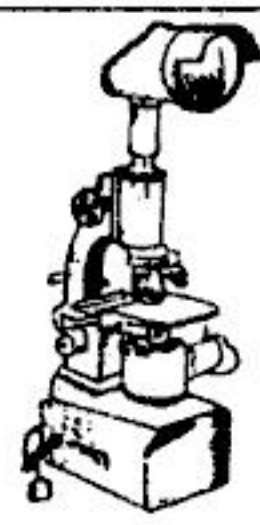


Fig. 1.6 F : Projection Microscope

Phase contrast microscope :

In this type of microscopy controlled illuminations of the specimen and special phase contrast objectives are used. By phase contrast, contrast can be added to normally invisible objects and hence more details can be observed.

In this technique, light passes through the annular diphragm of microscope. The rays passing through diaphragm are of two types i.e. deviated and undeviated. Amplitude (height of wave length) of deviated rays get reduced by the condenser by 1/4., while the undeviated rays do not get affected. In the second stage when the deviated rays pass further through diffraction plate (phase plate) of microscope, again 1/4 decrease in amplitude takes place and becomes actually half of the original amplitude. Brightness depends upon amplitude. Thus a contrast is seen between the deviated and undeviated rays which ultimately results in lowering the brightness. The change is due to diffraction, which is the cause of different densities of the material and two types of rays produced by annular diaphragm and diffraction plate.

Phase -contrast microscopy technique is largely utilised for studying living objects like cells and tissues and specifically the cytological details of the organelles. This technique has been discovered by Fritz Zernike, the Noble prize winner in 1953. Magnification upto 2000 is possible.

Fluorescence microscope :

Some chemical substances absorb light waves of one wavelength and emit visible waves of greater wavelength. So material under observation appears of one colour by ordinary light and of an entirely different colour by ultraviolet light.

The materials are known as fluorescent and the phenomenon is known as *fluorescence*.

By this method, cancer can be detected in early stages, while bacteria of various types and even antigen-antibody complexes be studied rapidly and also with accuracy.

The technique is used for cinchona, gambier etc.

Ultra-violet microscope :

When ultra-violet light having short wave length of 180–400° mμ is used as source of radiation, instead of visible light of 400-700° mμ more magnification can be obtained since it has greater resolution. (Resolution is the ability of the microscope to differentiate between adjacent objects as separate entities which decides the magnifying capacity of microscope.)

The absorption of ultraviolet radiation by certain substances enable them to locate or separate under the microscope. In ultra-violet microscopy the image is made visible by using photographic emulsion.

Electron microscope :

For the maximum magnification to the tune of 2,00,000–4,00,000 times, electron microscopy is used, now-a-days. In electron microscopy, beam of electrons is used instead of light waves to produce the magnified image. In case of light microscopy, the source of illumination is light bulb or natural light. But in electron microscope tungsten filament with high voltage of 80 kV is used . Instead of glass lenses the electro-magnetic lenses are used in electron microscope and focusing is done by varying the current. In case of electron microscope image is not observed by eye, it is projected onto a photographic plate or screen.

Electron microscope is installed in dust-free, vibration-free area without magnetic-fields and in air-conditioned room.

The material should be perfect dry for observation. Living organisms cannot be observed. However, it is very useful for understanding the ultra-structure of viruses and different types of animal and plant cells. It is being used since 1940.

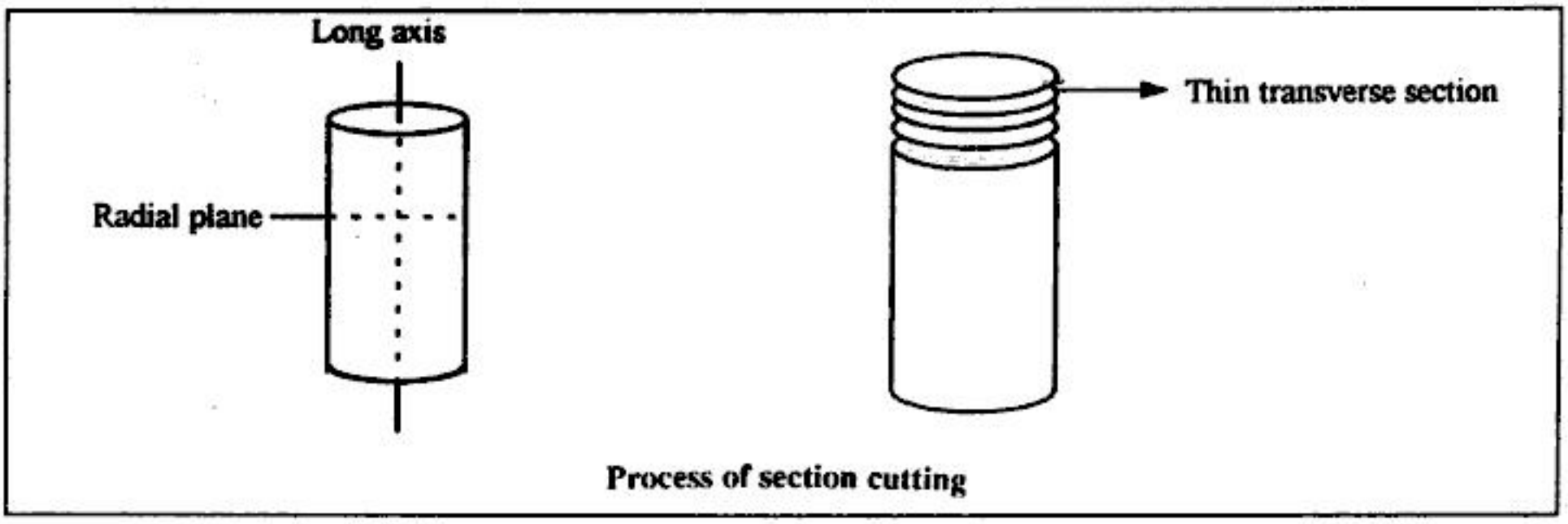
2 SECTION CUTTING TECHNIQUE

2.1 SECTION OF A STEM, ROOT, STOLON

Different sections can be obtained from a stem, root or stolon, depending on the plane of cutting, each section revealing details from a different angle.

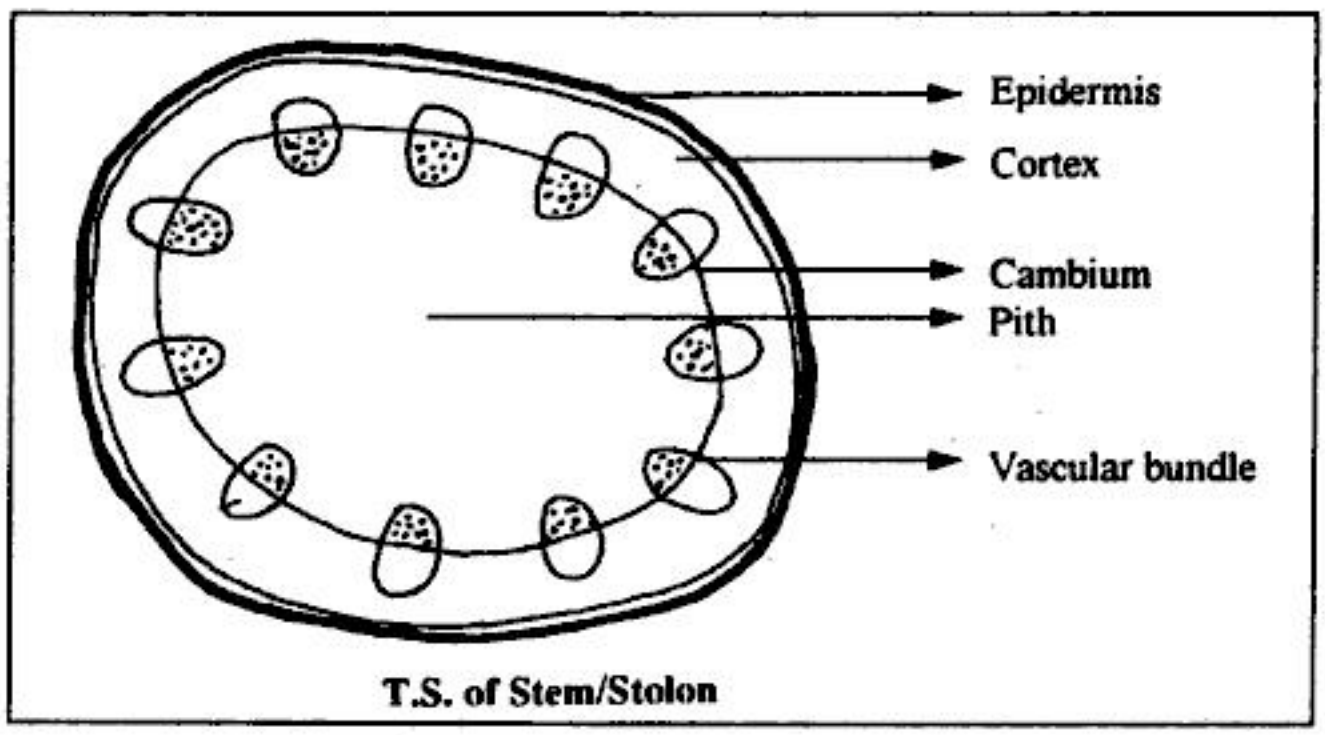
2.1.1 TRANSVERSE SECTION (T. S.)

Transverse section is obtained by cutting along the radial plane of a cylindrical portion of the stem/root/stolon and perpendicular to the long axis.



Process of section cutting

This section when prepared and observed under a microscope reveals the radial arrangement of tissues and shows concentric layers and vascular bundles.



T.S. of Stem/Stolon

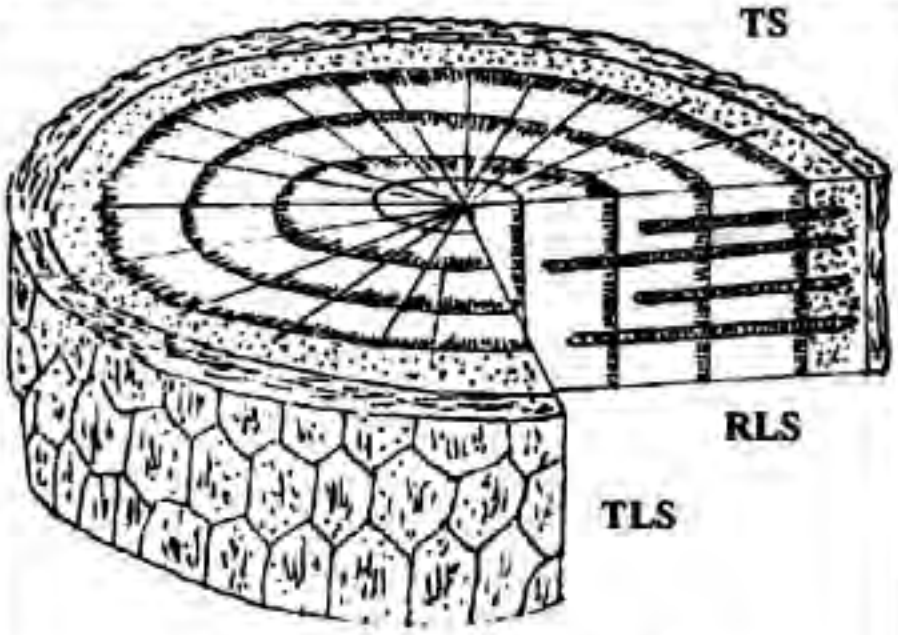
In order to reveal the tissue arrangement longitudinally viz. along the planes parallel to the long axis both radially and tangentially, we have to cut the longitudinal sections.

2.1.2 LONGITUDINAL SECTION

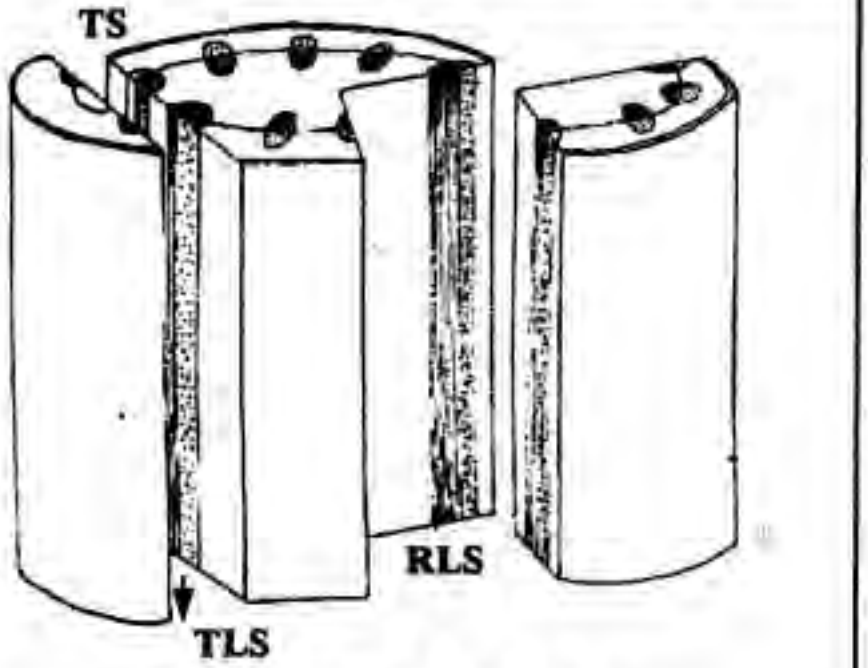
LONGITUDINAL SECTION

TANGENTIAL LONGITUDINAL SECTION
(A section cut along the long axis parallel to a tangent)

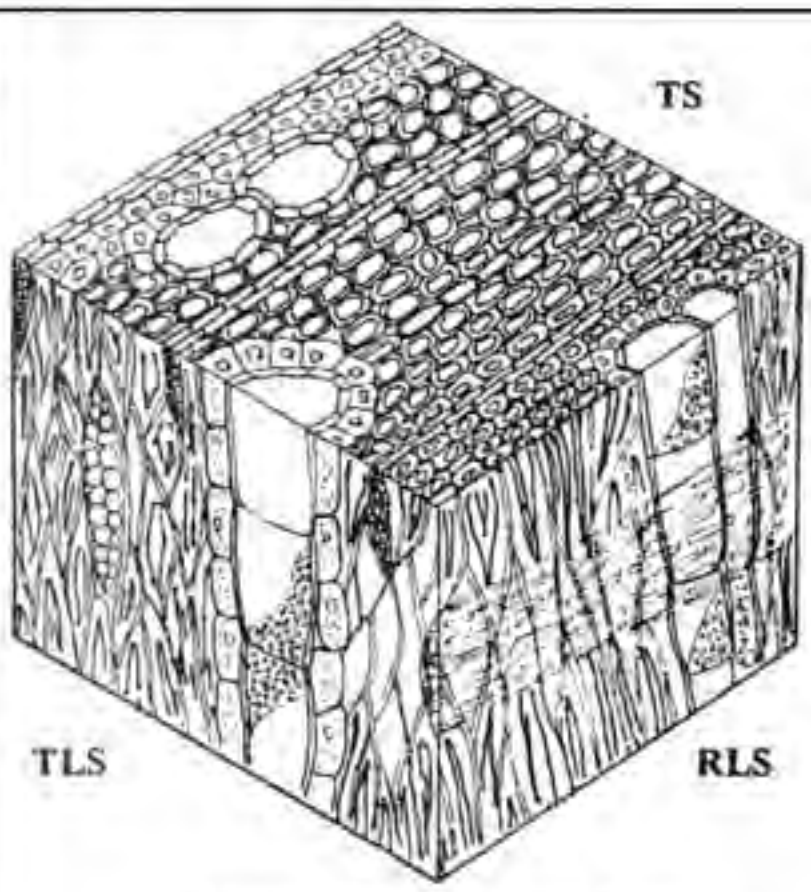
RADIAL LONGITUDINAL SECTION
(A section cut along the long axis and the cutting plane passing through the long axis and radius)



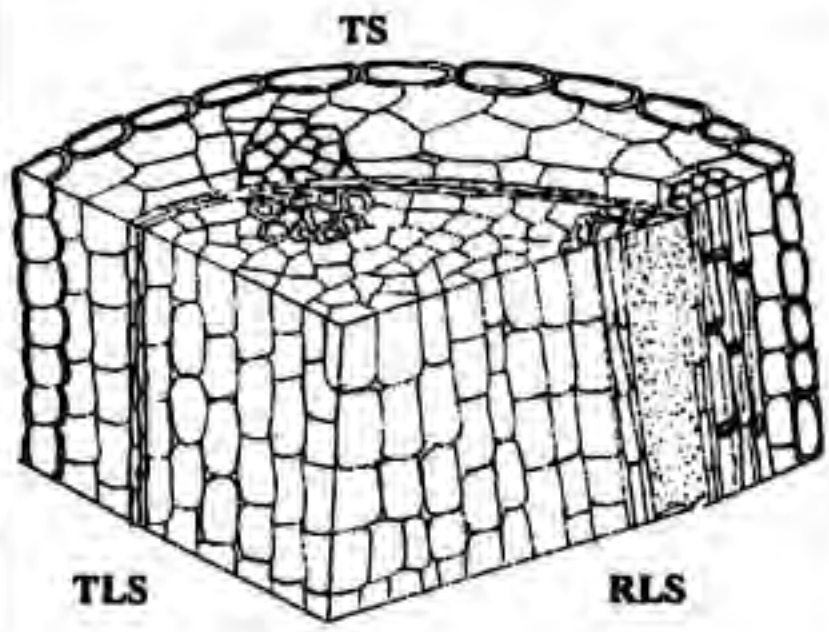
Stereogram of a woody stem



Stereogram of a stem



Stereogram of Quassia Wood



Stereogram of a Stem

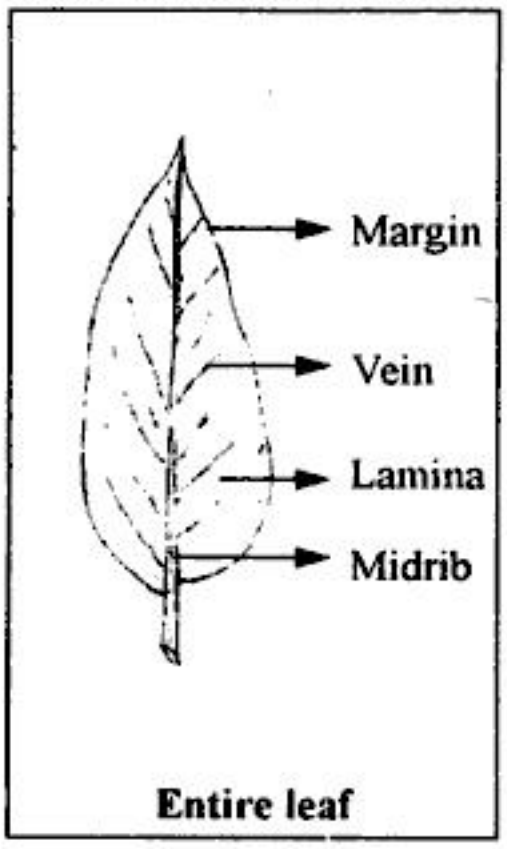
2.1.3 SIGNIFICANCE OF TS, TLS AND RLS

Observation of section of a stem/root/stolon in TS, TLS and RLS reveals the structure and morphology of a particular cell from all angles. At the same time arrangement of cells in a tissue is revealed from all angles as is evident from the figure showing a stereogram of Quassia wood.

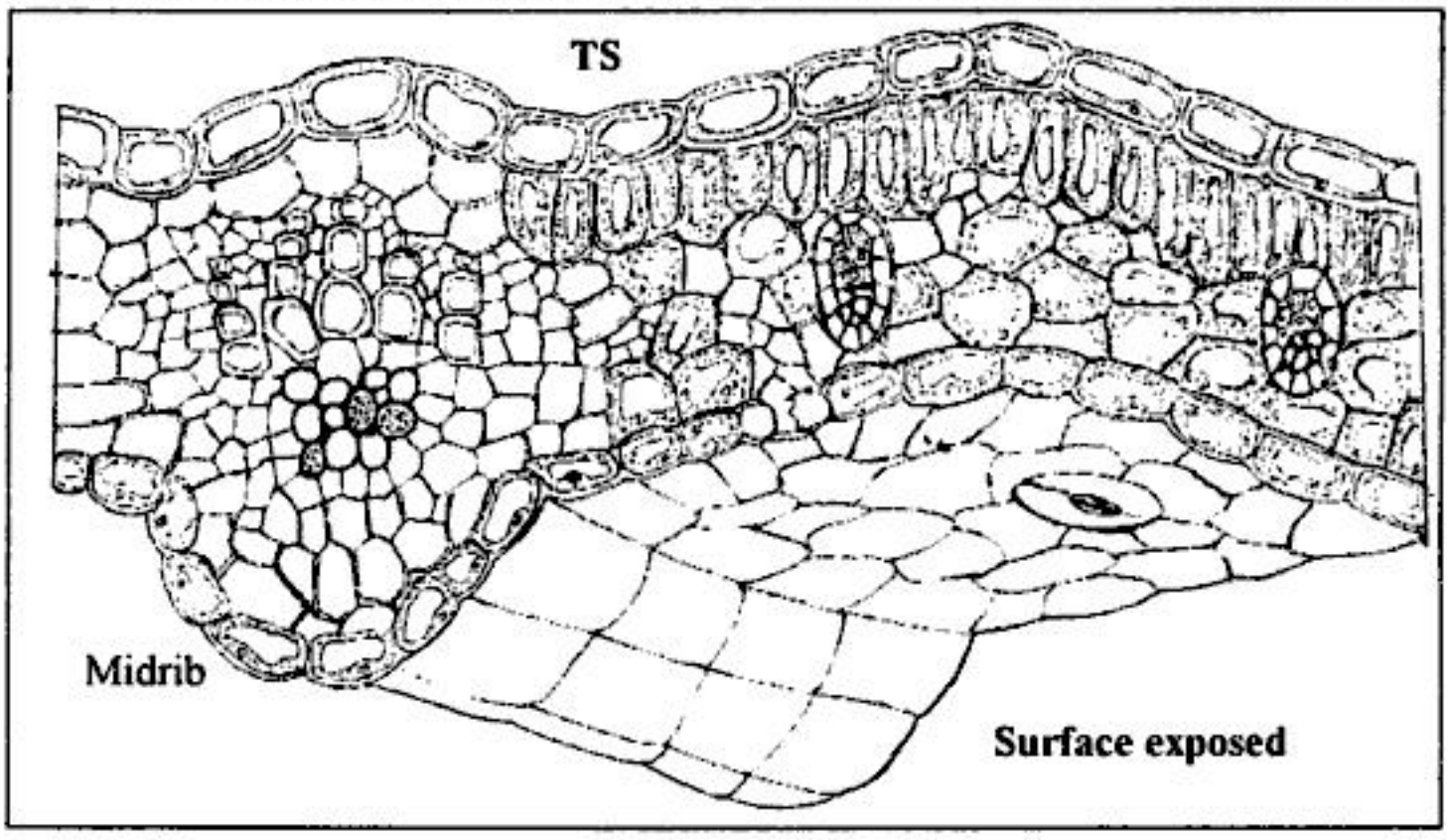
2.2 SECTION OF A LEAF

Unlike in stem/root/stolon, in case of a leaf the important aspects to study are a section through the midrib taken perpendicular to the midrib and observation of a surface preparation. In case of a bilateral leaf, either surface may be observed, but in case of a dorsiventral leaf, the lower epidermis is more important, bearing the stomata, guard cells and epidermal cells. Surface preparation (see the topic LEAVES) is, many a times, used as an identification tool by means of morphology and leaf constants.

