East Point College of Pharmacy

East Point Campus, Jnana Prabha, Virgo Nagar Post
Bengaluru – 560049, Karnataka

Approved
by
Pharmacy Council of India, New Delhi

Affiliated
to
Rajiv Gandhi University of Health Sciences
Karnataka
Bengaluru – 560 041
India

LAB MANUAL

PHARMACOGNOSY AND PHYTOCHEMISTRY I

B. PHARM 4th SEMESTER

EAST POINT COLLEGE OF PHARMACY
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Bengaluru – 560049, Karnataka

BPharm

Program Outcomes (PO's)

PO 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.

PO 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.

PO 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

PO 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.

PO 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 wellbeing.

PO 6- Professional Identity

Understand, analyse and communicate the value of their professional roles in society (e.g. health care professionals, promoters of health, educators, managers, employers, employees).

PO 7- Pharmaceutical Ethics

Honor personal values and apply ethical principles in professional and social contexts. Demonstrate behaviour 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

PO 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

PO 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.

PO 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.

PO 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-access and use feedback effectively from others to identify learning needs and to satisfy these needs on an ongoing basis.

Programme Specific Outcomes (PSO's)	
PSO 1	Acquire a thorough foundational knowledge in pharmaceutical sciences, including pharmacology, pharmaceutics, medicinal chemistry, and pharmacognosy, to excel in further academic pursuits
PSO 2	Gain expertise in the application of contemporary pharmaceutical techniques and technologies, enhancing employability across various sectors including the pharmaceutical industry, academia, and research institutions.
PSO 3.	Equip with entrepreneurial skills and knowledge of pharmaceutical business management, including market analysis, product development, regulatory affairs, and financial planning, to initiate and run successful ventures in the pharmacy Sector

Course Outcomes (CO's)
Code: BP409P Pharmacognosy and Phytochemistry I
CO1: To perform chemical tests and morphological evaluation to report on the phytochemical nature of the crude drugs.
CO2: To evaluate the phytochemical constants such as ash value, extractive value, moisture content, Swelling index, and Foaming index.
CO3: To evaluate the leaf constants.
CO4: To know the determination of purity by the Lycopodium spore method.

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SL NO	LIST OF THE EXPERIMENT
	Chemical Tests
1.	**Chemical Tests for Tragacanth**
2.	**Chemical Tests for Acacia**
3.	**Chemical Tests for Agar**
4.	**Chemical Tests for Starch**
5.	**Chemical Tests for Honey**
6.	**Chemical Tests for Castor Oil**
7.	**Chemical Tests for Gelatin**
	Physical Evaluation
8.	**Determination of Ash Value**
9.	**Extractive value determination**
10.	**Determination of moisture content-Loss on drying**
11.	**Determination of swelling index**
12.	**Determination of foaming index**
	Microscopical Evaluation
13.	**Determination of Fiber Length usingeyepiece micrometer**
14.	**Determination of Fiber Length usingcamera lucida**
15.	**Quantitative Microscopy- Lycopodium Spore method**
16.	**Determination of Size of StarchGrains by eyepiece micrometer**
17.	**Determination of Size of calciumoxalate crystals by eyepiece micrometer**
18.	**Determination of Stomata Number**
19.	**Determination of Stomatal index**
20.	**Determination of Vein islet number**
21.	**Determination of Vein terminationnumber**
22.	**Determination of the palisade ratio**

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CHEMICAL TESTS

Experiment No. 1

CHEMICAL TESTS FOR TRAGACANTH

Aim: To carry out the chemical tests for Tragacanth.

Reference:

1. Study of crude drugs by M.A.Iyengar, Pg.No. 119
2. Pharmacognosy by C.K. Kokate, 45th edn, Pg.No: 7.5.

Biological Source: It is the dried gummy exudation obtained from the stem and branches of *Astragalus gummifer*.

Family: Leguminosae.

Chemical constituents: Carbohydrates –complex Polysaccharides.

Bassorin (Water-insoluble) Tragacanthin (Water soluble)

Tragacanthin → Tragacanthic acid + Arabinogalactan

Tragacanthic acid →Galactouronic acid + Galactose + Fructose + Xylose.

Arabinogalactin → Arabinose + Galactose + Rhamnose.

Uses: Bulk laxative, emulsifying agent, stabilizing agent, Used in paper and textile industries.

Physical Tests

SL.No	Experiment	Observation	Inference
1	State	Flattened, lamellated	May be carbohydrate
2	Colour	White or faint yellow	May be carbohydrate
3	Odour	None	May be carbohydrate
4	Taste	Mucilaginous	May be carbohydrate
5	Solubility a) In cold water b) In hot water In alcohol	 Partially soluble Soluble Soluble	May be carbohydrate (Distinction from acacia and agar)
6	Litmus test	Acidic	May be carbohydrate

CHEMICAL TESTS

Sl.No.	Experiment	Observation	Inference
1	Molisch test: To a Small amount of sample solution add Molisch'sreagent, shake and add Conc. H_2SO_4, slowly from the sides.	A purple ring is formed at the junction of the two liquids.	Carbohydrate is present.
2	To the sample solution add lead sub acetate solution.	A White Precipitate is seen.	Distinction from acacia.
3	Take a small amount of the sample solution, add dilute HCl, and heat on the water bath for 30 min. Divide it into 2 parts, Part I: Neutralise it with dilute NaOH & add Fehlings A & B Soln. Part II: Add 10% $BaCl_2$ Solution.	Red Precipitate is obtained. No white ppt or turbidity is obtained.	Reducing sugar present.
4	Mucilage Test: To the sample powder add 1 drop of ruthenium red.	Particles take up no pink colour.	Mucilage is absent distinction from agar.
5	Boil the sample with freshly prepared $FeCl_3$ solution.	A deep yellow precipitate isobtained.	Indian tragacanth is present.
6	To the sample add strong Iodinesolution. Boil & transfer a few drops toa test tube filled with water.	A green colour solutionis obtained.	Tragacanth is confirmed.

