BP105T · B.PHARM THEORY

INTRODUCTION TO PHARMACOGNOSY

Unit III — Quality Control of Drugs of Natural Origin

Pills & Python Notes

From-zero teaching standard: every technical term is defined before use.

Syllabus coverage: Unit III (8 hours).

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UNIT III — QUALITY CONTROL OF DRUGS OF NATURAL ORIGIN

CHAPTER 11: ADULTERATION OF DRUGS OF NATURAL ORIGIN

11.1 Learning Objectives

After studying this chapter, the student will be able to:

1. Define adulteration and explain why it happens.
2. Distinguish **deliberate (intentional)** and **accidental (unintentional)** adulteration.
3. Give at least two examples of each type of adulteration.
4. Explain how adulteration harms patients, manufacturers, and honest traders.
5. List the basic strategies used to detect and prevent adulteration.

11.2 Introduction — When the Drug Is Not What It Claims to Be

In Unit II we learned how to grow, collect, and store medicinal plants properly. But a painful reality follows: what reaches the market is not always what the label says. A bag marked "senna leaves" may contain **leaves of a cheaper plant**, or senna leaves **mixed with stems, dust, and exhausted powder**. This is **adulteration** — and it is one of the biggest enemies of herbal medicine quality. A pharmacist who cannot spot adulteration cannot protect the patient. This chapter teaches you what adulteration is, the two families it falls into, and real examples examiners love.

11.3 Definitions

- **Adulteration:** the debasement of a drug — replacing it wholly or partly with another substance, or mixing inferior/foreign material into it — so that the drug is **no longer genuine, pure, or of the expected strength**.
- **Adulterant:** the foreign or inferior material added to (or substituted for) the genuine drug.
- **Genuine drug:** the correct species, correct plant part, and expected quality as defined by a pharmacopoeia.
- **Exhausted drug:** a drug from which the active constituents have already been removed (e.g., by a previous extraction) and which is then sold again as if fresh.

11.4 Why Adulteration Happens

Three pressures drive it:

1. **Money** — a cheaper material sold at the price of an expensive drug brings profit.
2. **Scarcity** — when the genuine drug is rare or out of season, substitutes fill the gap.
3. **Carelessness** — poor identification, bad harvesting, or sloppy handling (the accidental side).

11.5 Types of Adulteration

11.5.1 Deliberate (intentional) adulteration — done on purpose for profit

Method	What happens	Example
Substitution with an inferior variety or species	A cheaper, related plant sold as the costly one	*Cassia* species or other leaves sold as genuine senna; inferior cinnamon varieties sold as true cinnamon
Addition of exhausted drug	Material already extracted (active constituents removed) mixed back in	Exhausted ginger or exhausted fennel mixed with fresh stock — looks similar, works poorly
Addition of foreign matter	Sand, stones, dust, or stems added to increase weight	Earthy matter in powdered drugs; excess stems in leaf drugs
Harmful/adventitious adulteration	Toxic material added or present — the most dangerous kind	Artificial colouring of poor-quality drugs to look rich; addition of synthetic compounds to "strengthen" a weak natural product

11.5.2 Accidental (unintentional) adulteration — done without dishonest intent

Method	What happens	Example
Faulty collection	The wrong plant (or wrong part) collected by mistake	A similar-looking weed collected along with the drug plant because the collector could not tell them apart
Imperfect preparation	Stems, stalks, or other parts left in when only one part is wanted	Excess stem in a leaf drug; bark mixed into a wood drug
Deterioration during storage	The drug itself degrades and becomes substandard	Mouldy, insect-attacked, or moisture-damaged stock sold without checking (links to Chapter 7's storage enemies)
Confusion of common names	Two different plants sharing one local name get mixed	Different regional plants sold under the same vernacular name

11.5.3 Comparison table (exam favourite)

Feature	Deliberate adulteration	Accidental adulteration
Intent	dishonest, for profit	no dishonest intent — ignorance or carelessness
Typical forms	substitution, exhausted drug, added foreign matter	wrong species collected, wrong part, storage damage
Prevention	strict testing, legal punishment, supplier audits	training of collectors, correct identification, GACP
Moral/legal view	fraud	negligence — still makes the drug substandard

Terminology alert. "Sophistication" is an older textbook synonym for deliberate adulteration. If you see it in old question papers, it means the same thing.

11.6 Consequences of Adulteration

1. **Patient harm** — a weak drug fails to cure; a toxic adulterant can poison.
2. **Loss of trust** — one scandal damages the whole herbal industry's reputation.
3. **Economic loss** — manufacturers pay genuine-drug prices for worthless material; honest farmers are undercut.
4. **Regulatory action** — batches fail pharmacopoeial tests and are rejected or recalled.

11.7 Detection and Prevention — the Big Picture

Adulteration is fought with the **evaluation methods of Chapter 12** (looking, microscopy, chemistry) and the **parameters of Chapter 13** (ash, extractives, foreign matter). Prevention rests on three pillars:

1. **Correct identification** at collection (trained collectors, botanical verification).
2. **GACP compliance** (Unit II, Chapter 6) — clean, documented supply chains.
3. **Routine quality testing** of every consignment before it enters manufacture.

Flowchart description — "The adulteration decision tree." A single question at the top: *"Was the foreign material added on purpose?"* → YES branch leads to "Deliberate: substitution / exhausted drug / added foreign matter / harmful additives." → NO branch leads to "Accidental: faulty collection / imperfect preparation / storage deterioration / name confusion." Both branches converge at the bottom box: "Result: drug is not genuine → detect by evaluation (Ch 12–13) → reject the batch."

11.8 Analogy

Adulteration is like **watering down milk** — deliberate adulteration is the shopkeeper who adds water on purpose; accidental adulteration is the assistant who grabs the wrong container. Either way, the customer does not get pure milk.

11.9 Pharmacy Relevance

- The **QC pharmacist** is the gatekeeper: every raw-material consignment is tested for identity and purity before manufacture — adulteration detection is daily work.
- A **community pharmacist** must buy herbal products only from licensed, reputable manufacturers; cheap "bargain" herbal stock is a red flag.
- In **regulatory roles**, pharmacists enforce the Drugs and Cosmetics Act provisions against misbranded and adulterated drugs.

