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Diploma in Pharmacy 1st Year
Pharmacognosy
Chapter 4 : Secondary Metabolites

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PHARMACOGNOSY
Chapter 4
Secondary Metabolites
Alkaloids

- Alkaloids are heterocyclic nitrogenous compounds. Generally, they are basic in nature due to the presence of amino nitrogen. They can be defined as basic nitrogenous compounds of plant origin which are physiologically active. Chemically, at least one nitrogen atom is present in its cyclic structure.
- Ladenburg stated alkaloids as, 'compounds originated from plants with a basic nature, having one or more nitrogen atom in its ring structure'. The basic nature of alkaloids is due to the presence of N-atom in the 5- or 6-membered ring structure.
- Hence, based on the above findings, alkaloids can now be defined as, 'physiologically active basic compounds of plant origin in which at least one nitrogen atom forms part of a cyclic system.

Occurance and Distribution

- McKee in 1962 reported that about 1000 investigated alkaloids belong to almost 100 families, 500 genera, and 1200 species.
- Alkaloids are found in any part of the plant. In a species, Only one or two specific parts (and not all) form alkaloids.

For example

- In opium poppy (*Papaver somniferum*), alkaloids are present in fresh latex of the fruit, while the poppy seeds bear no alkaloids.
- In cinchona the bark of the tree has quinine alkaloids.

Isolation

In general, alkaloids may be extracted by any of the following three well-defined and widely accepted processes:

- 1) **Stas-Otto Process:** Alkaloids extracted are according to their basicity and solubility. Extraction is basically carried out by Stas-Otto process in which the moistened drug is treated with alkali to set free the base existing in salt form and then the free base is separated with an organic solvent
- 2) **Kippenberger's Process:** In this Process the powdered and sieved plant substance is first digested with solution of tannin in glycerol at a constant temperature of 40°C for 48 hours. The resultant mixture is further heated to 50°C to complete coagulation of proteinous substance, and finally filtered.

- 3) **Steam Distillation:** This method is specially employed for volatile liquid alkaloids like coniine, nicotine, and serpentine. This process is not suitable for alkaloids with high molecular weight.

Purification of Crude Extract of Alkaloids

Different alkaloidal crude extracts are purified by different ways:

- ✚ Direct Crystallisation from Solvent
- ✚ Steam Distillation
- ✚ Chromatographic Technique
- ✚ Gradient pH Technique

Identification Tests

- ♣ In case of qualitative estimation the chemical tests are performed for different class of compounds Following are some of the important chemical tests for alkaloids :
- ♣ **Mayer's Reagent (Potassium Mercuric Iodide Solution):** It gives white or pale yellow coloured precipitate except with alkaloids of purine groups.
- ♣ **Dragendorff's Reagent (Potassium Iodide + Bismuth Nitrate):** It gives orange red coloured precipitate. This reagent is also used as a spray reagent for thin layer chromatography of alkaloids.
- ♣ **Wagner's Reagent (Iodine Solution):** It gives a brown or reddish brown coloured precipitate.
- ♣ **Hager's Reagent (Saturated Solution of Picric Acid):** It gives a characteristic crystalline precipitate.
- ♣ **Freshly Prepared Aqueous Solution of Tannic Acid (5% w/v):** It gives a buff coloured precipitate which is soluble in dilute acid or ammonia solution.

Therapeutic Activity

Alkaloids are pre-infectious compounds which prevent bacterial or fungal infection.

Thus, alkaloids in medicine are used as follows:

- 1) They are used as analgesics or pain relievers, e.g., morphine and codeine.
- 2) Atropine and its synthetic derivatives are used in eye examinations for eye dilation.
- 3) Tropicamide is used for diagnosing Alzheimer's disease.
- 4) Quinine (isolated from *Cinchona officinalis*) is an antimalarial drug

Pharmaceutical Applications

- ❖ Presence of a wide-spectrum and complete diversity of complex structures in alkaloids allow them to show broad-range of pharmacological activities on cardiovascular and central nervous system.
- ❖ Alkaloids have their specific and definite pharmacological action.

Terpenoids

- Terpenoids are the volatile and aromatic substances present in plants and giving them their characteristic fragrance or odour.
- Generally, most of the terpenoids are a group of compounds present naturally in plants with some exceptions of i.e., (obtained from other sources) including civet, musk, etc
- In plants, terpenoids are present in leaves and fruits of higher plants, conifers, citrus plants, and other plants.

Occurrence and Distribution

- I. Terpenoids are volatile substances which give plants and flowers their fragrance. They occur widely in the leaves and fruits of higher plants, conifers, citrus and eucalyptus.
- II. Terpenoids comprise tens of thousands of small molecule natural products that are widely distributed across all domains of life. Plants produce by far the largest array of terpenoids with various roles in development and chemical ecology.

