ST JOSEPH'S COLLEGE OF PHARMACY

(Affiliated to KUHS and approved by, PCI and AICTE)

PHARMACOGNOSY AND PHYTOCHEMISTRY - II

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INTRODUCTION

STUDY OF COMPOUND MICROSCOPE

AIM

To study the different partsand handling of a compound microscope.

Parts of the compound microscope:

1. The support system (The frame work)
2. The illumination system
3. The magnification system
4. The adjustment system

1. THE SUPPORT SYSTEM

The support system is the framework of the microscope that holds its components.

- **Base**

  The base supports the microscope and holds it components.

- **Pillars**

  Two upright pillars project upward from the base and the handle of the microscope hinged to the pillars.

- **Handle (arm)**

  The arm supports the magnifying and adjusting system. It is also the handle by which the microscope can be carried without damaging the delicate parts.

- **Body tube**

  The body tube is the part through which, the passes to the eye piece.

- **Stage**

  a) Fixed stage (Platform)

  It is a horizontal platform, on which the object being observed is placed. There is an aperture on its centrethrough which the converging cone of light passes.

  b) Mechanical stage

  Most microscopes have a mechanical stage, which makes it easy to manipulatethe object being observed. It is calibrated and fitted on the fixed stage. There is a spring-mounted clip to hold the slide or the counting chamber in position, and two screws for moving the object transversely, or forward and backwards.

- **Nose piece**

The fixed nosepiece is attached to the lower end of the body tube and the revolving nosepiece is mounted under it. The revolving nose piece carries the objective lenses of different magnifying powers.

2. THE ILLUMINATION SYSTEM

The illumination system of a compound microscope consists of a light source, condenser and iris diaphragm.

- **The light source**

This may be internal or external

a) Internal source

In the microscope there is a built-in light source with an electric lamp, which provides better control of illumination.

b) External source

These microscopes use an external source of light. This can be form an electric lamp housed in a lampbox with a window, orfrom the sun.

- **Mirror**

The mirror is located at the base of the microscope. The rays of light are reflected by the mirror towards the object. It has two surfaces, a plane mirror and a concave mirror. The plane mirror is used for doing with oil-immersion objective whereas the concave mirror is used for low and high-power objectives.

- **Condenser**

The condenser focuses the rays of light reflected from the mirror onto the object under examination. It is mounted below the stage of the microscope with a rack and pinion mechanism for adjusting its focus.

- **Iris Diaphragm**

The Iris diaphragm regulates the amount of light that passes though the materials under observation. It is located at the bottom of condenser.

3. THE MAGNIFICATION SYSTEM

- **Eye piece**

It fits into the top of the body tube. There are different types of eyepieces of magnification powers of like 10x, 15x, 25x etc…

- **Objectives**

There are three objectives and they are screwed into the revolving nosepiece in a compound microscope.

a) Low power objective(10X)

This lens magnifies the image 10 times. It provides the maximum field view.

b) High power objective (45X)

It magnifies the image 45 times.

c) Oil immersion objective (100X)

It magnifies the image 100 times. It requires a special type of oil called immersion oil, which is placed between the objective and the slide.

4. THE ADJUSTING SYSTEM

The adjusting system consists of two adjustments, the coarse adjustment and the fine adjustment. The coarse adjustment is used to obtain an appropriate focus whereas the fine adjustment is used to obtain an exact focus of the object after the prior coarse adjustment.

Calculation of total magnification:

Although the light passed through a complex system of lenses, the total magnification can be calculated by multiplying the magnification power of the lens to that of the eyepiece.

E.g. If the eyepiece is 10x, the total magnification power of low power objective with= 10x magnification is

10X x 10X = 100 times.

FUNDAMENTAL CONCEPTS OF PLANT ANATOMY

The living things are composed of essential living substances. Protoplasm, which is decribed as the physical basis of life. Bits of protoplasm occur in small amount of units known as cells. The term cell was coined by an English man, Robert Hook in 1665. It is derived from Latin word 'cellular' means a small compartment. A number of cells united to perform a particular junction constitute a tissue. The tissue is aggregated to form tissue system and tissue systems together form an organ.

A well-accepted classification of system is as follows:

Dermal tissue system, Ground tissue system and Vascular tissue system

DERMAL TISSUE SYSTEM

Epidermis is the outermost layer of the plant consisting normally of a single layer of flattened cells. The walls may be straight, wavy or bedded and often covered with a layer of cuticle made up to cutin.

STOMATA: Stomata are minute epidermal openings present an arrival parts of the plant, with the following characteristics.

1)A central pore

2)Two kidney shaped similar cells containing chloroplast known as guard cells and varying number of subsidiary cells (epidermal) covering the guard cells.

Function:-

*The primary function is gaseous exchange

*secondary function is transpiration.

TYPES OF STOMATA:

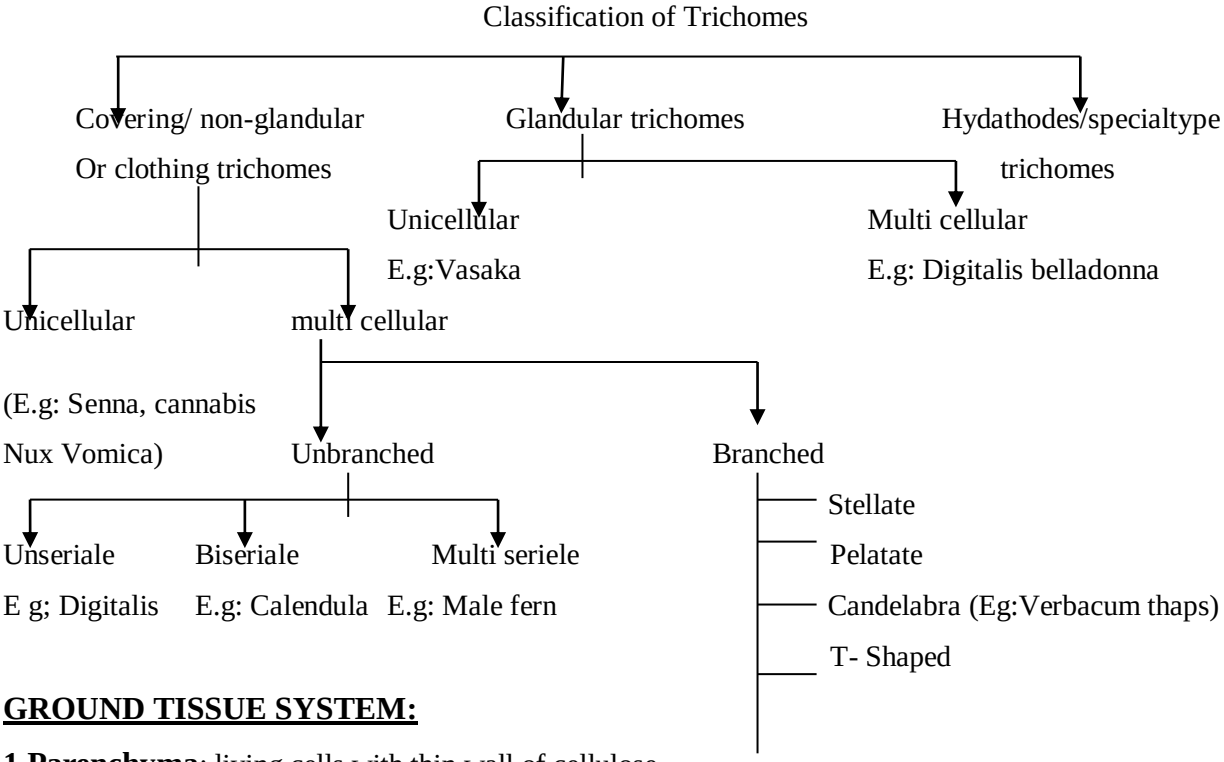
Depending upon the type of the guard cell and arrangement of subsidiary cells, stomata are divided into four types:

1). Moss type 2). Gymnospermous Type 3).Gramineous & 4). Dicotyledonous Type

In this type fourth type of stomata are of diagnostic significant, Dicotyledonous stomata are classified into following types depending upon form and arrangement of subsidiary cells.

1. **Paracytic or rubiaceous or parallel-celled stomata**: This type of stomata comprises to guard cells covered by two subsidiary cells, the axis of which are parallel to that of stomata. E.g. coca, senna .
2. **Diacytic, caryophyllaceous, or cross-celled stomata**: The guard cells are covered by two subsidiary cells but the arrangement of subsidiary cells is at right angle to that of stomata.
   E.g pepermint and Vasaka..
3. **Anisocytic or cruciferous or unequal celled stomata**: The number of guard cells is two, but the guard cells covered by three subsidiary cells of which one markedly smaller than the other two. E.g. Belladonna, Datura and Stramonium
4. **Anomocytic or ranumculaceous or irregular celled stomata**: In this type, stomata is surrounded by varying number of subsidiary cells. E.g. Digitalis
   In it, one more type of stomata known as Actinocytic stomata.
5. **Actinocytic stomata or radiate celled stomata**: The two guard cells are surrounded by radiating subsidiary cells

TRICHOMES: are elongated tubular outgrowths of an epidermal cell is termed as trichomes.



