ST. JOSEPH'S COLLEGE OF PHARMACY

CHERTHALA

Record Book

BACHELOR OF PHARMACY

FOURTH SEMESTER

PHARMACOGNOSY & PHYTOCHEMISTRY - I

Name……

Roll No……

Reg.No……

ST. JOSEPH'S COLLEGE OF PHARMACY

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BACHELOR OF PHARMACY

FOURTH SEMESTER

PHARMACOGNOSY & PHYTOCHEMISTRY - I

PRACTICAL RECORD

NAME……

REG.NO……

Certified that this is a bonafide record of the practical work done by the candidate during the period 20….

EVALUATED BY **FACULTY IN-CHARGE**

Examiner (Sign)

Date…….

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Practical work completed on

……/……/20….

Expt no:1

Date:

STUDY OF COMPOUND MICROSCOPE

Microscope may be defined as an optical instrument comprising of lens or a combination of lenses which enables to view the magnified images of a minute object, which is otherwise not visible to naked eye.

It is a most important instrument for the histological examination of a very large range of materials such as vegetable drugs, fibres, mineral substances, food products etc.

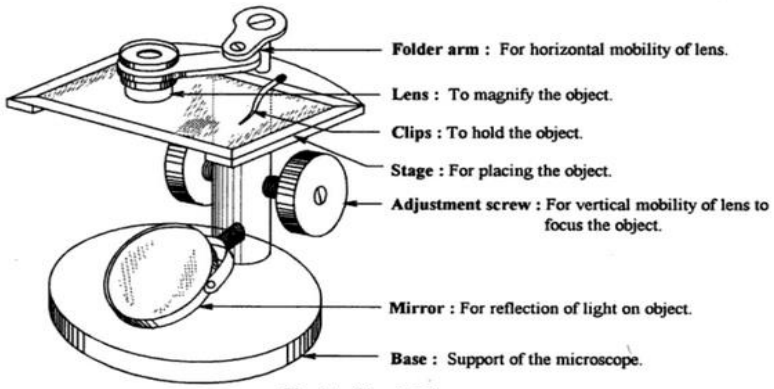
This history of microscope begins with ZacharisJansens invention and the first light microscope in 1950's. Anton Van Lewen hook later made significant improvements to compound microscope. By griding and polishing he was able to make small lens with great dirvatures and these lenses magnify up to 270X. He then observed blood cells, bacteria, and yeast cells. He has been since called father of microscopy.

The microscope in which ordinary light is used as the source of illumination is known as light microscope. The light microscopes are of two types.

1. Simple microscope
2. Compound microscope

Simple Microscope:

It consists of only one set of lenses. It helps to reveal the morphological characterestics of the object



. **Fig. 1.1 : Simple Microscope**

Compound Microscope

A light microscope with a system of two or more lenses is said to be compound microscope. It is an important analytical tool of the biologist today was developed to give gather magnification of lenses, the eyepiece and the objective lens. It has 3 major components.

I. Supporting System
II. Illuminating system
III. Magnifying system

I. Supporting system:

a) Base: It is the foundation of microscope which is horse shoe in shape.
b) Stage: It is a square metallic platform with a central opening called stage aperture and clips to hold the slide. It is also provided with
c) Body: The whole assembly is held in position by the body, which consist of arm to hold the instrument and coarse adjustment & fine adjustment.
d) Body tube: It is a long tube which holds the revolving nose piece at the bottom and eye piece at the top.

