WELCOME

PHARMACOGNOSY

- The word Pharmacognosy is derived from two words;

Pharmakon: *medicine (drug)*

Gignosco/gnosis: *to acquire knowledge of something*

- **"*Pharmacognosy is systematic study of crude drugs obtained from natural origin like plant, animal and minerals".***
- Pharmacognosy can be defined as branch of science which involves detailed study of drugs obtained from natural origin including name, habitat, collection, cultivation, macroscopy, microscopy, physical properties, chemical constituents, therapeutic actions, uses and adulterants."
- The **American Society of Pharmacognosy (ASP)** defines it as "***the study of the physical, chemical, biochemical and biological properties of drugs, drug substances, or potential drugs or drug substances of natural origin as well as the search for new drugs from different natural sources"***

- In the early period, primitive man went in search of food and ate at random, plants or their parts like tubers, fruits, leaves, etc.
- As no harmful effects were observed, he considered them as edible materials and used them as food.
- If it caused diarrhoea it was used as purgative, if vomitting it was used as memtic and if it was found poisonous and death was caused, he used it as arrow poison.
- The knowledge was empirical and was obtained by trial and error. He used drugs as such or as their infusions and decoctions.
- The results were passed on from one generation to the other, and new knowledge was added similarly.

HISTORY OF PHARMACOGNOSY

- **C.A. Seydler**, a medical student of Germany, given the name Pharmacognosy from his doctoral thesis entitled ***Analectca Phrmacognostica*** in **1815.**
- The physician **J.A. Schmidt** used this name previously in his book ***Lehrbuch der materia medica*** in **1811** to describe the study of medicinal plants.
- The ***Papyrus-ebers*** **(Ebers papyrus),** a famous document found in tomb of a mummy indicates that Egyptians were having the knowledge of medicinal effects of plants.
- Father of Pharmacognosy: **DIOSCORIDES**

It is mainly the Greek scientists who enriched the natural sciences;

- ✔ ***Hippocrates*** (460-370 BC) dealt with **Anatomy and Physiology**.
- ✔ ***Aristotle*** (384-322 BC) wrote about **Animal Kingdom**.
- ✔ ***Theopharastus*** (370-287 BC) described mainly the **Plant Kingdom**.
- ✔ ***Dioscorides*** described various Medicinal Plants in 78 AD.
- ✔ ***Pliny the Elder*** (23-78 AD) complied 37 volumes on **Natural History**.
- ✔ ***Galen*** (131-200 AD) complied 20 volumes, describing the methods of preparing formulae from plant and animal drugs.

Ancient China

- ***Shen Nung*** (about 2700 B.C.), the emperor who sought out and investigated the medicinal value of several hundred herbs.
- He reputed to have tested many of them on himself, and to have written the first ***Pen T-Sao, or Native Herbal***, recording **365 drugs**.
- These were subdivided as follows;
  - ✔ **120 emperor herbs** of high, food grade quality which are non-toxic and can be taken in large quantities to maintain health over a long period of time.
  - ✔ **120 minister herbs**, some mildly toxic and some not, having stronger therapeutic action to heal diseases.
  - ✔ **125 servant herbs** that having specific action to treat diseases. Most of those in the last group, being toxic, are not intended to be used daily over a prolonged period of weeks and months.

- ***Shang Hang Lun*** (Treatise on the Treatment of Acute Diseases Caused by Cold) written by **Chang Chung-Ching** (142–220).
- ***Chin Kuei Yao Lueh*** (Prescriptions from the Golden Chamber): most important classical herbal formulas that have become the basis of Chinese and Japanese-Chinese herbalism (called 'Kampo').
- ***Tao Hong Jing's*** (456–536) compilation of the Pen T'sao ***Jing Ji Zhu*** (Commentaries on the Herbal Classic): 730 herbs were described.
- ***Li Shi Zhen*** (1518–1593) began work on the monumental Pen T'sao ***Kan Mu*** (Herbal with Commentary): The book lists 1892 drugs, 376 described for the first time with 1160 drawings. It also lists more than 11,000 prescriptions.

Ancient Egypt

- ✔ The most complete medical documents existing are the ***Ebers Papyrus*** (1550 B.C.), a collection of **800 prescriptions**, mentioning **700 drugs**.
- ✔ ***Edwin Smith Papyrus*** (1600 B.C.), which contains **surgical instructions and formulas for cosmetics.**
- ✔ The ***Kahun Medical Papyrus*** is the oldest (1900 B.C.) and deals with the **health of women, including birthing instructions.**

Ancient India

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- In India, knowledge of medicinal plants is very old, and medicinal properties of plants are described in **Rigveda and in Atharvaveda** (3500–1500 B.C.) from which Ayurveda has developed.
- The basic medicinal texts in this world region— The Ayurvedic writings—can be divided in three main ones (**Charaka Samhita, Susruta Samhita, Astanga Hrdayam Samhita**) and three minor ones (**Sarngadhara Samhita, Bhava Prakasa Samhita, Madhava Nidanam Samhita**).

DEVELOPMENT OF PHARMACOGNOSY

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During the period 1934-1960, the knowledge of pharmacognosy has been greatly enriched by the application of principles of plant chemistry (organic and Bio-chemistry), Pharmacology, and modern methods of analysis.

Important events during this period have been-

1. Isolation of Penicillin (1928) by A. Fleming and its large-scale production by Florey and Chain in 1941.
2. Isolation of Alkaloids from Rauwolfia (Reserpine), Vinca (Vincristine and Vinblastine) and Opium (Morphine).
3. Isolation of various antibiotics like Streptomycin, Chloramphenicol and Tetracyclines.
4. Semi-synthetic drugs obtained from natural products.
5. Certain plant drugs served as models for synthetic drug e.g. Morphine for potent analgesics like Apomorphine, Pethidine. Salicin served as model for Aspirin.
6. Biosynthetic pathways for bio-synthesis of primary and secondary metabolites, studied. Examples are- Calvin Cycle (photosynthesis) Shikimic Acid Pathway (for aromatic compounds) Acetate Mevalonate pathway (for biosynthesis of terpenoid compounds)

PROGRESS FROM 1960 ONWARDS

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- During this period much newer compounds have not been discovered, but newer and better methods for production of Antibiotics, Hormones and Anti-tumour compounds have been devised.
- From 6- Amino Penicillanic Acid (6-APA), important broad spectrum Antibiotics like Ampicillin, Oxacillin, Methicillin, Cloxacillin and Phenethicillin.
- These developments have helped the pharmacognosy to develop to a wellestablished subject of pharmaceutical science from its descriptive botany stage.

SCOPE OF PHARMACOGNOSY

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- ✔ Pharmacognosy is important branch of pharmacy which is playing key role in new drug discovery and development by using natural products. Pharmacognosy has given many leads for new drug discovery and development.
- ✔ It is an important link between modern medicine systems (allopathy) and traditional system of medicine. It is part medicinal system which is affordable as well as accessible to common man. As part of integrative system of medicine, pharmacognosy can help to increase effectiveness of modern medicine system.
- ✔ It is acting as bridge between pharmacology, medicinal chemistry and pharmacotherapeutics and also pharmaceutics.
- ✔ Pharmacognosy can provide safe and effective drugs in combination with modern medicine system.
- ✔ Pharmacognosy includes knowledge about safe use of herbal drugs including toxicity, side effects, drug interaction thereby increasing effectiveness of modern medicine.

- Pharmacognosy is an important link between pharmacology and medicinal chemistry. As a result of rapid development of phytochemistry and pharmacological testing methods in recent years, new plant drugs are finding their way into medicine as purified phytochemicals, rather than in the form of traditional galenical preparations.
- Pharmacognosy is the base for development of novel medicines. Most of the compounds obtained from natural product serve as prototype or base for development of new drug which are more active and less toxic.
- By means of pharmacognosy, natural products can be dispensed, formulated and manufactured in dosage forms acceptable to modern system of medicine.
- There are vast number of plant and animal species which are not studied systematically.
- Development of pharmacognosy also leads to development of botany, taxonomy, plant biotechnology, plant genetics, plant pathology, pharmaceutics, pharmacology, phytochemistry and other branches of science.

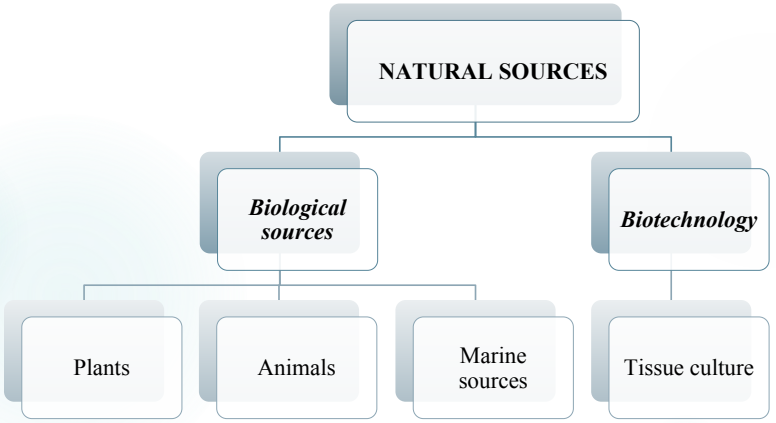
SCOPE OF PHARMACOGNOSY

- Plant Identification and Classification
- Extraction and Isolation of Natural Products
- Characterization of Natural Products
- Synthesis of Natural Products
- Evaluation of Biological Activity
- Drug Discovery and Development
- Quality Control and Standardization
- Cultivation and Domestication of the Medicinal Plants
- Analysis of Phytochemicals
- Preparation of General Tonics and Stimulants

CRUDE DRUG

- ✔ A crude drug is any **naturally occurring**, unrefined substance derived from organic or inorganic sources such as plant, animal, bacteria, organs or whole organisms intended for use in the **diagnosis, cure, mitigation, treatment, or prevention of disease** in humans or other animals.
- ✔ Crude drugs comprise of whole plants and herbs, their morphological or anatomical parts, saps, extracts, secretions and other constituents; whole animals, their anatomical parts, glands or other organs, extracts, secretions and other constituents of their organ.
- ✔ Examples: Acacia, Agar, Benzoin, Beeswax, Cinchona, Cinnamon, Digitalis, Datura, Ephedra, Linseed, Fennel, Mustard, Ginger, Isapagol, Nutmeg, etc.

SOURCES OF CRUDE DRUGS



PLANT DRUGS

DRUG	SOURCES	USES
Vinca (Vinblastine, vincristine)	Leaves of *Catharanthus roseus*, Apocynaceae	Anticancer agent
Rauwolfia (Reserpine)	Roots of *Rauwolfia serpentine*, Apocynaceae	Antihypertensive agent
Cinchona (Quinine)	Barks of Cinchona sps, Rubiaceae	Antimalarial agent
Ashwagandha (Withanolides)	Roots of *Withania somnifera*, Solanaceae	Sedative, Antirheumatic
Momordica (Momordicin, charantin)	Fruits of *Momordica charantia*, Cucurbitaceae	Hypoglycemic agent
Aloes (Barbaloin)	Juice of leaves of *Aloe barbadensis*, Liliaceae	Purgative
Black pepper (Piperine)	Fruits of *Piper nigrum*, Piperaceae	Aromatic, Stimulant
Tulsi (Eugenol)	Leaves of *Ocimum sanctum*, Labiatae	Anti-bacterial, Insecticidal, immunomodulatory agent

ANIMAL DRUGS

DRUG	SOURCES	USES
Honey	***Apis mellifera*** (honey bees), Apidae	Demulcent, nutrient, sweetening agent
Cantharides	Dried beetles, ***Cantharis vasicatoria***, Meloidae	Counter irritant, rubefacient
Cod liver oil	Extracted from the livers of ***Gadus morrhua***, Gadidae	Source of vitamins, Used in treatment of rickets & TB
Shark liver oil	Extracted from the livers of ***Hypoprion brevirostris***	Used in Vit. A deficiency, used in sunburn ointments
Spermaceti	Special cavity in the head of sperm whale, ***Physeter macrocephalus***, Physeteridae	Preparation of cosmetic creams
Gelatine	Skin, ligaments, tendons and bones of animals	Hard and soft gelatin capsules
Cobra venom	Secretions of poisonous glands of ***Naga naga***, Colubridae	Local haemostatic
Lanolin	Fat from the wool of ***Ovis aries*** (Sheep), Bovidae	Absorbable Ointment base

MICROBIAL DRUGS

1. **Bacitracin** from *Bacillus subtilis*
2. **Chloramphenicol** from *Streptomyces venezuelae*
3. **Streptomycin** from *Streptomyces griseus*
4. **Neomycin** from *Streptomyces fradiae*
5. **Penicillin** from *Penicillium notatum*
6. **Griseofulvin** from *Penicillium griseofullivum*
7. **Amphotericin B** from *Streptomyces nodosus*
8. **Tetracycline** from *Streptomyces aureofacein*

MARINE DRUGS

DRUG	SOURCES	USES
Palythoxin	***Palythoa sps.***	Vasoconstriction
Halitoxin	***Haliclona viridis*** (sponges)	Antitumour
Laminine	***Laminaria aungustata*** (Marine algae	Hypotensive agent
Saxitoxin	***Saxidomus giganteus***	Hypotensive agent
Manolide	Sponges	Anti-inflammatory
Tholpin	Annelida	Anti-microbial agent
Holotoxin	Sea cucumbers	Anti-microbial agent
Tetradotoxin	Liver and ovaries of puffer fishes	Anti- spasmodic agent

RECOMBINANT DNA TECHNOLOGY/ TISSUE CULTURE

- ✔ Recombinant DNA technology involves cleavage of DNA by enzyme restriction endonucleases.
- ✔ The desired gene is coupled to rapidly replicating DNA (viral, bacterial or plasmid).
- ✔ The new genetic combination is inserted into the bacterial cultures which allow production of vast amount of genetic material.
- ✔ Important example is **Human insulin** is produced by modification of porcine insulin or by bacteria using recombinant DNA technology.
- ✔ Few others include **Somatotrophin, Erythropoietin, Human blood coagulation factors** etc.
- ✔ **Scopolamine, Podophyllotoxin, Paclitaxel, Rosmarinic acid, Vanillin and Shikonin** are some of the examples of compounds produced from plant cell cultures.

S. No	Secondary Metabolites	Plant Source	Types of Culture
1	Reserpine	*Rawolfia serpentina*	Suspension Culture
2	Vinblastine	*Catharanthus roseus*	Cell Culture
3	Atropine	*Atropa belladona*	Hairy root Culture
4	Rhein	*Cassia angustifolia*	Callus Culture
5	Nicotine	*Nicotina tobacum*	Suspension Culture
6	Morphine	*Papaver somniferum*	Suspension Culture
7	Quinine	*Cinchona Ledgeriana*	Root Culture
8	Digitoxin	*Digitalis lanata*	Suspension Culture

ORGANISED CRUDE DRUGS

- Organized drugs consist of the cellular organization in the form of anatomical features.
- Plant or animal origin.
- These are mostly the crude drugs from plant sources.
- Almost all of the morphological plant parts or the entire plant itself can be called as an organized drugs.
- Directly used for extraction of active constituents from it.
- Eg., Cinchona bark, Sandalwood, Quassia wood, Senna, Digitalis leaves, Nux vomica seeds, Rauwolfia roots etc.

Leaves:

- The leaves of *Digitalis Purpurea* are the source of Digitoxin and Digoxin, which are cardiac glycosides.
- Leaves of Eucalyptus give oil of Eucalyptus, which is an important component of cough syrup.
- Tobacco leaves give nicotine.
- Atropa belladonna gives atropine.

Flowers:

- Poppy: *Papaver somniferum* gives morphine (opioid)
- *Vinca rosea* gives vincristine and vinblastine
- Rose gives rose water used as tonic.

Fruits:

- Senna pod gives anthracine, which is a purgative (used in constipation)
- Calabar beans give physostigmine, which is cholinomimetic agent.

Seeds:

- Seeds of Nux Vomica give strychnine, which is a CNS stimulant.
- Castor oil seeds give castor oil.

Roots:

- Ipecacuanha root gives Emetine, used to induce vomiting as in accidental poisoning. It also has amoebicidal properties.
- *Rauwolfia serpentina* gives reserpine, a hypotensive agent.

Bark:

- Cinchona bark gives quinine and quinidine, which are antimalarial drugs. Quinidine also has antiarrhythmic properties.
- *Hyoscyamus Niger* gives Hyosine, which is also anticholinergic.

Stem:

- *Chondrodendron tomentosum* gives tubocurarine, which is a skeletal muscle relaxant used in general anesthesia.

Rhizome:

- *Curcuma longa* (turmeric): management of oxidative and inflammatory conditions, metabolic syndrome, arthritis, anxiety, and hyperlipidemia

UNORGANISED CRUDE DRUGS

- They are crude drugs of plant or animal origin and have no cellular structure.
- They are either a mixture of chemical substances or they are decomposition products.
- They are classified into several groups: **dried latex, dried juices, dried extracts, gums, mucilage, oleoresins, and oleo-gum-resins.**

Gums and Mucilage

Gum	Mucilage
1. Gums are produced by plant when it in injured of diseased by a process " Gummosis"	1. Mucilage is the normal products of plant growth.
2. Gum is produced outside the plant cell.	2. Mucilage is produced inside the cell.
3. Gums are soluble in water to form adhesive solution.	3. Mucilage are not soluble in water, they form slimy solution with water.
4. Gums are made up of sugar, salts of uronic acid e.g. Gum acacia, gum Tragacanth gum.	4. Mucilage is made up of ester & Sulphuric acid. e. g. Mucilage is present in Agar, Senna, Isapgol.

Resins

- These are amorphous mixtures of essential oils, oxygenated products of terpenes and carboxylic acids
- Resins are of two types;
  - ✔ Synthetic resins
  - ✔ Natural resins
- They are a mixture of organic acids, alcohols, and esters.
- They are amorphous, non-crystallizable transparent, solids or semi-solids.
- They are insoluble in water & and soluble in organic solvents.
- When heated they melt and burn with sooty flame.
- They are produced in plants in special types of glands.
- On boiling with alkalis they form resin soap.
- They are non nitrogenous.
- They are hard, heavier than water and non-conductive.

According to the principle components present they have been classified as follows:

Class	Drug	Example
1 Acid Resin	Colophony, Myrrh	Abietic acid, Commiphoric acid
2. Ester Resin	Benzoin, Storax	Benzyl benzoate
3. Resin alcohol	Balsam of Peru, Gurjanbalsam	Peruresinotannol, Gurjuresinol..

Resin combinations

- ***Oleoresins***: - Homogenous mixture of volatile oil & resin is called as oleoresin. e.g. Oleoresin of Ginger.
- ***Oleo- gum- Resin:*** - Homogenous mixture of volatile oil, gum & resin. e.g. Myrrh, Asafoetida.
- ***Natural resins***: Homogenous mixture of sugar & resin Ex. Jalap, resin of Ipomoea.
- ***Balsam:***- Aromatic resinous substances. If the resin contains balsamic acid, cinnamic & or benzoic acid it is called balsam. Ex. Benzoin, balsam of Tolu.

Dried juices

- The juices obtained from fleshy leaves (aloes) or from the stems of the trees (kino).

Dried latex

- Latex is a product present in special tissue of plant it is white, aqueous, suspension, the suspended particles are protein, sugar, minerals, alkaloids, resin, or starch.
- Under microscope, small particles of oil globules are seen.
- E.g. opium, papain, Gutta-percha.

Dried extracts

- Pharmacognostic origin drugs are obtained by treating the part of plant with water or distillation followed by concentration.
- Pharmaceutical-origin drugs are obtained by alcoholic or hydro-alcoholic solutions and adjusting the product to a standard strength.

e.g. Agar, black catechu, gelatin

Organized drugs	Unorganized drugs
They are the sources from plants and animals.	They are the sources of plants, animals, and minerals.
They procured directly from the above sources.	They are products of plants and animals and obtained by extraction, distillation, incision methods.
They have proper cellular structures like, leaves, flowers, fruits, barks, roots, woods etc.	They do not have well defined cellular structure like gum, mucilage, resin etc.
They are identified by morphological characters.	They are identified by organoleptic properties.
They are solid in nature.	They are solid, semi-solid and liquid in nature.
To study their characters, transverse section is used for drugs under microscope.	To study their characters, physical parameters like density, optical rotation, viscosity, refractive index, chemical tests are important.
Examples: **Woods:** Quassia, sandal wood. **Leaves:** Digitalis, Eucalyptus, Mint, Senna,	**Examples:** **Dried Latex:** Opium, Papain. **Dried Juice:** Aloe, Kino.

EVALUATION OF CRUDE DRUGS

❖***Drug evaluation may be defined as the determination of a drug's identity, purity and quality.***

✓***Identity*** – Identification of biological source of the drug.

✓***Quality*** – quality of the active constituents present.

✓***Purity*** – the extent of foreign organic material present in a crude drug.

Importance of evaluation of crude drugs:

✓Determination of Biochemical variation in the drugs

✓Identification of deterioration due to treatment and storage

✓Reporting Substitution and adulteration, as a result of carelessness, ignorance, and fraud

METHODS OF DRUG EVALUATION

- ***Morphological evaluation***
- ***Microscopical evaluation***
- ***Physical evaluation***
- ***Chemical evaluation***
- ***Biological evaluation***

MORPHOLOGICAL EVALUATION

- Organoleptic evaluation, study of morphology and other sensory characters.
- Based on color, odour, taste, size, shape, texture etc.

Part of drug	Examples
Bark	Cinnamon
Underground part	Ginger, turmeric
Leaves	Digitalis
Flowers	Saffron
Fruits	Fennel
Seeds	Nuxvomica
Entire plant	Vinca

Character	Example
Aromatic odour	Umbelliferous fruits
Sweet taste	Liquorice
Fractured surface	Cinchona
Pungent taste	Ginger
Brown colour	Cinnamon
Wavy shape	Rauwolfia
7-8 mm width, 25-60 mm length	Senna leaf

Study of sensory characters

Flowers: Floral parts: stigmas, corollas, anther, ovary, receptacle.

Leaves and leaflets: Length, width, apex, margin, base, venation, the texture of the leaf and the hairs in upper and lower surface. The feel of the surface described as soft, hairy smooth.

Bark: Flat or curved pieces, Single quill, Double quills, Barks have two surfaces, an outer and inner, The inner surface is usually lighter in color than the outer surface

- **Odor and taste:** distinct, indistinct, aromatic, balsamic, spicy
- **Taste:** Acidic (sour), Saccharine (sweet), Saline (salty), Alkaline, Bitter, Tasteless, Distinctive sensations to the tongue like Mucilaginous and oily (soft feeling), Astringent, Pungent (warm biting sensation), Acrid (irritant sensation), Nauseous (those tending to excite vomiting).

COLOUR AND EXTERNAL MARKINGS	
White	Starch
Pale yellow	Ginger, Squill, White pepper
Deep yellow	Peeled liquorice
Pale green	Lobelia
Greenish brown	Most of the leaf herbs
Light brown	Nuxvomica, fennel
Dark brown	Clove buds
Dark reddish brown	Cinchona
Brick red	Cinnamon bark inner

MICROSCOPIC OR ANATOMICAL EVALUATION

- This method allows a more detailed examination of a drug and it can be used to identify organized drugs by their known histological characters.
- Before examination through a microscope the material must be suitably prepared.
- This can be done by powdering, cutting thin sections of the drug or preparing a macerate.
- This method is used to identify organized drug by their known histological characters through Transverse section (T.S.) or Longitudinal Section (L.S.) or Radial Longitudinal Section (R.L.S.) or Tangential Longitudinal Section (T.L.S.).

LEAF CONSTANTS

- Palisade Ratio
- Stomatal Number
- Stomatal Index
- Vein- islet number
- Vein termination number
- Trichomes
- Calcium oxalate crystals
- Lycopodium spore method

Examples:

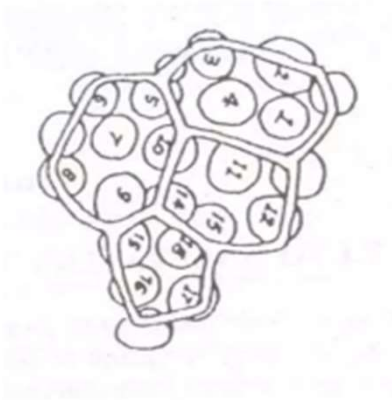
- ✓ Powder of clove stalk contain sclereids and calcium oxalate crystals, but cloves does not.
- ✓ Sclerenchyma absent in *Rauwolfia Serpentina* root but present in *Rauwofia micratha*, *R. densiflora*.
- ✓ Stone cell present in frangula bark and absent in cascara bark.
- ✓ Lignified Trichome and plasmodesmeta in nux-vomica.

1. Palisade ratio

- The average number of palisade cells beneath each epidermal cell is known as the palisade ratio.
- It is an important parameter for determination and characterization of leafy drugs.
- Determined using Camera lucida.

Eg;

- *Atropa belladonna*: 5-10
- *Adhatoda vasica*: 5.5-6.5
- *Cassia angustifolia*: 5.5-10
- *Digitalis lanata*: 2.5-6.5



2. Stomatal number

- It is defined as average number of stomata per square millimeter area of epidermis.

Eg;

- *Atropa belladonna*: UE: 7-10, LE: 77-115
- *Datura metel*: UE:147-160, LE: 200-209
- *Ocimum sanctum*: UE: 64-72, LE: 175-250

Stomata

- Stomata are minute pores or openings in the epidermis layer of the leaves, young stems, and some other parts of green plants which form an important constituent of their functioning.
- There are several types of stomata, distinguished by the forms and arrangement of the Surrounding cells.

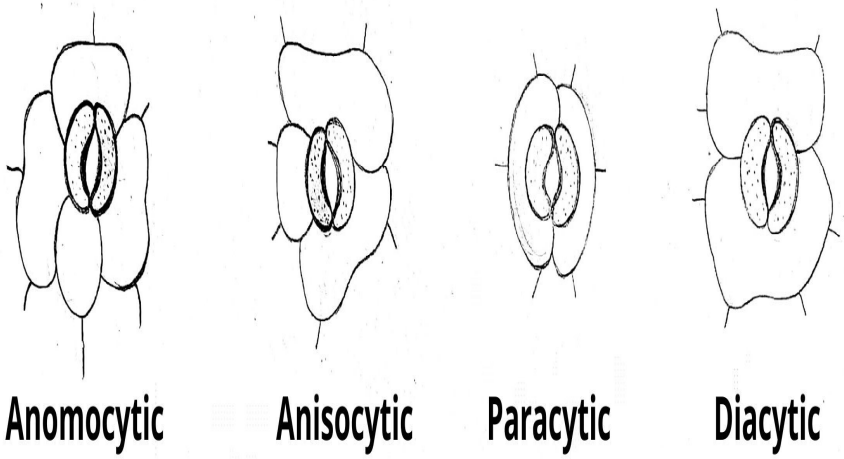
e.g.:

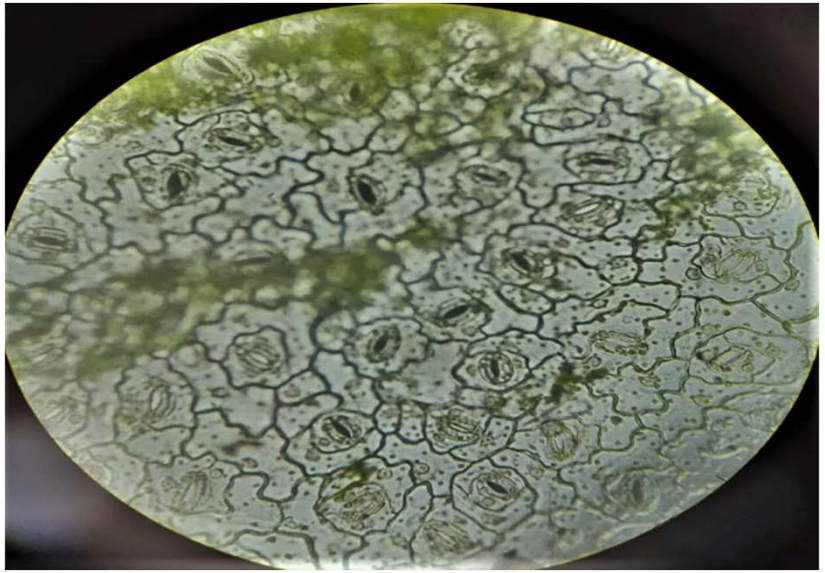
✓ **Anomocytic** (Ranunculaceous) irregular celled: Digitalis

✓ **Anisoytic** (Cruciferous) unequal – celled: Datura

✓ **Diacytic** (Caryophyllaceous) cross – celled: Mentha

✓ **Paracytic** (Rubiaceous) parallel celled: Senna





3. Stomatal index

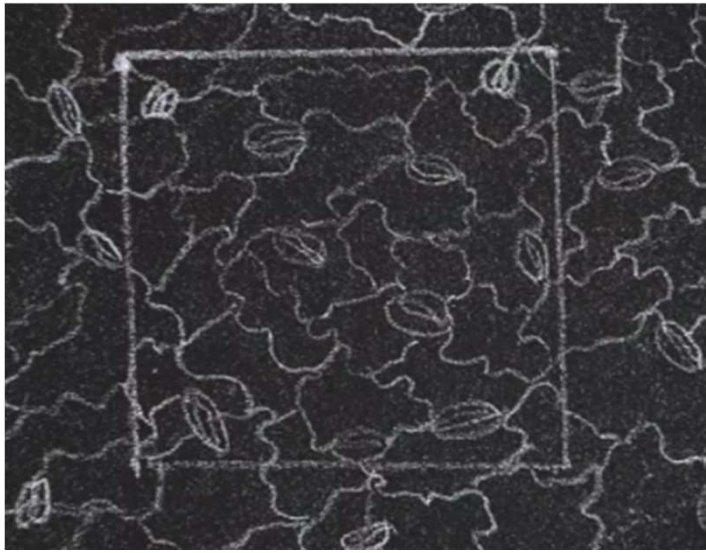
- Stomatal index (I) is the average number of stomata cells present per square millimeter of the epidermis of the leaf.
- It is the percentage proportion of the number of stomata to the total number of epidermal cells.
- Stomatal index can be calculated by using the following equation:

$$S.I = \frac{S}{E+S} * 100$$

- S= Number of stomata per unit area
- E= Number of epidermal cells in the same unit area.

