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UGC RECOGNISED UNIVERSITY

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PRACTICAL LAB MANUAL

Pharmacognosy & Phytochemistry - II

B.Pharm Vth Semester

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INTRODUCTION

“Pharmacognosy” derives from two Greek words, “Pharmakon” or drug, and “gnosis” or knowledge. Pharmacognosy is the study of medicinal drugs derived from plant source, Animal source and Marine source. Its scope includes the study of the physical, chemical, biochemical and biological properties of drugs, drug substances, or potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources. Research problems in pharmacognosy include studies in the areas of phytochemistry, microbial chemistry, bio synthesis, bio transformation, chemotaxonomy, and other biological and chemical sciences.

Plant Anatomy

Plant anatomy is a basic core subject in the study of biology, especially plant biology. In the study of plant structure, it is important to recognize that there is a fundamental difference between plant and animal development. In plants, the environment plays a greater role in regulating development. As a result, plant cells are more adapted to changes. The internal structure of the same plant can be slightly different when grown in different environments.

General procedure for Transverse Section cutting

Most plants parts are too thick to be mounted intact and viewed with a microscope. In order to study the structural organization of the plant body, section has to be made so that enough light can be transmitted through the specimen to resolve cell structures under the microscope. A free hand section is the simplest method of preparing specimens for microscopic viewing. This method allows one to examine the specimen in a few minutes. It is also suitable for a variety of plant materials, such as soft herbaceous stems and small woody twigs.

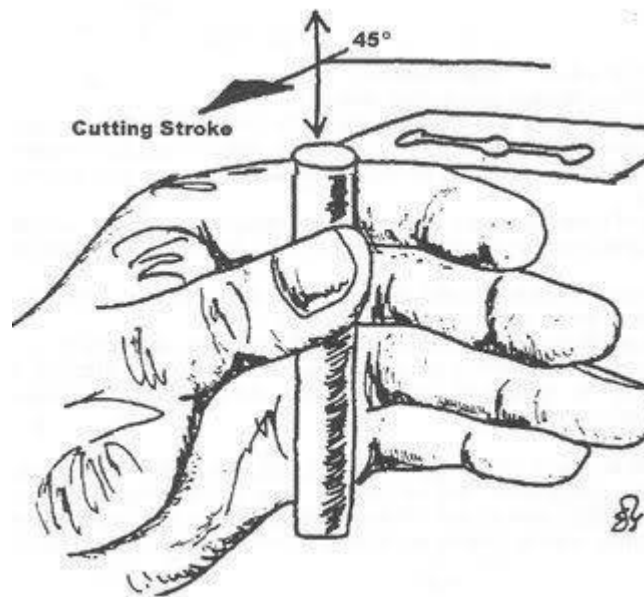
Procedures

1. Obtain a new double edge razor blade. To minimize the risk of cutting oneself, cover one edge of the razor blade with masking tape. Rinse the blade with warm tap water to remove traces of grease from the surface of the blade if necessary.
2. Hold the plant material firmly. The material should be held against the side of the first finger of the left hand or right hand by means of the thumb. The first finger should be kept as straight as possible, while the thumb is kept well below the surface of the material out of the way of the razor edge. Relax. It is not that easy to cut your own finger.
3. Flood the razor with water. This will reduce the friction during cutting as sections can float onto the surface of the blade. Take the razor blade in the right hand or left hand and place it on the first finger of the left hand or right hand, more or less at a right angle to the specimen.
4. Draw the razor across the top of the material in such a way as to give the material a drawing cut (about 45° in the horizontal direction). This results in the less friction as the razor blade passes through the specimen. Cut several sections at a time. Sections will certainly vary in thickness. However, there will be usable ones among the “thick” sections.

5. Transfer sections to water, always using a brush, not a forceps or needle.
6. Select and transfer the thinnest sections (the more transparent ones) onto a glass slide and stain.

Histological and Histochemical staining techniques

Section staining is the most fascinating part in the preparation of specimens for microscopy. In general, most biological tissues have very little contrast, and cellular details are hard to discern with the ordinary light microscope. Stains can enhance and improve the visibility of the specimen. In addition, different stains have different affinities for various organelles and macromolecules. Therefore, the careful selection and utilization of stains can also suggest the chemical nature of the substances within the cell.



Phloroglucinol-HCL test for lignin

Lignin is a common constituent in the secondary wall of plant cells; e.g; the walls of xylem elements and sclerenchyma tissue. The cinnamaldehyde end groups of lignin appear to react with Phloroglucinol-HCL to give Red-violet colour.

1. MORPHOLOGY, HISTOLOGY (T.S), POWDER CHARACTERISTICS AND DETECTION OF CINCHONA, CINNAMON, SENNA, CLOVE, EPHEDRA, FENNEL AND CORIANDER

CINCHONA

MORPHOLOGY OF CINCHONA BARK.

Aim: To identify the morphological characters of given organised drug.

Synonym: Jesuit's bark, Peruvian bark, cinchona bark

Biological source:

Dried bark of cinchona species *cinchona calisaya wedd*, *C.Ledgeriana Mocns*, *C.Officinalis L.*, *C.Succirubra Pav* or hybrids of either of the last two species with either of the first two. It contains not less than 6% of total alkaloids of cinchona.

Family: **Rubiaceae**

Morphology

Organoleptic characters:

Colour: Brown or reddish brown

Odour: slight and characteristics

Taste: Intensely bitter and slightly astringent

Shape: quill, double quill, curved

Fracture: short in outer surface and fibrous in inner part

Chemical constituents: Quinine, quinidine

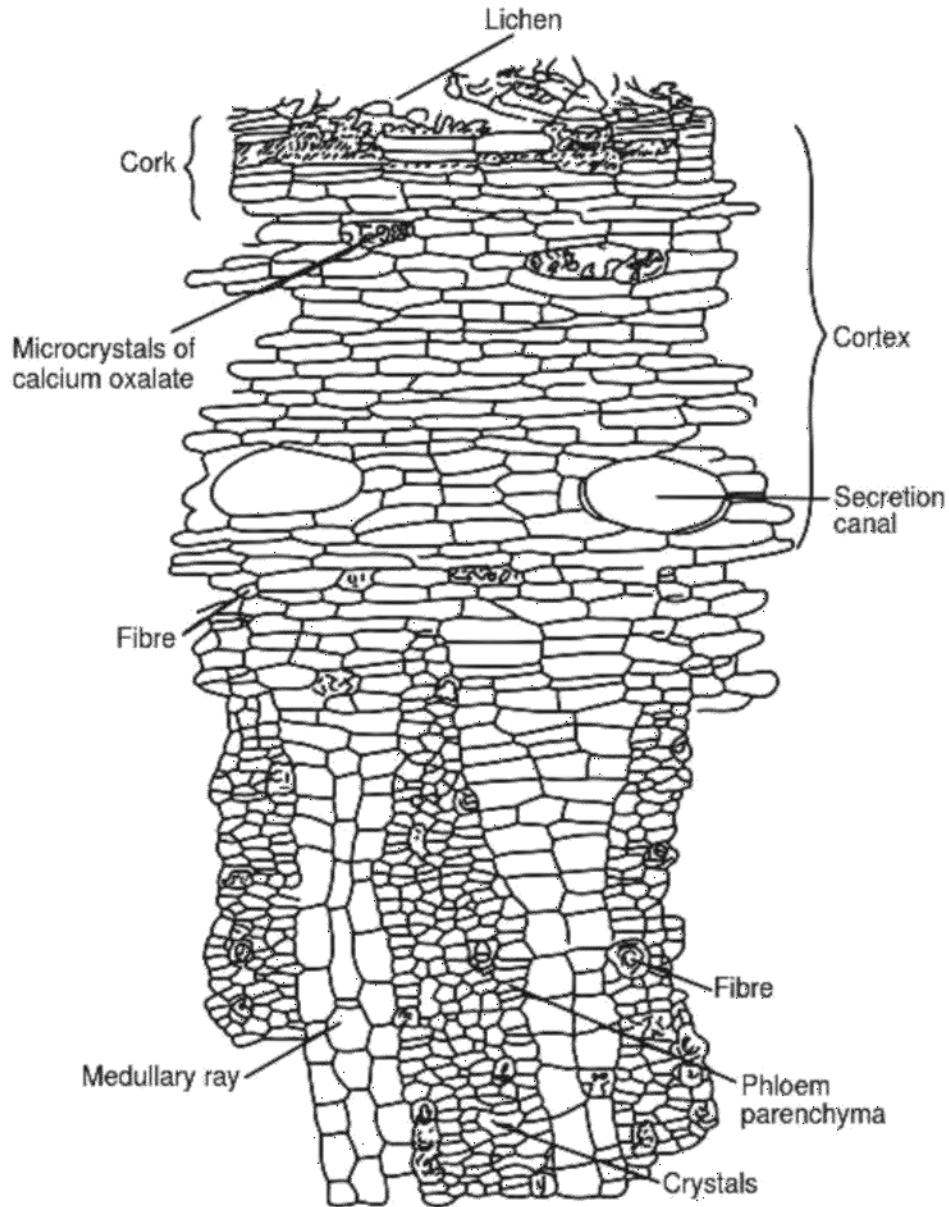
Uses: Anti malarial, Bitter tonic and Anti pyretic

Report:

The given organised drug was identified as.....

TRANSVERSE SECTION OF CINCHONA BARK

Transverse section of Cinchona bark



A. Transverse Section of Cinchona bark

Aim: To identify the Transverse section of Cinchona bark

Materials required: Hydrochloric acid, Phloroglucinol, Glycerin, Watch Glass, microscope slide. Cover slip, Blade, Brush and Compound Microscope.

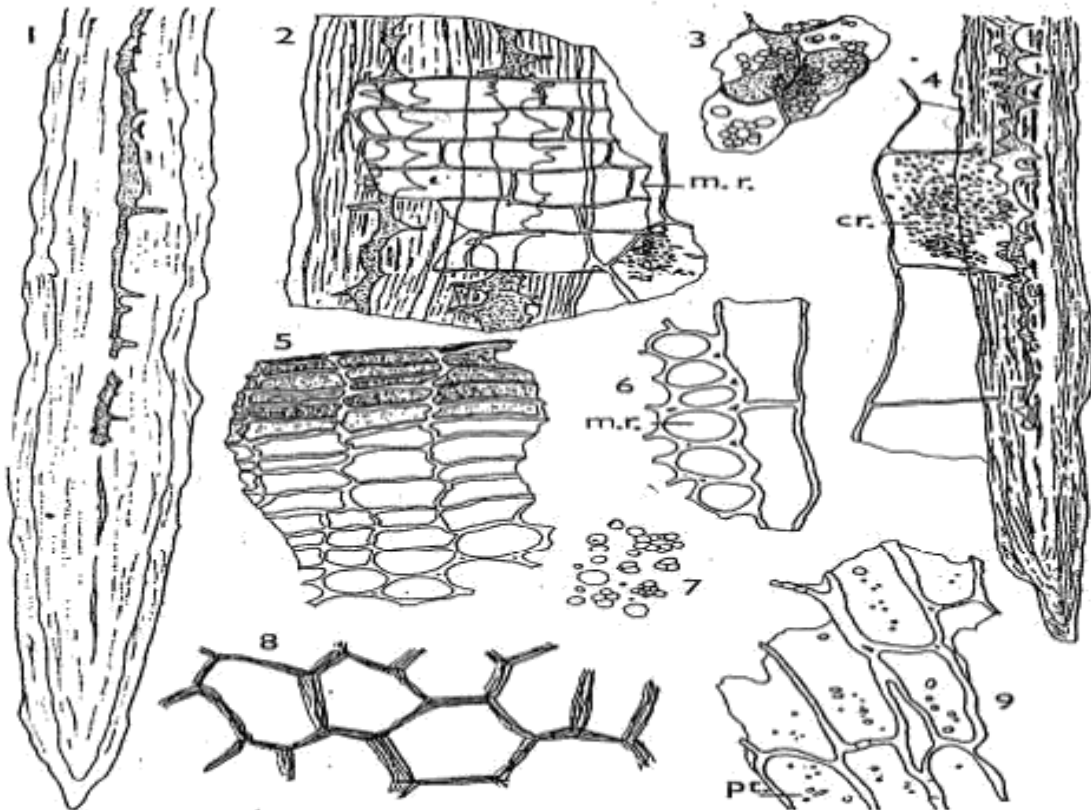
Description

- i- The cork consists of several layers of thin-walled flat cells occurring in rows and filled with reddish-brown content.
- ii- The cortex is made of brown thin walled parenchyma containing small starch grains with occasional idioblast filled with micro crystals of calcium oxalate. Near the inner margin of the cortex are large oval secretion canals at intervals.
- iii- The phloem which forms the greater bulk of the bark is traverses longitudinally by 1-3 seriate medullary rays. The phloem fibers are very diagnostic and are large fusiform thick-walled lignified with striated walls and funnel-shaped pits. The fibers occur singly or in small radial groups of two to four cells. Sclereids are absent.

POWDER MICROSCOPICAL CHARACTER OF CINCHONA BARK

Characteristic elements:

1. Cork (8).
2. Single fiber (1).
3. Cork and phelloderm in sectional view (5).
4. Parenchymatous cells containing starch granules and brown pigment (3).



× 300

1. Part of a single fiber.
2. Part of a group of fibers and phloem parenchyma with overlying medullary ray (m.r.) in radial longitudinal section.
3. Parenchymatous cells containing starch granules and brown pigment.
4. Part of a fiber with phloem parenchyma, one cell containing calcium oxalate micro-crystals (Cr.).
5. Cork and phelloderm in sectional view.
6. Phloem parenchyma and part of a medullary ray (m.r.) in tangential longitudinal section.
7. Starch granules.
8. Cork in surface view.
9. Phloem parenchyma with pits (pt.).

B. Powder Microscopical characters of Cinchona bark

Aim: To identify the Powder Microscopical characters of Cinchona bark

Biological Source- It consists of dried stem or root bark of the plant *Cinchona succirubra*, *C. ledgeriana*, *C. calisaya*, *C. officinalis* and other species of Cinchona.

Family: Rubiaceae

Description:

Reddish brown, showing fragments of:

1. Cork cells thin walled suberized with brown content.
2. Phloem fibre, long, fusiform, thick wall, lignified with narrow lumen, pointed apex and showing funnel-shaped pits.
3. Parenchyma with starch granules.
4. Idioblasts contain microprisms of calcium oxalate.