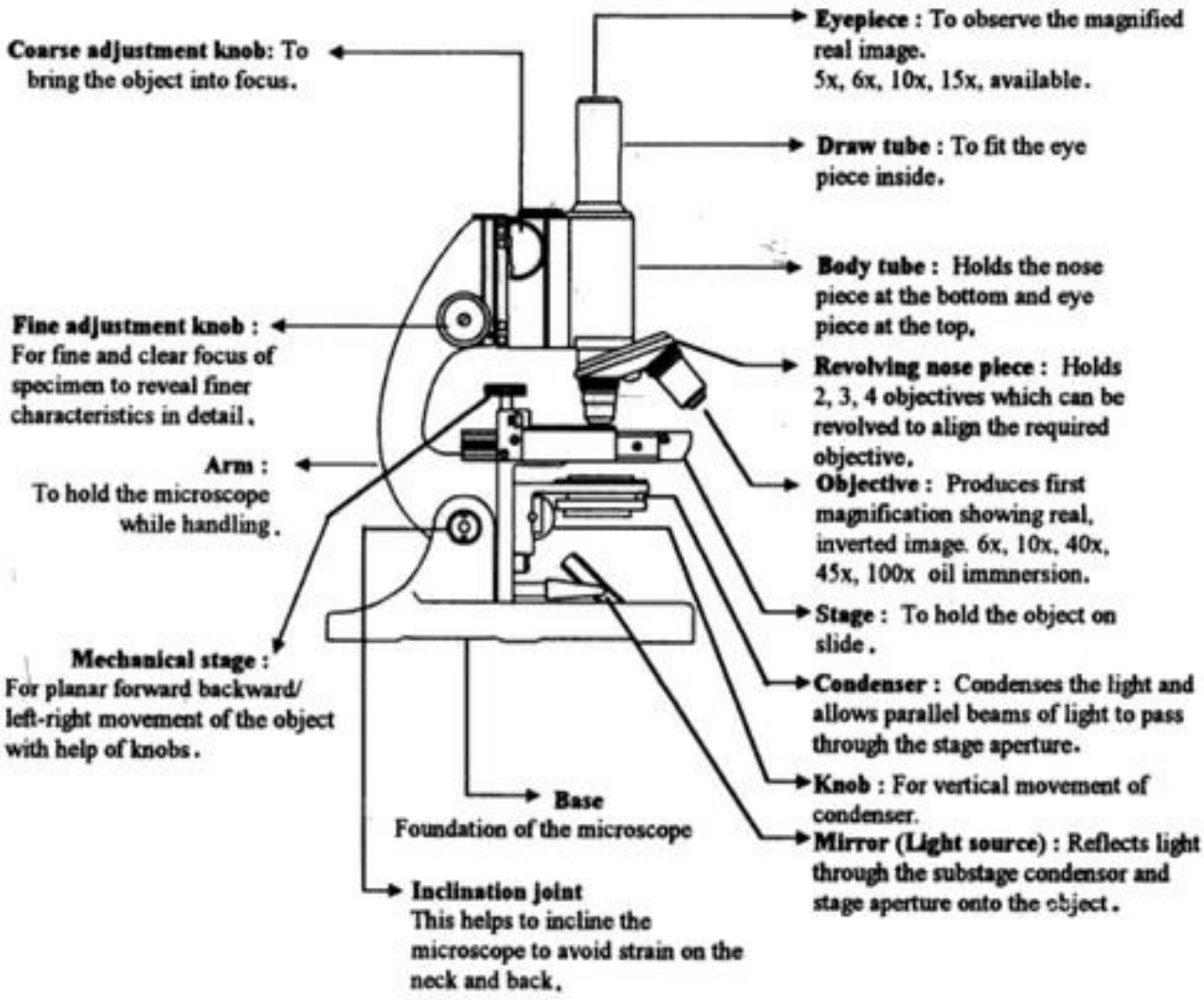
II. Illuminating system:

It is used for reflection of light into the object for proper viewing. It comprises of light source or mirror, iris diaphragm and condenser.

a) Mirror: It reflects light through condenser and stage aperture to the object. Mirror may be plane or concave. Plane mirror is used when a fixed source of light is used while concave mirror is used when sunlight is used as the light source.
b) Diaphragm: It controls the amounts of light entering to the microscope by opening and closing and hence making the object brighter or less bright.
c) Condenser: The light rays on the objects can be altered in two ways by means of condenser. It can be moved upwards with the knob so as to make the objects brighter. Condenser can be move downward to make the object less bright.

III. Magnifying System: It includes a set of lenses aligned in such a manner so that a magnified real image can be viewed. It consists of objective lenses and eye piece lenses.

a) Objective lenses: -It produces first magnification showing real inverted image. The objectives of various magnification are: - 10 X (low power), 40X (high power) and 100X(oil immersion lens)
b) Eye piece lenses: It is so called, since it is the lenses through which the image is visualized.



FACTS AND FIGURES ABOUT MICROSCOPE

A. Magnifying Power (M.P):

M.P. = Magnification of objective X Magnification of eye piece

B. Resolving power of objective:

It is defined as the ability to separate distinctly two small elements of an object which are situated a short distance apart. Resolving power 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.

D. Focusing:

Focusing an object while viewing through an eyepiece 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 object in field of view and 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.

WORKING OF COMPOUND MICROSCOPE

1. Clean all parts of microscope before working with a tissue.
2. Turn the power objective into position.
3. Illuminate the microscope suitably depending upon the type of experiment using mirror, condenser and diaphragm while viewing through the eyepiece.
4. Fix the slide with an object on the stage with stage clips.
5. Bring down the body tube by looking the slides so that objective lens become very close to the object.
6. By looking through the eyepiece turn the coarse adjustment knob and move the body tube towards you till a rough image is seen.
7. Make the image clear by using fine adjustment.

8. To focus the object to a high power, turn the objective and solve the higher magnification in to the position.
9. Turn the fine adjustment so as to get a clear and highly magnified image.

PRECAUTIONS TO BE FOLLOWED WHILE HANDLIN A COMPOUND MICROSCOPE

1. Always keep the microscope in a clean, dust free environment.
2. Hold the instrument firmly with both hands while handling the compound microscope.
3. Clean all the parts of microscope including the lenses.
4. If the lenses are not clear, clean them with Xylene. Never use alcohol since the cementing material between the glass and the metal is soluble in alcohol.
5. Handle the instrument carefully so as to prevent any damage to it.