2.2.1 SECTION THROUGH MIDRIB (T.S.)

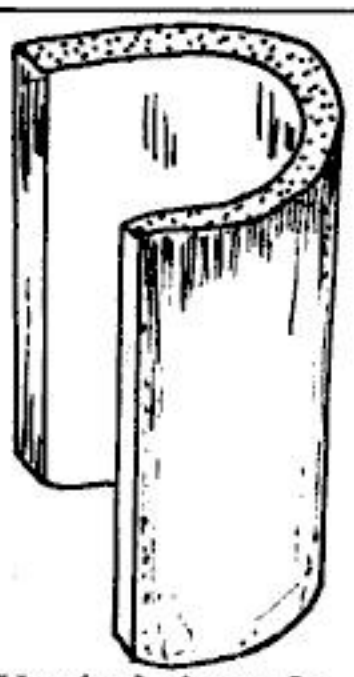


Entire leaf

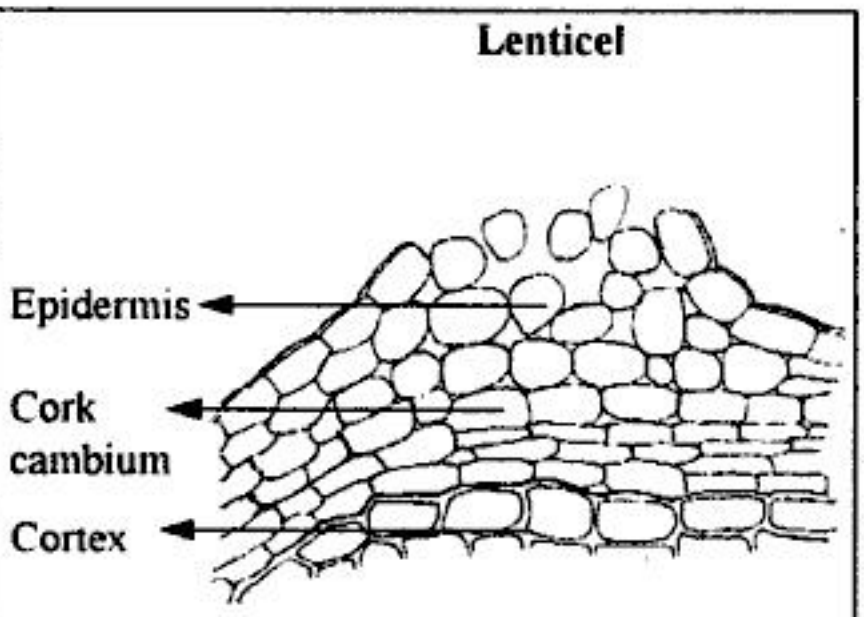


2.3 SECTION OF A BARK

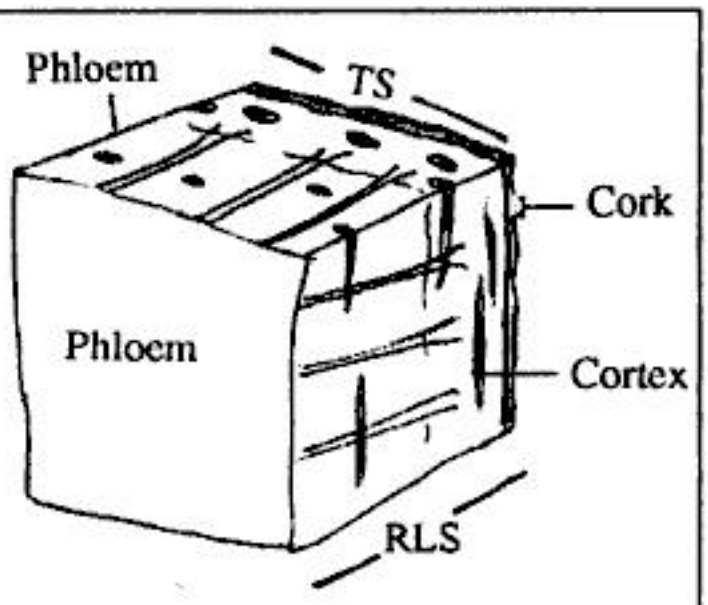
In case of a bark transverse section is important as it reveals the horizontal arrangement of cells and shows lenticels. RLS helps to reveal the arrangement and thickness of the cells.



Vertical piece of a curved bark



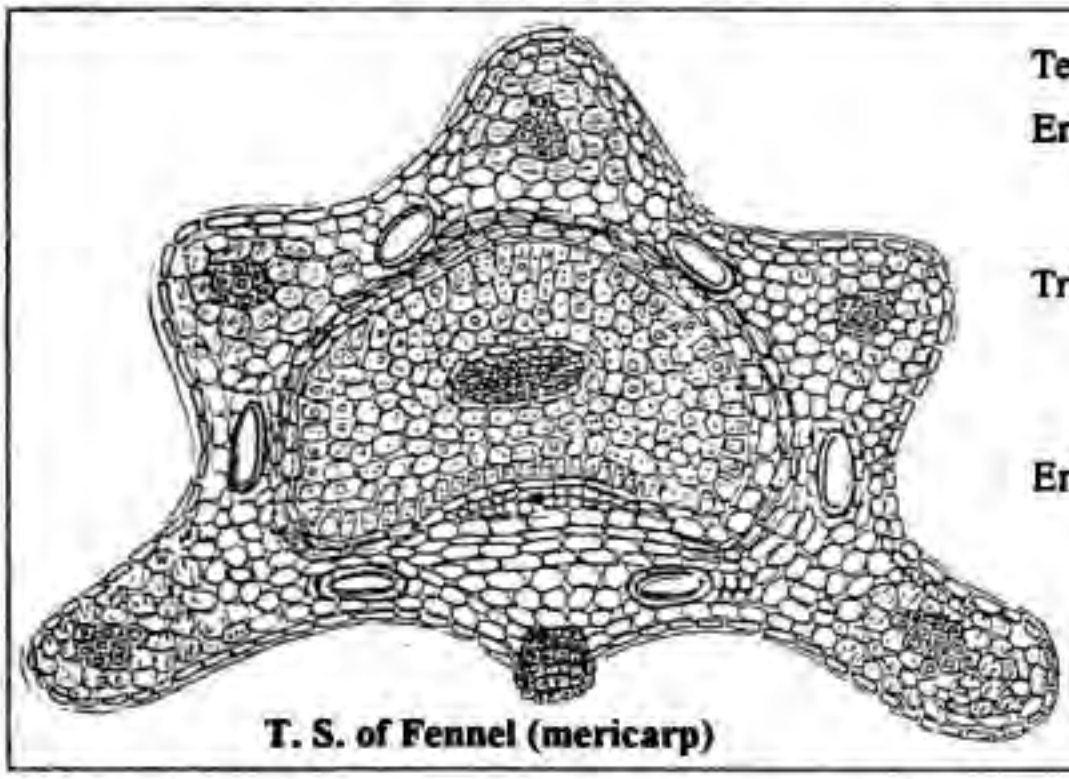
Section of a bark showing lenticel



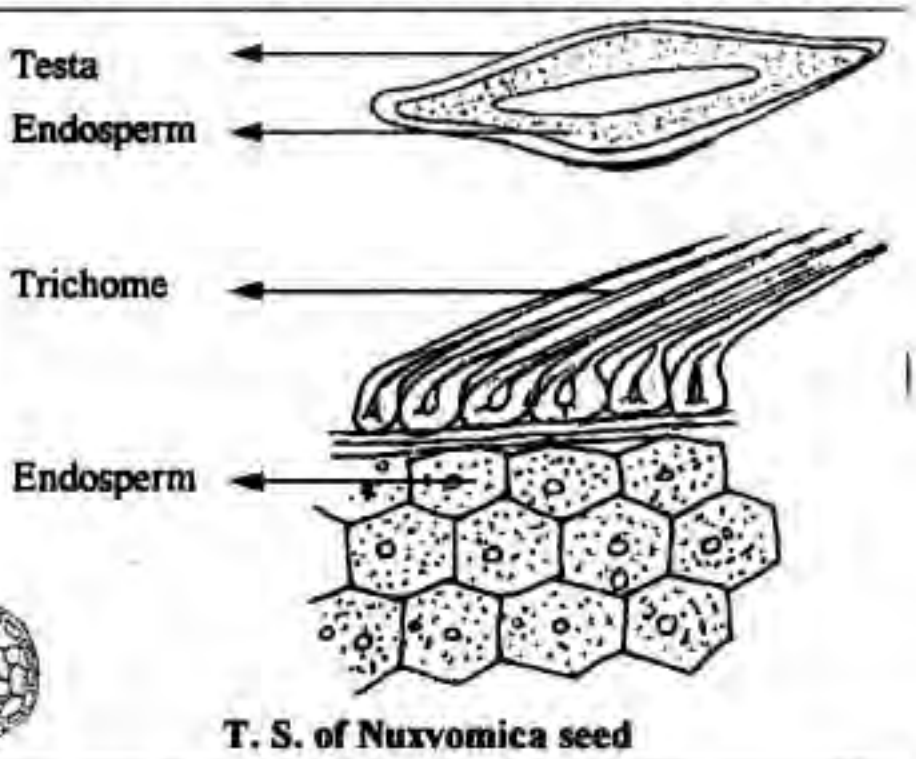
Stereogram of a bark

2.4 SECTION OF FRUIT, SEED

In case of fruits and seeds, generally T.S. of various parts are observed under microscope e.g. in case of umbelliferrous fruits like fennel, the T.S. of mericarp is observed, while in cardamom, T.S. of pericarp and seed is



T. S. of Fennel (mericarp)



T. S. of Nuxvomica seed

observed and in nux–vomica T.S. of seed is studied. Thus, in case of fruit and seed drugs, separate sectioning technique is required for individual drug. Sometimes, longitudinal sections cut through the centre of the seed the fruit have been found useful in investigation.

2.5 SECTION CUTTING TECHNIQUE

Section cutting is an art which every pharmacognosist must acquire. Thinner the sections, more clearly the tissues can be observed.

2.5.1 MATERIALS

First of all keep the following material ready on your laboratory table.

1.	Napkin	10.	A dropper
2.	Watch glass	11.	Filter paper/blotting paper
3.	Test tubes	12.	Stains
4.	Painting brush (thin)	13.	Drug sample
5.	Bunsen burner	14.	Forceps
6.	A sharp razor blade	15.	Test tube holder
7.	Micro-slides	16.	Test tube stand
8.	Cover slips	17.	Needle
9.	A beaker full of water	18.	Camel hair brushes (two)

2.5.2 SELECTION OF DRUG SAMPLE FOR SECTION CUTTING

Selection of appropriate size, part and shape of a crude drug sample is very important in obtaining a good section.

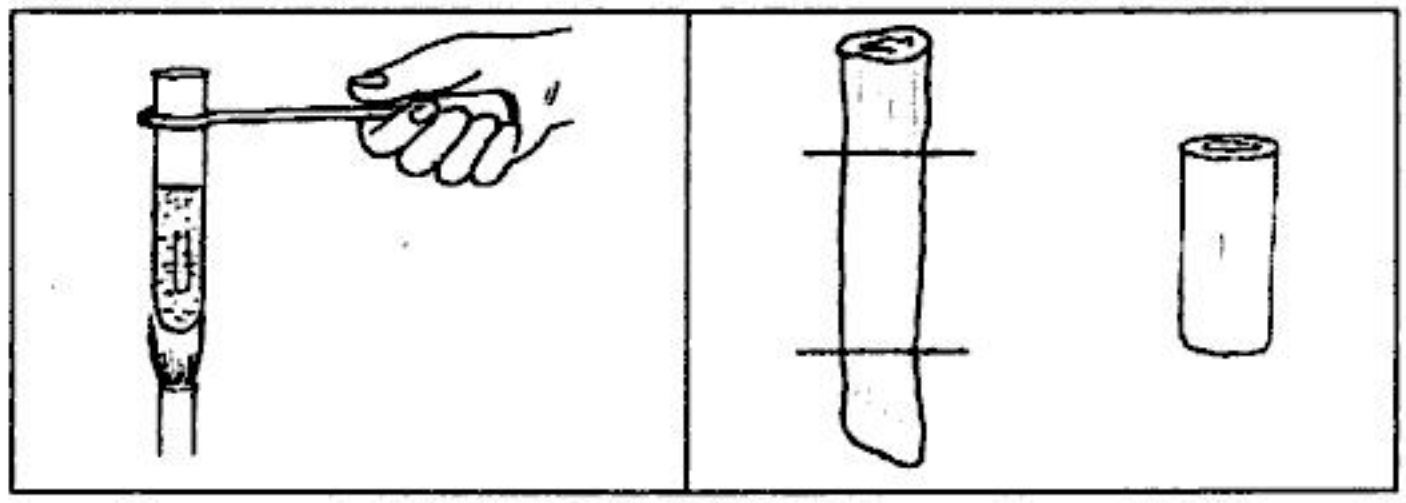
In case of a stem/root/stolon, select a portion of the drug having a diameter of 3 to 5 mm and a length of 2½ cms. A sample shorter in length will be difficult to hold and a sample thicker in diameter may give rise to thick and wedge shaped sections.

In case of a leaf, try to obtain a young leaf as it does not have a thick midrib and lamina, hence it is more convenient to obtain fine sections.

2.5.3 PREPARATION OF SAMPLE FOR SECTIONING

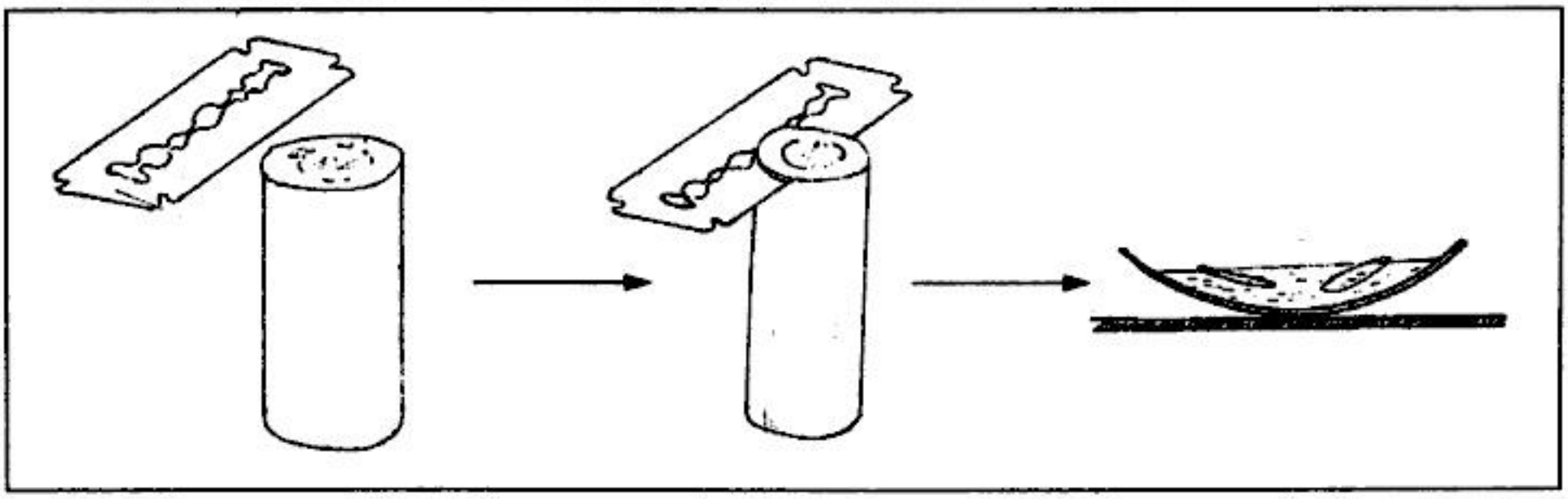
Put the sample selected in a test tube and add sufficient chloral-hydrate solution or water so that the sample remains submerged. Boil the sample in water over a bunsen flame for a few minutes. This will soften the hard drug sample and will help in obtaining fine sections. In case of a leaf, this step may not be necessary.

For a **stem/root/stolon** drug, cut a cylindrical portion which is almost straight and cut off both edges so as to make the edge surface smooth. This sample is ready for section cutting.



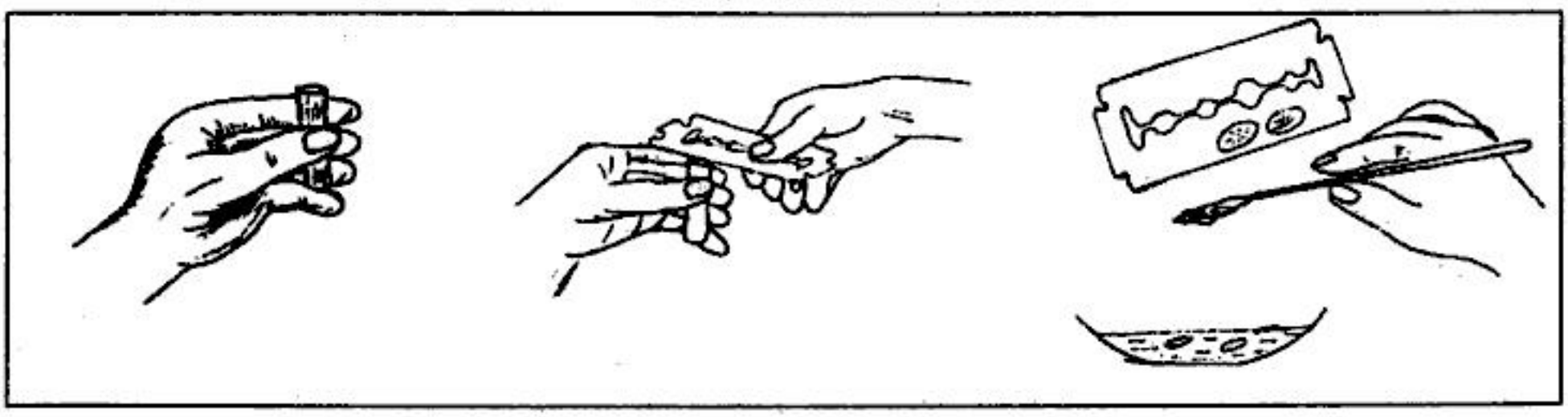
Hold the sample vertical between the first, second finger and the thumb and move the blade back and forth from one end to the other, obtaining fine slices. Take sufficient number of sections, as all sections will not be very fine and uniform.

Transfer the sections to a watch glass containing water with the help of a brush. Reject thick and oblique one.

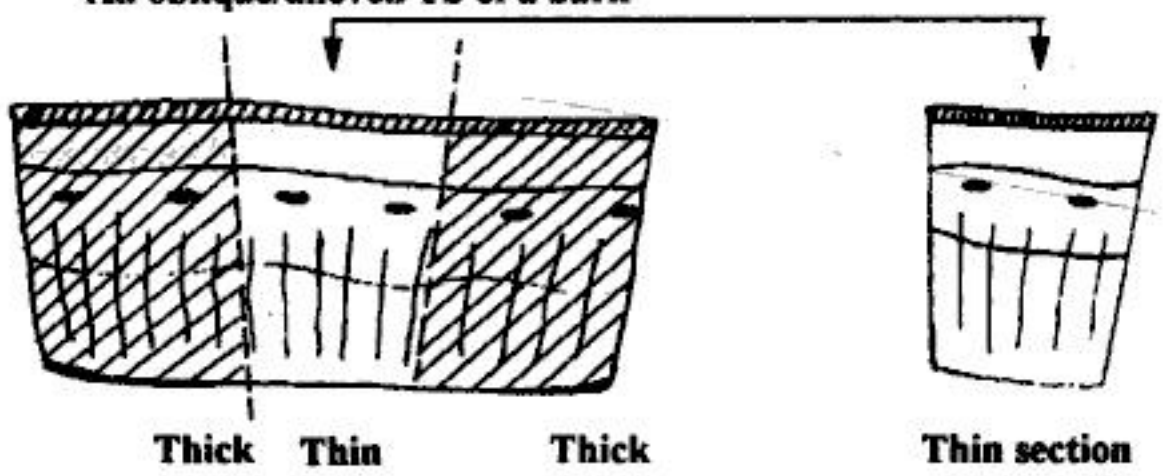


Similarly, cut sections of the leaf in the block of pith which shall give sections of the leaf when separated from the pith. Transfer the sections to a watch glass with a brush.

