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# PHARMACOGNOSY AND PHYTOCHEMISTRY - II

## UNIT 3

TOPIC :

- **c) Alkaloids:** Atropine,Quinine,Reserpine,Caffeine

# Alkaloids

- Alkaloids are one of the most important and diverse groups of naturally occurring nitrogen-containing compounds found mainly in plants.
  They usually have strong physiological and pharmacological effects on humans and animals.
- The name “alkaloid” comes from “alkali-like”, referring to their basic (alkaline) nature.
- Alkaloids are basic, nitrogenous organic compounds of plant origin, usually having heterocyclic nitrogen, and possessing definite physiological activity.
- Alkaloids are naturally occurring organic bases containing nitrogen, which show marked pharmacological actions.

## General Characteristics

- Contain one or more nitrogen atoms (usually in a ring structure).
- Generally bitter in taste.
- Alkaline in nature — form salts with acids.
- Mostly solid, crystalline substances (except nicotine and coniine, which are liquids).
- Soluble in organic solvents but form water-soluble salts with acids.
- Show specific physiological actions (e.g., morphine – analgesic, quinine – antimalarial).

## Classification of Alkaloids

### A. Based on Chemical Structure

| Type | Example | Source | Uses |
|---|---|---|---|
| Pyridine & Piperidine Alkaloids | Nicotine, Coniine | Tobacco, Hemlock | Stimulant, Toxic |
| Tropane Alkaloids | Atropine, Hyoscyamine | *Atropa belladonna*, *Datura* | Anticholinergic, Mydriatic |
| Quinoline Alkaloids | Quinine, Quinidine | *Cinchona bark* | Antimalarial, Antipyretic |
| Isoquinoline Alkaloids | Morphine, Codeine | *Papaver somniferum* (Opium) | Analgesic, Antitussive |
| Indole Alkaloids | Reserpine, Vincristine | *Rauwolfia*, *Vinca rosea* | Antihypertensive, Anticancer |
| Imidazole Alkaloids | Pilocarpine | *Pilocarpus jaborandi* | Miotic, Treats glaucoma |
| Steroidal Alkaloids | Solanine | *Solanum* species | Antifungal |
| Purine Alkaloids | Caffeine, Theobromine | *Tea, Coffee, Cocoa* | CNS stimulant |
| Quinazoline Alkaloids | Vasicine | *Adhatoda vasica* | Bronchodilator |

### B. Based on Pharmacological Action

| Action | Example | Source |
|---|---|---|
| Analgesic | Morphine, Codeine | *Opium* |
| Antimalarial | Quinine | *Cinchona* |
| Antihypertensive | Reserpine | *Rauwolfia serpentina* |
| CNS Stimulant | Caffeine | *Tea, Coffee* |
| Mydriatic | Atropine | *Belladonna* |
| Anticancer | Vincristine, Vinblastine | *Vinca rosea* |
| Antitussive | Codeine | *Opium* |

## Therapeutic Uses

- Analgesic and Narcotic – Morphine, Codeine
- Antimalarial – Quinine
- Anticancer – Vincristine, Vinblastine
- CNS Stimulant – Caffeine
- Antihypertensive – Reserpine
- Anticholinergic (Mydriatic) – Atropine
- Bronchodilator – Vasicine
- Local Anesthetic – Cocaine

## Commercial Applications

- Used in pharmaceutical preparations (pain relief, cough syrups, heart drugs).
- Used in chemical and forensic studies.
- Serve as precursors for semisynthetic drugs (e.g., heroin from morphine).
- Used in research and standardization of herbal medicines.

# Isolation, Identification, and Analysis of Atropine

- Atropine is a tropane alkaloid obtained mainly from Atropa belladonna (Deadly Nightshade) and other Solanaceous plants such as Datura stramonium and Hyoscyamus niger.
- It is a tertiary amine alkaloid with potent anticholinergic (parasympatholytic) activity.

