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Semester 2 - Practical Notes

Pharmacognosy and Phytochemistry

Complete Subject Manual

16 practical files and activities. Use the PDF bookmarks to navigate. Page numbers restart within each practical; content page counts below exclude promotion pages.

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Chemical Identification of Agar Acacia Tragacanth and Honey

Experiment 1 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To compare the physical and chemical responses of four carbohydrate-rich natural materials.

Principle

Polysaccharides differ in hydration, swelling and gel formation. General carbohydrate and reducing-sugar tests support constituent classes, but identity and purity require several observations together.

Requirements

- Authenticated agar, acacia, tragacanth and honey; water and labelled prepared test reagents.
- Water bath, test tubes, pipettes, iodine and instructor-prepared Molisch, ruthenium-red and Benedict reagents.

Procedure

1. Prepare portions

Label separate portions for hydration and each chemical test. Record the quantities and reagent strengths prescribed by the laboratory.

2. Agar

Warm the aqueous dispersion until properly hydrated, then allow it to cool. Observe gel formation. An iodine check for blue-black starch contamination is a separate screening observation.

3. Acacia

Observe dissolution or dispersion in water to form a mucilage. Perform the prescribed general carbohydrate reaction with a known control.

4. Tragacanth

Observe the swollen insoluble fraction and the soluble mucilage fraction. Record the ruthenium-red response of acidic mucilage; this stain is not unique to tragacanth.

5. Honey

Prepare the assigned aqueous dilution. Heat a separate portion with Benedict reagent in a water bath and record reducing-sugar response against controls.

6. Compare

Combine hydration, gel behaviour and chemical reactions. Honey containing reducing sugars is not automatically authentic honey; official adulteration/quality testing remains separate.

Pharmacognosy and Phytochemistry

Observations and Record

Material	Supporting response	Actual observation
Agar	Hot hydration; gel on cooling	_____
Acacia	Water-compatible gum/mucilage; carbohydrate response	_____
Tragacanth	Swelling fraction; mucilage staining	_____
Honey	Reducing-sugar reaction	_____

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- If all four give a carbohydrate response, that result alone cannot distinguish the four materials. Gel formation and swelling supply additional class evidence.

Result

State the material supported by the combined observations and any unresolved purity question.

Precautions

- Use authenticated, labelled teaching material and instructor-prepared reagents. Wear the protection specified for the chemical method.
- Compare with a known control and record actual observations. A single colour, precipitate or odour description cannot authenticate a crude drug.
- Do not taste botanical material or directly inhale vapours; dispose of solvents and staining reagents in their assigned waste containers.

Viva Questions and Answers**1. Why is Molisch not an identity test?**

It detects a broad carbohydrate class.

2. What causes tragacanth swelling?

Its water-swelling insoluble fraction contributes strongly to hydration behaviour.

3. Does a positive Benedict test prove pure honey?

No. Other sugar mixtures can also be positive.

Chemical Identification of Castor Oil Olive Oil Wool Fat and Beeswax

Experiment 2 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To compare two fixed oils and two wax-like natural materials.

Principle

Fixed oils contain mainly triacylglycerols. Wool fat is a complex wax-like mixture rich in sterol esters, while beeswax contains long-chain esters and other components. Solubility, consistency, emulsification and prescribed chemical values together support differentiation.

Requirements

- Labelled castor oil, olive oil, wool fat and beeswax.
- Prepared solvent/reagent systems, warming bath, small vessels and the laboratory identification worksheet.

Procedure

1. Appearance

Record liquid or semi-solid state, colour and consistency at the stated room temperature. Read any odour description from the material specification.

2. Oil comparison

Compare castor and olive oil using the assigned alcohol-solubility test at fixed solvent composition and temperature. The hydroxylated fatty-acid component in castor oil influences its solvent behaviour.

3. Fat/ester reaction

Apply the instructor's prescribed saponification demonstration to a small portion and controls. Ester hydrolysis supports a lipid class, not a unique identity.

4. Wool fat

Observe the teacher-demonstrated uptake of water during mixing and the formation of a water-containing ointment-like mass. Record amounts; do not assume unlimited uptake.

5. Beeswax

Observe controlled softening/melting and behaviour on cooling. Use the supplied monograph's chemical checks or acid/ester-value results where available.

6. Identify

Use the complete pattern and the applicable material specification. Additive-containing commercial products may differ from the pure raw material.

Pharmacognosy and Phytochemistry**Observations and Record**

Material	Distinguishing study	Record
Castor oil / olive oil	Assigned alcohol-solubility comparison	_____
Wool fat	Consistency and measured water uptake	_____
Beeswax	Controlled melting and chemical specification	_____
All materials	Prescribed ester/lipid check with controls	_____

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- Water uptake = incorporated water mass / initial wool-fat mass × 100. If 2.0 g incorporates 1.0 g under the stated test, uptake is 50% w/w for that test.

Result

Record the observed outcome and calculated value, with units. State whether the stated laboratory specification was met; do not copy the worked example as your result.

Precautions

- Use authenticated, labelled teaching material and instructor-prepared reagents. Wear the protection specified for the chemical method.
- Compare with a known control and record actual observations. A single colour, precipitate or odour description cannot authenticate a crude drug.
- Do not taste botanical material or directly inhale vapours; dispose of solvents and staining reagents in their assigned waste containers.

Viva Questions and Answers**1. Why can castor oil behave differently in alcohol?**

Its major fatty-acid residues contain hydroxyl groups.

2. Are fixed oils and waxes identical chemically?

No. Their main ester structures and associated components differ.

3. Why control the temperature?

Solubility, consistency and melting behaviour change with temperature.

Chemical Identification of Asafoetida Catechu and Aloe

Experiment 3 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To compare oleo-gum-resin, tannin-rich material and dried aloe latex using class-supporting tests.

