

# PHARMACOGNOSY AND PHYTOCHEMISTRY PRACTICAL BOOK

**BP-110 P**

**SEMESTER I**

As per PCI New Syllabus 2026 (NEP 2020)



**AUTHORS**

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# **PHARMACOGNOSY AND PHYTOCHEMISTRY PRACTICAL BOOK**

**(For First Year B. Pharmacy Candidates)**

**Semester - I**

**Course Code - BP 110 P**

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# PREFACE

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Pharmacognosy and Phytochemistry form the foundation of the study of medicinal plants and natural products in pharmaceutical sciences. Practical knowledge of crude drugs, their identification, evaluation, phytochemical analysis, and standardization is essential for pharmacy students to understand the importance of herbal medicines and their therapeutic applications.

This practical book has been specially designed according to the syllabus prescribed for First Year B. Pharmacy students (Semester-I, BP 110 P). The book includes detailed practical experiments related to chemical identification tests, quantitative microscopy, powder microscopy, phytochemical evaluation, and pharmacopoeial standardization of crude drugs. The experiments have been presented in a simple, systematic, and student-friendly manner to facilitate better understanding and practical application in laboratory work.

Special emphasis has been given to organoleptic, morphological, microscopic, and chemical evaluation methods of medicinal plants and herbal materials. The book also includes principles, requirements, procedures, observations, results, precautions, viva questions, and references to strengthen conceptual understanding and practical skills among students.

The objective of this book is not only to help students perform practical experiments accurately but also to develop scientific thinking, observational skills, and interest in the field of herbal drug research and quality control.

We sincerely hope that this book will serve as a useful guide for students, teachers, and researchers in the field of Pharmacognosy and Phytochemistry. Suggestions for further improvement of this book will always be welcomed and highly appreciated.

Mr. Utkarsh R. Mandage  
Dr. Shital J. Patil

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## Experiment No. 1: Chemicals test for identification of Asafoetida

**Title:** Detailed Chemical and physical Identification Test for Asafoetida (Oleo-Gum-Resin).

**Aim:** To systematically perform and interpret chemical tests confirming the identity, purity, and quality of Asafoetida sample as per pharmacopoeial standards.

### **Principle:**

Asafoetida is an oleo-gum-resin containing gum (polysaccharides), resin (ferulic acid derivatives, coumarins) and volatile sulphur-containing oil.

- Concentrated  $\text{H}_2\text{SO}_4$  reacts with sulphur compounds producing reddish brown colour.
- Nitric acid oxidizes ferulic acid derivatives giving green colour.
- Phloroglucinol reacts with coumarin derivatives producing cherry red colour.
- Water forms stable emulsion due to gum content.

These reactions help differentiate genuine Asafoetida from adulterants such as galbanum or starch.

### **Requirements:**

**Chemicals-** Asafoetida commercial sample (2g, powdered), Conc.  $\text{H}_2\text{SO}_4$  (AR grade, 2 ml), Dil.  $\text{HNO}_3$  (10%, 5 ml), Dil.  $\text{HCl}$  (5%, 10 ml), Phloroglucinol (0.1g in alcohol), Distilled water (50 ml), sand (washed, 2g)

**Instruments-** Analytical balance (0.01g), mortar-pestle (porcelain), Bunsen burner, test tube holder

**Glassware-** Test tubes (10x75 mm, 5 nos.), beakers (50 ml, 100 ml), Buchner funnel, Whatman No.1 filter paper.

### **Theoretical Data:**

**Synonyms:** Hing (Hindi), Devil's dung, Food of the gods, Gum asafoetida

**Biological Source:** Asafoetida is an oleo-gum-resin obtained from incision of living rhizomes and roots of *Ferula asafoetida*, *Ferula foetida* Regel, *Ferula rubricaulis* Boiss., and some other species of *Ferula*. (**Family:** Apiaceae/ Umbelliferae).



**Figure 1: Morphological forms of Asafoetida (Hing) – Resin mass and Powder form.**

**Geographical Source:** Asafoetida is mainly obtained from Iran and Afghanistan. It is largely imported into India, with limited cultivation in cold desert regions.

**Morphological Characters:**

- Occurs as irregular masses or rounded/flattened tears (5–30 mm in diameter).
- Colour: Grayish-white when fresh; gradually becomes yellowish or reddish-brown on exposure to air.
- Surface: Smooth or slightly wrinkled; fresh tears may be coated with whitish powder.
- Consistency: Soft and plastic when fresh; hard and brittle on ageing.
- Fracture: Conchoidal and granular.
- Odour: Strong, characteristic alliaceous (garlic-like).
- Taste: Bitter and acrid, followed by mucilaginous sensatio

**Chemical Constituent:**

Component	Approx. %	Important Compounds
Gum	40–65%	Arabinose, galactose, glucuronic acid
Resin	20–30%	Ferulic acid, umbelliferone
Volatile oil	10–15%	Disulphides (sec-butyl propenyl disulphide)

Also: Polysaccharides, sesquiterpenes.

**Uses:**

- Carminative – Relieves flatulence, abdominal distension and colic.
- Digestive stimulant – Enhances appetite and alleviates indigestion.
- Antispasmodic – Reduces intestinal and bronchial spasms.

- Expectorant – Used in cough, bronchitis and asthma.
- Anthelmintic – Helps in expulsion of intestinal worms.
- Mild laxative – Useful in constipation.
- Antimicrobial – Exhibits activity against certain pathogenic microorganisms.
- Flavouring agent – Used as condiment in culinary preparations.

#### Chemical Tests:

Sr. No.	Test	Procedure	Observation / Result	Inference
1	Sulphuric Acid Colour Reaction	Take a small pea-sized piece of asafoetida in a test tube. Add 1–2 ml concentrated H <sub>2</sub> SO <sub>4</sub> on the fractured surface.  Warm gently if required.	Instant reddish-brown colour develops and persists.	Due to presence of sulphur compounds and resin. Persistent colour confirms oleo-gum-resin nature; no change may indicate adulteration.
2	Water Emulsion Test	Crush 0.5 g asafoetida in mortar. Add 5 ml water and triturate well. Filter if required.	Forms stable yellowish-orange milky emulsion. Emulsion breaks slowly on standing.	Indicates presence of gum and volatile oil. Helps distinguish from pure gums.
3	Nitric Acid Test	Take 0.2 g powdered drug. Add 2 ml dilute HNO <sub>3</sub> and shake gently.	Solution turns bright green immediately; colour may fade on standing.	Due to oxidation of ferulic acid derivatives. Green colour is diagnostic for asafoetida.
4	Phloroglucinol Differentiation Test	Triturate 0.5 g drug with sand and 5 ml dilute HCl. Filter. Add few drops of	Cherry red colour develops in true asafoetida.	Confirms presence of resinous coumarins. Helps

		phloroglucinol solution to filtrate.		differentiate from galbanum (which remains pale).
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**Result:**

The given sample showed characteristic positive reactions such as reddish-brown colour with concentrated sulphuric acid, green colour with nitric acid and formation of stable milky emulsion with water. These tests confirm the presence of oleo-gum-resin containing sulfur compounds and ferulic acid derivatives. Hence, the sample was identified as Asafoetida.

**Conclusion:**

Based on morphological characters and specific chemical tests, the given sample was confirmed as Asafoetida (Hing), an oleo-gum-resin used for medicinal and culinary purposes.

**Marketed Preparations:**

Asafoetida is commercially available as powder, compounded hing, and tablet formulations. It is widely marketed as hing powder for culinary as well as medicinal purposes. Tablet preparations are generally available in strengths of 250–500 mg and are used in digestive formulations. It is also included in polyherbal syrups and digestive tonics for the management of flatulence, abdominal colic, and indigestion. In retail markets, compounded hing (asafoetida mixed with starch or gum base) is commonly sold for ease of use.

**Precautions:**

- Use concentrated acids carefully and add dropwise.
- Perform colour reactions in small quantities.
- Use freshly prepared reagents for accurate results.
- Avoid overheating during acid tests.
- Observe colour change immediately after adding reagents.

**References:**

1. Indian Pharmacopoeia. 2022. Vol. III. Government of India, Ministry of Health and Family Welfare, New Delhi.
2. Kokate, C.K., Purohit, A.P., Gokhale, S.B. Pharmacognosy. 58th ed. Nirali Prakashan, Pune, 2023.
3. Evans, W.C. Trease and Evans Pharmacognosy. 16th ed. Elsevier, London, 2009.

### **Viva Questions & Answers (5–6)**

**Q1:** Define oleo-gum-resin with example.

**A:** Oleo-gum-resin is a natural exudate containing resin, gum and volatile oil. Example: Asafoetida.

**Q2:** Why does Asafoetida give reddish-brown colour with concentrated  $H_2SO_4$ ?

**A:** Due to oxidation of sulphur-containing volatile compounds and resin components.

**Q3:** What is the reason for green colour in Nitric acid test?

**A:** Oxidation of ferulic acid derivatives present in resin fraction.

**Q4:** What is the major active constituent responsible for garlic-like odour?

**A:** Sec-butyl propenyl disulphide (sulphur-containing volatile oil).

**Q5:** Name common adulterants of Asafoetida.

**A:** Galbanum, starch, chalk powder and inferior *Ferula* species.

**Q6:** What are the main therapeutic uses of Asafoetida?

**A:** Carminative, anti-flatulent, expectorant and antispasmodic.

## Experiment No. 2: Chemicals test for identification of Boswellia.

**Title:** Detailed Chemical and physical Identification Test for Boswellia.

**Aim:** To systematically perform and interpret chemical tests confirming the identity, purity, and quality of Boswellia sample as per pharmacopoeial standards.

**Principle:**

Boswellia is an oleo-gum-resin containing resin (boswellic acids), gum (polysaccharides) and volatile oil.

- Sequential solvent extraction confirms oleo-gum nature.
- Ferric chloride reacts with phenolic groups of boswellic acids producing violet-red colour.
- Concentrated  $H_2SO_4$  does not produce characteristic red colour (differentiates from benzoin).

These reactions help confirm identity and purity of Boswellia resin.

**Requirements:**

**Chemicals-** Boswellia tears 1gm, Petroleum ether 20ml, Chloroform 20ml, Ethanol 20ml  
5%  $FeCl_3$  solution, Conc.  $H_2SO_4$ , Distilled water

**Instruments-** Water bath

**Glassware-** Test tubes (5), mortar pestle, beaker 50ml, Separatory funnel

**Theoretical Data:**

**Synonyms:** Salai guggul, Indian olibanum, Indian frankincense, Shallaki

**Biological Source:** Boswellia consists of oleo-gum-resin obtained by making incisions in the stem bark of

*Boswellia serrata* Roxb. ex Colebr. (**Family:** Burseraceae).



**Figure 2: Boswellia (Salai guggul) showing resin tears and powdered form.**

**Geographical Source:** Boswellia is indigenous to India and commonly found in dry deciduous forests of Madhya Pradesh, Rajasthan and Gujarat.

**Chemical Constituents:**

Component	Approx. %	Important Compounds	Component
Resin	60–70%	Boswellic acids, AKBA	Resin
Gum	20–30%	Arabinose, galactose	Gum
Volatile oil	5–10%	$\alpha$ -thujene, p-cymene	Volatile oil

**Morphological characters:**

- Occurs as pale yellow, greenish-white or light brown resin tears.
- Tears are rounded or irregular, about 5–25 mm in diameter.
- May occur singly or in agglomerated masses.
- Hard and brittle when dry; becomes soft and slightly plastic on warming.
- Fracture: Brittle and conchoidal; translucent at edges.
- Odour: Pleasant, balsamic and aromatic.
- Taste: Slightly bitter and acrid.

**Uses:**

- Anti-inflammatory – Used in osteoarthritis and rheumatoid arthritis.
- Analgesic – Reduces joint pain and swelling.
- Anti-asthmatic – Helps in bronchial asthma by reducing inflammation.
- Anti-ulcer / Gastroprotective – Protects gastric mucosa.
- Wound healing agent – Promotes healing of minor wounds.
- Antimicrobial – Shows activity against certain microorganisms.

**Chemical Tests:**

Sr. No.	Test	Procedure	Observation / Result	Inference
1	Solubility Profile Test	Powder 1 g resin. Extract sequentially	Petroleum ether and chloroform extracts	Sequential solubility

		with petroleum ether (for resins), chloroform (for resins + gums), and alcohol (for gums). Evaporate each extract separately.	appear oily/waxy. Alcohol extract appears gummy. No complete dissolution occurs in a single solvent.	confirms oleo-gum-resin nature. Lack of complete dissolution indicates purity of resin.
2	Ferric Chloride Test	Dissolve 0.1 g resin in alcohol. Add 2–3 drops of 5% FeCl <sub>3</sub> solution.	Deep red-violet colour develops and remains stable.	Indicates presence of phenolic boswellic acids. Helps differentiate from benzoin (which gives yellow colour).
3	Sulphuric Acid Test	Add concentrated H <sub>2</sub> SO <sub>4</sub> dropwise to powdered resin.	No distinct red colour develops.	Absence of characteristic colour reaction differentiates Boswellia from benzoin; confirms resinous nature.

### Results:

The given sample showed characteristic positive reactions such as violet-red colour with ferric chloride and sequential solubility in organic solvents confirming its oleo-gum-resin nature. Hence, the sample was identified as Boswellia.

### Conclusion:

Based on morphological characteristics and specific chemical identification tests, the given sample was authenticated as Boswellia (Salai guggul) suitable for medicinal use.

**Marketed Preparation:**

Boswellia is commercially available in the form of capsules, tablets, and powder preparations standardized for boswellic acids. It is commonly marketed in strengths such as 250 mg, 300 mg, and 500 mg per tablet or capsule. It is widely used in anti-inflammatory and joint-care formulations indicated for osteoarthritis and rheumatoid arthritis. It is also incorporated into Ayurvedic polyherbal preparations for musculoskeletal disorders.

**Precautions:**

- Use freshly prepared ferric chloride solution.
- Handle concentrated sulphuric acid carefully.
- Avoid excess heating during solubility testing.
- Use dry glassware for solvent extraction.
- Perform tests in small quantities to avoid wastage.

**References:**

1. Indian Pharmacopoeia. 2022. Vol. III. Government of India, Ministry of Health and Family Welfare, New Delhi.
2. Kokate, C.K., Purohit, A.P., Gokhale, S.B. Pharmacognosy. 58th ed. Nirali Prakashan, Pune, 2023.
3. Evans, W.C. Trease and Evans Pharmacognosy. 16th ed. Elsevier, London, 2009.

**Viva Questions & Answers (5-6):**

**Q1:** What is the major active constituent of Boswellia?

**A:** Boswellic acids, especially 3-O-acetyl-11-keto- $\beta$ -boswellic acid (AKBA).

**Q2:** What is the main pharmacological action of Boswellia?

**A:** Anti-inflammatory action by inhibition of 5-lipoxygenase enzyme.

**Q3:** Which test confirms presence of phenolic compounds?

**A:** Ferric chloride test.

**Q4:** To which family does Boswellia belong?

**A:** Burseraceae.

**Q5:** Why is sequential solvent extraction performed?

**A:** To confirm presence of resin, gum and volatile oil in oleo-gum-resin.

**Q6:** Name one therapeutic use of Boswellia.

**A:** Used in osteoarthritis and rheumatoid arthritis

### Experiment No. 3: Chemicals test for identification of Aloes.

**Title:** Detailed Chemical and physical Identification Test for Aloes.

**Aim:** To systematically perform and interpret chemical tests confirming the identity, purity, and quality of Aloes sample as per pharmacopoeial standards.

**Principle:**

Aloes contains anthraquinone glycosides (mainly aloin/barbaloin) and related anthracene derivatives.

- Borntrager's test detects free anthraquinones by producing pink to red colour in alkaline medium.
- Modified Borntrager's test confirms C-glycosides (barbaloin) after hydrolysis.
- Nitric acid produces yellow to orange coloration due to oxidation of anthraquinone derivatives.
- Solubility characteristics help confirm its resinous nature.

These chemical reactions help in identification and authentication of Aloes.

**Requirements:**

**Chemicals-** Aloes powder – 1 g, Dilute HCl (2N), Ferric chloride solution (1%), Chloroform (10 ml), dilute ammonia solution, concentrated nitric acid.

**Glassware-** Test tubes (5–6), Mortar and pestle, Measuring cylinder, Whatman filter paper.

**Instruments-** Water bath.

**Theoretical data:**

**Synonyms:** Aloe, Kumari, Ghritkumari, Musabbar (Unani name for dried latex), Barbados Aloe (commercial drug)

**Biological Source:** Aloe consists of the dried latex obtained from the leaves of various species of Aloe such as Aloe barbadensis (Curacao Aloe), Aloe ferox (Cape Aloe), Aloe perryi (Socotrine Aloe), Aloe africana and Aloe spicata. **Family:** Asphodelaceae (formerly placed under Liliaceae).



**Figure 3: (A) Fresh plant, (B) Cut leaf, (C) Leaf segments, (D) Extracted gel.**

**Geographical Source:** Aloe is indigenous to North and East Africa and is widely cultivated in tropical and subtropical regions of the world.

Commercial varieties are mainly obtained from:

- Barbados (West Indies) – Curacao Aloe
- South Africa – Cape Aloe
- Socotra Island – Socotrine Aloe

In India, Aloe is cultivated in Rajasthan, Gujarat, Maharashtra, Tamil Nadu and Andhra Pradesh.

**Chemical Constituent:**

Sr. No.	Constituents	Approximate %	Remarks
1	Aloin (Barbaloin)	10–30%	Major anthraquinone glycoside
2	Isobarbaloin	—	Isomer of aloin
3	Aloe-emodin	Trace	Anthraquinone derivative
4	Resin	40–60%	Gives purgative action
5	Polysaccharides	Small amount	Present in gel
6	Chromones	Trace	Minor constituents

**Morphological Characters:**

- Occurs as dark brown to black, hard and brittle masses (dried latex).
- Surface smooth, glossy and resinous.
- Fracture conchoidal and glassy.
- Odour slight and characteristic.
- Taste intensely bitter.
- When dissolved in water, produces yellowish solution.

**Uses:**

- Stimulant laxative – Acts on large intestine and increases peristalsis.
- Purgative – Used in severe constipation.
- Bitter tonic – Improves digestion and appetite.
- Wound healing agent – Gel promotes epithelial regeneration.
- Anti-inflammatory – Reduces skin irritation and burns.
- Used in cosmetic preparations – Creams, lotions and shampoos.

**Chemical tests:**

Sr. No.	Test	Procedure	Observation	Inference
1	Borntrager's Test	Boil drug with dilute H <sub>2</sub> SO <sub>4</sub> , filter, extract with benzene, add ammonia	Pink to red colour in ammoniacal layer	Presence of anthraquinone glycosides
2	Modified Borntrager's Test	Boil with ferric chloride + HCl, extract with benzene, add ammonia	Rose-pink colour	Confirms C-glycosides
3	Nitric Acid Test	Add conc. HNO <sub>3</sub> to powder	Yellow to orange colour	Presence of anthraquinone derivatives

**Result:**

The given sample showed positive Borntrager's reaction with development of pink colour in ammoniacal layer confirming presence of anthraquinone glycosides. Hence, the sample was identified as Aloe.

**Conclusion:**

Based on morphological examination and positive chemical tests, the given drug sample was confirmed as Aloe containing aloin as the chief active constituent responsible for its laxative action.

**Marketed Preparations:**

- Aloe vera juice
- Aloe tablets (laxative formulations)
- Aloe gel (topical use)

- Aloe-based creams and ointments

**Precautions:**

- Avoid excessive heating during hydrolysis.
- Use freshly prepared reagents.
- Handle acids carefully.
- Perform test in small quantity to avoid wastage.

**References:**

1. Indian Pharmacopoeia. 2022. Vol. III. Government of India, New Delhi.
2. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 58th ed. Nirali Prakashan, 2023.

**Viva Questions & Answers (5-6):**

**Q1.** What is the chief constituent of Aloe?

**A:** Aloin (anthraquinone glycoside).

**Q2.** Why is Modified Borntrager's test performed?

**A:** To detect C-glycosides present in Aloe.

**Q3.** What is the main pharmacological action?

**A:** Stimulant laxative.

**Q4.** Which part of plant is used?

**A:** Dried leaf latex.

## Experiment No. 4: Chemicals test for identification of Guggul.

**Title:** Detailed Chemical and physical Identification Test for Guggul.

**Aim:** To systematically perform and interpret chemical tests confirming the identity, purity, and quality of Guggul sample as per pharmacopoeial standards.