11.10 India Context

India's huge herbal raw-material trade — thousands of tonnes moving through mandis — makes adulteration a live national problem. The **Indian Pharmacopoeia** and the **Ayurvedic Pharmacopoeia of India** lay down identity and purity tests (foreign matter, ash, extractive values) precisely to catch it. The **National Medicinal Plants Board** promotes cultivated, traceable supply chains that squeeze out the room for substitution.

11.11 High-Yield Points

- **Adulteration** = debasement of a drug with foreign/inferior material or substitution.
- Two types: **deliberate** (profit-driven: substitution, exhausted drug, added matter) vs **accidental** (faulty collection, wrong part, storage damage, name confusion).
- **Exhausted drug** = already-extracted material resold as fresh.
- Detection = evaluation methods (Ch 12) + physicochemical parameters (Ch 13); prevention = identification + GACP + testing.

11.12 Common Confusions

- **Confusion:** adulteration vs deterioration. **Clarification:** adulteration involves **foreign material or substitution**; deterioration is the drug itself degrading (mould, insects) — though badly deteriorated stock sold as good is treated as adulterated quality.
- **Confusion:** "substitution" vs "addition." **Clarification:** substitution **replaces** the drug; addition **mixes into** it. Both are deliberate adulteration.

11.13 Common Mistakes

- **Mistake:** writing only deliberate examples when the question asks for "types of adulteration" — the two-type classification (deliberate vs accidental) is the expected framework.
- **Mistake:** calling exhausted drug "genuine but weak." It is an **adulterant** when mixed into or sold as fresh drug.

11.14 Mnemonic

"DASH" — Deliberate Adulteration for profit: **S**ubstitution, **A**dded matter, ex**H**austed drug (read D-A-S-H: Deliberate = Substitution, Addition, exHausted). Accidental = **"C-P-S-N"**: **C**ollection error, **P**reparation error, **S**torage damage, **N**ame confusion.

11.15 Chapter Summary

- **Adulteration** is the debasement of a drug by substitution or admixture, making it non-genuine or substandard.
- **Deliberate adulteration** (fraud for profit): substitution with cheaper species, exhausted drugs, added foreign matter, harmful additives.
- **Accidental adulteration** (no dishonest intent): faulty collection, imperfect preparation, storage deterioration, vernacular-name confusion.
- Consequences: patient harm, lost trust, economic loss, regulatory action.
- Defence: correct identification, GACP, and routine evaluation testing (Chapters 12–13).

11.16 One-Minute Revision

- Adulteration = drug debased by substitution/admixture.
- Deliberate (profit): substitution, exhausted drug, added matter.
- Accidental (carelessness): wrong collection, wrong part, storage damage, name confusion.
- Fight it with: identification + GACP + testing.

11.17 Questions

MCQs

1. An already-extracted drug resold as fresh is called: (a) genuine drug (b) exhausted drug (c) synthetic drug (d) formulated drug
2. Which is an example of deliberate adulteration? (a) Mouldy stock from bad storage (b) Cheaper species substituted for the genuine drug (c) Wrong plant collected by mistake (d) Stems left in a leaf drug accidentally
3. Accidental adulteration is caused by: (a) greed (b) ignorance or carelessness (c) high prices (d) export demand
4. Adding sand to powdered drugs to increase weight is: (a) accidental (b) deliberate (c) deterioration (d) value addition
5. The best prevention of accidental adulteration at the field level is: (a) lower prices (b) training collectors in correct identification (c) faster transport (d) attractive packing

Viva

1. Define adulteration and adulterant.
2. Differentiate deliberate and accidental adulteration.
3. What is an exhausted drug? Give an example.
4. List four consequences of adulteration.
5. Name the three pillars of adulteration prevention.

Application-based

1. A consignment labelled "senna leaves" contains 30% stems and dust plus leaves of another plant. Classify each problem as deliberate or accidental, with reasoning.
2. A manufacturer finds a batch of ginger powder with almost no pungency but normal appearance. What type of adulteration do you suspect, and which Chapter 13 parameter would confirm it?

> **Answers:** 1-(b), 2-(b), 3-(b), 4-(b), 5-(b)

11.18 References

- Evans WC. *Trease and Evans Pharmacognosy.* 16th ed. London: Saunders Elsevier; 2009.
- Kokate CK, Purohit AP, Gokhale SB. *Pharmacognosy.* Pune: Nirali Prakashan.
- WHO. *WHO Guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants.* Geneva: WHO; 2003.

CHAPTER 12: EVALUATION OF DRUGS — ORGANOLEPTIC, MICROSCOPIC, PHYSICAL, CHEMICAL AND BIOLOGICAL METHODS

12.1 Learning Objectives

After studying this chapter, the student will be able to:

1. Define **evaluation** of crude drugs and state its purpose.
2. Describe **organoleptic** evaluation with the five senses.
3. Explain **qualitative and quantitative microscopy**, including stomatal index, vein-islet number, palisade ratio, and the lycopodium spore method — with formulae.
4. List **physical, chemical, and biological** evaluation methods with one example each.
5. Choose the right evaluation method for a given adulteration problem.

12.2 Introduction — Proving the Drug Is Genuine

Chapter 11 showed the many ways a drug can be debased. **Evaluation** is the answer: the set of scientific tests that prove a crude drug is **what it claims to be, pure, and of proper strength**. Think of it as the drug's identity parade plus fitness certificate. Pharmacopoeias prescribe these tests in every monograph. There are five families of methods — and examiners expect you to name all five and detail each.

12.3 Definition

- **Evaluation (of crude drugs):** the assessment of a drug's **identity, purity, and quality** by defined tests, to confirm it matches the pharmacopoeial standard.
- **Monograph:** the official "report card" of a drug in a pharmacopoeia — its description, identification tests, purity limits, and assay.

12.4 The Five Families of Evaluation (overview table)

Method family	What it tests	Tools	Example
Organoleptic	identity by senses	eyes, nose, tongue, touch	colour, odour, taste of the drug
Microscopic	identity by cell structures	microscope	stomata, trichomes, fibres in leaf powder
Physical	purity/quality by measurable constants	instruments	ash values, extractive values, moisture
Chemical	identity/strength by chemical tests	reagents, chromatography	alkaloid precipitation tests, TLC
Biological	activity by living-system response	animals, microbes, tissues	antibiotic potency on bacteria

Flowchart description — "Choosing the evaluation method." Start: *"Question about the drug?"* → "Is it the right drug?" → organoleptic + microscopic + chemical identity tests. → "Is it pure?" → physical constants (ash, foreign matter) + microscopy for adulterants. → "Is it strong enough?" → chemical assay + biological assay. All three questions answered = batch accepted.