Note : Before taking the section, ensure that the blade is having enough amount of water on its edge, if a dry blade is used, it shall entrap air bubbles in the section, which are difficult to remove.

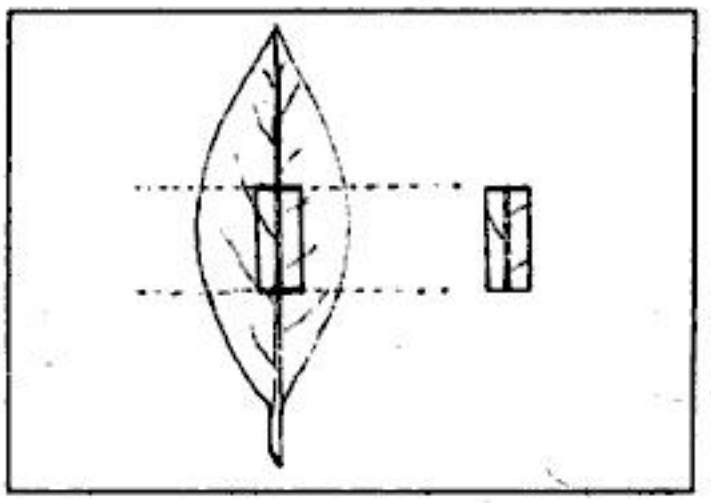


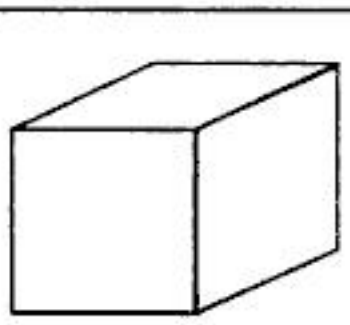
Bark :



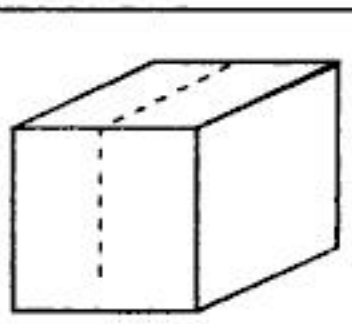
From an oblique/uneven section of a bark, thin part of the section, showing all the cellular structures in sequence can be separated and observed as shown above.

In case of a leaf drug, cut a part of the leaf passing through midrib as shown in diagram. This cut off portion may or may not be boiled. (boil it only if it is dry and requires boiling with water for softening). Since the lamina of a leaf is very thin, section cutting is difficult. The surface area of the surface to be cut has to be increased. This is done by embedding the sample in a block of pith. This pith is obtained from red pumpkin (Bhopla) or raw papaya or potato. A cubical portion of the pith is cut off and used as shown in the figure.

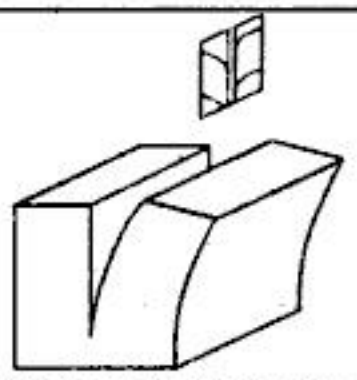




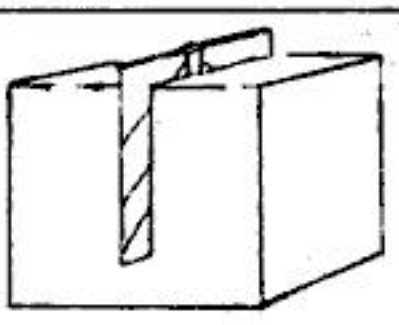
Cube of pith.



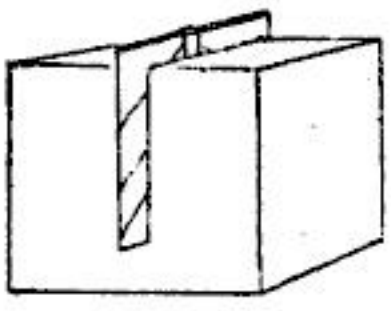
With the help of a blade give vertical cut upto 2/3rd height.



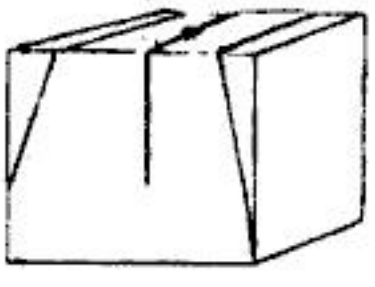
Slightly pull one side of the cube apart to make a wedge opening. Do not separate two parts.



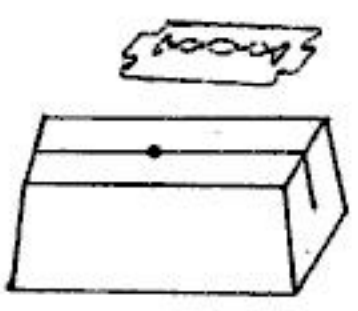
Then insert the leaf sample prepared into the wedge and press two sides of pith.



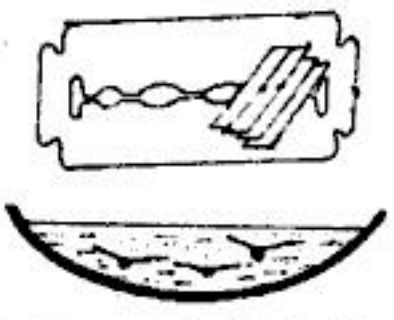
Now, cut off the portions of leaf protruding of the surfaces of the pith. The block is ready for section cutting.



The vertical side of pith may be tapered off upwards for convenience in section cutting.



Take sections by moving the blade back and forth.



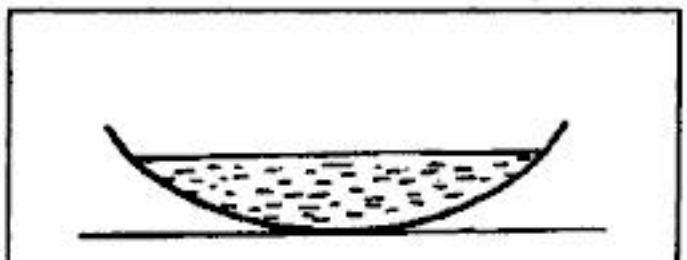
Sections ready for staining

3 STAINING AND MOUNTING OF SECTIONS

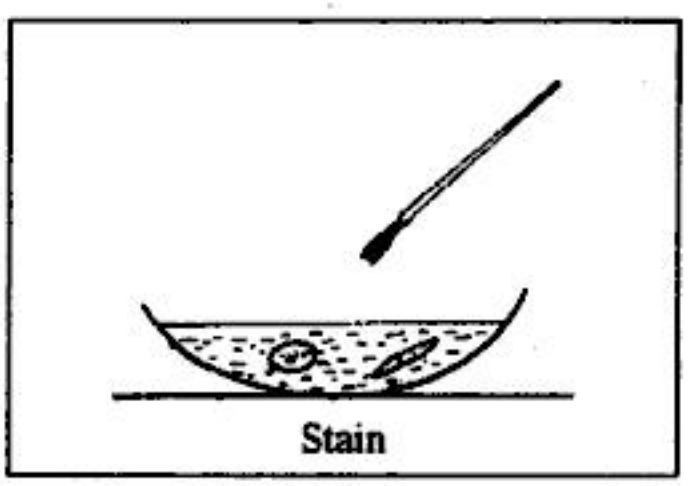
Staining is a process in which chemical dyes are used to impart colour to various tissues in a section of drug sample, which enables to distinguish the arrangement of various tissues in the sample. A STAIN is a chemical dye (colorant) which combines chemically or physically with a cell content to impart colour to it. e.g. safranin combines with the lignin present in cell wall and vessels and imparts a red colour to the lignified tissues. Iodine solution combines with starch grains to give a blue colour. Sudan Red III dissolves in the fixed oil present in the oilseeds to impart red colour.

For detail, refer *reactions of cell walls* and *reactions of cell contents* in the topic **PLANT CELL.**

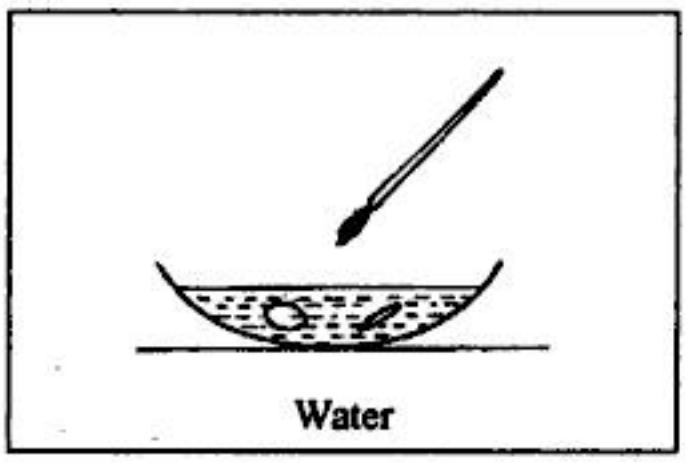
(1) Take a clean watch glass and add the staining solution to it.



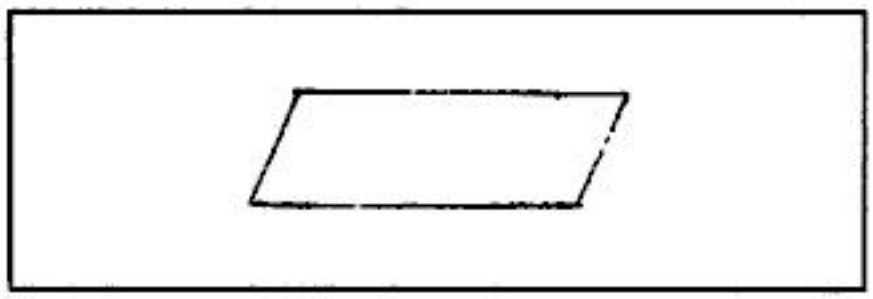
(2) With the help of a brush, transfer the section taken from water to stain solution and keep for 2-3 minutes.



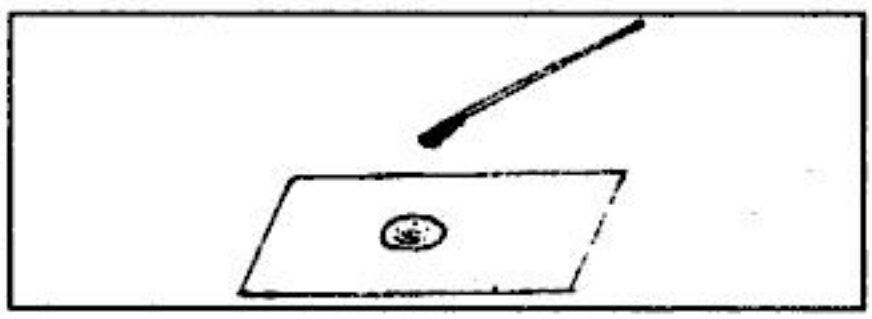
(3) Pick up the section after 2-3 minutes and transfer it to watch glass containing plain water, so that excess stain is washed away. This section is ready for mounting on a slide.



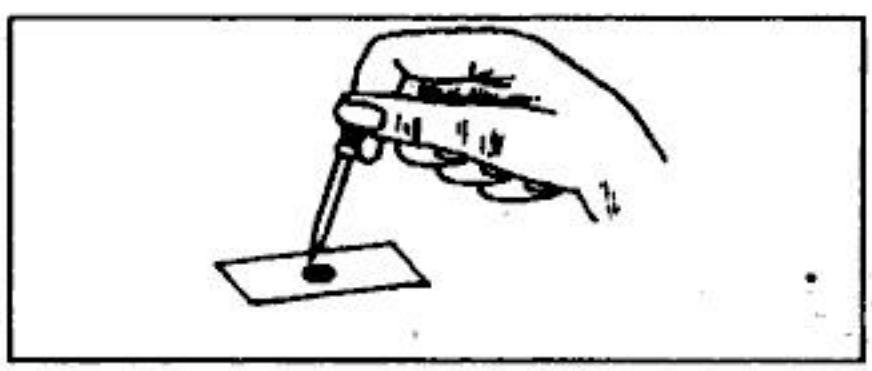
(1) Take a clean glass microslide.



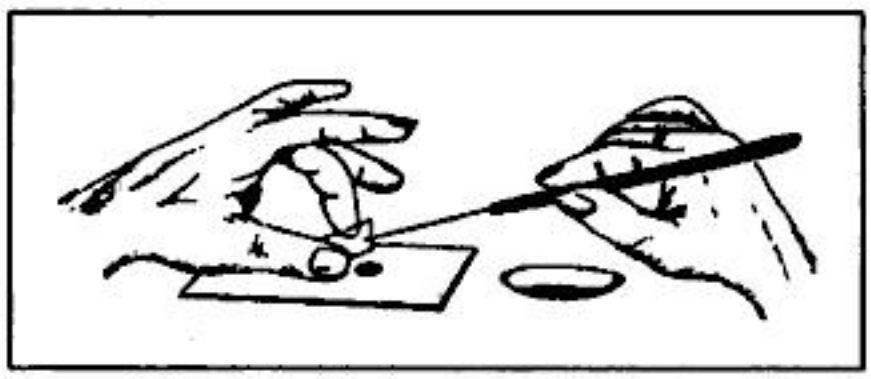
(2) On this slide transfer the section to be mounted, with the help of a brush.



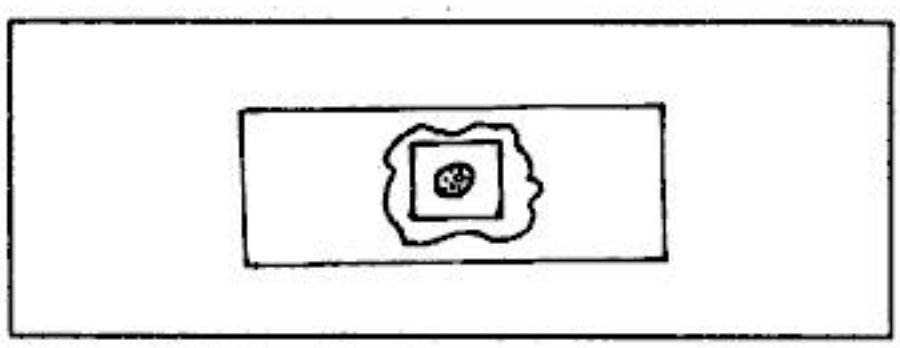
(3) Add one or two drops of water on the section with a dropper.
See that the section is submerged in the water.



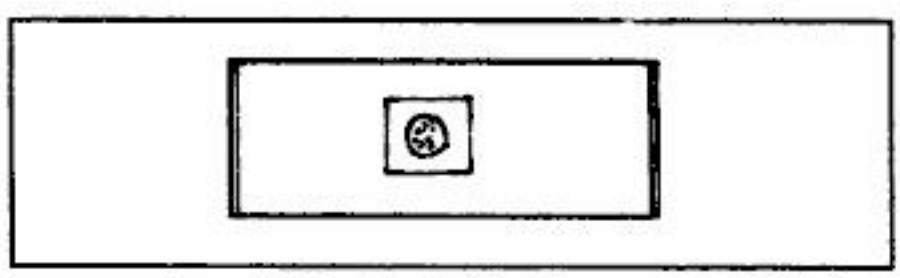
(4) Take a clean cover slip with the help of a forcep and needle.
Place the cover slip on the section gently.



(5) If any air bubbles are seen, slightly lift the cover slip and add a drop of water and replace the cover slip till the air bubble is removed.



(6) With the help of a blotting paper, wipe off excess water present outside the cover slip. The slide is ready for observation.



This procedure described above is the routine laboratory technique and the slide prepared will not last long. To avoid evaporation of water and drying of section, glycerine water can be used instead of water. In order to prepare a permanent mount, a special process is adopted.

3.3 DOUBLE STAINING TECHNIQUE

A permanent preparation is useful for preservation of good sections for study and for preparation of standards, with which the samples can be compared. This process generally involves staining with two reagents, hence is called double staining technique. One of the stains imparts colour to the lignified tissue and the other to the cellulose part. Two different techniques are involved in the preparation of a permanent slide.

3.3.1 METHOD 1

In this method **safranin** and **haematoxylin** are used.

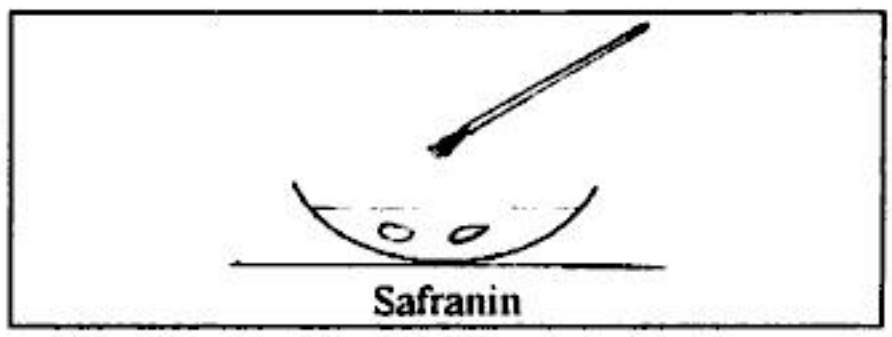
SAFRANIN SOLUTION : Prepare a 0.5-1% solution of safranin in water or 1% solution in 50% alcohol.

LIGNIN + SAFRANIN ⟶ DEEP RED

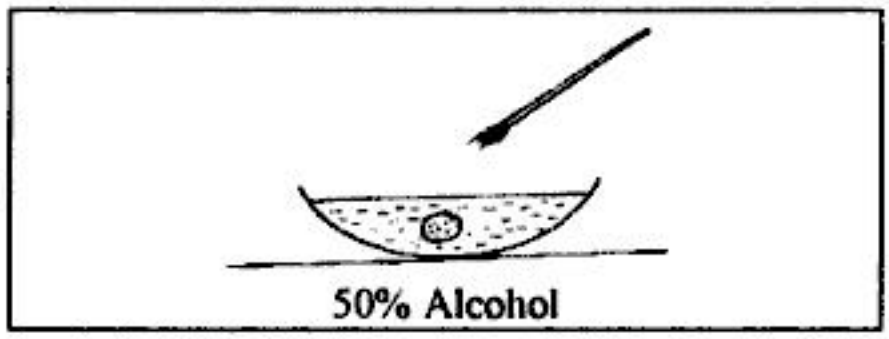
DELAFIELD'S HAEMATOXYLIN :

CELLULOSE + HAEMATOXYLIN ⟶ PURPLISH VIOLET

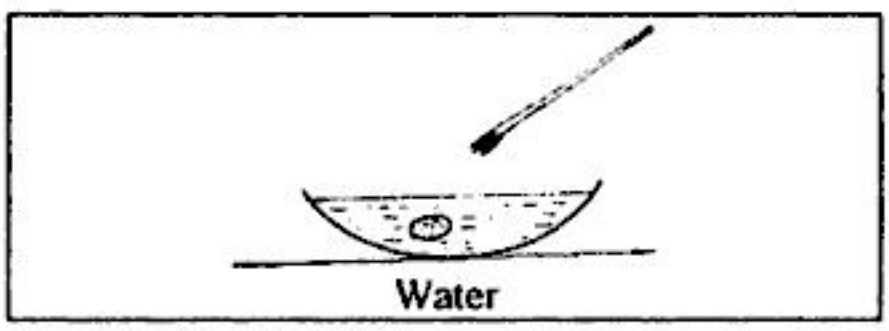
1. Take a clean watch glass, Add safranin solution to it, and transfer a thin uniform section to this solution
   Treat for 10 minutes



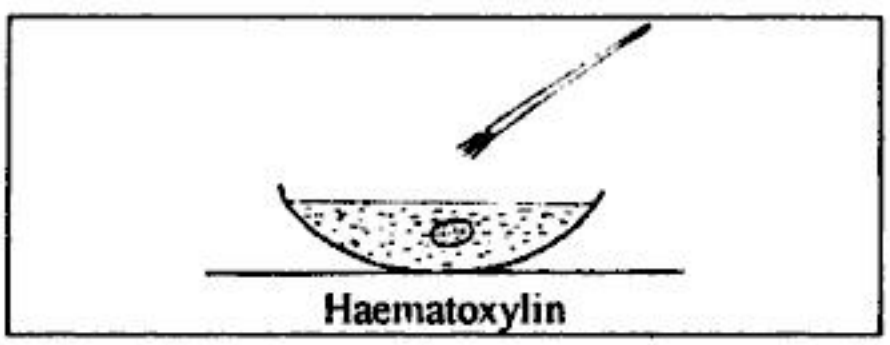
2. Take one watch glass containing 50% alcohol.
   Tansfer the section from safranin to 50 % alcohol, keep for 5 minutes.



