MARRI LAXMAN REDDY GROUP OF INSTITUTIONS

MLR INSTITUTE OF PHARMACY

(Approved by AICTE & PCI, New Delhi and Affiliated to JNTUH, Hyderabad)
Dundigal, Quthubullapur Mandal, Hyderabad-500043.

PHARMACOGNOSY-I

Practical Manual

ACADEMIC YEAR

NAME : _____

REGD.NO : _____

YEAR : _____

Prepared By:

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About MLRIP

VISION

To be an educational Institute of par excellence and produce competent pharmacy professionals to serve the community through research and the ever-increasing needs of Industry.

MISSION

1. Imparting quality education and innovative research for various career opportunities.
2. Creating conducive academic environment to produce competent pharmacy professionals.
3. Indoctrination of students adorned with high human values and make them aware of their responsibility as health care professionals.

Program Educational Objectives

PEO 1: To produce graduates with sound theoretical knowledge and technical skills required for their career opportunities in various domains.

PEO 2: To incite the students towards research and to address the challenges with their innovative contributions for the benefit of the mankind.

PEO 3: To instill the essence of professionalism, ethical commitment to become a health care professional with sound integrity and adherence to the core human values in the service of the society.

PROGRAM OUTCOMES

1. **Pharmacy Knowledge:** Possess knowledge and comprehension of the core and basic knowledge associated with the profession of pharmacy, including biomedical sciences; pharmaceutical sciences; behavioral, social, and administrative pharmacy sciences; and manufacturing practices.
2. **Planning Abilities:** Demonstrate effective planning abilities including time management, resource management, delegation skills and organizational skills. Develop and implement plans and organize work to meet deadlines.
3. **Problem analysis:** Utilize the principles of scientific enquiry, thinking analytically, clearly and critically, while solving problems and making decisions during daily practice. Find, analyze, evaluate and apply information systematically and shall make defensible decisions.
4. **Modern tool usage:** Learn, select, and apply appropriate methods and procedures, resources, and modern pharmacy-related computing tools with an understanding of the limitations.
5. **Leadership skills:** Understand and consider the human reaction to change, motivation issues, leadership and team-building when planning changes required for fulfillment of practice, professional and societal responsibilities. Assume participatory roles as responsible citizens or leadership roles when appropriate to facilitate improvement in health and well-being.
6. **Professional Identity:** Understand, analyze and communicate the value of their professional roles in society (e.g. health care professionals, promoters of health, educators, managers, employers, employees).
7. **Pharmaceutical Ethics:** Honour personal values and apply ethical principles in professional and social contexts. Demonstrate behavior that recognizes cultural and personal variability in values, communication and lifestyles. Use ethical frameworks; apply ethical principles while making decisions and take responsibility for the outcomes associated with the decisions.
8. **Communication:** Communicate effectively with the pharmacy community and with society at large, such as, being able to comprehend and write effective reports, make effective presentations and documentation, and give and receive clear instructions.
9. **The Pharmacist and society:** Apply reasoning informed by the contextual knowledge to assess societal, health, safety and legal issues and the consequent responsibilities relevant to the professional pharmacy practice.
10. **Environment and sustainability:** Understand the impact of the professional pharmacy solutions in societal and environmental contexts, and demonstrate the knowledge of, and need for sustainable development.
11. **Life-long learning:** Recognize the need for and have the preparation and ability to engage in independent and life-long learning in the broadest context of technological change. Self-assess and use feedback effectively from others to identify learning needs and to satisfy these needs on an ongoing basis.

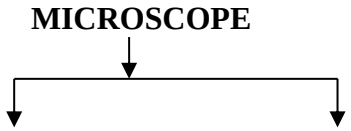
EXPERIMENT - 1

INTRODUCTION OF PHARMACOGNOSY LABORATORY AND EXPERIMENTS

MICROSCOPE

A microscope is an important analytical instrument for the histological examination of a very large range of materials such as vegetable drugs, fibres, mineral substances, food products and spices.

The microscope (micro means small and skopein means to see) is an optical instrument for the study of small objects and can 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.



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

SIMPLE MICROSCOPE (DESSECTING MICROSCOPE)

It helps to reveal the morphological characteristics of the object.

COMPOUND MICROSCOPE

The compound microscope essentially consists of three major systems.