Report

Experiment No. 2

CHEMICAL TESTS FOR ACACIA

Aim: To carry out the chemical tests for acacia.

Reference:

1. Study of crude drugs by M.A.Iyengar, Pg.No. 7
2. Pharmacognosy by C.K. Kokate, 45th edn, Pg.No: 7.1.

Biological Source: Acacia consists of the dry gummy exudation obtained from the stem and branches of *Acacia arabica* belonging to the **Family-** Leguminosae

Chemical constituents: Arabin (a mixture of Ca, Mg, and K salt of Arabic acid).

Arabin hydrolysis→→ D-galactose + L-arabinose + L-rhamnose + D-glucuronic acid.

Uses:

Demulcent, emulsifying agent, Suspending agent, Binding agent.

SL.No	Experiment	Observation	Inference
1	State	Rounded solid	May be carbohydrate
2	Colour	Colourless to slight yellow	May be carbohydrate
3	Odour	None	May be carbohydrate
4	Taste	Mucilaginous	May be carbohydrate
5	Solubility a. In cold water b. In hot water c. In alcohol	Partially solubleSoluble Insoluble	May be carbohydrate

CHEMICAL TEST

Sl.No.	Experiment	Observation	Inference
1	Molisch test: To a Small amount of sample solution add Molisch'sreagent, shake and add Conc. H_2SO_4, slowly from the sides.	A purple ring is formed at the junction of the two liquids.	Carbohydrate is present.
2	Take a small amount of the sample solution, add dilute HCl and heat on the water bath for 30 min. Divide it into 2 parts, Part I: Neutralise it with dilute NaOH & add Fehlings A & B Soln. Part II: Add 10% $BaCl_2$ Solution.	The red precipitate of cuprous oxide is formed. No Precipitate is obtained.	Reducing Sugar is present.
3	To the sample solution add lead sub acetate solution.	Flocculent white the precipitate is obtained.	Agar is absent. Acacia is confirmed.
4	To the given drug powder add N/50 Iodine solution.	No crimson colour.	Agar, tragacanth & starch absent. Therefore, acacia is confirmed.
5	To 2 ml solution, add 50 mg of borax.	Stiff translucent mass is formed.	Acacia is present.
6	Oxidase enzyme test/ Benzidine test: Tothe sample solution add 0.5 ml of 1% solution of benzidine in alcohol. Shake well and allow to stand for sometimes.	An unstable blue colour is obtained.	Oxidase enzyme present. Therefore, acacia is confirmed.

Report

Experiment No.3

CHEMICAL TESTS FOR AGAR

Aim: To carry out the chemical tests for agar.

Reference:

1) Study of crude drugs by M.A.Iyengar, Pg.No. 9
2) Pharmacognosy by C.K. Kokate, 45th ed, Pg.No: 7.25.

Biological Source: Agar consists of the dried gelatinous product obtained from different speciesof red algae like

Gelidium amansi- Fam: Gelidaceae

Pterocladia- Fam: Gelidaceae

Gracilaria- Fam: Gracilariaceae.

Chemical constituents: Polysaccharides.

Agarose (responsible for gel strength of agar)

Agaropectin (responsible for viscosity of agar solution) sulphated salt of galactose and uronic acid.

Uses: Bulk laxative, emulsifying agent, tablet excipient, bacteriological culture mediumpreparation.

SL.No.	Experiment	Observation	Inference
1	State	Thin membranous strips	May be carbohydrate
2	Colour	Colourless, greyish yellow	May be carbohydrate
3	Odour	None	May be carbohydrate
4	Taste	Mucilaginous	May be carbohydrate
5	Solubility a. In cold water b. In hot water c. In alcohol	 Powder swells Soluble Soluble	May be carbohydrate

CHEMICAL TEST

Sl.No.	Experiment	Observation	Inference
1	Molisch test: To Small amount of sample solution add Molisch'sreagent,shake and add Conc. H_2SO_4, slowly from the sides.	Purple ring is formedat the junction of the two liquids.	Carbohydrate is present.
2	Take the small amount of the sample solution, add dilute HCl and heat on the water bath for 30 min. Divide it into 2 parts, Part I: Neutralise it with dilute NaOH & add Fehlings A & B Soln.	The red precipitate of cuprous oxide is formed.	Reducing Sugar is present.
	Part II: Add 10% $BaCl_2$ Solution.	A White Precipitate is formed.	Agar is present.
3	To the sample solution add lead sub acetate solution.	No precipitate is obtained.	Tragacanth is absent. May be agar.
4	To the given drug powder add N/50 Iodine solution.	Crimson to brown colour is obtained.	Agar is present.
5	Test for Mucilage – Drug powder + solution of Ruthenium red. The test is to be performed on a slide.	The particles acquire a pink colour.	Mucilage is present, therefore agar may be present.
6	Gel Test: Boil 1gm of the sample with10 ml of water until the solution is affected. Cool to room temperature.	A jelly mass is formed.	Agar is confirmed.

Report

Experiment No. 4

CHEMICAL TESTS FOR STARCH

Aim: To carry out the chemical tests for Starch.

Reference:

1) Study of crude drugs by M.A.Iyengar, Pg.No. 116.
2) Pharmacognosy by C.K. Kokate, 45th edn, Pg.No: 7.36.

Synonym: Amylum.

Biological Source: Starch consists of polysaccharide granules obtained from the grains of maize (*Zea mays*); rice (*Oryza sativa*); Wheat (*Triticum aestivum*); **Family**: Gramineae

Tubers of Potato (*Solanum tuberosum*) Family: Solanaceae.

