B. Pharm. 4th Semester

BP405T

Pharmacognosy & Phytochemistry - I

INTRODUCTION TO PHARMACOGNOSY

Pharmacognosy, known initially as *materia medica*, may be defined as the study of crude drugs obtained from plants, animals and mineral kingdom and their constituents. There is a historical misinformation about who created the term *pharmacognosy*. According to some sources, it was C. A. Seydler, a medical student at Halle, Germany, in 1815; he wrote his doctoral thesis titled *Analectica Pharmacognostica*. However, The physician J. A. Schmidt (Vienna) used that one in his *Lehrbuch der materia medica* in 1811, to describe the study of medicinal plants and their properties. The word *pharmacog-nosy* is derived from two Latin words *pharmakon*, 'a drug,' and *gignoso*, 'to acquire knowledge of'. It means 'knowledge or science of drugs'.

Crude drugs are plants or animals, or their parts which after collec-tion are subjected only to drying or making them into transverse or lon-gitudinal slices or peeling them in some cases. Most of the crude drugs used in medicine are obtained from plants, and only a small number comes from animal and mineral kingdoms.

Drugs obtained from plants consist of entire plants, whereas senna leaves and pods, nux vomica seeds, ginger rhizome and cinchona bark are parts of plants. Though in a few cases, as in lemon and orange peels and in colchicum corm, drugs are used in fresh condition, and most of the drugs are dried after collections. Crude drugs may also be obtained by simple physical processes like drying or extraction with water. Therefore, aloe is the dried juice of leaves of *Aloe* species, opium is the dried latex from poppy capsules and black catechu is the dried aqueous extract from the wood of *Acacia catechu*. Plant exudates such as gums, resins and balsams, volatile oils and fixed oils are also considered as crude drugs.

Further drugs used by physicians and surgeons or phar-macists, directly or indirectly, like cotton, silk, jute and nylon in surgical dressing or kaolin; diatomite used in filtration of turbid liquid or gums; wax, gelatin, agar used as pharmaceutical auxiliaries of flavouring or sweetening agents or drugs used as vehicles or insecticides are used in pharmacognosy.

Drugs obtained from animals are entire animals, as can-tharides; glandular products, like thyroid organ or extracts like liver extracts. Similarly, fish liver oils, musk, bees wax, certain hormones, enzymes and antitoxins are products obtained from animal sources.

Drugs are organized or unorganized. Organized drugs are direct parts of plants and consist of cellular tissues. Unorganized drugs, even though prepared from plants are not the direct parts of plants and are prepared by some intermediary physical processes, such as incision, drying or extraction with water and do not contain cellular tissue. Thus aloe, opium, catechu, gums, resins and other plant exudates are unorganized drugs.

Drugs from mineral sources are kaolin, chalk, diatomite and other bhasmas of Ayurveda.

ORIGIN OF PHARMACOGNOSY

Views on the beginning of life on planet Earth have forever remained controversial and an unending subject of debate. Nevertheless, we can say with certainty that the vegetable kingdom was already there when man made his appearance on Earth. As man began to acquire closure acquaintance with his environment, he began to know more about plants, as these were the only curative agents he had. As he pro-gressed and evolved, he was not only able to sort on as to which plant served for eating and which did not, but he went beyond and began to associate curative characteristics with certain plants, classifying them as painkillers, febri-fuge, antiphlogistics, soporific and so on. This must have involved no doubt, a good deal of trial and error, and pos-sibly some deaths in the beginning also, but as it happened antidotes against poisons were also discovered. As we shall see later, drug substitutes were also forthcoming. All these states of affairs indicate that the origin of pharmacognosy, i.e. the study of natural curative agents points towards the accent of human beings on mother earth, and its historical account makes it clear that pharmacognosy in its totality is not the work of just one or two continental areas but the overall outcome of the steadfast work of many of the bygone civilizations like the Chinese, Egyptian, Indian, Persian, Babylonian, Assyrian and many more. Many of today's wonderful modern drugs find their roots in the medicines developed by the tribal traditions in the various parts of the world.

SCOPE OF PHARMACOGNOSY

Crude drugs of natural origin that is obtained from plants, animals and mineral sources and their active chemical constituents are the core subject matter of pharmacognosy. These are also used for the treatment of various diseases besides being used in cosmetic, textile and food industries. During the first half of the nineteenth century apothecaries stocked the crude drugs for the preparation of herbal tea mixtures, all kinds of tinctures, extracts and juices which in turn were employed in preparing medicinal drops, syrups, infusions, ointments and liniments.

The second half of the nineteenth century brought with it a number of important discoveries in the newly developing fields of chemistry and witnessed the rapid progress of this science. Medicinal plants became one of its major objects of interest and in time, phytochemists succeeded in isolating the pure active constituents. These active constituents replaced the crude drugs, with the development of semisynthetic and synthetic medicine, they became predominant and gradually pushed the herbal drugs, which had formerly been used, into the background. It was a belief that the medicinal plants are of no importance and can be replaced by man-made synthetic drugs, which in today's scenario is no longer tenable. The drug plants, which were rapidly falling into disuse a century ago, are regaining their rightful place in medicine. Today applied science of pharmacognosy has a far better knowledge of the active constituents and their prominent therapeutic activ-ity on the

human beings. Researchers are exploiting not only the classical plants but also related species all over the world that may contain similar types of constituents. Just like terrestrial germplasm, investigators had also diverted their attention to marine flora and fauna, and wonderful marine natural products and their activities have been studied. Genetic engineering and tissue culture biotech-nology have already been successful for the production of genetically engineered molecules and biotransformed natural products, respectively.

Lastly, crude drugs and their products are of economi-cal importance and profitable commercial products. When these were collected from wild sources, the amount collected could only be small, and the price commanded was exorbitantly high. All this has now changed. Many of the industrially important species which produced equally large economic profits are cultivated for large-scale crop production. Drug plants, standardized extracts and the therapeutically active pure constituents have become a significant market commodity in the international trade. In the light of these glorious facts, scope of pharmacognosy seems to be enormous in the field of medicine, bulk drugs, food supplements, pharmaceutical necessities, pesticides, dyes, tissue culture biotechnology, engineering and so on.

Scope for doctoral graduates in pharmacognosy is going to increase in the coming years. The pharmacognosist would serve in various aspects as follows:

Academics: Teaching in colleges, universities, museums and botanical gardens.

Private industry: Pharmaceutical companies, consumer products testing laboratories and private commercial testing laboratories, the herbal product industries, the cosmetic and perfume industries, etc.

Government: Placement in federal agencies, such as the Drug Enforcement Agency, the Food and Drug Admin-istration, the U.S. Department of Agriculture, Medicinal plant research laboratories, state agencies like forensic laboratories, environmental laboratories, etc.

Undoubtedly, the plant kingdom still holds large number of species with medicinal value which have yet to be discovered. Lots of plants were screened for their pharmacological values like, hypoglycaemic, hepatoprotective, hypotensive, antiinflammatory, antifertility, etc. pharmacognosists with a multidisciplinary background are able to make valuable contributions in the field of phytomedicines.

Sources of Natural Drugs

The following are the natural sources of Drugs:

- Biological sources (i.e. from Terrestrial and Marine living things)

- o Plants
- o Animals
- o Microorganisms: Fungi, Algae, Bacteria

- Mineral sources
- Biotechnology: Tissue culture/ Recombinant DNA Technology

Plant Sources

Plant source is the oldest source of drugs. Most of the drugs in ancient times were derived from plants. Almost all parts of the plants are used i.e. leaves, stem, bark, fruits and roots.

The number of species of flowering plants is estimated to be 2 to 2.5 lakhs falling in about 300 families and 10000 genera. Only a small percentage of the total species have been studied scientifically for the presence of any therapeutic activity and isolation of the responsible bioactive compound isolated.

Investigators face numerous hurdles and problems in the systematic investigation of all the species and as a result thousands of species are still not investigated thoroughly.

In many areas of the world, plants used in folklore medicine have been recorded. In other regions of the world such information has not been recorded or lost. Ethno botanists across the world have been trying to gather and record such valuable information before it is completely lost or forgotten.

The search for new drug needs a team work of experts from various domains such as botany, pharmacognosy, pharmacology, phytochemistry, medicine etc.

Majority of the natural drugs from plant sources are derived from Spermatophytes (seed bearing plants). Thy phyla Angiosperme is the dominant one while the phyla Gymnospermae yields few useful drugs such as Turpentine oil, Colophony, ephedrine etc. Male Fern from Pteridophyta provides Taenicidal (tape worm killing) agents

In Angiospermae, Dicotyledon plants provide more drugs than the Monocotyledon plants which yield limited drugs such as Squill, Lemon grass oil, Aloes etc.

Examples of drugs obtained from plants include Quinine, Atropine, Cocaine, Morphine, Codeine, Ergotamine, Reserpine, Caffeine, Sennosides, Glycyrrhizin, volatile oils, fixed oils etc.

Animal Sources:

Gelatin is obtained from ox and sheep, Wool fat from sheep, Beeswax from honeycomb, Cochineal from insects are some examples of drugs obtained from land animals.

Spermaceti, Shark liver oil, Cod liver oil, halibut liver oil are some of the drugs obtained from marine animals.

Microbial Sources:

Well-known antibiotics produced by a group of microorganisms known as actinomycetes yielding antibiotics such as actinomycin, amphotericin, chloramphenicol, erythromycin, kanamycin, neomycin, gentamicin, streptomycin and tetracycline.

Aspergillate group of fungi produce antibiotics such as penicillin, griseofulvin and cephalosporin. Among the bacteria, genus Bacillusproduces antibiotics such as polymyxin B and bacitracin. Ergot alkaloids also are obtained from a resting stage of a fungus.

Algae are source of limited number of drugs such as Agar and Alginate

Mineral Sources:

Several silicates such as Kaolin, Bentonite, Diatomite and compounds of Na, K, Al, Ca, Mg etc. are obtained from Mineral sources including Sulphur and Iodine.

Biotechnology:

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.

Classification of Drugs of Natural Origin

INTRODUCTION

The flora and fauna of mother earth has a great diversity. The number of plant species divided in about 300 families and 10,500 genera are supposed to be about 2–2.5 lacs. At least 100–150 species of medicinal plants are currently cultivated and about 30–40 of them are the large-scale field corps. Drugs of the animal and mineral origin have also been used since the beginning and even today many such crude drugs are important, commercial products. All these drugs of natural origin have been used as the curative agents and even in this age of scientific discoveries and invention, natural drug have been the primary choice as a source of drug. Human inquisitiveness has gone beyond the terrestrial regions and exploited the seas and oceans which contain about 5 lacs species of marine organisms. Therapeutically active constituents found in these organ-isms open yet another great natural source of drugs of unending search.

Crude drugs can be regarded as the substances either used directly or indirectly as a drug which have not been changed or modified in its chemical composition.

The crude drugs of natural origin can be divided into two main categories as organized crude drugs and unorganized crude drugs.

Organized Drugs

Organized drugs consist of the cellular organization in the form of anatomical features. 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. A long list can be made of such crude drugs. To mention few of them, like, Cinchona bark, Sandalwood, Quassia wood, Senna, Digitalis leaves, Nux vomica seeds, Rauwolfia roots and many other examples of above-mentioned groups or crude drugs exemplified by some other morphological organs can be quoted as the example of organized crude drugs

Microscopical and anatomical studies are preeminent for such crude drugs. These can be used directly in medicine or can be used by modifying or by extracting the active ingredient from it. The simple medicines prepared from these drugs are herbal teas, extracts, tinctures, etc., and it may be extensively processed for the isolation and purification of pure therapeutically active constituent which is ultimately responsible for the action of the drug.

Unorganized Drugs

The unorganized drugs do not have the morphological or anatomical organization as such. These are the products which come directly in the market but their ultimate source remains the plants, animals or minerals. Microscopical studies are not required for such crude drugs. These includes products like plant exudates as gums, oleogums, oleogumresins, plant lattices like that of opium, aloetic juices like aloes or dried extracts of black and pale catechu, agar, alginic acid, etc., are products coming under this group. Other products like essential oils, fixed oils, fats and waxes obtained from vegetable or animal sources, although hydro-distilled or extracted from plant, become the direct commodity for use. Unorganized crude drugs may be miscellaneous mineral products like *shilajit*. These products may be solid, semisolid or liquid and the physical, chemical and analytical standards may be applied for testing their quality and purity.

CLASSIFICATION OF CRUDE DRUGS

The most important natural sources of drugs are higher plant, microbes and animals and marine organisms. Some useful products are obtained from minerals that are both organic and inorganic in nature. In order to pursue (or to follow) the study of the individual drugs, one must adopt some particular sequence of arrangement, and this is referred to a system of classification of drugs. A method of classification should be:

a) simple,

b) easy to use, and

c) free from confusion and ambiguities.

Because of their wide distribution, each arrangement of classification has its own merits and demerits, but for the purpose of study the drugs are classified in the following different ways:

1.Alphabetical classification

2.Taxonomical classification

3.Morphological classification

4.Pharmacological classification

5.Chemical classification

6.Chemotaxonomical classification

Alphabetical Classification

Alphabetical classification is the simplest way of classifica-tion of any disconnected items. Crude drugs are arranged in alphabetical order of their Latin and English names (common names) or sometimes local language names (ver-nacular names). Some of the pharmacopoeias, dictionaries and reference books which classify crude drugs according to this system are as follows:

1.Indian Pharmacopoeia

2.British Pharmacopoeia

3.British Herbal Pharmacopoeia

4.United States Pharmacopoeia and National Formu-lary

5.British Pharmaceutical Codex

6.European Pharmacopoeia

In European Pharmacopoeia these are arranged according to their names in Latin where in United States Pharmaco-poeia (U.S.P.) and British Pharmaceutical Codex (B.P.C.), these are arranged in English.

Merits

·It is easy and quick to use.

·There is no repetition of entries and is devoid of con-fusion.

·In this system location, tracing and addition of drug entries is easy.

Demerits

There is no relationship between previous and successive drug entries.

Examples: Acacia, Benzoin, Cinchona, Dill, Ergot,Fennel, Gentian, Hyoscyamus, Ipecacuanha, Jalap, Kurchi, Liquorice, Mints, Nux vomica, Opium, Podophyllum, Quassia, Rauwolfia, Senna, Vasaka, Wool fat, Yellow bees wax, Zeodary.

Taxonomical Classification

All the plants possess different characters of morphologi-cal, microscopical, chemical, embryological, serological and genetics. In this classification the crude drugs are classified according to kingdom, subkingdom, division, class, order, family, genus and species as follows.

Class: Angiospermae (Angiosperms) are plants that produceflowers and Gymnospermae (Gymnosperms) which don't produce flowers.

Subclass: Dicotyledonae (Dicotyledons, Dicots) are plantswith two seed leaves; Monocotyledonae (Monocotyledons, Monocots) with one seed leaf.

Superorder: A group of related plant families, classified in theorder in which they are thought to have developed their dif-ferences from a common ancestor. There are six superorders in the Dicotyledonae (*Magnoliidae, Hamamelidae, Caryophyl-lidae, Dilleniidae, Rosidae, Asteridae*), and four superorders in the Monocotyledonae (*Alismatidae, Commelinidae, Arecidae*, and *Liliidae*). The names of the superorders end in *–idae.*

Order: Each superorder is further divided into several orders.