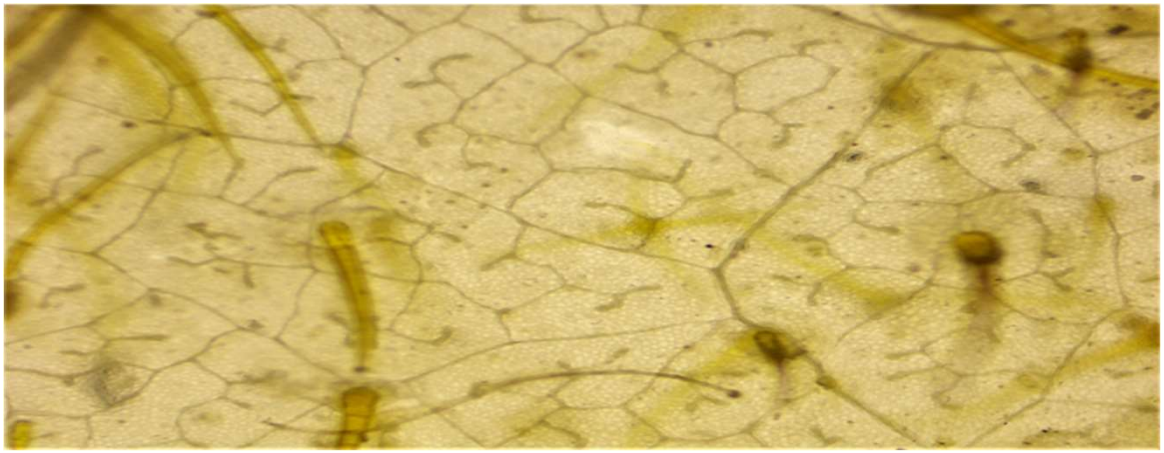
Examples:

- *Atropa belladonna*:-2.3-3.9 to 10.5 (Upper Surface), 20.2 to 23.0 (Lower Surface)
- *Digitalis Purpurea*- 2.7 to 4.0 (Upper Surface), 19.2- 25.2 (lower Surface).



4. Vein- islet number

- It is defined as average number of Vein Islet per square millimeter of the leaf surface midway between midrib and the margin.
- It is constant for a given species of the plant and is used as a character for the identification of the allied species.

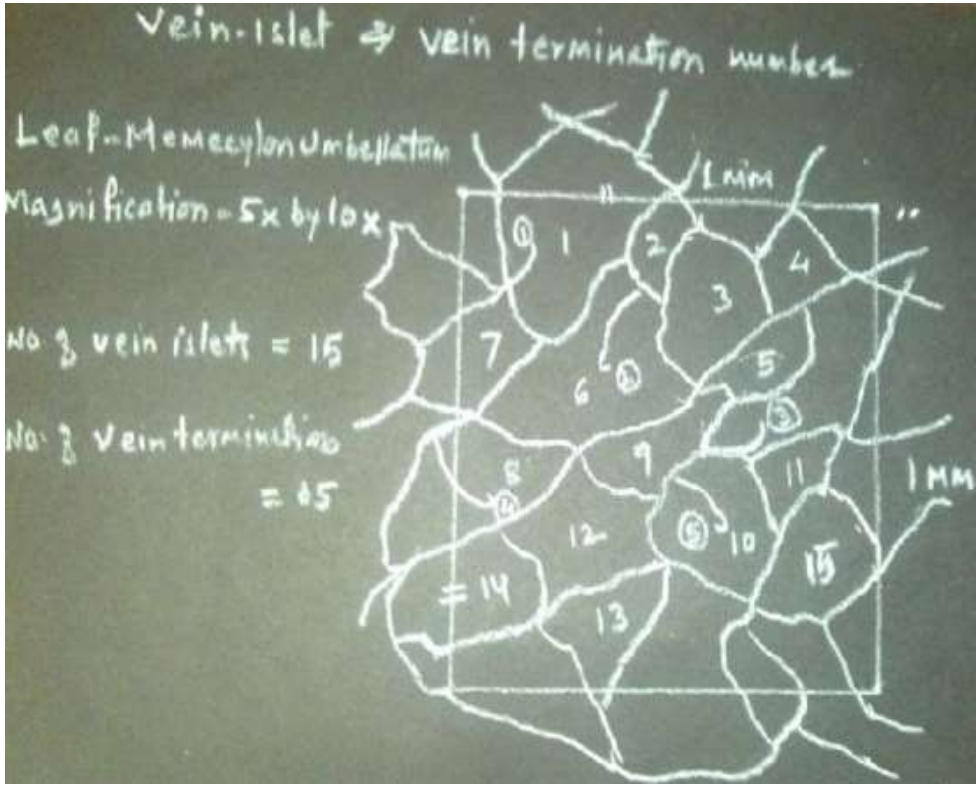


Examples:

NAME OF DRUG	Vein-islet Range
Andrograohis paniculata	9-12
Bacopa monniera	6-13
Cannabis sativa	18-24
Digitalis purpurea	2.5-3
Eucalpytus globules	8-13.5

5. Vein- termination number

- It is defined as average number of Vein terminations per square millimeter of the leaf surface midway between midrib and the margin.

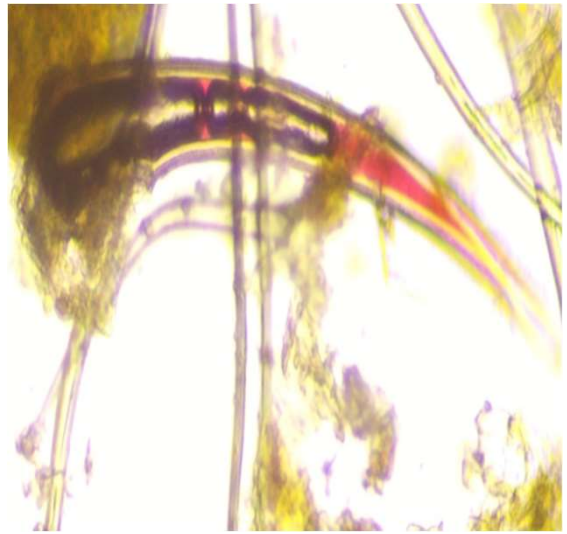


Examples:

- *Atropa belladonna* — 6.3-10.3
- *Atropa acuminate* — 1.4-3.5

6. Trichomes

- Trichomes are the tubular elongated or glandular outgrowth of the epidermal cells. Trichomes are also called as plant hairs. Trichomes consists of two parts root and body. Trichomes present in most of plant parts and are function less but some times perform secretory function.

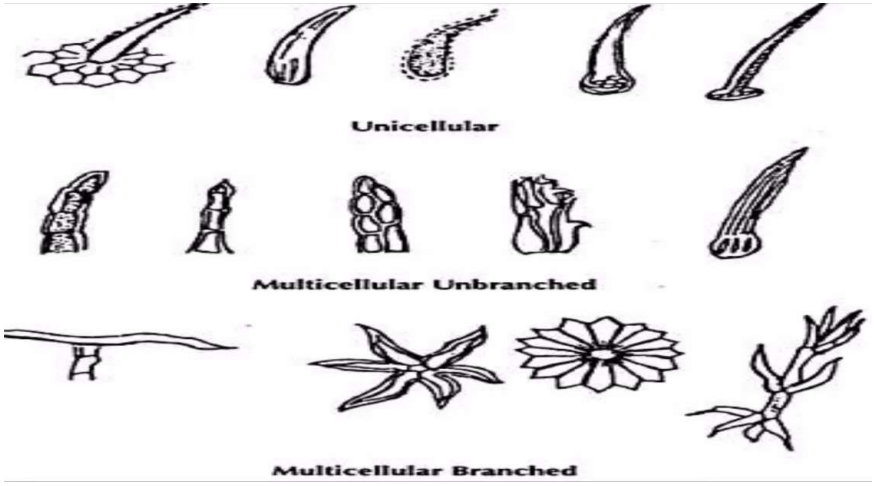


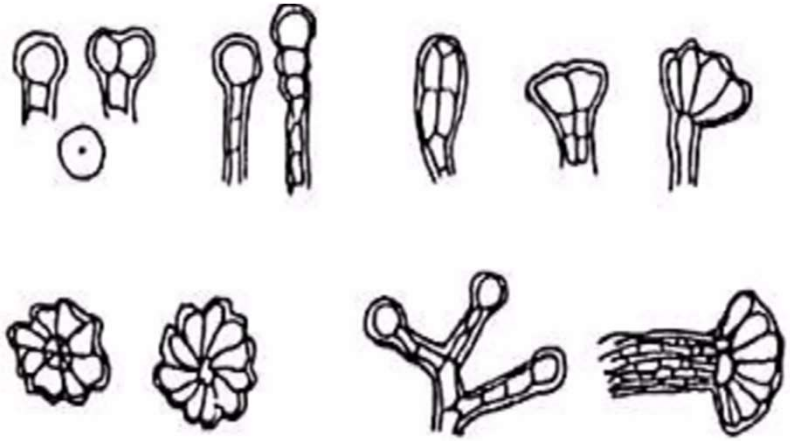
- Trichomes are divided and subdivided as follows;
- **Covering Trichomes:**
  - ✓ Unicellular Trichomes: Nux vomica, Cannabis
  - ✓ Uniseriate Multicellular Unbranched Trichomes: Datura
  - ✓ Biseriate Multicellular unbranched Trichomes: *Calendula officinalis*
  - ✓ Multiseriate Multicellular unbranched Trichomes: Male fern
  - ✓ Multicellular branched Trichomes: *Verbascum Thapsus*

- **Glandular Trichomes:**
  - ✓ Unicellular Glandular Trichomes: Vasaka
  - ✓ Multicellular Glandular Trichomes: *Digitalis purpurea*

Other types are;

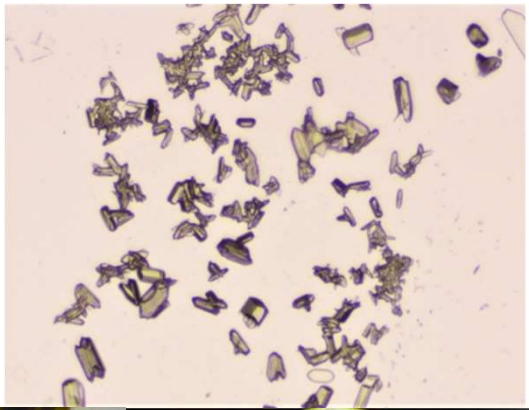
Glabrous, glabrate, hirsute, hispid, articulate, downy, pilose, puberulent, pubescent, strigillose, strigose, tomentellous, tomentose, villosulous, villous.





7. Calcium oxalate crystals

- The inorganic crystalline compounds by virtue of their specific shapes can be utilized for the identification of herbal drugs.
- Due to this reason they are called as **diagnostic characters of the plant**.



✓ **Cubical** (cube shape): Senna, Glycyrrhiza.

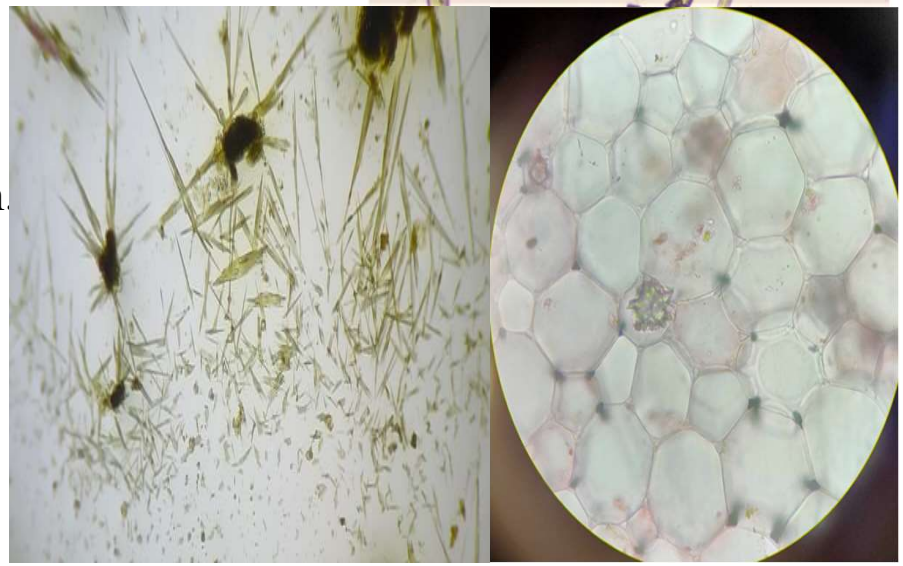
✓ **Rhombic** (diamond shape)

✓ **Tetragonal**: onion.

✓ **Acicular** (long slender, pointed, bundles): Squill, Cinnamon.

✓ **Rosettes** –clusters (aggregation of crystals): Clove, Arjuna.

✓ **Microsphenoidal** (minute in structures): Henbane.

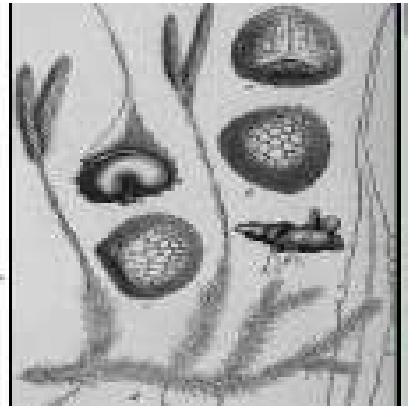
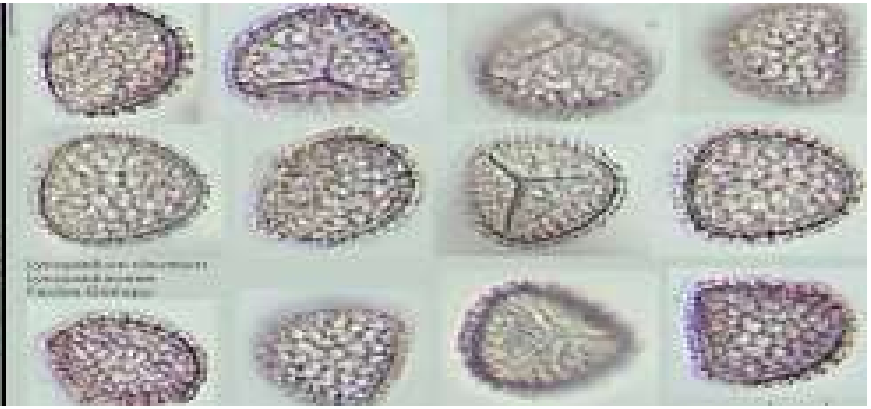


8. LYCOPODIUM SPORE METHOD

- Quantitative method
- It is used when especially chemical and other methods of evaluation of drugs fail to determine quality.
- Lycopodium spores are obtained from club moss, *Lycopodium clavatum* Linn., belonging to family Lycopodiaceae.
- The spores are yellow in colour, spheroidal, tetrahedral in shape with reticulate surface.
- They have uniform average diameter of 25 microns. One milligram contains average 94000 spores.
- They have uniform moisture content, hence the weight remains the same.
- This is the reason, why these spores are used to evaluate powdered drugs by comparison. The spores are also resistant to pressure.

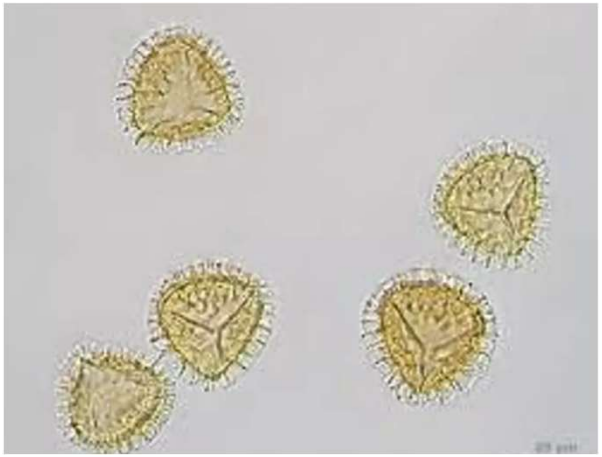
A powdered drug can be evaluated by comparison with lycopodium spores if it contains;

1. Well defined characteristic particles which can be counted (starch grains, pollen grains etc.)
2. Single layered cells or tissues, the area of which may be traced under suitable magnification and actual area calculated (eg: epidermal area of senna leaf).
3. The objects of uniform thickness, the length of which can be measured under suitable magnification and actual area calculated (eg: trichomes).

PROCEDURE

- Dry the powdered drug at 105^0 C and determine its steady weight at room temperature.
- Weigh accurately 200 mg of test material and 100 mg of lycopodium spore powder and mix with a glass rod or spatula uniformly.
- Make a thin smooth paste by adding 5 ml suspending medium (oil).
- Place a drop of above suspension on a glass slide, spread with a needle and put coverslip and observe under microscope in 10X.
- Count the characteristic particles of test and spores in the field.
- Take a count of 25 fields.
- Repeat the step in four times to get an average set of counts.



- Calculate the percentage purity using the formula;

$$\%\ purity = \frac{n * w * 94000 * 100}{s * m * p}$$

n= number of characteristic particles in 25 fields

s= number of lycopodium spores in the same 25 fields

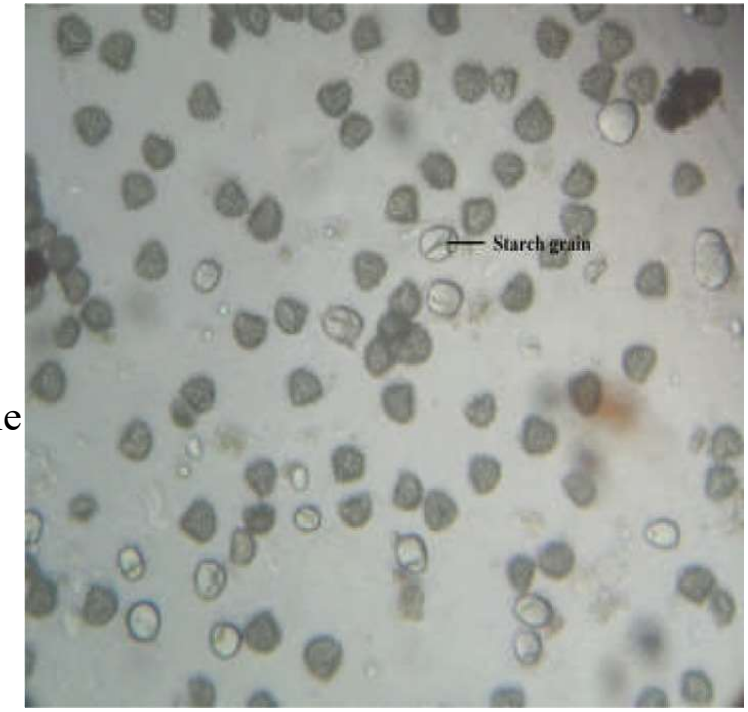
w= weight in mg of lycopodium spore powder taken

m= weight of the test sample taken in mg

p= standard value for number of characteristic particles per mg of pure sample

Eg: in case of starch grain it is 286000

94000= number of spores per mg of lycopodium spore powder



Applications

- Determine the size of the particles in powder such as fragments of leaves, isolated fibers.
- Lycopodium spore method can be used to evaluate powdered clove, ginger, cardamom, nutmeg, umbelliferous fruits etc.
- Determine the percentage of each powder particles in a mixture
- Determine the length of lignified ribs of trichomes in Nuxvomica
- Determine the Nuxvomica content of two veterinary medicines.

PHYSICAL EVALUATION

- For determining physical standards of a crude drug.
- In crude plant evaluation, physical methods are often used to determine the ***solubility, specific gravity, optical rotation, viscosity, refractive index, melting point, water content, degree of fibre elasticity***, and other physical characteristics of the herb material.

Foreign matter	**Loss on drying**	Refractive Index	Optical Rotation
Solubility	Specific Gravity	Moisture Content	Viscosity
Ash values	Ultraviolet Light	Melting Point	**Extractive values**

1. Solubility

- Drugs-specific behavior towards solvents is taken into consideration. This is useful for the examination of many oils, oleoresins, etc.

DRUG	SOLUBILITY
Castor oil	Soluble in 3 volumes of alcohol
Balsam of Peru	Soluble in chloral hydrate solution
Asafoetida	Soluble in carbon disulphide
Alkaloid bases	Soluble in chloroform
colophony	Soluble in light petroleum

2. Optical Rotation

- Many substances of biological origin, having a chiral centre, can rotate the plane of polarised light either to right (dextro rotatory)or to the left (laevo).
- The extent of rotation is expressed in degrees, plus(+) indicating rotation to the right and minus(-) indication rotation in the left. Such compound are optically active and hence called optical rotation.

Drugs	Angles of Optical Rotation
Caraway oil	+ 75° to +80°
Clove oil	0° to +6.0°
Honey	+3° to -15°

3. Refractive Index

- When a ray of light passes from one medium to another medium of different density, it is bent from its original path.
- Thus, the ratio of velocity of light in vaccum to its velocity in the substance is said to the Refractive index of the second medium.
- It is measured by means of refractometer. RI of a compound varies with the wavelength of the incident light, temperature and pressure.

Eg: **Arachis oil: 1.4678- 1.470**

Clove oil: 1.527-1.535

4. Specific Gravity

- It is also known as relative density.
- The ratio of the mass of a solid or liquid to the mass of an equal volume of distilled water at 4°C (39°F) or of a gas to an equal volume of air or hydrogen under prescribed conditions of temperature and pressure.
    - Eg: **cottonseed oil: 0.88–0.93**

Coconut oil: 0.925

Castor oil: 0.95

5. Viscosity

- Viscosity is the resistance of a fluid to flow.
- The viscosity of a liquid is constant at a given temperature and is an index of its composition.
- Hence, it is used as a means of standardizing liquid drugs.
    - Eg: **Liquid paraffin: not less than 64 centistokes**

      Pyroxylin: 1100-2450 centistokes

6. Melting Point

- The melting point of a solid is the temperature at which it changes state from solid to liquid.
- Plant constituents have very sharp and constant melting points.
- It is one of the parameters to judge the purity of crude drugs containing lipids as constituents.
- The purity of the crude drugs can be ascertained by determining their melting points in the range shown against each of them.

Eg: **Beeswax: 62–65°C**

Wool fat: 34–44°C

Agar: 85°C

7. Moisture content

- The moisture content of a drug will be responsible for the decomposition of crude drugs either producing chemical change or microbial growth.
- So the moisture content of a drug should be determined and controlled.
- The moisture content is determined by heating a drug at 105°C in an oven to a constant weight.

Eg: **Digitalis: 5% w/w**

Ergot: 8% w/w

8. Foreign Organic Matter

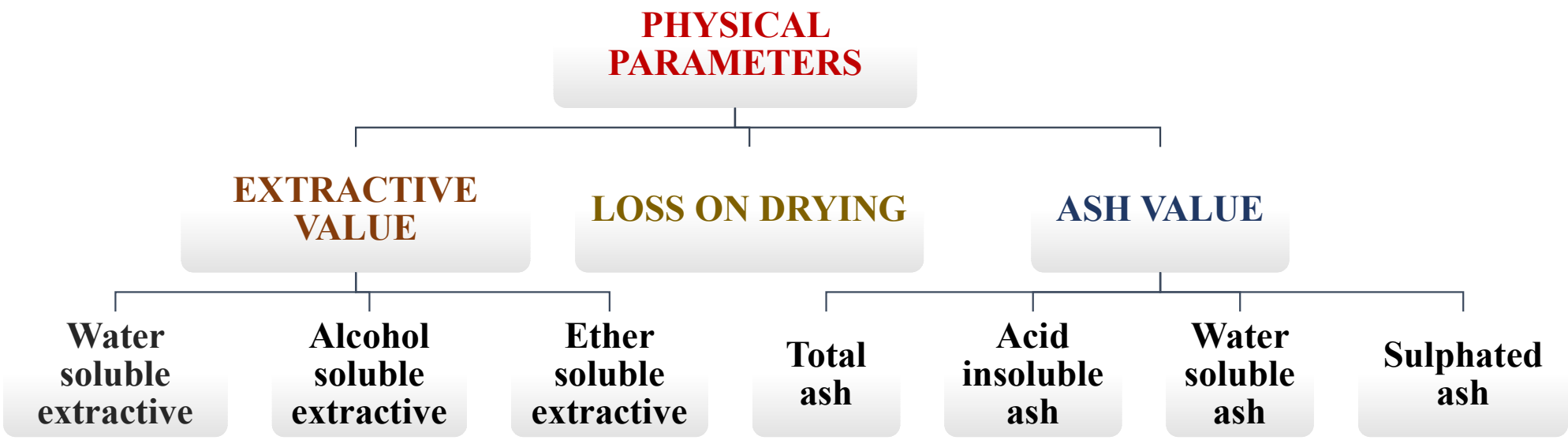
- The parts of the organ or organs other than those parts of drugs mentioned in the definition and description of the drug are known as foreign organic matters.
- They may be insect, molds, earthy material, animal excreta, etc.
- Each and every vegetable drug has its own limits.

Eg: **Garlic should not contain more than 2%**

Saffron should not contain more than 2%

Satavari should not contain more than 1%, etc.

IMPORTANT PHYSICAL PARAMETERS



ASH VALUE

- The residue left after incineration of the drug contains inorganic elements present in the plant is called as physiological ash.
- The determination of ash is useful for detecting low-grade products, exhausted drugs, and excess sandy or earthy matter.
- Ash values are helpful in determining the quality as well as purity of a crude drug, especially when the drug is present in powdered form.
- The object of ashing crude drugs is to remove the traces of organic matter which may interfere in an analytical determination.
- On incineration, the crude drugs normally produce ash which is usually consists of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium.
- The total ash of a crude drug reveals the care taken during its preparation. A higher limit of acid-insoluble ash is incorporated especially in cases where silica may be present or when the calcium oxalate content of the drug is very high.
- Ash value is a criterion to judge the identity or purity of the crude drug.
- The ash value varies within wide limits and is therefore an important parameter for the purpose of evaluation of crude drugs.

1. Total Ash value

- Organic and carbon matter present in the drug is converted to ash at temperature of 450°C or above.
- It mostly contains carbonates, Phosphate, Silicate and Silica.
- Total ash value can be used further study water soluble and acid insoluble ash.

Procedure

Take about 2 or 3 g, accurately weighed, of the ground drug in a tarred platinum or silica dish previously ignited and weighed.

Incinerate by gradually increasing the heat until free from carbon, cool, and weigh.

If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water

Calculate the percentage of ash with reference to the air dried drug.

2. Water-soluble ash value

- It is introduced by separating the **water soluble material** which is direct to yield water soluble ash.
- In this case most of water insoluble salt may contribute in total ash, or remove to find out the water soluble contents.

Procedure

The total ash obtained was boiled for 5 minutes with 25ml of water.

↓

Collected the insoluble matter on an ashless filter paper, washed with hot water, and ignited for 15 minutes at a temperature not exceeding 450°c.

↓

Subtracted the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash.

↓

Calculated the percentage of water-soluble ash with reference to the air-dried drug.

3. Acid insoluble ash value

- Total ash may be treated with **dilute hydrochloric acid** which removes any inorganic salts to yield many silica in the residue at acid-insoluble ash.

Procedure

The total ash obtained was boiled for 5 minutes with 25 ml of dilute hydrochloric acid.

Collected the insoluble matter on an ashless filter paper, washed with hot water, and ignited for 15 minutes at a temperature not exceeding 450°c.

Subtracted the weight of the insoluble matter from the weight of the ash; the difference in weight represents the acid insoluble ash.

Calculated the percentage of acid insoluble ash with reference to the air-dried drug.

4. Sulphated ash value

- The crude drug is incinerated at a temperature about 600°C with dilute sulphuric acid before ignition. This process converts all amides and carbonates to sulphate salt.

Procedure

Heated a platinum crucible to redness for 10 minutes, allowed to cool in a desiccator and weighed.

Put 3g of the powdered drug, accurately weighed, into the crucible, ignited gently at first, until the substance is thoroughly charred..

Cooled and moistened the residue with 1 ml sulphuric acid, heated gently until white fumes are no longer evolved and ignited at 800±25 ^{o} C until all black particles have disappeared.

The crucible was cooled, few drops of sulphuric acid was added and again heated.

The ignition was carried out as before, cooled and weighed to get a constant weight. Calculated the percentage of sulphated ash with reference to the air-dried drug

EXTRACTIVE VALUE

- Extractive values are used for evaluation of crude drugs when they cannot be estimated by any other method.
- Extractive values by different solvents are used to assess quality, purity and to detect adulteration due to exhausted and incorrectly processed drugs.
- Crude drugs contain a number of constituents and these have a selective solubility in different solvents.
- ***Alcohol, water and ether are used as solvents to prepare ethanol-soluble extractive, water soluble extractive (chloroform water), ether soluble extractive*** respectively.
- Extractive values indicate the presence of different constituents and TLC fingerprints can be developed for identification and semi-quantitative analysis from these extracts.

Procedure

5 g of accurately weight powdered air-dried drug was macerated with 100 ml of alcohol/water/ether in a closed flask for twenty-four hours, shaking frequently during six hours and allow standing for eighteen hours

It was then filtered rapidly, taking precautions against loss of solvent.

25 ml of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish and dried at 105°C to a constant weight and weighed.

The percentage of alcohol/water/ether soluble extractive was calculated with reference to the air-dried drug.

Significance

- ✓ Extractive values are also useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in particular solvents.
- ✓ Extractive values are primarily useful for the determination of exhausted or adulterated drugs and determines the quality as well as purity of the drug.
- ✓ Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage or formulating.