Chemical Constituents: Polysaccharides

Amylose (β- amylose)- water soluble.

Amylopectin (d-amylose)- water-insoluble (responsible for the gelatinizing property of starch)

Uses: Nutritive, Demulcent and protective, Absorbent, Used as a dusting powder.

CHEMICAL TESTS

SL.No.	Experiment	Observation	Inference
1	Molisch test: To a Small amount of sample solution add Molisch's reagent, shake and add Conc. H_2SO_4, slowly from the sides.	A purple ring is formed at the junction of the two liquids.	Carbohydrate is present.
2	Treat the sample solution with FehlingsA & B Soln. and heat on a water bath.	No precipitate isobtained.	May not hydrolyse. Not a reducing sugar.
3	Repeat the above procedure by hydrolyzing first with Conc. HCl and neutralized with NaOH solution.	The red precipitate of cuprous oxide is formed.	Hydrolysis has been taken; polysaccharides are present.
4	Boil the sample powder with water then cool. Add a few drops of Iodine solution.	Translucent viscousfluid or jelly is obtained. Blue colour, disappears on warming and reappears on cooling.	Starch is confirmed. Starch is confirmed.

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Experiment No. 5

CHEMICAL TESTS FOR HONEY

Aim: to carry out the chemical tests for Honey.

Reference:

1)Pharmacognosy by C.K. Kokate, 45th edn, Pg.No: 7.39.

Biological Source: Honey is a sugar secretion deposited in a honeycomb by the Honey bees, *Apis mellifera, Apis dorsata* and other species of Apis.

Family: Apidae.

Physical Characters

State: Viscous liquid

Colour: Pale yellow to yellowish

Taste: Sweet

Odour: Characteristic

Solubility: soluble in water, insoluble in alcohol.

Chemical constituents:

Carbohydrates – 70- 80% of invert sugar (equimolar mixture of glucose and fructose) and 2% sucrose.

Enzymes, Vitamins, Microelements, Mineral substances, Organic acids (Succinic acid) etc.

Uses:

Nutrient and demulcent, Laxative, As a pill excipient, in the preparation of cough drops,creams, lotions etc.

CHEMICAL TEST

Sl.No.	Experiment	Observation	Inference
1	0.5 g of Honey + 10 ml water, add Fehlings A & B Soln. and heat on a water bath for 15 mins.	Heavy red precipitate of copper oxide is obtained.	Reducing sugars present.
2	Selvinoff's test: To the test solution a crystal of resorcinol is added and equal volume of Conc. HCl, warm on water bath.	Rose colour is obtained.	Honey is present.
3	Test for adulteration – Fiehe's test: Shake 10 ml of honey thoroughly with 5 ml of ether separate ether layer evaporated to dryness in a porcelain dish, add 1 drop of 1% resorcinol in Hcl.	Transient red coloration. Red colour persists.	No Adulteration Indicated adulteration (Artificial invertsugar)

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Experiment No. 6

CHEMICAL TEST FOR CASTOR OIL

Aim: To carry out the chemical test for castor oil.

Reference:

1) Study of crude drugs by M.A. Iyengar, Pg.No. 35.
2) Pharmacognosy by C.K.Kokate, 45th Edn, Pg.No. 10.7.

Biological Source: Castor oil is the fixed oil obtained by cold expression of the seeds of *Ricinus communis*

Family: Euphorbiaceae.

Chemical Constituents: Triglycerides of Ricinoleic acid (80%, responsible for viscosity of the oil), Linoleic acid, Stearic acid, Isostearic acid.

Uses:

1) Used in the contraceptive jelly, creams.
2) Bactericide in tooth paste formulation.
3) Cathartic (it acts by irritant action of Ricinoleic acid)
4) It imparts transparency in the soap.

Physical tests

SL.No.	Experiment	Observation	Inference
1	State	Viscous liquid	May be lipid
2	Colour	Colourless transparent	May be lipid
3	Odour	Faint	May be lipid
4	Taste	Acrid & Nauseating	May be lipid
5	Solubility		
	In cold water	Insoluble	May be lipid
	In hot water	Insoluble	May be lipid
	In alcohol	Soluble	May be lipid
6	Litmus test	Neutral	May be lipid

CHEMICAL TEST

SLNo.	Experiment	Observation	Inference
1	To the equal quantity of the castoroil add an equal quantity of dehydrated alcohol.	Miscible.	May be castor oil
2	To the 10 ml of castor oil add 5 ml of light petroleum. If the light petroleum is increasedto 15 ml.	Clear solution is obtained. The mixture becomes turbid.	May be castor oil Other oil does notshow this test and adulteration can be detected by this test method.

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Experiment No. 7

CHEMICAL TESTS FOR GELATIN

Aim: To carry out the chemical tests for Gelatin.

Reference:

1)Practical Pharmacognosy, Khandelwal KR, Nirali Prakashan, 12th Edition, 2004. Pg.No: 174-175.

Biological Source: A protein extracted by partial hydrolysis of animal collagenous tissue like skin, tendons, ligaments and bones with boiling water.

Physical Characters:

Colour: Colourless or pale-yellow

Taste: Characteristic

Odour: Very slight

State: Translucent sheets, flakes, shreds or a course to fine powder.

Solubility: Insoluble in cold water but swells and softens, absorbs water but soluble in hot water forms jelly on cooling.

Chemical constituents: Amino acids like lysine etc.