I. Support system:

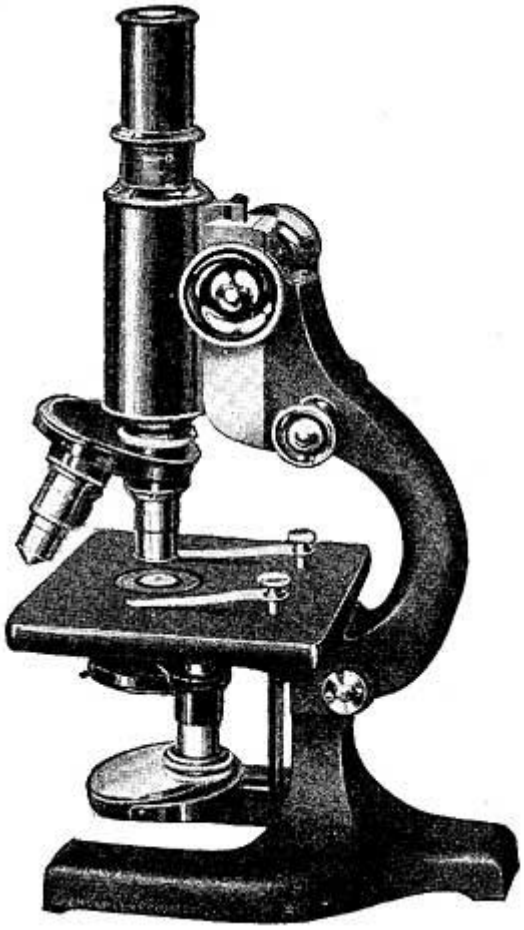
It comprises of base, stage and body tube.

II. Illumination system:

It throws light on the object for proper viewing. It comprises of light of 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.



DIFFERENT ILLUMINATION SYSTEMS USED IN MICROSCOPES

(A) Plain Mirror

Use plain mirror when the fixed source of light is used.

(B) Concave Mirror

When skylight is used, concave mirror helps to converge the beam onto the condenser.

(C) Sub stage lamp interchangeable with mirror

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

(D) **Build-in sub stage lamp (Tungsten – Filament or Halogen Lamp)** with intensity adjustment.

LIGHT ADJUSTMENT IN A MICROSCOPE

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

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

(B) 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.

FACTS AND FIGURES ABOUT 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 times.

Thus, the object viewed is magnified 50 times.

(B) Resolving power of objective (R.P)

Resolving power of objective is defined as the ability to separate distinctly two small elements of an object which is 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 shown as working distance

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

(D) Focusing:

Focusing an object while viewing through an eye piece requires 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 can view through the eye piece is the field of view. The field of view narrows and magnification increases.

(F) Objectives: Different objectives used in microscopy.

- 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.
- 10X – Low power objective; with the help of this one identifies the part of the observed in high power. This does not reveal many details.
- 40x – 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 I protect the front lens, as the working distance is low in high magnification and the lens may touch the slide while focusing. The spring does not allow pressure on the front lens when it touches the slide.
- 100X – Oil impression 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 hence allows very high resolving power.

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.

II. PREPATATION OF SAMPLE FOR SECTIONNING

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 pitch 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.

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.

III. STAINING AND MOUNTING OF SECTIONS

Staining is a process in which chemical dyes are used to impart 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 color to it. e.g., safranin combines with the lignin present in cell wall and vessels and imparts a red color to the lignified tissues. Iodine solution combines with starch grains to give a blue color. Sudan Red III dissolves in the fixed oil present in the oilseeds to impart red color.

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

STAINING PROCESS

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 side.

MOUNTING PROCESS

1. Take a clean glass micro slide.
2. On this slide transfer the section to be mounted, with the help of 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.

DOUBLE STAINING TECHNIQUE

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

METHOD I

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. Transfer the section 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, staring 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.

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 xylot.

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.

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

The section is then transferred 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.

Lignified tissues nuclei and cutinized 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 where as Delafield's haematoxylin deposits badly and requires frequent filtering.

