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BP105T

INTRODUCTION TO PHARMACOGNOSY

UNIT III — QUALITY CONTROL OF DRUGS OF NATURAL ORIGIN (WHO GUIDELINES)

Adulteration of Natural Drugs • Organoleptic, Microscopic, Physical, Chemical & Biological Evaluation

Physicochemical Parameters — Extractive Values, Ash Values, Bitterness, Foaming & Haemolytic Index and More

DNA Barcoding for Species Authentication

B.Pharm — Semester I

PCI Regulations, ER-2020 Syllabus

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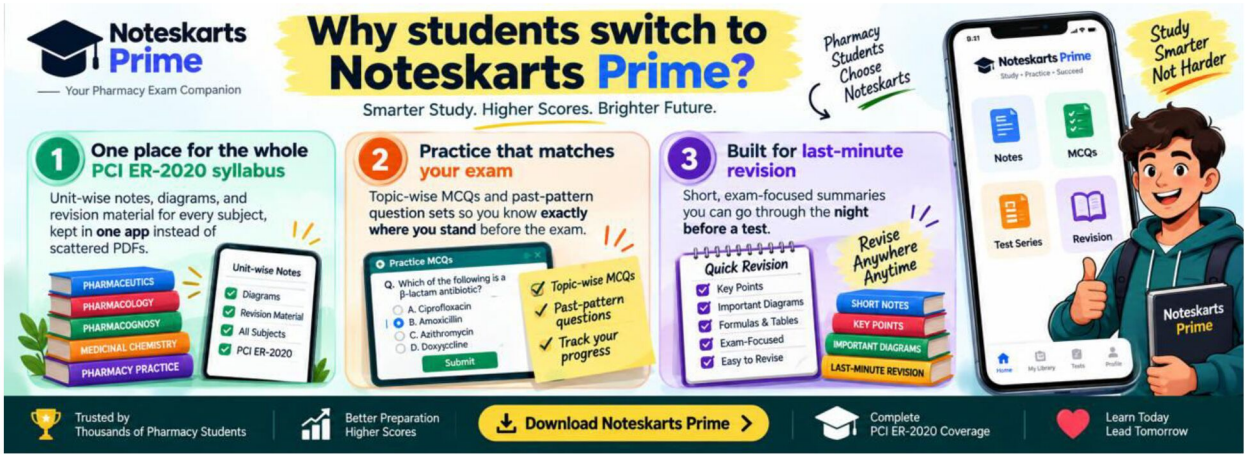

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1. Quality Control of Drugs of Natural Origin (WHO Guidelines)

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- **Quality Control (QC)** means checking a crude drug for:
  - **Identity** – Is it the correct drug?
  - **Purity** – Is it free from impurities and contamination?
  - **Strength** – Does it contain the required amount of active constituents?
- The main WHO reference is **"Quality Control Methods for Medicinal Plant Materials" (1998)**, published as **WHO Technical Report Series No. 882**.
- It provides internationally accepted methods for checking the **quality of medicinal plant materials**.
- WHO later updated these guidelines as **"Quality Control Methods for Herbal Materials."**
- Another WHO guideline, **"Guidelines on Assessing Quality of Herbal Medicines with Reference to Contaminants and Residues" (2007)**, deals with:
  - Heavy metals
  - Pesticide residues
  - Microbial contamination
  - Radioactive contamination

Why is QC of natural drugs difficult?

- Natural drugs are **complex mixtures** of many substances.
- Their composition can change due to:
  - Source of the plant
  - Season
  - Soil and climate
  - Collection and storage
  - Processing after harvesting
- Therefore, QC uses different types of tests:
  - **Organoleptic tests** – colour, odour, taste, etc.
  - **Microscopic tests** – cellular and tissue characteristics
  - **Physical tests** – moisture, ash, etc.
  - **Chemical tests** – active constituents and chemical markers
  - **Biological tests** – biological activity or potency

1.1 General Principles — Identity, Purity, Strength

- **Identity** — confirming the material genuinely is the species (and plant part) it is claimed to be, ruling out substitution (Section 2).
- **Purity** — confirming the material is free of excessive foreign matter, contamination, or adulterants, within pharmacopoeial limits.
- **Strength/potency** — confirming the material contains an adequate, quantifiable amount of the constituent(s) responsible for its therapeutic effect.
- Every method in this unit — organoleptic through to DNA barcoding — exists to answer one or more of these three questions; keeping this three-way framework in mind makes it easy to explain *why* a given test is used, not just *how* it is performed.