LOSS ON DRYING

- It is the loss of weight expressed as percentage w/w. It determines the amount of volatile matter of any kind (including water) that can be driven off under the conditions specified.

$$\text{Loss on drying (\%)} = \frac{\text{initial weight of sample - weight of sample after drying x 100}}{\text{Initial weight of sample}}$$

PROCEDURE

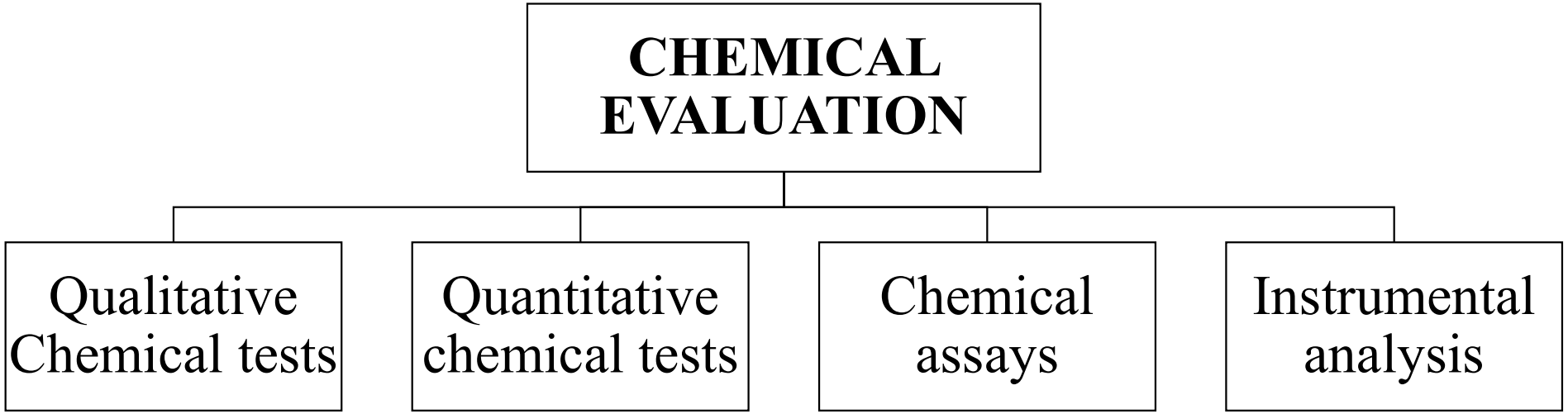
Accurately weighed about 1.5 g of powdered drug in a tarred porcelain dish and dried in an oven at 105°C to constant weight, cooled in desiccator and weighed. From the difference in weights, percentage loss on drying was calculated.

SIGNIFICANCE

Loss on drying is a widely used test method to determine the moisture content of a sample, although occasionally it may refer to the loss of any volatile matter from the sample.

CHEMICAL EVALUATION

- Evaluation by chemical means;



2. Quantitative chemical tests

a) Acid value
b) Saponification value
c) Ester value
d) Acetyl value
e) Peroxide value
f) Hydroxyl value

These tests are helpful in the evaluation of drugs like volatile oil (acetyl and ester value), balsams (acid, esters, saponification value), resins (acid value) etc.

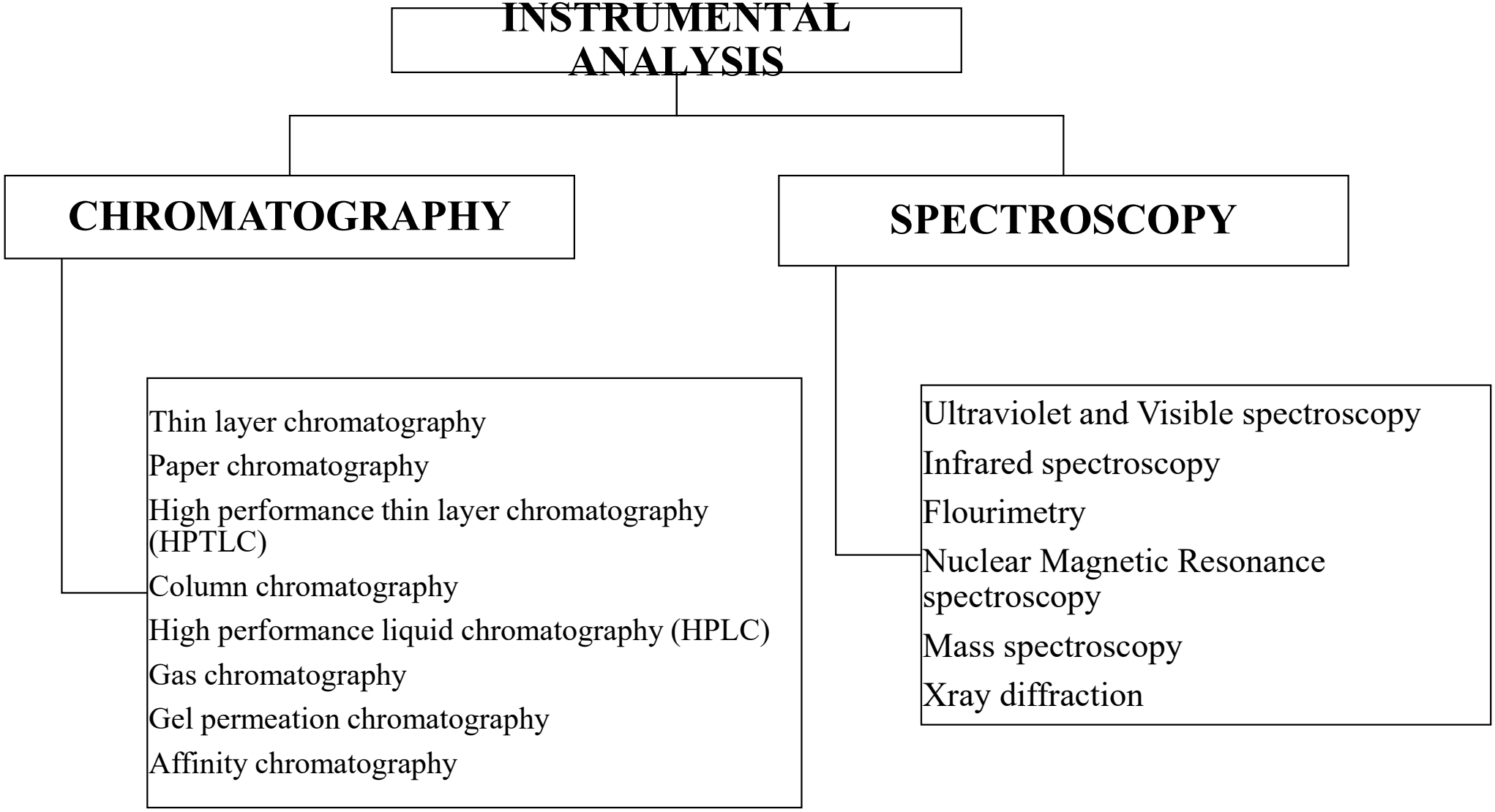
3. Chemical assays

- Assayed for a particular group of constituents.
- Commonly used techniques are **titrimetric and gravimetric methods**.

By titrimetric methods;

✓ Alkaloids can be estimated from alkaloidal drugs; eg: quinine from cinchona, reserpine from rauwolfia, atropine from belladonna
✓ Anthraquinone and cardiac glycosides.
✓ Estimation of carvone in dill oil, cineole in eucalyptus oil, aldehydes in lemon oil.

4. Instrumental analysis



BIOLOGICAL EVALUATION

- ✓ For the drugs cannot be evaluated using chemical & physical methods.
- ✓ Response produced on a test drug is compared with standard drug solution.
- ✓ Performed on living animals, isolated organ and tissue, animal preparations & micro-organisms.

Indication of Biological Evaluation:

- ✓ When the chemical nature of the drug is not known but is has an biological action.
- ✓ When chemical methods are not available.
- ✓ When the quantity of the drug is small, it cannot be evaluated chemically.
- ✓ Drugs which have different chemical composition but same biological activity. Example: Cardiac glycosides are evaluated by this method on cats, frogs or pigeons.

Examples;

- Antipyretic activity
- Anti-inflammatory activity
- Hypoglycaemic activity
- Antiulcer activity
- Analgesic activity
- Microbiological assays
- Anthelminthic activity

PHARMACOLOGICAL EVALUATION

- **Bitterness value**: Units equal to bitterness of std solution of quinine hydrochloride.
- **Heamolytic activity**: Ox blood comparison with std saponin.
- **Swelling index**: In water.
- **Foaming index**: Foam height produced by 14 gm material under std. conditions.

TOXICOLOGICAL EVALUATION

- **Pesticide residue**: estimation of chloride, phosphorus, lead, inorganic pesticides.
- **Arsenic & heavy metals**: limit tests
  - **Arsenic**: Stain produced in the $HgBr_2$ paper compared to std. stain.
  - **Heavy metals**: Atomic emission spectroscopy.
- **Microbial contamination**: maximum possible limits are determined. (E.coli, Salmonella, Enterobacteria)
- **Aflatoxins**: naturally occurring mycotoxins produced by Aspergillus species, determined by chromatographic methods using std aflatoxins B_1, B_2, G_1, G_2 mixtures.

11. Determination of bitterness value

Medicinal plant materials that have a strong bitter taste ("bitters") are employed therapeutically, mostly as appetizing agents. Their bitterness stimulates secretions in the gastrointestinal tract, especially of gastric juice.

Bitter substances can be determined chemically. However, since they are mostly composed of two or more constituents with various degrees of bitterness, it is first necessary to measure total bitterness by taste.

The bitter properties of plant material are determined by comparing the threshold bitter concentration of an extract of the materials with that of a dilute solution of quinine hydrochloride R. The bitterness value is expressed in units equivalent to the bitterness of a solution containing 1g of quinine hydrochloride R in 2000 ml.

Safe drinking-water should be used as a vehicle for the extraction of plant materials and for the mouth-wash after each tasting. Taste buds dull quickly if distilled water is used. The hardness of water rarely has any significant influence on bitterness.

Sensitivity to bitterness varies from person to person, and even for the same person it may be different at different times (because of fatigue, smoking, or after eating strongly flavoured food). Therefore, the same person should taste both the material to be tested and the quinine hydrochloride solution within a short space of time. The bitter sensation is not felt by the whole surface of the tongue, but is limited to the middle section of the upper surface of the tongue. A certain amount of training is required to perform this test. A person who does not appreciate a bitter sensation when tasting a solution of 0.058 mg of quinine hydrochloride R in 10 ml of water is not suitable to undertake this determination.

The preparation of the stock solution of each individual plant material (S_T) should be specified in the test procedure. In each test series, unless otherwise indicated, the determination should start with the lowest concentration in order to retain sufficient sensitivity of the taste buds.

Recommended procedure

Caution: This test should not be carried out until the identity of the plant material has been confirmed.

Preparation of solutions

Stock and diluted quinine hydrochloride solutions
Dissolve 0.100g of quinine hydrochloride R in sufficient safe drinking-water to produce 100 ml. Further dilute 5 ml of this solution to 500 ml with safe drinking-

Table 1
Determination of bitterness value: serial dilution for the initial test

	Tube no.								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
S_q (ml)	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8
Safe drinking-water (ml)	5.8	5.6	5.4	5.2	5.0	4.8	4.6	4.4	4.2
Quinine hydro-chloride in 10 ml of solution *(= c)* (mg)	0.042	0.044	0.046	0.048	0.050	0.052	0.054	0.056	0.058

S_q, stock solution of quinine hydrochloride.

Table 2
Determination of bitterness value: serial dilution for the second test

	Tube no.									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
S_T (ml)	1.00	2.00	3.00	4.00	5.00	6.00	7.00	8.00	9.00	10.0
Safe drinking-water (ml)	9.00	8.00	7.00	6.00	5.00	4.00	3.00	2.00	1.00	-

S_T, stock solution of the plant material being examined.

water. This stock solution of quinine hydrochloride (S_q) contains 0.01 mg/ml. Use nine test-tubes for the serial dilution for the initial test as indicated in Table 1.

Stock and diluted solutions of the plant material
Prepare the solution as specified in the test procedure for the given plant material (S_T). Use 10 test-tubes for the serial dilution for the test as indicated in Table 2.

Method
After rinsing the mouth with safe drinking-water, taste l0ml of the most dilute solution swirling it in the mouth mainly near the base of the tongue for 30 seconds. If the bitter sensation is no longer felt in the mouth after 30 seconds,

spit out the solution and wait for 1 minute to ascertain whether this is due to delayed sensitivity. Then rinse with safe drinking-water. The next highest concentration should not be tasted until at least 10 minutes have passed. The threshold bitter concentration is the lowest concentration at which a material continues to provoke a bitter sensation after 30 seconds. After the first series of tests, rinse the mouth thoroughly with safe drinking-water until no bitter sensation remains. Wait for at least 10 minutes before carrying out the second test.

In order to save time in the second test, it is advisable to ascertain first whether the solution in tube no. 5 (containing 5 ml of S_T in 10 ml) gives a bitter sensation. If so, find the threshold bitter concentration of the material by tasting the dilutions in tubes 1-4. If the solution in tube no. 5 does not give a bitter sensation, find the threshold bitter concentration by tasting the dilutions in tubes 6-10.

All solutions and the safe drinking-water for mouthwashing should be at 20-25 °C.

Calculate the bitterness value in units per g using the following formula:
2000 x c

$$\frac{2000 \text{ x } c}{\boldsymbol{a} \text{ x } \boldsymbol{b}}$$

where a = the concentration of the stock solution (S_T) (mg/ml),
b = the volume of S_T (in ml) in the tube with the threshold bitter concentration,
c = the quantity of quinine hydrochloride R (in mg) in the tube with the threshold bitter concentration.

12. Determination of haemolytic activity

Many medicinal plant materials, especially those derived from the families Caryophyllaceae, Araliaceae, Sapindaceae, Primulaceae, and Dioscoreaceae contain saponins. The most characteristic property of saponins is their ability to cause haemolysis: when added to a suspension of blood, saponins produce changes in erythrocyte membranes, causing haemoglobin to diffuse into the surrounding medium.

The haemolytic activity of plant materials, or a preparation containing saponins, is determined by comparison with that of a reference material, saponin R, which has a haemolytic activity of 1000 units per g. A suspension of erythrocytes is mixed with equal volumes of a serial dilution of the plant material extract. The lowest concentration to effect complete haemolysis is determined after allowing the mixtures to stand for a given period of time. A similar test is carried out simultaneously with saponin R.

Procedures proposed for the determination of the haemolytic activity of saponaceous medicinal plant material are all based on the same principle although the details may vary, e.g. the source of erythrocytes, methods for the preparation of the erythrocyte suspension and the plant material extract, the defined haemolytic activity of the reference material of saponin, and the experimental method. In order to obtain reliable results, it is essential to standardize the experimental conditions, and especially to determine the haemolytic activity by comparison with that of saponin R.

Recommended procedure

To prepare the erythrocyte suspension fill a glass-stoppered flask to one-tenth of its volume with sodium citrate (36.5 g/l) TS, swirling to ensure that the inside of the flask is thoroughly moistened. Introduce a sufficient volume of blood freshly collected from a healthy ox and shake immediately. Citrated blood prepared in this way can be stored for about 8 days at 2-4°C. Place 1 ml of citrated blood in a 50-ml volumetric flask with phosphate buffer pH 7.4 TS and carefully dilute to volume. This diluted blood suspension (2% solution) can be used as long as the supernatant fluid remains clear and colourless. It must be stored at a cool temperature.

To prepare the reference solution, transfer about 10 mg of saponin R, accurately weighed, to a volumetric flask and add sufficient phosphate buffer pH 7.4 TS to make 100ml. This solution should be freshly prepared.

The extract of plant material and dilutions should be prepared as specified in the test procedure for the plant material concerned, using phosphate buffer pH 7.4 TS.

Table 3
Determination of haemolytic activity: serial dilution for the preliminary test

	Tube no.			
	1	2	3	4
Plant material extract (ml)	0.10	0.20	0.50	1.00
Phosphate buffer pH 7.4 TS (ml)	0.90	0.80	0.50	-
Blood suspension (2%) (ml)	1.00	1.00	1.00	1.00

Preliminary test
Prepare a serial dilution of the plant material extract with phosphate buffer pH 7.4 TS and blood suspension (2%) using four test-tubes as shown in Table 3.

As soon as the tubes have been prepared, gently invert them to mix, avoiding the formation of foam. Shake again after a 30-minute interval and allow to stand for 6 hours at room temperature. Examine the tubes and record the dilution at which total haemolysis has occurred, indicated by a clear, red solution without any deposit of erythrocytes. Proceed as follows.

- If total haemolysis is observed only in tube no. 4, use the original plant material extract directly for the main test.
- If total haemolysis is observed in tubes 3 and 4, prepare a two-fold dilution of the original plant material extract with phosphate buffer pH 7.4 TS.
- If total haemolysis is observed in tubes 2, 3 and 4, prepare a five-fold dilution of the original plant material extract with phosphate buffer pH 7.4 TS.
- If, after 6 hours, all four tubes contain a clear, red solution, prepare a ten-fold dilution of the original plant material extract with phosphate buffer pH 7.4 TS and carry out the preliminary test again as described above.
- If total haemolysis is not observed in any of the tubes, repeat the preliminary test using a more concentrated plant material extract.

Main test
Prepare a serial dilution of the plant material extract, undiluted or diluted as deter-mined by the preliminary test, with phosphate buffer pH 7.4 TS and blood suspension (2%) using 13 test-tubes as shown in Table 4.

Carry out the dilutions and evaluations as in the preliminary test but observe the results after 24 hours. Calculate the amount of medicinal plant material in g, or of the preparation in g or ml, that produces total haemolysis.

To eliminate the effect of individual variations in resistance of the erythrocyte suspension to saponin solutions, prepare a series of dilutions of saponin R in the same manner as described above for the plant material extract. Calculate the quantity of saponin R in g that produces total haemolysis.

Table 4
Determination of haemolytic activity: serial dilution for the main test

	Tube no.												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Plant material extract (diluted if necessary) (ml)	0.40	0.45	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95	1.00
Phosphate buffer pH 7.4 TS (ml)	0.60	0.55	0.50	0.45	0.40	0.35	0.30	0.25	0.20	0.15	0.10	0.05	
Blood suspension (2%) (ml)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Calculate the haemolytic activity of the medicinal plant material using the following formula:

$$1000 \times \frac{a}{b}$$

where 1000 = the defined haemolytic activity of saponin R in relation to ox blood,
a = quantity of saponin R that produces total haemolysis (g),
b = quantity of plant material that produces total haemolysis (g).

13. Determination of tannins

Tannins (or tanning substances) are substances capable of turning animal hides into leather by binding proteins to form water-insoluble substances that are resistant to proteolytic enzymes. This process, when applied to living tissue, is known as an "astringent" action and is the reason for the therapeutic application of tannins.

Chemically, tannins are complex substances; they usually occur as mixtures of polyphenols that are difficult to separate and crystallize. They are easily oxidized and polymerized in solution; if this happens they lose much of their astringent effect and are therefore of little therapeutic value.

Recommended procedure

To prepare the plant material extract, introduce the quantity specified in the test procedure for the plant material concerned, previously powdered to a known fineness and weighed accurately, into a conical flask. Add 150ml of water and heat over a boiling water-bath for 30 minutes. Cool, transfer the mixture to a 250-ml volumetric flask and dilute to volume with water. Allow the solid material to settle and filter the liquid through a filter-paper, diameter 12cm, discarding the first 50ml of the filtrate.

To determine the total amount of material that is extractable into water, evaporate 50.0ml of the plant material extract to dryness, dry the residue in an oven at 105°C for 4 hours and weigh (T_1).

To determine the amount of plant material not bound to hide powder that is extractable into water, take 80.0ml of the plant material extract, add 6.0g of hide powder R and shake well for 60 minutes. Filter and evaporate 50.0 ml of the clear filtrate to dryness. Dry the residue in an oven at 105°C and weigh (T_2).

To determine the solubility of hide powder, take 6.0g of hide powder R, add 80.0 ml of water and shake well for 60 minutes. Filter and evaporate 50.0 ml of the clear filtrate to dryness. Dry the residue in an oven at 105°C and weigh (T_0). Calculate the quantity of tannins as a percentage using the following formula:

$$\frac{[T_1 - (T_2 - T_0)]x500}{w}$$

where w = the weight of the plant material in grams.

14. Determination of swelling index

Many medicinal plant materials are of specific therapeutic or pharmaceutical utility because of their swelling properties, especially gums and those containing an appreciable amount of mucilage, pectin or hemicellulose.

The swelling index is the volume in ml taken up by the swelling of 1 g of plant material under specified conditions. Its determination is based on the addition of water or a swelling agent as specified in the test procedure for each individual plant material (either whole, cut or pulverized). Using a glass-stoppered measuring cylinder, the material is shaken repeatedly for 1 hour and then allowed to stand for a required period of time. The volume of the mixture (in ml) is then read.

The mixing of whole plant material with the swelling agent is easy to achieve, but cut or pulverized material requires vigorous shaking at specified intervals to ensure even distribution of the material in the swelling agent.

Recommended procedure

Carry out simultaneously no fewer than three determinations for any given material. Introduce the specified quantity of the plant material concerned, previously reduced to the required fineness and accurately weighed, into a 25-ml glass-stoppered measuring cylinder. The internal diameter of the cylinder should be about 16mm, the length of the graduated portion about 125 mm, marked in 0.2-ml divisions from 0 to 25 ml in an upwards direction. Unless otherwise indicated in the test procedure, add 25 ml of water and shake the mixture thoroughly every 10 minutes for 1 hour. Allow to stand for 3 hours at room temperature, or as specified. Measure the volume in ml occupied by the plant material, including any sticky mucilage. Calculate the mean value of the individual determinations, related to 1 g of plant material.

15. Determination of foaming index

Many medicinal plant materials contain saponins that can cause a persistent foam when an aqueous decoction is shaken. The foaming ability of an aqueous decoction of plant materials and their extracts is measured in terms of a foaming index.

Recommended procedure

Reduce about 1 g of the plant material to a coarse powder (sieve size no. 1250), weigh accurately and transfer to a 500-ml conical flask containing 100ml of boiling water. Maintain at moderate boiling for 30 minutes. Cool and filter into a 100-ml volumetric flask and add sufficient water through the filter to dilute to volume.

Pour the decoction into 10 stoppered test-tubes (height 16cm, diameter 16mm) in successive portions of 1 ml, 2 ml, 3 ml, etc. up to 10 ml, and adjust the volume of the liquid in each tube with water to 10ml. Stopper the tubes and shake them in a lengthwise motion for 15 seconds, two shakes per second. Allow to stand for 15 minutes and measure the height of the foam. The results are assessed as follows.

- If the height of the foam in every tube is less than 1 cm, the foaming index is less than 100.
- If a height of foam of 1 cm is measured in any tube, the volume of the plant material decoction in this tube (a) is used to determine the index. If this tube is the first or second tube in a series, prepare an intermediate dilution in a similar manner to obtain a more precise result.
- If the height of the foam is more than 1 cm in every tube, the foaming index is over 1000. In this case repeat the determination using a new series of dilutions of the decoction in order to obtain a result.

Calculate the foaming index using the following formula:

$$\frac{1000}{a}$$

where a = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1 cm is observed.

16. Determination of pesticide residues

Medicinal plant materials are liable to contain pesticide residues which accumulate from agricultural practices, such as spraying, treatment of soils during cultivation, and administration of fumigants during storage. It is therefore recommended that every country producing medicinal plant materials (naturally grown or cultivated) should have at least one control laboratory capable of performing the determination of pesticides in accordance with the procedure outlined below.

Since many medicinal preparations of plant origin are taken over long periods of time, limits for pesticide residues should be established following the recommendations of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) which have already been established for food and animal feed (9). These recommendations include the analytical methodology for the assessment of specific pesticide residues.

Classification of pesticides
Different classifications of pesticides exist (*10, 11*). A classification based on the chemical composition or structure of the pesticide is most useful for analytical chemists, for example:

- chlorinated hydrocarbons and related pesticides: aldrin, HCH (hexachlorocy-clohexane or benzene hexachloride, BHC), hexachlorobenzene (HCB), chlordane, DDT (clofenotane), dieldrin, endrin, heptachlor, lindane, methoxychlor, camphechlor (toxaphene);
- chlorinated phenoxyalkanoic acid herbicides: 2,4-D; 2,4,5-T;
- organophosphorus pesticides: carbophenothion (carbofenotion), coumaphos (coumafos), demeton, dichlorvos, dimethoate, ethion, fenchlorphos (fenclofos), malathion, methyl parathion, parathion;
- carbamate insecticides: carbaryl (carbaril);
- dithiocarbamate fungicides: ferbam, maneb, nabam, thiram, zineb, ziram;
- inorganic pesticides: aluminium phosphide, calcium arsenate, lead arsenate;
- miscellaneous: bromopropylate, chloropicrin, ethylene dibromide, ethylene oxide, methyl bromide;
- pesticides of plant origin: tobacco leaf and nicotine; pyrethrum flower, pyrethrum extract and pyrethroids; derris root and rotenoids.

Only the chlorinated hydrocarbons and related pesticides (e.g. aldrin, chlordane, DDT, dieldrin, HCH) and a few organophosphorus pesticides (e.g. carbophenothion) have a long residual action. Most other pesticides have very short residual actions. Therefore it is suggested that, where the length of exposure to pesticides is unknown, the medicinal plant material should be tested for the presence of organically bound chlorine and phosphorus, or the content of these two substances should be determined. Alternative classifications of pesticides may be based on their intended use, for example:

- insecticides;
- fungicides and nematocides;

- herbicides;
- other pesticides (e.g. acaricides, molluscicides, rodenticides);
- fumigants (e.g. ethylene oxide, ethylene chlorohydrin, methyl bromide).

Methods for the determination of pesticide residues

Chromatography (mostly column and gas) is recommended as the principal method for the determination of pesticide residues. Samples are extracted by a standard procedure, impurities are removed by partition and/or adsorption, and the presence of a moderately broad spectrum of pesticides is measured in a single determination. However, these techniques are not universally applicable. Some pesticides are satisfactorily carried through the extraction and clean-up procedures, others are recovered with a poor yield, and some are lost entirely. In chromatography, the separations may not always be complete, pesticides may decompose or metabolize, and many of the metabolic products are still unknown. Consequently, as a result of limitations in the analytical technique and incomplete knowledge of pesticide interaction with the environment, it is not yet possible to apply an integrated set of methods that will be satisfactory in all situations.

It is therefore desirable to test plant materials of unknown history for broad groups of compounds rather than for individual pesticides. A variety of methods meet these requirements. Chlorinated hydrocarbons and other pesticides containing chlorine in the molecule, for example, can be detected by the measurement of total organic chlorine; insecticides containing phosphate can be measured by analysis for total organic phosphorus, while pesticides containing arsenic and lead can be detected by measurement of total arsenic or total lead, respectively. Similarly, the measurement of total bound carbon disulfide in a sample will provide information on whether residues of the dithiocarbamate family of fungicides are present.

If the pesticide to which the plant material has been exposed is known or can be identified by suitable means, an established method for the determination of that particular pesticide residue should be employed.

General aspects of analytical methodology

The samples should be tested as quickly as possible after collection, before any physical or chemical changes occur. If prolonged storage is envisaged, the samples should preferably be stored in air-tight containers under refrigeration.

Light can cause degradation of many pesticides, and it is therefore advisable to protect the samples and any extracts or solutions from undue exposure.

The type of container or wrapping material used should not interfere with the sample or affect the analytical results.

Solvents and reagents used in the analytical method should be free from substances that may interfere with the reaction, alter the results or provoke degradation of the pesticide residue in the sample. It is usually necessary to employ specially purified solvents or to distil them freshly in an all-glass

apparatus. Blank determinations with the solvents should be carried out, concentrating and testing them as specified in the test procedure for the plant material concerned.

The simplest and quickest procedure should be used to separate unwanted material from the sample (clean-up procedure) in order to save time when many samples have to be tested.

The process of concentrating solutions should be undertaken with great care, especially during the evaporation of the last traces of solvent, in order to avoid losses of pesticide residues. For this reason, it is often not advisable to remove the last traces of solvent. Agents, such as mineral oil or other oils of low volatility, that may help to preserve the solution could be added to retard the loss of the relatively volatile pesticides, especially when the last traces of solvent are being evaporated. However, these agents, while satisfactory in colorimetric procedures, are usually not desirable in gas chromatographic methods. It may be necessary to evaporate heat-labile compounds using a rotary vacuum apparatus.

Maximum limit of pesticide residues for medicinal plant materials

The toxicological evaluation of pesticide residues in medicinal plant materials should be based on the likely intake of the material by patients. In general, the intake of residues from medicinal plant materials should account for no more than 1% of total intake from all sources, including food and drinking-water. Certain plant materials may contain extremely high levels of pesticide residues, but the levels remaining after extraction are usually much lower, because of the low solubility in water or ethanol. It is therefore important to determine the actual quantity of residues consumed in the final dosage form.

Where the nature of the pesticide to which the plant material has been exposed is unknown, it is sufficient to determine the content of total chlorine and to base the calculation on the acceptable residue level (ARL) of the most toxic chlorine-containing pesticide (e.g. aldrin or dieldrin).

An ARL (in mg of pesticide per kg of plant material) can be calculated on the basis of the maximum acceptable daily intake of the pesticide for humans (ADI), as, recommended by FAO and WHO, and the mean daily intake (MDI) of the medicinal plant material.

Some countries have established national requirements for residue limits in plant materials. Where such requirements do not exist, the following formula may be used:

$$\text{ARL} = \frac{\text{ADI x E x 60}}{\text{MDI x100}}$$

where ADI = maximum acceptable daily intake of pesticide (mg/kg of body weight);

E = extraction factor, which determines the transition rate of the pesticide from the plant material into the dosage form;

MDI = mean daily intake of medicinal plant product.

The 60 in the numerator represents mean adult body weight, while the denominator incorporates a consumption factor of 100 reflecting the fact that no more than 1% of the total pesticide residue consumed should be derived from medicinal plant material.

This formula is based on the acceptable daily intake (ADI) determined by FAO and WHO.

Determination of total chlorine and phosphorus
Most pesticides contain organically bound chlorine or phosphorus.