REFERENCE:

1. Practical Pharmacognosy – Techniques and experiments by Dr. K.R. Khandelwal. Page no:1-5
2. Practical Pharmacognosy by C.K.Kokate. Page no:1-4

Chemical tests

Expt no:2

Date:

CHEMICAL TEST FOR AGAR

Aim: To perform the chemical test for Agar

Identification tests:

Experiment	Observation	Inference
1. Solubility test: a) In cold water b) Boil 1.5g Agar with 100ml water. Cool at room temperature. c) In organic solvents	In Soluble but swells Soluble & form stiff jelly Insoluble	Characteristic of agar Due to gelatinizing property Characteristic of agar
2. Test for mucilage: Agar powder is mounted in ruthenium red solution and observed under microscope	Particle acquires pink color	Due to mucilage
3.To 0.2% solution of agar, tannic acid is added	No ppt	Absence of Protein
4. Add 0.5ml HCl to 0.5% aqueous solution and heat on water bath for 30 minutes and divided into two parts. • Add to first part 3ml of 10% Caustic soda and 2ml of Fehling's solution &heat on a water bath • Add Barium chloride solution to	Reddish brown precipitate White precipitate of	Presence of reducing sugar (reduction take place) Presence of

second part (Sulphate test)	$BaSO_4$	sulphate ion
5. Agar on warming with alcoholic KOH	A canary yellow color is formed	Presence of agar
6. Agar solution + N/20 Iodine solution	A deep crimson to brown color	Presence of agar (distinction from Acacia &Tragacanth)

RESULT

The given sample was found to be

REFERENCE

Expt no:3

Date:

CHEMICAL TEST FOR ACACIA

Aim: To perform the chemical test for Acacia

Identification tests:

Experiment	Observation	Inference
IDENTITY TEST		
1. Solubility test: a) In water b) In alcohol c) Mount the powder on the Ruthenium red	 Soluble & viscous Insoluble No pink colour	 Characteristic of acacia Characteristic of acacia Absence of mucilage in Acacia
2. To an aqueous solution of drug (5ml), Borax (0.1g) is added	A stiff translucent mass is obtianed	Presence of gum acacia
3. Lead subacetate test: To an aqueous solution of drug add aqueous solution of Lead sub acetate	White precipitate is formed which is of Lead sulphate	Presence of gum acacia
4. Benzidine test: To an aqueous solution of drug add few drops of H_2O_2	Blue colour is produced indicates the presence of Oxidase & Peroxidase	Presence of gum acacia (distinction from Tragacanth)

solution and 0.5ml alcoholic solution of 1% Benzidine& shake well	enzymes	
5. <u>Test for reducing sugar:</u> Hydrolyse the aqueous solution of gum in the presence of dil. HCl by boiling on water bath for 10 minutes. Few drops of NaOH are added to neutralize the excess acid. To this add Fehling's solution A &B (1ml)	Red precipitate of Cuprous oxide is formed	Presence of reducing sugar (Liberation of reducing sugar as the product of hydrolysis)
6. To the boiled solution of gum in dil.HCl, $BaCl_2$ is added	White precipitate	Presence of sulphate ion
PURIYT TESTS		
7. Add $FeCl_2$ to the aqueous solution of gum	No blue/ black precipitate	Absence of tannins
8. Add Iodine solution to a solution of gum	No blue/ brown colour	Absence of starch or dextrose

9. Aqueous solution + Lead acetate	No precipitate	Distinction from Indian gum, Tragacanth gum

RESULT

The given sample was found to be

Expt no:4

Date:

CHEMICAL TEST FOR TRAGACANTH

Aim: To perform the chemical test for Tragacanth

Identification tests:

Experiment	Observation	Inference
1. Solubility test a) In water b) In alcohol	Partially soluble in water & swells to homogenous adhesive gelatinous mass Insoluble	Characterstic of Tragacanth Characterstic of Tragacanth
2. Tragacanth is heated with alcoholic Potash	Canary yellow colour	Characterstic of Tragacanth
3. Tragacanth solution is boiled with strong Iodine solution	Olive green colour	Presence of Tragacanth
4. Mount the powder on the Ruthenium red	No pink colour	Mucilage is absent
5. Test for reducing sugar: Hydrolyse the aqueous solution of gum in the presence of dil. HCl by boiling on water bath for	Red precipitate of Cuprous oxide is formed	Presence of reducing sugar (Liberation of reducing sugar as

10 minutes. Few drops of NaOH are added to neutralize the excess acid. To this add Fehling's solution A &B (1ml) & heat		the product of hydrolysis)
6. To the boiled solution of gum in dil.HCl, $BaCl_2$ is added	No White precipitate	Absence of sulphate ion
7. Aqueous gum solution + Lead acetate	White precipitate	Presence of Tragacanth
8. Solution of Tragacanth is boiled with few drops of $FeCl_2$	Deep yellow precipitate	Characterstic of Tragacanth

RESULT

The given sample was found to be

Expt no:5

Date:

CHEMICAL TEST FOR WOOL FAT

Aim: To perform the chemical test for Wool fat

Identification tests:

Experiment	Observation	Inference
1. Add 5ml of Chloroform to 0.5g of wool fat. Then treat it with 1ml of Acetic acid acidified with 2 drops of sulphuric acid	Deep green colour	Presence of fatty acid

RESULT

The given sample was found to be

Expt no:6

Date:

CHEMICAL TEST FOR BEES WAX

Aim: To perform the chemical test for Bees wax

Identification tests:

