TECHNOCRATS

Lab Work Book of

Pharmacognosy and Phytochemistry-II

(BP- 508)

Department of Pharmacy

Lab Manual of

Pharmacognosy and Phytochemistry-II

(BP-508)

Price : ₹ ...../-

Edition :

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Printed & Published by :

TECHNOCRATS
PUBLICATIONS

Arera Colony, Bhopal.
e-mail : technocratspublications@gmail.com

TECHNOCRATS
PUBLICATIONS

Lab Work Book
of

PHARMACOGNOSY AND PHYTOCHEMISTRY-II

(BP-508)

Name	:	.....
Enrollment No.	:	.....
Institute	:	.....
Academic Session	:	.....

Department of Pharmacy

TECHNOCRATS
PUBLICATIONS

Vision of the Institute

To grow as an institute of Excellence for Pharmacy Education and Research and to serve the humanity by sowing the seeds of intellectual, cultural, ethical, and humane sensitivities in the students to develop a scientific temper, and to promote professional and technological expertise.

Mission of the Institute

M 1: To inculcate ethical, moral, cultural and professional values in students

M 2: To provide state of art infrastructure facilities to the staff and students so as to enable them to learn latest technological advancements

M 3: State of art learning of professionalism by the faculty and students

M 4: To produce well learned, devoted and proficient pharmacists

M 5: To make the students competent to meet the professional challenges of future

M 6: To develop entrepreneurship qualities and abilities in the students

PROGRAM OUTCOMES (POs)

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

PEOs

PEO 1: To inculcate quality pharmacy education and training through innovative Teaching Learning Process.

PEO 2: To promote professionalism, team spirit, social and ethical commitment with effective interpersonal communication skills to boost leadership role assisting improvement in healthcare sector.

PEO 3: To enhance Industry-Institute-Interaction for industry oriented education and research, which will overcome healthcare problems of the society.

PEO 4: To adapt and implement best practices in the profession by enrichment of knowledge and skills in research and critical thinking

PEO 5: To generate potential knowledge pools with interpersonal and collaborative skills to identify, assess and formulate problems and execute the solution in closely related pharmaceutical industries and to nurture striving desire in students for higher education and career growth.

Course Outcomes (COs):

Student will be able to:

- CO1: Define Primary metabolites. Explain about shickmic acid path way.
- CO2: Explain about biogensis of Atropine and Morphine.
- CO3: List out factors effecting tracer technique.
- CO4: Define Alkaloids and glycosides with extraction procedure.
- CO5: Define tannins and resins.

Index

General Introduction:

[A] THE MICROSCOPE

A microscope (from the Greek: mikrós, "small" and, skopeîn, "to look" or "see") is an instrument designed to make fine details visible. The science of investigating small objects using such an instrument is called microscopy. Microscopic means invisible to the eye unless aided by a microscope. It is an invaluable tool in today's research and education. It is used in a wide range of scientific fields, where major discoveries in biology, medicine and materials research are based on advances in microscopy.

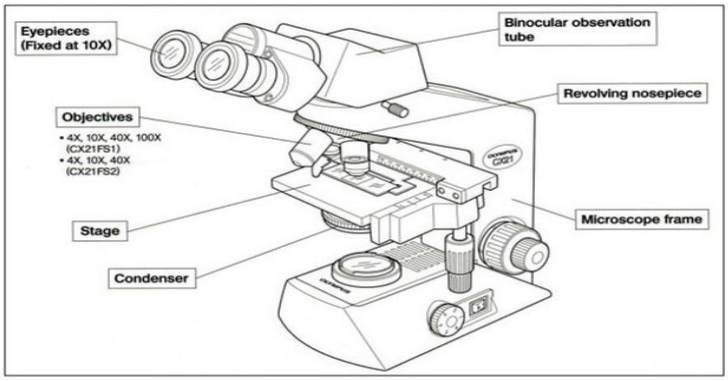
The microscope must accomplish three tasks: produce a magnified image of the specimen (magnification), separate the details in the image (resolution), and render the details visible to the eye, camera or other imaging devices.

COMPOUND LIGHT MICROSCOPE:

A compound microscope is an optical instrument consisting of two convex lenses of short focal lengths which is used for observing the highly magnified images of tiny objects. The compound microscope can magnify the image of a tiny object up to 1000.

PRINCIPLE OF COMPOUND MICROSCOPE: A compound microscope works on the principle that when a tiny object to be magnified is placed just beyond the focus of its objective lens, a virtual, inverted and highly magnified image of the object is formed at the least distance of distinct vision from the eye held close to the eye piece.

INSTRUMENTATION AND PARTS OF A COMPOUND MICROSCOPE:



Photograph of Compound Microscope

EYEPIECE: It is the top part of the microscope; it is the lens you look through to see your specimen.

Arm: It is the large smetal band attaching the base to the lens and eyepiece. When you carry a microscope, use one hand to hold the arm, and place the other hand under the base.

Coarse Adjustment Knob: Of the two knobs on the side of a microscope, it is the largest. It is used to focus on the specimen; it may move either the stage or the upper part of the microscope (in a relative up and down motion). Always focus with the coarse knob first.

Fine Adjustment Knob: It is the smaller round knob on the side of the microscope used to fine-tune the focus of your specimen after using the coarse adjustment knob.

Objective Lenses: Most microscopes have two, three or more lenses, typically made of glass to collect light from the sample, that magnify at different powers. Always start with the lowest power and work your way up to the strongest when examining a specimen. The shortest lens is usually the lowest power.

On a typical compound optical microscope, there are three objective lenses: a scanning lens (4X), low power lens (10X) and high power lens (ranging from 20 to 100X). Higher magnification lenses must be physically closer to the specimen itself, which poses the risk of jamming the objective into the specimen. Be very cautious when focusing. Some microscopes have a fourth objective lens, called an oil immersion lens. To use this lens, a drop of immersion oil is placed on top of the cover slip, and the lens is very carefully lowered until the front objective element is immersed in the oil film. Such immersion lenses are designed so that the refractive indexes of the oil and of the cover slip are closely matched so that the light is transmitted from the specimen to the outer face of the objective lens with minimal refraction. An oil immersion lens usually has a magnification of 50 to 100X.

The actual power or magnification of an optical microscope is the product of the powers of the ocular (eyepiece), usually about 10X and the objective lens being used. The magnified image seen by looking through a lens is known as a virtual image, whereas an image viewed directly is known as a real image.

Stage: It is where the sample or specimen is placed for examination. The stage usually has arms to hold slides (rectangular glass plates with typical dimensions of 25 mm by 75 mm, on which the specimen is mounted).

Iris Diaphragm: It is what allows you to control the amount of light on the specimen that comes through the stage.

Light Source: It can be a bulb or a mirror, and is usually found near the base of the microscope shining up through the stage.

Aperture: It is the hole in the stage that allows light through for better viewing of the specimen.

APPLICATIONS:

- Optical microscopy is used extensively in microelectronics, nanophysics, biotechnology, pharmaceutical research and microbiology.
- It is used for medical diagnosis, the field being termed histopathology when dealing with tissues, or in smear tests on free cells or tissue fragments.
- Microscopy is also becoming an important tool for forensic scientists who are constantly examining hairs, fibers, clothing, blood stains, bullets, and other items associated with crimes.
- The smallest objects that are considered to be living are the bacteria. The smallest bacteria can be observed and cell shape recognized at a 100X magnification.
- Optical microscopy is best suited to viewing stained or naturally pigmented specimens such as stained prepared slides of tissue sections or living photosynthetic organisms.

[B] MICROSCOPIC EVALUATION OF CRUDE DRUGS

Introduction:

- This method is used for identification of drugs on cellular level.
- It is used to determine structure of organised drugs by their histological characters. It includes examination of whole, certain parts or powdered crude drugs.

Histological characters:

- Size , shape and relative position of cells and tissues.
- Chemical nature of cell wall.
- Fragments of plant cells or tissues

Importance: It is necessary in:

- Initial identification of herbs
- Identification of small fragments of crude or powderd drug.
- Detection of adulterants (insects, molds, fungi)

Types:

- Transverse microscopy
- Powdered microscopy

B (A) TRANSVERSE SECTIONING MICROSCOPY: It is used to determine:

- Size of starch grain
- Length and width of fibers
- Size of stomata
- Diameter of phloem fibers

METHODS OF TRANSVERSE SECTIONING:

Free hand mounting: It is used for temporary slides. In this method material is cutted with help of blade and sliced smoothly from upper left towards lower right in a single motion. Keep the specimen and blade lubricated with water.

Glide mounting: It is used for solid material sectioning. It is composed of specimen feed, knife , holder, and specimen orientation. It's main quality is that it gives excellent sectioning results.

Cryology Mounting: This method is used to make slides of fresh and young herb tissues. Cut the sample into small pieces and embed them with cryomatrix on a crycasste. Freeze them, slice them, mount on glass slide and seal.

Paraffin Mountining: In this method specimen is embedded in paraffin and then slicing the block. The steps include: Sampling, Fixation, Dehydration, Vitrification, Slicing , Removing the paraffin, staining with safranin and fast green.

PROCEDURE FOR SECTION CUTTING AND STAINING: Cut fine and complete transverse section of given drug with help of sharp blade.

- Keep them soak in water.
- Take a watch glass. Pour about 1ml of 10% alcohol in it and cover it with another watch glass.
- Take a finest section of sample. Transfer it into watch glass containing 10% alcohol ans leave it for 2 min
- After 2 min. transfer the section into another watch glass containing 20% alcohol and leave it for 2 min.
- Repeat the same procedure for 30% , 40% and 50% alcohol. • 7: After 50% alcohol take another watch glass. Pour 1-2ml 50% Alcohol. Add 1-2 drops of safranin solution and transfer the section into it and leave it for 2min.
- Transfer the section into 60% alcohol and leave it for 2min.
- Repeat the same procedure for 70 and 80% alcohol.
- Take 80% alcohol in a watch glass. Add a drop of malachite green and leave it for half minute into it.
- Transfer the section in 90% and 100% alcohol and leave it for 2 minute.
- Transfer the section into another watch glass and pour 1-2 drops of clove oil which act as emollient.
- Transfer the section on neat and clean slide. Pour 1-2drops of canada balsam. Cover it with a cover slip making sure no air bubbles are present.
- This slide is permanent and can be observed under microscope

REAGENTS:

- Toludine blue
- Phloroglucinol
- Methyl orange
- Iodine
- 5% NaOH
- Dilute HCL
- 1% Chloral hydrate
- Conc. Nitric acid

B (b) LEAF CONSTANTS: Procedure for observing characteristic features of leave

Leaf surfaces are scrapped and peeled off from upper and lower surface.

- Epidermis is removed carefully.
- Wash it with chloral hydrated solution which is used as a cleaning agent to see the cellular components of leaf accurately.
- Observe under microscope.

Stomatal Index: It is a % which the no. of stomata form to totsal no. of epidermal cells.(each stomata being counted as 1 cell) Stomatal index= S/E+S X 100 S= No. of stomata per unit area. E= no. of epidermal cells in the same unit area

Vein Islet number: It is defined as no. of vein islet per square ml of leaf surface midway b/w midrib and margin.

Vein Termination number: It is defined as : No. of veinlet termination per square meter of leaf surface midway b/w midrib of leaf and its margin.

Palisade Ratio: It is the average no. of palisade cells beneath each epidermal cell.

B (c) POWDER MICROSCOPY:

Powder analysis plays a significant role in identification of crude drug. These characters will help in the identification of right variety and search for adulterants. Powder microscopy is one of the simplest and cheapest methods to start with for establishing the correct identity of the source materials. It is useful for further pharmacological and therapeutic evaluation along with the standardization of plant material. Preliminary examination and behavior of the powder with different chemical reagents was carried out and microscopical examination was carried out after treatment with different reagents like Phloroglucinol, Conc. HCl, Ruthenium red, Acetic acid and Iodine solution.

[C] PHYTOCHEMICALS SCREENING OF MEDITIONAL PLANT:

Phytochemical screening is a process of submitting plant parts to various chemical test in order to extract secondary plant constituents in them, it also gives us basic information concerning the medicinal importance of the plant extract. After every chemical reaction that takes place in plants, secondary metabolites are produced. phytochemical screening and quantitative estimation of the chemical constituents of indicates the presence of various metabolites consisting of reducing compounds, free radicals and other chemical constituents scavenging compounds such as gums, flavonoids, alkaloids, reducing sugars, terpenoids, saponins, coumarins, tannins, cardiac glycosides anthraquinones, and phlobatinins and other phenolic compounds. plants have high medicinal value. These plants have a wide range of uses by all sections of the society either directly or indirectly in traditional medicine and pharmaceutical preparation of modern medicine. Phytochemicals having biological activity have had great utility as pharmaceuticals and pharmacological actions. As time goes on, statistics indicate the high increase in various diseases and ailments due to improper intake of diets. Diests taken of late mostly contain high values of fats, proteins and carbohydrates which are all which are all processed and have a less value of natural products. Eating much natural plant food give a good amount of fibre, vitamins natural proteins and many more beneficial nutrients from organic sources. analysis and research indicates that plant leaves are a good source of metallic elements, protein and sugar. Due to the possession of these constituents and their characteristic

effects, users have more benefits when they use these as a substitute of sugar in various food preparations. A combination of these phyto chemicals can be found in the same plant with different functional groups, molecular structure and active ingredients. this compounds can be extracted using chemical additives and various extraction and separation mechanisms. This lab is aimed at identifying phytochemicals in a given plant sample and extracting them if present.