Recommended procedure

Preparation of samples
Reduce the plant material to a fine powder, and extract with a mixture of water and acetonitrile R. Most pesticides are soluble in this mixture, while most cellular constituents (e.g. cellulose, proteins, amino acids, starch, fats and related compounds) are sparingly soluble and are thus removed. A number of polar and moderately polar compounds may also be dissolved; it is therefore necessary to transfer the pesticides to light petroleum R. For pesticides containing chlorine, further purification is seldom required, but for those containing phosphorus, further purification by column chromatography may be necessary, eluting with mixtures of light petroleum R and ether R.

Preparation of the column
Use Florisil R grade 60/100 PR (or equivalent), activated at 650°C, as the support. If this material is obtained in bulk, transfer it immediately after opening to a 500-ml glass jar or bottle with a glass stopper or foil-lined, screw-top lid. Store in the dark. Before use, heat at not less than 130°C, cool in a desiccator to room temperature and heat once again to 130°C after 2 days.

Prepare a Florisil column (external diameter, 22 mm) which contains, after settling, 10 cm of activated Florisil topped with about 1 cm of anhydrous sodium sulfate R. Pre-wet the column with 40-50 ml of light petroleum R. Place a graduated flask under the column to receive the eluate.

Method
Grind the material to pass through a sieve no. 710 or 840 and mix thoroughly. Place 20-50 g of the ground sample into a blender, add 350 ml of acetonitrile R with a water content of 35% (to 350 ml of water add sufficient acetonitrile R to produce 1000 ml). Blend for 5 minutes at a high speed. Filter under vacuum

through an appropriate funnel, diameter 12 cm, fitted with filter-paper, into a 500-ml suction flask.

Transfer the filtrate to a 250-ml measuring cylinder and record the volume. Transfer the measured filtrate to a 1-litre separating funnel and carefully add 100ml of light petroleum R. Shake vigorously for 1-2 minutes, add 10 ml of sodium chloride (400 g/l) TS and 600 ml of water. Hold the separating funnel in a horizontal position and mix vigorously for 30-45 seconds. Allow to separate, discard the aqueous layer and gently wash the solvent layer with two 100-ml portions of water. Discard the washings, transfer the solvent layer to a 100-ml glass-stoppered cylinder, and record the volume. Add about 15 g of anhydrous sodium sulfate R and shake vigorously. The extract must not remain in contact with this reagent for longer than 1 hour. Transfer the extract directly to a Florisil column; if necessary, reduce the volume first to 5-10 ml. Allow it to pass through the column at a rate of not more than 5 ml per minute. Carefully rinse the cylinder with two portions, each of 5 ml, of light petroleum R, transfer them to the column, rinse with further small portions of light petroleum R if necessary, and then elute at the same rate with 200 ml of ether/light petroleum TS1. Change the receiver and elute with 200 ml of ether/light petroleum TS2. Again change the receiver and elute with 200 ml of ether/light petroleum TS3. Evaporate each eluate to a suitable volume, as required, for further testing.

- The first eluate contains chlorinated pesticides (aldrin, DDE, TDE (DDD), o,p'-and p,p'-DDT, HCH, heptachlor, heptachlor epoxide, lindane, methoxychlor), polychlorinated biphenyls (PCB), and phosphated pesticides (carbophenothion, ethion, and fenchlorphos).
- The second eluate contains chlorinated pesticides (dieldrin and endrin) and phosphated pesticides (methyl parathion and parathion).
- The third eluate contains phosphated pesticide (malathion).

Combustion of the organic matter

Combustion of the organic matter in oxygen is the preparatory step for the determination of chlorine and phosphorus. The pesticide is extracted from the sample and purified, if necessary. The extract is concentrated, evaporated to dryness, transferred to a sample holder, and burned in a suitable conical flask flushed with oxygen. The gases produced during combustion are then absorbed in a suitable solution. The absorbed chlorine is determined as chloride and the absorbed phosphorus as orthophosphate, both colorimetrically.

Apparatus

The combustion is carried out in a 1-litre conical flask made of borosilicate glass, into the stopper of which is fused one end of a piece of platinum wire about 1 mm in diameter. To the free end of the wire is attached a piece of platinum gauze measuring about 1.5 x 2 cm to provide a means of holding the sample clear of the absorbing liquid during combustion.

Sample holder for chlorine-containing residues. For a small quantity of solid material, use a sample holder made from a piece of halide-free filter-paper about 5 cm long and 3 cm wide; for a small volume of liquid, preferably use a sample

holder in the form of a cone made from cellulose acetate film. Prepare the cone as follows: wearing cloth gloves and using a suitable cardboard template cut the film in a circle of 4 cm radius.

Manually pin the two edges together to form a cone. Seal the joined edges using heat to form a seam about 5 mm wide. Immerse the seam in acetone R to about one half of its width for 10 seconds. Remove and dry it immediately in a stream of hot air. Using forceps, wash the cone by dipping in a 1-litre beaker containing warm sodium hydroxide (~240 g/l) TS for 10 seconds at a temperature of about 60°C. Rinse the cone thoroughly with water and allow to drain dry on a piece of aluminium foil. Place each cone in a clean funnel (diameter 65 mm).

Sample holder for phosphorus-containing residues. Use a piece of halide-free filter-paper about 4 cm square as the sample holder.

Combustion of chlorine-containing residues
Transfer an aliquot of the extract as prepared above onto the sample holder which is placed in a funnel using a solvent that will not dissolve the sample holder. Allow the solvent to evaporate. Wearing rubber gloves, remove the sample holder and its dry contents from the funnel, and fold it over and up to form a small packet, about 1 cm^2 in area, and secure it in the centre of the platinum gauze. Insert a narrow strip of filter-paper, about 1 x 3 cm, as a fuse into the top of the holder, between the folds of the packet. Add 30 ml of water to the combustion flask. Moisten the neck of the flask with water. Fill the flask thoroughly with oxygen by means of a tube with its end just above the liquid. Ignite the free end of the paper strip and immediately insert the stopper. Hold the stopper firmly in place. When vigorous burning has begun, tilt the flask to prevent incompletely burned material from falling into the liquid. Immediately after combustion is completed, shake the flask vigorously for 10 minutes to dissolve the combustion products. Place a small quantity of water around the rim of the flask, and carefully withdraw the stopper. Rinse the stopper, platinum wire, platinum gauze and sides of the flask with water. Transfer the liquid and liquids used for rinsing to a 50-ml volumetric flask and dilute to volume with water.

Combustion of phosphorus-containing residues
Dip the sample holder made from filter-paper into methanolic sodium hydroxide TS, then suspend it in a current of heated air. Immediately transfer about 0.2 ml of an aliquot of the extract as prepared above to the sample holder with the aid of 0.2-ml portions of chloroform R using a micropipette. Allow the solvent to evaporate from the paper, fold it to form a small packet about 1 cm^2 in area and place it in the centre of the platinum gauze. Insert a strip of filter-paper, about 1 x 3 cm, as a fuse into the top of the holder, between the folds of the packet. Add 10 ml of sulfuric acid (~37 g/l) TS to the combustion flask and continue with the combustion as described above. Transfer the solution and the liquid used for rinsing to a 25-ml volumetric flask and dilute to volume with water.

Determination of chlorides

Apparatus
The determination is made with a spectrophotometer capable of measuring absorbance at 460 nm using absorption cells with path-lengths of 2 cm and 10 cm.

Method
Place 15 ml of the solution obtained after combustion in a 50-ml conical flask together with 1 ml of ferric ammonium sulfate (0.25 mol/l) VS and 3 ml of mercuric thiocyanate TS. Swirl the contents of the flask and allow to stand for 10 minutes. Transfer a portion of the solution to a 2-cm cell and measure the absorbance at 460 run using water in the reference cell. The reading should be made promptly to minimize absorption of chloride from the air.

Prepare a standard solution of sodium chloride R containing 5 µg of chloride per ml. Transfer aliquots of this solution (0 ml, 2 ml, 4 ml, 6 ml, 8 ml, and 10 ml) into a series of 50-ml conical flasks and dilute to 15 ml with water. Develop the colour and measure the absorbances as described above. Plot the absorbances against the chloride content of the dilutions in µg per ml and interpolate the chloride content of the solutions of the material tested.

Determination of phosphates
The phosphomolybdate method is based on the reaction of phosphate ions with ammonium molybdate to form a molybdophosphate complex, which is subsequently reduced to form a strongly blue-coloured molybdenum complex. The intensity of the blue colour is measured spectrophotometrically. This method is applicable for the determination of any phosphates that have undergone a prior separation procedure.

Naturally occurring phosphates are present in most samples, and are often not removed during the clean-up procedure. In order to obtain background values, therefore, it is necessary to proceed with the determination for all samples, even those with no phosphate-containing pesticides. These background values should be subtracted from the results obtained on testing pesticide residues. Extracts of most uncontaminated materials contain about 0.05-0.1 mg/kg of phosphorus. Therefore, no contamination with organophosphate pesticides can be assumed for results in this range.

Apparatus
The determination is made with a spectrophotometer capable of measuring absorbance at 820 nm using an absorption cell with a path-length of 1 cm.

Method
Place 7 ml of the solution obtained after combustion in a calibrated 10-ml test-tube. Add 2.2 ml of sulfuric acid (300 g/l) TS and mix the solution well. Add 0.4 ml of ammonium molybdate (40 g/l) TS and swirl the mixture. Then add 0.4 ml of aminonaphtholsulfonic acid TS and swirl again. Heat the solution to 100°C for

12 minutes (±2 minutes), cool, and transfer a portion to a 1-cm cell. Measure the absorbance at 820 nm using water in the reference cell.

Prepare standard dilutions with a known content of phosphate and measure the absorbance as described above. Plot the absorbances against the phosphate content of the dilutions in µg per ml and interpolate the phosphate content of the solutions of the material tested.

Qualitative and quantitative determination of organochlorine pesticides

Recommended procedure

Preparation of sample
Place 20 g of powdered plant material (sieve no. 180), accurately weighed, in a 500-ml beaker (tall form), mix with 98 ml of water and allow to macerate for at least 30 minutes. Add 200 ml of acetone R; the resulting volume of extraction solvent will be 295 ml. Extract for 5 minutes, while cooling and using a high-speed mixer. Filter the homogenized mixture through a porcelain filter (Büchner funnel, diameter 70mm) fitted with a filter-paper, using a slight vacuum, into a 250-ml graduated cylinder, allowing the process to last no longer than 1 minute, and then measure the volume *(V)* of the filtrate in ml.

Method
Transfer the filtrate prepared as above to a 500-ml separating funnel. Add a quantity of sodium chloride R equivalent in grams to one-tenth of the volume of the filtrate, then add 100 ml of dichloromethane R. Shake vigorously for 5 minutes, allow the phases to separate and discard the lower (aqueous) layer. Dry the acetone-dichloromethane phase, transfer it to a 500-ml conical flask, add 25 g of anhydrous sodium sulfate R and swirl occasionally. Next, filter the solution into a 500-ml flask with a ground-glass stopper using a glass funnel (diameter 100 mm) containing purified glass-wool and anhydrous sodium sulfate R. Rinse the separating funnel, the conical flask and the glass funnel twice with 10 ml of ethyl acetate R. Add 5 ml of 2,2,4-trimethylpentane R, and concentrate the crude extract to about 2 ml in a rotary vacuum evaporator in a water-bath at 30-40°C. Expel the remaining solvent in a gentle stream of air.

To purify by gel chromatography, macerate 50g of suitable beads (e.g. S-X3 bio-beads) in an elution mixture of cyclohexane R and ethyl acetate R (1:1) and pour them into a chromatographic column (length 600 mm, diameter 25 mm) adapted for use with a vacuum pump. Rinse the gel bed with the elution mixture under air-free conditions. Dissolve the extract in the flask with 5.0 ml of ethyl acetate R. Add 2 g of anhydrous sodium sulfate R, swirl gently and add 5.0 ml of cyclohexane R. Filter the completely dissolved crude extract through a rapid filter into a 10-ml test-tube with a ground-glass stopper and close the tube immediately. Then transfer 5.0 ml of the filtrate onto the gel column. Elute with the elution mixture at an average rate of 5.0 ml/minute. Plant material components leave the gel column first, followed by the active ingredients of pesticides. Fractionation must be determined for each column, using appropriate reference substances.

Discard the first fraction (about 100 ml) containing the impurities. Collect the organochlorine pesticides appearing in the next eluate (about 70ml) in a flask with a ground-glass stopper. Add l0ml of 2,2,4-trimethylpentane R and concentrate the solution to about 5 ml in a rotary vacuum evaporator and a water-bath at 30-40°C. Pipette another 5 ml of 2,2,4-trimethylpentane R into the flask and carefully evaporate the solution to about 1 ml (do not allow to become completely dry).

Calculate the amount of plant material in g in the purified extract using the following formula:

$$\frac{V}{590} \text{ x sample weight in g}$$

where V = volume of filtrate.

To purify further, transfer 1 g of previously deactivated silica gel for column chromatography (70-230 mesh) containing 1.5% of water, to a chromatographic column (length 25 cm, internal diameter 7 mm). Put 10 mm of anhydrous sodium sulfate R on top of the content of the column and cover with purified glass-wool. Before use rinse the column with 5 ml of hexane R. Allow the solvent to reach the surface of the column filling, then transfer quantitatively, by means of a pipette, the purified extract obtained by gel chromatography from the flask to the prepared silica gel column and rinse with 1 ml of hexane R. Set the flask aside for subsequent elutions.

Using a 10-ml volumetric flask as the receiver, elute any residues of polychlorinated biphenyls from the column with 10 ml of hexane R (eluate 0). Add 2 ml of an elution mixture composed of toluene R/hexane R (35:65) to the flask and swirl. Quantitatively transfer the solution to the column. Using another 10-ml volumetric flask as the receiver, elute the majority of the organochlorine pesticides from the silica gel column using 6 ml of the same elution mixture. Dilute the contents of the flasks to volume with the elution mixture (eluate 1).

Rinse the flask with 2 ml of toluene R and transfer it quantitatively to the column. Collect the eluate in a third 10-ml volumetric flask. Add 8 ml of toluene R to the flask, swirl and transfer the solution to the silica gel column; elute the remaining organochlorine pesticides using the same receiver. Dilute the contents of the flask to volume with toluene R (eluate 2).

Evaluate the test solutions by capillary gas chromatography using an electron capture detector (ECD). Confirm the findings obtained for the main column (first separation system) with a second capillary column of different polarity (second separation system).

Determination by gas chromatography
A capillary gas chromatograph with an ECD is used for the measurement. Helium R is used as the carrier gas and a mixture of argon R and methane R (95:5) as an auxiliary gas for the detection.

First separation system
Use a vitreous silica column, 30 m long with an internal diameter of 0.25 mm, packed with a chemically bound phase of 5% phenyl, 95% methyl-polysiloxane. Use the following temperature programme:

- heat at 60°C for 0.5 minutes;
- increase the temperature at a rate of 30°C per minute to 160°C and maintain this temperature for 2 minutes;
- increase the temperature at a rate of 2°C per minute to 250°C and maintain this temperature for 5 minutes.

Use a "split/split-free" injector to inject the sample solution and maintain the injection port at a temperature of 240°C. Inject a volume of 1 µl at a rate of 30 seconds ("split-free"). The detector temperature should be 300°C.

Second separation system
Use a vitreous silica column, 15 m long with an internal diameter of 0.25 mm, packed with a chemically bound phase of 7% cyanopropyl, 7% phenyl, 86% methyl-polysiloxane. Use the following temperature programme:

- heat at 60°C for 0.2 minutes;
- increase the temperature at a rate of 30°C per minute to 180°C and maintain this temperature for 1 minute;
- increase the temperature at a rate of 2°C per minute to 250°C and maintain this temperature for 5 minutes.

Use an on-column injector to inject a volume of 1 µl of the sample solution. The detector temperature should be 300°C.

Use the "external standard" method for the qualitative and quantitative evaluation of the organochlorine pesticides in the test solutions with reference solutions of the following pesticides: α-, β-, γ- and δ-hexachlorocyclohexane (HCH); hexachlorobenzene; quintozene; aldrin; dieldrin; endrin; α- and β-endosulfan; endosulfan sulfate; heptachlor, heptachlorepoxide; camphechlor; TDE, DDE and DDT (both *o,p'*- and *p,p'*-isomers); methoxychlor.

Measure the peak height of the pesticides obtained in the chromatograms and calculate the concentration of the residues in mg/kg using the following formula:

$$\frac{h_t \text{ x10}}{w} x \frac{w_r}{h_r}$$

where h_t = peak height obtained for the test solution in mm,
w = quantity of sample in the purified extract (g),
w_r = quantity of pesticide in ng in the reference solution injected,
h_r = peak height obtained for the reference solution in mm.

Analysis of esters of organophosphorus compounds

The extraction and the clean-up procedures can be performed as described above, but the detection requires a phosphorus flame ionization detector (P-FID).

Determination of specific pesticide residues in plant material

General recommendations
For the total determination, mix thoroughly 1 kg of plant material.

In order to obtain reliable chromatographic results, do one or more of the following:

- repeat the separation using another column;
- use a different separation system;
- use a different detector system;
- apply a coupling technique;
- prepare a derivative;
- perform chromatography with a mixture of the sample and a reference substance;
- change the sample preparation;
- use a fractionated elution during the column-chromatography clean-up procedure of the plant extract and test every fraction by chromatography; and
- compare the distribution coefficient of the material with that of a reference substance.

Prior to the quantitative determination of the material to be tested, check whether there is a linear relationship between the values obtained for the reference substance and its concentration over the range 0.1-2 times the standard concentration. Otherwise, prepare another concentration range or evaluate the results using a reference curve. Use any suitable mechanical or manual technique for the chromatographic determination.

Store the reference solutions protected from light to prevent decomposition. Use glass vessels closed with glass stoppers and keep them in a container saturated with the solvent employed to avoid any increase in concentration due to evaporation. Check the loss by evaporation by interim weighing of the vessels.

Use concentrated reference solutions within 6 months and diluted reference solutions within 4 weeks.

Rate of recovery
The rate of recovery (R) is the percentage of the reference material, originally added to the plant material, that is determined using the method described below.

Determination of desmetryn, prometryn, and simazine residues

Preparation of the plant material extract
Place 10.0 g of powdered plant material in a 500-ml conical flask and add 125.0 ml of chloroform R. Shake the mixture for 60 minutes and filter under reduced pressure through a filter-paper (medium grade) into a round-bottomed flask. Wash the residue with 3 successive volumes each of 25.0 ml of chloroform R.

Method
Concentrate the combined filtrates to a volume of 3-5 ml using a rotary vacuum evaporator and a water-bath at 40°C. Transfer the extract to a chromatographic column as prepared below, rinsing the round-bottomed flask twice with 5.0 ml of chloroform R.

Preparation of chromatographic column
Use a glass tube (internal diameter 20-22 mm) with a restricted orifice and protected with a sintered-glass plate (e.g. P10 or P16, glass filter G4; or P40, glass filter G3). Fill the column with chloroform R, then pour purified aluminium oxide R into it to form a 100-mm thick layer. The support material should remain covered with chloroform R. After transferring the extract and the rinsing liquids to the column, elute with 150.0 ml of chloroform R, at a rate of 1-2 drops per second, collecting the eluate in a round-bottomed flask. The first purifying process is completed when no further eluate drips from the column.

Evaporate the eluate to dryness using a rotary vacuum evaporator and a water-bath at 40°C. To the residue add 10.0 ml of light petroleum R and transfer the mixture to a chromatographic column containing a layer of purified aluminium oxide R, 50 mm thick, in light petroleum R. Elute the mixture with 90.0 ml of light petroleum R, using this to rinse the round-bottomed flask, at a rate of 1-2 drops per second. Discard the eluate. Dissolve any remaining residue which has not dissolved in light petroleum R in 10.0 ml of a mixture composed of 60 volumes of chloroform R and 40 volumes of light petroleum R and transfer the solution to the column. Rinse the round-bottomed flask twice more with 10.0 ml of the solvent mixture. Transfer the liquid used for rinsing to the column. Elute with 120.0 ml of the same solvent mixture, at a rate of 1-2 drops per second and collect the eluate in a round-bottomed flask. The second purifying process is completed when no further eluate drips from the column.

Evaporate the eluate to dryness using a rotary vacuum evaporator and a water-bath at 40°C. To prepare a purified extract for the determination by gas chromatography, dissolve the residue in sufficient acetone R to produce a volume of 10.0 ml. If an especially purified extract is required, proceed as described below.

To the residue add 10.0 ml of light petroleum R and 10.0 ml of dimethyl sulfoxide R. Shake the mixture and transfer it to a separating funnel. Extract the dimethyl sulfoxide layer twice with 10.0 ml of light petroleum R. Discard the petroleum ether extract. Then add 100 ml of water to the dimethyl sulfoxide layer and extract 3 times, each with 20.0 ml of chloroform R. Extract the combined chloroform extracts twice with 20.0 ml of water and evaporate them to dryness using a rotary vacuum evaporator and a water-bath at 40°C. Transfer the residue along with a mixture of 10.0 ml of light petroleum R and 10.0 ml of hydrochloric acid (1 mol/l) VS to a separating funnel and extract the mixture first with 10.0 ml and then with 5.0 ml of hydrochloric acid (1 mol/l) VS. Discard the petroleum ether layer and adjust the pH of the combined aqueous solutions to a value between 7 and 8 using sodium hydroxide (1 mol/l) VS. Extract the solution 3 times, each with 20.0 ml of chloroform R. Dry the combined chloroform extracts with anhydrous sodium sulfate R and filter into a round-bottomed flask, rinsing the funnel 3 times with 10.0-ml portions of chloroform R. Evaporate the filtrate to dryness using a rotary vacuum evaporator and a water-bath at 40°C. Dissolve the residue in sufficient acetone R to produce 10.0 ml of especially purified extract to be used for the determination by gas chromatography.

Use the extracts as indicated below for the following plant materials:

No.	Material	No.	Material
1	Flores Calendulae	10	Fructus Foeniculi
2	Flores Chamomillae	11	Herba Millefolii
3	Folia Melissae	12	Herba Plantaginis anceolatae
4	Folia Menthae piperitae	13	Radix Althaeae
5	Folia Salviae	14	Radix Angelicae
6	Folia Thymi	15	Radix Levistici
7	Fructus Carvi	16	Radix Petroselini
8	Fructus Coriandri	17	Radix Valerianae
9	Fructus Cynobasti		

For materials no. 1 and 2, use an especially purified extract (see page 58); for materials no. 3-17, use a purified extract (see page 58).

Determination of the rate of recovery

Prepare five individual samples using each of the following procedures:

1. To prepare solution S_2, first dissolve separately 0.040 g of each of the reference substances, desmetryn R, prometryn R and simazine R, in sufficient acetone R to produce 100.0 ml. Then place 5.0 ml of each solution into a 100-ml volumetric flask and dilute the mixture to volume with acetone R (S_2). Place 10.0 g of powdered plant material into a 500-ml conical flask and add 1.0 ml of solution S_2. Shake this mixture

mechanically for 60 minutes; if necessary, repeat the operation manually and then proceed as described under "Preparation of the plant material extract". Use either the purified or especially purified extract for the determination by gas chromatography, as specified in the test procedure for the plant material concerned.

2. Treat 10.0 g of powdered plant material as described under "Preparation of the plant material extract". Use either the purified extract or the especially purified extract for the determination by gas chromatography, as specified in the test procedure for each individual plant material.

Calculate the rate of recovery (R) in % using the following formula:

$$\frac{2\,(a - b)}{c}$$

where a = average quantity in mg/kg of the 5 residues obtained using procedure 1,
b = average quantity in mg/kg of the 5 residues obtained using procedure 2,
c = quantity of reference substances in mg contained in solution S_2 during procedure 1.

The rate should be within the range 70-120%. It is specific for each drug.

Determination by gas chromatography
Perform the determination as described in Volume 1 of *The international pharmacopoeia* (5).

Apparatus
The equipment consists of:

- a glass column 1.2 m long, internal diameter 2 mm;
- a suitable stationary liquid phase;
- a suitable diatomaceous support.

Use nitrogen R as the carrier gas with a flow rate of 30.0 ml/min. The sample injection block should be maintained at 230°C, the column at 190°C and the detector, which should be nitrogen-selective, at 300°C. In addition:

- volume of sample solution to be injected: 2.0 µl;
- separation characteristics: $h \leq 1.2 \times 10^{-3}$ for desmetryn R; $R_S \geq 1.2$ for prometryn R and simazine R;
- relative standard deviation (precision of chromatographic system): $s_r \leq 0.05$ for desmetryn R, prometryn R and simazine R.

Method
Chromatogram T. To determine the separation characteristics, inject solution S_2 (for the preparation of solution S_2 see "Determination of the rate of recovery" above). Chromatograms A_1-A_5. To determine the relative standard deviation, inject solution S_2 and repeat the determination 5 times.

Chromatogram S_2. Inject 1.0 ml of solution S_2 for the determination of the rate of recovery. Dilute 1.0 ml of solution S_2 to 10.0 ml with acetone R and inject it for the chromatographic determination. On the chromatogram the peaks occur in the following sequence: prometryn, simazine, desmetryn.

Chromatogram P_2. Inject the purified extract or the especially purified extract. Determine using an external standard: $a = 0.0005$To convert the values obtained to percentage by weight, multiply the concentration in mg/kg by 10^4.

The total maximum permissible amount of residues due to desmetryn, prometryn and simazine is 2 mg per kg of plant material.

17. Determination of arsenic and heavy metals

Contamination of medicinal plant materials with arsenic and heavy metals can be attributed to many causes including environmental pollution and traces of pesticides.

Limit test for arsenic

The amount of arsenic in the medicinal plant material is estimated by matching the depth of colour with that of a standard stain.

Recommended procedure

Preparation of the sample by acid digestion
Place 35-70g of coarsely ground material, accurately weighed, in a Kjeldahl flask, capacity 800-1000 ml. Add 10-25 ml of water and 25-50 ml of nitric acid (~1000 g/l) TS and then carefully add 20 ml of sulfuric acid (~1760g/l) TS. Heat cautiously so that no excessive foaming takes place. Gradually add nitric acid (~10008/l) TS, drop by drop, until all the organic matter is destroyed. This is achieved when no further darkening of the solution is observed with continued heating, and a clear solution with copious vapours of sulfur trioxide is obtained. Cool, and add 75 ml of water and 25 ml of ammonium oxalate (25 g/l) TS. Heat again until sulfur trioxide vapours develop. Cool, transfer with the help of water to a 250-ml volumetric flask, and dilute to volume with water.

Apparatus
A suitable type of apparatus is constructed as follows. A wide-mouthed bottle of about 120-ml capacity is fitted with a rubber bung through which passes a glass tube. The latter, made from ordinary glass tubing, has a total length of about 200mm and an internal diameter of exactly 6.5 mm (external diameter about 8 mm). The lower end of the tube is drawn out to an internal diameter of about 1 mm, and there is a hole not less than 2 mm in diameter blown in the side of the tube, near the constricted part. The tube is positioned so that when the bottle contains 70ml of liquid the constricted end is above the surface of the liquid and the hole in the side is below the bottom of the bung. The upper end of the tube has a flat, ground surface at right-angles to the axis of the tube, with slightly rounded-off edges.

One of two rubber bungs (about 25 mm x 25 mm), each with a central hole of exactly 6.5 mm diameter, is fitted at the upper end of the tube. The other bung is fitted with a piece of glass tube about 3 mm long and with an internal diameter of exactly 6.5 mm and with a similar ground surface. One end of each of the tubes is flush with the larger end of the bungs, so that when these ends are held tightly together with a rubber band or a spring clip, the openings of the two tubes meet to form a true tube. Alternatively, the two bungs may be replaced by any suitable construction satisfying the conditions described in the test.

Method
Moisten some cotton-wool with lead acetate (80g/l) TS, allow to dry, and lightly pack into the tube which fits into the wide-mouthed bottle to not less than 25 mm from the top. Between the flat surfaces of the tubes, place a piece of mercuric bromide paper AsR that is large enough to cover their openings (15 mm x 15 mm). The mercuric bromide paper AsR can be fitted by any other means provided that:

- the whole of the evolved gas passes through the paper;
- the portion of the paper in contact with the gas is a circle 6.5 mm in diameter; and
- the paper is protected from sunlight during the test.

Place an aliquot (25-50ml) of the solution being tested, prepared as described above, in the wide-mouthed bottle, add 1 g of potassium iodide AsR and l0g of granulated zinc AsR, and place the prepared glass tube assembly quickly in position. Allow the reaction to proceed for 40 minutes. Compare any yellow stain that is produced on the mercuric bromide paper AsR with a standard stain produced in a similar manner with a known quantity of dilute arsenic AsTS. Examine the test and standard stains without delay in daylight; the stains fade with time.

The most suitable temperature for carrying out the test is generally about 40°C but, as the rate of evolution of the gas varies somewhat with different batches of granulated zinc AsR, the temperature may have to be adjusted to obtain an even evolution of gas. The reaction may be accelerated by placing the apparatus on a warm surface, care being taken to ensure that the mercuric bromide paper AsR remains dry throughout.

Between successive tests, the tube must be washed with hydrochloric acid (~250g/l) AsTS, rinsed with water and dried.

Preparation of standard stain
Add 10 ml of stannated hydrochloric acid (~250g/l) AsTS and 1 ml of dilute arsenic AsTS to 50 ml of water. The resulting solution, when treated as described in the general test, yields a stain on mercuric bromide paper AsR referred to as the standard stain (10 μg of As).

Limit test for cadmium and lead
The method of determination is left to the analyst. Nevertheless, the determination must be consistent and sensitive enough to allow comparison with a reference material.

Recommended procedure

Apparatus
The equipment consists of a digestion vessel, consisting of a vitreous silica crucible (DIN 12904), "tall form", height 62mm, diameter 50mm, capacity 75ml, with a vitreous silica cover.

Materials used are:

- *digestion mixture*: 2 parts by weight of nitric acid (~1000g/l) TS and 1 part by weight of perchloric acid (~1170g/l) TS.
- *reference materials*: olive leaves (*Olea europaea*)[1] and hay powder[2].