Experiment	Observation	Inference
1. Boil 5g of wax for 10minutes in 8ml of 10%w/v solution of Sodium hydroxide. Replace the water by evaporation. Cool, filter the solution through glass wool or asbestose and acidify with hydrochloric acid.	A precipitate is formed	Indicate the presence of fatty acids, fats and resins.
2. Heat the wax with aqueous solution of Sodium hydroxide. Cool and acidify.	Turbidity is produced.	Indicate the presence of bees wax
3. 1g of drug is treated with 10ml of alcoholic Potassium hydroxide and 10ml of alcohol. Reflux for one hour. Stir well.	Solution becomes cloudy between 59-61^{0}C	Presence of Bees wax

RESULT

Thegiven sample was found to be

Expt no:7

Date:

CHEMICAL TEST FOR CASTOR OIL

Aim: To perform the chemical test for Castor oil

Identification tests:

Experiment	Observation	Inference
1.Drug is treated with half of its volume of Petroleum ether	Completely miscible	Presence of fatty acids.
2. Add an equal volume of alcohol to the solution of Castor oil. A clear liquid is obtained. Cool it 0^0C for 3 hrs	Liquid remains clear	The presence of Ricinolic acid

RESULT

The given sample was found to be

Expt no:8

Date:

CHEMICAL TEST FOR SHARK LIVER OIL

Aim: To perform the chemical test for Shark liver oil

Identification tests:

Experiment	Observation	Inference
1. One drop of shark liver oil is dissolved in 1ml of Chloroform. Add 0.5ml of Sulphuric acid and shake well.	Pale violet colour which finally changes to brown or blue	Indicate the presence of Vitamin A
2. 1ml of shark liver oil is dissolved in 10ml of chloroform and then treated with a saturated solution of Antimony trichloride in chloroform. Shake well.	Blue colour is produced.	Indicate the presence of Vitamin A

RESULT

The given sample was found to be

Expt no:9

Date:

CHEMICAL TEST FOR SESAME OIL

Aim: To perform the chemical test for Sesame oil

Identification tests:

Experiment	Observation	Inference
1. To 2ml of Sesame oil add 1ml of 1% solution of sucrose in Hydrochloric acid.	A pink or red colour	Presence sesamol

RESULT

The given sample was found to be

Expt no:10

Date:

CHEMICAL TEST FOR HONEY

Aim: To perform the chemical test for Honey

Identification tests:

Experiment	Observation	Inference
1. Organoleptic characters Colour Odour Taste	White to pale yellow brown coloured liquid Pleasant and characterestic Sweet	Characteristic of honey
2. TEST FOR ADULTERATION: a) Fiehe's test (Furfural test) Shake 10ml of honey and 5ml of ether till them both become miscible. Separate the upper ethereal layer and evaporate it in the porcelain dish. To the residue add 1drop of 10% Resorcinol in Hydrochloric acid. • If natural honey	Transcient red colour	Absence of furfural (honey not adultered with

		invert sugar)
• Artificial honey	Red colour persists for some time	Presence of furfural
b) Cotton wick is soaked in honey and then ignified	It burns without	
• If natural honey	noise or any burnt sugar smell	Presence of pure honey
• Artificial honey	Burned sugar smell onburning	Adultered honey
c) 1ml of honey is mixed with 4ml of alcohol	No turbidity A slight turbidity	Presence of pure honey Presence of dextrin from added glucose
3. <u>TEST FOR PURITY</u>		
i. Put a drop of honey in a glass of water	Reaches the bottom without readily dissolving	Presence of pure honey
ii. Place 2 drops of honey on the floor and ignite with a match stick		Presence of pure honey
iii. Mix honey and equal amount of methylated spirit and shake.	Honey burns immediately	
• Pure honey	Honey settles down the bottom without	Presence of pure honey

• Impure honey	any changes Colour changes to milky white	Presence of impure honey
4. <u>Test for reducing sugar</u> Hydrolyse the aqueous solution of honey in presence of dilute hydrochloric acid by boiling on waterbath for 10minutes. Few drops of Sodium hydroxide is added to neutralize the excess acid. To this add equal amounts of Fehling's solution A&B and boil.	A heavy red precipitate of Copper oxide is formed	Presence of reducing sugar
5. <u>Selivanoff's test</u> To the test solution a crystal of resorcinol is added and also equal volume of con.HCl. warm it on a water bath.	Rose colour	Presence of ketose sugar

RESULT

The given sample was found to be

Micrometrics

MICROMETRICS

Micrometry is a scale used to measure microscopic object. It is expressed in microns. The technique of micrometric evaluation is useful for the measurement of dimension of starch gains,calcium oxalate. Crystals,fibres,oilcells,other cells and cell contents in the powered or unground crude drugs.

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

i. Stage micrometers
ii. Eye-piece micrometers.

STAGE MICROMETER

It is a slide. It contains a standard scale length of 1mm which is divided into 100divisions.

1division of stage micrometer = 1/100mm =0.01mm OR

1000/100 =10

EYE-PIECE MICROMETER

It is a circle of glass with a scal;e etched on the surface. It is suitable for insersion inside the ocular and used during the peration of measurement. (A convenient from is a linear scale of 1mm divided into 100divisions)

The value of its division varies with a combination of eye-piece and objective lenses,hencelalibration of one division of it is essential during practical work. In order to get exact value of its one divisions interms of microns.