[D] EXTRACTION AND IDENTIFICATION OF PHYTO-CONSTITUENTS

Extraction is the process of efficiently dissolving and separating the desired constituent from crude drug with the use of solvent the choice of solvent depends on charachteristics of the secondary metabolites like polarity, pH, thermal stability. The solvent should non toxic,inflammable, easy to remove.

FACTORS EFFECTING EXTRACTION:

- Quantity and chemical nature
- Size of powder of crude drug
- Moisture content
- Nature and volume of solvent
- Mixing ration of solvent and sample
- Temperature of extraction process
- pH of extraction solvent
- Lipophilicity of solvent mixture and sample

TYPES OF EXTRACTIONS

- Maceration
- Percolation
- Soxhlet extraction
- Infusion
- Decoction
- Water distillation
- Steam distillation
- Micro wave assisted extraction
- Sonication
- Sublimation
- Counter current extraction

- Supercritical fluid extraction

METHODS OF PURIFICATION

- Chromatography
- Distillation
- Evaparation
- Crystalisation
- Sublimation
- Preceptation physicalphysical chemicalchemical
  - » Hydrolisis
  - » Salt formation
  - » Acetylation

[E] CHROMATOGRAPHY

The word chromatography originated from two greek words 'chroma' meaning 'colour' and 'graphine' meaning 'to write'. Chromatography means colour writing and it was first employed by a Russian scientist Mikhail Tsvet. This method was first used for the separation of coloured substances in plants.

In the chromatographic technique, the mixture of substances is applied onto a phase called the stationary phase. The stationary phase may be solid or liquid. A moving phase that can be a pure solvent or a mixture of solvents, or a gas is allowed to move slowly over the stationary phase. This moving phase is called the mobile phase. When the mobile phase is moved over the mixture on the stationary phase, the components of the mixture gradually separates from one another.

CLASSIFICATION OF CHROMATOGRAPHY Depending on the basic principle involved in chromatography, it is mainly classified into two.

1. Adsorption chromatography:

It is based on the differential adsorption of the components on the adsorbent (stationary phase). This means that different compounds are adsorbed on an adsorbent at different degrees. Following are the two main types of chromatographic techniques based on the principle of differential adsorption.

- Column Chromatography
- Thin layer Chromatography

Thin Layer Chromatography: Thin layer chromatography is another type of adsorption chromatography, which involves the separation of a mixture of substances over a thin layer of an adsorbent coated on a glass plate. In this case, the stationary phase is a glass plate of suitable size coated with a thin layer of stationary phase usually silica gel or alumina. This plate is known as thin layer chromatography plate (TLC plate) or chromaplate. The solution of mixture to be separated is applied as a small spot about 2 cm above one end of the TLC plate. The glass plate is then placed in a closed jar (chromatography chamber)

containing the mobile phase. As the mobile phase rises up the plate by capillary action, the components of the mixture move up along with the solvent to different distances depending on their degree of adsorption and separation takes place. The relative adsorption of each component of the mixture is expressed in terms of its Retardation factor (Rf) (Retention factor).

$$R_f = \frac{\text{Distance travelled by the component from the original line}}{\text{Distance travelled by the solvent from the original line}}$$

2. Partition Chromatography

The basic principle of partition chromatography is the continuous differential partitioning of components of a mixture between the stationary phase and the mobile phase. An important partition chromatography is Paper Chromatography.

Paper Chromatography: In paper chromatography, the stationary phase is a special quality paper called chromatography paper. Mobile phase is a solvent or a mixture of solvents. A solution of the mixture is spotted on a line about 2 cm above from the bottom of the paper, called original line or base line and then suspended in a chromatography chamber containing suitable solvent. The solvent rises up the paper by capillary action and flows over the spot. The paper selectively retains different components according to their differing partition in the two phases. The paper strip so developed is called Chromatogram. The spots of the separated coloured compounds are visible at different heights from the position of initial spot on the chromatogram. The spots of the separated colourless components may be observed either under ultraviolet light or by the use of an appropriate spray reagent. The distance travelled by the solvent from the original line is called solvent front. The relative adsorption of each component of the mixture is expressed in terms of its Retardation factor (Rf) (Retention factor).

$$R_f = \frac{\text{Distance travelled by the component from the original line}}{\text{Distance travelled by the solvent from the original line}}$$

[F] APPLICATION OF TLC IN ANALYSIS OF PLANT EXTRACT

Thin-layer chromatography (TLC) is an important and simple technique used to rapidly separate and qualitatively analyse a few of substances.

Herb extract is a kind of products that using herbs as the raw material, through the process of extraction and separation, directional obtaining and extracting one or more effective ingredients in plants, and no changing its effective components structure at the same time in accordance with the extraction purpose. Herb extract is natural and little side effect, thus it has important value in research of medicine, food and chemical industry. It usually takes morphological identification, physical and chemical identification and spectral chromatographic analysis to identify authenticity of herb extract. And TLC is a kind of solid-liquid adsorption chromatography, it has both the advantages of column chromatography and paper chromatography. On the one hand, it is suitable for the separation of a few of samples. On the other hand, it can also be used to refine samples, just by thickening the adsorption layer when preparing thin layer plate. This method is particularly suited to the herb extract which is less volatile or changes easily under higher temperature and can't be analyzed with the gas chromatography. In recent years, TLC is widely applied to the analysis and identification of organic compounds, separation and refining, organic synthesis, structural analysis, biometrics, etc. of effective parts of plant medicine. Especially in the research and development of effective parts of plant medicine and quality control of patent medicine, it is the simplest scientific method of qualitative and quantitative analysis.

Experiment No. 1

Morphology, histology and powder characteristics & extraction & detection of: Cinchona, Cinnamon, Senna, Clove, Ephedra, Fennel and Coriander

OBJECTIVE 1.1

Chinchona bark

Scientific Name: Cinchona spp. (C. *officinalis*, C. *ledgeriana*, C. *succirubra*).

Family: Rubiaceae

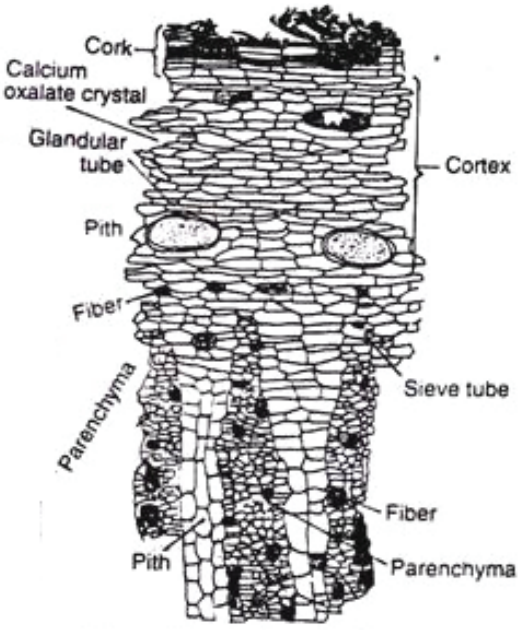
Photograph of Chinchona bark

ORGANOLEPTIC CHARACTERS:

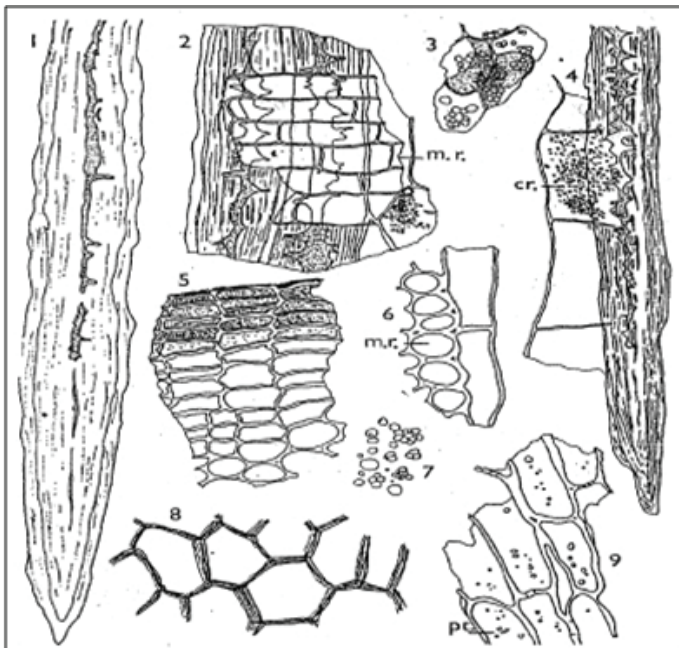
1. Pernnial trees 10 m.
2. The dried bark is curved ridges, greenish gray to brown.
3. Odour: Slight and characteristic
4. Taste: Intensely bitter and slightly astringent

MICROSCOPIC CHARACTERS:

Transverse Section: In T.S. of bark prominent cork cells noticed, cortex cells bear characteristic secretory canals, starch grains and oxalate crystals prominent in cortical cells; phloem fibre — long, spindle-like; xylem vessels smaller and simple; pith tissue arranged in two to three rows.



T.S. of Chinchona bark



Powder Microscopy of Chinchona bark

Powder Characteristic elements

1. Part of single Fibre
2. Part of a group of fibres and phloem parenchyma with overlying medullary ray in radical longitudinal section.
3. Parenchymatous cells containing starch granules and brown pigments.
4. Part of fibre with phloem parenchyma one cell containing calcium oxalate micro- crystal
5. Cork and phelloderm in sectional view.
6. Phloem parenchyma and part of a medullary ray in tangential longitudinal section.
7. Starch granules.
8. Cork in surface view.
9. Pholem parenchyma with pits.
10. **Constituents:** Alkaloids (quinine, quinidine, chinchonine)
11. **Uses:** Quinine: Antimicrobial, Night muscles cramps

Quinidine: Arrhythmias

IDENTIFICATION TESTS:

1. **Fluorescence Test:** Quinine gives a distinct and strong blue fluorescence when treated with an oxygenated acid, such as: acetic acid, sulphuric acid. This test is very marked and pronounced even to a few mg concentration of quinine.
   Note: The hydrochloride and hydroiodide salts of quinine do not respond to this fluorescence test.

2. **Thalleioquin Test:** Add to 2-3 ml of a weakly acidic solution of a quinine salt a few drops of bromine-water followed by 0.5 ml of strong ammonia solution, a distinct and characteristic emerald green colour is produced. The coloured product is termed as thalleioquin, the chemical composition of which is yet to be established. This test is so sensitive that quinine may be detected to a concentration as low as 0.005%.
   Notes: Quinidine and cupreine (a Remijia alkaloid) give also a positive response to this test; but cinchoninine and cinchonidine give a negative test.

RESULT:

The morphological, microscopical and chemical evaluation of given crude was successfully performed.