12.5 Organoleptic Evaluation — Testing by the Senses

Organoleptic = judged by the sense organs (*organ* + *leptic*, "taken by"). The oldest method — and still the first step.

- **Sight:** colour, size, shape, surface markings (e.g., the characteristic leaflets of senna).
- **Smell (odour):** aromatic drugs are recognised at once (clove, fennel, mint).
- **Taste:** sweet, bitter, pungent, astringent — but tasted **cautiously and in tiny amounts**, and never for unknown or potentially toxic material.
- **Touch:** texture — smooth, rough, fibrous, mucilaginous (isabgol swells and feels slippery).
- **Sound** is rarely used, but breaking a bark and hearing its fracture character is a traditional touch-point.

Strengths: instant, no equipment, catches crude substitution. **Limitations:** subjective; useless for powders of mixed drugs; cannot measure strength.

12.6 Microscopic Evaluation — The Drug Under the Lens

12.6.1 Qualitative microscopy — "what structures are present?"

Thin sections or powders are examined for **characteristic cell structures**: types of **stomata**, **trichomes** (plant hairs), **fibres**, **vessels**, **calcium oxalate crystals**, and **starch grains**. Each drug has a microscopic fingerprint. Example: senna leaflets show characteristic trichomes and stomata; an adulterant leaf shows different ones.

Key structures to know:

- **Stomata:** pores for gas exchange, each flanked by guard cells; their **type and arrangement** differ between species.
- **Trichomes:** hair-like outgrowths — covering, glandular, etc.; highly characteristic.
- **Calcium oxalate crystals:** prisms, rosettes, or raphides (needle bundles) — their form is diagnostic.
- **Starch grains:** shape and size help identify powdered drugs.

12.6.2 Quantitative microscopy — "how much of each structure?" (exam hotspot)

Some leaf drugs are identified by **counting** microscopic features per unit area. Three standard parameters:

1. Stomatal index (SI) The percentage of stomata among all epidermal cells in a given area: **Stomatal index = [S / (E + S)] × 100** where **S** = number of stomata per unit area, **E** = number of ordinary epidermal cells in the same area. Because it is a **ratio**, it stays constant regardless of leaf age or size — that is why it is so valuable.

2. Vein-islet number The **average number of vein islets per square millimetre** of leaf surface. A *vein islet* is the small area of green tissue surrounded by veinlets. Count islets in several fields, average them, and express per mm^2. It is constant for a species.

3. Palisade ratio The **average number of palisade cells beneath each upper epidermal cell**. Count palisade cells under several epidermal cells and take the average. Also constant for a species.

Worked mini-example (stomatal index). In one microscope field you count 20 stomata (S) and 180 epidermal cells (E). SI = 20/(180+20) × 100 = **10%**. A genuine drug has a defined SI range; an adulterant leaf gives a different number.

12.6.3 The lycopodium spore method — counting purity in powders

Principle: *Lycopodium* spores are uniform in size, and — this is the key — **one milligram of lycopodium powder contains about 94,000 spores** (the standard textbook figure). If you mix a **known weight** of lycopodium with your powdered drug and count spores and drug particles under the microscope, you can calculate how much genuine drug is present.

Procedure (step by step):

1. Weigh a known amount of the powdered drug sample and a known weight of lycopodium powder; mix uniformly.
2. Mount on slides and, in representative microscope fields, count: **S** = lycopodium spores, **D** = characteristic particles of the drug (e.g., pollen grains, trichomes, starch).
3. Do the same counting on an **authentic (genuine) reference sample** to find **P** = characteristic particles per mg of pure drug.
4. Calculate:

Weight of genuine drug in the mixture (mg) = (D × W × 94,000) / (S × P)

where **W** = weight of lycopodium added (mg), **94,000** = spores per mg of lycopodium, **D** = drug particles counted, **S** = spores counted, **P** = particles per mg of authentic drug.

Use: detects **powdered adulterants** — e.g., how much genuine powdered drug is actually in a suspicious mixture. It is the classical quantitative method for powdered crude drugs.

12.7 Physical Evaluation — Measurement Without Chemistry

Physical constants reveal impurity and quality without chemical reactions:

- **Ash values, extractive values, moisture content, foreign organic matter** — the Chapter 13 parameters (detailed there).
- **Melting point, boiling point, density, solubility** — for isolated constituents and volatile oils.
- **Viscosity, refractive index, optical rotation** — also in Chapter 13; listed here to show they belong to physical evaluation.

12.8 Chemical Evaluation — Tests and Assays

- **Qualitative chemical tests:** colour/precipitation reactions for classes of constituents — e.g., Mayer's and Dragendorff's reagents give precipitates with **alkaloids**; ferric chloride gives colour with **tannins**; foam test for **saponins**.
- **Quantitative assays:** titration or instrumental measurement of the active constituent (e.g., acid–base titration of alkaloids).
- **Chromatography: TLC (thin-layer chromatography)** and HPLC fingerprints — the modern identity card of a herbal drug; spots are compared with a reference standard.

12.9 Biological Evaluation — Testing on Living Systems

Used when the drug's activity **cannot be measured chemically** — or when chemical strength does not fully predict biological effect.

- **Microbiological assays:** antibiotic potency measured by the **zone of inhibition** on bacteria-seeded agar plates (compare with a standard antibiotic).
- **Animal/tissue tests (classical):** e.g., testing cardiac glycosides on isolated heart preparations; oxytocic activity on uterine muscle. *(Modern practice increasingly replaces animal tests with alternatives, but the principle — measuring effect on living tissue — is what examiners ask.)*
- **Toxicity checks:** e.g., haemolytic test for saponin-containing drugs (detailed as haemolytic potential in Chapter 13).

Strengths: measures the actual **therapeutic effect**. **Limitations:** expensive, time-consuming, needs living material and ethical approvals.