For the purpose of micrometric evaluation,following instructions should be followed,

i. Remove the eyepiece from the microscope,unscrew its lens,place the eye piece micrometer on the ridge inside replace the lens and put the eyepiece bark into the body tube of the microscope.

ii. Place the stage micrometer on the stage of the microscope and focus the scale under high power with eyepiece scale super imposed.

iii. Calculate the factor from the super imposed image. The shorter lines of the numbered scale represent the ruling of the eyepiece,Micrometer, while the lines extending across the field represent the scale of the stage micrometer.

iv. Replace the stage micrometer with the slide of object.Adjust slide in such away that the object is focused on the lines of the eyepiece micrometer. Note the number of divisions of eyepiece micrometr being covered by the object.

v. Multiply the value by calibration on factor so as to get the actual dimension of the object in microns. Take the average of atleast twenty five readings for establishing a value of micrometric determination.

Expt no:11

Date:

DETERMINATION OF LENGTH AND WIDTH OF PHLOEM FIBERS IN CINNAMON POWDER

AIM:

To measure the length and width of fibres present in the given sample to powdered Cinnamon.

MATERIALS AND REAGENTS:

Compound microscope, eyepiece micrometer, stage micro meter, potassium hydroxide solution,Phlouroglucinol, Hydrochloric acid, Glycerine.

PROCEDURE

1) Calibrate the eyepiece micrometer using the stage micrometer and calculate the factor.
2) Boil a little quality of powered drug with KOH solution. Remove clear powder in a watch glass and stain with 1drop of Phlouroglucinol and concentrated hydrochloric acid.
3) Mount a little of powered drug in dil.glycerin and observe slide under low power microscope.
4) Measure the length and width of fibre by focusing them on the lines of eyepiece micrometer. Note the number of divisions covered by the width of a fibre.
5) Calculate the values of fibres and multiply them by the factor. Calculate the average value and indicate the range for the width of fibres.

The powders of Ceylon cinnamon and cassia cinnamon can be distinguished from each other on the basis of the average value for the width of fibres.

REPORT

Average length of phloem fibres of cinnamon powder present in the given sample of cinnamon powder was found to be

Average width of phloem fibres present in the given sample of cinnamon powder was found to be

Expt no:12

Date:

DETERMINATION OF DIAMETER OF STARCH GRAINS

AIM

To determine the diameter of starch grains

MATERIALS AND REAGENTS

Compund microscope, eyepiece micrometer, stage micrometer, Nl_{20} Iodine solution

PROCEDURE

Calibrate eyepieces micrometer by using stage micrometer and calculate the factor. Add a drop of Iodine solution to a little of powder of crude drug.

Measure the diameter of isolated starch grains in the mount by focussing them on the scale of the eyepiece micrometer. Note the number of divisions being covered by starch grain.

Calculate the dimension of 50 starch grains. Multiply the value by the factor so as to get the actual dimension of the starch grains in microns.

Calculate the average value and give the range for the dimensions of the starch grains.

RESULT

Average diameter of starch grain present in given sample was found to be

Expt no:13

Date:

DETERMINATION OF DIMENSIONS OF CALCIUM OXALATE CRYSTALS IN POWDERED CRUDE DRUG

AIM- To measure the dimensions of calcium oxalate crystals in the cassia powder.

PRINCIPLE

Calcium oxalate crystals are considered as excretory products of plant metabolism. They occur in different forms and provide valuable information for identification of crude drugs in entire and powdered forms.

a) Microsphenoidal or sandy crystals eg, belladonna
b) Single acicular crystals eg, cinnamon, gentian
c) Prisms eg,quassia, senna ,cascara
d) Rosetts eg,stramonium ,senna, cascara
e) Bundles of acicular crystals. -squill ,ipecac

The sections to be examined for calcium oxalate should be cleared with caustic alkali and chloral hydrate. These reagents very slowly dissolve the crystals, so the observation should be made immediately after clearing the section. The polarizing microscope is useful in the detection of small crystals.

PROCEDURE

Calibrate the eye piece micrometer using stage micrometer. Mount a little quantity of powdered drug in glycerin water and measure the size of calcium oxalate

crystals .Note the number of division covered by the calcium oxalate crystals. Repeat for further 19 calcium oxalate crystals .Multiply each value by calibration factor. Calculate the average value and give the range for the dimensions.

REPORT

The minimum diameter of calcium oxalate crystals is…….. µm.

The maximum diameter of calcium oxalate crystals is……. µm.

Average diameter of calcium oxalate crystals is…… µm.

REFERENCE

Practical Pharmacognosy , Dr.C.K.Kokate, 4th ed, page no. 25-26,114.

DETERMINATION OF ASH

ASH VALUES

- Used to determine quality and purity of a crude drug and to establish the identity of it.
- Ash contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc. these are present in definite amount in a particular crude drug hence, quantitative determination in terms of various ash values helps in their standardization.
- Used to determine foreign inorganic matter present as an impurity
- The ash remaining following ignition of herbal materials is determined by three different methods which measure total ash, acid-insoluble ash and water-soluble ash.