PRACTICAL WORKS

1. Examine the physical characters of Chinchona bark powder
    - Colour
    - Odour
2. Describe the barks and the powder

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3. Identification test of Quinine

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OBJECTIVE 1.2

Cinnamon bark: Cortex *cinnamomi*

SCIENTIFIC NAME:

Cinnamomum *zeylanicum* Nees. (Ceylon Cinnamon) (Sri Lanka) Cinnamomum cassia Nees. (Chinese Cinnamon)

Family: Lauraceae

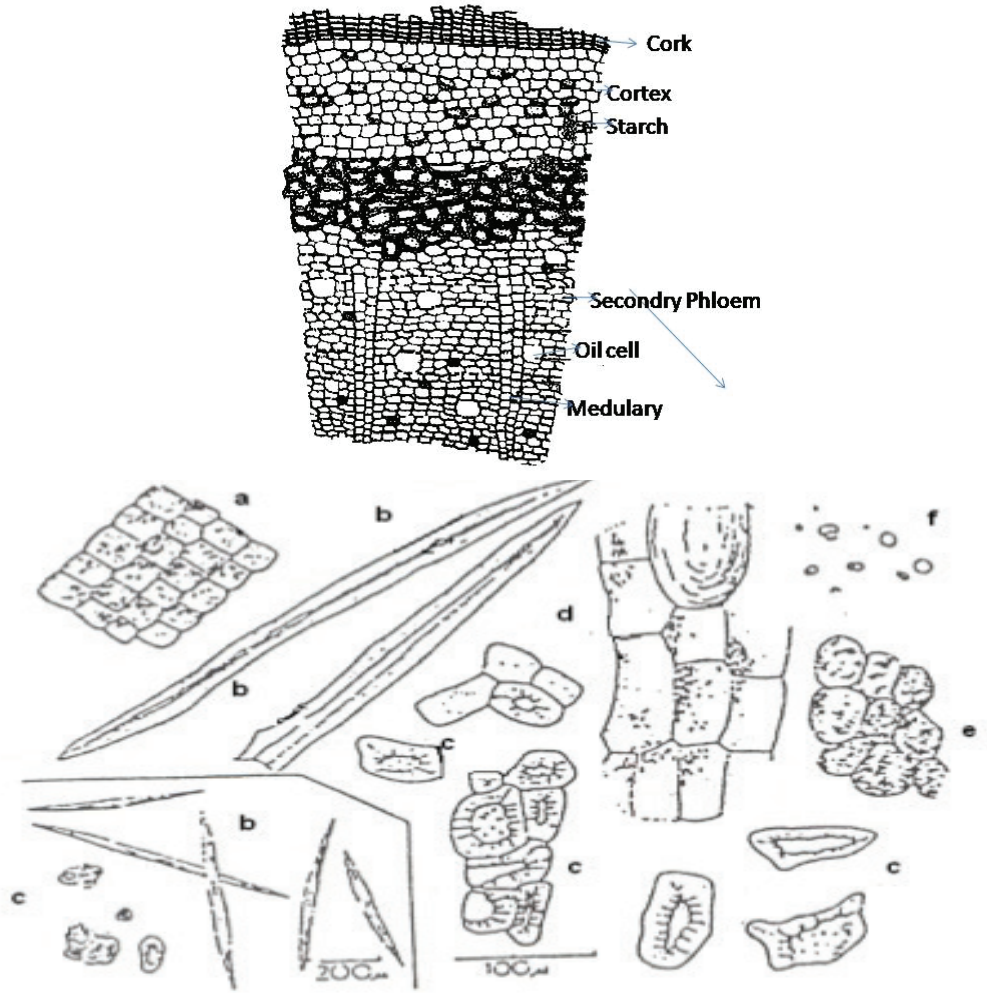
Photograph of Cinnamon bark

ORGANOLEPTIC CHARACTERS:

1. Perennial handsome evergreen large trees.
2. The bark external surface: yellowish brown.
3. The bark inner surface dark brown.
4. Odor: aromatic.
5. Taste: warm, sweet and aromatic.
6. Origin: Sri Lanka & China.

MICROSCOPIC CHARACTERS:

Transverse Section:



T.S. and Powder microscopy of Cinnamon bark

- Cork:It consists of several layers of radially arranged rows of thin walled cells with dark brown contents. The cork cells are impregnated with suberin.
- Phellogen: It consists of 2-3 layers of thin walled rectangular cells without any cellular contents.
- Phelloderm: 6 to 8 layers of thin walled rectangular cells without any cellular contents. Like cork, they are arranged at times in radial rows.
- Cortex: They consist of 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.
- Secondary phloem: It consists of phloem parenchyma, phloem fibres and medullary rays.
- Medullary rays: Medullary rays transverse radially the phloem parenchyma; 1 to 3 cells wide, extend up to cortex, cells radially elongated and contain starch grains.

Powder Characteristic elements

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

CONSTITUENTS:

Cinnamon oil (volatile oil): a. Cinnamic aldehyde 60-70%. b. Eugenol. Mucilage (mannitol), Sugars, Starch and Tannins (phlobatannin). Cinnamon cassia has the same constituents except eugenol. Contains Cinnamic aldehyde 80%.

Uses: Carminative, Flavor, Antiseptic, Antidiarrhea, Powerful germicide.

CHEMICAL TEST:

When extract (volatile oil) from Cinnamon oil is treated with a drop of Ferric Chloride solution it yields a pale green color. This pale green color is achieved as cinnamic aldehyde on reacting with ferric chloride gives brown color and eugenol on reacting with ferric chloride solution produces blue color. A pale green color is the result of combination of these brown and blue colors.

The same above test when done with Oil of Cassia (also called as Chinese cassia obtained from Cinnamomum cassia) yields only brown color due to absence of eugenol and with cinnamon leaf oil yields only blue color having eugenol as the main constituent.

RESULT:

The morphological, microscopical and chemical evaluation of given crude was successfully performed.

PRACTICAL WORKS

1. Examine the physical characters of the Cinnamon bark powder:

    a. Colour:

    b. Odour:

    c. Taste:

2. Describe the barks and the powder:

    .....

    .....

    .....

    .....

3. Identification of Tannins: Little powder + drops of FeCL3 solution ---------- dark green colour.

4. Test for Lignin: Little powder on a clean slide + one drop of phloroglucine.HCl solution ------ red Colour (examine under the microscope).

5. Examine the characteristic particles under the microscope using chloral hydrates as an amount.

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OBJECTIVE 1.3

Seena: Cassia *acutifolia*

Scientific Name: Dried leaflets of Cassia senna (Cassia acutifolia) also known commercially as Alexandrian senna or khartoum senna and Cassia angustifolia, which is commercially known as Tinnevelly senna or Indian senna. Should not be confused with Cassia which is a common name for cinnamon.

Family: Leguminosae

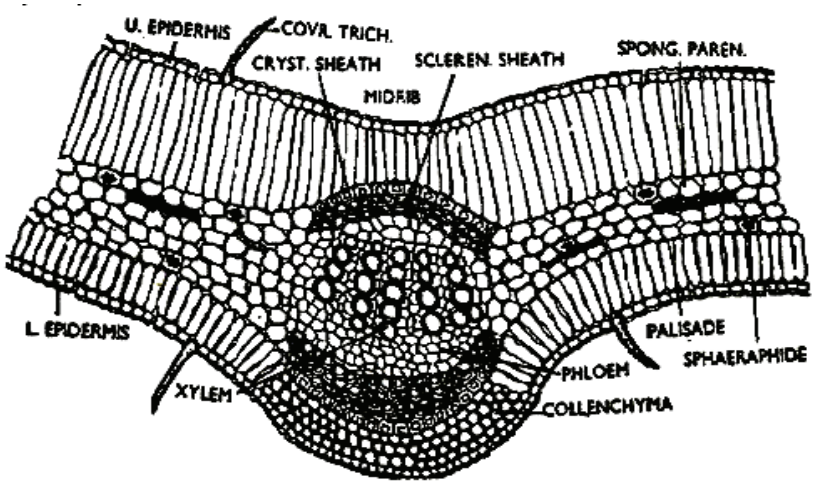
Photograph of Cassia acutifolia

ORGANOLEPTIC CHARACTERS:

- Senna plants are low branching shrubs (3 feet) with a straight woody stem and yellow flowers.
- Leaflets of senna have stout petiolules, entire margin lamina with an asymmetric base and an acute apex.
- It has a characteristic odor (faint) and bitterish unpleasant taste.

MICROSCOPIC CHARACTERS:

Transverse Section:



TS of Seena leaves

- Senna leaflets have an isobilateral structure with straight walled epidermal cells.
- 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.
- 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).
- Prismatic and cluster calcium oxalate crystals can be observed.
- Vein islet numbers for Alexandrian Senna is about 25-29.5 while that for Indian/Tinnevelly Senna is 19.5-22.5.
- 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 collenchyma.

CONSTITUENTS:

- Dianthrone glycosides (1.5% – 3%), Sennosides A and B (rhein dianthrones containing the aglycone Sennidin A and Sennidin B respectively), Sennosides C and D (gylcosides of heterodianthrones rhein and aloe emodin).
- Free anthraquinones are also present and several other glycosides such as palmidin A and aloe-emodin dianthrone diglycosides are also present.
- Senna also contains flavanols such as kaempferol (yellow color) and isorhamnetin. Traces of chrysophanic acid, saponin, salicylic acid and volatile oils have also been found.

Uses: Senna is a useful laxative for either occasional use or habitual constipation. It is a popular laxative specially amongst the elderly and is devoid of the astringent after effects as observed in Rhubarb. It is reviewed to be useful during pregnancy and lactation.

CHEMICAL TEST:

1. Treat powdered drug with acid solution and extract with ether. When the organic layer is treated with methanolic magnesium acetate solution Alexandrian Senna gives pink color in daylight, while Indian/Tinnevelly Senna gives orange color in daylight.

2. The same solution above in UV light gives a pale greenish-orange color for Alexandrian Senna, while the Indian Senna gives a yellowish-green color.

3. TLC test for both can be performed. Alexandrian senna contains 6-hydroxymusizin glycosides, while Indian Senna contains Tinnevellin glycoside.

RESULT:

The morphological, microscopical and chemical evaluation of given crude was successfully performed.

PRACTICAL WORKS

1. Examine the physical characters of the Cinnamon bark powder:

    a. Colour:

    b. Odour:

    c. Taste:

2. Identification test of Alexandrian Senna

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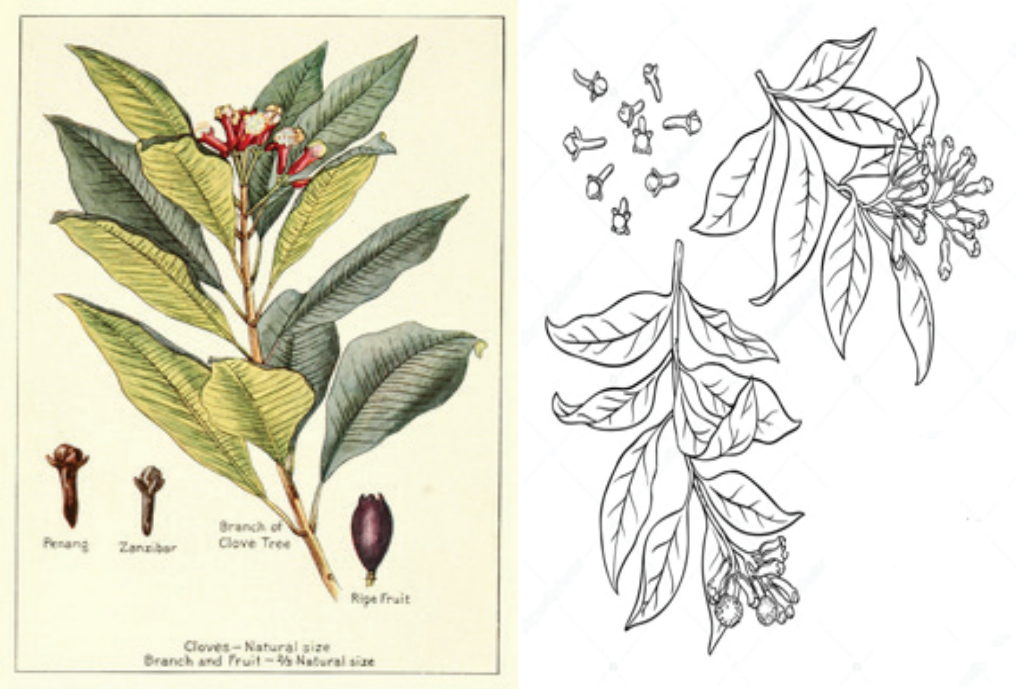
OBJECTIVE 1.4

Clove buds: Flos *Caryophylli*

Scientific Name: Eugenia *caryophyllus* (Sprengel) or Eugenia *caryophyllata* (Thunberg)

