Industrial Pharmacognosy

Unit IV

n, Characterization, Commercial Production and Analysis of Bioactive Phytocons

Pills & Python Notes

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

Syllabus coverage: Unit IV (8 hours).

CHAPTER 7

Isolation & Characterization of Six Phytoconstituents

7.1 Learning Objectives

1. Name the six phytoconstituents and their plant sources.
2. Describe the isolation workflow for each.
3. Explain how each is characterized/identified.

DEFINITIONS

- **Isolation:** Separating one pure compound from the complex plant mixture.
- **Characterization:** Proving what the isolated compound is (structure, purity) using chemical tests and spectroscopy.
- **Phytoconstituent:** A chemical compound naturally present in a plant.

7.3 Terminology

Term	Simple meaning
Marker	Compound used for QC (may or may not be the active)
Active constituent	The compound mainly responsible for the effect
Crystallization	Purifying by forming pure crystals
Column chromatography	Separation on a tall silica column — the classic isolation tool

7.4 The six phytoconstituents — master table

Constituent	Plant source	Chemical class	Simple use
Artemisinin	Artemisia annua (sweet wormwood) leaves	Sesquiterpene lactone (has a rare peroxide bridge)	Antimalarial (Nobel Prize 2015)
Sennosides (A & B)	Senna leaves/pods (Cassia angustifolia)	Anthraquinone glycosides (dianthrones)	Stimulant laxative
Withanolides	Ashwagandha roots/leaves (Withania somnifera)	Steroidal lactones	Adaptogen/antistress markers
Boswellic acid	Shallaki gum resin (Boswellia serrata)	Pentacyclic triterpenic acids	Anti-inflammatory (joints)
Atropine	Belladonna/Atropa (Atropa belladonna)	Tropane alkaloid	Anticholinergic (eye drops dilate pupil; pre-anaesthetic)
Lycopene	Tomato (Solanum lycopersicum) and other red fruits	Carotenoid (acyclic)	Antioxidant (prostate/heart health supplements)

7.5 General isolation workflow (applies to all six)

1. **Extraction:** Dried powdered plant + suitable solvent (ethanol/water for glycosides; non-polar for lycopene; etc.)

2. **Concentration** under vacuum.
3. **Fractionation:** liquid–liquid partition (separate by polarity) or precipitation.
4. **Column chromatography** on silica gel — collect fractions, monitor by TLC.
5. **Crystallization** — pure crystals of the constituent.
6. **Characterization:** melting point, TLC/HPLC vs standard, IR/NMR/MS for structure, optical rotation where relevant.

7.6 Individual notes (simple)

- **Artemisinin:** extracted with petroleum ether/low-polarity solvent (it is heat-sensitive — avoid prolonged boiling); crystallized; identified by TLC/HPLC and the characteristic peroxide confirmed spectroscopically.
- **Sennosides:** extracted with water/alcohol; purified by precipitation and column; identified by Bornträger reaction (anthraquinones give pink/red colour with alkali) + HPLC assay.
- **Withanolides:** alcoholic extraction of roots; column chromatography; characterized by HPLC fingerprint (withaferin A, withanolide A as markers) + NMR/MS.
- **Boswellic acid:** gum resin extracted with alcohol; acids separated; AKBA (3-O-acetyl-11-keto-β-boswellic acid) is the key marker; HPLC assay.
- **Atropine:** alkaloid — extracted with acid (alkaloids form water-soluble salts), basified, extracted into organic solvent, crystallized as salt; identified by alkaloid tests (Mayer's/Dragendorff's) + HPLC.
- **Lycopene:** extracted from tomato paste with non-polar solvents; protected from light/oxygen (it oxidizes easily); identified by deep red colour, UV-Vis (carotenoid bands) and HPLC.

7.7 Diagrams (describe for drawing)

- **Diagram 1 — Isolation funnel workflow:** plant powder → solvent extraction → partition → column → TLC-monitored fractions → crystals.
- **Diagram 2 — Six-constituent wheel:** centre "bioactive constituents", six spokes with plant + use.