TOTAL ASH VALUE

It is the total amount of material remaining after ignition. This includes both **"physiological ash",** which is derived from the plant tissue itself, and **"non-physiological" ash,** which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface.

ACID-INSOLUBLE ASH VALUE

The residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth.

Sometimes, inorganic variables like calcium oxalate, silica, carbonate content of the crude drug affects" total ash value". Such variables are removed by treating

with acid (as they are insoluble in hydrochloric acid) and acid insoluble ash value is determined.e.g. Rhubarb, liquorice etc.

WATER-SOLUBLE ASH VALUE

Water-soluble ash is the difference in weight between the total ash and the residue after treatment of the total ash with water.

Expt no:14

Date:

DETERMINATION OF ASH VALUE

Aim: To determine the Totalash value of Digitalis leaf

Requirements: Silica crucible, powdered Digitalis leaf

Reference:

1. Indian pharmacopoeia 2007, Vol I, 78
2. Practical pharmacognosy by Dr. Madhu C Divakar, 4th edition, 123-124
3. Text book of Pharmacognosy and phytochemistry- Biren shah & A.K. Seth; Elsever publications, First edition,113

Principle:

The ash of any organic material is composed of their non-volatile inorganic components. Controlled incineration of crude drugs results in an ash residue consisting of an inorganic material (metallic salts and silica). This value varies within fairly wide limits and is therefore an important parameter for the purpose of evaluation of crude drugs.

The determination of ash is useful for detecting low-grade products, exhausted drugs and excess of sandy or earthy matter. More direct contamination, such as by sand or earth, is easily detected by the ash value. Different types of ash values are used in detection of crude drugs like total ash, acid insoluble ash, water-soluble ash and sulphated ash

Total ash is designed to measure the total amount of ash produced after complete incineration of the ground drug at a temperature (less than 450^{o}C) to remove all the carbons. The total ash usually consists of carbonates, phosphates, silicates and silica which include both physiological (derived from plant tissue

itself) and non-physiological ash (it is the residue of the adhering material to the plant surface e.g. Sand and soil)

Procedure:

Place about 2-3 g of the ground material, accurately weighed, or the quantity specified in the monograph, in a suitable tared dish (for example, of silica or platinum), previously ignited, cooled and weighed. Incinerate the material by gradually increasing the heat, not exceeding 450 °C, until free from carbon; cool, and weigh. If a carbon-free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ashless filter-paper, incinerate the residue and filter-paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 450 °C. Calculate the content in mg of ash per g of air-dried material.

If carbon-free ash cannot be obtained in this manner, cool the crucible and moisten the residue with about 2 ml of water or a saturated solution of ammonium nitrate R. Dry on a water-bath, then on a hot-plate and ignite to constant weight. Allow the residue to cool in a suitable desiccator for 30 minutes, and then weigh without delay. Calculate the content of total ash in mg per g of air-dried material.

Report:

Total ash value of the given sample was found to be

Expt no:15

Date:

DETERMINATION OF ACID-INSOLUBLE ASH VALUE

AIM: To determine the acid insoluble ash value of the given sample

PROCEDURE

- Transfer the crucible containing the total ash into a 100 ml beaker; add 25 ml of dilute hydrochloric acid.
- Place mere gauze over a Bunsen burner and boil gently for 5 minutes.
- Collect the insoluble matter on an ashless filter-paper and wash with hot water until the filtrate is neutral.
- Ignite a crucible in the flame, cool and weigh.
- Transfer the filter-paper containing the insoluble matter to the weighed empty crucible, ignite to constant weight.
- Allow the residue to cool in a suitable desiccator for 30 minutes, then weigh without delay.
- Calculate the content of acid-insoluble ash with reference to the air-dried sample of the crude drug.

Report:

Acid insoluble ash value of the given sample was found to be

Expt no:16

Date:

DETERMINATION OF WATER-SOLUBLE ASH VALUE

AIM: To determine the water soluble ash value of the given sample

PROCEDURE

1. To the crucible containing the total ash, add 25 ml of water and boil for 5 minutes.
2. Collect the insoluble matter in a sintered-glass crucible or on an ashlessfilterpaper.
3. Wash with hot water and ignite in a crucible for 15 minutes at a temperature not exceeding 450 °C.
4. Subtract the weight of this residue in mg from the weight of total ash.
5. Calculate the content of water-soluble ash in mg per g of air-dried material.

Report:

Water soluble ash value of the given sample was found to be

SWELLING INDEX

Many herbal materials are of specific therapeutic or pharmaceutical utility because of their swelling properties − especially gums and those containing an appreciable amount of mucilage, pectin or hemicellulose.

The swelling index means volume in ml occupied by 1 g of swollen material under specified conditions.

Its determination is based on the addition of water or a swelling agent as specified in the test procedure for each individual herbal material (either whole, cut or pulverized). Using a glass-stoppered measuring cylinder, the material is shaken repeatedly for 1 hour and then allowed to stand for a required period of time. The volume of the mixture (in ml) is then read. The mixing of whole herbal material with the swelling agent is easy to achieve, but cut or pulverized material requires vigorous shaking at specified intervals to ensure even distribution of the material in the swelling agent.