12.10 Which Method for Which Problem? (summary table)

Problem	Best method
Wrong species substituted (whole drug)	organoleptic + qualitative microscopy
Adulterant mixed into powder	quantitative microscopy (lycopodium spore method)
Exhausted drug (weak)	extractive values + chemical assay
Sand/earth added	total ash + acid-insoluble ash
Strength/potency in doubt	chemical assay + biological assay

12.11 Analogy

Evaluation is the **airport security check** for drugs: organoleptic is the officer's glance at your face, microscopy is the fingerprint scan, physical tests are the baggage X-ray, chemical tests are the document verification, and biological assay is the final interview — only genuine passengers board the plane.

12.12 Pharmacy Relevance

- **QC pharmacist:** runs these tests daily on incoming raw material — microscopy of powders and TLC fingerprints are routine.
- **Manufacturing pharmacist:** evaluation results decide whether a batch is accepted, rejected, or reprocessed.
- **Analyst careers:** government drug-testing laboratories employ pharmacists specifically for pharmacognostic evaluation.

12.13 India Context

The **Indian Pharmacopoeia** monographs prescribe macroscopic/microscopic descriptions and chemical identification tests for herbal drugs; the **Ayurvedic Pharmacopoeia of India** includes powder microscopy and TLC identification for AYUSH raw materials. India's **government drug-testing laboratories** (e.g., the pharmacopoeial laboratory network) perform exactly these evaluations on market samples.

12.14 High-Yield Points

- Five evaluation families: **organoleptic, microscopic, physical, chemical, biological**.
- Qualitative microscopy = characteristic structures (stomata, trichomes, crystals, starch).
- Quantitative microscopy: **stomatal index = S/(E+S) × 100**; **vein-islet number** (islets per mm^2); **palisade ratio** (palisade cells per epidermal cell).
- **Lycopodium spore method:** ~94,000 spores/mg; formula **(D × W × 94,000)/(S × P)** — detects adulteration in powders.
- Biological evaluation measures **actual therapeutic effect**; used when chemistry alone is insufficient.

12.15 Common Confusions

- **Confusion:** qualitative vs quantitative microscopy. **Clarification:** qualitative = *which* structures (identification); quantitative = *how many* per unit area (stomatal index etc.).
- **Confusion:** vein-islet number vs veinlet termination number. **Clarification:** the syllabus names **vein-islet number** — number of islets per mm^2. (Some books also list veinlet termination number; know the syllabus term first.)
- **Confusion:** why stomatal index is better than stomatal number. **Clarification:** the index is a **ratio**, so it does not change with leaf age or size.

12.16 Common Mistakes

- **Mistake:** writing the stomatal index formula without × 100 (it is a **percentage**).
- **Mistake:** calling the lycopodium method a chemical test — it is **quantitative microscopy**.
- **Mistake:** saying biological evaluation is obsolete. It is still the referee method when chemical assays cannot capture full activity.

12.17 Mnemonic

"O-M-P-C-B: Observe, Magnify, Physically-check, Chemically-test, Biologically-prove" — the five evaluation families in order.

12.18 Chapter Summary

- **Evaluation** proves a crude drug's identity, purity, and quality against the pharmacopoeial monograph.
- **Organoleptic** (senses) is first and fastest; **microscopic** (qualitative structures; quantitative indices) is the identity specialist.
- **Stomatal index = S/(E+S) × 100**; vein-islet number and palisade ratio are species-constant counts.
- The **lycopodium spore method** (~94,000 spores/mg; formula (D×W×94,000)/(S×P)) quantifies genuine drug in powders.
- **Physical** constants, **chemical** tests/assays/TLC, and **biological** assays complete the picture — activity measured on living systems when chemistry is not enough.

12.19 One-Minute Revision

- 5 families: O-M-P-C-B.
- Stomatal index = S/(E+S)×100 (a ratio — age-independent).
- Vein-islet no. per mm^2; palisade ratio per epidermal cell.
- Lycopodium: 94,000 spores/mg; (D×W×94,000)/(S×P).
- Biological = measures real therapeutic effect.

12.20 Questions

MCQs

1. Stomatal index is calculated as: (a) S/E × 100 (b) S/(E+S) × 100 (c) E/S × 100 (d) (E+S)/S × 100
2. One mg of lycopodium powder contains about: (a) 9,400 spores (b) 94,000 spores (c) 940,000 spores (d) 940 spores
3. Vein-islet number is expressed per: (a) cm^2 (b) mm^2 (c) $inch^2$ (d) field
4. Biological evaluation is preferred when: (a) chemical assay is cheaper (b) chemical tests cannot fully measure therapeutic activity (c) the drug is synthetic (d) microscopy fails
5. Palisade ratio is the average number of palisade cells beneath each: (a) stoma (b) upper epidermal cell (c) trichome (d) vein islet

Viva

1. Define evaluation of crude drugs.
2. Name the five families of evaluation methods.
3. Why is stomatal index more reliable than stomatal number?
4. State the principle of the lycopodium spore method.
5. Give one example each of chemical and biological evaluation.

Application-based

1. A powdered leaf drug is suspected of containing 40% of another leaf powder. Which quantitative microscopy method would you use, and what reference would you need?
2. In one field you count 25 stomata and 225 epidermal cells. Calculate the stomatal index and explain what a different value in a market sample would mean.

Answers: 1-(b), 2-(b), 3-(b), 4-(b), 5-(b)

12.21 References

- Evans WC. *Trease and Evans Pharmacognosy.* 16th ed. London: Saunders Elsevier; 2009.
- Kokate CK, Purohit AP, Gokhale SB. *Pharmacognosy.* Pune: Nirali Prakashan.
- Wallis TE. *Textbook of Pharmacognosy.* (Classical reference for quantitative microscopy.)

CHAPTER 13: PHYSICOCHEMICAL PARAMETERS — THE NUMBERS THAT CERTIFY QUALITY

13.1 Learning Objectives

After studying this chapter, the student will be able to:

1. Explain what physicochemical parameters are and why pharmacopoeias prescribe them.
2. Describe all **13 syllabus parameters** — what each measures and how it is done (in principle).
3. Distinguish **total ash, acid-insoluble ash, and water-soluble ash**.
4. Interpret what an abnormal value suggests (adulteration? exhaustion? poor storage?).
5. Match each parameter to the drug type it suits best.