CHEMICAL TESTS

SL.No.	Experiment	Observation	Inference
1	Heat with soda lime	Ammonia evolves.	May be protein
2	Gelatin with 10% Picric acid	Yellow precipitate	May be protein
3	Gelatin is heated with Millon's reagent	Red coloured precipitate	May be protein
4	Dilute an aqueous solution of Gelatin with a solution of tannic acid and a solution of trinitrophenol.	Gelatin precipitates.	May be gelatin.
5	Alum/lead acetate/acids	Gelatin does not precipitate.	May be gelatin.
6	Biuret test – Take 3 ml of the test solution + add NaoH (1 ml of 5%) + 2 drops of 1%CuSo4	A violet or pink colour is developed	Gelatin is confirmed

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PHYSICAL EVALUATION

Experiment No. 8

ASH VALUE DETERMINATION

Aim: To determine the ash value of the given sample

The determination of physicochemical parameters such as Total Ash, Acid insoluble ash,Water soluble ash, Ethanol soluble extractive and Water-soluble extractives and loss on drying were determined and estimated in percentage by using methods recommended by Indian Pharmacopoeia.

Ash value:

The residue remaining after the incineration of a known weight of the plant material is calledthe ash value of a crude drug. When vegetative drugs are incinerated, they leave inorganic ash which in the case of many drugs varies within fairly wide limits and these values play a significantrole in plant drug evaluation. Ash values are of prime importance in the examination of the purity of powdered drugs. It is used to ensure the absence of an abnormal proportion of extraneous minerals incorporated accidentally and to check the calcium oxalate content of the drug. The Ash value of the air-dried powdered samples of plant species taken for our study was subjected to the following studies.

Total Ash

Acid insoluble ash

Water soluble ash.

Procedure:

Determination of Total Ash:

About 2 g of powdered drug was weighed accurately in a silica crucible, which was previously ignited, cooled in a desiccator and weighed. The drug was scattered into a fine even layer on the bottom of the dish and incinerated by gradually increasing the heat, not exceeding dullred heat until free from carbon. It was cooled and weighed.

The procedure was repeated to a constant weight. The percentage of the total ash was calculated with reference to the air-dried drug.

Determination of acid-insoluble ash:

The ash obtained as described in the determination of total ash was boiled with 25 ml of dilute hydrochloric acid for 5 minutes. It was filtered through an ashless filter paper, and washed withhot water. The insoluble ash was transferred into a pre-weighed silica crucible. It was ignited, cooled and weighed. The procedure was repeated until to get a constant weight. The percentage ofacid-insoluble ash was calculated with reference to the air-dried drug.

Determination of water-soluble ash:

The ash obtained as described in the determination of total ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on an ash-less filter paper and washed with hot water. The insoluble ash was transferred into a pre-weighed silica crucible and ignited for 15 minutes at a temperature not exceeding 450 ºC.

The procedure was repeated to get constant weight. The weight of the soluble matter was obtained by subtracting the weight of the total ash from the water insoluble ash value. The percentageof water-soluble ash was calculated with reference to the air-dried drug.

Report

Experiment No. 9

EXTRACTIVE VALUE DETERMINATION

Aim: To determine the extractive value of the given sample.

Extractive value:

The determination of extractable matter refers to the amount of plant material extracted with solvents. Such extractive values provide an indication of the extent of polar, medium polar and nonpolar components present in medicinal plant materials. It is used to identify the adulterants present in the drug. Air-dried powdered samples of plant species were subjected to the following studies.

Ethanol soluble extractive

Water soluble extractive

Procedure:

1. Macerate 5gm of the air-dried drug, coarsely powdered, with 100ml of ethanol (water) of the specified strength in a closed flask for 24hrs, shaking frequently during the first 6hrs and allowing tostand for 18hrs.
2. Filter rapidly taking precautions against loss of ethanol.
3. Evaporate 25ml of the filtrate to dryness in a tared flat-bottomed shallow dish, dry at 1050. Cool ina desiccator and weigh
4. Calculate the percentage of ethanol-soluble extractives with reference to the air-dried drug.
5. Proceed as directed for the determination of ethanol soluble extractive, using water instead ofethanol.

Calculation:

25ml of alcoholic extract gives =

100ml of alcoholic extract gives =5g of air-dried drug gives =100g of air-dried drug gives … =

Report

Experiment No. 10

DETERMINATION OF MOISTURE CONTENT OF CRUDE DRUGS

Aim: Determination of moisture content of the given sample of crude drug

Requirements: Porcelain dish, oven, desiccator, powdered drug

Discussion: Loss on drying is the loss in weight in %w/w resulting from water and volatile matter of any kind that can be driven off under specified conditions. The test is carried out on a well-mixed sample of the substance. If the substance is in the form of large crystals reduce the size by rapid crusting to a powder.

The moisture content of a drug should be determined and controlled because

a) The percentage of active chemical constituents in the crude drug is mentioned on air air-dried basis.

b) The moisture content of a drug should be minimized to prevent decomposition of crudedrugs either due to chemical change or microbial contamination.

Procedure:

1. Weigh about 1.5 gm of the powdered drug into a weighed flat and thin porcelain dish.
2. Dry in the oven at 100^0 or $105^{0.}$
3. Cool in a desiccator and weigh.
4. The loss in weight is usually recorded as moisture.
5. Dry until two consecutive weighs do not differ by more than 5 mg unless otherwise specified inthe test procedure. Calculate the loss of weight in mg per g of air-dried material.

Note: Heating should proceed slowly and material must not be allowed to catch fire or to give off, smoke as dense fumes. Heating is carried out till one gets constant weight at 100^0c for substances containing loss of weight at a temperature of 100^0c other methods are used for materials of this type.

Report

Experiment No. 11

DETERMINATION OF SWELLING INDEX

Aim: To determine the swelling index of the given sample.

Definition: The term swelling factor gives an idea about the mucilage content of the seed. Hence it is useful in the evaluation of crude drugs containing mucilage.

Procedure:

i) Take 1 g of the seeds in a 25 ml stoppered cylinder.

ii) Add water up to 25 ml marking.

iii) Shake occasionally during 23 hours.

iv) Keep aside for one hour.

v) Measure the volume occupied by the swollen seeds.