Principle

Asafoetida contains gum, resin and volatile components. Catechu is rich in polyphenolic tannins. Dried aloe latex contains hydroxyanthracene derivatives; it is different from the clear inner-leaf gel.

Requirements

- Authenticated asafoetida, catechu and dried aloe latex.
- Water, prescribed extracts, prepared ferric-chloride/gelatin reagents and instructor-pretreated aloe extract.

Procedure

1. Record source

Identify the exact drug part and extract used. Confirm that the aloe teaching material is dried latex, rather than a cosmetic gel product.

2. Asafoetida dispersion

Triturate the assigned portion with water and observe the milky dispersion/emulsion. Record insoluble residue and compare with authentic material.

3. Asafoetida resin study

Observe the prescribed prepared resin extract or staff-demonstrated coumarin-related fluorescence reaction. Treat this as supporting evidence; extraction and pretreatment affect the result.

4. Catechu

Apply ferric chloride to a suitable aqueous extract and gelatin to a separate portion. Record colour and precipitation alongside reagent and known-tannin controls.

5. Aloe

Use the instructor-pretreated extract for the modified Borntrager branch where the prescribed method requires oxidation of anthrone-type components. Observe the alkaline-layer pink/red response and identify the layer.

6. Interpret

List the combined evidence for each drug. A phenolic response can occur in many plant extracts and does not establish identity by itself.

Pharmacognosy and Phytochemistry**Observations and Record**

Material	Supporting pattern	Observed response
Asafoetida	Milky water dispersion; resin-related study	_____
Catechu	Tannin colour and protein-precipitation reactions	_____
Aloe dried latex	Prescribed hydroxyanthracene reaction	_____

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- Two extracts both reacting with ferric chloride are not thereby the same drug. Check source, physical behaviour and a second appropriate class test.

Result

Record the observed outcome and calculated value, with units. State whether the stated laboratory specification was met; do not copy the worked example as your result.

Precautions

- Use authenticated, labelled teaching material and instructor-prepared reagents. Wear the protection specified for the chemical method.
- Compare with a known control and record actual observations. A single colour, precipitate or odour description cannot authenticate a crude drug.
- Do not taste botanical material or directly inhale vapours; dispose of solvents and staining reagents in their assigned waste containers.
- Extract pretreatment and volatile-solvent work are performed in the assigned hood; do not substitute an untreated extract into a modified test.

Viva Questions and Answers**1. Is aloe gel the same as dried aloe latex?**

No. They come from different leaf tissues and differ in composition.

2. Why use gelatin in a tannin study?

Suitable tannins can precipitate proteins.

3. Why specify modified Borntrager pretreatment?

Some anthrone-related material needs the prescribed conversion before a useful reaction is observed.

Transverse Section and Powder Microscopy of Vinca Leaf

Experiment 4 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To examine a transverse section and powder of vinca leaf and record diagnostic features.

Principle

Authentication combines the arrangement of tissues in a section with diagnostic fragments in powder. *Catharanthus roseus* belongs to Apocynaceae. Material age, organ and processing can change the appearance; the complete pattern is more useful than one isolated cell.

Section Features

- Dorsiventral lamina with upper and lower epidermis, palisade tissue and spongy mesophyll.
- Midrib vascular region: distinguish xylem, phloem and surrounding ground tissue.
- Observe the stomatal pattern in an epidermal preparation. Anomocytic and anisocytic patterns have been reported; use the authenticated teaching specimen rather than imposing one universal pattern.

Powder Features

- Epidermal fragments with stomata.
- Palisade/mesophyll fragments and vessel elements from the veins.
- Other diagnostic inclusions actually demonstrated on the authentic slide.

Requirements

- Authenticated vinca leaf, labelled comparison slides and the prescribed microscope.
- Fresh razor used with a holder, slides, coverslips, water/glycerol mountant and instructor-prepared stains.

Procedure

1. Prepare a section

Use the instructor's safe cutting method to obtain a thin transverse section through the assigned organ. Transfer to water and select an intact section.

2. Mount

Mount in the assigned water/glycerol medium without folds or air bubbles. If staining is required, use the prepared reagent and prescribed rinse.

3. Low-power map

Observe the outline and tissue arrangement at low power. Draw the complete section with orientation and a clear title.

4. Diagnostic structures

Move to higher power to label the structures listed below. Record objective magnification; use stage calibration for any dimensions.

Pharmacognosy and Phytochemistry

5. Powder study

Mount a small amount of powder without a dense opaque layer. Scan several fields and draw at least three characteristic fragments.

6. Compare and conclude

Compare the section and powder with authenticated slides. Do not substitute a similarly shaped ornamental leaf for authenticated *C. roseus*. Record what was actually visible rather than copying every expected feature.

Observations and Record

Record	Entry
Botanical name / family / part	Catharanthus roseus / Apocynaceae / vinca leaf
Section, objective and stain	_____
Three powder fragments and magnification	_____
Diagnostic agreement / limitations	_____

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- Magnification alone does not give a fragment length. If calibration is 4 μm per eyepiece division and a fragment spans 12 divisions, its length is 48 μm .

Result

State whether the observed section and powder support the stated botanical material, naming the features seen and any unresolved difference.

Precautions

- Use authenticated, labelled teaching material and instructor-prepared reagents. Wear the protection specified for the chemical method.
- Compare with a known control and record actual observations. A single colour, precipitate or odour description cannot authenticate a crude drug.
- Do not taste botanical material or directly inhale vapours; dispose of solvents and staining reagents in their assigned waste containers.
- Use teacher-prepared staining/clearing reagents. Do not make chloral-hydrate or corrosive staining mixtures independently.

Viva Questions and Answers

1. Why study both section and powder?

The section preserves tissue arrangement, while powder reveals fragments retained after grinding.

2. Why examine several fields?

A single field may miss a diagnostic feature or overrepresent a contaminant.

3. How should an uncertain feature be recorded?

Describe it as uncertain and request comparison; do not force an identification.