13.2 Introduction — Quality You Can Measure

Chapters 11–12 taught *what* adulteration is and *how* to look for it. This chapter gives you the **numbers**: thirteen standard laboratory parameters that pharmacopoeias prescribe as pass/fail quality gates. Each one answers a simple question — *how much mineral impurity? how much extractable medicine? how much water?* — and each abnormal result points at a likely culprit. Learn them as thirteen small stories, not thirteen definitions to memorise.

13.3 Definitions

- **Physicochemical parameter:** a measurable physical or chemical constant of a drug (ash %, moisture %, extractive %) used as a **quality standard**.
- **Limit test (idea):** the pharmacopoeia fixes an acceptable range; a batch outside it is rejected. *(Exact pharmacopoeial limits vary by drug and edition — learn the principle and procedure; look up the number in the monograph when you need it.)*
- **Constant weight:** drying or igniting until two successive weighings differ negligibly — the standard "done" signal in gravimetric tests.

13.4 Ash Values — How Much Mineral Matter?

Principle: burn the drug completely; the **ash** left behind is the inorganic (mineral) residue. Genuine drugs leave a characteristic amount of ash. Extra ash = added earth/sand or contamination.

Procedure (in principle): weigh powdered drug → ignite in a crucible to red heat until free from carbon → cool → weigh ash → calculate % w/w.

The three ash values (must-know table)

Ash type	How obtained	What it detects
Total ash	ash from complete ignition	total mineral content — natural + added
Acid-insoluble ash	boil total ash with dilute HCl, filter, ignite the residue	**silica/sand** — earthy adulteration insoluble in acid
Water-soluble ash	(total ash) − (water-insoluble residue)	minerals soluble in water; detects **exhausted drug** (which has lost soluble matter)

Interpretation logic: high total ash → possible added minerals; high **acid-insoluble** ash → **sand/earth adulteration** (exam favourite); low water-soluble ash → drug may be **exhausted**.

13.5 Extractive Values — How Much Medicine Comes Out?

Principle: extract the powdered drug with a solvent; evaporate; weigh the residue. The **extractive value** (% w/w) tells how much soluble active matter the drug contains.

- **Water-soluble extractive:** for drugs whose actives dissolve in water.
- **Alcohol-soluble extractive:** for drugs whose actives need alcohol.
- **Ether-soluble extractive (non-volatile):** for resins, fixed oils.

Procedure (in principle): macerate a weighed quantity with the solvent for the prescribed time → filter → evaporate a measured volume → dry to constant weight → calculate %.

Interpretation logic: low extractive value → exhausted or weak drug (the medicine has already been taken out or was never there). This is the parameter that catches the "exhausted ginger" of Chapter 11.

13.6 Moisture Content (Loss on Drying)

Principle: water in a drug invites **mould, fermentation, and hydrolysis** (Chapter 7's storage enemies). **Procedure (in principle):** weigh → dry at the prescribed temperature to constant weight → loss in weight = moisture (+ volatile matter) %.

Interpretation logic: high moisture → bad drying/storage → rejection; also the drug is sold partly as water.

13.7 Foreign Organic Matter

Definition: any matter **not part of the drug as defined** — other plant parts, other plants, insects, animal matter, stones. **Procedure (in principle):** spread a weighed sample, pick out and weigh the foreign matter, calculate %. Pharmacopoeias fix maximum limits.

Interpretation logic: high foreign matter → **careless collection/preparation** (accidental adulteration, Chapter 11).

13.8 Bitterness Value — For the Bitters

Principle: bitter drugs (chirata, kutki, gentian) are valued *for* their bitterness. **Definition:** the bitterness value is the **volume of water (in ml) in which the bitterness of 1 g of drug can still be detected** by taste comparison against a standard bitter substance (classically quinine). Higher value = stronger bitter.

Interpretation logic: low bitterness value → weak or exhausted bitter drug.

13.9 Foaming Index — For the Saponin Drugs

Principle: saponins foam when shaken with water (like soap — the name comes from *sapo*, Latin for soap). **Procedure (in principle):** shake graded dilutions of the drug decoction in test tubes, measure foam height after standing; the **foaming index** is derived from the dilution that still gives a standard foam height.

> **Interpretation logic:** abnormal foaming index → wrong saponin strength → possible substitution or exhaustion.

13.10 Haemolytic Potential — Saponins Meet Blood Cells

Principle: saponins **rupture red blood cells (haemolysis)**. The test measures the drug's haemolytic activity compared with a reference saponin, typically on a blood-agar or RBC suspension basis as per pharmacopoeial procedure.

> **Interpretation logic:** unexpected haemolytic strength → wrong species or wrong saponin content; also a **safety** signal since haemolytic saponins must be controlled.

13.11 Swelling Index — For the Mucilage Drugs

Principle: mucilage drugs (isabgol/psyllium, tragacanth) swell enormously in water — that swelling *is* their therapeutic action (bulk laxatives). **Procedure (in principle):** place a weighed quantity in a graduated cylinder with water, let stand, and read the **volume occupied** — the swelling index.

> **Interpretation logic:** low swelling index → exhausted or substituted mucilage → the laxative will not work.

13.12 Viscosity — For Gums and Mucilages

Principle: gums and mucilages form viscous (thick) solutions; viscosity reflects their quality and concentration behaviour. Measured with a **viscometer** (e.g., Ostwald viscometer — the U-tube you met in physical pharmacy).

> **Interpretation logic:** abnormal viscosity → degraded or adulterated gum.

13.13 Optical Rotation — For Chiral Drugs

Principle: some drug molecules are **optically active** — they rotate plane-polarised light. A **polarimeter** measures the angle and direction (+ dextro, − laevo). Each pure optically active substance has a characteristic **specific rotation**.

> **Interpretation logic:** wrong rotation → wrong enantiomer, impurity, or substitution. Classic for sugars, volatile-oil components, and alkaloids.

13.14 Refractive Index — For Volatile Oils

Principle: light bends when entering a liquid; the **refractive index** (measured on a refractometer, typically with sodium light) is characteristic for a pure volatile oil.

Interpretation logic: wrong refractive index → oil **diluted or adulterated** with cheaper oils or solvents.