Family: Myrtaceae



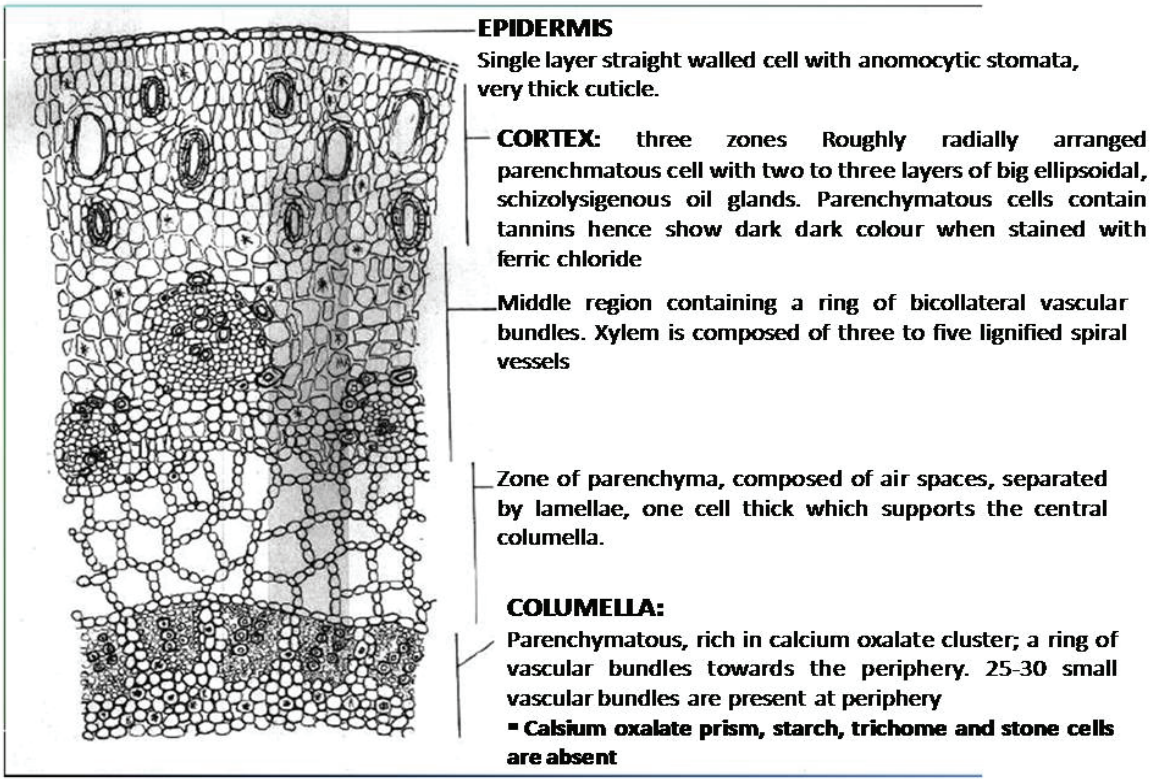
Photograph of Flos Caryophylli

ORGANOLEPTIC CHARACTERS:

1. Perennial large trees (15) m height.
2. The buds collected when their colour changed from green to red.
3. Dried clove buds colour: reddish brown.
4. Taste: pungent & acrid.
5. Odor: aromatic like the odor of Pepper and Cinnamon together.
6. Origin: Madagascar & Sumatra.

MICROSCOPIC CHARACTERS:

Transverse Section:



T.S. of Clove buds

Powder Characteristic elements

1. Sclerenchymatose fiber from the bud (f).
2. Secretory glands (b2).
3. Pollen grains with 3 emergences(C).

PowderMicroscopy of Clove buds

a1) Anther fragment in side view 1. Epidermis 2. Fiber cells. a2) Anther fragment, fiber cells of anther in top view. b1, b2) Fragments from the bud parenchyma with secretory glands (b2) & residues of two Secretory glands (b1) in the center of (b2). c) Pollen grains with 3 emergences. d) Epidermis fragment with 2 large somatal apertures in top view. e) Tissue fragment from the bud with vascular bundle & neighboring crystal cell layer. f) Schlerenchyma fiber from the bud. g) Tissue fragment with numerous secretory glands & vessels, left fragment with schlerenchyma fibers. h) Fragment from the bud wall, residue from a secretory gland. i) Style fragment with central vascular bundle & 2 secretory glands. k) Anther (torn) with numerous pollen grains in the interior.

CONSTITUENTS:

1-Volatile oil (Clove oil) 14%-20%. A- Eugenol 70%-90%. B-Vanillin. C- Caryophyllene1%. D-Acetyl eugenol 4%. 2- Tannin: Gallotannic acid 10-13%.

Uses: 1. Condiment. 2. Carminative. 3. Clove oil which contains high percentage of eugenol used commercially to produce Vanillin. 4. Antiseptic. 5. Flavoring agent. 6. Dental uses: as filling material with ZnO. 7. Local anesthetic (dental analgesic).

Drugs: 1. Dento drops. 2. Dentin drops

IDENTIFICATION TESTS:

1. Treat a thick section of hypanthium's of clove with 50% potassium hydroxide solution. Needle shaped crystals of potassium eugenate are seen.
2. Place a drop of chloroform extract of clove or clove oil or eugenol on a slide and add to it a drop of 30% aqueous solution of sodium hydroxide saturated with sodium bromide. Needle and pear-shaped crystal of sodium euginate arranged in rosette are seen almost immediately.
3. Dissolve a drop of clove oil in 5 ml alcohol and add a drop of ferric chloride solution. Blue colour is seen because of phenolic OH group of eugenol.
4. Prepare a decoction of clove and add to it ferric chloride solution. Blue-black colour is formed because of the tannins.

RESULT:

The morphological, microscopical and chemical evaluation of given crude was successfully performed.

PRACTICAL WORKS

1. Observe the physical characters of the powder and the buds and describe them below:

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2. Write the name of each part of this Clove bud section.

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3. Prepare a chloral hydrate mount of Clove buds powder, then observe the characteristic features under the microscope (draw these features below).

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4. Identification of Tannins: Powder + FeCl3 ---------- must produce dark green colour

OBJECTIVE 1.5

Ephedra: Ephedra *gerardiana*

Scientific Name: It consists of dried young stem of Ephedra gerardiana Wall.

Family: Ephedraceae

Photograph of Ephedra gerardiana

ORGANOLEPTIC CHARACTERS:

- Shape: cylindrical
- Colour: greenish yellow
- Odour: agreeable and slightly aromatic.
- Taste: astringent and bitter
- Length: 12-33 cm Thickness: 0.8 – 1.5 mm.

MICROSCOPIC CHARACTERS:

Transverse Section:

- Epidermis: single layer cell and thick wall with smooth cutical.
- Cortex: Many layers of thin walled cellulosic parenchyma.
- Unilignified fibers: like bunch of grapes, occur below the ridges.
- Fibers are scattered, isolated or in group of 2-4 cells.

- Vascular bundle: around 10 ,contain xylem and phloem.
- Pith: large thin walled cell and some cell contain dark brownish mucilaginous substance.

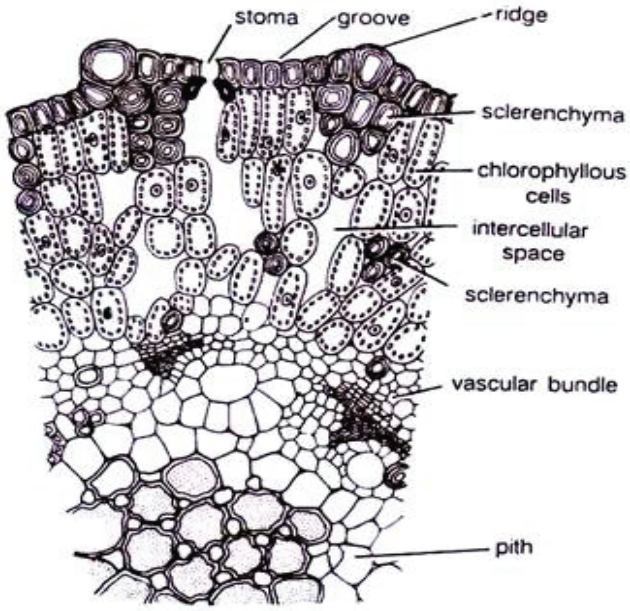

Fig. 14.2. *Ephedra trifurca* T. S. young stem

T.S. of Ephedra gerardiana

Powder Characteristic elements:

- Lignified and non lignified fibres.
- Tracheids with bordered pits.
- Epidermis with ridged outer walls.
- Dark brown pigmented cells

CONSTITUENTS:

Alkaloids, ephedrine, d-pseudoephedrine, N-methyl ephedrine, methyl ephedrine.

Uses: it is used in Asthma, bronchitis, whooping cough.

IDENTIFICATION TESTS:

Mayer's test: Ephedra give cream colour precipitate with Mayer's reagent (Potassium mercuric iodide solution).

Dragandroff's test: Ephedra give orange brown precipitate with Dragandroff's reagent (Potassium bismuth iodide solution).

Hager's test: Ephedra give yellow color precipitate with Hager's reagent (Saturated solution of picric acid).

Wagner's test: Ephedra give a reddish brown precipitate with wager's reagent (Solution of iodide in potassium iodide).

RESULT:

The morphological, microscopical and chemical evaluation of given crude was successfully performed.

PRACTICAL WORKS

1. Examine the physical characters of the Cinnamon bark powder:

    a. Colour:

    b. Odour:

    c. Taste:

2. Identification test of Alexandrian Senna

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OBJECTIVE 1.6

Fennel fruits:

Foeniculi *fructus*

Scientific Name: Foeniculum *vulgare* L

Family: Umbelliferae

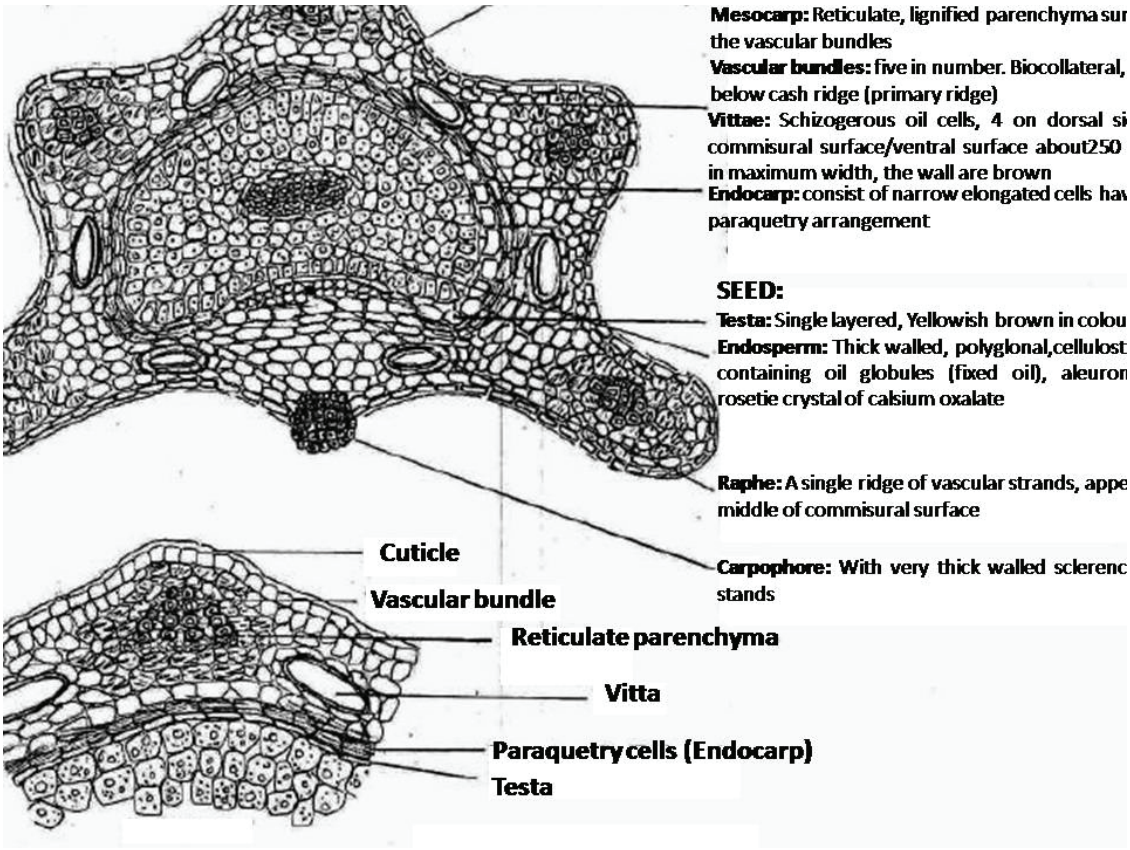
Photograph of Foeniculi fructus

ORGANOLEPTIC CHARACTERS:

- Perennial herb with yellow flowers.
- Fruits colour: greenish yellow or greenish brown.
- Odor: aromatic.
- Taste: Aromatic and sweet.
- Origin: Mediterranean region.

MICROSCOPIC CHARACTERS:

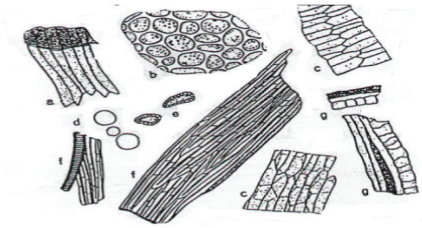
Transverse Section:



T.S. of Fennel fruits

Powder Characteristic elements

1. Sclerenchymatose fiber from the bud (f).
2. Secretory glands (b2).
3. Pollen grains with 3 emergences(C).



Powder Characteristic elements of Fennel fruits

1. Pitted parenchyma (a).
2. Corner collenchyma (c).
3. Yellowish oil droplets, numerous (d).
4. Endosperm with minute calcium oxalate rosettes (e).