Micro chemical Test

| Sr.no | Reagents | Observations | Characteristics |
|-------|------------------------------|-------------------------------------|--------------------------|
| 1 | Phloroglucinol+conc.HCl(1:1) | Pink | Lignified phloem fibres |
| 2 | Dil.Iodine solution | Blue | Starch |
| 3 | Dil.Acetic acid | Insoluble | Calcium oxalate crystals |
| 4 | Dil.HCl | Soluble | Calcium oxalate crystals |
| 5 | Sulphuric acid(6%) | Calcium sulphate crystals formation | Calcium oxalate crystals |

Chemical Test

| Sr.no | Tests | Observations |
|-------|---|---|
| 1 | Heat the powder with glacial acetic acid | Reddish brown fumes |
| 2 | Treat the bark with conc.sulphuric acid and observe under UV light | Blue fluorescence |
| 3 | Thalloquin test: Heat powder with few drops of bromine water,shake and strong ammonia | Emerald green colour developed, changed to blue on neutralization |

Uses: Antimalarial, Analgesic

Report

The given crude drug was identified as _____ with the help of various Histological characters (T.S), powder microscopical characters and chemical test.



CINNAMON

Morphology of Cinnamon bark.

Aim: To identify the morphological characters of given organised drug.

Biological source:- Obtained from dried inner bark of the tree *Cinnamomum zeylanicum*.

Family:-Lauraceae.

Description:

Cinnamon plant is a 15-20 m evergreen plant. Leaves on cinnamon plants are oval lanceolate, rough textured and short. The greenish flowers on the plant have a characteristic odor whereas the fruit is one-seeded berry of 1 cm size. Cinnamon is obtained in the form of quills (single or double) with longitudinal striations. Externally cinnamon bark is yellowish brown while the inner surface is more darker. The length of cinnamon quills is variable whereas the diameter of quills is around 6-10 mm. Good quality cinnamon is usually not more than 0.5 mm in thickness, aromatic odor and fragrant

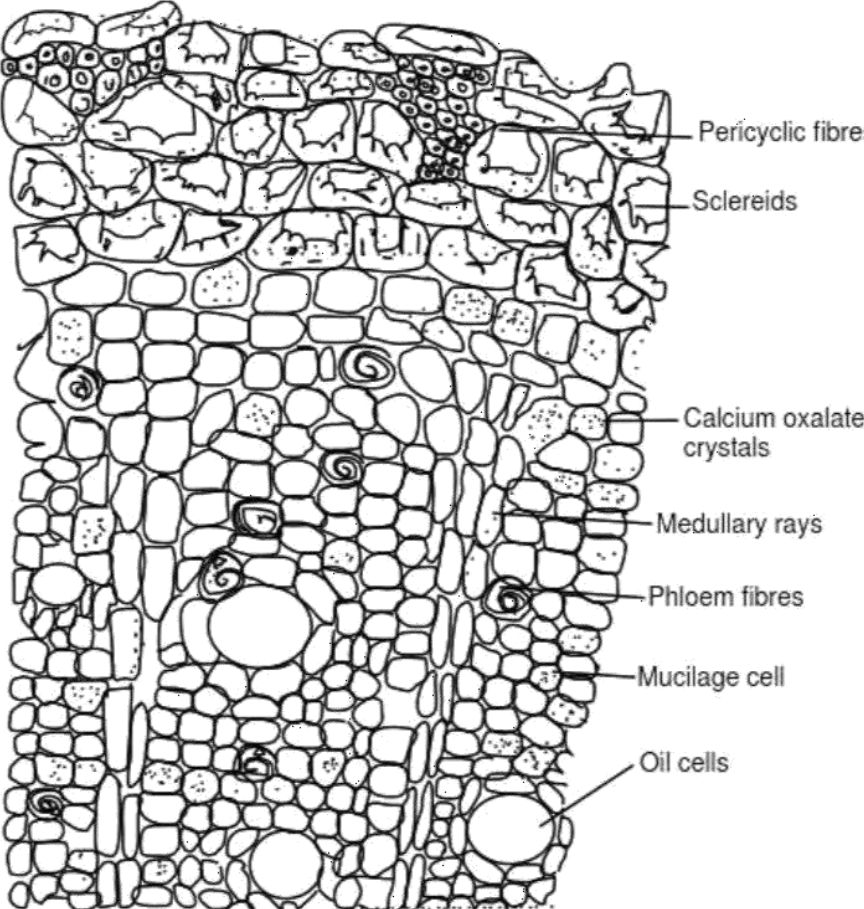
Chemical constituents:-Eugenol, Cinnamic acid &Cinnamic aldehyde.

Uses:-Flavouring agent, Germicide, Somachic & Diaphoretic.

Report:

The given organised drug was identified as.....

Transverse section of Cinnamon bark



A. TRANSVERSE SECTION OF CINNAMON BARK

Aim: To identify the Transverse section of Cinnamon bark.

Materials required: Hydrochloric acid, Phloroglucinol, Glycerin, Watch Glass, microscope slide. Cover slip, Blade, Brush and Compound Microscope.

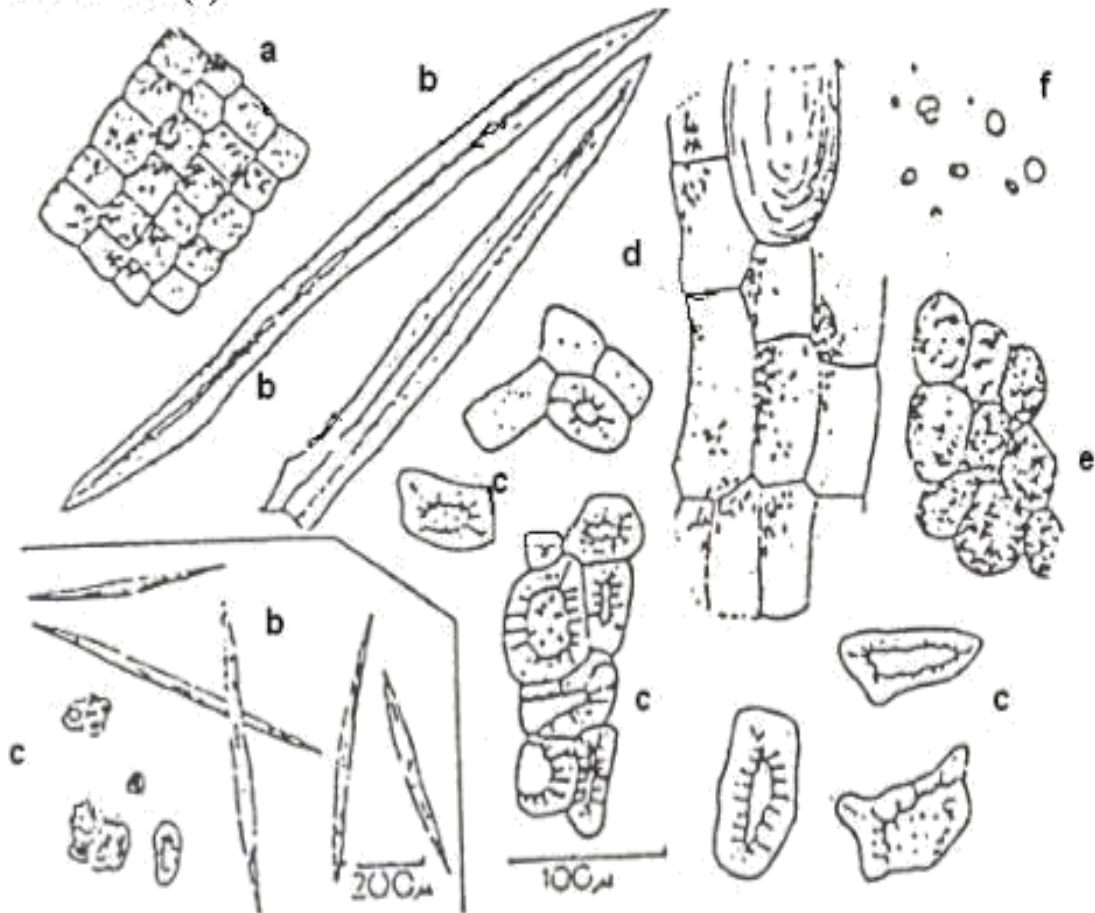
Description:

- i- The cork consists of several layers of thin-walled flat cells occurring in rows and filled with reddish-brown content.
- ii- The cortex is made of brown thin walled parenchyma containing small starch grains with occasional idioblast filled with micro crystals of calcium oxalate. Near the inner margin of the cortex are large oval secretion canals at intervals.
- iii- The phloem which forms the greater bulk of the bark is traversed longitudinally by 1-3 seriate medullary rays. The sieve tubes are usually collapsed with the small companion cell and phloem parenchyma, some of which are idioblast with micro prismatic crystals of calcium oxalate. The phloem fibers are very diagnostic and are large fusiform thick-walled lignified with striated walls and funnel-shaped pits. The fibers occur singly or in small radial groups of two to four cells. Sclereids are absent.
- iv- The root bark is similar to the stem bark but is mainly composed of phloem. The fibers are similar to those of the stem but are generally forked. There are occasional thickwalled striated pitted sclereids. Secretion tubes are absent.

Powder microscopical characters of cinnamon powder

Microscopical examination

1. Modularly ray tissue with calcium oxalate needles (a).
2. Fibers (b).
3. Stone cells(c).



- a) Cells of modularly ray tissue with calcium oxalate needles.
- b) Fibers & fiber fragments.
- c) Stone cells from primary bark.
- d) Cells from the cortical parenchyma with crystal needles and occluded excretory cell.
- e) Cells from cortical parenchyma.
- f) Starch grains.

B. Powder Microscopical characters of Cinnamon bark

Aim: To identify the Powder Microscopical characters of Cinnamon bark.

Biological source:-Obtained from dried inner bark of the tree *Cinnamomum zeylanicum*.

Family:-Lauraceae.

Description:

- 1) Since cinnamon is a bark, there should be no microscopic epidermis and cork in the transverse section. However, residual patches of cork cells may be observed.
- 2) The outer layer of the bark is made up of pericycle fibres 1000-2500 μm long with lignified walls having pit canals.
- 3) Presence of starch grains (both compound and simple) is visible under the microscope, sized around 10 μm and can be found in the phloem. It is difficult to distinguish the primary phloem, however, the secondary phloem contains phloem parenchyma with the oil and mucilage cells which produce the volatile oil constituents of the plant.
- 4) There are also phloem fibres and medullary rays.
- 5) Acicular (radiating needle like) calcium oxalate crystals can be found about 5-8 μm in size.
- 6) The phloem parenchyma contains tannins. Presence of medullary rays in the secondary phloem is prominent which are uni- or biseriate near the cambium but become broader towards the periphery.
- 7) There is also presence of sclereid layers of cells along the pericyclefibres.

Micro chemical Test

| Sr.no | Reagents | Observations | Characteristics |
|-------|------------------------------|-------------------------------------|--|
| 1 | Phloroglucinol+conc.HCl(1:1) | Pink | Lignified cells:Pericyclic fibres,stone cells,cork cells |
| 2 | Dil.Iodine solution | Blue | Strach |
| 3 | Dil.Acetic acid | Insolube | Calcium Oxalate crystals |
| 4 | Dil.HCl | Soluble | Calcium Oxalate crystals |
| 5 | Sulphuric acid(6%) | Calcium sulphate crystals formation | Calcium Oxalate crystals |
| 6 | Ruthenium red | Pink | Mucilage cells |
| 7 | 1% osmic acid solution | Brown or pale | Volatile oil |
| 8 | Dil tincture alkana | Red on standing for 30min | Volatile oil |

Chemical Test

| Sr.no | Tests | Observations | Inference |
|-------|--|--|---------------------------|
| 1 | Alcoholic extract of drug+one drop of ferric chloride solution | Green colour | Cinnamic aldehyde,eugenol |
| 2 | Chloroform extract of the drug + 10% aqueous solution of phenylhydrazine hydrochloride | Rod shaped crystals of hydrozone of cinnamaldehyde | Cinnamic aldehyde |
| 3 | Aqueous extract + 5% FeCl ₃ | Dark colour | Tannins |
| 4 | Aqueous extract + lead acetate reagent | White precipate | Tannins |
| 5 | Aqueous extract + potassium permanganate solution | Decolourisation | Tannins |

Uses:-Flavouring agent, Germicide, Somachic & Diaphoretic.

Report

The given crude drug was identified as _____ with the help of various Histological characters (T.S), powder microscopical characters and chemical test.

SENNA

MORPHOLOGY OF SENNA

Aim: To identify the morphological characters of given organised drug.

Synonym: senna ki patti, sonamukhi, Indian senna, Tinnevelley senna

Biological source:

It consist of dried leaflets of *Cassia angustifolia* Valh. Family: **Leguminosae**

It contains Not less than 2.0% of glycosides calculated as sennoside B.

Organoleptic characters:

Colour: Yellowish green

Odour: slight

Taste: mucilaginous, slightly bitter

Shape: lanceolate with stout petioles, entire margin with acute apex and having asymmetrical base. Venation is reticulate, anastomosing towards margin.

Size: 2.5 to 5 cm long and 3-8 mm wide

Extra features: Isobilateral, thin, pubescent (hairy) with trichomes on both surfaces.

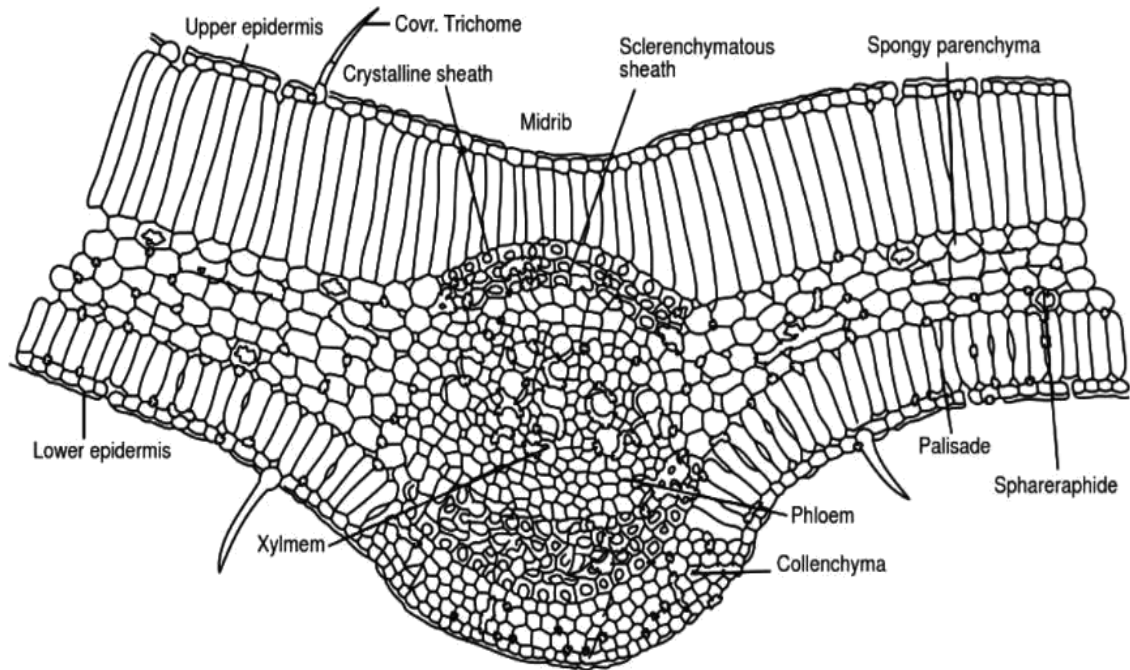
Chemical constituents: Anthroquinone glycosides mainly A,B,C and D.