13.15 Acid Value and Saponification Value — For Fats, Oils and Resins

Acid value: the **mg of potassium hydroxide (KOH) needed to neutralise the free acids in 1 g** of the fat/oil/resin. High acid value = more free fatty acids = **rancid or degraded** oil.

Saponification value: the **mg of KOH needed to saponify (convert to soap) 1 g** of fat/oil. It is **inversely related to molecular weight** — shorter fatty-acid chains give higher values. Used to check identity and purity of fixed oils and fats.

Interpretation logic: abnormal acid value → rancidity; abnormal saponification value → wrong oil or admixture.

13.16 Master Table — All 13 Parameters at a Glance (exam favourite)

#	Parameter	Measures	Drug types	Abnormal value suggests
1	Total ash	mineral residue	all drugs	added minerals
2	Acid-insoluble ash	silica/sand	all drugs	**earthy adulteration**
3	Water-soluble ash	soluble minerals	all drugs	exhaustion
4	Extractive values	soluble active matter	all drugs	**exhausted/weak drug**
5	Moisture content	water %	all drugs	bad drying, mould risk
6	Foreign organic matter	non-drug matter %	all drugs	careless collection
7	Bitterness value	bitter strength	bitters (chirata, kutki)	weak bitter
8	Foaming index	saponin frothing	saponin drugs	wrong saponin strength
9	Haemolytic potential	RBC rupture	saponin drugs	wrong species/safety issue
10	Swelling index	water-swelling volume	mucilages (isabgol)	exhausted mucilage
11	Viscosity	solution thickness	gums, mucilages	degraded gum
12	Optical rotation	light rotation	chiral drugs	wrong isomer/impurity
13	Refractive index	light bending	volatile oils	diluted/adulterated oil
(+2)	Acid value / Saponification value	free acids / soap-forming capacity	fixed oils, fats, resins	rancidity / wrong oil

(Acid and saponification values are listed together as the syllabus does — "acid value and saponification value".)

Flowchart description — "Reading a lab report." Boxes in sequence: *High acid-insoluble ash?* → YES: "sand/earth added." *Low extractive value?* → YES: "exhausted drug." *High moisture?* → YES: "bad storage — mould risk." *High foreign matter?* → YES: "careless collection." *Wrong refractive index?* → YES: "oil adulterated." All NO → "batch meets pharmacopoeial quality."

13.17 Analogy

Physicochemical parameters are the **blood-test panel** of a crude drug: ash values are the "mineral profile," extractives are the "protein levels," moisture is "hydration status" — and just like a doctor reads the panel to diagnose, the pharmacist reads these numbers to diagnose adulteration.

13.18 Pharmacy Relevance

- **QC pharmacist:** these are the daily pass/fail tests on every consignment — ash, extractives, moisture, foreign matter first.
- **Manufacturing pharmacist:** extractive values decide batch blending; moisture decides drying before grinding.
- **Regulatory pharmacist:** pharmacopoeial limits are legal standards — a batch failing them cannot be sold.

13.19 India Context

The **Indian Pharmacopoeia** prescribes ash values, extractive values, foreign matter, and loss on drying in herbal monographs; the **Ayurvedic Pharmacopoeia of India** similarly includes these parameters for AYUSH raw materials. *(Specific limit values differ per drug and edition — always read the current monograph; this chapter teaches the procedures and interpretation.)*

13.20 High-Yield Points

- **Acid-insoluble ash → sand/earth**; **low extractive → exhausted drug**; **high moisture → mould risk**.
- Three ashes: **total** (all minerals), **acid-insoluble** (silica), **water-soluble** (soluble minerals).
- **Bitterness value** = ml of water in which 1 g still tastes bitter — for bitters.
- **Swelling index** = volume after swelling — for mucilages (isabgol).
- **Acid value** = mg KOH per g to neutralise free acids (rancidity); **saponification value** = mg KOH per g to saponify (∝ 1/molecular weight).

13.21 Common Confusions

- **Confusion:** the three ash values. **Clarification:** total = everything; acid-insoluble = **sand** (the forensic one); water-soluble = soluble minerals (falls when drug is exhausted).
- **Confusion:** acid value vs saponification value. **Clarification:** acid value neutralises **free** acids only (rancidity signal); saponification value saponifies **all** fat (identity signal).
- **Confusion:** foaming index vs haemolytic test. **Clarification:** both relate to **saponins** — foaming measures froth, haemolysis measures RBC rupture.

13.22 Common Mistakes

- **Mistake:** saying high total ash always means adulteration — some genuine drugs are naturally mineral-rich; compare with the **monograph limit** and check the acid-insoluble fraction.
- **Mistake:** mixing up swelling index (volume) with viscosity (thickness) — both are for mucilages/gums but measure different things.
- **Mistake:** quoting pharmacopoeial limit numbers from memory in exams — limits vary by drug and edition; write "as per the monograph limit."

13.23 Mnemonic

"AEM FoBHaS VORAS" — **A**sh, **E**xtractive, **M**oisture, **Fo**reign matter, **B**itterness, **Ha**emolytic, **S**welling, **V**iscosity, **O**ptical rotation, **R**efractive index, **A**cid value, **S**aponification value. (13 parameters — count them on your fingers.)

13.24 Chapter Summary

- **Ash values** reveal mineral/earthy adulteration (acid-insoluble ash = sand).
- **Extractive values** reveal exhaustion (low = weak drug); **moisture** reveals storage quality; **foreign matter** reveals collection care.
- **Bitterness value, foaming index, haemolytic potential** serve bitter and saponin drugs; **swelling index and viscosity** serve mucilages and gums.
- **Optical rotation and refractive index** check chiral drugs and volatile oils; **acid and saponification values** check fats/oils/resins.
- Read the panel like a diagnosis: each abnormal number points at a culprit.

13.25 One-Minute Revision

- Ash trio: total / acid-insoluble (sand) / water-soluble.
- Low extractive = exhausted; high moisture = mould risk.
- Bitters → bitterness value; saponins → foaming + haemolytic; mucilage → swelling index.
- Chiral → optical rotation; volatile oil → refractive index; fats → acid & saponification values.