Report

Experiment No. 12

DETERMINATION OF FOAMING INDEX

Aim: Determination of swelling factor and foaming index of the given drug

Discussion: Saponins give persistent foam when shaken with water. Hence, plant material/ extract containing saponins is evaluated by measuring the foaming ability in terms of the foaming index.

Procedure:

1. Take 1g of coarse powder of the plant material in a 500 conical flask
2. Add 100ml of boiling water and maintain moderate boiling for 30 minutes
3. cool and filter
4. Collect the filtrate or decoction in a 100-volumetric flask and adjust the volume to 100 ml byadding sufficient water.
5. Pour decoction into 10 stoppered test tubes (height 16cm, diameter 16mm) as 1ml, 2ml, 3ml, etc. upto 10ml
5. Adjust the volume of the liquid in each test tube to 10ml by adding a sufficient quantity of water andstopping the tubes.
6. Shake test tubes in a lengthwise motion for 15 seconds (two shakes per second)
7. Allow test tubes to stand for 15 minutes and measure the height of the foam

Assessment:

If the height of the foam in every tube is less than 1cm it means the foaming index is less than 100. If the height of the foam is more than 1cm in every test tube; the foaming index is over 1000. In this case, repeat the experiment using a new series of dilutions of the decoction to get a result.
If the height of foam is of 1cm in any tube, the volume of the plant material decoction in this tube(a) isused for the determination of the foaming index using the following formula.

$$\text{Foaming index} = \frac{1000}{a}$$

a= volume in ml of the decoction in the test tube showing 1cm foam height

Note: If the test tube showing 1cm foam height is the first or second in the series, prepare an intermediatedilution similarly to obtain a precise result.

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MICROSCOPICAL EVALUATION

Camera Lucida:

Camera Lucida, when attached to a compound microscope, helps draw microscope images of objects on paper. It works on a simple optical principle reflecting a beam of light through a prism and aplane mirror.

The microscopic image of the object is reflected by the prism onto the plane mirror and fromthe image is reflected onto the plane paper. The observer moves the pencil on the lines of the image and draws a correct and faithful figure of the object on the paper.

There are three main parts of a camera lucida the attachment ring, the prism, and the mirror. The attachment ring attaches the camera lucida to the body tube of the microscope. The prism restsjust above the eyepiece when the instrument is attached to the microscope.

The observer now views the image of the object under the microscope through the prism which reflects the image horizontally to the plane mirror. The plane mirror, attached at the tip of an arm rotates and is set at an angle of 45° with reference to the prism and the plane paper.

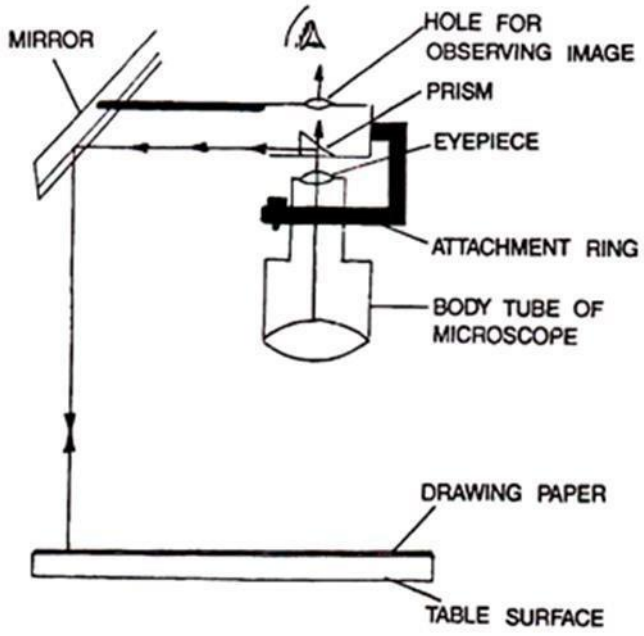
Micrometry:

The eyepiece micrometer is a circular glass disc with a scale of 1cm divided into 1/100 of centimeters engraved on it. This disc can be placed on the circular diaphragm between the field lens and the eye lens of the ocular. The stage micrometer has an engraved scale of 1 or 1.1 mm long and is divided into 0.1 and 0.01 partsof millimeters on a glass slide

For measurement, the eyepiece micrometer must be calibrated for every magnification. This may becarried out with the help of a stage micrometer

1. Remove the eyepiece from the microscope, unscrew its lens, place the eyepiece micrometer onthe ridge inside, replace the lens, and put the eyepiece back into the body of the microscope.
2. Place the stage micrometer on the stage of the microscope and focus the scale under high powerwith the eyepiece scale superimposed.
3. Calculate the factor from the superimposed image. The shorter lines of the numbered scale represent the rulings of the eyepiece micrometer, while the lines extending across the field represent the scale of the stage micrometer.

4.Replace the stage micrometer with the slide of the object. Adjust the slide in such a way that theobject is focused on the lines of the eyepiece micrometer. Note the number of the eyepiece micrometer being covered by the object.



Diagrammatic representation of a camera lucida.

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Experiment No. 13

DETERMINATION OF FIBRE LENGTH USING EYEPIECE MICROMETER

Aim: To determine the fiber length of a given powder drug sample.

Reference:

Practical Pharmacognosy, Khandelwal KR, Nirali Prakashan, 12th Edition, 2004. P. No: 41-44

Material and Chemicals used: eyepiece micrometer, stage micrometer, chloralhydrate, phloroglucinol, glycerin, coverslip, microscope, microscope slide, etc.