**Principle:**

Guggul is an oleo-gum-resin containing resin, gum and volatile oil along with biologically active guggulsterones. Identification of Guggul is based on its characteristic morphological features and specific chemical reactions that indicate the presence of resinous and phenolic constituents.

Ferric chloride test detects phenolic compounds, while solubility behaviour in organic solvents confirms its oleo-gum-resin nature. The presence of guggulsterones is responsible for its pharmacological activity.

**Requirements:**

**Chemicals-** Concentrated sulphuric acid, Ferric chloride solution (5%), Ethanol / Alcohol, Distilled water, Petroleum ether (if solubility test performed)

**Glassware-** Test tubes, Beaker (50–100 ml), Conical flask, Measuring cylinder, Glass rod, Dropper, Funnel, Watch glass

**Instruments-** Weighing balance, Mortar and pestle, Water bath (if heating required)

**Theoretical data:**

**Synonyms:** Indian Bdellium, Mukul myrrh, Guggulu (Ayurvedic name), Mahishaksha

**Biological Source:** Guggul consists of the oleo-gum-resin obtained by incision of the stem bark of *Commiphora mukul*. (**Family:** Burseraceae.)



**Figure 4: Morphological forms of Guggul – Resin masses and powdered drug.**

**Geographical Source:** Guggul is indigenous to India and is widely distributed in Rajasthan, Gujarat and Madhya Pradesh. It is also found in Pakistan and parts of Arabia. The plant grows in arid and semi-arid rocky regions.

**Chemical Constituents:**

Sr. No.	Constituents	Approx. %	Significance
1	Guggulsterones (E & Z)	1–2%	Hypolipidemic activity
2	Resin	60–70%	Anti-inflammatory action
3	Gum	20–30%	Emulsifying property
4	Volatile oil	0.5–1%	Characteristic odour
5	Sterols & Diterpenes	Present	Anti-arthritic activity

**Morphological Characters:**

- Occurs as irregular, rounded or agglomerated tears.
- Colour: Pale yellow, brown or dark reddish-brown.
- Surface rough and dusty.
- Soft and sticky when fresh; becomes hard and brittle on ageing.
- Fracture: Irregular and resinous.
- Odour: Aromatic and balsamic.
- Taste: Bitter and slightly acrid.

**Uses:**

- Hypolipidemic agent – Reduces cholesterol and triglycerides.
- Anti-inflammatory – Used in rheumatoid arthritis and osteoarthritis.
- Anti-obesity agent – Enhances lipid metabolism.
- Thyroid stimulant – Mild thyroid stimulating effect.
- Antioxidant – Protects against oxidative stress.
- Used in Ayurvedic formulations like Yogaraj Guggulu.

**Chemical Tests:**

Sr. No.	Test	Procedure	Observation	Inference
1	Solubility Test	Shake powdered drug with alcohol	Partial solubility	Confirms oleo-gum-resin nature
2	Ferric Chloride Test	Add FeCl <sub>3</sub> to alcoholic extract	Brownish-green colour	Presence of phenolic compounds
3	Sulphuric Acid Test	Add conc. H <sub>2</sub> SO <sub>4</sub>	Reddish-brown colour	Presence of resin

**Result:**

The given sample showed characteristic morphological features and positive chemical reactions confirming presence of resin and guggulsterones. Hence, the sample was identified as Guggul.

**Conclusion:**

Based on morphological examination and chemical tests, the given drug sample was confirmed as Guggul, an oleo-gum-resin containing guggulsterones responsible for its hypolipidemic and anti-inflammatory activity.

**Marketed Preparations:**

- Guggul tablets (cholesterol control)
- Yogaraj Guggulu tablets
- Guggul capsules
- Anti-arthritis herbal formulations

**Precautions:**

- Store in airtight container (absorbs moisture easily).
- Avoid contamination with dust and sand.
- Use freshly prepared reagents for tests.
- Handle acids carefully during testing.

**References:**

1. Indian Pharmacopoeia. 2022. Vol. III. Government of India.
2. Kokate CK. Pharmacognosy. 58th ed. Nirali Prakashan, 2023.

3. Trease & Evans Pharmacognosy. 16th ed. Elsevier, 2009.

**Viva Questions & Answers (5-6):**

**Q1.** What is the chief active constituent of Guggul?

**A:** Guggulsterone (E & Z isomers).

**Q2.** To which family does Guggul belong?

**A:** Burseraceae.

**Q3.** What is the major therapeutic use?

**A:** Hypolipidemic and anti-inflammatory.

**Q4.** What type of drug is Guggul?

**A:** Oleo-gum-resin.

**Q5.** Which test confirms phenolic compounds?

**A:** Ferric chloride test.

**Q6.** In which disorder is Guggul commonly used?

**A:** Hyperlipidemia and arthritis.

## Experiment No. 5: Chemicals test for identification of Catechu.

**Title:** Detailed Chemical and physical Identification Test for Catechu.

**Aim:** To systematically perform and interpret chemical tests confirming the identity, purity, and quality of Catechu sample as per pharmacopoeial standards.

**Principle:** Catechu contains a high percentage of tannins, mainly catechin and catechu-tannic acid, which are responsible for its strong astringent property. Identification of Catechu is based on its characteristic morphological features and its chemical reactions with specific reagents used for tannin detection.

Tannins react with ferric chloride to produce a greenish-black colour and with lead acetate to form a white precipitate. These reactions confirm the presence of phenolic compounds in the drug. Thus, the identification of Catechu depends upon its tannin content and characteristic chemical behaviour.

**Requirements:**

**Chemicals-** Ferric chloride solution (5%), Lead acetate solution, Dilute hydrochloric acid, Distilled water

**Glassware-** Test tubes, Beaker (50–100 ml), Measuring cylinder, Conical flask, Glass rod, Dropper, Funnel, Watch glass

**Instruments-** Weighing balance, Mortar and pestle, Water bath (if heating required)

**Theoretical data:**

**Synonyms:** Cutch, Katha, Khadira (Ayurvedic name), Black Catechu

**Biological Source:** Catechu is the dried aqueous extract obtained from the heartwood of *Acacia catechu*. (**Family:** Fabaceae).

**Geographical Source:**

- Indigenous to India
- Widely distributed in Uttar Pradesh, Bihar, Madhya Pradesh, Assam
- Also found in Sri Lanka and Myanmar
- Grows mainly in dry deciduous forests

## TYPES OF CATECHU:

### Type 1: Pale Catechu (Katha)

1. **Source:** Obtained from the concentrated aqueous extract of Acacia catechu heartwood and allowed to crystallize slowly.
2. **Appearance:**
  - Light brown to pale reddish-brown
  - Porous and brittle
  - Occurs as irregular masses or cubes
  - Surface dull and rough
3. **Fracture:**
  - Brittle
  - Crystalline appearance
4. **Taste & Odour:**
  - Odourless
  - Strongly astringent taste
5. **Chemical Constituents:**
  - High amount of Catechin (10–20%)
  - Small amount of catechu-tannic acid
  - Quercetin
  - Gum and ash
6. **Uses:**
  - Used in pan (betel quid)
  - Astringent in diarrhea and dysentery
  - Used in mouth ulcers and sore throat
  - Dental preparations
  - Antioxidant property



**Figure 5. Pale Catechu**

## 7. Identification Tests:

Test	Observation	Inference
Ferric Chloride	Greenish-black colour	Presence of tannins
Lead Acetate	White precipitate	Presence of tannins
Matchstick Test	Faint pink	Presence of catechin (less amount)

### Type 2: Black Catechu (Cutch)

1. **Source:** Obtained from further concentration and evaporation of the decoction without crystallization.

2. **Appearance:**

- Dark brown to black
- Hard, compact and heavy
- Occurs as solid blocks

3. **Fracture:**

- Hard
- Shiny appearance

4. **Taste & Odour:**

- Odourless
- Strongly astringent

5. **Chemical Constituents:**

- Higher percentage of Catechu-tannic acid (20–50%)
- Less catechin
- Phlobatannins (responsible for dark colour)

6. **Uses:**

- Dyeing industry
- Leather tanning
- Textile coloring
- Sometimes medicinal use as astringent



Figure 6. Black Catechu

## 7. Identification Tests:

Test	Observation	Inference
Ferric Chloride	Greenish-black colour	Presence of tannins
Lead Acetate	White precipitate	Presence of tannins
Matchstick Test	Faint pink	Presence of catechin (less amount)

### Morphological Characters (General)

- Irregular cubes or masses
- Brittle texture
- Brown to black colour
- Odourless
- Strongly astringent taste
- Soluble in hot water forming brown solution

### Chemical Constituents (General Table)

Sr. No.	Constituents	Approx. %	Significance
1	Catechin	10–20%	Astringent, antioxidant
2	Catechu-tannic acid	20–50%	Strong astringent
3	Quercetin	Present	Antioxidant
4	Phlobatannins	Present	Colour property
5	Gum & Ash	Small quantity	Structural component

### Uses:

- Astringent – Controls diarrhea and dysentery
- Mouth ulcers & sore throat – Used as gargle
- Dental preparations – Strengthens gums
- Antioxidant – Due to catechin
- Dyeing & tanning industry – Mainly black catechu
- Pan preparation – Pale catechu (katha)

**Chemical Tests:**

Sr. No.	Test	Procedure	Observation	Inference
1	Ferric Chloride Test	Add 5% FeCl <sub>3</sub> to aqueous extract	Greenish-black colour	Tannins present
2	Lead Acetate Test	Add lead acetate solution	White precipitate	Tannins present
3	Matchstick Test	Apply extract on matchstick, add conc. HCl	Pink/red colour	Catechin present

**Result:**

The given sample showed characteristic morphological features such as brown brittle masses with strongly astringent taste. It produced greenish-black colour with ferric chloride and white precipitate with lead acetate confirming the presence of tannins and catechin. Hence, the sample was identified as Catechu.

**Conclusion:**

Based on macroscopic evaluation and positive chemical tests for tannins, the crude drug sample was confirmed as Catechu obtained from the heartwood extract of *Acacia catechu*. The major active constituents present are catechin and catechu-tannic acid which are responsible for its astringent property.

**Marketed preparations:**

- Catechu lozenges
- Herbal mouth gargles
- Dental powders and tooth pastes
- Astringent syrups
- Khadira preparations (Ayurvedic formulations)
- Used in preparation of pan (betel quid)

**Precautions:**

- Use freshly prepared ferric chloride solution for accurate results.

- Avoid contamination of the sample with moisture.
- Perform chemical tests with small quantities of the drug.
- Handle acids carefully during matchstick test.
- Store the drug in an airtight container away from humidity.

**References:**

1. Indian Pharmacopoeia. 2022. Vol. III. Government of India.
2. Kokate C.K. Pharmacognosy. 58th ed. Nirali Prakashan, 2023.
3. Trease & Evans Pharmacognosy. 16th ed. Elsevier, 2009.
4. C.S. Rama Murthy. Practical Pharmacognosy, 2021.

**Viva questions & answers:**

**Q1.** What is the biological source of Catechu?

**A:** It is the dried aqueous extract obtained from the heartwood of *Acacia catechu*.

**Q2.** To which family does Catechu belong?

**A:** Fabaceae.

**Q3.** What are the two types of Catechu?

**A:** Pale catechu (Katha) and Black catechu (Cutch).

**Q4.** What are the chief constituents of Catechu?

**A:** Catechin and catechu-tannic acid.

**Q5.** Which test confirms the presence of tannins?

**A:** Ferric chloride test.

**Q6.** What is the main pharmacological action of Catechu?

**A:** Astringent action.

**Q7.** What is the colour produced with ferric chloride?

**A:** Greenish-black colour.

## **Experiment No. 6: Quantitative microscopy for determination of size of starch grains, calcium oxalate crystals, fibre length and width using eyepiece micrometre**

**Aim:** To determine the microscopic dimensions of starch grains, calcium oxalate crystals, fibre length and fibre width of a crude drug sample using an eyepiece micrometer under a compound microscope.

**Requirements:**

**Apparatus-** Compound microscope, Eyepiece micrometer, Stage micrometer, Glass slides, Cover slips, Dropper, Forceps, Needle, Brush

**Materials-** Powdered crude drug, Distilled water, Iodine solution, Glycerin / suitable mounting medium

**Chemicals-** Iodine solution (for starch identification)

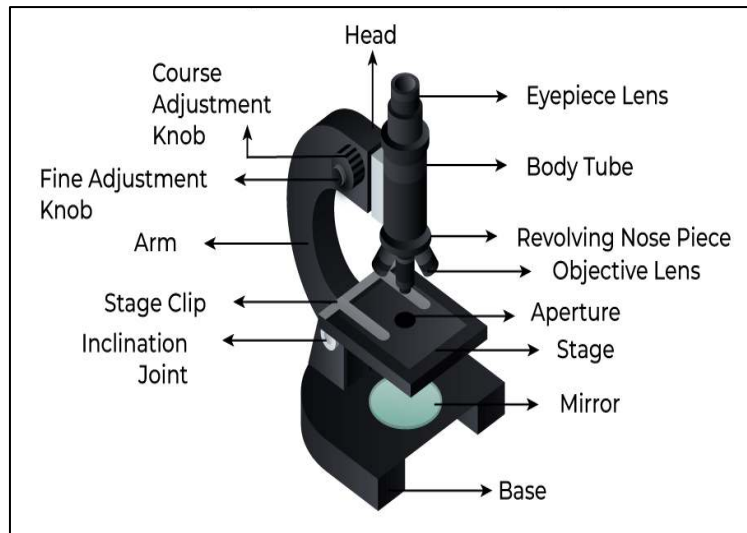
**Principle:**

Quantitative microscopy is a microscopic analytical technique used for the measurement of quantitative parameters of crude drugs. It is mainly employed for the evaluation, authentication and standardization of powdered crude drugs, especially in cases where chemical analysis is difficult or not possible.

In this method, an eyepiece micrometer (a calibrated glass scale fitted inside the microscope eyepiece) is used along with a stage micrometer to measure microscopic structures such as:

- Diameter of starch grains
- Size of calcium oxalate crystals
- Fibre length
- Fibre width

The eyepiece micrometer is first calibrated using a stage micrometer to determine the actual value of one division. After calibration, microscopic structures of the sample are measured and converted into real units ( $\mu\text{m}$ ). This technique plays an important role in the identification of crude drugs and detection of adulteration by providing measurable microscopic characteristics.



**Figure 7: Labeled diagram of compound microscope used for quantitative microscopy.**

**Procedure:**

**A. Calibration of Eyepiece Micrometer-**

**Purpose:** To determine the actual value of each division of the eyepiece micrometer using a stage micrometer.

**Steps:**

1. Switch on the compound microscope and clean the lenses properly using lens paper.
2. Insert the eyepiece micrometer into the eyepiece of the microscope.
3. Place the stage micrometer slide on the microscope stage.
4. Fix the slide with stage clips.
5. Select the low power objective (10×) and focus properly.
6. Adjust the stage micrometer scale so that both scales (eyepiece and stage) overlap clearly.
7. Align one zero division of the eyepiece scale with one zero division of the stage micrometer scale.
8. Count how many divisions of the eyepiece micrometer coincide with known divisions of the stage micrometer.
9. Note the number of divisions matched.
10. Calculate the calibration factor using the formula:  

$$\text{Value of 1 Eyepiece Division} = \frac{\text{Known value of Stage Micrometer Division}}{\text{Number of Eyepiece Divisions}}$$
11. Repeat the calibration at different magnifications if required.

12. Record the calibration value in the observation table.

This value will be used to convert microscopic readings into micrometers ( $\mu\text{m}$ ).

**Observations:**

**Table 1: Calibration of Eyepiece Micrometer**

Stage Micrometer Division	Eyepiece Division	Known Value ( $\mu\text{m}$ )	Value of 1 Eyepiece Division ( $\mu\text{m}$ )

**Calculation:**

Calibration Factor = Stage Micrometer Value / Number of Eyepiece Divisions

Calibration Value = \_\_\_\_\_  $\mu\text{m}$

**B. Preparation and Measurement of Starch Grains-**

**Steps:**

1. Take a small amount of powdered crude drug on a clean glass slide.
2. Add 1–2 drops of iodine solution to stain starch grains.
3. Gently place a cover slip over the sample without trapping air bubbles.
4. Remove excess stain using blotting paper.
5. Place the slide on the microscope stage.
6. Focus under low power and then high power ( $40\times$ ).
7. Identify starch grains they appear blue-black in colour after iodine staining.
8. Adjust the eyepiece micrometer scale so that it overlaps the starch grain.
9. Measure the diameter of the starch grain.
10. Record measurements of at least 10 starch grains.
11. Multiply the observed eyepiece divisions with the calibration factor to get actual size in micrometers.
12. Calculate the average size.

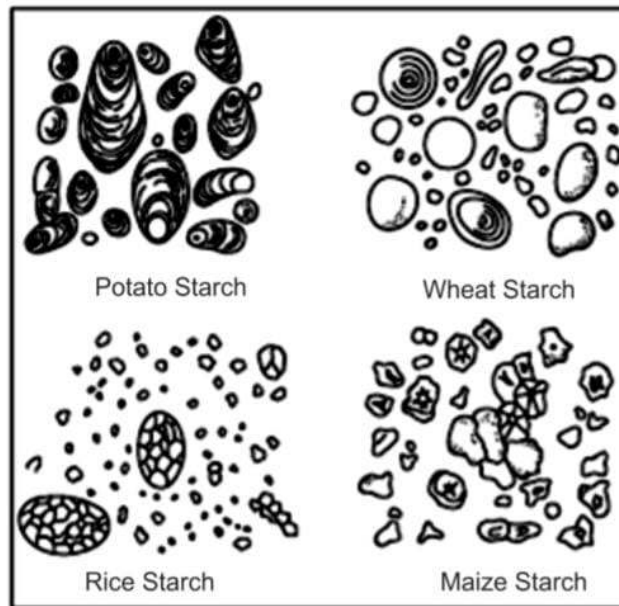
**Table 2: Characteristics of Some Starch Grains**

Sr. No	Characteristic	Maize	Rice	Wheat	Potato
1	Colour	White	White	Faint grey	Yellowish tint
2	Shape	Simple grains, angular; hilum central; rarely compound grains	Simple or compound grains; polyhedral with sharp angles	Mostly simple grains; faint striations; hilum appears as line	Flattened ovoid or subspherical; well-marked striations; hilum eccentric
3	Size	5–30 $\mu\text{m}$	2–10 $\mu\text{m}$	Small: 2–9 $\mu\text{m}$ ; Large: 10–45 $\mu\text{m}$	10–100 $\mu\text{m}$
4	pH	Neutral	Alkaline	Acidic	Acidic
5	Moisture Content (% w/w)	13%	13%	13%	20%

**Observations:****Table 3: Starch Grain Size**

Sr. No	Eyepiece Division	Actual Size ( $\mu\text{m}$ )

Average Size = \_\_\_\_\_ ( $\mu\text{m}$ )



**Figure 8: Starch grains observed under microscope after iodine staining showing hilum and concentric rings.**

### **C. Preparation and Measurement of Calcium Oxalate Crystals**

#### **Steps:**

1. Take powdered drug sample on a clean slide.
2. Add a drop of distilled water or suitable mounting medium.
3. Place cover slip carefully.
4. Observe under microscope using low power and then high power.
5. Identify calcium oxalate crystals:
  - Prism crystals
  - Raphide crystals
  - Cluster crystals
  - Rosette crystals
6. Align the crystal with the eyepiece micrometer scale.
7. Measure length or diameter of the crystal.
8. Record at least 10 readings.
9. Convert readings into micrometers using the calibration factor.
10. Calculate the mean value.

**Observations:**

**Table 4: Calcium Oxalate Crystals**

Sr. No	Type of Crystal	Eyepiece Division	Actual Size ( $\mu\text{m}$ )

Average Size = \_\_\_\_\_ ( $\mu\text{m}$ )

**D. Measurement of Fibre Length and Width**

**Steps:**

1. Prepare a slide of powdered drug with distilled water or glycerin.
2. Spread the fibres evenly to avoid overlapping.
3. Place cover slip carefully.
4. Observe under microscope.
5. Identify fibres clearly.
6. Align the fibre parallel to the micrometer scale.
7. Measure:
  - Fibre Length
  - Fibre Width
8. Take minimum 10 observations for both parameters.
9. Convert divisions into micrometers using calibration value.
10. Calculate average length and width.

