

M.Phil Pharmaceutical Chemistry (Session 2020-2022)

First Semester (Feb.2020-June 2020)

Chemistry of Natural Product Course code: PHC-703

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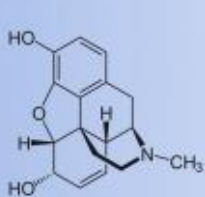
Course Contents:

- 1. Introduction to Natural Products and Naturoceuticals**
- 2. Classification of Natural Products**
- 3. Overview of the Methodologies to study Natural Products**
- 4. Chemistry and Chemical Modification of Natural Drug Substances In Modern Drug Synthesis**
- 5. Developments in the discovery and chemical studies of plant-derived anticancer agents**
- 6. Chemistry and Biological Activity of Alkaloids, Glycosides, Terpenoids, Saponins and Flavonoids**
- 7. Chemistry and mechanism of action of cardio active plant constituents**
- 8. Immunomodulator Activities of Medicinal Plants**
- 9. Chemistry of Natural Products Possessing Anti-Viral, Anti Hiv, Anti Fungal Activities**
- 10. Chemistry of Fertility Regulatory Activities Obtained From Plants**

TOPIC 1

Introduction to Natural Products and Naturoceuticals

Alkaloids

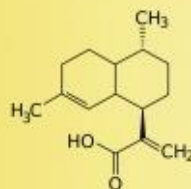


Morphine
(analgesic)



Papaver somniferum
(Opium Poppy)

Terpenoids

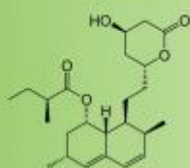


Artemisinic Acid
(antimalarial drug)



Artemisia annua
(Sweet Wormwood)

Polyketides

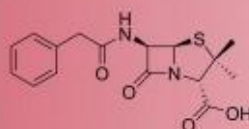


Lovastatin
(hypolipidemic agent)



Pleurotus ostreatus
(Oyster Mushroom)

Nonribosomal peptides



Penicillin
(antibiotic)



Penicillium chrysogenum

Natural Products

Chemical compound or substance produced by a living organism—that is, found in nature are called natural products. Natural products include any substance produced by life. They have pharmacological/ Biological activity which is used in pharmaceutical drug discovery and design.

Sources of Natural Products

- Microbial World
- Plant Source
- Marine Source
- Animal Source

Natural Products from Micorbial world

Antibiotics	Source	Mode of action	Spectrum
Penicillins	Penicillium notatum	Blocks cell wall synthesis	Gram-ve bacteria
Cephalosporins	Cephalosporium notatum	Blocks cell wall synthesis	Gram+ve bacteria,some gram _ve and spirochetes
Bacitracin	Bacillus subtilus	Blocks cell wall synthesis	Gram-ve bacteria

Natural Products from Plants

Drug	Source	Activity
Morphine	Poppy plant (Papaver somniferum)	Analgesic
Atropine	Atropa belladonna	Antimuscranic
Strychnine	Strychnos nux-vomica	Poison to kill pesticides
Taxol	Pacific yew tree	Anti-cancer (ovarian & breast)

Natural products from Marine Organisms

Drug	Source	Activity
Spongouridine	Sponge (Cryptotheca crypta)	Anti-cancer, Anti-viral
Spongothymidine	Sponge (Cryptotheca crypta)	Anti-cancer, Anti-viral
Discodermolide	Sponge (Discodermia dissoluta)	Anti-cancer

Natural Products from Animals

Drug	Source	Activity
Epibatidine	Skin of Ecuadorian poison frog	Analgesic
Cilazapril	Brazilian viper	Anti-hypertensive
Captopril	Brazilian viper	Anti-hypertensive

Naturoceuticals

Naturoceuticals are food or part of food or nutrient that provides health benefits including treatment and prevention of disease. These are also known as functional food. Naturoceuticals allow consumer to take enriched food.

Categories of Naturoceuticals

Nutrients	Substances with established nutritional functions e.g. Vitamins, Amino acids, Fatty acids
Herbal/Phytochemicals	Herbs or botanical products
Dietary supplements	Probiotics , Enzymes, Prebiotics

Nutrients:

Vitamins

- Fat soluble
- Water soluble

Fat Soluble Vitamins

	Vitamin A (Retinol)	Vitamin D	Vitamin E (Tocopherol)	Vitamin K
Sources	Sweet potatoes, Carrots, Dark leafy greens, Dried apricots	Fatty fish, Dairy products, Cereals, Beef liver, produced in skin on exposure to sunlight	Vegetable oil, egg, meat, nuts	Bacteria in intestine, Green vegetables
Functions	Antioxidant, Anti-cancer,	Promotes growth of bones	Antioxidant, Supports immune function, regulates blood pressure	Blood clotting, bone formation
	maintains healthy vision, treats skin disorders	Supports immune function, regulates blood pressure	Improves cognitive function	Cell growth

Water Soluble Vitamins

	Vitamin C (Ascorbic Acid)	Vitamin B1 (Thiamine)	Vitamin B2 (Riboflavin)	Vitamin B3 (Niacin)
Sources	Peppers, Citrus Fruits, Papaya, Green Vegetables, Tomatoes	Brewer's yeast Whole grains, cereals, and beans	Cheese, Beef, Fish, Mushrooms, Eggs and Almonds	Cheese, Beef, Fish, Mushrooms
Functions	1. Synthesis of collagen 2. Promote Wound Healing 3. Prevent Heart Diseases, stroke,	1. Helps in Carbohydrate metabolism 2. Essential for neurological functions	1. Helps in Carbohydrate metabolism 2. Essential for neurological functions	1. Metabolism 2. Brain Functions

	Vitamin B5 (Panthonic acid)	Vitamin B6	Vitamin B12 (Cyanocobalmine)	Folic Acid
Sources	Cheese, Beef, Fish, Mushroom, and Sweet Potatoes	Beef & Fish liver Potatoes, Citrus fruits	Eggs, Milk, Cheese, Meat, Fish, Shellfish and Poultry	Dried Beans and Peas Dark Green Vegetables
Functions	1. Synthesis of Cholesterol, steroids, and Fatty acids 2. Crucial for intraneuronal synthesis of acetylcholine	1. Helps to produce essential proteins 2. convert proteins to energy	1. Help in producing genetic material 2. Formation of RBC 3. Maintenance of CNS 4. Synthesis of amino acids	1. RBC formation 2. Formation of genetic material cell 3. Essential during pregnancy

Dietary Supplements

Probiotics

Probiotics are bacterial preparations that impart clinically verified beneficial effects on health of the host when consumed orally.

Source:

Major source is cultured dairy products such as cheese, butter milk and yogurt.

- 95% of all the bacteria found in the colon, some are harmful and some are useful and their balance plays a very important role.
- They improve gut health.
- Probiotics help to reduce certain food allergies

Prebiotics:

Prebiotics are non-digestible food ingredients that stimulate the growth and/or activity of bacteria in the digestive system in ways claimed to be beneficial to health.

Prebiotics are also called as synbiotics.

Herbs and Phytochemicals

Herbs:

An herb is a plant or plant part used for its scent, flavor, or therapeutic properties. Herbal medicines are one type of dietary supplement. They are sold as powders, teas, extracts, and fresh or dried plants. People use herbal medicines to try to maintain or improve their health.

- Aloe vera: Anti-inflammatory, wound healing, emolient.
- Garlic: Antibacterial, Antifungal, Antithrobotic, Anti-inflammatory
- Ginger: Carminative, Antiemetic, treatment of dizziness
- Green Tea: Antioxidant, reduce risk of CVD, enhance Humoral and cell mediated immunity.
-

Phytochemicals:

Phytochemicals	Lycopene	Lutein	Zeaxanthine
Sources	Red Fruits and Vegetables Particularly tomato	Spinach, Peas Orange juice	Stereoisimer present in Butter squash
Benefits	1. Antioxidant and free radical scavenging activity 2. Cancer decreased with Lycopene 3. Lower Prostate cancer (intake of tomato)	1. Beneficial for Heart disease 2. Maintenance of eye health make screening pigment known as macular pigment 3. Reduce risk of age related macular degenertaion	Same effect on eyes as Lutien

References:

Pharmacognosy by Trease and Evans 15th edition

TOPIC 2

"Classification of Natural Products"

NATURAL SUBSTANCES

The term “Natural substances” refers to those substances found in nature & that comprise:

- ✓ Whole plant and herbs and anatomic parts thereof; vegetable saps, extracts, secretions.
- ✓ whole animals and anatomic parts thereof; glands or other animal organs, extracts, secretions
- ✓ Other constituents thereof; that have not had changes made in their molecular structure as found in nature.

Sources of Natural Substances:

The most important natural sources of drugs are **higher plants, microbes and animals**. Some useful products are obtained from **minerals that are both organic and inorganic in nature**.

Crude Drugs:

Crude drugs are **vegetables or animal drugs** that consist of **natural substances** that have undergone only the processes of **collection and drying**.

e.g., Acacia, Bees Wax, Cinnamon, Digitalis, Ephedra, Fennel, Ginger, Ispaghul, Mustard, Nutmeg etc.

METHODS OF CLASSIFICATION

Systematic method of arrangement of crude drug into different group based on the similarities and differences. A method of classification should be:

- a) Simple
- b) Easy to use
- c) Free from confusion and ambiguities

The drugs are classified in following different ways:

1. Alphabetical classification
2. Morphological classification
3. Taxonomical classification
4. Pharmacological classification
5. Chemical classification
6. Chemo-taxonomical classification
7. Sero-taxonomical classification

1) Alphabetical Classification

Alphabetical classification is the **simplest way** of classification of any disconnected items.

Crude drugs are arranged in alphabetical order of their **Latin** and **English names** or sometimes **local language names**.

Some of the pharmacopoeias and reference books which classify crude drugs according to this system are;

1. Indian pharmacopoeia
2. British pharmacopoeia

3. United states pharmacopoeia and National Formulary
4. British Pharmaceutical Codex
5. European Pharmacopoeia

Examples:

Acacia, Benzoin, Cinchona, Dill, Ergot, Fennel, Ginger, Hyoscyamus, Ipecac, Jalap etc.

Merits:

- It is quick and easy to use.
- It should be devoid of confusion.
- There is no repetition of entries.
- In this system; addition, location and tracing of drug entries is easy.

Demerits:

- There is no relationship between previous and successive drug entries.

2) Morphological Classification

Under morphological classification, the drugs are arranged according to the **morphological or external characters of the plant parts and animal parts.**

i.e. which part of plant is used as a drug such as leaves, roots, barks, flowers etc.

Organized drugs: The drugs obtained from the **direct parts of the plants** and **containing cellular tissues** are called as organized drugs. e.g., rhizomes, bark, leave, flower, fruits etc.

Unorganized drugs: The drugs which are prepared from **plants by some intermediate physical processes** such as incision, drying or extraction with solvent and **not containing any cellular plant tissues** are called as unorganized drugs. e.g., aloe juice, agar, opium latex, resins, gum and oils etc.

Merits:

- It is more helpful to identify and detect adulteration.
- It is more practical for convenient study especially when chemical nature of drug is not clearly understood.

Demerits:

- Repetition of plants and drugs can occur.
- During collection, drying & packing; morphology of drug changes.

3) Taxonomical Classification

Taxonomical classification is purely a **botanical classification** which is based on Phylogenetic similarity of plant or principles of natural relationship and evolutionary development.

In this system drugs are classified according to Kingdom, Subkingdom, Division, Class, Order, Family, Genus and Species.

Example,

Phylum	-	Spermatophyta
Division	-	Angiospermae

Class	-	Dicotyledons
Sub-class	-	Sympetalae
Order	-	Tubiflorae
Family	-	Solanaceae
Genus	-	Atropa, Datura, Hyoscyamus
Species	-	<i>Hyoscyamus niger</i> , <i>Datura stramonium</i> , <i>Atropa belladonna</i>

Merits:

- It is helpful for studying evolutionary developments.

Demerits:

- No-correlation between chemical constituents and biological activity of drugs.

4) Pharmacological Classification

Grouping of drug according to their **pharmacological action** or **of most important constituent** or **their therapeutic use** is termed as pharmacological or therapeutic classification of drug.

Examples;

- CNS stimulant (Tea, Coffee)
- Vasodilators (Veratrum alkaloid, Reserpine, Papaverine)
- Vasoconstrictors (Ephedrine, Nicotine)
- Anticholinergic drugs (Hyoscine, Hyoscyamine)
- Antiemetic (Ginger, Cannabis)
- Carminatives (Fennel, Peppermint)
- Expectorants (Ipecacuanha, Tolu balsam)
- Anti-cancer (Vinca, Taxus)
- Antimalarial drugs (Quinine)
- Analgesics (Morphine, Codeine)

Merits:

- It can be used for suggesting for substitutes of drugs if they are not available at particular place or point of time.

Demerits:

- Drugs having different actions on the body gets classified separately in more than one group that cause ambiguity and confusion. e.g., Nux-vomica.
- No idea whether the drug is organized and unorganized.

5) Chemical Classification

The crude drugs are classified according to the **chemical nature** of their most important chemical constituent.

1. Carbohydrates

2. Glycosides
3. Alkaloids
4. Volatile oils
5. Tannins
6. Lipids
7. Resins
8. Protein

CARBOHYDRATES

The word carbohydrate is derived from “Carbon Hydrates“

These are aldehyde or ketone alcohols containing carbon, hydrogen and oxygen in which the hydrogen and oxygen are in generally the same ratio as in water.

Carbohydrates are polyhydroxy alcoholic compounds with at least 3 carbon atoms with potentially active carbonyl group which may either be an aldehyde or ketone group.

They have the generic formula = $C_n (H_2O)_m$

Glucose.... $C_6H_{12}O_6$

Sucrose..... $C_{12}H_{22}O_{11}$

Classification of carbohydrates:

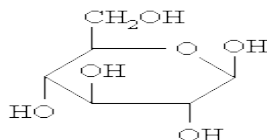
Carbohydrates are classified as,

- a) Monosaccharide b) Disaccharides c) Polysaccharides

a) **Monosaccharides**

Saccharide is a term derived from the Latin word meaning sugar.

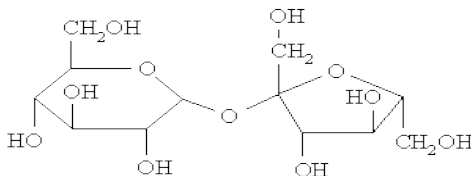
A **monosaccharide** contains a single carbohydrate. They are sweet in taste and soluble in water. For example: Glucose



b) **Disaccharides**

A disaccharide gives two carbohydrate units on hydrolysis. They are sweet in taste and less soluble in water.

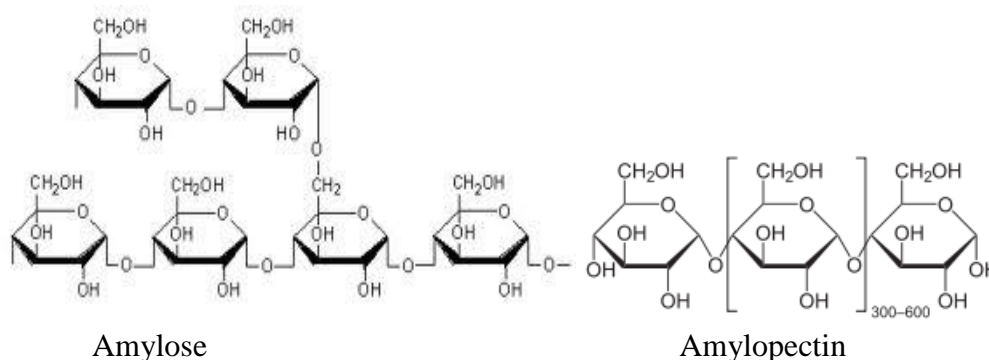
Example: Sucrose



c) **Polysaccharides**

A polysaccharide gives more than two carbohydrate units on hydrolysis. They are tasteless and insoluble in water. For example; Starch and Glycoproteins.

Starch



GLYCOSIDES

Non-reducing substances that yield one or more sugars on hydrolysis linked with a non-sugar moiety through a glycosidal linkage.

Glycosides → Sugar component + Non-sugar component

Glycone

Aglycone

CLASSIFICATION BASED ON THE NATURE OF SUGARS:

All kinds of sugars are present in glycoside which are classified as,

1. Glucoside → glucose as glycone
2. Fructoside → fructose as glycone
3. Galactoside → galactose as glycone
4. Rhamnoside → rhamnose as glycone

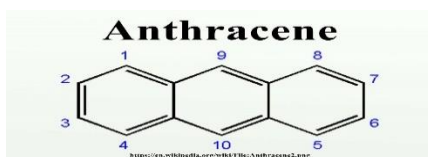
CLASSIFICATION BASED ON AGLYCONE GROUP:

1. Anthraquinone glycoside
2. Saponin glycoside
3. Cardiac glycosides
4. Cyanophore glycoside Alcohol glycoside
5. Aldehyde glycoside
6. Flavonol glycoside
7. Isothiocyanate glycoside
8. Phenol glycoside

1. Anthraquinone Glycosides:

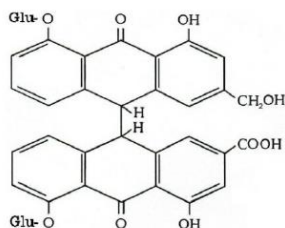
These glycosides upon hydrolysis produce di, tri or tetra hydroxyl anthraquinons. In plants, number of glycosides whose aglycone are related to anthracene are present.

e.g., Aloe, Senna, Cascara.

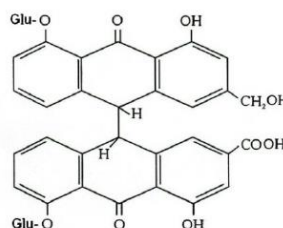


Senna:

- ▶ Botanical origin: *Cassia acutifolia*
- ▶ Family: Leguminosae
- ▶ Part used: Dried leaves
- ▶ Use: Cathartic, laxative, Stimulate the muscular coat of intestine
- ▶ Constituents: Sennoside A, Sennoside B, Sennoside C, Sennoside D



Sennoside A



Sennoside B

2. Saponin Glycosides:

Toxic surfactant glycosides that occur in higher plants and are characterized by producing soapy solution are known as Saponin glycosides. e.g. Quillaia, Arjuana, Glycyrrhiza,

a) Steroidal Saponin (neutral saponin)

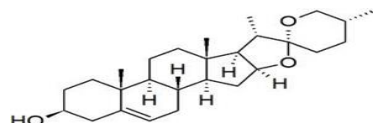
b) Triterpenoid Saponin (acid saponin)

a) Steroidal Saponins (Neutral Saponins)

Also commonly called tetracyclic triterpenoids. e.g., Sarsaponin, Digitonin, Dioscin etc.

Dioscin:

- ▶ Botanical source: *Dioscorea deltoidea*
- ▶ Family: Dioscoreaceae
- ▶ Part used: Dried rhizomes
- ▶ Use: Starting material for production of steroids hormones.
- ▶ Constituents: Diosgenin, Yammogenin

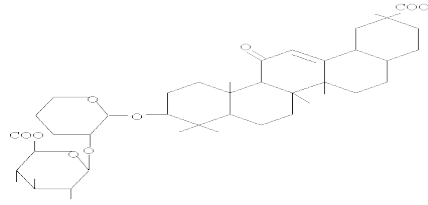


Diosgenin

b) Triterpenoid Saponins (Acid Saponins)

Glycyrrhiza:

- ▶ Botanical origin: *Glycyrrhiza glabra*
- ▶ Family: Leguminosae
- ▶ Part used: Dried unpeeled roots and rhizomes
- ▶ Uses: Expectorant
- ▶ Constituents: Glycyrrhizin, liquiritin, Glycyrrhetic acid.



Structure of Glycyrrhizin

3. Cardiac Glycosides:

They are steroidal glycosides.

They show highly specific and powerful action upon the cardiac muscles.

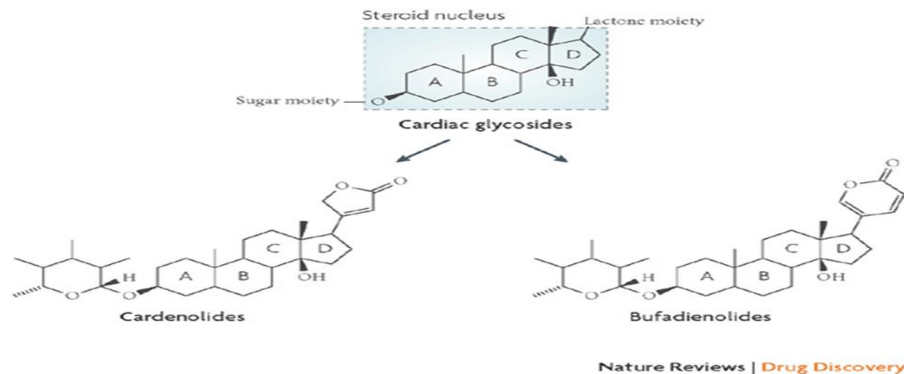
The steroidal aglycones are of two types:

Cardenolides: (Five membered lactone ring)

e.g., Digitalis, Strophanthus etc.

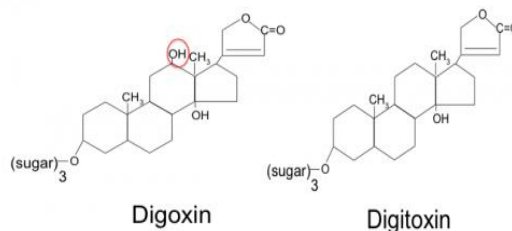
Bufadienolides: (Six membered lactone ring)

e.g., Scilla, White Squill etc.



Digitalis:

- ▶ Botanical origin: *Digitalis purpurea*
- ▶ Family: Scrophulariaceae
- ▶ Part used: Dried leaves
- ▶ Use: Cardiotonic, Atrial fibrillation, Supraventricular tachycardia
- ▶ Constituents: Digitoxin, Digoxin



4. Cyanophore Glycosides:

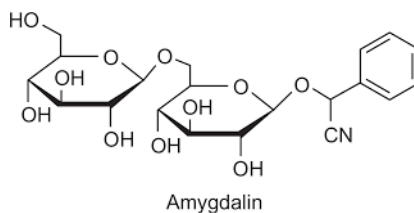
These glycosides upon hydrolysis yields hydrogen cyanide.

Amygdalin group is well known Cyanophore glycosides. e.g., Bitter almond.

Prusin is also widely distributed Cyanophore glycoside. e.g., Wild cherry bark

Bitter almond:

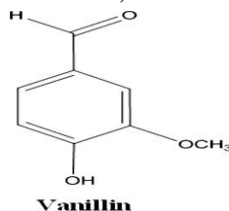
- ▶ Botanical origin: *Prunus amygdalus*
- ▶ Family: Rosaceae
- ▶ Part used: Seeds
- ▶ Use: diuretic, fungicidal, bactericidal
- ▶ Constituents: Amygdalin



5. Aldehyde Glycoside:

Vanilla:

- ▶ Botanical origin: *Vanilla planifolia*
- ▶ Family: Orchidaceae
- ▶ Part used: fully grown Unripened fruit
- ▶ Use: Flavouring agent, in vanilla tincture.
- ▶ Constituents: Vanillin, Glucovanillin, Glucovanillic alcohol

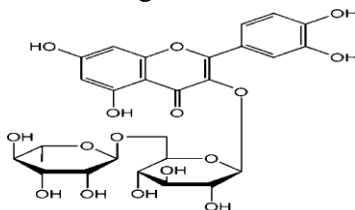


6. Flavonol Glycosides:

The flavonoids are a group of compounds comprising the derivatives of flavone, isoflavone, flavanones, isoflavanone and flavanols.

Rutin:

- ▶ Aglycone: Quercetin
- ▶ Sugar: Rutinose
- ▶ Botanical origin: *Fagopyrum esculentum*
- ▶ Family: Polygonaceae
- ▶ Part used: Rind of green citrus fruits
- ▶ Use: Antihaemorrhage

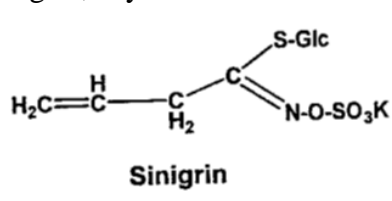


7. Isothiocyanate Glycosides:

They contain Sulphur and on hydrolysis, they yield Isothiocyanate aglycones which may be aliphatic or aromatic. Also known as glucosinolates.

Black Mustard:

- ▶ Botanical origin: *Brassica nigra*
- ▶ Family: Brassicaceae
- ▶ Part used: Dried ripe seeds
- ▶ Use: Local irritant and an emetic
- ▶ Constituents: Singrin, Myrosin

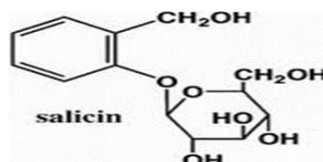


8. Alcohol Glycoside:

Glycosides having alcoholic aglycone part are called alcohol glycosides.

Salicin:

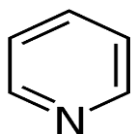
- ▶ Botanical origin: *Salix purpurea*
- ▶ Family: Salicaceae
- ▶ Part used: Bark and leaves
- ▶ Use: Anti rheumatic and analgesic
- ▶ Constituents: Salicortin



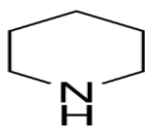
ALKALOIDS

Alkaloids are the basic nitrogenous compounds of plant origin having definite pharmacological activities.

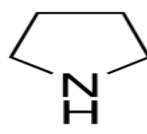
Classified according to nature of the basis of chemical structure.



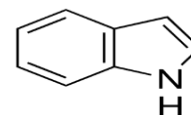
Pyridine



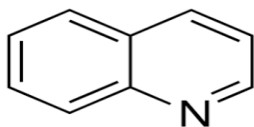
Piperidine



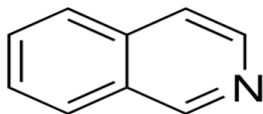
Pyrrolidine



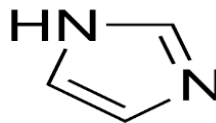
Indole



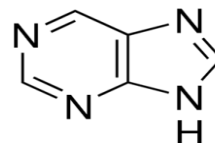
Quinoline



Isoquinoline



Imidazole



Purine Base

CLASSIFICATION OF ALKALOIDS:

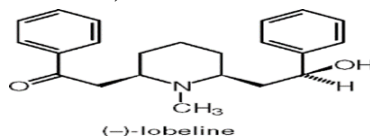
- A. Pyridine-piperidine alkaloids
 - Derivatives of piperidine
 - Derivatives of pyridine and Pyrrolidine
- B. Tropane alkaloids
- C. Quinoline alkaloids
- D. Isoquinoline alkaloids
- E. Indole alkaloids
- F. Imidazole alkaloids
- G. Alkaloidal Amines
- H. Purine bases

A. Pyridine-piperidine Alkaloids:

- **Derivatives of piperidine**

Lobelia:

- ▶ Botanical origin: *Lobelia inflata*
- ▶ Family: Lobeliaceae
- ▶ Part used: Dried leaves and flowering tops
- ▶ Uses: Antismoking preparation, expectorant
- ▶ Constituent: Lobeline, Lobelidine



- **Derivatives of Pyridine and Pyrrolidine**

Tobacco:

- ▶ Botanical origin: *Nicotiana tabacum*
- ▶ Family: Solanaceae
- ▶ Part used: Dried leaves
- ▶ Uses: Antismoking preparations
- ▶ Constituent: Nicotine, Nor-nicotine, Campharine



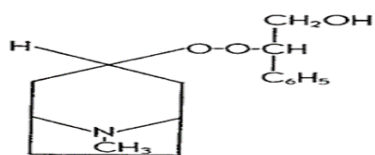
B. Tropane alkaloids:

Tropane is dicyclic compound formed by condensation of a pyrrolidine precursor (ornithine) with 3 acetate-derived carbon atom.

Ring is composed of pyrrolidine and piperidine rings.