Transverse Section and Powder Microscopy of Cinnamon Bark

Experiment 5 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To examine a transverse section and powder of cinnamon inner bark and record diagnostic features.

Principle

Authentication combines the arrangement of tissues in a section with diagnostic fragments in powder. *Cinnamomum verum* belongs to Lauraceae. Material age, organ and processing can change the appearance; the complete pattern is more useful than one isolated cell.

Section Features

- Phloem region with fibres, stone cells and parenchyma; medullary-ray arrangement.
- Oil/mucilage-containing cells and calcium-oxalate inclusions where visible.
- Scraping and processing remove much outer tissue, so the retained cork/cortex varies.

Powder Features

- Bast fibres and thick-walled stone cells.
- Oil/mucilage cells and parenchyma fragments.
- Calcium-oxalate crystals and starch where present in the assigned material.

Requirements

- Authenticated cinnamon inner bark, labelled comparison slides and the prescribed microscope.
- Fresh razor used with a holder, slides, coverslips, water/glycerol mountant and instructor-prepared stains.

Procedure

1. Prepare a section

Use the instructor's safe cutting method to obtain a thin transverse section through the assigned organ. Transfer to water and select an intact section.

2. Mount

Mount in the assigned water/glycerol medium without folds or air bubbles. If staining is required, use the prepared reagent and prescribed rinse.

3. Low-power map

Observe the outline and tissue arrangement at low power. Draw the complete section with orientation and a clear title.

4. Diagnostic structures

Move to higher power to label the structures listed below. Record objective magnification; use stage calibration for any dimensions.

5. Powder study

Mount a small amount of powder without a dense opaque layer. Scan several fields and draw at least three characteristic fragments.

Pharmacognosy and Phytochemistry

6. Compare and conclude

Compare the section and powder with authenticated slides. Cassia and true cinnamon are different materials; state the supplied species and processing. Record what was actually visible rather than copying every expected feature.

Observations and Record

Record	Entry
Botanical name / family / part	Cinnamomum verum / Lauraceae / cinnamon inner bark
Section, objective and stain	_____
Three powder fragments and magnification	_____
Diagnostic agreement / limitations	_____

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- Magnification alone does not give a fragment length. If calibration is 4 μm per eyepiece division and a fragment spans 12 divisions, its length is 48 μm .

Result

State whether the observed section and powder support the stated botanical material, naming the features seen and any unresolved difference.

Precautions

- Use authenticated, labelled teaching material and instructor-prepared reagents. Wear the protection specified for the chemical method.
- Compare with a known control and record actual observations. A single colour, precipitate or odour description cannot authenticate a crude drug.
- Do not taste botanical material or directly inhale vapours; dispose of solvents and staining reagents in their assigned waste containers.
- Use teacher-prepared staining/clearing reagents. Do not make chloral-hydrate or corrosive staining mixtures independently.

Viva Questions and Answers

1. Why study both section and powder?

The section preserves tissue arrangement, while powder reveals fragments retained after grinding.

2. Why examine several fields?

A single field may miss a diagnostic feature or overrepresent a contaminant.

3. How should an uncertain feature be recorded?

Describe it as uncertain and request comparison; do not force an identification.

Transverse Section and Powder Microscopy of Fennel Fruit

Experiment 6 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To examine a transverse section and powder of fennel fruit mericarp and record diagnostic features.

Principle

Authentication combines the arrangement of tissues in a section with diagnostic fragments in powder. *Foeniculum vulgare* belongs to Apiaceae. Material age, organ and processing can change the appearance; the complete pattern is more useful than one isolated cell.

Section Features

- Five primary ridges in a mericarp, with vascular tissue associated with the ridges.
- Secretory canals (vittae): commonly four dorsal and two commissural in the teaching transverse section.
- Inner seed/endosperm tissue with storage contents.

Powder Features

- Fragments of vittae and fruit-wall tissues.
- Endosperm cells with oil/protein storage contents.
- Vascular elements and other fragments matching the section.

Requirements

- Authenticated fennel fruit mericarp, labelled comparison slides and the prescribed microscope.
- Fresh razor used with a holder, slides, coverslips, water/glycerol mountant and instructor-prepared stains.

Procedure

1. Prepare a section

Use the instructor's safe cutting method to obtain a thin transverse section through the assigned organ. Transfer to water and select an intact section.

2. Mount

Mount in the assigned water/glycerol medium without folds or air bubbles. If staining is required, use the prepared reagent and prescribed rinse.

3. Low-power map

Observe the outline and tissue arrangement at low power. Draw the complete section with orientation and a clear title.

4. Diagnostic structures

Move to higher power to label the structures listed below. Record objective magnification; use stage calibration for any dimensions.

5. Powder study

Mount a small amount of powder without a dense opaque layer. Scan several fields and draw at least three characteristic fragments.

Pharmacognosy and Phytochemistry

6. Compare and conclude

Compare the section and powder with authenticated slides. Orient the commissural surface before counting vittae; an oblique or incomplete section can mislead. Record what was actually visible rather than copying every expected feature.

Observations and Record

Record	Entry
Botanical name / family / part	Foeniculum vulgare / Apiaceae / fennel fruit mericarp
Section, objective and stain	_____
Three powder fragments and magnification	_____
Diagnostic agreement / limitations	_____

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- Magnification alone does not give a fragment length. If calibration is 4 μm per eyepiece division and a fragment spans 12 divisions, its length is 48 μm .

Result

State whether the observed section and powder support the stated botanical material, naming the features seen and any unresolved difference.

Precautions

- Use authenticated, labelled teaching material and instructor-prepared reagents. Wear the protection specified for the chemical method.
- Compare with a known control and record actual observations. A single colour, precipitate or odour description cannot authenticate a crude drug.
- Do not taste botanical material or directly inhale vapours; dispose of solvents and staining reagents in their assigned waste containers.
- Use teacher-prepared staining/clearing reagents. Do not make chloral-hydrate or corrosive staining mixtures independently.