The names of the orders end in *–ales.*

Family: Each order is divided into families. These are plantswith many botanical features in common, and are the highest classification normally used. At this level, the similarity between plants is often easily recognizable by the layman. Modern botanical classification assigns a type plant to each family, which has the particular characteristics that separate this group of plants from others, and names the family after this plant.

The number of plant families varies according to the botanist whose classification you follow. Some botanists recognize only 150 or so families, preferring to classify other similar plants as subfamilies, while others recognize nearly 500 plant families. A widely accepted system is that devised by Cronquist in 1968, which is only slightly revised today. The names of the families end in *–aceae.*

Subfamily: The family may be further divided into a numberof subfamilies, which group together plants within the family that have some significant botanical differences. The names of the subfamilies end in *–oideae.*

Tribe: A further division of plants within a family, based onsmaller botanical differences, bin still usually comprising many different plants. The names of the tribes end in *–eae.*

Subtribe: A further division based on even smaller botanicaldifferences, often only recognizable to botanists. The names of the subtribes end in *–inae.*

Genus: This is the part of the plant name that is most famil-iar; the normal name that you give a plant—Papaver (Poppy), Aquilegia (Columbine), and so on. The plants in a genus are often easily recognizable as belonging to the same group.

Species: This is the level that defines an individual plant.Often, the name will describe some aspect of the plant— the colour of the flowers, size or shape of the leaves, or it may be named after the place where it was found. Together, the genus and species name refer to only one plant, and they are used to identify that particular plant. Sometimes, the species is further divided into subspecies that contain plants not quite so distinct that they are classified as variet-ies. The name, of the species should be written after the genus name, in small letters, with no capital letter.

Variety: A variety is a plant that is only slightly differentfrom the species plant, but the differences are not so insig-nificant as the differences in a form. The Latin is *varietas*, which is usually abbreviated to var. The name follows the genus and species name, with var. before the individual variety name.

Form: A form is a plant within a species that has minorbotanical differences, such as the colour of flower or shape of the leaves. The name follows the genus and species name, with form (or f.) before the individual variety name.

Cultivar: A cultivar is a cultivated variety—a particularplant that has arisen either naturally or through deliberate hybridization, and can be reproduced (vegetatively or by seed) to produce more of the same plant.

The name follows the genus and species name. It is written in the language of the person who described it, and should not be translated. It is either written in single quotation marks or has cv. written in front of the name.

Kingdom	Plants
Subkingdom	Tracheobionta—Vascular plants
Superdivision	Spermatophyta—Seed plants
Division	Magnoliophyta—Flowering plants
Class	Magnoliopsida—Dicotyledons
Subclass	Asteridae
Order	Asterales
Family	Asteraceae—Aster family
Genus	*Tridax* L.—tridax

Merits

Taxonomical classification is helpful for studying evolution-ary developments.

Demerits

This system also does not correlate in between the chemical constituents and biological activity of the drugs.

Morphological Classification

In this system, the drugs are arranged according to the morphological or external characters of the plant parts or animal parts, i.e. which part of the plant is used as a drug, e.g. leaves, roots, stem, etc. The drugs obtained from the direct parts of the plants and containing cellular tissues are called as *organized* drugs, e.g. rhizomes, barks, leaves, fruits, entire plants, hairs and fibres. The drugs which are pre-pared from plants by some intermediate physical processes such as incision, drying or extraction with a solvent and not containing any cellular plant tissues are called *unorga-nized* drugs. Aloe juice, opium latex, agar, gambir, gelatin, tragacanth, benzoin, honey, beeswax, lemon grass oil, etc., are examples of unorganized drugs.

Organized drugs

Woods: Quassia, Sandalwood and Red Sandalwood.

Leaves: Digitalis, Eucalyptus, Gymnema, Mint, Senna,Spearmint, Squill, Tulsi, Vasaka, Coca, Buchu, Hamamelis, Hyoscyamus, Belladonna, Tea.

Barks: Arjuna, Ashoka, Cascara, Cassia, Cinchona, Cinnamon, Kurchi, Quillia, Wild cherry.

Flowering parts: Clove, Pyrethrum, Saffron, Santonica, Chamomile.

Fruits: Amla, Anise, Bael, Bahera, Bitter Orange peel,Capsicum, Caraway, Cardamom, Colocynth, Coriander, Cumin, Dill, Fennel, Gokhru, Hirda, Lemon peel, Senna pod, Star anise, Tamarind, Vidang.

Seeds: Bitter almond, Black Mustard, Cardamom, Colchi-cum, Ispaghula, Kaladana, Linseed, Nutmeg, Nux vomica,

Physostigma, Psyllium, Strophanthus, White mustard. **Roots and Rhizomes:** Aconite, Ashwagandha, Calamus, Calumba, Colchicum corm, Dioscorea, Galanga, Garlic, Gention, Ginger, Ginseng, Glycyrrhiza, Podophyllum, Ipecac, Ipomoea, Jalap, Jatamansi, Rauwolfia, Rhubarb, Sassurea, Senega, Shatavari, Turmeric, Valerian, Squill.

Plants and Herbs: Ergot, Ephedra, Bacopa, Andrographis,

Kalmegh, Yeast, Vinca, Datura, Centella.

Hair and Fibres: Cotton, Hemp, Jute, Silk, Flax.

Unorganized drugs

Dried latex: Opium, Papain

Dried Juice: Aloe, Kino

Dried extracts: Agar, Alginate, Black catechu, Pale catechu,Pectin

Waxes: Beeswax, Spermaceti, Carnauba wax

Gums: Acacia, Guar Gum, Indian Gum, Sterculia, Tra-gacenth

Resins: Asafoetida, Benzoin, Colophony, copaiba Gua-iacum, Guggul, Mastic, Coal tar, Tar, Tolu balsam, Storax, Sandarac.

Volatile oil: Turpentine, Anise, Coriander, Peppermint,Rosemary, Sandalwood, Cinnamon, Lemon, Caraway, Dill, Clove, Eucalyptus, Nutmeg, Camphor.

Fixed oils and Fats: Arachis, Castor, Chalmoogra, Coconut,Cotton seed, Linseed, Olive, Sesame, Almond, Theobroma, Cod-liver, Halibut liver, Kokum butter.

Animal Products: Bees wax, Cantharides, Cod-liver oil,Gelatin, Halibut liver oil, Honey, Shark liver oil, shellac, Spermaceti wax, wool fat, musk, Lactose.

Fossil organism and Minerals: Bentonite, Kaolin, Kiess-lguhr, Talc.

Merits

·Morphological classification is more helpful to identify and detect adulteration. This system of classification is more convenient for practical study especially when the chemical nature of the drug is not clearly understood.

Demerits

·The main drawback of morphological classification is that there is no corelation of chemical constituents with the therapeutic actions.

·Repetition of drugs or plants occurs.

Pharmacological Classification

Grouping of drug according to their pharmacological action or of most important constituent or their therapeutic use is termed as pharmacological or therapeutic classification of drug. This classification is more relevant and is mostly a followed method. Drugs like digitalis, squill and strophan-thus having cardiotonic action are grouped irrespective of their parts used or phylogenetic relationship or the nature of phytoconstituents they contain

Sl. No.	Pharmacological category	Example
1.	Drug acting on G.I.T.	
	Bitter	Cinchona, Quassia, Gentian
	Carminative	Fennel, Cardamom, Mentha
	Emetic	Ipecac
	Antiamoebic	Kurchi, Ipecac
	Laxative	Agar, Isabgol, Banana
	Purgative	Senna, Castor oil
	Cathartic	Senna
2.	Drug acting on Respiratory system	
	Expectorant	Vasaka, Liquorice, Ipecac
	Antitussive	Opium (codeine)
	Bronchodilators	Ephedra, Tea
3.	Drug acting on Cardiovascular system	
	Cardio tonic	Digitalis, Strophanthus, Squill
	Cardiac depressant	Cinchona, Veratrum
	Vasoconstrictor	Ergot
	Antihypertensive	Rauwolfia
4.	Drug acting on Autonomic nervous system	
	Adrenergic	Ephedra
	Cholinergic	Physostigma, Pilocarpus
	Anticholinergic	Datura, Belladonna
5.	Drug acting on Central nervous system	
	Central analgesic	Opium (morphine)
	CNS depressant	Belladonna, Opium, Hyoscyamus
	CNS stimulant	Tea, Coffee
	Analeptic	Nuxvomica, Camphor, Lobelia
6.	Antispasmodic	Datura, Hyoscyamus, Opium, Curare
7.	Anticancer	Vinca, Podophyllum, Taxus
8.	Antirheumatic	Aconite, Colchicum, Guggal
9.	Anthalmintic	Quassia, Vidang
10.	Astringent	Catechu, Myrobalans
11.	Antimalarial	Cinchona, Artemisia
12.	Immunomodulatory	Ginseng, Ashwagandha, Tulsi
13.	Immunizing agent	Vaccines, Sera, Anti toxin
14.	Drug acting on skin membrane	Beeswax, Wool fat, Balsam of Tolu, Balsam of Peru
15.	Chemotherapeutic	Antibiotics
16.	Local Anesthetic	Coca

Merits

This system of classification can be used for suggesting substitutes of drugs, if they are not available at a particular place or point of time.

Demerits

Drugs having different action on the body get classified separately in more than one group that causes ambiguity and confusion. Cinchona is antimalarial drug because of presence of quinine but can be put under the group of drug affecting heart because of antiarrhythmic action of quinidine.

Chemical Classification

Depending upon the active constituents, the crude drugs are classified. The plants contain various constituents in them like alkaloids, glycosides, tannins, carbohydrates, saponins, etc. Irrespective of the morphological or taxonomical char-acters, the drugs with similar chemical constituents are grouped into the same group. The examples are shown in this table.

Sl. No.	Chemical constituent group	Examples
1.	Alkalnids	Cinchnna, Datura, Vinca, Ipecac Nux vomica
2.	Glycosides	Senna, Aloe, Ginseng, Glycyrrhiza, Digitalis
3.	Carbohydrates and its derived products	Acacia, Tragacanth, Starch, Isabgol
4.	Volatile oil	Clove, Coriander, Fennel, Cinnamon, Cumin
5.	Resin and Resin combination	Benzoin, Tolu Balsam, Balsam of peru
6.	Tannins	Catechu, Tea
7.	Enzymes	Papain, Caesin, Trypsin
8.	Lipids	Beeswax, Kokum butter, Lanolin

Merits

It is a popular approach for phytochemical studies.

Demerits

Ambiguities arise when particular drugs possess a number of compounds belonging to different groups of compounds.

Chemotaxonomical Classification

This system of classification relies on the chemical similarity of a taxon, i.e. it is based on the existence of relationship between constituents in various plants. There are certain types of chemical constituents that characterize certain classes of plants. This gives birth to entirely a new concept of chemotaxonomy that utilizes chemical facts/characters for understanding the taxonomical status, relationships and the evolution of the plants.

For example, tropane alkaloids generally occur among the members of Solanaceae, thereby, serving as a chemot-axonomic marker. Similarly, other secondary plant metabo-lites can serve as the basis of classification of crude drugs. The berberine alkaloid in Berberis and Argemone, Rutin in Rutaceae members, Ranunculaceae alkaloids among its members, etc., are other examples.

It is the latest system of classification that gives more scope for understanding the relationship between chemical constituents, their biosynthesis and their possible action.

Adulteration of Drugs of Natural Origin

INTRODUCTION

Medicinal plants constitute an effective source of traditional (e.g. ayurvedic, chinese, homeopathy and unani) and modern medicine. Herbal medicine has been shown to have genuine utility. Germany and France, together represent 39% of the $14 billion global retail market. In India, about 80% of the rural population depends on medicinal herbs and/or indigenous systems of medicine. In fact today, approximately 70% of 'synthetic' medicines are derived from plants. Popularity among the common people increased the usage of medicinal plants/herbal drugs. Herbal adulteration is one of the common malpractices in herbal raw-material trade. Adulteration is described as intentional substitution with another plant species or intentional addition of a foreign substance to increase the weight or potency of the product or to decrease its cost. In general, adulteration is considered as an intentional practice. However, unintentional adulterations also exist in herbal raw-material trade due to various reasons, and many of them are unknown even to the scientific community. The present chapter deals with different intentional and unintentional adulterations, reasons behind them and methods for easy identification of the spurious plant and authentication of the authentic plant.

ADULTERATION

A treatise published two centuries ago (in 1820) on adulterations in food and culinary materials is a proof for this practice as an age-old one. Due to adulteration, faith in herbal drugs has declined. Adulteration in market samples is one of the greatest drawbacks in promotion of herbal products. Many researchers have contributed in checking adulterations and authenticating them. It is invariably found that the adverse event reports are not due to the intended herb, but rather due to the presence of an unintended herb. Medicinal plant dealers have discovered the 'scientific' methods in creating adulteration of such a high quality that without microscopic and chemical analysis, it is very difficult to trace these adulterations.

Definition: The term adulteration is defined as substituting original crude drug partially or wholly with other similar-looking substances. The substance, which is mixed, is free from or inferior in chemical and therapeutic property.

Types of Adulterants

Adulteration in simple terms is debasement of an article. The motives for intentional adulteration are normally commercial and are originated mainly with the intension of enhancement of profits. Some of the reasons that can be cited here are scarcity of drug and its high price prevailing in market. The adulteration is done deliberately, but it may occur accidentally in some cases. Adulteration involves different conditions such as deterioration, admixture, sophistication, substitution, inferiority and spoilage. Deterioration is impairment in the quality of drug, whereas admixture is addition of one article to another due to ignorance or carelessness or by accident. Sophistication is the intentional or deliberate type of adulteration. Substitution occurs when a totally different substance is added in place of original drug. Inferiority refers to any substandard drug, and spoilage is due to the attack of microorganisms.

Unintentional Adulteration

Unintentional adulteration may be due to the following reasons:

1. confusion in vernacular names between indigenous systems of medicine and local dialects
2. lack of knowledge about the authentic plant
3. nonavailability of the authentic plant

4. similarity in morphology and or aroma
5. careless collection
6. other unknown reasons

Name confusion

In ayurveda, 'Parpatta' refers to *Fumaria parviflora*. In siddha, 'Parpadagam' refers to *Mollugo pentaphylla*. Owing to the similarity in the names in traditional systems of medicine, these two herbs are often interchanged or adulterated or substituted. Because of the popularity of siddha medicine in some parts of south India, traders in these regions supply *M. pentaphylla* as Parpatta/Parpadagam and the north Indian suppliers supply *F. parviflora*. These two can be easily identified by the presence of pale yellow to mild brown-coloured, thin wiry stems and small simple leaves of *M. pentaphylla* and black to dark brown-coloured, digitate leaves with narrow segments of *F. parviflora*. *Casuarina equisetifolia* for *Tamarix indica* and *Aerva lanata* for *Bergenia ciliata* are some other examples of adulterations due to confusion in names.

Lack of knowledge about authentic source

'Nagakesar' is one of the important drugs in ayurveda. The authentic source is *Mesua ferrea*. However, market samples are adulterated with flowers of *Calophyllum inophyllum*. Though the authentic plant is available in plenty throughout the Western Ghats and parts of the Himalayas, suppliers are unaware of it. There may also be some restrictions in forest collection. Due to these reasons, *C. inophyllum* (which is in the plains) is sold as Nagakesar. Authentic flowers can be easily identified by the presence of two-celled ovary, whereas in case of spurious flowers they are single celled.