**Observations:**

**Table 5: Fibre Length & Width**

Sr. No	Fibre Length ( $\mu\text{m}$ )	Fibre Width ( $\mu\text{m}$ )

Average Length = \_\_\_\_\_ ( $\mu\text{m}$ )

Average Width = \_\_\_\_\_ ( $\mu\text{m}$ )

**Result:**

Quantitative microscopic measurements of the given crude drug sample were successfully

performed using an eyepiece micrometer after proper calibration with a stage micrometer. The average size of starch grains, calcium oxalate crystals, fibre length and fibre width were determined and recorded in micrometers ( $\mu\text{m}$ ). The obtained values help in standardization and identification of the crude drug.

### **Conclusion:**

From the present experiment, it is concluded that quantitative microscopy is an effective method for the determination of microscopic parameters such as starch grain size, crystal size, fibre length and fibre width. The measured values can be used for authentication and quality control of crude drugs. This technique plays an important role in detection of adulteration and standardization of herbal materials.

### **References:**

1. Kokate C.K., Purohit A.P., Gokhale S.B. Pharmacognosy. Nirali Prakashan, Pune.
2. Trease G.E., Evans W.C. Pharmacognosy. 16th Edition. Elsevier Health Sciences.
3. Wallis T.E. Textbook of Pharmacognosy. CBS Publishers & Distributors, New Delhi.

### **Viva questions & answers:**

**Q1.** What is quantitative microscopy?

**A:** It is a microscopic method used to measure the size of microscopic plant structures such as starch grains, calcium oxalate crystals and fibres for standardization and identification of crude drugs.

**Q2.** Why is calibration of the eyepiece micrometer necessary?

**A:** Calibration is necessary to determine the actual value of one division of the eyepiece micrometer in micrometers ( $\mu\text{m}$ ) using a stage micrometer.

**Q3.** What is the role of iodine in this experiment?

**A:** Iodine stains starch grains blue-black, which helps in their clear identification under the microscope.

**Q4.** Why are at least 10 readings taken during measurement?

**A:** To improve accuracy, reduce experimental error and obtain a reliable average value.

**Q5.** What is the importance of this experiment in pharmacognosy?

**A:** It helps in authentication, standardization and detection of adulteration in crude drugs by measuring microscopic characteristics.

## **Experiment No.7: Quantitative microscopy for determination of stomatal number and index, vein islet number and vein termination number, palisade ratio using camera lucida**

**Aim:** To determine the stomatal number, stomatal index, vein islet number, vein termination number and palisade ratio of a given leaf drug using quantitative microscopy with the help of a camera lucida.

**Requirements:**

**Apparatus-** Compound microscope, Camera lucida, Glass slides, Cover slips, Watch glass, Forceps, Needle, Brush, Ruler, Drawing paper, Pencil

**Chemicals-** Chloral hydrate solution, Distilled water, Safranin (if required)

**Sample-** Fresh or dried leaf of given crude drug

**Principle:**

Quantitative microscopy is used for the identification and standardization of crude drugs based on measurable microscopic constants of leaves.

Certain leaf constants remain fairly constant for a particular species and are helpful in detecting adulteration. These include:

- **Stomatal Number** – Average number of stomata per square millimeter of leaf surface.
- **Stomatal Index** – Percentage proportion of stomata to total epidermal cells.
- **Vein Islet Number** – Number of small areas of green tissue surrounded by veins per square millimeter.
- **Vein Termination Number** – Number of veinlet terminations per square millimeter.
- **Palisade Ratio** – Average number of palisade cells beneath one epidermal cell.

A camera lucida is attached to the microscope to draw the microscopic field accurately on paper for counting and calculation.

These parameters are important for pharmacognostical standardization of leaf drugs.

## Procedure:

### A. Preparation of Leaf Sample-

Steps:

1. Take a fresh leaf or a small piece of the given crude drug leaf.
2. Boil the leaf piece in chloral hydrate solution for 5–10 minutes to remove chlorophyll and make it transparent.
3. Wash the cleared leaf thoroughly with distilled water.
4. Separate the lower epidermis carefully using forceps and needle.
5. Place the epidermal peel on a clean glass slide.
6. Add 1–2 drops of glycerin as mounting medium.
7. Place a cover slip gently to avoid air bubbles.
8. Observe under microscope under low power and adjust focus properly.

### B. Determination of Stomatal Number-

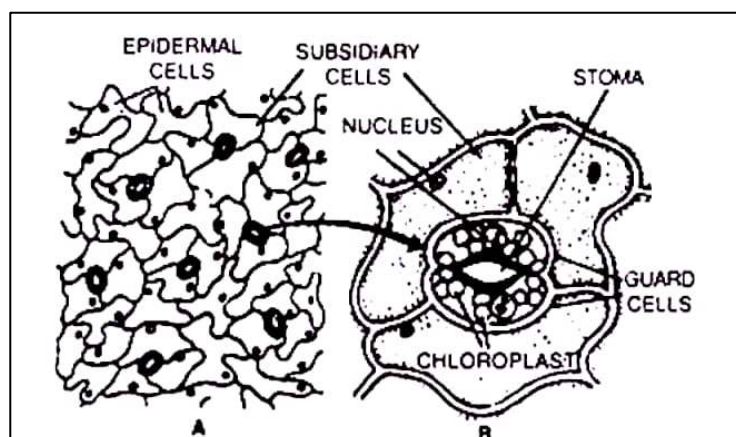
Definition: Stomatal number is defined as the average number of stomata present per square millimeter of the epidermis of a leaf.

Formula:

$$\text{Stomatal Number} = \frac{\text{Total Stomata}}{\text{Number of Fields}}$$

Steps:

1. Attach the camera lucida to the eyepiece of the microscope.
2. Adjust light properly so that both microscopic field and drawing paper are visible.
3. Focus the epidermal peel clearly.
4. Place drawing paper on the working table under camera lucida.
5. Draw a square of known area (usually 1 mm<sup>2</sup>) using stage micrometer calibration.
6. Trace the microscopic field inside the square.
7. Count the number of stomata present within the square area.
8. Repeat the counting in at least 5 different fields.
9. Record the number of stomata in each field.
10. Calculate the average stomatal number per square millimeter.



**Figure 9. (A) A portion of lower epidermis of leaf magnified to show stomata (B) A stomata magnified.**

**Observation Table:**

**Table 6. Stomatal Number**

Field No	Number of stomata
1	
2	
3	
4	
5	

Average Stomatal Number = \_\_\_\_\_

**C. Determination of Stomatal Index-**

Definition: Stomatal index is the percentage proportion of the number of stomata to the total number of epidermal cells (including stomata) present in a given area of the leaf.

Steps:

1. In the same drawn area used for stomatal number:
2. Count the number of stomata (S).
3. Count the number of epidermal cells (E) in the same area.
4. Apply the formula:

$$\text{Stomatal Index} = \frac{S}{E + S} \times 100$$

Where,

S = Number of stomata per unit area

E = Number of epidermal cells in the same area

5. Repeat for 5 fields.

6. Calculate the average stomatal index.

**Observation Table:**

**Table 7. Stomatal Index**

Field	No. of Stomata (S)	No. of Epidermal Cells (E)	Stomatal Index (%)
1			
2			
3			
4			
5			

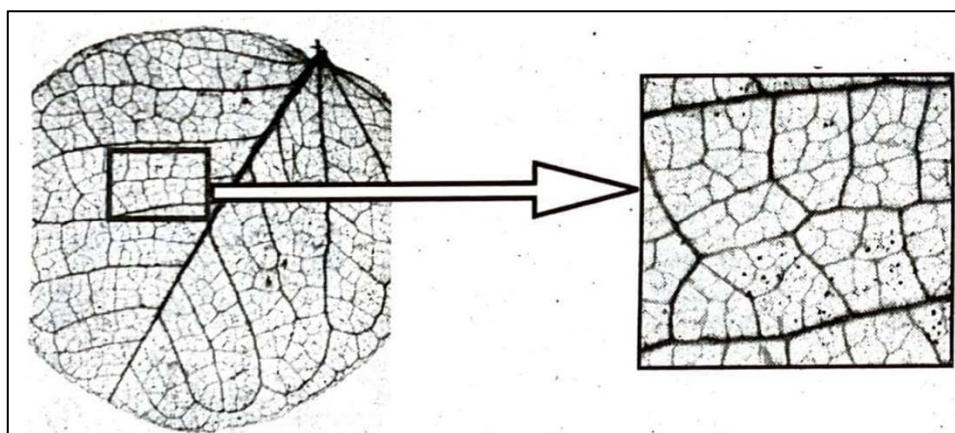
Average Stomatal Index = \_\_\_\_\_%

**D. Determination of Vein Islet Number-**

Definition: Vein islet number is defined as the average number of small areas of green tissue (vein islets) surrounded by veins per square millimeter of leaf surface.

Steps:

1. Take a cleared leaf sample.
2. Mount it on slide using glycerin.
3. Focus under low power.
4. Attach camera lucida.
5. Draw a square of 1 mm<sup>2</sup> area.
6. Trace the venation pattern inside the square.
7. Count the number of vein islets (small green areas surrounded by veins).
8. Repeat for 4–5 different fields.
9. Calculate the average vein islet number per square millimeter.



**Fig. 10: Cleared leaf showing closed vein meshes (vein islets) within a 1 sq. mm area for quantitative microscopy.**

**Observation Table:**

**Table 8. Vein Islet Number**

Field No.	Vein Islets
1	
2	
3	
4	
5	

Average Vein Islet Number = \_\_\_\_\_

**E. Determination of Vein Termination Number-**

Definition: Vein termination number is the average number of veinlet terminations (free endings of veins) present per square millimeter of leaf surface.

Steps:

1. Using the same drawn field:
2. Count the number of veinlet terminations (free endings of veins).
3. Repeat in at least 5 fields.
4. Record readings.
5. Calculate the average vein termination number per square millimeter.

**Observation Table:**

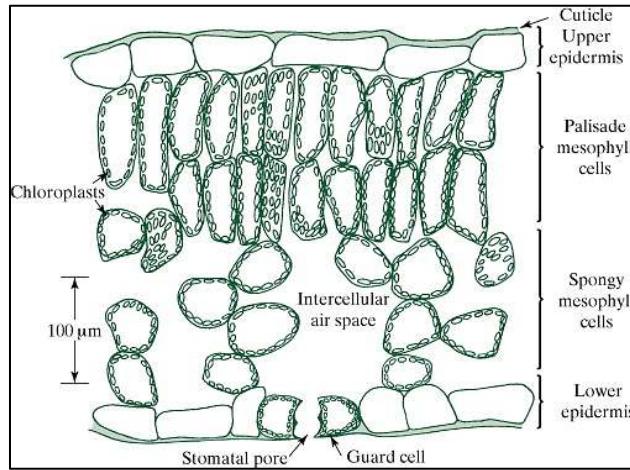
**Table 9. Vein Termination Number**

Field No	Vein Terminations
1	
2	
3	
4	
5	

Average Vein Termination Number = \_\_\_\_\_

**F. Determination of Palisade Ratio-**

Definition: Palisade ratio is defined as the average number of palisade cells present beneath one epidermal cell of the leaf.



**Figure 11: T.S. of leaf showing palisade cells beneath upper epidermis.**

Steps:

1. Prepare a transverse section (T.S.) of the leaf through lamina.
2. Clear the section using chloral hydrate if necessary.
3. Mount with glycerin.
4. Focus on upper epidermis under high power.
5. Attach camera lucida.
6. Draw four contiguous epidermal cells.
7. Count the number of palisade cells present beneath each epidermal cell.
8. Add total palisade cells counted.
9. Divide by number of epidermal cells observed.

$$\text{Palisade Ratio} = \frac{\text{Total Palisade Cells}}{\text{Number of Epidermal Cells}}$$

10. Record the calculated value.

**Observation Table:**

**Table 10. Palisade Ratio**

Epidermal Cell No.	Palisade Cells Counted
1	
2	
3	
4	

**Table 10.1. Palisade Ratio of Selected Leaf Drugs**

Plant Name	Palisade Ratio (Range)	Surface (if specified)
<i>Adhatoda vasica</i>	5.5 – 6.5	—
<i>Andrographis paniculata</i>	3.0 – 4.5	—
<i>Atropa belladonna</i>	5.0 – 7.0	—
<i>Azadirachta indica</i>	3.0 – 3.5	—
<i>Bacopa monnieri</i>	1.5 – 2.25	—
<i>Cassia angustifolia</i>	5.5 – 10.0 (upper) / 4.0 – 7.4 (lower)	Upper & Lower
<i>Centella asiatica</i>	3.5 – 5.75	—
<i>Datura metel</i>	5.0 – 6.5	—
<i>Datura stramonium</i>	4.2 – 6.5	—
<i>Digitalis lanata</i>	2.5 – 6.5	—
<i>Digitalis purpurea</i>	3.7 – 4.2	—
<i>Eucalyptus globulus</i>	5.5 – 6.5 (upper) / 3.5 – 5.0 (lower)	Upper & Lower
<i>Hyoscyamus niger</i>	3.0 – 6.0	—
<i>Lawsonia inermis</i>	7.0 – 8.5 (upper) / 4.5 – 5.7 (lower)	Upper & Lower
<i>Mentha arvensis</i>	3.5 – 5.0	—
<i>Mentha piperita</i>	6.1 – 8.5	—
<i>Momordica charantia</i>	2.5 – 3.8	—
<i>Nicotiana tabacum</i>	3.5 – 4.0	—
<i>Ocimum sanctum</i>	0.2 – 3.5	—
<i>Thymus vulgaris</i>	1.7 – 3.2	—

**Result:**

The stomatal number, stomatal index, vein islet number, vein termination number and palisade ratio of the given leaf drug were determined successfully using quantitative microscopy with the help of camera lucida. The calculated leaf constants can be used for identification and standardization of the leaf drug.

**Conclusion:**

Leaf constants such as stomatal number, stomatal index, vein islet number, vein termination number and palisade ratio are important pharmacognostical parameters. These values remain fairly constant for a particular species and help in authentication and detection of adulteration in crude drugs. Quantitative microscopy using camera lucida is a reliable method for evaluating leaf drugs.

**Precautions:**

- Leaf should be properly cleared before observation.
- Avoid air bubbles while mounting.
- Calibration must be accurate before drawing square area.

- Count only clearly visible stomata and cells.
- Take readings from different fields for accuracy.
- Do not count incomplete or overlapping cells.

**References:**

1. Kokate C.K., Purohit A.P., Gokhale S.B. Pharmacognosy. Nirali Prakashan, Pune.
2. Trease G.E., Evans W.C. Pharmacognosy. 16th Edition. Elsevier.
3. Wallis T.E. Textbook of Pharmacognosy. CBS Publishers.
4. Indian Pharmacopoeia Commission. Indian Pharmacopoeia. Latest Edition.

**Short viva questions and answers:**

1. What is stomatal number?

It is the average number of stomata per square millimeter of leaf surface.

2. Define stomatal index.

It is the percentage ratio of stomata to total epidermal cells.

3. What is vein islet number?

It is the number of small areas of leaf tissue enclosed by veins per square millimeter.

4. What is palisade ratio?

It is the average number of palisade cells beneath one epidermal cell.

5. Why is camera lucida used?

It is used to accurately draw and measure microscopic structures

## **Experiment No. 8: Sensory, Morphological, Chemical and Microscopical (Powder Microscopic) identification of Ashwagandha, Cinnamon, Fennel, Senna, Tulsi, Kalmegh and Nux-vomica**

**Aim:** To identify the given crude drugs Ashwagandha, Cinnamon, Fennel, Senna, Tulsi, Kalmegh and Nux-vomica on the basis of sensory characters, morphology, chemical tests and powder microscopy.

### **Principle:**

Crude drugs are natural substances obtained from plants and they possess characteristic physical, chemical and microscopic properties. Proper identification of crude drugs is essential to ensure their purity, quality and safety. In pharmacognosy, identification is carried out by using different evaluation methods which help in authentication and detection of adulteration.

- Sensory evaluation (Organoleptic characters):

This method involves identification of the drug by using sense organs such as sight, smell, taste and touch. The colour, odour, taste, size, shape and texture of the drug provide preliminary information about its identity.

It is the simplest and quickest method of evaluation.

- Morphological study (External features):

In this method, the external characteristics of the crude drug such as size, shape, surface, fracture, margin, apex, arrangement and other visible features are studied carefully. These morphological characters are specific for each drug and help in distinguishing genuine drugs from adulterants.

- Chemical tests (Detection of specific constituents):

Certain crude drugs contain specific chemical constituents such as alkaloids, glycosides, tannins, volatile oils, etc. When treated with suitable reagents, they produce characteristic colour changes or precipitates. These chemical reactions confirm the presence of active constituents and help in identification.

- Powder microscopy (Diagnostic microscopic characters):

When crude drugs are in powdered form, their identification is done by microscopic examination. Diagnostic features such as starch grains, fibres, vessels, trichomes, stomata, crystals and oil glands are observed

under the microscope. These microscopic characters are unique for each drug and serve as reliable criteria for authentication.

Thus, by combining sensory, morphological, chemical and microscopical evaluation, crude drugs can be accurately identified and their quality can be assured. This comprehensive approach helps in preventing substitution and adulteration in herbal medicines.

## **ASHWAGANDHA**

**Biological Source:** Dried mature roots of *Withania somnifera*

**Family:** Solanaceae

**Common name:** Indian ginseng / Winter cherry



**Figure 12: Dried Mature Roots and Powder of Ashwagandha (*Withania somnifera*)**

**Geographical Source:** Ashwagandha is widely distributed in India, especially in Madhya Pradesh, Rajasthan, Gujarat and Maharashtra. It is also found in Pakistan and other tropical regions. It grows in dry and semi-arid climates.

**Chemical Constituents:**

<b>Sr. No.</b>	<b>Class of Constituents</b>	<b>Specific Compounds</b>	<b>Nature</b>	<b>Pharmacological Importance</b>
1	Steroidal lactones	Withanolides (Withaferin A, Withanolide D)	Active principle	Adaptogenic, anti-inflammatory, anticancer
2	Alkaloids	Withanine, Somniferine,	Nitrogenous compounds	CNS stimulant, sedative effect

		Anaferine		
3	Saponins	Sitoinosides	Glycosidic compounds	Immunomodulatory activity
4	Starch	—	Carbohydrate	Nutritive component
5	Iron	—	Mineral element	Hematinic property
6	Amino acids	Various free amino acids	Organic compounds	Nutritional role

### Uses of Ashwagandha:

- Adaptogen – Helps the body to cope with stress and improves resistance against physical and mental fatigue.
- General Tonic – Used as a rejuvenator to improve strength, stamina and vitality.
- Immunomodulator – Enhances immune response and increases body resistance to infections.
- Anti-inflammatory – Useful in conditions like arthritis and inflammatory disorders.
- Nervine Tonic – Improves memory, concentration and is beneficial in anxiety and insomnia.
- Aphrodisiac – Traditionally used to improve reproductive health and sexual weakness.

### Sensory (Organoleptic) Identification:

Organoleptic evaluation is the first step in identification of Ashwagandha. The crude drug is examined using sense organs.

- Colour: Buff to light brown externally; inner surface creamish white
- Odour: Characteristic horse-like odour (hence name Ashwagandha – “smell of horse”)
- Taste: Slightly bitter and acrid
- Texture: Hard, cylindrical and fibrous
- Fracture: Short and starchy

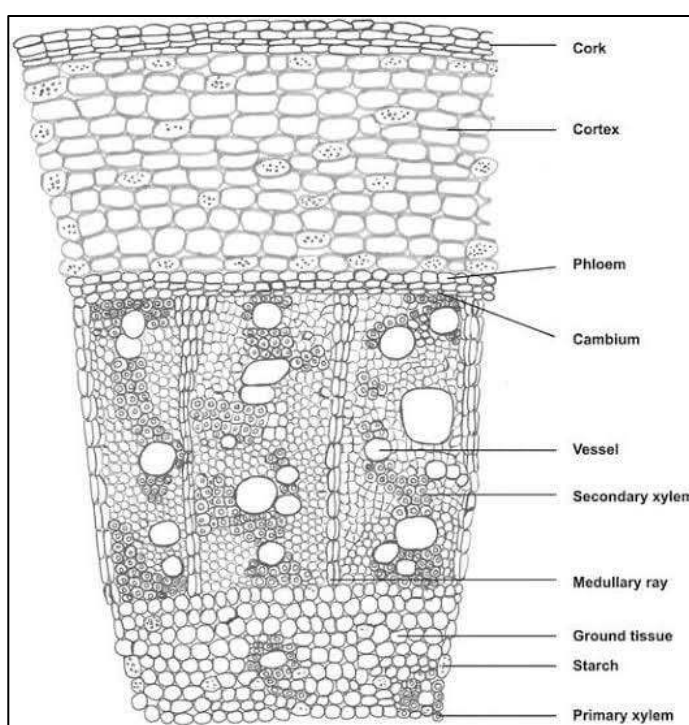
These features help in preliminary identification before further tests.