INFOGRAPHIC CONCEPT

The Isolation Detective Path Plant → Extract → Partition → Column → Crystals → Proof! **Proof tools:** TLC/HPLC (matches standard?) → MP (sharp?) → IR/NMR/MS (structure?) → chemical test (Bornträger for sennosides, alkaloid reagents for atropine). **Remember the six: A**rtemisinin (malaria), **S**ennosides (laxative), **W**ithanolides (stress), **B**oswellic acid (joints), **A**tropine (eye), **L**ycopene (antioxidant) — "**A S**uper **W**ise **B**otanist **A**lways **L**earns".

EXAMPLES

1. Artemisinin's peroxide bridge is unusual — that is why its structure proof needed MS + NMR.
2. Senna pods vs leaves: both contain sennosides; pods often preferred for milder action.
3. Tomato oleoresin standardized for lycopene % is sold as antioxidant capsules.

ANALOGIES

- **Isolation = finding one friend in a crowded mela:** extraction gathers the crowd, chromatography separates them one by one, crystallization picks your friend out.
- **Characterization = Aadhaar verification:** TLC is the photo match; NMR/MS is the full biometric.

PHARMACY RELEVANCE

These six are industrial raw materials — artemisinin for antimalarials, sennosides for laxatives, withanolides for adaptogen brands. A pharmacist must know their sources, tests and handling (e.g., atropine is potent/toxic; lycopene needs light protection).

INDIA CONTEXT

India cultivates Artemisia annua (including CIMAP varieties) for artemisinin; Senna is grown in Tamil Nadu/Rajasthan; Boswellia is collected in central India; ashwagandha in MP/Rajasthan.

KEY POINTS

- Six constituents: artemisinin, sennosides, withanolides, boswellic acid, atropine, lycopene.
- Workflow: extract → partition → column → crystallize → characterize.
- Characterization: TLC/HPLC + MP + IR/NMR/MS + specific chemical tests.
- Alkaloids need acid–base extraction; carotenoids need light protection.

COMMON CONFUSIONS

- **Isolation vs characterization:** isolation = getting it pure; characterization = proving what it is.
- **Sennosides vs senna:** sennosides are the chemicals; senna is the plant.
- **Atropine vs belladonna:** atropine is the alkaloid; belladonna is the plant.

COMMON MISTAKES

- Heating artemisinin extracts strongly — it is heat-sensitive.
- Exposing lycopene to light/air during isolation.
- Calling withanolides "alkaloids" — they are steroidal lactones.

MNEMONIC

- Six: **ASWBAL** — "**A S**uper **W**ise **B**otanist **A**lways **L**earns".
- Sources: Artemisia→Artemisinin; Senna→Sennosides; Withania→Withanolides; Boswellia→Boswellic acid; Atropa→Atropine; Tomato (Lycopersicon)→Lycopene — the plant name hides inside the chemical name!

EXAM SUMMARY

Six bioactive phytoconstituents: artemisinin (Artemisia annua, antimalarial), sennosides (senna, laxative), withanolides (ashwagandha, adaptogen), boswellic acid (Boswellia serrata, anti-inflammatory), atropine (Atropa belladonna, anticholinergic), lycopene (tomato, antioxidant). Isolation follows extract → partition → column chromatography → crystallization; characterization uses TLC/HPLC, melting point, IR/NMR/MS and class-specific chemical tests.

ONE-MINUTE REVISION

- ASWBAL: six constituents.
- Workflow: extract → partition → column → crystals → proof.
- Proof: TLC/HPLC + MP + spectra + chemical tests.
- Plant name ≈ chemical name.

7.19 Exam questions

Short answer:

1. Give the source and use of artemisinin and lycopene.
2. Outline the general isolation workflow of a phytoconstituent.
3. Which chemical test identifies anthraquinone glycosides?
4. Why is atropine extracted via acid–base treatment?

Long answer:

5. Describe the isolation, characterization and uses of any two: artemisinin, sennosides, withanolides.
6. Write notes on boswellic acid, atropine and lycopene: source, class, isolation hints, uses.