1.1 The WHO Quality-Control Toolkit — What It Covers

WHO guidelines include tests to check the **identity, purity, strength, and safety** of herbal drugs.

- **Sampling** – Proper sample collection.
- **Macroscopic & microscopic tests** – Identify the drug.
- **Foreign matter** – Detect unwanted materials.
- **TLC** – Confirm identity and chemical constituents.
- **Ash & extractive values** – Check purity and chemical content.
- **Moisture & volatile oil** – Determine water and oil content.
- **Bitterness, haemolytic, swelling & foaming indices** – Measure specific properties.
- **Tannin content** – Determine tannins.
- **Contaminant tests** – Check pesticides, heavy metals, microbes, and radioactivity.
- **DNA barcoding** – Helps confirm plant identity.

2. Adulteration of Drugs of Natural Origin

- Adulteration means mixing or replacing a genuine crude drug with inferior, impure, or wrong materials.
- It may be intentional or accidental.
- It reduces the drug's quality, purity, and potency.
- Quality control helps detect and prevent adulteration.

Figure 2.1 — Five recognised types of adulteration in drugs of natural origin.

2.1 Types of Adulteration

- **Substitution –** Genuine drug is replaced with a cheaper or inferior drug/species.
- **Foreign Matter –** Sand, stones, soil, stems, leaves, etc. are mixed with the drug.
- **Exhausted Drug –** Drug from which active constituents have already been removed is reused.
- **Artificial/Spurious Matter –** Synthetic or fake materials are used instead of the genuine drug.
- **Contamination –** Drug contains harmful substances such as heavy metals, pesticides, microbes, or mycotoxins.

2.2 Classic Examples Used in Teaching

Genuine drug	Common adulterant / problem
Senna leaves	Leaves of other Cassia species with lower sennoside content
Clove	Clove stalks or exhausted (oil-extracted) cloves mixed with whole clove buds
Ergot	Grains/seeds or other fungal sclerotia mixed with genuine ergot
Powdered spices (e.g. chilli)	Non-permitted colourants (e.g. Sudan dyes) added to mask poor colour
Ginseng	Substitution with cheaper Panax/related species of lower ginsenoside content

2.3 Consequences and Detection

Consequences

- Reduced or no therapeutic effect
- Unpredictable dose
- Possible toxicity due to wrong or contaminated drugs

Detection

- **Organoleptic tests** – Check colour, odour, taste, etc.
- **Microscopy** – Detect wrong plant parts or foreign matter.
- **Physicochemical tests** – Detect dilution or exhausted drugs.
- **Chemical tests & chromatography** – Confirm expected compounds.
- **DNA barcoding** – Confirms the **correct plant species**, even in powdered drugs.

3. Evaluation of Drugs — Organoleptic, Microscopic, Physical, Chemical and Biological Methods

Drugs of natural origin are evaluated using **five main methods**.