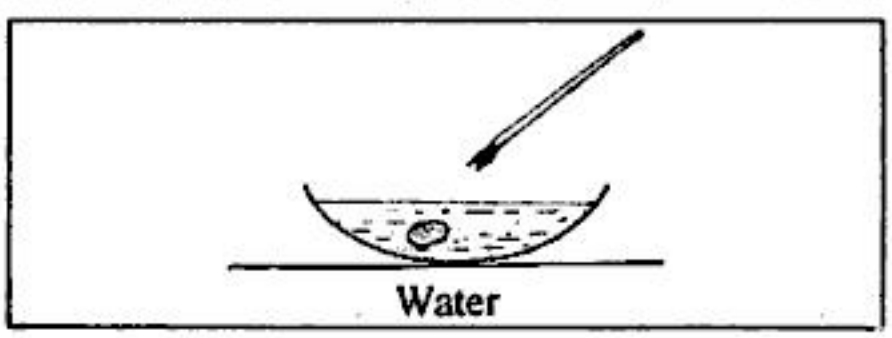
3. Transfer the section in watch glass containing water; keep for 5 minutes. This washing shall remove the stain from cellulose part.



4. Transfer this safranin stained section to a watch glass containing dilute haematoxylin treat for 2 minutes.

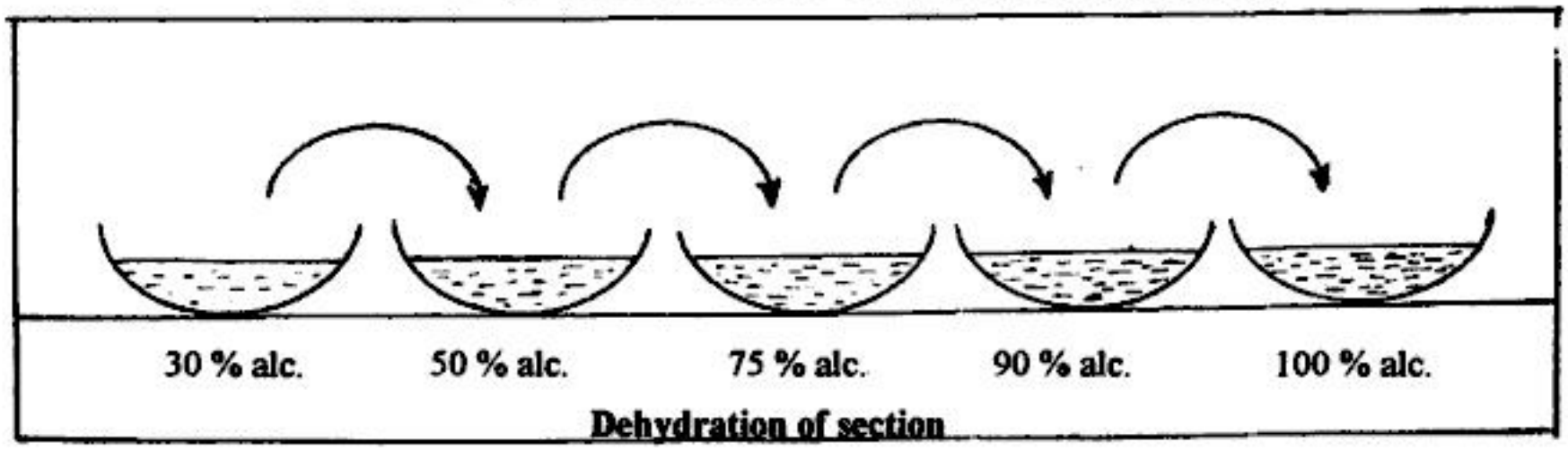


5. Transfer to a watch glass containing water for washing.

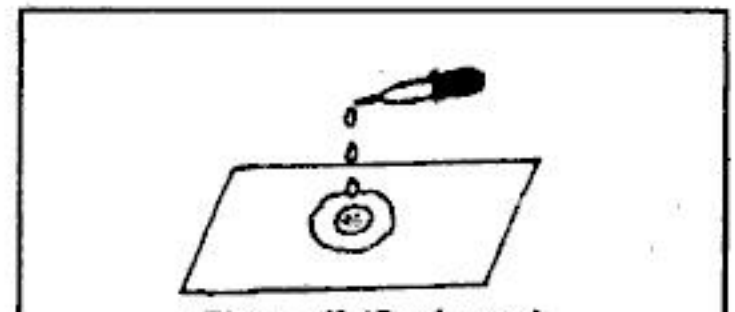


This section is double stained and now requires dehydration otherwise, over a period of time it may develop a foggy appearance and the observations shall not be clear.

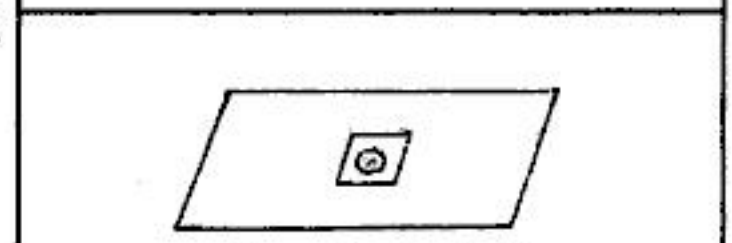
For **dehydration** double stained and washed section is treated with increasing strengths of alcohol for 1 minute in each strength, starting with 30% alcohol, followed by 50%, 75%, 90% and 100%. This removes all moisture from the section. This dehydrated section is now ready for a permanent mount.



Dehydration of section



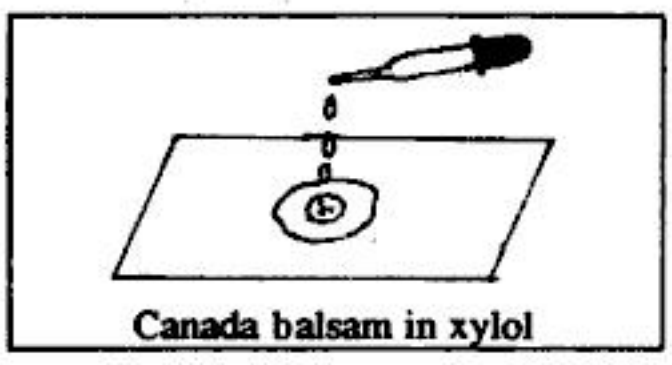
Clove oil (5 minutes)



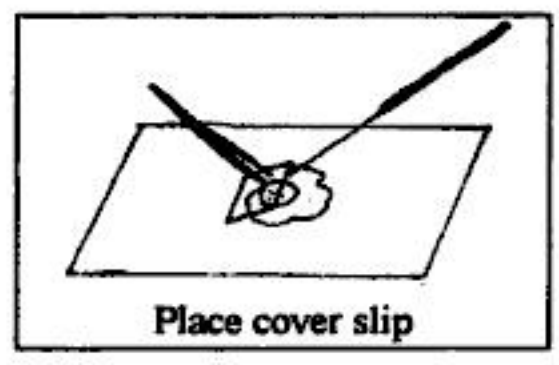
Clove oil wiped off

For **mounting**, select a 1 to 1.2 mm thick glass slide and a thin cover slip. Place the section in the centre of the slide and add few drops of clove oil. This makes the section clear, as it removes unwanted debris. After 5 minutes, dry the section with a blotting paper.

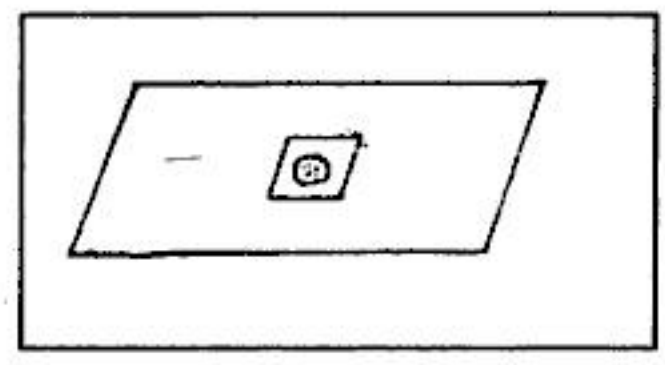
To this section, now add few drops of **Canada balsam** dissolved in xylol.



Canada balsam in xylol



Place cover slip



Slightly warm the slide or keep for drying in sun in a dust–free place. Natural drying is a time consuming process and takes 2–3 days for completion. The solvent evaporates and the balsam fixes the section. Label the slide, accordingly.

3.3.2 METHOD II

In this method, the stains used are **safranin** and **Fast green** solution.

SAFRANIN + LIGNIN ⟶ DEEP RED

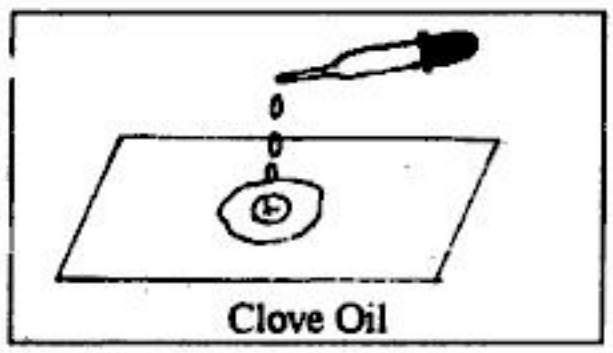
FAST GREEN + CELLULOSE ⟶ BRIGHT GREEN

First stain the section with safranin and treat with 50 % alcohol as in METHOD I (steps 1 and 2). Later, dehydrate the section as in METHOD I.

The second stain is added after dehydration. Fast green is dissolved in clove oil and treated with the section for 2 – 3 minutes.


Fast Green in Clove Oil

The section is then transfered to a clean slide, treated with plain clove oil for 5 minutes for clearing the section. Remove excess of clove oil and fix the section in canada balsam as in **Method I.** Dry and store in a slide box until required for observation.


Clove Oil

Lignified tissues, nuclei and cutinised walls get stained red; cytoplasm and cellulose walls get stained green.

This is a good double stain and has the merit that the fast green solution keeps well, wheras Delafield's haematoxylin deposits badly and requires frequent filtering.

3.3.3 PREPARATION OF CANADA BALSAM SOLUTION FOR FIXING

Canada balsam is a semi–solid, resinous substance, and translucent in nature.

Take small quantity of Canada balsam in a test tube, warm on a water–bath to remove volatile matter. Warm until a hard mass is left behind, cool and dissolve by constant stirring in xylol or benzene. A clear transparent viscous fluid is obtained which may be filtered to remove particle matter. This fluid is used for fixing the section.

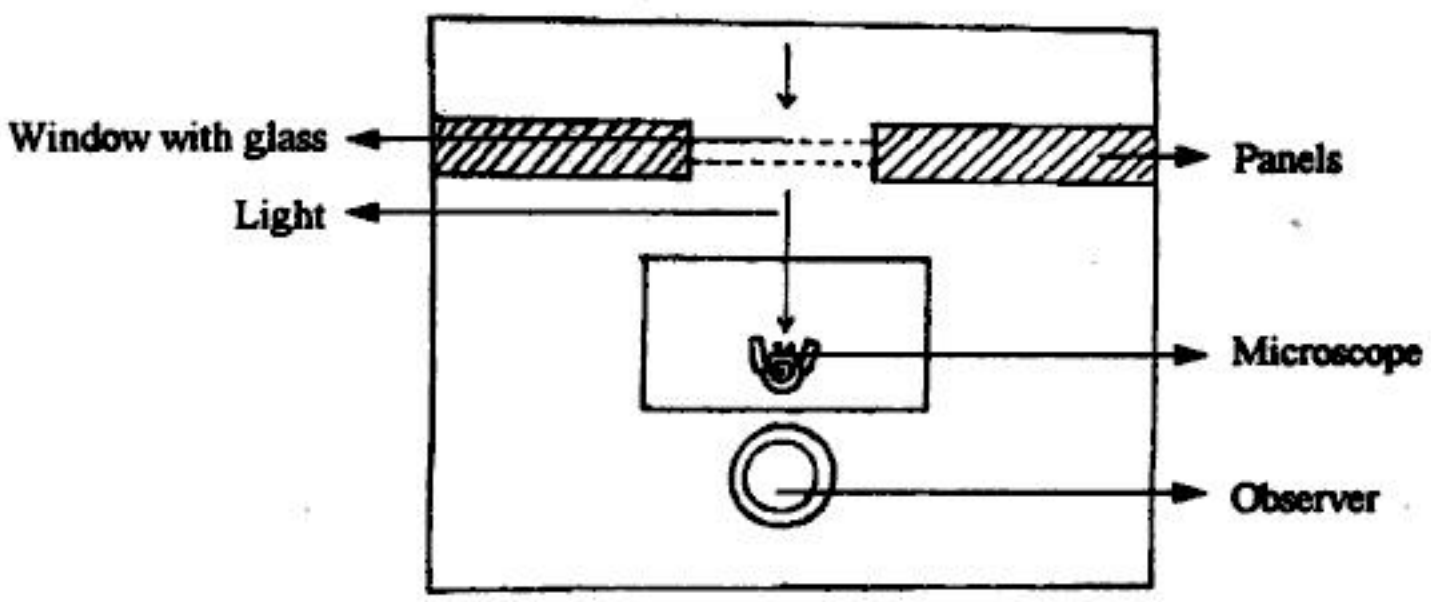
Thus, sections are ready for observation under a microscope; no matter which technique is used for preparation of a slide.

Usually, the routine laboratory technique discussed in 3.1 and 3.2 are used unless, the section has to be preseved for observation or evaluation in the future.

4 OBSERVATION OF A SLIDE UNDER MICROSCOPE

4.1 Select a place in the laboratory where sufficient light is available. See that direct sunlight does not fall on the place of work. Always have the window panes closed so that the direct sunlight does not fall onto the microscope. If the microscope has a built in or external light source, any place would be suitable.

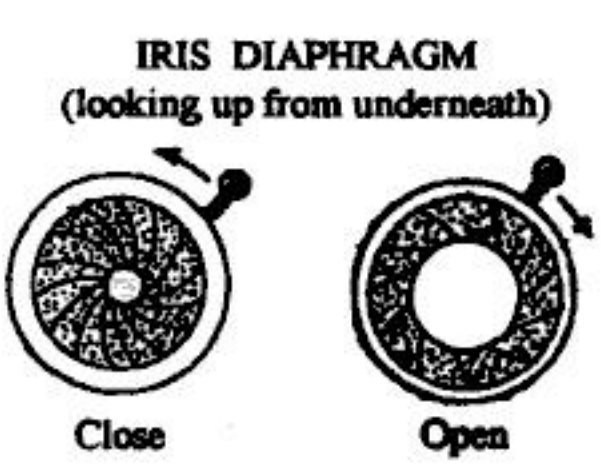
4.2 Remove the microscope from the box and place on the table before you, with the C-Arm towards you and the objectives and mirror facing the light.



4.3 Take a piece of cotton cloth and wipe the microscope to remove any dust. With xylol, wipe all the lenses so that grease or dust could be removed with the help of a tissue paper. This shall make all lenses clear and no undesired foreign matter would be observed.

4.4 Once the microscope is thoroughly cleaned, it is ready for use. Observing through the eyepiece, align the low power objective (5x) or 4x scanner. Open the diaphragm completely and with the help of the substage mirror adjust the position so that the field of view is sufficiently illuminated. While doing this, see that the working distance is about 3/4" to 1".

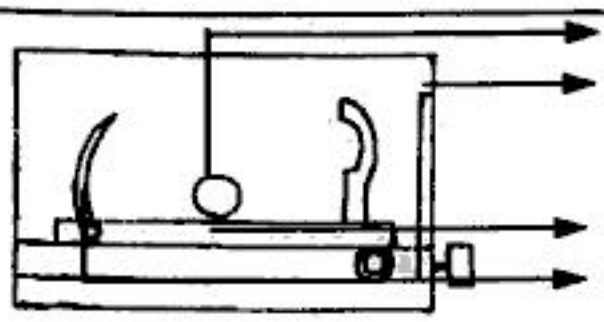
Adjustment of substage mirror

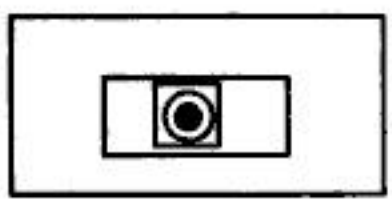


Adjustment of substage diaphragm

The mirror and diaphragm are so adjusted, as to get the required illumination.

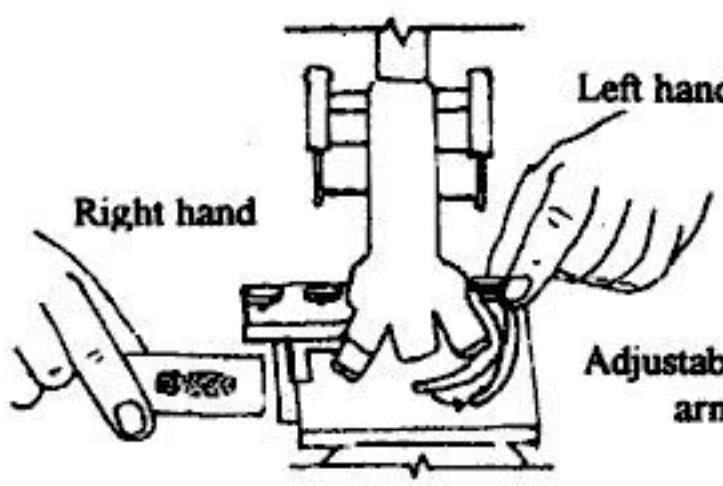
4.5 Place the slide prepared on the stage of the microscope at the centre, with the section placed exactly in line with the stage window lying above the condenser.

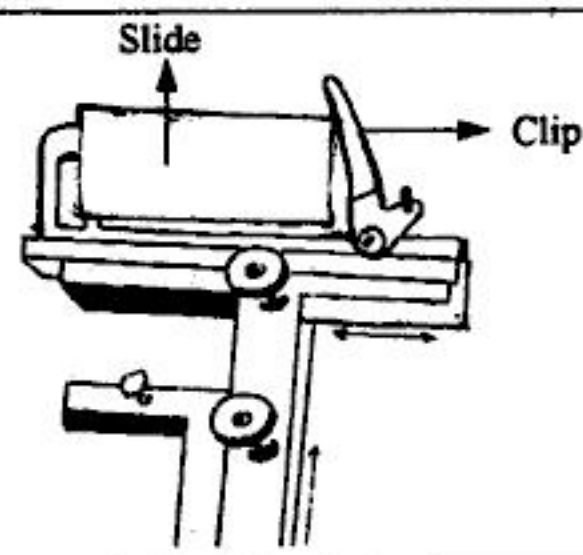




Slide placed such that the section lies above the stage window.

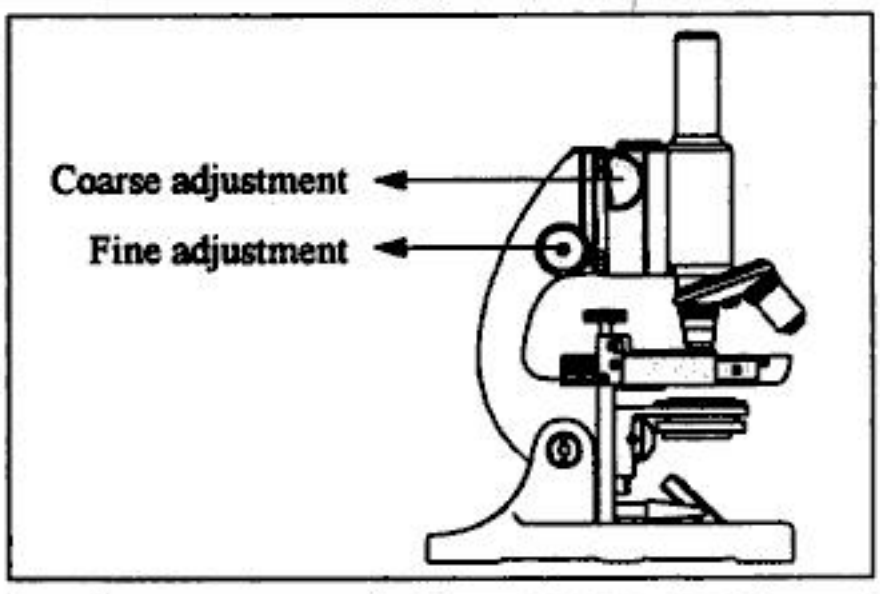
Fix the slide between the clips. Now the slide can be moved forward, backward or sideways above the stage with the help of two screws provided on the mechanical stage.





4.6 With the help of coarse adjustment screw (while observing through the eyepiece), bring the object into focus.Try to get as clear an image as possible by moving this screw. If the light in the field is too much, cut off the beam by slightly closing the diaphragm. If less light is available, illuminate by further opening the diaphragm.

Now, leave the coarse adjustment and with the fine adjustment knob, try to obtain a sharp image.

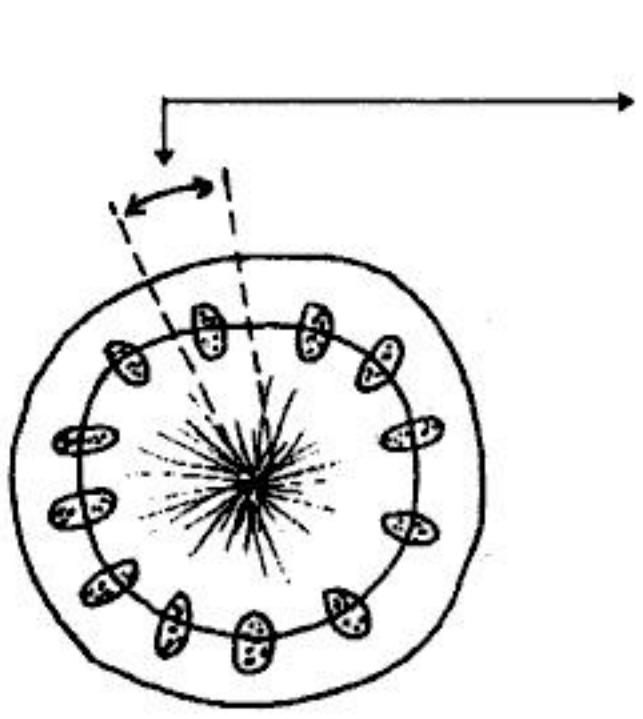


4.7 Note the observation under low power, if further elaboration is required,rotate the nose piece and align the high power objective (40x or 45x). Do not move the slide or coarse adjustments. **The working distance in this case is low and greater illumination is required.** Thus, by observing through the eye piece,adjust the diaphragm to obtain sufficient illumination. Now with the help of fine adjustment knob,' obtain a sharp focus and image. This is a magnified image and reveals more details with higher magnification as compared to low power.