### Morphological Identification:

The drug consists mainly of dried roots.

- Roots are cylindrical, tapering, and straight or slightly curved
- Surface shows longitudinal wrinkles
- Length: 5–20 cm
- Diameter: 0.5–2 cm
- Outer surface light brown
- When broken, inner surface shows white starchy appearance
- Fracture is short and granular

These macroscopic characters help differentiate genuine Ashwagandha from substitutes.



**Figure 12.1: Morphological characters of Ashwagandha root**

### Chemical Identification:

Ashwagandha contains-

- Withanolides (steroidal lactones)
- Alkaloids (withanine, somniferine)
- Saponins
- Starch

**Chemical Test:**

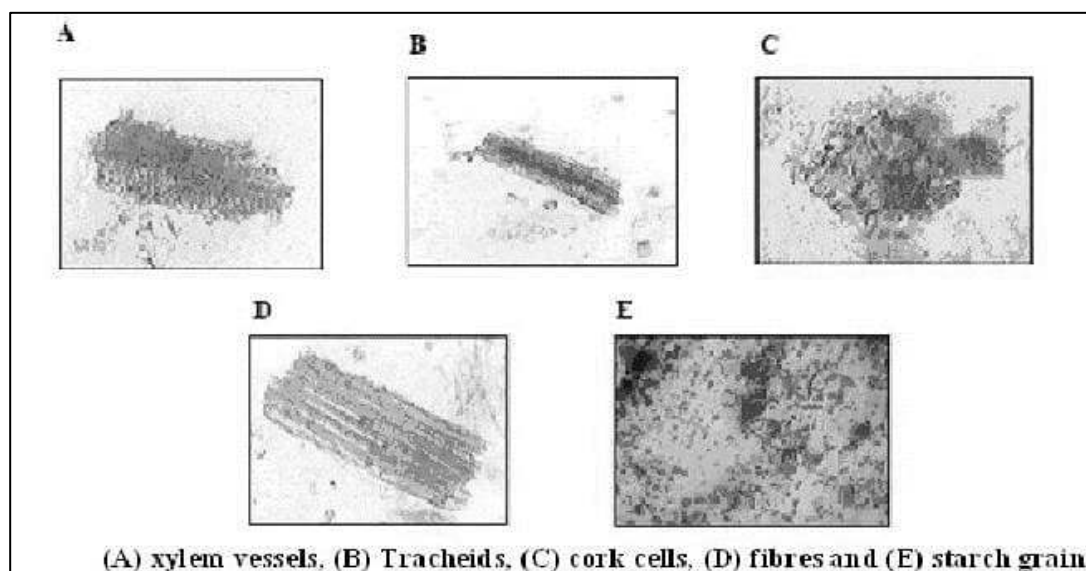
Sr. No.	Test Name	Reagent Required	Procedure (Detailed)	Observation
1	Dragendorff's Test (Alkaloids)	Dragendorff's reagent	Prepare alcoholic extract of powdered drug. Filter. To 2 ml filtrate add few drops of Dragendorff's reagent.	Orange or reddish-brown precipitate forms
2	Wagner's Test (Alkaloids)	Wagner's reagent (Iodine + Potassium iodide)	Take 2 ml extract and add few drops of Wagner's reagent.	Brown or reddish precipitate
3	Liebermann–Burchard Test (Steroidal compounds)	Acetic anhydride + Conc. H <sub>2</sub> SO <sub>4</sub>	To 2 ml extract add acetic anhydride. Carefully add few drops of conc. H <sub>2</sub> SO <sub>4</sub> along side of test tube.	Formation of green or bluish-green colour
4	Foam Test (Saponins)	Distilled water	Shake 1 g powdered drug with 10 ml water vigorously for 2–3 minutes.	Persistent froth for 10–15 minutes
5	Iodine Test (Starch)	Iodine solution	Add few drops of iodine solution to small quantity of powder.	Blue colour appears
6	Hager's Test (Alkaloids)	Hager's reagent (Saturated picric acid)	Add few drops of reagent to extract.	Yellow precipitate

**Microscopical Identification (Powder Microscopy):**

Powder of Ashwagandha root was mounted in glycerin and observed under microscope.

**Diagnostic Microscopic Characters:**

1. Fibres- Lignified, Thick-walled, Yellowish, Present in bundles
2. Xylem Vessels- Large, With bordered pits, Lignified walls
3. Calcium Oxalate Crystals- Prism shaped, Present in parenchyma
4. Starch Grains- Simple, Oval/round, 2–10  $\mu\text{m}$ , No striations
5. Cork Cells- Brown coloured, Polygonal, Thick walled



**Figure 12.2: Powder microscopic characters of Ashwagandha**

**Micro-Chemical / Staining Tests:**

Sr. No.	Reagent Used	Short Procedure	Observation	Inference
1	Iodine solution	Place small quantity of powder on slide, add 1 drop iodine, mount with cover slip	Blue colour	Presence of starch grains
2	Phloroglucinol + Conc. HCl	Add few drops to powder on slide	Pink/red colour	Lignified fibres and vessels
3	Dragendorff's reagent	Add 1–2 drops to powdered drug extract on slide	Orange precipitate	Alkaloids present
4	Sudan III	Treat powder with Sudan III solution	Red staining	Presence of fixed oil content
5	5% $\text{FeCl}_3$	Add drop to powder extract	Greenish coloration	Phenolic compounds

**Result:**

The given crude drug sample was identified as Ashwagandha based on its characteristic sensory features, morphological characters, positive chemical reactions for alkaloids and steroidal compounds, and diagnostic microscopical features observed in powder form.

**Conclusion:**

From the above study, the crude drug was authenticated as Ashwagandha on the basis of:

- Characteristic odor and slightly bitter taste
- Cylindrical, tapering root morphology
- Presence of withanolides and alkaloids
- Diagnostic powder characters such as lignified fibres, xylem vessels, starch grains and calcium oxalate crystals

Thus, the drug complies with standard pharmacognostical identification parameters.

**Precautions:**

- Use clean and dry glassware during chemical tests.
- Handle concentrated acids (HCl, H<sub>2</sub>SO<sub>4</sub>) carefully.
- Observe colour changes immediately after adding reagents.
- Prepare thin powder slides for clear microscopic observation.
- Use proper staining reagents in correct concentration.
- Avoid contamination of crude drug sample.

**References:**

1. Khandelwal K.R., Practical Pharmacognosy Techniques and Experiments.
2. Kokate C.K., Purohit A.P., Gokhale S.B., Pharmacognosy.
3. Indian Pharmacopoeia Commission. Indian Pharmacopoeia. Latest Edition.

**Viva question & answers:**

**Q1.** What is the biological source of Ashwagandha?

**A:** It consists of dried roots of *Withania somnifera* belonging to family Solanaceae.

**Q2.** What are the major chemical constituents of Ashwagandha?

**A:** Withanolides, withaferin A, alkaloids (somniferine, anaferine), steroidal lactones and starch.

**Q3.** Why is Ashwagandha called Indian ginseng?

**A:** Because it acts as an adaptogen and rejuvenator similar to ginseng.

**Q4.**What are the diagnostic powder characters of Ashwagandha?

**A:** Lignified fibres, bordered pitted xylem vessels, simple starch grains and calcium oxalate crystals.

**Q5.** Which chemical test confirms presence of alkaloids?

**A:** Dragendorff's test gives orange precipitate confirming alkaloids.

**Q6.**What is the main therapeutic use of Ashwagandha?

**A:** It is used as a tonic, adaptogen, anti-stress and immunomodulatory agent

## CINNAMON

**Biological Source:** Cinnamon consists of the dried inner bark of *Cinnamomum verum* (syn. *Cinnamomum zeylanicum*), belonging to **family** Lauraceae.

**Common Name:** Cinnamon, Dalchini, True Cinnamon, Ceylon Cinnamon



**Figure 13: Dried Inner Bark and Powder of Cinnamon (*Cinnamomum verum*)**

**Geographical Source:** Cinnamon is mainly cultivated in tropical regions with warm and humid climate. The chief commercial source of true cinnamon is Sri Lanka, which is considered the world's finest producer of Ceylon cinnamon. It is also grown in India (particularly in Kerala and Tamil Nadu), as well as in Myanmar, Indonesia, and Madagascar. The plant thrives well in well-drained sandy soil and humid climatic conditions.

**Chemical Constituents:** Cinnamon contains 0.5–1% volatile oil, the major component being cinnamaldehyde which imparts characteristic aroma and therapeutic activity. It also contains eugenol, tannins, mucilage, resins and small amounts of coumarin.

Sr. No	Chemical Constituent	Chemical Nature / Class	Approx. Presence	Significance
1	Cinnamaldehyde	Aromatic aldehyde (major volatile oil constituent)	60–75% of volatile oil	Responsible for characteristic aroma and flavour
2	Eugenol	Phenolic compound	Minor amount	Antiseptic and analgesic property
3	Cinnamic acid	Aromatic organic acid	Small amount	Oxidation product of cinnamaldehyde
4	Cinnamyl acetate	Ester	Trace	Contributes to aroma
5	Tannins	Polyphenolic compounds	Moderate amount	Astringent property
6	Mucilage	Polysaccharide	Small quantity	Soothing effect
7	Coumarin	Benzopyrone derivative	Trace (very low in true cinnamon)	Flavor component
8	Resins	Complex organic compounds	Small amount	Contributes to medicinal activity

**Uses:**

- Carminative – Relieves flatulence and abdominal discomfort by promoting expulsion of gas.
- Stomachic – Improves appetite and aids digestion.
- Aromatic & Flavouring Agent – Widely used to improve taste and aroma in pharmaceutical preparations and food products.
- Antimicrobial – Exhibits antibacterial and antifungal activity due to presence of cinnamaldehyde and eugenol.
- Antidiabetic – Helps in regulation of blood glucose levels.
- Mild Astringent – Used in diarrhoea and gastrointestinal disturbances.

### **Sensory Identification (Organoleptic Evaluation):**

Cinnamon is identified initially by observing its colour, odour, taste and texture using sensory organs.

- Colour: The outer surface is light yellowish-brown to reddish-brown, while the inner surface appears darker brown.
- Odour: It possesses a strong, pleasant, sweet and characteristic aromatic odour due to the presence of volatile oil (cinnamaldehyde).
- Taste: The taste is sweet, warm, aromatic and slightly pungent, producing a mild burning sensation in the mouth.
- Texture: The bark pieces are thin, smooth and brittle to touch.

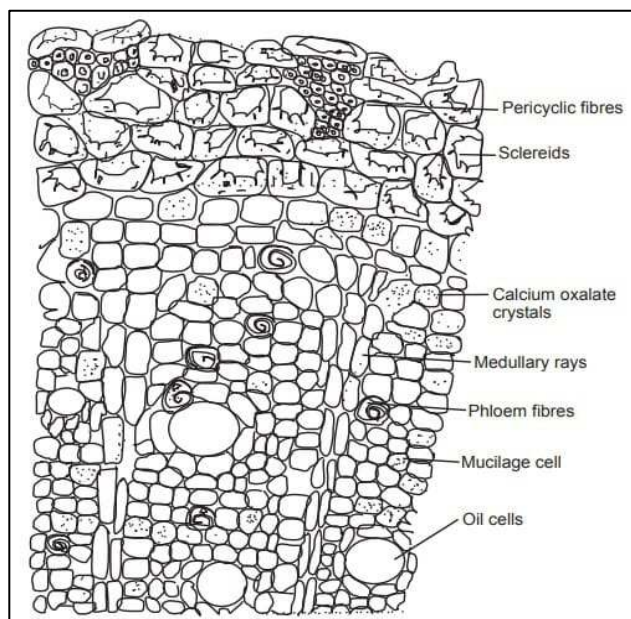
These organoleptic characters help in rapid preliminary identification and detection of adulteration.

### **Morphological Identification (External Characters):**

Cinnamon occurs as dried inner bark in the form of quills.

- Shape: Thin, cylindrical, compound quills formed by several layers of bark rolled together.
- Size: Usually 5–10 cm in length and about 0.2–0.8 mm in thickness.
- Outer Surface: Smooth, light brown with fine longitudinal striations and occasional scars.
- Inner Surface: Darker brown, smooth and slightly glossy.
- Fracture: Short and splintery.
- Structure: The bark is thin, flexible when fresh but becomes brittle on drying.

These characteristic external features help in distinguishing true cinnamon from other species and adulterants.



**Figure 13.1: Morphological characters of Cinnamon**

**Chemical Identification:**

Presence of volatile oil mainly cinnamaldehyde is confirmed by:

- Characteristic aromatic smell
- Positive reaction with alkali
- Formation of cinnamic acid on oxidation

**Chemical Test:**

Sr. No.	Test Name	Reagents Required	Procedure	Observation	Inference
1	Ferric Chloride Test (Tannins)	5% Ferric chloride solution	Prepare aqueous extract of powdered cinnamon by boiling 1 g powder in 10 ml distilled water. Filter. Add 2–3 drops of 5% FeCl <sub>3</sub> solution to 2 ml filtrate.	Greenish-black colour develops	Presence of tannins
2	Lead Acetate Test (Tannins)	Lead acetate solution	To 2 ml aqueous extract, add few drops of lead acetate solution.	White precipitate forms	Tannins present
3	Sudan III Test (Volatile Oil)	Sudan III solution	Place small quantity of powder on slide. Add	Red staining of	Presence of volatile oil

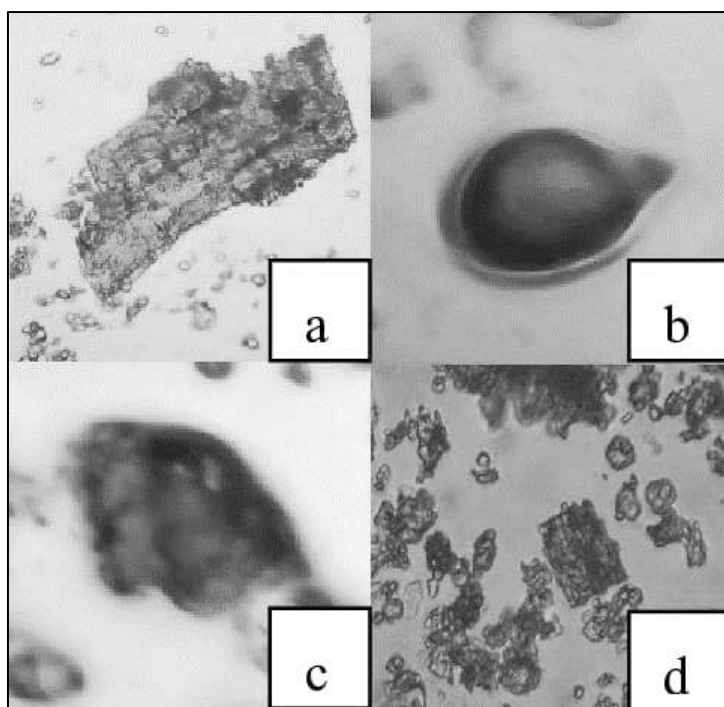
			few drops of Sudan III solution and observe.	oil cells	
4	Sodium Hydroxide Test (Cinnamaldehyde)	Dilute NaOH solution	Add few drops of NaOH to alcoholic extract of cinnamon.	Yellow colour appears	Presence of cinnamaldehyde
5	Shinoda Test (Phenolic compounds)	Magnesium ribbon + Conc. HCl	To alcoholic extract add small piece of magnesium ribbon followed by few drops of conc. HCl.	Pink/red colour develops	Presence of phenolic compounds

### **Microscopical Identification (Powder Microscopy):**

When powdered cinnamon is examined under microscope, the following diagnostic characters are observed:

- Cork cells: Polygonal, thick-walled brown cells forming outer protective tissue.
- Stone cells (Sclereids): Thick, lignified cells with narrow lumen providing mechanical strength.
- Phloem fibres: Long, slender and lignified fibres.
- Oil cells: Rounded cells containing volatile oil responsible for characteristic aroma.
- Calcium oxalate crystals: Small prismatic crystals present in parenchymatous tissue.

These microscopic features help in identification of cinnamon and detection of adulterants.



**Figure 13.2: . Microscopic characterization of Cinnamomum sp. bark powder (a) parenchyme cells with starch (b) resin cell (c) cells with reddish brown contents (d) crystal bearing cell.**

**Micro-Chemical / Staining Tests:**

Sr. No.	Reagent	Short Procedure	Observation	Inference
1	Phloroglucinol + Conc. HCl	Place small quantity of powder on slide, add 1–2 drops of reagent and cover with cover slip.	Pink / red colour	Presence of lignified fibres and sclereids
2	Iodine solution	Mount powder in iodine solution on slide.	Blue coloration (if present)	Starch grains (trace)
3	Sudan III	Treat powder with Sudan III solution and observe under microscope.	Red staining of cells	Volatile oil present in oil cells
4	5% Ferric chloride solution	Add few drops to powder mounted in water.	Green coloration	Presence of tannins
5	Dilute HCl	Mount powder with dilute HCl and observe.	Effervescence absent; crystals visible	Calcium oxalate crystals present

**Result:**

The given crude drug sample was identified as Cinnamon based on its characteristic aromatic odour, quilled bark morphology, presence of volatile oil (cinnamaldehyde) and tannins, and diagnostic microscopical characters such as cork cells, stone cells, phloem fibres and oil cells observed in powdered form.

**Conclusion:**

From the above study, the crude drug was authenticated as Cinnamon on the basis of:

- Pleasant aromatic odour and warm, sweet taste
- Thin, quilled bark morphology
- Presence of cinnamaldehyde and tannins
- Diagnostic powder characters like sclereids (stone cells), lignified fibres and oil cells

Thus, the drug complies with standard pharmacognostical identification parameters

**Precautions:**

- Use fresh powdered sample to detect volatile oil.
- Store the drug in an airtight container to prevent loss of aroma.
- Handle concentrated reagents carefully during chemical tests.
- Observe colour reactions immediately after adding reagents.
- Prepare a thin powder mount for clear microscopic observation.
- Avoid contamination of the crude drug sample.

**Reference:**

1. Khandelwal K.R., Practical Pharmacognosy Techniques and Experiments.
2. Kokate C.K., Purohit A.P., Gokhale S.B., Pharmacognosy.
3. Indian Pharmacopoeia Commission. Indian Pharmacopoeia. Latest Edition.

**Viva questions & answers:**

**Q1.**What is the biological source of Cinnamon?

**A:** It consists of dried inner bark of *Cinnamomum verum* belonging to family Lauraceae.

**Q2.**What is the major active constituent of Cinnamon?

**A:** Cinnamaldehyde.

**Q3.**What type of drug is Cinnamon morphologically?

**A:** It is a bark drug.

**Q4.**What are the diagnostic powder characters of Cinnamon?

**A:** Cork cells, stone cells (sclereids), phloem fibres and oil cells.

**Q5.**Which chemical test confirms the presence of tannins?

**A:** Ferric chloride test gives greenish-black colour.

**Q6.**What are the main uses of Cinnamon?

**A:** It is used as carminative, flavouring agent and mild antiseptic.

## **FENNEL**

**Biological Source:** Fennel consists of the dried ripe fruits of *Foeniculum vulgare* Mill., belonging to **family** Apiaceae (Umbelliferae).

**Common Name:** Fennel, Saunf, Sweet Fennel



**Figure 14: Dried Ripe Fruits of Fennel (*Foeniculum vulgare*)**

### **Geographical Source:**

Fennel is cultivated widely in Mediterranean countries and temperate regions. Major producers include India (especially Gujarat, Rajasthan, Uttar Pradesh and Punjab), Egypt, Turkey, Italy and Iran. The plant grows well in mild climate with well-drained loamy soil and adequate sunlight.