GROUND TISSUE SYSTEM:

1.Parenchyma: living cells with thin wall of cellulose.

Isodiametric with intercellular spaces.
Function: - absorption and storage of food, water, gases and photosynthesis.
Site:-present in all soft parts of cortex of root, cortex and pith of stem and mesophyll of a leaf.
Types: - Chlorenchyma: parenchyma-containing chloroplast
Aerenchyma, parenchyma containing air spaces
2.**Collenchyma:** living cells, resemble parenchyma
Thickened walls, deposited with cellular
Pectin: Due to thick wall air space tiny on absent
Function: - mechanical strength
Site: - occurs mostly in cortical region of stem bark, leaf but not in root.
3.Sclerenchyma: is a hardtissue with thickened lignified walls.
Function: mechanical strength
Site: found in all hardwood parts of plant whenever mechanical strength is needed
Type: - Scleride or stone cells: iso diametrical, irregular, lignified, pitted or stratified cells, occurring simply or in hand with varying lumen abundant in cortex
Sclerenchymatous fibers: narrow, often elongated, lignified cells and pointed end

VASCULAR SYSTEM:

The xylem, phloem, cambium, parenchyma and sclenchyma are arranged together to form a vascular bundle. There are different types of vascular bundles depending on the arrangement of conducting tissues. These types can prove to be diagnostic characters of individual drugs.

RADIAL VASCULAR BUNDLE:

The xylem and phloem are observed in separate patches placed on the alternate radial on the axis. Xylem is exarch meaning that protoxylem is pointing towards periphery.
e.g; Podophyllum root
Conjoint vascular bundles:
This is a type in which xylem and phloem are present in the same vascular bundle.
a**). Collateral**: In this case, xylem and phloem are placed on trhe same radius, xylem lies towards the center and phloem towards periphery. These are two types of vascular bundles.
i). **Open collateral**: When cambium is placed in between xylem and phloem occur in dicotyledonous plants
ii). **Closed collateral**: When no cambium is present in between xylem and phloem. This type is found in monocotyledonous plants.
b**)bicollateral** : in this type, the xylem is in the middle and phloem and cambium lie on either side of the xylem

CONCENTRIC VASCULAR BUNDLES

Vascular bundles, where there is no cambium
a). either the xylem lies in the center surrounded by phloem (centroxylic)
b). phloem in the center surrounded by xylem (centrophloric)

MORPHOLOGICAL STUDY OF CRUDE DRUGS

Exp.no:1 Date:

CINNAMON

SYNONYM: Ceylon Cinnamon

BIOLOGICAL SOURCE: It consists of dried inner bark of shoots of Coppiced trees of ***Cinnamomum Zeylanicum***

FAMILY: Lauraceae

MORPHOLOGY:

- Colour : Outer surface is yellowish brown, inner surface is darker.
- Odour : aromatic and fragrant
- Taste : aromatic, sweet and warm
- Shape : Single, double or compound quills Fracture: splintery
- Size : Varying length, 6-10mm (d), 0.5mm (thicknesss)
- Surface : outer surface dull yellowish brown and inner surface darker in colour Bark is free of cork, closely packed compound quills.
- Fracture: Short and splintery
- Condition : dry

CHEMICAL CONSTITUENTS

- Volatile oil (0.5-1%)- Cinnamic aldehyde, eugenol, safrol Terpinene, pinene, phellandrene, linalool, cumin aldehyde.
- Starch, Fixed oil, Tannins-Phlobatannins, Calcium oxalate and mucilage.

USES:

- ✓ carminative and aromatic, mild astringent, powerful germicide and flavouring agent

REPORT:

REFERENCE:

Study of crude drugs by M.A Iyengar, 14th edition. Pageno: 42-43

Exp.no:2 Date:

CORIANDER

SYNONYM: Coriander fruit

BIOLOGICAL SOURCE: it consists of dried ripe fruits of ***Coriandrum sativum***

FAMILY: Umbelliferae

MORPHOLOGY:

- Color : Straw yellow or brownish yellow
- Odour : aromatic
- Taste : spicy and characteristic
- Shape : sub globular or oval with 5 calyx teeth. Short stylopod at the apex and short stalk (pedicel) at the base.
- Size : 2.3-4.3mm (d)
- Type : cremocarp. A cremocarp is made up of 2 hemispherical mericarps. Each mericarp has 2 surfaces. A flat surface is called commissural surface and rounded surface called the dorsal surface. Dorsal surface of each mericarp shows 5 primary and 4 secondary ridges. The primary ridges are wavy, inconspicuous and alternate with prominent straight secondary ridges. The commissural surface shows the carpophore, a thin elongated structure attached to the pedicel below and continued up to the upper end of mericarp and holds both the mericarps together.

CHEMICAL CONSTITUENTS:

- Volatile oil (.2-1%) –coriandrol(linalool), Terpinene (20%), geraniol, borneol, citronellol. Fixed oil and proteins

USES:

- ✓ carminative and aromatic, stimulant and flavouring agent

REPORT:

REFERENCE:

Study of crude drugs by M.A Iyengar, 14th edition. Page no: 48-49.

Exp.no:3 Date:

CLOVE

SYNONYM: Clove bud

BIOLOGICAL SOURCE: It consists of dried flower buds of ***Eugenia caryophyllus***

FAMILY: Myrtaceae

MORPHOLOGY:

- Color : Dark brown
- Odour : Aromatic, strong and characteristic
- Taste : Spicy, pungent and characteristic
- Shape : Spherical head and a sub cylindrical hypanthium, tapering at the lowering end
- Type : Actinomorphic, bisexual, epigynous.
- Calyx : Polysepalous, 4 hard and thick sepal with oil glands
- Corolla : Polypetalous, 4 petals, imbricate, enclosed the stamens and form the head of bud
- Androecium: Numerous stamens, free, introrse
- Gynoecium: Bilocular, inferior ovary with many ovules and axile placentation
- Style : Single, erect

CHEMICAL CONSTITUENTS:

- Volatile oil (3-8%)- Eugenol, isoeugenol, methyl and dimethyl furfural, α and β caryophyllene. Tannins.

USES:

- ✓ carminative and aromatic, stimulant, antiseptic, dental analgesic and flavouring agent

REPORT:

REFERENCE:

Study of crude drugs by M.A Iyengar, 14th edition. Page no: 44

Exp.no:4 Date:

FENNEL

SYNONYM: Fennel fruit

BIOLOGICAL SOURCE: It consists of dried ripe fruits of ***Foeniculum vulgare***
FAMILY: Umbelliferae

MORPHOLOGY:

- Color : greenish or yellow brown
- Odour : aromatic and characteristic
- Taste : aromatic and characteristic
- Shape : Straight or slightly curved, oblong, laterally compressed. Tapering towards the base and apex. At the apex, a short curved bifid structure called as stylopod is present. A thin pedicel is seen at the base.
- Size : 5-10mm (l), 2-4mm(w)
- Type : cremocarp. consisting of two mericarps connected by a central stalk called carpophore, with a short stylopod at the apex and a long pedicel. Each mericarp has five primary ridges, which are prominent, straight and pale coloured.
  Each mericap has 2 surfaces. The dorsal and commissural surface. Dorsal surface is glabrous, with 5 straight prominent primary ridges and stylopod at the apex. Commisural surface is flat and shows the carpophore, which holds the 2 mericarps together.

CHEMICAL CONSTITUENTS:

- Volatile oil (4-6%) Anethole (50-60%), fenchone (10%) Fixed oil (12-18 %) and proteins

USES:

- ✓ carminative and aromatic, respiratory stimulant and flavouring agent

REPORT:

REFERENCE:

Study of crude drugs by M.A Iyengar, 14th edition. Page no: 58-61

Exp.no:5 Date:

CINCHONA

SYNONYMS: Jesuit's bark, Peruvian bark

BIOLOGICAL SOURCE: It consist of dried bark of cultivated trees of ***Cinchona officinalis***, ***Cinchona calisaya***, ***Cinchona ledgeriana,Cinchona succirubra*** *belonging* to the family **Rubiaceae.**

MORPHOLOGY:

Colour : Yellowish brown or reddish brown
Odour : Characteristic
Taste : Bitter and astringent
Shape : Flat, channeled, single quill or double quill
Size : Varies
Surface: Outer surface is rough due to longitudinal and transverse ridges, fissures and wrinkles Inner surface is dark reddish brown or pale yellowish brown with longitudinal striations.

- Fracture: Short and fibrous

CHEMICAL CONSTITUENTS:

Alkaloids (6-7%) – Quinoline alkaloids. quinine, quinidine, cinchonine, cinchonidine. Quinovin – a bitter glycoside, which on hydrolysis yields quinovic acid and quinovose- sugar derivative. Tannins – phlobatannins

USES: Treatment of malarial fever (Quinine), preparation of tonic water, quinidine is a cardiac depressant.

