

PHARMACOGNOSY AND PHYTOCHEMISTRY (PRACTICAL)

A Laboratory Manual for B.Pharm Semester II

BP210P

As per the new PCI Syllabus 2026 (NEP 2020)

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Preface

This manual accompanies the practical course in Pharmacognosy and Phytochemistry (BP210P) prescribed for the second semester of the Bachelor of Pharmacy programme. Its purpose is to take the student through every experiment the syllabus lists, not as a set of steps to be copied, but as a piece of reasoning to be understood. Each experiment is therefore presented as a principle, a procedure, an observation, and an inference, so that the bench work and the theory behind it are learned together.

The crude drugs and constituents treated here are the ones the syllabus names, and the methods are the standard methods of the *Indian Pharmacopoeia* and the prescribed reference texts. Where a precise pharmacopoeial value depends on the current edition of a monograph, the text states the standard range and marks the figure for confirmation against the compendium in use at the institution, since exact limits are revised from edition to edition and must not be quoted from memory. The student is encouraged to record every observation as it is seen and to read it against the official standard, which is the discipline the whole subject builds toward.

The manual serves three readers from the same pages: the student preparing for the laboratory session, the student revising for the viva, and the student preparing for the written examination. The self-assessment at the end of each chapter mirrors the university question-paper pattern of short notes, long-answer questions, and objective questions, with model answers that show what earns the marks.

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Syllabus

Course objectives

The objectives of this course are to enable the student to:

1. Perform chemical tests for the identification of gums, resins, oils, fats, waxes, and unorganized drugs.
2. Examine crude drugs using transverse section and powder microscopy for correct identification.
3. Apply suitable extraction techniques and study their effect on the yield of crude extracts.
4. Isolate and identify important phytoconstituents and volatile oils using standard procedures.
5. Evaluate herbal raw materials collected from field and market sources as per pharmacopoeial standards.

Course outcomes

Upon successful completion of this course, the student will be able to:

1. CO1: Identify unorganized crude drugs using specific chemical tests.
2. CO2: Analyse organized crude drugs through microscopy and powder analysis.
3. CO3: Select and perform appropriate extraction methods for herbal drugs.
4. CO4: Isolate and characterise phytoconstituents using laboratory techniques and thin-layer chromatography.
5. CO5: Assess and compare the quality and purity of herbal raw materials with official standards.

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List of practicals

A minimum of twelve experiments must be performed; this manual covers all sixteen so that any selection is supported.

1. Chemical tests for identification of agar, acacia, tragacanth, and honey.
2. Chemical tests for identification of castor oil, olive oil, wool fat, and beeswax.
3. Chemical tests for identification of asafoetida, catechu, and aloe.
4. Transverse section and powder microscopy of a vinca leaf.
5. Transverse section and powder microscopy of a cinnamon bark.
6. Transverse section and powder microscopy of a fennel fruit.
7. Transverse section and powder microscopy of a clove flower bud.
8. Transverse section and powder microscopy of a tulsi stem.
9. Transverse section and powder microscopy of a liquorice root.
10. Study of the effect of different extraction techniques on the yield of crude extract (maceration, decoction, soxhletation).
11. Isolation of volatile oil and its thin-layer chromatography.
12. Isolation of caffeine from tea.
13. Isolation of hesperidin from lemon peel.
14. Isolation of glycyrrhizin from liquorice roots.
15. Isolation of pectin from orange peel.
16. Experiential learning experiment: evaluation and comparison of field- or market-collected herbal raw materials with pharmacopoeial standards.

Chapter 1. Chemical Identification of Unorganized Crude Drugs

Learning outcomes

After this chapter the student will be able to:

1. Perform the standard chemical identity tests for agar, acacia, tragacanth, and honey, and use them to tell the three gums apart.
2. Carry out the identity tests for the fixed oils, the wax, and the wool fat of the second experiment, and interpret the analytical constants that describe them.
3. Identify asafoetida, catechu, and aloe by their characteristic chemical reactions, naming the constituent each test detects.
4. Record observations in the pharmacopoeial form and read each result against the identity expected of a genuine drug.

1.1 Why unorganized drugs are identified chemically

An unorganized drug, has no cellular structure, so the microscope has nothing to read. The gum exuded from an acacia tree, the oil pressed from a castor seed, the wax secreted by a bee, and the dried juice of an aloe leaf are all products in which the original cells have been left behind. Identity therefore rests on how the material behaves: its solubility, the colours it gives with defined reagents, and, for the oils and waxes, the analytical constants that fix the kinds and amounts of fatty acids present. The three experiments of this chapter group the drugs by chemical nature: first the carbohydrate gums and a sugar product, then the lipids, and finally a set of resinous and glycosidic drugs. Each test in the chapter targets a named constituent, and the student should state that target before performing the test.