Similarity in morphology

Mucuna pruriens is the best example for unknown authentic plant and similarity in morphology. It is adulterated with other similar papilionaceae seeds. *M. utilis* (sold as white variety) and *M. deeringiana* (sold as bigger variety) are popular adulterants. Apart from this, *M. cochinchinensis, Canavalia virosa* and *C. ensiformis* are also sold in Indian markets. Authentic seeds are up to 1 cm in length with shining mosaic pattern of black and brown colour on their surface. *M. deeringiana* and *M. utilis* are bigger (1.5–2 cm) in size. *M. deeringiana* is dull black, whereas *M. utilis* is white or buff coloured.

Lack of authentic plant

Hypericum perforatum is cultivated and sold in European markets. In India, availability of this species is very limited. However, the abundant Indo-Nepal species *H. patulum* is sold in the name of *H. perforatum*. Market sample is a whole plant with flowers, and it is easy to identify them taxonomically. Anatomically, stem transverse section of *H. perforatum* has compressed thin phloem, hollow pith and absence of calcium oxalate crystals. On the otherhand, *H. patulum* has broader phloem, partially hollow pith and presence of calcium oxalate crystals.

Similarity in colour

It is well known that in course of time, drug materials get changed to or substituted with other plant species. 'Ratanjot' is a recent-day example. On discussion with suppliers and nontimer forest product (NTFP) contractors, it came to be known that in the past, roots of *Ventilago madraspatana* were collected from Western Ghats, as the only source of 'Ratanjot'. However, that is not the practice now. It is clearly known that *Arnebia euchroma var euchroma* is the present source. Similarity in yielding a red dye, *A. euchroma* substitutes *V. madraspatana*. The description to identify these two is unnecessary because of the absence of *V. madraspatana* in market. Whatever is available in the market, in the name of Ratanjot, was originated from *A. euchroma.*

Careless collections

Some of the herbal adulterations are due to the carelessness of herbal collectors and suppliers. *Parmelia perlata* is used in ayurveda, unani and siddha. It is also used as grocery. Market samples showed it to be admixed with other species (*P. perforata* and *P. cirrhata*). Sometimes, Usnea sp. is also mixed with them. Authentic plants can be identified by their thallus nature.

Unknown reasons

'Vidari' is another example of unknown authentic plant. It is an important ayurvedic plant used extensively. Its authentic source is *Pueraria tuberosa*, and its substitute is *Ipomoea digitata*. However, market samples are not derived from these two. It is interesting to know that an endangered gymnosperm *Cycas circinalis* is sold in plenty as Vidari. The adulterated materials originated from Kerala, India. Although both the authentic plant and its substitute are available in plenty throughout India, how *C. circinalis* became a major source for this drug is unknown. *P. tuberosa* can be easily identified by the presence of papery flake-like tubers, *I. digitata* by the presence of its concentric rings of vascular bundles and their adulterant *C. circinalis* by its leaf scars and absence of vessel elements.

Intentional Adulteration

Intentional adulteration may be due to the following reasons:

1. adulteration using manufactured substances
2. substitution using inferior commercial varieties
3. substitution using exhausted drugs
4. substitution of superficially similar inferior natural substances
5. adulteration using the vegetative part of the same plant
6. addition of toxic materials
7. adulteration of powders
8. addition of synthetic principles

Adulteration using manufactured substances

In this type of adulteration the original substances are adulterated by the materials that are artificially manufactured. The materials are prepared in a way that their general form and appearance resemble with various drugs. Few examples are cargo of ergot from Portugal was adulterated with small masses of flour dough moulded to the correct size and shape and coloured, first using red ink, and then into writing ink. Bass-wood is cut exactly the required shape of nutmegs and used to adulterate nutmegs. Compressed chicory is used in place of coffee berries. Paraffin wax is coloured yellow and is been substituted for beeswax, and artificial invert sugar is used in place of honey.

Substitution using inferior commercial varieties

In this type, the original drugs are substituted using inferior quality drugs that may be similar in morphological characters, chemical constituents or therapeutic activity. For example hog gum or hog tragacanth for tragacanth gum, mangosteen fruits for bael fruits, Arabian senna, obovate senna and Provence senna are used to adulterate senna, ginger being adulterated with Cochin, African and Japanese ginger. *Capsicum annuum* fruits and Japanese chillies are used for fruits of *C. minimum.*

Substitution using exhausted drugs

In this type of substitution the active medicaments of the main drugs are extracted out and are used again. This could be done for the commodities that would retain its shape and appearance even after extraction, or the appearance and taste could be made to the required state by adding colouring or flavouring agents. This technique is frequently adopted for the drugs containing volatile oils, such as: clove, fennel etc. After extraction, saffron and red rose petals are recoloured by artificial dyes. Another example is balsam of tolu that does not contain cinnamic acid. The bitterness of exhausted gentian is restored by adding aloes.

Substitution of superficially similar inferior natural substances

The substituents used may be morphologically similar but will not be having any relation to the genuine article in their constituents or therapeutic activity. Ailanthus leaves are substituted for belladona, senna, etc. saffron admixed with saff flower; peach kernels and apricot kernels for almonds; clove stalks and mother cloves with cloves; peach kernel oil used for olive oil; chestnut leaves for hamamelis leaves and Japan wax for beeswax are few examples for this type of adulteration.

Adulteration using the vegetative part of the same plant

The presence of vegetative parts of the same plant with the drug in excessive amount is also an adulteration. For example, epiphytes, such as mosses, liverworts and lichens that grow over the barks also may occur in unusual amounts with the drugs, e.g. cascara or cinchona. Excessive amount of stems in drugs like lobelia, stramonium, hamamelis leaves, etc. are few example for this type of adulteration.

Addition of toxic materials

In this type of adulteration the materials used for adulteration would be toxic in nature. A big mass of stone was found in the centre of a bale of liquorice root. Limestone pieces with asafetida, lead shot in opium, amber-coloured glass pieces in colophony, barium sulphate to silvergrain cochineal and manganese dioxide to blackgrain cochineal, are few examples in this adulteration.

Addition of synthetic principles

Synthetic pharmaceutical principles are used for market and therapeutic value. Citral is added to lemon oil, whereas benzyl benzoate is added to balsam of Peru. Apart from these, the herbal products labelled to improve sexual performance in men, when analysed, contained sildenafil. Brand names included Actra-Rx, Yilishen, Hua Fo, Vinarol and Vasx, Sleeping Buddha containing estazolam, Diabetes Angel containing glyburide and phenformin are few examples under this category.

Adulteration of powders

Powdered drugs are found to be adulterated very frequently. Adulterants used are generally powdered waste products of a suitable colour and density. Powdered olive stones for powdered gentian, liquorice or pepper; brick powder for barks; red sanders wood to chillies; dextrin for powdered ipecacuanha, are few adulterants.

Evaluation of Plant Drugs

INTRODUCTION

Evaluation of a drug ensures the identity of a drug and determines the quality and purity of drugs. The main reasons behind the need for evaluation of crude drugs are biochemical variation in the drug, effect of treatment and storage of drugs, and the adulterations and substitutions.

Improvements in analytical methods have definitely led to improvements in harvesting schedules, cultivation techniques, storage, activity, stability of active compounds, and product purity. All of these gains have resulted in tremendous improvements in the quality of herbal preparations now available.

Methods currently employed in evaluating herbs are organoleptic, microscopic, physical, chemical, and biological parameters.

ORGANOLEPTIC EVALUATION

Organoleptic evaluation means the study of drugs using organs of senses. It refers to the methods of analysis like colour, odour, taste, size, shape, and special features, such as: touch, texture, etc. Obviously, the initial sight of the plant or extract is so specific that it tends to identify itself. If this is not enough, perhaps the plant or extract has a characteristic odour or taste. Organoleptic analysis represents the simplest, yet the most human form of analysis.

Talka gum, which is used as a substitute for acacia gum could be identified by its colour and form. Talka gum is usually broken and also some tears are brown in colour and other colourless, whereas acacia is white to yellow in colour. Mangosteen fruits are a substitute for bael fruits and can be identified by darker rind and the wedgeshaped radiate stigmas. Cuprea Bark *(Remijia pedupiculata)* differs in its morphological character with cinchona. Blood Root used as an adulterant for hydrastis is dark reddishbrown in colour, whereas hydrastis is yellow in colour. *Rheumrhaponticum* are much smaller than those of the Chineserhubarb and are easily distinguished.

Ginger and capsicum have pungent taste, whereas gentian and chirata have bitter taste. Morphological differentiation of leaves and pods of Indian senna and Alexandrian senna, sweet taste of liquorice, odours of umbelliferous fruits, discshaped structure of nux vomica, conical shape of aconite, quills of cinnamon, etc. are few examples of this organoleptic evaluation.

MICROSCOPICAL EVALUATION

Microscopic evaluation is indispensable in the initial identification of herbs, as well as in identifying small fragments of crude or powdered herbs, and in the detection of adulterants (e.g. insects, animal faeces, mold, fungi, etc.) as well as identifying the plant by characteristic tissue features. Every plant possesses a characteristic tissue structure, which can be demonstrated through study of tissue arrangement, cell walls, and configuration when properly mounted in stains, reagents, and media. Lignin stains red or pink with a drop of phloroglucinol and concentrated hydrochloric acid. Mucilage is stained pink with rhuthenium red, and N/50 iodine solution stains starch and hemicellulose blue.

The characteristic features of cell walls, cell contents, starch grains, calcium oxalate crystals, trichomes, fibres, vessels, etc. have been studied in details. Surinam quassia is recognized by the

absence of calcium oxalate and presence of uniseriate medullary rays, crystal fibres, and wavy medullary rays of cascara bark, lignified trichomes, and plasmodesma in nux vomica. Stone cells are absent in the frangula bark, whereas they are present in cascara. Presence of pith in rhizomes and absence in roots, warty trichomes of senna, and presence or absence of crystals of aloin indicates different varieties of aloes, glandular trichomes of mint, etc. The powder of clove stalks contains sclereids and calcium oxalate crystals, but cloves do not contain these two. *Rauwolfia micrantho, R. densiflora,* and *R. perokensis* are found to serve as an adulterant for *R. serpentine.* The roots of these species can bedifferentiated from *R. serpentine* by the presence of sclerenchyma in the above species which is absent in *R. serpentine.*

CHEMICAL EVALUATION

The chemical evaluation includes qualitative chemical tests, quantitative chemical tests, chemical assays, and instrumental analysis. The isolation, purification, and identification of active constituents are chemical methods of evaluation. Qualitative chemical tests include identification tests for various phytoconstituents like alkaloids, glycosides, tannins, etc. The procedures for the identification tests of various phytoconstituents are given under their respective chapters in the text, where it could be referred. Examples of identification of constituents are: copper acetate used in the detection of colophony present as an adulterant for resins, balsams, and waxes; Holphen's test for cottonseed oil and Baudouin's test for sesame oil in olive oil; the test with acetic and nitric acids for Gurjun balsam in copaiba; Van Urk's reagent for ergot; Vitali's morins reaction for tropane alkaloids; iodine for starch; murexide test for purine bases, etc. are examples of this evaluation.

Quantitative chemical tests such as acid value (resins, balsams), saponification value (balsams), ester value (balsams, volatile oils), acetyl value (volatile oils), etc. are also useful in evaluation of a drug by means of chemical treatment.

Chemical assays include assays for alkaloid, resin, volatile oil, glycoside, vitamins, or other constituent. Few examples are the assay of total alkaloid in belladonna herb, the total alkaloid and nonphenolic alkaloid in ipecacuanha, the alkaloid strychnine in nux vomica, the resin in jalap, and the vitamins in codliver oil. The results obtained can conclude the presence of inferior or exhausted drug and, by proving absence of the assayed constituent, it will suggest complete substitution of a worthless article.

Instrumental analyses are used to analyse the chemical groups of phytoconstituents using chromatographic and spectroscopic methods. Chromatographic methods include paper chromatography, thinlayer chromatography, gas chromatography, highperformance liquid chromatography, and highperformance thinlayer chromatography. Spectroscopic methods include ultraviolet and visible spectroscopy, infrared spectroscopy, mass spectroscopy, and nuclear magnetic spectroscopy.

PHYSICAL EVALUATION

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.

Solubility

Drugs specific behaviours towards solvents are taken into consideration. This is useful for the examination of many oils, oleoresins, etc. Few examples are the solubility of colophony in light petroleum, the solubility of balsam of Peru in solution of chloral hydrate, the solubility of castor oil in half its volume of light petroleum and the turbidity produced with two volumes of the solvent; the solubility of balsam of Peru in an equal volume of alcohol, 90%, and the production of a turbidity with a larger volume; castor oil is soluble only in three volumes of 90% alcohol, while the adulterated form it shows good solubility in alcohol. Alkaloidal bases are soluble in organic solvents and alkaloidal salts are soluble in polar solvents.

Optical Rotation

Anisotropic crystalline solids and samples containing an excess of one enantiomer of a chiral molecule can rotate the orientation of planepolarized light. Such substances are said to be optically active, and this property is known as optical rotation. The enantiomer that rotates light to the right, or clockwise when viewing in the direction of light propagation, is called the dextrorotatory (d) or (+) enantiomer, and the enantiomer that rotates light to the left, or counterclockwise, is called the levorotatory (l) or ({) enantiomer. Few examples of drugs with this property are eucalyptus oil (0° to +10°), honey (+3° to {15°), Chenopodium oil ({30° to {80°), etc.

Refractive Index

Refractive index is defined as the property of a material that changes the speed of light, computed as the ratio of the speed of light in a vacuum to the speed of light through the material. When light travels at an angle between two different materials, their refractive indices determine the angle of transmission refraction of the light beam. In general, the refractive index varies based on the frequency of the light as well; thus, different colours of light travel at different speeds. High intensities can also change the refractive index. This could be used as a parameter in evaluating the herbal drugs; for example castor oil 1.4758 to 1.527, clove oil 1.527 to 1.535, etc.

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. Some examples of specific gravity of drugs are cottonseed oil 0.88–0.93, coconut oil 0.925, castor oil 0.95, etc.

Viscosity

Viscosity is the resistance of a fluid to flow. This resistance acts against the motion of any solid object through the fluid and also against motion of the fluid itself past stationary obstacles. Viscosity of a liquid is constant at a given temperature and is an index of its composition. Viscosity also acts internally on the fluid between slower and fastermoving adjacent layers. Since it is constant at a given temperature, it is used as an evaluation parameter; for example, pyroxylin kinematic viscosity, 1100–2450 centistokes.

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. As far as crude drugs are concerned,

melting point range has been fixed due to mixed chemicals. The following drugs could be evaluated using this parameter; for example, beeswax 62–65°C, wool fat 34–44°C, agar melts at 85°C, etc.

Moisture Content

The moisture content of a drug will be responsible for 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. Following are the examples of two crude drugs with their moisture content limit: the moisture content of Digitalis and Ergot should not be more than 5% w/w and 8% w/w, respectively.

Ultraviolet Light

Certain drugs fluoresce when the cut surface or the powder is exposed to ultraviolet radiation, and it is useful in the identification of those drugs. Some pieces of rhapontic, Indian, and Chinese rhubarb are very difficult to distinguish, and it is very difficult in powdered form, but examination in ultraviolet light gives such marked differences in fluorescence that the varieties can be easily distinguished from each other.