Belladonna:

- ▶ Botanical origin: *Atropa belladonna*
- ▶ Family: Solanaceae
- ▶ Part used: Dried leaves and flowering tops
- ▶ Uses: Spasmolytic agent, anticholinergic properties, Antidote for mushroom and heavy metal poisoning
- ▶ Constituents: Atropine, Scopolamine, Hyoscyamine

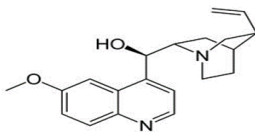


Atropine

C. Quinoline Alkaloids:

Cinchona:

- ▶ Botanical origin: *Cinchona succirubra*
- ▶ Family: Rubiaceae
- ▶ Part used: Dried bark of stem and roots
- ▶ Uses: Quinine: antimalarial, antipyretic
Quinidine: to treat cardiac arrhythmia

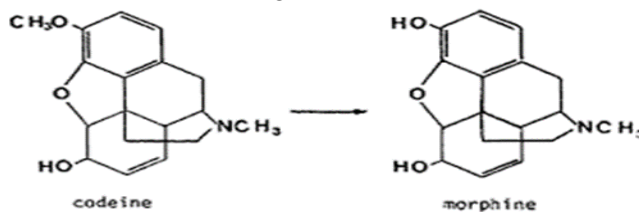


Quinine

D. Isoquinoline Alkaloids:

Opium:

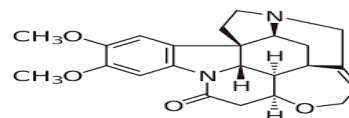
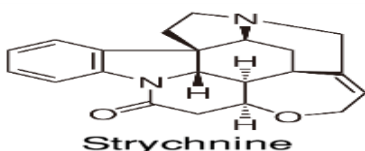
- ▶ Botanical origin: *Papaver somniferum*
- ▶ Family: Papaveraceae
- ▶ Part used: Raw opium obtained by the incision of the unripe capsules
- ▶ Constituents and Uses:
 - Ø Morphine: narcotic analgesics, hypnotic
 - Ø Codeine: narcotic analgesic and antitussive, sedative



E. Indole Alkaloids:

Nux vomica:

- ▶ Botanical origin: *Strychnos nux-vomica*
- ▶ Family: Loganiaceae
- ▶ Part used: Dried ripe seeds
- ▶ Uses: Strychnine: Central stimulant, Rodenticides, Indigestion
Brucine: less toxic than strychnine and used as alcohol denaturant

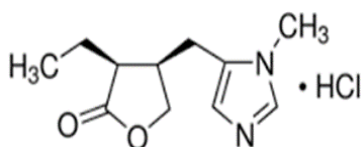


Brucine

F. Imidazole Alkaloids:

Pilocarpine:

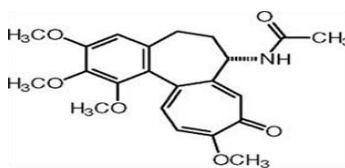
- ▶ Botanical origin: *Pilocarpus jaborandi*
- ▶ Family: Rutaceae
- ▶ Part used: Dried leaflets
- ▶ Use: in glaucoma (cholinergic), antagonist of atropine
- ▶ Constituents: Pilocarpine, Isopilocarpine, Pilocarpidine



G. Alkaloidal Amines:

Colchicum:

- ▶ Botanical origin: *Colchicum autumnale*
- ▶ Family: Liliaceae
- ▶ Part used: Dried seeds & corms
- ▶ Use: in gout, produce polyploidy, antineoplastic properties
- ▶ Constituents: Colchicine



Colchicine

VOLATILE OIL

Odorous principles which are the mixtures of hydrocarbons and oxygenated compounds derived from the hydrocarbons, evaporate when exposed to air at room temperature are called volatile oils, ethereal oils or essential oils.

Classification of Volatile Oils

The following are the divisions in which volatile oils and volatile oils containing drugs are placed.

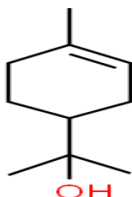
1. Hydrocarbon volatile oil
2. Alcoholic volatile oil
3. Aldehyde volatile oil
4. Ketonic volatile oil
5. Phenolic volatile oil
6. Phenolic Ether volatile oil
7. Oxide volatile oil
8. Ester volatile oil

1. Hydrocarbon Volatile Oil:

e.g., Turpentine oil, Cubeb

Turpentine oil:

- ▶ Botanical origin: *Pinus palustris*
- ▶ Family: Pinaceae
- ▶ Part used: Volatile oil distilled from oleo-resins
- ▶ Uses: as counter-irritant, rubifacient, solvent for waxes
- ▶ Constituents: alpha pinene, beta pinene, Terpeneol



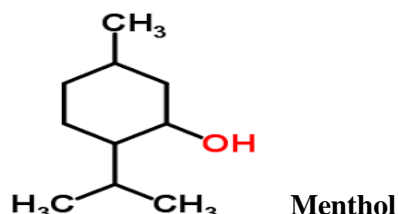
Terpineol

2. Alcoholic Volatile Oil:

e.g., Coriander, Cardamom, Peppermint

Peppermint:

- ▶ Botanical origin: *Mentha piperita*
- ▶ Family: Labiatae
- ▶ Part used: Dried leaves and flowering tops
- ▶ Uses: as carminative and flavorant and in mouthwashes
- ▶ Constituents: Menthol, Menthone

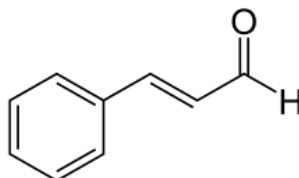


3. Aldehyde Volatile Oil:

e.g., Cinnamon, lemon oil, Bitter almond oil and orange oil.

Cinnamon bark:

- ▶ Botanical origin: *Cinnamomum loureirii*
- ▶ Family: Lauraceae
- ▶ Part used: Dried inner bark of coppiced shoots
- ▶ Uses: as carminative, as flavorant, antiseptic
- ▶ Constituents: Cinnamaldehyde, Cinnamyl acetate



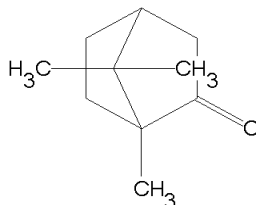
Cinnamaldehyde

4. Ketonic Volatile Oil:

e.g., Camphor, Caraway, Buchu, Spearmint.

Camphor:

- ▶ Botanical origin: *Cinnamomum camphora*
- ▶ Family: Lauraceae
- ▶ Part used: Ketonic crystalline substance in wood of tree
- ▶ Uses: Topical antipruritic, rubefacient, anti-infective
- ▶ Constituents: Camphor, Cineol, Borneol, Camphene



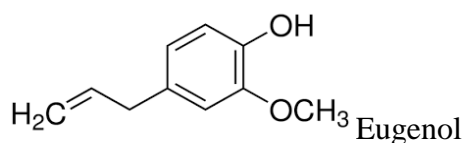
5. Phenolic Volatile Oil:

e.g., Clove, Thyme.

Clove:

- ▶ Botanical origin: *Eugenia caryophyllus*
- ▶ Family: Myrtaceae
- ▶ Part used: Dried ripe flowering buds

- ▶ Uses: in toothache, for platelet aggregation inhibition
- ▶ Constituents: Vanillin, Eugenol, Acetyl eugenol

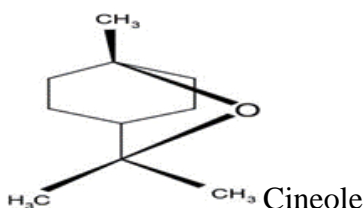


6. Oxides Volatile Oil:

e.g., Eucalyptus, Chenopodium

Eucalyptus Oil:

- ▶ Botanical origin: *Eucalyptus globulus*
- ▶ Family: Myrtaceae
- ▶ Part used: Dried leaves
- ▶ Uses: flavor, expectorant, antiseptic, diaphoretic
- ▶ Constituents: Eucalyptol commonly known as Cineole

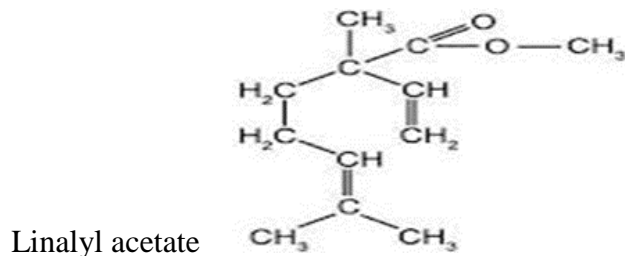


7. Esters Volatile Oil:

e.g., Lavender oil, Pine needle oil, Mustard oil, Wintergreen oil

Lavender oil:

- ▶ Botanical origin: *Lavandula angustifolia*
- ▶ Family: Labiatae
- ▶ Part used: flowering top
- ▶ Uses: in perfume
- ▶ Constituents: Linalool, Linalyl acetate



TANNINS

Tannins are complex organic, non-nitrogenous derivatives of polyhydroxy benzoic acids. Chemically tannins are complex substances that occur as mixture of polyphenols in in plant and they are difficult to separate because they do not crystallize.

Classification of Tannins:

1. Hydrolysable tannins
2. Condensed tannins or non-hydrolysable
3. Complex tannins
4. Pseudotannins

1. Hydrolysable Tannins:

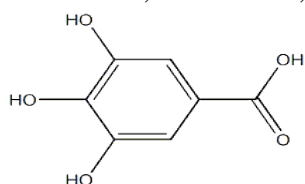
These are hydrolyzed by acids or enzyme (Tannase) into phenolic acid and sugar.

Formally known as Pyrogallol Tannins.

e.g., Nut gall, Clove, Eucalyptus leaves.

Nutgall:

- ▶ Botanical origin: *Quercus infectoria*
- ▶ Family: Fagaceae
- ▶ Part used: Excrescence produced on young twigs
- ▶ Use: astringent, hemostatic, as heavy metal antidote
- ▶ Constituents: Tannic acid, Gallic acid, Ellagic acid



Tannic acid

Eucalyptus:

- ▶ Botanical origin: *Eucalyptus globulus*
- ▶ Family: Myrtaceae
- ▶ Part used: leaves
- ▶ Uses: Flavor, Diaphoretic, Expectorant
- ▶ Constituents: Eucalyptol, Pinene, Limonene



2. Condensed Tannins:

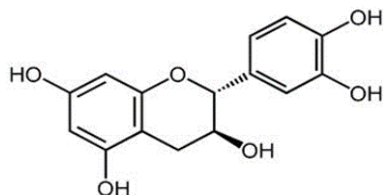
These tannins are resistant toward hydrolysis by enzymes so called non-hydrolysable or condensed tannins.

On treatment with acids or enzymes converted into red insoluble compounds known as Phlobaphenes.

Examples; Pale and black catechu, Cinchona, Cinnamon and Wild cherry bark.

Pale Catechu:

- ▶ Botanical origin: *Uncaria gambier*
- ▶ Family: Rubiaceae
- ▶ Part used: Dried extract of leaves and young twigs of plant
- ▶ Uses: Powerful astringent, Douche in leucorrhoea
- ▶ Constituents: Catechin, Catechu tannic acid, Catechu red



Catechin

LIPIDS

Lipids are ester of long chain fatty acids and alcohols or of closely related derivatives.

Classification of lipids:

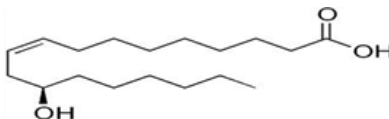
- 1) Fixed oils
- 2) Fats
- 3) Waxes

i. Fixed oils:

In fixed oils; fatty acid is unsaturated & liquid at room temperature.
e.g., Castor oil, Cotton seed oil, Linseed oil.

Castor oil:

- ▶ Botanical origin: *Ricinus communis*
- ▶ Family: Euphorbiaceae
- ▶ Part used: Oil obtained from the ripe seed
- ▶ Uses: Manufacturing of soap, paints and varnishes
- ▶ Constituents: Ricine, Ricinoleic acid, Oleic acid



Ricinoleic acid

ii. Fats:

In fats, fatty acid is saturated & solid at room temperature.
e.g., Theobroma oil, Lanolin.

Theobroma:

- ▶ Botanical origin: *Theobroma cocoa*
- ▶ Family: Sterculiaceae
- ▶ Part used: Roasted seeds
- ▶ Uses: in making cocoa syrup, suppository base, in petroleum jelly

- Constituents: oleic acid, Linoleic acid, Palmitic acid, Stearic acid



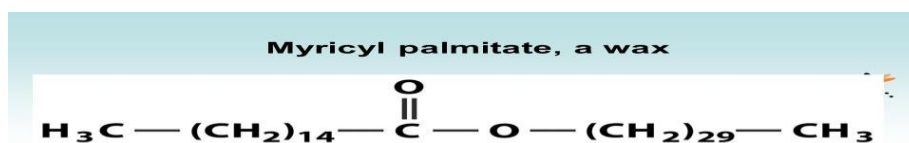
iii. **Waxes:**

Waxes are defined as esters resulting from the condensation of high molecular weight primary straight chain alcohol and straight chain acid.

e.g., Beeswax, Spermaceti, Jojoba oil, Carnauba wax.

Bees Wax:

- Biological source: *Apis mellifera*
- Family: Apidae
- Part used: Wax obtained from honey comb of the bee.
- Uses: To prepare plasters, yellow ointments and in cosmetics.
- Constituent: Myricyl palmitate, Myricyl stearate.



RESINS

Resins are amorphous products of complex chemical substances which are formed in schizogenous or schizolysigenous ducts or cavities in the plants.

Chemical composition of resins:

- Resin acid
- Resin alcohol
- Resenes
- Glycoresins
- Esters

CLASSIFICATION OF RESINS:

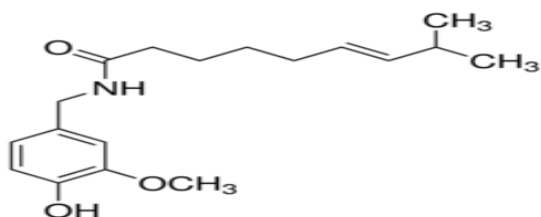
1. Oleoresins:

Oleoresins are homogenous mixture of resins and volatile oil. e.g., Turpentine, Ginger, Capsicum.

Capsicum:

- Botanical origin: *Capsicum frutescens*
- Family: Solanaceae
- Part used: Dried ripe fruit
- Uses: as an irritant and carminative, rubefacient.

- Constituents: Capsaicin, Capsacutin



Capsaicin

2. Oleo gum resins:

These are resins in combination with gum & volatile oil. e.g., Myrrh and Asafetida.

Myrrh:

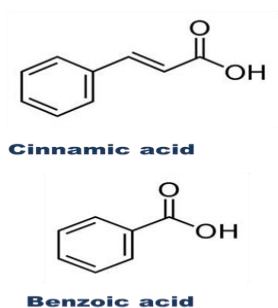
- Botanical origin: *Commiphora molmol*
- Family: Burseraceae
- Part used: Oleo gum resin obtained from stem
- Use: As tonic, stomachic and in mouth washes as astringent
- Constituents: Eugenol, Camphoric acid, Myrrholic acid



3. Balsam:

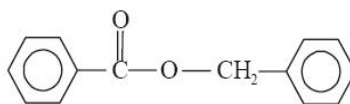
Resinous mixture that contains large proportion of benzoic acid, cinnamic acid or both or ester of these acids.

e.g., Tolu balsams, storax, benzoin.



Tolu Balsam:

- Botanical origin: *Myroxylon balsamum*
- Family: Leguminosae
- Part used: Incision of stem
- Uses: benzoin tincture, expectorant, flavoring in syrup, perfumery
- Constituents: Benzyl benzoate, Benzyl cinnamate, Styrene, Eugenol.



Benzyl Benzoate

Merits:

- Helpful for Phytochemical analysis.

Demerits:

- Difficulty to place the drug containing two or more main chemicals belonging to different categories. e.g., Nutmeg.

6) Chemo-taxonomical classification:

The biological classification is based on the chemical similarity of a Taxon means it is based on the existence of relationship between chemical constituent in various plant.

e.g., Tropane alkaloid (Solanaceae), Rutin (Rutaceae)

Merits:

- Helpful for understanding the relation between chemical constituent, their biosynthesis and their possible action.

7) Sere-taxonomical classification:

The application or utility of Serology in solving taxonomic problems is called Serotaxonomy.

Serology: Study of antigen-antibody reaction. It determines the degree of similarity between species, genus, family etc. by comparing the reaction with antigen (protein) from various Taxa with antibody present against a given Taxa.

TOPIC 3

Overview of the Methodologies to study Natural Products

Contents:

- Introduction
- Extraction Process
- Methods for Separation and Isolation
- Characterization of isolated compounds
- References

Introduction

A natural product is a chemical compound or substance produced by a living organism. They may be extracted from plants, animals, marine organisms or micro-organisms.

The products obtained from plant source are relatively complex mixtures of metabolites in liquid or semisolid state or in dry powder form, intended to be widely used in nutraceutical and pharmaceutical applications.

Extraction Process

It is defined as the treatment of the plant or animal tissues with solvent, whereby the medicinally active constituents are dissolved and most of the inert matter remains undissolved.

Solvents used in extraction:

Alcohol,

Water immiscible solvents; ether, chloroform, light petroleum.

Water	Ethanol	Methanol	Chloroform	Ether	Acetone
Anthocyanins	Tannins	Anthocyanins	Terpenoids	Alkaloids	Phenol
Starches	Polyphenols	Terpenoids	Flavonoids	Terpenoids	Flavonols
Tannins	Polyacetylenes	Saponins		Coumarins	
Saponins	Flavonol	Tannins		Fatty acids	
Terpenoids	Terpenoids	Xanthoxylines			
Polypeptides	Sterols	Totarol			
Lectins	Alkaloids	Quassinoids			
		Lactones			
		Flavones			
		Phenones			
		Polyphenols			

Extraction types

Extraction procedure choice depends upon the nature of plant material and type of component to be isolated.

Maceration: In this process, the whole or coarsely powdered crude drug is placed in a stoppered container with the solvent, allowed to stand at room temp. for a period of atleast 3 days with frequent agitation.

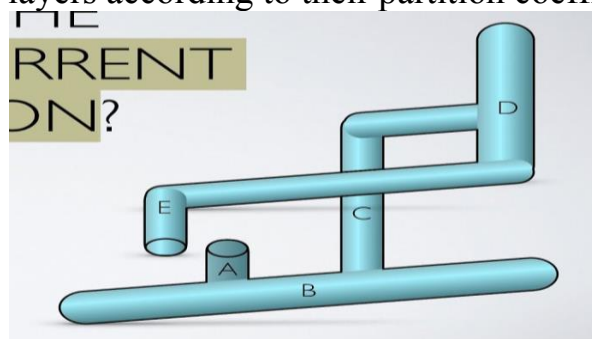
The mixture is then strained, marc (the damp material) is then pressed and the liquid is clarified by filtration or decantation after standing.

- **Infusion:** Infusions are prepared by macerating the crude drug for short period of time with cold or boiling water. These are dilute soln. of readily soluble constituents of crude drugs.
- **Digestion:** Form of maceration in which gentle heat is used during the process of extraction. Efficiency is increased.
- **Decoction:** In this process, the crude drug is boiled in a specified volume of water for a defined time; it is then cooled, strained or filtered. This is suitable for extracting water soluble, heat stable constituents. Starting ratio of crude drug to water is fixed. i-e; 1:4 or 1:16 the volume is then brought to one fourth to it's original volume by boiling, then this conc. Extract is filtered and used as such or processed further.
- **Percolation:** It is the continuous flow of solvent through the bed of the crude drug material to get the extract. The powdered drug is moistened with an appropriate amount of the specified solvent and allow to stand for approximately 4 hour in a closed container. After this the mass is packed and top of the percolator is closed. Additional solvent is added and form the layer on top of mass and allow to macerate for 24 hours.

The outlet of percolater is then opened and the liquid is allowed to drip slowly. Additional solvent is allowed to pass if required. The collected liquid is clarified by filtration or by standing followed by decanting.

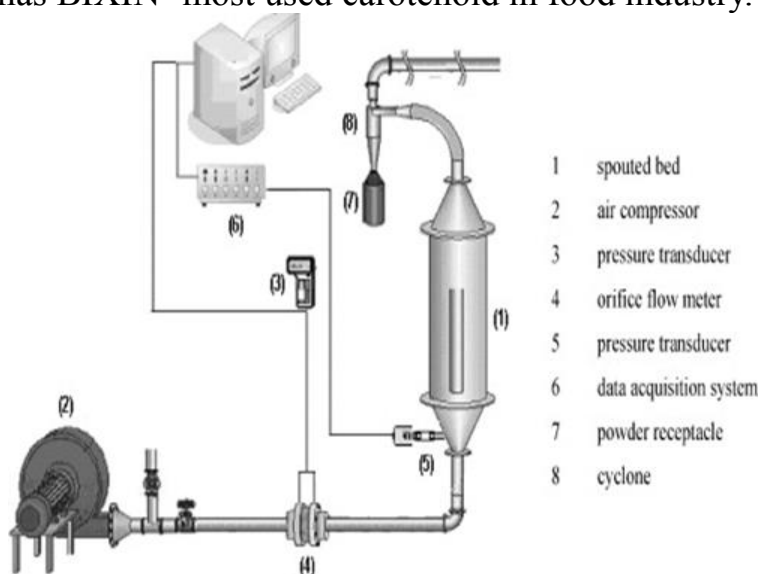
- **Counter current extraction:** This is a liquid-liquid extraction process which is used to separate, identify and quantify the chemical components of a mixture. A lower stationary phase is contained in a series of tubes and an upper moving immiscible liquid is transferred from tube to tube along the series. The immiscible liquids shaken and allowed to separate between each transference. The mixture to be fractionated is placed in the first tube

containing immiscible liquids and apparatus is agitated and the layers are allowed to separate. The components of the mixture will be distributed between the two layers according to their partition coefficients.



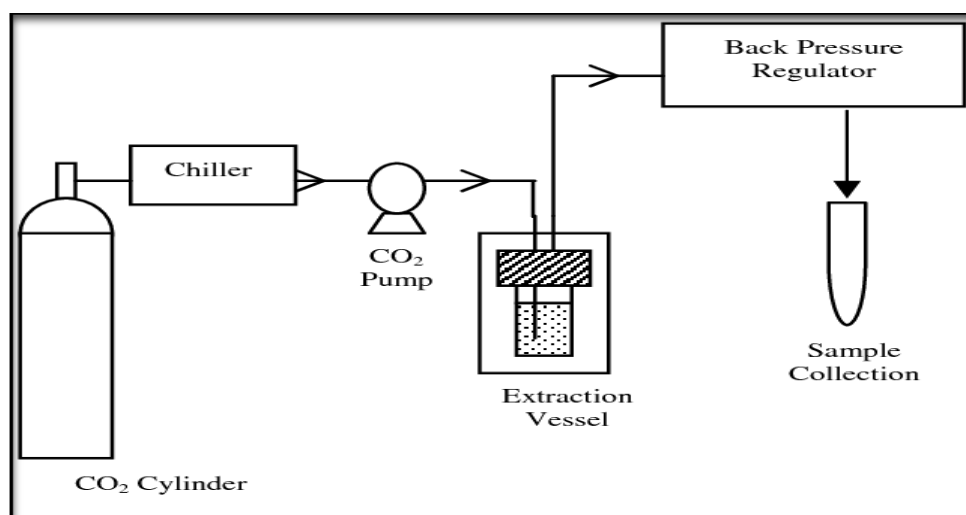
- **Spouted bed extraction:** It is used where solvent extraction is preferred over coat removal of seed. It consists of a cylinder tapered at both ends, containing seeds at the lower end through which a jet of hot air is pushed. Fine particles (loaded with seeds and pigments) are propelled into space. Seeds move back for recirculation and powder moves to a cyclone to be collected.

Example: Production of annatto powder from seeds of *Bixa orellana*.
80% of annatto has BIXIN- most used carotenoid in food industry.



- **Super critical fluid extraction:** It is the process of separating one component (the extractant) from another (the matrix) using super critical fluids as the extracting solvent.
Carbon dioxide (CO₂) is the most used supercritical fluid, sometimes modified by co-solvents such as ethanol or methanol.

Phytochemicals	Products
Alkaloids	Decaffeination of green coffee
Diterpene	Taxol from <i>Taxus brevifolia</i>
Fixed oils	Oil from Evening primrose oil
Pigments	Annatto seeds
Volatile oils	Rose petals



- Solid phase microextraction:** technique that involves the use of a fiber coated with an extracting phase, that can be a liquid (polymer) or a solid (sorbent), which extracts different kinds of analytes (including both volatile and non-volatile) from different kinds of media, that can be in liquid or gas phase. The quantity of analyte extracted by the fibre is proportional to its concentration in the sample as long as equilibrium is reached or, in case of short time pre-equilibrium, with help of convection or agitation.
 This is suitable for drugs containing volatile oils.

Methods for Separation and Isolation

Sublimation: IUPAC-The direct transition of a solid to a vapour without passing through a liquid phase. It may be possible on the whole drug.

Example: Isolation of caffeine from tea or purification of crude extract.

Distillation: The process of separating the constituents of a liquid mixture by heating it and condensing separately the components according to their different boiling points. Steam distillation-isolation of volatile oils/ hydrocyanic acid(HCN) from plant material.

- **Fractional liberation:** Some groups separate out from the mixture by fractional distillation.

Mixture of alkaloid salts in aq soln + Treated with Alkali aliquots



Weak base in free state



Followed by base liberation in increasing order of basicity



If the mixture is shaken with an organic solvent after each addition, then a fractionated series of bases will be obtained.

Similar in the case with organic acids in water immiscible solvents.

Starting with acid salts, acids can be liberated by addition of mineral acids.

- **Fractional crystallization:** It is a method of refining components on the basis of solubility. Components have different solubilities on the same temperature.

Principle: least soluble components will crystallize out first and most soluble ones at the last.

Example: Picrates of alkaloids and Osazones of sugars.

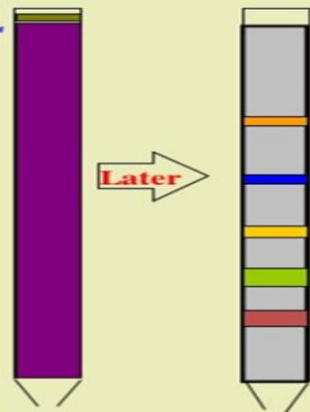
Glucose + Fructose dissolves in Ethanol, fructose crystallizes out first.

- **Adsorption Chromatography:** It is form of chromatography in which separation depends on differences between the components of the mobile phase and their affinity for adsorption on the surface of the stationary (solid) phase.

Original Chromatography Experiment

Start: A glass column is filled with powdered limestone (CaCO_3).

An EtOH extract of leaf pigments is applied to the top of the column. EtOH is used to flush the pigments down the column.



End: A series of colored bands is seen to form, corresponding to the different pigments in the original plant extract. These bands were later determined to be chlorophylls, xanthophylls and carotenoids.

Principle: selective adsorption of components

Assembly:

- It consists of a vertical glass tube into which adsorbent has been packed.
- Small plug of glass wool or sintered glass disc is placed at the bottom for support.
- In a column with nonpolar stationary phase, nonpolar components are retained and polar are eluted while the reverse happens when polar column is used.
- Distinct bands are obtained upon percolation with more pure solvent.

Common adsorbing materials: Alumina, kaolin, magnesium oxide, calcium carbonate, charcoal and sugars.

Applications:

- Isolation and purification of vitamins, hormones, many alkaloids, cardiac glycosides, anthraquinones
- Cleanup technique for removal of unwanted materials from plant extracts prior assay.

- **Partition Chromatography:** A process of separation of solutes utilizing the partition of solutes between two liquid phases.

The method has now been largely superseded by the more sophisticated HPLC but it retains the advantage of being inexpensive to set up and operate.

Principle: The separation of the components of a mixture depends on differences in the partition coefficients of the components between an aqueous and an immiscible organic liquid.

Setup:

- The aqueous phase usually the stationary phase is mixed with a suitable 'carrier' such as silica gel, purified kieselguhr or powdered glass and packed in a column as in adsorption chromatography.
- The mixture to be fractionated is introduced on the column, in a small volume of organic solvent and the chromatogram is developed with more solvent or successively with different solvents of increasing eluting power depending on their partition coefficient between the two liquid phase.

- **HPLC- High Performance Liquid Chromatography:**

- Liquid column chromatography system which employs relatively
- **Columns** -5 mm diameter
- **T**- ambient-temperature or up to 200°C
- **Pressure**-to 200 atm (20 000 kPa).
- **Normal flow rates** of eluate-2-5 ml/min but can be up to 10ml min⁻¹.
- Detection of the often very small quantities of solute in the eluate is possible by **continuous monitoring**.
- HPLC can give much improved and more rapid separation than can be obtained with the other liquid chromatography methods.
- Mostly used stationary phase is silica base which has porous particles of 5-10µm.
- The silanol groups (Si-OH) afford a polar surface which can be exploited in separations using an organic mobile phase.
- Racemic mixtures can also be separated.
- The separation of aromatics and the resolution of mixtures of (+)- and (-)-epicatechin and other proanthocyanidin enantiomers of *Cassia fistula* and *C.javanica*.

- **TLC- Thin Layer Chromatography:** Thin-layer chromatography (TLC) is a chromatography technique used to separate non-volatile mixtures.

Method: The method consists of preparing, on suitable glass plate, a thin layer of material, the sorbent, which may be either an adsorbent as used in column adsorption chromatography or an inert support which holds an aqueous phase as in column partition chromatography.

- The mixture to be resolved are dissolved in a suitable solvent.

- Mixture placed as a series of spots on the film towards one end of the plate.
- This end is then dipped in a suitable solvent mixture.
- The whole is enclosed in an air tight container.
- The solvent front travels up the film and after a suitable time the plate is removed, the solvent front is marked.
- Then the solvent is allowed to evaporate.
- Then the positions of the separated compounds are determined by suitable means.