- Pitted parenchyma (denated cells) from the mesocarp.
- Parquet cells of the inner integument epidermis with subjacent parenchyma, on the right, residual oil duct (fragment of a schizogenous duct by epithelial cells).
- Corner collenchyma near conducting vascular bundle with brown- red cell walls.
- Yellowish oil droplets, numerous.
- Endosperm fragment with minute calcium oxalate rosettes, hyaline, thick cell walls.
- Fragment of broken sclerenchymal fiber from the carophore, rare, not characteristic.
- Fragments from the parquet cell tissue with dark yellowish secretory ducts, evident even under low magnification.

Constituents: Volatile oil: Fenchone , Anethole and limonene.

Uses: 1. Carminative. 2. Antispasmodic. 3. Flavoring agent. 4. Sedative for the menstrual pains. 5. Treatment of inflammated eyes.

Result: The morphological, microscopical and chemical evaluation of given crude was successfully performed.

PRACTICAL WORKS

1. Observe the physical characters of the powder and the fruits and describe them below:

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2. Prepare a chloral hydrate mount of Fennel fruits powder, then observe the characteristic features under the microscope (draw these features below).

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3. Prepare a transverse section of the cremocarp and observe this section under the microscope.

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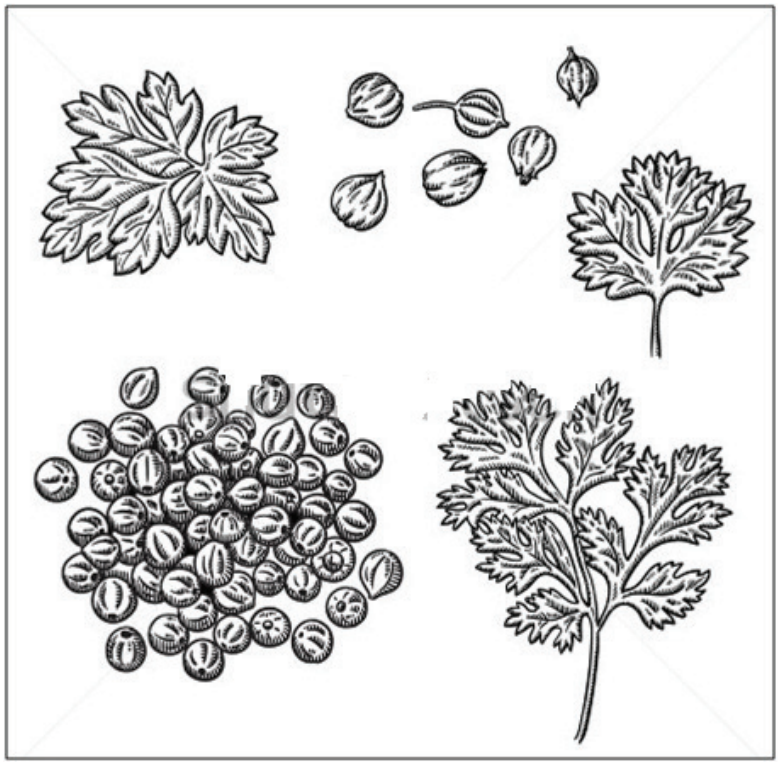
OBJECTIVE 1.7 :

Coriander: Coriandrum sativum L

Scientific Name: Coriandrum *sativum* L

Family: Umbelliferae



Photograph of Coriandrum sativum L

ORGANOLEPTIC CHARACTERS:

- Entire cremocarps.
- 3-4 mm long, subspherical.
- Yellowish brown in colour.
- Aromatic odour with aromatic spicy test.
- Five wavy inconspicuous primary ridges and four prominent secondary ridges.
- Seed-coelospermous.

MICROSCOPIC CHARACTERS:

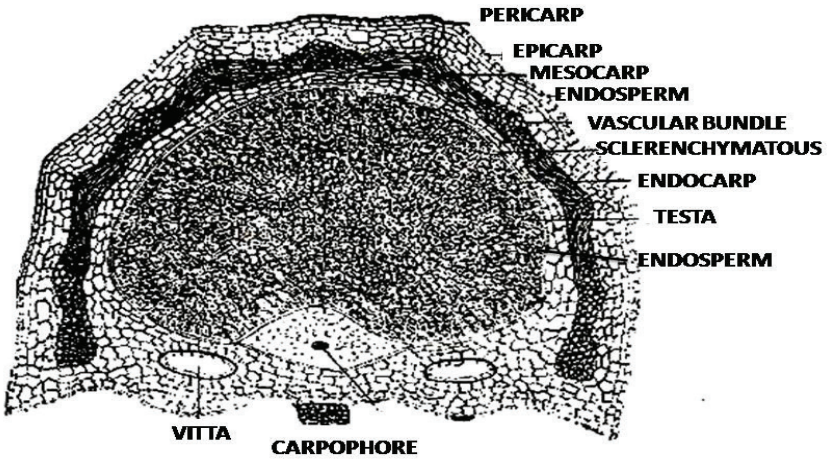
Transverse Section:

Epicarp: Polygonal cells with occasional stomata and calcium oxalate crystal.

Mesocarp: Inner and outer layer of parenchyma with sclerenchyma in between. Sclerenchyma in tangential and longitudinal bands. Two vittae on the commissural surface and four lacunae on the dorsal surface.

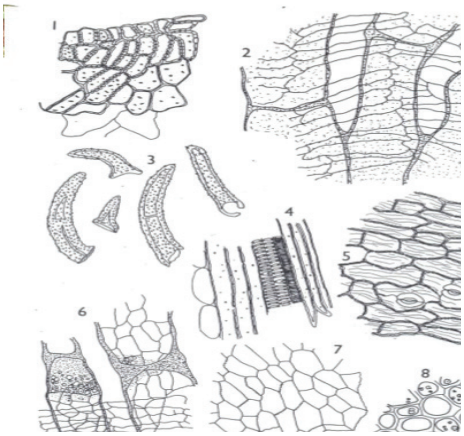
Endocarp: Elongated cells forming parquetry layer.

Endosperm: Cellulosic parenchyma containing oil globules and aleurone grains.



T.S. of Coriander seed

Powder Characteristic elements:



Powder Microscopy of Coriander Fruits

1. A group of sclereids from the mesocarp with adjacent unlignified parenchyma
2. Branching vittae (shown in outline only) and underlying endocarp in surface view.
3. Covering trichomes.

4. Part of a group of fibro vascular tissue.
5. Epicarp in surface view showing stomata and striated cuticle.
6. Part of vittae showing transverse septa and part of the underlying endocarp in surface view
7. Testa in surface view.
8. Endosperm containing microspheroidal crystal of calcium oxalate.

CONSTITUENTS:

1. Volatile oil: (i) Main (+) linalool (coriandrol) and α-pinene. (ii) Limonene (iii) α and γ-telpinene, (iv) P-cymene, (v) Camphor, (vi) Geraniol, (vii) Borneol
2. Fixed oil
3. Malic acid
4. Tannin
5. Vitamin A.

Uses:

1. Carminative.
2. Flavouring agent.
3. Anthelmintic.
4. Aromatic.
5. Diuretic.
6. Stimulant.
7. Stomachic.
8. Aphrodisiac..
9. Oil is used along with purgatives to prevent gripping

IDENTIFICATION TESTS:

1. Wagner's Test: 2 ml of extract was treated with wagner's reagent (Iodine and Potassium iodide in 100 ml water) and formation of reddish brown precipitate indicates of the presence of alkaloids.
2. Molisch's Test Few drops of molisch's reagent is added to each of the portion dissolved in distilled water, this was then followed by addition of 1 ml of Conc. H2SO4 by the side of the test tube. The mixture was then allowed to stand for two minutes and then diluted with 5ml of

   distilled water. Formation of red or dull violet colour at there was interphase of the two layers was a positive test indicates of the presence of carbohydrates.

3. Liebermann – Burchard Test 1ml of extract was treated with chloroform, acetic anhydride and few drops of H2SO4 was added and observed the formation of dark green colour indicates of the presence of terpenoids.

RESULT:

The morphological, microscopical and chemical evaluation of given crude was successfully performed.

PRACTICAL WORKS

1. Observe the physical characters of the powder and the fruits and describe them below:

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2. Identification of terpenoids:

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3. Identification of carbohydrates.

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4. Observe Powder characteristic fetures of Coriander Fruits

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Experiment No- 2

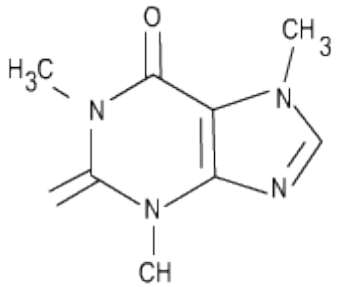
EXERCISE INVOLVING ISOLATION AND DETECTION OF ACTIVE PRINCIPALES; CAFFEINE FROM TEA DUST, DIOSGENIN FROM DIOSCOREA, ATROPINE FROM BELLADONA, SENNOSIDE FROM SENNA

OBJECTIVE 2.1

To extract caffeine from tea powder using polar - nonpolar solvent extraction technique.

Theory:

The technique used to separate an organic compound from a mixture of compounds is called Extraction. Extraction process selectively dissolves one or more of the mixture compounds into a suitable solvent. The solution of these dissolved compounds is referred to as the Extract. Here the organic solvent dichloromethane is used to extract caffeine from an aqueous extract of tea leaves because caffeine is more soluble in dichloromethane (140 mg/ml) than it is in water (22 mg/ml). However, the tannins that are slightly soluble in dichloromethane can be eliminated by converting it to their salts (phenolic anions by adding sodium carbonate) (tannins are phenolic compounds of high molecular weight and being acidic in nature can be converted to salts by deprotonation of the -OH group) which remain in the water. Bevarages cover a vast variety of addictive drinks out of which Tea and Coffee are the most popular acceptable drinks. Tea powder is extracted from tea leaves which contain tannins, which are acidic in nature, a number of colored compounds and a small amount of unrecompensed chlorophyll and an important stimulant called Caffeine. Because of the presence of Caffeine, tea and coffee are gaining popularity as an addictive stimulant. An average 30g of tea can contain 20-ll0 mg of caffeine thereby making tea a significant source of caffeine compared to other beverages. Caffeine can stimulate nervous system and can cause relaxation of respiratory and cardiac muscles. Caffeine is well known to increase both the alertness level and attention span. But like all other addictives, tea also shows withdrawal symptoms like headache, nervousness and insomnia for a regular consuming person.



Caffeine

Caffeine, 1,3,7 - trimethylxanthine, belongs to a wide class of compounds known as alkaloids. These are plant derived compounds with complex structure containing nitrogen, and usually have roles in physiological activity. The melting point of Caffeine is 238°C.

PRINCIPLE:

Extraction is a method used for the separation of organic compound from a mixture of compound. This technique selectively dissolves one or more compounds into an appropriate solvent. The solution

of these dissolved compounds is referred to as the extract. In the case of Caffeine extraction from tea powder, the solubility of caffeine in water is 22mg/ml at 25°C, 180mg/ml at 80°C, and 670mg/ml at 100°C. Here the organic solvent Dichloromethane is used to extract caffeine from aqueous extract of tea powder because caffeine is more soluble in dichloromethane (140mg/ml) than it is in water (22mg/ml).The dichloromethane - caffeine mixture can then be separated on the basis of the different densities of dichloromethane and water because dichloromethane is much denser than water and insoluble in it. Residual water is separated from dichloromethane by drain out the dichloromethane through separating funnel, thus dichloromethane passed through the funnel while polar solvents such as water is still remains in the funnel. Water and dichloromethane is slightly soluble in each other. So, after separating the solvents, residual water will remain the organic layer. Mainly anhydrous sodium sulfite is used for the removal of water from organic layer. Anhydrous sodium sulfite is an insoluble inorganic solid which will absorb water, thus drying it.

REAGENTS REQUIRED:

Dichloromethane, Anhydrous sodium sulfite, Distilled water.

MATERIALS REQUIRED:

Tea bags, Beaker (500ml), Hot plate, Separating funnel, Melting point apparatus,

PROCEDURE:

1. Tea bags are used as the source of caffeine for this experiment.
2. Take 5 tea bags and record the weight of these tea bags.
3. Take 500 ml beaker add 200 ml of distilled water to it. Now place the 5 tea bags in this beaker.
4. Boil the contents in the beaker vigorously using a hot plate.
5. Allow the mixture to cool for 5 minutes and then decant the mixture into another beaker.
6. Gently squeeze the tea bags to liberate the rest of the water.
7. Cool the aqueous solution to near room temperature.
8. Continue cooling in an ice box, the tea must be cool (20° C) before coming in contact with dichloromethane (boiling point = 40° C).
9. Extract the solution three times with 30-mL portions of dichloromethane (CH2Cl2). Do not get dichloromethane on your hands.