REPORT

REFERENCE

Study of crude drugs by M.A Iyengar, 14th edition. Page no: 40-42

Exp.no:6 Date:

EPHEDRA

SYNONYM: Ma- Huang

BIOLOGICAL SOURCE: It consist of dried young stems of ***Ephedra gerardiana***, ***Ephedra nebrodensis***

FAMILY: **Ephedraceae (Gnetaceae).**

MORPHOLOGY:

Color : Older plants are straw colored and younger grayish green.
Odour : Fresh stems are aromatic and have no odour when dry.
Taste : Bitter and astringent.
Shape : Stems are cylindrical and much branched. Nodes and internodes are seen clearly.
Size : Varies
Surface : Main stem is woody and young twigs are slender. Both surface shows striations
Leaves : Reduced to minute 2 toothed sheets
Fracture : Fibrous in the cortical region whereas pith shows brown powdery mass.

CHEMICAL CONSTITUENTS:

Alkaloids- aminoalkaloids, ephedrine (30-90%), pseudo ephedrine, nor ephedrine, dimethyl ephedrine. It also contains macrocyclic alkaloids like ephedrodienes.

USES:

Ephedrine is used for asthmatic condition and hay fever. It produces lasting increase of blood pressure, causes mydriasis and diminishes hyperemia.

REPORT:

REFERENCE

Study of crude drugs by M.A Iyengar, 14th edition. Page no: 54-55

Exp.no:7 Date:

SENNA

SYNONYM: Folia senna

BIOLOGICAL SOURCE: It consists of the dried leaflets of ***Cassia angustifolia*** (Alexandrian senna) and ***Cassiaacutifolia***(Indian senna) belonging to the family **Leguminosae.**

MORPHOLOGY:

Colour : Pale green or yellowish green
Odour : None
Taste : Mucilaginous, bitter and characteristic.
Size : 7-8mm width, 2.5-60 cm in length.
Shape : Leaves are lanceolate or ovate, entire margin, apex is mucronate(acute with spine at the top). Bases of the leaflets are asymmetrical with transverse lines, more prominent on lower surface. Small petiole. Pinnately reticulated venation
Surface : Glabrous
Condition : Dry
Type : Leaflet of paripinnately compound leaf

CHEMICAL CONSTITUENTS:

Glycosides- Anthraquinone glycosides-Sennoside A, Sennoside B, Sennoside C, Sennoside D, rhein, aloe emodin, palmidin, kaempferol, isorhamnetin, phytosterol, myricyl alcohol, salicylic acid, chrysophanic acid etc

USES:

Purgative

REPORT:

REFERENCE

Study of crude drugs by M.A Iyengar, 14th edition. Page no: 110-114

HISTOLOGICAL STUDY OF CRUDE DRUGS

Exp.no:8 Date:

TRANSVERSE SECTION OF CORIANDER

AIM

To carry out transverse section of Coriander fruit

MATERIALS AND REAGENTS

Crude drug, conc.Hcl, Phloroglucinol, glass slide, coverslip, test tube

TRANSVERSE SECTION

I.PERICARP

Epicarp: Consist of single row of small, but thick walled cells. Few cells contain calcium oxalate crystals, covered by smooth cuticle.

Mesocarp: Divided into 3 layers

i) Outer layer contains loosely arranged tangentially elongated non lignified parenchyma. Lacunae at the dorsal side

ii) Middle layer contain fusiform lignified sclerchymatous cells. Sclerchymatous cells are of 2 types. a) Tangentially elongated sclerchyma b) Longitudinally elongated sclerchyma. 5 vascular bundles at the dorsal side present above longitudinally elongated sclerenchyma. 2 vittae at ventral side, no vittae at dorsal side

iii) Inner layer contain large, irreregular , hexagonal, lignified parenchyma.

Endocarp: Inner pericarp shows typical parquetry arrangement of the cells

II.TESTA: single layer of yellowish cells.

III. ENDOSPERM: Thick walled, polygonal colorless parenchyma containing fixed oils and aleurone grains

REPORT

REFERENCE

Anatomy of crude drugs by M.A Iyengar and S.G.K Nayak, 11th edition. Page no:64

Exp.no:9 Date:

TRANSVERSE SECTION OF FENNEL

AIM

To carry out transverse section of Fennel fruit

MATERIALS AND REAGENTS

Crude drug, conc.Hcl, Phloroglucinol, glass slide, coverslip, test tube

TRANSVERSE SECTION

T.S of a mericarp shows two prominent surfaces – the commissural and the dorsal. The commissural surface is flat with 2 pronounced ridges (primary ridges) and carpophore in the middle. The dorsal surface is also ridged (3 ridges). Thus altogether a mericarp shows 5 primary ridges.

Mericarp can broadly be divided into pericarp, testa and endosperm.

I.PERICARP

The epicarp or the exocarp of the pericarp, surrounding the entire mericarp consists of a layer of polygonal, tangentially elongated cells with a smooth cuticle.

Mesocarp is made of parenchyma. Bicollateral vascular bundles appear in the mesocarp below the primary ridges. Surrounding the vascular bundles, reticulate and lignified parenchyma appear. Besides, yellowish brown and elliptical vittae (schizogenous oil ducts) are seen ,4 on the dorsal surface and 2 on the commissural surface, which are also important feature of the mesocarp.

Endocarp: Another typical umbelliferous feature is the presence of parquetry arrangement (groups of parallel cells arranged in different directions) of cells of endocarp which however is seen as a single layer between mesocarp and testa.

II. TESTA: single layer of yellowish cells.

III. ENDOSPERM: Thick walled, polygonal colourless parenchyma containing oil globules and aleurone grains. A crescent shaped embryo is seen in the sections passing through the apical region of mesocarp. Raphae, a ridge of vascular strand, appears in the middle of commissural surface just in front of capophore, as the ovule is anatropous (inverted) here.

REPORT

REFERENCE

Anatomy of crude drugs by M.A Iyengar and S.G.K Nayak, 11th edition. Page no:12

Exp.no:10 Date:

TRANSVERSE SECTION OF CLOVE

AIM

To carry out the transverse section of Clove bud

MATERIALS AND REAGENTS

Crude drug, conc.Hcl, Phloroglucinol, alcoholic KOH, glass slide, coverslip, test tube

TRANSVERSE SECTION

T.S of the hypanthium (below the ovary) shows a

I. Epidermis: single layer cells with straight walls as the outer layer; it has a very thick cuticle. Epidermal layer gets intercepted by Ranunculaceous type of stomata.

II. Cortexhaving 3 distinct zones.

(a)The peripheral region of 2-3 layers of big ellipsoidal, schizolysigenous oil glands, embedded in the radially elongated parenchymatous cells.

(b)The middle region consisting of 2-3 layers of bicollateral vascular bundles associated with a few pericyclic fibres embeded in thick walled parenchyma and

(c)The inner region made of loosely arranged aerenchyma. Aerenchyma is a lacunous region, consisting of air spaces separated by lamella of one cell thickness, and enclosing central columella.

III. Columella forms the central cylinder containing thick walled parenchyma with a ring of bicollateral vascular bundles (15-17) towards the periphery of the cylinder. Numerous cluster crystals (sphaeraphides) of calcium oxalate (5-25µ in diameter) are seen scattered throughout the columella and to a certain extent in the middle cortical zone.

T.S of hypanthium in the region of ovary shows ovarian tissue instead of central columella. Ovary consists of many ovules attached to an axile placenta and surrounded by the wall which is very strongly thickened and cellulosic in nature.

REPORT

REFERENCE

Anatomy of crude drugs by M.A Iyengar and S.G.K Nayak, 11th edition. Page no: 58

Exp.no:11 Date:

TRANSVERSE SECTION OF CINNAMON

AIM

To carry out transverse section of Cinnamon bark

MATERIALS AND REAGENTS

Crude drug, conc.Hcl, Phloroglucinol, alcoholic KOH, glass slide, coverslip, test tube

TRANSVERSE SECTION

Transverse section (TS) does not show any cork cell as it is the inner bark.

I. **PERICYCLE (Stone cell layer)** : The outermost layer is consisting of about 3-4 layers of sclerenchymatous cells or sclerides, with small groups of lignified **pericyclic fibres** occurring at intervals on the outer side.

Sclerides have thickened lignified walls with well-defined pit-canals. The thickening on the outer wall is often less pronounced than on the radial and inner tangential walls giving them characteristic U-shaped appearance.

II. **SECONDARY PHLOEM:** It consists of phloem parenchyma, phloem fibres and medullary rays.

Phloem parenchyma contains numerous starch grains and small acicular crystals of calcium oxalate.

Oil cells are scattered in phloem parenchyma; cells are axially elongated, 2-3 times the diameter of the phloem fibres.

Mucilage cells can be identified after staining with ruthenium red, shows pink or red colour

Medullary rays are composed of radially elongated cells, 7-14 cells high, uni or bi-seriate near cambium and widening slightly as they approach the pericycle.