MCQs:

7. Artemisinin contains a rare: (a) Peroxide bridge (b) Sugar unit (c) Steroid ring (d) Carotenoid chain — **Answer: (a)**
8. Bornträger test is for: (a) Alkaloids (b) Anthraquinones (c) Carotenoids (d) Triterpenes — **Answer: (b)**
9. Withanolides are: (a) Alkaloids (b) Steroidal lactones (c) Flavonoids (d) Tannins — **Answer: (b)**
10. Lycopene is obtained from: (a) Belladonna (b) Tomato (c) Senna (d) Artemisia — **Answer: (b)**

Viva:

11. Why must lycopene be protected from light?
12. What is AKBA?

Application-based:

13. Your column fractions of ashwagandha extract show three TLC spots matching withanolide standards. How do you decide which to pool?

CHAPTER 8

Commercial Production & Analysis of Phytoconstituents

8.1 Learning Objectives

1. Describe how the six phytoconstituents are produced at commercial scale.
2. List the analytical methods used for their quality control.
3. Explain stability and storage considerations.

DEFINITIONS

- **Commercial production:** Making a phytoconstituent in tonnes/kilos (not milligrams) — needs high-yield plant varieties, efficient extraction and cost control.
- **Good Agricultural and Collection Practices (GACP):** WHO guidelines for growing/collecting medicinal plants so the raw material is consistent and contaminant-free.
- **Batch-to-batch consistency:** Every production lot meeting the same specification.

8.3 Terminology

Term	Simple meaning
Yield	Amount of pure compound per kg of plant (e.g., 0.8% artemisinin)
Mother liquor	The leftover liquid after crystallization (still contains product — recycled)
Specification	The written quality limits a batch must meet
Stability study	Testing how the product degrades over time/heat/humidity

8.4 Explanation — Commercial production (per constituent, simple)

Common commercial flow:

1. High-yield variety cultivation under GACP (contract farming).
2. Harvest at the right stage (actives peak at specific times).
3. Industrial extraction (large percolators/counter-current extractors).
4. Concentration, purification (industrial columns or crystallization tanks).
5. Drying, milling, blending to specification.
6. QC release → packing (light/moisture-proof) → market.

Constituent-specific notes:

- **Artemisinin:** high-artemisinin Artemisia annua varieties; gentle extraction (heat-sensitive); crystallization; stored cool and dark.
- **Sennosides:** senna leaf/pod cultivated in Tamil Nadu/Rajasthan; aqueous-alcoholic extraction; spray-dried standardized powder (e.g., 20% sennosides — typical industry grade; confirm spec).
- **Withanolides:** ashwagandha roots from MP/Rajasthan; alcoholic extraction; HPLC-standardized extract (withanolide %).

- **Boswellic acid:** gum resin tapped from Boswellia trees; alcohol extraction; enriched to boswellic acids/AKBA %.
- **Atropine:** cultivated belladonna; strict control (potent alkaloid — narcotic/poison regulations apply); produced as atropine sulphate in licensed facilities.
- **Lycopene:** tomato oleoresin; extraction under nitrogen/dark; formulated in oil/beadlets to prevent oxidation.

8.5 Analysis / QC of the six (table)

Constituent	Identity tests	Assay method	Special QC point
Artemisinin	TLC/HPLC vs standard	HPLC	Heat & light protection; peroxide intact
Sennosides	Bornträger + TLC/HPLC	HPLC	Microbial limits (leaf products)
Withanolides	HPLC fingerprint	HPLC (withanolide %)	Withaferin A/withanolide A markers
Boswellic acid	TLC/HPLC	HPLC (AKBA %)	Resin adulteration check
Atropine	Alkaloid tests + HPLC	HPLC/titration	Potency & poison-law compliance
Lycopene	UV-Vis + HPLC	HPLC/UV-Vis	Oxidation, light protection

Plus universal tests: loss on drying, ash values, heavy metals (AAS), microbial limits, residual solvents.

8.6 Flowchart — Commercial batch release

Raw herb (GACP) → extraction → purification → drying → blending to spec → QC (identity, assay, safety tests) → stability data → batch release → dispatch.

8.7 Diagrams (describe for drawing)

- **Diagram 1 — Industrial extractor:** large vertical percolator with solvent in, extract out, plant bed inside.
- **Diagram 2 — Stability chamber:** shelves of packed samples at 30°C/65% RH with calendar — "test at 0, 3, 6 months".

INFOGRAPHIC CONCEPT

Tonnes, Not Test Tubes Right variety → right harvest time → big extractor → purify → dry → blend to spec → test everything → pack against light/moisture → ship. **Golden rules:** GACP at farm + GMP at factory + HPLC at lab = batch-to-batch consistency.