Note: Swelling index of Isapgol seeds is not less than 10 ml

Expt no:17

Date:

DETERMINATION OF SWELLING INDEX OF ISAPGOL SEEDS

AIM: To determine the swelling index of the given sample

PROCEDURE

1. Take 1 g of the seeds into a 25-ml glass-stoppered measuring cylinder.
2. Add 25 ml of water and shake the mixture thoroughly every 10 minutes for 1 hour.
3. Allow to stand for 3 hours at room temperature, or as specified.
4. Measure the volume in ml occupied by the swollen seeds.
5. Calculate the mean value of the individual determinations, related to 1 g of herbal material.

RESULT

Swelling index of Isapgol seeds was found to be

FOAMING INDEX

Many herbal materials contain saponins that can cause persistent foam when an aqueous decoction is shaken. The foaming ability of an aqueous decoction of herbal materials and their extracts is measured in terms of a foaming index.

ASSESSMENT:

If the height of the foam in every tube is less than 1 cm, the foaming index is less than 100. If a height of foam of 1 cm is measured in any tube, the volume of the herbal material decoction in this tube (a) is used to determine the index. If this tube is the first or second tube in a series, prepare an intermediate dilution in a similar manner to obtain a more precise result.

If the height of the foam is more than 1 cm in every tube, the foaming index is over 1000. In this case repeat the determination using a new series of dilutions of the decoction in order to obtain a result.

Calculate the foaming index using the following formula:

$$\text{FOAMING INDEX} = 1000 / a$$

Where a = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1 cm is observed.

Expt no:18

Date:

DETERMINATION OF FOAMING INDEX

AIM: To determine the foaming index of the given sample

PROCEDURE

1. Take 1 g of coarse powder of the herbal material in a 500-ml conical flask.
2. Add 100 ml of boiling water and Maintain at moderate boiling for 30 minutes.
3. Cool and filter into a 100-ml volumetric flask and add sufficient water through the filter to dilute to volume.
4. Pour the decoction into 10 stoppered test-tubes (height 16 cm, diameter 16 mm) in successive portions of 1 ml, 2 ml, 3 ml, etc. up to 10 ml.
5. Adjust the volume of the liquid in each tube with water to 10 ml. Stopper the tubes and shake them in a lengthwise motion for 15 seconds, two shakes per second.
6. Allow to stand for 15 minutes and measure the height of the foam.

RESULT

Foaming index of the given sample was found to be

Expt no:19

Date:

DETERMINATION OF EXTRACTIVE VALUES OF CRUDE DRUGS

Aim: to determine the alcohol soluble and water-soluble extractive value

Requirements: alcohol (90%), distilled water, stoppered flask, china dish.

Reference:

1. Indian pharmacopoeia- 2007, Vol.I, 191
2. Practical pharmacognosy by Dr. Madhu C Divakar, 4th edition, 124-5
3. Text book of Pharmacognosy and phytochemistry- Biren shah & A.K. Seth; Elsever publications, First edition,113

Principle:

The extracts obtained by exhausting crude drugs with different solvents are approximate measure of their chemical constituents. Various solvents are used according to the type of the constituents to be analyzed.

- **Water soluble extractive value: It** plays an important role for the evaluation of crude drugs. Water soluble extractives are applied to drugs containing water-soluble active constituents such as tannins, sugars, plant acids, mucilage, glycosides etc.
- **Alcohol soluble extractive value:** Alcohol soluble extractives are applied to drugs containing alcohol soluble active constituents such as lignans, alkaloids etc.

The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent.

Procedure:

Water soluble extractive value

The air dried, coarsely powdered Lobelia plant (5gm) was macerated with 100ml of chloroform water in a stoppered conical flask for 24hrs, shaken frequently and allowed to stand for 18hrs. This extract was filtered and filtrate was collected; 25ml of the filtrate was transferred to an evaporating dish of known weight. Then it was dried in an oven at 105^0C, cooled and dried. The percentage w/w of alcohol soluble extractive value was calculated with reference to air dried drug.

Alcohol soluble extractive value

The air dried, coarsely powdered Lobelia plant (5gm) was macerated with 100ml of 95%alcohol in a stoppered conical flask for 24hrs, shaken frequently and allowed to stand for 18hrs. This extract was filtered rapidly taking precautions against loss of alcohol into a 50ml glass beaker. The filtrate was collected; 25ml of the filtrate was transferred to an evaporating dish of known weight. Then it was dried in an oven at 105^0C, cooled and dried. The percentage w/w of alcohol soluble extractive value was calculated with reference to air dried drug.

Report:

Alcohol soluble extractive value of Lobelia powder was found to be

Water soluble extractive value of Lobelia powder was found to be

Expt no:20

Date:

DETERMINATION OF STOMATAL INDEX AND STOMATAL NUMBER

Aim: To determine stomatal number and stomatal index of the given leaf

Requirements: compound microscope, stage micrometer, eye piece micrometer, camera lucida, glycerine, drawing paper, 5% KOH

Reference:

1. Practical pharmacognosy by Dr. Madhu C Divakar, 4th edition, 124-5
2. Text book of Pharmacognosy and phytochemistry- Biren shah & A.K. Seth; Elsever publications, First edition,111

Principle:

Stomatal number is defined as the average number of stomata per sq. mm of epidermis of leaf. It has significance in determining the quality of crude drugs; this number varies depending on the environmental condition and geographical sources where the plants are grown.