Ash Values

The determination of ash is useful for detecting lowgrade products, exhausted drugs, and excess of sandy or earthy matter. Different types of ash values are used in detection of crude drugs like, total ash, acidinsoluble ash, watersoluble ash, and sulphated ash.

Total ash is useful in detecting the crude drugs that are mixed with various mineral substances like sand, soil, calcium oxalate, chalk powder, or other drugs with different inorganic contents to improve their appearance, as is done with nutmegs and ginger. The maximum temperature used for total ash should be not more than 450°C because alkali chlorides that may be volatile in higher temperatures would be lost.

Acidinsoluble ash means the ash insoluble in dilute hydrochloric acid. It is often of more value than the total ash. The majority of crude drugs contain calcium oxalate, and the quantity of calcium oxalate varies very frequently. So total ash of a crude drug vary within wide limits for specimens of genuine drug, for example, rhubarb, total ash range from 8 to 40%. In this case, the total ash is useless to detect earthy matter adherent to such a drug. So acidinsoluble ash would be preferable for rhubarb. The calcium oxide or carbonate, yielded by the incinerated oxalate, will be soluble in hydrochloric acid when the ash is treated with hydrochloric acid; the remaining ash is weighed, which is known as the acidinsoluble ash. By this we can detect the presence of excessive earthy matter, which is likely to occur with roots and rhizomes and with leaves which are densely pubescent, like those of foxglove, clothed with abundant trichomes secreting resin, as in henbane, and tend to retain earth matter splashed on to them during heavy rainstorms.

The watersoluble ash is used to detect the presence of material exhausted by water. Sulphated ash is done by addition of sulphuric acid in order to get sulphate salts, and the percentage ash is calculated with reference to the airdried drug. The temperature used for this is above 600°C. The total ash and acidinsoluble ash values of Guduchi are not more than 16 and 3%, respectively. The total ash value and watersoluble ash values of ginger are 6 and 1.7%, respectively.

Extractive Values

The extracts obtained by exhausting crude drugs with different solvents are approximate measures of their chemical constituents. Various solvents are used according to the type of the constituents to be analysed. Watersoluble extractive is used for crude drugs containing watersoluble constituents like glycosides, tannins, mucilage, etc.; alcoholsoluble extractive is used for crude drugs containing tannins, glycosides, resins, etc.; and ethersoluble extractives are used for drugs containing volatile constituents and fats.

Extractive Values of Some Crude Drugs

Water-soluble extractive (% w/w)		Alcohol-soluble extractive (% w/w)	
aloe	Not less than 25.0	aloe	Not less than 10.0

Foreign Organic Matters

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, moulds, earthy material, animal excreta, etc. Each and every vegetable drug has their own limits. Few examples of such limits are: garlic should not contain more than 2%, saffron should not contain more than 2%, satavari should not contain more than 1%, etc.

BIOLOGICAL EVALUATION

The plant or extract can then be evaluated by various biological methods to determine pharmacological activity, potency, and toxicity. The biological evaluation would serve better than the physical and chemical evaluation for drugs that could not be satisfactorily assayed by these last two methods. Moreover, this is an important method, the crude drugs are considered important only because of their biological effects and this evaluation would conclude the effect. These methods are considered to be less precise, more timeconsuming and more expensive. Bioassays should be as simple as possible, and attempts should be made to have access to a large number of different tests so that many biological properties can be screened. The bioassay methods are of three types they are, toxic, symptomatic and tissue or organ methods. Different animals are used in toxic and symptomatic method and isolated organ or tissue is used in the third method.

These assays are conducted by determining the amount of drug of known potency required to produce a definite effect on suitable test animals or organs under standard conditions. Reference standard are used in certain bioassay procedures to minimize errors.

Toxicity studies are performed in suitable animal models to decide the lethal dose and effective dose of crude drags. Mice are used to test the effects of various vaccines.

Oxytocic activity of vasopressin injection is tested on guinea pigs, and oxytocic injection is assayed on young domestic chickens by injecting into an exposed crural or brachial vein and noticing the

changes in blood pressure. Pigeons are used to assay Digitalis glycosides by transfusing the drug through the alar vein to the blood stream and observing the lethal effects. Depressor activities and mydriatic effects of certain drugs are tested in cats and cat's eye, respectively. Anthelmintic drugs are evaluated on worms.

The drugs that have an effect in eyes are assayed on rabbit's eyes. Dogs are used to assay the drugs that exhibit cardiac and gastrointestinal activities. Effects of Ergot are carried out on cock's comb or rabbit's intestine or its uterus. Next to the animals, the studies are carried out in human beings also. In some instances, the effects that are observed from animal studies would be different when tested in humans. The tested biological activities include hepatoprotective activity, hypoglycaemic activity, antiinflammatory activity, antiulcer activity, immunomodulatory activity, etc.

Microbiological assays are carried out to determine the effects of drug in various microorganisms, and this is employed in the identification of antimicrobial drugs. The methods used in this type of assays are agar welldiffusion method, discdiffusion method, and turbidimetric method. In other microbiological methods, the living bacteria yeast moulds are used for assaying vitamins.

Quantitative Microscopy:

The techniques like microscopic linear measurements, determination of leaf constants and quantitative microscopy are also used in this evaluation.

Linear measurements include size of starch grains, length and width of fibres, trichomes, etc. The diameter of starch grains present in ipecacuanha assists in distinguishing its varieties. The diameter of starch grains in cassia bark distinguishes from cinnamon and detects senna stalk in powdered senna leaf. The size of the stomata in leaves of *Barosma betulina* distinguishes it from other species of Barosma. The diameter of phloem fibres aids the detection of cassia in cinnamon, and the width of the vessel helps to detect clove stalks in powdered cloves. Measurements of diameter for the identification of commercial starches and for the detection in them of foreign starch are few examples of linear measurements.

Determination of leaf constants include: stomatal number, stomatal index, vein islet, vein termination number, and palisade ratios. Stomatal number is average number of stomata per sq. mm of epidermis of the leaf.

Stomatal index: It is the percentage which the numbersof stomata form to the total number of epidermal cells, each stoma being counted as one cell. Stomatal index can be calculated by using the following formula:

Stomatal Index (S.I.) = S / E + S × 100

where, S = number of stomata per unit area and

E = number of epidermal cells in the same unit area.

Veinislet number: It is defined as the number of veinislets per sq. mm of the leaf surface midway between the midrib and the margin. It is a constant for a given species of the plant and is used as a characteristic for the identification of the allied species. Levin in 1929 determined veinislet numbers of several dicot leaves.

Veinlet termination number: It is defined as thenumber of veinlet termination per sq. mm of the leaf surface midway between midrib and margin. A vein termination is the ultimate free termination of veinlet. Hall and Melville in 1951 determined veinlet termination number of distinguishing between Indian and Alexandrian Senna.

Palisade ratio: It is defined as the average number of palisade cells beneath each epidermal cell. Unlike veinislet number for the determination of which an unbroken portion of the leaf is required, palisade ratio can be determined with the powdered drug. The technique of palisade ratio determination was introduced by Zorning and Weiss (1925) in their studies on Compositae.

One example is veinislet number of Alexandrian senna is 25–29.5, whereas Indian senna is 19.5–22.5. Stomatal index of Alexandrian senna is 10–15, whereas that of Indian Senna is 14–20.

Quantitative Microscopy (Lycopodium Spore Method)

This is an important technique employed in identification of crude drug when chemical and physical methods are inapplicable. Using this, one can determine the proportions of the substances present by means of the microscope, using the Lycopodium spore method.

The powdered drugs with welldefined particles which may be counted—for example, starch grains or singlelayered cells or tissues—the area of which may be traced under suitable magnification or the objects of uniform thickness, and the length of which, can be measured under suitable magnification and actual area calculated are usually evaluated using this method.

Adulterated starchy drugs can be determined by counting the number of starch grains per mg and calculating the amount from the known number of starch grains per mg of the pure starch or starchy material.

Thus, if spent ginger is the adulterant, one knows that ginger contains 286,000 starch grains per mg, and the amount used as an adulterant can be calculated by using this figure. The percentage purity of an authentic powdered ginger is calculated using the following equation:

$$[N \times W \times 94{,}000 \times 100] / [S \times M \times P] = \% \text{ purity of drugs}$$

where,

N = number of characteristic structures (e.g. starch grains) in 25 fields;

W = weight in mg of lycopodium taken;

S = number of lycopodium spores in the same 25 fields;

M = weight in mg of the sample, calculated on basis of sample dried at 105°C; and

P = 2,86,000 in case of ginger starch grains powder.

If the material is one for which a constant is not available, it is necessary to determine one by a preliminary experiment.

Plant Drug Quality Control – Introduction, significane, status and challenges

Traditional herbal medicine and their preparations have been widely used for the thousands of years in developing and developed countries owing to its natural origin and lesser side effects or dissatisfaction with the results of synthetic drugs. Despite its existence and continued use over many centuries, and its popularity and extensive use during the last few decades, traditional medicine has not been officially recognized in most countries.

Unlike in olden days, herbal medicines are being manufactured on large scale where manufacturers come across many problems such asavailability of good quality raw material, authentication of raw material, availability of standards, proper standardization methodology of single drugs and formulation, quality control parameters etc; hence the concept of quality from very first step is paramount factor must get good attention.

Variations in Plant drug materials and its effect on Quality

The inherent variability of the constituents of herbal materials, is the main reason why quality control of oriental herbal drugs is more difficult than that of western drug. Consistency in composition and biological activity are essential requirements for the safe and effective use of therapeutic agents. However, botanical preparations rarely meet this standard, as a result of problems in identifying plants, genetic variability, variable growing conditions, differences in harvesting procedures and processing of extracts, and the lack of information about active pharmacologic principles.

Environmental conditions such as sunlight, rainfall, altitude, temperature, soil, storage conditions as well as different harvesting procedures, time and method of collection, manufacturing processes such as selecting, drying, purifying, extracting, and genetic variability can create substantial variability in product quality and in the concentration of plant chemicals within different products.

Ecological conditions like insect feeding; microbial infections may affect secondary metabolites and in turn chemical composition of the plant. Also different parts of same plant (example roots, stem and leaves) contain different concentration of chemical constituents. At the same time diurnal variations (for example paclitaxel, opium alkaloids) and seasonal changes also account for variability in Herbal Medicines. The therapeutic or toxic components of plant vary depending on the part of the plant used as well as stages of ripeness. Products from different manufacture vary considerably and it is not possible to control all the factors that affect the plants chemical composition.

More over, as botanicals are prone to contamination and deterioration there may be batch to batch variation in composition. Each herb contains large number of diverse compounds and it is not possible to analyze for presence or absence for all compounds. Modern chromatographic techniques use chemical markers, which may not be therapeutically active. Due to over exploitation of certain plants, habitat loss and fragmentation of the forest, many medicinal plants have reached to the level of the endangered or rare species. These and many other factors (like cost of raw material) cause problem for availability of genuine drug, which encourages the adulteration of plant by substitution with inferior commercial varieties, artificially manufactured substances, exhausted drugs or cheaper plant or by another vegetative part. Several reports suggest that many herbal products contain undisclosed pharmaceuticals and heavy metals. The intentional use of pharmaceutical adulterant is possible. Agrochemicals are used to protect the plant from infections and insects, which occur as contaminant in the crude plant material.

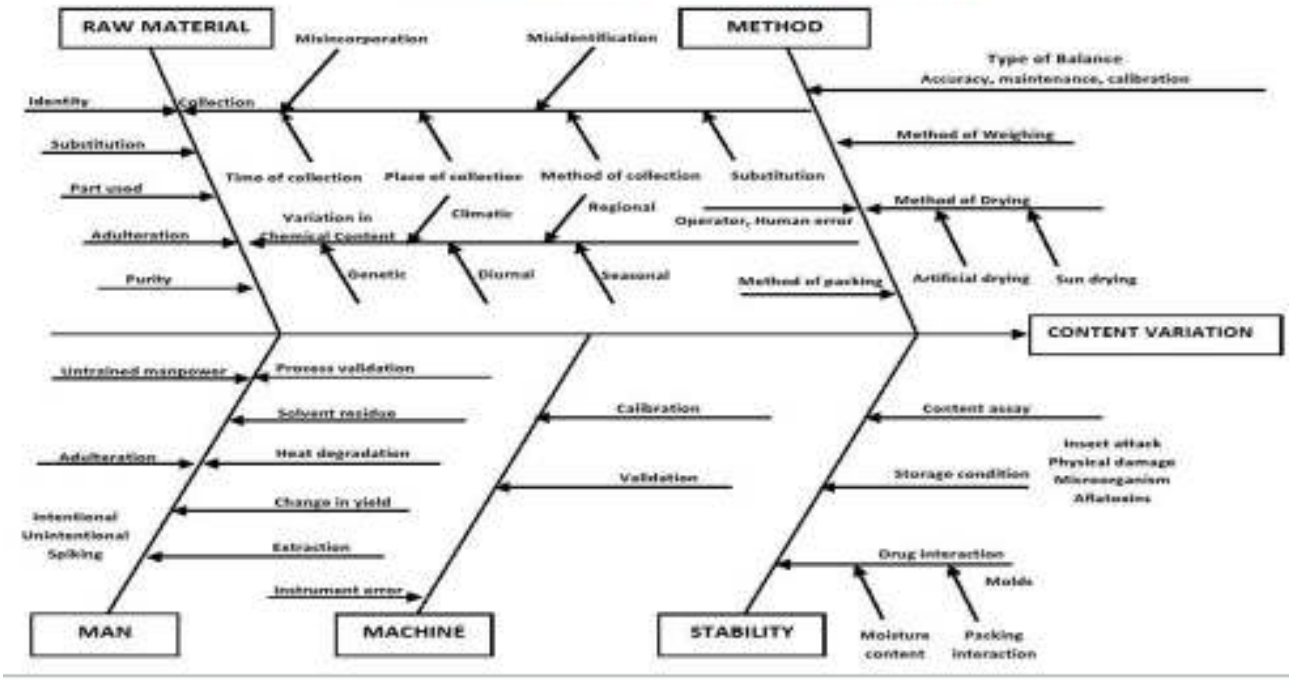


Figure 1: The Cause and effect diagram for content variation of herbal drugs:

Due to the inherent variability of the constituents of herbal products, it is difficult to establish quality control parameters, and batch-to-batch variation. In the absence of reference standards for identification, variability can start from the collection of raw material, and increase during storage and further processing. Generally, it is believed that the risk associated with herbal drugs is very low, but several reports on serious reactions are indicating the need for the development of safety profiles and stringent quality control systems for authentication, isolation, and standardization of herbal medicine. Reliable and consistent quality is the basis for efficacy and safety of herbal medicinal products. Proper validation of herbs used in traditional medicines needs to be done for their promotion and development.

The chemistry of plants involves the presence of therapeutically important constituents usually associated with many inert substances (coloring agents, cellulose, lignin etc). The active principles are extracted from the plants and purified for therapeutic utility for theirselective pharmacological activity. So quality control of herbal plant drug materialss and their constituents is of great importance in modern system of medicine. Lack of proper standard parameters for the standardization of herbal preparation and several instances of substandard herbs, adulterated herbs come into existence.