Clean scrupulously with nitric acid (~1000g/l) TS the digestion vessel and all other equipment to be used for the determination, rinse thoroughly several times with water and dry at 120°C.

Preparation of the sample
For the wet digestion method in an open system, place 200-250mg of air-dried plant material, accurately weighed, finely cut and homogeneously mixed, into a cleaned silica crucible. Add 1.0ml of the digestion mixture, cover the crucible without exerting pressure and place it in an oven with a controlled temperature and time regulator (computer-controlled, if available).

Heat slowly to 100°C and maintain at this temperature for up to 3 hours; then heat to 120°C and maintain at this temperature for 2 hours. Raise the temperature very slowly to 240°C, avoiding losses due to possible violent reactions especially in the temperature range of 160-200°C, and maintain at this temperature for 4 hours. Dissolve the remaining dry inorganic residue in 2.5 ml of nitric acid (~1000g/l) TS and use for the determination of heavy metals.

Every sample should be tested in parallel with a blank.

Method
The contents of lead and cadmium may be determined by inverse voltametry or by atomic absorption spectrophotometry.

The following maximum amounts in dried plant materials, which are based on the ADI values, are proposed:
- lead, 10 mg/kg;
- cadmium, 0.3 mg/kg.

[1] BCR reference material CRM No. 62 Community Bureau of Reference, obtainable from BCR, Directorate-General X11, Commission of the European Communities, 200 rue de la Loi, B-1049 Brussels, Belgium.
[2] Obtainable from IAEA/V-10, International Atomic Energy Agency, Analytical Quality Control Services, Laboratory Geibersdorf, P.O. Box 1'00, A-Vienna, Austria.

18. Determination of microorganisms

Medicinal plant materials normally carry a great number of bacteria and moulds, often originating in soil. While a large range of bacteria and fungi form the naturally occurring microflora of herbs, aerobic spore-forming bacteria frequently predominate. Current practices of harvesting, handling and production may cause additional contamination and microbial growth. The determination of Escherichia coli and moulds may indicate the quality of production and harvesting practices.

Methods for decontamination are restricted. For example, the use of ethylene oxide has been forbidden within countries of the European Union. Treatment with ionizing irradiation is also forbidden or requires a special registration procedure in some countries.

In addition, the presence of aflatoxins in plant material can be hazardous to health if absorbed even in very small amounts. They should therefore be determined after using a suitable clean-up procedure.

Test for specific microorganisms

The conditions of the test for microbial contamination are designed to minimize accidental contamination of the material being examined; the precautions taken must not adversely affect any microorganisms that could be revealed.

Recommended procedure

Pretreatment of the material being examined

Depending on the nature of the crude medicinal plant material, grind, dissolve, dilute, suspend or emulsify the material being examined using a suitable method and eliminate any antimicrobial properties by dilution, neutralization or filtration.

Water-soluble materials

Dissolve or dilute 10 g or 10 ml of plant material, unless otherwise specified in the test procedure for the material concerned, in lactose broth or another suitable medium proven to have no antimicrobial activity under the conditions of the test, adjust the volume to 100ml with the same medium. (Some materials may require the use of a larger volume.) If necessary, adjust the pH of the suspension to about 7.

Non-fatty materials insoluble in water

Suspend 10g or 10ml of material, unless otherwise specified in the test procedure for the material concerned, in lactose broth or another suitable medium proven to have no antimicrobial activity under the conditions of the test; dilute to 100ml with the same medium. (Some materials may require the use of a larger volume.) If necessary, divide the material being examined and homogenize the suspension mechanically. A suitable surfactant, such as a solution of polysorbate

80 R containing 1 mg per ml may be added. If necessary, adjust the pH of the suspension to about 7.

Fatty materials
Homogenize 10g or 10ml of material, unless otherwise specified in the test procedure for the material concerned, with 5g of polysorbate 20R or polysorbate 80R. If necessary, heat to not more than 40°C. (Occasionally, it may be necessary to heat to a temperature of up to 45°C, for the shortest possible time.) Mix carefully while maintaining the temperature in a water-bath or oven. Add 85 ml of lactose broth or another suitable medium proven to have no antimicrobial activity in the conditions of the test, heated to not more than 40°C if necessary. Maintain this temperature for the shortest time necessary until an emulsion is formed and, in any case, for not more than 30 minutes. If necessary, adjust the pH of the emulsion to about 7.

Enterobacteriaceae and certain other Gram-negative bacteria

Detection of bacteria
Homogenize the pretreated material appropriately and incubate at 30-37°C for a length of time sufficient for revivification of the bacteria but not sufficient for multiplication of the organisms (usually 2-5 hours). Shake the container, transfer 1g or 1ml of the homogenized material to 100ml of Enterobacteriaceae enrichment broth-Mossel and incubate at 35-37°C for 18-48 hours. Prepare a subculture on a plate with violet-red bile agar with glucose and lactose. Incubate at 35-37°C for 18-48 hours. The material passes the test if no growth of colonies of Gram-negative bacteria is detected on the plate.

Quantitative evaluation
Inoculate a suitable amount of Enterobacteriaceae enrichment broth-Mossel with quantities of homogenized material prepared as described under "Detection of bacteria" above, appropriately diluted as necessary, containing 1.0g, 0.1g and 10µg, or 1.0ml, 0.1 ml and 10µl, of the material being examined. Incubate at 35-37°C for 24-48 hours. Prepare a subculture of each of the cultures on a plate with violet-red bile agar with glucose and lactose in order to obtain selective isolation. Incubate at 35-37°C for 18-24 hours. The growth of well-developed colonies, generally red or reddish in colour, of Gram-negative bacteria constitutes a positive result. Note the smallest quantity of material that gives a positive result. Determine the probable number of bacteria using Table 5.

Escherichia coli
Transfer a quantity of the homogenized material in lactose broth, prepared and incubated as described above, and containing 1 g or 1 ml of the material being examined, to 100 ml of MacConkey broth and incubate at 43-45°C for 18-24 hours.

Table 5
Determination of Enterobacteriaceae and certain other Gram-negative bacteria

Result for each quantity or volume			Probable number of bacteria per g of material
1.0 g or 1.0 ml	0.1g or 0.1 ml	0.01 g or 0.01 ml	
+	+	+	More than 10^2
+	+	–	Less than 10^2 but more than 10
+	–	–	Less than 10 but more than 1
–	–	–	Less than 1

Prepare a subculture on a plate with MacConkey agar and incubate at 43-45°C for 18-24 hours. Growth of red, generally non-mucoid colonies of Gram-negative rods, sometimes surrounded by a reddish zone of precipitation, indicates the possible presence of E. coli. This may be confirmed by the formation of indole at 43.5-44.5°C or by other biochemical reactions. The material passes the test if no such colonies are detected or if the confirmatory biochemical reactions are negative.

Salmonella spp.
Incubate the solution, suspension or emulsion of the pretreated material prepared as described above at 35-37°C for 5-24 hours, as appropriate for enrichment.

Primary test
Transfer 10 ml of the enrichment culture to 100 ml of tetrathionate bile brilliant green broth and incubate at 42-43°C for 18-24 hours. Prepare subcultures on at least two of the following three agar media: deoxycholate citrate agar; xylose, lysine, deoxycholate agar; and brilliant green agar. Incubate at 35-37°C for 24-48 hours. Carry out the secondary test if any colonies are produced that conform to the description given in Table 6.

Table 6
Description of Salmonella colonies appearing on different culture media

Medium	Description of colony
Deoxycholate citrate agar	Well developed, colourless
Xylose, lysine, deoxycholate agar	Well developed, red, with or without black centres deoxycholate agar
Brilliant green agar	Small, transparent and colourless, or opaque, pink or white (frequently surrounded by a pink to red zone)

Secondary test
Prepare a subculture of any colonies showing the characteristics described in Table 6 on the surface of triple sugar iron agar using the deep inoculation technique. This can be achieved by first inoculating the inclined surface of the culture medium followed by a stab culture with the same inoculating needle and incubating at 35-37°C for 18-24 hours. The test is positive for the presence of Salmonella spp. if a change of colour from red to yellow is observed in the deep culture (but not in the surface culture), usually with the formation of gas with or without production of hydrogen sulfide in the agar. Confirmation is obtained by appropriate biochemical and serological tests.

The material being examined passes the test if cultures of the type described do not appear in the primary test, or if the confirmatory biochemical and serological tests in the secondary test are negative.

Pseudomonas aeruginosa
Pretreat the material being examined as described on pages 64-65 but using buffered sodium chloride-peptone solution, pH 7.0, or another suitable medium shown not to have antimicrobial activity under the conditions of the test, in place of lactose broth. Inoculate 100 ml of soybean-casein digest medium with a quantity of the solution, suspension or emulsion thus obtained containing 1g or 1 ml of the material being examined. Mix and incubate at 35-37°C for 24-48 hours. Prepare a subculture on a plate of cetrimide agar and incubate at 35-37 °C for 24-48 hours. If no growth of microorganisms is detected, the material passes the test. If growth of colonies of Gram-negative rods occurs, usually with a greenish fluorescence, apply an oxidase test and test the growth in soybean-casein digest medium at 42°C. The following method may be used. Place 2 or 3 drops of a freshly prepared 0.01 g/ml solution of N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride R on filter-paper and apply a smear of the suspected colony; the test is positive if a purple colour is produced within 5-10 seconds. The material passes the test if cultures of the type described do not appear or if the confirmatory biochemical test is negative.

Staphylococcus aureus
Prepare an enrichment culture as described for *Pseudomonas aeruginosa*. Prepare a subculture on a suitable medium such as Baird-Parker agar. Incubate at 35-37°C for 24-48 hours. The material passes the test if no growth of microorganisms is detected. Black colonies of Gram-positive cocci often surrounded by clear zones may indicate the presence of *Staphylococcus aureus*. For catalase-positive cocci, confirmation may be obtained, for example, by coagulase and deoxyribonuclease tests. The material passes the test if cultures of the type described do not appear or if the confirmatory biochemical test is negative.

Validation of the tests for specific microorganisms
If necessary, grow separately the test strains listed in Table 7 on the culture media indicated at 30-35°C for 18-24 hours. Dilute portions of each of the cultures using buffered sodium chloride-peptone solution pH 7.0 so that the test suspensions contain about 10^3; microorganisms per ml. Mix equal volumes of

each suspension and use 0.4 ml (approximately 10^2 microorganisms of each strain) as an inoculum in tests for *Escherichia coli, Salmonella spp., Pseudomonas aeruginosa and Staphylococcus aureus*, in the presence and absence of the material being examined, if necessary. The test method should give a positive result for the respective strain of microorganism.

Table 7
Test strains and culture media for use in validating the tests for specific microorganisms

Microorganism	Strain number[a]	Medium
Escherichia coli	e.g. NCIMB 8545 (ATCC 8739, CIP 53.126)	lactose broth
Pseudomonas aeruginosa	e.g. NCIMB 8626 (ATCC 9027, CIP 82.118)	soybean-casein digest medium
Salmonella typhimurium	No strain number is recommended. Species not pathogenic for humans, such as Salmonella abony (NCTC 6017, CIP 80.39), may be used	lactose broth
Staphylococcus aureus	e.g. NCIMB 8625 (ATCC 6538 P, CIP 53.156) or NCIMB 9518 (ATCC 6538, CIP 4.83)	soybean-casein digest medium

[a]See section 20, page 78.

Total viable aerobic count

The total viable aerobic count of the material being examined is determined, as specified in the test procedure, for the plant material concerned using one of the following methods: membrane-filtration, plate count or serial dilution.

Pretreatment of the material being examined
Pretreat the material as described in the "Test for specific microorganisms", page 64, but in place of lactose broth use buffered sodium chloride-peptone solution pH 7.0, or another suitable medium shown not to have antimicrobial activity under the conditions of the test.

Membrane filtration
Use membrane filters with a nominal pore size of not greater than 0.45 μm, the effectiveness of which in retaining bacteria has been established. For example, cellulose nitrate filters are used for aqueous, oily and weakly alcoholic solutions, and cellulose acetate filters for strongly alcoholic solutions. The technique described uses filter discs of about 50mm in diameter. For filters of a different diameter, adjust the volumes of the dilutions and washings accordingly. Sterilize the filtration apparatus and the membrane by appropriate means. They are

designed to permit the solution being examined to be introduced and filtered under aseptic conditions, and the membrane to be transferred to the culture medium.

Transfer 10ml or a solution containing 1g of the material to each of two membrane filters and filter immediately. If necessary, dilute the pretreated material to obtain an expected colony count of 10-100. Wash each membrane, filtering three or more successive quantities of approximately 100ml of a suitable liquid such as buffered sodium chloride-peptone solution, pH 7.0. For fatty materials, a suitable surfactant may be added, such as polysorbate 20R or polysorbate 80R. Transfer one of the membrane filters, intended primarily for the enumeration of bacteria, to the surface of a plate with casein-soybean digest agar and the other, intended primarily for the enumeration of fungi, to the surface of a plate with Sabouraud glucose agar with antibiotics. Incubate the plates for 5 days, unless a more reliable count can be obtained otherwise, at 30-35°C for the detection of bacteria and at 20-25°C for the detection of fungi. Count the number of colonies formed. Calculate the number of microorganisms per g or per ml of the material tested, if necessary counting bacteria and fungi separately.

Plate count
For bacteria. Use Petri dishes 9-10 cm in diameter. To one dish add a mixture of 1 ml of the pretreated material and about 15 ml of liquefied casein-soybean digest agar at a temperature not exceeding 45°C. Alternatively, spread the pretreated material on the surface of the solidified medium in a Petri dish. If necessary, dilute the pretreated material as described above to obtain an expected colony count of not more than 300. Prepare at least two dishes using the same dilution and incubate them at 30-35°C for 5 days, unless a more reliable count is obtained in a shorter period of time. Count the number of colonies formed and calculate the results using the plate with the largest number of colonies, up to a maximum of 300.

For fungi. Use Petri dishes 9-10 cm in diameter. To one dish add a mixture of 1 ml of the pretreated material and about 15 ml of liquefied Sabouraud glucose agar with antibiotics at a temperature not exceeding 45°C. Alternatively, spread the pretreated material on the surface of the solidified medium in a Petri dish. If necessary, dilute the pretreated material as described above to obtain an expected colony count of not more than 100. Prepare at least two dishes using the same dilution and incubate them at 20-25°C for 5 days, unless a more reliable count is obtained in a shorter period of time. Count the number of colonies formed and calculate the results using the dish with not more than 100 colonies.

Serial dilution
Prepare a series of 12 tubes each containing 9-10ml of soybean-casein digest medium. To each of the first three tubes add 1 ml of the 1:10 dilution of dissolved, homogenized material prepared as described on pages 64-65. To the next three tubes add 1 ml of a 1:100 dilution of the material and to the next three tubes add 1 ml of a 1:1000 dilution of the material. To the last three tubes add 1 ml of the diluent. Incubate the tubes at 30-35°C for at least 5 days. No microbial growth

should appear in the last three tubes. If the reading of the results is difficult or uncertain owing to the nature of the material being examined, prepare a subculture in a liquid or a solid medium, and evaluate the results after a further period of incubation. Determine the most probable number of microorganisms per g or ml of the material using Table 8.

If, for the first column, the number of tubes showing microbial growth is two or less, the most probable number of microorganisms per g or per ml is less than 100.

Effectiveness of the culture medium and validity of the counting method
The following strains are normally used (see also section 20):

Staphylococcus aureus	NCIMB 8625 (ATCC 6538-P, CIP 53.156) or NCIMB 9518 (ATCC 6538, CIP 4.83)
Bacillus subtilis	NCIMB 8054 (ATCC 6633, CIP 52.62)
Escherichia coli	NCIMB 8545 (ATCC 8739, CIP 53.126)
Candida albicans	ATCC 2091 (CIP 1180.79) or ATCC 10 231 (NCPF 3179, CIP 48.72)

Allow the test strains to grow separately in tubes containing soybean-casein digest medium at 30-35°C for 18-24 hours, except for Candida albicans which needs a temperature of 20-25°C for 48 hours.

Dilute portions of each of the cultures using buffered sodium chloride-peptone solution pH 7.0 to obtain test suspensions containing about 100 viable microorganisms per ml. Use the suspension of each microorganism separately as a control of the counting methods, in the presence and absence of the material being examined, if necessary.

Table 8
Determination of total viable aerobic count

Number of tubes 100mg or 0.1 ml per tube	with microbial 10mg or 0.01 ml per tube	growth[a] 1mg or 0.001 ml per tube	Most probable number of microorganisms per g or ml
3	3	3	>1100
3	3	2	1100
3	3	1	500
3	3	0	200
3	2	3	290
3	2	2	210
3	2	1	150
3	2	0	90
3	1	3	160
3	1	2	120
3	1	1	70
3	1	0	40
3	0	3	95
3	0	2	60
3	0	1	40
3	0	0	23

[a] Amounts in mg or ml are quantities of original plant material.

To validate the method, a count for the test organism should be obtained differing by not more than a factor of 10 from the calculated value for the inoculum. To test the sterility of the medium and the diluent, as well as aseptic performance, carry out the total viable aerobic count using sterile buffered sodium chloride-peptone solution pH 7.0 as the test preparation. There should be no growth of microorganisms.

Microbial contamination limits in medicinal plant materials

Different limits are set according to the use of the material and the material itself.

- For contamination of "crude" plant material intended for further processing (including additional decontamination by a physical or chemical process) the limits, adapted from the provisional guidelines established by an international consultative group (12), are given for untreated plant material harvested under acceptable hygienic conditions:
  - *Escherichia coli*, maximum 10^4 per gram;
  - mould propagules, maximum 10^5 per gram.

- For plant materials that have been pretreated (e.g. with boiling water as used for herbal teas and infusions) or that are used as topical dosage forms:
  - aerobic bacteria, maximum 10^7 per gram;

- yeasts and moulds, maximum 10^4 per gram;
- Escherichia coli, maximum 10^2 per gram;
- other enterobacteria, maximum 10^4 per gram;
- salmonellae, none.

- For other plant materials for internal use:
  - aerobic bacteria, maximum 10^5 per gram;
  - yeasts and moulds, maximum 10^3 per gram;
  - Escherichia coli, maximum 10 per gram;
  - other enterobacteria, maximum 10^3, per gram;
  - salmonellae, none.

Test for aflatoxins

This test is designed to detect the possible presence of aflatoxins B_1, B_2, G_1 and G_2, which are highly dangerous contaminants in any material of plant origin.

Recommended procedure

Preparation of samples
Grind or reduce not less than 100g of crude medicinal plant material to a moderately fine powder (sieve no. 355/180). The larger the sample size, i.e. 500g-1kg or more, the greater the possibility of detecting pockets of contamination.

Weigh 50g of the powdered material, transfer to a conical glass-stoppered flask, and add 170 ml of methanol R and 30 ml of water. Using a mechanical device, shake vigorously for not less than 30 minutes. Filter through a medium-porosity filter-paper. If a special clean-up procedure is required (see below), collect 100ml of filtrate (A) from the start of flow; otherwise discard the first 50ml and collect 40ml of filtrate (B).

In order to eliminate interfering plant pigments use a special clean-up procedure: transfer 100 ml of filtrate A to a 250-ml beaker and add 20 ml of zinc acetate/aluminium chloride TS and 80 ml of water. Stir, allow to stand for 5 minutes, add 5 g of a filter aid, such as diatomaceous earth, mix and filter through a medium-porosity filter-paper. Discard the first 50ml and collect 80ml of filtrate (C).

Transfer either filtrate B or C to a separating funnel. Add 40ml of sodium chloride (100 g/l) TS and 25 ml of light petroleum R, and shake for 1 minute. Allow the layers to separate and transfer the lower layer to a second separating funnel. Extract twice with 25 ml of dichloromethane R and shake for 1 minute. Allow the layers to separate and combine each of the lower layers in a 125-ml conical flask. Add several boiling chips and evaporate almost to dryness on a water-bath. Cool the residue, cover the flask and keep it for the determination by thin-layer chromatography or for a further clean-up procedure by column chromatography.

If necessary, remove further interfering compounds using a column 300 mm long with an internal diameter of 10 mm, a stopper and either a medium-pore sintered disc or a glass-wool plug. Prepare a slurry by mixing 2 g of silica gel R with 10 ml of a mixture of 3 volumes of ether R and 1 volume of light petroleum R, pour into the column and wash with 5 ml of the same solvent mixture. Allow the adsorbent to settle and add to the top of the column a layer of 1.5 g of anhydrous sodium sulfate R. Dissolve the residue from above in 3 ml of dichloromethane R and transfer it to the column. Rinse the flask twice with 1-ml portions of dichloromethane R and add them to the column, eluting at a rate not faster than 1 ml/min. Then add successively to the column 3 ml of light petroleum R, 3 ml of ether R and 3 ml of dichloromethane R, and elute at a rate not faster than 3 ml/min. Discard the eluates. Add to the column 6 ml of a mixture of 9 volumes of dichloromethane R and 1 volume of acetone R and elute at a rate not faster than 1 ml/min, preferably without using vacuum. Collect this eluate in a small vial, add a few boiling chips and evaporate just to dryness on a water-bath.

Method

To either of the residues obtained above, add 0.2 ml of a mixture of 98 volumes of chloroform R and 2 volumes of acetonitrile R, close the vial and shake vigorously until the residues are dissolved, preferably using a vortex mixer.

Carry out the test as described in section 6, "Thin-layer chromatography", using silica gel G as the coating substance and a mixture of 85 volumes of chloroform R, 10 volumes of acetone R and 5 volumes of 2-propanol R as the mobile phase. Apply separately to the plate 2.5 µl, 5 µl, 7.5 µl and 10 µl of aflatoxin mixture TS, then apply three volumes, each of 10 µl, of the sample residues. Further superimpose on one of these spots 5 µl of aflatoxin mixture TS. Place the plate in an unsaturated chamber and develop. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in a dark room under ultraviolet light (365 nm).

Four clearly separated blue fluorescent spots are obtained from the aflatoxin mixture. Observe any spot obtained from the solutions of the residues that coincides in hue and position with those of the aflatoxin mixture. Any spot obtained from the solutions of the residues with the superimposed aflatoxin mixture should be more intense than the corresponding spot for the test solution, and should show no sign of separation or tailing, which would be a sign of dissimilar compounds.

Interpretation of results

No spots corresponding to aflatoxin should be obtained from any of the sample residues. If any such spot is obtained, compare its position with the spots obtained from the aflatoxin mixture to identify the type of aflatoxin present. An approximate estimation of the concentration of aflatoxin in the sample may be obtained by comparing the intensity of the spots with those of the aflatoxin mixtures.

19. Radioactive contamination

A certain amount of exposure to ionizing radiation cannot be avoided since there are many sources, including radionuclides occurring naturally in the ground and the atmosphere. These sources are described extensively in the booklet Facts about low-level radiation (13).

Dangerous contamination may be the consequence of a nuclear accident. The World Health Organization, in close collaboration with several other international organizations, has developed guidelines for use in the event of widespread contamination by radionuclides resulting from a major nuclear accident (14). This publication emphasizes that the health risks from food accidentally contaminated by radionuclides depend not only on the specific radionuclide and the level of contamination but also on the quantity of food consumed.

The range of radionuclides that may be released into the environment as the result of a nuclear accident might include long-lived and short-lived fission products, actinides, and activation products. The nature and the intensity of radionuclides released may differ markedly and depend on the source (reactor, reprocessing plant, fuel fabrication plant, isotope production unit, etc.).

The amount of exposure to radiation depends on the intake of radionuclides and other variables such as age, metabolic kinetics, and weight of the individual (also known as the dose conversion factor).

Even at maximum observed levels of radioactive contamination with the more dangerous radionuclides, significant risk is associated only with consumption of quantities of over 20 kg of plant material per year so that a risk to health is most unlikely to be encountered given the amount of medicinal plant materials that would need to be ingested. Additionally, the level of contamination might be reduced during the manufacturing process. Therefore, no limits for radioactive contamination are proposed.

Method of measurement
Since radionuclides from accidental discharges vary with the type of facility involved, a generalized method of measurement is so far not available. However, should such contamination be of concern, suspect samples can be analysed by a competent laboratory. Details of laboratory techniques are available from the International Atomic Energy Agency (IAEA).[3]

[3] International Atomic Energy Agency (IAEA), Analytical Quality Control Services, Laboratory Seibersdorf, PO Box 100, Vienna, Austria.

20. Culture media and strains of microorganisms

Culture media

The following media are satisfactory, but other media may be used if they have similar nutritive and selective properties for the microorganisms to be tested.

Baird-Parker agar
Procedure. Dissolve 10.0 g of pancreatic digest of casein R, 5.0 g of beef extract R, 1.0g of water-soluble yeast extract R, 5.0 g of lithium chloride R, 20.0 g of agar R, 12.0 g of glycine R and 10.0 g of sodium pyruvate R in sufficient water to produce 950 ml. Heat to boiling for 1 minute, shaking frequently and adjust the pH to 6.6-7.0 using sodium hydroxide (0.5 mol/l) VS. Sterilize in an autoclave at 121°C for 15 minutes, cool to 45-50°C and add 10 ml of a sterile 0.01 g/ml solution of potassium tellurite R and 50 ml of egg-yolk emulsion.

Brilliant green agar
Procedure. Dissolve 10.0 g of dried peptone R (meat and casein), 3.0 g of water-soluble yeast extract R, 5.0 g of sodium chloride R, 10.0 g of lactose R, 10.0 g of sucrose R, 20.0 g of agar R, 0.08 g of phenol red R and 12.5 mg of brilliant green R in sufficient water to produce 1000 ml. Heat to boiling for 1 minute. Using sodium hydroxide (0.05 mol/l) VS adjust the pH to 6.7-7.1. Immediately before use, sterilize in an autoclave at 121°C for 15 minutes, cool to 50°C and pour into Petri dishes.

Buffered sodium chloride-peptone solution pH 7.0
Procedure. Dissolve 3.56 g of potassium dihydrogen phosphate R, 7.23 g of disodium hydrogen phosphate R, 4.30 g of sodium chloride R and 1.0 g of dried peptone R (meat and casein) in sufficient water to produce 1000ml. Polysorbate 20 R or polysorbate 80 R may be added, 0.001-0.01 g per ml. Sterilize in an autoclave at 121°C for 15 minutes.

Casein-soybean digest agar
Procedure. Dissolve 15.0g of pancreatic digest of casein R, 3.0g of papaic digest of soybean meal R, 5.0 g of sodium chloride R and 15.0 g of agar R in sufficient water to produce 1000 ml. Using sodium hydroxide (0.05 mol/l) VS adjust the pH to 7.1-7.5. Sterilize in an autoclave at 121°C for 15 minutes.

Cetrimide agar
Procedure. Dissolve 20.0 g of pancreatic digest of gelatin R, 1.4g of magnesium chloride R, 10.0 g of potassium sulfate R, 0.3 g of cetrimide R, 13.6 g of agar R and 10.0 ml of glycerol R insufficient water to produce 1000 ml. Heat to boiling for 1 minute with shaking. Using sodium hydroxide (0.05 mol/l) VS adjust the pH to 7.0-7.4. Sterilize in an autoclave at 121°C for 15 minutes.

Deoxycholate citrate agar
Procedure. Dissolve 10.0 g of beef extract R, 10.0 g of dried peptone R (meat), 10.0 g of lactose R, 20.0 g of sodium citrate R, 1.0 g of iron(III) citrate R, 5.0 g of sodium deoxycholate, 13.5 g of agar R and 20 mg of neutral red R in sufficient

Resins and resin combinations

- The term ‘resin’ is applied to more or less solid, amorphous products of complex chemical nature. These are amorphous mixtures of essential oils, oxygenated products of terpenes and carboxylic acids

- Resins and related resinous products are produced in plants during normal growth or secreted as a result of injury to the plants
- They are usually occur in schizogenous or schizolysigenous cavities or ducts

General properties

- Physical characters –
- All resins are heavier than water , they are usually amorphous ,hard, and brittle solids.
- They are insoluble in water and usually insoluble in petroleum ether but dissolve more or less completely in alcohol, chloroform and ether.

- Chemically, resins are complex mixtures of resin acids, resin alcohols (resinols), resin phenols (resinotannols), esters and chemically inert compounds known as resenes.
- Many resins ,when boiled with alkalies yield soaps

- By the action of heat they soften yielding clear, adhesive fluids , Resins burn with a characteristic, smoky flame.
- Resins are often associated with volatile oils (*oleoresins*), with gums (*gum-resins*) or with oil and gum (*oleo-gum-resins*).
- Resins may also be combined in a glycosidal manner with sugars.

Chemical nature :

- Chemically resins are not pure substances but complex mixtures of several resinous substances as resin acids, resin alcohols, resin esters, and neutral resins.
- Resins do not contain nitrogen elements
- (Non nitrogenous compounds)

Classification of resins

- Resins are classified in different three ways:

1. Taxonomical classification, i.e. according to botanical origin, e.g. *Berberidaceae* resins.
2. Classification according to predominating chemical constituent; e.g. acid resins, resene resins, glycosidal resins; etc.

3.*Resins may be classified according to the portion of the main constituents of the resin or resin combination*; e.g. resins, oleoresins, oleo-gum-resins, balsams.

Classification of resins

- 1. Acid resins –
- Here the resins occur their along with acids. Examples –
- Colophony - Abietic acid
- Sandrac - Sandracolic acid
- Myrrh - Commiphoric acid
- Copaiba - Copaivic acid

2. Ester resins –

- This group contains esters as the chief constituents of the resins- Examples
- Benzoin and Storax ,
- Benzoin contains benzyl benzoate,
- Storax contains cinnamyl cinnamate

- 3.Resin alcohols –
- They occurs as in free state or as esters , examples –
- Balsam of peru with perru resino tannol
- Guaiaccum resin with guaic resinol

- They are also further classified into –
  Resins: colophony, cannabis.

- Oleoresins: copaiba, ginger.
- Oleo-gum-resins: asafoetida, myrrh.
- Balsams: balsam of Tolu, balsam of Peru.
- Glycoresins : jalap
- Resenes : Asafoetida, colophony

Balsams

Balsams are resinous mixtures that contain large proportions of cinnamic acid, benzoic acid or both or esters of these acids.