Advantages:

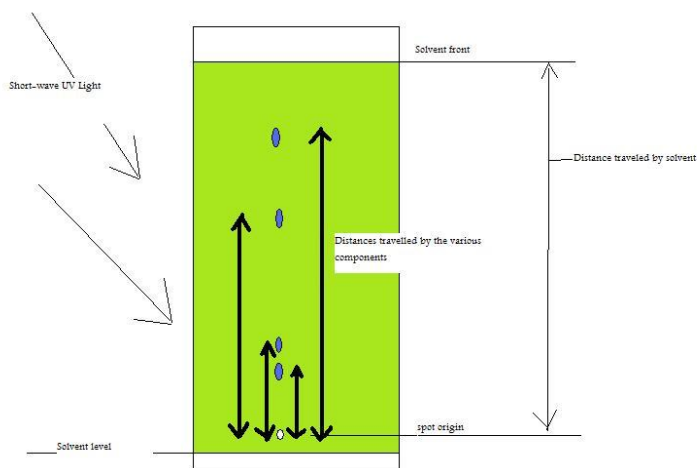
- Fractionations can be effected more rapidly with smaller quantities of mixture.
- The separated spots are usually more compact and more clearly demarcated from one another.
- The nature of the film is such that drastic reagents, as concentrated H_2SO_4 can be used for location of separated substance.

Adsorption TLC:

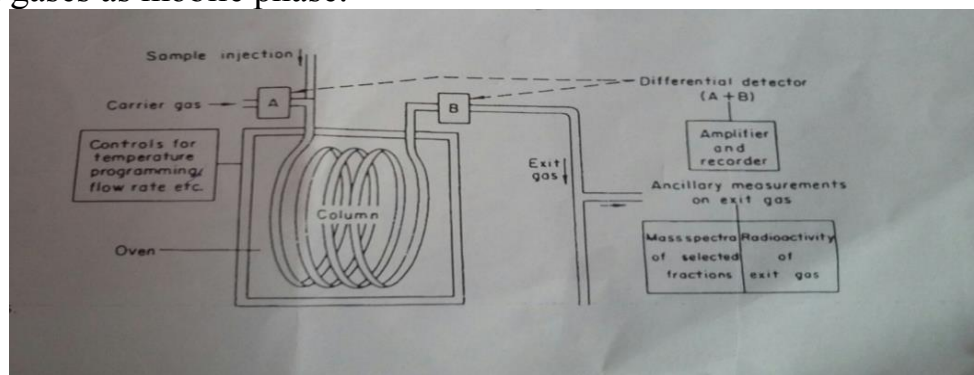
- With a adsorption TLC various substances exhibit different adsorptive capacities and any one material may vary in its activity according to the pre treatment.
- The adsorbent must be chosen in relation to the properties of the solvent and the mixture to be fractionated.
- Alumina is commonly used of different activity grades.
- To produce a film with reasonable handling, the adsorbent may be mixed with about 12% of its weight of $\text{CaSO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$ to act as a binder.
- Solutions of substances to be examined are applied the film with aid of capillary tubes or with microsyringe.
- The drug sample is placed in a cartridge.
- Then inserted in the TAS oven
- Compounds resolved on TLC plate are visualized using either general or specific methods; thus ultraviolet light will indicate fluorescent compounds.
- Iodine and Dragendorff's reagents are used in the form of sprays for the general detection of alkaloids.

- Ammonia vapour can be used for free anthraquinone compounds and fast blue salt B 'Merck' for cannabinoids and phloroglucides.

Preparative TLC: As mentioned above, thicker layers of sorbent are employed for preparative work and the separated bands of compounds are scraped from the plate and subjected to solvent extraction.



- **Gas Liquid Chromatography:** used by chemists to separate and identify the compounds in a mixture. This technique uses liquid as stationary phase and gases as mobile phase.



Method:

- The empty columns are made of glass or metal and often up to about 1.3m in length.
- James and Martin used a tube of 4mm internal diameter.
- The choice of stationary phase is governed by the temperature at which the column is to operate and the nature of the material to be fractionated.

- One method of dispersing the stationary phase over the inert support is to dissolve it in a low boiling point solvent such as ether, mix thoroughly with support and spread out the powder to allow the solvent to evaporate.
- The powder is then packed into the empty column a little at a time and as evenly as possible and then enclosed in a uniformly heated oven.
- The mobile phase is a gas which is inert as the other components.
- The choice of the gas is depend on the detector system. commonly gases are Hydrogen, Nitrogen, Argon.
- The flow rate of the gas is important: too high a flow rate will give incomplete separations and too slow a rate will give high retention time and diffuse peaks.
- Typical flow rates for short columns are 10-50ml/min.
- By means of suitable injection device, the sample to be analysed is introduced on the top of the column; 1.0-5.0ul.
- The mixture to be analysed should volatilize immediately it comes into contact with stationary phase.
- Some compounds, not themselves volatile, may be converted into volatile derivatives before chromatography.
- The detector system analyses the effluent gas from the column.
- All the differential detectors give an electrical signal which is recorded graphically by a suitable recorder. Not all the detectors give same relative response to given compounds under same conditions, so connected with double detector system.

Applications:

- Examination of many volatile oils e.g. clove oils, camphor, plant acids, some alkaloids(opium, tobacco and conium and tropane derivatives), The resins of the convolvulaceae and of cannabis, and steroidal compounds such as sapogenins and cardio active glycosides and aglycones.
- The detection and estimation of cocaine and its metabolites in the body is an important forensic application.
- The estimation of pesticide residues on crops is of utmost importance, and here the sensitivity of detector systems, such as the electron capture detector, has made possible the determination of the chlorinated pesticides down to the parts-per-billion range.

- **Capillary-Column Gas Chromatography:** As the name implies, capillary bore columns are used rather than the standard columns described above. They afford marked improvements in resolving power and in speed of analysis.
 - The internal diameters of the columns range from about 0.15mm to about 0.53mm and the columns can be 1 to 60m in length.
 - They were made of stainless steel and then glass, but fused silica columns are now considered the obvious choice.
 - As they are strong
 - Easy to use, highly inert and give excellent performance.

Columns hold the stationary phase in a number of ways;

- Wall-coated open tubular column(WCOT).
- Support-coated open tubular column(SCOT).
- Micro packed columns(MP).

1. These columns have the inner wall of the tube coated with stationary phase up to 1µm in thickness. Thicker layers, and hence increased sample capacity, can be achieved with silica columns having specially bounded phase. **WCOT columns** have the highest efficiency but are low sample capacity.

Greater thickness leads to column bleeding in which the stationary phase moves down the columns and eventually leaks into the detector.

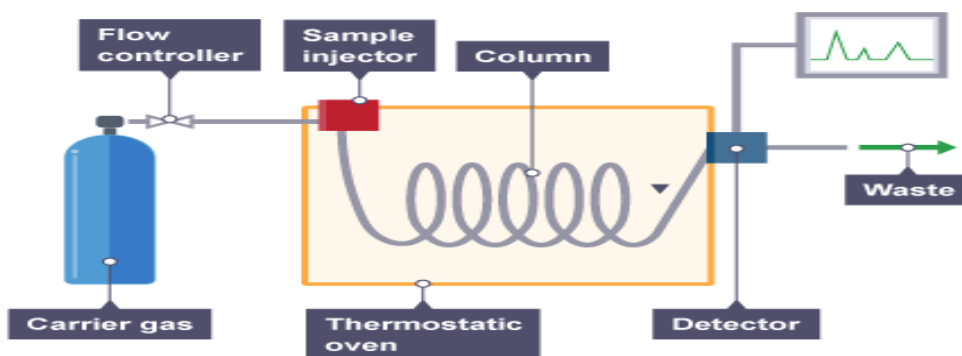
2. **SCOT columns** have the inner wall lined with a thin layer of support material coated with immobile phase.

This has the effect of increasing the available area of immobile phase, affording the column a greater load capacity.

3. **Micro packed columns** involve a coated support packed into narrow-bore columns.

Being more efficient than the normal packed columns but having the same problem in that, column length is restricted by the high back-pressure.

In CCGC, the volume of the sample dissolved in solvent for analysis by the capillary method is too small for a micro syringe and so special injection heads are necessary which either split the sample or are called split less-injector, which are able to accommodate large volume of solvent and deliver dissolved solute to the column.



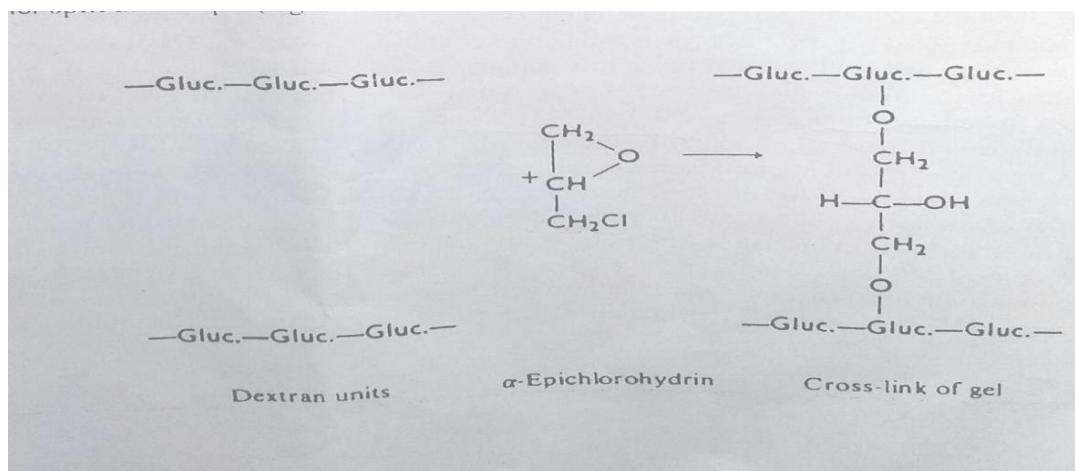
Applications:

- Separation of two enantiomers of linalool enabling the detection of reconstituted bergamot oil in the genuine oil.
- Detection of added reconstituted lemon oil in the genuine cold-pressed essential oil.

● Gel Filtration:

- Hydrophilic gels such as those prepared from starch, agar, polyacrylamide and cross linked dextrans have been used for the fractionation of proteins, peptides, amino acids and polysaccharides.
- The particles of these gels possess pores formed by the molecular structure of the gel.
- And when packed into a column and percolated with a solution, they permit large molecules of solute, which do not enter the pores, to pass rapidly down the column with solvent through the intergranular interstices.
- Thin layer of the sorbent can be used in TLC.
- Dextran gels are formed by the cross linking dextrans.

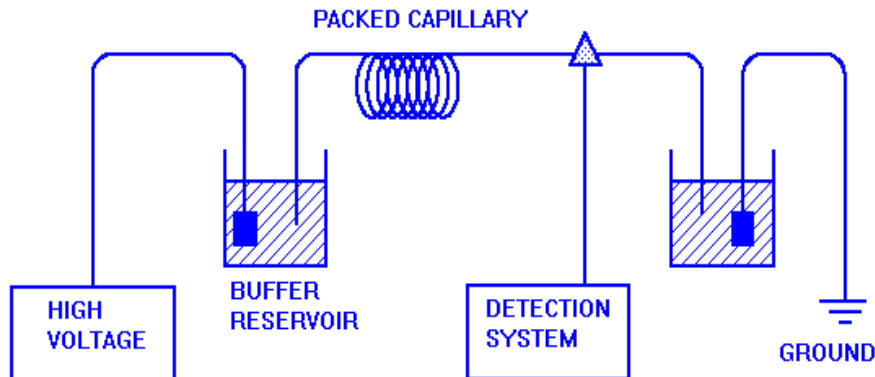
Each gel type possess a range of pore size, so that below the size limit of complete exclusion of the large molecules, different-sized solute molecules will enter the gel to a greater or lesser extent and so will vary in their elution rates.



Applications:

- Mixtures containing large molecules of various sizes and to separate the large molecules from the small ones. e.g. as in desalting operations on partially hydrolyzed proteins.
- **Electrochromatography:** In this technique, large molecules are fractionated by applying the electric field.
 - For the electrophoretic separation of mixtures, a filter paper strip is impregnated with solution of an electrolyte and supported in the center.
 - Its two ends are dipped into solutions in which electrodes are immersed.
 - A spot of the material to be fractionated is placed on the paper.
 - The whole apparatus sealed and a potential difference of 2-10 volts per centimeter applied along the paper.
 - Some separations are carried out at much higher voltages than the above.
 - The migration velocity for a given substance depends on the;
 - Magnitude of the ionic charge
 - Size and shape of molecule.
 - In this chromatography, the paper can be replaced by the thin layers of the gels as described in 'gel filtration'.
 - A development which combines the advantages of both gel filtration and electrophoresis is that of **polyacrylamide gradient gel electrophoresis**.
 - It is two dimensional electrophoresis system which separates according to mobility of solutes in one direction and according to size in the other.
 - **Capillary electrophoresis** is a technique of relatively recent introduction and can give separation efficiencies of the order of 4×10^4 theoretical plates.

- It provides a more rapid analysis than gel electrophoresis.



Applications:

Alkaloidal mixtures, plant acids, component sugars of cardiac active glycosides, anthraquinone derivatives can be separated and analysis of flavonoids in capillary electrophoresis.

- **Affinity Chromatography:**

- This method has been developed largely for the resolution of protein mixtures, and it depends on the specific, reversible binding of individual proteins with a particular ligand such as enzyme substrate or inhibitor.
- Excess ligand is removed by washing and the material is packed in a column.
- A protein mixture in a suitable buffer solution is passed down the column and protein with sufficient affinity for the bound ligand is retarded and may later be eluted in a purified state by a change in ionic strength or pH of the column buffer.

Applications:

- Applied to the purification of enzymes for potential clinical applications.
- For the isolation of antibodies.
- For specific fractionation of different types of cells e.g. erythrocytes and lymphocytes.

Characterization of Isolated Compounds

It is sufficient to state that the chemists are coming to rely more and more on the use of physical techniques to establish structure of new compounds and to identify known compounds in plant sources.

Various modifications of mass spectrometry MS have become of increasing importance for the structural characterization and determination of the active constituents of plants;

- Electron ionization MS
- Chemical ionization MS
- Field desorption MS
- Fast atom bombardment MS
- Electro spray ionization MS
- Sequential tandem MS

For example; electro spray MS and sequential MS combined to the investigation of the steroidal saponin mixture of the Chinese and indian drug *Tribulus terrestris*.

References:

- Page 137-149, General methods associated with phytochemical investigation of herbal products, Trease and Evans Pharmacognosy.
- Slideshare.

TOPIC 4

“CHEMISTRY AND CHEMICAL MODIFICATION OF NATURAL DRUG SUBSTANCES IN MODERN DRUG SYNTHESIS”

Introduction of Chemical modification

Drug innovation is characterized by painstaking molecular-level syntheses and modifications as the basic components of research and development. Similarly, natural products are chemically tailored and modified based upon their structural and biological properties

Chemical compounds usually derived from plants and other natural sources, have been used by human for thousands of years to alleviate pain, diarrhea, infection and various other maladies.

Until 19 century, the “remedies” were primarily crude preparation of plant material of unknown constitution. During 19 century structure of active constituents of naturally derived medicines were identified. Early study of relationship between chemical structure and biologic activity were conducted by Crum-Brown and Fraser (1869). They found that the compound containing tertiary amine groups’ exhibit muscle relaxant activity when converted to quaternary ammonium compound. It was concluded that specific chemical groups or nuclei were responsible for specific biological effects.

Modification strategies of natural products

Individualized manipulation based on the molecular size and complexity

For large and complicated natural products, simplified operations are normally carried out to eliminate structurally-unnecessary factors. Molecular dissection is a useful method. The linear molecules or peptides might be sheared along the chain consecutively. For compounds with fused rings, the scaffolds could be segmented into four quadrants to separately modify the structures or groups. Maintaining the structure-activity relationship (SAR) is absolutely necessary.

2. Analyzing SAR and designing novel structures

In the absence of information on target structures, classical medicinal chemistry methods are normally applied to the modification of natural products. SARs or quantitative SAR (QSAR) are explored to reveal and assign the pharmacophores, which guide the design of novel compounds with simplified or different scaffolds

4. Removal of unnecessary chiral centers

Chirality in drug molecules yields diploid characteristics. The positive side involves an increase of activity strength and selectivity because of the appropriate binding to steric ally-complementary and asymmetrical targets. The negative side is the difficulty in synthesis, separation, and resolution of single eutomers. In fact, not all chiral centers in natural products are necessary for binding and activity.

Chemical modification

It is chemical alteration of a known and previously characterized compound for the purpose of enhancing its usefulness as a drug. This could mean enhancing its specificity for a particular body target site, increasing its potency, improving its rate and extent of absorption, modifying to advantage its time course in the body, reducing its toxicity, changing its physical or chemical properties (like solubility) to provide desired features

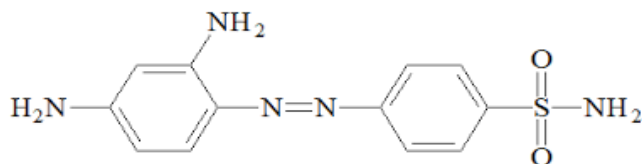
Chemical Modification of various Drugs:

SULPHA DRUGS

In 1935 Domagk healed his daughter from a streptococcal infection using the dye prontosil. In 1936, Ernest Fourneau in Paris demonstrated that Prontosil breaks down in the human body to produce sulphanilamide, the active agent against streptococci

HISTORY OF PRONTOSIL

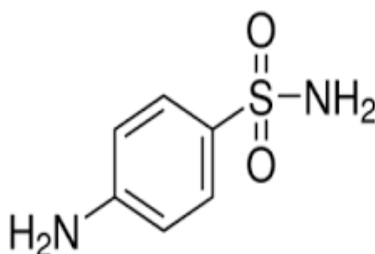
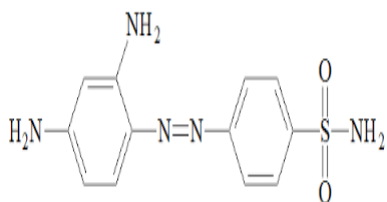
Prontosil is a red dye that was thought by the Bayer as similar to common dyes Structure:



- ❑ French scientists, Trefouel and Bovet, took the urine of a patient that received prontosil, the drug was metabolized into active form known as Sulphanilamide

Structure of prontosil

sulphanilamide

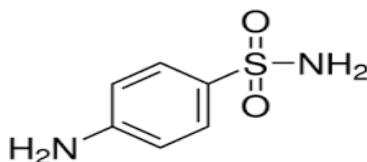
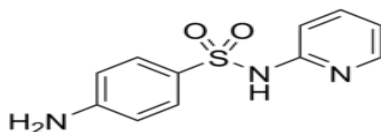


This discovery led to develop Sulphonylureas as hypoglycemic agents and carbonic anhydrase inhibitor as diuretic

- ❑ Sulphonamides have been an important part of our antibacterial history

- ❑ Since 1930's over 4500 different sulphonamides have been synthesized e.g **sulfanilamide**

sulfa pyridine:



CLASSIFICATION

Sulphonamides used for the treatment of systemic infections

- ❑ **SHORT ACTING SULPHONAMIDE**
Sulphanilamide, Sulphadiazine
- ❑ **INTERMEDIATE ACTING SULPHONAMIDE**
Sulphamethoxazole, Sulphaphenazole
- ❑ **LONG ACTING SULPHONAMIDE**
Sulphamethoxypyridazine, Sulphadimethoxine
- ❑ **ULTRA LONG ACTING**

Sulphadoxine

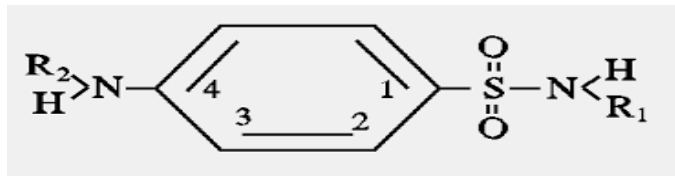
S.A.R OF SULPHONAMIDE

1. Sulphonamides consist of a benzene ring, an amine moiety ($-NH_2$), and a sulfonamide group ($-SO_2NH_2$).
2. Relatively insoluble in water but their sodium salts are readily soluble.
3. $-NH_2$ and $-SO_2NH_2$ must be *para* to one another (possess antibacterial properties)
4. P.amino group must be unsubstituted and only mono substitution is allowed on the sulphonamide group
5. Pka for amino group should be 4.7 and sulphonamide moiety for antibacterial activity
6. Monosubstitution on the sulfonamide group is the basis for pharmacokinetic variation within the class of antibacterial
7. The addition of different functional groups manipulate the properties of the drug

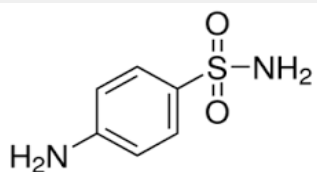
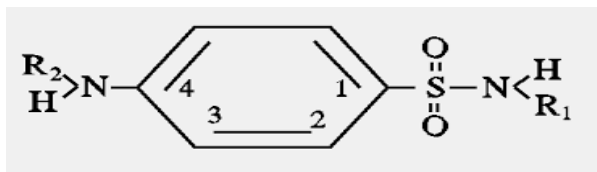
DISADVANTAGE OF SULPHONAMIDE

Renal damage due to crystallization in kidneys

BASIC RING STRUCTURE OF SULPHONAMIDE



Substitution of Hydrogen on R1 and R2 GIVE SULFANILAMIDE

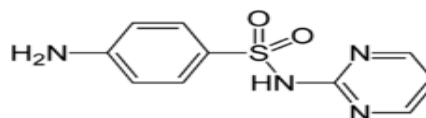


SUBSTITUTION ON R1

8. Substitution of 2-amino pyrimidine at R1 gives Short acting sulphonamide named SULPHADIAZINE

9. Chemically it is 4-amino-N-2-pyridinyl benzene sulphonamide

Structure of SULPHADIAZINE



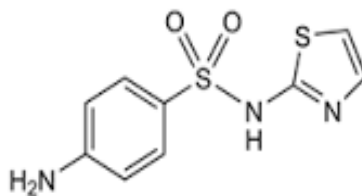
SUBSTITUTION OF 4-amino-n-(thiazole-2-yl) on r1 gives SULPHATHIAZOLE

Short acting

Chemically it is 4-amino-N-(2-thiazolyl)benzene sulphonamide

Used topically to treat skin infection

SULPHATHIAZOLE



SUBSTITUTION OF 3-AMINO-5-METHYL ISOXAZOLE GIVES SULPHAMETHOXAZOLE

Intermediate acting

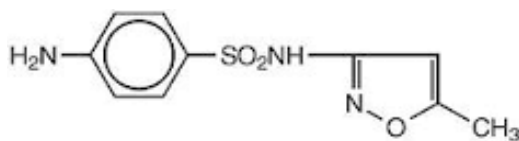
Chemically it is 4-amino-N-(5-methyl-3-isoxazolyl) benzene sulphonamide

USES

Mainly used to treat acute, uncomplicated urinary tract infections caused by E.Coli.

With trimethoprim in 5:1 proportions is used as bactericidal or bacteriostatic against gram-positive and gram-negative organisms

SULPHAMETHOXAZOLE



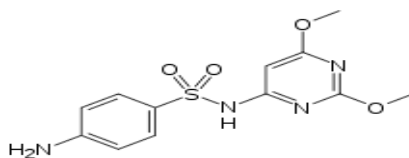
SUBSTITUTION OF 4-AMINO-N-(2, 6-DIMETHOXY-4-PYRIMIDINE GIVES SULPHADIMETHOXINE

Chemically it is 4-amino-N-(2,6-dimethoxy-4-pyrimidine)benzene-sulphonamide

Long acting sulphonamide

USES

Treat respiratory, genitourinary tract, enteric and soft tissue infections against streptococci, staphylococci, escherichia, salmonella, klebsella or shigella



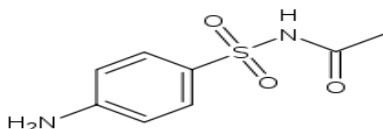
ACETYLATION OF SULFANILAMIDE GIVES SULFACETAMIDE

Acetyl derivative of sulfanilamide

USES

Used in form of injection or eye drops or eye ointments to treat eye infections

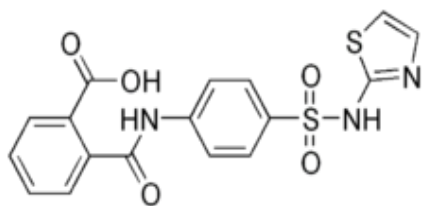
STRUCTURE



OTHER EXAMPLES

Sulphaguanidine formed by alkaline hydrolysis and condensing p-amino benzene sulphonyl chloride with guanidine nitrate

Effective against Gram +ve and Gram -ve bacteria.

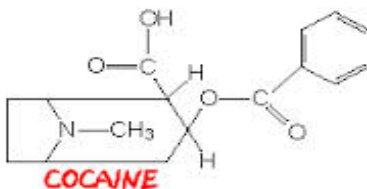


Local Anesthetics

Can be traced back to naturally alkaloid cocaine isolated from *Erythroxylon coca* by Swedish chemist Albert Niemen at Gothenburg University in 1860

Koller found that Cocaine numbed the tongue, thus he discovered a local anesthetic

STRUCTURE OF COCAINE



USES OF COCAINE

- Exerts a strong local anesthetic activity
- Vasoconstriction activity due to longer duration of activity

DISADVANTAGES

- Addiction liability
- High toxicity
- Cortical stimulation

Due to legal restriction on its production and sale, search for newer non-habit forming, cheaper and better local anesthetic was carried out. The search has yielded good local anesthetics which are used in clinical practice. However cocaine is still used in ophthalmic because of its mydriatic effect

SAR:

HYDROLYSIS OF CARBOMETHOXY AND BENZOATE GROUP

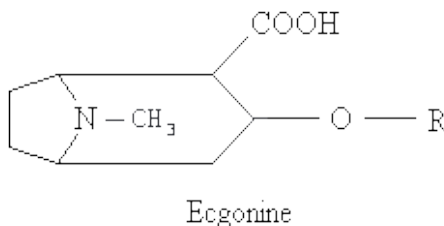
Hydrolysis of carbomethoxyl group and benzoate group leading to ecgonine

Results in loss of activity

REMOVAL OF CARBOXYLIC GROUP

However decarboxylated cocaine, tropococaine possessed local anesthetic activity

STRUCTURES OF ECGONINE



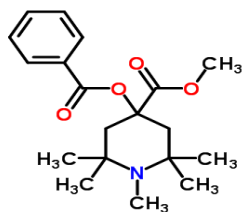
TROPOCOCAINE



LOCAL ANESTHETIC ACTIVITY OBSERVED IN PIPERIDINE ANALOGUES

Observance of local anesthetic activity in piperidine analogs, a. eucaïne and b. eucaïne showed that tropane moiety was not necessary for local anesthetic-activity

STRUCTURES



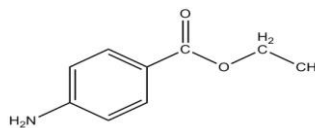
Alpha-eucaine



beta-eucaine

LOCAL ANESTHETIC ACTIVITY OBSERVED IN BENZOCAINE

Local anesthetic activity was also observed in ethyl p-aminobenzoate which is known as benzocaine. An effective compound. Retained in medicine being used as dusting powder.



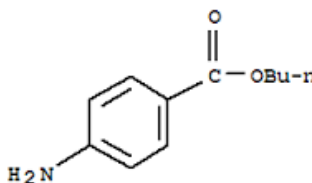
Benzocaine
[Benzoic acid, 4-amino-, ethyl ester]

STRUCTURE

BUTYL ANALOGUE OF BENZOCAINE GIVES BUTESIN

Butyl analog of benzocaine butesin used as picrate salt on denuded surfaces.

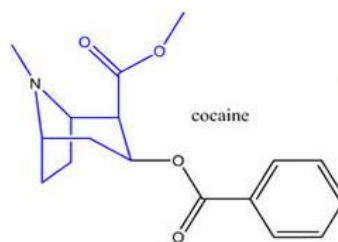
STRUCTURE



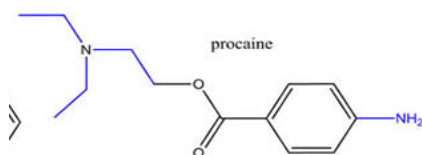
DIALKYLAMINO ESTERS OF AROMATIC ACIDS

From SAR of cocaine and the activity of alkyl p-aminobenzoate led to a new series of local anesthetics called dialkylamino esters of aromatic acid.

So anesthesophore group in cocaine consisted of aromatic acids esterified with tertiary amino alcohol.



Procaine had the most favorable therapeutic ratio among these type of esters having local anesthetic activity Its monochloride is known as Novocain.