Hence every single herb needs to be quality checked to ascertain that it confirms to quality requirement and delivers the properties consistently. Standardization assures that products are reliable in terms of quality, efficacy, performance and safety. It is however observed that the drugs in commerce are frequently adulterated and do not comply with the standards prescribed for authentic drug.

Evaluation means confirmation of its identity and determination of quality and purity of the herbal drug. Evaluation of plant drug materials is necessary because of three main reasons: biochemical variations in the drug, deterioration due to treatment and storage, substitution and adulteration as a result of carelessness, ignorance or fraud or variability caused by differences in growth, geographical location, and time of harvesting. For the quality control of a traditional medicine, the

traditional methods are procured and studied, and documents and the traditional information about the identity and quality assessment are interpreted in terms of modern assessment or monograph in herbal pharmacopoeia

In general, quality control is based on three important pharmacopoeias definitions:

Identity: Is the herb the one it is claimed to be?

Purity: Are there contaminants, e.g., in the form of other herbs which should not be there?

Content or assay: Is the content of active constituents within the defined limits.

It is obvious that the content is the most difficult one to assess, since in most herbal drugs the active constituents are unknown. Sometimes markers can be used which are, by definition, chemically defined constituents that are of interest for control purposes, independent of whether they have any therapeutic activity or not. To prove identity and purity, criteria such as type of preparation sensory properties, physical constants, adulteration, contaminants, moisture, ash content and solvent residues have to be checked. The correct identity of the crude herbal material, or the botanical quality, is of prime importance in establishing the quality control of herbal drugs

Identity can be achieved by macro- and microscopical examinations. Voucher specimens are reliable reference sources. Outbreaks of diseases among plants may result in changes to the physical appearance of the plant and lead to incorrect identification.

Purity is closely linked with the safe use of drugs and deals with factors such ash values, contaminants (e.g. foreign matter in the form of other herbs), and heavy metals. However, due to the application of improved analytical methods, modern purity evaluation includes microbial contamination, aflatoxins, radioactivity, and pesticide residues.

Content or assay is the most difficult area of quality control to perform, since in most herbal drugs the active constituents are not known. Sometimes markers can be used. In all other cases, where no active constituent or marker can be defined for the herbal drug, the percentage extractable matter with a solvent may be used as a form of assay, an approach often seen in pharmacopeias. The choice of the extracting solvent depends on the nature of the compounds involved, and might be deduced from the traditional uses.

The plant drug materials can be evaluated or identified by five methods:

- Organoleptic evaluation or morphological evaluation
- Microscopic evaluation
- Physical & Proximate values evaluation
- Chemical evaluation
- Chromatography and chemical fingerprints of herbal medicines
- Biological evaluation
- Radioimmunoassay

Standardization

The standardization of Herbal Medicine for quality, emphasises the standardization of herb medicinal substances/materials and preparations and that of Herbal Medicine substance. The morphological identification and microscopic identification are utilized to determine the authenticity of Herbal Medicine s whereas the physical and chemical characters are used to evaluate the quality of herbs in the existing quality standards. The chemical complexity of Herbal Medicine s cannot be elaborated only by above methods.

So far, during the production and circulation of Herbal Medicines, there is no comprehensive and integrated quality control measure to reflect the variations of Herbal Medicine products, and to

effectively control the quality in the whole process. The research and establishment of fingerprints contributed much to solving the problem. It can evaluate the integrative and holistic properties of Herbal Medicines by comparing the similarity and correlation of the analytes among the whole producing process, such as manufacture, processing and storage of raw materials for preparation, intermediate products, finished products and distribution products. The fingerprint analysis has been internationally accepted as one of the efficient methods to control the quality of Herbal Medicines. Chemical fingerprint is used to analyze the chemical constituents in Herbal Medicines, consisting chromatographic fingerprint, such as thin layer chromatography (TLC), high performance liquid chromatography (HPLC), gas chromatography (GC).

Analytical Evaluation

Botanical parameters:

- **Sensory evaluation**: including visual macroscopy/ touch/odour/taste
- **Foreign matter**: including foreign plants, foreign animals, foreign minerals, etc.
- **Microscopy**: including histological observation and measurements

Physico-chemical methods:

- **Ash values**: Total, acid insoluble, water soluble
- **Extractive values**: in hot water, cold water and ethanol,
- **Volatile matter**: Loss on drying,
- **Volatile oils**: by steam distillation.
- Determination of crude fibers,
- Determination of moisture content.

Biological parameters:

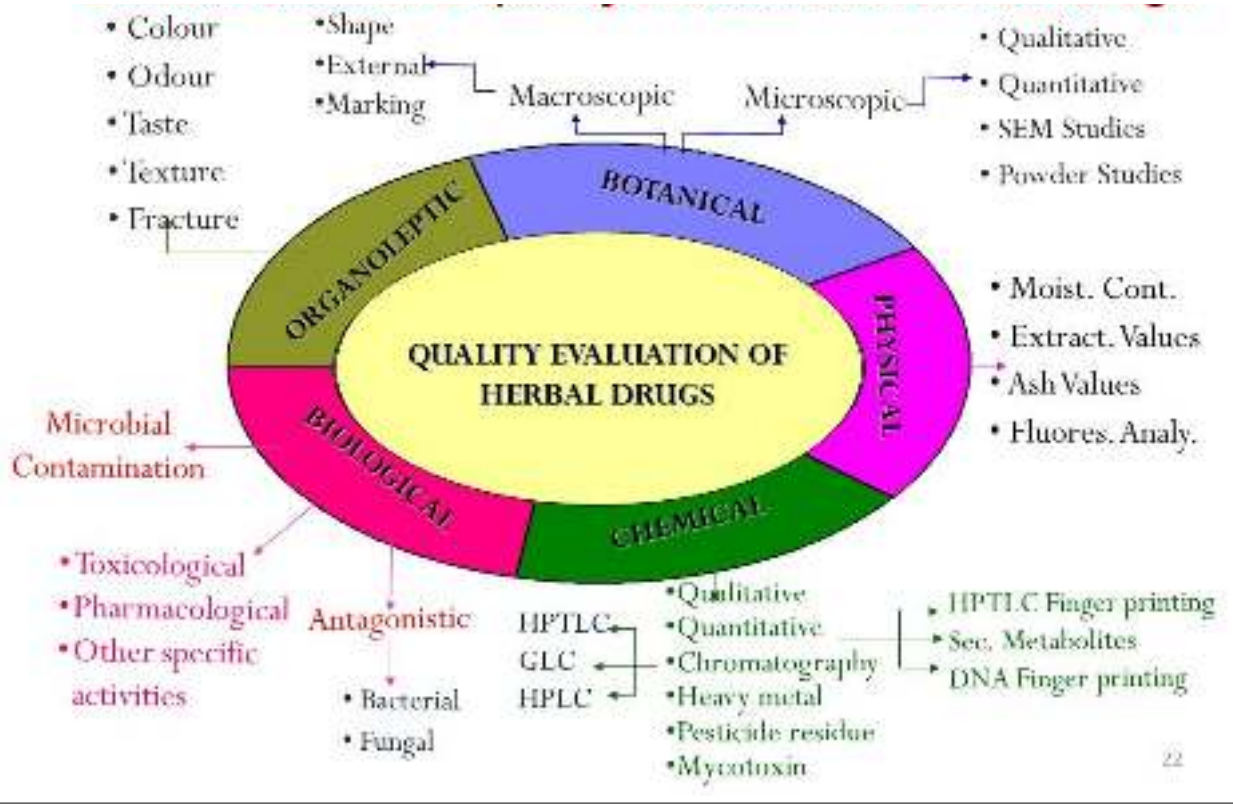
- **Bitterness value**: unit equivalent bitterness of standard solution of Quinine HCl
- **Hemolytic property**: on ox blood by comparison with standard reference solution of saponin
- **Astringent property**: tannins that bind to standard Frieberg Hide powder
- **Swelling index**: in water
- **Foaming index**: foam height produced by 1g material under specified conditions

Toxicological parameters:

These include the various identification procedures:

- **Arsenic**: stain produced on $HgBr_2$ paper in comparison to standard stain
- **Pesticide residues**: including total organic chloride and total organic phosphorus
- **Heavy metals**: like cadmium, mercury and lead
- **Microbial contaminations**: total viable aerobic count of pathogens; viz. *Enterobacteriaceae*, *E. Coli*, *Salmonella*, *P. aeruginosa*, *S. aureous*, (e.g. *Mellilotus officinalis)**
- **Aflatoxins**: by TLC using standard aflatoxins (B_1, B_2, G_1 and G_2)
- Radioactive contaminations

Summary Diagram of Quality Evaluation of Herbal Drugs



Cultivation, Collection and Processing of Herbal Drugs

INTRODUCTION

The crude drugs which reach the market and pharmaceutical industries will have passed through different stages that have some effect in the nature and amount of active constituents responsible for therapeutic activity. Those stages are to be concerned more in order to make a drug useful to the mankind by all means. This chapter concerns regarding such parameters which has some effect over plants.

Cultivation produces improved quality of plants. It helps in selecting the species, varieties or hybrids that have the desired phytoconstituents due to the controlled environmental growth better plant product is obtained and makes the collection and 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 purposes.

The advantages of cultivation may be briefly summarized as follows:

1. It ensures quality and purity of medicinal plants. Crude drugs derive theirutility from chemical contents in them. If uniformity is maintained in all operations during the process of cultivation, drugs of highest quality can be obtained. Cultivation of rhizomes demands an adequate quantity of fertilizers and proper irrigation. Systematic cultivation results in raising a crop with maximum content of volatile oil and other constituents. The examples of ginger, turmeric and liquorice can be cited to illustrate this point. If the cultivated plants are kept free of weeds, the contamination of crude drugs can be conveniently avoided.

2. Collection of crude drugs from cultivated plants gives a better yield and therapeutic quality. However, it is a skilled operation and requires some professional excellence, if the collection of crude drugs for market is done from cultivated plants by skilled and wellexperienced personnel, the high yield and therapeutic quality of drugs can be maintained. For example, collection of latex from poppy capsules and oleoresins from Pinus species, if done by experienced persons, can result in better yield of crude drugs. Preservation of green colour of senna leaves and minimizing the deterioration of cardiac glycosides in freshly collected leaves of digitalis can be achieved only by highly skilled labour.

3. Cultivation ensures regular supply of a crude drug. In other words, cultivation is a method of cropplanning. Planning a crop cultivation regularizes its supply and as a result the industries depending upon crude drugs do not face problem of shortage of raw material.

4. The cultivation of medicinal and aromatic plants also leads to industrialization to a greater extent. The cultivation of coffee and cocoa in Kerala has given rise to several cottage and small scale industries. The cultivation of cinchona in West Bengal has led to the establishment of the cinchonaalkaloid factory near Darjeeling. The government owned opium factory at Ghaziabad is an eloquent testimony to the significance of well planned cultivation of poppy.

5. Cultivation permits application of modern technological aspects such as mutation, polyploidy and hybridization.

SOILS, SEEDS AND PROPAGATION MATERIAL

The physical, chemical and microbiological properties of the soil play a crucial role in the growth of plants. Water holding capacity of different sizes of soil too affects the plants. The calcium present in the soil would be very much useful for some plants where as the others does not require calcium. The seed to be used for cultivation should be identified botanically, showing the details of its species, chemotype and origin. The seeds should be 100% traceable. The parent material should meet standard requirements regarding the purity and germination. It should be free from pests and diseases in order to guarantee healthy plant growth. Preference should be given to the resistant or tolerant species. Plant materials or seeds derived from genetically modified organisms have to comply with national and European Union regulations. Season

when the seeds should be sown and at what stage a seed should be sown should be predetermined. Few seeds such as cinnamon losses its viability if stored for long period and the percentage of germination would be less for the seeds which were long stored.

Methods of Plant Propagation

Medicinal plants can be propagated by two usual methods as applicable to nonmedicinal plants or crops. These methods are referred as sexual method and asexual method. Each of these methods has certain advantages, and also, disadvantages.

1. Sexual method (seed propagation)

In case of sexual method, the plants are raised from seeds and such plants are known as seedlings. The sexual method of propagation enjoys following advantages:

1.Seedlings are longlived (in case of perennial drugs) and bear more heavily (in case of fruits). Plants are more sturdier.

2.Seedlings are comparatively cheaper and easy to raise.

3.Propagation from seed has been responsible for production of some chanceseedlings of highly superior merits which may be of great importance to specific products, such as orange, papaya, etc.

4.In case of plants where other vegetative methods cannot be utilized, propagation from seeds is the only method of choice.

Sexual method suffers from following limitations

1.Generally, seedling trees are not uniform in their growth and yielding capacity, as compared to grafted trees.

2.They require more time to bear, as compared to grafted plants.

3.The cost of harvesting, spraying of pesticides, etc. is more as compared to grafted trees.

4.It is not possible to avail of modifying influence of root stocks on scion, as in case of vegetatively propagated trees.

For propagation purpose, the seeds must be of good quality. They should be capable a high germination rate, free from diseases and insects and also free from other seeds, used seeds and extraneous material. The germination capacity of seeds is tested by rolled towel test, excised embryo test, etc. The seeds are preconditioned with the help of scarcification to make them permeable to water and gases, if the seeds are not to be germinated in near future, they should be stored in cool and dry place to maintain their germinating power. Long storage of seeds should be avoided.

Before germination, sometimes a chemical treatment is given with stimulants like gibberellins, cytokinins, ethylene, thiourea, potassium nitrate or sodium hypochlorite. Gibbereilic acid (GA3) promotes germination of some type of dormant seeds and stimulates the seedling growth. Many freshly harvested dormant seeds germinate better after soaking in potassium nitrate solution. Thiourea is used for those seeds which do not germinate in dark or at high temperatures.

Methods of sowing the seeds

Numerous methods of sowing the seeds of the medicinal plants are in practice. Few of them using seeds for cultivation are described:

Broadcasting: If the seeds are extremely small the sowing is done by broadcasting method. In this method the seeds are scattered freely in well prepared soil for cultivation. The seeds only need raking. If they are deeply sown or covered by soil, they may not get germinated. Necessary thinning of the seedlings is done by keeping a specific distance, e.g. Isabgol, Linseed, Sesame, etc.

Dibbling: When the seeds of average size and weight are available, they are sown byplacing in holes. Number of seeds to be put in holes vary from three to five, depending upon the vitality, sex of the plants needed for the purpose and the size of the plant coming out of the seeds.

For example, in case of fennel four to five fruits are put in a single hole keeping suitable distance in between two holes. In case of castor, only two to three seeds are put. In case of papaya, the plants are unisexual and only female plants are desired for medicinal purposes. Hence, five to six seeds are put together and after the sex of the plants is confirmed, healthy female plant is allowed to grow while male plants and others are removed.

Miscellaneous: Many a times the seeds are sown in nursery beds. The seedlings thus produced are transplanted to farms for further growth, such as cinchona, cardamom, clove, digitalis, capsicum, etc.

Special treatment to seeds: To enhance germination, special treatments to seeds may be given, such as soaking the seeds in water for a day e.g. castor seeds and other slowgerminating seeds. Sometimes, seeds are soaked in sulphuric acid e.g. henbane seeds. Alternatively, testa is partially removed by grindstone or by pounding seeds with coarse sand, e.g. Indian senna. Several plant hormones like gibberellins, auxins are also used.