## Chemical nature:

- Class: Tropane alkaloid
- Chemical formula: $C_{17}H_{23}NO_3$
- Molecular weight: 289.37
- Structure: Ester of tropic acid and tropine

## Source

| Plant Name | Family | Part Used | Major Alkaloid |
|---|---|---|---|
| *Atropa belladonna* | Solanaceae | Leaves and roots | Atropine |
| *Datura stramonium* | Solanaceae | Seeds and leaves | Atropine, Hyoscyamine |
| *Hyoscyamus niger* | Solanaceae | Leaves | Hyoscyamine |

# Isolation of Atropine

## Steps for Isolation:

1. **Collection and Drying:**
   - Leaves or roots of *Atropa belladonna* are collected, cleaned, and shade-dried.
2. **Powdering:**
   - Dried material is finely powdered to increase surface area for extraction.
3. **Extraction:**
   - Powdered drug is moistened with dilute ammonia (to free the alkaloid base) and extracted with ether or chloroform using a Soxhlet apparatus.
   - Alkaloids dissolve in the organic solvent.
4. **Filtration and Concentration:**
   - The extract is filtered and concentrated under reduced pressure to remove the solvent.

5. **Acid Extraction:**
    - The concentrated extract is shaken with dilute sulfuric acid.
    - Alkaloids form soluble alkaloid sulfate salts, which move to the aqueous layer.
6. **Basification:**
    - The acidic aqueous layer is made alkaline with dilute ammonia to liberate free atropine base.
7. **Re-extraction:**
    - The liberated free base is extracted again with chloroform.
8. **Evaporation and Crystallization:**
    - The chloroform extract is evaporated, and the residue is recrystallized from ethanol or ether to obtain pure atropine crystals.

## Identification of Atropine

Identification confirms the presence and purity of atropine using physical, chemical, and instrumental methods.

### A. Physical Characteristics:

- Appearance: Colorless, odorless crystals
- Taste: Bitter
- Melting point: 115–120°C
- Solubility: Soluble in alcohol, chloroform, ether; slightly soluble in water

### B. Chemical Tests:

1. Vitali–Morin Test (specific for tropane alkaloids):
    - Dissolve atropine in fuming nitric acid and evaporate to dryness.
    - Add alcoholic KOH → a violet color appears.
      → Confirms presence of tropane nucleus.
2. Mayer's Test:
    - Add Mayer's reagent ($K_2HgI_4$) to aqueous solution of atropine.

- Formation of a creamy white precipitate indicates presence of alkaloid.

3. Dragendorff's Test:
   - Add Dragendorff's reagent ($KBiI_4$) → orange precipitate appears — confirms alkaloids.
4. Wagner's Test:
   - Add Wagner's reagent (iodine in KI) → reddish-brown precipitate forms.

## C. Spectroscopic Identification:

1. UV-Visible Spectroscopy:
   - Atropine shows absorption maxima (λmax) around 255 nm.
2. IR Spectroscopy:
   - C=O (ester) stretching: 1735 $cm^{-1}$
   - O–H (alcohol) stretching: 3400 $cm^{-1}$
   - C–N stretching: 1200 $cm^{-1}$
3. NMR Spectroscopy:
   - Distinct signals for tropine ($C_8H_{15}NO$) and tropic acid components.
4. Mass Spectrometry:
   - Molecular ion peak at m/z 289, confirming the molecular weight of atropine.

# Analysis of Atropine

## A. Qualitative Analysis:

- Confirmed by Vitali–Morin test, TLC, and Dragendorff's reagent.
- Helps identify atropine in plant extracts or pharmaceutical preparations.

## B. Quantitative Analysis:

1. UV Spectrophotometric Method:
   - Atropine is dissolved in ethanol or methanol.

- Absorbance measured at λmax 255 nm.
- Concentration calculated using Beer–Lambert's law.

2. HPLC (High-Performance Liquid Chromatography):
   - Column: C18 reverse-phase
   - Mobile phase: Acetonitrile : Water (60:40), adjusted to pH 3.0 with phosphoric acid
   - Flow rate: 1.0 mL/min
   - Detector: UV detector at 254 nm
   - Retention time: Around 3–5 minutes
   - Used for accurate estimation of atropine in formulations.
3. Titrimetric Method:
   - Aqueous atropine solution is titrated with 0.02 N perchloric acid using methyl orange as an indicator.
   - End point: color change from yellow to red.

## Applications

- Mydriatic: Dilates pupils for ophthalmic examinations.
- Antispasmodic: Relieves smooth muscle spasms of GI tract and urinary system.
- Pre-anesthetic agent: Reduces salivary and bronchial secretions.
- Antidote: Used in organophosphate and carbamate poisoning.
- Antiparkinsonian and bronchodilator effects.

# Isolation, Identification and Analysis of Quinine

- Quinine is a natural alkaloid obtained from the bark of Cinchona species (*Cinchona officinalis*, *C. ledgeriana*, *C. succirubra*).
- It is a bitter-tasting crystalline alkaloid belonging to the quinoline group.
- Quinine and its derivatives (like quinidine) are used as antimalarial agents, antipyretics, and analgesics.