Viva Questions and Answers

1. Why study both section and powder?

The section preserves tissue arrangement, while powder reveals fragments retained after grinding.

2. Why examine several fields?

A single field may miss a diagnostic feature or overrepresent a contaminant.

3. How should an uncertain feature be recorded?

Describe it as uncertain and request comparison; do not force an identification.

Transverse Section and Powder Microscopy of Clove Flower Bud

Experiment 7 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To examine a transverse section and powder of unopened clove flower bud and record diagnostic features.

Principle

Authentication combines the arrangement of tissues in a section with diagnostic fragments in powder. *Syzygium aromaticum* belongs to Myrtaceae. Material age, organ and processing can change the appearance; the complete pattern is more useful than one isolated cell.

Section Features

- Hypanthium with epidermis, prominent oil cavities and vascular tissues.
- Associated floral structures in the appropriate part of the bud; section level changes the appearance.
- Sclereids and calcium-oxalate clusters where visible.

Powder Features

- Oil-cavity tissue fragments and sclereids.
- Pollen grains from the flower bud.
- Calcium-oxalate clusters and vascular fragments.

Requirements

- Authenticated unopened clove flower bud, labelled comparison slides and the prescribed microscope.
- Fresh razor used with a holder, slides, coverslips, water/glycerol mountant and instructor-prepared stains.

Procedure

1. Prepare a section

Use the instructor's safe cutting method to obtain a thin transverse section through the assigned organ. Transfer to water and select an intact section.

2. Mount

Mount in the assigned water/glycerol medium without folds or air bubbles. If staining is required, use the prepared reagent and prescribed rinse.

3. Low-power map

Observe the outline and tissue arrangement at low power. Draw the complete section with orientation and a clear title.

4. Diagnostic structures

Move to higher power to label the structures listed below. Record objective magnification; use stage calibration for any dimensions.

5. Powder study

Mount a small amount of powder without a dense opaque layer. Scan several fields and draw at least three characteristic fragments.

Pharmacognosy and Phytochemistry

6. Compare and conclude

Compare the section and powder with authenticated slides. A stalk-only or exhausted material lacks the complete authentic bud pattern. Record what was actually visible rather than copying every expected feature.

Observations and Record

Record	Entry
Botanical name / family / part	Syzygium aromaticum / Myrtaceae / unopened clove flower bud
Section, objective and stain	_____
Three powder fragments and magnification	_____
Diagnostic agreement / limitations	_____

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- Magnification alone does not give a fragment length. If calibration is 4 μm per eyepiece division and a fragment spans 12 divisions, its length is 48 μm .

Result

State whether the observed section and powder support the stated botanical material, naming the features seen and any unresolved difference.

Precautions

- Use authenticated, labelled teaching material and instructor-prepared reagents. Wear the protection specified for the chemical method.
- Compare with a known control and record actual observations. A single colour, precipitate or odour description cannot authenticate a crude drug.
- Do not taste botanical material or directly inhale vapours; dispose of solvents and staining reagents in their assigned waste containers.
- Use teacher-prepared staining/clearing reagents. Do not make chloral-hydrate or corrosive staining mixtures independently.

Viva Questions and Answers

1. Why study both section and powder?

The section preserves tissue arrangement, while powder reveals fragments retained after grinding.

2. Why examine several fields?

A single field may miss a diagnostic feature or overrepresent a contaminant.

3. How should an uncertain feature be recorded?

Describe it as uncertain and request comparison; do not force an identification.

Transverse Section and Powder Microscopy of Tulsi Stem

Experiment 8 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To examine a transverse section and powder of tulsi stem and record diagnostic features.

Principle

Authentication combines the arrangement of tissues in a section with diagnostic fragments in powder. *Ocimum tenuiflorum* (syn. *O. sanctum*) belongs to Lamiaceae. Material age, organ and processing can change the appearance; the complete pattern is more useful than one isolated cell.

Section Features

- Young stem has a quadrangular outline with supporting collenchyma prominent toward the corners.
- Epidermis with hairs, cortex and collateral vascular regions: phloem outward, xylem inward.
- Central pith; secondary growth changes the appearance of older stems.

Powder Features

- Covering/glandular hair fragments where retained.
- Fibres, vessel fragments and cortical/pith parenchyma.
- Epidermal fragments matching the authentic stem material.

Requirements

- Authenticated tulsi stem, labelled comparison slides and the prescribed microscope.
- Fresh razor used with a holder, slides, coverslips, water/glycerol mountant and instructor-prepared stains.

Procedure

1. Prepare a section

Use the instructor's safe cutting method to obtain a thin transverse section through the assigned organ. Transfer to water and select an intact section.

2. Mount

Mount in the assigned water/glycerol medium without folds or air bubbles. If staining is required, use the prepared reagent and prescribed rinse.

3. Low-power map

Observe the outline and tissue arrangement at low power. Draw the complete section with orientation and a clear title.

4. Diagnostic structures

Move to higher power to label the structures listed below. Record objective magnification; use stage calibration for any dimensions.

5. Powder study

Mount a small amount of powder without a dense opaque layer. Scan several fields and draw at least three characteristic fragments.

Pharmacognosy and Phytochemistry

6. Compare and conclude

Compare the section and powder with authenticated slides. Record stem age; a woody older stem need not look identical to a young four-angled section. Record what was actually visible rather than copying every expected feature.

Observations and Record

Record	Entry
Botanical name / family / part	Ocimum tenuiflorum (syn. O. sanctum) / Lamiaceae / tulsi stem
Section, objective and stain	_____
Three powder fragments and magnification	_____
Diagnostic agreement / limitations	_____

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- Magnification alone does not give a fragment length. If calibration is 4 μm per eyepiece division and a fragment spans 12 divisions, its length is 48 μm .