2. Asexual method

In case of asexual method of vegetative propagation, the vegetative part of a plant, such as stem or root, is placed in such an environment that it develops into a new plant.

Asexual propagation enjoys following advantages:

1. There is no variation between the plant grown and plant from which it is grown. As such, the plants are uniform in growth and yielding capacity. In case of fruit trees, uniformity in fruit quality makes harvesting and marketing easy.
2. Seedless varieties of fruits can only be propagated vegetatively e.g. grapes, pomegranates and lemon.
3. Plants start bearing earlier as compared to seedling trees.
4. Budding or grafting encourages diseaseresistant varieties of plants.
5. Modifying influence of rootstocks on scion can be availed of.
6. Inferior or unsuitable varieties can be overlooked.

It suffers from following disadvantages:

1. In comparison to seedling trees, these are not vigorous in growth and are not longlived.
2. No new varieties can be evolved by this method.

Asexual method of vegetative propagation consists of three types:

a) Natural methods of vegetative propagation.
b) Artificial methods of vegetative propagation.
c) Aseptic method of micropropagation (tissueculture).

a) Natural methods of vegetative propagation: It is done by sowing various parts of the plants in well prepared soil. The following are the examples of vegetative propagation:

- Bulbs: Squill, Garlic
- Tubers: Aconite, Jalap, Potato
- Rhizomes: Ginger, Trumeric
- Stolons: Liquorice

b) Artificial methods of vegetative propagations: Themethod by which plantlets or seedlings are produced from vegetative part of the plant by using some technique or process is known as artificial method of vegetative propagation. These methods are classified as under:

1.Cuttings

i.Stem cuttings

a)Soft wood cuttings: Berberry.

b)Semi hard wood cuttings: Citrus, camellia.

c)Hard wood cuttings: Orange, rose and bougainvillea.

ii.Root cuttings: Brahmi.

iii.Leaf cuttings: Bryophyllum.

iv.Leaf bud cuttings.

2.Layering

i.Simple layering: Guava, lemon

ii.Serpentine layering: jasmine, clematis

iii.Air layering (Gootee): Ficus, mango, bougainvillea, cashew nut

iv.Mount layering

v.Trench layering

vi.Tip layering

3.Grafting

i.Whip grafting: Apple and rose

ii.Tongue grafting

iii.Side grafting: Sapota and cashew nut

iv.Approach grafting: Guava and Sapota

v.Stone grafting: Mango

(c) Aseptic methods of micropropagation (tissue culture)

It is a novel method for propagation of medicinal plants. In micropropagation, 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 Beds

For various genuine reasons, seeds cannot be sown directly into soil i.e. very small size (Isabgol, tulsi) high cost, poor germination rate and long germination time (Cardamom, Coriander). Under such circumstances, seeds are grown into the nursery bed which not only is economical, but one can look after the diseases (if any) during germination period. Small size of beds can be irrigated conveniently along with fertilizers, as and when necessary. There are four types of nursery beds.

1.Flat bed method

2.Raised bed method

3.Ridges and furrow method

4.Ring and basin method

Taking into consideration the amount of water and type of soil required for a particular seed one should select the type.

Methods of Irrigation

Water is essential for any type of cultivation. After studying the availably and requirement of water for a specific crop, one has to design his own irrigation system at the reasonable cost.

Following methods of irrigation are known traditionally in India. The cultivation has an option after giving due consideration to the merits and demerits of each.

1.Hand watering: economical and easy to operate.

2.Flood watering: easy to operate, results in wastage of water.

3.Boom watering: easy to operate, but restricted utility.

4.Drip irrigation: Scientific, systematic and easy to operate; costly.

5.Sprinkler irrigation: Costly, gives good results.

GOOD AGRICULTURAL PRACTICES

Depending on the method of cultivation different Standard Operating Procedures for cultivation should be followed by the cultivators. A suitable area for the cultivation and the standard operation procedures for the cultivation should be developed depending upon the needs of the plants. Medicinal and aromatic plants should not be grown in soils which are contaminated by sludge and not contaminated by heavy metals, residues of plant protection products and any other unnatural chemicals, so the chemical products (pesticide and herbicide) used should be with as minimum negative effect as possible, human faeces should be avoided. Depending upon the soil fertility and the nutritional requirement of medicinal plants the type of the fertilizer and the amount of the fertilizer to be used is determined. Products for chemical plant protection have to conform to the European Union's maximum residue limits. Proper irrigation and drainage should be earned out according to the climatic condition and soil moisture. The soil used for cultivation should be well aerated. The use of pesticides and herbicides has to be documented. Irrigation should be minimized as much as possible and only be applied according to the needs of the plant. Water used for irrigation should be free from all possible forms of contaminants and should comply with national and European Union quality standards. The area for cultivation should be strictly prohibited from the contaminations like house garbage, industrial waste, hospital refuse and feces. Field management should be strengthened and proper measures like pruning, shading etc. should be provided for increasing the yield of the active constituent and maintain the consistency of the yield. The pests used should give high efficacy, hypotoxicity, and low residue at the minimum effective input so that the residue of pesticides are also reduced and protected from ecological environment.

Application and storage of plant protection products have to be in conformity with the regulations of manufacturers and the respective national authorities. The application should only be earned out by qualified staff using approved equipment. The nutrient supply and chemical plant protection, should secure the marketability of the product. The buyer of the batch should be informed about the brand, quantity and date of pesticide use in written.

Though several countries in the world have a rich heritage of herbal drugs, very few have their claim for their procurement of crude drugs only from cultivated species. Our reliance on wild sources of crude drugs and the lack of information on the sound cultivation and maintaining technology of crude drugs have resulted in gradual depletion of raw material from wild sources. Though the cultivation of medicinal plants offers wide range of advantages over the wild sources, it can be an uneconomical process for some crude drugs which occur abundantly in nature e.g. nux vomica, acacia etc. On the other hand, crude drugs like cardamom, clove, poppy, tea, cinchona, ginger, linseed, isabgol, saffron, peppermint, fennel, etc. are obtained majorly from cultivated plants. The cultivation of crude drugs involves keen knowledge of various factors from agricultural and pharmaceutical sphere, such as soil, climate, rainfall, irrigation, altitude, temperature, use of fertilizers and pesticides, genetic manipulation and biochemical aspects of natural drugs. When all such factors are precisely applied, the new approach to scientific cultivation technology emerges out.

COLLECTION OF CRUDE DRUGS

Collection is the most important step which comes after cultivation. Drugs are collected from wild or cultivated plants and the tasks for collection depends upon the collector, whether he is a skilled or unskilled labour. Drugs should be collected when they contain maximum amount of constituents in a highly scientific manner. The season at which each drug is collected is so important, as the amount, and sometimes the nature, of the active constituents could be changed throughout the year. For example, Rhubarb is collected only in summer seasons because no anthraquinone derivatives would be present in winter season but

anthranols are converted to anthraquinones during summer. Not only the season but also the age of the plant should be taken in to great consideration since it governs not only the total amount of active constituents produced in the plants but also the proportions of the constituents of the active mixture. High proportion of pulegone in young plants of peppermint will be replaced by menthone and menthol and reduction in the percentage of alkaloids in datura as the plant ages are examples of the effect of aging in plants.

Moreover the composition of a number of secondary plant metabolites varies throughout the day and night, and it is believed that some inter conversion would happen during day and night.

Generally the leaves are collected just before the flowering season, e.g. vasaka, digitalis, etc., at this time it is assumed that the whole plant has come to a healthy state and contain an optimum amount of metabolites, flowers are collected before they expand fully, e.g. clove, saffron, etc., and underground organs as the aerial parts of plant cells die, e.g. liquorice, rauwolfia, etc. Since it is very difficult to collect the exact medicinally valuable parts, the official pharmacopoeia's has fixed certain amount of foreign matter that is permissible with drug. Some fruits are collected after their full maturity while the others are collected after the fruits are ripe. Barks are usually collected in spring season, as they are easy to separate from the wood during this season. The barks are collected using three techniques, felling (bark is peeled off after cutting the tree at base), uprooting (the underground roots are dug out and barks are collected from branches and roots) and coppicing (plant is cut one metre above the ground level and barks are removed).

Underground parts should be collected and shaken, dusted in order to remove the adhered soil; water washing could be done if the adhered particles are too sticky with plant parts. The unorganized drugs should be collected from plants as soon as they oozes out, e.g. resins, latex, gums, etc. Discoloured drugs or drugs which were affected by insects should be rejected.

HARVESTING OF CRUDE DRUGS

Harvesting is an important operation in cultivation technology, as it reflects upon economic aspects of the crude drugs. An important point which needs attention over here is the type of drug to be harvested and the pharmacopoeial standards which it needs to achieve. Harvesting can be done efficiently in every respect by the skilled workers. Selectivity is of advantage in that the drugs other than genuine, but similar in appearance can be rejected at the site of collection. It is, however, a laborious job and may not be economical. In certain cases, it cannot be replaced by any mechanical means, e.g. digitalis, tea, vinca and senna leaves. The underground drugs like roots, rhizomes, tubers, etc. are harvested by mechanical devices, such as diggers or lifters. The tubers or roots are thoroughly washed in water to get rid of earthymatter. Drugs which constitute all aerial parts are harvested by binders for economic reasons. Many a times, flowers, seeds and small fruits are harvested by a special device known as seed stripper. The technique of beating plant with bamboos is used in case of cloves. The cochineal insects are collected from branches of cacti by brushing. The seaweeds producing agar are harvested by long handled forks. Peppermint and spearmint are harvested by normal method with mowers, whereas fennel, coriander and caraway plants are uprooted and dried. After drying, either they are thrashed or beaten and the fruits are separated by winnowing. Sometimes, reaping machines are also used for their harvesting.

DRYING OF CRUDE DRUGS

Before marketing a crude drug, it is necessary to process it properly, so as to preserve it for a longer time and also to acquire better pharmaceutical elegance. This processing includes several operations or treatments, depending upon the source of the crude drug (animal or plant) and its chemical nature. Drying consists of removal of sufficient moisture content of crude drug, so as to improve its quality and make it resistant to the growth of microorganisms. Drying inhibits partially enzymatic reactions. Drying also facilitates pulverizing or grinding of a crude drug. In certain drugs, some special methods are required to be followed to attain specific standards, e.g. fermentation in case of Cinnamomum zeylanicum bark and gentian

roots. The slicing and cutting into smaller pieces is done to enhance drying, as in case of glycyrrhiza, squill and calumba. The flowers are dried in shade so as to retain their colour and volatile oil content. Depending upon the type of chemical constituents, a method of drying can be used for a crude drug. Drying can be of two types (1) natural (sun drying) and (2) artificial.

Natural Drying (SunDrying)

In case of natural drying, it may be either direct sundrying or in the shed. If the natural colour of the drug (digitalis, clove, senna) and the volatile principles of the drug (peppermint) are to be retained, drying in shed is preferred. If the contents of the drugs are quite stable to the temperature and sunlight, the drugs can be dried directly in sunshine (gum acacia, seeds and fruits).

Artificial Drying

Drying by artificial means includes drying the drugs in (a) an oven; i.e. traydryers; (b) vacuum dryers and (c) spray dryers.

a. Tray dryers

The drugs which do not contain volatile oils and are quite stable to heat or which need deactivation of enzymes are dried in tray dryers. In this process, hot air of the desired temperature is circulated through the dryers and this facilitates the removal of water content of the drugs (belladonna roots, cinchona bark, tea and raspberry leaves and gums are dried by this method).

b. Vacuum dryers

The drugs which are sensitive to higher temperature are dried by this process, e.g. Tannic acid and digitalis leaves.

c. Spray dryers

Few drugs which are highly sensitive to atmospheric conditions and also to temperature of vacuumdrying are dried by spraydrying method. The technique is followed for quick drying of economically important plant or animal constituents, rather than the crude drugs. Examples of spray drying are papaya latex, pectin, tannins, etc.

GARBLING (DRESSING)

The next step in preparation of crude drug for market after drying is garbling. This process is desired when sand, dirt and foreign organic parts of the same plant, not constituting drug are required to be removed. This foreign organic matter (extraneous matter) is removed by several ways and means available and practicable at the site of the preparation of the drugs. If the extraneous matter is permitted in crude drugs, the quality of drug surfers and at times, it dose not pass pharmacopoeial limits. Excessive stems in case of lobelia and stramonium need to be removed, while the stalks, in case of cloves are to be deleted. Drugs constituting rhizomes need to be separated carefully from roots and rootlets and also stem bases. Pieces of iron must be removed with the magnet in case of castor seeds before crushing and by shifting in case of vinca and senna leaves. Pieces of bark should be removed by peeling as in gum acacia.

PACKING OF CRUDE DRUGS

The morphological and chemical nature of drug, its ultimate use and effects of climatic conditions during transportation and storage should be taken into consideration while packing the drugs. Aloe is packed in goat skin. Colophony and balsam of tolu are packed in kerosene tins, while asafoetida is stored in well closed containers to prevent loss of volatile oil. Cod liver oil, being sensitive to sunlight, should be stored in such containers, which will not have effect of sunlight, whereas, the leaf drugs like senna, vinca and others are pressed and baled. The drugs which are very sensitive to moisture and also costly at the same time need special attention, e.g. digitalis, ergot and squill. Squill becomes flexible; ergot becomes susceptible to the microbial growth, while digitalis looses its potency due to decomposition of glycosides, if brought in contact

with excess of moisture during storage. Hence, the chemicals which absorb excessive moisture (desiccating agents) from the drug are incorporated in the containers. Colophony needs to be packed in big masses to control autooxidation. Cinnamon bark, which is available in the form of quills, is packed one inside the other quill, so as to facilitate transport and to prevent volatilization of oil from the drug.

The crude drugs like roots, seeds and others do not need special attention and are packed in gunny bags, while in some cases bags are coated with polythene internally. The weight of certain drugs in lots is also kept constant e.g. Indian opium.

STORAGE OF CRUDE DRUGS

Preservation of crude drugs needs sound knowledge of their physical and chemical properties. A good quality of the drugs can be maintained, if they are preserved properly. All the drugs should be preserved in well closed and, possibly in the filled containers. They should be stored in the premises which are waterproof, fire proof and rodentproof. A number of drugs absorb moisture during their storage and become susceptible to the microbial growth. Some drugs absorb moisture to the extent of 25% of their weight. The moisture, not only increases the bulk of the drug, but also causes impairment in the quality of crude drug. The excessive moisture facilitates enzymatic reactions resulting in decomposition of active constituents e.g. digitalis leaves and wild cherry bark. Gentian and ergot receive mould infestation due to excessive moisture. Radiation due to direct sunlight also causes destruction of active chemical constituents, e.g. ergot, cod liver oil and digitalis. Form or shape of the drug also plays very important role in preserving the crude drugs. Colophony in the entire form (big masses) is preserved nicely, but if stored in powdered form, it gets oxidized or looses solubility in petroleum ether. Squill, when stored in powdered form becomes hygroscopic and forms rubbery mass on prolonged exposure to air. The fixed oil in the powdered ergot becomes rancid on storage. In order to maintain a good quality of ergot, it is required that the drug should be defatted with lipid solvent prior to storage. Lard, the purified internal fat of the abdomen of the hog, is to be preserved against rancidity by adding siam benzoin. Atmospheric oxygen is also destructive to several drugs and hence, they are filled completely in well closed containers, or the air in the container is replaced by an inert gas like nitrogen; e.g. shark liver oil, papain, etc.

