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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	<i>Atropa belladonna</i> , <i>Datura</i>	Anticholinergic, Mydriatic
Quinoline Alkaloids	Quinine, Quinidine	<i>Cinchona bark</i>	Antimalarial, Antipyretic
Isoquinoline Alkaloids	Morphine, Codeine	<i>Papaver somniferum</i> (Opium)	Analgesic, Antitussive
Indole Alkaloids	Reserpine, Vincristine	<i>Rauwolfia</i> , <i>Vinca rosea</i>	Antihypertensive, Anticancer
Imidazole Alkaloids	Pilocarpine	<i>Pilocarpus jaborandi</i>	Miotic, Treats glaucoma
Steroidal Alkaloids	Solanine	<i>Solanum</i> species	Antifungal
Purine Alkaloids	Caffeine, Theobromine	Tea, Coffee, Cocoa	CNS stimulant
Quinazoline Alkaloids	Vasicine	<i>Adhatoda vasica</i>	Bronchodilator

B. Based on Pharmacological Action

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

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.

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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
<i>Atropa belladonna</i>	Solanaceae	Leaves and roots	Atropine
<i>Datura stramonium</i>	Solanaceae	Seeds and leaves	Atropine, Hyoscyamine
<i>Hyoscyamus niger</i>	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 (λ_{max}) 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 ($\text{C}_8\text{H}_{15}\text{NO}$) 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 λ_{max} 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.

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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 ($\text{HgCl}_2 + \text{KI}$) → 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
- R_f 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 R_f value with standard quinine confirms its presence.

B. Quantitative Analysis:

1. UV Spectrophotometric Method:
 - Prepare a standard solution of quinine in dilute H₂SO₄.
 - 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: $265-268^\circ\text{C}$ (decomposes)

B. Chemical Tests:

1. Van Urk's Test (for Indole group):
 - Reserpine solution + p-dimethylaminobenzaldehyde (Ehrlich's reagent) + $\text{H}_2\text{SO}_4 \rightarrow$ Violet or blue color confirms indole nucleus.
2. Mayer's Test:
 - Add Mayer's reagent ($\text{HgCl}_2 + \text{KI}$) $\rightarrow$ White or cream precipitate confirms alkaloids.
3. Dragendorff's Test:
 - Add Dragendorff's reagent (Bi-KI complex) $\rightarrow$ Orange or brown precipitate forms $\rightarrow$ 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
- R_f value: Around 0.55

D. Spectroscopic Identification:

1. UV-Visible Spectroscopy:
 - $\lambda_{max} \approx 268 \text{ nm}$ (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.

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Analysis of Reserpine

A. Qualitative Analysis:

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

B. Quantitative Analysis:

1. Spectrophotometric Method:
 - Reserpine shows maximum absorption at λ_{max} 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 $\rightarrow$ Purple or violet color appears (due to formation of ammonium purpurate).
☒ Confirms caffeine (xanthine alkaloid).
2. Tannic Acid Test:
 - Add tannic acid solution $\rightarrow$ 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 $\rightarrow$ 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:
 - $\lambda_{\text{max}} = 272 \text{ nm}$ (due to conjugated purine system).
2. IR Spectroscopy:
 - Characteristic absorption bands:
 - C=O (amide): 1690 cm^{-1}
 - C-N stretch: $1330\text{--}1400 \text{ cm}^{-1}$
 - N-H: $3100\text{--}3500 \text{ cm}^{-1}$
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 $\lambda_{\text{max}} 272 \text{ nm}$ 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 $\approx$ 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.

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