A practical caution applies to the whole chapter. Many of these tests are comparative: a single drug seen alone may give an ambiguous colour, but the same test run side by side on two drugs separates them cleanly. Where a test is diagnostic only by comparison, the text says so, and the comparison tables collect the distinctions that matter for the viva and the written paper.

Experiment 1. Chemical tests for the identification of agar, acacia, tragacanth, and honey

Aim. To identify agar, acacia, tragacanth, and honey by chemical tests, and to distinguish the three gums from one another.

Requirements. Test tubes and rack, water bath, microscope and slides, measuring cylinders and droppers; the four samples; the reagents ruthenium red, dilute and basic lead acetate solution, benzidine in alcohol, hydrogen peroxide, Molisch reagent (alpha-naphthol in alcohol) with concentrated sulphuric acid, N/50 iodine solution, ferric chloride solution, resorcinol in concentrated hydrochloric acid for honey, and Fehling's solution.

Principle

Agar, acacia, and tragacanth are polysaccharide drugs, and the tests separate them by three properties: their behaviour in water, the precipitate they give with lead acetate, and the enzymes they carry. Acacia (gum arabic), obtained from *Acacia* species, dissolves almost completely in water to a clear, slightly acidic solution and carries oxidase and peroxidase enzymes. Tragacanth, from *Astragalus* species, does not dissolve but swells in water to a stiff, opalescent mucilage, and carries no oxidase. Agar, from red algae of the genus *Gelidium* and related genera, swells in cold water and dissolves in hot water to a solution that sets to a firm gel on cooling. Honey is not a gum but a sugar product, chiefly fructose and glucose; its identity tests confirm reducing sugars and, by Fiehe's test, detect the hydroxymethylfurfural that signals overheating or adulteration with invert sugar.

Procedure and observations

A general carbohydrate test is run first, then the discriminating tests.

1. **Molisch test (general carbohydrate).** To a little of the aqueous solution or mucilage of each gum add two drops of alpha-naphthol solution, then pour concentrated sulphuric acid gently down the side of the tube. A reddish-violet ring at the junction of the two liquids confirms a carbohydrate. All three gums give the test.

2. **Behaviour in water.** Shake a small quantity of each gum with water. Acacia dissolves to a clear, viscous, faintly acidic solution. Tragacanth does not dissolve; it swells into a stiff, translucent, opalescent gel. Agar swells in cold water, and on warming dissolves to a solution that sets to a firm gel when cooled. This single observation already separates the three.

3. **Lead acetate test.** To a dilute aqueous solution of each gum add dilute lead acetate solution. Acacia gives no precipitate with dilute lead acetate, whereas tragacanth gives a flocculent precipitate; with basic (subacetate) lead acetate, acacia gives a white precipitate. The contrast in behaviour toward lead acetate is the classical chemical distinction between acacia and tragacanth.

4. **Benzidine test for oxidase (the key test for acacia).** To the aqueous solution of the gum add a few drops of benzidine in alcohol and a few drops of hydrogen peroxide. Acacia develops a distinct blue colour because it contains an oxidase enzyme. Tragacanth and agar give no blue colour, having no oxidase. This is the most reliable single test for acacia.

5. **Ruthenium red.** Mount a little of the powdered gum in ruthenium red and examine under the microscope. Mucilaginous material takes a pink to red stain. The test confirms the mucilage character and helps distinguish acacia, whose particles do not stain pink, from Indian (false) tragacanth, whose particles do.

6. **Iodine test.** Add N/50 iodine solution to a little of the powdered gum. Tragacanth acquires an olive-green colour, a useful distinction from acacia and agar, which do not.

7. **Tests for honey.** Confirm reducing sugars by warming honey solution with Fehling's solution: a brick-red precipitate of cuprous oxide forms. Then run **Fiehe's test** for adulteration: shake honey with solvent ether, decant and evaporate the ether layer in a dish, and add resorcinol dissolved in concentrated hydrochloric acid to the residue. A cherry-red colour indicates hydroxymethylfurfural, which forms when honey is overheated or adulterated with commercial invert sugar; genuine, unheated honey gives a negative or only faint reaction.

Result and inference

Record the behaviour in water, the lead acetate result, the benzidine result, and the iodine colour for each gum, and the Fehling's and Fiehe's results for honey. The combination identifies each drug: acacia by its solubility and positive benzidine test, tragacanth by its swelling and olive-green iodine colour, agar by its hot-water solution that gels on cooling, and honey by its reducing sugars with a negative Fiehe's test in a genuine sample. Table 1.1 collects the distinctions.