Apart from protection against adverse physical and chemical changes, the preservation against insect or mould attacks is also important. They can be prevented by drying the drugthoroughly before storage and also by giving treatment of fumigants. The common fumigants used for storage of crude drugs are methyl bromide, carbon disulphide and hydrocyanic acid. At times, drugs are given special treament, such as liming of the ginger and coating of nutmeg. Temperature is also very important factor in preservation of the drugs, as it accelerates several chemical reactions leading to decomposition of the constituents. Hence, most of the drugs need to be preserved at a very low temperature. The costly phytopharmaceuticals are required to be preserved at refrigerated temperature in well closed containers. Small quantities of crude drugs could be readily stored in airtight, moisture proof and light proof containers such as tin, cans, covered metal tins, or amber glass containers. Wooden boxes and paper bags should not be used for storage of crude drugs.

FACTORS INFLUENCING CULTIVATION OF MEDICINAL PLANTS

(Exogenous or External Factors)

Cultivation of medicinal plants offers wide range of advantages over the plants obtained from wild sources. There are few factors to concern which have a real effect on plant growth and development, nature and quantity of secondary metabolites. The factors affecting cultivation are altitude, temperature, rainfall, length of day, day light, soil and soil fertility, fertilizers and pests. The effects of these factors have been studied by growing particular plants in different environmental conditions and observing variations. For example, a plant which is subjected to a particular environment may develop as a small plant which, when analysed shows high proportion of metabolite than the plants attained the required growth. Nutrients have the ability to enhance the production of secondary metabolites, at the same time they may reduce the metabolites as well.

Altitude

Altitude is a very important factor in cultivation of medicinal plants. Tea, cinchona and eucalyptus are cultivated favourably at an altitude of 1,000–2,000 metres. Cinnamon and cardamom are grown at a height of 500–1000 metres, while senna can be cultivated at sea level. The following are the examples of medicinal and aromatic plants indicating the altitude for their successful cultivation (Table below).

Plant	Altitude (m
Tea	1,000–1,50
Cinchona	1,000–2,00
Camphor	1,500–2,00
Cinnamon	250–1,000
Coffee	1,000–2,00

Temperature

Temperature is a crucial factor controlling the growth, metabolism and there by the yield of secondary metabolites of plants. Even though each species has become adapted to its own natural environment, they are able to exist in a considerable range of temperature. Many plants will grow better in temperate regions during summer, but they lack in resistance to withstand frost in winter.

Table: Optimum Temperature for Drug Cultivation

Plant	Optimum Temperature (°F)
Cinchona	60–75
Coffee	65–70
Tea	70–90
Cardamom	50–100

Rainfall

For the proper development of plant, rainfall is required in proper measurements. Xerophytic plants like aloes do not require irrigation or rainfall. The effects of rainfall on plants 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 the plants.

Day Length and Day Light

It has been proved that even the length of the day has an effect over the metabolites 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. For example peppermint has produced menthone, menthol and traces of menthofuran in long day conditions and only menthofuran in short day condition.

The developments of plants vary much in both the amount and intensity of the light they require. The wild grown plants would meet the required conditions and so they grow but during cultivation we have to fulfill the requirements of plants. The day light was found to increase the amount of alkaloids in belladonna, stramonium, cinchona, etc. Even the type of radiation too has an effect over the development and metabolites of plants.

Soil

Each and every plant species have its own soil and nutritive requirements. The three important basic characteristics of soils are their physical, chemical and microbiological properties. Soil provides mechanical support, water and essential foods for the development of plants. Soil 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. The type and amount of minerals plays a vital role in plant cultivation. Calcium favours the growth of certain plants whereas with some plants it does not produce any effects. The plants are able to determine their own soil pH range for their growth; microbes should be taken in to consideration which grows well in certain pH. Nitrogen containing soil has a great momentum in raising the production of alkaloids in some plants.

Depending upon the size of the mineral matter, the following names are given to the soil (Table 6.3).

Table : Type of soil on the basis of particle size.

Particle size (diametre)	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

Depending upon the percentage covered by clay, soils are classified as under (Table below.).

Table : Type of soil on the basis of percentage covered by clay.

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

Soil Fertility

It is the capacity of soil to provide nutrients in adequate amounts and in balanced proportion to plants. If cropping is done without fortification of soil with plant nutrients, soil fertility gets lost. It is also diminished through leaching and erosion. Soil fertility can be maintained by addition of animal manures, nitrogenfixing bacteria or by application of chemical fertilizers. The latter is time saving and surest of all above techniques.

Fertilizers and Manures

Plant also needs food for their growth and development. What plants need basically for their growth are the carbon dioxide, sunrays, water and mineral matter from the soil. Thus, it is seen that with limited number of chemical elements, plants build up fruits, grains, fibres, etc. and synthesize fixed and volatile oils, glycosides, alkaloids, sugar and many more chemicals.

(a) Chemical fertilizers

Animals are in need of vitamins, plants are in need of sixteen nutrient elements for synthesizing various compounds. Some of them are known as primary nutrients like nitrogen, phosphorus and potassium. Magnesium, calcium and sulphur are required in small quantities and hence, they are known as secondary nutrients. Trace elements like copper, manganese, iron, boron, molybdenum, zinc are also necessary for plant growths are known as micronutrients. Carbon, hydrogen, oxygen and chlorine are provided from water and air. Every element has to perform some specific function in growth and development of plants. Its deficiency is also characterized by certain symptoms.

(b) Manures

Farm yard manure (FYM/compost), castor seed cake, poultry manures, neem and karanj seed cakes vermin compost, etc. are manures. Oilcake and compost normally consists of 3–6% of nitrogen, 2% phosphates and 1–1.5% potash. They are made easily available to plants. Bone meal, fish meal, biogas slurry, blood meal and press mud are the other forms of organic fertilizers.

(c) Biofertilizers

Inadequate supply, high costs and undesirable effects if used successively are the demerits of fertilizers or manures and hence the cultivator has to opt for some other type of fertilizer. Biofertilizers are the most suitable forms that can be tried. These consist of different types of micro organisms or lower organisms which fix the atmospheric nitrogen in soil and plant can use them for their day to day use. Thus they are symbiotic. Rhizobium, Azotobactor, Azosperillium, Bijericcia, Bluegreen algae, Azolla, etc. are the examples of biofertilizers.

Pests and Pests Control

Pests are undesired plant or animal species that causes a great damage to the plants. There are different types of pests; they are microbes, insects, non insect pests and weeds.

Microbes

They include fungi, bacteria and viruses. Armillaria Root Rot (Oak Root Fungus) is a disease caused by fungi *Armillaria mellea* (Marasmiaceae) and in this the infected plantbecome nonproductive and very frequently dies within two to four years. Plants develop weak, shorter shoots as they are infected by the pathogen. Dark, rootlike structures (rhizomorphs), grow into the soil after symptoms develop on plants. The fungus is favoured by soil that is continually damp. Powdery mildew is another disease caused by fungus *Uncinula necato* on leaves, where chlorotic spots appear onthe upper surface of leaf. On fruit the pathogen appears as white, powdery masses that may colonize the entire berry surface. Summer Bunch Rot is a disease in which masses of black, brown, or green spores develop on the surface of infected berries caused by a variety microbes like *Aspergillusniger, Alternaria tennis, Botrytis cinerea, Cladosporium herbarum, Rhizopus arrhizus,* Penicillium sp., and others.

Fomitopsis pinicola (Sw.) P. Karst. Belonging to familyFomitopsidaceae causes a diseases known as redbelted fungus. Several other fungi attacks the medicinal plants, like *Pythium pinosurn* causes pythium rhizome rot, *Septoriadigitalis* causing leaf spot, little leaf disease by *Phywphthora cinnamomi* Rands (Pythiaceae), etc.

Crown gall disease caused by *Agrobacterium tumefaciens* (Rblzobiaceae). Galls may be produced on canes, trunks, roots, and cordons and may grow to several inches in diameter. Internally galls are soft and have the appearance of disorganized tissue. The pathogen can be transmitted by any agent that contacts the contaminated material. Galls commonly develop where plants have been injured during cultivation or

pruning. *Xylella fastidiosa* is a bacterium causes Pierce's Disease, in this leaves become slightly yellow or red along margins and eventually leaf margins dry or die.

Many viruses are also reported to cause necrosis of leaves, petioles and stems, they are tobacco mosaic virus, mosaic virus, cucumber mosaic virus, tobacco ring spot virus, yellow vein mosaic, etc.

Controlling techniques: Chemical fumigation of thesoil, fungicide, bactericide, pruning, proper water and fertilizer management, good sanitation, heat treatment of planting stock, cut and remove the infected parts, genetically manipulating the plants for producing plants to resist fungi and bacteria are practices that are used to prevent or minimize the effects produced by microbes.

Insects

Ants, they are of different varieties, Argentine ant: *Linepi thema humile,* Gray ants: *Formica aerata* and *Formica perpilosa,* Pavement ant: *Tetramorium caespitum.,* Southern fire ant: *Solenopsis xyloni,* Thief ant: *Solenopsis molesta,* they spoilthe soil by making nest and they feed honey dew secreted in plants.

Branch and Twig Borer *(Melalgus confertus)* burrow into the canes through the base of the bud or into the crotch formed by the shoot and spur. Feeding is often deep enough to completely conceal the adult in the hole. When shoots reach a length of 10–12 inches, a strong wind can cause the infected parts to twist and break. The click beetle *(Limonius canus)* can feed on buds. Cutworms *(Peridroma saucia) (Amathes cnigrum) (Orthndes rufula)* injures the buds andso the buds may not develop. Leafhoppers *(Erythroneuraelegantuhi) (Erythroneum variabilis)* remove the contents ofleaf cells, leaving behind empty cells that appear as pale yellow spots.

Oak twig pruners *(Anelaphiis* spp. Linsley) are known as shoot, twig and root insects that affects the above mentioned parts.

Controlling techniques: Tilling the soil will also affectsthe nesting sites of ants and help to reduce their populations, collection and destruction of eggs, larvae, pupae and adults of insects, trapping the insects, insecticides, creating a situation to compete among males for mating with females, cutworms can be prevented by natural enemies like, predaceous or parasitic insects, mammals, parasitic nematodes, pathogens, birds, and reptiles,

Non insect pests

They are divided in to vertebrates and invertebrates. Vertebrates that disrupt the plants are monkeys, rats, birds, squirrels, etc. Non vertebrates are, Webspinning Spider Mites *(Tetranychuspacificus) (Eotetranychus willamettei) (Tetranychus urticae)* causes discoloration in leaves and yellow spots.Nematodes *(Meloidogyne incognita) (Xiphinema americanutri)(Criconemella xenoplax)* produces giant cell formation, disturbs the uptake of nutrients and water, and interferes with plant growth, crabs, snails are the other few invertebrates that causes trouble to the plant.

Controlling techniques: Construction of concrete warehouses, traps, biological methods, rodenticides, etc.

Weeds

Weeds reduce growth and yields of plants by competing for water, nutrients, and sunlight. Weed control enhances the establishment of new plants and improves the growth and yield of established plants. The skilled persons have many weed management tools available to achieve these objectives; however, the methods of using these tools vary from year to year and from place to place.

Soil characteristics are important to weed management. Soil texture and organic matter influence the weed species that are present, the number and timing of cultivations required, and the activity of herbicides. Annual species, such as puncturevine, crabgrass, horseweed, and Panicum spp., or perennials like johnsongrass, nutsedge, and bermudagrass are more prevalent on lighttextured soil while perennials such as curly dock, field bindweed, and dallisgrass are more common on heaviertextured soils. Less preemergent

herbicide is required for weed control on sandy, light soils, but residual control may be shorter than on clay or clay loam soils. Use low rates of herbicide on sandy soils or those low in organic matter. Clay soils are slower to dry for effective cultivation than sandy loam soils; thus, more frequent cultivation is practiced on lighter soils than heavy soils.

Apart from these, Parasitic and Epiphytic Plants like dodder (Cuscuta spp. L.), mistletoe (Phoradendron spp. Nutt.), American squawroot (*Conopholis americana*), etc., too affects the growth of plants,

Controlling techniques: Use of low rates of herbicides:Herbicides are traditionally discussed as two groups: those that are active against germinating weed seeds (preemergent herbicides) and those that are active on growing plants (postemergent herbicides). Some herbicides have both preand postemergent activity. Herbicides vary in their ability to control different weed species.

Preemergent herbicides are active in the soil against germinating weed seedlings. These herbicides are applied to bare soil and are leached into the soil with rain or irrigation where they affect germinating weed seeds. If herbicides remain on the soil surface without incorporation, some will degrade rapidly from sunlight. Weeds that emerge while the herbicide is on the surface, before it is activated by rain or irrigation, will not be controlled. Postemergent herbicides are applied to control weeds already growing in the vineyard. They can be combined with preemergent herbicides or applied as spot treatments during the growing season. In newly planted plants, selective postemergent herbicides are available for the control of most annual and perennial grasses, but not broadleaf weeds.

Frequent wetting of the soil promotes more rapid herbicide degradation in the soil. Herbicide degradation is generally faster in moist, warm soils than in dry, cold soils.

General Methods of Pest Controls

- Cultural: Changing the time of sowing and harvesting, maintenance of storage, special cultivation methods, [roper cleaning, using trap crops and resistant varities.
- Physical: Mechanical control, utilization of physical factors such as temperature, humidity
- Biological: Using natural predators, parasites, pathogens, sterilization, genetic manipulation, pheromones etc.
- Chemical: Use of Pesticides, herbicides and antifeedants etc.

Other Factors that Affect the Cultivated Plants

Air Pollution

Chemical discharges into the atmosphere have increased dramatically during this century, but the total effect on plants is virtually unknown. It has been demonstrated that air pollutants can cause mortality and losses in growth of plants. Nearly all species of deciduous and coniferous trees are sensitive to some pollutants. There are many chemicals released into the atmosphere singly and as compounds. In addition, other compounds are synthesized in the atmosphere. Some chemicals can be identified through leaf tissue analysis and by analysing the air. Generally, pollution injury first appears as leaf injury. Spots between the veins, leaf margin discoloration, and tip burns are common. These symptoms can also be influenced by host sensitivity, which is effected by genetic characteristics and environmental factors.

Herbicide

Herbicides should be handled very carefully; misapplication of herbicides can often damage nontarget plants. The total extent of such damage remains unclear, but localized, severe damage occurs. Symptoms of herbicide injury are variable due to chemical mode of action, dosage, duration of exposure, plant species, and environmental conditions. Some herbicides cause growth abnormalities such as cupping or twisting of foliage while others cause foliage yellowing or browning, defoliation, or death.

Plant Growth Regulators And Their Applications

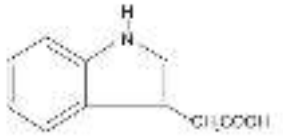
Plant growth regulators (also called as hormones / phytohormones) are physiological inter-cellular messengers that control the complete plant lifecycle, including germination, rooting, growth, flowering, fruit ripening, foliage and death. In addition, plant hormones are secreted in response to environmental factors such as excess of nutrients, drought conditions, light, temperature and chemical or physical stress. So, levels of hormones will change over the lifespan of a plant and are dependent upon season and environment.