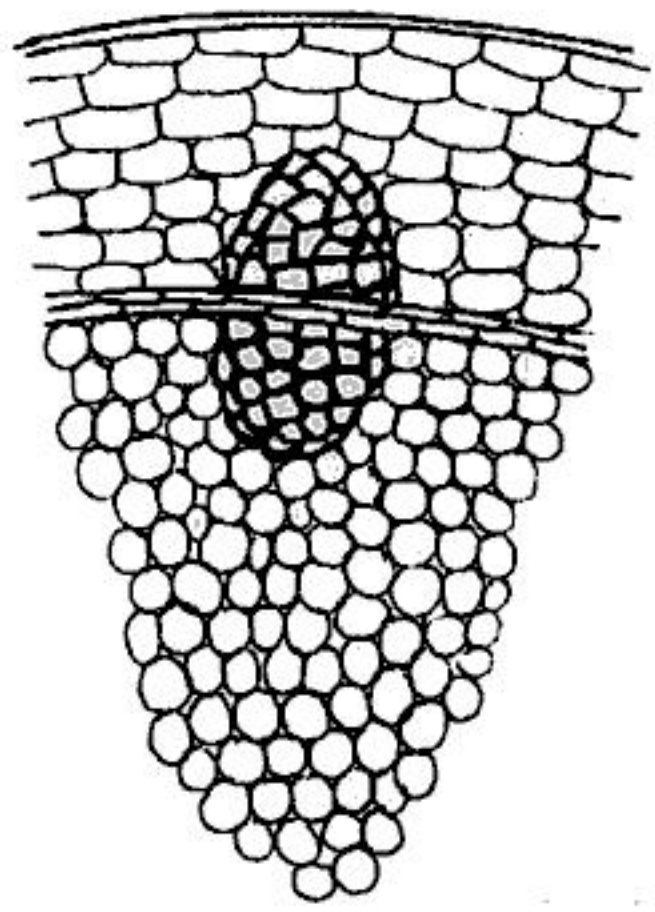
Revolving nose piece

Make the necessary observations of the whole section, moving the slide with the help of the mechanical stage.

4.8 The low power observation helps to draw a schematic diagram, as minute details of cells are not visible or not clear.



Schematic diagram

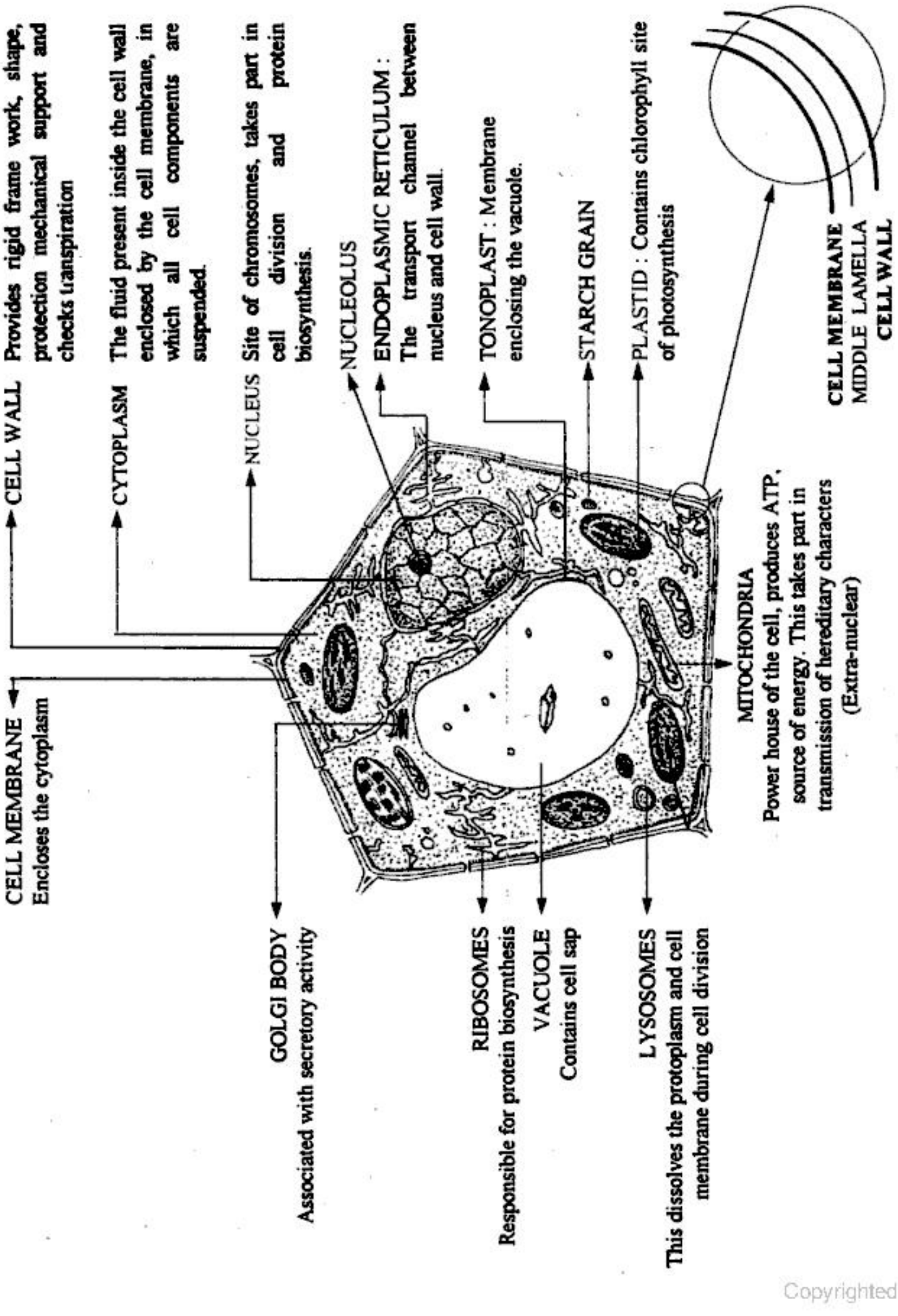


Sketch as observed under high power

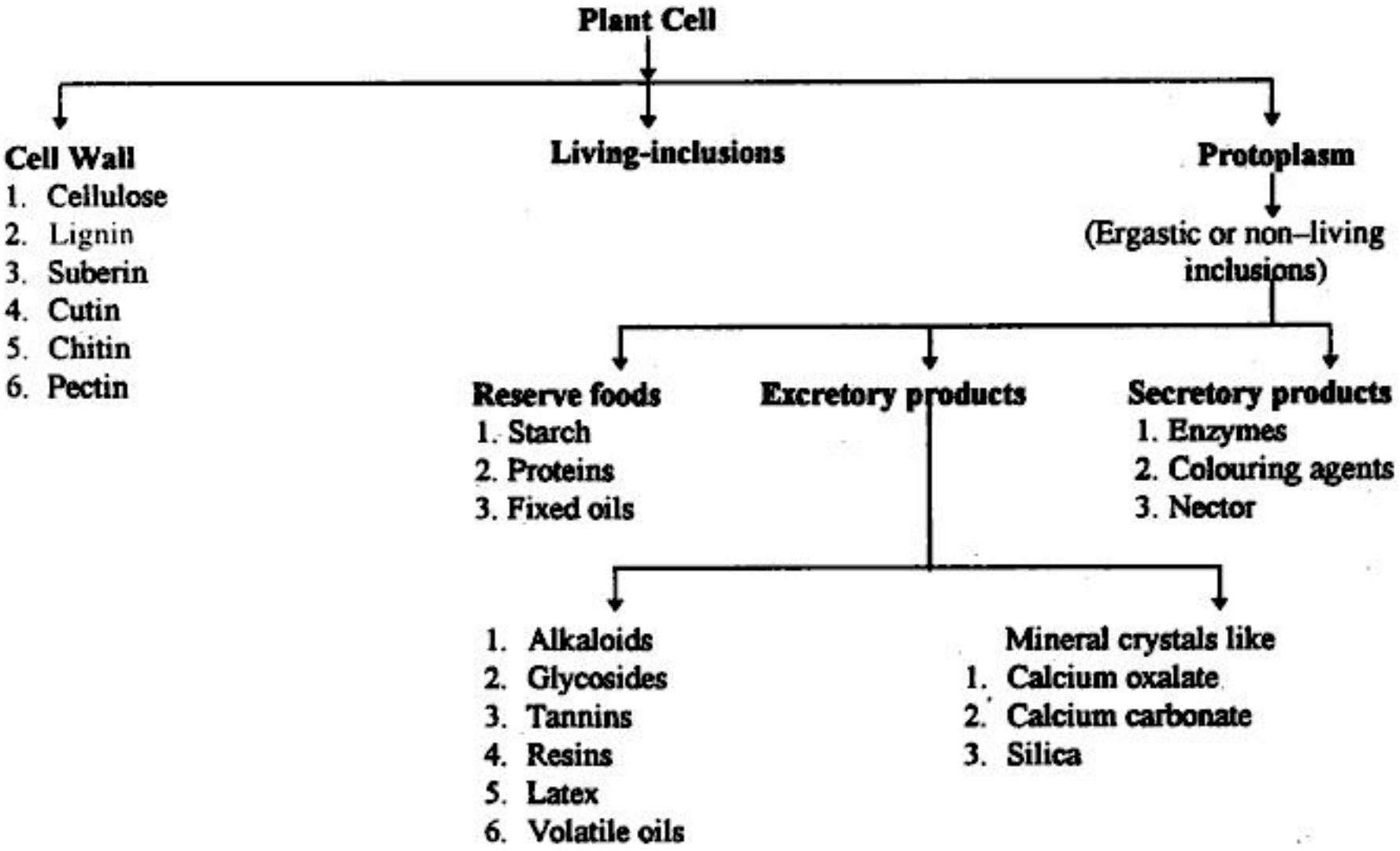
4.9 PRECAUTIONS TO BE TAKEN WHILE USING MICROSCOPE :

1. Always clean the microscope before and after using.
2. Do not touch the lenses with hand. This makes them greasy and blur the image.
3. While lowering the objective to bring the object in focus, watch from the side to avoid the objective touching the slide.
4. Do not use high power objective unless the object is covered with a cover glass so that the lens does not touch the specimen.
5. While keeping the microscope back, in the box always put an eyepiece in the body tube so that the dust does not enter the body tube.
6. Do not spill water or chemicals on the stage. This may lead to corrosion.
7. Always store the microscope covered in a plastic cover or in a wooden box.

5 PLANT CELL



STUDY OF PLANT CELL INCLUSIONS



REACTIONS OF CELL WALLS

COMPONENTS	CHARACTERISTICS	TEST REAGENTS *	OBSERVATION UNDER MICROSCOPE
Cellulose : Chemically carbohydrate in nature	It is a fibrous material of cell wall and together with lignin responsible for structural rigidity of plant.	1. Iodine solution 2. Iodine solution + Sulphuric acid (60% v/v) or zinc chloride 3. Chlor-zinc-Iodide solution 4. Cuoxam solution (Schweitzer's reagent) 5. Delafields haematoxylin.	Pale yellow Bright blue Blue or purple Swells and dissolve Violet
Lignin : A complex phenyl propanoid polymer	Hard, permeable to water. Lignified tissues provide mechanical rigidity of plant body	1. Iodine solution 2. Iodine solution + sulphuric acid (60% v/v) or zinc chloride 3. Chlor-zinc- iodide solution 4. Aniline hydrochloride 5. Phloroglucinol + conc. HCl (1:1) 6. Mount in sulphuric acid (conc) and warm 7. Safranin (1% safranin in 50% alcohol)	Deep blue Brown Yellow Bright yellow Red Lignified cells dissolve Red

* For preparation of reagents refer appendix-I

COMPONENT	CHARACTERISTICS	TEST REAGENTS	OBSERVATIONS
Suberin : A mixture of oxygenated or condensed products of fatty acid, suberogenic acid. ***Cutin :*** A mixture of oxygenated or condensed products of cutionogenic acid.	Impervious to water, checks evaporation of water. Waxy, relatively impermeable to water and gases, checks evaporation of water.	(Tests for suberin and cutin) 1. Iodine solution 2. Iodine solution + sulphuric acid (60% v/v) or zinc chloride 3. Chlor-zinc-iodide solution 4. Mount in dil. tincture of alkanna (diluted with 95% Alcohol) 5. Treat with Sudan red III, mount in glycerin and warm slightly 6. Concentrated sulphuric acid 7. Strong chlorophyll solution 8. Heat gently with potassium chlorate and nitric acid on slide. 9. Warm with 20% (potassium chloride) solution	 Deep yellow Deep brown Yellowish brown Pinkish red Red Does not dissolve cell walls Deep green Cell wall changes into droplets soluble in organic solvents Yellow
Chitin : Chemically made up of n-acetyl-2-glucose amine units $(C_6H_{13}O_5N)_n$	Chitin forms major part of cell walls of fungi (i.e. ergot) and insects.	1. Sudan red III, 2. Chlor-zinc-iodide solution 3. Alcoholic chlorophyll solution 4. Iodine solution + sulphuric acid	Red Yellowish brown Deep green Violet colour
Pectin : Carbohydrates consisting of methoxylated polygalacturonic acid	Swells in water to form stiff jellies, present mostly in fruits	1. Pectin solution (1%) + 2% NaOH Sol. solution $\xrightarrow{20\ min}$ dil. HCl	Gelatinous ppt, on boiling becomes white.

REACTIONS OF CELL CONTENTS

COMPONENTS	CHARACTERISTICS	TEST REAGENTS	OBSERVATIONS
Starch (Insoluble polysaccharide)	TYPES OF GRAINS : Simple or Compound Eccentric or concentric	1. Iodine solution 2. Sodium hydroxide solution (5%) 3. Water	Blue Swell and ultimately dissolves. Insoluble in cold, gelatinsed on heating.
Mucilage : Polymers of monosaccharide and many of them are combined with uronic acid	Absorbs water and forms viscous mass. After drying, it becomes hard and horny	1. Iodine solution + sulphuric acid or zinc chloride 2. Methylene blue 3. Ruthenium red solution 4. Corallin soda solution 5. Water or aqueous potassium hydroxide solution	violet Deep blue Pink Red Swells considerably in the cold.
Resins	Occurs in granular masses	1. Chloral hydrate 2. Sudan red III or tincture of alkana	Soluble Stains red
Proteins (Complex nitrogenous compounds)	Aleurone grains consist of amorphous mass of protein enveloped by denser protein membrane Inclusions : 1. Crystalloids : Well formed crystals of proteins. 2. Globoids : Sphere crystals consist of calcium, magnesium organophosphoric acid. 3. Calcium oxalate : Rosettes form or prisms, and needles are rare	1. Iodine solution 2. Eosin aqueous solution 3. Alcoholic picric acid solution 4. Millions reagent, warm 5. Pectin + 2% caustic soda 6. Section + dil.acetic acid	Crystalloids yellow in colour Red coloured crystalloids Yellow coloured crystalloids Brick red Aleurone grains dissolve Globoids dissolve
Fixed oil and Fats (Glyceryl esters of fatty acids)	Reserve food, abundance in seeds. Insoluble in water, soluble in ether, chloroform and benzene.	1. Pressed against a paper 2. Sudan red III or tincture alkana 3. Osmic acid 4. Ether, benzene, chloroform 5. Alcohol (90 percent)	Permanent greasy Red Brown or black Soluble Insoluble, except castor oil

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CLEARING REAGENTS

These reagents remove cell contents such as starch, protein, resin, volatile oil, chlorophyll etc., which obscure other more important characteristics of cell structure. Clearing may be done by mixing, shaking, washing or boiling with the reagents. Removal of such cell contents makes remaining part more transparent and reveals details of the other characteristics.

Chloral hydrate solution [Chloral hydrate + water (5 : 2)]	**Removes many common cell contents including chlorophyll.** **No marked distortion of tissues.** **Very useful mountant for cal. oxalate crystals, as it attacks them very slowly (about 20 days for complete dissolution).** **Induce reswelling of cell walls.** **On heating, tends to crystallize (for prevention, add 1-2 drops of glycerine).**
Oily clearing reagents i) Cedar wood oil, ii) Clove oil	**Dissolves fixed oil.** **Used for preparation of permanent mounts.** **Great penetrating power.** **No swelling of tissues, some times cause shrinkage.** **Does not tend to crystallize.**
Phenolic clearing reagents (alone or in combination) **i) Cresol** **ii) Lactophenol**	**Removes many common cell contents.** **Makes the starch almost transparent.** **Useful** mountant for Nux-vomica hairs, **chalk, kieselguhr, pollens,** etc. **Does not tend to crystallize.** **Useful for mounting silk, aloes, etc.** **Does not tend to crystallize.**
Sodium hydroxide and potassium hydroxide Solution	**Removes starch and protein.** **Disintegrates certain cellulose tissues.** **Causes distortion of cell walls.**
Hydrochloric acid (20% v/v)	**Removes starch by hydrolysis on boiling.**
Organic solvents Lipid solvents like ether, pet. ether, etc.	**Removes fixed oil/fat.** **Ethyl alcohol due to dehydration causes hardening of tissues.**

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5.6 FUNDAMENTAL OR GROUND TISSUE SYSTEM

The ground tissue is restricted usually to the hypodermis, cortex, pith and in case of leaves to the mesophyll and a portion of midrib. This system comprises of 3 types of tissues.

	PARENCHYMA	COLLENCHYMA	SCLERENCHYMA
	Living cells with thin wall of cellulose, isodiametric ; intercellular spaces present.	Living cells, resemble parenchyma but have thickened walls, deposited with cellulose and pectin. Due to thick walls air spaces are tiny or absent.	Sclerenchyma is a hardened, dead, tissue with thickened and lignified walls.
Observations in TS	Isodiametric, slightly elongated, oval or polygonal cells. Some parenchyma also show pitted and reticulate thickenings.	Cells generally appear oval or polygonal. Highly refractile due to thickenings.	Cells are generally long, narrow and pointed at both ends like fibres and in T. S. it appears polygonal with thickening and without intercellular.
Function	Absorption and storage of food, water, gases and photosynthesis.	Mechanical strength.	Mechanical strength.
Site	Present in all soft parts of a cortex of root, cortex and pith of stem, and mesophyll of leaf.	Occurs mostly in the cortical region of stem, bark and midrib of leaf. and not in Root.	Found in all hard wood parts of the plant, wherever mechanical strength is needed.
Chemical Test and Observation under Microscope	Treat with chlor-zinc-iodine, cellulose containing cells appear blue or violet.	Treat with chlor-zinc-iodine, collenchymatous cells appear blue or violet.	Phloroglucinol+Hydrochloric acid.(1 : 1) Under microscope sclerenchyma appears red.
Types	**1. Chlorenchyma :** Parenchyma containing chloroplast. **2. Aerenchyma :** Parenchyma containing air spaces		**1. Sclereids or stone cells :** Isodiametric or irregular, lignified,pitted or stratified cells, occurring simply or in bands, with varying lumen (the middle space left over after the lignification) Generally abundant in cortex, phloem of stem and roots, may be in the seeds (cardamom) or flesh of fruits (pears); in cinnamon bark they show typical U shaped thickening.