Isolation

- Fractional Distillation
- Chromatographic Method
- Chemical Method

Identification Tests

- ✚ **Noller's Test:** The sample to be analysed is mixed with tin and thionyl chloride and subjected to warmth. Pink colouration appears, if tri-terpenoids are present.
- ✚ **Test for Terpenoids:** In a test tube, the plant extract (0.5gm) is taken along with chloroform (2ml). Conc. H₂SO₄ (5ml) is added carefully in the tube in such a manner that it forms a separate layer.
- ✚ Appearance of reddish brown colouration at the junction of two layers confirms the presence of terpenoids.

Pharmaceutical Application

- ♠ Some plants produce volatile terpenes for attracting insects for pollination or to repel certain animals feeding on that plant
- ♠ This bitter taste protects the plants from getting devoured by animals and are also used as antifeedants.
- ♠ They also possess medicinal properties like perilla alcohol acts as antineoplastic agent, artemisinin has antimalarial property, glycyrrhizin has antiulcer, hepaticidal, antimicrobial and diuretic properties, and taxol (diterpenoid) has anticancer property

Glycosides

- ♣ In a glycoside molecules , sugar group is bonded through its anomeric carbon to another group via glycosidic bond A glycosidic bond is a covalent bond that binds the sugar molecule to another molecule (that may or may not be sugar molecules.) Thus a substance containing a glycosidic bond is called a glycoside
- ♣ Glycosides may be defined as “the organic compounds of plants or animals origin which on enzymatic or acid hydrolysis give one or more sugar moieties along with non-sugar moiety”
- ♣ The sugar group is known as the glycone and the non-sugar group as aglycone or genin part of the glycoside

Occurrence and Distribution

- ◆ Glycosides widely occurs in root, bark, fruits, and to a small extent in leaves

Isolation

- Sta-Otto method

Identification Tests

- ❖ Borntrager's Test : 1 gm of drug is boiled with 5-10 ml dilute HCL for 10 Min on a water bath. After boiling the solution is filtered and the filtrate is extracted with CCl_4 / benzene.
- ❖ The obtained extracted solution is shaken with an equal quantity of ammonia solution . A pink or red colour appears in the ammoniacal layer Which confirms the presence of anthraquinone moiety.

Therapeutics Activity

- Cardiac glycosides are compounds derived from the foxglove plant (*Digitalis purpurea*). Initially, digitalis was used to treat dropsy (i.e., oedema). Later studies revealed that digitalis may treat oedema caused by a weakened heart (i.e., heart failure)

Volatile Oils

- ♣ Volatile oils are obtained from various plant parts. They are basically the mixture of hydrocarbon sesquiterpenes, and polyterpenes and their oxygenated derivatives.
- ♣ Volatile oils carry the plant's active constituents and hence are also called essential oils. Mostly the volatile oils are already present in special secretory tissues in the Plant, e.g., the oil ducts in umbelliferous fruits
- ♣ Volatile or ethereal oils are defined principles of plant as, "odorous volatile principle of plants and animal origin which evaporate when exposed to air at ordinary temperature"

Occurrence and Distribution

- Aromatic plants contain essential oils
- Volatile oils can be formed by two methods, i.e., by hydrolysis of some glycosides, and directly by the Protoplasm

Isolation

- The amount of volatile oils present in plants reaches its maximum at some particular time, e.g., jasmine has the greatest concentration of volatile oil at sunset, and thus the plant parts containing volatile oils should be collected at this particular time.
- Extraction by Steam Distillation:
- Extraction by Scarification:

Identification Tests

- 1) A thin section of drug is treated with an alcoholic solution of Sudan III. A red colour appears that confirms the presence of volatile oils.
- 2) A thin section of drug is treated with a tincture of alkane. A red colour appears that confirms the presence of volatile oils.

Therapeutic Activity

volatile oil molecules enter the body, they correlate with physiological functions through the following three modes of action:

- A. **Biochemical (Pharmacological):** They chemically interact in the bloodstream with hormones and enzymes (like farnesene).
- B. **Physiological:** They interact by participating in specific physiological function; For example, the volatile oil present in fennel contains oestrogen-like compounds which prevent female problems (like lactation and menstruation).
- C. **Psychological:** On inhaling the volatile oil molecules, the olfactory area of the brain (limbic system) gets triggered and releases chemical and neurotransmitter messengers

Pharmaceutical Applications

- Volatile oils are employed as flavouring agent, perfuming agent in pharmaceutical products, foods, beverages, and in cosmetics

Tannins

- The term tannin was first used by Seguin in 1976 to denote substances present in plant extracts which are able to combine with proteins of animal hides, prevent their putrefaction, and convert them into leather.
- On this basis tannin is a substance which is detected qualitatively by a tanning test (the Goldbeater's skin test) and is determined quantitatively by its absorption on standard hide powder.
- Tannins are defined as, "complex substances that usually Occur as mixtures of polyphenols that are very difficult to separate since they do not crystallise".
- Tannins are present in the aerial parts, e.g., leaves, fruits, barks, or stem, generally occurs in immature fruits, but disappears during the ripening process.