### **Chemical Constituents:**

Fennel contains about 1–6% volatile oil. The chief constituent is anethole, responsible for its characteristic aroma and flavour. Other constituents include fenchone, estragole, limonene, flavonoids, fixed oil, proteins

Sr. No	Chemical Constituent	Chemical Nature / Class	Approx. Presence	Significance
1	Anethole	Phenylpropanoid (major volatile oil constituent)	50–70% of volatile oil	Characteristic sweet aroma, carminative action
2	Fenchone	Ketone	10–20%	Bitter taste, digestive stimulant
3	Estragole	Phenolic ether	Small amount	Aromatic property
4	Limonene	Monoterpene	Trace	Flavor component
5	Fixed oil	Fatty oil	8–12%	Nutritional value
6	Flavonoids	Polyphenolic compounds	Small amount	Antioxidant property

**Uses:**

- Carminative – Relieves flatulence and abdominal discomfort by reducing gas formation.
- Digestive stimulant – Improves appetite and promotes digestion.
- Aromatic flavouring agent – Used in pharmaceutical preparations, syrups and food products to improve taste.
- Expectorant – Helpful in cough and bronchitis by promoting expulsion of mucus.
- Antispasmodic – Relieves intestinal and stomach spasms.
- Galactagogue – Traditionally used to promote lactation in nursing mothers.

**Sensory Identification (Organoleptic Evaluation):**

Fennel fruits are identified initially by their characteristic sensory features:

- Colour: Greenish to yellowish-brown
- Odour: Strong, sweet, aromatic and pleasant (due to volatile oil, mainly anethole)
- Taste: Sweet, slightly spicy and cooling
- Texture: Smooth externally with ridges
- Size & Shape: Small, elongated and slightly curved fruits

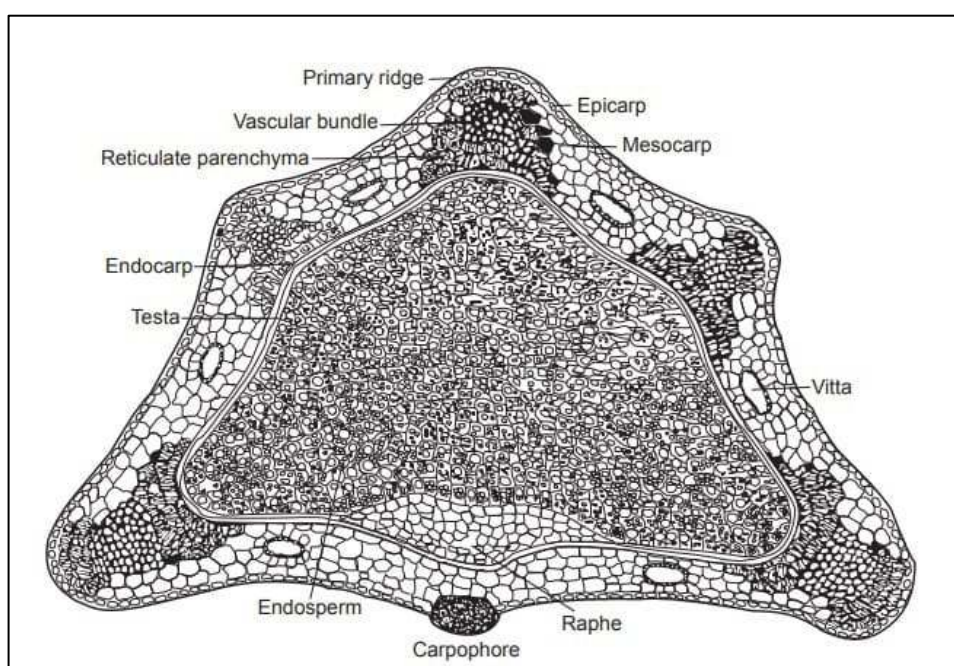
The sweet aromatic smell is the most important diagnostic sensory character of fennel.

### Morphological Identification (External Characters):

Fennel consists of dried ripe fruits (cremocarp).

- Type of drug: Fruit drug
- Size: 4–10 mm long
- Shape: Oblong, cylindrical and slightly curved
- Surface: Five prominent longitudinal ridges
- Structure: Fruit splits into two mericarps
- Colour: Yellowish-green to brown

The presence of five ridges and characteristic aroma helps in distinguishing fennel from other umbelliferous fruits.



**Figure 14.1: Morphological characters of Fennel**

### Chemical Identification:

Fennel contains 1–6% volatile oil, mainly anethole, along with fenchone and flavonoids. The presence of volatile oil is confirmed by aromatic odour and specific staining reactions.

Flavonoids give colour reactions with ferric chloride and lead acetate solutions.

Thus, chemical identification confirms authenticity and helps detect adulteration.

### Chemical Test:

Sr. No.	Test	Short Procedure	Observation	Inference
1	Sudan III Test	Treat powdered drug with few drops of Sudan	Red staining	Presence of volatile oil

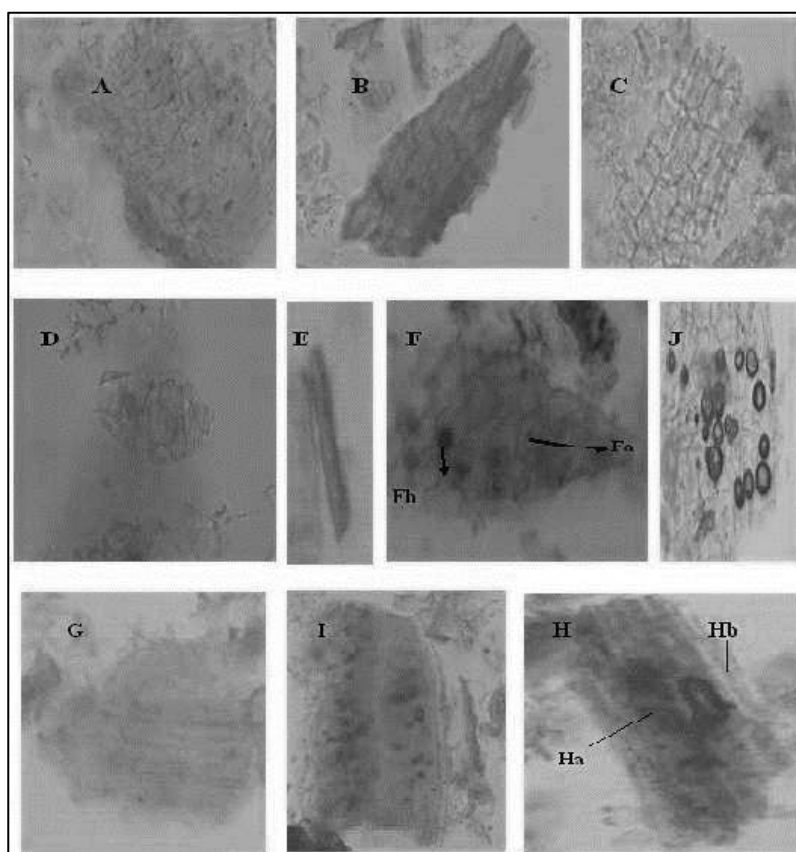
		III solution		
2	Ferric Chloride Test	Add few drops of 5% FeCl <sub>3</sub> to aqueous extract	Green colour	Presence of flavonoids
3	Lead Acetate Test	Add lead acetate solution to aqueous extract	Yellow precipitate	Flavonoids present
4	Shake Test	Shake powder with water in test tube	Persistent aroma	Volatile oil present
5	NaOH Test	Add few drops of NaOH to extract	Yellow coloration	Flavonoid compounds

#### **Microscopical Identification (Powder):**

When powdered fennel is examined under microscope, the following diagnostic characters are observed:

- Oil ducts (Vittae): Large oval or circular oil ducts containing volatile oil (important diagnostic feature).
- Epidermal cells: Polygonal cells with thick walls.
- Parenchyma cells: Thin-walled cells forming ground tissue.
- Endosperm cells: Contain aleurone grains and fixed oil globules.
- Calcium oxalate crystals: Small prismatic crystals present in parenchyma.

Presence of vittae (oil ducts) is the most important microscopic feature of fennel.



**Figure 14.2: Microscopic Characters of Fennel (*Foeniculum vulgare*) Fruit Powder**  
 (A – Epidermal cells (polygonal, thick-walled), B – Fragment of pericarp tissue, C – Parenchymatous mesocarp cells, D – Vittae (oil ducts) in transverse view, E – Lignified fibre, F – Endosperm cells containing fixed oil, G – Fragment of vascular bundle, H – Oil globules within vittae, I – Thick-walled sclerenchymatous cells, J – Calcium oxalate crystals)

**Micro-chemical Tests (Powder):** Micro-chemical tests help confirm the presence of specific cellular components directly under microscope.

Reagent	Short Procedure	Observation	Characteristic Confirmed
Sudan III	Mount powder with reagent on slide	Red stained oil ducts	Volatile oil
Phloroglucinol + HCl	Add reagent to slide	Pink colour	Lignified tissues
Iodine Solution	Mount powder with iodine	No prominent blue colour	Starch absent
Dilute HCl	Mount with dilute HCl	Crystals visible	Calcium oxalate

**Result:**

The given crude drug sample was identified as Fennel based on its sweet aromatic odour, characteristic ridged fruits, presence of volatile oil (anethole), and diagnostic microscopical characters such as vittae (oil ducts), endosperm cells with fixed oil and lignified fibres.

**Conclusion:**

From the above pharmacognostical evaluation, the crude drug was authenticated as Fennel on the basis of:

- Sweet aromatic odour and taste
- Oblong, ridged cremocarp morphology
- Presence of volatile oil (anethole)
- Diagnostic powder characters like oil ducts (vittae) and endosperm cells

Thus, the drug complies with standard identification parameters.

**Precautions:**

- Use freshly powdered drug for volatile oil detection.
- Store in airtight container away from light and moisture.
- Avoid overheating during extraction to prevent loss of volatile oil.
- Handle chemical reagents carefully.
- Prepare thin powder mount for microscopic study.

**References:**

1. Khandelwal K.R., Practical Pharmacognosy Techniques and Experiments.
2. Kokate C.K., Purohit A.P., Gokhale S.B., Pharmacognosy.
3. Indian Pharmacopoeia Commission. Indian Pharmacopoeia. Latest Edition.

**Viva Questions & Answers:**

**Q1.** What is the biological source of Fennel?

**A:** It consists of dried ripe fruits of *Foeniculum vulgare* belonging to family Apiaceae.

**Q2.** What is the major active constituent of Fennel?

**A:** Anethole.

**Q3.** What type of drug is Fennel morphologically?

**A:** It is a fruit drug (cremocarp).

**Q4.** What are the diagnostic powder characters of Fennel?

**A:** Vittae (oil ducts), endosperm cells, lignified fibres and epicarp cells.

**Q5.** Which test confirms volatile oil in Fennel?

A: Sudan III test and spot test on filter paper.

Q6. What are the main uses of Fennel?

A: Carminative, stomachic, expectorant and flavouring agent.

## SENNA

**Biological Source:** Senna consists of the dried leaflets of *Senna alexandrina* Mill. (syn. *Cassia angustifolia* and *Cassia acutifolia*), belonging to **family** Fabaceae (Leguminosae).

**Common Name:** Senna, Indian Senna, Tinnevelly Senna



Figure 15: Dried Leaflets of Senna (*Senna alexandrina* Mill.)

**Geographical Source:** Senna is mainly cultivated in India (Tamil Nadu, Rajasthan, Gujarat), Sudan and Egypt. It grows well in warm, arid regions with sandy soil and low rainfall.

### **Chemical Constituents:**

Senna contains 2–5% anthraquinone glycosides known as **sennosides** (major active constituents). It also contains aloe-emodin, rhein, kaempferol, mucilage and flavonoids.

Sr. No	Chemical Constituent	Chemical Nature / Class	Approx. Presence	Significance
1	Sennosides A & B	Anthraquinone glycosides	2–5%	Laxative action
2	Rhein	Anthraquinone derivative	Small amount	Purgative property

3	Aloe-emodin	Anthraquinone	Trace	Stimulant laxative
4	Kaempferol	Flavonoid	Small amount	Antioxidant
5	Mucilage	Polysaccharide	Small quantity	Soothing effect

**Uses:**

- Stimulant Laxative – Senna is mainly used for relieving constipation by stimulating peristaltic movement of intestine.
- Bowel Evacuant – Used before diagnostic procedures like colonoscopy.
- Purgative – In higher doses, produces strong purgative action.
- Detoxifying Agent (Traditional use) – Used in traditional medicine for cleansing bowel.
- Hemorrhoids & Fissure – Used to ease bowel movement in painful conditions.

**Sensory Identification (Organoleptic Evaluation):**

- Colour: Yellowish-green to greyish-green.
- Odour: Slight, characteristic.
- Taste: Bitter and slightly mucilaginous.
- Texture: Thin, brittle leaflets.

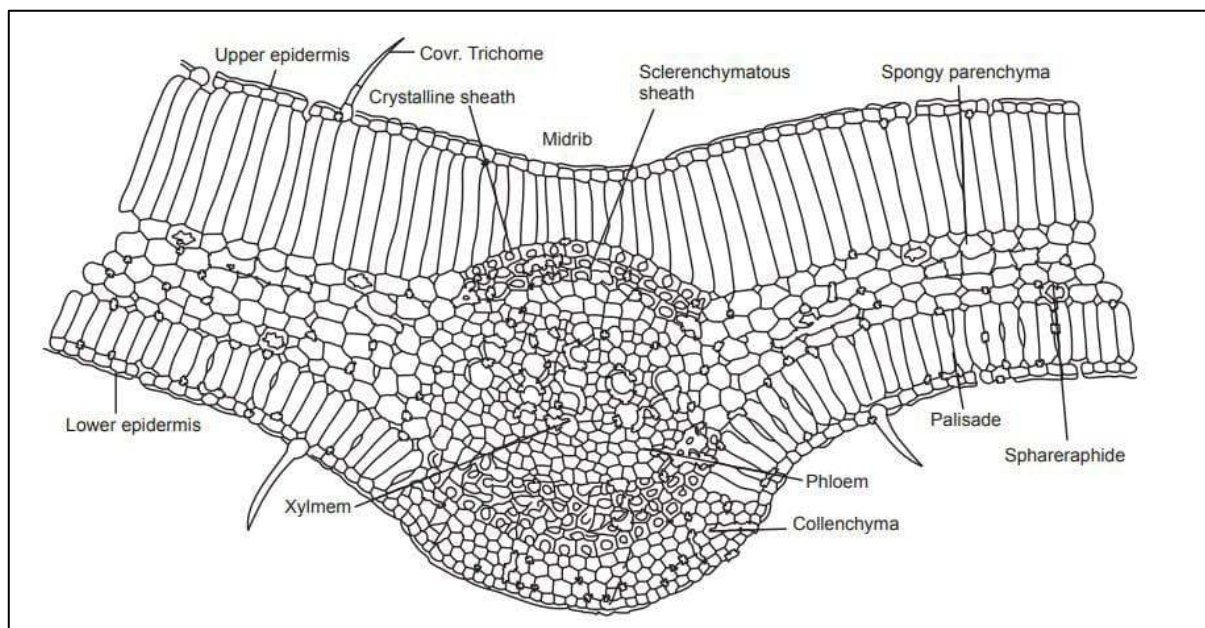
These features help in primary identification of crude drug.

**Morphological Identification (External Characters):**

Consists of dried leaflets

- Shape: Lanceolate or ovate-lanceolate
- Length: 2–5 cm long, 0.5–2 cm wide.
- Apex: Acute
- Base: Asymmetrical
- Margin: Entire
- Venation: Reticulate
- Surface: Smooth

These morphological features differentiate Senna from adulterants.



**Figure 15.1: Morphological characters of Senna**

**Chemical Identification:**

Senna contains anthraquinone glycosides mainly sennosides A & B, which are responsible for laxative action.

Presence of anthraquinone glycosides is confirmed by Borntrager's test and modified Borntrager's test.

**Chemical Test:**

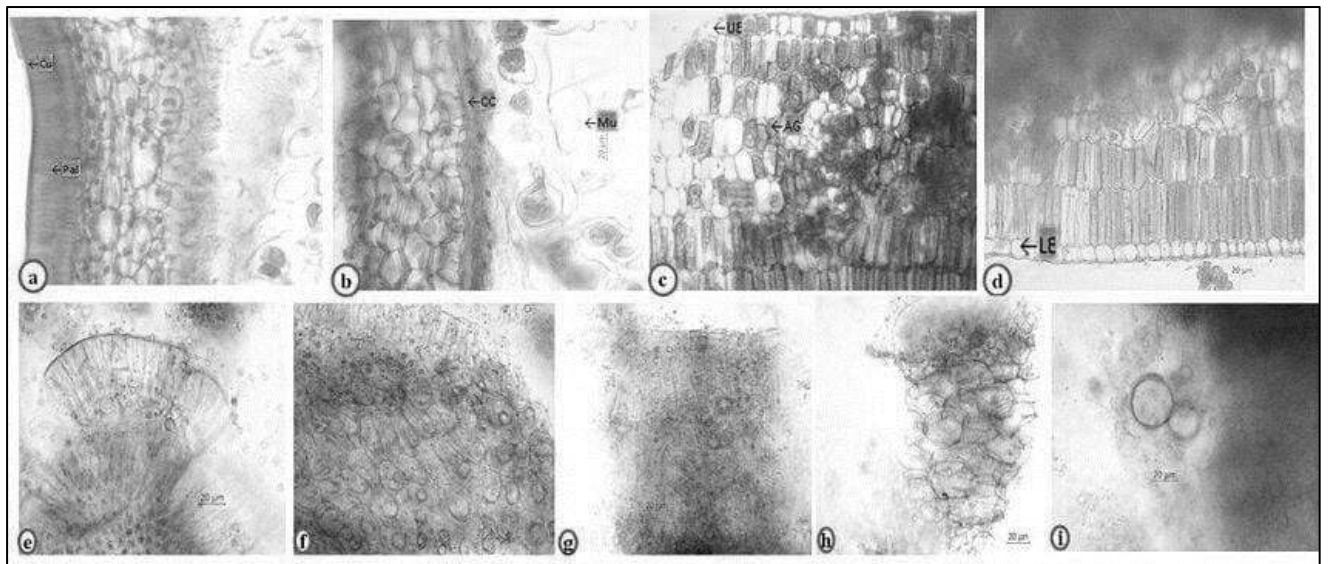
Sr. No	Test	Short Procedure	Observation	Inference
1	Borntrager's Test	Boil powder with dilute HCl, filter, extract with benzene, add ammonia	Pink to red colour in ammoniacal layer	Anthraquinone glycosides present
2	Modified Borntrager's Test	Boil with $\text{FeCl}_3 + \text{HCl}$ , extract with benzene, add ammonia	Rose-pink colour	C-glycosides present
3	Ferric chloride test	Add 5% $\text{FeCl}_3$ to extract	Greenish colour	Phenolic compounds
4	Powder + NaOH	Add NaOH solution	Reddish colour	Anthraquinone derivatives

### Microscopical Identification (Powder):

When powdered Senna is examined under microscope, the following diagnostic characters are observed:

- Epidermal cells: Wavy walls with stomata
- Paracytic stomata: Characteristic stomatal type
- Trichomes: Unicellular, thick-walled covering hairs
- Calcium oxalate crystals: Prismatic crystals
- Vascular elements: Spiral and reticulate vessels
- Mesophyll fragments: Showing palisade cells

These features confirm authenticity and help detect adulteration



**Figure 15.2: Microscopic Characters of Senna (*Senna alexandrina*) Leaf**

(a – Transverse section of leaflet showing cuticle (Cu), palisade cells (Pal), b – Collenchyma (CC) and mucilage (Mu) cells, c – Vascular bundle with xylem and phloem, d – Lower epidermis (LE), e – Paracytic stomata, f – Calcium oxalate crystals, g – Epidermal cells with straight anticlinal walls, h – Covering trichomes (unicellular), i – Prism-shaped calcium oxalate crystals)

**Micro-chemical Tests (Powder):**

Sr. No.	Reagent	Short Procedure	Observation	Inference
1	Phloroglucinol + HCl	Mount powder with reagent	Pink colour	Lignified tissues
2	Iodine solution	Mount with iodine	No blue colour	Starch absent
3	Dilute NaOH	Add on slide	Red coloration	Anthraquinone derivatives
4	Dilute HCl	Mount with HCl	Crystals visible	Calcium oxalate present

**Result:**

The given crude drug sample was identified as Senna based on its characteristic leaf morphology, positive Borntrager's test and presence of diagnostic microscopical characters.