B) PREPARATION OF COMMONLY USED REAGENTS AND SOLUTIONS IN MICROSCOPIC WORK

REAGEGNTS	PREPARATION AND USES
Acetic acid (1-5 %)	Dilute aqueous solution (1-5 %). It does not affect calcium oxalate crystals, however, dissolves globoids of aleurone grains.
Ammonium -vanadate (Sulphuric acid)	Dissolve 1.62g of anhydrous ammonium vanadate in 125ml of concentrated sulphuric acid, cool and add into 125ml of ice-cold water. After dilution to 10 fold, used to detect alkaloids, phenolic compounds and steroids.
Aniline blue	Staining reagent for cellulosic tissue. 1% aqueous solution stains callus. whereas the alcoholic solution is as a counter stain with safranin.
Aniline sulphate	It is saturated aqueous solution of aniline sulphate acidified with concentrated sulphuric acid. it stains lignified cell walls to yellow in colour.
Bleaching solution	Prepare a solution by dissolving 75g of crystalline sodium carbonate in 125ml of distilled water, add it to a mixture of 50g of chlorinated lime in 375ml of distilled water. Shake occasionally and filter.
Canada balsam	Heat Canada balsam on a water - batch to remove all the volatile matter. Dissolve the residue in xylene or benzene to form a thin viscous fluid. The reagent is used to prepare permanent mounts.
Chloral hydrate	Dissolve 50g in 20 ml of distilled water. It is used as clearing agent. For the removal of chlorophyll, boil material with a little amount of this reagent over flame for a short while.
Chlor-zine-iodide (Schulze's solution)	Dissolve 30g zinc chloride, 5g potassium iodide and 1g iodine in 14ml distilled water. It is stains cellulose-blue or violet; lignin-yellow, cutin and suberin-yellow or brown, starch-blue and protein-brown.
Chromic acid	It is the saturated aqueous solution of chromic acid or mixture of 10% chromic acid in 10% nitric acid. Strong solution dissolves cellulose and lignified walls.
Corallin soda	Prepare a solution by dissolving 25g of crystalline sodium carbonate in 100ml of water and second solution by dissolving 5g of Corallin in 100ml of 90% alcohol. At the time of use mix both in 21 : 1 ration. It stains callus of sieve - tubes, starch grains and liquefied walls.
Cuoxam	Mix 0.5g of copper carbonate in a mortar with 10ml of distilled water and gradually add to it 10ml of strong solution of ammonia with constant stirring. It dissolves cellulose walls.
Dragendorff's reagent	Boil 14g of sodium iodide with 5.2g basic bismuth carbonate in 50ml glacial acetic acid for a few minutes. Allow it to stand overnight and filter off the precipitate of sodium acetate crystals to 40ml of the rd-brown filtrate add 160ml of acetate and 1ml water. Preserve the stock solution in amber-coloured bottle. When needed add 20ml of acetic acid to 10ml of this stock solution and make up to 100ml with water. Used for detection of alkaloids (orange brown precipitate).
Fehling's solution	Dissolve 34.66 g of copper sulphate in distilled water and make volume up to 500ml (solution A). Dissolve 173g of potassium sodium tartarate and 50g of sodium hydroxide in distilled water and make volume up to 500ml (solution B). Mix two solutions in equal volume prior to use for detection of reducing sugars.

EXPERIMENT-2

PHARMACOGNOSTIC STUDY OF ISAPGOL SEED

AIM: To Study the pharmacognostic characteristics of Isapgol seed.

REQUIREMENTS:

GLASSWARE: Microscope, glass slide, cover slip, camel brush.

CHEMICALS: Phloroglucinol , conc.Hcl, picric acid, ruthenium red.

SYNONYM: Isapgula, Isapgol, Isabgul, Psyllium seeds / flaxseeds.

BIOLOGICAL SOURCE: It consists of dried seeds of *Plantago ovata* Forsk belonging to the family. *Plantaginaceae.*

MORPHOLOGICAL CHARECTERS:

COLOR**:** Pinkish gray to brown in color**.**

ODOUR: Odour less / None.

TASTE: Blank / Mucilaginous.

SHAPE: Ovoidcymbiform**.**

SIZE: 10 - 3.5 mm length, 1 – 1.75 mm width

MICROSCOPICAL CHARECTERS:-

EPIDERMIS (HUSK): Single layer, color less, radially elongated thin walled cells, full of mucilage.

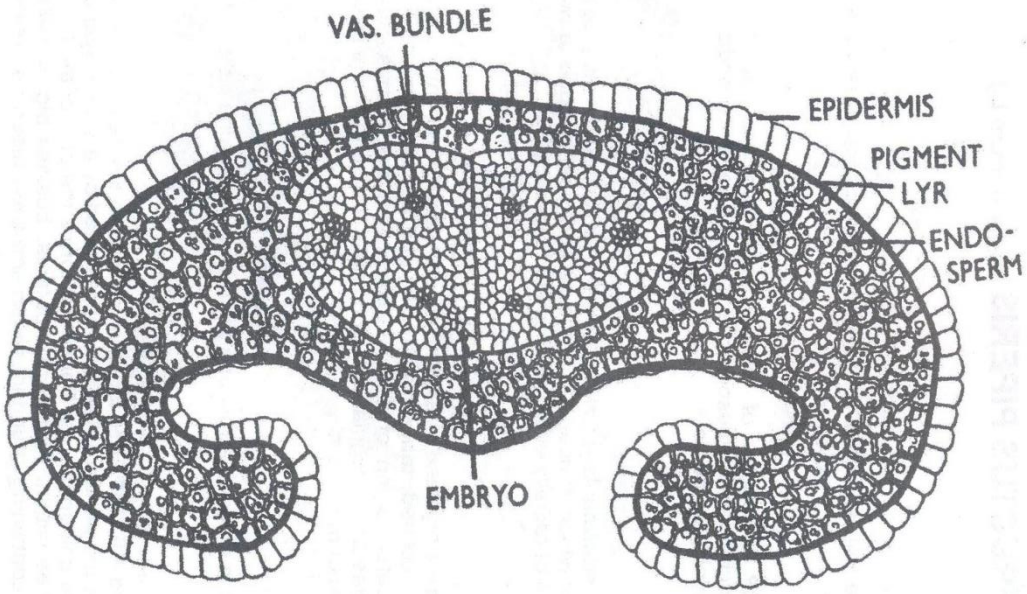
PIGMENT LAYER: Single layered yellowish in color.