ADVANTAGES

- Low toxicity
- Low cost of manufacture
- Stability of its solutions

DISADVANTAGES

- Weak anesthetic effect
- Short duration of action for prolonged duration it is used in oil or with polyethylene glycol propylene glycol

INCREASE IN SIZE OF ALKYL CHAIN ON PROCAINE RESULTING IN BUTACAINE AND AMETHOCAINE

Increase in the size of alkyl group on the amino nitrogen and increase in size of the chain leads to prolongation of duration of action

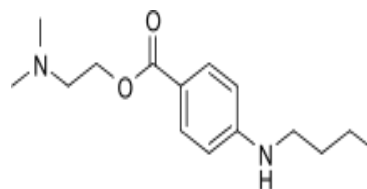
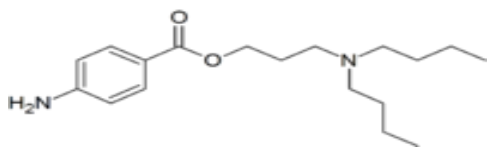
Addition of butyl chains on Nitrogen yields butacaine

Amethocaine has butyl group substituted at amino group

USES

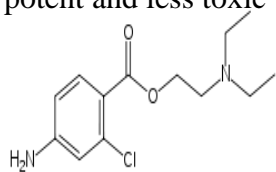
- For infiltration
- Surface anesthesia.

STRUCTURE OF BUTACAINE AND AMETHOCAINE

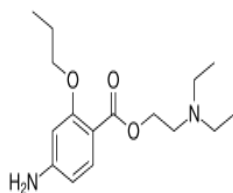


SUBSTITUTION ON AROMATIC NUCLEOUS AT OTHER POSITON

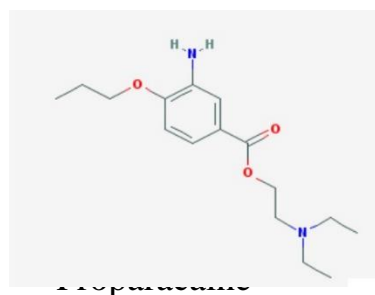
Compounds where the aromatic nucleus containing substitution at other position also possess local anesthetic activity i.e Chloroprocaine used as infiltration anesthesia Propoxycaine, benoxinate and propoxycaine, proparacaine(structure isomer of propoxycaine)found as more potent and less toxic than cocaine



Chloroprocaine



Propoxycaine



REPLACING PHENYL RING WITH OTHER AROMATIC RING

As basic ester of naphthanoic acid,naphthacaine has found use as infiltration anesthetic Other compounds found as local anesthetic during this study include amylocaine, hexylcaine, isobucaine., meprylcaine and cyclomethylcaine etc

DESIGNING DRUGS TO COMBATE CANCER

The drugs used to treat cancer belong to two categories

1. Cytotoxic drugs (cell killing drugs)
2. Cytostatic drugs (cell stabilizing drugs)

Both categories lead to reduction in size of tumor

CYTOTOXIC DRUGS

- Cytotoxic drugs work by interfering the DNA replication because cancer cells are rapidly dividing and rapidly synthesizing the new DNA
- 3 main groups of drugs that can interfere with DNA replication .

1. Antimetabolites
2. Alkylating agents
3. DNA binding agents
- 4.

ANTIMETABOLITES

- Molecules that appear to be nucleotides so incorporated into the DNA leading to non-functional DNA .
- The drugs that interfere one or more enzymes or their reactions that are necessary for DNA synthesis.
- **EXAMPLE :methotrexate, mercaptopurine, 5-flourouracil**

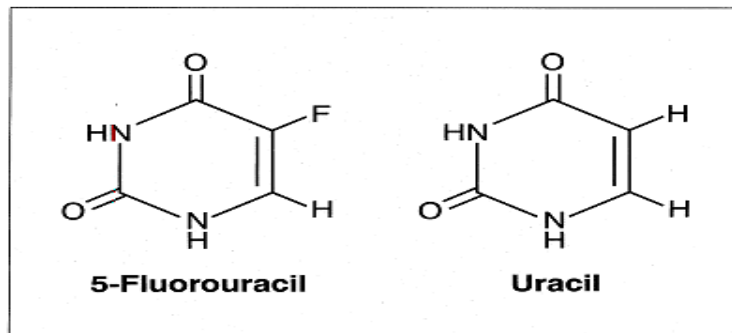


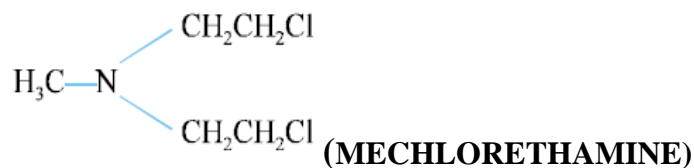
Figure 1: Clinical structures of 5-FU and uracil. Adapted with permission from Valeriotte et al.[2]
5-FU = 5-fluorouracil.

ALKYLATING AGENTS

- These are the compounds that works by adding the alkyle group to protienstha make up the DNA molecule,preventing the strands of double helix from linking as they should .
- This causes the breakage of the DNA strands affecting the ability of cancer cells to multiply.
- Unfortunately these are attaches to many other molecules in cells.

EXAMPLES :

- nitrogen mustards e.g.Mechlorethamine, cyclophosphamide
- nitroso urease
- alkylesulphonates



DNA BINDING AGENTS

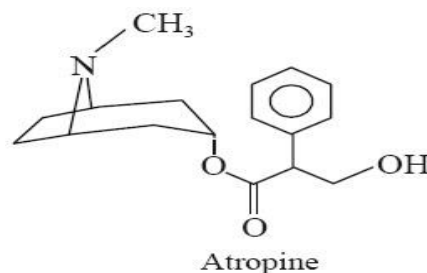
- Molecules that attach to DNA chain breaks it, disengage from it and then attach to another chain to repeat the process .
- They usually function with enzyme.
- These are currently the most effective drugs currently in use.
- EXAMPLES: Anthracyclines like; Doxorubicin, Epirubicin.

Antimuscarinic Agents

Commonly known as antimuscarinics, these agents (for example, *atropine* and *scopolamine*) block muscarinic receptors., causing inhibition of all muscarinic functions. In addition, these drugs block the few exceptional sympathetic neurons that are cholinergic, such as those innervating salivary and sweat glands.

Atropine:

- Atropine a tertiary amine belladonna alkaloid
- has a high affinity for muscarinic receptors

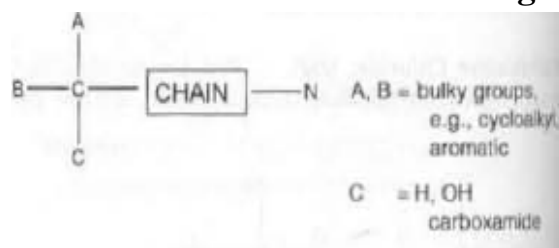


Mechanism of action:

- Binds competitively, with muscarinic receptor, preventing acetylcholine from binding to those sites
- *Atropine* acts both centrally and peripherally.
- Its general actions last about 4 hours except when placed topically in the eye, where the action may last for days.

Structure activity relationship:

General structure of anticholinergics:



- The A or B groups must be carbocyclic or heterocyclic, but if both are cyclic it gives maximal antagonist potency. The rings may be same or different. One of is generally aromatic and other is saturated ring or olefinic group (ie it has a C-C double bond). Rings may be same or different. The benzene could be any type Cyclohexane (non-aromatic carbocyclic or pyridine (aromatic heterocyclic) or Pyrrolidine (non-aromatic heterocyclic A and C
- The C group can be hydrogen, hydroxyl (-OH), hydroxymethyl (-CH₂OH), amide. Best potency is seen with hydroxyl or hydroxymethyl.
- The N substituent can be both quaternary ammonium salt and tertiary amine with different alkyl groups. Most potent derivatives have quaternary ammonium salt. The alkyl group is not restricted to only methyl. It can be ethyl, propyl or isopropyl.
- The distance between the ring substituted carbon and nitrogen is not fixed ie it can vary the no. of alkyl units between that carbon and nitrogen can vary from 2-4, with most potency in case of two CH₂ units.

ANTIBIOTICS:

Antibiotics are the metabolites of microorganisms which in low concentration destroy or inhibit the growth of other microorganisms.

Discovery of antibiotics:

➤ The era of antibiotics started with the discovery of antibiotics properties of penicillin by Fleming in 1929. Subsequently, many therapeutically used antibiotics have been reported.

Chemical Classification

This classification includes:

- B-lactam antibiotics
- Peptide antibiotics

- Polyene antibiotics
- Macrolide antibiotics
- Aminoglycoside antibiotics
- Antibiotics with fused rings
- Miscellaneous antibiotics

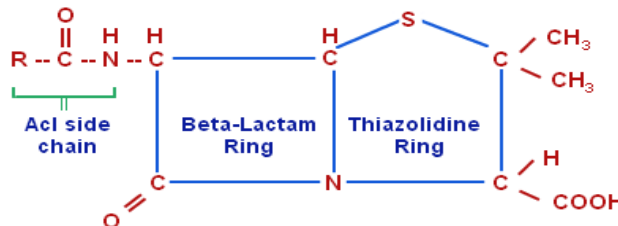
B-lactam antibiotics

➤ Antibiotics belong to this class include penicillin and cephalosporins. Both of these class of compounds contain a B-lactam ring fused to a heterocyclic ring. In case of penicillin B-lactam ring fused to thiazolidine ring and in case of cephalosporins B-lactam ring fused to dihydrothiazine ring.

Penicillin (wonder drug)

➤ Penicillin was discovered by Alexander Fleming in 1928 when he was working with cultures of staphylococcus aureus. An air borne fungus contaminated the culture and produce clear zone of inhibition in culture showing antimicrobial effect of this fungus. Later on fungus penicillium-notatum and p-chrysogenum were discovered. Penicillins are derived from a number of strains of these fungus.

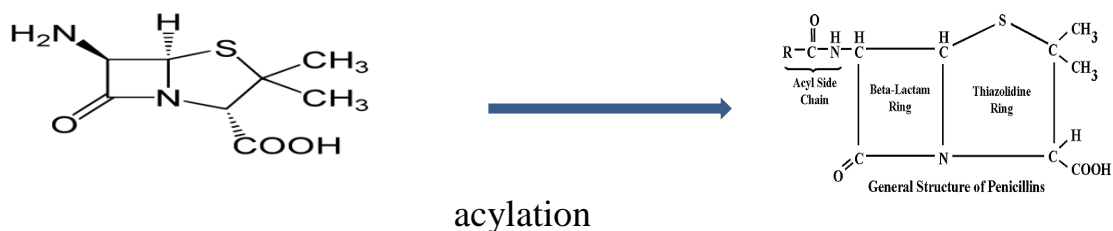
Structure of penicillin's



General Structure of Penicillins

Chemical modifications in penicillin

Penicillins comprises of entire group of natural and semi-synthetic penicillins and all are derived from 6-aminopenicillanic acid and differ only in R substituent attached to 6,APA. 6,APA is converted into penicillin by the acylation in the prescence of amidase with acetyl chloride or acetic anhydride and then by adding different functional groups in the side chain of carbonyl carbon ,newer antibiotics are obtained. So main precursor is 6,APA.



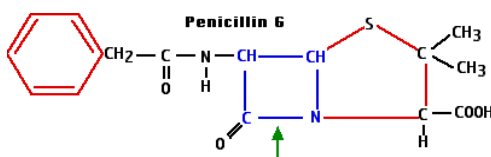
Penicillins are classified into

1. Natural penicillins
2. Semisynthetic penicillins

Natural penicillins: The two Natural penicillins obtained from the cultures of *p.notatum* and *p.chrysogenum* are penicillin G and more acid resistant penicillin V.

Penicillin G (natural penicillin)

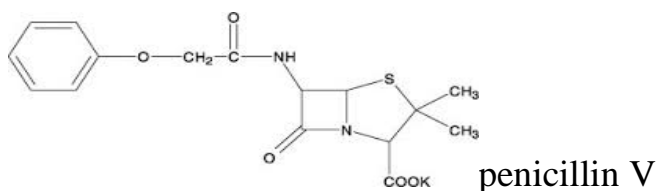
➤ By altering side chain of general penicillin, penicillin G is obtained as shown below...



Penicillin G also known as benzyl penicillin contains all qualities of an “ideal antibiotic” and is effective against a wide variety of organisms including fungi. It is obtained from the fermentation of *p-chrysogenum*.

Penicillin V

➤ Penicillin V or phenoxymethyl penicillin is obtained by adding 2- phenoxyethanol to *p.notatum* culture. structure as shown below...

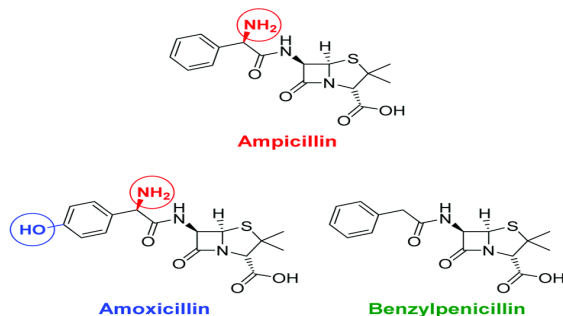


- Aminopenicillins (ampicillin and amoxicillin)

The major difference in the ampicillin and amoxicillin is their higher oral absorption and high serum level.

- Ampicillin: amino subst. Of penicillin G and known as p-amino benzyl penicillin and the corresponding product from the acylation with 2-azido-4-hydroxyl acetyl chloride is **amoxicillin** .

➤ **Amoxicillin** differs from **ampicillin** from p.hydroxyl group on benzene ring.



- Bacampicillin is another prodrug of ampicillin. Which is the ester prodrug of ampicillin

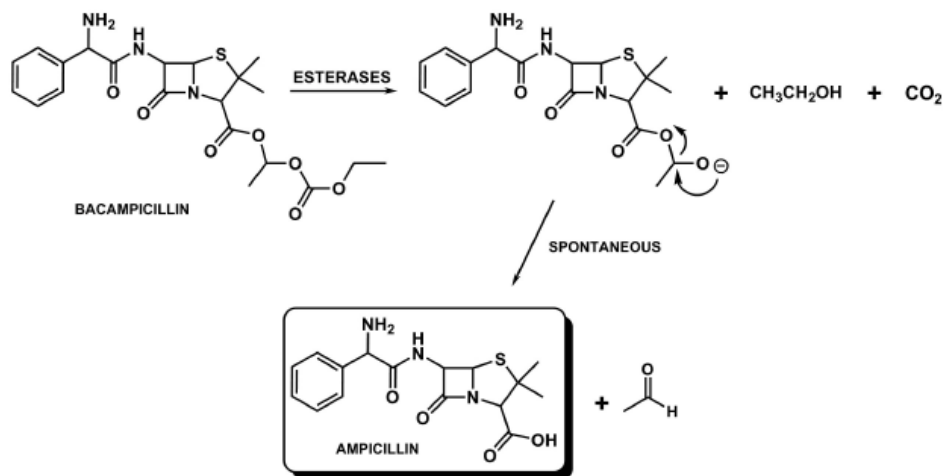


FIGURE 20 - Bacampicillin structure and its conversion into ampicillin.

Semisynthetic Penicillins

➤ Natural penicillins can be further modified chemically by substitution of 6-amino group of 6,APA that confer new properties. The new acylated products produced are termed as semi synthetic penicillins. There are three types of semisynthetic penicillins...

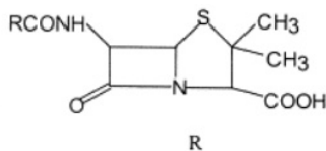
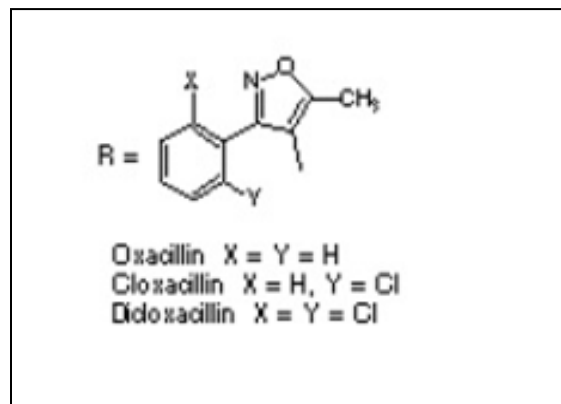
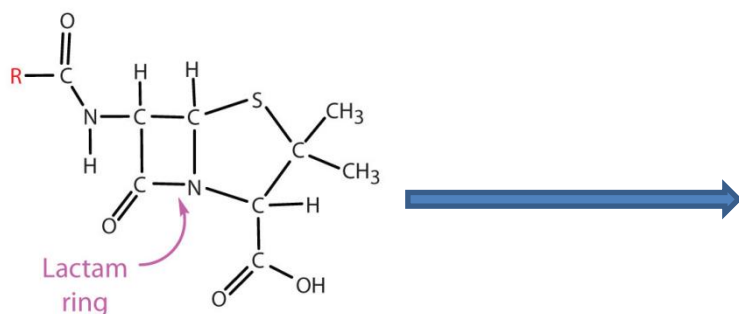
- Penicillinase resistant penicillins
- Broad spectrum penicillins
- Increased acid resistant penicillins

Penicillinase resistant penicillins

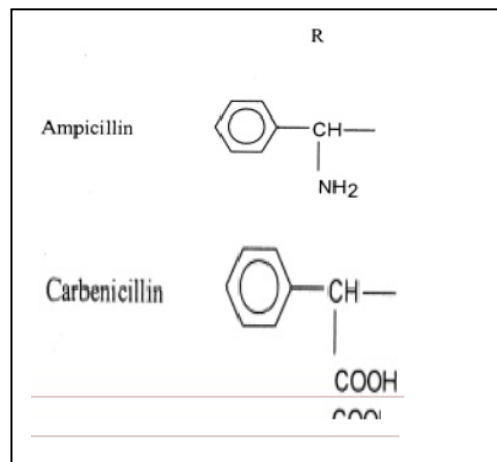
➤ Penicillin resistant penicillins are effective against staphylococcus infection. The resistance to penicillinase can be achieved by substitution on the carbon adjacent to amide carbonyl.

➤ This is achieved by incorporating the alpha carbon atom of side chain into an heterocyclic aromatic ring and placing appropriate susbs. At ortho position. some of the examples of penicillinase resistant penicillins are

- Methicillin
- Nafcillin
- Oxacillin
- Cloxacillin
- Dicloxacillin



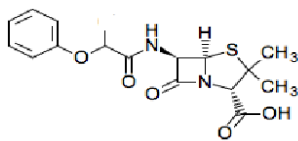
main nucleus



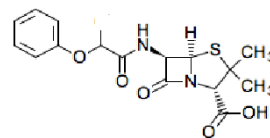
Increased acid resistant penicillin's

➤ Incorporation of the alpha aryloxyalkyl moiety in the amide portion imparts acid resistant properties and they were the first semisynthetic penicillins to be prepared from 6,APA. Some of these includes...

- Phenoxyethylpenicillin or penicillin V
- Alpha phenoxyethylpenicillin or phenethicillin
- Alpha phenoxypropylpenicillin or propicillin etc



C2H5



C3H7

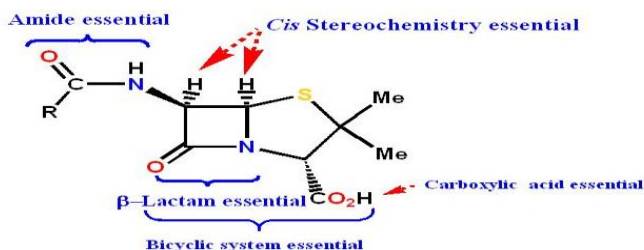
Phenithicillin (phenoxyethylpenicillin)

Propicillin (phenoxypropylpenicillin)

SAR of Penicillins

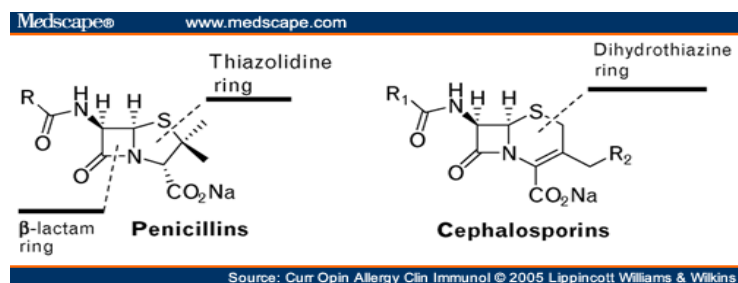
- Oxidation of sulphur group decrease activity

- The methyl group, carbonyl and nitrogen at position 7 are essential for activity.
- Esterification of -COOH group at position 3 decrease activity
- Any subs. At position 5 results in decreased activity
- Any modification in penam ring or the cleavage of beta lactam ring results in decreased activity.



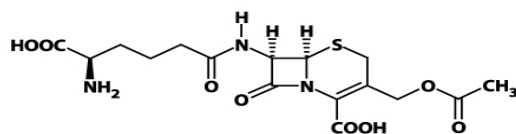
CEPHALOSPORINS

- Cephalosporins are beta lactam antibiotics isolated from the cephalosporium spp. Or prepared semisynthetically.
- In 1945 Brotzu discovered that cultures of fungus “cephalosporium acremonium” inhibit growth of a wide range of gram positive and gram negative bacteria.
- In 1961 Abraham and Newton isolated three principle antibiotic components...
- **Cephalosporin P** was a steroid with minimal antibiotic activity.
- **Cephalosporin C** contains dihydrothiazine ring instead of thiazolidine ring of penicillins.



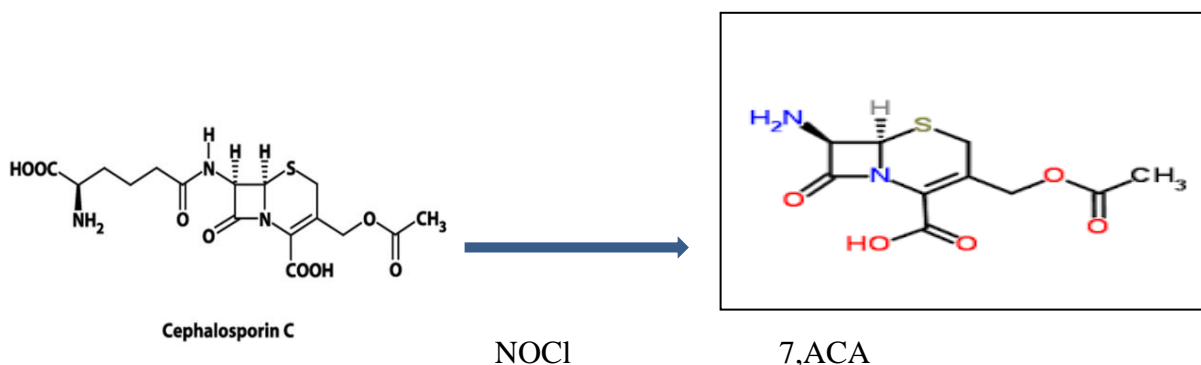
CHEMICAL MODIFICATIONS OF CEPHALOSPORINS

➤ The discovery that the alpha amino adipyl side chain could be removed to efficiently produce 7-aminocephalosporanic acid (7,ACA) led to semisynthetic cephalosporins of medicinal values.

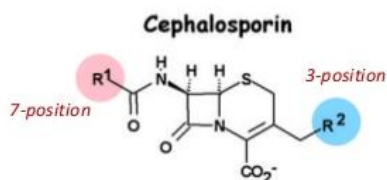


Cephalosporin C

➤ Like 6,APA, 7-aminocephalosporanic acid has been obtained by the degradation of cephalosporin C with NOCl.



- This coupled with modifications of substituents at position 3 and introduction of substituents at position 7. so 7,ACA is the precursor from which we can produce series of semisynthetic cephalosporins.

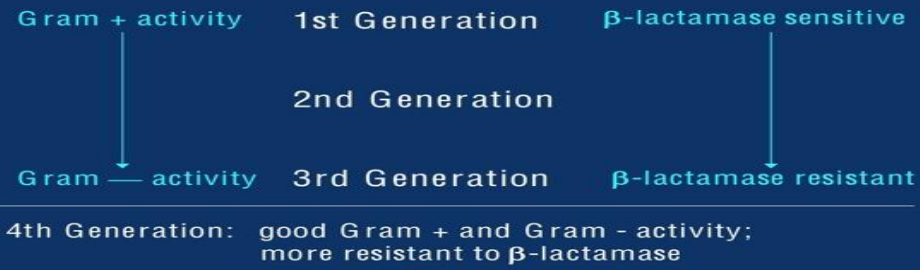


main nucleus of cephalosporins

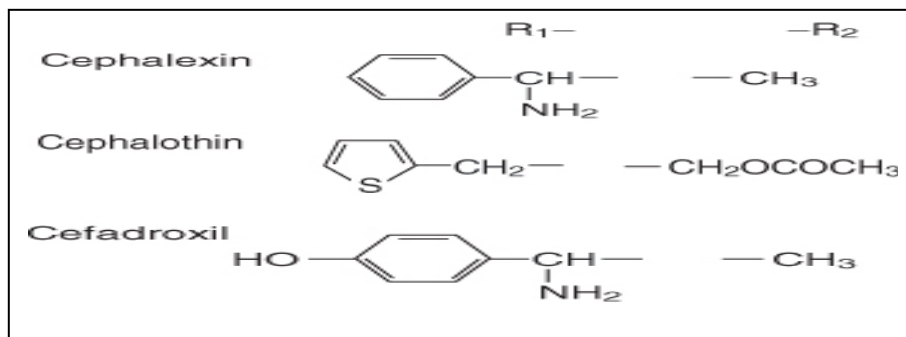
CLASSIFICATION OF CEPHALOSPORINS:

➤ There are four generations of cephalosporins and categorized in progression from 1st to fourth generation.

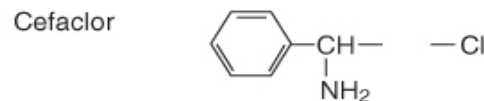
Cephalosporins



First generation cephalosporins

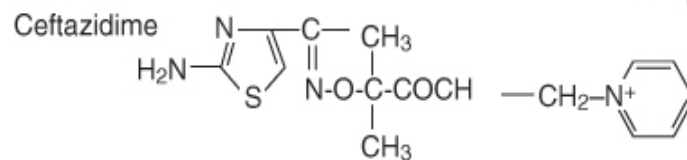
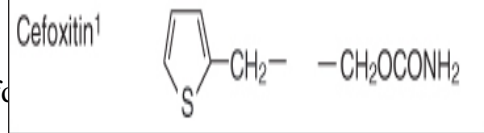


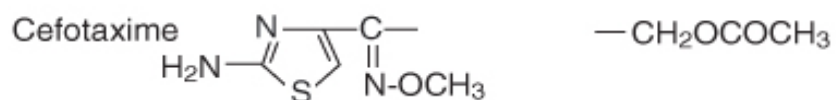
2. Second generation cephalosporins



3. Third generation cephalosporin's

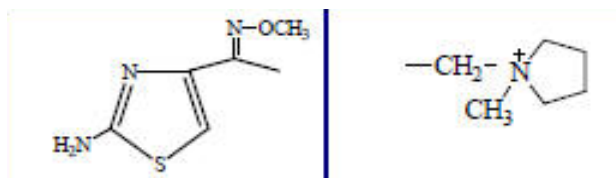
Ceftazidime, cefotaxime, cefixime, cefprozone, ceftriaxone, cefepime





4. Fourth generation cephalosporins

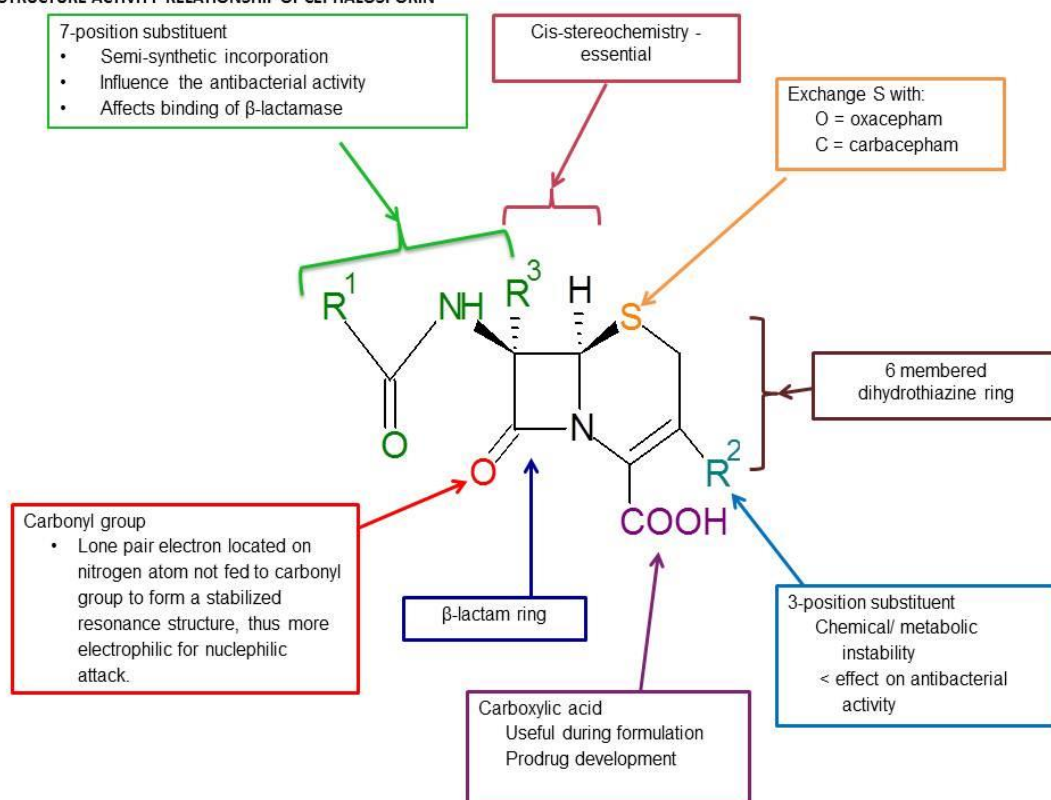
➤ Cefepime and cefpirome



Cefepime

SAR OF CEPHALOSPORINS

STRUCTURE ACTIVITY RELATIONSHIP OF CEPHALOSPORIN



TOPIC 5

**“Developments in the discovery and chemical studies
of plant-derived anticancer agents”**

Definition:

Cancer is disease in which there is uncontrolled proliferation and spread with in the body of abnormal form of body own cell.