- The tea solution is poured into a separating funnel and 20ml of dichloromethane is added to it. The mixture will separate into two layers - the top layer is the tea layer and bottom layer is the dichloromethane since it is denser than tea.
- Remove the funnel from the stand and keep your fingers on the stopper and carefully shake the separating funnel.
- Vent the separating funnel periodically (every 30 sec) to relieve vapour pressure created inside the funnel.

- When the contents have been sufficiently shaken place the separating funnel back on the ring stand and let the two layers separate.
- Drain the bottom layer into a conical flask because now the caffeine is extracted into the dichloromethane layer. Cover the mouth of the conical flask to avoid evaporation of solution.
- Repeat steps a) through e) twice.

10. Dry the combined dichloromethane solutions with anhydrous Sodium sulfite. Add about 1 teaspoon of the drying agent until it no longer clumps together at the bottom of the flask. Mix well and leave it for 10 minutes.
11. Decant the dichloromethane into a conical flask (100ml). Evaporate the dichloromethane solvent in a hot water bath.
12. When all the solvent is removed you observe a residue of yellowish green - white crystalline caffeine.

- Take the conical flask containing crystalline caffeine.
- Sublime the crude caffeine at atmospheric pressure by placing the flask directly on a pre-heated hot plate. Caffeine melts at 238°C and sublimes at 178°C.
- Collect your sublimed caffeine by keeping a test tube on the mouth of the conical flask.

13. White vapour of caffeine sticking onto the test tube and the walls of the conical flask is observed.
14. Now cool the conical flask.
15. Take a clean watch glass and record its weight in a weigh balance.
16. Now strip off the caffeine from the conical flask and the walls of the test tube into the watch glass using a spatula.
17. Record the weight of the watch glass + caffeine in a weigh balance and then find out the weight of extracted pure caffeine.
18. The melting point of the extracted caffeine is determined using the melting point apparatus.

CHEMICAL TESTS:

Few crystal of caffeine are treated with 3-4 drops of nitric acid in porcelain dish and evaporated to dryness. Addition of 2 drops of ammonium hydroxide solution to the residue gives a purple colour.

PERCENTAGE CALCULATION:

Weigh of tea dust = Known quantity taken during experiment

Weigh of caffeine = X

% yield of caffeine = weigh of caffeine(X) / weigh of tea dust x 100.

RESULT:

Hence, the percentage yield of caffeine was found to be…….....%w/w.

PRACTICAL WORK

1. Melting point of caffeine is:

   A) 450C

   B) 900C

   C) 1800C

   D) 2300C

2. Role of sodium carbonate in extracting caffeine is:

   A) Diluent

   B) To react with coffee extract to form water soluble substances

   C) To dissolve methylene chloride

   D) None of the above

3. Caffeine is an:

   A) Alkaloid

   B) Flavinoid

   C) Terpanoid

   D) All the above

4. The sublimation point of caffeine is:

   A) 1780C

   B) 1600C

   C) 1000C

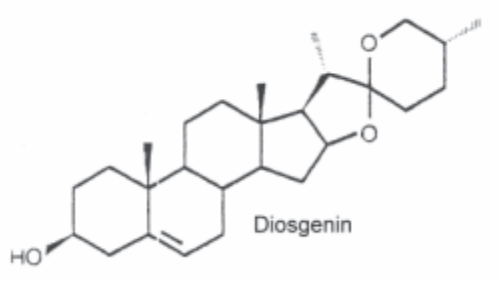
   D) 2000C

OBJECTIVE 2.2

To extract Diosgenin from Dioscorea alata powder using hydrolysis technique.

THEORY

Diosgenin is a bioactive steroidal sapogenin belonging to the triterpene group and is of great interest to the pharmaceutical industry. It is the aglycone formed by the hydrolysis of saponin dioscin, a compound found in Dioscorea spp. It serves as an important starting material for the production of corticosteroids, sexual hormones, oral contraceptives as well as other steroidal drugs via hemisynthesis. Different chemical and biological protocols are reported to extract diosgenin.



Diosgenin

REAGENTS REQUIRED

Methanol, HCl or H2SO4, distilled water, alcohol, sodium bicarbonate, Benzene, Diethylether, Silica gel G

MATERIALS REQUIRED

Tea bags, Beaker (500ml), Hot plate, Separating funnel, Melting point apparatus,

PROCEDURE

1. Alcoholic extraction method:
2. Dioscorea tubers are cut into small pieces & dried under sun.
3. Dried tubers are powdered , extracted with ethanol / methanol , twice for 6-8 hrs
4. Filter & filterate is concentrated to a syrupy liquid.
5. The concentrated liq. Is then hydrolysed using an acid , HCl or H2SO4 for 2 -12 hrs.
6. 85% of diosgenin is ppted.
7. Ppts are filtered , washed with water.
8. Purification with alcohol.

Acid hydrolysis method

1. Dried rhizomes are powdered (20#) and first subjected to hydrolysis by refluxing with 5% HCl for 2 hours.
2. The hydrolyzed mass is filtered, washed twice with water and then twice with 5% sodium bicarbonate solution.
3. It is finaly washed with water till the washing are neutral. The residue thus obtained is dried and futher extracted with toluene for 8 hours.
4. The toluene extract concentrated during which diosgenin gets precipitated.
5. Diosgenin filtered, washed with little hexane and dried (40-60 o c) to yield about 95% pure product.

Fermentation cum acid hydrolysis

1. The fresh green roots are collected & smashed in a hammer mill . the mesh is placed in the fermentation bin & allowed for fermentation for 2 days .
2. The fermented mesh is dried in sun to reduce the moisture content to 7-8 %. It is then subjected to hydrolysis with a mineral acid at reduced temp.
3. Resulting solution is extracted with heptane to obtain diosgenin.

Incubation cum acid hydrolysis method

1. The fresh plant material is incubated in H2O at 370 C for few days.
2. It is later subjected to acid hydrolysis.
3. The hydrolysed liq is concentrated & extracted with hydrocarbon solvent to obtain diosgenin Purification of Diosgenin: Crystallise the diosgenin from acetone and Chromatograph it on Al 2 O 3 and elute with Benzene : Diethylether (9:1), then crystallize it from methanol.

Identification tests Thin Layer Chromatography

Identification tests Thin Layer Chromatography Stationary phase: silica gel G Solvent system: Toluene: Ethyl acetate (7:3) Spraying reagent: Anisaldehyde in sulfuric acid Standard solution: Dissolve Std .diosgenin 1mg in 1 ml chloroform. Test solution : Dissolve residue obtained through isolation in chloroform Rf (for diosgenin) : 0.62.

Chemical Tests

Libermann-Burchard test: Treat the extract with few drops of acetic anhydride , boil and cool. Then add conc .sulphuric acid from the sides of test tube , brown ring is at the junction of two layers &upper layer turns green (steroids) and formation of deep red colour (triterpenoids) 2. Libermann ‘s reaction : mix 3 ml extract + 3 ml acetic anhydride heat & cool add few drops of conc H2SO4 Blue color obtained .

PERCENTAGE CALCULATION:

Weigh of tea dust = Known quantity taken during experiment

Weigh of diosgenin = X

% yield of diosgenin = weigh of diosgenin (X) / weigh of Dioscorea x 100.

Result: Hence, the percentage yield of diosgenin was found to be…… %w/w.

PRACTICAL WORK

Q.1. Write about Acid hydrolysis method of isolation of Diosgenin from Dioscorea alata

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Q.2. What was the mobile phase compostion and Rf value of Diosgenin on TLC

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Q.3. Write the name of chemical test used for detection of Diosgenin

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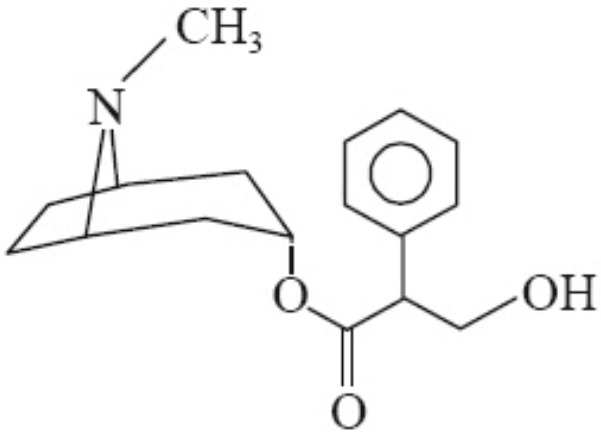
OBJECTIVE 2.3

To isolation and identification of atropine from Atropa *belladonna*.

INTRODUCTION

Atropine is a tropane alkaloid from the members of the Solaneaceae family. It is present in Atropa belladonna, Datura stramonium and Hyocyamus niger, other important solaneaceous alkaloids are hyocyamine, hyoscine (scopolamine), apoatropine, belladonine and norhyoscyamine. Atropine is an optically inactive laevorotatory isomer of hyoscyamine.



Atropine

Atropine alkaloid is mainly found in member of solanaceae family in different concentration:

1. Atropa belladona (0.4 – 1%)
2. Datura stromonium (0.2%)
3. Hyosymus niger (0.05%)
4. Other solanaceous alkaloid are Hyosyamine, Hyoscine (Scopolamine), Apoatropine, Belladonine

REAGENTS REQUIRED

K2CO3, CHCl3, Methanol, H2SO4, Distilled water, alcohol, Calcium chloride, KOH, Methyl alcohol, Ammonia, Diethylether, Acetic acid, Ethyl acetate and Silica gel G

Materials Required: Atropa belladona, Beaker (500ml), Hot plate, Separating funnel, Melting point apparatus,

PROCEDURE:

Powdered drug/ juice + moisten drug with aqueous solution of K2CO3 Extract with CHCl3, Evaporate solvent. Re extract with Dil. H2SO4 Acidic extract made alkaline by K2CO3 Atropine precipitate out, Crystallize by use of alcohol.

Chemical Tests:

Vitali – Morin reaction: - Alkaloid/ atropine (1µg) + Drop of H2SO4 Evaporate to dryness add 0.3ml of 3% solution of KOH in methyl alcohol which produce bright purple color Indicates presence of atropine. On addition of AgNO3 solution to solution of hyoscine hydrobromide, Yellowish white ppt Insoluble – HNO3 Soluble – Dil. NH3

Identification by TLC:

1% solution of atropine dissolved in 2N acetic acid is spotted over silica gel G plate and eluted in the solvent system of strong NH3 solution – methanol (! : 5 : 100).• TLC plate is spread with an acidified iodoplatinate solution.•Rf – 0.18.•Solvent system – Acetone – 0.5 sosium chloride solution.•Spraying reagents – Dragondroff's reagent

PERCENTAGE CALCULATION:

Weigh of tea dust = Known quantity taken during experiment

Weigh of atropine = X

% yield of atropine = weigh of atropine (X) / weigh of Atropa belladona x 100.

RESULT:

Hence, the percentage yield of atropine was found to be…… %w/w.

PRACTICAL WORK

Q.1. Atropine is a tropane alkaloid from the members of the Solaneaceae family. It is present in.

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Q.2. Write about theoretical % yield of atropine from Datura stromonium

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Q.3. Write the name of reaction or chemical test used fir identification of atropine.

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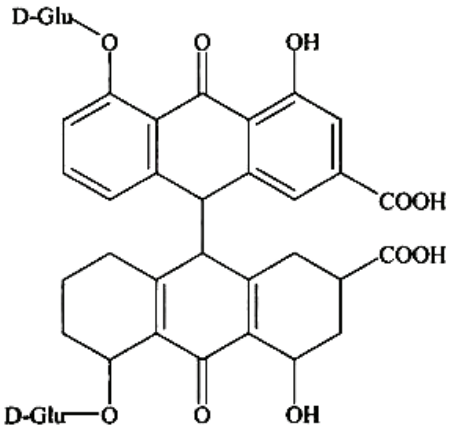
OBJECTIVE 2.4

To isolate and identification of sennosides from senna leaves

INTRODUCTION

Sennosides are obtained from the dried leaflets of Cassia senna (Cassia acutifoliaj Delile and Cassia angustifolia Vahl (Fam: Leguminosae).



Sennoside

REAGENTS REQUIRED

Benzene, Methanol HCL, Distilled water, Calcium chloride, Ammonia, Diethylether, Ethyl acetate , Silica gel G

Materials Required: Seena leaves, Beaker (500ml), Hot plate, Separating funnel, Melting point apparatus,

PROCEDURE

Senna leaves are dried and powdered to coarse powdered senna is extracted with benzene in electric shaker 2 hrs Filter the extract and dried with 70% methanol for 5hrs and again extract marc with fresh methanol for 2 hrs Filter and combine with extracts and concentrate up to 1/4 th of its.