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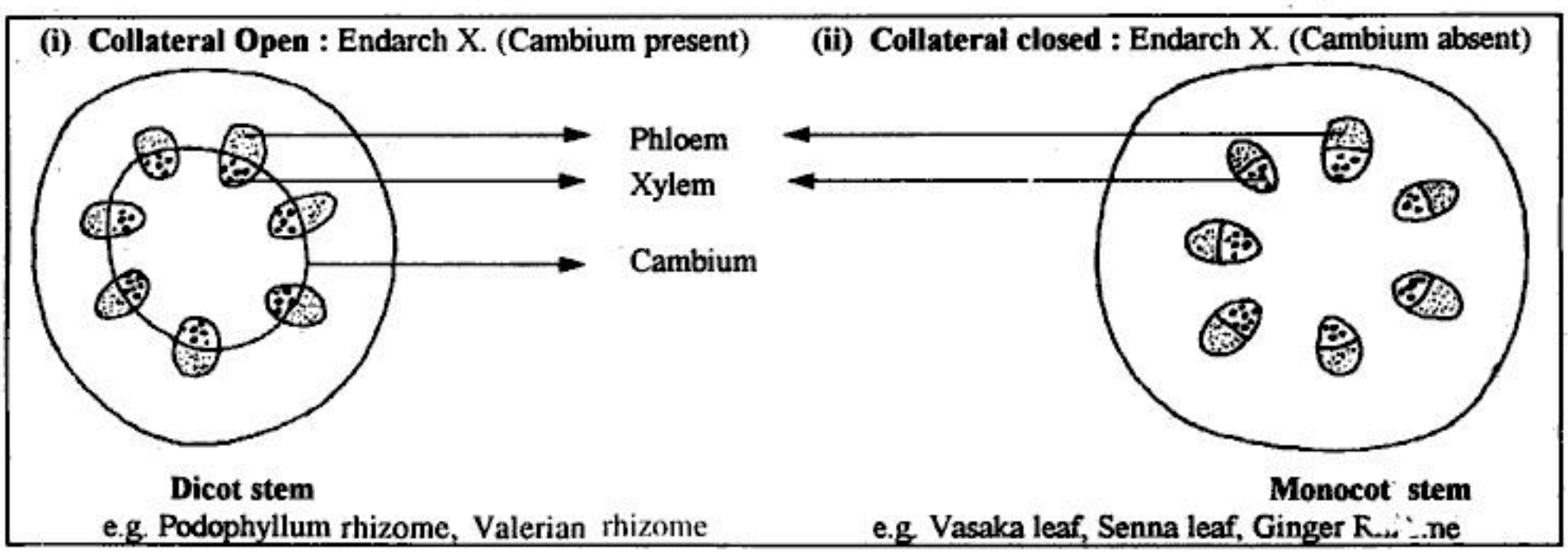
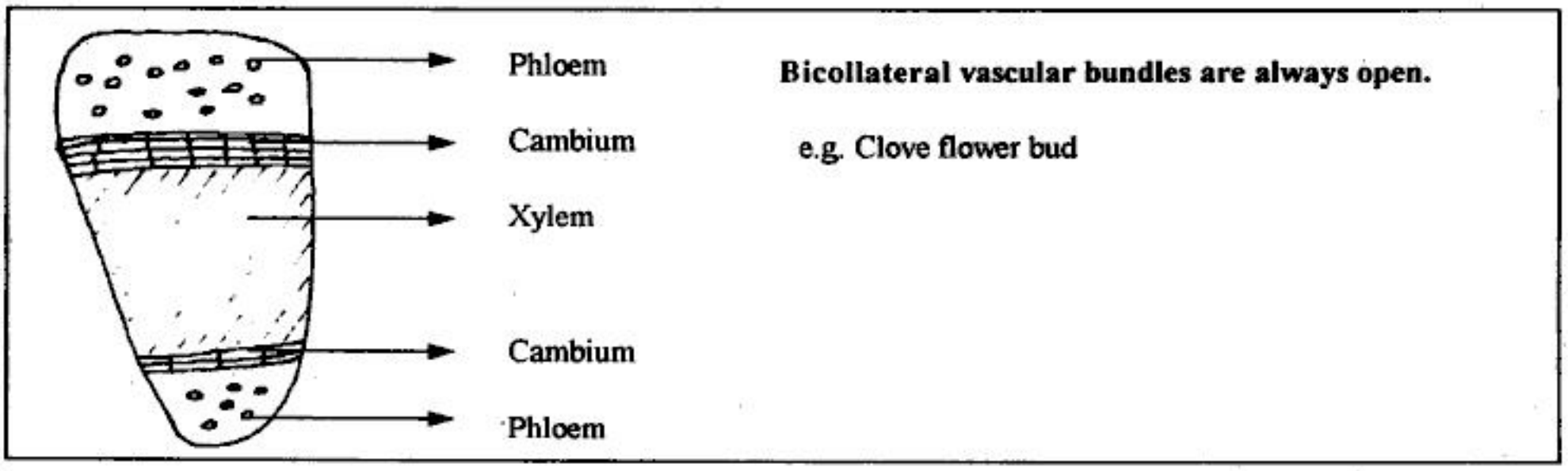
6.8.3 CONJOINT VASCULAR BUNDLE

This is a type in which xylem and phloem are present in the same vascular bundle.

(A) Collateral : Xylem and phloem are present on the same radius, side by side, in the same V. B.



(B) Bicollateral : In this type,the xylem is in the middle and phloem and cambium lie on either side of the xylem.

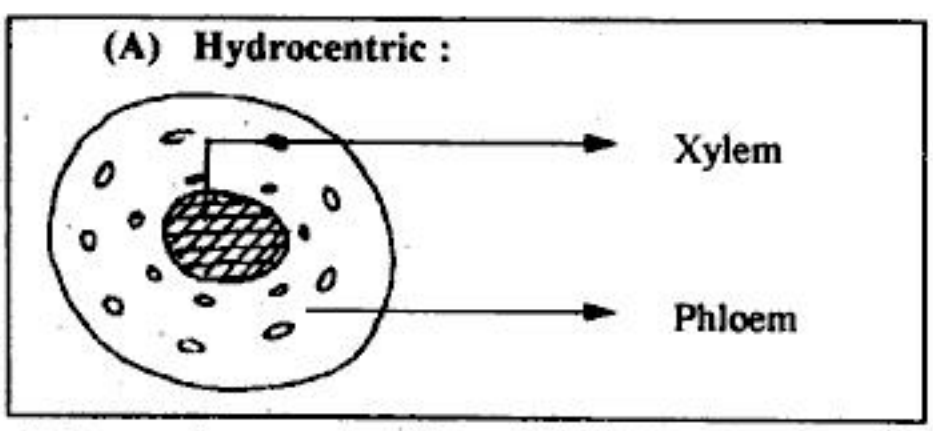


Bicollateral vascular bundles are always open.

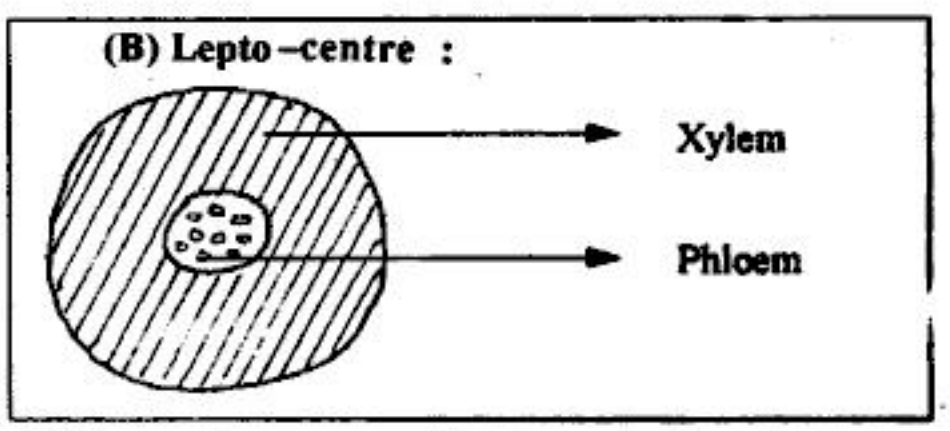
e.g. Clove flower bud

6.8.4 CONCENTRIC VASCULAR BUNDLE

In this,one vascular tissue totally surrounds the other. Concentric vascular bundles are always closed.



Xylem at the centre and is surrounded by phloem.



Phloem at the centre and is surrounded by xylem.

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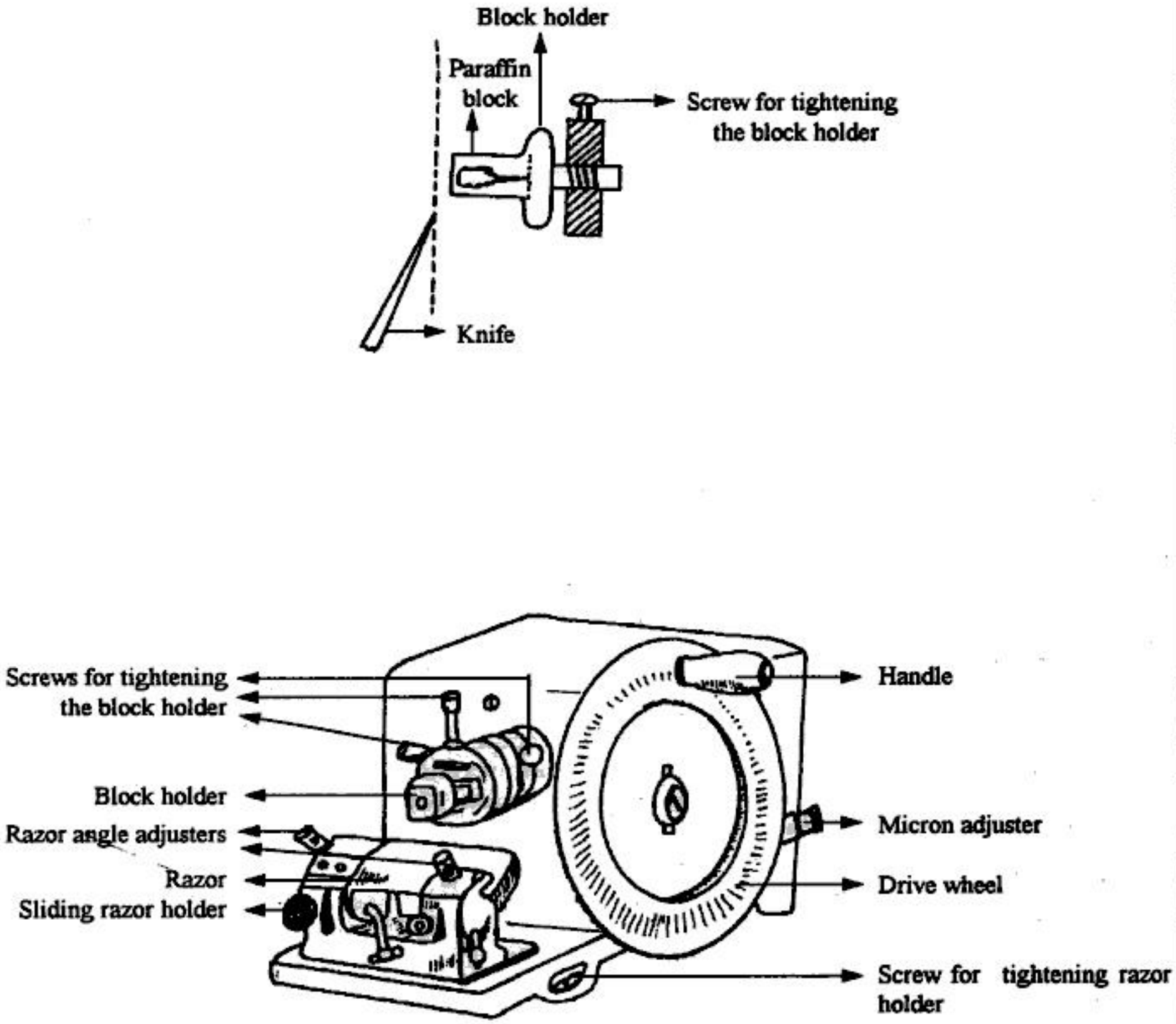

Spencer type rotary microtome

Advantages of using a microtome :

i) Sections are of uniform thickness, which can't be regulated in free-hand sections. There it may be thick or thin and also oblique.

ii) The sections can be obtained at any desired thickness (10,15 , 20 or 25 μ etc.)

iii) Sections obtained are not oblique. (If processed systematically)

iv) Sections of entire tissue can be obtained, which is sometimes necessary for studying the development of a plant organ.

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The drawing paper should be supported by a drawing board and if necessary, tilt it at correct angle to avoid distortion. Test the equal magnification on all the parts of board by placing a stage micrometer on the stage of the microscope and tracing its division on paper. If the distance between two divisions are not equal, the angle of tilt of the board is adjusted until equality is obtained. Now trace the image of the scale on paper and measure the distance between two lines.

For example, 0.4 mm of the stage micrometer = 13.2 cm on the drawing board

$\therefore$ 1 mm of the stage micrometer scale = 330 mm on the drawing board.

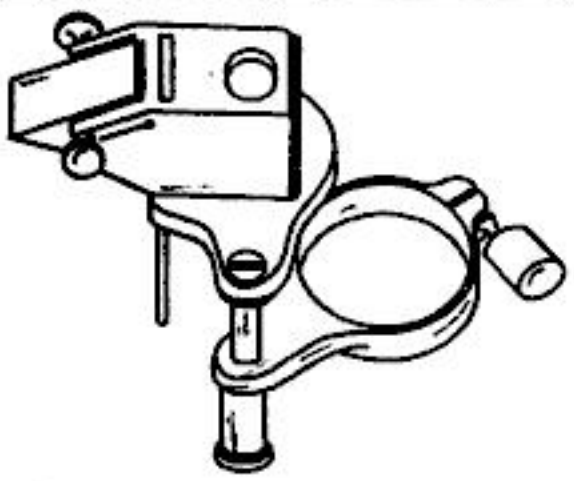
For the measurement of object, replace the stage micrometer by the mount of the object without disturbing other adjustments. Trace the outline of object and measure the dimensions of object.

If length of image of the object on the paper = 5.1 cm (51 mm)

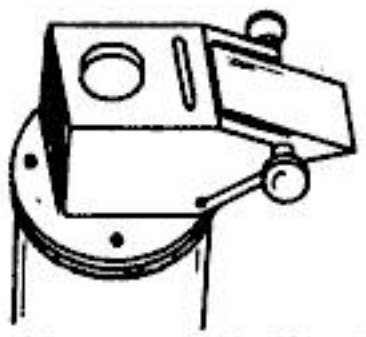
$$\therefore \text{Actual length of object} = \frac{51}{330} \text{ mm} = 0.154 \text{ mm}$$

$$= 154\ \mu$$

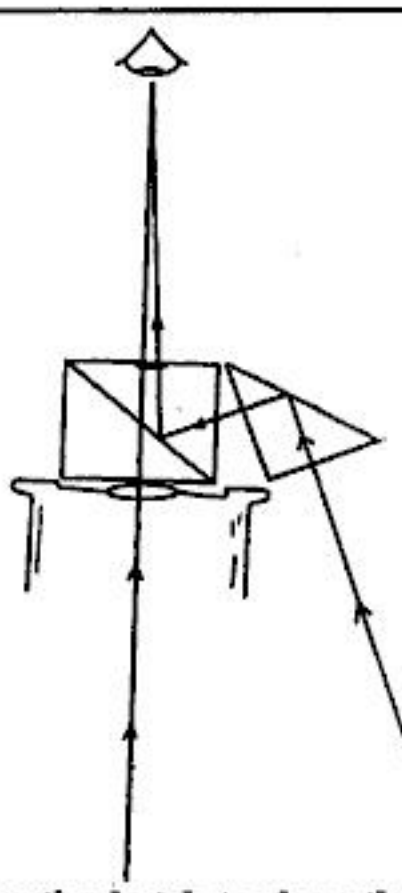
Alternatively, the drawing may be super-imposed on the traced outline of the stage micrometer and the length read off directly.



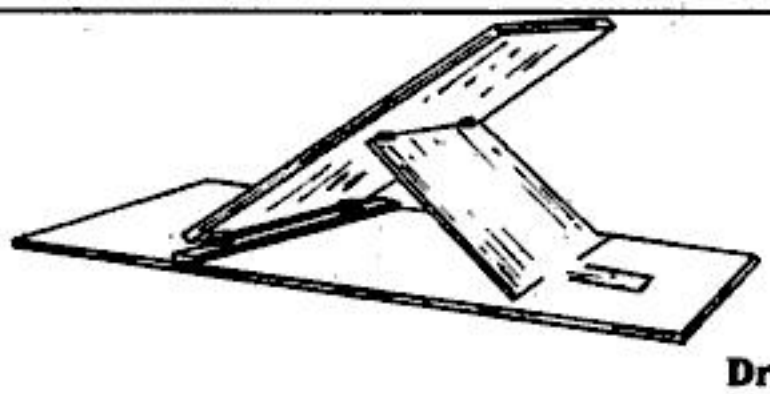
A : Swift Ives model camera lucida



B : Camera lucida mounted directly on a special eyepiece



C : Diagrammatic sketch to show the path of light rays through the camera lucida and through the microscope to converge in the eye. (In actual practice the eye is nearer the camera lucida that is shown in the figure.



Drawing board

1. Drawing board set up at an angle.

2. Drawing board in position of rest.

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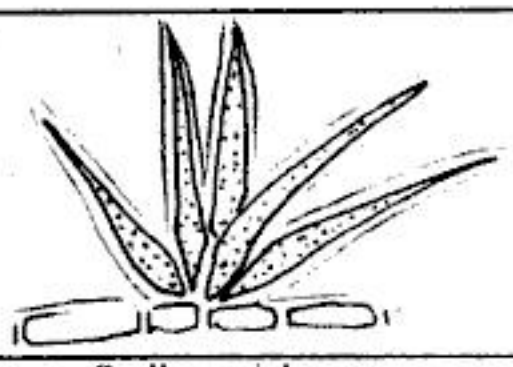
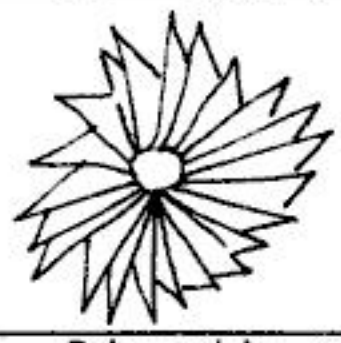
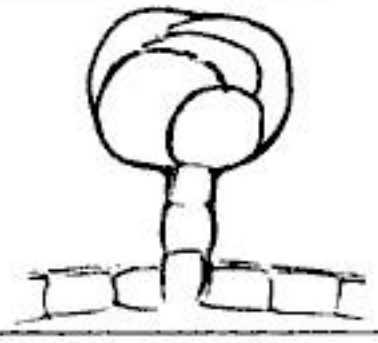
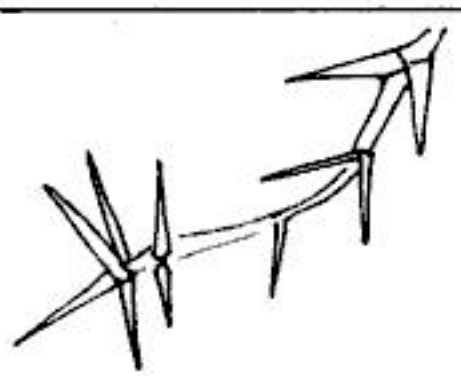
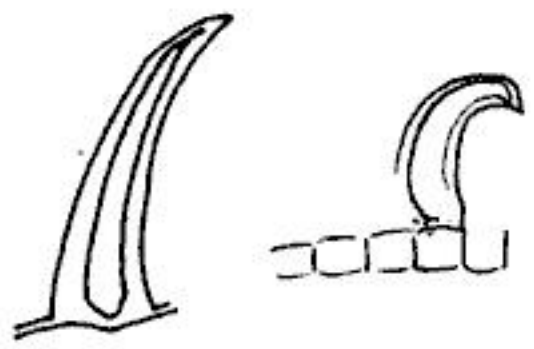
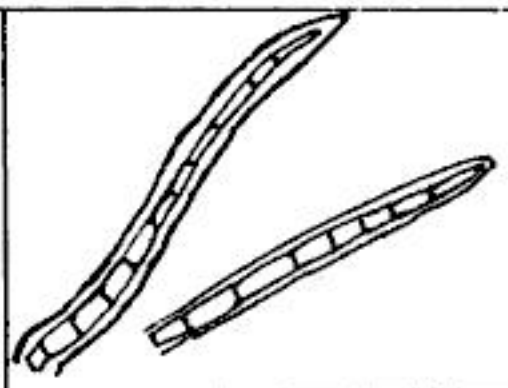
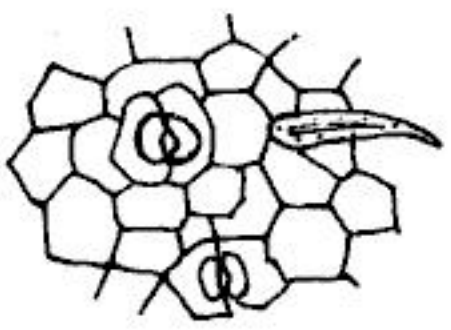
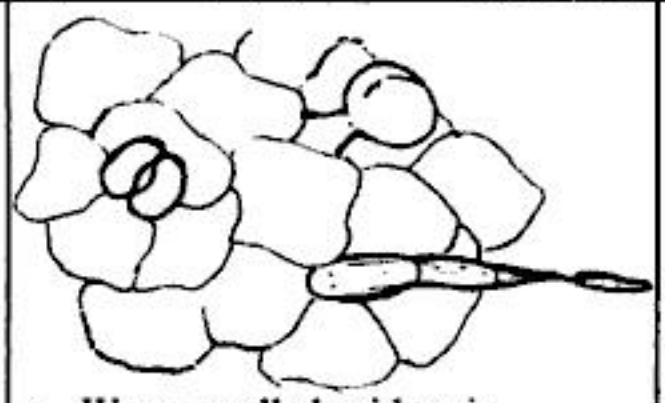
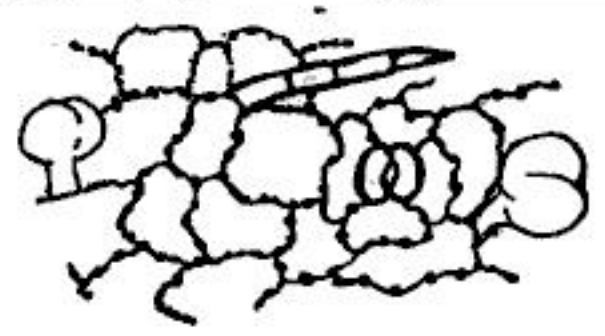
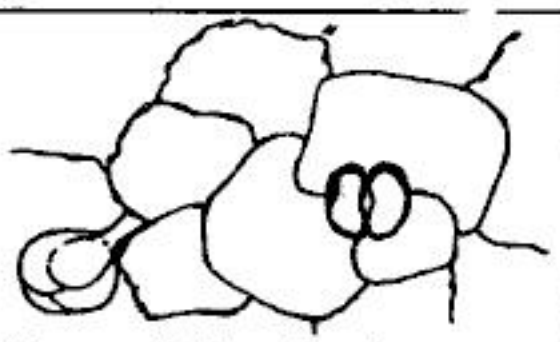
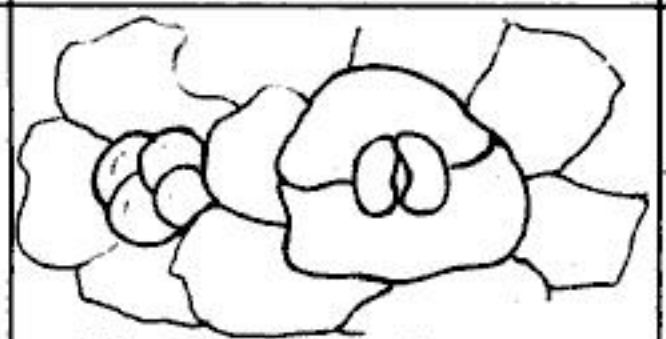
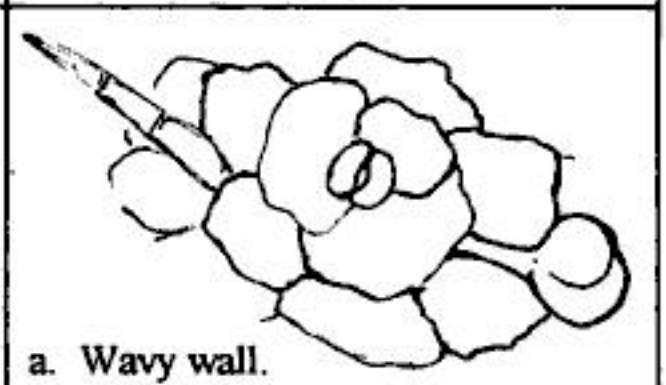
TYPICAL TRICHOMES :