ENDOSPERM: Hard cells thick walled containing numerous pits & granular contents (Aleurone grains and oil globules).

The outer layer consists of palisade like cells.

EMBRYO: It contains two polyhedral cotyledons; cells contain aleurone grains and oil globules.

3 – 5 vascular bundles are present in each cotyledon.



Ispaghula Seed. T. S. (X 50)

CHEMICAL CONSTITUENTS:-

Mucilage: 10%

Carbohydrates: Fixed oil proteins.

MICROCHEMICAL TEST:

S.NO.	REAGENTS	OBSERVATION	CHARECTERISTICS
1.	Phluroglucinol + conc. Hcl (1 : 1)	Pink color	Vascular bundles
2.	Ruthenium red	Red color	Mucilage present in epidermis
3.	Alcoholic picric acid	Yellow color aleurone grains present in the cells of endosperm and embryo.	Aleurone grains present in the cells of endosperm and embryo.
4.	Sudan red – III	Red color	Oil globules present in the cells of endosperm and embryo.

CHEMICAL TEST:-

SWELLING FACTOR:

1 gm of Isapgol reds are taken in 20ml of water in 25ml capacity measuring cylinder by constant shaking.
Then the volume occupied by mucilage is observed after 24 hrs.

Swelling factor for Isapgol is 10 to 14.

USES:

1. It is used as Bulk laxative.
2. It is used as demulcent and emollient.
3. It is used as an Anti-rheumatic agent.

Note:

- Anti-rheumatic agent: drugs used in rheumatoid arthritis to slow down disease progression.
- Demulcent: a substance that relieves inflammation or irritation.
- Emollient: a drug having the quality of softening or soothing the skin.

REPORT:

EXPERIMENT-3

ISOLATION OF STARCH FROM POTATOES

AIM: To isolate starch from potatoes.

APPARATUS:

Mortar and Pestle, Sieves, Muslin Cloth, Watch Glass, and Test Tube.

MATERIALS AND REAGENTS: fresh Potato, Iodine solution and Distilled Water etc.

PRINCIPLE: Potatoes contain starch, mineral salts (inorganic), soluble proteins and vegetable tissues but does not contain gluten (insoluble protein) unlike other starch sources. Potato starch is easily isolated by blending small pieces of potatoes followed by separating the cell debris from the starch by sieves. Subsequently, starch settles down when allowed to stand for sufficient period of time.

Starch is an important polysaccharide found in plant sources. It is present in the form of grains in different parts of the plant. Ex: Aleurone grains in the endosperm of many seeds.

Starch is chemically consists of Amylose and Amylopectin.

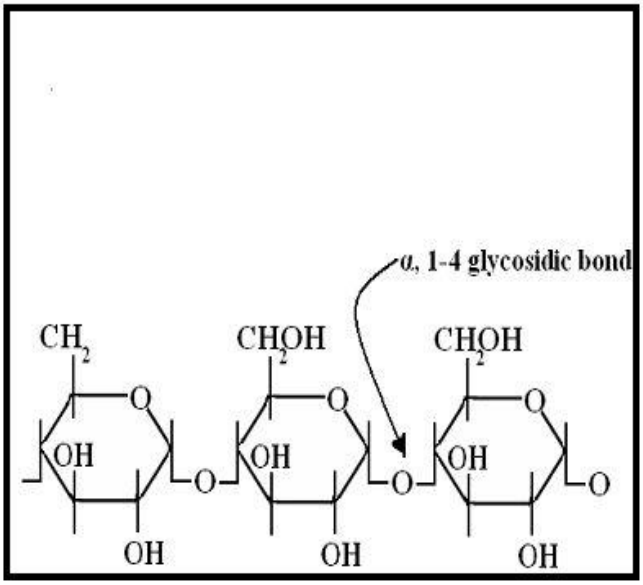
Amylose:

It is a straight chain polymer of D-glucose units linked by alpha 1, 4-glycosidic linkage. It constitutes about 20% of starch. It is more soluble in water and stains blue with iodine.