Add sufficient quantity of HCL to make pH 3.2 and keep a side for 2 hrs at 5°C Filter and add sufficient quantity of alcoholic anhydrous calcium chloride with continuous stirring Add ammonia to bring pH 8 and kept a side for 2hrs filter and collect ppt of calcium Sennosides Dry the ppt in desiccators and calculate percentage yield

Chemical Tests

Borntragers test: Take a little quantity of a solution of sample +water +CCL4 or ether Separate organic layer and shake with dilute ammonia Rose pink color of ammonia layer appearss.

Identification by TLC

TLC/HPTLC: Stationary phase silica gel G; Mobile phase : ethyl acetate : Methanol: water (100:16.5:13.5

v/v); Detection: Red colour spots will appear when the spots are sprayed with 25% nitric acid and turn to yellow when sprayed after drying with alcoholic potassium hydroxide solution.

PERCENTAGE CALCULATION

Weigh of tea dust = Known quantity taken during experiment

Weigh of diosgenin = X

% yield of diosgenin = weigh of Seenosiode (X) / weigh of seena x 100.

RESULT

Hence, the percentage yield of Seenosiode was found to be…… %w/w.

PRACTICAL WORK

Q.1. Write the common name and biological source of crude drug used for isolation of seenosides

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Q.2. Write the name of different menstrum used during isolation of seenosides.

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Q.3. Write the composition of mobile phase and spraying reagent used for identification of seenosides.

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Experiment No: 3

SEPRATION OF SUGAR BY PAPER CHROMATOGRAPHY

OBJECTIVE 3.1

To perform paper chromatography for separation of sugar.

PRINCIPLE

Distribution of solute (sugar) between the stationary and mobile phases, that is the partition process is the major factor in the PC separation of sugars. Their partition coefficients are substantially in favour of the aqueous phase. Therefore, with non-aqueous developers, sugars appear on the paper choromatogram with low Rf values, whereas with developer containing larger aqueous ratio, the Rf values of sugars are much higher. This is because a sugar molecule containing larger number of hydroxyl groups which is readily soluble in water and makes the partition coefficient in favour of the aqueous phase. Further, the Rf values of sugars are affected by their structural formulae, their molecular mass, the number of-OH ' groups, and presence of other kinds of groups such as aldehydes. or ketones etc.

MATERIALS

Paper (Usually whatman No. 1 filter paper), Boiling tubes, Measuring cylinder, Spotting Capillaries, Spraying-bottle,

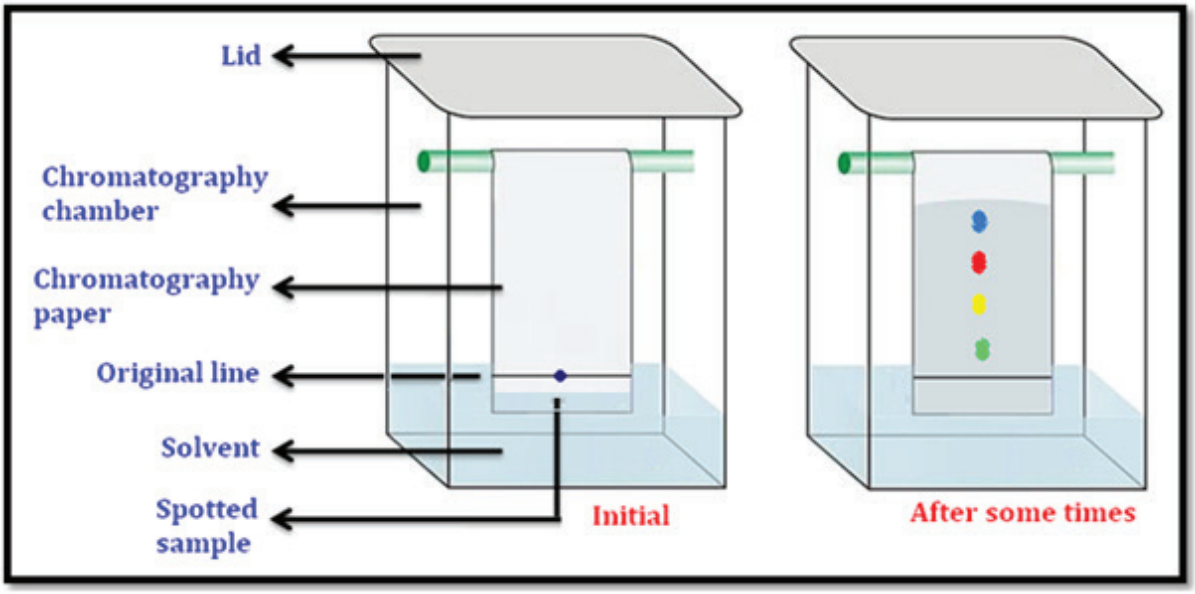
SOLVENTS

1. Water-saturated phenol + 1% ammonia
2. n-butanol-acetic acid-water (4:1:5 v/v)
3. Isopropanol-pyridine-water-acetic acid (8:8:4:1 v/v)

SPRAY REAGENT

1. **Ammoniacal silver nitrate:** add equal volumes of NH4OH to a saturated solution of AgNO3 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.
2. **Alkaline permanganate:** Prepare aqueous solution of KMNO4 (1%) containing 2 % NaCO3.After spraying with this mixture, the chromatograms are kept at 100C for a few minutes, when the sugar spots appear as yellow spots in purple background.
3. **Aniline diphenylamine reagent:** Mix 5 volumes of 1% aniline and 5 volumes of 1% diphenylamine in acetone with 1 volume of 85% phosphoric acid .after spraying the dried chromatograms with this solution the spots are visualized by heating the paper at 100C for a few minutes.
4. **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 90C.

PROCEDURE:



Photograph of experimental Paper Chromatography

1. Place sufficient solvent into the bottom of the tank. Cover the led and allow the tank to be saturated with the solvent.
2. Take a sheet of whattman 1 chromatography paper (about 9 x 10 cm) 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 spots the mixture contained.

OBSERVATION:

Observe the colour of the spots of various sugars. The colour depends on the detector used. Measure the distance travelled by the centre of the solute zone (ds) and thc distance lravelled by the solvenl front (dm) on the paper choromatogram. Calculate the Rf values of each sugar by the relation RF = &/dm.

RESULT:

Sugars present in the unknown sample are:.....with RFvalue of.....

PRACTICAL WORK

Q.1. Draw a sketch of your chromatogram.

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Q.2. Calculate Rf values for each spot of the mixture being separated.

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Q.3. Comparing the RF values of the mixture along with those for the standards, state what sugars does this mixture contain?

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Experiment No: 4

TLC OF HERBAL EXTRACT

OBJECTIVE 4.1

To separate the chlorophyll pigments by Thin Layer Chromatography (T.L.C.).

INTRODUCTION

Mixtures of compounds are very common in Organic Chemistry. Most reactions produce more than one product. Naturally occurring materials are only rarely 100% pure. It is therefore desirable to have a simple, fast and efficient way to determine the purity of Organic mixtures. The separation of a mixture by passing it, in solution, over an adsorbent (such as Alumina or Silica Gel) is the basic idea of Chromatography.

THEORY

In thin layer chromatography, a solid phase, the adsorbent (the stationary phase) is a powder which is coated onto a solid support, as a thin layer (about 0.25 mm thick). Thin plates of glass are the most common support, but plastic and aluminum can also be used. In TLC, the mixture starts as a small spot near the bottom of the plate and the solvent (mobile phase) carries the compounds up the plate as it travels up from the bottom by capillary action. In most cases, the stationary phase (adsorbent) is very polar and the mobile phase (eluant) is fairly non-polar. Molecules that are more polar stick to the polar stationary phase more than fairly non-polar molecules which are carried along in the mobile phase. Keep in mind that this is an equilibrium, all molecules do a little of both. Separation occurs because some things spend a higher percentage of the time standing still, adsorbed on the stationary phase than others do. Several factors determine the efficiency of a chromatographic separation. The adsorbent and solvent system chosen are the easiest factors to change. Silica gel (SiO2) is a very commonly used, strongly polar adsorbent material that you will be using for these experiments. Other common polar adsorbents include alumina, charcoal and Florisil. Non-polar adsorbents may also be used with relatively polar solvent systems, this is known as “reverse phase” chromatography because the nature of the stationary and mobile phases is inverted. Drug purification frequently employs this technique.

The most common factor that is adjusted to achieve good separation is the solvents used in the mobile phase. As you can see in the list provided below, there are many choices for solvents and solvent mixtures are quiet common. The substances being separated are adsorbed onto the stationary phase, but polar solvent molecules are also adsorbed by the stationary phase.

Molecules that are already adsorbed are displaced and “pushed along” by polar solvent molecules. Thus, everything moves up the plate faster in more polar solvent systems. The “Eluting power” of a solvent is largely a measure of how well it is adsorbed onto the stationary phase, displacing other molecules.

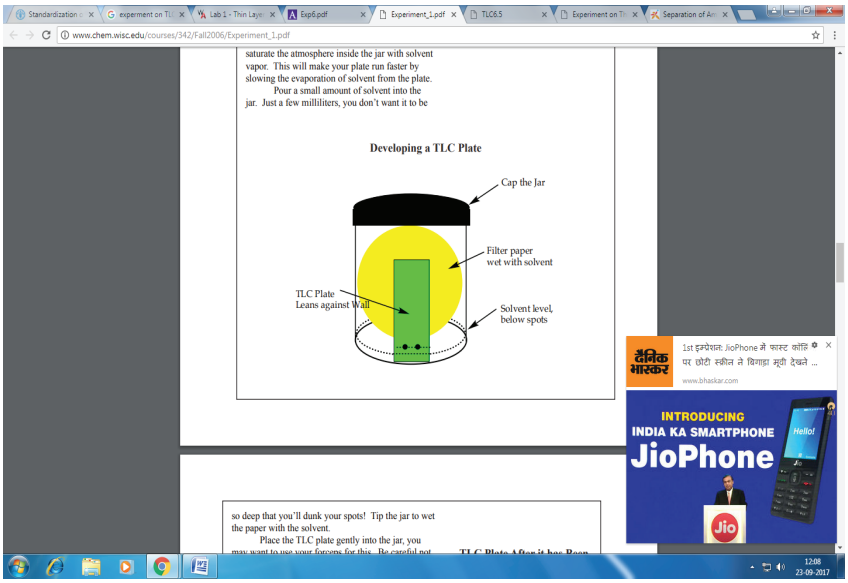
MATERIAL

Spinach leaves, slides, staining tube, acetone, benzene, mortar, pastel, Silica gel G, drier, pencil, sand, distilled water and oven.

PREPARATION OF T.L.C. SLIDES

1. Prepare slurry of Silica gel by suspending about 2 gm. of Silica gel in 10 ml. of distilled water.
2. Take 4 clean microscopic slides and place them in a horizontal position.
3. Spread the suspension over these slides very quickly m such a manner that a homogenous layer of Kieselgel is formed. Wait for 10 to 15 minutes.
4. Keep these slides in an oven for about 30 minutes at 120°C.
5. The slides become dry and are now ready for the further treatment.

PROCEDURE:



Photograph of TLC chromatogram development

1. Take a TLC slide and draw a small circle at a distance of about 1.5 cm from one edge.
2. Put a spot of chlorophyll extract in the circle and make it dry with a drier. Make the spot concentrated by applying the extract 2-3 times.
3. Take a beaker having the benzene-acetone (8.5:1.5) solvent and put the lower edge of the TLC slide (having the chlorophyll spot) in the solvent.
4. Cover the beaker with a petri-dish and keep it in an undisturbed condition for about 20-30 minutes.

OBSERVATIONS AND RESULTS:

Different pigments separate at different levels on T.L.C. slide.

THE SEQUENCE OF PIGMENTS FROM TOP TO BOTTOM IS AS UNDER:

Carotene — Orange yellow

Xanthophylls — Yellow bands

Chlorophyll a — Bluish green

Chlorophyll b — Yellowish green

CALCULATE THE RF VALUES WITH THE FOLLOWING FORMULA:

$$Rf = \frac{\text{Distance travelled by a given spot}}{\text{Distance travelled by solvent front}}$$

RESULT:

The Rf value of separated pigments from Spinach leaves was found

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PRACTICAL WORK

Q.1. What is thin layer chromatography and how does it work?

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Q.2. What is the purpose of the thin layer chromatography lab.

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Q.3. How do you calculate Rf values for TLC?

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Q.4. What is a TLC spotter.

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Experiment No: 5

DISTILLATION OF VOLATILE OIL AND DETECTION OF PHYTOCONSTITUENTS BY TLC

OBJECTIVE 5.1

To isolate and detection of volatile oil from different parts of lemon basil (Ocimum basilicum) using steam distillation and TLC.