Uses: Irritant purgatives

Report:

The given organised drug was identified as.....

TRANSVERSE SECTION OF SENNA LEAF



Transverse section of senna leaflet

A. TRANSVERSE SECTION OF SENNA LEAF

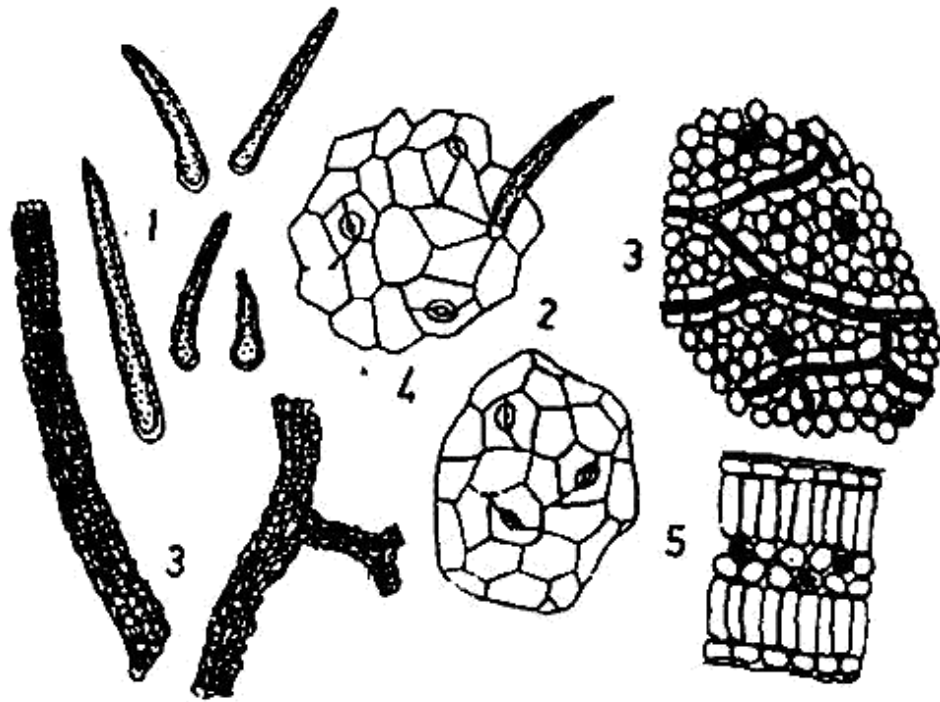
Aim: To identify the Transverse section of senna leaf.

Materials required: Hydrochloric acid, Phloroglucinol, Glycerin, Watch Glass, microscope slide. Cover slip, Blade, Brush and Compound Microscope.

Description:

- A. **Lamina:** Isobilateral
- B. **Upper epidermis:** single layered, polygonal, straight, anti clinal walls, few cells contain mucilage. Epidermis covered with cuticle.
- C. **Mesophyll**
 - a. **Upper palisade:** single layered; elongated, compactly arranged, narrow thin walled parenchyma, continued over midrib region.
 - b. **Spongy parenchyma:** Thin walled, loosely arranged, large intracellular spaces. Spherulites present.
 - c. **Lower palisade:** loosely arranged, wavy walls, cells smaller than upper palisade
- D. **Lower epidermis:** similar to upper epidermis
 - a. **Trichome:** conical, unicellular, thick walled, covering trichome.
 - b. **Stomata:** Rubiaceous (paracytic)
- E. **Midrib**
 - a. **Palisade parenchyma:** single layer
 - b. **Crystal sheath:** present at dorsal and ventral side. Parenchymatous layer containing calcium oxalate prism.
 - c. **Sclerenchymatous sheath:** lignified, thick walled cells, covering the vascular bundle.
- F. **Vascular bundle:**
 - a. **Xylem:** lignified cells present at ventral surface
 - b. **Phloem:** non lignified cells present at dorsal side
- G. **Collenchyma:** Multilayered, thick walled parenchyma containing cellulose. present only at ventral side.

POWDER MICROSCOPICAL CHARACTERS OF SENNA POWDER



Identifying characters of Senna powder

1. Broken hair
2. Epidermal fragment with paracytic stomata
3. Sclerenchyma fibers
4. Epidermal fragment with broken hair
5. Palisade mesophyll

B. POWDER MICROSCOPICAL CHARACTERS OF SENNA LEAF

Aim: To identify the Powder Microscopical characters of Senna leaf

Biological Source:-Obtained from dried ripe seeds of *Cassia angustifolia*

Family:-Leguminoceae.

Description:

- 1) Senna leaflets have an isobilateral structure with straight walled epidermal cells.
- 2) Nonlignified, unicellular warty hairs (trichomes) (upto 260 μm long) are scattered on both the surfaces. These hairs are more abundant in Alexandrian senna with three epidermal cells between hairs, while in Tinnevelly senna it is less frequent with about six epidermal cells between hairs.
- 3) Paracytic stomata are present with Alexandrian senna having two subsidiary cells, while Indian senna has two or three subsidiary cells (in a ratio of 7:3).
- 4) Prismatic and cluster calcium oxalate crystals can be observed.
- 5) Vein islet numbers for Alexandrian Senna is about 25-29.5 while that for Indian/TinnevellySenna is 19.5-22.5.
- 6) The transverse section contains upper and lower epidermis, upper and lower palisade cells with an inner mesophyll. The mesophyll contains vascular bundles, xylem, fiber groups, and calcium oxalate crystals. Below the midrib is the collenchymas.

Micro chemical Test

| Sr.no | Reagents | Observations | Characteristics |
|-------|------------------------------|-------------------------------------|--|
| 1 | Phloroglucinol+conc.HCl(1:1) | Red/Pink | Lignified tissue:xylem(Vascular bundles) |
| 2 | Ruthenium red | Pink | Mucilage cells |
| 3 | Dil.Acetic acid | Crystals Insoluble | Calcium Oxalate crystals |
| 4 | Dil.HCl | Crystals Soluble | Calcium Oxalate crystals |
| 5 | Sulphuric acid(6%) | Calcium sulphate crystals formation | Calcium Oxalate crystals |
| 6 | Sudan red III | Pink | Cutin/cuticle |

Identification Test

| Sr.no | Tests | Observations |
|-------|---|---|
| 1 | Borntrager test for anthraquinone: Boil drug with dil sulphuric acid(hydrolysis) filter and cool.Add benzene or CCl ₄ (immiscible organic solvents) shake and separate organic solvent layer in another test tube.Add strong ammonia solution,shake slightly and keep the test tube aside. | Lower ammonical layer shows pink or red colour. |

Uses:

Purgative

Report

The given crude drug was identified as _____ with the help of various Histological characters(T.S), powder microscopical characters and chemical test.

CLOVE

MORPHOLOGY OF CLOVE

Aim: To identify the morphological characters of given organised drug.

Synonym: Lavang

Biological source:

It consists of dried flower buds of *Eugenia caryophyllus*

Family: *Myrtaceae*

The oil contain Not less than 15% V/W of clove oil.

Organoleptic character

Colour: Dark brown or crimson red

Odour: Aromatic

Taste: spicy pungent followed by numbness

Shape: sub- cylindrical, slightly flattened

Size: 1-1.3 cm long, 0.4 cm wide, 0-2 cm thick

Extra features: **Hypanthium** is a lower body of flower bud which is sub cylindrical , slightly flattened. Inferior, binocular ovary is situated in the upper portion of the hypanthium having numerous ovules attached by axile placentation.

Crown or head is upper body of clove bud consisting of 4 crimson, unopened petals enclosing numerous incurved stamens and central erect style.

Chemical constituents: Eugenol, iso eugenol, methyl and dimethyl furfuryl , α and β caryophylline, hydrolysable tannins.

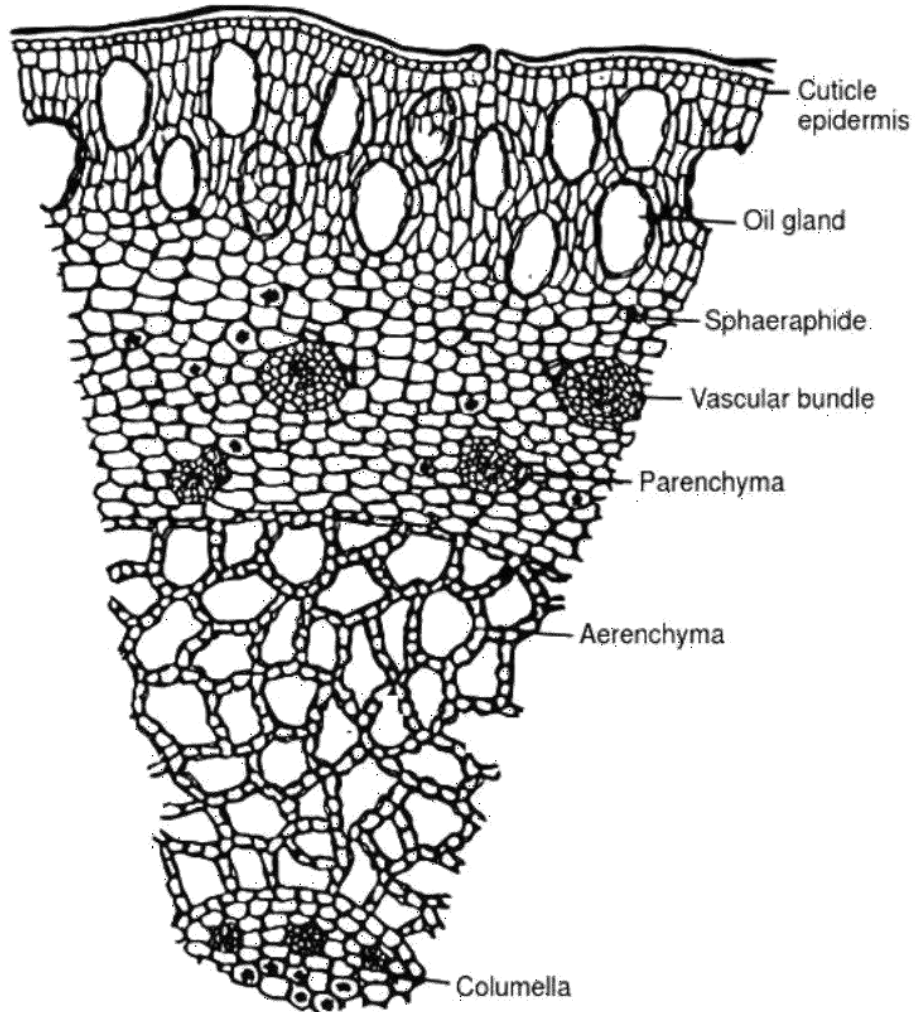
Uses: carminative, aromatic, stimulant, anti septic, flavouring agent, dental analgesic oil.

Report:

The given organised drug was identified as.....

TRANSVERSE SECTION OF CLOVE BUDS

Transverse section of Clove flower bud



A. TRANSVERSE SECTION OF CLOVE BUDS

Aim: To identify the Transverse section of Clove buds.

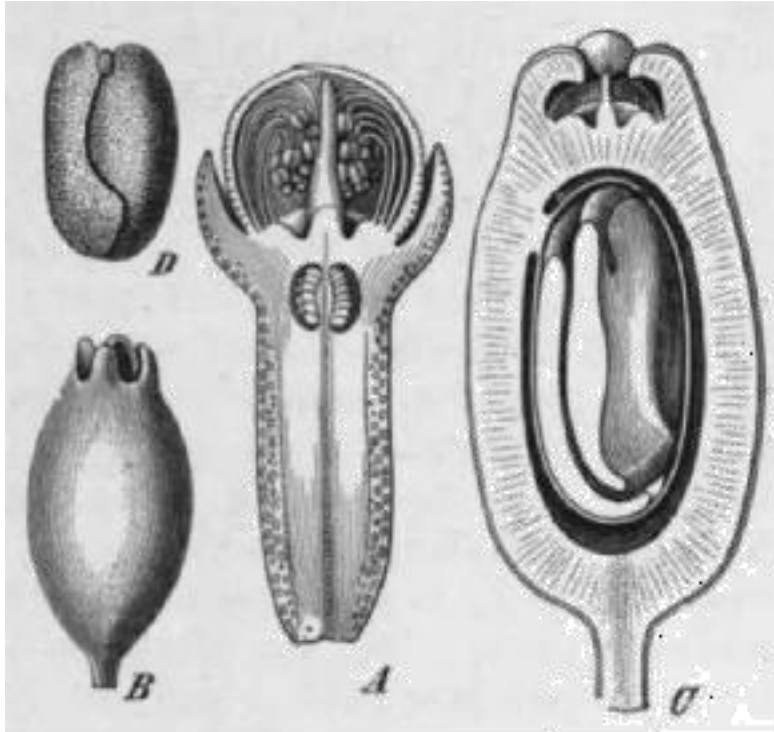
Materials required: Hydrochloric acid, Phloroglucinol, Glycerin, Watch Glass, microscope slide. Cover slip, Blade, Brush and Compound Microscope.

Description:

Transverse section of clove hypanthium below the ovary shows epidermis, cortex and columella:

1. Epidermis: Single layered small cells with straight walls and has a very thick cuticle. Epidermal layer gets intercepted by Ranunculaceous type of stomata.
2. Cortex: The three distinct zones or regions in the cortex can be made out.
 - (a) The peripheral region containing 2 to 3 layers of big, ellipsoidal, schizo-lysigenous oil glands embedded in the radially elongated parenchymatous cells.
 - (b) The middle region containing 1 or 2 rings of bicollateral vascular bundles associated with a few pericyclic fibres, embedded in thick walled parenchyma and
 - (c) The inner region made of loosely arranged aerenchyma.
3. Columella: Forms the central cylinder containing thick walled parenchyma with a ring of bicollateral vascular bundles towards the periphery of the cylinder. Numerous sphaeraphides are seen scattered throughout the columella and to a certain extent in the middle cortical zone.