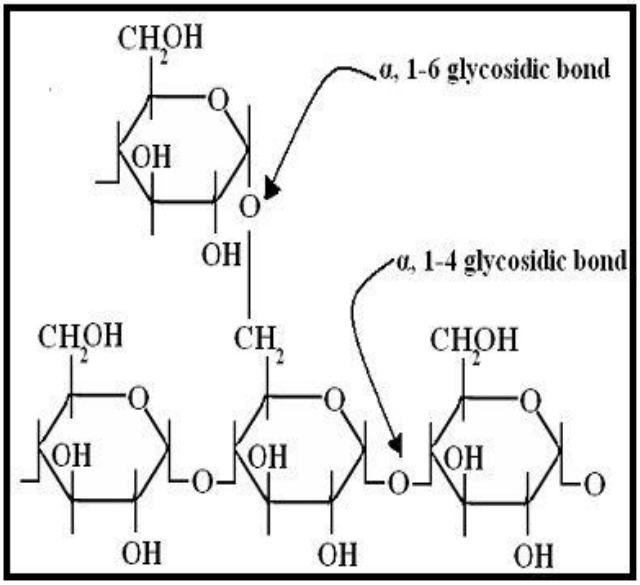
Amylopectin:

It is a branched chain polysaccharide of D-glucose units and constitutes about 80% of starch. It contains 1, 6 - glycosidic linkage by alpha-1, 4 glycosidic linkage and alpha-1, 6 glycosidic linkage.

It is less soluble in water but soluble in hot water with swelling. It stains reddish brown with iodine.



(a) amylose



(b) amylopectin

The different starches can be differentiated based on size, Shape and microscopical characters like hilum etc.

Source	**Size**	**Simple/compound**	**Hilum & Striations**
Potato	ovoid or spherical up to 100 µ	Compound	The hilum is a point at the narrow end of the granules and concentric striations are well marked.
Corn starch (maize)	fairly uniform in size measuring 5-25µ	Simple	In the center there is often a small cleft, or two or three radiating from the center, the position of the hilum.
Wheat	larger granules, 15-50 µ in diameter and smaller granules 6-7 µ	Simple	The larger granules are lenticular and the hilum appears as a central point, or when the granules are on their edges, as a line.
Rice	minute granules about 6 µ	Compound	They are polyhedral with sharp angles without concentric striations; a hilum is visible in the larger granules.

PROCEDURE:

- Wash potatoes thoroughly with water to remove adhering soil and earthy matter
- Reduce to fine slurry with water in a mixer grinder or grind them in a motor and pestle with sufficient water.
- Pass the slurry through shaking sieves in order to remove the cell debris and other impurities.
- Allow the milky liquid to settle down, decant the supernatant liquid. Wash 2-3 times with distilled water with constant stirring.
- Decant the upper layer and take the sediment into 2-3 folds of filter paper and press it and remove the water then transfer into fresh paper.
- Keep it in oven for 10minutes below 45 ^{0}C and weigh the powder and submit the yield.

  The yield of starch is approximately 10 percent.

Identification test:

Take a small quantity of test solution with a drop of 1 N HCl and then add two drops of iodine solution. Formation of blue colour indicates the presence of starch.

REPORT: The % yield of starch is _____

MICROSCOPICAL MEASUREMENT

To measure the dimensions of the cells such as fibres, stone cell, trichomes etc. Cell contents (starch grains) pollen grains etc of crude drugs both in entire and powdered forms can be measured by

(i) Using the micrometer (micrometry)
(ii) Using the camera Lucida

MICROMETER:

It is a scale used to measure microscopic objects it is expressed in microns. The technique of micrometric evaluation is useful for measurement of dimension is starch grains, calcium oxalate crystals, fibres, oil cells, other cells & cell content in the powdered ungrounded crude drug.

The size of an object or plant of it can be measured with the help of two types of micrometer.

(i) Stage micrometer
(ii) Eyepiece micrometer

STAGE MICROMETER:

A Stage Micrometer is simply a microscope slide with a finely divided scale marked on the surface. The scale is of a known true length and is used for calibration of optical systems with eyepiece graticule patterns it is a slide that contains a standard scale length of 1mm which is divided into 100 divisions. It is a fixed scale.