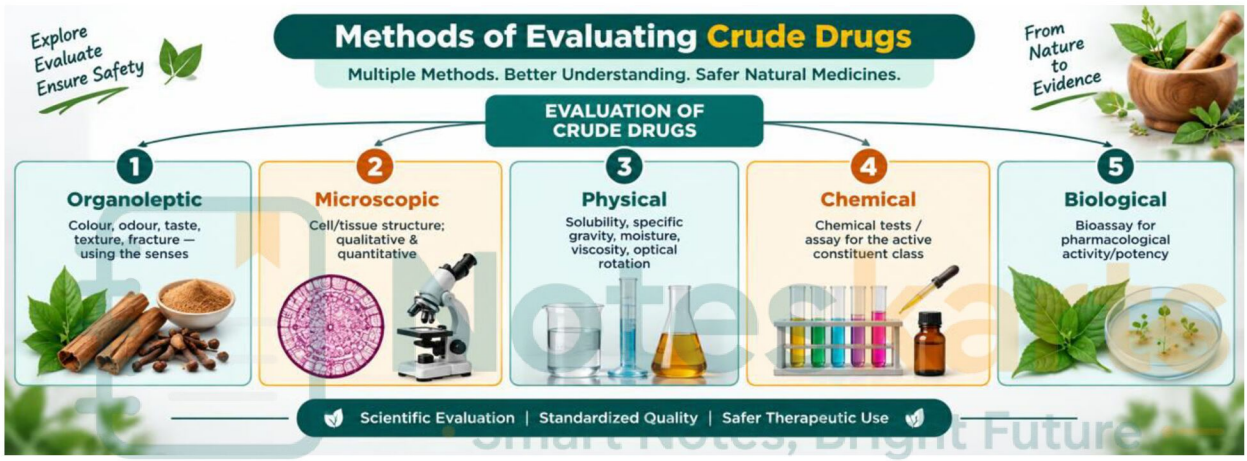

Figure 3.1 — The five recognised methods of evaluating a crude drug.

Method	Speed / cost	Equipment needed	Typical use
Organoleptic	Very fast, near zero cost	None	First-line field/market screening
Microscopic	Fast, low cost	Compound microscope	Identity confirmation, esp. powders
Physical	Fast–moderate, low–moderate cost	Simple instruments (refractometer, polarimeter)	Purity/identity checks on oils, extracts
Chemical	Moderate, moderate cost	Reagents; TLC/HPLC/GC	Marker-compound identity & potency
Biological	Slow, high cost	Animal/tissue/microbial assay setup	Potency where chemistry alone is insufficient

3.1 Organoleptic Evaluation

- Evaluation using the **senses** — the fastest, cheapest, first-line screening method, requiring no equipment.

- **Colour** — e.g. genuine saffron is a deep red-orange; a faded or unusually bright colour can signal adulteration or age.
- **Odour** — e.g. characteristic aromatic odour of clove or cinnamon; absence of odour can indicate an exhausted (oil-extracted) drug.
- **Taste** — e.g. the intense bitterness of Chirata or quinine bark is itself a quick identity check.
- **Size, shape, and texture** — comparing the material against a known reference description.
- **Fracture** — how the material breaks (e.g. short and even vs fibrous and splintery) can help distinguish drugs of similar appearance.
- **Limitation:** organoleptic evaluation is subjective, depends on the observer's experience, and cannot detect adulteration that has been deliberately disguised (e.g. powdered material, or a substitute chosen specifically because it looks/smells similar).

3.2 Microscopic Evaluation

- **Qualitative microscopy** — examining characteristic cellular and tissue features to confirm identity: cell shapes, presence of specific cell types (e.g. stone cells, laticifers, oil cells), crystal forms (calcium oxalate as prisms, rosettes, or raphides), and starch grain shape are all diagnostic. Essential for identifying **powdered drugs**, where macroscopic features are destroyed.
- **Quantitative microscopy** — assigns numerical values to microscopic features so that two samples can be objectively compared, rather than relying on subjective description alone.