POWDER MICROSCOPICAL CHARACTERS OF CLOVE BUDS



A, Clove Cut Vertically, Showing Calyx, Corolla, Stamens, Pistil, And Ovules; Near The Margin Oil-Glands; Magnified.

B, Fruit (Mother Clove), Natural Size.

C, The Same, Cut Vertically And Magnified.

D, Embryo, Natural Size.

B. Powder Microscopic character of Clove buds

Aim: To identify the Powder Microscopical characters of Clove buds

Biological source:-Obtained from dried flower buds of a tree of *Eugenia caryophyllus*.

Family:-Myrtaceae.

Description:

The cloves of commerce are therefore the dried flower-buds of the tree. Each of them consists of a nearly cylindrical, dark reddish-brown portion, slightly tapering at the base, which is sometimes regarded as a gynophore, sometimes as a fleshy calyx tube, but is perhaps most correctly interpreted as the solid lower portion of the ovary, crowned by four, thick, divergent calyx-teeth of a similar colour, from the centre of which arise four, paler, brown, unexpanded, imbricate petals. After soaking in water for twenty-four hours the petals can be removed, and they will be found to enclose a large number of stamens bending over a stiff erect style arising from a depression in the centre of a small disc. Just below the disc is the two-celled ovary with its numerous ovules; it can be found by cutting the clove either longitudinally or transversely.

The lower part of the ovary is solid and fleshy, spongy near the centre. It contains, especially near the periphery, a large number of oil-glands, visible, when the transverse section is examined under the lens, as dark shining points or small cavities. Similar glands can be seen both in the calyx-teeth and petals; in the latter they appear as translucent dots by transmitted light. Cloves are strongly aromatic and have a pungent aromatic taste. Good cloves should be plump and heavy, have a bright, reddish-brown colour.

Micro chemical Test

| Sr.no | Reagents | Observations | Characteristics |
|-------|------------------------------|---|-----------------------------|
| 1 | Phloroglucinol+conc.HCl(1:1) | Red/pink | Vascular bundles and fibres |
| 2 | Strong KOH solution | Needle shaped potassium eugenate crystals | Eugenol |
| 3 | Dil.HCl | Crystals soluble | Calcium oxalate crystals |
| 4 | Sulphuric acid(60%) | Calcium sulphate crystals formation | Calcium oxalate crystals |
| 5 | Sudan red III | Pink | Cuticle/oil gland |

Chemical Test

| Sr.no | Tests | Observations | Characteristics |
|-------|--------------------------------------|-------------------|-----------------|
| 1 | Aq.extract + lead acetate solution | White precipitate | Tannins |
| 2 | Clove oil+alcohol+5% ferric chloride | Blue colouration | Eugenol |
| 3 | Aq.extract + 5% ferric chloride | Dark colour | Tannins |

Uses:-Antiseptic, Stimulant, Carminative, Antimicrobial & Treatment of Toothache

Report:

The given crude drug was identified as _____ with the help of various Histological characters (T.S), powder microscopical characters and chemical test.



EPHEDRA

Morphology of Ephedra stem

Aim: To identify the morphological characters of given organised drug.

Biological source:-Obtained from dried young stems of *Ephedra gerardiana* Wall stapf, and also of *E. nebrodensis* (Tineo) stapf collected in autumn.

It contains not less than 1.0% of total alkaloids calculated as ephedrine.

Family:-Ephedraceae.

Description: It may grow up to 4 feet. Nearly leafless, the plant has slender, cylindrical, yellow-green branches and underground runners. In August, the flowers bear poisonous, fleshy, red cones resembling berries.

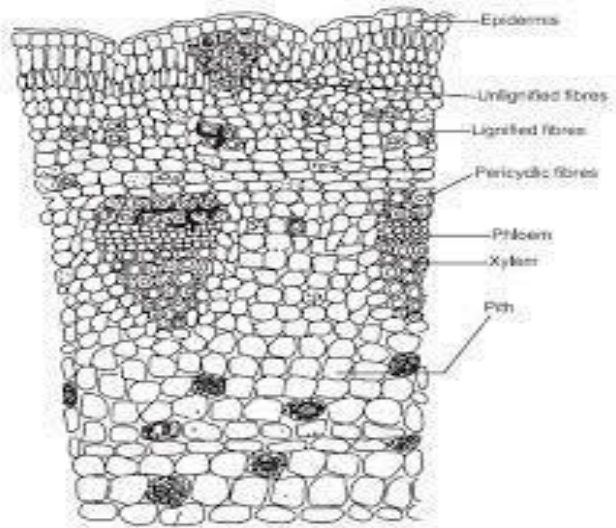
Chemical constituents:-Ephedrine & Pseudoephedrine.

Uses: Treatment of asthma, hay fever, and the common cold.

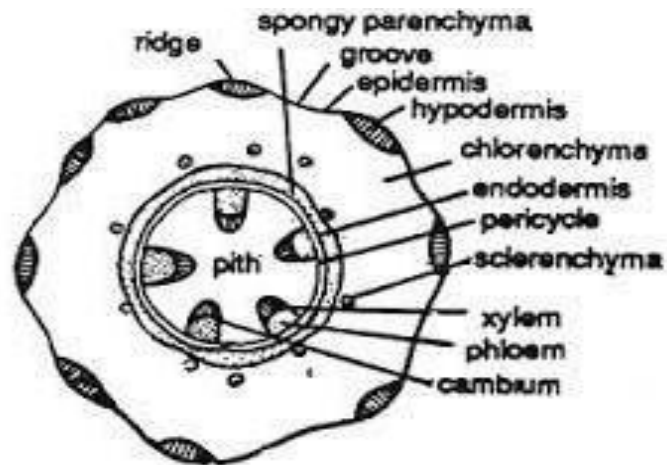
Report:

The given unknown organised drug was identified as.....

TRANSVERSE SECTION OF EPHEDRA



Transverse section of Ephedra



Ephedra T.S young stem (schematic diagram)

A. TRANSVERSE SECTION OF EPHEDRA

Aim: To identify the Transverse section of Ephedra.

Materials required: Hydrochloric acid, Phloroglucinol, Glycerin, Watch Glass, microscope slide. Cover slip, Blade, Brush and Compound Microscope.

Histology:

A. Epidermis: It is the outermost single layered, quadrangular, thick walled cells, covered with a thick and smooth cuticle. Sunken stomata are present on the slopes of the ridges in the circular pits.

B. Cortex: It is present between the thick walled sclerenchyma and vascular cylinder. It can be differentiated into outer and inner cortex. The outer cortex contains 2-3 layers of radially elongated palisade tissue and inner cortex consists of 2-3 layers of spongy parenchyma.

The cells of outer and inner cortex are loosely arranged with large intercellular spaces and are provided with chlorophyll to perform the function of photosynthesis. A few patches of sclerenchymatous cells may also occur in the cortex to provide mechanical support to the young axis.

C. Pericyclic Fibres: Lignified, crown the phloem on its outer side. (strengthening cells)

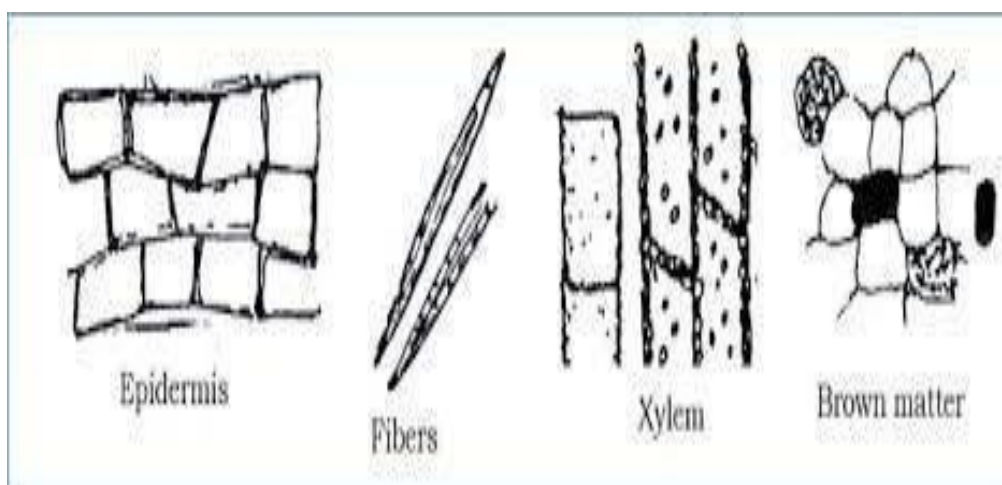
D. Vascular cylinder: Around 10, collateral, conjoint, open and arranged in rings. Contains phloem and xylem.

a. Phloem: containing sieve tubes and companion cells.

b. Xylem: well developed, consist of vessel, tracheids, fibrotracheids and parenchyma

Pith: Large, thin walled, lignified big polygonal parenchyma with intercellular space. Some cells contain dark brownish mucilaginous substance.

POWDER MICROSCOPICAL CHARACTERS



Epidermis: Fragments of epidermis, quadrangular cells, and outer walls are ridged.

Fibres: Lignified and non lignified, long, slender and cylindrical.

Xylem: Tracheids with bordered pits.

Brownish matter: Abundant, dark brownish mucilaginous substance from pith.

Microchemical Test:

| Sr.no | Reagents | Observations | Characteristics |
|-------|------------------------------|--------------|---|
| 1 | Phloroglucinol+conc.HCl(1:1) | Red/pink | Lignified fibres,vascular bundles and pericyclic fibres |

Chemical Test

| Sr.no | Tests | Observations |
|-------|--|---|
| 1 | 10mg drug+1ml water+0.2 ml dil HCl+0.1 ml copper sulphate+1ml sodium hydroxide;solution becomes violet add 1ml solvent ether,shake | Ether layer becomes purple Aqueous layer is blue |
| 2 | Drug+Dragendorff's reagents | Orange brown Precipitate |
| 3 | Drug+Mayer's reagents | Precipitate |
| 4 | Drug+Hager's reagents | Yellow Precipitate |
| 5 | Drug+Wagner's reagents | Reddish brown Precipitate |

Report

The given crude drug was identified as _____ with the help of various Histological characters(T.S), powder microscopical characters and chemical test.

FENNEL

MORPHOLOGY OF FENNEL

Aim: To identify the morphological characters of given organised drug.

Synonym: Bari sauf, fructus foeniculi

Biological source:

Dried ripe fruits of cultivated species, *Foeniculum vulgare* Miller

Family: umbeliferae

It contains not less than 1.4% of volatile oil.

Organoleptic characters:

Shape: Straight or slightly curved

Colour: Greenish or yellowish brown

Odour: sweet aromatic and characteristic

Taste: sweet, mucilaginous, aromatic

Size: Mericarp are 5-10 mm long and 1.3-4 mm broad

Extra features: Glabrous bearing 5 straight prominent ridges and bifid stylopod at the apex.

Chemical constituents:

Volatile oil (4-6%), Anethole (50-60% of volatile oil), d-fenchone (10% of volatile oil), fixed oil (12-18%), proteins (14-22%). Minor constituents of the fennel include limonene, anisaldehyde and methyl chavicol.

Uses:

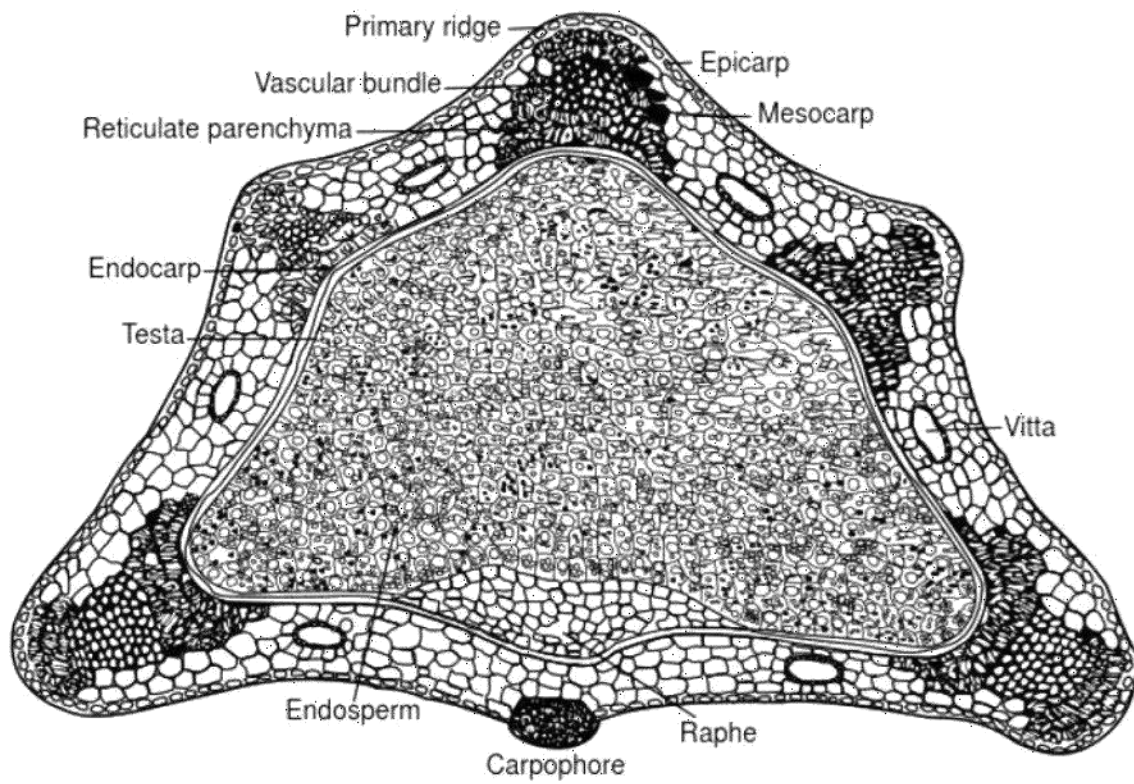
Carminative, respiratory stimulant, aromatic, flavouring agent.

Report:

The given organised drug was identified as.....