Molecular Formula: $C_{20}H_{24}N_2O_2$
Molecular Weight: 324.42 g/mol

## Isolation of Quinine

### Source:

- Plant: *Cinchona officinalis* or *Cinchona ledgeriana*
- Family: Rubiaceae
- Part used: Bark

### Procedure:

1. Collection and Drying:
    - Cinchona bark is collected, cleaned, and dried in shade.
2. Powdering:
    - The dried bark is powdered finely to increase surface area.
3. Extraction:
    - The powdered bark is treated with dilute acid ($H_2SO_4$) to form quinine sulfate, which is soluble in water.
    - The solution is filtered to remove impurities.
4. Basification:
    - The filtrate is made alkaline with ammonia or sodium hydroxide to liberate free quinine base, which is insoluble in water and separates out.
5. Solvent Extraction:
    - The free base is extracted using chloroform or ether.
    - The organic layer is separated and concentrated under reduced pressure.
6. Purification:

- The crude quinine is purified by crystallization from ethanol or ether, yielding pure quinine crystals.

## Identification of Quinine

Identification is done using physical, chemical, and instrumental methods.

### A. Physical Properties:

- Appearance: White, needle-like crystals
- Taste: Very bitter
- Solubility: Soluble in alcohol, ether, and chloroform; sparingly soluble in water
- Melting point: 177°C

### B. Chemical Tests:

1. Thalleioquin Test (Green Fluorescence Test):
   - Add few drops of bromine water and then ammonia to the quinine solution.
   - A green fluorescence appears — confirms the presence of quinine (quinoline ring).
2. Fluorescence Test:
   - Quinine solution in dilute acid shows blue fluorescence under UV light.
3. Mayer's Test:
   - Add Mayer's reagent ($HgCl_2$ + KI) → White or cream precipitate indicates alkaloid presence.
4. Dragendorff's Test:
   - Orange or reddish-brown precipitate forms → confirms alkaloid.

### C. TLC Identification:

- Stationary phase: Silica gel G
- Mobile phase: Chloroform : Methanol (9:1)
- Detection: Under UV light (365 nm) — blue fluorescence spot

- Rf value: Around 0.3–0.4

## Analysis of Quinine

### A. Qualitative Analysis:

- Detection of quinine in plant extracts using TLC or fluorescence under UV.
- Comparison of Rf value with standard quinine confirms its presence.

### B. Quantitative Analysis:

1. UV Spectrophotometric Method:
    - Prepare a standard solution of quinine in dilute $H_2SO_4$.
    - Measure absorbance at 330 nm.
    - Use Beer-Lambert's law to calculate concentration.
2. Fluorimetric Method:
    - Based on the blue fluorescence of quinine sulfate in dilute acid.
    - Intensity is proportional to concentration (highly sensitive method).
3. HPLC Method:
    - Column: C18 (reverse phase)
    - Mobile phase: Methanol : Water (70:30)
    - Detector: UV detector at 330 nm
    - Flow rate: 1 mL/min
    - Used for accurate quantification in Cinchona extracts or formulations.

### Applications of Quinine

- Antimalarial: Acts on the asexual erythrocytic stage of *Plasmodium falciparum*.
- Antipyretic and Analgesic: Reduces fever and pain.
- Cardiac stimulant: Used in small doses to stimulate heart function.
- Antispasmodic: Helps relieve muscle cramps and leg pains.

# Isolation, Identification and Analysis of Reserpine

- Reserpine is an indole alkaloid obtained from the roots of *Rauwolfia serpentina* (Indian snakeroot).
- It was one of the first alkaloids used for the treatment of hypertension and mental disorders.
- It acts by depleting catecholamines (dopamine, norepinephrine, serotonin) from nerve endings, leading to sedation and blood pressure reduction.

Source: *Rauwolfia serpentina*
Family: Apocynaceae
Molecular Formula: $C_{33}H_{40}N_2O_9$
Molecular Weight: 608.68 g/mol

## Isolation of Reserpine

### Plant Source:

- Roots of *Rauwolfia serpentina* (Sarpagandha).
- Contains several indole alkaloids: reserpine, rescinnamine, ajmaline, serpentine, etc.