**Conclusion:**

The drug was authenticated as Senna on the basis of:

- Yellowish-green lanceolate leaflets
- Presence of anthraquinone glycosides
- Positive Borntrager's reaction
- Diagnostic microscopic features

**Precautions:**

- Use freshly powdered drug for chemical testing.
- Perform Borntrager's test carefully with proper heating.
- Handle acids and ammonia solution cautiously.
- Prepare thin powder mount for clear microscopic observation.
- Avoid contamination and moisture exposure of the crude drug.

**Reference:**

1. Khandelwal K.R., Practical Pharmacognosy Techniques and Experiments.
2. Kokate C.K., Purohit A.P., Gokhale S.B., Pharmacognosy.
3. Indian Pharmacopoeia (latest edition).

### **Viva questions and answers:**

**Q1.** What is the biological source of Fennel?

**A:** It consists of dried ripe fruits of *Foeniculum vulgare* belonging to family Apiaceae.

**Q2.** Which family does Fennel belong to?

**A:** Apiaceae (Umbelliferae).

**Q3.** What are the active constituents of Fennel?

**A:** Volatile oil mainly containing anethole, along with fenchone and estragole.

**Q4.** Which test is used to detect volatile oil in Fennel?

**A:** Sudan III test and spot test on filter paper.

**Q5.** What is the morphological type of Fennel drug?

**A:** It is a fruit drug (cremocarp).

**Q6.** What are the diagnostic microscopic characters of Fennel?

**A:** Vittae (oil ducts), endosperm cells with fixed oil, lignified fibres and epicarp cells.

**Q7.** What is the main therapeutic use of Fennel?

**A:** It is used as a carminative and stomachic to relieve flatulence and improve digestion.

## **TULSI**

**Biological Source:** Tulsi consists of the fresh or dried leaves and flowering tops of *Ocimum tenuiflorum* (syn. *Ocimum sanctum*), belonging to **family** Lamiaceae.

**Common Name:** Tulsi, Holy Basil, Sacred Basil



**Figure 16: Fresh or Dried Leaves and Flowering Tops of Tulsi (*Ocimum sanctum*)**

### Geographical Source:

Tulsi is widely cultivated throughout India and other tropical and subtropical regions such as Sri Lanka, Thailand and parts of Africa. In India, it is commonly grown in household gardens and temples due to its medicinal and religious importance. It grows well in warm climate with moderate rainfall and well-drained soil.

### Chemical Constituents:

Tulsi contains volatile oil (0.5–1.5%) as the major active principle. The important constituents include eugenol, methyl eugenol, ursolic acid, rosmarinic acid, flavonoids and tannins.

Sr. No	Chemical Constituent	Chemical Nature / Class	Approx. Presence	Significance
1	Eugenol	Phenolic compound (volatile oil)	Major constituent	Antimicrobial, analgesic
2	Methyl eugenol	Phenylpropanoid	Moderate	Aromatic property
3	Ursolic acid	Triterpenoid	Small amount	Anti-inflammatory
4	Rosmarinic acid	Phenolic acid	Small amount	Antioxidant
5	Flavonoids	Polyphenolic compounds	Moderate	Immunomodulatory
6	Tannins	Polyphenols	Small quantity	Astringent property

### Uses:

- Antimicrobial – Used in infections due to its antibacterial and antifungal properties.
- Antitussive & Expectorant – Useful in cough, cold and bronchitis.
- Adaptogen – Helps in stress management and improves immunity.
- Anti-inflammatory – Used in inflammatory conditions.
- Digestive stimulant – Relieves indigestion and flatulence.
- Immunomodulator – Enhances body resistance.

### Sensory Identification (Organoleptic Evaluation):

Tulsi can be identified easily by using our senses:

- Colour: Green to brownish-green leaves.
- Odour: Strong, clove-like aromatic smell due to eugenol.
- Taste: Slightly pungent, bitter and aromatic.

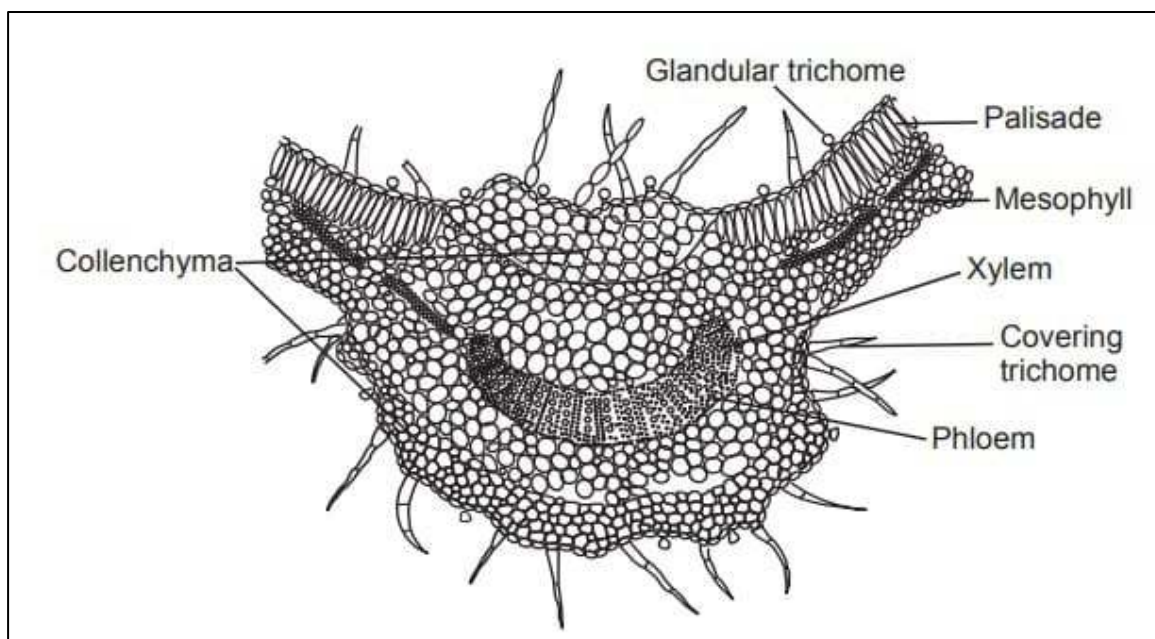
- Texture: Soft, hairy (pubescent) leaves.

These features help in quick preliminary identification.

**Morphological Identification (External Characters):**

- Type: Leaf drug.
- Shape: Ovate or elliptic.
- Size: 2–5 cm long.
- Margin: Serrated (toothed).
- Apex: Acute.
- Base: Rounded.
- Surface: Hairy on both sides.
- Venation: Reticulate.
- Stem: Quadrangular (typical of Lamiaceae).

These characters help distinguish Tulsi from other basil species.



**Figure 16.1: Morphological characters of Tulsi (*Ocimum Tenuiflorum*)**

**Chemical Identification:**

Tulsi contains volatile oil rich in eugenol, along with flavonoids and tannins. Presence of volatile oil is identified by aromatic odour and Sudan III test. Phenolic compounds like eugenol give colour reaction with ferric chloride.

**Chemical Test:**

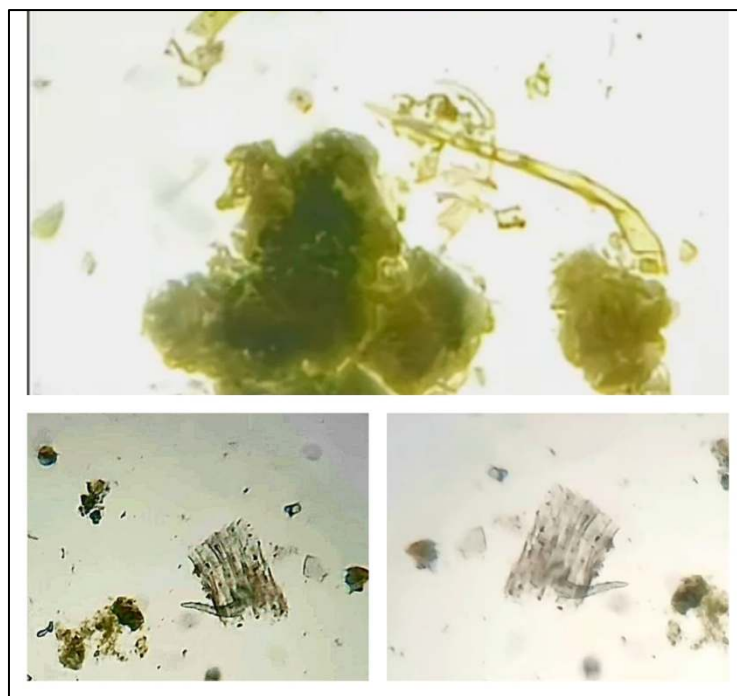
Sr. No.	Name of Test	Reagent Used	Detailed Procedure	Observation	Inference
1	Ferric Chloride Test	5% FeCl <sub>3</sub> solution	Prepare aqueous extract of Tulsi leaves. Add 2–3 drops of 5% ferric chloride solution to the extract.	Greenish-black / dark green colour	Presence of phenolic compounds (Eugenol)
2	Lead Acetate Test	Lead acetate solution	Add few drops of lead acetate solution to aqueous extract.	Yellow precipitate	Presence of flavonoids
3	Sodium Hydroxide Test	Dilute NaOH solution	Add few drops of dilute NaOH to alcoholic extract of Tulsi.	Yellow colour which becomes colourless on addition of acid	Presence of flavonoids
4	Sudan III Test	Sudan III solution	Mount powdered drug on slide and add Sudan III reagent.	Red staining of oil glands	Presence of volatile oil
5	Foam Test	Distilled water	Shake powdered drug with water in test tube for few minutes.	Persistent froth (if present)	Presence of saponins (trace)

**Microscopical Identification (Powder):**

When powdered Tulsi leaf is examined under microscope, following characters are observed:

- Epidermal cells: Wavy-walled cells
- Stomata: Diacytic type (characteristic of Lamiaceae)
- Covering trichomes: Multicellular, uniseriate hairs
- Glandular trichomes: Oil-containing glands
- Vascular tissues: Spiral xylem vessels
- Calcium oxalate crystals: Small prismatic crystals

These features help in authentication and detection of adulteration.



**Figure 16.2 Powder microscopic characters of Tulsi; (a) Trichomes (b) Upper Epidermis (c) Lower Epidermis**

**Micro-chemical Tests (Powder):** Micro-chemical tests are performed directly on powdered leaf mounted on slide to identify specific cellular components by characteristic colour reactions under microscope.

Sr. No.	Reagent	Detailed Short Procedure	Observation	Inference
1	Sudan III	Place a small quantity of Tulsi powder on a clean slide. Add 1–2 drops of Sudan III solution. Warm gently if required. Cover with cover slip and observe under microscope.	Glandular trichomes and oil globules stain red	Presence of volatile oil (eugenol)
2	Phloroglucino 1 + Conc. HCl	Mount powder in phloroglucinol solution and add a drop of conc. HCl. Observe immediately.	Pink to red coloration of vessels	Presence of lignified xylem elements

3	5% Ferric Chloride Solution	Add few drops of ferric chloride to powder mounted in water. Observe under microscope.	Greenish colour	Presence of phenolic compounds (eugenol)
4	Dilute NaOH	Mount powder with dilute NaOH solution. Observe colour change.	Yellow coloration	Presence of flavonoids
5	Iodine Solution (if needed)	Mount powder in iodine solution and observe.	Blue colour (if starch present)	Presence of starch grains (trace)

**Result:**

The given crude drug sample was identified as Tulsi based on its characteristic aromatic odour, ovate serrated leaves, presence of volatile oil (eugenol), and diagnostic microscopical features such as diacytic stomata, glandular trichomes and covering trichomes observed in powdered form.

**Conclusion:**

From the above study, the crude drug was authenticated as Tulsi on the basis of:

- Strong aromatic (clove-like) odour
- Ovate, serrated and pubescent leaves
- Presence of volatile oil rich in eugenol
- Diagnostic powder characters like diacytic stomata and glandular trichomes

Thus, the drug complies with standard pharmacognostical identification parameters.

**Precautions:**

- Use fresh powdered sample to detect volatile oil.
- Avoid overheating during chemical tests.
- Handle chemical reagents carefully.
- Prepare a thin powder mount for proper microscopic observation.
- Store the drug in an airtight container to prevent loss of aroma.
- Avoid contamination of the sample.

### Reference:

1. Khandelwal K.R., Practical Pharmacognosy Techniques and Experiments.
2. Kokate C.K., Purohit A.P., Gokhale S.B., Pharmacognosy.
3. Indian Pharmacopoeia (latest edition).

### Viva questions & answers:

**Q1.** What is the biological source of Tulsi?

**A:** Tulsi consists of dried leaves of *Ocimum sanctum* (syn. *Ocimum tenuiflorum*) belonging to family Lamiaceae.

**Q2.** What is the major active constituent of Tulsi?

**A:** Eugenol.

**Q3.** What type of stomata are present in Tulsi leaf?

**A:** Diacytic stomata.

**Q4.** Which family does Tulsi belong to?

**A:** Lamiaceae.

**Q5.** What are the diagnostic powder characters of Tulsi?

**A:** Glandular trichomes, covering trichomes and diacytic stomata.

**Q6.** What are the main uses of Tulsi?

**A:** Antimicrobial, antitussive, immunomodulator and anti-inflammatory agent.

## KALMEGH

**Biological Source:** Kalmegh consists of the dried whole plant or aerial parts of *Andrographis paniculata* (Burm.f.) Nees, belonging to **family** Acanthaceae.

**Common Name:** Kalmegh, Creat, Green Chiretta, King of Bitters



**Figure 17: Dried Whole Plant or Aerial Parts of Kalmegh (*Andrographis paniculata*)**

**Geographical Source:**

Kalmegh is widely distributed in India, Sri Lanka, Pakistan, Malaysia and other tropical Asian countries. In India, it is commonly found in plains and cultivated in states like West Bengal, Tamil Nadu and Uttar Pradesh. It grows well in moist, shady places and well-drained soil.

**Chemical Constituents:**

Kalmegh is rich in diterpene lactones, mainly andrographolide which is responsible for its intense bitter taste and pharmacological activity.

<b>Sr. No</b>	<b>Chemical Constituent</b>	<b>Chemical Nature / Class</b>	<b>Approx. Presence</b>	<b>Significance</b>
1	Andrographolide	Diterpene lactone	Major constituent	Hepatoprotective, anti-inflammatory
2	Deoxyandrographolide	Diterpene lactone	Moderate	Antimicrobial
3	Neoandrographolide	Diterpene glycoside	Small amount	Immunomodulatory
4	Flavonoids	Polyphenolic compounds	Small amount	Antioxidant
5	Bitter principles	Lactones	Significant	Characteristic bitter taste

**Uses:**

- Hepatoprotective – Used in liver disorders like jaundice and hepatitis.
- Bitter tonic – Improves appetite and digestion.
- Antipyretic – Used in fever.
- Anti-inflammatory – Useful in inflammatory conditions.
- Immunomodulator – Enhances body resistance.
- Antimicrobial – Effective against infections.

### Sensory Identification (Organoleptic Evaluation):

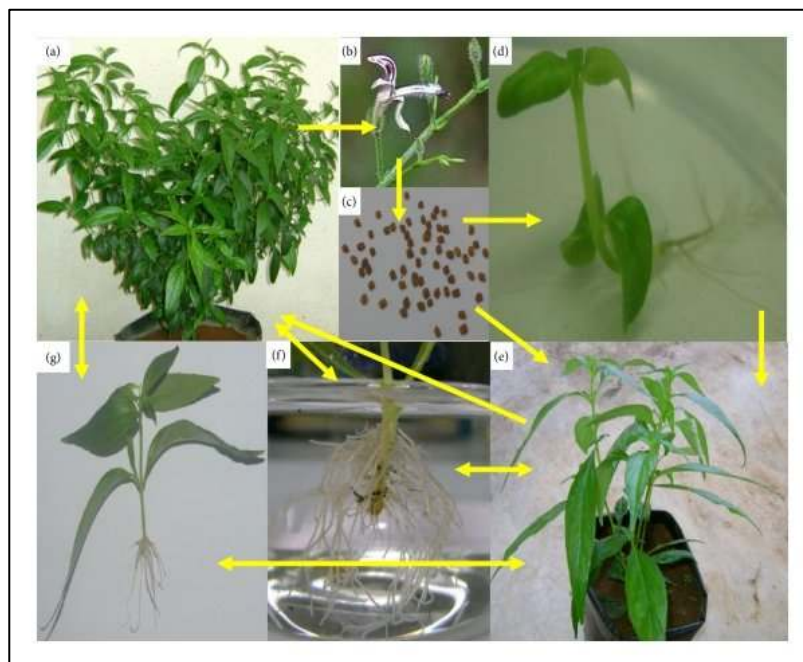
- Colour: Green stem and leaves.
- Odour: Slight, characteristic.
- Taste: Extremely bitter (diagnostic feature).
- Texture: Thin stems, smooth leaves.

The intense bitterness is the most important identifying character/feature of kalmegh.

### Morphological Identification (External Characters):

- Type: Whole plant drug (herb).
- Stem: Erect, green, quadrangular with longitudinal furrows.
- Leaves: Opposite, lanceolate, glabrous.
- Size of leaves: 4–8 cm long.
- Flowers (if present): Small, white with purple spots.
- Fruit: Capsule containing yellowish seeds.

These features help distinguish Kalmegh from other bitter herbs.



**Figure 17.1: Morphological Characters of Kalmegh (*Andrographis paniculata*)**

**(a) Whole plant showing erect habit, (b) Flowering twig with characteristic small white flowers having purple markings, (c) Seeds of Kalmegh, (d) Germinating seed (seedling stage), (e) Young cultivated plant, (f) Root system showing well-developed tap root with lateral roots, (g) Early seedling with opposite leaves**

### Chemical identification:

Kalmegh contains diterpene lactones, mainly andrographolide, which is responsible for its

bitterness and hepatoprotective activity.

Chemical reactions confirming presence of diterpenes and flavonoids help in authentication.

**Chemical Test:**

Sr. No.	Test	Procedure (In Detail)	Observation	Inference
1	Sodium hydroxide test	Take alcoholic extract of Kalmegh in a test tube and add few drops of 5% NaOH solution. Shake gently.	Yellow colour appears	Presence of diterpene lactones (andrographolide)
2	Concentrated sulphuric acid test	Add 1–2 drops of conc. H <sub>2</sub> SO <sub>4</sub> to small quantity of powder on a porcelain tile.	Reddish-brown colour develops	Presence of diterpene compounds
3	Ferric chloride test	Add few drops of 5% FeCl <sub>3</sub> solution to aqueous extract of the drug.	Greenish colour formation	Presence of phenolic compounds
4	Lead acetate test	Add few drops of lead acetate solution to aqueous extract.	Yellow precipitate forms	Presence of flavonoids
5	Shinoda test	Add magnesium turnings and few drops of conc. HCl to alcoholic extract.	Pink to red colour develops	Presence of flavonoids
6	Keller–Killiani test (modified)	Add glacial acetic acid containing trace of FeCl <sub>3</sub> , then carefully add conc. H <sub>2</sub> SO <sub>4</sub> along side of test tube.	Brown ring at junction	Indicates presence of glycosidal nature compounds

**Microscopical Identification (Powder Microscopy):**

When Kalmegh powder is observed under microscope, the following characters are seen:

- Epidermal cells: Polygonal with straight walls.
- Diacytic stomata: Present (typical of Acanthaceae).
- Covering trichomes: Multicellular, uniseriate hairs.
- Xylem vessels: Spiral and reticulate thickening.
- Calcium oxalate crystals: Present in parenchyma.
- Fibres: Lignified fibres in vascular bundles.

These characters help in identifying genuine drug and detecting adulteration.