Neoplasia:

Neoplasia is an abnormal mass of tissue the growth of which exceeds (uncontrolled) and is uncoordinated with that of the normal tissues and persist in the same excessive manner after the cessation of stimuli which evoked the change.

- In common medical usage Neoplasia is often referred to as tumor.
- **Benign Tumor**- Which is not malignant and favorable for recovery.
- **Malignant Tumor**-Tendency to become worse and end in death..

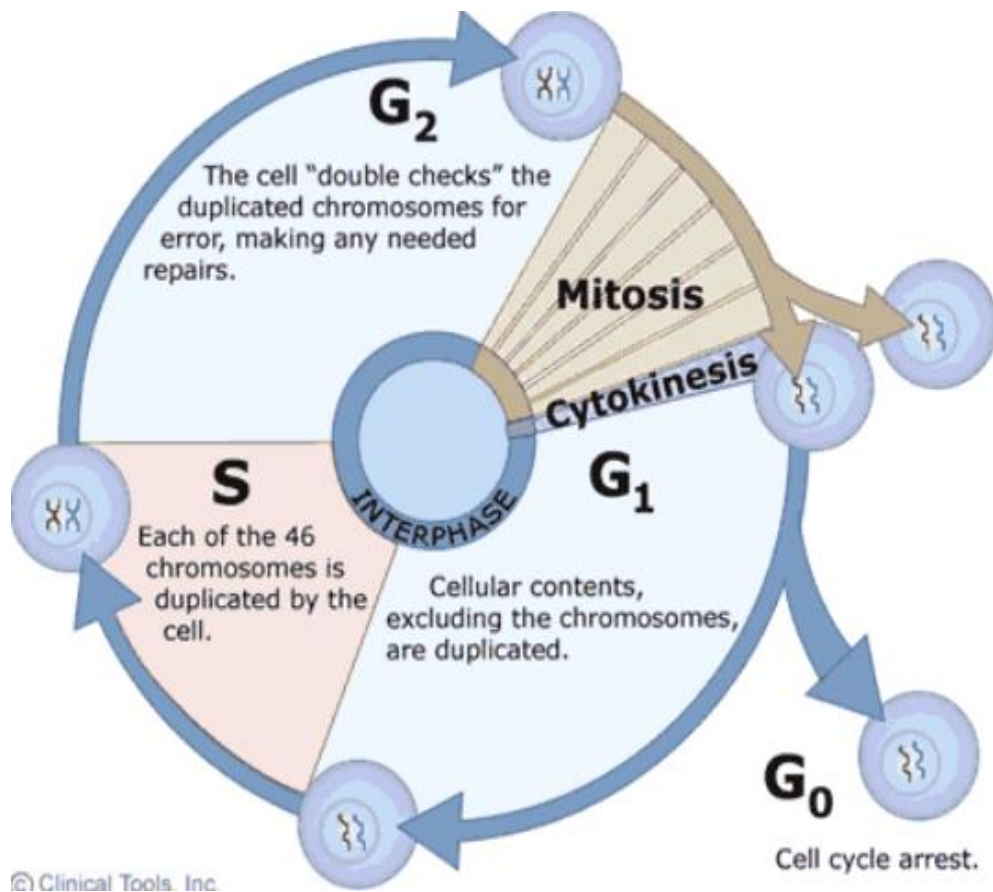
Cancer pathogenesis and cancer chemotherapy: general principles

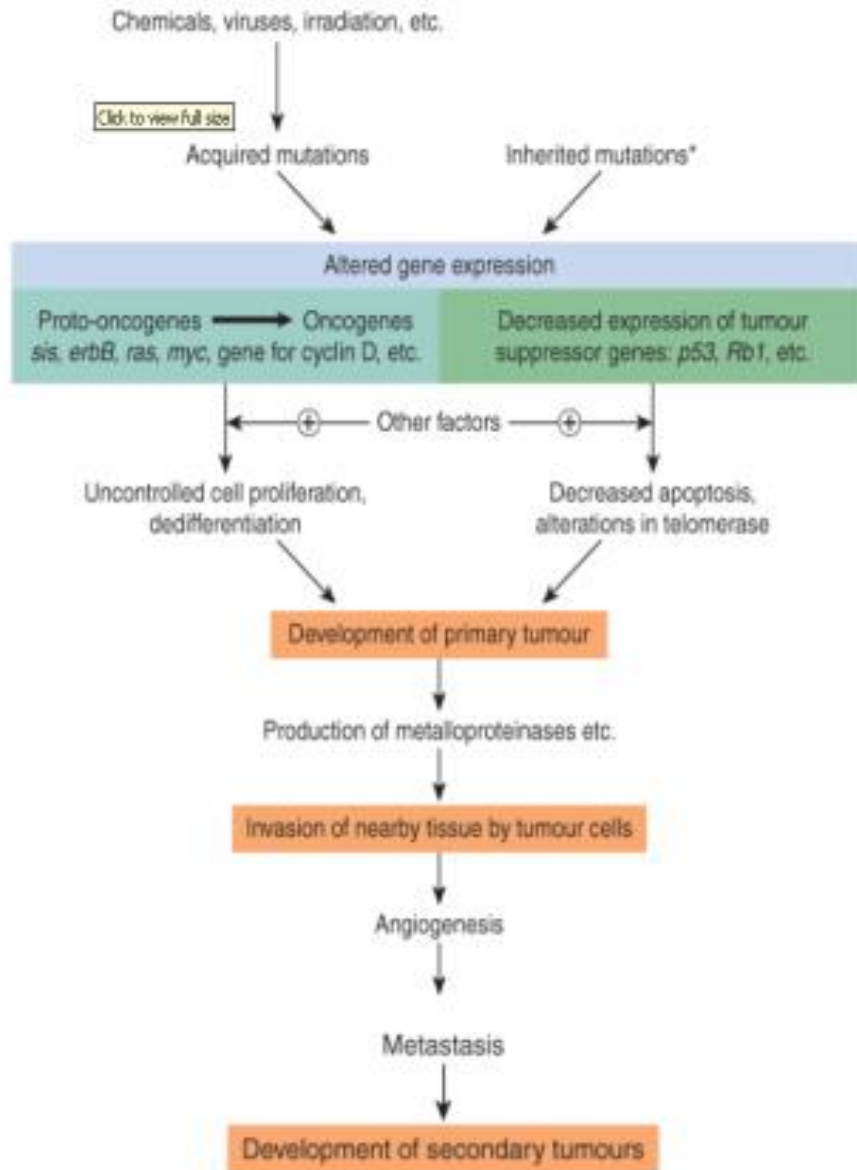
- The term *cancer* refers to a malignant neoplasm (new growth).
- Cancer arises as a result of a series of genetic and epigenetic changes, the main genetic lesions being:
 1. Inactivation of tumour suppressor genes.
 2. The activation of oncogenes (mutation of the normal genes controlling cell division and other processes).
- **Cancer cells have four characteristics that distinguish them from normal cells:**
 1. Uncontrolled proliferation
 2. Loss of function because of lack of capacity to differentiate
 3. Invasiveness
 4. The ability to metastasise.
- Cancer cells have uncontrolled proliferation because of changes in:
 1. Growth factors and/or their receptors
 2. Intracellular signalling pathways, particularly those controlling the cell cycle and apoptosis
 3. Telomerase expression
 4. Tumour-related angiogenesis.

- Most anticancer drugs are antiproliferative-most damage DNA and thereby initiate apoptosis. They also affect rapidly dividing normal cells and are thus likely to depress bone marrow, impair healing and depress growth. Most cause nausea, vomiting, sterility, hair loss and teratogenicity.

Cell cycle:

Refers to series of stages through which cell passes during its development.





CANCER WARNING SIGNS

- *C*hange in bowel or bladder habit
- *A* sore that does not heal
- *U*nusual bleeding or discharge
- *T*hickening or lump in breast or else where
- *I*ndigestion or difficulty in swelling
- *O*bvious change in wart or mole
- *N*agging cough or hoarseness

PRECAUTIONARY MEASURES

- *Cancel smoking*
- *Avoid alcohol*
- *Reduce stress*
- *Checkup & pap smear every year*
- *Investigate skin changes*
- *Never neglect nodes*
- *Overcome laziness*
- *Measure suspicious stains*
- *Appetite & hunger*

FACTORS

1. Age
2. Sex
3. Chemical carcinogens
4. Environmental factors e.g smoke
5. Viruses
6. Radiations
7. Immune factors
8. Hormones
9. Food

TREATMENT STRATEGIES

1. **Chemotherapy**
2. **Radiotherapy**
3. **Surgery**

1. Chemotherapy:

The main anticancer drugs can be divided into the following general categories.

1. **Cytotoxic drugs:**

- *Alkylating agents* and related compounds, which act by forming covalent bonds with DNA and thus impeding replication
- *Antimetabolites*, which block or subvert one or more of the metabolic pathways involved in DNA synthesis

- ***Cytotoxic antibiotics***, i.e. substances of microbial origin that prevent mammalian cell division
- ***Plant derivatives*** (vinca alkaloids, Taxans, camptothecins) -most of these specifically affect microtubule function and hence the formation of the mitotic spindle.

2. **Hormones:**

The most important are steroids, namely glucocorticoids, estrogens and androgens, as well as drugs that suppress hormone secretion or antagonize hormone action

3. **Miscellaneous agents**

These do not fit into the above categories. This group includes a number of recently developed drugs designed to affect specific tumour related targets.

Developments in the discovery and chemical studies of plant-derived anticancer agents

In the endeavour to discover effective drugs for the treatment of various cancerous diseases, the natural kingdoms, especially the plant kingdom, have been extensively researched. The research involved has been enormous and although the number of successful outcomes appears very modest, the effective drugs produced rank among the most common chemotherapeutic agents employed. Also, the wide diversity and complexity of the compounds isolated have afforded valuable material for the manufacture of semi-synthetic derivatives, often less toxic and clinically superior to the original isolate.

Anticancer Drugs:

It has been estimated (2005) that over 60% of the anticancer drugs in current use are in some way derived from plants and microorganisms; marine products are in the process of evaluation. A successful anticancer drug should kill or incapacitate cancer cells without causing excessive damage to normal dividing cells. This ideal is difficult, or perhaps impossible, to attain and is why cancer patients frequently suffer unpleasant side-effects when undergoing treatment.

Plant materials have been used in the treatment of malignant diseases for centuries; a comprehensive survey of the literature describing plants used against cancer listed over 1400

genera. Recent phytochemical examination of plants which have a suitable history of use in folklore for the treatment of cancer has indeed often resulted in the isolation of principles with antitumour activity.

There are few plants which are used now-a-days that are discussed later in this chapter are following:

1. Podophyllum
2. Catharanthus
3. Colchicine

PODOPHYLLUM

Synonyms:

Mapple root, umbrella plant

Botanical origin:

Podophyllumpeltatum (American variety)

Podophyllumemodi & P. hexandrum (Indian variety)

Family:

Berberidaceae

Part used:

Dried tuberous roots and rhizomes

Habit & Habitat:

Plant is a perennial herb.

- American variety: America & Northern Carolina
- Indian variety: Pakistan, India, Afghanistan & Tibet.

Extraction:

Podophyllin resin is extracted with alcohol, precipitated with acidified water, then washed with twice of water, then dried and powder.

Constituents:

They contain resin whose active principle are lignans (phenolic compounds):

- Podophyllin 2-8% (American variety)

Podophyllin 6-12% (Indian variety)

- Podophyllotoxins:

Podophyllotoxin 20% (American variety)

Podophyllotoxin 40% (Indian variety)

Two semisynthetic derivatives of podophyllotoxins:

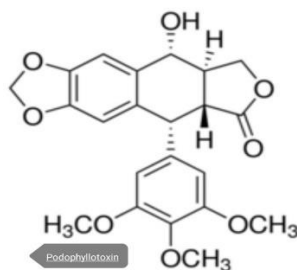
- i. Etoposide is used for the treatment of small cell lung cancer and testicular cancer.
- ii. Teniposide is used for pediatric cancer.

They also contain:

- α -peltatin 10%
- β -peltatin 5%
- Starch
- Calcium oxalate crystals Indian variety contain more thicker resin than American variety.

All these components possess cytotoxic or antitumor activity but this activity is lost on mild base treatment.

Structure:



Uses:

1. Suspension of podophyllum resin 25% in mineral oil and ointment containing the resin is administered for the removal of soft warts.
2. It is beneficial in some types of tumours.
3. Semisynthetic variety of podophyllotoxin name as Etoposide is used in lung cancer, testicular cancer, lymphoma and leukaemia.
4. Other derivative like Teniposide is used in neuroblastoma, leukaemia and brain cancer in children.
5. Podophyllotoxin is used in the treatment of soft venereal and other warts.
6. Hydrogogue cathartic.
7. Purgative.

CATHARANTHUS

Synonym:

Vincarosea

Botanical origin:

Catharanthus roseus

Family:

Apocynaceae

Part used:

Dried whole herb.

Habit & Habitat:

Plant is an erect perennial herb (herbaceous subshrub).

Native to Madagascar but it is Cosmopolitan (present all over the world).

Cultivated as ornamental plant in Southern Florida, Africa, Europe, Thailand, Taiwan, and Australia.

Production:

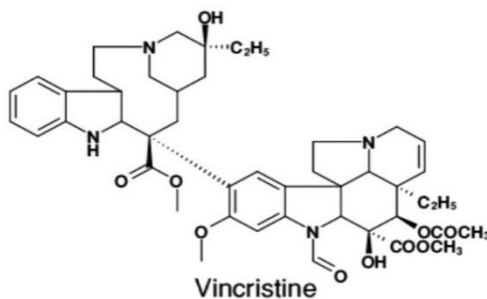
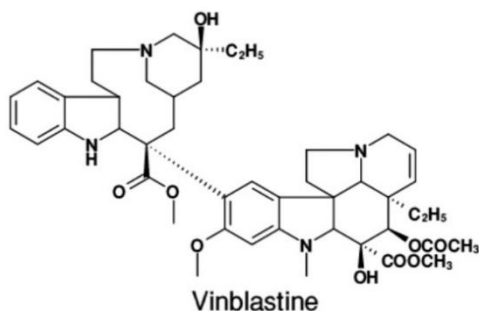
500kg of plant will produce 1gm of vinblastine.

Constituents:

It contains about 150 alkaloids. They are basically bisindole (Indole + Indoline) which are obtained for anticancerous activity. Major alkaloids having neuroplastic activity are:

- Vinblastine (vincaleukoblastin)
- Vincristine (leurocristine) 0.0002% in plant
- Vincasidine
- Vincryptine (oncorin)
- Vinrosidine
- Vincalurosine.
- Vindesine (semi-synthetic derivative of Vinblastine).
- Vinorelbine (newer semi-synthetic drug with broader anti-tumor activity and less neurotoxic effect).

Structure:



The mechanism of action is De-polymerization of microtubules.

Both vincristine and vinblastine bind tightly to tubulin and interfere with the functioning of the microtubule system which is a component of the mitotic spindle and inhibit the polymerization of tubulin into microtubules.

Clinical Uses:

1. Hodgkin's disease:
Vinblastin is used to treat Hodgkin's disease.
2. Non-Hodgkin's disease:
Vincristine is used to treat non-hodgkin's disease.
3. Neoplasm:
Vinblastin sulphate and vincristine sulphate i.e. obtain from catharanthus is used to treat neoplasm.
4. Lung cancer:
Vinorelbin Tartrate semisynthetic derivative of vinblastin given as single agent or in combination with cisplatin for 1st line treatment of small cell lung cancer.
5. Chemotherapy regimens:
Vincristine is delivered via intravenous infusion for use in various types of chemotherapy regimens.
6. Acute Lymphoid Leukemia:
Vindesine is used in acute lymphoid leukemia in children.
7. Vinblastine is used to treat:
 - i. Histolytic lymphoma
 - ii. Renal cell carcinoma
 - iii. Testicular cancer
 - iv. Breast cancer
 - v. Carcinoma of testis
8. Vincristine is used to treat:
 - i. Breast cancer
 - ii. Lymphosarcoma
 - iii. Neuroblastoma
 - iv. Lung cancer
 - v. Cervical cancer
 - vi. Acute lymphocytic leukemia in children.

COLCHICINE

Botanical Origin:

Colchicum autumnale

Family:

Liliaceae

Part Used:

Dried seeds & corms

Habit & Habitat:

Plant is a herb.

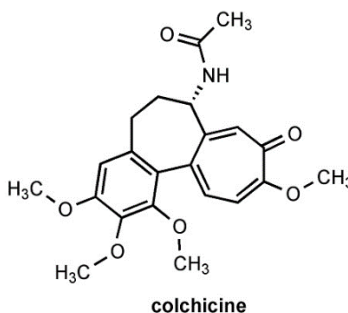
Cultivated in England, Central Europe & Northern Africa

Commercial supply comes from Yugoslavia & Italy.

Constituents:

Drug contains about 0.6-1.2% alkaloids in which major alkaloid is Colchicine.

Structure:



Mechanism of Action:

Colchicine is mitotic inhibitor prevents polymerization and assembly of microtubules.

Bleomycin acts by binding to DNA which results in single strand and double strand breaks following free radicals formations and inhibition of DNA-biosynthesis.

Uses:

1. Treatment of Gout.
2. Anti-inflammatory in gouty inflammation.
3. It produce polyploidy (doubling of chromosomes) experimentally antineoplastic properties have been reported.
4. In large doses cause GIT irritation.
5. It is now being used experimentally in the treatment of neoplastic diseases.

Table 27.1 Some antitumour compounds from plants.

Class	Compound	Plant Source	Family
Monoterpene	Allamandin	<i>Allamanda cathartica</i>	Apocynaceae
	4-Ipomeanol	<i>Ipomoea batatas</i>	Convolvulaceae
Sesquiterpene	Penstimide	<i>Penstemon deustus</i>	Scrophulariaceae
	Baccharin	<i>Baccharis megapotamica</i>	Compositae
	Elephantopin	<i>Elephantopus elatus</i>	Compositae
	Helenalin	<i>Helenium autumnale</i>	Compositae
	Liatrin	<i>Liatris chapmanii</i>	Compositae
	Phyllanthoside	<i>Phyllanthus acuminatus</i>	Euphorbiaceae
	Phyllanthostatin 1	<i>P. acuminatus</i>	Euphorbiaceae
	Vernolepin	<i>Vernonia hymenolepis</i>	Compositae
Diterpene	Gnidin	<i>Gnidia lamprantha</i>	Thymelaeaceae
	Jatrophone	<i>Jatropha gossypifolia</i>	Euphorbiaceae
	Mezerein	<i>Daphne mezereum</i>	Thymelaeaceae
	Taxodione	<i>Taxodium distichum</i>	Taxodiaceae
	Taxol	<i>Taxus brevifolia</i>	Taxaceae
	Triptdiolide	<i>Tripterygium wilfordii</i>	Celastraceae
	Triptolide	<i>T. wilfordii</i>	Celastraceae
Quassinoid/Simaroubolide	Bruceantin	<i>Brucea antidysenterica</i>	Simaroubaceae
	Glaucarubinone	<i>Simarouba glauca</i>	Simaroubaceae
	Holacanthone	<i>Holacantha emoryi</i>	Simaroubaceae
Triterpenoid, Steroid, etc.			
Cucurbitacin	Cucurbitacin E	<i>Marah oreganus</i>	Cucurbitaceae
Saponin	Acer saponin P	<i>Acer negundo</i>	Aceraceae
Cardenolide	Strophanthidin	<i>Parquetina nigrescens</i>	Asclepiadaceae
Bufadienolide	Hellebrigenin acetate	<i>Bersama abyssinica</i>	Melianthaceae
Withanolide	Withaferin A	<i>Acnistus arborescens</i>	Solanaceae
Stilbene	Combretastin A-4	<i>Combretum caffrum</i>	Combretaceae
Lignan	α - and β -Peltatin	<i>Podophyllum peltatum</i>	Berberidaceae
	Podophyllotoxin	<i>P. hexandrum</i> , <i>P. peltatum</i>	Berberidaceae
		<i>Juniperus chinensis</i>	Cupressaceae
Quinone	Steganacin	<i>Steganotaenia araliacea</i>	Umbelliferae
	Jacaranone	<i>Jacaranda caucana</i>	Bignoniaceae
	Lapachol	<i>Stereospermum suaveolens</i>	Bignoniaceae
Alkaloid			
Pyrrolizidine	Monocrotaline	<i>Crotalaria spectabilis</i>	Leguminosae
	Indicine-N-oxide	<i>Heliotropium indicum</i>	Boraginaceae
Isoquinoline	Emetine	<i>Cephaelis acuminata</i>	Rubiaceae
Bis-isoquinoline	Tetrandrine	<i>Cyclea peltata</i>	Menispermaceae
	Thalicarpine	<i>Thalictrum dasycarpum</i>	Ranunculaceae
Benzophenanthridine	Fagaronine	<i>Fagara zanthoxyloides</i>	Rutaceae
	Nitidine	<i>F. macrophylla</i>	Rutaceae
Phenanthroindolizidine	Tylocrebine	<i>Tylophora crebiflora</i>	Asclepiadaceae
Acridone	Acronycine	<i>Acronychia baueri</i>	Rutaceae
Pyridocarbazole	Ellipticine	<i>Ochrosia elliptica</i> , <i>O. moorei</i>	Apocynaceae
	9-Methoxyellipticine	<i>O. maculata</i>	Apocynaceae
Pyrroloquinoline	Camptothecin	<i>Camptotheca acuminata</i>	Nyssaceae
		<i>Nothapodytes foetida</i> (formerly <i>Mappia foetida</i>)	Icacinaeae
Cephalotaxine	Harringtonine	<i>Cephalotaxus harringtonia</i>	Cephalotaxaceae
	Homoharringtonine	<i>C. harringtonia</i>	Cephalotaxaceae
Bis-indole	Leurosine	<i>Catharanthus lanceus</i> , <i>C. roseus</i>	Apocynaceae
	Vinblastine	<i>C. roseus</i>	Apocynaceae
	Vincristine	<i>C. roseus</i>	Apocynaceae
Maytansinoid/Ansa macrolide	Maytanacine	<i>Maytenus buchananii</i>	Celastraceae
	Maytansine	<i>M. buchananii</i> , <i>M. serrata</i>	Celastraceae
		<i>Putterlickia verrucosa</i>	Celastraceae
		<i>Maytenus buchananii</i>	Celastraceae
Non-heterocyclic Peptide	Maytanvaline	<i>Colchicum speciosum</i>	Liliaceae
	Colchicine	<i>Bouvardia ternifolia</i>	Rubiaceae
	Bouvardin	<i>B. ternifolia</i>	Rubiaceae
	Deoxybouvardin		

TOPIC 6

CHEMISTRY AND BIOLOGICAL ACTIVITY OF

1. **ALKALOIDS,**
2. **GLYCOSIDES,**
3. **TERPENOIDS,**
4. **SAPONINS,**
5. **FLAVONOIDS**

1. Alkaloids:

The term alkaloid was introduced by German Chemist Carl F.W. Meissnesin.

Alkaloids are alkali like” (derived from word alkali).

Definition:

“Group of naturally occurring organic compounds which are basic in nature containing one or more Nitrogen atom normally of heterocyclic nature possess specific pharmacological action on human or animal body.”

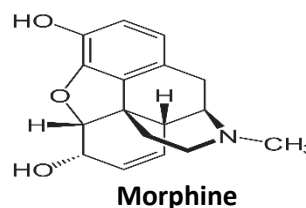
Classification on the basis of origin:

True Alkaloids:

- Contain Heterocyclic N-atom (N in heterocyclic ring)
- Derived directly from amino acid (tryptophan, tyrosine, histidine, ornithine etc.
- Basic in nature, form water soluble salts.

Example:

Morphine synthesized in plants by
Using precursor tyrosine (an amino acid).

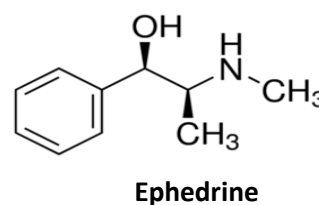


Proto Alkaloids:

- Also known as amino alkaloids.
- Contain Nitrogen but not in the ring system.
- Derived from amino acids sometimes considered as biological amines.
- Basic in nature.

Example:

Ephedrine is synthesized in plants by using
An amino acid precursor Phenylalanine



Pseudo Alkaloids:

- Contain heterocyclic N-atom.
- Not derived from amino acid precursor (derived from non-amino acid precursor)
- Weakly basic nitrogenous compound.

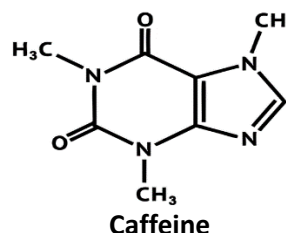
Example

Purine alkaloids.

e.g. Caffeine Alkaloids

Steroidal Alkaloids:

e.g. Solanidine, Conessine.



Pharmacological Classification:

Based on Pharmacological response:

CNS Stimulant:

Caffeine (Tea)

Strychnine (Nuxvomica) etc

Anti-Cancer:

Taxol (Taxus)

Vincristine, Vinblastine (Vinca)

Bronchodilator:

Ephedrine (Ephedra)

Vasicinone (Vasaka)

Narcotic Analgesic:

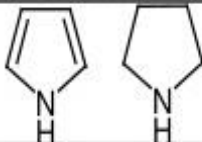
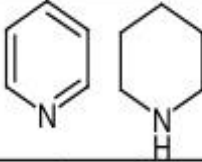
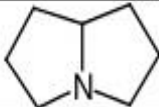

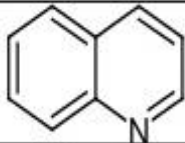
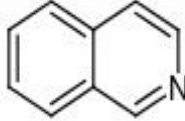
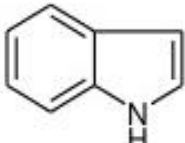
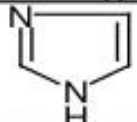
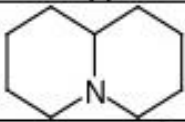
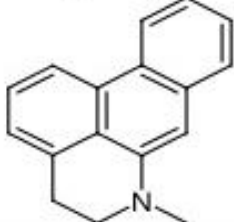
Morphine (opium)

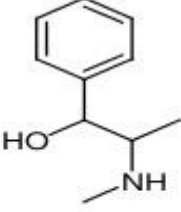
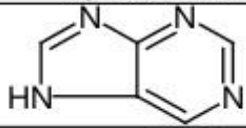
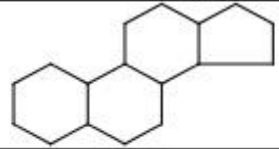
Codeine (opium)

Anti-hypertensive:

Reserpine (Rauwolfia)

Chemical Classification of Alkaloids:

A) True alkaloids			
Sr. no.	Type	Structure	Examples
1.	Pyrrole and pyrrolidine		e.g. Hygrine, coca species
2.	Pyridine and piperidine		e.g. Arecoline, anabasine, lobeline, conine, trigonelline
3.	Pyrrolizidine		e.g. Echimidine, senecionine, seneciphylline
4.	Tropane		e.g. Atropine, hyoscyne, hyoscyamine, cocaine, pseudopelletirine
5.	Quinoline		e.g. Quinine, quinidine, cinchonine, cupreine, camptothecine
6.	Isoquinoline		e.g. Morphine, codeine, emetine, cephaline, narcotine, narceine, d-tubocurarine
7.	Indole		e.g. Erotamine, ergotametriene, reserpine, vincristine, vinblastine, strychnine, brucine
8.	Imidazole		e.g. Pilocarpine, isopilocarpine, pilosine
9.	Norlupinane		e.g. Cystisine, laburinine
10.	Piporphine (reduced isoquinoline naphthalene)		e.g. Boldine

B) PROTOALKALOID		
1.	Alkyalamine	Ephedrine, Pseudoephedrine
		
C) Pseudoalkaloid		
1.	Purine	e.g. Caffeine, thophylline, theobromine
		
2.	Steroidal	e.g. Solanidine, conessine, protoveratrine
		
3.	Diterpene	$C_{20}H_{32}$ e.g. Aconitine, aconine, hypoaconine

Pharmacological activities of alkaloids

Many alkaloids act on the nervous system, one of two important information systems in animals. Plants that contain protoberberine alkaloids are reported to be used as analgesics, antiseptics, sedatives, and stomatics in Chinese folk medicine.