EYEPIECE MICROMETER:

An ocular micrometer is a glass disk that fits in a microscope eyepiece that has a ruled scale, which is used to measure the size of magnified objects. The physical length of the marks on the scale depends on the degree of magnification. The value of its division varies with the combination of eyepiece & objective lens. Hence calibration of 1 division of it is essential during practical work in order to get exact value of one division in terms of microns.

CAMERA LUCIDA:

Swift ives camera lucida **Abbe's camera lucida**

The camera Lucida or drawing ocular is useful for maintaining for tracing a magnified image of the object under microscopical study with proper adjustments of camera Lucida and illumination, it is possible to see simultaneously the drawing paper, the pencil point & the object under microscope & it is then easy to trace the required outlines.

This is much quicker & more accurate then the free hand drawing, but it requires the subsequent addition of details by free hand.

EXPERIMENT-4

IDENTIFICATION OF STARCH GRAINS IN DIFFERENT POWDERED DRUGS

Aim: To identify and draw the structure of starch grains in given different powder samples

R**equirements:**

Apparatus:

Camera lucida, Microscope, Stage micrometer, Occular micrometer, Slide, Watch Glass

Chemicals:

Iodine solution, Potato starch, Maize powder, Ginger powder

Principle:

The camera Lucida or drawing ocular is useful for maintaining for tracing a magnified image of the object under microscopical study. It is possible to see simultaneously the drawing paper, the pencil point & the object under microscope & it is then easy to trace the required outlines.
On addition of Iodine solution to the sample powder, it stains the starch grains present in the powder to blue colour. By this reaction starch grains can be easily identified.

Procedure

- Take little quantity of sample powder on the slide add Iodine solution to it.
- Make a thin smear using cover slip and place the cover slip over it.
- Focus the starch grains containing area.
- Place the camera lucida on to the microscope and place a black chart to the right side of microscope.
- Start tracing the starch grains diagram on the black chart in selected area.
- Repeat the same procedure with other powdered drugs.
- Report it.

Report:

EXPERIMENT: 5

MEASUEMENT OF DIMENSIONS OF STARCH GRAINS IN POWDERED GINGER

Aim: To draw the structure and measure the length of starch grains in given Ginger powder

R**equirements:**

Apparatus:

Camera lucida, Microscope, Stage micrometer, Occular micrometer, Slide, Watch Glass

Chemicals:

Iodine solution, Ginger powder

Principle:

The camera Lucida or drawing ocular is useful for maintaining for tracing a magnified image of the object under microscopical study. It is possible to see simultaneously the drawing paper, the pencil point & the object under microscope & it is then easy to trace the required outlines.
On addition of Iodine solution to the sample powder, it stains the starch grains present in the powder to blue colour. By this reaction starch grains can be easily identified.

starch grains

Procedure

- Take little quantity of ginger powder on the slide add Iodine solution to it.
- Make a thin smear using cover slip and place the cover slip over it.
- Calibrate stage micrometer using ocular micrometer and calculate the calibration factor.
- Remove the stage micrometer and place the slide.
- Focus the starch grains containing area.
- Place the camera lucida on to the microscope and place a black chart to the right side of microscope.
- Start tracing the starch grains diagram on the black chart and simultaneously calculate the length of the starch grains by rotating ocular micrometer.
- Note the values and calculate the actual length in micrometers by multiplying with calibaration factor.
- Take around 10 values and calculate the average length of the starch grains and report it.
- The length of starch grains in ginger powder is in the range of 5µ to 50µ

Calculation:

Report:

EXPERIMENT - 10

ISOLATION OF EUCALYPTUS OIL USING CLAVENGER APPARATUS

AIM:

To isolate the volatile oil from eucalyptus leaves using hydrodistillation.

APPARATUS:

Clavenger's apparatus.

PRINCIPLE:

Hydrodistillation is done by using clavenger apparatus based on the densities of oil. Clavenger apparatus consists of two different set ups:

1) Lighter than water.

2) Heavier than water.

CLAVENGER APPARATUS:

The apparatus consists of a spherical glass vessel charged with plant material. The device is with a narrow opening and the vessel is put in to a heating mantle.

The vapors produced pass through a long vertical glass tube (delivery tube) through a long vertical glass condenser having a long vertical glass tube.

Cool water enters the jacket of the condenser having a tube by an inlet and is circulated through an inlet and is circulated through an outlet to a graduated collection funnel.

The eucalyptus oil is immiscible in water and being less dense separates on upper layer.

A return convict connects the base of the measuring tube to vertical tube and allows the recycling of the aqueous port of vapours.