TRANSVERSE SECTION OF FENNEL FRUIT

Transverse Section of Fennel fruit



A TRANSVERSE SECTION OF FENNEL FRUIT

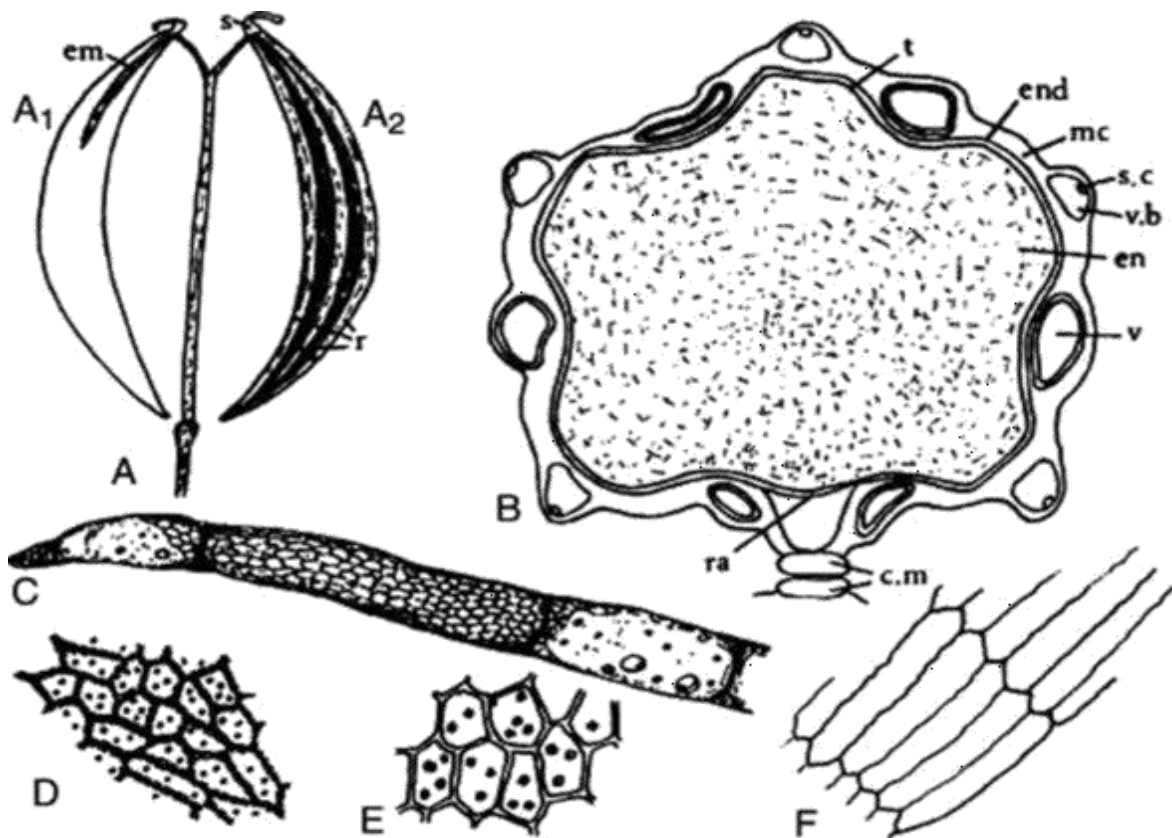
Aim: To identify the Transverse section of Fennel fruit.

Materials required: Hydrochloric acid, Phloroglucinol, Glycerin, Watch Glass, microscope slide. Cover slip, Blade, Brush and Compound Microscope.

Description:

- i- Pericarp a- Epicarp consists of thick-walled rectangular polygonal cells with smooth cuticle showing few anomocytic stomata and hairs.
- ii- Mesocarp is formed of rather thick-walled parenchyma traversed by 6 large vittae appearing elliptical in T.S. and having epithelial cells, and in the ridges by vascular bundles, each having 2 lateral phloem strands and an inner xylem accompanied by an upper and lower groups of characteristic lignified reticulate parenchyma. These thickened cells have large oval or rounded pits.
- iii- Endocarp it is formed of a single layer of narrow elongated cells arranged in groups of 6 or more cells, with their axes parallel but set obliquely to the long axes of the adjoining groups forming parquetry arrangement. II-Seed a- Seed-coat is thin formed of brownish tangentially elongated cells, within it is a collapsed hyaline layer. b- Endosperm is formed of thick-walled polygonal cellulosic parenchyma containing fixed oil, several aleurone grains enclosing a globoid and one or more micro rosette crystals of calcium oxalate.
- iv- Carpophore often not splitted, showing very thick-walled sclerenchyma in two strands

POWDER MICROSCOPICAL CHARACTER OF FENNEL FRUIT



A. mericarps showing attachments to carpophore;

A₁, mericarp sectioned longitudinally to show position of embryo;

A₂, mericarp side view (×8).

B, transverse section of mericarp (×50);

C, portion of vitta

D, sclereids of mesocarp;

E, Endosperm cells with micro-rosette crystals of calcium oxalate;

F, Endocarp layer in surface view.

B. POWDER MICROSCOPICAL CHARACTERS OF FENNEL FRUIT

Aim: To identify the Powder Microscopical characters of Fennel fruit.

Biological source:-Obtained from dried ripe fruits of *Foeniculumvulgare*

Family:-Umbelliferae.

Description:

1. Mesocarp: Lignified and reticulate nature of the 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.
5. Organoleptic characters:
 - (i) Colour: Yellowish- brown to greenish- brown powder.
 - (ii) Odour: Pleasant and aromatic odour.
 - (iii) Taste: Pleasant and aromatic.

Micro chemical Test

| Sr.no | Reagents | Observations | Characteristics |
|-------|------------------------------|--------------|---|
| 1 | Phloroglucinol+conc.HCl(1:1) | Red/pink | Lignified fibres,vascular bundles and pericyclic fibres |
| 2 | Alcoholic picric acid | Yellow | Aluerone grains |
| 3 | Sudan red III | Pink | Oil globules in the cells of endosperm and cuticle |

Uses: Stomachic, Aromatic, Diuretic, Carminative, Diaphoretic, Digestive, Pectoral, Antipyretic, Antimicrobial & Anti inflammtory.

Report:

The given crude drug was identified as _____ with the help of various Histological characters (T.S), powder microscopical characters.

CORIANDER

MORPHOLOGY OF CORIANDER

Aim: To identify the morphological characters of given organised drug.

Synonym: Dhania, coriander fruit

Biological source:

Dried ripe fruits of *Coriandrum sativum* Linn.

Family: Umbelliferae

It contains not less than 0.3% of volatile oil.

Organoleptic characters:

Shape: sub-spherical (globular)

Colour: Brownish yellow

Odour: Aromatic

Taste: spicy characteristics

Size: 2.3 to 4.3 mm diameter

Extra Features: cremocarps with a pedicel at the base, caelospermous fruit. There are ten wavy primary ridges alternating with eight straight secondary ridges on the surface. A short stylopod present at the apex.

Chemical constituents:

Volatile oil (0.2 -1%), coriandrol (D-Linalol 60-70%), Terpenes (20%), Fixed oil (13-20%), proteins (70%), small amount of borneol, geraniol, p-cymene and α -pinene are also present. Vitamin A also found in the coriander leaves.

Uses:

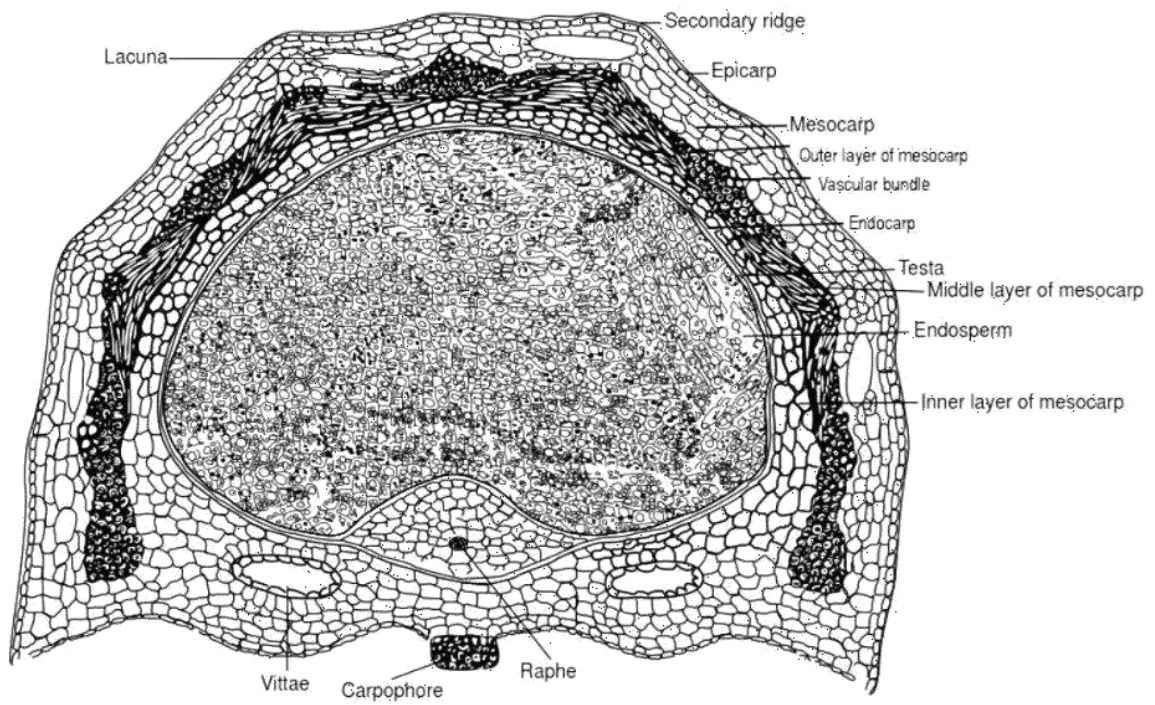
Carminative, aromatic, stimulant, spice, flavouring agent.

Report:

The given organised drug was identified as.....

TRANSVERSE SECTION OF CORIANDER FRUIT

Transverse Section of Coriander fruit



A. TRANSVERSE SECTION OF CORIANDER FRUIT

Aim: To identify the Transverse section of Coriander fruit.

Materials required: Hydrochloric acid, Phloroglucinol, Glycerin, Watch Glass, microscope slide. Cover slip, Blade, Brush and Compound Microscope.

Description:

The drug usually consists of the whole cremocarp, which are sub-spherical, 3-5mm in diameter, nearly glabrous, brownish– yellow or brown in color, each is crowned by 5 small sepals and a short conical stylopod. The mericarps are usually united by their margins. The dorsal surface of each mericarp shows 5 inconspicuous wavy primary ridges and 4 more prominent straight secondary ridges. The transverse section of fullyripe mericarp shows only 2 vittae in the commissural side but no vittae in the dorsal one, an almost complete ring of sclerenchyma in the dorsal side, a large oily endosperm and a small curved apical embryo. Coriander has aromatic odour and aromatic spicy and characteristic taste.

I-PERICARP

a- Epicarp is composed of polygonal tubular thick- walled cells and showing occasional small prismatic crystals of calcium oxalate, few anisocytic stomata and no hairs.

b- Mesocarp is formed of 3 different zones, the outer zone consists of few layers of tangentially elongated parenchymatous cells usually collapsed, showing degenerated vittae as tangentially flattened cavities and longitudinally traversed by 10 vascular stands with small spiral vessels. The middle zone is formed of a broad layer of sclerenchyma consisting of strongly lignified pitted fusiform fibres in 2 sinous bands crossing each other at right angles, the outer 5 to 6 rows run longitudinally while the inner, 1 to 3 rows run tangentially, in the secondary ridges almost all the cells run tangentially. The inner zone is composed of 2-3 rows of large tangentially elongated thin walled parenchyma. The inner most layer of the

mesocarp consists of flattened hexagonal thin – walled sclerenchyma. Mesocarp on the commissural side shows no sclerenchyma but two large elliptical yellowish brown vittae.

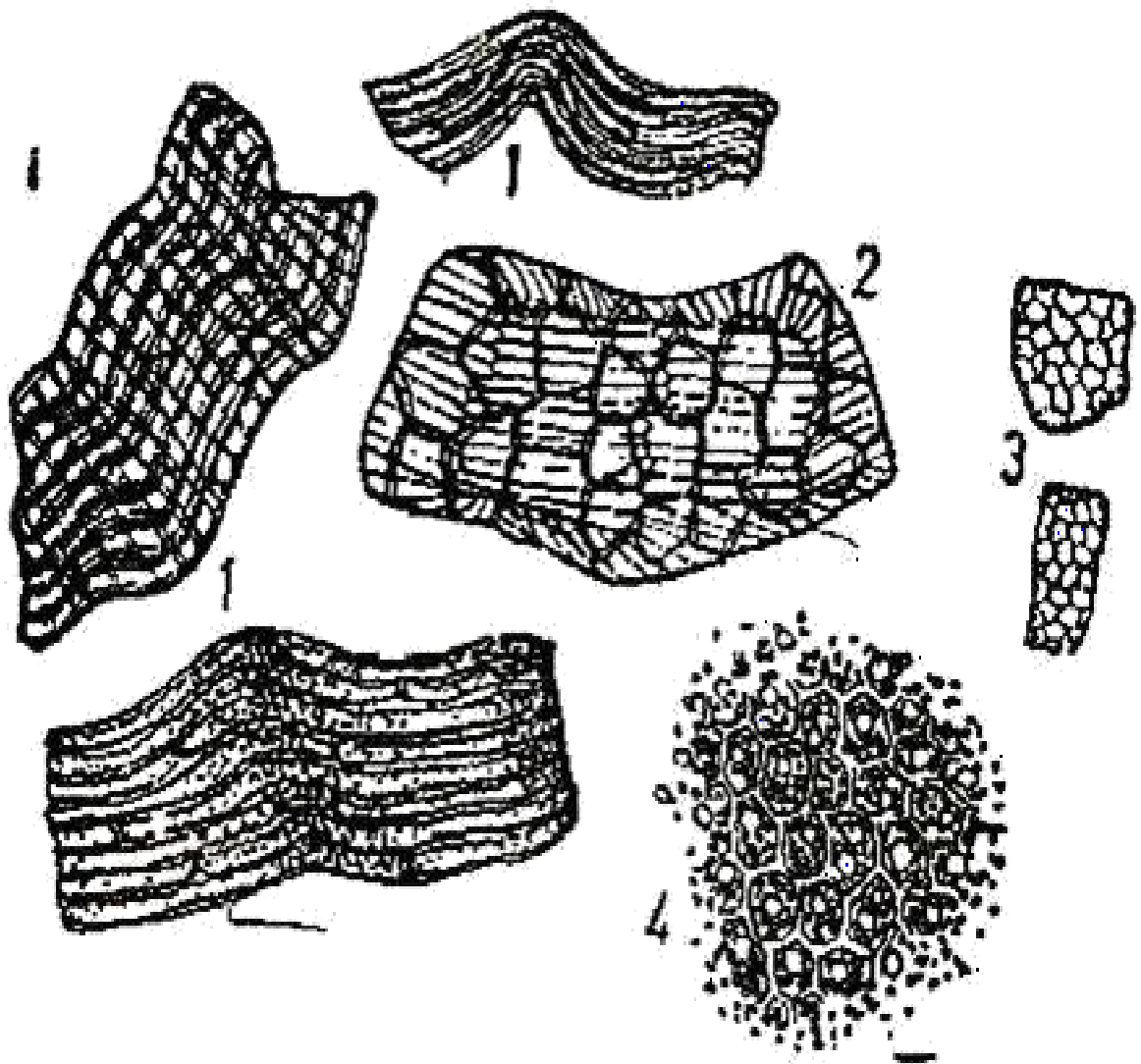
c- Endocarp is formed of very narrow elongated thin – walled cells, arranged in variously oriented groups i.e., parquetry arranged.