In Indian and Islamic folk medicine, such plants are used for bleeding disorders and eye diseases, and antiseptics, sedatives, stomatics, and uterine muscle depressants.

In china, tetrahydropalmatine is used as an analgesic, and has been reported to exhibit bradycardial, hypotensive, and sedative activities. Among many thousands of modern drugs, about 40% are of natural origin.

The alkaloids zephyrantine and narcyclasine-glucoside exhibit antitumor activity. Even in low doses, these alkaloids inhibit the growth of the epithelium carcinoma

The alkaloid norberbamine-2 turned out to be a strong inhibitor of reverse transcriptase, while the alkaloid noscapine, an anti-cough remedy.

Berberine, an isoquinoline alkaloid isolated from *C. chinensis*, inhibits the proliferation and migration of breast cancer.

Berberine also possesses anti-HIV, anti-fungal, cardioprotective, immunoregulative, anti-malarial, anti-inflammatory, antioxidant, cerebro-protective, anti-mutagenic, vaso-relaxing, anxiolytic and analgesic activities.

Actions on the immunologic system are also described for these alkaloids; induction and inhibition of gene expression, anti-inflammatory, anti-proliferative, anti-system complement, and apoptosis induction.

At the level of the digestive system, some relevant actions were reported such as anti-diarrhetic, electrolyte transport inhibition, and antiulcer activities.

Naturally occurring alkaloid of *Papaver somniferum*, and used as substitution therapy in the treatment of opioid dependence), undergoes extensive first-pass metabolism and therefore has very low oral bioavailability.

2. Glycosides:

- An organic compound usually of plant origin.
- Composed of a sugar moiety linked to a non-sugar moiety by glycosidic bond
- The sugar portion is called glycone. And the non-sugar portion is called aglycone or genin.
- Generally the pharmacological action is due to the aglycone part.
- All natural glycosides are hydrolyzed into a sugar and other organic Compound by boiling with mineral acid however most easily hydrolyzed by Enzymes that occur in same plant tissue.

Classification:

- Based on atoms involved in the glycosidic linkage.
- Based on the stereo configuration of the glycosidic linkage.
- Based on the sugar moiety.
- Based on the aglycone.

BASED ON ATOMS INVOLVED IN THE GLYCOSIDIC LINKAGE

1- O-glycosides:

Formed through Oxygen links

2- C-glycosides:

Formed through Carbon links.

3- S-glycosides:

Formed through Sulphur links.

4- N-glycosides:

Formed through Nitrogen links.

BASED ON THE CONFIGURATION OF THE GLYCOSIDIC LINKAGE:

This classification depends on whether the glycosidic bond lies above or below the plane of the sugar part of the glycoside.

Two Types:

1- β -GLYCOSIDES:

- Here the sugar involved has the β configuration at the anomeric carbon.
- The majority of plant glycosides isolated are β -glycosides.

2- α -GLYCOSIDES:

- Here the sugar involved has the α configuration at the anomeric carbon.
- There are only few medicinal α -glycosides known, especially the rhamnosides

ACCORDING TO THE SUGAR MOIETY:

1-Glucosides:

- Containing glucose at its sugar portion.

2- Ribosides:

- Containing ribose at its sugar portion.

3- Rhamnosides:

- Containing rhamnose at its sugar portion.

ACCORDING TO THE AGLYCONES:

1. Cardioactive Glycosides:

- These are steroidal glycosides and show highly specific powerful action on the cardiac muscles. Two types of cardiac glycosides are present in nature.
- Cardenolide is a type of steroid. (These are C23 steroids having an alpha, beta unsaturated five membered lactone ring attached at 17 beta position.)
- These compounds are present in Digitalis, Strophanthus and Oleander.
- Digoxin is used as cardiotonic.

2. Bufadienolides:

- These are C24 Steroids having double unsaturated six membered lactone ring at the 17 alpha position.
- These are a type of cardiac glycoside. e.g. Scillaren A, B

3. Flavanol Glycosides:

- The flavonoids are a group of compounds comprising the derivatives of flavone, isoflavone, flavanones, isoflavanone, and flavanols. e.g. quercetin is obtained from rutin used as Antihemorrhagic.

4. Anthraquinone Glycosides:

- In these glycosides sugar moiety is attached to an anthracene aglycone, which is derivative of anthraquinone.
- They occur as pharmacologically active constituent of various cathartic plant e.g. cascara, senna, aloe, rhubarb.
- Sennoside A and B obtained from senna used as cathartic and laxative.

5. Saponin Glycosides:

- Saponin ring is present in these glycosides. e.g. Dioscin, glycyrrhizin

- **Two types** on the basis of aglycone.
- **Steroidal saponin.** e.g. Dioscin
- **Triterpenoid saponin.** e.g. Glycyrrhizin used as expectorant

6. Cyanophore Glycosides

- On hydrolysis it yields hydrocyanic acid.
- They are commonly found in rosaceous plants.
- Amygdalin is well known and widely distributed cyanophore glycosides. e.g., Bitter almond, Wild cherry bark
- Amygdalin obtained from bitter almond used as demulcent

7. Alcoholic glycosides:

- Possessing alcoholic aglycones. e.g. Salicin
- It converted in body into salicylic acid
- Have analgesic, antipyretic, anti-inflammatory effect.

8. Aldehyde glycosides:

- Possessing an aldehyde aglycone. e.g. Glucovanillin.
- Vanillin-D-glucoside. It is obtained from the green fruit of vanilla used as flavor.

9. Isothiocynate glycosides:

- They contain sulphur and on hydrolysis they yield isothiocyanate aglycones which may be aliphatic or aromatic.
- Also known as glucosinolates.
- Example. Sinigrin from black mustard.

PHARMACOLOGICAL OR THERAPEUTIC ACTIVITY

1. **Cardiac glycosides** of Digitalis, Strophanthus, and Squill are cardiotonic agents useful in congestive heart failure.
2. **Anthracene glycosides** of Cascara, Frangula, Senna, Rhubarb, and Aloes have a laxative effect.
3. **Flavonoid glycosides** e.g. Rutin and Hesperidin are used to decrease capillary fragility and permeability. Also have antioxidant property.

4. In case of Cyanogenic or Thioglycosides, where aglycone is the active component used. The aglycone (e.g. HCN & Benzaldehyde) is useful as a sedative and flavoring agent, and aglycone (e.g. thiocyanate) is useful as a rubifacient and counterirritant.
5. **Saponin glycosides** Medicinal value is due to their expectorant, anti-inflammatory, anti-hepatotoxic, anti-bacterial, anti-rheumatic and antiviral. Treatment in gastric and duodenal ulcer.
6. **Phenolic glycosides** of bearberry has urinary antiseptic effect. Used as skin lightning agent.
7. **Alcoholic glycosides** of salix have analgesic, antipyretic, anti-inflammatory effect.

3. Terpenoids:

- Terpenoids are secondary metabolites synthesized by plants, marine animals and fungi by head to tail joining of isoprene units. They are found to occur in rocks, fossils and animal kingdom.
- Many volatile oils consist of largely terpenes. Terpenes are defined as natural products may be divided into isoprene units. These units arise from acetate via mevalonic acid and are branched chain, 5 carbon units containing 2 unsaturated bonds.



2-methyl-1, 3-butadiene

Classification of terpenoids on the basis of isoprene units:

The terpenoids have general formula $(C_5H_8)_n$. Based on the value of 'n' the terpenoids are classified into following:

Value of 'n'	No: of carbon atoms	Class	Molecular formula
1	5	Hemiterpene or isoprene	C_5H_8
2	10	Monoterpenes or terpenes	$C_{10}H_{16}$
3	15	Sesquiterpenes	$C_{15}H_{24}$
4	20	Diterpenes	$C_{20}H_{32}$
5	25	Sesterterpenes	$C_{25}H_{40}$
6	30	Triterpenes	$C_{30}H_{48}$
8	40	Tetraterpenes	$C_{40}H_{64}$
>8	>40	Polyterpenes	$(C_5H_8)_n$

Terpenoid skeleton occur as open chain as well as in various cyclised form. They may be classified on the basis of no of rings present in their structure.

Classification on the basis of no of rings present in their structure:

- In monocyclic terpenoids there is one ring in structure.
- Bicyclic terpenoids contains two rings.
- Tricyclic terpenoids contains three rings.
- Tetracyclic terpenoids contains four rings.

And acyclic terpenoids have no ring

Monoterpenoids:

- **Geraniol** (present in rose oil, lemon and lavender oil)
Used in perfume and as flavour
- **Carvone** (50-60% present in caraway oil
Also in spearmint oil)
Used as aromatic, carminative, stomachic, flavouring agent and as a spice.
- **Menthol** (obtained from peppermint oil)
- Used as Topical antipruritic , Counterirritant, Antiseptic and stimulant
Anasthetic property

Diterpenoids:

- **Taxol** : Excellent anticancer activity against breast and ovarian cancer.
Belonging to taxane skeleton and isolated from the bark of Taxus brevifolia

Sesquiterpenoids:

- **Zingiberene- α** Zingerberine forms the major constituent of ginger oil.
- Used as a condiment ,aromatic stimulent and a carminative

Tetraterpenoids:

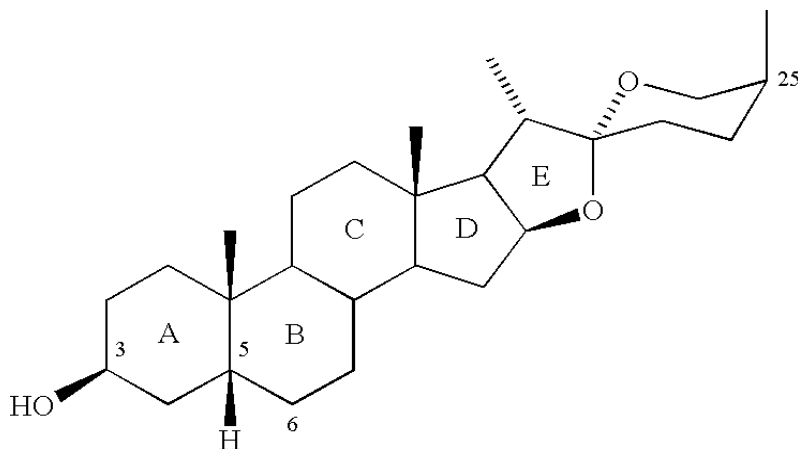
β -carotenes

- The name β – carotenes was derived from the source of its isolation i.e carrots .It occurs relatively in high concentration in carrots, alfalfa and palm oil.

4. Saponins:

1. DEFINITION

Are highly complex glycosides and containing high %age of glycosides. The general formula is ($C_nH_{2n-8}O_{10}$)



GENERAL FORMULA

- Saponins are high molecular weight triterpene glycosides containing a sugar group attached to either a sterol or other triterpene
- Saponins comes from Latin word sapo meaning soap
- When taken orally, saponins are harmless.

CHARACTERISTICS

- Highly toxic when injected into blood causes Haemolysis
- When taken Orally less toxic
- Widely Distributed in the higher plants
- Commercially prepared from Yucca plant or from Quillaja
- Makedly toxic (Sapotoxins) use for fish poison.

PROPERTIES OF SAPONINS: Highly mol.wt, high polanit.

- Greater portion, when pure are colourless or white optically active, soluble in Alcohol or dilute alcohol.
- Readily soluble in water producing a froth (foaming) on vigorous shaking. Produce stable emulsion on shaking with oils & fats.
- In pure form, isolation of glycoside is difficult & laborious.
- Acid in taste. Powder form causes sneezing.
- Acid Hydrolysis, Aglycone (Sapogenin) Which may be steroid or triterpenoid, Various sugars, & Uronic Acid is produces.
- Use to enhance the absorption of Insulin (Takeda) from the application of Nasal Aerosoles.
- Extracted from the plant by water Alcohol or mixture of both.
- Production & purification process, vary according to the nature of the materials & glycosides.
- Sapogenins are insoluble in water but soluble in weak Alcohol
-

2 Types of saponins are recognized:

- Pentacyclic triterpenoid.
- Steroidal saponins.
 - Soluble in water, alcohol and mixture of them.
 - Form persistent froth with water.
 - Used as detergent and emulsifying agents.
 - Aglycone are called Sapogenin.

- Cause hemolysis of RBC's if reach the blood.
- Form complex with cholesterol.
- Only small part absorbed when taken orally.
- Enhance the absorption of other drugs.

CLASSIFICATION:

According to the nature of the aglycone saponins are classified into Steroidal and Triterpenoid saponins

Liquorice:

- Dried root of *Glycyrrhiza glabra* contain triterpenoidal saponins Glycyrrhizinic acid
- Sweet taste due to glycyrrhizin
- Yellow colour due to flavonoids
- Actions
 - Demulcent
 - Expectorant
 - Anti-microbial/anti-viral
 - Anti-spasmodic
 - Antiulcerogenic

Dioscorea (yam)

- Diosgenin is obtained from tubers of various *dioscorea spp.* (e.g. *D. villosa*, *D. deltoidea*)
- Family: dioscoreaceae

Uses:

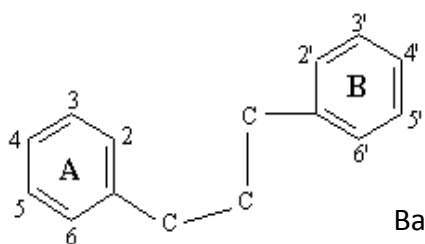
- Various steroidal drugs like progesterone and cortisol
- Disperse swelling.
- As tonic

5. Flavonoids:

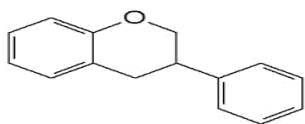
Flavonoids are polyphenol **antioxidant** compounds that occur both in free state and as glycoside. These are largest group of naturally occurring pigments which are responsible for colors of many fruits and flowers.

Chemistry:

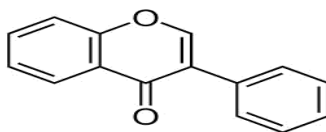
The term “flavonoid” is generally used to describe a broad collection of natural products that include a $C_6-C_3-C_6$ carbon framework, or more specifically a phenylbenzopyran functionality.



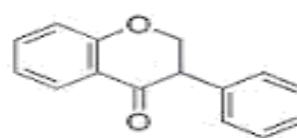
Basic skeleton of flavonoids



Isoflavan



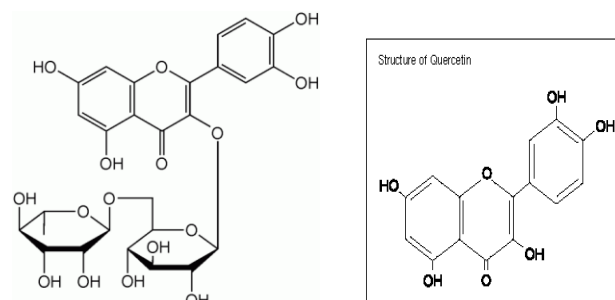
Isoflavon



Isoflavanone

Distribution

- Flavonoids are found in most plant material.
- The most important dietary sources are fruits, tea and soybean.
- Green and black tea contains about 25% percent flavonoids.
- Other important sources of flavonoids are apple (quercetin), citrus fruits (rutin and hesperidin).



Rutin and Quercetin

Pharmacological activity:

The main pharmacological activities include:

- Anti-inflammatory
- Anti-allergic
- Antithrombotic
- Vasoprotective,
- Anti-tumor,
- Protective for gastric mucosa

Biosynthesis

- Flavonoids are synthesized by the phenylpropanoid metabolic pathway in which the amino acid phenylalanine is used to produce 4-coumaroyl-CoA.
- This can be combined with malonyl-CoA to yield the true backbone of flavonoids, a group of compounds called chalcones, which contain two phenyl rings. Conjugate ring-closure of chalcones results in the familiar form of flavonoids, the three-ringed structure of a flavone.
- The metabolic pathway continues through a series of enzymatic modifications to yield

flavanones → dihydroflavonols → anthocyanins

- Along this pathway, many products can be formed, including the flavonols, flavan-3-ols, proanthocyanidins (tannins) and a host of other various polyphenolics.
- Flavonoids can possess chiral carbons. Methods of analysis should take this element into account especially regarding bioactivity or enzyme stereospecificity.
- The biosynthesis of flavonoids involves several enzymes.
- Dihydrokaempferol 4-reductase

- Flavanone 4-reductase
- Anthocyanidin reductase
- Flavanone 3-dioxygenase
- Flavone synthase
- Flavonoid 3'-monooxygenase
- Flavonol synthase

Important dietary sources

1. Green tea:

Botanical Origin: *Camellia sinensis*

Family: Theaceae

Part Used: Dried leaves

Constituents: Catechins, Epicatechins, Flavonols, Theaflavin

Uses: External genital warts, Prostate cancer

Also high consumption associated with lower risk for bladder, esophageal and pancreatic cancers. Improves cholesterol levels

- Green tea flavonoids are potent antioxidant compounds in vitro, with potential to reduce incidence of cancer and heart disease.
- The major flavonoids in green tea are kaempferol and catechins (catechin, epicatechin, epicatechin gallate (ECG), and epigallocatechin gallate (EGCG)).

2. Buckwheat:

Botanical Origin: *Fagopyrum esculatum*

Family: Polygonaceae

Part Used: seed

Constituents: Rutin

Uses:

Rutin can strengthen blood vessels, so they use it for internal bleeding, hemorrhoids and to prevent strokes. Rutin is also used for osteoarthritis.

OTHERS:

1. Citrus

- A variety of flavonoids are found in citrus fruits, including grapefruit.

- The citrus bioflavonoids include hesperidin (a glycoside of the flavanone hesperetin), quercitrin, rutin (two glycosides of the flavonol quercetin), and the flavone tangeritin.
- In addition to possessing in vitro antioxidant activity and an ability to increase intracellular levels of vitamin C, rutin and hesperidin may have beneficial effects on capillary permeability and blood flow.
- They also exhibit anti-allergy and anti-inflammatory benefits of quercetin from in vitro studies.

2. Wine

- Grape skins contain significant amounts of flavonoids as well as other polyphenols.
- Both red and white wine contain flavonoids; however, since red wine is produced by fermentation in the presence of the grape skins, red wine has been observed to contain higher levels of flavonoids, and other polyphenolics such as resveratrol

3. Dark chocolate

- Flavonoids exist naturally in cacao, but because they can be bitter, they are often removed from chocolate, even the dark variety.
- While the flavonoids are present in milk chocolate, studies have shown that they are not as readily taken up by the body; nor are they easily taken up when dark chocolate is consumed alongside milk.

Topic 7

Chemistry and mechanism of action of cardio active plant constituents

Cardio active plants:

Cardiac Plants are a group of plants that increase the output force of the heart and increase its rate of contractions. They act directly on the heart muscle. They are used in the treatment of cardiovascular diseases.

A considerable number of plants scattered throughout the plant kingdom which exert on the failing heart a slowing and strengthening effect.

Cardiovascular Diseases:

Cardiovascular disease generally refers to conditions that involve narrowed or blocked blood vessels that can lead to a heart attack, chest pain (angina) or stroke..

NATURAL PLANTS

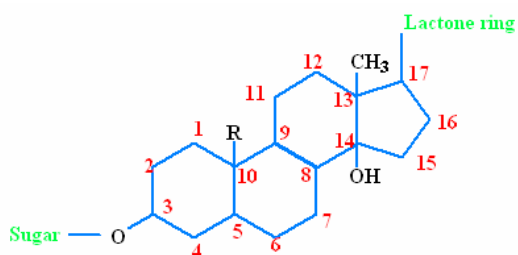
- Cardio active Glycosides
- Garlic
- Ginkgo
- Tea
- Rauwolfia
- Horse Chestnut
- Olive
- Hawthorn

A. Cardio active glycoside:

Cardiac glycosides are a class of organic compounds that increase the output force of the heart and increase its rate of contractions by acting on the cellular [sodium-potassium ATPase pump](#).

Pharmacologic activity is due to genin part.

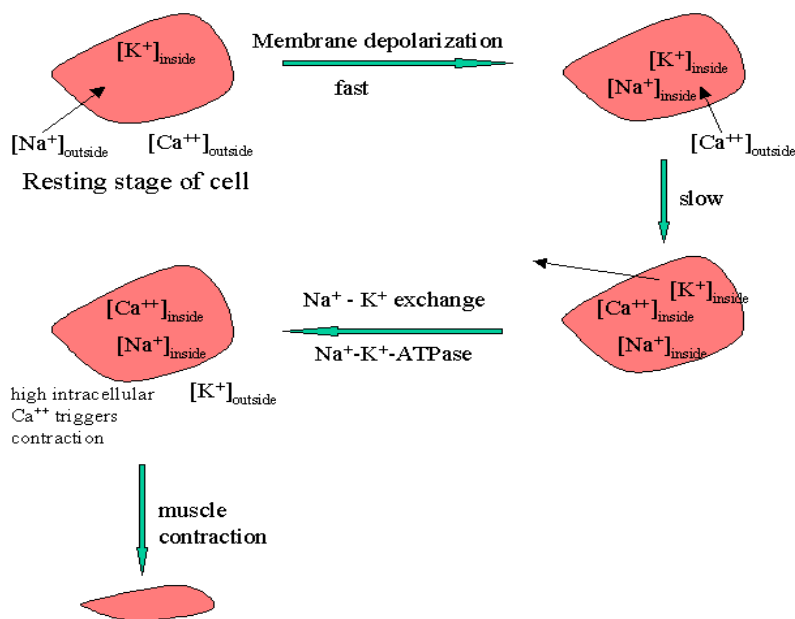
Basic STRUCTURE of Cardiac Glycosides



For Maximum Activity of Cardioactive agents these points are important:

- 3b-OH group involve in glycosidic linkage
- 14b-OH group at C-14
- A/B ring junction cis
- B/C ring junction trans
- C/D ring junction cis
- Cis means on the same side
- Trans means on the opposite side
- **All cardio active glycosides are characterized by the following structural features:**
- The presence of β -OH at position C-3, which is always involved in a glycosidic linkage to a mono, di, tri, OR tetra saccharide.
- The presence of another β -OH group at C-14.
- The presence of unsaturated 5 or 6- membered **lactone ring** at position C-17, also in the β configuration.

Mechanism of Action

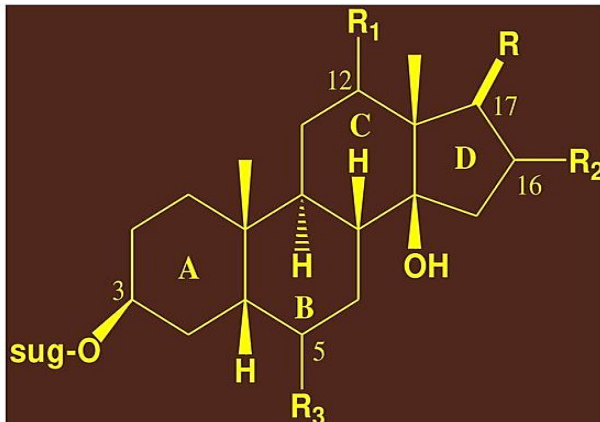


Mechanism of Action

- Cardiac Glycosides inhibits the Sodium Pump ($Na, K - ATPase$),
- Which results in increase Na^+ ,
- Which inhibits the Na^+ / Ca^{+2} exchange
- Which results in increase Ca^{+2} ,
- Results in Stimulation of Contraction of Cardiac muscles,
- Hence weakened heart to function more efficiently

MEDICINAL IMPORTANCE

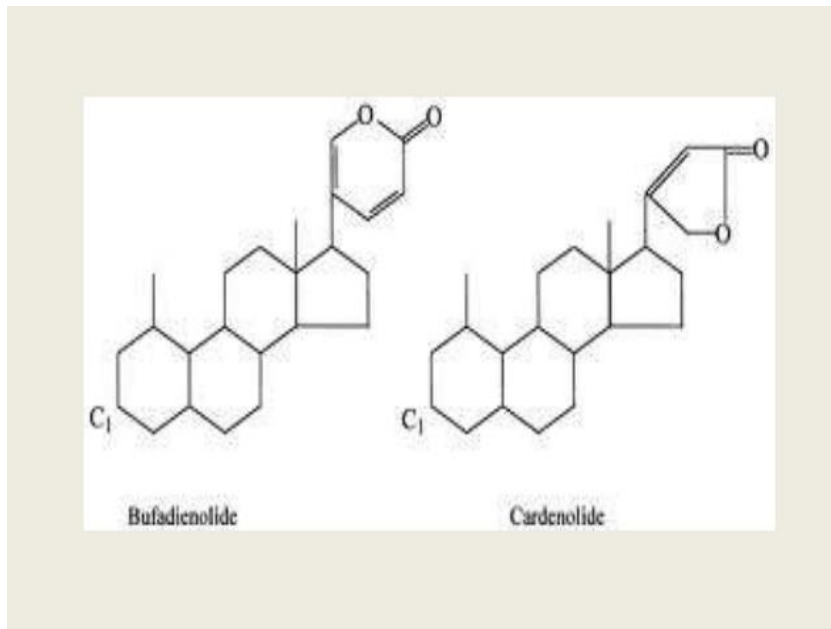
- Cardiotonics, CHF, rheumatic heart disease, atherosclerosis, HTN.
- Diuretics (capillary of the kidneys are dialated).



The presence of lactone at 17-β

According to the type of lactone ring cardiac glycoside is classified into

1. Cardenolides: cardiac active glycosides 5-membered ring medicinally more significant
2. Bufadienolide: Bufanolides or Bufadienolides 6-membered ring.



Plants from which cardenolides can be derived

- *Convallaria majalis* (Lily of the Valley): convallotoxin^[7]
- *Antiaris toxicaria* (upas tree): antiarin
- *Strophanthus kombe* (*Strophanthus* vine): ouabain (g-strophanthin) and other strophanthins
- *Digitalis lanata* and *Digitalis purpurea* (Woolly and purple foxglove): digoxin, digitoxin
- *Nerium oleander* (oleander tree): oleandrin

- *Asclepias sp.* (milkweed): oleandrin
- *Adonis vernalis* (Spring pheasant's eye): adonitoxin
- *Kalanchoe daigremontiana* and other *Kalanchoe* species

Organisms from which bufadienolides can be derived

- *Leonurus cardiaca* (motherwort): scillarenin^[7]
- *Drimys maritima* (squill): proscillaridine A
- *Bufo marinus* (cane toad): various bufadienolides
- *Kalanchoe daigremontiana* and other *Kalanchoe* species: daigremontianin and others

- A small group of plant glycosides act directly on the heart muscle.
- Cardenolides are steroidal glycosides that exert a slowing and strengthening effect on the failing cardiac muscle.
- Effectiveness depends on both the aglycones and the sugar attachments. Medicinal action depends on the aglycone.
- But the sugars make the compound more soluble in increases the fixation of the glycoside to the heart muscle. The overall action of Digitalis glycosides is complicated by the number of different effects produced.

MECHANISM OF ACTION OF CARDIAC GLYCOSIDES

- The mechanism whereby cardiac glycosides cause a positive inotropic effect and electrophysiologic changes is still not completely clear.
- However several mechanisms have been proposed, but the most widely accepted involves the ability of cardiac glycosides to inhibit the membrane bound $\text{Na}^+\text{-K}^+\text{-ATPase}$ pump responsible for $\text{Na}^+\text{-K}^+$ exchange.
- The process of membrane depolarization / repolarization is controlled by the movement of three cations, Na^+ , Ca^{+2} , and K^+ in and out of the cell.
- At the resting stage, the concentration of Na^+ is high on the outside of cell.

When heart beats a wave of membrane depolarization occur causes sodium flux-in leading to an immediate elevation of the action potential.

- Cardiac glycosides are proposed to inhibit this enzyme with a net result of an increase in sodium and a decrease in potassium within the cell which in turn stimulate a secondary $\text{Na}^+ \text{Ca}^{++}$ exchange mechanism that cause increase in intracellular Ca^{++} that causes a series of intracellular biochemical events. The positive inotropic action or muscle contraction enhancement due to cardiac glycosides occur by this mechanism.
-

I. Cardenolides

- Among cardioactive glycosides, those of cardenolide group are most important medicinally.

Examples:

- Digitalis
- Strophanthus
- Nerium Oleander

1. Digitalis

Botanical origin: *Digitalis purpurea* and *Digitalis lanata*

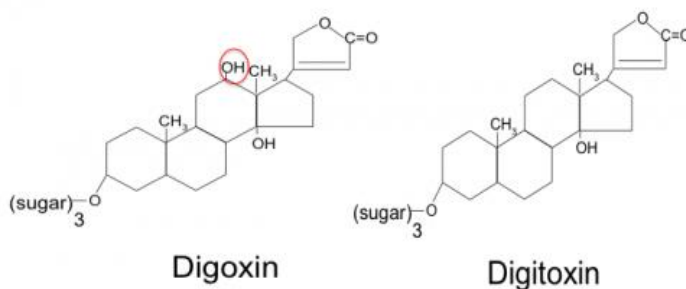
Family: Scrophulariaceae

Part used: Leaves

Active constituents: digoxin, digitoxin

Uses: It is used to treat some heart conditions such as atrial fibrillation.