REPORT

REFERENCE

Practical Pharmacognosy by Khandelwal K.R , Page no: 104

Exp.no:12 Date:

TRANSVERSE SECTION OF EPHEDRA

AIM

To carry out transverse section of Ephedra stem

MATERIALS AND REAGENTS

Crude drug, conc.Hcl, Phloroglucinol, alcoholic KOH, glass slide, coverslip, test tube

TRANSVERSE SECTION

T S of the stem is more or less circular. The margin is prominently wavy due to the ridges which number from 30 to 40. Following are number of tissues from the periphery to the centre.

EPIDERMIS: single row of rectangular cells with a very thick and smooth cuticle.

Distinct stomata of sunken type are restricted obviously to furrows.

CORTEX: outer most 2-3 layers of cortical parenchyma appearing like loosely arrangedpalisade cells contain chloroplasts. Groups of un-lignified fibers appearing like a bunch of grapes occur below the ridges where no palisades like cells are found. Scattered lignified either isolated or a groups of 2 to 4occur in the inner layers of oval cortical parenchyma. The latter also show chloroplast.

VASCULAR BUNDLES: around 10, collateral, conjoint, open and arranged in a ring.Phloem is towards the outer side and distinct. Groups of lignified pericyclic fibers crown the phloem on its outer side. Cambium is indistinguishable. Well-developed xylem consists of vessels, tracheids, fibrotracheids and parenchyma. Xylem in mature stem shows a well-developed continuous band.

PITH: is large and is made of thin walled, lignified, big polygonal parenchyma withintercellular spaces. Some cells of the pith contain brownish matter.

REPORT

REFERENCE

Anatomy of crude drugs by M.A Iyengar and S.G.K Nayak, 11th edition. Page no:76

Exp.no:13 Date:

TRANSVERSE SECTION OF CINCHONA

AIM

To carry out transverse section of Cinchona bark

MATERIALS AND REAGENTS

Crude drug, conc.Hcl, Phloroglucinol, alcoholic KOH, glass slide, coverslip, test tube

TRANSVERSE SECTION

TS show a well-developed periderm, a wide cortex and a large secondary phloem.

PERIDERM:

Cork- consists of several layers of radially arranged rows of thin walled cells with dark brown contents. The cork cells are impregnated with suberin.

Phellogen- 2 to 3 layers of thin walled rectangular cells without any cell contents.

Phelloderm- 6 to 8 layers of thin walled rectangular cells without any cell contents, like cork, they are arranged at times in radial rows.

CORTEX:

Several layers of thin walled and tangentially elongated cells containing yellowish brown matter. Some of the cortical cells are filled with microsphenoidal crystals of calcium oxalate and the rest with minute starch grains. Besides, isolated secretion cells are also found in the cortical parenchyma.

SECONDARY PHLOEM:

This region comprises of phloem parenchyma, phloem fibres and medullary rays. Phloem fibres, characteristics of cinchona bark occur intermingled with phloem parenchyma and in between medullary rays. Fibres are numerous mostly isolated, at times in groups of 2 or 3, rounded to oval, in various sizes, yellow in colour, thick walled, and strongly lignified with a small lumen and stratification.

MEDULLARY RAYS: Transverse radially the phloem parenchyma; 1 to 3 cells wide, extend up to cortex, cells radially elongated and certain starch grains.

REPORT

REFERENCE

Anatomy of crude drugs by M.A Iyengar and S.G.K Nayak, 11th edition. Page no:36

Exp.no:14 Date:

TRANSVERSE SECTION OF SENNA

AIM

To carry out the transverse section of Senna leaf

MATERIALS AND REAGENTS

Crude drug, conc.Hcl, Phloroglucinol, alcoholic KOH, glass slide, coverslip, test tube

TRANSVERSE SECTION

T.S of the leaflet shows isobilateral nature. It is divided into lamina and midrib

I. LAMINA

a) **Upper epidermis:** Single layered with polygonal cells covered on the outer side by thick warty cuticle. Some epidermal cells contain mucilage. Only covering trichomes emerge from the epidermal layer. Paracytic or rubiaceous stomata are seen at the regular intervals.

b) **Mesophyll:** It is differentiated into palisade abd spongy parenchyma. Being a isobilateral leaf, palisade is further differentiated into upper and lower palisade.

- **i) Upper palisade:** single layered, compact with elongated, narrow, columnar cells and this continues also over the midrib region.
- **ii) Spongy parenchyma:** Thin walled, loosely arranged between upper and lower palisade. Spheraphides are present.
- **iii) Lower palisade:** It is restricted to laminar region only. Cells are smaller than those of upper palisade, loosely arranged and their walls are wavy

c) **Lower epidermis:** Similar to upper epidermis

II. MIDRIB

The epidermal layers are continuous over the midrib. Cells of the lower epidermis are small with thick cuticle. The cells of the upper palisade which appears below the upper epidermis in the midrib region are relatively smaller. Instead of lower palisade, a patch of collenchyma is seen.

Parenchymatous layer containing calcium oxalate prism present at the dorsal and ventral side.

Collateral vascular bundle is prominent occupying the central portion of the midrib. Xylem is towards the ventral surface and phloem towards the dorsal surface. The vascular bundle is covered on the both side by patches of sclerenchymatous fibres.

REPORT

REFERENCE

Anatomy of crude drugs by M.A Iyengar and S.G.K Nayak, 11th edition. Page no:50

POWDER MICROSCOPY OF CRUDE DRUGS

Exp.no:15 Date:

POWDER MICROSCOPY OF CORIANDER

AIM:

To carry out the powder microscopy of given crude drug

MATERIALS AND REAGENTS

Powdered drug, conc. Hcl, phloroglucinol, glass slide, cover slip

BIOLOGICAL SOURCE: it consists of dried ripe fruits of ***Coriandrum sativum***

FAMILY: Umbelliferae

IDENTIFYING CHARACTERS

- Color : Straw yellow or brownish yellow
- Odour : aromatic
- Taste : spicy and characteristic

1. **Sclerenchymatous layer** : Groups of fusiform fibres of Sclerenchyma running wavy and at times crossing with each other or with thin walled lignified cells of the mesocarp.
2. **Endocarp:** fragments of parquetry arrangement of thin walled lignified cells with the polygonal cells of the mesocarp.
3. **Vittae:** few brown fragments of vittae
4. **Endosperm:** fragments of endosperm with aleurone grains and oil globules

REPORT

REFERENCE

Pharmacognosy of powdered crude drugs by M.A Iyengar, 9th edition, Page no: 40

Exp.no:16 Date:

POWDER MICROSCOPY OF FENNEL

AIM:

To carry out the powder microscopy of given crude drug

MATERIALS AND REAGENTS

Powdered drug, conc. Hcl, phloroglucinol, glass slide, cover slip

BIOLOGICAL SOURCE: It consists of dried ripe fruits of ***Foeniculum vulgare***

FAMILY: Umbelliferae

IDENTIFYING CHARACTERS

1. **Mesocarp:** lignified and reticulate nature of parenchyma
2. **Endocarp:** cells showing parquetry arrangement
3. **Endosperm:** polyhedral, thick walled cells containing aleurone grains, minute calcium oxalate crystals and oil globules.
4. **Vittae:** many in the form of yellowish brown fragments.

REPORT

REFERENCE

Pharmacognosy of powdered crude drugs by M.A Iyengar, 9th edition, Page no:42

Exp.no:17 Date:

POWDER MICROSCOPY OF CLOVE

AIM:

To carry out the powder microscopy of given crude drug

MATERIALS AND REAGENTS

Powdered drug, conc. Hcl, phloroglucinol, glass slide, cover slip

BIOLOGICAL SOURCE: It consists of dried flower buds of ***Eugenia caryophyllus***

FAMILY: Myrtaceae

IDENTIFYING CHARACTERS

- Color : Dark brown
- Odour : aromatic, strong and characteristic
- Taste : spicy, pungent and characteristic

1. **Oil glands:** fragments of parenchyma containing entire or a portion of oil glands.
2. **Aerenchyma:** portion of loose parenchyma
3. **Fibres:** sclerenchymatous fibres associated with parenchymatous cell
4. **Sclereids:** from the stalk, oval to sub-rectangular, thickened walls having numerous simple or branched pits.
5. **Calcium oxalate:** in the form of cluster crystal

REPORT

REFERENCE

Pharmacognosy of powdered crude drugs by M.A Iyengar, 9th edition, Page no: 32

Exp.no:18 Date:

POWDER MICROSCOPY OF CINNAMON

AIM:

To carry out the powder microscopy of given crude drug

MATERIALS AND REAGENTS

Powdered drug, conc. Hcl, phloroglucinol, glass slide, cover slip

BIOLOGICAL SOURCE: It consists of dried inner bark of shoots of Coppiced trees of ***Cinnamomum Zeylanicum***

FAMILY: Lauraceae

IDENTIFYING CHARACTERS

- Colour : Outer surface is yellowish brown; inner surface is darker.
- Odour : aromatic and fragrant
- Taste : aromatic, sweet and warm

1. **Fibres:** isolated bast fibres measure 250-600µ (l) and 15-30µ (b)
2. **Stone cells:**almost U-shaped as one wall is thinner than the other three
3. **Starch grains:** abundant starch which doesnot measure more than 10µ
4. **Calcium oxalate crystals:** presence of small acicular raphides in the parenchyma.
5. **Oil cells:** big and isolated.