II- Seeds

The seed coat is formed of polygonal brown cells with narrow collapsed layer underneath. The endosperm is composed of thick- walled cellulosic cells containing fixed oil and aleurone grains including globoids and micro rosette crystals of calcium oxalate.

III- Carpophore, splits, passing at the apex of each mericarp into the raphe and at the base to the pedicel.

POWDER MICROSCOPICAL CHARACTERS OF CORIANDER POWDER



Identifying characters of coriander powder

B. POWDER MICROSCOPIC CHARACTERS OF CORIANDER FRUIT

Aim: To identify the Powder Microscopical characters of Coriander fruit

Biological source: It consists of the dried ripe fruits of *Coriandrum sativum*

Family: Umbelliferae

Description:

1. Sclerenchymatous layer: Groups of fusiform fibres of sclerenchyma running way 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 mesocarp.
3. Vittae: Few brown fragments of vittae.
4. Endosperm: Fragments of endosperm with aleurone grains and oil globules.
5. Organoleptic characters:
 - a. Colour: Brown powder
 - b. Odour: Characteristic, aromatic
 - c. Taste: Spicy

Micro chemical Test

| Sr.no | Reagents | Observations | Characteristics |
|-------|------------------------------|--------------|---|
| 1 | Phloroglucinol+conc.HCl(1:1) | pink | Lignified sclerenchyma,vascular bundles |
| 2 | Alcoholic picric acid | Yellow | Aluerone grains |
| 3 | Sudan red III | Pink | Oil globules and cuticle |

USES: - Carminative, Stimulant.

Report

The given crude drug was identified as _____ with the help of various Histological characters(T.S), powder microscopical characters.

ISOLATION AND DETECTION OF ACTIVE PRINCIPLES

ISOLATION AND DETECTION OF CAFFEINE FROM TEA DUST

Introduction

Caffeine is one of the most important naturally occurring methyl derivatives of xanthine. In black tea the concentration of caffeine is 0.5%, theobromine 0.17% and theophylline 0.13%. Tea consist of fresh, dried or prepared leaves and leaf buds of *Thea sinensis*.

Family: Theaceae.

Aim

To extract caffeine from tea dust

Materials required

Processed tea leaf powder, sodium carbonate, 10% sulphuric acid, chloroform, water, beaker, stirrer, funnel, separating funnel, filter paper, muslin cloth.

Principle

The tea leaves or dust extracted with water and treated with sodium carbonate solution. Tannins precipitated at sodium tannate the excess of sodium can be removed as sodium sulphate by treatment with 10% sulphuric acid. Then filtrate contain caffeine in extract with chloroform evaporated to get crystal of caffeine.

Uses: CNS stimulant

Procedure:

About 50 g of finely powdered tea leaves are taken in a beaker. Add 10 g of sodium carbonate and 250 ml of water. The mixture is boiled till a decoction of tea is formed. Take care to avoid the thickening of liquid while boiling filter the hot solution and neutralise itwith 10% sulphuric acid. The neutralised filtrate is then extracted with chloroform in successive quantities and care must be taken to avoid emulsion formation. The chloroform extracts are combined and concentrate the solvent.

Chemical test:

1. Murexide Test:

In porcelain dish add caffeine and 3 drops of nitric acid. Evaporate to dryness and add 2 drops of ammonium hydroxide solution in the residue, a purple colour is produced.

TLC

Dissolve 1 mg caffeine in chloroform or methanol. Spot the sample on TLC plate and elute it in ethyl acetate- methanol- acetic acid (8:1:1). Visualize the dried TLC plate by exposure to iodine vapour. Caffeine develops a spot at R_f value 0.41.

Calculation:

Weight of the processed tea leaf powder =

Weight of the isolated caffeine =

$$\text{Percentage yield of isolated caffeine} = \frac{\text{Weight of the isolated caffeine}}{\text{weight of the tea powder taken}} \times 100$$

R_f Value of the isolated caffeine

$$R_f \text{ Value calculation} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Report:

1. The Percentage yield of isolated caffeine was found to be _____
2. The R_f Value of the isolated caffeine was found to be _____

ISOLATION AND DETECTION OF DIOSGENIN FROM DIOSCOREA

Introduction

Diosgenin is a steroidal saponin obtained by initial hydrolysis of dioscin which is present in the tubers of various dioscorea species such as *D. deltoidea*, *D. floribunda*, *D. composite* Family: Dioscoreaceae. It is present to the extent of 2-5% in dioscorea tubers. Diosgenin is an important starting material for the synthesis of steroidal hormones. It is converted to 16-dehydropregnenolone acetate which is used as a substrate for various types of steroidal drugs such as corticosteroids, sex hormones, oral contraceptives, spiranolactones etc. Diosgenin is used as a pharmaceutical aid for the synthesis of various steroidal drug.

Properties:

Colour: white crystalline powder

Solubility: organic solvents and acetic acid

Chemical constituents: Dioscin, Diosgenin

Aim:

To extract Diosgenin from Dioscorea

Requirements:

Chemicals: Rhizomes powder, HCL, sodium bi carbonate, toluene, hexane

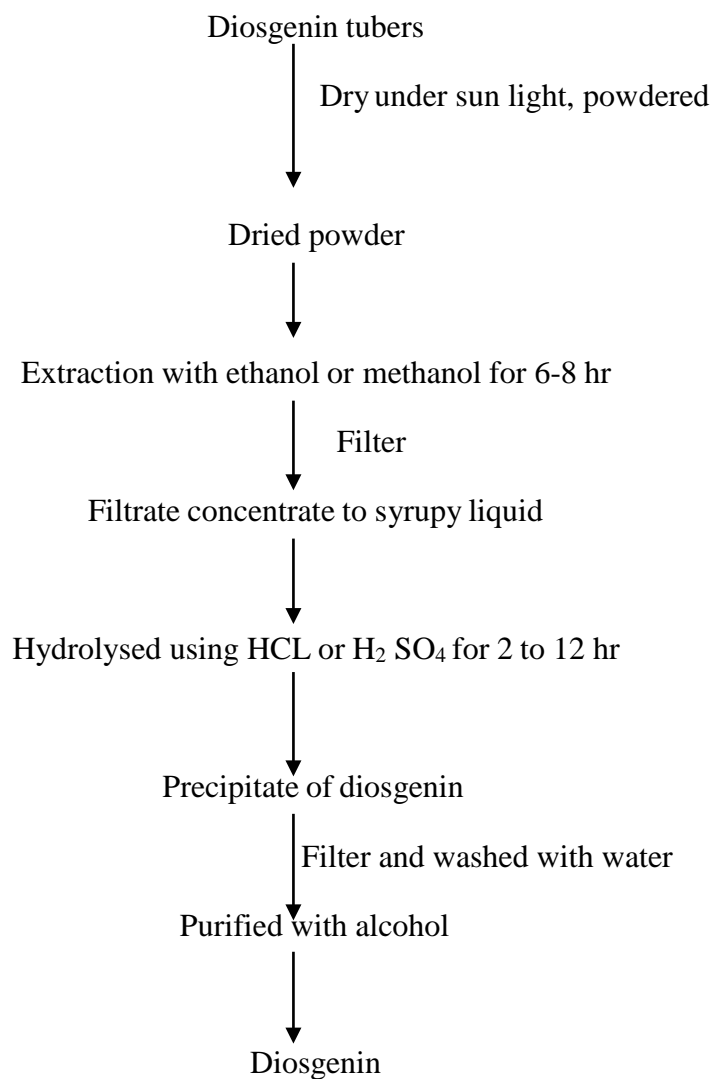
Apparatus: funnel, Round bottom flask, filter paper

Procedure:

Alcoholic extraction method:

1. The diosgenin tubers are cut into small pieces and dried under sun. The dried tubers are powdered, extracted with ethanol or methanol twice for 6-8hr.
2. Filter the extract and filtrate is concentrate to syrupy liquid which is then hydrolysed using HCL or H₂SO₄ for 2 to 12 hr. About 85% of the crude diosgenin is precipitate.
3. The precipitates are filtered, washed with water and purified with alcohol.

FLOW CHART DIAGRAM OF DIOSGENIN ISOLATION



TLC:

Dissolved 1 mg diosgenin in 1 ml methanol. silica gel-G plates spotted with the sample are eluted in solvent system Toluene : ethyl acetate (7:3). The dried plates are sprayed with anisaldehyde- sulphuric acid reagent and heated at 110° C for 10 min. Dark spot is observed in day light at R_f Value 0.37

Calculation:

1. Percentage yield of isolated diosgenin

Weight of the dried powder =

Weight of the isolated diosgenin =

$$\text{Percentage yield of isolated diosgenin} = \frac{\text{Weight of the isolated diosgenin}}{\text{weight of the dried powder taken}} \times 100$$

2. R_f Value of the isolated diosgenin

$$\text{R}_f \text{ Value calculation} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Report:

3. The Percentage yield of isolated diosgenin was found to be _____

4. The R_f Value of the isolated diosgenin was found to be _____

ISOLATION AND DETECTION OF ATROPINE FROM BELLADONA

Introduction

Atropine is a tropane alkaloid from the members of the solanaceae family. It is present in *Atropa belladonna*, *Datura stramonium*, and *hyoscyamus niger*. Tropanealkaloids also known as solanaceous alkaloids, belladonna alkaloids which includes atropine, scopolamine (hyoscine), belladonine, hyoscyamine, apoatropine and norhyoscyamine.

Atropine isolated from the juice or the powdered drug. *Hyoscyamus muticus* having high alkaloidal content hence preferred for manufacturing of atropine and then *D. stramonium* is next in order.

Uses: Atropine is used as an antispasmodic, mydriatic and anti cholinergic.

Atropine first stimulates and then depresses the CNS.

Atropine dilates the pupils of the eyes hence used in ophthalmology.

Properties:

Atropine is an optically inactive levorotatory isomer of hyoscyamine.

Colour: colourless needle like crystals or white crystalline powder

Odour: odourless

Taste: strong poison with sharp bitter test

Solubility: alcohol, benzene, dilute acids and sparingly soluble in water.

Aim

To extract Atropine from belladonna.

Requirement

Chemicals: Belladonna leaves, sodium carbonate, ether, benzene, acetic acid

Apparatus: Beaker, round bottom flask, vacuum filtration assembly.

Procedure:

1. The powdered drug material is thoroughly moistened with an aqueous solution of sodium carbonate and then extracted with ether or benzene.
2. The alkaloidal free bases are extracted from solvent with water acidified with acetic acid.
3. The acid solution is then shaken with solvent ether or acetone and dehydrated with anhydrous sodium sulphate before filtration.
4. Concentrate the filtrate which yields crude crystals of hyoscyamine and atropine from the solution.

- The crude crystalline mass is dissolved in alcohol and sodium hydroxide solution is added and the mixture is allowed to stand until hyoscyamine is completely racemized to atropine which is indicated by the absence of optical activity.
- Crude atropine is purified by crystallization from acetone. Atropine sulphate is the most important salt of atropine.

Chemical Test:

1. Vitali-morin reaction

Treat Dil atropine solution with con nitric acid and the mixture evaporated to dryness on the steam bath, produces a pale yellow residue. The residue gives a violet colouration when a drop of freshly prepared solution of potassium hydroxide is added.

2. TLC

1% atropine solution dissolved in 2N acetic acid is spotted over silica gel G plate and eluted in the solvent system of strong ammonia solution-methanol (1.5:100). TLC plates is sprayed with an acidified iodoplatinate solution. Atropine gives R_f value 0.18.

Atropine sulphate shows the R_f value 0.70, in the solvent system acetone 0.5 M sodium chloride and spraying with Dragendorffs reagent.

Calculation:

1. Percentage yield of isolated Atropine

Weight of the dried powder =

Weight of the isolated Atropine =

$$\text{Percentage yield of isolated Atropine} = \frac{\text{Weight of the isolated Atropine}}{\text{weight of the dried powder taken}} \times 100$$

2. R_f Value of the isolated Atropine

$$R_f \text{ Value calculation} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Report:

- The Percentage yield of isolated Atropine was found to be _____
- The R_f Value of the isolated Atropine was found to be _____

ISOLATION AND DETECTION OF SENNOSIDES FROM SENNA

Introduction

Sennosides are obtained from *cassia angustifolia* (Tinnevely senna), *cassia acutifolia* (Alexandrian senna). Sennosides are the dimeric anthroquinone glycosides. Sennoside A and B is a pair of stereoisomer containing rhein dianthrone (sennedine A and B) as the aglycon. Sennoside D and E are the dianthrone of aloe-emodin and rhein.

Purgative activity of senna is mainly due to sennoside A and sennoside B while sennoside C and D exerts a powerful synergistic effect upon the purgative activity.

Properties:

Colour: Brownish powder

Solubility: Alcohol, sparingly soluble in acetone.

Chemical constituent: sennoside A, sennoside B.

Aim:

To extract sennoside from senna.

Requirements:

Chemicals: senna leaves or pods, benzene, ethanol, methanol.

Apparatus: vacuum filter unit, electric shaker, desiccators

Procedure:

Method 1:

1. Extract coarsely powdered senna leaves or pods with benzene and 1-5% ethanol to remove pigments and resins.
2. The dried marc extracts with ethanol and concentrates under at 40°C.
3. Alcoholic extract mixed with the solution of calcium chloride in methanol and the solution is filtered.
4. Add methanolic ammonia in the filtrate until red brown colour disappears.
5. The precipitated material is filtered and washed with methanol and dried.
6. The precipitate containing calcium sennosides is suspended in methanol and acidified with gluconic acid at 40°C.
7. Filter the acidified extract which gives yellow mass of sennoside A.
8. The filtrate when treated with methanolic hydrobromic acid and subsequently evaporated to produce sennoside B.