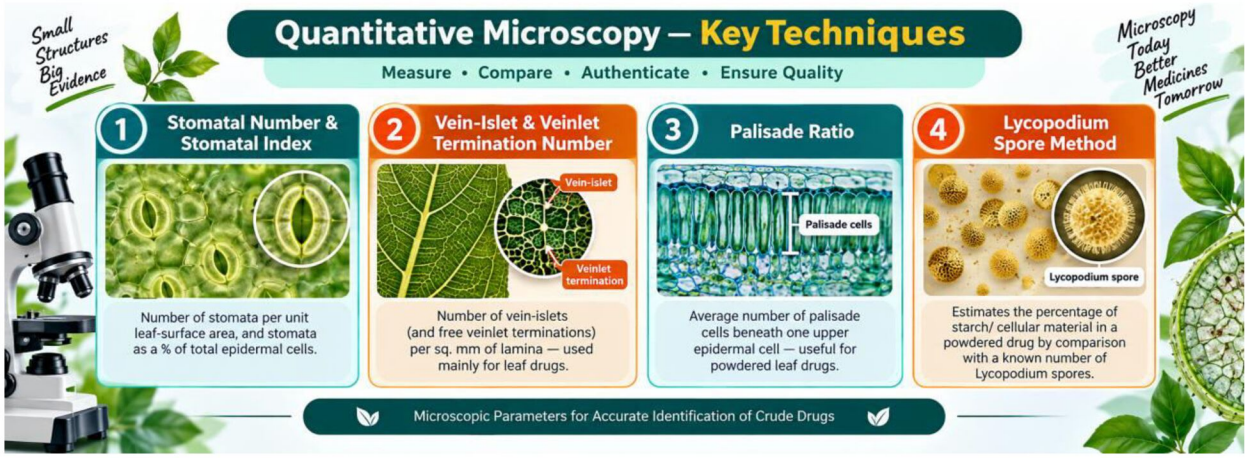

Figure 3.2 — The four Imp quantitative microscopy techniques used in pharmacognosy.

- **Stomatal number** — average number of stomata per square millimetre of leaf epidermis; **stomatal index** expresses stomata as a percentage of the total number of epidermal cells (stomata + ordinary epidermal cells) in the same area, which is more reliable than stomatal number alone since it is independent of leaf size/expansion.

- **Vein-islet number** — number of vein-islets (areas of leaf lamina enclosed by veinlets) per square millimetre, determined over the middle region of the lamina midway between the midrib and the margin; the **veinlet termination number** counts the free termination points of veinlets within the same area. Both are primarily used for leaf drugs.
- **Palisade ratio** — the average number of palisade cells lying directly beneath a single upper epidermal cell; useful for identifying powdered leaf material, since it survives powdering even when the whole-leaf structure is lost.
- **Lycopodium spore method** — estimates the percentage of starch (or other cellular material) in a powdered drug by comparing it, under the microscope, against a mixture containing a known number of Lycopodium spores per unit weight.

3.3 Physical Methods of Evaluation

- Based on measurable **physical properties** of the drug or its extract rather than its chemistry.
- **Solubility** — in water, alcohol, or other solvents; a genuine drug has a characteristic solubility profile.
- **Specific gravity** — particularly relevant for volatile oils and fixed oils.
- **Moisture content, viscosity, optical rotation, refractive index** — covered in detail as physicochemical parameters in Section 4, since these are the specific, standardised physical tests actually used in pharmacopoeial monographs.
- **Melting point/boiling point** — relevant for isolated constituents or oils rather than whole crude drugs.

3.4 Chemical Methods of Evaluation

- Uses **chemical reactions or analytical chemistry** to detect or quantify the constituent class responsible for the drug's identity or activity.
- **Qualitative chemical tests** — colour reactions and precipitation tests (e.g. Dragendorff's reagent for alkaloids, Liebermann–Burchard test for steroids/triterpenoids, ferric chloride test for tannins).
- **Chromatography** — thin-layer chromatography (TLC) is explicitly recommended in the WHO guidelines as a rapid identity-confirmation method, comparing the sample's chromatographic fingerprint against a genuine reference sample or marker compound.
- **Quantitative assay** — titrimetric, spectrophotometric, or chromatographic (HPLC/GC) quantification of a specific marker compound, giving a numeric potency value that can be checked against a pharmacopoeial limit.

3.5 Biological Methods of Evaluation (Bioassay)

- Measures a drug's **pharmacological activity directly**, using a living system (whole animal, isolated tissue/organ, or microorganism) rather than measuring a chemical constituent.
- Used particularly when the exact chemical basis of activity is not fully known, or when biological potency does not correlate simply with the amount of one named constituent (e.g. digitalis glycoside mixtures, where potency is traditionally expressed in biological units via a cat or pigeon bioassay historically, now largely replaced by chemical assay where possible).
- **Microbiological assay** — measuring the zone of inhibition an antibiotic drug produces against a standard test organism, compared with a reference standard.
- **Limitation:** bioassays are typically slower, more expensive, and show more biological variability than chemical assays, so they are used mainly where a validated chemical alternative does not fully capture the drug's activity.

4. Physicochemical Parameters for Evaluation of Crude Drugs

Beyond the general methods of Section 3, pharmacopoeias lay down a specific set of numeric physicochemical parameters for individual crude drugs. Each targets a different kind of quality problem — purity, correct processing, or the presence of a specific functional constituent.