Discussion:

Sclerenchyma is a dead tissue containing more thickened cell-type fibers, providing support in older tissues. The cell walls are deposited with lignin. Two types of sclerenchyma, more or less, iso-diametrically, occur either singly or in bonds or in void masses. The thickening may be uniform or irregular and sometimes stratification and pitting may be seen. Sclerenchymatous fibers are thick-walled with narrow lumen and pointed ends. Fibers are named according to the typeof tissue they are found.

Procedure:

1)Calibrate the eyepiece micrometer and stage micrometer and calculate the calibration factor.

2)Mount powder on the slide and stain with phloroglucinol and HCl. Add glycerin to it and mount the cover slip on it gently.

Place the slide on the microscope and observe it. Measure the length of fiber with an eyepiece micrometer

Observation:

$$\text{Calibration value} = \frac{\text{stage micrometer value}}{\text{eyepiece micrometer value}} \times 10 = \_\_\_\_\_ \mu m$$

SL.No	Size of eyepiece micrometer division	Actual size(µm)

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Experiment No. 14

DETERMINATION OF FIBRE LENGTH USING CAMERA LUCIDA

Aim: To determine the fiber length using camera lucida.

Reference:

Practical Pharmacognosy, Khandelwal KR, Nirali Prakashan, 12th Edition, 2004. P. No: 41-44

Requirements: camera lucida, black paper, white pencil, stage micrometer, microscope.

Discussion:

Fibers of the xylem are mechanical or supporting elements and each consists of a single parenchymatous cell. They are polygonal in the transverse section and are thirty to fifty times as longas their width with very long tapering ends. The cavity is narrow, thick-walled simple pits, and slit-shaped. Typically, thick-walled fibers occur in guaicum, but in quassia, the walls are comparatively thin and the lumen is large. Fibers sometimes have delicate transverse partitions formed by cellulosic or pectic material and are then called septate fibers. Such fibers occur in teak wood and also in the fibrous bundle sheath of ginger. Cell forms intermediate between the tracheids and fibers occur in ephedra and in calumba root, they have walls thicker than those of ordinary tracheids, while the pits are larger than those of typical fibers and the ends are less tapering. These fibers/tracheids do not contain protoplasm or starch.

Observation:

1. Take a small quantity of powder on a glass slide and stain it with phloroglucinol and con Hcl.

Sl.No	Actual distance (mm)	Distance after magnification (mm)	Magnification

Sl.No	Magnified size	Actual size

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Experiment No. 15

QUANTITATIVE MICROSCOPY- LYCOPODIUM SPORE METHOD

Aim: To determine the number of starch grains in ginger powder using the lycopodium spore method. To find out the percentage purity of the given sample of ginger.

Reference:

Practical Pharmacognosy, Khandelwal KR, Nirali Prakashan, 12th Edition, 2004. P. No: 190-191

Materials and reagents required:

Microscope, Stoppered tube, Flexible spatula, Glass slide, Glass plate, Coverslip, Suspending fluid, Iodine solution, Glycerin.

Theory: A microscope can be used for the quantitative analysis. When physical and chemical methods cannot be utilized for quantitative purposes, in certain cases, microscopy can be utilized. Therefore, this is an important analytical tool, particularly for powdered drugs.

Lycopodium is used as a standard reference in quantitative microscopy. Thus, it is frequently referred to as the lycopodium spore method.

The characteristics by which lycopodium spores are used in quantitative microscopy are:

1. Characteristic shape (tetrahedral shape or quarter of a ball)
2. Uniform in size. (About 25 µ).
3. Not affected by common reagents used in pharmacognosy labs.
4. Very light.
5. Float in water.

1 mg of lycopodium spore powder contains on average 94,000 spores. This is themost important consideration for its use in quantitative microscopy.

Procedure:

1. Weigh accurately about 100 mg of given ginger powder and 50 mg of lycopodium spore powder. Mix them with a flexible spatula with the help of a small quantity of suspended fluid.Suspended fluid may be glycerin: mucilage of tragacanth: water- 2:1:2 or olive oil.

2. Incorporate more quantity of suspending fluid until a smooth paste results. Transfer it totallyto a stoppered tube with an excess quantity of suspending fluid.
3. Oscillate the stoppered tube gently to get a uniform suspension.
4. Place 1 drop of suspension on a glass slide and spread it uniformly with a needle. Apply coverslip carefully. Add a dilute solution of Iodine at the border of the coverslip. Allow iodine to diffuse inside and leave the slide for a few minutes for settling of the contentsunder the coverslip. Similarly, prepare another slide from the same suspension.
5. Prepare another suspension and from that prepare two more slides. (a total of 4 slides). Count the starch grains and lycopodium spores in each of the 25 fields and enter a tabular column.

Field No.	No. of starch grains	No. of spores

Calculation:

The no. of starch grains per mg of ginger powder is calculated by using the formula:

$$\% \text{ purity} = \frac{N \times W \times 94.000 \times 100}{S \times M \times P}$$

Where,

N= The no. of starch grains in 20 fields

S= The no. of lycopodium spores in the same 20 fields.

W= Weight of lycopodium spore powder in mg.

M= Weight of ginger powder in mg.

P = 284000

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Experiment No. 16

DETERMINATION OF SIZE OF STARCH GRAINS

Aim: To determine the size of the starch grain of a given powder drug sample.

Reference:

Practical Pharmacognosy, Khandelwal KR, Nirali Prakashan, 12th Edition, 2004. P. No: 41-44

Materials and Chemicals used: Eyepiece micrometer, stage micrometer, starch iodine solution, and glycerin.

Discussion:

Starch is the most important source of carbohydrates in the human diet. It is found in most plants, particularly seeds. Starch occurs in the form of granules which vary in size and shape depending upon their plant source.

Sources of starch*:* maize, rice, cereals, wheat, potato.

Chemical composition: starch is not a pure compound. It is a mixture of two polysaccharides:

i)Amylose

ii)Amylopectin.