REPORT

REFERENCE

Pharmacognosy of powdered crude drugs by M.A Iyengar, 9th edition, Page no:15

Exp.no:19 Date:

POWDER MICROSCOPY OF EPHEDRA

AIM:

To carry out the powder microscopy of given crude drug

MATERIALS AND REAGENTS

Powdered drug, conc. Hcl, phloroglucinol, glass slide, cover slip

BIOLOGICAL SOURCE: It consist of dried young stems of ***Ephedra gerardiana***,***Ephedra nebrodensis***

FAMILY:**Ephedraceae(Gnetaceae).**

IDENTIFYING CHARACTERS

- Color : Older plants are straw colored and younger grayish green.
- Odour : Fresh stems are aromatic and have no odour when dry.
- Taste : Bitter and astringent.

1. **Epidermis**: fragments of epidermal cells whose outer walls are ridged.
2. **Fibres**: both lignified and non-lignified fibres of uniform thickness, long, slender and cylindrical (like glass rods) appear either entire or in fragments.
3. **Wood elements**: consisting of only tracheids with bordered pits.
4. **Brownish matter**: abundant and possess regular shape and form. They originate from pith.

REPORT

REFERENCE

Pharmacognosy Of Powdered Crude Drugs – M.A.Iyengar, 9th edition, page no: 53.

Exp.no:20 Date:

POWDER MICROSCOPY OF CINCHONA

AIM:

To carry out the powder microscopy of given crude drug

MATERIALS AND REAGENTS

Powdered drug, conc. Hcl, phloroglucinol, glass slide, cover slip

BIOLOGICAL SOURCE: It consist of dried bark of cultivated trees of ***Cinchona officinalis***, ***Cinchona calisaya***, ***Cinchona ledgeriana,Cinchona succirubra*** or hybrids of either of the last 2 species with either of the 2 belonging to the family **Rubiaceae**

IDENTIFYING CHARACTERS

- Colour : Yellowish brown or reddish brown
- Odour : Characteristic
- Taste : Bitter and astringent

1. **Fibers:** phloem fibers or bast fibers, numerous either entire or in fragments, spindle shaped, yellowish, thick walled, strongly lignified, porous walls having simple pores or branched pores and measure around 500-1350 microns in length and 50-130 microns in width.
2. **Cork:** typical thin walled cork cells which appear reddish brown in color.
3. **Calcium oxalate crystals:** microsphenoidal crystals in dark colored parenchyma.
4. **Starch grains:** minute, both simple and compound (2-5) and the individual grains measure 3 to 10 microns in diameter.
5. **Test:** powdered cinchona when heated in a dry test tube in a slanting position, purple condensation is seen on the upper side of the test tube.

REPORT

REFERENCE

1. Pharmacognosy Of Powdered Crude Drugs – M.A.Iyengar, 9th edition, page no: 16.

Exp.no:21 Date:

POWDER MICROSCOPY OF SENNA

AIM:

T o carry out the powder microscopy of given crude drug

MATERIALS AND REAGENTS

Powdered drug, conc. HCl, phloroglucinol, glass slide, cover slip

BIOLOGICAL SOURCE: It consists of the dried leaflets of ***Cassia angustifolia*** (Alexandrian senna) and ***Cassiaacutifolia***(Indian senna) belonging to the family**Leguminosae.**

IDENTIFYING CHARACTER

- Colour : Pale green or yellowish green
- Odour : None
- Taste : Mucilaginous, bitter and characteristic.

1. **Trichomes:** only covering type, short, thick, unicellular, warty and frequently curved near the base.
2. **Stomata:** rubiaceous or paracytic type meaning thereby the two subsidiary cells are parallel to the stomal pore.
3. **Calcium oxalate:** occurring as cluster crystals in the cells of the mesophyll and as prisms in a sheath of cells around the fibers and as well freely distributed in powder.

Attention: cortex Cascara, Cortex Quillaia, Radix Licorice.

4. **Epidermis:** with polygonal epidermal cells in surface view.
5. **Mesophyll:** fragments of leaf showing isobilateral arrangement.

REPORT

REFERENCE

1. Pharmacognosy Of Powdered Crude Drugs – M.A.Iyengar, 9th edition, page no: 27.

ISOLATION OF ACTIVE PRINCIPLES

Date :

EXTRACTION

Extraction is the process in which the active fraction of a crude drug is separated by means of suitable solvents.Extractions carried out in the laboratory often result in constituents of less purity. Therefore, a process of purification and identification may also be conducted on the isolated product. There are various techniques of extraction like infusion, decoction, digestion, maceration, percolation, supersonic extraction etc. In the process of extraction, many stages take place within the plant cell. They are,

⇒ Penetration of solvent in to plant cell.

⇒ Dissolution of extractive substances.

⇒ Diffusion of dissolved extractive substance out of plant cell

Factors affecting extraction:

1. Quantity and nature of drug.
2. Degree of size reduction.
3. Nature and volume of solvent.
4. Drug solvent ratio.
5. Temperature
6. PH of extraction solvent.
7. Extraction time.
8. Solubility of active constituents.
9. Moisture content of drug.
10. Extraction methods involved.

Few terms used commonly in the process of extraction

1. Menstruum => Solvent or solvent mixture used for extraction.
2. Miscelle => Solution containing extracted substance.
3. Marc => Complete extraction of constituents from crude drugs.

Exp.no:22 Date:

ISOLATION OF STARCH FROM POTATO

AIM:

To isolate starch from the given sample of potato.

REQUIREMENTS:

Potatoes, Distilled water, Blender, Stirrer, Sieves, Oven

PRINCIPLE:

Starch is obtained from the grains of *Oryza sativa* (Rice), *Triticum aestivum* (Wheat), *Zea mays* (Maize), all belonging to the family Graminae and from the tubers of Potato, *Solanum tuberosum* belonging to the family Solanaceae. It is present in the form of grains in different parts of plants and is a reserved food material. It chemically consists of amylose and amylopectin, and final product of hydrolysis is glucose.

Starch can be qualitatively identified by its reaction with strong iodine solution producing a bluish violet color. In the pharmaceutical industry, starch is used as a binder, disintegrant and diluent in tablet preparation and as demulscent in many other preparations.

PROCEDURE:

Potatoes were peeled to remove adhering soil and then reduced to fine slurry with water in a blender; this slurry was passed through shaking sieves in order to remove cell debris and other impurities. The milky liquid was allowed to settle down. Decanted the supernatant liquid, washed the settled starch 2 to 3 times with distilled water with constant stirring, the milk liquid was centrifuged. The product was dried in an oven at low temperature and powdered. The yield of starch from potato was calculated on % w/w basis.

REPORT:

The practical yield of starch from potato was found to be……

REFERENCE:

Practical Pharmacognosy by Dr. C K Kokate. page no: 141

Exp.no:23 Date:

ISOLATION OF CALCIUM CITRATE FROM LEMON

AIM:

To isolate citric acid as calcium citrate from the fresh juice of lemon

REQUIREMENTS:

10 % calcium chloride solution, 15 % sodium hydroxide solution, Muslin cloth, Litmus paper, Filtration assembly, Lemon fruit, Glass wares.

PRINCIPLE:

Citric acid is one of the most common plant acid used in pharmacy. It is obtained from the juice of Citrus fruits like Lemon (*Citrus limon*) and Orange (*Citrus aurantium*) belonging to the family Rutaceae.

The fresh juice of lemon contains 6.5 to 8.5 % of citric acid along with some amount of vitamin C. The acidity of both juice and fruit diminishes on keeping as citric acid gets gradually decomposed to glucose and carbonic acid.

For the isolation of citric acid as calcium citrate, lemon juice in alkaline media is treated with calcium chloride solution. This cause the formation of calcium citrate which is insoluble in hot water and thus precipitated by boiling.

PROCEDURE:

1. To about 25 ml of lemon juice, 15% sodium hydroxide solution was added drop wise with constant stirring until the mixture was slightly alkaline.
2. The mixture was passed through a muslin cloth for removing the pulp particle, and 5 ml of 10 % calcium chloride solution was added for each 10 ml of filtrate.
3. The solution was heated to boiling and filtered off while hot. The copious precipitate of calcium citrate was washed with small volume of boiling water.
4. The precipitate was resuspended in minimum volume of cold water, heated to boiling. It was then filtered and the salt was allowed to air dry. The percentage yield was calculated on a W/V basis

REPORT:

The Practical yield of calcium citrate from the fresh juice of lemon fruit was found to be……..