Occurrence and Distribution

- ♣ Rhubarb, red rose petals, bearberry leaves, Chinese galls, Turkish gall, hamamelis, chestnut, and maple are rich in gallitannins. Pomegranate rind, pomegranate myrobolans, eucalyptus leaves, some Australian kinds and Oak bark are rich in ellagitannins
- ♣ parts of plants are rich in condensed tannins
 - 1) Barks: Cinnamon, wild cherry, cinchona, willow, acacia, oak, and hamamelis.
 - 2) Roots and Rhizomes: Krameria and male fern.
 - 3) Flowers: Lime and hawthorn.
 - 4) Seeds: Cocoa, guarana, kola, and areca.
 - 5) Leaves: Hamamelis, howathron, and tea.
 - 6) Extracts and Dried Juice: Catechu, acacia, mangrove cutches, East Indian Kino, butea gum, eucalyptus, and kino.

Isolation

The steps involved in extracting tannins are :

- 1) 100gm of the powdered sample is refluxed for 15 minutes in a 500ml Erlenmeyer flask. During the reflux process, the entire sample should be covered with 70% acetone. The flask is removed from the hot plate and filtered in another flask.
- 2) The residue on the filter paper is washed off using a sufficient amount of 70% acetone.
- 3) The washed solution is combined with the filtrate and the resultant mixture is subjected to extraction using diethyl ether.
- 4) This process is repeated five times till the diethyl ether and tannin separate out completely as distinct upper and lower layer, respectively.
- 5) The tannin layer is separated via separatory funnel. The tannin so collected is evaporated till a residue, known as the extracted tannin, is obtained which is the final product.
- 6) The tannin percentage is calculated with the following formula: % tannin = $\frac{\text{Wt. of dried tannin}}{\text{Wt. of the powdered sample}} \times 100\%$
- 7) After extracting the tannin, the aqueous and ethereal layers are concentrated, dried, further iso-lated, and purified using various chromatography techniques.

Identification tests:

- **Gelatin Test:** To a solution of tannin (0.5-1%), aqueous solutions of gelatin (1%) and sodium chloride (10%) are added. A white buff-coloured precipitate is formed.

Therapeutic Activity

The medicinal uses of tannins are:

- I. Anti-Diarrhoeal
- II. Anti-Tumour
- III. Antidote

Resins

- Resins can be defined as, "the hydrocarbon secretion of various plants (especially the coniferous trees)".
- Apart from this, resins are also used as a raw material for synthesising organic compounds.

Occurrence and Distribution

- ✚ Below are the examples of different plants whose different structures or secretion zone contain resins:
- ✚ Ginger (*Zingiber officinale* Roscoe) belonging to family Zingiberaceae has resin cells
- ✚ Cannabis (*Cannabis sativa* Linn.) belonging to family- Moraceae contain resins in their glandular hair.

Identification Tests

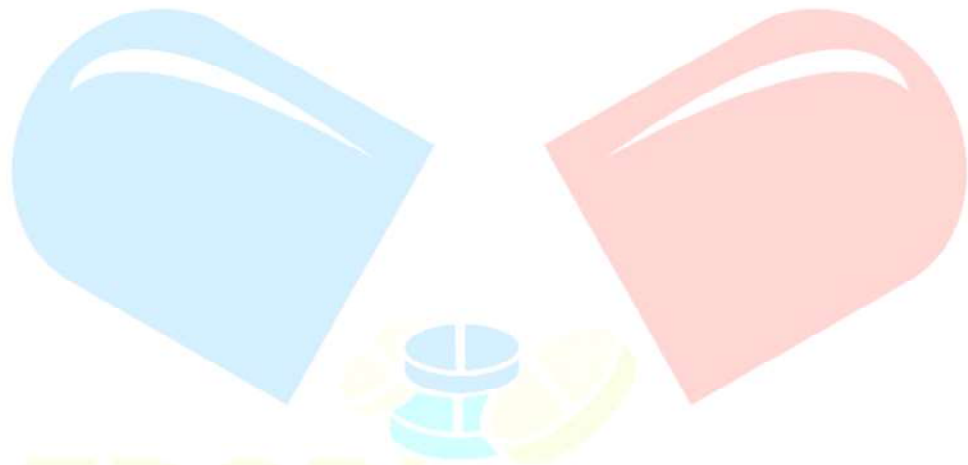
- i. Colophony resins are The tests recommended for colophony resins are
- ii. 100mg of resin powder is dissolved in 10ml acetic anhydride. To this solution, few drops of sulphuric acid is added which results in purple violet colouration of the solution.
- iii. **Guaiacum Resin:** A solution of resin is prepared in ethanol and to this solution ferric chloride is added. This results in deep blue colouration of the solution.

Therapeutic Activity

- Resins possess mild antiseptic property, hence are used topically in the form of plaster, ointments, and cerates.
- They are also used for preparing emulsions.

Pharmaceutical applications

Resins are being used since ages in various cultures and are used as herbal medicine for several diseases in the Eastern countries.



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