Amylose: is a water-soluble fraction. It is a straight-chain polysaccharide composed entirely of D-glucose units. The units are joined by α – glycosidic linkages betweenC1 of one glucose and C4 of the next. Amylose gives deep blue colour with iodine.

Amylopectin: is water insoluble fraction. It is a branched chain polysaccharide. It iscomposed of chains of 24 to 30 D-glucose units joined by α – glycosidic linkages between C1 of one glucose and C4 of the next. Amylopectin gives no colour with iodine.

Procedure:

1) The calibration value was determined by using an eyepiece and stage micrometer.
2) Starch was mounted on a glass slide and was treated with a drop of iodine solution.
3) Glycerin was added and the mount cover slip and was observed under a microscope.
4) The diameter of the starch grain was measured by focusing them on the scale of the eyepiece micrometer.
5) The number of divisions being covered by starch grains was noted. The diameter of 25 starchgrains was calculated by multiplying the division by a factor to get the actual dimension.

Observation:

of eyepiece micrometer value = ...of stage micrometer value

$$\text{Calibration value} = \frac{\text{stage micrometer value}}{\text{eyepiece micrometer value}} \quad X\ 10 = \_\_\_\_\_\ \mu m$$

Sl.No	Size of eyepiecemicrometer division	Actualsize (µm)

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Experiment No. 17

DETERMINATION OF SIZE OF CALCIUM OXALATE CRYSTALS

Aim: To determine the size of Calcium oxalate crystals for the given drug sample.

Reference:

Practical Pharmacognosy, Khandelwal KR, Nirali Prakashan, 12th Edition, 2004. Pg. No: 41-44

Materials and Chemicals used: eyepiece micrometer, stage micrometer, starch iodine solution, and glycerin.

Discussion:

Formation of crystals: Protein metabolism and other metabolism give oxalic acid which is harmful to the plant. To remove the harmful effect of oxalic acid, the plant forms harmless calcium oxalate with calcium (obtained from soil).

Oxalic acid + Ca^{++} → Calcium oxalate

i) Deposited in different tissues, in different forms.

ii) Harmless to the plant.

iii) Doesn't take part in metabolism, hence called excretory product.

Calcium oxalate occurs in two different crystal systems.

- Tetragonal/ Quadratic system:

  Crystals contain three molecules of water for crystallization.

  Crystals have 3 axes, which are at right angles to each other. Two axes (lateral) are equaland the third axis may be smaller or larger than the lateral axes, called vertical or principal axes.Less common in plants.

- Monoclinic system:

  One molecule of water of crystallization

Crystals have 3 axes. One vertical/principal axes and two lateral axes are at rightangles to each other but only one axis is at right angle to the principal axes.
Most common in plants.

Due to irregular development of axes, adhesion, or twinning of crystals or belonging to differentcrystal systems, calcium oxalate crystals occur in different shapes. eg; Single crystal/ prism, Cluster crystals/ spheraphides, Rosette, Acicular crystal/ raphides, Microcrytal/ crystal sand/ microsphenoids crystal.

Procedure:

i) Take uniformly thin sections of the drug.

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

iii) Remove and treat them with 1% solution of silver nitrate in 15% hydrogen peroxidefor 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 the usual technique, mount the sections and observe under the microscope.

Calcium oxalate crystals appear black against red background. Dimension of crystal can be measured using camera lucida.

Sl.No	Magnified size	Actual size

$$\text{Actual size} = \frac{\text{Magnified size} \quad \text{x } 1000}{\text{Magnification}}$$

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Experiment No. 18

DETERMINATION OF STOMATAL NUMBER

Aim: To determine the stomata number of the given leaf drug.

Reference:

Practical Pharmacognosy, Khandelwal KR, Nirali Prakashan, 12th Edition, 2004. Pg. No: 41-44

Materials required: camera lucida, microscope, black paper, white pencil

Discussion:

A stoma is a minute epidermal opening present on aerial parts of the plant, with acentral spore and two kidney-shaped similar cells containing chloroplast known as guard cells. The primary function of stomata is gaseous exchange and the secondary function is transpiration.Generally, stomata are present in green parts of plants but are also present in the stems, flowers,and fruits. However, it is generally observed that stomata are present in dicot leaves. In some cases, they are present on the upper surface of leaves while in others on the lower surface only. Insome, stomata are present on both surfaces of leaves.

Types: Depending upon the type of guard cells and arrangement of subsidiary cells, stomata aredivided into:

1. *Paracytic stomata:* This type of stomata comprises two guard cells covered by twosubsidiary cells, the long axis of which is parallel to that of the stoma. Eg: coca, and senna leaves.
2. *Diacytic stomata:* The guard cells are covered by two subsidiary cells but the arrangement of subsidiary cells on guard cells is at a right angle to that of the stoma. Eg:peppermint.
3. *Anisocytic stomata:* The number of guard cells is two, but the guard cells are coveredby three subsidiary cells of which one is smaller than the other two.
4. *Anomocytic stomata:* Stoma is surrounded by a varying number of subsidiary cells resembling other epidermal cells. Eg: digitalis.
5. *Actinocytic stomata:* Two guard cells are surrounded by a circle of radiating subsidiary cells.

Procedure:

1. Fragments of leaves from the middle of the lamina is cleaned with chloral hydrate solution.
2. The leaves were boiled in a standard chloral hydrate solution for decolorisation.
3. The portion of the leaf between the midrib and origin was cut.
4. The portion is made transparent by scratching with a blade and mounted on the slide.
5. To this glycerin is added and a coverslip is placed.
6. Using black paper, the stomata and epidermis are neatly drawn with white pencil.
7. The traced stomata were counted and the stomata number was calculated using thegiven formula.

$$\text{Stomata number} = \frac{\text{total number of stomata}}{\text{area of field}} \times 1 \text{ sq. mm}$$

Observations:

Sl.No	Field no.	No. of stomata	Area of field	Stomata no.