REFERENCE:

Practical Pharmacognosy by Dr. C K Kokate: page no: 139

Exp.no:24 Date:

ISOLATION OF PECTIN FROM LEMON PEEL

AIM:

To isolate pectin from the fresh peels of lemon

REQUIREMENTS:

Lemon peels, Isopropanol/acetone/ethanol, Citric acid/tartaric acid or any dilute acid, Demineralised water, Nylon cloth, Glass wares

PRINCIPLE:

Pectin is a natural hydrophilic colloid consisting of partially methoxylated poly galacto uronic acid. It is obtained from the inner rind of citrous fruits like lemon and orange, from apple pomace or from other vegetative matter such as raw papaya and sun flower heads. Pectin gives flexibility to plant parts and along with cellulose, is responsible for the mechanical strength.

Among the various natural sources lemon peel is the best because it yields maximum amount of pectin. Pectin is present in the middle lamella of the cell in an insoluble form called proto pectin. It is converted to the soluble pectin by heating with dilute acid. Maximum extraction of pectin takes place at P^H 3.5-4 and dilute acid is used for this purpose. Heating at high temperature deactivates the enzyme. Charcoal may be added for the decolorisation of the solution. Pectin is insoluble in alcohol, isopropanol, acetone etc and can be precipitated by adding the filtrate to any of these solvents.

Pectin is used as adsorbent in the treatment of diarrhoea. It is also used as haemostatic for internal or external hemorrhage. Pectin is used as a thickening agent in the preparation of jams, jellies and fruit beverages.

PROCEDURE:

1. About 20 g of lemon peel was weighed and size reduced to small pieces, thoroughly washed with water and immersed in 80 ml of demineralised water.
2. Adjusted the P^H to 3.5 - 4 by adding citric acid.
3. Heated at 85 to 90^oC with constant stirring for about one hour.
4. The mixture was filtered immediately while hot; the filtrate was cooled and poured slowly to three times its volume of isopropanol.
5. The solution was stirred thoroughly for the precipitation of Pectin.
6. The solution was then filtered through a nylon sieve, washed the precipitate several times with small volume of isopropanol / acetone / ethanol in order to make it free from acidic ions.
7. The product was dried and weighed, the Practical yield was calculated.

REPORT:

The practical yield of Pectin was found to be …….

REFERENCE:

1. Practical Pharmacognosy by Dr. C K Kokate, page no: 137

Exp.no:25 Date:

ISOLATION OF CASEIN FROM MILK

AIM:

To isolate Casein from Milk.

REQUIREMENTS:

Milk, Glacial acetic acid, Ethanol, Ether, Distilled water, Beakers(100 ml), Glass rod, Funnel

PRINCIPLE:

Milk is a mixture of many types of proteins, most of them are present in very small amount .Milk proteins are classified into three main groups of proteins on the basis of their widely different behaviours and forms of existence. They are caseins(80%), Whey proteins and Minor proteins. Casein is a heterogenous mixture of phosphorous containing proteins in milk. Caasein is present in milk as calcium salt and calcium caseinate.It is a mixture of alpha, beta and gamma caseins to form a cluster called micelle. These micelles are responsible for white opaque appearance of milk.

Casein like proteins are made up of hundreds of individual aminoacids .Each may have a positive or a negative charge,depending on the P^H of the system (Milk). At some P^H value all the positive charges and all the negative charges on the protein will be in balance, so that the net charge on the protein will be zero.The p^H value is known as isoelectric point of the protein and is generally the p^H at which the protein is least soluble.For casein, the I.E.P is approximately 4.6 and it is the p^H value at which acid casein is precipitated . In milk , which has a P^H of about 6.6 , the casein micelle have a net negative charge and are quiet stable. During the addition of acid to milk, the negative charges on the outer surface of the micelle are neutralized and the neutral protein (casein) precipitates. Finally purification of casein is obtained by treating the isolated casein with ethanol and ether to remove the fatty impurities.

PROCEDURE:

1. 25 ml of milk was taken into 100 ml beaker and warmed at 40ºC.
2. Glacial acetic acid was added dropwise with continous stirring and adjusted p^H to 4.6
3. The precipitated casein was separated and dispersed in ethanol to solubilize the unwanted fatty materials.
4. Filtered and treated against ether to remove fractions of alcohol insoluble fatty contents.
5. It was then dried at 40ºC and stored in air tight containers.
6. Calculate the practical yield.

REPORT:

The practical yield of casein was found to be …….

REFERENCE:

Madhu .C.Divakar, Plant Drug Evaluation –A Laboratory guide , pg no:82

Exp.no:26 Date:

ISOLATION OF CURCUMIN FROM TURMERIC

AIM:

To isolate curcumin from the given sample of turmeric powder.

REQUIREMENTS:

Turmeric powder, Petroleum ether, Chloroform, Glass wares.

PRINCIPLE:

Turmeric consists of the dried rhizomes of *Curcuma longa* belonging to the family Zingiberaceae. Turmeric contains a group of diaryl heptanoid compounds called curcuminoids out of which curcumin is the major one. In addition to this turmeric contain volatile oil, resin and starch.

For the isolation of curcumin from turmeric powder, simple extraction procedure (simple maceration) is adopted. Petroleum ether is used in the first step to remove the fatty matter. Chloroform is used for subsequent extraction because of the excellent solubility of curcumin in Chloroform. Curcumin is used as an indicator in analytical chemistry, as a dye in textile industry and as a coloring agent in food and pharmaceuticals.

PROCEDURE:

1. About 20 g of turmeric powder was taken in a glass stoppered flask and 50 ml of petroleum ether was added, closed the flask and shaken for 30 minutes.
2. Rapidly filtered through a funnel and discarded the filtrate.
3. The residue left back in the funnel was collected and dried by spreading on a sheet of filter paper.
4. It was then taken in to a glass stoppered flask and 60 ml of chloroform was added, shaken intermittently, yet thoroughly for a period of one hour.
5. This extract was filtered through a filter paper and the filtrate was collected in a tared Petri dish.
6. The filtrate was evaporated to get the free flowing solids of curcumin.

REPORT:

The practical yield of curcumin was found to be …….

REFERENCE:

Dr. C K Kokate, Practical Pharmacognosy: page no: 138

Exp.no:27 Date:

ISOLATION OF LAWSONE FROM HENNA

AIM

To Isolate lawsone from henna leaves

REQUIREMENTS

Henna leaves, sodium bicarbonate solution, 20% ditute HCl, ammonium hydroxide benzene.

PRINCIPLE

Lawsone, 2- hydroxy -1,4 -naphiquinone is the active constituents lawsone (henna) of lythraceae. It is reported to posses various medical properties such as anti haemorshage antifungal, antiviral, anti tuberculotic. It is a yellowish brown coloured crystal having melting point 192-193°c.

PROCEDURE

About 50 g of crushed fresh leaves of henna are extracted by agitation for 2 hrs with 20% Na_2C03 200ml (solution). The extract is refiltered and is re-exracted with 100ml of same solution for 1 hr. Filtered and the alkaline is acidified are pooled together. The extract is acidified with dilute HCl and crude product obtained on standing was re-extracted with sufficient quantity of ammonium hydroxide and again acidified with dilute HCl. The product is finally extracted with 2 successive quantity of benzene (40ml) and fitered. The filtrate is dried to yield lawsone. The yield of the product was calculated.

REPORT

Dried crystals of lawsone was isolated and submitted.

REFERENCE

Practical pharmacognosy by Dr. C.k Kokate, Page no: 196-197

Experiment pharmacognosy by Biren Shah and B.S Nayak, Page no: 249

Exp. No: 28 Date:

ISOLATION OF CAFFEINE FROM TEA DUST

AIM

To isolate caffeine from tea dust and calculate its percentage yield.

REQUIREMENTS

Beaker, funnel, glass rod, con. NH3, dichlorome thane, petridish

PRINCIPLE

Caffeine is obtained from prepared leaf and leaf buds of Thea sinesis of family Theaceae and from the plant Coffea arabica, family Rubiaceae. Tea leaves contain 1-4% of caffeine while coffee seeds contains 1-2% of caffeine. Caffeine is a xanthine alkaloid and is one of the most important methyl derivatives of xanthine present in tea and coffee. It is chemically 1,3,7-trimethyl xanthine and occurs as white silky needles with a melting point 235^{o}c. Its molecular formula is $C_8H_{10}N_4O_2$. It posses bitter taste without any odour. It is widely accepted and used as a CNS stimulant due to cerebral vasoconstriction effects. Medicinally it is used in the form of citrates or hydrochlorides as diuretics and heart stimulant. It is given along with ergotamine tartarate to potentiate the action of later as a potential analgesic in migraine.

Caffeine is liberated as alkaloid base on treatment with alkali. Then the alkaloid, caffeine is separated by extraction using organic solvents like chloroform and the step also helps to remove the water like chloroform and the step also helps to remove the water soluble tannins. Decolourisation step is done with activated charcoal.