The term "balsam" is often wrongly applied to oleoresins and should be reserved for such substances as *balsam of Peru*, *balsam of Tolu* and *storax*, which contain a high proportion of aromatic balsamic acids.

Preparation of resins

- Two general classes of resinous substances are recognized and this classification is based on the method used in preparing them:

1. Natural resins, occur as exudates from plants, produced normally or as result of pathogenic conditions

- Example by artificial punctures e.g. mastic; or deep cuts in the wood of the plant e.g. turpentine, or by hammering and scorching, e.g. balsam of Peru.

2. Prepared resins; are obtained by different methods. The drug containing *resins* is powdered and extracted with alcohol till exhaustion. The Concentrated alcoholic extract is either evaporated, or poured into water and the precipitated resin is collected, washed and carefully dried.

In the preparation of *oleoresins*; ether or acetone having lower boiling point are used. The volatile oil portion is removed through distillation.

When the resin occurs associated with gum (*gum-resins*), the resin is extracted with alcohol leaving the gum insoluble.

IDENTIFICATION TEST

Physical test

Such as solubility, taste, odor and examination of powder under the microscope .

Chemical test – for the identification of resins, Acid value, Saponification value , Iodine value

Specific chemical test – for specific constituents such as Cinnamic acid, Benzoic acid in Benzoin, Tolu balsam and Peru Balsam

CHEMICAL TESTS

IDENTIFICATION TEST	REAGENTS USED	POSITIVE RESULT	COMPOUNDS POSITIVE FOR THE TEST
HCl TEST	HCl	Formation of pink color	Presence of resins
FeCl3 TEST	Fecl3	Greenish blue color	Presence of resins
Combined Umbelliferone Test (Specific test for Asafoetida)	HCl + conc. NH4OH Nitric acid Sulfuric acid Washed with water	→Blue fluorescence →Green color →Red color →Violet	Umbelliferone
Copper Acetate test	Petroleum ether	Emerald green	Abietic acid

USES

- It reflects light. This decreases the heat on the flowers, thereby protecting them.
- They are used in the preparation of emulsions.
- Solid resenes are available as adhesives.
- They are used externally as mild antiseptic agents in the form of ointments and plasters.
- Resins are local irritant and hence act as local cathartics (e.g. Jalap and Ipomea)
- As anti cancer (Podophyllum)
- In bronchial asthma (e.g. Cannabis)
- Used externally as mild antiseptic in the form of tinctures (Benzoin), ointment and plasters (Turpentine and Colophony)

Tannins

Introduction

*The term tannin was first time coined by **Seguin** in 1796. This term was used to denote substances present in plant extract which react with protein of animal hide, prevent their putrefaction and convert hide and skin into leather.*

Definition

"Complex substances that usually occur as mixtures of polyphenols that are very difficult to separate since they don't crystallize, are called tannins."

OR

"Tannins are polyhydroxy phenolic compounds."

Physical Properties

Color:

Dark brown or reddish brown

Taste:

Puckering or astringent taste

State:

Non-crystalline

Solubility:

Soluble in water, alcohol, dilute alkalis, glycerols and acetone.

Physical properties

- Tannins are **non-crystallizable** compounds.
- They are **soluble in water** forming colloidal solutions with acidic reaction and sharp astringent taste.
- They are soluble in dilute alkalis, alcohol, glycerol and acetone, but only sparingly soluble in other organic solvents.
- Their solutions precipitate heavy metals, alkaloids, glycosides and protein (e.g. gelatin).

Chemical properties

(i) Precipitation

(ii) Anti-oxidizing properties

(iii) Astringent

(iv) Carcinogenicity

(v) Reaction with salts

(vi) Reaction with potassium ferricyanide and ammonia

(i) Precipitation

Tannins have ability to precipitate solutions of;

Gelatin

Alkaloids

Glycosides

Heavy metals

Proteins

(ii) Anti-oxidizing properties

Because of accumulation of OH group on small size nucleus, these agents have anti-oxidant nature.

(iii) Astringent

Tannins have property to react with protein of mucous membrane and cause precipitation.

(iv) Carcinogenicity

Prolong use of tannin containing plant material is hazardous because it causes cancer. Habitual use of Areca catechu can cause oral and esophageal cancer.

(v) Reaction with salts

Hydrolysable tannins + ferric salt ⟶ Blue black precipitates

Condensed tannins + ferric salt ⟶ Brownish green precipitates

(vi) Reaction with potassium ferricyanide and ammonia

Tannins + Potassium ferricyanide/ ammonia ⟶ Deep red color formation

Importance of tannins

Medicinal Uses:

Antidote
Antiseptic
Algicidals
Astringents
Anti-carcinogenic

Industrial Uses:

Ink manufacture

Vegetable tanning

Preservatives

Biological Activities:

Inhibition of lipid per oxidation

Decrease in blood urea nitrogen content

Inhibition of plasmin

Lipolysis in fat cells

Medicinal and biological properties

- Tannin-containing drugs **precipitate proteins** and have been traditionally used as **styptics** (stop hemorrhage) and internally for the **protection of inflamed surfaces** of mouth and throat.
- They play an important role in the **treatment of burns**. They form a mild antiseptic protective layer on the surface of the injured skin below which regeneration of new tissue takes place.

- They act as **anti-diarrheals**, although not recommended in this respect as they usually delay elimination of bacterial toxins from the body.

- Tannins have been employed as **antidote** in **poisoning by heavy metals**, **alkaloids** and **certain glycosides** due to their **precipitation as tannates**.
- Recently **tannins as most polyphenols** were proved to have a potent **antioxidant effect**.

CLASSIFICATION OF TANNINS

True Tannins

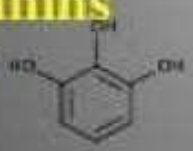
(High molecular weight compounds)

Pseudotannins

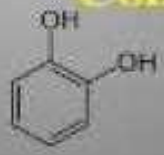
Low molecular weight compounds

e.g. Gallic acid, Flavan-3,4-diol

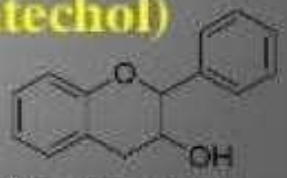
Hydrolysable Tannins (Pyrogallol)



Condensed Tannins (Catechol)

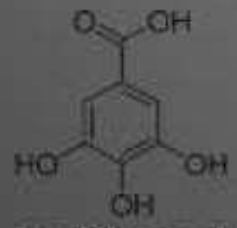


Complex tannins



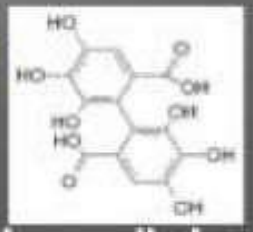
Flavan-3-ol

Gallitannins



Gallic acid

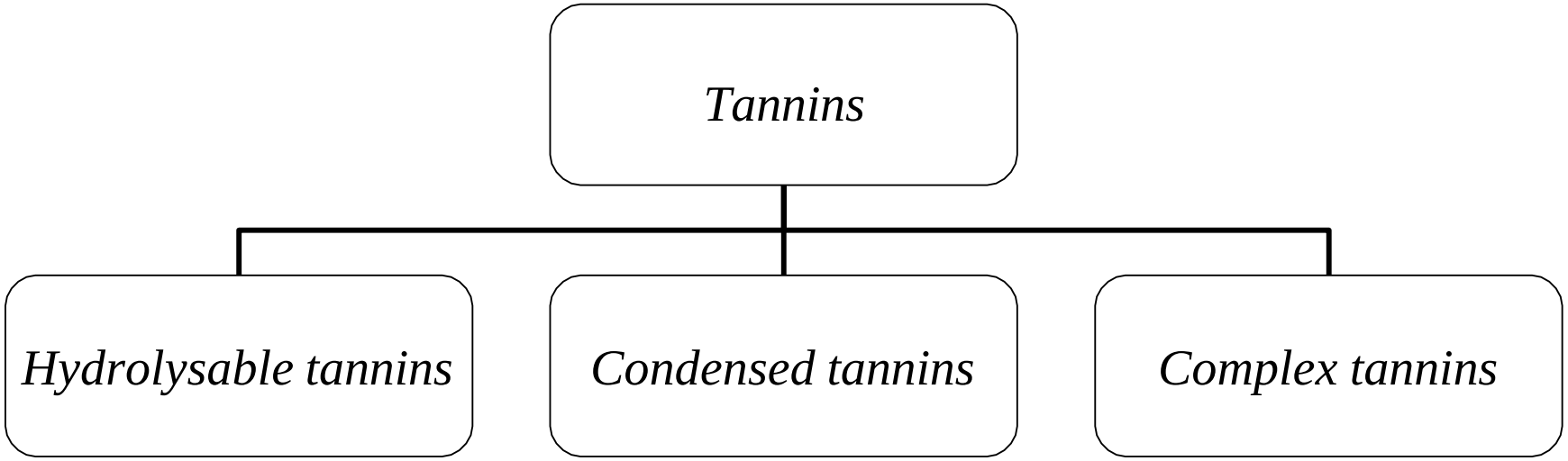
Ellagitannins



Hexahydroxydiphenic acid

Chemical Classification

Based on identity of phenolic nuclei involved and on the way they are joined.



(i) Hydrolysable tannins

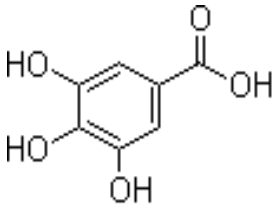
These tannins are hydrolyzed by enzymes or acids.

Precursors:

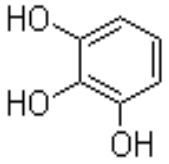
Phenolic acid (Gallic acid, Ellagic acid)

Glucose residue

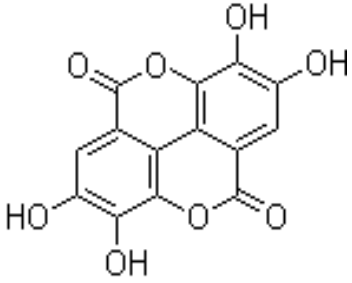
Between phenolic acids and glucose sugar, there is ester linkage



Gallic acid



Pyrogallol



Ellagic acid

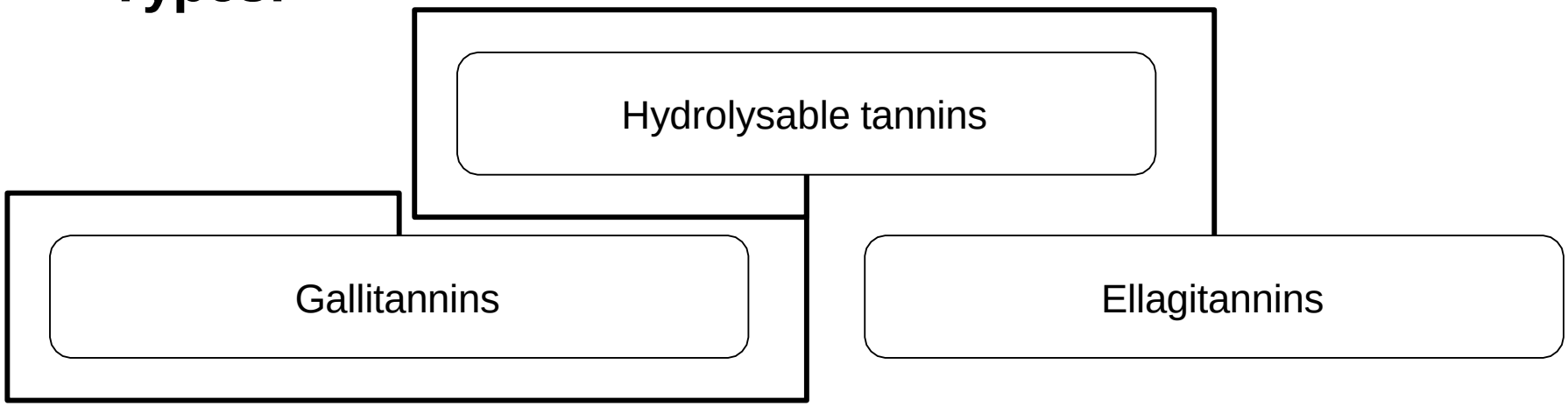
Properties:

Gallic acid + Iron salts ⟶ Blue flourescence

Ellagic acid + Iron salts ⟶ Blue flourescence

Hydrlysable tannins $\xrightarrow[\text{distillation}]{\text{dry}}$ Pyrogallols + Phenolic compounds

Types:



	Gallitannins	Ellagitannins
Occurrence	*Rhubarb, Clove* *Amla,* *chestnut*	*Pomegranate* *Eucalyptus, oak, myrobalan*
Hydrolysis	*Upon acid hydrolysis of Gallitannins, Gallic acid is produces.*	*Upon acid hydrolysis of Ellagitannins, Ellagic acid is produces.*
Properties	* *Rapidly soluble in water.* * *Free Gallic acid, in plant, is converted to gluco Gallitannins.*	* *Slowly soluble in water.* * *Present in plants in open and ring forms as Hexa hydroxy diphenic acid.*

(ii) Condensed tannins

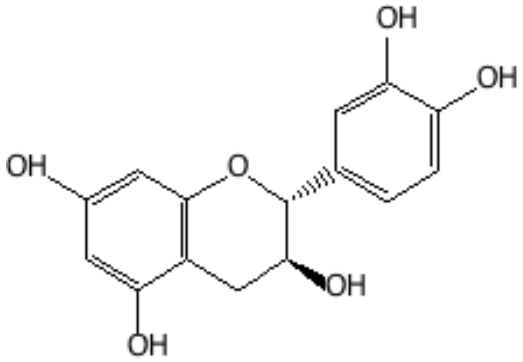
These tannins are derivatives of Flavonoid, catechin, flavonol-3-4-diol.

Precursors:

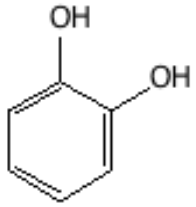
Flavonoid

Catechin

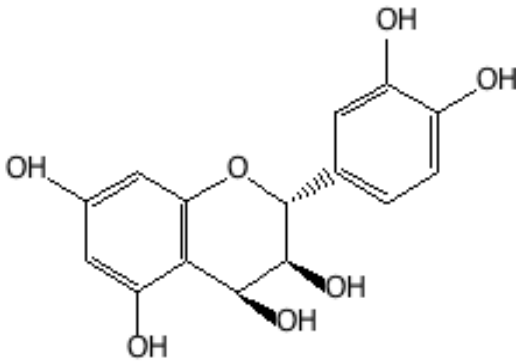
Flavonol-3-4-diol



catechin



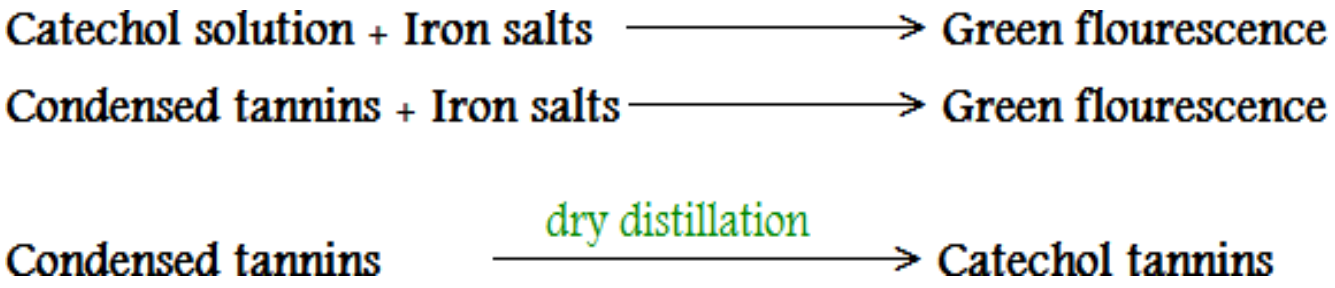
catechol



flavan–3,4–diol

Properties:

When heated with acids, these are self condensated, polymerized and converted to insoluble red colored complexes, called Phlobaphenes.



Examples: *Hamamelis, Green tea, wild cherry bark, pale and black catechu, Cinchona, Cinnamon*

(iii) Complex tannins

These tannins are mixtures of both, hydrolysable and condensed tannins

Examples:

Tea

Quercus

Castanea

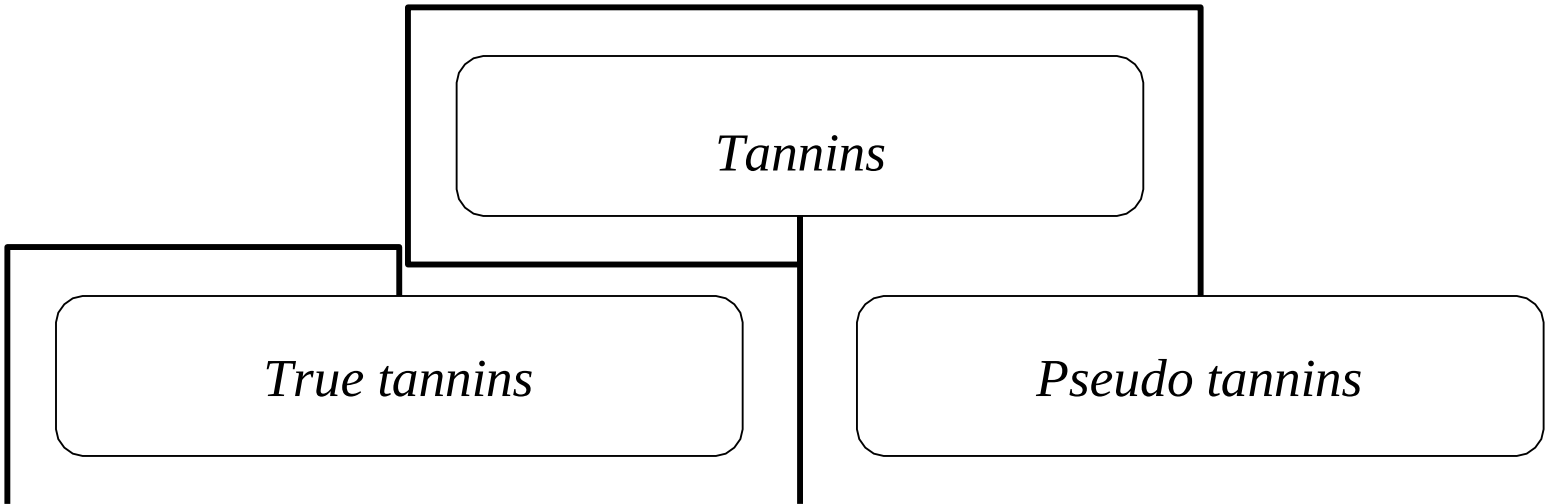
2nd Classification

Tannin is a substance which is detected qualitatively by tanning test (The Gold beater's skin test) and quantitatively by its adsorption on standard hide powder.

Depending upon this, tannins are of two types;

True tannins

Pseudo tannins



True tannins	Pseudo tannins
Polyhydroxy phenolic compounds which convert animal hide to leather by precipitating proteins and give positive Gold beater's skin test, are called true tannins.	*Phenolic compounds of plant origin that don't convert animal hide to leather but do give positive Gold beater's skin test, are called pseudo tannins.*
Molecular weight is 1000-5000	*Molecular weight is less than true tannins.*

Pseudo tannins- *This is not as such a separate group of tannins, but may be treated as a* ***subgroup*** *because they do not obey to goldbeater's skin test and are low molecular weight compounds.*

Eg: Chlorogenic acid in coffee, nuxvomica, ipecacuanhic acid in ipecacuanha, and catechins in cocoa are examples of pseudo tannins.

Extraction and Isolation of tannins

The various types of the methods of extraction depending upon the source of tannins are employed.

As the tannins are high molecular weight compounds it becomes difficult to isolate the tannins in pure form. Thus the solvents used are a mixture of polar, non-polar and semi-polar solvent like alcohol, ether, water, acetone etc.

Identification tests

Color reaction:

Tannins give color reaction with iron.

Catechol solution + Iron salts ⟶ Green flourescence

Condensed tannins + Iron salts ⟶ Green flourescence

Gallic acid + Iron salts ⟶ Blue flourescence

Ellagic acid + Iron salts ⟶ Blue flourescence

Identification tests

Matchstick test:

Dip matchstick in plant extract.

Dry it.

Moisten it with hydrochloric acid. And warm near flame.

Wood will turn pink or red in color due to phloroglucinol.

Gelatin test:

Solution of tannin (0.5%-1%) precipitates 1% solution of gelatin containing 10% sodium chloride.

Identification tests

Phenazone test:

Take 5ml of aqueous extract of drug.

Add 0.5grams of sodium acid phosphate.

Warm it and cool.

Filter solution.

To the filtrate, add 2%solution of Phenazone.

Tannins will be precipitated.

Precipitates will be bulky and colored.

Identification tests

Gold beater's skin test:

Gold beater's skin is a membrane prepared from intestine of Ox and I behaves similarly to un tanned skin.

Soak a small piece of Gold beater's skin in 2% hydrochloric acid.

Rinse it with distilled water.

Place it in solution to be tested for 5 minutes.

Wash in water and transfer to 1% solution of ferrous sulphate.

Black or brown color of skin indicates presence of tannins.

It is a quantitative test and +ive only for true tannins

Identification tests

Catechin test:

Catechin when heated with acid produce phloroglucinol. Phloroglucinol can be detected with matchstick test.

Test for chlorogenic acid:

Treat extract containing chlorogenic acid with aqueous ammonia and expose it to air. Green color will appear gradually.

Volatile Oils

- Volatile oils are the odorous liquid obtained from various plant parts.
- Volatile oils **evaporate** on exposure to air at ordinary temperature.
- As volatile oils are responsible for the essence or odour of the plant they are also known as **essential oils**.

- All volatile oils consist of complex chemical mixtures.
- they vary in their chemical composition that they consist of many organic compounds as (hydrocarbons, alcohol, ketone, aldehydes, oxides, esters and others), and only a few possess a single component in a high percentage e.g.

1. Volatile mustard oil yields not less than 93% of allylisothiocyanate

2. Clove oil contains not less than 85% of phenolic substances, chiefly eugenol.

Properties of volatile oils

- Volatile oils are colorless, particularly when they are fresh, but on long standing they may oxidize and resinify, thus **darkening in color**. **To prevent this darkening, they should be stored in a cool, dry place**.
- They possess characteristic odors, immiscible with water, but they are sufficiently soluble to impart their odor to water as aromatic waters. Volatile oils, are soluble in ether, alcohol, and most organic solvents.

Differences between volatile oils and fixed oils

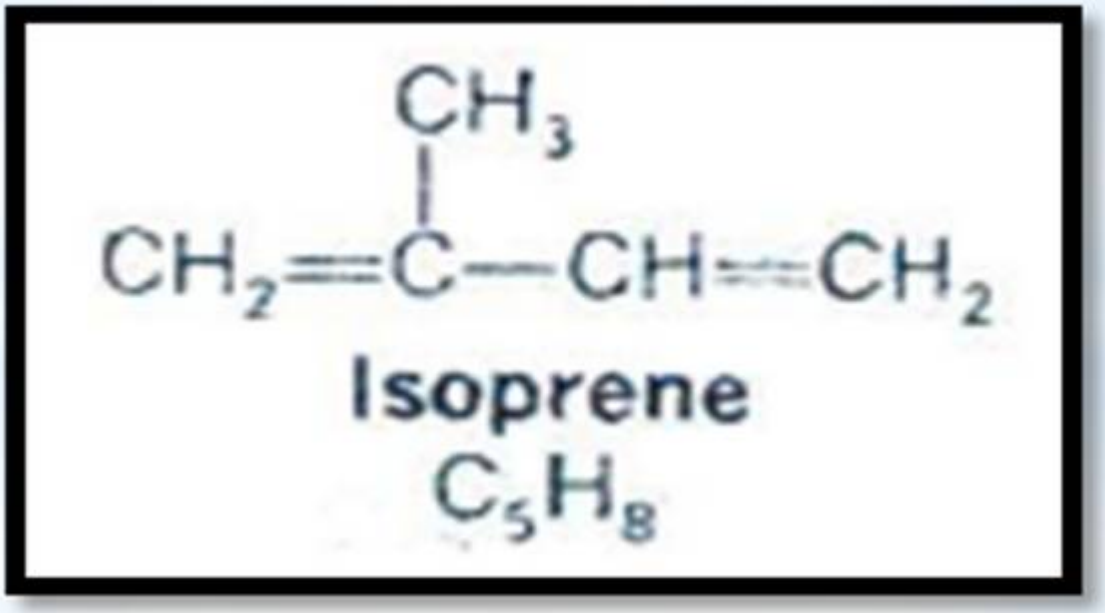
1. Volatile oils can be **distilled** from their natural sources
2. They **do not** consist of **glyceryl esters of fatty acids**
3. They **do not leave permanent grease** spot on paper and **cannot be saponified with alkalies**
4. They **do not become rancid** as do the fixed oils but instead, on exposure to light and air they **oxidize and resinify**

Chemistry of volatile oil

- Chemical constituents of volatile oils may be divided into two classes based on their biosynthetic origin,
- **terpene derivative** (formed via the **acetate-mevalonic acid pathway,**
- and **aromatic compounds** (phenyl propanoids) (formed via the **shikirnic acid-phenylpropanoid route**).

1. Terpenes

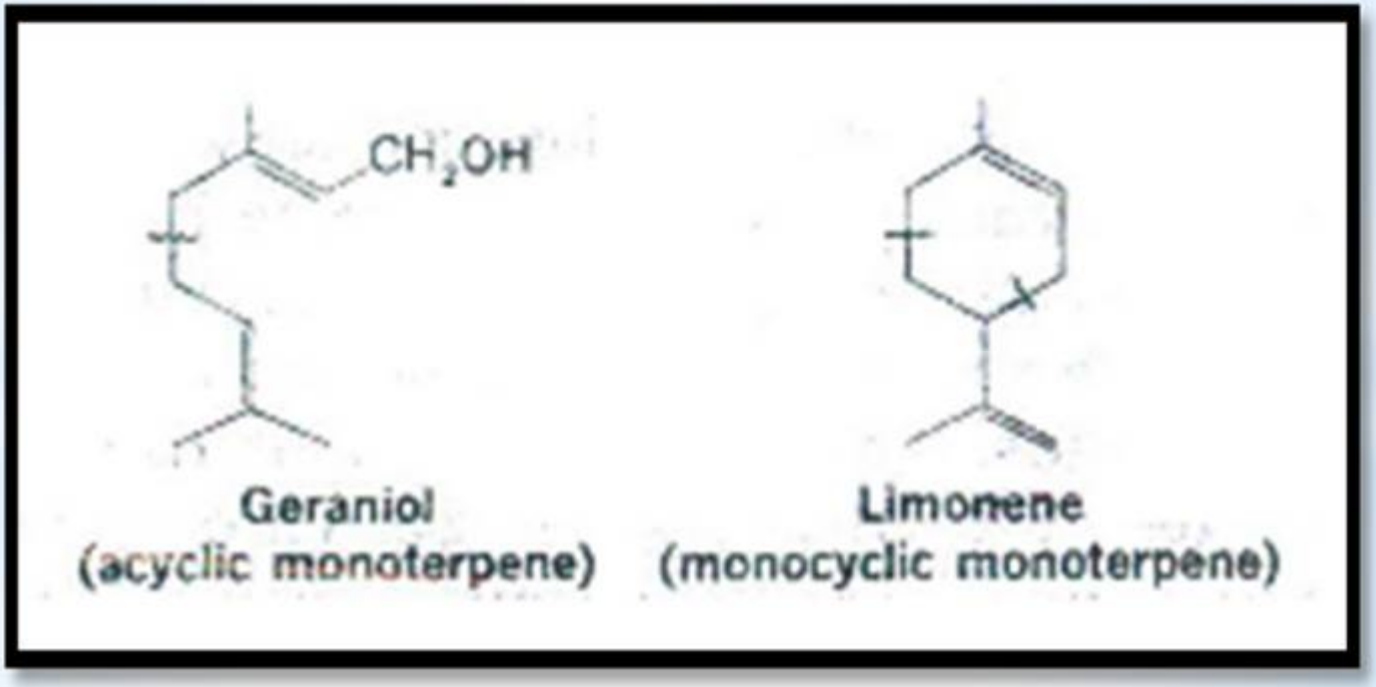
- Terpenes are defined as natural products whose structures consist of isoprene units.
- These units arise from acetate via mevalonic acid and are branched-chain, 5-carbon units containing 2 unsaturated bonds.



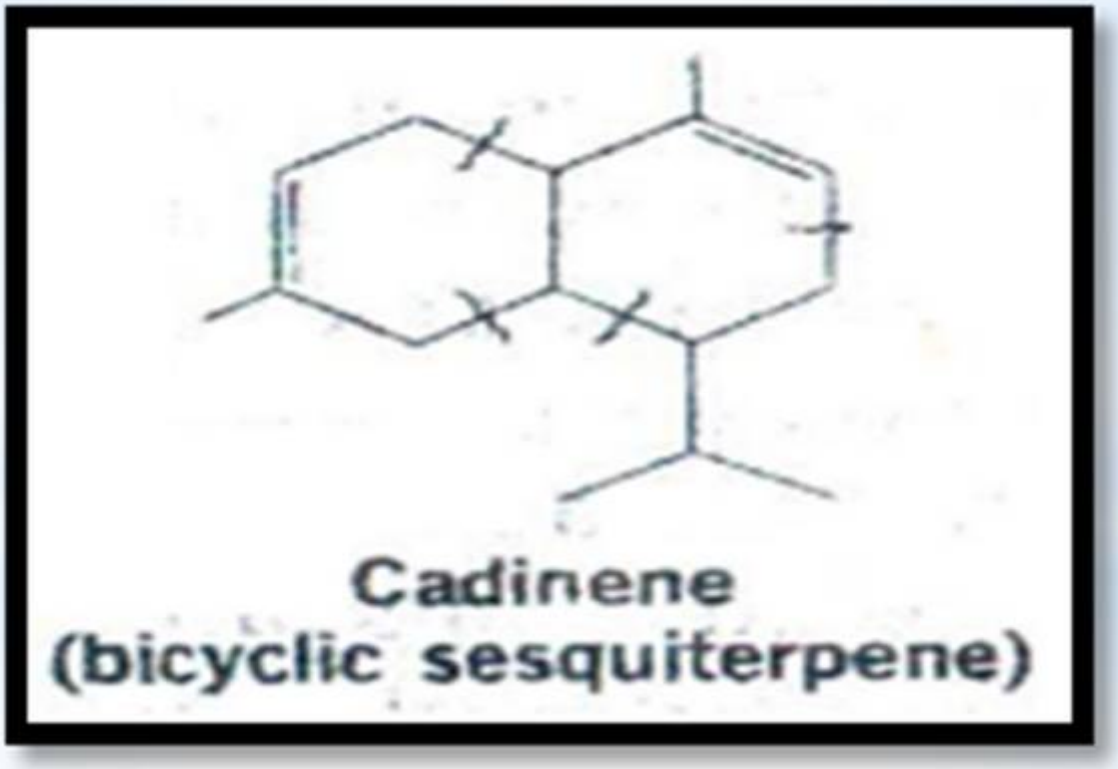
1. Terpenes Classification

Depends on the number of isoprene units, terpenes can be classified into:

1.Monoterpenes: are composed of 2 isoprene units example geraniol and limon



2. Sesquiterpenes: contain 3 isoprene units.
Example cadinene



3. Diterpenes: are consist of 4 isoprene units

4. Triterpenes: are composed of 6 isoprene units.

The terpenes found most often in volatile oils are monoterpenes.