The oil is collected at the outlet by opening a valvssse provided.

PROCEDURE: About 100 g of leaves of the eucalyptus species are dried and subjected to hydro distillation for two hours using clavenger apparatus. The oil is separated from water by decantation and dry by filtration oSver anhydrous sodium sulphate. The oil yield is 2%.

REPORT: The volatile oil from eucalyptus by hydro distillation was found to be_____%v/w

Experiment

DETERMINATION OF ACID VALUE

AIM: To determine the acid value of given substance.

APPARATUS: Dry flask, reflux condenser, water bath burette,etc.

CHEMICALS REQUIRED:- potassium hydroxide, phenophthalein,alkali,ether.

PRINCIPLE:fats are generally estimated by the values like saponification value.it represents the number of milligrams of KOH required to saponify one gram of fat as conditions specify, acid value and ester value etc.saponification value is the measure of all the fatty acids present.

$$\text{No. of moles} = \frac{\text{mass of sample}}{\text{Relative atomic mass}}$$

It is no. of milligram of KOH required to neutralize free fatty acids in 1 gm of a fat.

PROCEDURE:

1. 10 gms of substance is weighed to it add 50 ml of mixture of equal volume of ethanol ether are added.
2. Add 1 ml of phenolphthalein solution and titrate with 0.1 M KOH until the solution remains faintly pink.

$$\text{Acid value} = \frac{\text{volume of 1M KOH consumed}}{\text{Weight of thse sample}}$$

REPORT: the acid value of the given sample was found to be

Note:

- The acid number is used to quantify the amount of acid present in fats and oils.
- The higher the amount of acid value, the higher the deterioration or rancidity of the oils and fats (undergonedeterioration or rancid). As the rancidity increases, the oil achieves a foul smellalongwitha sour taste.
- Cause for rancidity is the hydrolytic or oxidative cleavage of triglycerides causing the formation of free fatty acids in oils or fats.

CHEMICAL TESTS FOR ACACIA

Aim: Identification of unorganized drug Acacia by studying physical and chemical characters.

Synonym: Babool ki gond.

Physical characters:

Physical form: Rounded or ovoid tears.

Colour and fluorescence:opaque white with yellowish tint.

Hardness and fracture:hard and brittle.

Solubility: insoluble in alcohol but soluble in water and produce translucent viscous liquid.

Chemical tests:

1. Aqueous solution (2%) w/v + a few drops of lead subacetate= white precipitate.
2. On boiling with dil.HCl for 10 min, hydrolysis which gives positive test with Fehling's solution.
3. It gives positive test with Molischs reagent.
4. It gives negative test with N/50 Iodine solution.(distinction from agar and tragacanth).

Use:demulcent and emulsifying agent.

Biological source:the given sample of crude drug consists of dried exudation of acacia Senegal (fam: Leguminosae)

CHEMICAL TESTS FOR AGAR

Aim: identification of unorganized drug Agar by studying physical and chemical characters.

Synonym: agar-agar, gelose.

Physical characters:

Physical form: solid long thick strips up to 60 cms long, thickness 0.5 to 1 cm and width 2.5 cm..

Colour and fluorescence: grayish white, translucent with yellowish tint.

Hardness and fracture: tough difficult to break.

Solubility: very slightly soluble in water and swells in water and forms jelly on boiling.

Odour and taste: characteristic and slight with mucilaginous taste.

CHEMICAL CONSTITUENTS: It contains chief active constituents is Arabin which is a mixture of calcium, magnesium, and potassium salts of Arabic acid. It also contains an enzyme oxidase & peroxidase. Products obtained after hydrolysis are Arabinose, Rhamnose,, D- galactose, D-galacto uronic acid.

Chemical tests:

1. It gives positive test with Molischs reagent and fehlings solution.
2. It gives red colour with ruthenium red.
3. It gives positive test with N/50 Iodine solution. (Crimson to bown).
4. 1% solution on boiling gives jelly like consistency
5. On heating with potassium hydroxide gives yellow colored solution.

Use: use d for preparation of bacteriological culture.

Biological source: the given sample of crude drug consists of dried decoction of various species of algae of family Geladiaceae.

CHEMICAL TESTS FOR TRAGACANTH

Aim: identification of unorganized drug Agar by studying physical and chemical characters.

Synonym: Anjira.

Physical characters:

Physical form: thin flattened curved flakes.

Colour and fluorescence: translucent.

Hardness and fracture: hard and fractures are short.

Solubility: very slightly soluble in water and form tenacious gelatinous mass.