The stomatal index is the percentage of the number of stomata formed by the total number of epidermal cells including the stoma being counted as one cell.Stomatal index can be calculated by using the formulae;

$$\text{Stomatal index, S.I.} = \frac{S \times 100}{E+S}$$

Where S = Total number of stomata in a given area of leaf

E = No. of epidermal cell in the same area of leaf

Procedure:

1. A piece of the leaf is cut from the middle portion of the lamina avoiding midrib and margin.
2. It is then taken in a test tube and boiled with 5% KOH solution on a water bath, until it is clean enough for observation.
3. Prepare the mounts of lower and upper epidermis separately in glycerin water
4. Draw a square of known dimension by means of a stage micrometer and camera lucida on a drawing paper.
5. Replace the stage micrometer by the cleared leaf preparation, focus under the same magnification and trace the epidermal cells and stomata by looking through the microscope when a superimposed image of the leaf is seen at the same time. Count the number of the epidermal cells and stomata within the square, a cell being counted if at least half of its area lies within the square provided two adjacent sides are considered for purpose of calculation.
6. Calculate the number of stomata and stomatal index.

Report:

Stomatal index of the given sample was found to be

Stomatalnumber of the given sample was found to be

Expt no:21

Date:

DETERMINATION OF PALISADE RATIO

Aim: To determine the palisade ratio of the given leaf

Requirements: compound microscope, stage micrometer, eye piece micrometer, camera lucida, glycerin, drawing paper

Reference:

1. Practical pharmacognosy by Dr. Madhu C Divakar, 4th edition, 120-21
2. Text book of Pharmacognosy and phytochemistry- Biren shah & A.K. Seth; Elsever publications, First edition,111

Principle:

It is defined as the average number of palisade cells beneath each epidermal cell. This value remains constant within a range for a given plant species and is of diagnostic value in differentiating the species. This value does not alter based on geographical variation and differs from species to species. Palisade ratio is an important data for leaf drug evaluation and can be successfully applied for the studies of several dicot leaves of medicinal importance.

Procedure:

Small pieces of leaf from the apex were taken from the young leaves; the lower epidermis was peeled off and then cut into pieces. It was boiled gently for 15minutes with 5ml 5% KOH solution to clear the green colour. The pieces were kept in a glass slide and observed under high power objective with 10X or 15 X eyepieces. After tracing four continuous epidermal cells, moving the fine

adjustment of the microscope, palisade cells were focused and the cells inside all the 4 epidermal cells were drawn. The palisade cells inside the boundary and those that are 50% or more inside the outer boundary of 4 epidermal cells were taken into account. The number of total palisade cells is divided by 4, which gives the average number of palisade cell under each epidermal cell.

Report:

Palisade ratio of the given leaf was found to be

Expt no: 22

Date:

DETERMINATION OF VEIN ISLET NUMBER & VEIN TERMINATION NUMBER

Aim

To determine the Vein-Islet and Veinlet termination number of Leaf.

Requirements

Compound microscope, Stage micrometer, Camera lucida, Drawing paper, Lactophenol solution and Glycerin water.

Principle

The mesophyll of the leaf in dicot leaves is divided into small portions by branching of the veins throughout the tissues. The small areas of the green tissue outlined by the veinlets are termed as veinislets. Vein-islet Number: Vein-islet number is defined as the number of vein-islets per sq mm of the leaf surface midway between the midrib and the margin. It is a constant for a given species of the plant and is used as a characteristic for the identification of the allied species. Veinlet Termination Number: Veinlet termination number is defined as the number of veinlet termination per sq mm of the leaf surface midway between midrib and margin. A vein termaination is the ultimate free termination of veinlet.

Procedure

a) Boil a few leaves in lactophenol solution in test-tube placed in a boiling water bath. If the leaves are difficult to clear in this manner, soak them in water, treat successively with chlorinated soda for bleaching, 10% hydrochloric acid for the removal of calcium oxalate and finally lactophenol.

b) Mount the preparation in glycerin water.

c) Set up the camera lucida and divide the paper into squares of 4 sq mm by means of the stage micrometer.

d) Replace the stage micrometer by the cleared leaf preparation and trace the veins in four continuous squares, either in square of 4 mm X 4 mm or rectangle of 1 mm X 4 mm. Trace the vein-islet and veinlet termination by looking through the microscope when a superimposed image of the leaf portion and paper is seen at the same time.

e) Count the number of vein-islets and veinlet termination present within the square of rectangle and also by taking into consideration incomplete vein-islets and any two adjacent sides of the square or rectangle. Record the observation in the form of range and also indicate the mean value.

Report

1. The Vein-Islet number of given leaf is found to be…….
2. The Veinlet termination number of the given leaf is found to be ……..

Reference

1. Kokate, CK. 2013. Practical Pharmacognosy, Vallabh Prakashan, 116-117.
2. Khandelwal, KR and Vrunda, S. 2014. Practical Pharmacognosy, Nirali Praksshan, 24.3-24.4.