**Figure 17.2. Powder Microscopic Characters of Kalmegh (*Andrographis paniculata*)**

#### Micro-Chemical / Staining Tests:

Micro-chemical tests are performed directly on powdered drug mounted on a slide to identify specific cellular components by characteristic colour reactions under microscope.

Sr. No.	Reagent	Short Procedure (On Slide)	Observation	Inference
1	Phloroglucinol + Conc. HCl	Place a small quantity of powder on slide, add 1–2 drops of phloroglucinol solution followed by conc. HCl, cover with cover slip and observe.	Pink to red coloration of cells	Presence of lignified tissues (xylem vessels, fibres)
2	Dilute HCl	Mount powder in dilute HCl and observe under microscope.	Crystals remain intact and clearly visible	Presence of calcium oxalate crystals
3	5% Ferric chloride	Add few drops to powder mounted in water.	Greenish coloration	Presence of phenolic

				compounds
4	Iodine solution	Mount powder with iodine solution.	No blue coloration	Starch absent (important feature)

**Result:**

The given crude drug sample was identified as Kalmegh based on its extremely bitter taste, quadrangular stem, presence of andrographolide and diagnostic microscopic features such as diacytic stomata and lignified fibres.

**Conclusion:**

From the above pharmacognostical evaluation, the crude drug was authenticated as Kalmegh on the basis of:

- Characteristic intense bitterness
- Opposite lanceolate leaves and quadrangular stem
- Presence of diterpene lactones (andrographolide)
- Diagnostic microscopic features

Thus, the drug complies with standard pharmacognostical parameters.

**Precautions:**

- Use fresh and properly dried powdered drug for analysis.
- Prepare a thin and uniform powder mount for clear microscopic observation.
- Handle concentrated acids (HCl, H<sub>2</sub>SO<sub>4</sub>) carefully while performing chemical and micro-chemical tests.
- Observe colour changes immediately after adding reagents.
- Use clean and dry glassware to avoid contamination.
- Store the crude drug in an airtight container to prevent moisture absorption.

**Reference:**

1. Khandelwal K.R., Practical Pharmacognosy Techniques and Experiments.
2. Kokate C.K., Purohit A.P., Gokhale S.B., Pharmacognosy.
3. Indian Pharmacopoeia (latest edition).

### **Viva questions & answers:**

**Q1.** What is the biological source of Kalmegh?

**A:** It consists of dried whole plant of *Andrographis paniculata* belonging to family Acanthaceae.

**Q2.** What is the major active constituent of Kalmegh?

**A:** Andrographolide (a diterpene lactone).

**Q3.** Why is Kalmegh known as “King of Bitters”?

**A:** Because of its extremely bitter taste due to andrographolide.

**Q4.** What are the diagnostic microscopic characters of Kalmegh?

**A:** Diacytic stomata, multicellular trichomes, lignified vessels and calcium oxalate crystals.

**Q5.** What is the main therapeutic use of Kalmegh?

**A:** It is mainly used as hepatoprotective and antipyretic drug.

**Q6.** Which family does Kalmegh belong to?

**A:** Acanthaceae.

### **Nux-Vomica**

**Biological Source:** Nux vomica consists of the dried ripe seeds of *Strychnos nux-vomica* Linn., belonging to **family** Loganiaceae.

**Common Name:** Nux vomica, Kuchla, Poison nut, Crow fig



**Figure 18: Dried Ripe Seeds of Nux Vomica (*Strychnos nux-vomica* Linn.)**

### Geographical Source:

Nux vomica is native to India, Sri Lanka and Southeast Asia. In India, it is commonly found in tropical and subtropical regions such as Tamil Nadu, Kerala, Karnataka and West Bengal. It grows well in dry deciduous forests and well-drained soil.

**Chemical Constituents:** Nux vomica contains 2–5% indole alkaloids, which are highly toxic but pharmacologically active in controlled doses.

Sr. No	Chemical Constituent	Chemical Nature / Class	Approx. Presence	Significance
1	Strychnine	Indole alkaloid	1–1.5%	Central nervous system stimulant (toxic in high dose)
2	Brucine	Indole alkaloid	1.5–2%	Bitter principle
3	Loganin	Iridoid glycoside	Small amount	Medicinal activity
4	Fixed oil	Fatty oil	2–4%	Nutritional component
5	Proteins	Organic compounds	Moderate	Structural component

### Uses:

- Central nervous system stimulant (in very small therapeutic doses).
- Bitter tonic – stimulates appetite and digestion.
- Used in treatment of atonic dyspepsia and constipation.
- Acts as circulatory stimulant.
- Used in some homeopathic preparations.

Note: Contains toxic alkaloids (strychnine and brucine); hence used in controlled doses only.

### Sensory Identification (Organoleptic Evaluation):

- Colour: Greyish-green to ash colour.
- Odour: Odourless.
- Taste: Intensely bitter.
- Texture: Hard, tough and silky to touch due to closely appressed hairs.

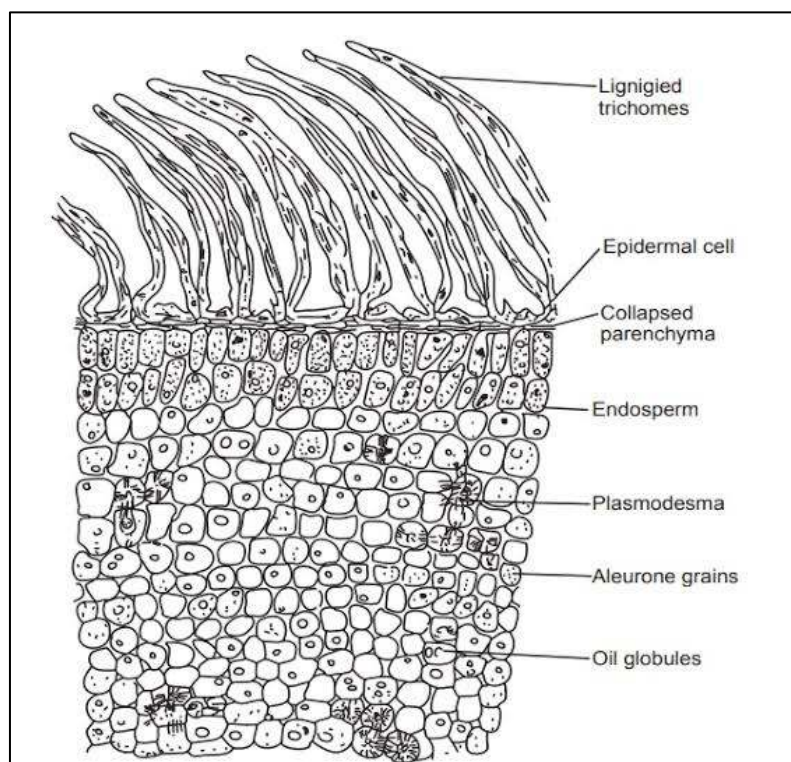
The intense bitterness and silky surface are key identifying features.

### Morphological Identification (External Characters):

- **Type:** Seed drug.
- **Shape:** Disc-shaped, circular, slightly convex on one side and concave on the other.

- **Size:** 2–3 cm in diameter; about 4–6 mm thick.
- **Surface:** Covered with fine silky hairs radiating from the centre.
- **Colour:** Greyish or greenish-grey.
- **Hilum:** Small depression at the centre.
- **Fracture:** Hard and horny.
- **Internal structure:** Contains large endosperm and small embryo.

These characters help distinguish genuine *Nux vomica* seeds from adulterants.



**Figure 18.1: Morphological characters of Nux-Vomica**

### **Chemical Identification:**

*Nux vomica* contains two major indole alkaloids:

- Strychnine
- Brucine

Presence of alkaloids is confirmed by general alkaloidal reagents and specific colour reactions.

### **Chemical Test:**

*Nux vomica* contains toxic indole alkaloids mainly strychnine and brucine. These alkaloids give characteristic precipitates and colour reactions with specific reagents.

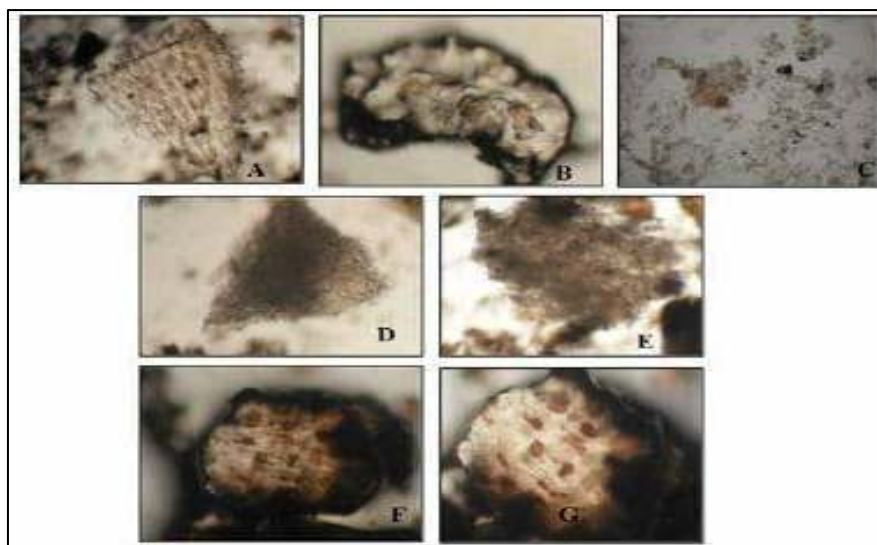
Sr. No.	Test Name	Short Procedure	Observation	Inference
1	Dragendorff's Test	Prepare acidic extract of powdered drug using dilute HCl. Filter and add few drops of Dragendorff's reagent.	Orange or reddish-brown precipitate	Presence of alkaloids
2	Mayer's Test	To acidic extract, add Mayer's reagent dropwise.	Cream or pale yellow precipitate	Alkaloids present
3	Wagner's Test	Add Wagner's reagent to acidic extract.	Reddish-brown precipitate	Alkaloids confirmed
4	Hager's Test	Add Hager's reagent (picric acid solution) to acidic extract.	Yellow precipitate	Presence of alkaloids
5	Strychnine Confirmatory Test	Add conc. H <sub>2</sub> SO <sub>4</sub> to small quantity of extract, then add a crystal of potassium dichromate.	Violet or blue colour which fades slowly	Presence of strychnine
6	Brucine Test	Treat powder with conc. HNO <sub>3</sub> .	Blood red colour formation	Presence of brucine

#### Microscopical Identification (Powder Microscopy):

When powdered Nux vomica seed is examined under microscope, the following features are observed:

- Epidermal cells: Thick-walled with lignified hairs.
- Lignified fibres: Present in testa.
- Endosperm cells: Large polygonal cells containing fixed oil and aleurone grains.
- Oil globules: Present within endosperm.
- Calcium oxalate crystals: Occasionally present.

These diagnostic features help in confirming authenticity and detecting adulteration.



**Figure 18.2: Powder Microscopic Characters of Nux Vomica**

(A: Lignified epidermal cells with thick, pitted walls, B: Endosperm cells containing fixed oils and aleurone grains, C: General powder view showing lignified trichome bases, D: Fragment of thick-walled endosperm tissue, E: Mass of parenchymatous cells from inner seed layers, F & G: Epidermal sections showing lignified trichomes with rod-like thickenings.)

**Micro-Chemical / Staining Tests:**

Micro-chemical tests are performed directly on powdered drug mounted on slide or small quantity taken on tile. These tests help in confirming presence of lignified tissues and characteristic alkaloids like strychnine and brucine.

Sr. No.	Reagent	Short Procedure	Observation	Inference
1	Phloroglucinol + Conc. HCl	Place small quantity of powder on slide, add 1–2 drops of phloroglucinol followed by conc. HCl and observe under microscope.	Pink to red colour in cell walls	Presence of lignified tissues (sclerenchyma, fibres)
2	Conc. Nitric Acid (HNO <sub>3</sub> )	Add 1–2 drops of conc. HNO <sub>3</sub> to small amount of powder on tile.	Red coloration	Presence of brucine
3	Conc. H <sub>2</sub> SO <sub>4</sub> + Potassium	Treat powder with conc. H <sub>2</sub> SO <sub>4</sub> and add	Violet to blue colour	Presence of strychnine

	dichromate	small crystal of $K_2Cr_2O_7$ .		
4	Dilute HCl	Mount powder in dilute HCl and observe microscopically.	Crystals remain intact and visible	Calcium oxalate crystals present

**Result:**

The given crude drug sample was identified as Nux Vomica based on its characteristic disc-shaped seeds, extremely bitter taste, presence of alkaloids (strychnine and brucine), and diagnostic microscopical features such as sclerenchymatous cells, aleurone grains and calcium oxalate crystals observed in powdered form.

**Conclusion:**

From the above study, the crude drug was authenticated as Nux Vomica on the basis of:

- Circular, flat, silky seeds
- Extremely bitter taste
- Positive alkaloidal tests (Dragendorff's, Mayer's)
- Specific colour reactions for strychnine and brucine
- Diagnostic powder characters like lignified cells and aleurone grains

Thus, the drug complies with standard pharmacognostical identification parameters.

**Precautions:**

- Handle the drug carefully as it contains toxic alkaloids.
- Use gloves while handling powdered drug.
- Avoid inhalation of powder.
- Handle concentrated acids ( $HNO_3$ ,  $H_2SO_4$ ) carefully during chemical tests.
- Use clean glassware to avoid false positive reactions.
- Perform alkaloidal tests using properly prepared acidic extract.

**Reference:**

1. Khandelwal K.R., Practical Pharmacognosy Techniques and Experiments.
2. Kokate C.K., Purohit A.P., Gokhale S.B., Pharmacognosy.
3. Indian Pharmacopoeia (latest edition).

**Viva question & answer:**

**Q1.** What is the biological source of Nux Vomica?

**A:** It consists of dried ripe seeds of *Strychnos nux-vomica* belonging to family Loganiaceae.

**Q2.** What are the major active constituents?

**A:** Strychnine and brucine (indole alkaloids).

**Q3.** Why is Nux Vomica considered dangerous?

**A:** Because it contains highly toxic alkaloids, especially strychnine.

**Q4.** What is the characteristic morphological feature of Nux Vomica seed?

**A:** Disc-shaped, flat seeds with silky hairs.

**Q5.** Which test confirms presence of strychnine?

**A:** Violet colour reaction with concentrated sulphuric acid and potassium dichromate.

**Q6.** What are the diagnostic powder characters?

**A:** Lignified sclerenchymatous cells, aleurone grains and calcium oxalate crystals.

## **Experiment No. 9: Gravimetric determination of content of alkaloid, glycoside, saponin and resin**

### **Aim:**

To determine the percentage content of alkaloids, glycosides, saponins and resins present in the given crude drug sample by gravimetric method.

### **Principle:**

Gravimetric analysis is a quantitative method in which the constituent of interest is separated from the crude drug, isolated in pure form, dried and weighed.

The percentage of the constituent is calculated based on the weight of dried extract obtained from a known quantity of crude drug.

Each class of phytoconstituent is extracted using a suitable solvent:

- Alkaloids → Extracted in acidic medium and precipitated using alkaloidal reagents.
- Glycosides → Extracted using hydroalcoholic solvent.
- Saponins → Extracted with alcohol and partitioned using organic solvents.
- Resins → Extracted with non-polar solvents like petroleum ether or acetone.

The dried residue obtained after evaporation gives the gravimetric value.

### **A. Gravimetric Determination of Total Alkaloids**

**Requirements:** Crude drug powder (5 g), 10% Acetic acid in ethanol, Ammonia solution, Whatman filter paper, Evaporating dish, Water bath, Analytical balance.

### **Principle:**

Alkaloids are basic nitrogen-containing compounds. They are extracted using dilute acid and precipitated using ammonium hydroxide or Dragendorff's reagent. The precipitate is collected, dried and weighed.

### **Procedure:**

1. Accurately weigh 5 g of air-dried powdered crude drug.
2. Add 100 ml of 10% acetic acid in ethanol.
3. Cover and allow to stand for 4 hours, shaking occasionally.
4. Filter the extract.
5. Concentrate the filtrate on a water bath to about one-fourth of original

volume.

6. Add concentrated ammonia solution dropwise until precipitation is complete.
7. Allow the precipitate to settle.
8. Filter and wash with dilute ammonia.
9. Dry the precipitate in a hot air oven at 60°C.
10. Cool in desiccator and weigh.

**Calculation:**

$$\text{Percentage of Alkaloids} = \frac{\text{Weight of Residue}}{\text{Weight of Sample}} \times 100$$

**Observation table:**

Sr. No.	Particulars	Symbol	Observation
1	Weight of empty filter paper	$W_1$	_____ g
2	Weight of filter paper + alkaloid precipitate (after drying)	$W_2$	_____ g
3	Weight of alkaloid obtained	$(W_2 - W_1)$	_____ g
4	Weight of crude drug taken	$W$	_____ g
5	% of Alkaloids	$\frac{(W_2 - W_1)}{W} \times 100$	_____ %

**B. Gravimetric Determination of Total Glycosides**

**Requirements:** Powdered drug (5 g), 80% Ethanol, Dilute acid, Separating funnel, Water bath, Evaporating dish

**Principle:**

Glycosides are extracted using alcohol. After hydrolysis (if required), the aglycone portion is separated, dried and weighed.

**Procedure:**

1. Weigh 5 g powdered drug.
2. Extract with 80% ethanol by heating for 30 minutes.
3. Filter and concentrate the extract.
4. Hydrolyze with dilute acid (if required).

5. Separate aglycone portion using suitable organic solvent.
6. Evaporate solvent and dry residue.
7. Weigh the dried residue.

**Calculation:**

$$\text{Percentage of Glycosides} = \frac{\text{Weight of dried residue}}{\text{Weight of Sample}} \times 100$$

**Observation table:**

Sr. No.	Particulars	Symbol	Observation
1	Weight of empty evaporating dish	$W_1$	_____ g
2	Weight of dish + dried glycoside residue	$W_2$	_____ g
3	Weight of glycosides obtained	$(W_2 - W_1)$	_____ g
4	Weight of crude drug taken	$W$	_____ g
5	% of Glycosides	$\frac{(W_2 - W_1)}{W} \times 100$	_____ %

### **C. Gravimetric Determination of Total Saponins**

**Requirements:** Powdered drug (5 g), 20% Ethanol, Water bath, Separating funnel, Diethyl ether

**Principle:**

Saponins are extracted with aqueous alcohol. On concentration, they form residue which can be dried and weighed.

**Procedure:**

1. Weigh 5 g powdered drug.
2. Add 100 ml of 20% ethanol.
3. Heat over water bath for 4 hours with continuous stirring.
4. Filter and re-extract residue.
5. Combine extracts and concentrate to 40 ml.
6. Transfer to separating funnel and extract with diethyl ether to remove impurities.
7. Collect aqueous layer and evaporate to dryness.
8. Dry and weigh the residue.

**Calculation:**

$$\text{Percentage of Saponins} = \frac{\text{Weight of Residue}}{\text{Weight of Sample}} \times 100$$

**Observation table:**

Sr. No.	Particulars	Symbol	Observation
1	Weight of empty evaporating dish	$W_1$	_____ g
2	Weight of dish + dried saponin residue	$W_2$	_____ g
3	Weight of saponins obtained	$(W_2 - W_1)$	_____ g
4	Weight of crude drug taken	$W$	_____ g
5	% of Saponins	$\frac{(W_2 - W_1)}{W} \times 100$	_____ %

**D. Gravimetric Determination of Total Resins**

**Requirements:** Powdered drug (5 g), Petroleum ether / Chloroform, Soxhlet apparatus (if available), Water bath, Evaporating dish

**Principle:**

Resins are soluble in organic solvents like ether or chloroform. After extraction, the solvent is evaporated and resin is weighed.

**Procedure:**

1. Weigh 5 g powdered drug.
2. Extract with petroleum ether using Soxhlet apparatus for 6 hours.
3. Collect extract and evaporate solvent.
4. Dry residue in evaporating dish.
5. Weigh the resin content.

**Calculation:**

$$\text{Percentage of Resins} = \frac{\text{Weight of dried resin}}{\text{Weight of Sample}} \times 100$$

**Observation table:**

Sr. No.	Particulars	Symbol	Observation
1	Weight of empty evaporating dish	$W_1$	_____ g
2	Weight of dish + dried resin residue	$W_2$	_____ g
3	Weight of resin obtained	$(W_2 - W_1)$	_____ g
4	Weight of crude drug taken	$W$	_____ g
5	% of resin	$\frac{(W_2 - W_1)}{W} \times 100$	_____ %

**Result:**

The gravimetric analysis of the given crude drug sample showed the presence of alkaloids, glycosides, saponins and resins.