13.26 Questions

MCQs

1. High acid-insoluble ash indicates: (a) water adulteration (b) sand/earthy adulteration (c) exhausted drug (d) high oil content
2. A low alcohol-soluble extractive value suggests: (a) fresh drug (b) exhausted drug (c) mineral adulteration (d) good storage
3. Swelling index is the key parameter for: (a) bitters (b) mucilage drugs like isabgol (c) volatile oils (d) alkaloid drugs
4. Acid value is expressed as mg of KOH required to neutralise free acids in: (a) 100 g (b) 1 g (c) 10 g (d) 1 ml of the oil
5. Refractive index is most useful for detecting adulteration in: (a) leaf powders (b) volatile oils (c) barks (d) seeds

Viva

1. Distinguish total ash, acid-insoluble ash, and water-soluble ash.
2. What does a low extractive value tell you?
3. Define bitterness value.
4. How do acid value and saponification value differ?
5. Why is moisture content a critical parameter?

Application-based

1. A lab report shows: acid-insoluble ash 8% (monograph limit 2%), extractive value normal. Diagnose the likely adulteration and explain your reasoning.
2. Two isabgol samples: A swells to 12 ml, B to 4 ml per gram. Which would you accept for a bulk-laxative formulation, and why?

Answers: 1-(b), 2-(b), 3-(b), 4-(b), 5-(b)

13.27 References

- Evans WC. *Trease and Evans Pharmacognosy.* 16th ed. London: Saunders Elsevier; 2009.
- Kokate CK, Purohit AP, Gokhale SB. *Pharmacognosy.* Pune: Nirali Prakashan.
- Indian Pharmacopoeia (current edition) — general tests for ash, extractives, foreign matter, loss on drying.
- Ayurvedic Pharmacopoeia of India — quality parameters for AYUSH raw materials.

CHAPTER 14: DNA BARCODING — THE GENETIC IDENTITY CARD

14.1 Learning Objectives

After studying this chapter, the student will be able to:

1. Define DNA barcoding in simple words.
2. Explain the principle: a short standard DNA sequence as a species "barcode."
3. List the steps of DNA barcoding from sample to identification.
4. State which DNA regions are used for plants and why.
5. Explain how DNA barcoding detects adulteration that older methods miss.

14.2 Introduction — When the Microscope Is Not Enough

Chapters 12–13 gave us powerful tools — but they have a blind spot. Two closely related species can look **identical** under the microscope, and a **powdered mixture** can defeat even quantitative microscopy. What if the drug carried an identity card written in a language no adulterant can forge? That language is **DNA**, and reading a short standard stretch of it to name the species is **DNA barcoding** — the newest quality-control tool in the syllabus.

14.3 Definitions

- **DNA (deoxyribonucleic acid):** the molecule carrying a living thing's genetic instructions; every species has a characteristic DNA sequence.
- **DNA barcoding:** identifying a species by reading a **short, standardised DNA region** (the "barcode") and matching it against a reference database — exactly like scanning a product barcode at a shop counter.
- **Barcode region (locus):** the specific short DNA stretch agreed upon as the standard identifier for a group of organisms.
- **Reference database:** a library of barcode sequences from correctly identified species, used for matching (examples: GenBank, BOLD — the Barcode of Life Data System).

14.4 Principle — One Short Sequence, One Species

The idea has three parts:

1. **Choose a standard region** — the same short DNA stretch is read for every plant, so results are comparable.
2. **It varies between species but not within a species** — individuals of one species share nearly identical barcodes; different species differ enough to tell apart.
3. **Match against the library** — the unknown sample's barcode is compared with reference barcodes; the closest match names the species.

> **Analogy.** A supermarket barcode does not describe the whole product — it is just a short strip that uniquely *identifies* it at the counter. A DNA barcode is the same: a short strip of DNA that uniquely identifies the species.

14.5 The Plant Barcode Regions

For **plants**, the scientific community uses regions from the chloroplast and nuclear DNA. The standard plant barcodes examiners expect:

Barcode region	Source	Note
rbcL	chloroplast gene	easy to amplify; good at family/genus level
matK	chloroplast gene	evolves faster — better species-level discrimination
ITS2	nuclear ribosomal region	widely used for medicinal plants; good species resolution

(The rbcL + matK combination is the formally proposed standard plant barcode; ITS2 is additionally popular in herbal-drug work. New markers continue to be evaluated — the principle matters more than the list.)

14.6 Procedure — Step by Step

1. **Sample collection:** take the crude drug (even powder works — DNA survives grinding).
2. **DNA extraction:** break open cells and purify the DNA, removing polysaccharides and polyphenols that interfere (medicinal plants are rich in these — extraction is the tricky step).
3. **Amplification (PCR):** the **polymerase chain reaction** makes millions of copies of just the barcode region, using primers that flank it.
4. **Sequencing:** read the exact A–T–G–C letter order of the amplified region.
5. **Database matching:** compare the sequence with reference barcodes (BLAST search against GenBank/BOLD); the best match identifies the species.
6. **Interpretation:** a ≥99% match to the genuine species = authentic; a match to another species = substitution/adulteration detected.

Flowchart description — "The barcoding pipeline." Five boxes left to right: *Sample (powder/leaf)* → *Extract DNA* → *PCR amplify barcode* → *Sequence (A-T-G-C)* → *Match database* → *Species name*. A side note under PCR: "primers target rbcL/matK/ITS2." A final decision diamond: "Matches genuine species? YES → accept; NO → adulterated."

14.7 Advantages

1. **Works on powders and processed material** — where morphology and microscopy fail.
2. **Objective** — a sequence match, not a subjective opinion about colour or hairs.
3. **Detects substitution by close relatives** — the hardest adulteration to catch otherwise.
4. **Tiny sample needed** — PCR amplifies from traces.
5. **Standardised and shareable** — sequences can be compared worldwide.

14.8 Limitations

1. **Needs a laboratory** — equipment, reagents, and trained staff; costlier than microscopy.
2. **Depends on the reference database** — a species missing or mislabelled in the database gives a wrong answer ("garbage in, garbage out").
3. **DNA degrades** in badly stored or heavily processed material — very old or overheated samples may fail.
4. **Cannot measure quantity or strength** — it says *which* species, not *how much* active constituent (pair it with Chapters 12–13).
5. **Close hybrids** can occasionally confuse single-region barcodes.