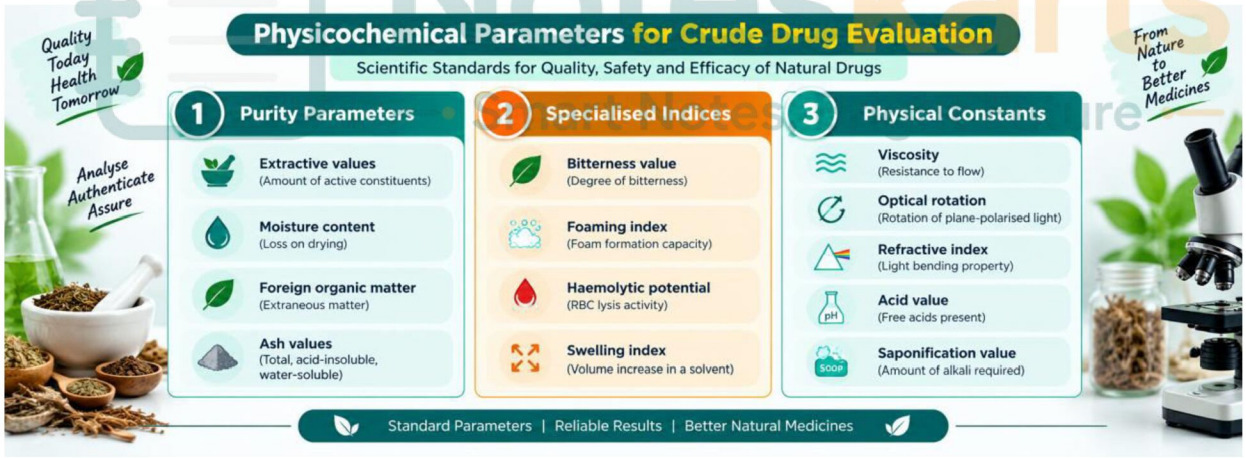

Figure 4.1 — The thirteen physicochemical parameters grouped by what they assess.

4.1 Extractive Values

- The amount of a drug that dissolves in a given solvent, expressed as a **percentage of the starting weight** — an indirect measure of how much extractable (potentially active) material the drug contains.
- **Water-soluble extractive** — material extracted by water; reflects water-soluble constituents such as sugars, mucilages, and some glycosides.

- **Alcohol-soluble extractive** — material extracted by ethanol (typically 90% or absolute, depending on the monograph); reflects alcohol-soluble constituents such as alkaloids, resins, and many glycosides.
- **Use:** an abnormally low extractive value compared with the pharmacopoeial limit is a classic sign of an **exhausted (spent) or heavily adulterated drug** (Section 2).

4.2 Moisture Content

- Also called **loss on drying** — the percentage weight lost when a sample is dried under specified conditions (oven-drying or azeotropic distillation).
- **Why it matters:** high moisture promotes enzymatic degradation, microbial growth, and mould (mycotoxin risk, Unit II); pharmacopoeias set an upper limit (commonly around 8–14% depending on the drug).

4.3 Foreign Organic Matter

- The percentage, by weight, of matter **other than the part of the plant specified** by the monograph — other plant parts, a different species entirely, or non-plant material (soil, stones).
- Determined by spreading a weighed sample and manually separating and weighing the foreign matter; pharmacopoeias set a maximum permissible percentage for each official drug.

4.4 Ash Values

- **Total ash** — the inorganic residue remaining after a sample is incinerated (ignited) at a controlled temperature; represents the total mineral/inorganic content, both physiological (naturally present in plant tissue) and non-physiological (adhering sand/earth).