### Steps of Isolation:

1. Collection and Drying:
    - Fresh roots of *Rauwolfia serpentina* are cleaned, cut, and dried under shade.
2. Powdering:
    - The dried roots are finely powdered to increase extraction efficiency.
3. Defatting:
    - Powdered root is first extracted with petroleum ether (40–60°C) to remove fats, oils, and chlorophyll.
4. Extraction of Alkaloids:
    - The defatted material is extracted with acidified alcohol (ethanol + dilute $H_2SO_4$) to dissolve alkaloids as their soluble salts.
5. Filtration and Concentration:
    - The extract is filtered and concentrated under reduced pressure to obtain a thick residue.

6. Basification:
    - The concentrated extract is made alkaline with ammonia or $Na_2CO_3$, which liberates the free alkaloids.
7. Solvent Extraction:
    - The liberated alkaloids are extracted with chloroform or benzene.
8. Purification:
    - The crude alkaloid mixture is separated using column chromatography on silica gel.
    - Elution solvent: Chloroform : Methanol (99:1)
    - The fraction containing reserpine is collected and crystallized from methanol or acetone to yield pure reserpine crystals.

## Identification of Reserpine

Identification is done using physical, chemical, and instrumental methods.

### A. Physical Properties:

- Appearance: White or slightly yellow crystalline powder
- Taste: Bitter
- Solubility: Soluble in chloroform, methanol, and alcohol; insoluble in water
- Melting Point: 265–268°C (decomposes)

### B. Chemical Tests:

1. Van Urk's Test (for Indole group):
    - Reserpine solution + p-dimethylaminobenzaldehyde (Ehrlich's reagent) + $H_2SO_4$ → Violet or blue color confirms indole nucleus.
2. Mayer's Test:
    - Add Mayer's reagent ($HgCl_2$ + KI) → White or cream precipitate confirms alkaloids.
3. Dragendorff's Test:
    - Add Dragendorff's reagent (Bi-KI complex) → Orange or brown precipitate forms → confirms alkaloid.

4. Wagner's Test:
    - Add Wagner's reagent ($I_2$ + KI) → Brown precipitate indicates alkaloid presence.

## C. TLC Identification:

- Stationary phase: Silica gel G
- Mobile phase: Chloroform : Methanol (9:1)
- Detection: Under UV light (365 nm) or by spraying with Dragendorff's reagent
- Rf value: Around 0.55

## D. Spectroscopic Identification:

1. UV-Visible Spectroscopy:
    - λmax ≈ 268 nm (due to indole chromophore).
2. IR Spectroscopy:
    - Characteristic peaks for:
        - –NH (indole): 3400 $cm^{-1}$
        - –C=O (ester): 1730 $cm^{-1}$
        - –C–O–C– (ether): 1100 $cm^{-1}$
3. Mass Spectrometry:
    - Molecular ion peak at m/z = 608 confirms molecular weight.
4. NMR Spectroscopy ($^1H$ and $^{13}C$):
    - Shows signals for indole ring and ester functional groups.

# Analysis of Reserpine

## A. Qualitative Analysis:

- Performed using TLC or HPTLC fingerprinting of Rauwolfia extract.
- Comparison of Rf value and fluorescence with standard reserpine confirms presence.

## B. Quantitative Analysis:

1. Spectrophotometric Method:
    - Reserpine shows maximum absorption at λmax 268 nm in methanol.
    - Concentration is determined using Beer-Lambert's law.
2. HPLC Method:
    - Column: C18 reverse-phase
    - Mobile phase: Methanol : Water : Acetic acid (65:30:5)
    - Flow rate: 1 mL/min
    - Detector: UV at 268 nm
    - Retention time: ~5–7 min
    - Used for accurate quantification of reserpine in *Rauwolfia* extract or tablets.
3. Fluorimetric Method:
    - Based on the fluorescence of reserpine solution under UV light (excitation at 260 nm, emission at 380 nm).

## Applications of Reserpine

- Antihypertensive: Used to lower blood pressure by depleting catecholamines.
- Antipsychotic: Used in the treatment of schizophrenia and mental disorders.
- Sedative: Induces calmness and relaxation by CNS depression.
- Research use: As a biochemical tool for studying neurotransmitter storage and release.

# Isolation, Identification and Analysis of Caffeine

- Caffeine is a purine alkaloid belonging to the xanthine alkaloid group.
- It is a CNS stimulant found naturally in tea leaves (Camellia sinensis), coffee beans (Coffea arabica), cacao beans (Theobroma cacao), and cola nuts (Cola acuminata).
- It is widely used as a stimulant, diuretic, and mild analgesic.