2. Phenyl propanoids

They are another major group of volatile-oil constituents. These compounds contain the C6 phenyl ring with an attached C3 propane side chain.

Methods of Obtaining Volatile Oils

1. **Distillation in water or steam**
2. **Expression**
3. **Extraction**
4. **Enzymatic hydrolysis**

Medicinal and Commercial Uses

1. Used as **flavoring agent**
2. **Local irritant** e.g. camphor
3. **Anesthetic** e.g. clove oil
4. **Prophylactic** against insects e.g. citronella oil
5. **Bactericidal and antiseptic** as in soap and gargles
6. **Against asthma**, the action is due to volatile oil which irritate the mucous membrane of the respiratory tract causing the expectorant action

7. **Carminatives** e.g. peppermint oil

8. **Urinary antiseptic** e.g. Buchu leaves

9. **Anthelmintic** e.g. ascaridol

10. **The manufacture of perfumes, soaps, and deodorizers** and for providing odor to household cleaners, polishes, and insecticides

Classification of volatile oils and volatile oil containing drugs

(1) Hydrocarbons (2) Alcohols (3) Aldehydes
(4) Ketones

(5) Phenols (6) Phenolic ethers (7) Oxides
(8) Esters

Evaluation of Volatile Oils

Several physicsl and chemical properties are used to evaluate the volatile oils.

-Physical properties

1.Solubility

2.Specific gravity

3.Refractive index

4.Optical rotation

-Chemical properties

1.Determination of acid

2.Determination of ester

3.Determination of alcohol

4.Determination of ketone and aldehyde

5.Determination of phenol

1. Hydrocarbon Volatile Oils

Hydrocarbons occur in practically all volatile oils, they occur as:

A.Monocyclic monoterpenes: e.g. Limonene

B.A dicyclic monoterpene: e.g. Pinene and Sabinen.

C.Acyclic monoterpene hydrocarbons: e.g. myrcene.

D.Sesquiterpene hydrocarbon: e.g. Cadinene

Turpentine Oil

Turpentine oil or spirits of turpentine is the volatile oil distilled from the oleoresin obtained from **Pinus palustris** and from other species of **Pinus Linné** (Fam. Pinaceae).

Uses: Turpentine oil is used as a **counterirritant** in Vicks Vaporub, and as **expectorant**.

2. Alcohol Volatile Oils

Alcohols found in volatile oils may be classified into:

A. Acyclic alcohols: e.g. gerarniol, linalool, citronellol

B. Monocyclic alcohols: e.g. menthol (from peppermint) and α-terpineol.

C. Dicyclic alcohols: e.g. Borneol

Among the important alcohol volatile oil drugs are **peppermint, cardamom oil, rose oil, orange flower oil, and pine oil.**

Peppermint

Peppermint consists of the dried leaf and flowering top of **Mentha piperita** (Farn. Labiatae). Peppermint oil contains menthol as a major constituent.

Uses: It has been used as a **carminative, a stimulant, and a counterirritant,** commercial use is as a **flavoring agent**, especially for chewing gum and in the **antacid products.**

3. Aldehyde Volatile Oils

Aldehydes occurring in volatile oils may be divided into acyclic and cyclic.

A. Acyclic aldehyde: Neral

B. Cyclic aldehyde: Cyclocitial.

C. Aromatic aldehyde: Cinnamaldehyde, vanillin and Anise oil.

Plants containing aldehyde volatile oil

1. Cinnamon: is the dried bark of **Cinnamonium loureirii**. (Fam. Lauraceae). Contains cinnamaldehyde and terpenes.

Uses: as **flavoring agent, carminative and antiseptic**.

2. Lemon Peel: Lemon is the fruit of **Citrus limon** (Fam. Rutaceae). Lemon peel is the outer yellow rind of the fresh ripe fruit of C. limon.

Uses: Lemon peel is a **flavoring agent, and stimulant**.

3. Lemon oil: is the volatile oil obtained by expression, without the aid of heat, from the fresh peel of the fruit of C. limon.

Uses: Lemon oil is a **flavoring agent. It has stimulant**, and **carminative**. It is also used in **cosmetics and liquid cleansers** because the aroma and flavor are widely accepted by consumers.

4. Bitter almond: is the dried ripe kernels of **prunus amygdalis**, F: Rosaceae.
Contains benzaldehyde.

4. Ketone Volatile Oils

Ketones occurring in volatile oils may be divided into:

A. Monocylic terpene ketones: including menthone.

B. Dicyclic ketones: including camphor.

Plants containing ketone volatile oils

1. Camphor: Camphor is a ketone obtained from Cinnamomum camphora

(F: Lauraceae)

Uses: Camphor is a **topical antipruritic, rubefacient, and anti-infective.**

2. Caraway: Consist of dried ripe fruit of Carum carvi (F: Umbellifarae).

Contains volatile oil from 5-7% and 2% fixed oil.

Uses: It used as **flavoring agent and carminative**.

3. Spearmint: Spearmint consists of the dried leaf and flowering top of **Mentha spicata** (Fam. Labiatae).

Uses: Spearmint is **flavoring agent and possesses carminative properties.**

5. Phenol Volatile Oils

- **Two kinds of phenols occur in volatile oils**: those that are present naturally and those that are produced as the result of destructive distillation of certain plant products.
- **Eugenol, thymol, and carvacrol** are the most important phenols occurring in volatile oils.

Plants containing phenol volatile oil

1. Thymol: is a phenol obtained from **thyme oil (Thymus vulgaris)** F: labiatae.

Uses: Thymol is an **antifungal and antibacterial agent**. It is employed topically in lotions, creams, and ointments. Thyrnol is used in the **feminine hygiene products**

2. Clove: Clove or cloves is the dried flower bud of **Eugenia caryophyllus** (Fam. Myrtaceac). Contains chiefly eugenol.

Uses: Clove oil is classed as a **flavor**. It is commonly employed as **a toothache** remedy that is applied topically to dental cavities as required. Clove oil also possesses **antiseptic, counterirritant, and carminative properties**.

6. Phenolic Ether Volatile Oils

- A number of phenolic ethers occur in volatile oils as anethole from anise, safrole from sassafras and nutmeg.
- myristicin (methoxysafrole) is a derivative of **safrole from nutmeg.**

Plants containing phenolic ether volatile oils

1. Nutmeg

Nutmeg or myristica is the dried, ripe seed of **Myristica fragrans** (Fam. Myristicacea). Contain volatile oil (myristicin) and safrol and fixed oils.

Uses: Myristica is a **flavor and a condiment**. it is a useful agent for controlling **diarrhea** associated with certain carcinomas. Has **CNS effects**, large quantity up to 15mg cause hallucination.

2. Anise: consists of dried ripe fruit of **pimpinella anisum** F:umbellifarae. Contains 1-3 % volatile oils consist of 90-93% anethol.

Uses: It used as **carminative and flavoring agent**.

7. Oxide Volatile Oils

Cineole (eucalyptol) is found in eucalyptus which consist of fresh leaves of **Eucalyptus globus** F: Myrtacea. contains about 70 to 85% cineole.

USES: Eucalyptus oil is classed as a **flavor**. It is frequently used as an **antiseptic, diaphoretic, and expectorant.**

8. Ester Volatile Oils

- A wide variety of esters occurs in volatile oils.
- The most common are the acetates of terpineol, borneol, and geraniol.
- Examples of esters in volatile oils are **allyl isothiocyanate in mustard oil and methyl salicylate in wintergreen oil**.

Methyl salicylate is obtained from the leaves of **Gultheria procunbens** F: Ericaceae.

Uses: It used as **analgesic, anti-inflammatory** drug, has **antiseptic, and antirheurnatic** properties. **Alleviate** fever, headache, sore throat and various ache pain. It's a common **flavoring agent** for chewing gums, candies, and for dental hygiene products such as mouth wash and paste.

CULTIVATION, COLLECTION, PROCESSING AND STORAGE OF CRUDE DRUGS

CULTIVATION:

Cultivation may be defined as the production of crops using prepared land in controlled environment by unskilled or skilled people.

Cultivation produce improved quality of plants. It helps in selecting the species, varieties or hybrids that have the desired phytoconsituents. Due to the controlled environmental growth better plant product is obtained and makes the collection & processing steps easier when compared to wild sources.

- Cultivation results in obtaining plants with maximum secondary metabolites.
- It leads to industrialization in the country by the regular supply of plants.
- Serves as a useful tool for research purpose.

Advantages:

1. It ensures quality & purity of medicinal plants.
2. Collection of crude drugs from the cultivated plants gives a better yield and therapeutic quality.
3. Cultivation ensures regular supply of crude drugs.
4. The cultivation of medicinal & aromatic plants leads to industrialization to a regular extent.
5. Cultivation permits application of modern technological aspects as mutation, polyploidy & hybridization.

METHOD OF PROPAGATION

Propagated by two usual methods:

I. Sexual methods (Seed propagation)
II. Asexual methods

I. SEXUAL METHODS:

Plants are raised from seeds and are known as seedlings.

Advantages:

a) Seedlings are long lived (in case of perennial drugs) & bears more heavily (in case of fruits). Plants are sturdier.
b) Seedlings are comparatively cheaper & easy to raise.
c) In case of plants where other vegetative methods cannot be utilized, propagation from seeds is the only method of choice.

Limitations:

a) Seedling trees are not uniform in their growth and yielding capacity as compared to grafted trees.
b) Require more time to bear as compared to grafted plants.
c) Cost of harvesting, spraying of pesticides, etc. is more as compared to grafted trees.

For propagation purpose:

- Seeds must be of good quality.
- Capable of high germination rate.
- Free from diseases & insects.
- Also free from other seeds, used seeds & extraneous materials.

METHODS OF SOWING THE SEEDS

a) Broadcasting Method:

If the seeds are extremely small the sowing is done by this method. Here the seeds are scattered freely in well prepared soil for cultivation. (If deeply sown / covered by soil may not germinate).

Eg --- Isabgol, Linseed, etc.

b) Dibbling :

When the seeds of average size & weight are available, they are sown by placing in holes.

Number of seeds to be put in holes vary from 3-5, depending on the vitality, sex of the plants needed for the purpose & the size of the plant coming out of the seeds.

Eg : **Fennel** --- 4-5 fruits put in a single hole keeping suitable distance in b/w two holes.

Castor --- 2-3 seeds are put

Papaya --- Plants are unisexual & only female plants are desired for medicinal purpose. Hence, 5-6 seeds are put together & after the sex of the plants is confirmed, healthy female plant is allowed to grow while male plants & others are removed.

c) <u>Miscellaneous:</u>

The seeds are sown in nursery buds, seedlings are thus produced are transplanted to farms for further growth.

Eg. Cinchona, Cardamom, Clove, Digitalis, Capsicum.

d) <u>Special treatment of seeds:</u>

To enhance germination, special treatments to seeds may be given.

- Eg. (i) Soaking in water for a day (eg: Castor seeds) and slow germinating seeds
- Soaked in sulphuric acid (eg. Henbane)
- Testa is removed partially by grindstone or by pounding seeds with coarse sand (eg. Indain Senna)
- Several plant hormones like Gibberillins, auxins are also used.

II. <u>ASEXUAL METHODS:</u>

a) Natural Methods of Vegetative Propagation
b) Artificial Methods Of Vegetative Propagation
c) Aseptic Method of Micropropagation (Tissue Culture Method)

a) <u>Natural Methods of Vegetative Propagation:</u>

It is done by sowing various parts of the plants in well prepared soil.

Eg.	Bulbs	-	Squill, Garlic
	Corm	-	Colchicum, Saffron
	Tubers	-	Potato, Jalap, Aconite
	Rhizomes	-	Ginger, Turmeric
	Runner	-	Peppermint
	Suckers	-	Mint, Banana

Offsets	-	Aloe, valerian
Stolons	-	Liquorice

b) Artificial Methods Of Vegetative Propagation:

Methods by which plantlets or seedlings are produced from vegetative part of the plant by using some technique or process is known as artificial methods of vegetative propagation.

a. Cutting

i. Soft wood cutting	:	Berberry
ii. Semi hard wood cutting	:	Citrus
iii. Hard wood cuttings	:	Orange, Rose
iv. Root cuttings	:	Brahmi
v. Leaf cuttings	:	Bryophyllum
vi. Leaf bud cuttings		

b. Layering

i. Simple layering	:	Lemon
ii. Serpentine layering	:	Jasmine
iii. Air layering (Gootee)	:	Mango, Cashew nut
iv. Mount layering		
v. Trench layering		
vi. Tip layering		

c. Grafting

i. Whip grafting	:	Apple, Rose
ii. Tongue grafting	:	
iii. Side grafting	:	Sapota, Cashew nut
iv. Approach grafting	:	Sapota
v. Stone grafting	:	Mango

c) Aseptic Method of Micropropagation (Tissue Culture Method):

The plants are developed in an artificial medium under aseptic conditions from fine pieces of plants like single cells, callus, seeds, embryos, root tips, shoot tips, pollen grains, etc. They are also provided with nutritional and hormonal requirements.

Preparation and Types Of Nursery Buds:

- For various reasons, seeds cannot be sown directly into soil i.e. very small size, high cost, poor germination rate and long germination time.
- Under such conditions, seeds are grown into the nursery buds which is not only is economical, but one can look after the diseases during germination period.
- Small size of beds can be irrigated conveniently along with fertilizers, as and when necessary.

Four types of nursery beds are there:

a) Flat bed method
b) Raised bed method
c) Ridges & furrow method
d) Ring & basin method

Methods of Irrigation:

1) Hand Watering : Easy to operate, Economical
2) Flood watering : Easy to operate, results in wastage of H_2O
3) Boom watering : Easy to operate, but restricted utility
4) Drip irrigation : Scientific, Systematic, Easy to operate, Costly
5) Sprinkler irrigation: Costly, Gives good results

FACTORS AFFECTING CULTIVATION

Cultivation of plants offers a wide range of advantages over the plants obtained from wild sources.

The factors are of 2 types:

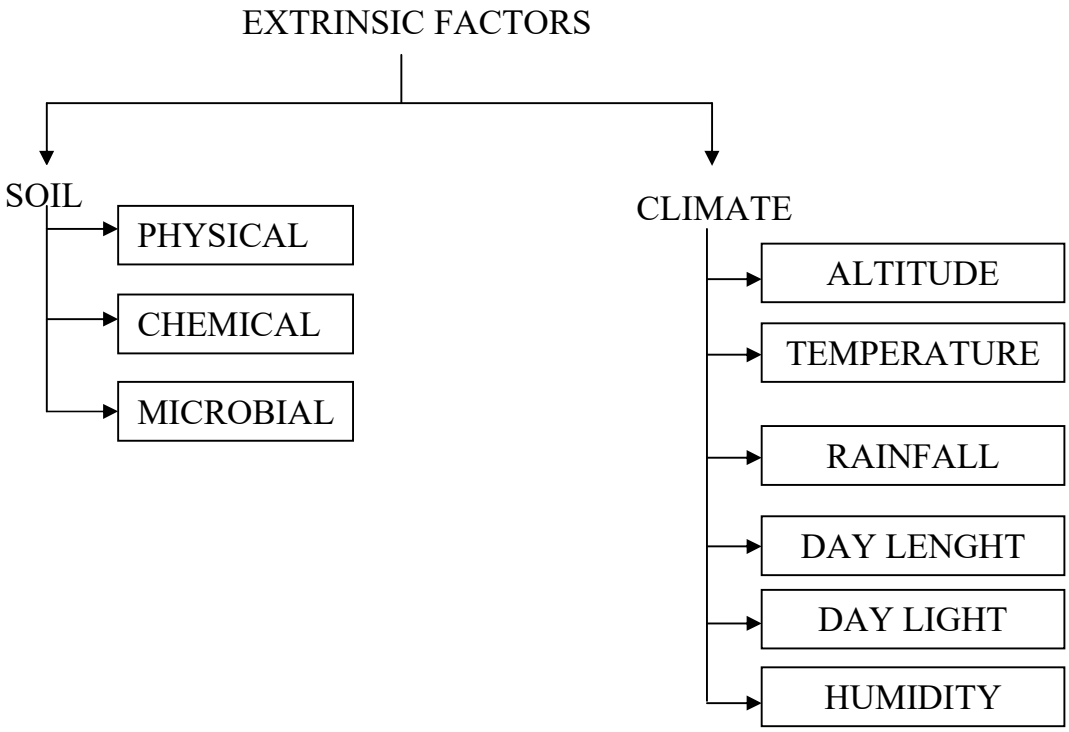
1) Intrinsic Factors
2) Extrinsic Factors

Intrinsic Factors ⟶ Genetic Factors

Extrinsic Factors ⟶ Soil
Extrinsic Factors ⟶ Climate

Examples of the process that affects genetic factors are

i. Polyploidy
ii. Hybridization
iii. Mutation



EXTRINSIC FACTORS

I. CLIMATE:

a) Altitude:

Altitude is a very important factor in cultivation of medicinal plants.

Tea, Cinchona, Eucalyptus	:	1000-2000 meters
Cinnamon, Cardamom	:	500-100 meters
Senna	:	at sea level

Eg.

Plants	Altitudes(m)
Tea	1000-1500
Cinchona	1000-2000
Camphor	1500-2000
Cinnamon	250-1000
Coffee	1000-2000
Clove	Up to 900
Saffron	Up to 1250
Cardomom	600-1600

b) Temperature:

Temperature is a critical factor controlling the growth, metabolism & there by the yield of secondary metabolites. Many plants will grow better in temperature regions during summer, but they lack in resistance to withstand frost in winter.

Eg: Optimum temperatures for drug cultivation:

Plants	Optimum Temperature
Tea	70-90
Cinchona	60-75
Coffee	55-70
Cardamom	50-100

c) Rainfall:

- For the proper development of plant, rainfall is required in proper measurement.
- The effects of rainfall on plant must be considered in relation to the annual rainfall throughout the year with the water holding properties of the soil.
- Variable results have been reported for the production of constituents under different conditions of rainfall.
- Excessive rainfall could cause a reduction in the secondary metabolites due to leaching of water soluble substances from plants.

- Xerophytes plants like Aloes do not require irrigation or rainfall.

d) <u>Day length</u>:

- Length of day has an effect on metabolite production
- The plants that are kept in long day conditions may contain more or less amount of constituents when compared to the plants kept in short day conditions.

  Eg: Peppermint produce menthone, menthol and traces of menthofuran in long day conditions but only menthofuran in short day conditions.

e) <u>Day light:</u>

- Developments of plants vary much in both the amount & intensity of the light they require.
- Wild plants meet the required conditions.
- In case of cultivated plants we have to fulfill the requirements.
- Radiation also affects the metabolite production & cultivation.

  Eg: Day light – Increases the amount of alkaloids in Belladonna stramonium, Cinchona, etc.

II. <u>SOIL</u>

Each and every plant species have its own soil & nutritive requirements. The three important basic characteristics of the soil are their physical, chemical & microbial properties.

a) <u>Physical properties:</u>

- Soil provides mechanical support, water and essential food for the development of plants.
- It consists of air, water, mineral matters and organic matters.
- Variations in particle size result in different soils ranging from clay, sand and gravel.
- Particle size influences the water holding capacity of soil.

Type of soil on the basis of particle size:

Particle size (diameter)	Type of soil
Less than 0.002 mm	Fine clay
0.002-0.02 mm	Coarse clay or silt
0.02-0.2 mm	Fine sand
0.2-2.0 mm	Coarse sand

Type of soil on the basis of % covered by clay:

Type of soil	% covered by clay
Clay	More than 50% of clay
Loamy	30-50% of clay
Silt loamy	20-30% of clay
Sandy loamy	10-20% of clay
Sandy soil	More than 70% of sand
Calcarious soil	More than 20% of lime

Soil Fertility---- It is the capacity of soil to provide nutrients in adequate amounts & in balanced proportions to plants.

b) <u>Chemical Properties:</u>

- Soil consists of inorganic elements & organic matters.
- It needs micro & macro nutrients in an optimum level.
- % of silica is important.

The inorganic matter consists of 1^o nutrients & 2^o nutrients & micronutrients.

1^o nutrients --- N, P & K

2^o nutrients --- Mg, Ca, S (Required in small quantity)

Micronutrients --- Cu, Mn, Fe, Boron, Molybdinum & Zn

Organic matters --- decayed matter of the plants.

c) <u>Microbial properties:</u>

The soil consists of different types of micro organism, which fix nitrogen from atmosphere in soil & the plants can use them for day to day activities.

Eg: Rhizobium, Azotobactor, Blue green algae, Azolla

FERTILIZERS AND MANURES

- Plants need food for their growth & development.
- They also need CO_2, sun-rays, water & mineral matter from soil.

a) Chemical Fertilizers:

- Plants are in need of 16 nutrient elements for synthesizing various compounds.
- Some of them are known as 1^o nutrients (P, K, N)
- Mg, Ca & S are required in small quantities, hence known as 2^o nutrients.
- Trace elements like Cu, Mn, Fe, Boron, Molybdenum, Zn are also necessary ⟶ Micronutrients.
- C, H, O_2 & Cl_2 are provided from H_2O & air.
- Every elements has its own function to perform, its deficiency is characterized by certain symptoms or disease conditions.

b) Manures:

- Farm yard manure (FYM/compost), castor seed cake, poultry manures, Neem & Karanj seed cakes, vermin compost, etc. are manures.
- Oil cakes & compost normally consists of 3-6% of Nitrogen, 2% Phosphate & 1-1.5% Potash.
- These are easily available for plants.
- Bone meal, fish meal, biogas slurry, blood meal and press mud are other forms of organic manures.

c) Biofertilizers:

- Inadequate supply, high cost & undesirable effects are the demerits of fertilizers & manures.
- So opted for some other types of fertilizers i.e. micro organisms & lower organisms which fix atmospheric nitrogen in soil.

Eg: Rhizobium, Blue green algae, Azolla, etc.

PEST MANAGEMENT & NATURAL PEST CONTROLLING AGENTS

Pests are undesirable plant or animal species that causes a great damage to the plants.

Pesticides are chemical derived from synthetic & natural sources effective in small concentration against pest.

TYPES OF PESTS:

1. **Microbes**
    a) Fungi
    b) Bacteria
    c) Virus
2. **Insects**
3. **Non-insects Pests**
4. **Weeds**

1. MICROBES:

They include fungi, bacteria & viruses.

a) FUNGI:

Phytophthora nicotianae is a dreadful disease occurring on Belladonna & other plants in which dropping of young leaves & branches, yellowing of older leaves & drying of whole apical portions occurs. This disease is known as **Phytophthora root rot**.

Pythium rhizome rot is caused by ***Pythium pinosum.***

Leaf spot caused by ***Septoria digitalis***

Little leaf disease caused by ***Phytophthora cinnamomi***.

Leaf spot on Datura species caused by ***Alternaria tennusima.***

b) BACTERIA:

Crown gall disease caused by ***Argobacterium tumefaciens***.

Pierce's disease caused by ***Xylella fastidiosa***, in this leaves become slightly yellow or red along margin, dry & dies.

c) <u>VIRUSES:</u>

The viruses shows disease symptoms on Rauwolfia, Tobacco, Datura, Vinca & Eucalyptus. The disease occurring on plants are:

- **Necrosis** of leaves, stems & petioles in different Solanaceous plants.
- **Tobacco mosaic virus, Cucumber mosaic virus** & **Tobacco ring spot** virus are observed on Digitalis.
- **Cucumber mosaic virus** is detected on Hyoscyamus.

Controlling techniques:

i. Fumigation of the soil.
ii. Fungicide, Bactericide
iii. Pruning
iv. Proper water & fertilizer management.
v. Good sanitation
vi. Heat treatment of plant stock
vii. Genetic improvement.

2. <u>INSECTS:</u>

Morphologically insects are two types:

a) Biting & chewing
b) Piercing & sucking

The insects affecting the medicinal plants are:

a) **<u>Ants:</u>** Argentine ant, Grey ant, Pavement ant, Southern fire ant, Thief ant: They spoil the soil by making nest & they feed honey dew secreted in plants.
b) **<u>Branch & Twig borer</u>:** Burrow into the canes through the base of the bud.
c) **<u>Click beetle</u>:** Can feed buds.
d) **Cut worms:** Injures the buds & they may not develop
e) **Leaf hoppers:** Removes the leaf contents of the leaf cell, appear as yellow spot.

Mentha species affected by ***Phytomyza atricornis***

Rauwolfia attacked by ***Diaphania nilgirica***

Dill affected by ***Papillo machon***

Belladonna losses leaves due to ***Gonocephalum*** Species.

Controlling Methods:

i. Tilling the soil
ii. Trapping the insects
iii. Insecticides
iv. Create competition for mating with females among males
v. Cutworms can be prevented by natural enemies.

3. NON-INSECTS PESTS:

They are grouped into 2 categories:

i. **Vertebrates:** like rats, monkeys, birds, rabbits, squirrels, deer, pig, etc.
ii. **Invertebrates:** like nematodes, crabs, snails, mites, etc.

The rodents have sharp & gnawing incisor teeth with which they cause considerable spoilage to stored crude drugs & the faecal matters of such animals causes serious contamination of crude drugs.

Controlling Techniques:

i. Construction of concrete ware houses, traps, biological methods rodenticides, etc.

4. WEEDS:

A weed is a undesired plant. Weed reduces growth & yields of plant by competing for water, nutrients & sunlight.

Few common weeds are

i. Bermudagrass
ii. Dallis grass
iii. Pigweeds
iv. Pineapple weeds

Some weeds causes allergies

Eg: Ragweed, Median tea produces hay fever.

Some causes dermatitis

Eg: Poison ivy, western poison oak, etc.

Controlling Techniques:

By using low rates of herbicides.

Two types of herbicides are available:

i. Pre emergent herbicides: (Active in soil against germinating weedings)
ii. Post emergent herbicides: (Control of most annual & perennial grasses)

METHODS OF PEST CONTROL

i. **Mechanical method**
ii. **Agricultural method**
iii. **Biological method**
iv. **Chemical method**

i. MECHANICAL METHOD:

- It employs manual labour along with different devices for collection & destruction of pest.
- Simple techniques ---- Hand picking, pruning, burning & trapping of pests.
- Collection & destruction of eggs, larvae, pupae and adults of insects.
- Rodents --- Construction of concrete warehouses.
- Flying insets --- Funnel shaped containers containing flavoured attractants.

ii. AGRICULTURAL METHODS:

- Plant breeding techniques --- genetic manipulations --- production of pest resistant species.
- Systemic insecticides --- adsorbed through the roots & reach to leaves by which all the foliage portion becomes distasteful for the insects.
- Crop rotation
- Changing the environment --- obstruction in life cycle.

iii. BIOLOGICAL CONTROL:

- By combating the pests, mostly insects with other living organisms.
- The chemical substances produced & released by some female insects are capable of eliciting a sexual response from the opposite sex, which could be properly exploited for biological control of pests. Such substance are called sex pheromones.
  Eg : 7,8-epoxy-2-methyloctadecane from Gypsy moth.

iv. CHEMICAL CONTROL:

a. Rodenticides	: Warfarin, Strychnine, Arsenic trioxide, Red squill, Thallium sulphate
b. Insecticides	: DDT, Parathion, Malathion, Carbamates, Pyrethroids, etc.
c. Acaricides	: Tetradifon, Chlorobenzoate
d. Fungicides	:Bordeaux mixture, Chlorophenols, antibiotics, 4^{o} ammonium compounds, etc.
e. Herbicides	: 2,4- dichlorophenoxy acetic acid, calcium acetate, H_2SO_4, etc.

PLANT HORMONES AND THEIR APPLICATIONS

PLANT GROWTH REGULATORS

The growth and development of plants is regulated by a number of chemical substances which together exert a complex interaction to meet the needs of the plants. The term plant growth regulators include both the native (endogenous) & synthetic (exogenous) substances which modify the plant growth.

Five groups of plant hormones are well established, they are:

I. Auxins
II. Gibberellins
III. Cytokinins
IV. Abscisic acid & its derivatives
V. Ethylene

They are,

a) Specific in action
b) Active in very low concentration
c) Regulate cell enlargement, cell division, cell differentiation, organogenesis & dormancy. (Dormancy is a period in an organism's life cycle when growth, development & physical activity are temporarily stopped. This minimizes metabolic activity & therefore, helps an organism to conserve energy)

The essential role of this substance is illustrated by cell & tissue culture. In the field of pharmaceuticals, these can be used for producing plants containing an enhanced proportion of active constituents.

AUXINS

The term auxin is derived from the greek word "auxein" which means to grow.

Generally compounds are considered as auxins if they are able to induce cell elongation in stems & otherwise resemble indolacetic acid (the first auxin isolated) in physiological activity.