Method 2:

1. Extract 100 g powdered leaves with 300 ml benzene for 2 hr on electric shaker, filter in vacuum and distill off the solvent.
2. The dried marc extract with 300 ml 70% methanol on shaker for 4-6 hrs, filter under vacuum and the marc re-extract with 200 ml of 70% methanol for 2 hr; filter and combine methanolic extract.
3. Concentrate methanolic extract and acidify to P^H 3.2 by addition of HCL with constant stirring. Set aside the mixture for 2 hr at $5^{\circ}C$. Filter under vacuum and add 1g anhydrous calcium chloride in 13 ml denatured spirit with vigorous shaking.
4. Adjust P^H of the solution to 8 by addition of ammonia solution and set aside for 2 hr; filter the solution by vacuum and dry precipitate over phosphorous pentoxide in a desiccator.

Chemical test:

Borntrager test:

Boil drug with dil sulphuric acid (hydrolysis). Filter and cool. Add benzene or CCL_4 (Immiscible organic solvents). Shake and separate organic solvent layer in another test tube. Add strong ammonia solution, shake slightly and keep the test tubes aside, lower ammoniacal layer shows pink or red colour.

TLC

Dissolve 1 mg sennoside in 1 ml solvent containing equal volumes of ethyl acetate, n-propanol and water (upper layer). The silica gel -G plates spotted with the sample and eluted in solvent system ethyl acetate: n-propanol: water (4:4:3). The dried plate is exposed to vapours of ammonia for 5 min till the colour develops. Cover the plate with glass and heat at $110^{\circ}C$ for 5-10 min sennosides A and B develop two prominent spots.

Calculation:

1. Percentage yield of isolated sennoside

Weight of the dried powder =

Weight of the isolated sennoside =

$$\text{Percentage yield of isolated sennosides} = \frac{\text{Weight of the isolated sennoside}}{\text{weight of the dried powder taken}} \times 100$$

2. R_f Value of the isolated sennosides

$$\text{R}_f \text{ Value calculation} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Report:

1. The Percentage yield of isolated sennoside was found to be _____
2. The R_f Value of the isolated sennoside was found to be _____

3.0 SEPERATION OF SUGARS BY PAPER CHROMATOGRAPHY

Principle

The term chromatography comes from the earlier times when the technique was used for the separation of colored plants pigments. chromatography is a technique for separation of closely related groups of compounds. The separation is brought about by differential migration along a porous medium and the migration is caused by the flow of solvent.

Within limits chromatography can be divided into two types: partition and adsorption chromatography.

Paper chromatography is an example of liquid-liquid chromatography.

In this type of chromatography separation is due to differential partition of solutes between two liquid phases.

One liquid phase is bound to the porous medium for example; the water bound in the cellulose paper, this phase is referred to as, the stationary phase. The other liquid phase, the mobile phase flows along the porous medium.

As the mobile phase flows over the solute mixture, the individual solutes partition themselves between the aqueous stationary phase and the organic mobile phase relative to their solubility in the two phases.

The more soluble a solute in the mobile phase, the faster it will travel along the paper, and conversely, the mobile phase must be a mixture in which the compounds to be separated are soluble or partially soluble.

In paper chromatography solute or solute mixture is spotted in solution along a base line on a sheet of filter (whatman NO.1).The mobile phase(solvent) is allowed to flow over the spots either ascending the paper by capillary action or descending te paper by gravity.

The separation is measured in terms of a unit called R_f(relative rates of flow)with respect to the solvent front.

$$R_f \text{ Value calculation} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

The R_f value of a compound in a particulare solvent system is constant under identical conditions of the experiment, e.g.temperature,pH,etc.

Because most compounds are colorless the spots are visualized after separation by specific reagent. The location reagent is applied by spraying the paper or rapidly dipping it in a solution of the reagent in a volatile solvent. viewing under ultraviolet light is also useful since some compound which absorbs it strongly show up as dark spots against the fluorescent background of the paper.

Materials:

Paper: Whatman filter paper

Solvents:

- a. Water saturated phenol+1% ammonia
- b. N-butanol-acetic acid-water(4:1:5 v/v)
- c. Isopropanol-pyridine-water-acetic acid(8:8:4:1 v/v)

Spray Reagent:

A. Ammonical silver nitrate:

Add equal volumes of NH_4OH to a saturated of AgNO_3 and dilute the methanol to give a final concentration of 0.3M. After spraying the developed chromatograms place them in an oven for 5-10 minutes, when the reducing sugars appear as brown spots.

B. Alkaline permanganate:

Prepare aqueous solution of KMnO_4 (1%) containing 2% NaCO_3 . After spraying with this mixture the chromatograms are kept at 100°C for a few minutes, when the sugar spots appear as yellow spots in purple background.

C. Aniline diphenylamine reagent:

Mix 5 volumes of 1% aniline and 5 volumes of 1% diphenylamine in acetone with 1 volume of 85/5 phosphoric acid. After spraying the dried chromatograms with this solution the spots are visualized by heating the paper at 100°C for a few minutes

D. Resorcinol reagent:

Mix 1% ethanolic solution of resorcinol and 0.2N HCl (1:1 v/v). spray the dried chromatograms and visualize spots by heating at 90°C .

Procedure:

1. Place sufficient solvent into the bottom of the tank. cover the lid and allow the tank to be saturated with the solvent.
2. Take a sheet of whattman 1 chromatography paper (about 9*10cm) and place it on a piece of clean paper on a bench.
3. Draw a fine line with a pencil along the width of the paper and about 1.5cm from the lower edge.
4. Along this line place four equality spaced (about 2cm apart) small circles with a pencil.
5. Label the paper at the top with the name of each of the sugars and label the last unknown.
6. Use a fine capillary or tooth pick to place the drops of the solutions of the sugars, glucose, fructose, maltose, lactose and the mixture.
7. After spotting dry the paper with hot air dryer for one minute, repeat this step again.
8. Place the spotted paper in the chromatographic tank and make the development by using the ascending technique.
9. Close the tank with lid allow the solvent to flow for about 30-45 minutes.
10. Remove the paper and immediately mark the position of the solvent front with a pencil.
11. After the chromatogram has dried spray the paper with the locating reagent.
12. You need to put the paper on the hot plate at low temperature or expose it to the hot air dryer, until the colored spots appear. The colors are stable for some weeks if kept in the dark and away from acid vapors.
13. Circle the position of each spot with pencil.
14. Calculate the Rf value for each spot and also for the spot the mixture contained.

General summary of the behaviour of the various sugars to these reagents are given below:

| Sugars | a | b | c | d |
|---------------|----------|----------|----------|------------|
| Aldohexoses | + | + | + | pink |
| Ketohexoses | + | + | + | red |
| Aldopentoses | + | + | + | blue,green |
| Ketopentoses | + | + | + | - |
| Deoxy sugars | - | + | + | - |
| Glycosides | + | - | - | - |

Amino sugars + + + -

The table below R_f values of some sugars in the solvents previously mentioned. They are only for comparative purposes, since R_f varies with physical parameters.

| Sugar | solvent a | solvent b | solvent c |
|--------------|-----------|-----------|-----------|
| Glucose | 0.39 | 0.18 | 0.64 |
| Galactose | 0.44 | 0.16 | 0.62 |
| Fructose | 0.51 | 0.25 | 0.68 |
| Ribose | 0.59 | 0.31 | 0.76 |
| Deoxy ribose | 0.73 | - | - |
| Lactose | 0.38 | 0.09 | 0.46 |
| Maltose | 0.36 | 0.11 | 0.50 |
| Sucrose | 0.39 | 0.14 | 0.62 |

Precaution:

1. Saturation of chamber has been done so as to avoid the unequal solvent evaporation losses from developed plate which leads to various types of random behaviour and edge effects.
2. The beaker is often lined with some filter paper soaked in solvent to help saturating the atmosphere in the beaker with vapours.
3. The solvent level should be kept below the spots. Generally ascending method is used.

Report:

1. The R_f value of the given sample was found to be _____

THIN LAYER CHROMATOGRAPHY OF HERBAL EXTRACTS
SEPERATION OF ACTIVE CONSTITUENTS OF CLOVE BY THIN LAYER
CHROMATOGRAPHY

Botanical source:

It consist of the dried flower buds of *Eugenia caryophyllus* belonging to the family:

Myrtaceae.

Active constituents:

It contains volatile oil, 14 to 20%, gallotannic acid, 10-13% caryophyllin, a white, odourless, tasteless, crystallizing silky needles, vanillin, eugenin. It contains more than 85% of eugenol.

Uses: Clove is an aromatic, flavouring agent, used as local anaesthetic agent in dentistry.

Procedure:

Preparation of plate:

Thin layer glass plates are used as supporting medium. Mix the adsorbent (30g) in a mortar to a smooth consistency with the required amount of water and spread the slurry on the plate so as to get a thickness of 1-2 mm. Allow it to dry and keep it for activation for 1 hr at 120° C. Cool and use the plates for separation.

Extraction of oil.

Take clove flower buds and powder them. Add toluene (5ml) to the powder (1g) and shake it for some times. Filter and concentrate the filtrate. Use this as sample.

| | |
|---------------------------|---|
| Standard sample | : Eugenol |
| Stationary phase | : silica gel G |
| Layer thickness | : 1 mm |
| Preparation and thickness | : Activate for 1 hr at 120°C. |
| Separation technique | : Adsorption |
| Mobile phase composition | : Toluene: Ethyl Acetate (93:7) |
| Amount applied | : 10 microlitre |
| Detecting agent | : 1. Vanillin in sulphuric acid 2. Ferric chloride solution |

Calculation:

$$R_f \text{ Value calculation} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Report:

The R_f Value of the different spots of the clove extract:

The R_f Value of the eugenol _____

SEPERATION AND IDENTIFICATION OF CURCUMINOIDS FROM CURCUMIN

Aim

To separate and identify the curcuminoid present in turmeric by thin layer chromatography.

Sample preparation:

The sample is extracted with methanol on a water bath. Cool and filter. The filtrate is used for TLC studies.

Adsorbent used for TLC studies is silica gel G coated on glass plate.

Mobile phase: chloroform: ethanol: glacial acetic acid (95:5:1)

Procedure:

The solvent system is prepared in the given ratio and then taken in a chamber and kept for saturation. The extract is placed as a spot on TLC plate and kept in the chamber at an angle of 45°. After 3/4th movement of solvent the plate is taken out and mark the solvent front. Then the plate is dried in air and detected in UV light.

Calculation:

$$R_f \text{ Value calculation} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Report:

The R_f Value of the given sample of turmeric extract was found to be _____

SEPERATION AND IDENTIFICATION OF PIPERINE FROM PEPPER

Aim

To identify piperine by performing thin layer chromatography.

Preparation of plate:

Thin layer glass plates are used as supporting medium. Mix the adsorbent (30g) in a mortar to a smooth consistency with the required amount of water and spread the slurry on the plate so as to get a thickness of 1-2 mm. Allow it to dry and keep it for activation for 1 hr at 120° C. Cool and use the plates for separation.

Extract: Aqueous and alcoholic extract of piper nigrum

Stationary phase: Silica gel – G

Mobile phase : Toluene : Ethyl acetate (7:3)

Spraying agent : vanillin in sulphuric acid

Procedure:

Solvent system is prepared and taken in the chamber and kept it for saturation about 30 min. sample is placed on the plate above 1 cm of the plate by using capillary tube. Place the plate into the chamber at an angle of 45°. Develop the chromatogram by ascending technique till the solvent front moved by 3/4th of the TLC plates. The plates are withdrawn from the chamber and air dried. The plate is sprayed with vanillin sulphuric acid and dried in hot air oven. Appearance of yellow colour spot indicates the presence of piperine.

Calculation:

$$R_f \text{ Value calculation} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Report:

The R_f Value of the piperine was found to be _____

5.0 DISTILLATION OF VOLATILE OILS AND DETECTION OF PHYTOCONSTITUENTS BY TLC

Volatile oil or essential oils or ethereal oils are the odorous principles of the plants. Being volatile at the temperature of boiling water or steam, mostly essential oils are hydro distilled or steam distilled for their separation from the crude drugs. colour, odour, taste, density, specific rotation, refractive index, boiling range and solubility are some important parameters used in determining the purity of volatile oils.

Methods of extraction of volatile oil

1. Distillation
2. Enfleurage
3. Expression
4. Maceration
5. solvent Extraction

Extraction by distillation

Most common method for the production of volatile oil is distillation>In general ,the term distillation applies to vaporization process in which the vapours evolved are recovered,usually by condensation.

Distillation apparatus is basically consists of three parts

- a. Distillation flask: Round bottom 1 litre boiling flask
- b. condenser: provide cooling to avoid reflux of the distillate
- c. Receiver: Allow separation of the oily layer from water in the distillate

Isolation of caraway oil from caraway fruit by hydro-distillation method. (volatile oil lighter than water)

Principle:

Hydro-distillation is based on distilling the drug with water and/or glycerine and collecting distillate in a graduated tube from which the aqueous portion of distillate is automatically returned to the distillation flask.

Biological source

The caraway oil obtained from the dried fruits of *carum carvi*. Family: *umbelliferae*. Dried ripe fruits of caraway should contain not less 2.5% v/w of volatile oil.

The characteristics of the caraway oil are as given below:

Colour: pale Yellow

Odour and taste: Aromatic and characteristic

Weight per ml: 0.90 to 0.91

Solubility: soluble in 8 parts of 80% alcohol

Optical rotation: At 250+700 + 800

Content of carvone: 53 to 63% w/w

Apparatus: Heating mantle, Volatile distillation unit of 1 litre capacity.

Chemicals: powdered drug

Procedure:

1. Take 50g of powdered drug in 1 litre of distillation flask together with 250 ml of water. Add few pieces of porcelain to it in order to avoid bumping.
2. place the distillation flask on the heating mantle and set the distillation assembly. fill the graduated receiver with water avoiding any air bubbles. do not tighten the outlet near the upper end of the receiver. instead loosely pack it with cotton.
3. Distillation should be done for 4 hours. Allow the distillation to be collected in the graduated receiver in which the aqueous portion of the distillate is automatically separated and returned to the distillation flask.
4. Measure the volume of volatile oil which separates out as the upper layer in the graduated tube and calculate the % v/w on a dry weight basis.

TLC of carvone:

Dissolve 1 mg of carvone in 1ml of methanol and apply the spot over silica gel- G plate. Elute the plate with toluene-ethyl acetate (93:7) as a solvent system. Spray the dried plate with vanillin sulphuric acid reagent carvone shows the spot with red violet colour at Rf 0.46.

Storage: volatile oil should be stored in well closed well filled containers away from light and in cool place

Precautions:

1. Add a few pieces of porcelain to it in order to avoid bumping during distillation.
2. Do not tighten the outlet near the upper end of the receiver. Instead loosely pack it with cotton.