The percentage content was calculated using the formula:

$$\text{Percentage} = \frac{(W_2 - W_1)}{W} \times 100$$

The calculated values are as follows:

- % Alkaloids = \_\_ %
- % Glycosides = \_\_ %
- % Saponins = \_\_ %
- % Resins = \_\_ %

(Actual values to be filled as per experimental observation.)

**Conclusion:**

From the above experiment, it can be concluded that the gravimetric method is a simple and reliable technique for quantitative estimation of major phytoconstituents present in crude drugs. The method is based on extraction, isolation and accurate weighing of dried residues. The percentage content obtained gives an idea about the phytochemical richness and quality of the crude drug sample.

**Precautions:**

- Weigh the crude drug accurately using analytical balance.
- Ensure complete extraction of phytoconstituents.
- Avoid loss of precipitate during filtration.
- Dry the residue completely before final weighing.

- Cool the evaporating dish in desiccator before taking weight.
- Do not overheat the extract to prevent decomposition.
- Use clean and dry glassware to avoid errors.

**Reference:**

1. Khandelwal K.R., Practical Pharmacognosy Techniques and Experiments.
2. Kokate C.K., Purohit A.P., Gokhale S.B., Pharmacognosy.
3. Indian Pharmacopoeia (latest edition).

**Viva question & answer:**

**Q1.** What is gravimetric analysis?

**A:** It is a quantitative analytical method in which the analyte is isolated and weighed to determine its amount.

**Q2.** On which principle does this experiment depend?

**A:** It depends on differences in solubility, precipitation and evaporation of phytoconstituents.

**Q3.** Why is desiccator used before weighing?

**A:** To remove moisture and avoid variation in weight.

**Q4.** Why is ammonia added in alkaloid estimation?

**A:** To precipitate alkaloids in free base form.

**Q5.** Why is drying important before final weighing?

**A:** Because moisture can give inaccurate higher weight.

**Q6.** What type of method is gravimetric method?

**A:** It is a quantitative method.

## **Experiment No. 10: Spectrophotometric determination of phenols and flavonoids**

**Aim:** To determine the total phenolic content and total flavonoid content present in the given crude drug extract using UV-Visible spectrophotometry.

### **Requirements:**

**Chemicals-** Folin–Ciocalteu reagent, Sodium carbonate solution (7.5%), Aluminium chloride (10%), Methanol, Gallic acid (standard), Quercetin (standard), Distilled water

**Apparatus-** UV–Visible spectrophotometer, Cuvettes, Volumetric flask, Pipettes, Test tubes, Analytical balance

**Principle:** Spectrophotometry is a quantitative analytical technique based on the measurement of absorbance of light by a colored solution at a specific wavelength.

According to Beer–Lambert’s Law, absorbance is directly proportional to the concentration of the analyte and path length of the solution.

### **Determination of Total Phenols-**

Phenolic compounds react with **Folin–Ciocalteu reagent** in alkaline medium to form a blue-colored complex. The intensity of the blue color is directly proportional to the concentration of phenolic compounds present in the sample.

Absorbance is measured at **765 nm** using UV-Visible spectrophotometer. Gallic acid is used as standard and results are expressed as mg Gallic Acid Equivalent (GAE) per gram of extract.

### **Determination of Total Flavonoids-**

Flavonoids form a yellow-colored complex with **aluminium chloride (AlCl<sub>3</sub>)**. The intensity of yellow color is proportional to flavonoid concentration.

Absorbance is measured at **415 nm**. Quercetin is used as standard and results are expressed as mg Quercetin Equivalent (QE) per gram of extract.

## **Part A: Determination of Total Phenolic Content (TPC)**

### **Preparation of Standard Solution (Gallic Acid):**

1. Prepare stock solution of gallic acid (100 µg/ml).
2. Prepare different concentrations (20, 40, 60, 80, 100 µg/ml).
3. These will be used for calibration curve.

**Procedure:**

1. Take 1 ml of standard or sample extract.
2. Add 5 ml of Folin–Ciocalteu reagent (diluted).
3. Allow to stand for 5 minutes.
4. Add 4 ml of 7.5% sodium carbonate solution.
5. Incubate for 30 minutes at room temperature.
6. Measure absorbance at 765 nm against blank.
7. Plot calibration curve of concentration vs. absorbance

**Observation Table (Phenols – Standard):**

Sr. No	Concentration of Gallic Acid (µg/ml)	Absorbance at 765 nm
1	20	_____
2	40	_____
3	60	_____
4	80	_____
5	100	_____

**Observation Table (Sample)-**

Sr. No	Sample Absorbance at 765 nm	Calculated Phenolic Content
1	_____	_____ mg GAE/g extract

(GAE = Gallic Acid Equivalent)

**Calculation:**

From calibration curve of gallic acid:

$$\text{Total Phenolic Content (mg GAE/g)} = \frac{C \times V \times DF}{M}$$

Where:

C = Concentration obtained from calibration curve

V = Volume of extract

DF = Dilution factor (if any)

M = Weight of extract

## **Part B: Determination of Total Flavonoid Content (TFC)**

### **Preparation of Standard Solution (Quercetin):**

Prepare stock solution (100 µg/ml) and dilute to obtain 20–100 µg/ml concentrations.

### **Procedure:**

1. Take 1 ml of standard or sample.
2. Add 1 ml of 10% aluminium chloride.
3. Add 1 ml potassium acetate.
4. Add 5 ml methanol.
5. Incubate for 30 minutes at room temperature.
6. Measure absorbance at 415 nm.
7. Plot calibration curve.

### **Observation Table (Flavonoids – Standard):**

<b>Sr. No</b>	<b>Concentration of Quercetin (µg/ml)</b>	<b>Absorbance at 415 nm</b>
1	20	_____
2	40	_____
3	60	_____
4	80	_____
5	100	_____

### **Observation Table (Sample)-**

<b>Sr. No</b>	<b>Sample Absorbance at 415 nm</b>	<b>Calculated Flavonoid Content</b>
1	_____	_____ mg QE/g extract

(QE = Quercetin Equivalent)

### **Calculation:**

$$\text{Total Flavonoid Content (mg QE/g)} = \frac{C \times V \times DF}{M}$$

Where:

C = Concentration from calibration curve

V = Volume of extract

DF= Dilution factor

M = Weight of extract

**Result:**

The total phenolic content and total flavonoid content of the given plant extract were determined spectrophotometrically.

Total Phenolic Content = \_\_ mg GAE/g

Total Flavonoid Content = \_\_ mg QE/g

**Conclusion:**

The intensity of color formed was directly proportional to concentration, confirming the reliability of UV-Visible spectrophotometric analysis for phytochemical estimation.

**Precautions:**

- Use freshly prepared reagents.
- Protect reagents from light.
- Use clean cuvettes without scratches.
- Avoid bubbles in cuvette during reading.
- Calibrate spectrophotometer before use.
- Prepare standard curve carefully for accurate results

**Reference:**

1. Khandelwal K.R., Practical Pharmacognosy Techniques and Experiments.
2. Kokate C.K., Purohit A.P., Gokhale S.B., Pharmacognosy.
3. Indian Pharmacopoeia (latest edition).

**Viva Questions & Answers:**

**Q1.** What is spectrophotometry?

**A:** It is a method of measuring absorbance of light by a solution at a specific wavelength.

**Q2.** Which reagent is used for phenol estimation?

**A:** Folin–Ciocalteu reagent.

**Q3.** At what wavelength is phenol content measured?

**A:** 765 nm.

**Q4.** Which reagent is used for flavonoid estimation?

**A:** Aluminium chloride.

**Q5.** At what wavelength is flavonoid content measured?

**A:** 415 nm.

## **Experiment No. 11: Experiential learning based experiments involving evaluation and comparison of field/ market collected herbal raw materials with pharmacopoeial standards.**

### **Aim:**

To evaluate and compare field or market collected herbal raw material with official pharmacopoeial standards using organoleptic, macroscopic, microscopic and physicochemical parameters.

### **Requirements:**

- Market collected crude drug sample
- Official pharmacopoeial monograph
- Analytical balance
- Compound microscope
- Glass slides and cover slips
- Chloral hydrate solution
- Muffle furnace
- Hot air oven
- Soxhlet apparatus (if required)
- Petroleum ether / Alcohol / Water

### **Principle:**

Herbal drugs are obtained from natural sources and their quality is greatly influenced by environmental and handling factors. Unlike synthetic drugs, crude drugs are biological materials and therefore show natural variability. The quality of herbal raw materials available in the market may vary due to several reasons such as:

- Geographical variation (soil type, altitude, region)
- Climatic conditions (temperature, rainfall, humidity)
- Time and method of harvesting
- Improper drying and processing
- Adulteration (intentional mixing with inferior substances)
- Substitution with closely related species
- Presence of foreign matter
- Poor storage conditions leading to moisture absorption, microbial growth or degradation

Such variations may affect the identity, purity, potency and safety of the crude drug. To ensure uniform quality and therapeutic efficacy, official pharmacopoeias establish standard parameters for evaluation. These include the Indian Pharmacopoeia and the guidelines provided by the World Health Organization for quality control of herbal medicines.

These official standards specify:

- Organoleptic and macroscopic characteristics
- Microscopic diagnostic features
- Physicochemical constants (ash values, extractive values, moisture content)
- Limits for foreign matter
- Other quality control parameters

In this experiment, the field- or market-collected crude drug is evaluated using standard laboratory methods and the results are compared with official pharmacopoeial limits. If the observed values fall within prescribed limits, the sample is considered authentic and of acceptable quality. If not, it may indicate adulteration, substitution, or poor handling. Thus, this comparative evaluation ensures standardization, quality assurance and safety of herbal raw materials before their use in pharmaceutical preparations.

**Drug Selected:**

(Write the name of drug given to you, e.g., Ashwagandha root / Senna leaf / Tulsi leaf / Fennel fruit)

**Procedure:**

**A. Collection and Authentication-**

The crude drug sample was collected from a local herbal market/field source. The collected material was carefully cleaned to remove dust, soil and extraneous matter. The sample was then properly dried (if required), packed and labeled with details such as:

- Name of the drug
- Date of collection
- Place of collection
- Part used

For authentication, the collected drug was compared with the standard description provided in the official pharmacopoeial monograph. Identification was carried out based on:

- Morphological characteristics

- Colour
- Odour
- Size and shape
- Distinguishing external features

The sample was confirmed to be genuine based on similarity with official standards.

### **B. Organoleptic Evaluation-**

The crude drug was evaluated using sensory organs to determine its organoleptic properties.

A small quantity of the drug was examined carefully and the following parameters were observed:

- Colour – observed under daylight
- Odour – determined by gentle smelling
- Taste – tested carefully (only if safe and permitted)
- Size – measured approximately
- Shape – observed visually
- Surface characteristics – smooth, rough, wrinkled, hairy, etc.
- Texture – brittle, hard, soft, fibrous, etc.

The observed characteristics were compared with those mentioned in the official pharmacopoeial description to check compliance.

### **C. Macroscopic Evaluation-**

The external morphological features of the crude drug were examined carefully with the naked eye or using a magnifying lens.

The following features were studied:

- Nature of outer surface
- Presence of wrinkles, ridges, lenticels or hairs
- Type of fracture (short, fibrous, splintery, smooth, etc.)
- Internal colour (after breaking or cutting)
- Arrangement of plant parts (leaf venation, fruit segments, root branching, etc.)
- Presence of foreign matter

These characteristics were compared with official pharmacopoeial standards for identification and quality assessment.

### **D. Microscopic Evaluation-**

Microscopic examination was carried out to identify characteristic internal structures of the crude drug.

Depending on the nature of the drug, either:

- Thin Transverse Section (T.S.) was prepared
- OR
- Powder microscopy was performed

**For T.S. Preparation:**

1. A thin transverse section of the drug was prepared using a sharp blade.
2. The section was cleared using chloral hydrate.
3. It was stained with suitable staining reagents if required.
4. The section was mounted on a glass slide with glycerin.
5. Observed under microscope (low and high power).

**For Powder Microscopy:**

1. Small quantity of powdered drug was taken on slide.
2. Treated with suitable reagents.
3. Covered with cover slip.
4. Observed under microscope.

**Diagnostic Characters Observed:**

- Type of stomata (anomocytic, paracytic, etc.)
- Presence and type of trichomes
- Calcium oxalate crystals (prismatic, rosette, acicular)
- Starch grains (simple or compound)
- Vascular bundles
- Fibres
- Cork cells
- Oil globules (if present)

These microscopic characters were compared with official monograph for confirmation of identity.

**E. Determination of Physicochemical Parameters:**

Physicochemical evaluation helps in determining the purity and quality of crude drugs. These parameters are useful for detecting adulteration, substitution, improper handling and presence of inorganic impurities.

**a) Determination of Foreign Matter-**

**Principle:**

Foreign matter consists of any part of the plant or extraneous material other than the drug described in the pharmacopoeial monograph. Excess foreign matter indicates poor quality and improper cleaning of the crude drug.

**Procedure:**

1. Weigh accurately 100 g of the crude drug sample.
2. Spread the sample on a clean white sheet or tray.
3. Carefully examine and separate visible foreign materials such as:
  - Soil
  - Stones
  - Sand
  - Insect parts
  - Other plant parts
4. Weigh the separated foreign matter accurately.

**Calculation:**

$$\% \text{ Foreign Matter} = \frac{\text{Weight of Foreign Matter}}{\text{Total Weight of sample}} \times 100$$

**Observation Table:**

Total Weight of Sample (g)	Weight of Foreign Matter (g)	% Foreign Matter

**b) Determination of Total Ash-**

**Principle:**

Total ash represents total inorganic residue remaining after incineration. It includes:

- Physiological ash (natural mineral content)
- Non-physiological ash (dirt, sand, contamination)

**Procedure:**

1. Accurately weigh 2–3 g of air-dried powdered drug in a previously ignited and tarred silica crucible.
2. Gradually increase the temperature to avoid sudden ignition.

3. Incinerate the sample in a muffle furnace at 450–600°C until it becomes white, indicating absence of carbon.
4. Cool the crucible in a desiccator.
5. Weigh the crucible containing ash.
6. Repeat heating until constant weight is obtained.

**Calculation:**

$$\% \text{ Total Ash} = \frac{\text{Weight of Ash}}{\text{Weight of Air – Dried Sample}} \times 100$$

**Observation Table:**

Weight of Sample (g)	Weight of crucible + ash (g)	Weight of ash (g)	% Total ash

**c) Determination of Acid Insoluble Ash-**

**Principle:**

Acid insoluble ash measures the amount of silica and earthy matter present in the crude drug. It indicates contamination with sand or siliceous materials.

**Procedure:**

1. Take the total ash obtained.
2. Add 25 ml dilute hydrochloric acid.
3. Boil gently for 5 minutes.
4. Filter through ashless filter paper.
5. Wash the residue with hot distilled water until neutral.
6. Dry the filter paper with residue.
7. Incinerate in muffle furnace until constant weight.
8. Cool in desiccator and weigh.

**Calculation:**

$$\% \text{ Acid Insoluble Ash} = \frac{\text{Weight of Acid Insoluble Residue}}{\text{Weight of Sample}} \times 100$$

**Observation Table:**

Weight of Sample (g)	Weight of acid insoluble residue (g)	% Acid insoluble ash

***d) Determination of Extractive Values-***

Extractive values indicate the amount of active constituents extracted with specific solvents. They help evaluate the presence of polar and non-polar constituents.

**I. Water Soluble Extractive**

***Procedure-***

1. Weigh accurately 5 g of air-dried powdered drug.
2. Macerate with 100 ml chloroform water in a closed flask for 24 hours.
3. Shake frequently during first 6 hours.
4. Allow to stand for remaining 18 hours.
5. Filter rapidly to prevent solvent loss.
6. Take 25 ml of filtrate in a tarred evaporating dish.
7. Evaporate to dryness on water bath.
8. Dry at 105°C to constant weight.
9. Cool in desiccator and weigh.

***Calculation-***

$$\% \text{ Water Soluble Extractive} = \frac{\text{Weight of residue} \times 4}{\text{Weight of Sample}} \times 100$$

(Multiplication by 4 because only 25 ml of 100 ml is taken)

**II. Alcohol Soluble Extractive**

***Procedure-***

Same procedure as water soluble extractive, but use 90% ethanol instead of chloroform water.

***Calculation:***

$$\begin{aligned} \% \text{ Alcohol Soluble Extractive} \\ = \frac{\text{Weight of residue} \times 4}{\text{Weight of Sample}} \times 100 \end{aligned}$$

**Observation Table:**

Weight of sample (g)	Solvent Used	Weight of residue (g)	% Extractive Value
	Water		
	Alcohol		

**Observation Tables:****Organoleptic Comparison**

Parameter	Market Sample	Pharmacopoeial Standard	Complies / Not Complies
Colour			
Odour			
Taste			
Shape			

**Physicochemical Comparison**

Parameter	Experimental Value	Official Limit	Within Limit (Yes/No)
Total Ash			
Acid Insoluble Ash			
Water Soluble Extractive			
Alcohol Soluble Extractive			
Foreign Matter			

**Result:**

The evaluated crude drug sample was compared with official pharmacopoeial standards. The sample was found to be (within / not within) prescribed limits.

**Conclusion:**

The experiment confirmed that pharmacopoeial standards are essential for ensuring quality and authenticity of herbal raw materials. The collected sample was found to be suitable / not suitable for medicinal use based on evaluation parameters.

**Precautions:**

- Use accurately weighed sample.
- Avoid contamination during handling.
- Maintain proper temperature in muffle furnace.
- Dry samples to constant weight before weighing.
- Use latest pharmacopoeial edition for comparison.

**References:**

1. Indian Pharmacopoeia, Latest Edition, Indian Pharmacopoeia Commission, Ghaziabad.
2. World Health Organization. Quality Control Methods for Herbal Materials, WHO Press, Geneva.
3. Kokate's Pharmacognosy by C.K. Kokate, A.P. Purohit, S.B. Gokhale.
4. Practical Pharmacognosy Techniques and Experiments by K.R. Khandelwal.

**Viva question & answer:**

**Q1.** Why is physicochemical evaluation important in crude drugs?

**A:** Physicochemical evaluation is important to ensure the identity, purity and quality of crude drugs. It helps in detecting adulteration, contamination and improper handling.

**Q2.** What is the difference between total ash and acid insoluble ash?

**A:** Total ash represents the total inorganic residue remaining after incineration of the drug. Acid insoluble ash is the portion of total ash that remains insoluble in dilute hydrochloric acid and indicates silica or sand contamination.

**Q3.** Why is acid insoluble ash determined?

**A:** It is determined to detect earthy materials such as sand and siliceous matter present in the crude drug.

**Q4.** What do extractive values indicate?

**A:** Extractive values indicate the amount of active constituents soluble in a particular solvent and help evaluate the chemical nature and quality of the drug.

**Q5.** What does a high ash value indicate?

**A:** A high ash value indicates possible adulteration, contamination with dirt or sand, or improper cleaning of the crude drug.

## About the Authors



**Mr. Utkarsh Ravindra Mandage** is a dynamic and forward-thinking academician, researcher, and author in the field of pharmaceutical sciences, currently serving as an Assistant Professor in the Department of Pharmacognosy at Ravindra Vidya Prasarak Mandal's Institute of Pharmacy, Nashik. Renowned for his passion for teaching and innovation, he actively mentors students while driving impactful research in natural products, medicinal plants, and herbal drug development. He has an impressive academic portfolio, having authored 6 pharmacy books, including 1 international publication, along with 17 research papers and over 40 review articles published in reputed national and international journals. An accomplished innovator, he holds 5 Indian patents and 1 UK patent in pharmaceutical sciences. His excellence in academia has been recognized through prestigious honors such as the Best Teacher Award, Young Researcher Award, and multiple Best Article Awards. Further strengthening his professional stature, he serves as an editorial board member for 7 journals and publication houses and has been nominated as an external expert scientist for two institutes. With a strong vision for advancing pharmaceutical education and research, Mr. Mandage continues to inspire, innovate, and contribute significantly to the scientific community.

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