14.9 DNA Barcoding vs Traditional Methods (comparison table)

Feature	Microscopy/chemistry	DNA barcoding
Works on fine powders	limited	yes
Subjectivity	observer-dependent	objective sequence
Tells species identity	often yes	yes, including close relatives
Measures strength/purity	yes (extractives, assays)	no
Cost and infrastructure	low	higher
Verdict	still essential	**complementary**, not a replacement

14.10 Pharmacy Relevance

- **QC of the future:** herbal companies and regulators increasingly add DNA-based identity tests for high-value or frequently adulterated drugs.
- **Pharmacist's role:** understanding the method lets pharmacists interpret DNA test reports on certificates of analysis and choose the right test for the right problem.
- **Research avenue:** building reference barcode libraries for Indian medicinal plants is active academic work pharmacognosy graduates can join.

14.11 India Context

India's vast medicinal-plant diversity — and its busy raw-drug markets — make DNA barcoding especially valuable for authentication of AYUSH raw materials. Indian research institutions are contributing barcode sequences of native medicinal plants to global databases, strengthening the reference library that the whole method depends on.

14.12 High-Yield Points

- **DNA barcoding** = species identification by a short standard DNA sequence matched to a reference database.
- Plant barcodes: **rbcL, matK** (chloroplast; the standard pair) and **ITS2** (nuclear; popular for herbs).
- Steps: **extract DNA → PCR amplify → sequence → database match**.
- Kills the blind spot: identifies **powders and close-relative substitutes**.
- Limitation: needs good **reference databases**; tells identity, **not strength**.

14.13 Common Confusions

- **Confusion:** DNA barcoding vs genetic fingerprinting of humans. **Clarification:** same idea (DNA identifies), different purpose — here it identifies **plant species**, not individuals.
- **Confusion:** "barcoding replaces microscopy." **Clarification:** it is **complementary** — microscopy and chemistry still measure purity and strength, which DNA cannot.

14.14 Common Mistakes

- **Mistake:** naming animal barcode regions for plants. The classic animal barcode is **COI** (cytochrome oxidase I) — for **plants**, write **rbcL/matK/ITS2**.
- **Mistake:** saying PCR "identifies" the species. PCR only **amplifies**; **sequencing + database matching** identifies.

14.15 Mnemonic

"EPSD — Extract, Photocopy (PCR), Spell-out (sequence), Detect (match)" — the four barcoding steps. And **"RMI — rbcL, matK, ITS2"** for the plant barcode regions.

14.16 Chapter Summary

- **DNA barcoding** identifies a species from a short standard DNA region, matched against a reference database — like scanning a shop barcode.
- Standard plant barcodes: **rbcL + matK** (chloroplast) and **ITS2** (nuclear).
- Pipeline: **sample → DNA extraction → PCR amplification → sequencing → database match → identification**.
- It succeeds where older methods fail: **powders and closely related substitutes**.
- Limits: needs labs, good databases, intact DNA — and it measures **identity, not strength**; it complements, never replaces, Chapters 12–13.

14.17 One-Minute Revision

- Barcode = short standard DNA identifying the species.
- Plant regions: rbcL, matK, ITS2 (COI is for animals).
- Steps: extract → PCR → sequence → match.
- Best for: powders, close-relative substitution.
- Can't do: measure strength; needs database + lab.

14.18 Questions

> **MCQs**

1. The standard plant DNA barcode pair is: (a) COI + 16S (b) rbcL + matK (c) ITS2 + COI (d) 18S + 28S
2. In DNA barcoding, PCR is used to: (a) identify the species (b) amplify the barcode region (c) extract DNA (d) build the database
3. DNA barcoding is most valuable for: (a) measuring alkaloid % (b) identifying powdered drugs and close substitutes (c) testing moisture (d) measuring ash
4. A limitation of DNA barcoding is that it: (a) cannot identify species (b) cannot measure drug strength (c) needs no equipment (d) works only on fresh leaves
5. ITS2 is a barcode region from: (a) chloroplast (b) nuclear ribosomal DNA (c) mitochondria of animals (d) plasmid

Viva

1. Define DNA barcoding.
2. Name the standard plant barcode regions.
3. List the steps of DNA barcoding.
4. Why is DNA barcoding better than microscopy for powdered drugs?
5. State two limitations of DNA barcoding.

Application-based

1. Two dried root powders look identical microscopically, but one is the genuine drug and the other a cheaper relative. Design a DNA-barcoding plan to resolve it, naming the regions you would try first.
2. A DNA test identifies the species correctly but the batch still fails clinically. Explain why, using this unit's concepts.

Answers: 1-(b), 2-(b), 3-(b), 4-(b), 5-(b)

14.19 References

- Evans WC. *Trease and Evans Pharmacognosy.* 16th ed. London: Saunders Elsevier; 2009.
- Hebert PDN, Cywinska A, Ball SL, deWaard JR. The DNA barcode concept is described in the primary literature (Proceedings of the Royal Society B, 2003) — consult the original paper for the founding principle.
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UNIT III INFOGRAPHIC CONCEPT — "THE QUALITY DETECTIVE'S TOOLKIT"

Layout: a detective's evidence board. Center: a magnifier over a crude-drug sample labelled "Is this drug genuine, pure, and strong?" Around it, five evidence folders:

1. 🔍 **SPOT THE FAKE** (Ch 11) — deliberate vs accidental adulteration; exhausted drugs; sand in powders.
2. 👁️ **LOOK & COUNT** (Ch 12) — organoleptic senses; microscopy; stomatal index = S/(E+S)×100; lycopodium 94,000 spores/mg.
3. 🧪 **THE NUMBERS PANEL** (Ch 13) — ash trio; extractives; moisture; bitterness/foaming/swelling; optical rotation; refractive index; acid & saponification values.
4. 🧬 **THE DNA CARD** (Ch 14) — rbcL · matK · ITS2; extract → PCR → sequence → match.
5. ⚖️ **VERDICT** — identity ✓ purity ✓ strength ✓ → batch accepted; any ✗ → batch rejected.

Bottom banner: "Quality is measured, not assumed."

UNIT III REFERENCES (COMBINED)

- Evans WC. *Trease and Evans Pharmacognosy.* 16th ed. London: Saunders Elsevier; 2009.
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