INTRODUCTION

The genus Ocimum of Lamiaceae family is comprised of 200 species differentiated from each other on the basis of morphological features and chemical constituents. Lemon basil and sweet basil is used mainly as spice in Thai cuisine. Basil is cultivated for its extraordinary essential oil which display many therapeutic usages such as in medicinal application, herbs, culinary, perfume for herbal toiletries, aromatherapy treatment and as flavouring agent. Use of the basil leaves in food is beneficial to health because of antifungal, anti-inflammatory, antimicrobial and antioxidant activities.

MATERIAL

Ocimum basilicum, distilled water, toluene, ethyl acetate, vanillin, Clevenger apparatus, separating funnel, beaker, TLC plate, TLC Chamber, Conical Flask, Weighing balance.

Extraction of essential oil: Essential oil was extracted from stems, leaves and flowers through hydro-distillation using Clevenger type apparatus for three hours Essential oil yield was calculated on dry weight basis by using following formula.

% yield of EO = Volume of essential oil in ml (X) / weigh of plant material x 100.

The essential oil was stored at 4ºC in amber glass vials for use in experiments.

THIN LAYER CHROMATOGRAPHY (TLC)

TLC was carried out for the screening of major components present in essential oil extracted from stem, leaves and flowers. TLC Silica gel 60 F254 (20x20 cm) glass plates were activated at 110ºC for 30 minutes. Mobile phase consisted of toluene and ethyl acetate (19:1) for resolution of components. Essential oils samples were prepared by dissolving 10µl in toluene (50µl). Samples (3µl) were applied at 1.5cm distance from the base of the plate. After air drying the developed TLC plates were sprayed with methanolic solution of vanillin and heated at 100ºC for fifteen minutes. Retention factor (Rf) values were recorded for visible spots.

$$Rf = \frac{\text{Distance travelled by a given spot}}{\text{Distance travelled by solvent front}}$$

RESULTS:

Percentage yield of essential oil from lemon basil was found

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Preliminary phyto-chemical screening through TLC of essential oil of different parts of lemon basil revealed the presence of phytocontituents in difrent Rf value like Stem……. Leaves……Flowers……

PRACTICAL WORK

Q.1. Write the name of method used for isolation of volatile oil

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Q.2. What is the biological source of lemon basil.

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Q.3. Write the name of spraying reagent used for detection of total essential oil ?

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Q.4. Write the formula used for calculation of % yield of extract or essential?

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Experiment No: 06

ANALYSIS OF CRUDE DRUGS BY CHEMICAL TEST: (I) ASAFOETIDA (II) BENZOIN (III) COLOPHONY (IV) ALOES (V) MYRRH

OBJECTIVE 6.1

To perform Preliminary phytochemical screening of given powdered crude drug ((Asafoetida, Benzoin, Colophony, Aloes, Myrrh) by using different chemical test.

REFERENCE:

Khandelwal K.R., 'Practical Pharmacongnosy Techniques and Experiments', 21st edition, Aug 2011, Nirali prakashan, Pune, Page no. 25.1-25.6

INTRODUCTION OF CRUDE DRUG:

Asafoetida (Hing):

Asafoetida is the dried latex (gum oleoresin) exuded from the living underground rhizome or tap root of several species of Ferula (three of which grow in India) belongs to family Apiaceae. In India it is grown in Kashmir and in some parts of Punjab. The major supply of asafoetida to India is from Afghanistan and Iran. There are two main varieties of asafetida.

BENZOIN (LOBAN):

Benzoin is the balsamic resin obtained from the incised stem of Styrax benzoin Dryander and Styrax paralleloneurus Perkins. belongs to family Styraceae. Styrax benzoin is cultivated as a main source of benzoin resin in Indonesia. It is also grown as an ornamental tree for shade in West Africa

COLOPHONY:

Colophony is a natural substance obtained by distillation of oil from trees of the pine (Pinaceae) family. Rosin, also called colophony or Greek pitch (Latin: pix græca), is a solid form of resin obtained from pines and some other plants.

ALOES (GHEEKUMARI):

Aloe Vera (or Aloe Barbadensis Miller) is a plant - its that simple! It is a member of the onion and lily family but grows two to three feet tall with large thick leaves. Aloe flourishes in hot dry climates such as the Caribean, Far East and parts of America. It takes around four years until it is ready for harvest and then, just like any vegetable juice, it must be stabilised before it can start to oxidize and loose its goodness.

MYRRH:

Commiphora myrrha, called myrrh, African myrrh, herabol myrrh, Somali myrrhor, common myrrh, or gum myrrh is a tree in the Burseraceae family. It is one of the primary trees used in the production of myrrh, a resin made from dried tree sap

MATERIALS REQUIRED:

powder crude drug, Beaker, test tube, glass rod and reagents.

PROCEDURE:

1. To take a pinch of powder in test tube, this consists of chemical reagent.
2. After addition of powder in test tube, it shows the change in color.
3. The changed color of solution shows the presence of drug.

CHEMICAL TESTS:

TEST FOR ALKALOIDS:

Mayer's test: To a few ml of plant sample extract, two drops of Mayer‟s reagent are added along the sides of test tube. Appearance of white creamy precipitate indicates the presence of alkaloids.

Wagner's test: A few drops of Wagner‟s reagent are added to few ml of plant extract along the sides of test tube. A reddish- Brown precipitate confirms the test as positive.

TEST FOR AMINO ACIDS:

The extract (100 mg) is dissolved in 10 ml of distilled water and filtered through Whatmann No. 1 filter paper and the filtrate is subjected to test for Amino acids.

Ninhydrin test: Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) are added to 2 ml of aqueous filtrate. Appearance of purple colour indicates the presence of amino acids.

TEST FOR CARBOHYDRATES:

Molish's test: To 2 ml of plant sample extract, two drops of alcoholic solution of α- naphthol are added. The mixture is shaken well and few drops of concentrated sulphuric acid is added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates.

Benedict' s test: To 0.5 ml of filtrate, 0.5 ml of Benedict‟s reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

TEST FOR FIXED OILS AND FATS

Spot test: A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

Saponification test A few drops of 0.5 N alcoholic potassium hydroxide solution is added to a small quantity of extract along with a drop of phenolphthalein. The mixture is heated on a water bath for 2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

TEST FOR GLYCOSIDES

For 50 mg of extract is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests.

Borntrager's test: To 2 ml of filtered hydrolysate, 3 ml of choloroform is added and shaken, choloroform layer is separated and 10% ammomia solution is added to it. Pink colour indicates presence of glycosides.

LEGAL'S TEST:

50 mg of extract is dissolved in pyridine, sodium nitroprusside solution is added and made alkaline using 10% NaOH. Presence of glycoside is indicated by pink colour.

Test for Phenolic compounds and Tannins

FERRIC CHLORIDE TEST:

The extract (50 mg) is dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution are added. A dark green colour indicates the presence of phenolic compound.

GELATIN TEST:

The extract (50 mg) is dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing 10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds.

LEAD ACETATE TEST:

The extract (50 mg) is dissolved in of distilled water and to this 3 ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

ALKALINE REAGENT TEST:

An aqueous solution of the extract is treated with 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

Magnesium and Hydrochloric acid reduction: The extract (50 mg) is dissolved in 5 ml of alcohol and few fragments of magnesium ribbon and concentrated hydrochloric acid (drop wise) are added. If any pink to crimson colour develops, presence of flavonol glucosides is inferred.

TEST FOR PHYTOSTEROLS:

Libermann-Burchards test: The extract (50 mg) is dissolved in of 2 ml acetic anhydride. To this, 1 or 2 drops of concentrated sulphuric acid are added slowly along the sides of the test tube. An array of colour change shows the presence of phytosterols.

TEST FOR PROTEINS:

The extract (100 mg) is dissolved in 10 ml of distilled water and filtered through Whatmann No. 1 filter paper and the filtrate is subjected to test for proteins.

MILLON'S TEST:

To 2 ml of filtrate few drops of Millon‟s reagent are added. A white precipitate indicates the presence of proteins.

BIURET TEST:

2 ml of filtrate is treated with 1 drop of 2% copper sulphate solution. To this 1 ml of ethanol (95%) is added, followed by excess of potassium hydroxide pellets. Pink colour ethanolic layer indicates the presence of protein.

TEST FOR SAPONINS:

The extract (50 mg) is diluted with distilled water and made up to 20 ml. The suspension is shaken in a graduated cylinder for 15 minutes. A two cm layer of foam indicates the presence of saponins.

TEST FOR GUM AND MUCILAGES:

The extract (100 mg) is dissolved in 10 ml of distilled water and to this 2 ml of absolute alcohol is added with constant stirring. White or cloudy precipitate indicates the presence of Gums and Mucilages.

TEST FOR VOLATILE OIL:

For volatile oil estimation 50 mg of powdered material (crude drug) is taken and subjected to hydro-distillation. The distillate is collected in graduate tube of the assembly, wherein the aqueous portion automatically separated out from the volatile oil.

SN	Crude drug in Powder form	Name of Chemical test	Detection of secondary metabolite	Observation Present/ Absent
1	**Asafoetida**	Mayer's test	**Alkaloids**	
		Ninhydrin test:	**Amino acids**	
		Molish's test	**Carbohydrates**	
		Spot test	**Fixed oils and Fats**	
		Borntrager's test:	**Glycosides**	
		Ferric Chloride test	**Phenolic and Tannins**	
		Libermann-Burchard's test	**phytosterols**	
		Millon's test:	**Proteins**	
		Foam test	**Saponins**	
		Distilled water and to this 2 ml of absolute alcohol	**Mucilages**	
		Methanolic solution of vanillin	**volatile oil**	

2	**Benzoin**	Mayer's test	**Alkaloids**	
		Ninhydrin test:	**Amino acids**	
		Molish's test	**Carbohydrates**	
		Spot test	**Fixed oils and Fats**	
		Borntrager's test:	**Glycosides**	
		Ferric Chloride test	**Phenolic and Tannins**	
		Libermann-Burchard's test	**phytosterols**	
		Millon's test:	**Proteins**	
		Foam test	**Saponins**	
		Distilled water and to this 2 ml of absolute alcohol	**Mucilages**	
		Methanolic solution of vanillin	**volatile oil**	

SN	**Crude drug in Powder form**	**Name of Chemical test**	**Detection of secondary metabolite**	**Observation** **Present/ Absent**
3	**Colophony**	Mayer's test	**Alkaloids**	
		Ninhydrin test:	**Amino acids**	
		Molish's test	**Carbohydrates**	
		Spot test	**Fixed oils and Fats**	
		Borntrager's test:	**Glycosides**	
		Ferric Chloride test	**Phenolic and Tannins**	
		Libermann-Burchard's test	**phytosterols**	
		Millon's test:	**Proteins**	
		Foam test	**Saponins**	
		Distilled water and to this 2 ml of absolute alcohol	**Mucilages**	
		Methanolic solution of vanillin	**volatile oil**	

4	**Aloes**	Mayer's test	**Alkaloids**	
		Ninhydrin test:	**Amino acids**	
		Molish's test	**Carbohydrates**	
		Spot test	**Fixed oils and Fats**	
		Borntrager's test:	**Glycosides**	
		Ferric Chloride test	**Phenolic and Tannins**	
		Libermann-Burchard's test	**phytosterols**	
		Millon's test:	**Proteins**	
		Foam test	**Saponins**	
		Distilled water and to this 2 ml of absolute alcohol	**Mucilages**	
		Methanolic solution of vanillin	**volatile oil**	

SN	Crude drug in Powder form	Name of Chemical test	Detection of secondary metabolite	Observation Present/ Absent
5	**Myrrh**	Mayer's test	**Alkaloids**	
		Ninhydrin test:	**Amino acids**	
		Molish's test	**Carbohydrates**	
		Spot test	**Fixed oils and Fats**	
		Borntrager's test:	**Glycosides**	
		Ferric Chloride test	**Phenolic and Tannins**	
		Libermann-Burchard's test	**phytosterols**	
		Millon's test:	**Proteins**	
		Foam test	**Saponins**	
		Distilled water and to this 2 ml of absolute alcohol	**Mucilages**	
		Methanolic solution of vanillin	**volatile oil**	

RESULT:

The analysis of given samples of crude drug was successfully performed chemical test

PRACTICAL WORK

Q.1. Preliminary phytochemical screening of Colophony shows presence of metabolites like

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Q.2. What is the biological source of Benzoin.

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Q.3. Write the name of chemical test used for detection of alkaloids?

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Q.4. Write the name of chemical test used for detection of carbohydrates?

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Q.5. Ferric chloride test stand for detection of

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Q.6. Borntrager's test stand for detection of

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