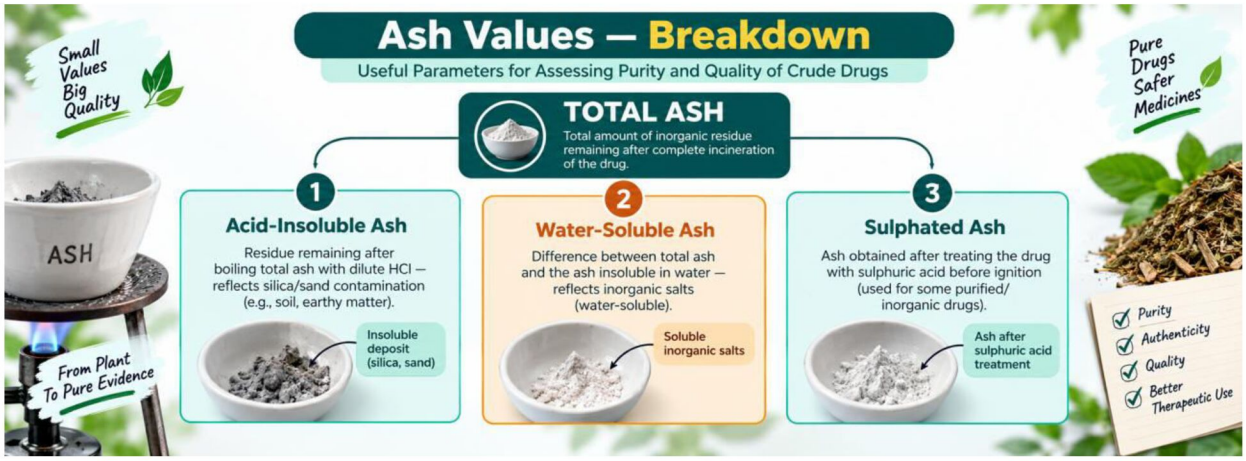

Figure 4.2 — Total ash and its three specialised sub-determinations.

- **Acid-insoluble ash** — the residue remaining after boiling total ash with dilute hydrochloric acid; specifically reflects **silica/sand contamination**, since most physiological plant-mineral ash dissolves in dilute acid.

- **Water-soluble ash** — the difference between total ash and the ash that is insoluble in water; reflects the proportion of inorganic salts genuinely present in the plant tissue.
- **Sulphated ash** — ash obtained after treating the sample with sulphuric acid before ignition; used for certain purified or largely inorganic materials, where it gives a more reproducible result than total ash alone.
- **Use:** a high acid-insoluble ash value is one of the most direct pharmacopoeial red flags for **soil/sand adulteration**.

4.5 Bitterness Value

- Expresses the **intensity of bitter taste** of a drug or extract, determined by a human taste panel comparing serial dilutions of the test sample against serial dilutions of a **quinine hydrochloride** reference standard.
- The bitterness value of quinine hydrochloride itself is fixed by convention at **200,000 units** (a 1-in-2,000 dilution of quinine hydrochloride is the faintest solution that still tastes reliably bitter to a trained panel); a test drug's bitterness value is calculated relative to this reference.
- **Relevance:** important for bitter tonics and appetite stimulants (e.g. Chirata, gentian), where bitterness itself is pharmacologically relevant, not merely an organoleptic curiosity.

4.6 Foaming Index

- Measures a drug's ability to produce a persistent **froth/foam** when shaken with water — a simple indirect indicator of **saponin content**, since saponins are surface-active glycosides.
- **Method (WHO-recommended):** a decoction of the drug is prepared and distributed into a series of tubes at increasing dilution; each tube is shaken for a fixed time, then left to stand; the foaming index is derived from the dilution at which a foam layer of a defined height (conventionally 1 cm) persists for a specified time.

4.7 Haemolytic Potential (Haemolytic Index)

- Measures a drug's ability to **rupture red blood cells (haemolysis)**, again used mainly as an indirect indicator of saponin content, since many saponins are haemolytic by disrupting the erythrocyte membrane.
- **Method:** the test drug's haemolytic activity on a standardised red-blood-cell suspension is compared against a reference saponin of known haemolytic activity, and expressed relative to that standard.

4.8 Swelling Index

- The **volume (in mL)** occupied by 1 g of a drug, including any adhering mucilage, after it has swollen in water for a specified time (commonly 4 hours), under specified conditions.
- **Relevance:** important for **mucilaginous drugs** used as bulk laxatives, e.g. isabgol (psyllium) husk and linseed, where the swelling/gel-forming capacity is directly linked to the drug's laxative mechanism, making this parameter a functional potency indicator, not just a purity check.