Chemical tests:

1. Powdered heated with 5% caustic potash to give yellow colour.
2. Treat 0.1 g of powder with N/50 Iodine solution to give olive green colour mixture.
3. To 0.5% W/V solution of the sample, add 20% W/V solution of lead acetate. a flocculent precipitate is obtained.
4. 0.5% W/V solution is heated with conc. HCl on a water bath for 30 min. make the solution alkaline with NaOH then add Fehling's solution and warm on water bath to give red ppt.

Use: pharmaceutical aid, thickening and suspending agent.

Biological source:the given sample of crude drug consists of dried gummy exudation of *Astragalus gummifer* (Leguminosae).

CHEMICAL TESTS FOR STARCH

AIM: identification of unorganized drug Gelatin **by** studying physical and chemical characters.

BIOLOGICAL SOURCE: MAIZE STARCH**:***Zea mays* Graminae

POTATO STARCH: *Solanum tuberosum* Solanaceae.

RICE STARCH: *Oryza sativa* Graminae

WHEAT STARCH: *Triticum vulgare* Graminae

PHYSICAL CHARECTERISTICS :-

CHAREACTERS	DESCRIPTION
Form	Fine powder, irregular angular mass or columnar masses readily reducible to powder maize starch usually occurs in powder, rice starch in masses.
Color	Rice and Maize : white Wheat : cream colored Potato : slightly yellowish
Odour	None
Taste	Mucilaginous
Solubility	Cold water : insoluble Alcohol 7 Organic solvent : insoluble Gives colloidal solution when boiled with water.
Specific gravity	Greater than 1 1.62 to 1065)
pH	Maize : Neutral ; Rice : Alkaline Wheat : Faint acidic Potato : Neutral / very faint acidic

CHEMICAL TEST :

1. Mount a few starch granules in water add a drop of iodine starch granules show bluish coloration when examined microscopically.
2. STARCH MUCILAGE: Make a suspension of 0.5g of starch with about 5ml of water and then boil it gently with 20ml of water for 2-3 minutes and cool.
3. Starch mucilage + Fehling's reagent + heat ⟶ No reduction takes place (no red coloration)

4. 5ml starch mucilage + 0.5ml HCl + heat (30min). After 30min. add sodium hydroxide until the mixture is alkaline to litmus and warm for few min. perform the test (3) ⟶ reduction takes place.
5. 5ml of starch mucilage + 4 drops of iodine water ⟶ deep blue coloration is produced which disappear an heating.

REPORT:

(i) Starch:

It is an insoluble carbohydrate of polysaccharide type formed by the condensation of simple sugars like glucose. The starch is usually found in the form of starch grains of various shapes. The starch grains are abundantly found in the storage organs of plants, e.g., tuberous roots, underground stems, cortex of stems, endodermis, grains of cereals, banana fruits, etc. The starch grains vary in their shape and may be used for the identification of plants.

The starch grains are not found in fungi and certain algal groups. The starch grains have different shapes that are characteristic of the plant types, e.g., they are oval-shaped in potato; flat in wheat; polygonal in maize; spherical in pulses and dumbell or rod-shaped in the latex cells of some Euphorbias. The starch grains of rice are smallest and those of Carina largest. The starch grains vary from 5-100μ in size. The starch is always derived either from chloroplasts of green cells or from leucoplasts (amyloplasts) of storage tissue.

The structure of the starch grain usually exhibits conspicuous concentric layers formed around a dark roundish spot, the hilum.

The layering may be conspicuous in some grains whereas inconspicuous in others. Most of the starch grains show this layering and are known as stratified starch grains. If the concentric layers

of the starch are formed on one side of the hilum of starch grain, the grain is said to be eccentric (e.g., potato) and when the layers are deposited concentrically around the hilum (e.g., wheat) the grain is known as concentric.

Concentric types of starch grains are quite common in most of the plants. If the starch grain possesses a single hilum, it is known as simple. Sometimes two, three or many grains, arranged in a group with as many hila as the starch grains, they are known as compound grains. Compound grains are commonly found in potato, sweet potato, rice and oats. The starch is turned blue or black in aqueous solution of iodine.

Viva questions

- Define magnification.
- What is a microscope?
- Classify different microscopes?
- How you define resolving power of a microscope?
- What are the different parts of a microscope?
- What are the types of objective lenses?
- What is staining?
- What is mounting?
- What are different staining techniques?
- Name some reagents used for staining cell wall components?
- Name some section cutting techniques.
- What is the reagent used to differentiate lignin from other tissues
- Why glycerin is used during mounting process?
- What is meant by clearing of tissue?
- What is the significance of significance of ts, tls and rls