- It slows atrioventricular conduction so that the heartbeat slows down and very slightly increases contraction power (positive inotropic effect).
- Because of the improved circulation in congestive heart failure caused by fast atrial fibrillation, the kidneys can function better, which stimulates the flow of urine, which lowers the volume of the blood and lessens the load on the heart.



2. Nerium

- **Botanical origin:** *Nerium Oleander*
- **Family:** Apocynaceae
- **Part used:** Leaves
- **Constituents:** Oleandrin, derivatives of gitoxigenins

- **Uses:** To treat Cardiac insufficiency

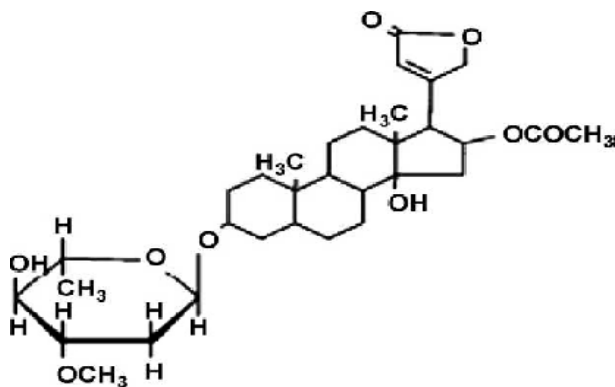


Figure 1. Chemical structure of oleandrin.

3. **Strophanthus:**

Botanical origin: *Strophanthus kombe*,

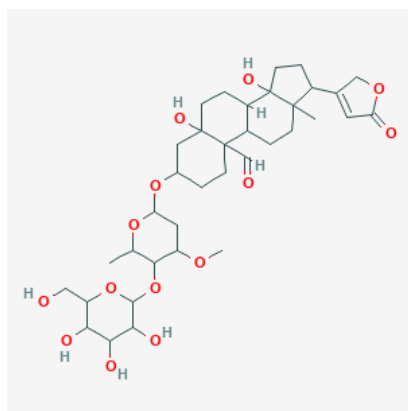
Family: Apocynaceae

Part used: Dried ripe seed

Constituents: Strophanthoside, strophanthin K, Cymarín,

Uses: used in the preparation of poison [arrowheads](#) used for hunting. Today, the seeds are used pharmaceutically for patients with certain heart conditions that affect blood circulation.

used medicinally to treat heart failure.



Structure of strophanthin K

II. **Bufadienolides:**

- Less commonly distributed in nature than cardenolides
- Occur in some Liliaceae and Ranunculaceae Species.

- Therapeutically there is not much value as the therapeutic index is low and production of side effects high

1) Squill:

Botanical origin: *Urginea maritima*

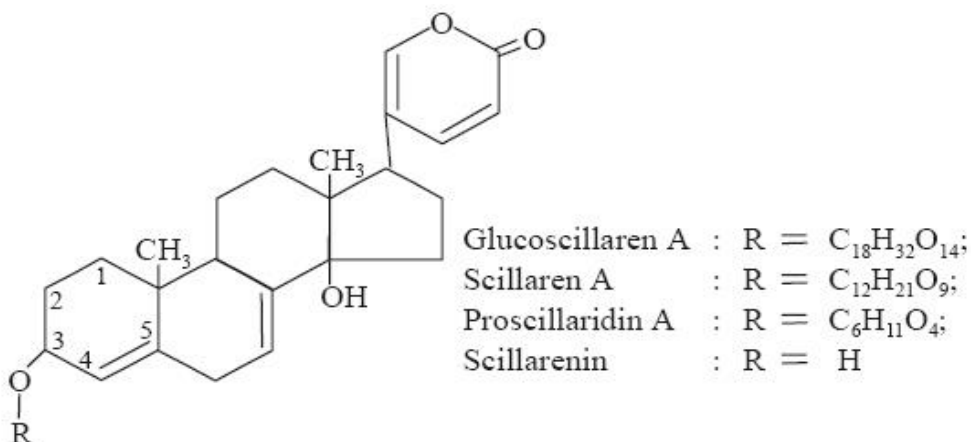
Family: Liliaceae

Part used: Dried Sliced Bulb

Constituents: scillaren A (a pure crystalline substance) and scillaren B , Scillipicrin, Scillitoxin, Calcium oxalate

Uses:

- it cause peripheral vasodilation and bradycardia
- a chemical in squill, might improve heart function in people with coronary heart disease.
- Used in abnormal heart rhythm and other heart problems.



2. Artichoke:

Botanical origin: *cynara cardunculus*

Family: Asteraceae

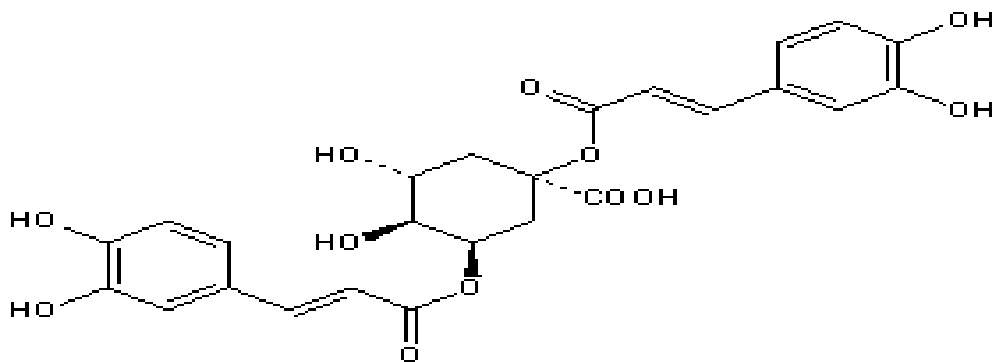
Part used: flower bud before the flower came into bloom

Constituents: cynarine, apigenin and luteolin

Uses: Aid in heart disease that is caused by high cholesterol level.

Inhibit cholesterol synthesis

Used in coronary heart diseases



Cynarine

B. Garlic:

Botanical origin: *Allium sativum*

Family: Liliaceae

Part used: Bulb or cloves

Constituents: Allin, Allicin Sulphur compounds, volatile & some essential oils.

Uses:

- antimicrobial activity
- lowers the lipids and cholesterol in plasma.
- Antiplatelet effects (due to the blockade of thromboxane synthesis)
- It can be used in high blood pressure, high cholesterol, coronary heart disease, heart attack, and “hardening of the arteries” (atherosclerosis).



Mechanism of Action:

Garlic's lipid lowering effects may occur via inhibition of HMG-CoA reductase or other enzymes, possibly by diallyl di- and trisulphide components of garlic, garlic extract and its constituents have been shown to inhibit Cu^{2+} -induced oxidative modification of low-

density lipoprotein. Aged garlic extract and its constituent S-allylcysteine have been found to protect vascular endothelial cells from injury caused by oxidized LDL.

C. Ginkgo:

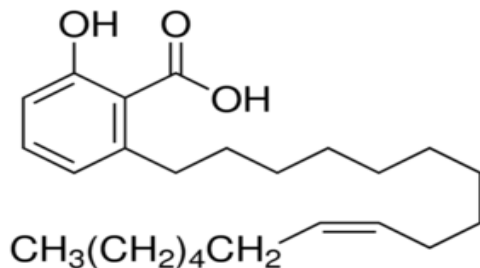
Biological origin: Ginkgo biloba

Family: Ginkgoaceae

Part used: Leaves

Consituents: ginkgolic acid, proanthocyanidins, ascorbic acid, carotenoids and flavonoids

Uses: Heart Diseases



Mechanism of action:

Antagonize platelets activating factor, to cause blood vessel to dilate.

Protection of vascular endothelium from free redicals and lipid peroxidation.

Possible protection against oxidation of LDL to oxysterol.

D. Rauwolfia:

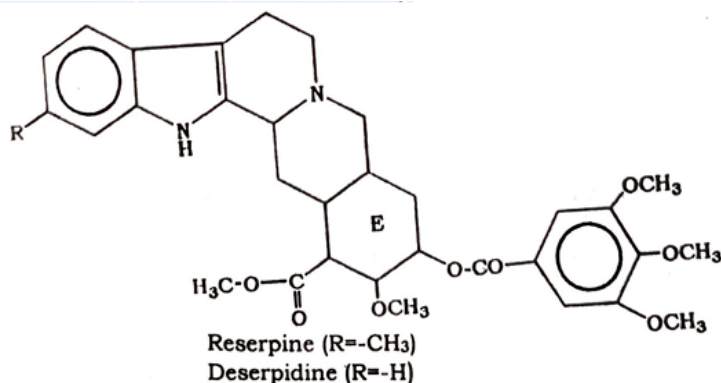
Botanical origin: Rauwolfia Serpentina

Family: Apocynaceae

Part used : Dried roots

Constituents: [indole alkaloid](#) family, including [ajmaline](#), [ajmalicine](#), [reserpine](#), and [serpentine](#),

Uses: Used in high Blood pressure



Mechanism of action/Effect:

Acts at postganglionic sympathetic nerve endings; depletes tissue and central nervous system (CNS) stores of catecholamines and serotonin; antihypertensive activity thought to be due to reduced cardiac output and possibly some decrease in peripheral resistance.

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TOPIC-8

Immunomodulator Activities of Medicinal Plants

Immune System

The immune system is a host defense system comprising many biological structures and processes within an organism that protects against disease. To function properly, an immune system must detect a wide variety of agents, known as pathogens, from viruses to parasitic worms, and distinguish them from the organism's own healthy tissue.

The immune system is made up of antibodies, white blood cells, and other chemicals and proteins that attack and destroy substances such as bacteria and viruses that they recognize as foreign and different from the body's normal healthy tissues. The immune system is designed to protect the host from invading pathogens and to eliminate diseases.

There are two major subsystems of the immune system:

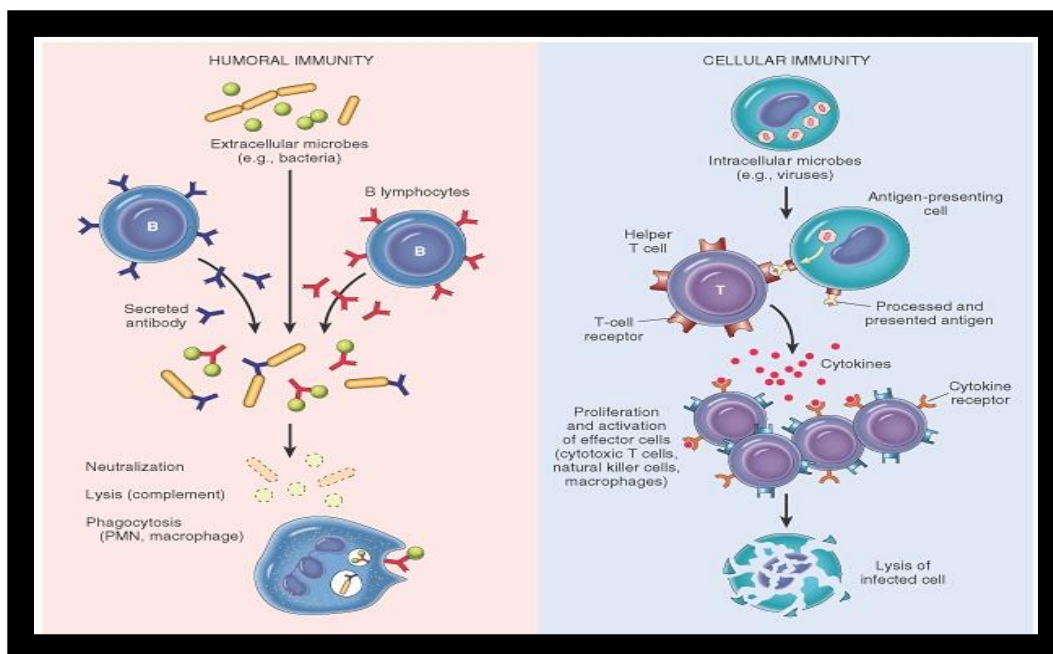
- **Innate immune system**

It is the first line of defense against an antigenic insult. Includes defenses like physical (skin), biochemical (complement, lysozyme, interferons) and cellular components (neutrophils, monocytes, macrophages).

- **Adaptive immune system.**

Humoral immunity: Antibody production – killing extracellular organisms.

Cell mediated immunity: cytotoxic / killer T cells – killing virus and tumor cells.



ABNORMAL IMMUNE RESPONSE

- **Hypersensitivity reactions**

- > Type 1 – Anaphylactic shock
- > Type 2 – mismatched blood transfusion
- > Type 3 – Serum Sickness, glomerulonephritis and arthritis.
- > Type 4 – TB, leishmaniasis.

- **Autoimmunity**

- > Autoimmune diseases arise when the body mounts an immune response against itself as a result of failure to distinguish self-tissues and cells from foreign antigens.
 - ✓ Rheumatoid Arthritis, S.L.E, Type 1 Diabetes Mellitus, Multiple Sclerosis etc....

Immunodeficiency Diseases

- > **Congenital** – Di George's syndrome, SCID due to ADA deficiency.
- > **Extrinsic** – HIV causing AIDS.

Immunomodulation

The term immunomodulation means alteration of immune response which may increase or decrease the immune responsiveness. Immunomodulation can be of two types

- Immunostimulation
- Immunosuppression

Enhancement in the immune response is called **immunostimulation**.

Reduction in the immune response is called **immunosuppression**.

Immunomodulators are the drugs which; either stimulate the immune system (**immunostimulators**) or suppress the immune system (**immunosuppressant**).

TYPES OF IMMUNOSUPPRESSANTS

- **Glucocorticoids** - Prednisolone.
- **Calcineurin inhibitors** – Cyclosporine, Tacrolimus.
- **Cytotoxic agents** – Azathioprine, Leflunomide, Cyclophosphamide, Methotrexate, Dactinomycin, Vincristine.
- **Antibodies** – ALG, ATG like Muromonab CD3, IGIV, Monoclonal antibodies like Daclizumab Basiliximab.
- **Others** – Interferons, Thalidomide, Sirolimus, Mycophenolate Mofetil.

TYPES OF IMMUNOSTIMULANTS

Cytokines

- like INF alpha, INF beta, INF Gamma IL-2.
- TNF alpha.

Levamisole

METHOD OF DETECTION OF IMMUNOMODULATING COMPOUNDS

Drugs may modulate the immune responses by one or more of the following steps:

- Antigen recognition or phagocytosis
- Lymphocyte proliferation
- Synthesis of antibodies
- Ag-Ab recognition
- Release of mediators due to immune responses
- Modification of target tissue response

MEDICINAL PLANTS WITH IMMUNOMODULATORY ACTIVITY

1. Plant name: *Withania somnifera*

- **Family:** Solanaceae
- **Part used:** Plant roots
- **Activity:** Extract of the powdered root of plant show immunomodulatory effect by enhancing the total white blood cell (WBC) count and circulating antibody titre.
- **Mechanism:** Enhance wbc and antibody production
- **Chemical Constituents:** Withanolide, Ergostane, Withaferin A, Cuscohygrin
- **Uses:**
 - Anti-inflammatory agent
 - Antiaging
 - General tonic

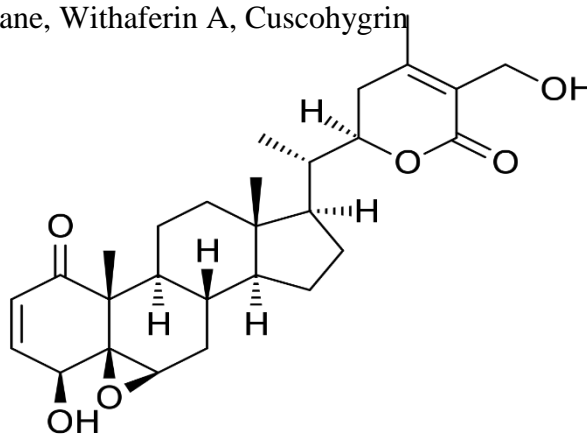


Figure 1: Withaferin A

2. Plant name: *Bidens pilosa*

- **Family:** Asteraceae
- **Part used:** Whole plant
- **Activity:** Aqueous extract of plant show immunomodulatory effect by enhancing the cytokines production and WBCs.
- **Mechanism:** Enhance the cytokines and WBCs production.
- **Chemical constituents:** Dimethyl quercetin, isopropyl phthalate, kaempferol and vanillic acid
- **Uses**
 - Anti-inflammatory activity, Antirheumatic activity
 - Anti malarial activity

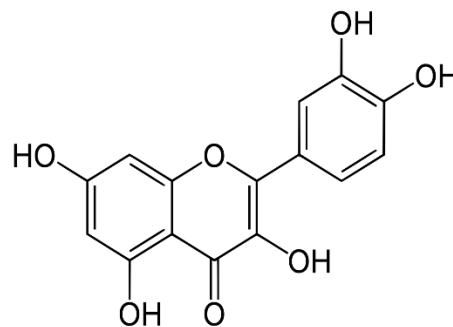


Figure 2: Quercetin

3. Plant name: *Camellia sinensis*

- **Family:** Theaceae
- **Part used:** Leaves
- **Activity:** The extract of *C. sinensis* in combination with low doses of cyclosporine increases the production of immunosuppressive cytokines.
- **Mechanism:** Enhance neopterin in peripheral mononuclear cells.
- **Chemical constituents:** Caffeine, Theophylline, Theobromine, and Xanthine.
- **Uses:**
 - Smooth muscle relaxant
 - Bronchial asthma
 - Diuresis

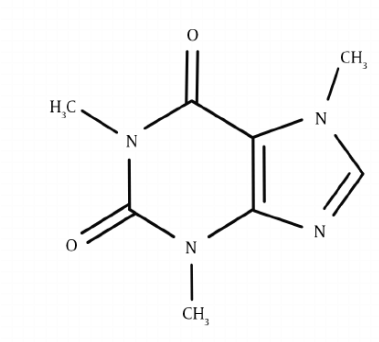


Figure 3: Caffeine

4. Plant name: *Curcuma longa*

- **Family:** Zingiberaceae
- **Part used:** Rhizom
- **Mechanism:** Curcumin modulate the activation of T-cells, B-cells, Macrophages, and Neutrophils.
- **Chemical constituents:** Curcumin, Dihydrocurcumin, and Volatile oils.
- **Uses:**
 - Treats indigestion
 - Reduces inflammation
 - Prevents atherosclerosis
 - Natural pain reliever
 - Antioxidant

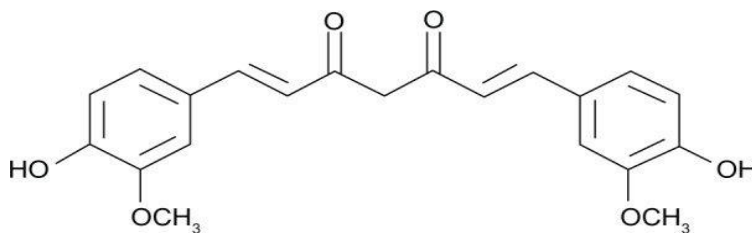


Figure 4: Curcumin

5. Plant name: *Aloevera*

- **Family:** Asphodelaceae

- **Part used:** Leaves
- **Activity:** Aqueous extract show immunomodulatory effect.
- **Mechanism:** Increases phagocytosis and stimulate the production of super oxide.
- **Chemical constituents:** Aloin, Emodin, Aloemodin

Uses:

- Emollient
- Stimulant
- Purgative
- Antiaging
- Anti-inflammation
- Wound healing

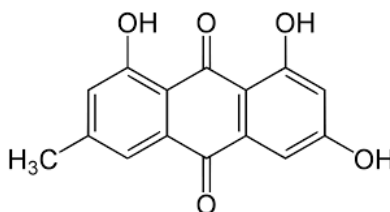


Figure 5: Emodin

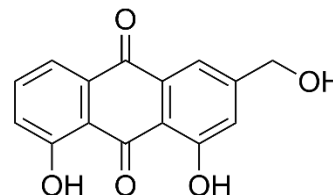


Figure 6: Aloe emodin

6. Plant name: Chrysanthemum indicum

- **Family:** Comositae
- **Part used** Aerial parts
- **Activity:** 70% ethanolic extract inhibited skin inflammation in mice.
- **Mechanism:** Increases antibody generation and phagocytosis.
- **Chemical constituents:** Cineol, Camphor, Borneol, Bornyl acetate, and Flavone glycosides.

Uses:

- Amenorrhea
- Digestive disorder
- Fever
- Headache
- Laxative

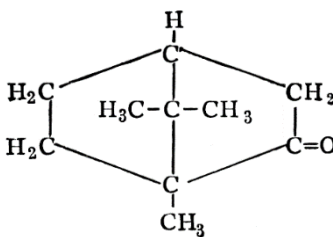


Figure 7: Camphor

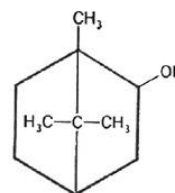


Figure 8: Borneol

7. Plant name: Allium sativum

- **Family:** Alliaceae

- **Part used:** Fruit
- **Mechanism:** Suppress the leukocyte inflammatory cytokine production.
- **Chemical constituents:** Disulphides such as allicin and allylpropyl disulphide.
- **Uses:**
 - Antimicrobial
 - Antihypertensive
 - Antioxidant
 - Anticancer

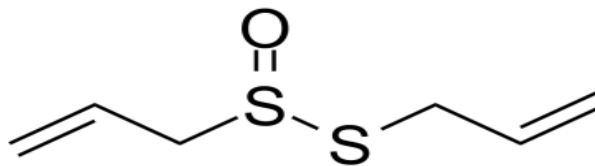


Figure 9: Allicin

8. Plant name: Acorus calmosus

- **Family:** Araceae
- **Part used:** Rhizome
- **Activity:** Ethanolic extract of rhizome possesses immunomodulatory properties.
- **Mechanism:** The extract inhibit the production of interleukin 2.
- **Chemical constituents:** Volatile oils, Acorin, Eugenol acetic acid, and Traces of tannins.
- **Uses:**
 - Antipyretic
 - Indigestion
 - Antifungal
 - Menstrual disorder
 - Analgesic
 - Antidiabetic

Topic-9

CHEMISTRY OF NATURAL PRODUCTS POSSESSING ANTI-VIRAL, ANTI HIV, ANTI FUNGAL ACTIVITIES

Virus may be defined as:

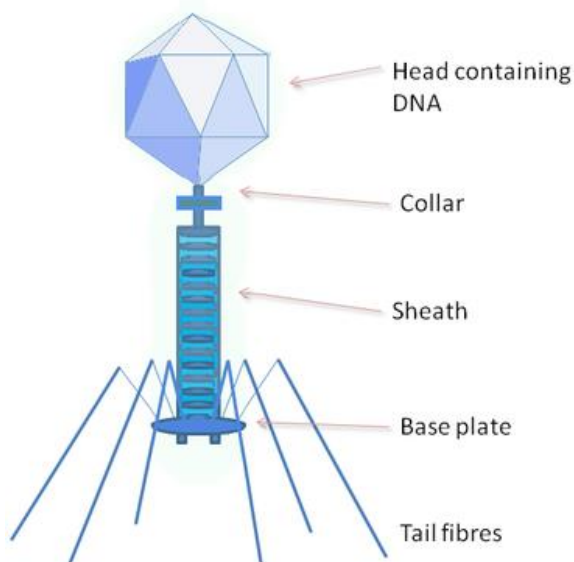
- An infective agent that typically consists of a nucleic acid molecule in a protein coat, is too small to be seen by light microscopy, and is able to multiply only within the living cells of a host.

Characteristics of Virus

- Viruses are obligate intracellular parasites.
- Lack both cell wall and cell membrane.
- Don't carry out metabolic processes.
- Use the metabolic machinery of their host cell.
- They replicate only inside host cell.
- Size range is considerable, ranging from an approximate diameter of 200 μm to 10 μm .

Composition of virus

- Virus is composed of
- Inner core: nucleic acid (double- or single-stranded DNA or RNA),
- Outer covering: protein coat (capsid), some virus also possess lipid envelop.



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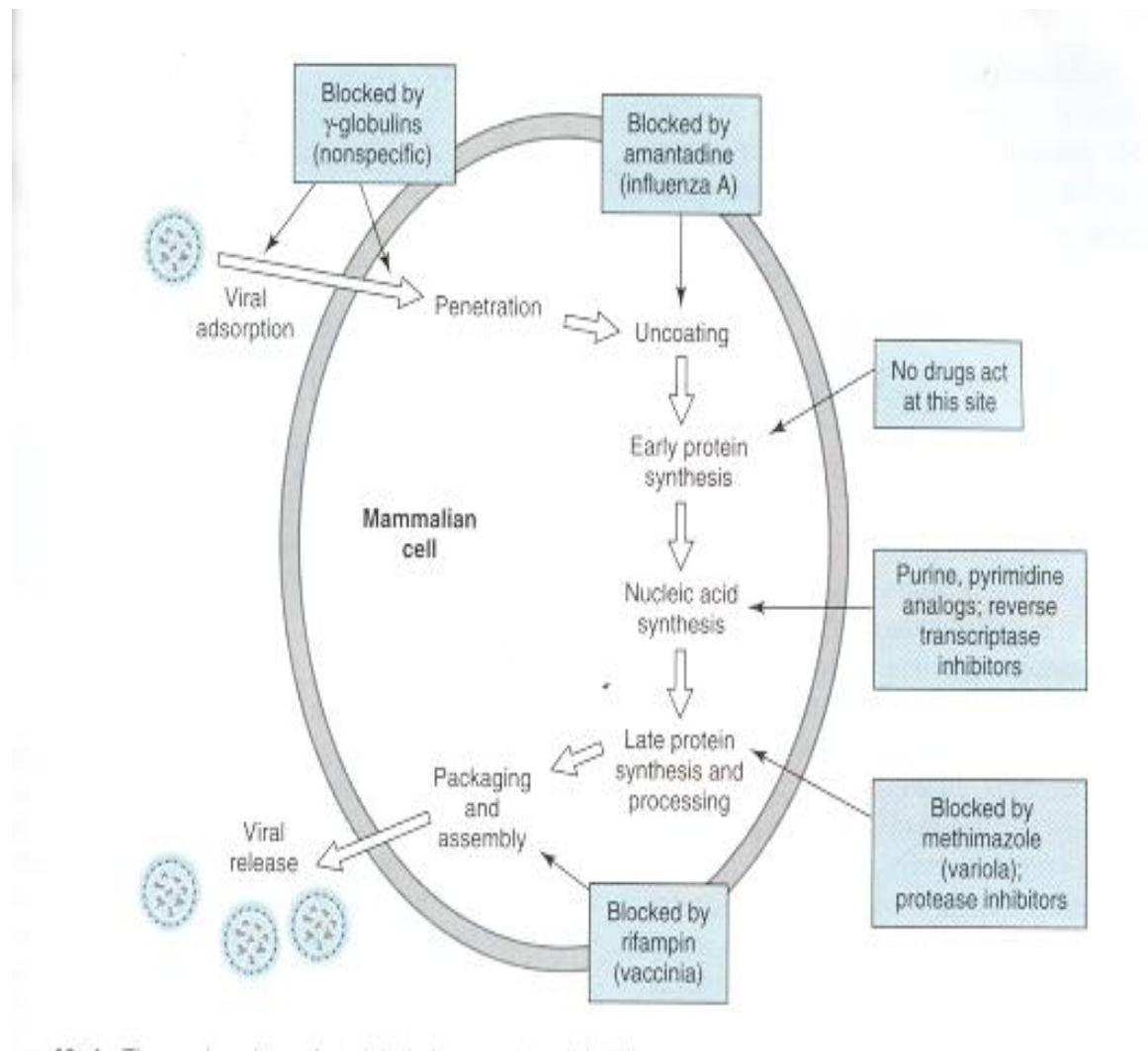
- **Infectious process**

- A typical DNA virus will enter the nucleus of the host cell
- Viral DNA is transcribed into messenger RNA by host cell RNA polymerase.
- mRNA is then translated into virus specific proteins that facilitate assembly, maturation, and release of newly formed virus into surrounding tissues.

HOW ANTIVIRAL DRUGS ACTS

Mostly antiviral drugs act by blocking any of these steps prior to the start of the replication cycle.

- Attachment of the virus to the host cell via its receptor complex
- Entry into the cell via endocytosis
- Release of the viral nucleic acid from the protein coat.