4.9 Viscosity

- A measure of a liquid's **resistance to flow**, determined using a viscometer.
- **Relevance:** important for mucilages and gums (e.g. tragacanth, acacia) used as suspending/thickening agents in pharmaceutical formulation, where consistent viscosity is essential for reproducible product performance.

4.10 Optical Rotation

- The angle through which a chiral (optically active) substance rotates the plane of **plane-polarised light**, measured with a polarimeter and expressed as **specific rotation [α]** under standard conditions of temperature and wavelength.
- **Relevance:** many natural constituents (sugars, alkaloids, terpenoids, volatile-oil components) are chiral; specific rotation is a quick, non-destructive identity and purity check — a value outside the expected range signals adulteration or degradation.

4.11 Refractive Index

- The ratio of the speed of light in a vacuum (or air) to its speed in the test substance, measured using a refractometer.
- **Relevance:** a standard identity/purity test for **fixed oils and volatile oils** — each oil has a characteristic refractive index range, and a value outside that range suggests adulteration with a different, cheaper oil.

4.12 Acid Value

- The number of **milligrams of potassium hydroxide (KOH)** required to neutralise the **free fatty acids** present in 1 g of a fat or oil.
- **Relevance:** a high acid value indicates a high proportion of free fatty acids, which usually means the oil has undergone hydrolytic rancidity/degradation during storage — making acid value a useful indicator of an oil's freshness and storage history.

4.13 Saponification Value

- The number of **milligrams of KOH** required to saponify (hydrolyse) the esters, and neutralise the free fatty acids, in 1 g of a fat or oil.

- **Relevance:** reflects the **average chain length/molecular weight** of the fatty acids esterified in the oil — oils rich in shorter-chain fatty acids have a higher saponification value than oils rich in long-chain fatty acids, so this parameter is used both for identity confirmation and for detecting adulteration with a chemically different oil.

4.14 Summary Table

Parameter	What it mainly detects	Typical relevant drug type
Extractive value	Exhaustion / dilution of active constituents	Any crude drug
Moisture content	Over-drying risk / under-drying (spoilage) risk	Any crude drug
Foreign organic matter	Admixture with unrelated plant/mineral matter	Any crude drug
Ash values	Mineral content; sand/soil contamination	Any crude drug, especially roots/rhizomes
Bitterness value	Bitter-principle strength	Bitter tonics (gentian, Chirata)
Foaming index	Saponin content (indirect)	Saponin-rich drugs (liquorice, senega)
Haemolytic potential	Saponin content (indirect)	Saponin-rich drugs
Swelling index	Mucilage / gel-forming capacity	Bulk laxatives (isabgol, linseed)
Viscosity	Consistency of gums/mucilages	Suspending/thickening agents (tragacanth)
Optical rotation	Identity/purity of chiral constituents	Volatile oils, alkaloids, sugars
Refractive index	Identity/purity of oils	Fixed and volatile oils
Acid value	Free fatty acid content (rancidity)	Fixed oils and fats
Saponification value	Average fatty-acid chain length; oil identity	Fixed oils and fats

5. DNA Barcoding

Every method covered so far — organoleptic, microscopic, physical, chemical, biological — examines the plant's **phenotype**: what it looks, smells, tastes, or reacts like. DNA barcoding instead examines the plant's **genotype** directly, offering a species-identification method that works even when morphological features have been destroyed by drying, powdering, or processing.

- **DNA barcoding** = identifying a species by sequencing a short, standardised region of its DNA (the "barcode") and comparing that sequence against a reference database of known, authenticated species.

- **Why it matters for pharmacognosy specifically:** many adulteration and substitution problems (Section 2) involve species that are extremely difficult, or impossible, to distinguish once the material is powdered, extracted, or otherwise processed — exactly the situation where morphological and even many chemical methods struggle, but DNA (if not too badly degraded) can still resolve the question.