Result

State whether the observed section and powder support the stated botanical material, naming the features seen and any unresolved difference.

Precautions

- Use authenticated, labelled teaching material and instructor-prepared reagents. Wear the protection specified for the chemical method.
- Compare with a known control and record actual observations. A single colour, precipitate or odour description cannot authenticate a crude drug.
- Do not taste botanical material or directly inhale vapours; dispose of solvents and staining reagents in their assigned waste containers.
- Use teacher-prepared staining/clearing reagents. Do not make chloral-hydrate or corrosive staining mixtures independently.

Viva Questions and Answers

1. Why study both section and powder?

The section preserves tissue arrangement, while powder reveals fragments retained after grinding.

2. Why examine several fields?

A single field may miss a diagnostic feature or overrepresent a contaminant.

3. How should an uncertain feature be recorded?

Describe it as uncertain and request comparison; do not force an identification.

Transverse Section and Powder Microscopy of Liquorice Root

Experiment 9 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To examine a transverse section and powder of liquorice root and record diagnostic features.

Principle

Authentication combines the arrangement of tissues in a section with diagnostic fragments in powder. *Glycyrrhiza glabra* belongs to Fabaceae. Material age, organ and processing can change the appearance; the complete pattern is more useful than one isolated cell.

Section Features

- Cork in unpeeled material; broad secondary phloem and xylem regions with radial rays.
- Phloem/xylem fibres, pitted vessels, starch-bearing parenchyma and prismatic crystals near fibre bundles.
- True roots lack a distinct central pith; rhizome/stolon material may have a pith.

Powder Features

- Pitted/reticulate vessel fragments and fibres with associated crystal-bearing cells.
- Starch grains and parenchyma.
- Cork fragments when the original material is unpeeled.

Requirements

- Authenticated liquorice root, labelled comparison slides and the prescribed microscope.
- Fresh razor used with a holder, slides, coverslips, water/glycerol mountant and instructor-prepared stains.

Procedure

1. Prepare a section

Use the instructor's safe cutting method to obtain a thin transverse section through the assigned organ. Transfer to water and select an intact section.

2. Mount

Mount in the assigned water/glycerol medium without folds or air bubbles. If staining is required, use the prepared reagent and prescribed rinse.

3. Low-power map

Observe the outline and tissue arrangement at low power. Draw the complete section with orientation and a clear title.

4. Diagnostic structures

Move to higher power to label the structures listed below. Record objective magnification; use stage calibration for any dimensions.

Pharmacognosy and Phytochemistry

5. Powder study

Mount a small amount of powder without a dense opaque layer. Scan several fields and draw at least three characteristic fragments.

6. Compare and conclude

Compare the section and powder with authenticated slides. Distinguish a root from rhizome/stolon material before interpreting the presence of a pith. Record what was actually visible rather than copying every expected feature.

Observations and Record

Record	Entry
Botanical name / family / part	Glycyrrhiza glabra / Fabaceae / liquorice root
Section, objective and stain	_____
Three powder fragments and magnification	_____
Diagnostic agreement / limitations	_____

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- Magnification alone does not give a fragment length. If calibration is 4 μm per eyepiece division and a fragment spans 12 divisions, its length is 48 μm .

Result

State whether the observed section and powder support the stated botanical material, naming the features seen and any unresolved difference.

Precautions

- Use authenticated, labelled teaching material and instructor-prepared reagents. Wear the protection specified for the chemical method.
- Compare with a known control and record actual observations. A single colour, precipitate or odour description cannot authenticate a crude drug.
- Do not taste botanical material or directly inhale vapours; dispose of solvents and staining reagents in their assigned waste containers.
- Use teacher-prepared staining/clearing reagents. Do not make chloral-hydrate or corrosive staining mixtures independently.

Viva Questions and Answers

1. Why study both section and powder?

The section preserves tissue arrangement, while powder reveals fragments retained after grinding.

2. Why examine several fields?

A single field may miss a diagnostic feature or overrepresent a contaminant.

3. How should an uncertain feature be recorded?

Describe it as uncertain and request comparison; do not force an identification.

Effect of Maceration Decoction and Soxhlet Extraction on Crude Yield

Experiment 10 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To compare dry crude-extract yields using three extraction approaches.

Principle

Diffusion, solvent renewal, temperature and contact time affect extraction. A fair comparison controls raw-material batch, dry mass, particle size and solvent. The measured yield is total extracted residue, not the concentration of a particular active constituent.

Requirements

- Three equal portions of one authenticated stable teaching drug; water or the common solvent selected by the instructor.
- Maceration vessel, decoction setup, Soxhlet assembly, filtration, evaporation and dry-residue weighing apparatus.

Procedure

1. Design

For a water-compatible stable drug, an illustrative design uses 5.0 g dry material and 50 mL water in each arm. If the Soxhlet arm uses a different solvent, explicitly report that solvent and method are both changing.

2. Maceration

Keep the first portion in a closed labelled vessel for the assigned period, for example 24 h, with the prescribed mixing. Filter and account for retained solvent.

3. Decoction

Heat the second aqueous portion gently for the assigned period, for example 15 min. Cool, filter and restore the extract to a measured volume where the method requires it.

4. Soxhlet

Staff check the condenser, thimble and vapour path. Run the third portion under the approved heating method; record solvent, duration and siphon cycles. Do not seal the system.

5. Dry residue

Take a known extract aliquot into a weighed dish. Evaporate under the assigned method and dry to the specified stable mass condition.

6. Compare

Scale aliquot residue to total measured extract volume and calculate dry-basis yield. Compare replicates, thermal exposure and possible degradation; the largest residue yield need not be the best selective extraction.