		
Stellate trichome *(Hamamelis virginiana* L.*)*	Peltate trichome of *Croton eleuteria* Benn. (Cacarilla)	Allanthus

Detection of adulterants of *Digitalis purpurea* by means of trichomes :

		
Mullelin leaves	Comfrey leaves	Primrose leaves

Typical Examples of Epidermis Showing Stomata, Trichomes and Epidermal Cell

Senna	**Digitalis purpurea**	**Digitalis lanata**
		
a. Straight wall epidermis b. Paracytic stomata c. Covering trichome	a. Wavy - walled epidermis b. Anomocytic stomata c. Covering and glandular trichomes. Some covering trichomes are with collapsed cell.	a. Beaded walled epidermis b. Anomocytic stomata c. Covering trichomes and glandular trichomes very few than D. purpurea.
Belladonna	**Vasaka**	**Datura**
		
a. Wavy walled epidermis b. Anisocytic stomata c. Glandular trichome	a. Wavy anticlinal wall b. Diacytic stomata c. Sessile glandular (quadricellular)	a. Wavy wall. b. Anisocytic stomata c. Covering and glandular trichomes

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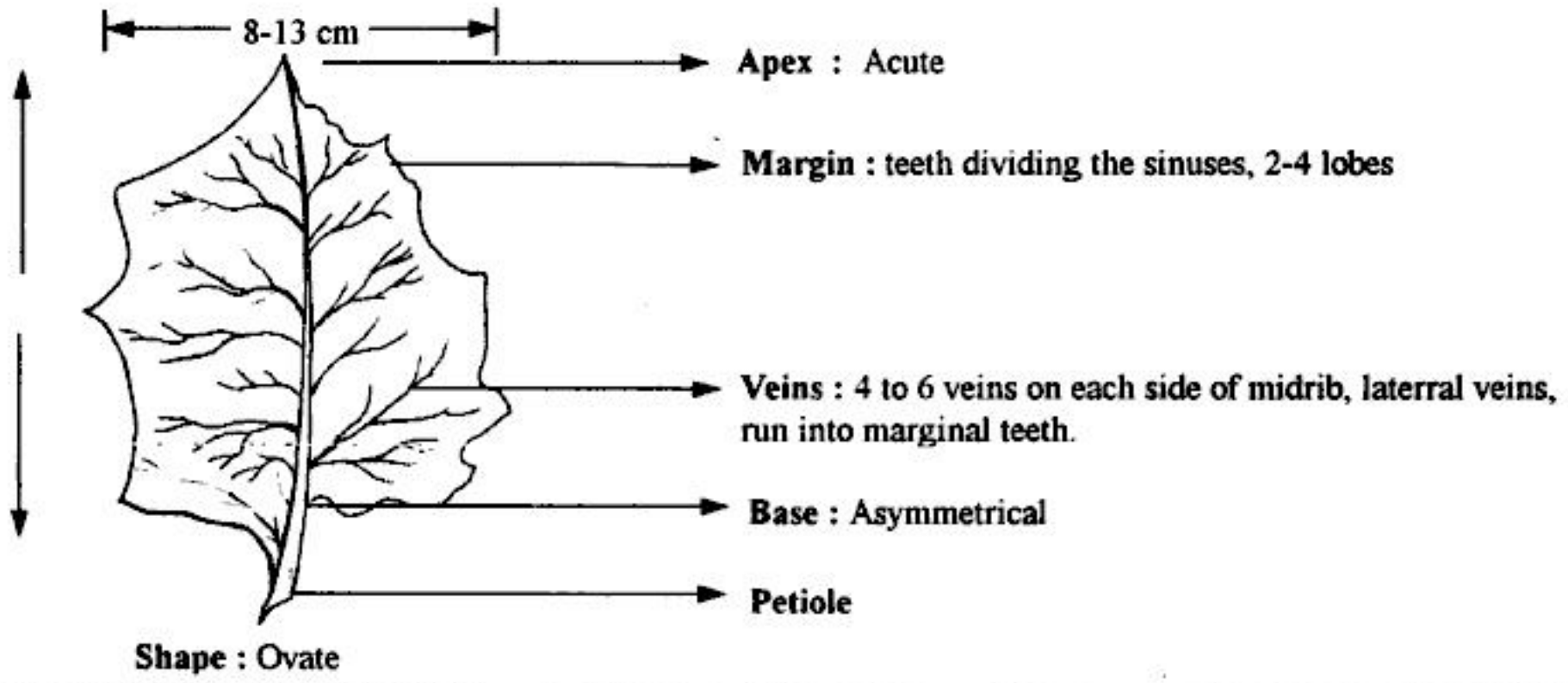
10 DATURA LEAF

SYNONYM : Datura herb

BIOLOGICAL SOURCE : Datura consists of dried leaves and flowering tops of *Datura metel* and D *metel* Var. *fastuosa* Safford belonging to family Solanaceae. It contains not less than 0.20% of total alkaloids calculated as hyoscyamine.

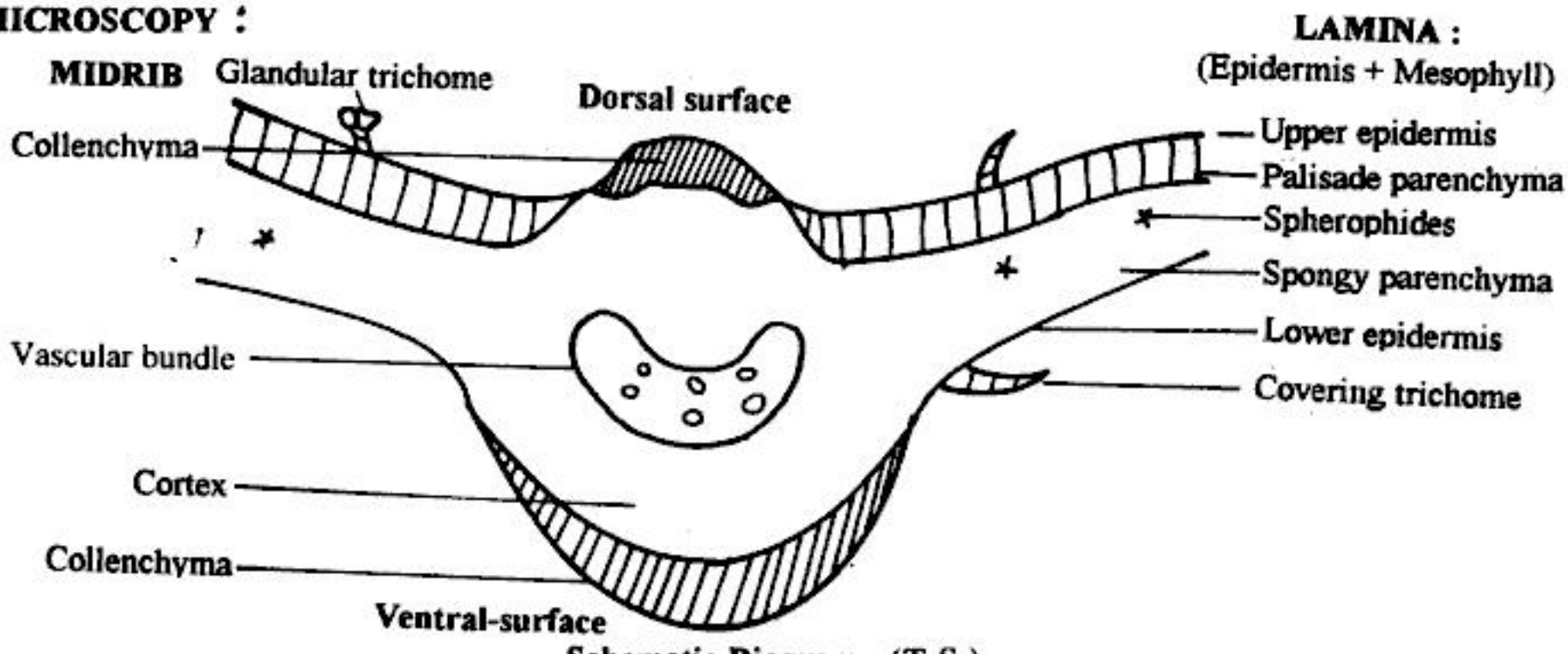
MACROSCOPY :



ORGANOLEPTIC CHARACTERS : Colour : Pale green. Odour : disagreeable characterstic, Taste : Unpleasant, bitter.

EXTRA FEATURES : Texture : Thin and minutely hairy. Upper epidermis : darker than lower. Midrib prominent on lower surface.

MICROSCOPY :



Schematic Diagram (T.S.)

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CHEMICAL TESTS :

Killer Killiani test for digitoxose :

Boil about 1g of finely powdered digitalis with 10 ml of 70% alcohol for two to three minutes, and filter the extract. To filtrate, add 15 ml of water and strong solution of lead acetate. Shake well and separate the filtrate. Treat the filtrate with equal volume of chloroform and filter. Evaporate to dryness. Dissolve the residue in glacial acetic acid and after cooling add 2 drops of ferric chloride solution. Transfer to tube containing 2ml of conc. Sulphuric acid. A reddish brown ring develops between two layers. Upper layer shows green colour. (This test is for deoxy sugar-digitoxose)

Legal's test :

Extract + pyridine + sodium nitroprusside solution and make it alkaline. Pink or red colour is produced. (This test is for 5 membered lactone ring present in cardenoloids).

Baljet test. See MICRO CHEMICAL TESTS.

CHEMICAL CONSTITUENTS :

Primary glycosides : Purpurea glycosides A and B, glucogitaloxin
Secondary glycosides : Digitoxin, gitoxin, gitaloxin.
Other minor glycosides : Odoroside H, verodoxin and glucoverodoxin.

Use : Cardiotonic.
Preparations : Prepared Digitalis I. P.; Digitalis tincture I. P.

ALLIED DRUGS :

(I) *Digitalis lanata* (Wooly foxglove) : (See ahead)

(II) *Digitalis lutea* (Straw foxglove) :

i) Leaves are sessile, about 15 cm in length and 2.5 cm. in width with an acuminate apex.
ii) Surface is less hairy than that of *D. purpurea.*
iii) More than one water pore may be present on each tooth.
iv) Vein islet number is lower than that of *D. purpurea.*

(III) *Digitalis thapsi* (Spanish foxglove) :

i) Leaves are elongated with decurrent lamina
ii) Pericyclic fibres and small prisms of calcium oxalate are present.
iii) Absence of non-glandular trichome and thick cuticle.
iv) Vein-islet number is higher than that of *D. purpurea.*

ADULTERANTS : (For diagrams of trichomes, see the topic : LEAVES).

(I) Comfrey leaves : (*Symphytum officinale.* Family. Boraginaceae)

i) Leaves are lanceolate or ovate in shape.
ii) Trichomes are thick-walled, tapering, with sharp apices, many are hooked near the tip. (hook at the top)

(II) Primarose leaves : (*Primula vulgaris.* Family. Primulaceae)

i) Leaves are nearly spatulate with straight lateral veins dividing near the margin.
ii) Trichomes are abundant at the lower surface and are of two types, covering trichomes uniseriate and 6 to 12 cells in length and small glandular trichomes with multicellular stalks and unicellular, spherical heads.

(III) Mullelin leaves : (*Verbascum thapsus.* Family. Scrophulariaceae)

i) Leaves are oblong in shape, 8 to 30 cm. long.
ii) Margin is somewhat toothed and apex is acute
iii) Surface is densely covered with large wooly branched Candelabra trichomes.

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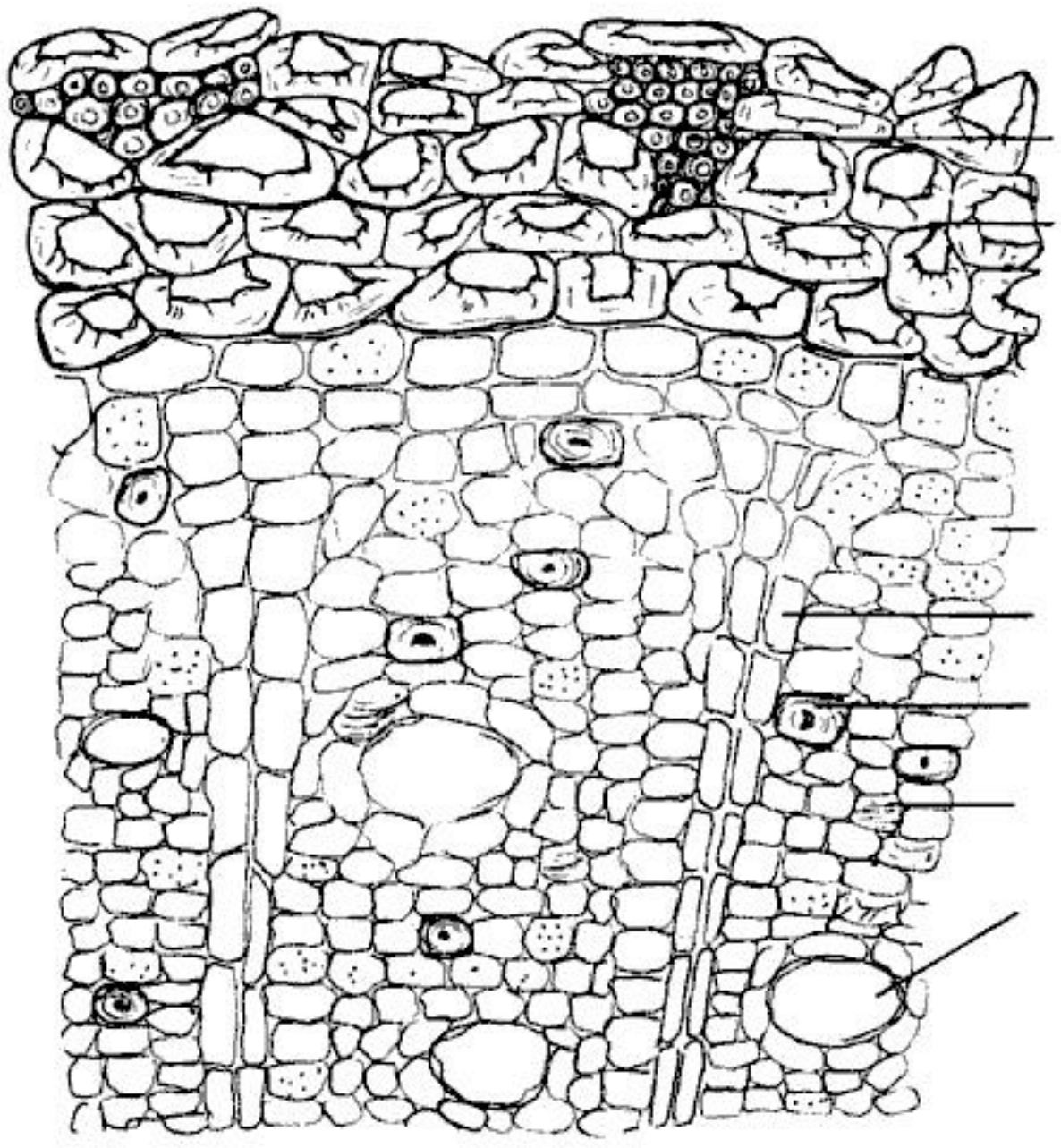

T. S. of Cinnamon Bark

PERICYCLE (STONE CELL LAYERS) :

Produce the light coloured wavy, longitudinal lines on the outside of the bark.

Pericyclic fibres : Small groups of about six to fifteen pericyclic fibres (lignified) occur at intervals.

Sclerides : Three to four layers of pitted sclerides, thickened lignified walls, isodiametric, slightly elongated tangentially (U-shaped thickening), with starch grains.

SECONDARY PHLOEM :

Parenchymatous. Few cells contain acicular calcium oxalate crystals and starch grains (diameter upto 10 μ).

Medullary rays : Biseriate, narrow at inner side, wider in the scleride band side, contains starch, acicular raphides.

Phloem fibres : Single, isolated, circular, lignified with stratification, being above 12 to 22 to 35 μ wide and 200 to 500 to 600 μ long.

Mucilage cells : Can be identified after staining with Ruthenium red (shows pink/red colour).

Oil cells : Big, isolated.

* **Cork and cortex are absent.**

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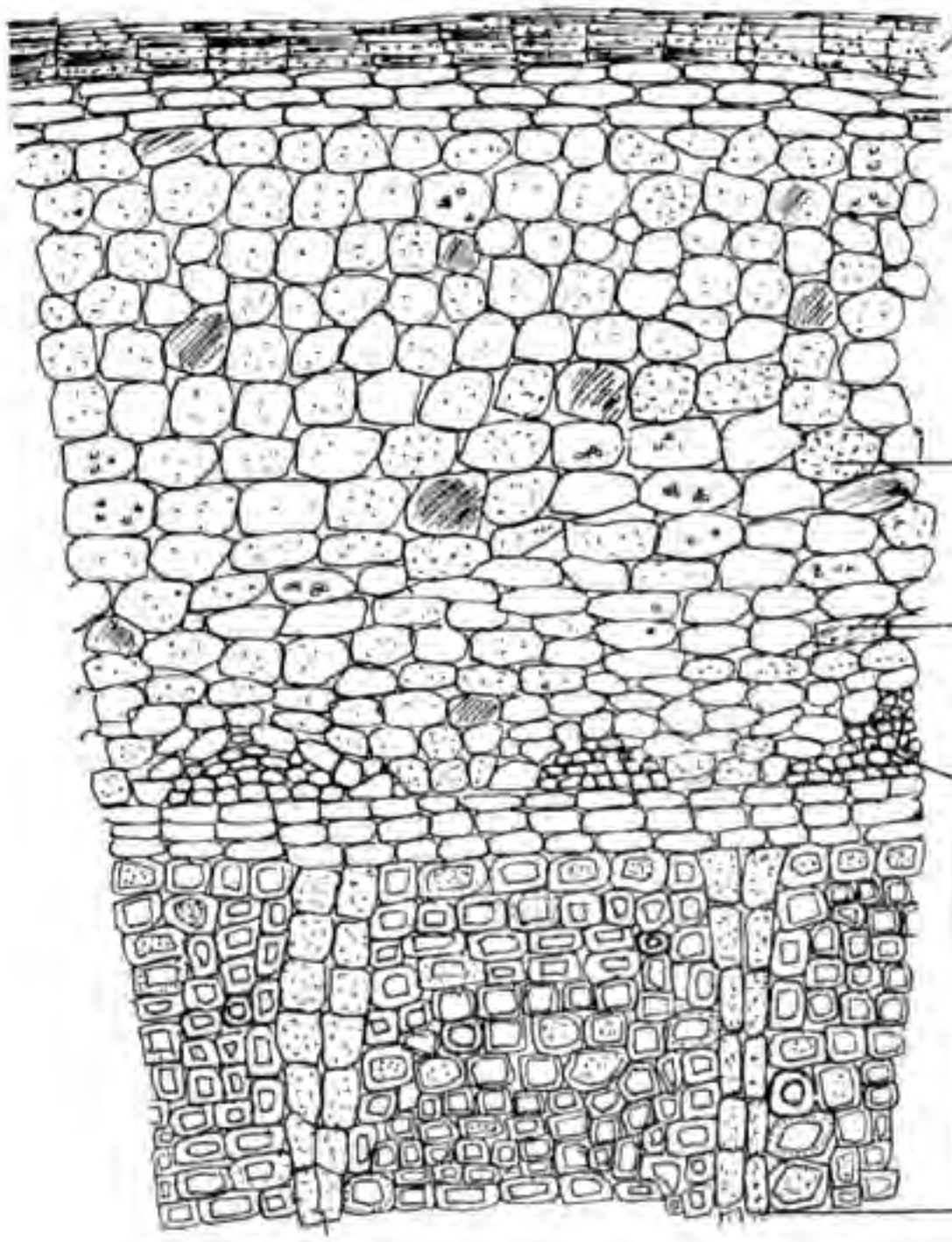

T. S. Ipecac root

CORK :
Narrow, tangentially elongated isodiametric cells, with dark brown granular matter, shape is not regular.

PHELLODERM :
Two or three rows of tangentially elongated thin walled cells. Few cells contain starch grains.