5.1 Choice of Barcode Region

- Unlike animals, where a single mitochondrial gene (COI) works well as a near-universal barcode, **no single gene region works reliably across all plants**, because plant plastid genes mutate more slowly and more unevenly across lineages.
- The **Consortium for the Barcode of Life (CBOL) Plant Working Group (2009)** evaluated seven candidate chloroplast regions and recommended a **two-locus core barcode: rbcL + matK**.
- **rbcL** — easy to amplify and sequence reliably across almost all plants (high universality), but has relatively low species-level discriminating power on its own.
- **matK** — evolves faster and gives better species-level discrimination, but is harder to amplify consistently across all plant groups (lower universality).
- **ITS / ITS2** (internal transcribed spacer, a nuclear ribosomal region) — often added as a supplementary marker, and specifically recommended by some researchers as especially well-suited for authenticating **medicinal plant and herbal-medicine material**, since it tends to show better species-level resolution for these applications than rbcL + matK alone.

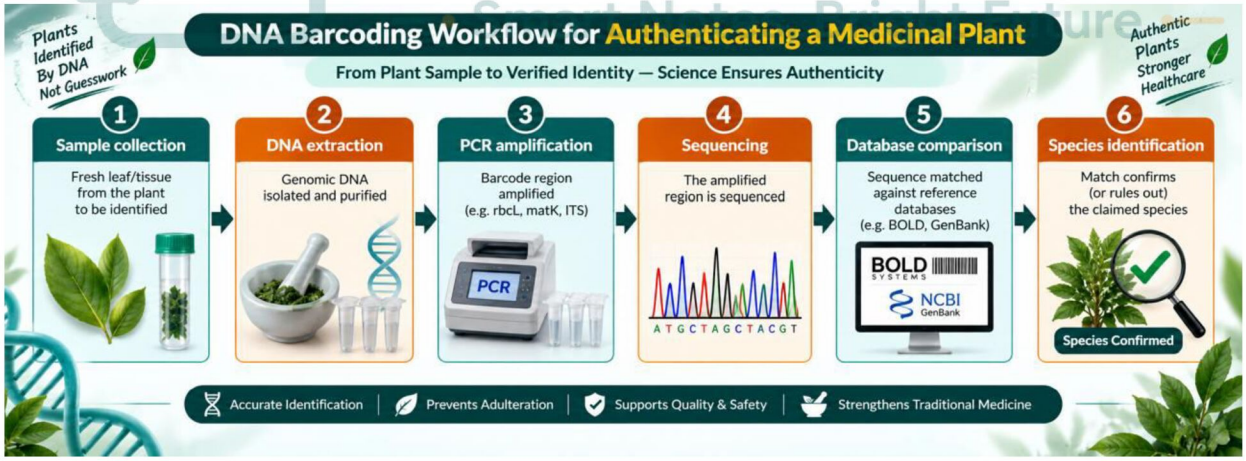

Figure 5.1 — The DNA barcoding workflow used to authenticate a medicinal plant sample.

5.2 Applications in Pharmacognosy and Quality Control

- **Authenticating raw material** — confirming that a herbal drug consignment is genuinely the labelled species, even from powdered, dried, or minimally processed material where morphological identification is impossible.

- **Detecting substitution and admixture** — identifying a cheaper substitute species mixed into, or entirely replacing, the genuine drug (directly addressing the Section 2 problem).
- **Authenticating multi-ingredient herbal formulations** — DNA metabarcoding (sequencing all the DNA present, not just one target species) can, in principle, confirm which plant species are actually present in a complex polyherbal product.
- **Supporting conservation and trade regulation** — confirming species identity in traded material helps enforce CITES and other trade restrictions on endangered medicinal plants (linking to the Kutki/Chirata discussion in Unit II).

5.3 Limitations

- **DNA degradation** – Heat, storage, and processing can damage DNA.
- **Incomplete databases** – Correct identification requires a **reliable reference sequence**.
- **Closely related species** – Standard **rbcL and matK** markers may not clearly distinguish some species.
- **High cost** – Requires **specialised equipment and laboratory facilities**.

Exam framing: DNA barcoding is best understood as the most **specific but least accessible** tool in the pharmacognosist's evaluation toolkit — organoleptic and microscopic methods remain the fast, low-cost front line; physicochemical parameters give standardised, pharmacopoeial numeric checkpoints; and DNA barcoding is the definitive method reached for when species identity absolutely must be confirmed beyond doubt.