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Experiment No. 19

DETERMINATION OF STOMATAL INDEX

Aim: To determine the stomatal index of a given leaf drug.

Reference:

Practical Pharmacognosy, Khandelwal KR, Nirali Prakashan, 12th Edition, 2004. Pg. No: 41-44

Materials Required: camera lucida, microscope, black paper, white pencil.

Discussion:

The stomatal index is the percentage proportion of the ultimate division of epidermis which have been converted to stomata. It is given by,

$$S.I = \frac{S}{E+S} \times 100$$

where S.I = stomatal index

E = epidermal cells per unit area

S = number of stomata per unit area.

While the stomatal number varies from the age of the leaf, the stomatal index is constant for a given species andmay be determined on either an entire or powdered sample. It is employed in IP 1964, to distinguish leaflets of Indian and Alexandrian senna.

Procedure:

1. Fragments of leaves from the middle of the lamina are cleaned with chloral hydrate solution.
2. The leaves were boiled in a standard chloral hydrate solution for decolorisation.
3. The portion of the leaf between the midrib and origin was cut.
4. The portion is made transparent by scratching with a blade and mounted on the slide.
   To this glycerin is added and a coverslip is placed.
5. Using black paper, the stomata and epidermis are neatly drawn with white pencil.
6. The traced stomata and epidermis were counted and the stomata index was calculatedusing the above formula.

Sl.No	Field no.	No. of stomata	No. of epidermal cells	Stomatal Index	Average

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Experiment No. 20

DETERMINATION OF VEIN-ISLET NUMBER

Aim: To determine the Vein-islet number for the given leaf drug.

Reference:

Practical Pharmacognosy, Khandelwal KR, Nirali Prakashan, 12th Edition, 2004. Pg. No: 41-44

Materials and Chemicals used: Stage micrometer, Microscope, and glycerin.

Discussion:

A vein islet is a small area of green tissue surrounded by the veinlets. The vein-islet number is the average number of vein-islets per square millimeter area 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:

1. Clear a piece of the leaf by boiling in chloral hydrate solution for about thirty minutes.
2. Arrange camera lucida and drawing board for making drawings to scale.
3. Place the stage micrometer on the microscope and using a 16 mm objective, draw a lineequivalent to 1 mm as seen through the microscope.
4. Construct a square on this line.
5. Move the paper so that the square is seen in the eyepiece, in the center of the field.
6. Place the slide with the cleared leaf.
7. Trace off the veins that are included within the square, completing the outlines of thoseislets that overlap two adjacent sides of the square.
8. Count the number of vein islets in the square millimeter. Where the islets are intersected by the sides of the square, include those on two adjacent sides and exclude those islets onthe other sides.

   Find the average number of vein islets from the four adjoining squares, to get the value for one sq. mm.

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Experiment No.21

DETERMINATION OF VEIN-TERMINATION NUMBER

Aim: To determine the Vein- termination number for the given leaf drug.

Reference:

Practical Pharmacognosy, Khandelwal KR, Nirali Prakashan, 12th Edition, 2004. P. No: 41-44

Materials and Chemicals used: Stage micrometer, Microscope, and glycerin.

Discussion: The vein termination number is defined as the number of veinlet terminations per sq.mm area of the leaf surface, midway between the midrib of the leaf and its margin. A vein termination is the ultimate free termination of a veinlet.

Procedure:

1. Clear a piece of the leaf by boiling in chloralhydrate solution for about thirty minutes.
2. Arrange camera lucida and drawing board for making drawings to scale.
3. Place the stage micrometer on the microscope and using a 16 mm objective, draw a lineequivalent to 1 mm as seen through the microscope.
4. Construct a square on this line.
5. Move the paper so that the square is seen in the eyepiece, in the center of the field.
6. Place the slide with the cleared leaf.
7. Trace off the veins that are included within the square, completing the outlines of thoseislets that overlap two adjacent sides of the square.
8. Count the number of veinlet terminations in the square millimeter.
9. Find the average number of vein termination numbers from the four adjoining squares, toget the value for one sq. mm.

Report:

Experiment No.22

DETERMINATION OF PALISADE RATIO

Aim: To determine the Palisade Ratio for the given leaf drug.

Reference:

Practical Pharmacognosy, Khandelwal KR, Nirali Prakashan, 12th Edition, 2004. Pg. No: 41-44

Materials and Chemicals used: Stage micrometer, Microscope, and glycerin.

Discussion: 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:

1. Clear a piece of the leaf by boiling in chloralhydrate solution.
2. Arrange the camera lucida and drawing board for making drawings.
3. Using the 4 mm objective, trace off the outlines of four cells of the epidermis.
4. Then, focus down to the 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 bythe epidermal walls.
5. Count the palisade cells under the four epidermal cells.
6. Calculate the average number of cells beneath a single epidermal cell; this figure is thePalisade ratio.
7. Repeat the determination for five groups of four epidermal cells from different parts ofthe leaf. Take the average of the results for the five groups.

Report:

Vision and Mission of the Institution

Vision

The East Point College of Pharmacy aspires to be a globally acclaimed institution, **recognized** for **excellence in** pharmaceutical education, research and nurturing students for **holistic development.**

Mission

M1 Create pharmacy graduates through **quality education**

M2 Promote innovation, **creativity**, and excellence **in teaching**, learning, and **research**

M3 **Inspire** integrity, teamwork, critical thinking**, personal** development, and ethics in **students** and lay **the** foundation for lifelong learning

M4 **Serve** the **healthcare, technological, scientific**, and **economic** needs of then **society**.