Pharmacognosy and Phytochemistry**Observations and Record**

Method	Solvent / time / cycles	Dry residue / yield
Maceration	_____	_____
Decoction	_____	_____
Soxhlet extraction	_____	_____

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- Total residue = aliquot residue $\times$ total extract volume / aliquot volume.
- A 5 mL aliquot from 50 mL gives 0.060 g dry residue: total residue = 0.600 g.
- For 5.0 g dry starting material, yield = $0.600 / 5.0 \times 100 = 12.0\%$.

Result

Record the observed outcome and calculated value, with units. State whether the stated laboratory specification was met; do not copy the worked example as your result.

Precautions

- Use authenticated, labelled teaching material and instructor-prepared reagents. Wear the protection specified for the chemical method.
- Compare with a known control and record actual observations. A single colour, precipitate or odour description cannot authenticate a crude drug.
- Do not taste botanical material or directly inhale vapours; dispose of solvents and staining reagents in their assigned waste containers.
- Check cooling-water flow before heating; use suitable electrical heating for flammable solvents.

Viva Questions and Answers**1. Does higher yield mean more active compound?**

No. Sugars, salts and other extractives can increase crude mass.

2. Why use dry-basis starting mass?

Different moisture content otherwise distorts the comparison.

3. What does Soxhlet renewal change?

Freshly condensed solvent repeatedly contacts the material.

Isolation of Volatile Oil and Thin-Layer Chromatography

Experiment 11 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To isolate an essential oil by hydrodistillation and compare its TLC pattern with an authentic marker.

Principle

Steam carries suitable volatile components from the hydrated drug, which condense and separate in the collection apparatus. TLC separates components by their relative affinity for the stationary phase and the chosen mobile phase.

Requirements

- Authenticated fennel or clove, water, Clevenger-type apparatus and labelled reference oil/marker.
- TLC plate, chamber, capillary spotters, assigned solvent system and authorised visualisation setup.

Procedure

1. Set up

Record dry drug mass and assemble the staff-checked hydrodistillation apparatus. Add the prescribed water amount so the material remains adequately wetted.

2. Distil

Heat at the specified rate while maintaining condenser cooling and an open vapour path. Continue to the assigned endpoint and monitor water/oil collection.

3. Measure oil

Cool the collector, allow separation and read oil volume at the stated temperature. Record emulsions or water carryover instead of counting them as pure oil.

4. Prepare TLC

Dilute a portion in the assigned solvent. Spot the oil, authentic marker and solvent blank on one baseline, keeping spots small.

5. Develop

Use the validated teaching solvent system and an equilibrated chamber. Keep solvent below the baseline; remove the plate and immediately mark the solvent front.

6. Visualise and compare

Use the approved UV or stain procedure. Measure band and front travel from the baseline; compare the whole pattern with the authentic marker. Matching R_f alone does not prove identity or purity.

Pharmacognosy and Phytochemistry**Observations and Record**

Record	Entry
Drug mass / oil volume / temperature	_____
Plate / mobile phase / visualisation	_____
Band travel / front travel / R _f	_____
Marker agreement / other bands	_____

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- Oil yield (% v/w) = oil mL / dry drug g × 100. A 1.0 mL yield from 25.0 g is 4.0% v/w.
- R_f = band-centre travel / solvent-front travel. A 3.2 cm band and 8.0 cm front give R_f = 0.40.

Result

Record the observed outcome and calculated value, with units. State whether the stated laboratory specification was met; do not copy the worked example as your result.

Precautions

- Use authenticated, labelled teaching material and instructor-prepared reagents. Wear the protection specified for the chemical method.
- Compare with a known control and record actual observations. A single colour, precipitate or odour description cannot authenticate a crude drug.
- Do not taste botanical material or directly inhale vapours; dispose of solvents and staining reagents in their assigned waste containers.
- Do not heat an oil/solvent mixture with an open flame or view UV lamps without the required shield.

Viva Questions and Answers**1. Why mark the front immediately?**

The solvent front can disappear as the plate dries.

2. Is R_f a universal compound constant?

No. It depends on the plate, solvent system and development conditions.

3. Does collected oil volume establish purity?

No. Water and other components can affect the reading.

Isolation of Caffeine from Tea

Experiment 12 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To obtain a caffeine-enriched fraction from tea and assess recovery and identity.

Principle

Caffeine can be extracted from an aqueous tea preparation into a suitable organic solvent. Mild alkalinity can reduce transfer of acidic polyphenols. Isolation mass is crude recovery until identity and purity have been assessed.

Requirements

- Tea, water, instructor-assigned carbonate and ethyl acetate extraction system.
- Controlled heater, filter, separatory funnel, drying agent, evaporation setup and identity-check materials.

Procedure

1. Extract tea

An illustrative microscale teaching setup uses 10 g tea and 100 mL hot water with the assigned carbonate quantity. Use the college's specified extraction time and filter away the leaves.

2. Cool

Cool the filtrate before liquid-liquid extraction. Transfer to a correctly supported separatory funnel with free headspace.

3. Partition

Extract with three small fresh ethyl-acetate portions, such as 10 mL each under the approved method. Mix gently and vent safely in the hood. Confirm phase identity; ethyl acetate is normally the upper layer.

4. Dry

Combine the appropriate organic fractions. Use the prescribed anhydrous drying agent, then separate it without transferring solids.

5. Recover

Evaporate through the authorised controlled setup. Weigh the dry recovered material; no open flame is used.

6. Confirm

Compare the TLC/other assigned identity test with authentic caffeine. If the class performs further purification, record its mass loss and separate purity assessment.

Pharmacognosy and Phytochemistry

Observations and Record

Record	Entry
Tea mass / water / carbonate / extraction time	_____
Organic solvent and extraction portions	_____
Dry recovered mass / identity result	_____

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- Crude recovery (% of tea mass) = recovered dry mass / tea mass $\times$ 100.
- From 10.0 g tea, 0.080 g dry isolate gives 0.80% recovery. This is not a measured purity or a percentage of the true caffeine content recovered.