CORTEX :
Many layers of thin walled cellulosic parenchyma, with very small intercellular spaces. Cells are packed with starch grains. Scattered idioblasts contain bundle of acicular raphides of calcium oxalate.

Starch grain : Rarely single, mostly compound with 2-4 grains. Few cells show more than 4 grains too. Individual diameter about 4 to 10μ. (not more than 15 μ in diameter)

Calcium oxalate crystals : 30 to 80 μ length.

In powdered drug, these raphides are generally broken and crystals are scattered singly throughout the powder.

PHLOEM :
Many patches of small groups of sieve tissues embedded in parenchymatous cells, above the cambium.

XYLEM :
Entirely lignified, consists of tracheids, tracheidal vessels, xylem parenchyma and medullary rays. Tracheids have pitted wall. Vessels are not easily distinguished from the tracheids. Xylem parenchymatous cells are packed with starch grains like those of cortex.

Medullary Rays : Consists of lignified, radially elongated cells arranged in radial rows. Cells are usually filled with starch grains.

There are no vessels or normal fibres.

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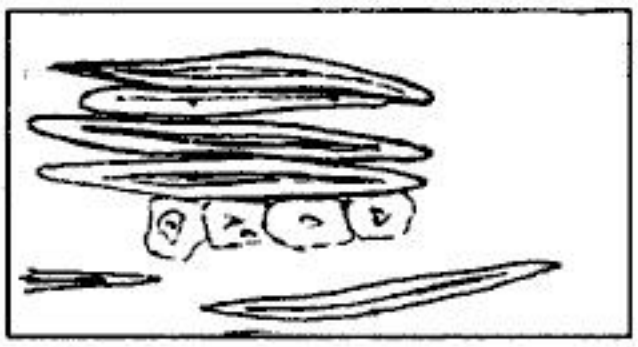
STAINING / DIAGNOSIS / MICRO-CHEMICAL TESTS:

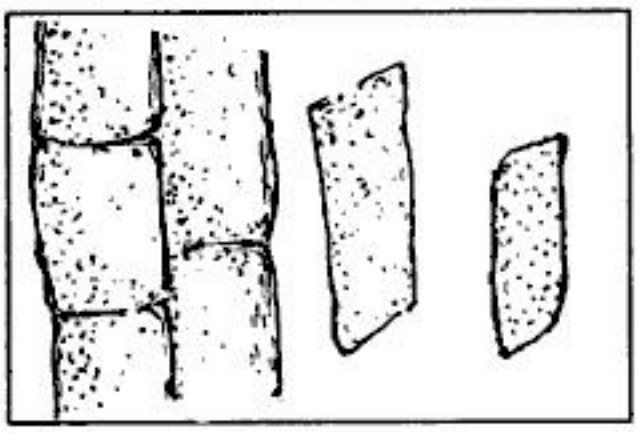
Sr. No.	Reagents	Observations	Characteristics
(1)	Phloroglucinol + Conc.HCl (1:1)	Pink	Lignified xylem fibres, phloem fibres
(2)	Iodine solution	Blue	Starch
(3)	Acetic acid	Insoluble	Calcium oxalate crystals
(4)	Hydrochloric acid	Soluble	Calcium oxalate crystals
(5)	Sulphuric Acid (60% w/w)	Soluble, needles of calcium sulphate on standing	Calcium oxalate crystals

MICROSCOPICAL CHARACTERISTICS OF POWDERED DRUG :



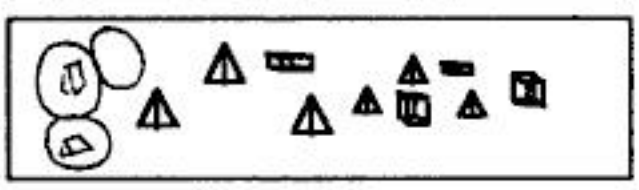
Fibres :

Lignified,thickened,yellow walls,in bundles of about10-50,with crystal sheath of parenchymatous cells each containing single prism of calcium oxalate.



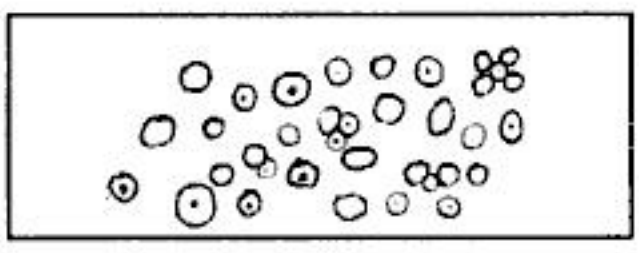
Xylem vessels :

Large, with numerous bordered pits.
Surface is lignified



Calcium oxalate crystals :

Individually prism shaped,10-15 to 25-35μ long.
Present in parenchymatous cells and many are scattered in the powder.



Starch grains :

Most are simple, oval or rounded, about 2-4 to 10-20μ long, showing no striations.

Cork :

Abundant fragments of orange–brown cork composed of thin walled polygonal cells.

CHEMICAL TESTS :

Tests	Observation	Inference
Aqueous extract + few drops of 5% $FeCl_3$ solution	Dark coloration	Phenolic compounds
5ml. dilute $KMnO_4$ solution + Few drops of aq. extract	Decolorisation of $KMnO_4$	Reducing compounds
Aq. extract + lead acetate reagent	White ppt.	Phenolic compounds, flavonoids
Aq. extract + mineral acid (HCl / H_2SO_4)	Reddish orange coloration which gets decolorized on addition of alkali and reappears on addition of acid again.	Flavonoids
Dried pieces / section / powder + a drop of sulphuric acid (80% v/v)	Orange yellow color	Flavonoids
Powder + water	Stable foam on shaking the test tube	Saponins

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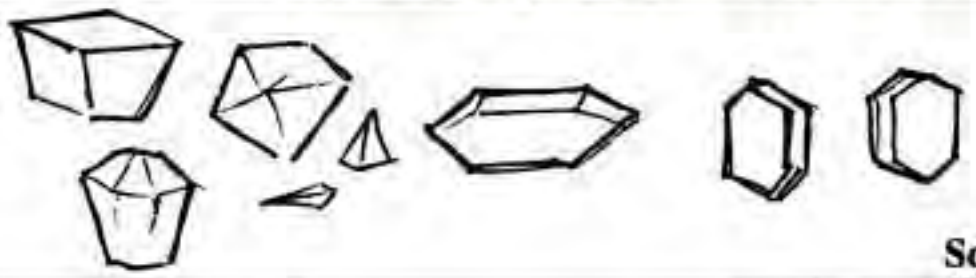
CRYSTAL SHEATH / CRYSTAL FIBRE

In crystal sheath or crystal fibre, parenchymatous cells surrounding the fibres contain calcium oxalate crystals. These parenchymatous cells containing crystals form crystal sheath. In each cell, one crystal is present and a large number of such cells are arranged in row, e.g. Liquorice, Cascara, Senna, Arjuna etc.

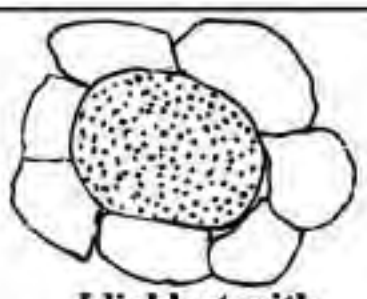
Generally, prisms are present in parenchymatous cells but in arjuna and other barks of the plants of family *Combretaceae* cluster crystals are present in crystal sheath or crystal fibres.



Squares and prisms

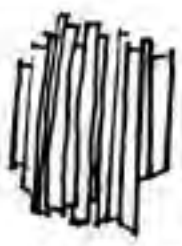
Cluster crystal

Rosette crystal



Idioblast with Microsphenoid crystals

Single acicular



Bundle of acicular

OBSERVATION OF CALCIUM OXALATE CRYSTALS BY PIZZOLOTO METHOD :

Procedure :

(i) Take uniformly thin sections of drug.

(ii) Treat them with 2N acetic acid for about 15 min.

(iii) Remove and treat them with 1% solution of silver nitrate in 15% hydrogen peroxide for about 15 min. (at 22°C).

(iv) Remove the sections and wash them with distilled water.

(v) Counterstain the sections with 2% safranin for 1 to 3 min.

(vi) Following usual technique, mount the sections and observe under microscope.

Calcium oxalate crystal appear black against red background. This method was designed by Pizzoloto, P. hence called as Pizzoloto method for observation of calcium oxalate crystals.

Dimensions of calcium oxalate crystals can be measured by the technique mentioned in the topic – MICROSCOPICAL DRAWINGS AND MEASUREMENT

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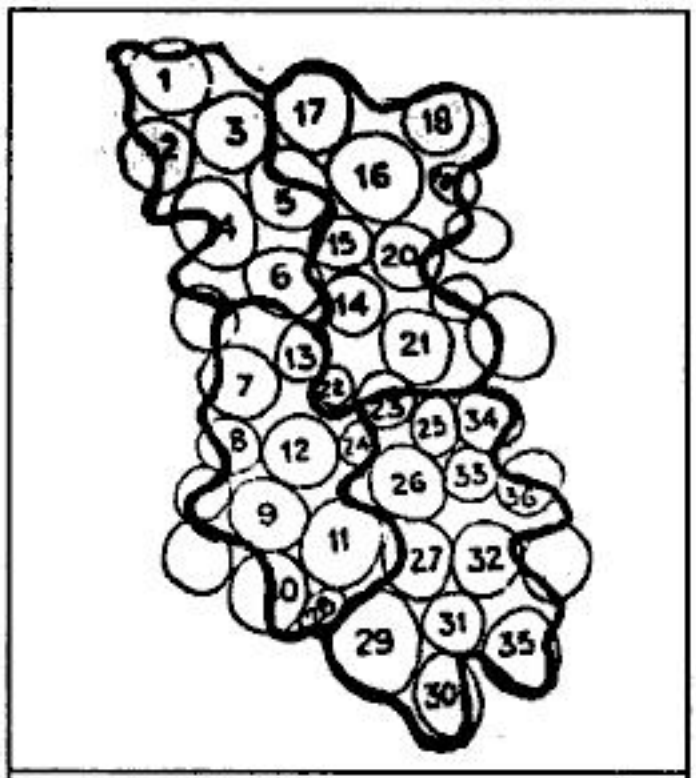
DETERMINATION OF PALISADE RATIO

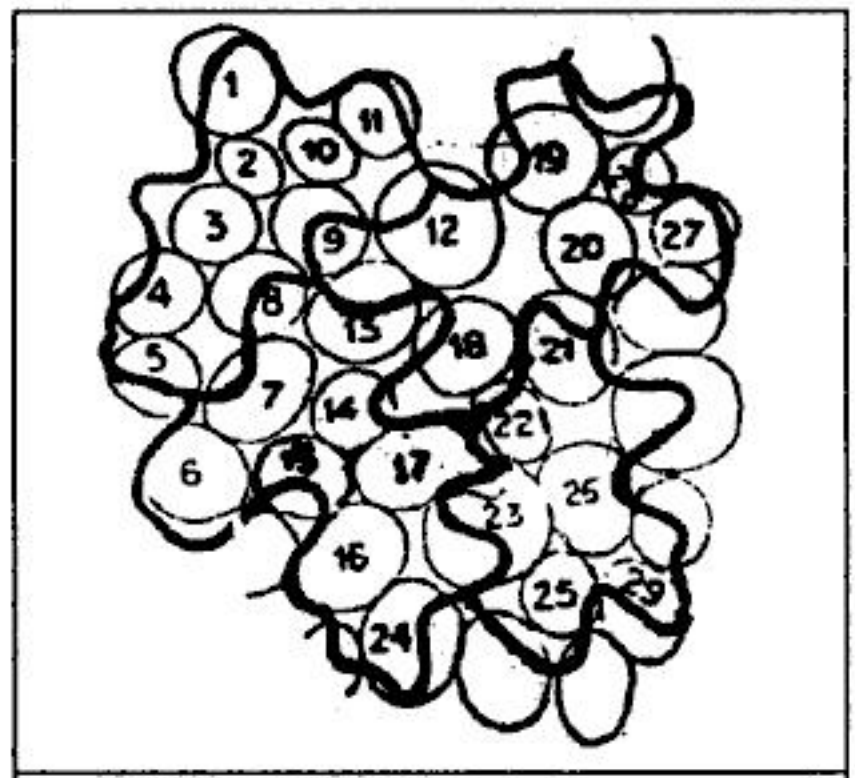
Definition : The palisade ratio is the average number of palisade cells beneath one epidermal cell of a leaf. It is determined by counting the palisade cells beneath four continuous epidermal cells.

Procedure :

(i) Clear a piece of the leaf by boiling in chloral hydrate solution.
(ii) Arrange the camera lucida and drawing board for making drawings.
(iii) Using the 4 mm objective, trace off the outlines of four cells of the epidermis.
(iv) Then, focus down to palisade layer and trace off sufficient cells to cover the tracings of the epidermal cells. Complete the outlines of those palisade cells which are intersected by the epidermal walls.
(v) Count the palisade cells under the four epidermal cells. (Include the palisade cell in the count when more than half is within the area of epidermal cell and exclude it when less than half is within the area of epidermal cells.)
(vi) Calculate the average number of cells beneath a single epidermal cell; this figure is the 'palisade ratio."
(vii) Repeat the determination for five groups of four epidermal cells from different parts of the leaf. Take the average of the results for the five groups. This average is the **'palisade ratio'** of the leaf.



Palisade ratio = 1 : 7
Belladonna Leaf



Palisade ratio = 1 : 9
Hyoscyamous Leaf

Atropa belladonna	6 to 10
Datura stramonium	4 to 7
Digitalis purpurea	3.7 to 4.2
Solanum nigrum	2 to 4

DETERMINATION OF VEIN-ISLET NUMBER

Definition : A vein-islet is the small area of green tissue surrounded by the veinlets. The vein-islet number is the average number of vein-islets per square millimeter of a leaf surface. It is determined by counting the number of vein-islets in an area of 4 sq. mm. of the central part of the leaf between the midrib and the margin.

Procedure :

(i) Clear a piece of the leaf by boiling in chloral hydrate solution for about thirty minutes.
(ii) Arrange camera lucida and drawing board for making drawings to scale.

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DETERMINATION OF FOREIGN ORGANIC MATTER

Foreign organic matter means the material consisting of any one or all of the following substances :

(a) Material not coming from the original plant source.
(b) Insects, moulds or other animal contamination.
(c) Parts of the organ or organs from which the drug is derived other than the parts named in the definition and description.
(d) Any other organ than those named in the definition and description.

Procedure :

(i) Weigh 100 to 500 g of the sample (or the quantity specified in the monograph of the drug).
(ii) Spread the sample on a white tile or a glass plate uniformly without overlapping.
(iii) Inspect the sample with naked eyes or by means of a lens (5x or above).
(iv) Separate the foreign organic matter (mentioned above) manually.
(v) After complete separation, weigh the matter and determine % w/w. present in the sample.

DETERMINATION OF CRUDE FIBRE BY THE DUTCH METHOD

Crude fibre is the residue of resistant tissues which can be obtained after giving treatment to the vegetable powder with dilute acid and alkali.

Advantages :

(1) Determination of crude fibre is useful in distinguishing between similar drugs or in the detection of adulteration.
(2) It also helps to remove the more resistant parts of plant organs which can be used for microscopical examination.
(3) The process removes starch and other cell contents (cellulose is not affected if dilute reagents are used). It also destroys lignin present in the cell wall of lignified tissue.

Procedure :

If crude drug contains appreciable amount of fat or oil, it must be removed first by extraction with suitable lipid solvent, before processing.

Steps :

(i) Weigh 2 grams of powdered drug in a beaker.
(ii) Add 50 ml. of 10% v/v nitric acid.
(iii) Heat to boil with constant stirring (till about 30 seconds after boiling starts).
(iv) Strain through fine cotton cloth on a Buchner funnel.
(v) Give washing to the residue with boiling water. (Suction may be used).
(vi) Transfer residue from the cloth to a beaker.
(vii) Add 50 ml. of 2.5% v/v sodium hydroxide solution.
(viii) Heat to boil. Maintain at boiling point for 30 seconds, stirring constantly.
(ix) Strain and wash with hot water as mentioned earlier.
(x) For quantitative determination, transfer the residue in a cleaned and dried crucible.
(xi) Weigh the residue and determine percentage crude fibres.

For microscopical examination, residue is suspended in water or alcohol (70%) until required for use.

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INSOLUBLE IN ALCOHOL

Test	Observation	Inference
1) Triturate with water	White emulsion	Asafoetida
	Yellowish emulsion	Myrrh
2) Mount in alcohol & irrigate with water	Particles dissolved without swelling	Acacia
	Particles swell and ultimately dissolve or become diffused	Tragacanth, agar, gelatin sterculia gum.
3) Mount in ruthenium red	Stains pink	Agar or sterculia gum
4) Heat with soda lime in a dry test tube.	Ammonical vapour	Albumen, gelatin
5) Boil about 1 part with 100 parts of water	Stiff jelly like mass on cooling.	Agar
	The liquid coagulates.	Albumen
6) Warm with caustic soda (5%)	Canary yellow	Agar, tragacanth
7) Mount in N/50 iodine	No colour reaction	Agar
	Blue specks appear	Tragacanth
SOLUBLE IN ALOCHOL		
Dilute solution + ferric chloride solution	Green colour	Gambier, tolu balsam
Dilute solution + potasium permanganate	Odour of benzaldehyde	Balsams
Apply test for hydroxymethyl anthaquinone derivative	Rose- pink colour	Aloe except zanzibar aloes
Dilute solution + borax	Green fluorescence	Aloe

Use remaining of the powder to perform specific chemical tests mentioned in the book.
Thus by means of physical and chemical tests, one can identify an unorganised drug in powder form.

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REAGENTS	PREPARATION AND USES
Ferric chloride (alcoholic)	A 5 % w/v solution of ferric chloride in 90 % alcohol and used for detection of phenols (blue colour).
Frohde's reagent	Dissolve 1 g of sodium molybdate in 100 ml concentrated sulphuric acid and use after few weeks. It gives bluish green colour with ipecacuanha alkaloids.
Iodine water	Dissolve 2 g of iodine and 3 g of potassium iodide in 100 ml of water. With starch grains it shows blue colour. Cellulose, proteins, lipids give yellow to brown colour.
Lactophenol	Dissolve 20 g each of phenol and lactic acid, 40 g of glycerin in 20 ml distilled water. Use as mountant for unorganised drug like starch, aloe etc.
Liebermann Burchard reagent	Mix 10 ml of acetic anhydride with 10 ml of concentrated sulphuric acid by cooling. To it, add 100 ml of absolute alcohol by cooling. Use it to detect triterpenes and steroids.
Mayer's reagent	Prepare a solution by dissolving 1.36 g of mercuric chloride in 60 ml distilled water, add it to a solution of 5 g of potassium iodide in 20 ml distilled water, make volume to 100 ml. With alkaloids,it shows cream precipitate.
Millon's reagent	Dissolve 1 g of mercury in 9 ml of fuming nitric acid, after cooling, add equal volume of distilled water. Protein is stained red on warning.
Molisch's reagent	Dissolve 10 g of α-napthol in 100 ml of 95 % alcohol. Use to detect carbohydrate.
Ninhydrin reagent	0.1 % solution in butanol. It is used to detect amino acids.
Phloroglucinol solution	Dissolve 2 g of phloroglucin in 100 ml of 90 % alcohol. Alongwith conc. hydrochloric acid (1 : 1). It stains lignified tissue to pinkish red.
Potash caustic	Dissolve 5 g of stick potash in 95 ml of water. A 50 % solution is used for macerating soften tissues. It acts as a clearing agent.
Ruthenium red	Dissolve 0.008 g of ruthenium red in 10 ml of 10 % solution of lead acetate. It stains mucilage to red colour.
Safranin	It is 1 % safranin solution in 50 % ethyl alcohol. It stains lignified cells red.
Sudan red III	Dissolve 0.04 g of Sudan red in 20 ml of alcohol (90 %) and 20 ml of glycerin. It stains fixed oil, volatile oil globules red.
Tannic acid solution	Dissolve 5 g of tannic acid in distilled water and adjust volume to 100 ml. It is used to detect alkaloids.
Tincture of alkana	Macerate alkana root with five volumes of 90 % alcohol for one week and filter. Use within six months to stain fixed oils, fats, suberin or cutin (pinkish red)
Vanillin-sulphuric acid	It is fresh solution of 0.5 % vanillin in sulphuric acid-ethanol (4 : 1) solvent system, used to detect steroids and terpenes.
Van Urk reagent	Dissolve 1 g of p-dimethyl-amino-benzaldehyde in 50 ml of hydrochloric acid and 50 ml of 95 % ethanol. It is used to detect indole type of alkaloids.
Wagner's reagent	Dissolve 1.27 g of iodine and 2 g of potassium iodide in 5 ml of water and make up the volume to 100 ml with distilled water. With alkaloids,it shows reddish brown precipitate.

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Dr. K. R. Khandelwal, Department of Pharmacognocy, Bharati Vidyapeeth Deemed University's Poona college of Pharmacy has obtained his Diploma in Pharmacy from Poona College of Pharmacy, graduation from Government College of Pharmacy - Karad, Post-graduation from Department of Pharmaceutical Sciences at Nagpur and Doctorate from University of Poona.

He is a recognised post-graduate teacher and research guide. His research area includes Pharmacognosy and Pharmacological studies on natural products, optimization of Ayurvedic formulations and formulation process development in Indian system of medicine. He has guided a number of post-graduate students and published several research papers in National and International Journals. Dr. Khandelwal is receipient of the Best Guide Award as well as Gold Medal for Best Thesis. He is a member of Board of studies, faculty of Pharmaceutical Sciences, University of Pune. He is also co-author of books- Drug Index and Health Education and Community Pharmacy.

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