Molecular Formula: $C_8H_{10}N_4O_2$
Molecular Weight: 194.19 g/mol

## Isolation of Caffeine

### Source:

- Tea leaves (*Camellia sinensis*, Family: Theaceae)
- Coffee beans (*Coffea arabica*, Family: Rubiaceae)

### Plant Part Used:

- Dried leaves or seeds

### Procedure (From Tea Leaves):

1. Collection and Drying:
    - Fresh tea leaves are collected and dried under shade.
2. Powdering:
    - Dried leaves are finely powdered to increase the extraction surface.
3. Extraction:
    - Powdered tea is boiled with distilled water for about 15–20 minutes to extract caffeine and tannins.
4. Filtration:
    - The hot mixture is filtered to remove insoluble materials.
5. Tannin Removal:
    - Add lead acetate to the filtrate to precipitate tannins.
    - The solution is filtered again to remove the precipitate.
6. Neutralization:
    - Add a few drops of sodium carbonate to neutralize excess acid and free the caffeine base.
7. Solvent Extraction:

- Extract the filtrate several times with chloroform or dichloromethane in a separating funnel.
- Caffeine dissolves in the organic layer.

8. Evaporation:
   - The chloroform layer is collected and evaporated under reduced pressure.
   - On cooling, pure caffeine crystals separate out.

## Identification of Caffeine

Identification is done by physical, chemical, and instrumental methods.

### A. Physical Properties:

- Appearance: White, silky crystals or powder
- Taste: Bitter
- Solubility: Soluble in hot water, alcohol, and chloroform
- Melting Point: 236°C

### B. Chemical Tests:

1. Murexide Test (for purine alkaloids):
   - Add a small amount of caffeine to a porcelain dish.
   - Add a few drops of concentrated $HNO_3$, evaporate to dryness.
   - Expose residue to $NH_3$ vapor → Purple or violet color appears (due to formation of ammonium purpurate).
     ✅ *Confirms caffeine (xanthine alkaloid).*
2. Tannic Acid Test:
   - Add tannic acid solution → white precipitate forms (indicates presence of alkaloid).
3. Sublimation Test:
   - When heated on a slide, caffeine sublimes and deposits needle-shaped crystals — a characteristic property.
4. Dragendorff's Test:

- Add Dragendorff's reagent → orange precipitate confirms alkaloid.

## C. TLC Identification:

- Stationary Phase: Silica gel G
- Mobile Phase: Chloroform : Ethanol (9:1)
- Detection: Under UV light (254 nm) — caffeine shows a bright blue spot.
- Rf Value: ≈ 0.45

## D. Spectroscopic Identification:

1. UV-Visible Spectroscopy:
   - λmax = 272 nm (due to conjugated purine system).
2. IR Spectroscopy:
   - Characteristic absorption bands:
     - C=O (amide): 1690 $cm^{-1}$
     - C–N stretch: 1330–1400 $cm^{-1}$
     - N–H: 3100–3500 $cm^{-1}$
3. Mass Spectrometry:
   - Molecular ion peak at m/z = 194 confirms molecular weight.
4. NMR Spectroscopy ($^1H$ NMR):
   - Signals correspond to three N-methyl groups and the purine ring protons.

# Analysis of Caffeine

## A. Qualitative Analysis:

- TLC or UV spectroscopy can confirm caffeine in plant extracts or beverages.
- Murexide test is a classical qualitative method.

## B. Quantitative Analysis:

1. UV Spectrophotometric Method:
    - Measure absorbance at λmax 272 nm in chloroform or water.
    - Use Beer-Lambert's law to calculate caffeine concentration.
2. HPLC Method:
    - Column: C18 (reverse-phase)
    - Mobile phase: Methanol : Water (60:40)
    - Flow rate: 1.0 mL/min
    - Detector: UV at 272 nm
    - Retention time ≈ 5–7 min
    - Used for accurate quantification in tea, coffee, or soft drinks.
3. Gravimetric Method:
    - After isolation, dried caffeine crystals are weighed — gives approximate yield of caffeine per gram of plant material.

## Applications of Caffeine

- CNS Stimulant: Reduces fatigue, increases alertness.
- Cardiac Stimulant: Mildly increases heart rate and cardiac output.
- Diuretic: Increases urine output.
- Analgesic adjunct: Enhances effect of pain relievers like aspirin and paracetamol.
- Pharmaceutical Use: Used in energy drinks, tablets, and cold medications.