Result

Record the observed outcome and calculated value, with units. State whether the stated laboratory specification was met; do not copy the worked example as your result.

Precautions

- Use authenticated, labelled teaching material and instructor-prepared reagents. Wear the protection specified for the chemical method.
- Compare with a known control and record actual observations. A single colour, precipitate or odour description cannot authenticate a crude drug.
- Do not taste botanical material or directly inhale vapours; dispose of solvents and staining reagents in their assigned waste containers.
- Cool before funnel extraction, vent away from people and verify the layer before discarding any fraction.

Viva Questions and Answers**1. Why use several fresh solvent portions?**

Repeated partitioning usually improves transfer compared with one equal total-volume portion.

2. Why dry the organic fraction?

Dissolved water can remain in the extract and distort mass or crystallisation.

3. Why call the first product caffeine-enriched?

Other soluble compounds may still be present.

Isolation of Hesperidin from Lemon Peel

Experiment 13 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To prepare a hesperidin-enriched fraction from authenticated lemon peel and assess its identity.

Principle

Hesperidin is a citrus flavanone glycoside. Solvent extraction followed by controlled precipitation/crystallisation can enrich it, but citrus species and extraction conditions affect the composition. A crude lemon-peel precipitate is not automatically pure hesperidin.

Requirements

- Clean dried lemon peel, an authentic hesperidin marker and the college's validated citrus-peel isolation method.
- Assigned defatting and extraction solvents, controlled heating, filtration, evaporation and TLC/identity apparatus.

Procedure

1. Prepare peel

Record botanical source, peel portion, dry mass and particle size. Select the laboratory method specifically approved for lemon material.

2. Optional defatting

If the method prescribes it, use the designated defatting step to reduce peel oils, then remove residual solvent under controlled conditions.

3. Extract

Use the assigned aqueous-alcohol or other validated extraction conditions, recording solvent composition, ratio, temperature and time. Do not substitute an orange-peel method without instructor validation.

4. Concentrate

Filter and concentrate without boiling to dryness. Record the resulting volume and any insoluble material.

5. Precipitate

Carry out the prescribed acidification/crystallisation stage with the method's controlled pH or acetic-acid system. Cool for the assigned period, collect and wash the solid.

6. Assess

Dry to the specified condition and weigh. Compare with authentic hesperidin using the assigned TLC and confirmatory test; report a crude enriched fraction when purity is not established.

Pharmacognosy and Phytochemistry

Observations and Record

Record	Entry
Dry lemon-peel mass / extraction conditions	_____
Precipitation pH or solvent / cooling time	_____
Dry fraction mass / recovery	_____
Marker comparison / purity limitation	_____

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- A 0.120 g dry fraction from 5.00 g peel is 2.40% crude mass recovery. That value includes any co-precipitated substances.

Result

Record the observed outcome and calculated value, with units. State whether the stated laboratory specification was met; do not copy the worked example as your result.

Precautions

- Use authenticated, labelled teaching material and instructor-prepared reagents. Wear the protection specified for the chemical method.
- Compare with a known control and record actual observations. A single colour, precipitate or odour description cannot authenticate a crude drug.
- Do not taste botanical material or directly inhale vapours; dispose of solvents and staining reagents in their assigned waste containers.

Viva Questions and Answers**1. Why specify lemon rather than generic citrus peel?**

Species and peel composition alter extraction and product identity.

2. What does a co-migrating TLC band establish?

It supports identity under that system but does not alone establish purity.

3. Why record precipitation conditions?

Solubility and co-precipitation depend on pH, solvent and temperature.

Isolation of Glycyrrhizin from Liquorice Roots

Experiment 14 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To obtain a glycyrrhizin-enriched fraction from liquorice and distinguish it from its aglycone.

Principle

Glycyrrhizin is a triterpenoid saponin glycoside. Aqueous alcohol extracts it along with other soluble constituents, and controlled acid precipitation can enrich the fraction. Glycyrrhetic acid is the aglycone and must not be reported as the same compound.

Requirements

- Authenticated dried liquorice root, authentic glycyrrhizin marker and the approved aqueous-alcohol/acid precipitation method.
- Ultrasonic or other assigned extraction setup, filters, controlled evaporator, instructor-supplied acid and identity-test apparatus.

Procedure

1. Prepare

Record dry root mass and grinding conditions. The teaching method must specify solvent composition and extraction ratio.

2. Extract

An illustrative literature-based extraction uses 70% aqueous methanol with brief repeated sonication. A 5 g portion can be studied with approximately 87 mL first extraction and two 65 mL repeat extractions, subject to the college's microscale method.

3. Pool

Filter each extract, combine and concentrate under the authorised reduced-temperature method. Record total recovered liquid rather than assuming no solvent loss.

4. Precipitate

Staff supervise addition of the assigned acid to the specified endpoint. Collect the resulting precipitate after the approved standing/cooling period; avoid prolonged strong-acid heating that changes the glycoside.

5. Wash and dry

Wash by the validated method and dry under controlled conditions. Record dry mass as crude enriched recovery.

6. Identify

Compare the fraction with glycyrrhizin and, where available, glycyrrhetic-acid markers. Report the identity evidence separately from crude mass; further purification may be required.

Pharmacognosy and Phytochemistry**Observations and Record**

Record	Entry
Root mass / solvent / extraction repetitions	_____
Concentration and precipitation endpoint	_____
Dry fraction mass / glycyrrhizin comparison	_____

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- From 5.00 g root, 0.250 g dry enriched fraction gives 5.00% crude recovery. It does not imply that the fraction is 100% glycyrrhizin.

Result

Record the observed outcome and calculated value, with units. State whether the stated laboratory specification was met; do not copy the worked example as your result.