EXAMPLES

1. A sennoside 20% powder lot is blended from 24% and 16% sub-lots to hit exactly 20%.
2. Lycopene beadlets in an amber bottle with nitrogen flushing — oxidation prevented.
3. Atropine sulphate is stored under lock as per poison regulations.

ANALOGIES

- **Commercial production = cooking for a wedding, not for two people:** same recipe, but you need bigger vessels, more helpers and strict timing.
- **Stability study = expiry-date science:** like checking how long milk lasts in the fridge vs outside.

PHARMACY RELEVANCE

Production pharmacists scale up extraction, validate cleaning, and release batches; regulatory pharmacists compile the quality dossier for buyers/regulators.

INDIA CONTEXT

India is a major producer of sennosides, withanolide extracts and boswellic acid for world markets; CIMAP develops high-yield varieties; exports need WHO-GMP and buyer-specific specs.

KEY POINTS

- Scale-up needs: high-yield varieties, GACP farming, industrial extractors, crystallization/purification trains.
- Each constituent has identity + assay + safety tests.
- Special care: artemisinin (heat), lycopene (light/oxygen), atropine (poison control).
- Stability studies decide shelf life and storage label.

COMMON CONFUSIONS

- **Extract vs pure constituent:** an extract is a mixture standardized to a %; the pure constituent (e.g., 99% artemisinin) is a single chemical.
- **Yield vs assay:** yield = how much you got; assay = how pure/strong it is.

COMMON MISTAKES

- Ignoring harvest timing — active content varies with season/stage.
- Storing lycopene in clear bottles.
- Forgetting residual-solvent testing for extracted products.

MNEMONIC

- Commercial chain: **"V H E P D B T"** — **V**ariety, **H**arvest, **E**xtract, **P**urify, **D**ry, **B**lend, **T**est.

EXAM SUMMARY

Commercial production of the six phytoconstituents uses GACP-grown high-yield varieties, industrial extraction, purification/crystallization, blending to specification and full QC (identity, HPLC assay, heavy metals, microbial limits, residual solvents). Special handling: heat (artemisinin), light/oxygen (lycopene), poison control (atropine). Stability studies fix shelf life.

ONE-MINUTE REVISION

- V H E P D B T: commercial chain.
- Six QC tables: identity + HPLC assay + special point.
- Universal tests: LOD, ash, AAS metals, micro, residual solvents.

8.19 Exam questions

Short answer:

1. What is GACP and why does industry need it?
2. How is a sennoside 20% powder commercially standardized?
3. List stability-indicating considerations for lycopene.
4. Why is atropine production specially controlled?

Long answer:

5. Describe the commercial production and quality control of any two phytoconstituents among the six.
6. Write a note on analytical methods for quality evaluation of isolated phytoconstituents.

MCQs:

7. Counter-current extraction is used for: (a) Lab testing (b) Industrial scale extraction (c) Tablet coating (d) Fermentation — **Answer: (b)**
8. Residual solvents are tested because: (a) They add flavour (b) They may be toxic (c) They increase yield (d) They preserve colour — **Answer: (b)**
9. Which needs nitrogen flushing? (a) Sennosides (b) Lycopene (c) Atropine (d) Boswellic acid — **Answer: (b)**
10. Batch-to-batch consistency is ensured mainly by: (a) Advertising (b) GMP + specifications (c) Low price (d) Big factory — **Answer: (b)**

Viva:

11. What is mother liquor and why is it recycled?
12. Difference between yield and assay.

Application-based:

13. Two ashwagandha extract lots assay 3.1% and 1.9% withanolides. How do you make a 2.5% batch? Show the blending logic.

UNIT IV — REFERENCES

1. Indian Drug Manufacturers' Association. Indian Herbal Pharmacopoeia.
2. Indian Pharmacopoeia Commission. Indian Pharmacopoeia. Government of India.
3. Ayurvedic Pharmacopoeia Committee. Ayurvedic Pharmacopoeia of India. Government of India, Ministry of AYUSH.
4. World Health Organization. Guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants.
5. International Council for Harmonisation. ICH Guidelines on Quality, Safety, Efficacy and Multidisciplinary Topics.