NATURAL PRODUCTS HAVING ANTIVIRAL ACTIVITY

Major classes are

- FLAVONOIDS
- TERPENOIDS
- ALKALOIDS
- POLYPHENOLS
- LIGANS
- COUMARINS

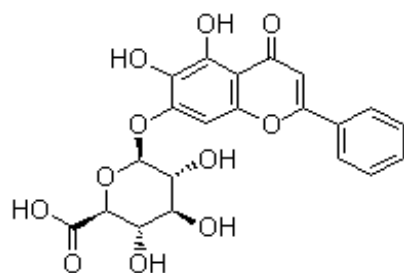
1. FLAVONOIDS

BAICALIN

Origin of plant:- *Scutellaria baicalensis*

Activity: having anti HIV activity target on Reverse transcriptas Infection/entry, replication

Baicalin



Biflavonoids (Ginkgetin)

- ***Origin of plant:*** *Ginkgo biloba* L.
- **Activity :** influenza virus

2. TERPENOIDS

- **Agastanol and Agastaquinone**
- *Origin of plan:* *Agastache rugosa*
- Activity :Protease

Uvaol and Ursolic acid

- *Origin of plant:* *Crataegus pinnatifi da*
- Activity: Protease

3. ALKALOIDS

- **Thalimonine**
- *Origin of plant: Thalictrum simplex L.*
- Activity: influenza virus replication

Indole alkaloid

- *Origin of plant: Uncaria rhynchophylla*
- Activity: influenza virus replication

4. POLYPHENOLS

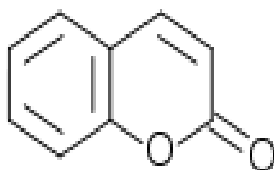
- *Origin of plant: Geranium sanguineum L.*
- Activity:
- Influenza virus
-

5. LIGANS:

- **Rhinacanthin E,F**
- *Origin of plant: Rhinacanthus nasutus*
- Activity:
- Influenza virus

6. COUMARINS

- **Calanolide A**
- *Origin of plant: Calophyllum lanigerum*
- Activity: Reverse transcriptase



VIRAL VACCINES :

- **Measles Virus Vaccines** is made from attenuated live virus grown in chicken Fibroblast.
- **Rubella Virus Vaccines** is made from attenuated live virus grown in Human diploid Cells.
- **Influenza Virus Vaccines** is made from inactivated whole virus or viral subunits grown in chicken embryo cells.
- **Hepatitis B Vaccines.** Two types are available.
 - Earlier vaccines is made from chemically inactivated Hepatitis Surface Antigen particles.
 - A recombinant Hepatitis B Vaccines

Anti Viral Agents

Antiviral drugs/agents are a group of medication used for treatment of viral infections.

It was formerly defined as “substances other than a virus or virus containing vaccine or specific antibody which can produce either a protective or therapeutic effect to the clear detectable advantage of the virus infected host” (Swallow, 1977).

SOME COMMON VIRAL DISEASES

Dengue Virus:

Chebulagic acid and punicalagin

Chemical Nature: TANNINS

Source: *Terminalia chebula*

Mechanism: Inactivate free virus particles and inhibit early viral entry including attachment and penetration phases; do not affect viral cell-to-cell transmission

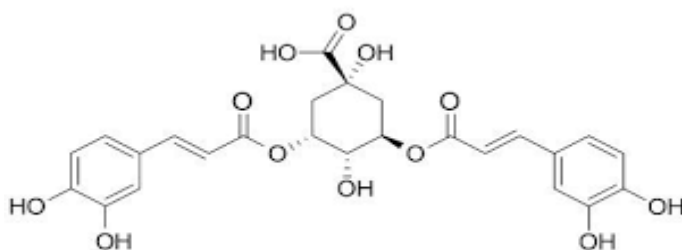
HEPATITIS B VIRUS:

Isochlorogenic acid A

Chemical Nature: PHENOLIC ACID

Source: *Lagdera alata*

Mechanism: Unclear

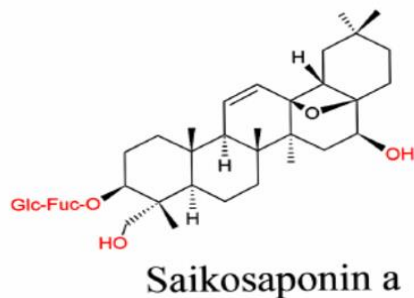
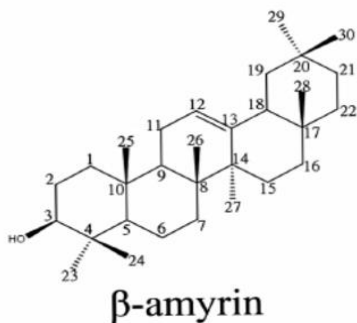


Saikosaponins (C, D)

Chemical Nature: saponons

Source: *Bupleurum species*

Mechanism: Saikosaponin C inhibits HBeAg expression and HBV DNA replication



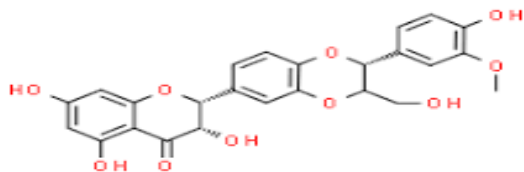
HEPATITIS C VIRUS

SILYMARIN

Chemical Nature: Flavonolignans (natural phenols composed of a part flavonoid and a part lignan)

Source: *Silybum marianum*

Mechanism: Antiviral effect probably related to antioxidant functions of the flavonolignans

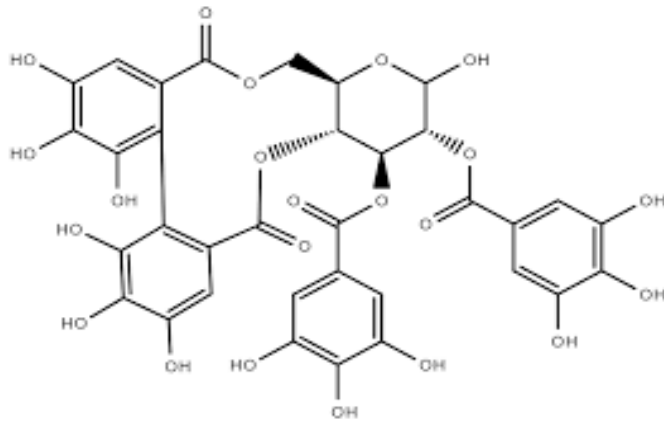


Tellimagrandin I

Chemical nature: ellagitannin

Source: *Rosae Rugosae*

Mechanism: HCV invasion inhibitor

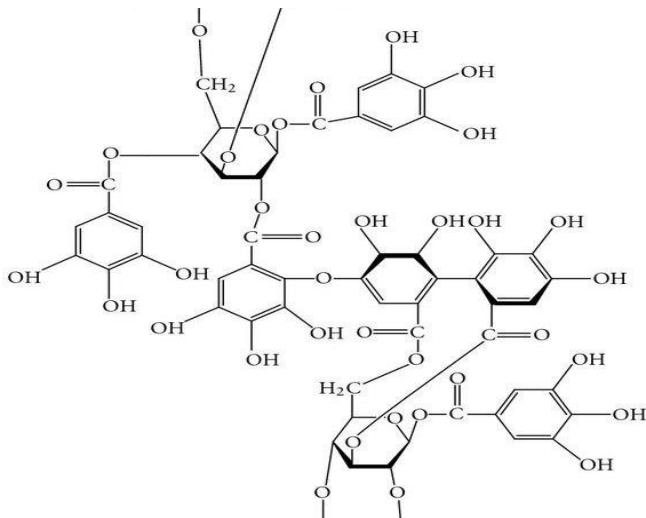


HERPES SIMPLEX VIRUS

Excoecarianin active against HSV-2

Source: *Phyllanthus urinaria*

Mechanism: Inactivation of virus particles



Chebulagic acid and punicalagin against HSV-1

Chemical nature: TANNINS

Source: *Terminalia chebula*

Mechanism: Cell surface GAG-competitors; inhibit viral entry (binding and fusion) and post-infection cell-to-cell spread

INFLUENZA VIRUS

Xanthones against IFA

Source: *Polygala karensium*

Mechanism: IFA NA inhibitors

Chalcones against IFA

Source: *Glycyrrhiza inflata*

Mechanism: IFA NA inhibitors

Homoisoflavonoids against IFA

Source: *Caesalpinia sappan*

Mechanism: IFA NA inhibitors

MEASLES VIRUS

Chebulagic acid and punicalagin

Chemical nature: TANNINS

Source: *Terminalia chebula*

Mechanism: Inactivate free virus particles and inhibit early viral entry including attachment and penetration phases; do not affect viral cell-to-cell transmission

HUMAN IMMUNODEFICIENCY VIRUS

***Artemisia annua* and *Artemisia afra* against HIV-1**

Mechanism: Unclear

Tricyclic coumarin against HIV-1

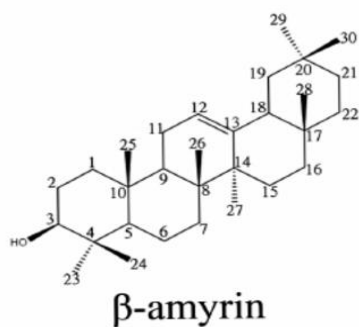
Source: stem bark of *Calophyllum Brasiliense*

Mechanism: Inhibits viral replication in both acute and chronic infections by suppressing NF- κ B activation

CORONAVIRUS

Saikosaponins (A, B2, C, D) against HCoV-22E9

Mechanism: Saikosaponin B2 inhibits viral attachment and penetration stages

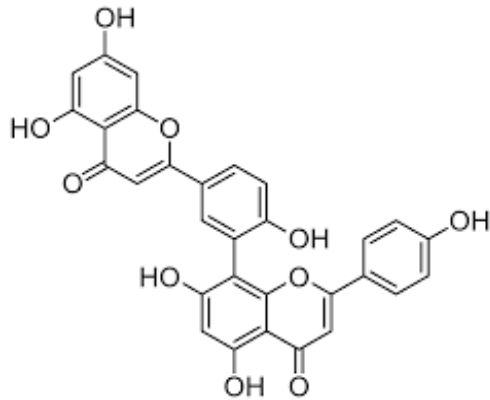


Amentoflavone against SARS-CoV

Chemical nature: Biflavonoid

Source: *Torreya nucifera*

Mechanism: SARS-CoV 3CL protease inhibitor



Fungus is a **Latin** word meaning mushroom.

A fungus is a member of the group eukaryotes.

Includes unicellular microorganisms such as yeasts and molds.

Also include multicellular fungi that produce familiar fruiting forms known as mushrooms.

FUNGAL DISEASES:

Human diseases caused by fungi are called **mycoses**.

The diseases are divided into three groups

These groups are:

- i. Superficial ► These infect the skin, nails and hair.
- ii. Subcutaneous ► These infect the deep layers of the skin.
- iii. Systemic ► An unsuspecting person may inhale the pathogenic fungal spores. Some spores stay in the lungs and grow while others enter the bloodstream, travel around the body and infect other organ.

Infectious diseases caused by fungi are called mycoses. Mostly they are chronic in nature.

WHAT ARE FUNGI ?

Fungi are eukaryotic (Nucleus enclosed in membrane), having rigid cell wall that determines their shape and are **Heterotrophs**. Many are **saprophytes** that digest dead organic matter and organic waste. Some are **Parasite** which obtained nutrients from the tissues of other organism.

STRUCTURE OF FUNGI

The body of fungi is called **Thallus**. Thallus of most multicellular fungi consist of a **Mycelium**. Cylindrical tubes or mass of thread like structure which loosely organized is called Hyphae. Mycelium is embeded in decaying organic matter soil or the tissue of a living organism. Mycelial cell release enzyme that digest the substrate and absorb small nutrients molecules.

Fungi grows in two basic forms.

- (i) Yeast (ii) Molds

YEAST

Yeast are single cell spherical to ellipsoid in shape and diameter from 3-15 μm . Yeast reproduced by budding. Yeast colonies are usually soft, opaque 1-3mm in size and cream coloured.

MOLDS

Molds produced multicellular filamentous colonies. These colonies consist of branching cylindrical tubules called hyphae of diameter 2-10 μm . Some hyphae are divided into cells by corss wall or **Septa**. Medically important mold is **Zygomycetes**.

STRUCTURE OF FUNGAL CELL WALL

Cell wall of fungi are composed of carbohydrates layers, polysaccharides, glycoprotein & lipids chitin. Chitin is a polymer of **N-ACETYL GLUCOSAMINE** rather than **PEPTIDOGLYCAN** which is the component of bacterial cell wall. The fungal cell membrane contain **ERGOSTEROL** rather than **CHOLESTEROL** found in mammalian membrane.

CLASSIFICATION

Human fungal diseases are classified into three well defined groups by the location or in the body where the infection occurs.

1. Superficial or cutaneous mycoses
2. Subcutaneous mycoses
3. Deep seated or systemic mycoses

1. SUPERFICIAL MYCOSIS

Mostly caused by homogenous group of fungi called Dermatophytes. These are superficial infections of the Keratinized epidermis & keratinized epidermis appendages. This include various forms of tinea & ringworm (infection of the hair or hair follicles), flat areas of harmless skin and nails.

2. **SUBCUTANEOUS MYCOSES**

This infection penetrates beneath the skin.

3. **DEEP SEATED OR SYSTEMIC MYCOSES**

When the infection is deep within the body and disseminated to internal organs. This type of infection is life threatening. These are frequently transmitted from one host to another. These diseases have a heterogeneous etiology. Systemic mycoses divided into two groups.

a. **True pathogenic fungi**

Fungi are capable to infect healthy individual.

b. **Opportunistic fungi**

These fungi infect initially those individuals having predisposing conditions such as immunodeficiency or debilitated diseases like diabetes, leukemia, Hodgkin & other lymphomas.

Following are the fungi which causes systemic mycoses.

- Histoplasmosis • Sporotrichosis • Blastomycosis
- Coccidioidomycosis • Cryptococcosis • Paracoccidioidomycosis

NAMES OF FUNGAL DISEASES:

Aspergillosis

Athletes foot

Candidiasis (thrush)

Mycosis

Tinea

Ring worm

PLANT DERIVED ANTIFUNGALS:

i) Alkaloids ii) Phenols iii) Flavonoids iv) Coumarins

v) Quinones vi) Saponins

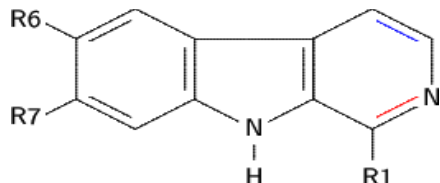
vii) Terpenoids and Essential oils.

1. Alkaloids:

Heterocyclic nitrogen compounds are called alkaloids.

β -carboline

A tryptamine alkaloid including harmine, harmaline, harmane.



2. Phenols and Phenolic acids:

The mechanisms include enzyme inhibition possibly through reaction with sulfhydryl group or through interactions with the proteins.

Caffeic acid:

Biological source: *Thymus vulgaris*

Family: Lamiaceae



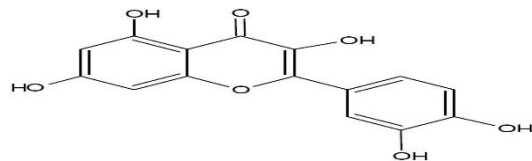
3. Flavonoids:

Their activity is due to their ability to complex with extracellular and soluble proteins and with fungal cell walls.

Quercetin

Biological source: *Adina cordifolia*

Family: Rubiaceae



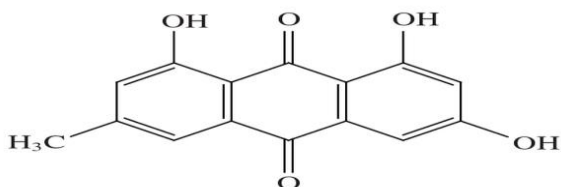
4. Quinones:

Quinones are known to complex irreversibly with nucleophilic amino acids in proteins.

Emodin

Biological source: *Rhamnusfrangula*

Family: Rhamnaceae



4. Saponins:

CAY-1

Biological source: *Capsicum frutescens*

Family: Solanaceae

Dioscin

Biological source: *Dioscorea cayenensis*.

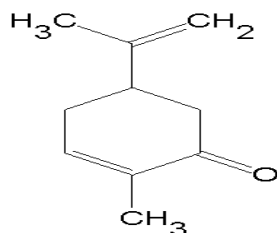
Family: Dioscoreaceae

5. Terpenoids and essential oils:

Carvone

Biological source: *Menthaspicata*

Family: Labiateae



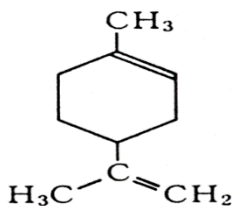
Limonene

Limonene occurs in two optically active forms, l-limonene and d-limonene. Both isomers have different odours:

l-limonene smells piny and turpentine like and d-limonene has a pleasing orange scent.

Biological source: *Citrus limon*

Family: Rutaceae



SOME OTHER NATURAL PLANT HAVING ANTIFUNGAL ACTIVITY

KAVA - PIPERACEAE

Const: Kavalactones (Yangonin)

Dosage: Dried rhizome– 1.5-3 g/day or 60-120 mg karalactine / day

Liquid extract 3-6 ml/day.



QUASSIA-SIMAROUBACEAE

Const: Indole Alkaloids is **canthin 6-one**

Dosage: Tincture of Quassia = 2-4 ml

Conc Quassia Infusion = 2-4 ml with water



THYME - LABIATAE/LAMIACEAE

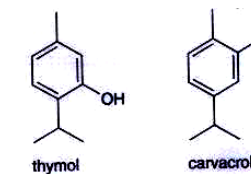
Const: Monoterpenes are active Thymol, carvacrol

& thyme oil have antifungal activity.

Dosage Dried herb 1-4 g TDs.

Liquid extract 0.6-4.0 ml of thyme

Tincture 2-6 ml (1:5 in 45% Alcohol) TDS or 4 drops.

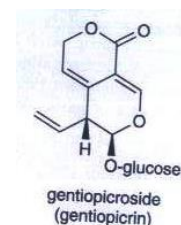


GENTIAN - GENTIANACEAE

Const: Gentiopicroside also known as gentiamarin & gentiopicrin

Dosage: Dried rhizom / roots = 0.6-2g (infusion) TDS

Tincture =1-4ml TDS (1:5 in 45% Alcohol)



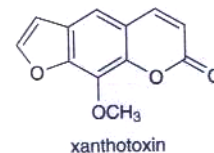
ANGELICA - APIACEAE/UMBELLIFERAE

Const: Angelicin, xanthotoxin

Dosage: Dried leaf = 2-5 g infusion TDS

Leaf extract = 2-5 ml TDS

Leaf tincture = 2-5 ml TDS (1:5 in 45% alcohol)



ANISEED-APIACEA / UMBELLIFERACE

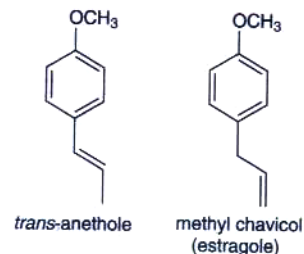
Const: Phenylpropanoids are trans anethole & methyl chavicol

Dosage: Dried fruit = 1-5g infusion TDS

Aniseed oil = 0.05-0.2 ml TDS

Tincture = 0.3- ml TDS

Distilled anis water = 15-30 ml TDS



ARNICA - ASTERACEAE/COMPOSITAE

Const: Sesquiterpene lactone is Helenalin (terpenoid)

Dosage: Tinc of Arinca flower 2-4 ml externally

Preparation= ointment - creams, gels, made with 5-25% v/v tinctures.



CALAMUS - ACOACEAE

Const: Phenylpropanoids is β -asarone

Dosage: Rhizome: = 1-3g infusion TDS.

Tincture = 1-3 ml TDS (1:5 in 60% Alcohol)

Liquid extract = 1-3 ml TDS (1:1 in 60% Alcohol)



CELANDINE PAPAVERACEAE

Const: Alkaloid is chelidonine

Dosage: Aerial parts 2-5g/12-30 mg chelidonine.

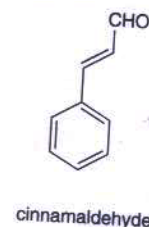


CINNAMON – LAURACEAE

Const: 4% cinnamaldehyde.

Dosage:

Liquid extract -0.5-1 ml (1:1 in 70% alcohol)



Tinc of cinnamon: 2-4 ml

ECHINACEA - ASTERACEAE/COMPISTAE

Const: Polycetylenic compounds.

Trideca 1-ene, 3,5,7,9,10-pentayne.

Dosage: E.purpureae roots: = 3 x 60 drops of Tincture (1:5 in 55% ethanol

Tincture =2-5 ml (1:5 in 45% Alcohol) TDS

GARLIC - ALLIACEAE / LILIACEAE

Const: Alliin and Allicin

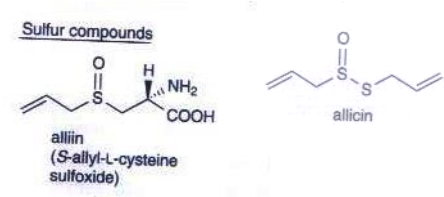
Dosage

Juice of Garlic = 2-4ml

Syrup of Garlic = 2-8 ml

Oil of Garlic = 0.03-0.12ml TDS

Tincture =2-4 ml TDS (1:5 in 45% Alcohol)



TOPIC 10

CHEMISTRY OF FERTILITY REGULATORY ACTIVITIES
OBTAINED FROM PLANTS

FERTILITY REGULATORS:

- Fertility Regulators can be used to either promote or inhibit fertility.
- Sex Regulating Hormones:
- Androgen and estrogen play a major role in the development of both sexes secondary characteristics.
- Although Luteinizing hormone (LH) and follicle Stimulating Hormone (FSH) are regulated Indirectly through steroidal hormone
- Growth regulators may regulate either the growth of entire body like Human growth hormone or of specific tissue like erythropoietin for hematopoiesis

•

SEX REGULATING HORMONES :

- **Androgen** or testosterone give the male its sex characteristics during puberty and for promoting tissue and muscle growth.
- **Estrogen** or estrone are synthesized in the ovaries, which control female secondary characteristics and regulation of the menstrual cycle.
- Another sex hormone is needed for preparing the uterus for implantation of the ovum, this hormone is **progesterone**.

INFERTILITY:

- **Infertility** primarily refers to the biological inability of a person to reproduce naturally.
- **Female infertility** is the inability to carry a pregnancy to full term. There are many biological causes of infertility, some which may be bypassed with medical intervention
- **Male infertility** is the inability to cause a pregnancy. Male infertility is often due to low sperm count.

Ratio of Infertility

- * 40% female
- * 40% male
- * 20% *unexplained*

Unexplained infertility may be associated with

- * toxins
- * immune reactions

- * nutritional deficiencies
- * inflammation
- * stress/HPA activations

FACTORS CONTRIBUTING TO INCREASE LEVEL OF INFERTILITY:

- Stress
- Hormonal Imbalance e.g. hypothyroidism
- Sexually transmitted diseases and genito-urinary infections
- Drugs e.g. Alcohol, caffien
- Radiations
- Previous use of contraception programmes
- Greater age of prospective parents
- Pollution
- Greater age of prospective parents
- Toxicity
- Immune system failure
- Poor Nutrition

GYNECOLOGICAL DISORDERS EFFECTING FEMALE FERTILITY:

These include a verity of conditions like

- Menopausal Symptoms
- Premenstrual syndrome including mastrodynia(breast pain) and Dysmenorrhea
- In female menopause, the period following the complete cessation of menstruation drops to about 10 percent of its premenopausal levels and progesterone production drops to nearly zero.

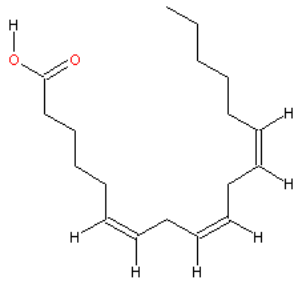
MEDICNAL PLANT USED TO TREAT GYNECOLOGICAL DISORDERS:

- Black Cohosh
- Chaste Berry
- Evening Prime rose Oil
- Raspberry Leaf

- Licorice
- Papaverine

EVENING PRIMROSE OIL:

Active Ingredients: Seeds contain about 14% of a fixed oil of which 70% is cis-linoleic acid and 9% is cis-gamma-linoleic acid (GLA).

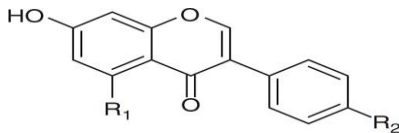


gamma- Linolenic acid

- Help for Premenstrual Syndrome (PMS)
- EPO has been used by to increase cervical mucous. Cervical fluid is necessary for allowing the sperm to swim freely through the cervix.

BLACK COHOSH:

- **Active Ingredients:** Isoflavone formononetin which may be the basis of estrogenic property. Steroidal triterpene glycosides, actein, hydroxy sterols .



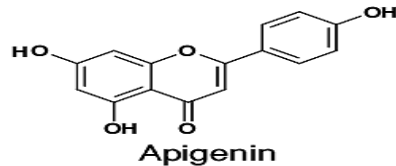
- 1 R₁=OH; R₂=OCH₃
- 2 R₁=H; R₂=OCH₃
- 3 R₁=R₂=OH
- 4 R₁=H; R₂=OH

Isoflavone formononetin

- Alcoholic extract of Black Cohosh suppressed hot flashes in menopausal women by reducing the secretion of luteinizing hormone (LH)
- Amenorrhea (absent period)
- Dysmenorrhea (painful menstruation)
- Relief of uterine contractions associated with threatened miscarriage
- Ovarian pain: Whether it is ovarian cyst pain or ovulation pain.

Chaste berry:

- **Active Ingredients:** Apigenin, a flavonoid, has been identified as an active phytoestrogen in chasteberry.



Uses:

- Chasteberry, may boost fertility and reduce symptoms of PMS and menopause.

Raspberry Leaf

- * **Botanical Name :** Rubus idaeus L. or R.strigosus Michx
- * **Family:** Rosaceae
- * **Part used:** Dried leaves
- * **Active Ingredients** The action of which mainly of antagonistic. These include
 - 1.a smoth muscle stimulent.
 - 2.an anticholineesterase.and
 - 3.a spasmolytic
- * **Pharmacology**

A traditional remedy for painful and profuse menstruation

MALE INFERTILITY:

- Male infertility is the inability to cause a pregnancy. Male infertility is often due to low sperm count.
- Main Cause of male infertility is Impotence
-

FACTORS

- Psychogenic factors: such as sexual anxieties, guilt, fear, feelings of inadequacy, and are responsible for 50-60 percent of erectile dysfunction.
- Vascular

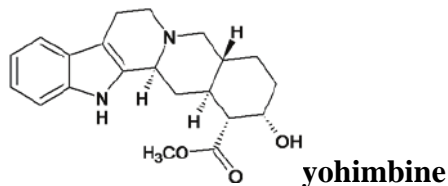
- Neurogenic
- Endocrine abnormalities (diabetes)
- Drug treatment e.g. (antihypertensive and antidepressants).

DRUGS USED IN MALE SEXUAL DYSFUNCTION:

- Yohimbe
- Papaverine
- Ginseng
- Epimedium SPP

YOHIMBE

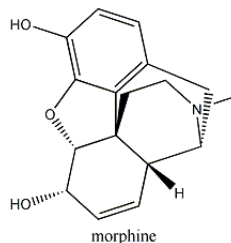
- **Active Ingredients:** it consist 6% mixture of alkaloid, principal of which is yohimbine.



- **Uses:** Yohimbe is primarily an α_2 -adrenergic antagonist. Its peripheral effect is to increase cholinergic and decrease adrenergic activity, in male it increases the penile blood flow.

PAPAVERINE:

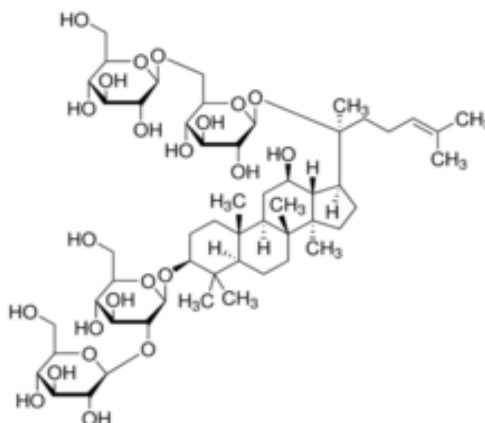
- **Constituents:** The most important constituents of opium are the alkaloids, The principal alkaloid is Morphine. Next to this, Narcotine and Codeine. Among the numerous remaining alkaloids, amounting in all to about 1 per cent of the drug, are Thebaine, Papaverine, Codamine



- **Uses:** Papaverine is used as an [erectile dysfunction](#) drug, alone or sometimes in combination. Papaverine, when injected in penile tissue causes direct [smooth muscle](#) relaxation and consequent filling of the [corpus cavernosum](#) (sponge like regions in penile tissues) with blood resulting in erection.

Ginseng:

- The dried roots and rhizomes of ginseng contain many physiologically important constituents. These include ginseng saponins, ginseng oils and phytosterol, carbohydrates and sugars, organic acids, nitrogenous substances, amino acids and peptides, vitamins and minerals, and certain enzymes that have been isolated and characterized. Among these, ginseng saponins are proven to be the principal and most active constituents



Ginsenoside- Rb1

Uses:

- Ginsenosides are triterpenoid saponins that structurally resemble the steroid hormones. Thus, it is tempting to speculate that the effects of ginsenosides on sexual function and spermatogenesis are a result of activation of steroid receptor.
- It has been shown that intake of American ginseng (500 mg/kg/day) can protect sperms, in particular by increasing the sperm count, reducing sperm death and abnormalities, and resuming sperm motility from CP insult in adult male.
- Ginseng is also found to help preserve the ejaculated sperms.
- Ginseng have positive effect on increasing sperm count.