These growth promoting substance were first studied in 1981 by the Dutch Scientist, Frits Warmolt Went who isolated growth regulating acids

(auxin – a & auxin – b), obtained from human urine & cereal products respectively.

Kenneth V Thimann determined its chemical structure as indole-3-acetic acid (IAA). They found that these had similar properties to indole-3-acetic acid (IAA), which found in actively growing tissues (coleopile tissues).

They are 2 types of auxins available,

- Natural (produced by plants themselves)
- Synthetic (have same action as natural auxins)

NATURAL AUXINS

a) Indole – 3 – acetic acid (IAA)
b) Indole – 3 – aceto nitrile (IAN)
c) 4 – chloro – indole – 3 – acetic acid
d) Indole pyruvic acid
e) Indole acetaldehyde

These 5 compounds are derived in plants from tryptophan.

SYNTHETIC AUXINS

a) Indole – 3 – butyric acid (IBA)
b) 2 – naphthyloxyacetic acid (NOA)
c) α- naphthyl acetic acid (NAA)
d) 1-naphthyl acetamide (NAD)
e) 2, 4- dichlorophenoxy acetic acid (2, 4- D)
f) 2, 4, 5- trichlorophenoxy acetic acid (2, 4, 5- T)

Effects of auxin or their functions:

1) Cell elongation giving an increase in the stem length.
2) Leaf growth
3) Inhibition of root growth
4) Initiation of vascular tissues
5) Adventious root production
6) Fruit setting in the absence of pollination.

Uses of auxins or its applications:

The main practical uses of auxins are:

1) **In low concentration to accelerate the rooting of woody & herbaceous cuttings:**

When placed for 24 hours in a 1: 500000 solution of NAA, cuttings will subsequently develop roots.

The Cinchona cuttings treated with IBA, saved 2 or 3 years compared with growth from seeds. The same effect obtained with the cuttings of Cassia, Coffee & Pinus.

2) **In higher concentration to act as selective herbicides or weed killers:**

Auxins when used in suitable concentration i.e. stronger than that of rooting cuttings selectively destroy some species of plants and leaves.

2, 4- D is toxic to dicotyledon plants.

3) **The auxins used for the development of 2^{o} metabolites:**

Seedlings and young plants of *Mentha piperita* when treated with derivatives of NAA, produce increased yield of oil (30-50%) & itself contains 4.5-9% more menthol.

In *Datura* species auxins increased the alkaloid content (i.e. Tropine)

When treated with 2, 4- D, *Datura stramonium* produces morphological changes.

GIBBERELLINS

These are a group of diterpenoid acids that functions as plant growth regulators influenzing a range of developmental processes such as stem elongation, germination, dormancy, flowering, sex expression, enzyme induction & leaf & fruit senescence.

Its origin is related to the causes of "bakanae" (foolish seedlings) disease which seriously affected rice crops and producing symptoms like pale yellow, elongated seedlings with slender leaves & slender roots. Causative organism is *Gibberella fujikuroi.*

Ellichi kurosawa found that culture filtrates from dried rice seedlings caused marked elongation in rice and other subtropical grasses. He concluded that bakanae fungus secretes a chemical that stimulates the shoot formation, inhibits chlorophyll formation & suppresses root growth.

Gibberellins are synthesized in leaves & they accumulate in relatively large quantities in the immature seeds & fruits of some plants.

Gibberellins are of many types GA_1, GA_2, GA_3, etc. GA_3 commonly referred to as gibberellic acid and produces commercially by fungal cultivation. There are 126 GA_3 are identified from plants, fungi & bacte

Functions of Gibberellins:

1) Stimulate stem elongation --- by stimulating cell division & elongation.
2) Flowering in biennial plants in controlled by GA.
3) Breaks seed dormancy in some plants that require stratification or light to induce germination.
4) Stimulate α- amylase in germinating cereals.
5) Induces maleness in dioecious flowers (sex expression)
6) Can cause parthenocarpic (seedless) fruit development or increase the size of seedless fruits (grapes).
7) Can delay senescence in leaves & citrus fruits.

Applications:

1) Dramatic effect of Gibberellins can be seen by their application to short node plants.
   Eg: Those plants producing rosettes of leaves (*Digitalis, Hyoscyamus*)

2) Dwarf varieties of many plants grow to taller varieties when treated with the hormones.

3) GA produces more secondary metabolites.

4) GA treatment of volatile oil containing plants such as *Citrus, Eucalyptus* & *Foeniculum* produces resultant changes in morphological characters.

5) GA spraying of the flowers of *Humulus lopules* advanced the maturity of the Hops by 10 days.

6) The volatile oil is increased by the GA treatment of *Chenopodium* species & *Anethum* species. Chenopodium afforded a 35% increase in volatile oil with no appreciable change in Ascaridole content.

   With *Anethum graveolens* species, when doses of GA increased, the oil content increased by 50%.

7) The seeds of Tropane alkaloid producing species (Atropa, Hyoscyamus & Datura) often exhibit dormancy & germination.

8) *Hyoscyamus niger* is a perennial and hormone effects included stem elongation giving 2 to 3 fold increase in height.

9) GA treatment in *Catharanthus roseus* produces change in the relative proportion of vinblastin to other alkaloids.
10) *Rauwolfia* produces alkaloids in roots, which will increase with the increase in dose.
11) Daily GA treatment of first young rosette *Digitalis purpurea* plants, produce flowering in the first year & the leaves become longer & more linear.

    An increase in cardiac glycoside was obtained but no increase in the Digitoxose content.
12) Digitalis lanata produces the above similar effects with bolting after the 12^{th} week.
13) As a result of GA treatment castor plants grows 5 times the height of Control. But no significant difference observed in the quality & quantity of the fixed oil of the seed.

CYTOKININS

These are the compounds with a structure resembling adenine which promote cell division (cytokinesis) and also regulate the pattern & frequency of organ production as well as position and shape. They have an inhibitory effect on senescence.

Kinetin was the first cytokinin identified. Though it is a natural compound it is not made in plants and is usually considered as a synthetic cytokinin.

The naturally occurring cytokinin in plants today is called zeatin, which are isolated from corn.

Cytikinin concentrations are more in meristematic regions & areas of continuous growth potential such as roots, young leaves, developing fruits & seeds.

Naturally occurring cytokinins:

1) Zeatin
2) N^6 dimethyl amino purine
3) Isopentanyl amino purine

Synthetic cytokines:

1) Kinetin
2) Adenine
3) 6- benzyl adenine benzimidazole
4) N,N^1- diphenyl urea

Functions:

1) Stimulate cell division
2) Stimulate morphogenesis in tissue culture
3) Stimulate leaf expansion
4) Many enhance stomatal opening
5) Stimulate the dark germination of light dependent seeds.
6) Delayed senescence

Applications:

1) Cytokinins reported to increase sennoside content in Tinnevely Senna leaves.
2) In Opium they cause formation of elongated capsule & reduced alkaloid content.
3) In Duboisia hybrids, the cytokinin activity present in extract of seaweeds shows marked increase in both leaf content & also hyoscine content.
4) Plays an important role in nucleic acid metabolism & protein synthesis.
5) Cytokinins used to promote the biosynthesis of Berberine, condensed tannins, etc.
6) Increase the growth & alkaloid content of Hyoscyamus when treated with kinetin.
7) Leaves of coffee plant after kinetic treatment developed a transient increase upto 10% in their caffeine content.

ETHYLENE

It is a simple organic molecule present in the form of volatile gas & shows profound physiological effects. It is present in ripening fruits, flowers, stems, roots, tubers & seeds.

It is present in very small quantity in plant, about 0.1ppm (part per million) and quality increase in local areas during the time of growth & development.

Ethylene is produced by incomplete burning of carbon rich substances like natural gas, coal & petroleum.

- Deny (1924) showed the yellowing of lemon due to stove gas.
- Gane (1935) found that a gas evolved from ripe apples can also effect the ripening of green apples & showed that it was ethylene gas which had a role in ripening of other fruits.

Ethylene shows a broad array of growth responses in plants, which include fruit ripening, leaf abscission, stem swelling, leaf bending, flower petal discolouration & inhibition of stem & root growth.

Commercially,

- For production of flowering
- Fruit ripening
- Induction of fruit abscission
- Breaking dormancy
- Stimulation of latex flow in rubber trees.

Functions:

1) Production stimulated during ripening
2) Regulator of cell death program in plants
3) Stimulates the release of dormancy
4) Stimulate shoot & root growth & differentiation
5) Regulate ripening of fruits.
6) Have role in adventitious root formation
7) Stimulate leaf & fruit abscission.

GROWTH INHIBITORS

Natural growth inhibitors are present in plants and affect bud opening, seed germination & development of dormancy. The physiological activities in plants like retaining or shedding of organs such as leaves, stems, flowers & fruits have led to finding of natural growth inhibitors.

Abscission accelerating substances isolated from cotton plants and named them as Abscisin I and Abscisin II.

ABSCISIC ACID

It is the natural growth inhibitor hormone isolated from the fungus, *Cenospora rosicola*. It was called as Abscisin II, because it plays a major role in abscission of plants. Also it is named as dormin, because it had a major role in dormancy of bud. Though it has inhibitory roles, it also posses promoting function as well.

Function:

1) Stimulates the closure of stomata.
2) Involved in abscission of buds, leaves, petals, flowers, & fruits.
3) Production is accentuated by stresses such as water loss & freezing temperatures.
4) Involved in bud dormancy.
5) Prolongs seed dormancy & delays germination.
6) Inhibition of elongation.
7) Promotes senescence
8) Can reserve the effects of growth stimulating hormones.

HYBRIDISATION

It is the mating or crossing o two genetically dissimilar plants having desired genes genotype and bringing them together into one individual called hybrid. The process through which hybrids are produced is called hybridization.

A hybrid is an organism which results from crossing of two species or varieties differing at least in one set of characters. It is of 3 types,

a) Mono hybrid ⟶1 pair of different characters
b) Di hybrid ⟶2 pair of different characters
c) Poly hybrid ⟶More than 2 pairs of different Characters

Steps:

1) Choice of parents:

   Select two parents one should be as well adopted and proven variety in the area. Other variety should have the characters that are absent in the first chosen variety.

2) Emasculation:

   Removed of anthers or stamens or killing the pollengrains of a flower without affecting the female reproductive organ (♀) is known as emasculation. It is essential in bisexual flowers.

3) Bagging:

Immediately after emasculation, the flowers or inflorescence are enclosed in bags of suitable sizes to prevent random cross pollination.

4) Pollination:

Mature, fertile and viable pollens are placed on a receptive stigma. Collect the pollens from fresh anthers and dusting them on the stigmas of emasculated flowers.

5) Raising F_1 plants:

After pollination, fertilization occurs. Seeds are developed. Harvested the seeds and dried, then stored. These seeds are grown to produced F_1 hybrids.

Applications:

1) Hybrids of **Chinchona** yield more quinine. Hybridization of *Cinchona succirubra* with *Cinchona Ledgeriana* produces 11.3% of alkaloids in the bark (parents posses 3.4% & 5.1 % respectively)
2) **Pyrethrum** yield more pyrethrin.
3) ***Withania somnifera*** chemotype II hybridized with W. somnifera south African chemotype produced new hybrid with new 3 withanolides.
4) *Digitalis purpurea* x *Digitalis lanata*, *D. purpurea* x *D. lutea* produces more lanatoside A glycoside alongwith lanatoside B & E.

POLYPLOIDY

In sexually reproducing organisms the cells have diploid (2n) number of chromosomes. During gamete formation (meosis occurs), this number is reduced to half (n), so that fertilization can restore diploidy.

In plant cells (2n) numbers of chromosomes are present. During fertilization, '1n' from pollen & '1n' from egg cell fertilized to form '2n' diploid zygote.

If the plant cell consists of more than '2n' number of chromosomes it is called polyploidy.

i.e. 3n - Triploid

4n - Tetraploid
8n - Octaploid

If polyploidy derived by multiplication of chromosomes of a single species is called autoploids.

If polyploidy derived as a result of multiplication of chromosomes following hybridization between 2 species is called alloploids. In alloploids, the 2 hybrids of 2 species itself infertile, the polyploidy may give rise to fertile species.

Tetraploidy can be induced by the treatment with colchicine. Colchicine inhibits spindle formation during cell division. Therefore, the divided chromosomes unable to separate and pass to the daughter cells. Two sets of chromosomes remain in one cell and develops to tetraploid plant. Colchicine produces effects in meristem.

Method:

The seeds are soaked in dilute solution of colchicines or the seedlings, the soil around the seedlings or the young shoots treated with colchicine. A 72 hours treatment with colchicines on onion root tip produces 256 chromosomes.

The typical effects of polyploidy compared with diploid stage are:

a) Larger flower
b) Larger pollengrain
c) Larger stomata
d) Chromosome count in root tip

Application of polyploidy in pharmacognosy:

1) Increased in alkaloids compared with diploid plants of ***Datura stramonium*** was 68% with a maximum increase of 211.6%
2) Increase in alkaloids in Belladonna is 93%
3) Percentage of volatile oil content of diploid Acorus calamus is 2.1% but in tetraploid is 6.8%.
4) Tetraploid plant of caraway found to be perennial and posses increased frost resistance. The volatile oil content is increased by 100%.
5) Tetraploid D. lanata posses high proportion of lanatosides A & B compared with diploid form.

MUTATION

Mutation is the sudden permanent heritable change in the structure of a gene, chromosome or chromosome number. It is caused due to changes in hereditary constitution.

Mutations are **5 types**,

1) Spontaneous & induced
2) Recessive & dominant
3) Somatic & germinal
4) Forward, backward & suppressor
5) Chromosomal, genomic & point

Mutation which occurs due to some unknown reason from nature is clled spontaneous mutation.

Chromosomal mutation otherwise called chromosomal aberration, which in many causes leads to changes in amount or position of genetic materials.

Artificial mutation can be produced by certain agents called mutagens or mutagenic agents. They are of 2 types:

a) Physical mutagens
b) Chemical mutagens

a) PHYSICAL MUTAGENS:

Two types of mutagens present

i. Ionizing radiations
ii. Non- ionizing radiations

i. Ionizing radiations:

X-rays, γ- rays, α- particles, β- rays and fast neutrons.

Due to ionizing radiations hydroperoxyl (HO_2) radical is formed. The hydroxyl & hydroperoxyl radicals impaired the sugar phosphate part of DNA leading to chromosoamal mutations like breaks, deletions, additions, inversions & translocations.

This can cause break in chromosomes and thus show abnormal cell division.

ii. Non- ionizing radiations:

UV radiations

These are absorbed by purines & pyramidines. The changed bases are known as photoproducts. UV radiation produces pyramidine hydrates and dimmers, thymidine dimmers. This will disturb DNA double helix & replications.

b) CHEMICAL MUTATION:

These are the following chemicals:

i. Alkylating & Hydroxylating agents:

N & S mustard, methyl & ethyl sulphonate, ethyl ethane sulphonate, sodium azide.

ii. Nitrous acid

iii. Acridines, cyanides, deoxyadenosine, etc.

The chemical mutagens have profound cellular effects like

a. Production of abnormal DNA (N mustard)
b. Inhibition of deoxyribonucleotide synthesis (deoxy adenine)
c. Inhibition of cytochrome oxidase (cyanides)

Application of mutation in Pharmacognosy:

1) The isolation of Penicillin from Penicillium notatum was very expensive and the yield was very poor. But the yield of Pencillin from P. chrysogenum (mutant strain) was 100 units of Penicillin per ml of culture medium.

Single spore isolation ---- 250 units/ml of culture medium
X- ray treatment ---- 500 units/ml of Culture Medium
UV treatment ---- 1000 units/ml of culture medium

2) Mutant strain of Capsicum annum yielding Capsicum (20-60%) from M_5 & M_4 generation originated from seed treated with sodium azide & ethylethane sulphonate.

3) Irradiation of Poppy seeds with ^{60}Co, a number of mutagens have been produced including one with increased morphine content.

4) Bitter Lupin seeds of an X-ray induced early- maturing mutant of Lupinnus digitalis were treated with ethyl methane sulphonate solution of the 440 progency, 11 were mutants which could be classed as sweet.

5) Higher Solasodine content achieved by applying radiation & chemical mutagens in Solanum khasianum.

6) The tuber yield & diosgenin content of Dioscorea bulbifera is increased by radiation.

7) γ - radiations on Mentha piperita produces dominant mutation for Verticillium (Wilt) resistance, a disease to which mints are particularly pron.

8) Economically, important characters of Atropa belladonna have been enhanced by radiations & chemical mutagens.

COLLECTION OF CRUDE DRUGS

Collection is the most important step which comes after cultivation. Drug should be collected from wild or cultivated plants when they contain maximum amount of constituents in a highly scientific manner.

1. The season at which each drug is collected is so important, as the amount, and sometimes the nature, of the active constituents could changed throughout the year.

   Eg: Rhubarb ---- Collected only in summer seasons because nu anthraquinone derivatives would be present in winter.

2. The age of the plant governs total amount of active constituents produced and also the proportion of the constituents of the active mixture.

   Eg: Peppermint ---- Increased proportion of pulegone in young plants replaces by menthone & menthol.
   Datura ---- Decreased in % of alkaloids when plant ages.

3. Leaves are collected just before flowering season.
   Eg: Vasaka, Digitalis, etc.

4. Flowers are collected before they expand fully.
   Eg : Clove, Saffron, etc.

5. Underground organs as the aerial part of plant cells dies.
   Eg : Liquorice, Rauwolfia, etc.

6. Fruits ---- Some are collected after their full maturity others are collected after the fruits are ripe.
7. Barks are collected in spring season, as they are easy to separate from the wood during this season.
8. Unorganized drugs should be collected from plants as soon as they oozes out.
   Eg: Reins, Latex, Gum, etc.

HARVESTING OF CRUDE DRUGS

- The underground drugs are harvested by mechanical devices, such as diggers or lifters.
  Eg: Root, rhizome, tubers, etc.
- Aerial parts are harvested by binders for economic reasons.
- Flowers, seeds & small fruits are harvested by a special device known as seed strippers.

Clove ---- Beaten with bamboos
Cochineal ---- Collected from branch by brushing
Fennel & Coriander ---- Uprooted & dried, trashed or beaten, winnowing.

DRYING OF CRUDE DRUGS

Before marketing a crude drug, it is necessary to process it properly to preserve it for a longer time.

- Drying consists of removal of sufficient moisture content of crude drug, so as to improve its quality & make it resistance to the growth of microorganisms.
- Inhibits partially enzymatic actions.
- Slicing and cutting into smaller pieces improve drying.

Depending upon the type of chemical constituents, a method of drying can be used for a crude drug.

Two types:

1. Natural drying (Sun drying)
2. Artificial drying

1. NATURAL DRYING

- Either direct sun drying or in the shade.
- If natural colour & volatile principles of the drug are to be retained ---- should be dried in shade.
- If the contents of the drug are quite stable to the temperature & sunlight ---- dried directly in sunlight.

2. ARTIFICIAL DRYING

a) An oven i.e. tray dyers
b) Vacuum dryers
c) Spray dryers

a) Tray dryers:

Drug which do not contain volatile oils & are stable to heat or which need deactivation of enzymes are dried here. Hot air of desired temperature is circulated through the dryers & this facilitates the removal of water content of drugs.

b) Vaccum dryers:

Drugs which are sensitive to higher temperature

Eg: Tannic acid & Digitalis leaves

c) Spray dryers:

Drugs which are highly sensitive to atmospheric conditions & also to temperature of vacuum drying are dried by this quick drying of economically important plant or animals.

GARBLING(DRESSING)

Here sand, dirt & foreign organic parts of the same plants, not constituting drug are required to be removed.

PACKING OF CRUDE DRUGS

- Aloe is packed in goat skin.
- Colophony & Tolu balsam in kerosene tins.
- Asafoetida is stored in well closed containers to prevent loss of volatile oils.
- Cod liver oil sensitive to light, should be stored in such containers which will not have effect of light.
- Digitalis loses potency due to decomposition of glycosides with excessive of moisture during storage.

STORAGE OF CRUDE DRUGS

- Preservation of crude drugs needs sound knowledge of their physical & chemical properties.
- A good quality of drugs can be maintained, if they are preserved properly.
- All drugs should be preserved in well closed and possibly in the filled containers.
- They should be stored in the premises which are water proof, fire proof & rodent proof.
- Apart from protection against physical & chemical changes, the preservation against insect or mould attacks is also important (drying thoroughly & giving treatment of fumigants)
  Eg: Methyl bromide, carbon disulphide & hydrocyanic acid.
- Drugs need to be preserved at very low temperature.
- Costly phytopharmaceuticals are required to be preserved at refrigerated temperature.
- Wooden boxes & paper bags should not be used for storage.

FIBRES

Fibres are elongated thick walled cells with pointed ends, cell walls of which may consist of cellulose and may or may not contain lignin.

Apart from the three natural sources (pants, animal and mineral), fibres are now synthesized chemically from various materials.

1. Plant fibres : Cotton, Flax, Jute
2. Animal fibres : Silk, Wool
3. Regenerated & Synthetic fibres :
   - Rayon, Fibrolin
   - Nylon, Orlon
4. Mineral fibres : Glass, Asbestos

COTTON

Synonyms: Cotton wool, purified cotton, Absorbent cotton, Surgical cotton

Biological Source: It consists of the epidermal trichomes or hairs of the seeds of cultivated species of the ***Gossypium (G. herbaceum, G. barbadense)*** belonging to the family ***Malvaceae.***

Geographical source : USA, Egypt, India.

Preparation:

The plant after flowering bears fruits known as ***capsules***
(fruits are 3-4 celled)

↓

Each capsule contains numerous seeds

↓

The seeds covered with hairs are known as ***bolls***

↓

The bolls are collected, dried and taken to ***ginning press***
(where trichomes are separated from the seeds)

↓

Various devices are used to separate short and long hiars

(Long hairs ---- used for manufacture of absorbent cotton
Short hairs ---- Known as linters)

↓

Raw cotton obtained by this way is full of impurities
(i.e. wax, fat, colouring matters, etc.)

↓

So, this raw cotton is then taken to machine known as ***Cotton opener***
(followed by treatment with dilute soda solution/ soda ash solution under pressure for about 10-15 hours)

↓

The wax, fatty material and colouring matter are removed by this treatment

↓ Washed with H_2O
Treated with suitable bleaching agent

Again washed with H_2O, dried and carded into flat sheets

↓

Finally packed in wrappers & sterilized

HISTOLOGICAL CHARACTERS:

The trichomes are unicellular, flattened and ribbon – like with slightly thickened and rounded apex. They are tubular and hollow.

DESCRIPTION :

Colour	:	White (due to bleaching)
Odour	:	Odourless
Taste	:	Tasteless
Size	:	Cotton fibre 2.5-4.5 cm (length) 25 - 35µ (diameter)

CHEMICAL CONSTITUENTS:

Raw cooton	-	90% cellulose 7 -8 % moisture Wax, fat and remains of protoplasm
Purified cotton	-	Entirely cellulose 6 -7 % of moisture

CHEMICAL TEST:

1. Cotton + iodinated zinc chloride solution ⟶ Violet coloured
2. Insoluble ---- dilute NaOH & HCl
   Soluble ---- 66% H_2SO_4
3. Soak cotton in iodine water & dry + few ml of 80% H_2SO_4 ⟶ trichomes assume purplish blue or bluish green colour.

4. **Cuoxam test:**
   - ❖ A few threads of cotton wool is moistened with alcohol & mounted in H_2O
   - ❖ The preparation is irrigated with ammoniacal solution of copper oxide (Cuoxam reagent) ⟶ Examine under microscope

     - ✓ Absorbent cotton ⟶ Swells uniformly & dissolves ultimately
     - ✓ Raw cotton ⟶ develops balloon like swellings which are separated by ring shape constrictions

Uses:

- ➢ Special types of sutures, sieves & ligatures.
- ➢ Filtering medium
- ➢ Surgical dressings
- ➢ Insulating materials.

SILK

Biological Source: These are the fibres obtained from the coccons of ***Bombyx mori*** & other species of Bombyx belonging to the family ***Bombycidae.***

Geaographical source: Manufactured in India, Japan, China, France, Italy.

Preparation:

The larva of silk worm secretes around itself a cocoon consisting of a continuous thread measuring up to 1200m

↓

This thread contains two silk fibres joined together by a glue which is secreted by another gland of the larva

↓

Larva then matures into a pupa (chrysalis).
The pupa is not allowed to develop into an insect & escape
(to prevent damage of silk)

↓

The cocoons are collected at the pupa stage

↓

They are killed by heating at 60-80^{o}C or by exposing to steam

↓

They are then put into hot H_2O
(This softens the glue and loosens fibres)

↓

The fibres of 2 – 15 cocoons are picked up and made into a single fibre

DESCRIPTION :

- Soft, smooth, fine and yellow in colour.
- Considerable tensile strength and elasticity.
- Soluble - Conc. Hcl, 66% H_2SO_4

CHEMICAL CONSTITUENTS:

- Contains a protein called **fibroin**
- Main aminoacids of fibroin are ***alanine*** & ***glycine***

CHEMICAL TEST:

1) When flamed ⟶ smell of brunt hair.
2) It does not contain sulphur and so the test with lead acetate does not gives black precipitate.

Uses:

- Special types of sutures, sieves and ligatures.

WOOL

Synonyms: Sheep's wool, Animal wool

Biological Source: It consists of hairs from the fleece of sheep ***Ovis aries*** belonging to the family ***Bovidae.***

Geographical source: The major producers of wool are Australia, America, Russia, Argentina and India.

Preparation:

The hairs forming the fleece of the sheep are removed at shearing time

↓

Then washed with soap and alkali carbonate
(This removes dirt and also wool grease which is the source of wool-fat)

↓

Then bleached with hydrogen peroxide or sulphur oxide

↓

Thoroughly washed

↓

Spread on wire netting

↓

Dried by hot air

DESCRIPTION :

- Wool hairs are smooth, elastic, lustrus, curly, hygroscopic and slippery to touch.
- Have a tendency to cling together.
- Insoluble : 66% H_2SO_4
  Conc HCl
  Cuoxam solution

CHEMICAL CONSTITUENTS:

- Chemically, wool contains sulphur containing protein known as ***keratin***
- Keratin is rich in sulphur containing aminoacid ***cystein***

CHEMICAL TEST:

1) Lead acetate + solution of wool in caustic soda ⟶ Black precipitate
(due to increased sulphur content)
(distinction from silk)

Uses:

- Filtering and straining medium.
- Manufacture of dressings, crepe bandages.

NYLON

Synonyms: Aralac

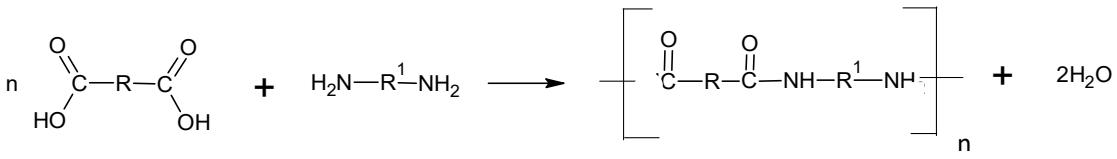
Nylon is a synthetic thermoplastic polymer invented in 1935 by Wallace Carothers at Du Pont.

- First commercially successful polymer
- First synthetic fibre made from inorganic ingredient like coal, water & air.
- It is made of repeating units linked by peptide bonds.
- It is a polymer of adipic acid and hexamethylene diamine.
- Nylon was intented to be a synthetic replacement for silk and sunbstituted for it in many different products after silk became scarce during II world war.

COMMERCIAL PRODUCTION:

Two common methods ---- nylon for fibre application

I. Molecules with an acid (COOH) group on each end are reacted with molecules containing amine (NH_2) groups on each end. The resulting nylon is named on the basis of the number of carbon atoms separating the two acid groups and the two amines. These are formed into monomers of intermediate molecular weight, which are then to form long polymer chains.



II. Other forms of nylon are through the chemical reaction between two monomers: adipoyl chloride and hexamethylene diamine.

DESCRIPTION:

Colour	:	Highly lustrous to dull, white or coloured
Insoluble	:	Acetone
Soluble	:	5M HCl, 90% formic acid & 90% phenol (distinguishes it from fibres of biological origin)

- When applied to flame, the fibre melts with formation of beads.
- Highly resistant to insects, fungi, animals, as well as molds, mildew, rot and many chemicals.

CHEMISTRY:

Nylons are condensation copolymers formed by reaction of equal parts of a diamine and a dicarboxylic acid, so that peptide bonds form at the both ends of each monomer in a process analogue to polypeptide biopolymers.

Uses:

- Important plastic
- Very wear resistant
- Used to build gears, bearings and bushings and other mechanical parts
- Non- absorbable sutures
- Used in carpets and nylon stockings
- Used in many military applications.

GLASS WOOL

- These fibres consists of sand (silica), mixed with oxides of aluminium, calcium, boron and magnesium.
- It is not affected by all usual reagents used in identification of fibres.
- Melt at high temperature and forms transparent beads.

Uses:

- Filter fabrics for insulation
- Splinting materials

ASBESTOS

Biological Source: It is a naturally occurring mineral which differs from other minerals in its crystal development. The crystal formation of asbestos is in the form of long thin fibres.

Geographical source: Through the world. Australia, Canada, South America, Russia, Karnataka, Bihar, UP

Preparation:

Asbestos is found as mineral in massive deposits. These often occur under a layer of soil or boulder clay. It is collected by open pit working up to 500-600 ft beyond this mining methods are adopted for collection.

DESCRIPTION :

- Contains mainly hydrated magnesium silicates.
- Rock asbestos – white, yellow or green.
- Do not fuse when heated

On the basis of crystalline structure asbestos are of 2 types - ***Serpentine & Amphibole.***

Serpentine **-** Fibrous form and friable & carcinogenic.

Amphibole **-** Have short or layered structure

Crystalline asbestos is the common type of asbestos among serpentine species.

CHEMICAL CONSTITUENTS:

It is a double silicate of Ca – Mg with little amount of Iron which give colour to asbestos.

USES:

- Preparation of filtering medium
- For bacterial filters
- Used as proof gloves, fire proof clothing, heat resistant insulators.