Calculation:**Percentage yield of isolated volatile oil**

Weight of the powder taken =

volume of the isolated volatile oil =

$$\frac{\text{volume of the isolated volatile oil}}{\text{weight of the powder taken}} \times 100$$

Percentage yield of isolated volatile oil =

R_f Value of the carvone

Distance travelled by the solute

R_f Value calculation = $\frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$

Report:

1. The Percentage yield of isolated volatile oil was found to be _____
2. The R_f Value of the carvone was found to be _____

ISOLATION OF CLOVE OIL FROM CLOVE BUDS(VOLATILE OIL HEAVIER THAN WATER)

Principle:

Eugenol is 4-allyl-2-methoxy phenol obtained from the essential oil of clove buds *Eugenia caryophyllus* Family *myrtaceae*. clove oil contain 80 to 90% of eugenol. Dried clove buds are hydro distilled to yield the clove oil. Being heavier than water it makes a layer beneath water

The characteristics of the Eugenol are as given below:

Colour: colourless or pale yellow liquid

Odour: odour of clove and spicy

Taste: Pungent

Solubility: Alcohol, chloroform, and ether

Insolubility: water

Specific gravity: 1.038 to 1.060

Refractive index: 1.527 to 1.535

Content of Eugenol: 15% w/w

Apparatus: Heating mantle, volatile distillation unit of litre capacity

Chemicals: powdered drug

Procedure:

1. Take 20gm powdered drug in a 1 litre of distillation flask together with 250ml of water. Add few pieces of porcelain to it in order to avoid bumping.
2. Place the distillation flask on the heating mantle and set the distillation assembly. Boil the mixture for 2 hours.
3. Separate the oil and add few grams of anhydrous sodium sulphate to remove water residue
4. Calculate the oil content in ml per 100g of plant material

TLC of Eugenol:

Dissolve 1 mg of eugenol in 1 ml of methanol and apply the spot over silica gel-G plate. Elute the plate with pure benzene as a solvent system. Spray the dried plate with 1% anisaldehyde-sulphuric acid reagent and heat the plate at 110°C for 10min. Eugenol shows the spot with dirty green colour at R_f 0.40 in case of normal chamber saturation at 24°C.

Storage: volatile oil should be stored in well closed well filled containers away from light and in cool place

Precautions: Add a few pieces of porcelain to it in order to avoid bumping during distillation.

Calculation:

Percentage yield of isolated volatile oil

Weight of the powder taken =

volume of the isolated volatile oil =

$$\text{Percentage yield of isolated volatile oil} = \frac{\text{volume of the isolated volatile oil}}{\text{weight of the powder taken}} \times 100$$

R_f Value of the eugenol

$$\text{R}_f \text{ Value calculation} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Report:

1. The Percentage yield of isolated volatile oil was found to be _____
2. The R_f Value of the eugenol was found to be _____

ANALYSIS OF CRUDE DRUGS BY CHEMICAL TEST

UNORGANIZED DRUG:

Unorganized drug are the material having a structure that is fairly uniform throughout and are not composed of cells. They are usually derived from parts of plant or animals by various processes of extraction, decoction, Expression or are natural secretion such as Bees wax and Myrrh

Unorganized drugs can be classified based upon their origin and nature:

- A. Dried latex: e.g. Opium
- B. Dried juice: e.g. Aloes'
- C. Extracts: e.g. Catechu
- D. Gum: e.g. .Acacia
- E. Resin: e.g. Colophony
- F. Gum Resins: e.g. Myrrh
- G. Oleo-resins: e.g. Copaiba
- H. Waxes: e.g. Bees wax
- I. Saccharine substances: e.g. Honey
- J. Oils and fats: e.g. Castor oil, Lard
- K. Volatile oil: e.g. clove oil

ASAFOETIDA

Synonyms: Devil's drug, Hing, Gum asafoetida

Biological source: It is an oleo-gum resin of living roots and rhizomes of *Ferula foetida*, *F. rubricalis*, *F. asafoetida* etc and other species of *Ferula* belongs to family *Umbelliferae*.

Physical Characteristics:

Colour: yellowish brown to reddish brown tears

Odour: Intense, penetrating, persistent alliaceous

Taste: Bitter, acrid, alliaceous

Shape: It occurs in two forms viz tears and masses

Tears are rounded or flattened more or less agglutinated together.

Mass consists of agglutinated tears with foreign mass like stone, earth, pieces of roots, calcium sulphate and it is of inferior quality as compared to tears

Size: 0.5×4.0cm in diameter

Solubility: Partly soluble in alcohol

Extra Feature:

Fresh tears are tough, dried are hard and brittle. Tears are internally milky white yellowish, translucent or opaque mass consists of agglutinated tears with foreign materials and impurities

Chemical constituents: Resin (40 to 60%) mainly Asaresinotannol in free or combined form with ferulic acid, pinene, vanillin and asaresene; gum (20 to 25%) and volatile oil (4 to 20%) contains isobutyl propanyl disulphide which gives alliaceous odour to drug.

Uses: carminatives, laxative, antispasmodic, Nervine tonic, anthelmintic and digestive

It is used to treat flatulence colic, constipation, asthma, bronchitis, whooping cough and epilepsy.

It is also used as flavouring agent in sauces, pickles and curries.

Precautions:

1. Avoid direct contact with any hazardous chemical.
2. Do not mouth pipette

Chemical tests/Identification tests:

| Sr.No | Test | Observations |
|-------|--|---------------------------------------|
| 1 | Fractured surface is treated with sulphuric acid now washed with water | Reddish brown colour Violet colour |
| 2 | Asafoetida treated with sulphuric acid | Reddish brown colour |
| 3 | Fractured surface treated with 50% nitric acid | Green colour |
| 4 | Asafoetida triturated with water | Yellowish orange emulsion |
| 5 | 10ml alcoholic extract of drug + conc HCl + phloroglucinol few drops | Pink colour |
| 6 | Asafoetida on burning | Yellow flame |
| 7 | Combined umbelliferone test: 0.5 g drug+ sand+3ml HCl +3ml water and triturated for several minutes and boil Add strong ammonia solution to the filtrate | Blue fluorescence |

Report:

From the above morphological characters and chemical test the given crude drug was identified as _____

BENZOIN

Synonyms: Sumatra benzoin, loban, siam benzoin

Biological source: Balsamic resin obtained from the incised stem of syntax benzoin or styrax paralleloneurus, s.tonkinensis belongs to family styraceae

Physical characteristics:

Colour: greyish brown or grey masses

Odour: agreeable and balsamic

Taste: sweetish and slightly acrid

Size: varying in size

Shape: tears, masses, lumps

Uses: Expectorant

| Characteristics | Sumatra benzoin | Siam benzoin |
|---|---|--|
| Physical: Odour Taste Extra features Solubility | Greyish brown or grey Aromatic and characteristics Sweetish and slightly acrid Lumps of varaying sizes and tears which are externally yellowish milky white Alcohol | Yellowish brown to rusty brown Vannila like Sweetish and slightly acrid Hard and brittle masses.soften and on heating becomes plastic. Alcohol |
| Standards: Acrid insoluble ash Benzoic acid extractive Loss on drying | Not more than 1% Not less than 6% Not less than 75% Not more than 10% | Not more than 0.5% Not less than 12% Not less than 90% Not more than 10% |
| Chemical constituents | Free balsamic acids(benzoic and cinnamic acid), Triterpenoid acids like summaresionlic acid | An ester coniferyl benzoate(75%) styol,vanillin and phenyl propyl cinnamate |

| | | |
|--|---------------------------|--------------------------|
| Chemical test 1. Warm 0.5g benzoin powder+10ml KMNO ₄ | Odour of benzaldehyde | |
| 2. 1ml ether extract of benzoin +2 -3 drops of H ₂ SO ₄ | Deep reddish brown | Deep purplish red colour |
| 3. Alcoholic extract benzoin powder+alcoholic solution of FeCl ₃ | Green colour | Negative |
| 4. Digest 0.5g of drug +5ml ether 5 min take 1 ml of ethereal solution in a porcelain dish+conc sulphuric acid | Deep reddish brown colour | Deep purplish red |

Chemical tests/Identification tests:

| Sr.no | Test | Observations |
|-------|---|---|
| 1 | 0.5g benzoin powder heated with 10ml of KMNO ₄ | Odour of benzaldehyde |
| 2 | Alcoholic solution of benzoin+water | Milky solution(acidic to litmus) |
| 3 | Heat 0.5g benzoin powder slowly in dry test tube | Evolves irritating whitish fumes which condense to form whitish crystalline sublimates at upper part of test tube |
| 4 | Sublimates after cooling | Crystals of cinnamic acids |

Report:

From the above morphological characters and chemical test the given crude drug was identified as _____

COLOPHONY

Synonym: Rosin, Rosina, colophonium, Amber resin, resin

Biological source: colophony is the solid residue obtained after distilling the oleo-resin from various species of pinus (p.palustris, p.longifolia, p.radiata etc)

Family: pinaceae

Physical characteristics:

Colour: amber or pale yellow

Odour: turpentine like

Taste: slightly bitter

Solubility: alcohol, ether, chloroform, and light petroleum

Insolubility: water

Extra features: brittle and readily fusible glassy masses

Standards:

Melting point: 75 to 85°C

Acid value: Not less than 150

Saponification value: 188 to 192

Ash value: NMT 0.125%

Chemical constituents: Colophony contains 90% of abietic acid (resin acid), 5 to 6 % of resene, and 0.5% of volatile oil. Other acid present are sapinic acid, pimaric acid.

Uses:

Colophony possesses stimulant and diuretic properties.

It is commonly used as ingredients of plasters and ointment.

Industrially it is used in manufacturing of varnishes, paint driers, printing ink, soaps, wood polishes, cements, paper, plastics and fireworks.

Storage: colophony should be stored in large pieces in well closed containers away from light.

Chemical Test

| Sl. No | Test | Observation |
|--------|--|--|
| 1 | Colophony mixed with alcohol | Forms milky white solution |
| 2 | Dissolve 0.1 g in 10 ml of acetic anhydride by means of gentle heat, cool and add a drop of con. Sulphuric acid | Bright red changes to violet (For abietic acid) |
| 3. | Dissolve 0.1 g in light petroleum and filter. To this add 2-3 times dil copper acetate solution. (For identification of adulteration of colophony) | Emerald green colour of petroleum layer (For abietic acid) |

Report:

From the above morphological characters and chemical test the given crude drug was identified as _____

ALOES

Synonym: musabbar, Ghritkumari, Aloe

Biological source: Aloe is the dried juice of the leaves of *Aloe barbadensis* mille (curacao aloes), *Aloe perrbaker*, (socotrine aloes) *Aloe ferox* miller and its hybrid with *Aloe africana* miller and *Aloe spiculata* Baker (cape aloes). Family: Liliaceae. It is obtained by incision of leaves at the base.

Physical characteristics:

Texture: solid waxy masses

Colour: Dark brown.

Odour: characteristic unpleasant odour

Taste: Bitter

Solubility: ethanol, alkali and glacial acetic acid; partly soluble in water, chloroform and ether.

Extra features: Hard and uneven porous fracture

Chemical constituents:

Aloes are the major sources of anthraquinone glycosides. Aloin is the mixture of three isomers namely barbaloin, β Barbaloin and isobarbaloin.

Aloe emodin, Aloe-resin, aloesone, aloetic acid, chrysophanic acid, chrysamminic acid, glactouronic acid, choline, saponins, coniferyl alcohol.

Uses:

Purgative and improve digestion, cosmetics.

Ointment of aloe is used in sun burns, thermal burns, radiation burns, abrasions and skin irritation.

At higher doses of aloes are abortifacient.

Used to prepare compound benzoin tincture in which it is pharmaceutical adjunct.

Stimulates immune system specially T4 cells.

It has greatest nutritional and therapeutic properties.

Adulterant and substitutes

Natel aloes, Mocha aloes

Chemical Test

| Sl.No | Test | Observation |
|-------|---|---|
| 1. | <p>Boil 0.5 g with 50 ml of water until nearly dissolved, cool and add 0.5 g kieselguhr and filter. Follow below test to the filtrate.</p> <ol style="list-style-type: none">1. Borax test: Heat 2.5 ml solution with 0.1 g borax add few drops of this in a test tube having water.2. 1 ml solution + 1 ml fresh solution of bromine3. Modified borntreger test: 5 ml filtrate +10 ml FeCl_3 5 ml dil HCL, heat for 10 min and filter. Filtrate + benzene, separate benzene layer + strong ammonia solution.4. Nitrous acid test Solution + NaNO_2 + Acetic acid, Heat | <p>Green fluorescence</p> <p>Pale yellow precipitate</p> <p>Pink to red colour to the ammonia layer</p> <p>Reddish brown colour</p> |
| 2. | <p>Cupraloin test for isobarbaloin: 10 ml of 0.1% solution of aloes in distilled water, add 1 drop of 5% solution of copper acetate, 0.5 ml of saturated solution of sodium chloride, 1ml of alcohol and warm.</p> | <p>Pale wine red colour</p> |
| 3. | <p>Bromine test: Add equal quantity of aloe to bromine solution</p> | <p>Bulky yellow precipitate of tetrabromaloin.</p> |

Report:

From the above morphological characters and chemical test the given crude drug was identified as _____

MYRRH

Synonym: Myrrha, Gum-myrrh, Bol

Biological source: myrrh is the oleo-gum-resin obtained from incision from the stem of commiphora molmol belonging to family: burseraceae.

Physical characteristics:

Colour: externally reddish internally brown

Odour: Agreeably aromatic

Taste: Aromatic, bitter, acrid

Size: 1.5-3.0 cm in diameter

Shape: irregular tears or lumps

Solubility: partly soluble in alcohol and ether

Insolubility: water

Extra features: fractured surface in granular, brittle, and translucent

Chemical constituents: volatile oil (10%) which are terpenes, cuminic aldehyde, eugenol, gum (60%), resin (25 to 40%) which contains ether soluble resin acids, α , β , and γ commiphoric acid.

Uses: carminatives, antiseptic, uterine stimulant, protective, used in gargles and mouth washes.

Substitutes and adulterants:

Yemen myrrh, Arabian myrrh, bissabol or perfumed bdellium, Indian bdellium, gum hotai.

Chemical tests/Identification test

| Sl.No | Test | Observation |
|-------|--|--------------------------------|
| 1. | Triturate with water | Forms yellowish brown emulsion |
| 2. | Ether extract of drug with bromine vapours | Red colour |
| | Moistened with nitric acid solution | Purple colour |

Report:

From the above morphological characters and chemical test the given crude drug was identified as _____