Precautions

- Use authenticated, labelled teaching material and instructor-prepared reagents. Wear the protection specified for the chemical method.
- Compare with a known control and record actual observations. A single colour, precipitate or odour description cannot authenticate a crude drug.
- Do not taste botanical material or directly inhale vapours; dispose of solvents and staining reagents in their assigned waste containers.
- Methanol extraction and acid handling use the designated hood and trained supervision; no tasting of the root or isolate is permitted.

Viva Questions and Answers**1. What is the difference between glycyrrhizin and glycyrrhetic acid?**

The former is a glycoside; the latter is its aglycone.

2. Why repeat extraction?

Fresh solvent can remove material left after the first extraction.

3. Why avoid prolonged strong-acid heating?

It can hydrolyse or otherwise change the intended glycoside.

Isolation of Pectin from Orange Peel

Experiment 15 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To recover a crude pectin-rich fraction from orange peel and calculate dry-basis recovery.

Principle

Acidified hot water releases pectic material from the plant cell wall. Alcohol lowers its solubility and promotes precipitation. Other polysaccharides or soluble substances may accompany the recovered material.

Requirements

- Clean dried orange peel, water, prepared citric-acid system and the assigned ethanol grades.
- Controlled water bath, filter, cooling facility, collection setup, drying oven and balance.

Procedure

1. Prepare

Weigh 5.00 g dried powdered peel for an illustrative scaled teaching method. Add 30 mL water and adjust to approximately pH 2 with the assigned citric-acid system.

2. Extract

Heat the stirred dispersion at approximately 90°C for 2 h in the supervised setup. Limit evaporation and record actual temperature, pH and liquid loss.

3. Clarify

Filter the hot extract using the approved apparatus, then cool before alcohol addition.

4. Precipitate

Add an equal measured volume of cold ethanol to the clarified extract as prescribed. Keep at approximately 4°C overnight in a suitable labelled container.

5. Wash

Collect the precipitate. Where this method is used, wash successively with the assigned 75%, 85% and 95% ethanol systems.

6. Dry and assess

Dry at the prescribed condition, for example 40°C overnight followed by the laboratory stable-mass check. Weigh and record the pectin-rich fraction; a gel test alone is not a full purity or esterification assay.

Pharmacognosy and Phytochemistry**Observations and Record**

Record	Entry
Peel dry mass / water / pH	_____
Extraction temperature / time / filtrate volume	_____
Alcohol volume / collection / drying	_____
Dry fraction mass / yield	_____

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- Dry recovery (%) = recovered dry fraction / dry peel mass × 100.
- A 0.800 g fraction from 5.00 g dry peel gives 16.0% crude recovery.

Result

Record the observed outcome and calculated value, with units. State whether the stated laboratory specification was met; do not copy the worked example as your result.

Precautions

- Use authenticated, labelled teaching material and instructor-prepared reagents. Wear the protection specified for the chemical method.
- Compare with a known control and record actual observations. A single colour, precipitate or odour description cannot authenticate a crude drug.
- Do not taste botanical material or directly inhale vapours; dispose of solvents and staining reagents in their assigned waste containers.
- Cool the extract before adding ethanol and keep alcoholic liquids away from flames; use an authorised drying/evaporation arrangement.

Viva Questions and Answers**1. Why acidify the extraction water?**

Controlled acidity helps release soluble pectic material.

2. Why is ethanol added?

It reduces solubility and promotes precipitation of the pectin-rich fraction.

3. Does recovered mass measure degree of esterification?

No. That needs a separate analytical determination.

Evaluation of Field and Market Herbal Materials

Experiment 16 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To compare authenticated and market/field material against the applicable pharmacopoeial monograph.

Principle

Herbal quality requires correct botanical identity, suitable plant part and compliance with defined analytical limits. Similar appearance or a traditional trade name does not alone establish identity or quality.

Requirements

- Permission-collected field material or purchased labelled material, authenticated comparison material and the current monograph supplied by the college.
- Record sheets, microscopy equipment and the apparatus/reagents for the selected official quality tests.

Procedure

1. Document origin

Record supplier/location, date, batch, trade name, stated species and plant part. Collect only with permission; maintain a voucher or authenticated record.

2. Check identity

Compare macroscopic and microscopic features and the specified chromatographic identity test. Record substitution, wrong organ and foreign material separately.

3. Apply tests

Follow the monograph's exact methods for applicable foreign matter, loss on drying, ash and extractive values. Record edition, method and basis of calculation in the laboratory record.

4. Read limits

Enter each actual monograph limit with its unit and dry/as-is basis. Do not use a generic limit borrowed from another drug.

5. Compare batches

Prepare a result-versus-limit table for each field/market lot and authenticated comparison. Consider storage, moisture, exhausted material and contamination.

6. Conclude

State supported identity, tests meeting limits, tests failing limits and tests not performed. Untested contaminant limits cannot be reported as compliant.

Pharmacognosy and Phytochemistry

Observations and Record

Test / method	Observed value	Actual monograph limit / decision
Identity / botanical part	_____	_____
Foreign matter / loss on drying	_____	_____
Ash / extractive value	_____	_____
Other required checks not performed	_____	Not assessed

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- Teaching example only: if an assigned maximum is 10.0% and a result is 12.0% on the same basis, that test fails the limit. These numbers are not a real limit for any named herb.

Result

Summarise identity and each tested quality requirement without claiming compliance for unperformed tests.

Precautions

- Use authenticated, labelled teaching material and instructor-prepared reagents. Wear the protection specified for the chemical method.
- Compare with a known control and record actual observations. A single colour, precipitate or odour description cannot authenticate a crude drug.
- Do not taste botanical material or directly inhale vapours; dispose of solvents and staining reagents in their assigned waste containers.

Viva Questions and Answers**1. Why keep a voucher record?**

It connects later tests to an authenticated botanical source.

2. Why must the calculation basis match?

Dry-basis and as-is percentages are not directly interchangeable.

3. Can an identity pass prove all quality requirements?

No. Purity, contaminants and other required tests remain separate.

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