PROCEDURE

Take out 25g of tea leaves in a beaker, add conc. NH_3 for moistening and stirr well. To that add 10-15ml of dochloromethane/ chloroform and shake vigorously for 5-10 min. Filter the solution while hot and separate in the petridish for crystallisation.

REPORT

The crude drug dried sample of coffeine was prepared and submitted. The percentage yield was found to be _ _ _ _ _ _ _ _ _ _

REFERENCE

1. Natural products, A laboratory guide by Raptel and Ihani, page no: 231

2. Organic Chemistry by Gurudeep Chatwal, page no: 177-178.

CHROMATOGRAPHY

CHROMATOGRAPHY

Chromatography is the separation of a mixture into individual components using a stationary phase and a mobile phase.

TYPES OF CHROMATOGRAPHY

Based upon the nature of stationary and mobile phase

There are different types of chromatography based on the type of stationary and mobile phase used. They are

1. Gas - Solid chromatography
2.Gas - Liquid chromatography
3.Solid-Liquid chromatography [Column chromatography, Thin layer chromatography, HPLC (High performance liquid chromatography)]
4.Liquid - Liquid chromatography
(Paper partition chromatography, column partition chromatography)

Based on the principal of separation

The principle of separation can be either adsorption or partition chromatography.

1. Adsorption chromatography

When a mixture of compounds (adsorbate) dissolved in the mobile phase(eluent) moves through a column of stationary phase(adsorbent), they travel according to the relative affinities towards stationary phase. The compound which has more affinity towards stationary phase travels slower and the compound which has lesser affinity towards stationary phase travels faster.Hence the compounds are seperated.

eg. Gas-solid chromatography, Thin layer chromatography, Column chromatography and HPLC(High performance liquid chromatography)

2. Partition chromatography

when two immiscible liquids are present, a mixture of solutes will be distributed according to their partition co-efficients. When a mixture of compounds are dissolved in the mobile phase anpassed through a column of liquid stationary phase, the component which is more soluble in the mobile phase travels faster. Thus the components are separated because of the differences in their partition co-efficients.

eg.Gas- Liquid chromatography, Paper partition chromatography, Column partition chromatography e.t.c

Based on the modes of chromatography

There are two types:

1. Normal Phase chromatography

In this the stationary phase is polar and mobile phase is non-polar.

2. Reverse phase chromatography

In this, stationary phase is non-polar and mobile phase is polar.

R_f VALUE

The R_f value(Retardation factor) is calculated for identifying the spots i.e in Qualitative analysis. R_f value is the ratio of distance travelled by the solute to the distance travelled by the solvent front.

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front.}}$$

The R_f value ranges from 0 to 1. But ideal values are from 0.3 to 0.8. R_f value is specific and constant for every compound in a particular combination of stationary and mobile phase. When the R_f value of a sample and reference compound is same, the compound is identified by its standard. When the R_f value differs, the compound may be different from its reference standard.

PAPER CHROMATOGRAPHIC ANALYSIS

INTRODUCTION

Chromatography is defined as a technique by which various components of a mixture are separated by means of their affinity difference for a stationary phase and mobile phase. Paper chromatography makes use of whatman's filter paper as the stationary phase and suitable solvent mixture as the mobile phase.

Principle:

Paper chromatography is an example of liquid-liquid type. The moisture present in the fibres of paper is the stationary phase, mixture of solvents suitable for the analyte is the mobile phase. When the analyte passes between these two phases various components of the mixture gets partitioned between these phases depending on their partition coefficient. As a result, they travel at different rates and occupy different positions on the paper. This helps to separate and identify the various components present in the mixture.

Preparation technique:

Very much like thin layer chromatography, Paper chromatography also can be Ascending, Descending, 2 dimensional, in addition ascending and descending, Circular and Radial technique also can be performed on paper. Ascending and Circular are the most popular techniques in Paper chromatography.

Detection:

Various components separated can be detected with the help of specific solvents.

Eg: Ninhydrin - Amino acids
Dragendorff's - Alkaloids

Evaluation:

Both quantitative and semi quantitative evaluation can be done in Paper chromatography.

Quantitative evaluation is based on R_f values to identify the compounds.

Semi quantitative evaluation is based on the spot size and spot color intensity. Alternatively separated spots can be carefully cut out from the chromatogram and treated with suitable solvents and can be colourimetrically analysed. However, such determinations are not accurate.

Application:

- Identification of compounds.
- Separation of mixtures.
- Detection of adulteration or determination of purity.
- Detection of approximate quantity.
- Standardizations.

Advantages:

- Flexible.
- Low requirement of sample.

- Good separation.
- Chromatogram can be preserved.

Disadvantages:

- Corrosive reagents cannot be used.
- Paper need to be handled carefully.
- Takes more time for development than TLC.

Exp.no:29 Date:

PAPER CHROMATOGRAHIC ANALYSIS OF AMINOACID

AIM

To perform paper chromatography and identify the given amino acid

REQUIREMENTS

Whatsmann chromatographic paper
Mobile phase: N-butanol : acetic acid : water (4:1:5)
Spraying reagent : Ninhydrin in acetone
Paper chocomatographic chamber.

PRINCIPLE

Seperation by paper charomatography is partition . Here the cellulose layer in filter paper contain moisture which acts as stationary phase. Organic solvents or buffers are used as mobile phase .The mobile phase used in this experiment is a hydrophilic mobile phase. The development technique used here is ascending technique.

Paper chromatography is mainly applicable to water soluble plant constituents. There are mainly two types of paper chromatography.

a) Paper partition

Paper is used as inert support with one solvent as mobile and other as stationary phase.

b)Paper adsorption

A modified paper is used as an adsorbent and single solvent is allowed to flows over unknown components.

When immiscible liquids are passed ,a mixture of solids are distributed in account to position coefficient. When a mixture of compounds are dissolved in mobile phase and they passes through the stationary phase. The component more soluble moves fast

PROCEDURE

The given sample of aminoacid was dissolved in little quantity of water. what manns filter paper of no. I grade is used for the study. The mobile phase was prepared in prescribed composition and placed in the solvent chamber, properly covered with a lid. The chamber was allowed to stand for 1 hr for complete saturation . The chromatographic paper was covered with amino acid using glass capillaries. All the spots should be in equal distance. Air dry the spot. The paper was then placed in the solvent chambers with the help of a thread in such a manner that the spotted area remains above the mobile phase layer After the solvent front passed for above 3/4 th length of paper. Remove it from the chamber, and mark the position of solvent front with pencil.Then the paper was dried followed by staining with ninhydrin and acetone as spraying reagent After spraying the paper was dried in oven and the purple colour spots were measured for Rf value.

$$\text{Rf value} = \frac{\text{Distance travelled by solute from origin}}{\text{distance travelled by the solvent..}}$$

REFERENCE

Experimental pharmacognosy by Biren shah, B. J Naik Textbook of pharmacognosy and phytochemistry by Dr. G'.S kumar and Dr. KM Jaya Page no: 31

REPORT

Date :

THIN LAYER CHROMATOGRAPHIC ANALYSIS

INTRODUCTION

The Thin Layer Chromatography is a technique by which various components of a mixture are separated with respect to their affinity to a set of stationary and mobile phase. In TLC, adsorbent coated on a glass plate serve as stationary phase and various solvents suitable for sample mixture serve as mobile phase. The coated glass plates are heated for about 30 minutes at 100-120^{o}C in a hot air oven (activation). Mobile phase is held in a TLC chamber for about 30 minutes (saturation). When sample mixture is introduced between stationary phase and mobile phase, various components of sample travel and occupy different positions on the glass plate based on their adsorption capacity ie, compound with least affinity to the stationary phase travels most and the component with most affinity to the stationary phase travels least. Thus, the components are separated. This is the basis of TLC.

Adsorbents:

There are two types of adsorbents. They are, Non modified and modified

Non-modified Eg: silica gel, aluminium oxide, cellulose, keisulghur, and polyamide.

Modified Eg: hydrophobically modified silica gel, hydro physically modified silica gel, surface modified cellulose etc….

Preparation and activation of TLC plates:

A wide range of readymade sorbents are nowadays available for TLC. As a rule, plates of 5X20, 10X20, 20X20 cm are coated after thorough cleaning and rinsing with distilled water. There are various methods of coating the glass plates such as spraying, spreading, dipping, pouring etc. The layer thickness varies between 0.1-2mm. Surface of glass plate must be completely free of grease before coating otherwise layer might not adhere. Coating should be performed rapidly because layer hardens very fast. Coating should be performed at very high speed. The above tips help to produce good TLC plate with uniform thickness.

There are numerous active sites present in the surface of the sorbent on to which substance gets adsorbed. Activation at high temperature helps to enhance the absorbance capacity. It also helps to remove moisture and other adsorbed impurities.

Sample application:

Sample can be applied as spots or as bands. Spot broadening in the direction of development is smaller in case of band wise application. Overloading of layer which can leads to spot tailing can be largely avoided. In this way, sample applied band wise are better separated and evaluated. Sample and reference substances should be dissolved in the same solvent. Sample volumes of 1-5µl are applied on TLC plates. 100-500µl is applied for HPTLC. The sample concentration generally lies in the range of 0.01-1%. The spot size should not exceed maximum of 5mm on application. Repeated application at the same spot should be avoided because the previously applied zone is chromatographed for each subsequent application. This results in Dog bone shaped spot in the finished chromatogram. When it is necessary to perform repeated application, the solvent should be evaporated off between the applications. On the TLC plate, the starting line should be marked a distance of 2cm from the bottom.

The solvent should ideally run at least 10-15cm from the starting line. All markings should only be made with soft pencil. Application of the sample can be done by using micro syringe or capillary tube.

Mobile phase:

The choice of solvent system or mobile phase exerts a decisive influence on separation. The solvent dissolves the substance to be separated and transport them across the plate. Some examples of mobile phase are Toluene: Ethyl acetate, Butanol: Glacial acetic acid: Water (4:1:5), Hexane: Ethyl acetate: Formic acid: Water (4:4:1:1).

Chamber saturation:

During separation, solvent evaporate from the plate mainly in the region of solvent front. More solvent is required for the front to travel a given distance and solvent front in the edges of plate travels faster than that in the centre causing edge effect. As a remedy, the chamber is lined with paper so that the solvent vapours are distributed uniformly and the tank gets saturated.

Development:

There are various methods for developing the plate such as ascending, descending, 1dimensional, 2 dimensional etc.

Types of chambers

In principle, two types of chambers mainly, normal chamber and sandwich chamber exist. In the normal chamber simultaneous development of 2 TLC plates is possible. The other chamber is divided into two compartments by ridge on the floor. This reduces solvent consumption. Other chambers for TLC are horizontal chambers, barrier chambers, AMB chambers etc…

Detection:

There are three methods of detection,

Visualization- colored substances

Eg: TLC of curcumin

UV detection- fluorescent compounds

Eg: Blue fluorescence- quinine

Spray detection- compounds where reaction products are colored (derivatization).

Eg: Amino acids with Ninhydrine

Evaluation:

Qualitative evaluation:

It is done by calculating the R_f-value. It is the ratio of distance travelled by a solute to the distance travelled by the solvent front. By comparing the R_f-value of sample with standard, the sample can be identified.

Semi quantitative evaluation:

Here evaluation is conducted by the visual evaluation of spot diameter or spot colour intensity. The accuracy factor is <10%.

Quantitative evaluation:

Both indirect and direct methods are available for measurement. In indirect evaluation, the sample and standard are scrapped from the adsorbent layer and subjected to analysis by suitable techniques. In the direct technique the chromatogram is scanned densitometrically.

Factors affecting TLC:

Stationary phase related parameters

Particle size of the adsorbent

Thickness of the adsorbent layer

Non-uniformity of layer thickness
Activation of stationary phase
Binder in the stationary phase
Angle at which plate is kept in the chamber
Adsorption capacity of stationary phase

Mobile phase related parameters

Relative ratio of mobile phase solvents
Flow rate of the mobile phase
Impurities in the mobile phase
Saturation of the chamber by mobile phase
Migration distance
Choice of mobile phase

Sample related parameters

Sample volume
Concentration of the sample
Spot size on application
Spot drying after application
Repeated application of spots

Exp.no:30 Date:

THIN LAYER CHROMATOGRAPHIC ANALYSIS OF TURMERIC EXTRACT

AIM:

To carry out the thin layer chromatographic analysis of turmeric extract in comparison with standard curcumin.

REQUIREMENTS:

Turmeric extract, Standard curcumin, Activated TLC plates, Mobile phase (Toluene: Ethyl acetate (93:7)),Capillary tubes, TLC chamber, Spray reagent (1% vanillin in sulphuric acid).

PRINCIPLE:

Thin layer chromatography is a separation technique by which various component of a mixture are separated with respect to their adsorption capacity on stationary phase. When a mixture can pass through a stationary phase by means of a mobile phase, the constituents travel and occupy different positions on the plate. The one with more affinity to the stationary phase travels slower and vice versa. Here in the TLC analysis of turmeric extract, silica gel G is used as adsorbent and chloroform: ethanol: glacial acetic acid in the ratio 95: 5: 1 is used as mobile phase.

Turmeric consists of the dried rhizomes of Curcuma longa belonging to the family Zingiberaceae. Turmeric contains a group of diaryl heptanoid compounds called curcuminoids out of which curcumin is the major one. In addition to this turmeric contain volatile oil, resin and starch.

PROCEDURE:

1. The TLC plate was coated with silica gel G and activated at $110^{o}C$ for 30 minutes.
2. The mobile phase was prepared, and poured in to TLC chamber and kept for saturation by inserting a filter paper in to it and the chamber was closed.
3. After a period of saturation for about 30 minutes, the sample and standard were spotted on the plate using capillary tubes at 2 cm above the plate bottom, and the spots were allowed to air dry at room temperature.
4. The plate was placed properly in the chamber; the chamber was closed and kept for analysis until the mobile phase reached $2/3^{rd}$ of the plate height.
5. The plate was then taken out, the solvent front was immediately marked and allowed to air dry.
6. The constituent separated was visualized by spraying with 1 % vanillin in sulphuric acid.
7. Rf value of spots developed were calculated using formula

$$R_F = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent front}}$$

REPORT:

REFERENCE:

1. Quality control of herbal drugs byDr Pulok mukherji.Page no:728
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CHEMICAL TESTS

Exp no: 31 Date:

I. MYRRH:-

Experiment	Observation	Inference
1. Triturate with water	Yellowish emulsion	Presence of Myrrh
2. Dry ethereal extract when treated with bromine vapours.	Reddish in colour	Presence of Myrrh

II. BENZOIN:-

Experiment	Observation	Inference
1. Alcoholic solution + water	Milky solution acidic to litmus	Presence of Benzoin
2. Heat 0.5g with 10ml solution of potassium permanganate	Bitter almond smell of benzaldehyde	Presence of Benzoin
3. Digest 0.25g drug + 5 ml ether + 1 ml ether decant + 2 drops of sulphuric acid.	Deep reddish brown colour (Sumatra), deep purplish red (Siam benzoin)	Presence of Benzoin

III. ASAFOETIDA:-

Experiment	Observation	Inference
1. Triturate with water	Yellowish orange emulsion	Presence of Asafoetida
2. Treat with 50% nitric acid	Green colour	Presence of Asafoetida
3. Treat fractured surface with sulphuric acid	Red or reddish brown colour	Presence of Asafoetida
4. Combined Umbelliferone Test: Triturate 0.5 g of drug + sand + 5 ml Hydrochloric acid, add 3 ml water, filter, filtrate + equal volume of ammonia.	Blue fluorescence (umbelliferone)	Presence of Asafoetida
5. Alcoholic extract + phlorogulucinol and conc. HCI	Pink colour	Presence of Asafoetida
6. On burning	Yellow flame	Presence of Asafoetida

IV. COLOPHONY

Experiment	Observation	Inference
1. Dissolve 0.1 g in 10 ml acetic anhydride by gentle heat, cool and add a drop of Conc.Sulphuric acid	A bright purplish red colour rapidly changing to violet is produced (reaction for abietic acid)	Presence of Colophony
2. Dissolve 0.1 g in light petroleum and filter. To this add 2 – 3 times dil.copper acetate solution is added	Petroleum layer shows emerald green colour.(reaction for abietic acid).	Presence of Colophony
3. Expose the alcoholic solution of colophony to litmus	Colour changes due to acidic reaction	Presence of Colophony

V. ALOE

Experiment	Observation	Inference
1. BORAX TEST Heat 5ml solution with 0.2g borax. Add few drops of this solution in a test tube filled with water	Green fluorescence is produced	Presence of Aloe
2. NITRIC ACID TEST Take 2.5ml Aq. Solution, add 2 ml Nitric acid to it.	i) Reddish- brown color ii) Pale yellow-brown color iii) Brown color changing to green	i) Curacao Aloe ii) Socotrine Aloe iii) Cape Aloe
3. NITROUS ACID TEST Take the extract solution and add Sodium nitrite and Acetic acid, heat	Reddish-brown colour	Presence of Aloe
4. CUPRALOIN (KLUNGES-ISOBARBALOIN TEST Take little quantity of Aq. Solution of Aloe, add a few drops of	i) Wine red color last for many hours	i) Curacao Aloe

saturated Copper sulphate, few mg of Sodium chloride and 5ml 90% alcohol	ii) No colour iii) Faint colour immediately changes to yellow iv) No colour	ii) Socotrine Aloe iii) Cape Aloe iv) Zanzibar Aloe

REFERENCE

Experimental Phytopharmacognosy- A comprehensive guide by Dr. S.S Khadabadi, Dr. S.L.Deore, 2nd edition, Page no: 3.18-3.23