The term 'plant growth regulator' (PGR) is usually employed for plant hormones or substances of similar effect that are administered to plants. Growth regulators (also referred to as factors) are widely used in industrialized agriculture to improve productivity. The application of growth regulators allows synchronization of plant development to occur. For instance, ripening fruits can be controlled by setting desired atmospheric ethylene levels. Using this method, fruits that are separated from their parent plant will still respond to growth regulators; allowing commercial plants to be ripened in storage during and after transportation. This way the process of harvesting can be run much more efficiently and effectively. Other applica-tions include rooting of seedlings or the suppression of rooting with the simultaneous promotion of cell division as required by plant cell cultures. Just like with animal hormones, plant growth regulators come in a wide variety, producing different and often antagonistic effects. In short, the right combination of hormones is vital to achieve the desired behavioural characteristics of cells and the produc-tive development of plants as a whole. The plant growth regulators are classified into synthetic and native. The synthetic regulators are also known as exogenous regulators and the native are called the endogenous,

Five major classes of plant hormones are mentioned: auxins, cytokinins, gibbereilins, abscisic acid and ethylene. However as research progresses, more active molecules are being found and new families of regulators are emerging; one example being polyamines (putrescine or spermidine). Plant growth regulators have made the way for plant tissue culture techniques, which were a real boon for mankind in obtaining therapeutically valuable secondary metabolites.

Auxins

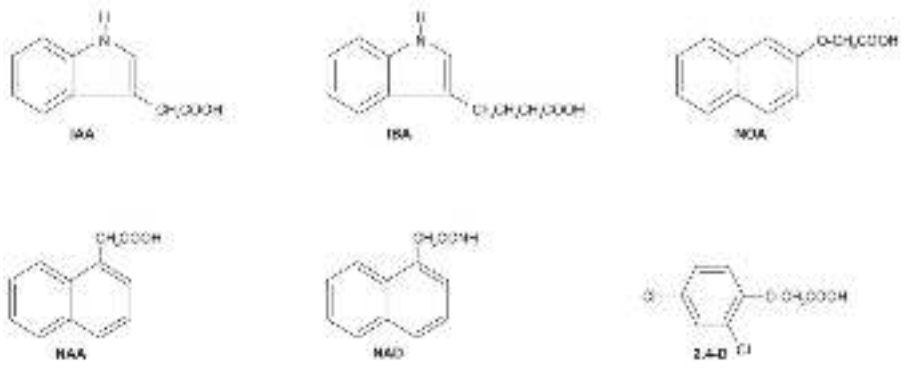
The term auxin is derived from the Greek word auxein which means to grow. Generally compounds are considered as auxins if they are able to induce cell elongation in stems and otherwise resemble indoleacetic acid (the first auxin isolated) in physiological activity. Auxins usually affect other processes in addition to cell elongation of stem cells but this characteristic is considered critical of all auxins and thus 'helps' define the hormone.



Auxins were the first plant hormones discovered. Charles Darwin was among the first scientists to pool in plant hormone research.

Indole acetic acid (IAA) is the principle natural auxin and other natural auxins are indole-3-acetonitrile (IAN), phenyl acetic acid and 4-chloroindole-3-acetic acid. The exogenous or synthetic auxins are indole-3-butyric acid (IBA), α-napthyl acetic acid (NAA), 2-napthyloxyacetic acid (NOA), 1-

napthyl acetamide (NAD), 5-carboxymeth-yl-N, N-dimethyl dithiocarbamate, 2,4-dichlorophenoxy acetic acid (2,4-D), etc.



Functions of auxin

Stimulates cell elongation.

The auxin supply from the apical bud suppresses growth of lateral buds. Apical dominance is the inhibiting influ-ence of the shoot apex on the growth of axillary buds. Removal of the apical bud results in growth of the axillary buds. Replacing the apical bud with a lanolin paste containing IAA restores the apical dominance. The mechanism involves another hormone - ethylene. Auxin (IAA) causes lateral buds to make ethylene, which inhibits growth of the lateral buds.

Differentiation of vascular tissue (xylem and phloem) is stimulated by IAA.

Auxin stimulates root initiation on stem cuttings and lateral root development in tissue culture (adventitious rooting).

Auxin mediates the tropistic response of bending in response to gravity and light (this is how auxin was first discovered).

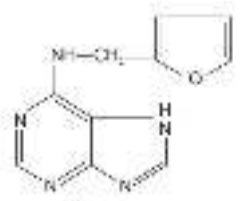
Auxin has various effects on leaf and fruit abscission, fruit set, development, and ripening, and flowering, depending on the circumstances.

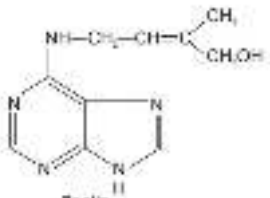
Cytokinins

Cytokinins are compounds with a structure resembling adenine which promote cell division and have other similar functions to kinetin. They also regulate the pattern and frequency of organ production as well as position and shape. They have an inhibitory effect on senescence. Kinetin was the first cytokinin identified and so named because of the compounds ability to promote cytokinesis (cell division). Though it is a natural compound, it is not made in plants, and is therefore usually considered a 'synthetic' cytokinin. The common naturally occurring cytokinin in plants today is called zeatin which was isolated from corn.

Cytokinin have been found in almost all higher plants as well as mosses, fungi, bacteria, and also in many prokary-otes and eukaryotes. There are more than 200 natural and synthetic cytokinins identified. Cytokinin concentrations are more in meristematic regions and areas of continuous growth potential such as roots, young leaves, developing fruits, and seeds.

The naturally occurring cytokinins are zeatin, N^6 dim-ethyl amino purine, isopentanyl aminopurine. The syn-thetic cytokinins are kineatin, adenine, 6-benzyl adenine benzimidazole and N, N'-diphenyl urea.





Functions of cytokinin

- Stimulate cell division (cytokinesis).
- Stimulate morphogenesis (shoot initiation/bud forma-tion) in tissue culture.
- Stimulate the growth of lateral (or adventitious) buds-release of apical dominance.
- Stimulate leaf expansion resulting from cell enlarge-ment.
- May enhance stomatal opening in some species.
- Promotes the conversion of etioplasts into chloroplasts via stimulation of chlorophyll synthesis.
- Stimulate the dark-germination of light-dependent seeds.
- Delays senescence.
- Promotes some stages of root development.

Gibberellins

Unlike the classification of auxins which are classified on the basis of function, gibberellins are classified on the basis of structure as well as function. All gibberellins are derived from the ent-gibberellane skeleton. The gibberellins are named GA_1. GA_n in order of discovery. Gibberellic acid was the first gibberellin to be structurally characterized as GA_3. There are currently 136 GAs identified from plants, fungi and bacteria.

They are a group of diterpenoid acids that functions as plant growth regulators influencing a range of developmental processes in higher plants including stem elongation, germination, dormancy, flowering, sex expression, enzyme induction and leaf and fruit senescence.

Functions of gibberellins

Stimulates stem elongation by stimulating cell division and elongation. GA controls internode elongation in the mature regions of plants. Dwarf plants do not make enough active forms of GA.

Flowering in biennial plants is controlled by GA. Bien-nials grow one year as a rosette and after the winter, they bolt (rapid expansion of internodes and formation of flowers).

Breaks seed dormancy in some plants that require strati-fication or light to induce germination.

Stimulates α-amylase production in germinating cereal grains for mobilization of seed reserves.

Juvenility refers to the different stages that plants may exist in. GA may help determine whether a particular plant part is juvenile or adult.

Stimulates germination of pollen and growth of pollen tubes.

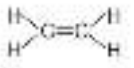
Induces maleness in dioecious flowers (sex expres-sion).

Can cause parthenocarpic (seedless) fruit development or increase the size of seedless fruit (grapes).

Can delay senescence in leaves and citrus fruits. May be involved in phytochrome responses.

Ethylene

Ethylene has been used in practice since the ancient times, where people would use gas figs in order to stimulate ripening, burn incense in closed rooms to enhance the ripening of pears.



Ethylene

Crocker proposed that ethylene was the plant hormone responsible for fruit ripening as well as inhibition of vegetative tissues. Ethylene is now known to have many other functions as well.

Functions of ethylene

Production stimulated during ripening, flooding, stress, senescence, mechanical damage, infection.

Regulator of cell death programs in plants (apoptosis). Stimulates the release of dormancy.

Stimulates shoot and root growth and differentiation (triple response).

Regulates ripening of climacteric fruits.

May have a role in adventitious root formation. Stimulates leaf and fruit abscission.

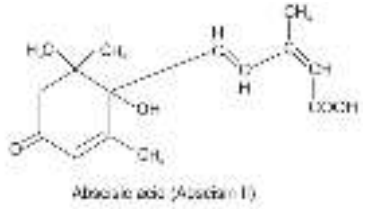
Flowering in most plants is inhibited by ethylene. Mangos, pineapples and some ornamentals are stimu-lated by ethylene.

Induction of femaleness in dioecious flowers. Stimulates flower opening.

Stimulates flower and leaf senescence.

Abscisic Acid

Natural growth inhibiting substances are present in plants and affect the normal physiological process of them. One such compound is abscisic acid, a single compound unlike the auxins, gibberellins, and cytokinins. It was called 'absci-sin II' originally because it was thought to play a major role in abscission of fruits. At about the same time another group was calling it 'dormin' because they thought it had a major role in bud dormancy. Though abscisic acid gener-ally is thought to play mostly inhibitory roles, it has many promoting functions as well.



In 1963, when Frederick Addicott and his associates were the one to identify abscisic acid. Two compounds were isolated and named as abscisin I and abscisin II. Abscisin II is presently called abscisic acid (ABA). At the same time Philip Wareing, who was studying bud dormancy in woody plants and Van Steveninck, who was studying abscission of flowers and fruits discovered the same compound.

Production and occurrence

ABA is a naturally occurring sesquiterpenoid (15-carbon) compound in plants, which is partially produced via the mevalonic pathway in chloroplasts and other plastids. Because it is synthesized partially in the chloroplasts, it makes sense that biosynthesis primarily occurs in the leaves. The production of ABA is by stresses such as water loss and freezing temperatures. The biosynthesis occurs indi-rectly through the production of carotenoids. Breakdown of these carotenoids occurs by the following mechanism: Violaxanthin (forty carbons) is isomerized and then splitted via an isomerase reaction followed by an oxidation reaction. One molecule of xanthonin is produced from one molecule of violaxanthonin and it is not clear what happens to the remaining byproducts. The one molecule of xanthonin produced is unstable and spontaneously changed to ABA aldehyde. Further oxidation results in ABA. Activation of the molecule can occur by two methods. In the first, method, an ABA-glucose ester can form by attachment of glucose to ABA. In the second method, oxidation of ABA can occur to form phaseic acid and dihyhdrophaseic acid. Both xylem and phloem tissues carries ABA. It can also be translocated through parenchyma cells. Unlike auxins, ABA is capable of moving both up and down the stem.

Functions of abscisic acid

The abscisic acid stimulates the closure of stomata (water stress brings about an increase in ABA synthesis) (Figure 6.3).

Involved in abscission of buds, leaves, petals, flowers, and fruits in many, if not all, instances, as well as in dehiscence of fruits.

Production is accentuated by stresses such as water loss and freezing temperatures.

Involved in bud dormancy.

Prolongs seed dormancy and delays germination (vivipary).

Inhibits elongation.

ABA is implicated in the control of elongation, lateral root development, and geotropism, as well as in water uptake and ion transport by roots.

ABA coming from the plastids promotes the metabolism of ripening.

Promotes senescence.

Can reverse the effects of growth stimulating hormones.

Closure of stomata and water stress brings about an increase in ABA synthesis

Salicylic Acid

Salicylic acid has been known to be present in some plant tissues for quite some time, but has only recently been recognized as a potential PGR. Salicylic acid is synthesized from the amino acid phenylalanine. SA is thought by some to be a new class of plant growth regulator. It is a chemically characterized compound, ubiquitously found in the plant kingdom and has an effect on many physiological processes in plants at low concentrations. Further molecular studies on SA signal

transduction should yield insights into the mechanism of action of this important regulatory compound.

Functions of salicylic acid

Promotes flowering.

Stimulates thermogenesis in *Arum* flowers.

Stimulates plant pathogenesis protein production (systemic acquired resistance).

May enhance longevity of flowers.

May inhibit ethylene biosynthesis.

May inhibit seed germination.

Blocks the wound response. Reverses the effects of ABA.

Jasmonates

Jasmonates are represented by jasmonic acid and its methyl ester. They were first isolated from the jasmine plant in which the methyl ester is an important product in the perfume industry. Jasmonic acid is synthesized from lino-lenic acid, which is an important fatty acid. Jasmonic acid is considered by some to be a new class of plant growth regulator. It is a chemically characterized compound and has been identified in many plant species. It has physiological effects at very low concentrations and indirect evidence suggests that it is transported throughout the plant.

Functions of jasmonates

Inhibition of many processes such as seedling longitu-dinal growth, root length growth, mycorrhizial fungi growth, tissue culture growth, embryogenesis, seed germination, pollen germination, flower bud formation, carotenoid biosynthesis, chlorophyll formation, rubisco biosynthesis, and photosynthetic activities

Promotion of senescence, abscission, tuber formation, fruit ripening, pigment formation, tendril coiling, differentiation in plant tissue culture, adventitious root formation, breaking of seed dormancy, pollen germination, stomatal closure, microtubule disruption, chlorophyll degradation, respiration, ethylene biosynthesis, and protein synthesis

Polyploidy, Mutation and Hybridization in Cultivation of Medicinal Plants

Polyploidy:

Plants whose cells contain two sets of chromosomes, derived at fertilization from the union of one set from the pollen and one set from the egg cells, are described as diploids and denoted by “2n”. The term polyploidy is applied to plants with more than two sets of chromosomes in the cells; when four sets are present the plants are described as tetraploids and denoted by “4n”.

Tetraploidy is induced by treatment with colchicine, which inhibits spindle formation during cell division, so that the divided chromosomes are unable to separate and pass to the daughter cells. The two sets of chromosomes remain in one cell and this develops to give tetraploids plant.

Treatment with colchicine may be applied in various ways, but all depend on the effects produced in the meristem. The seeds may be soaked in a dilute solution of colchicine, or the seedlings, the soil around the seedling or the young shoot treated with colchicine solution. Fertile seed and robust, healthy tetraploid plants were obtained, the tetraploid condition being indicated by the increased size of the pollen grains and stomata; chromosome counts in root-tip preparations confirm the tetraploid condition.

The average increase in alkaloids content compared with diploid plants of Datura stromonium and Datura tatula was 68%, with a maximum increase of 211.6%. Similar results were obtained with Atropa belladonna and Hyoscyamus niger, the average increase in belladonna being 93%. Increased Alkaloidal content of tetraploids plants has been confirmed for Datura stromonium and Datura tatula. The diploid of Acorus calamus is 2.1% of volatile oil content but they are converted into tetraploid, they produce 6.8% of volatile oil contents.

Mutation:

Sudden heritable change in the structure of a gene on chromosome or change the chromosome number.

Type of mutations:

1. Spontaneous and induced mutations.
2. Recessive and dominant mutations.
3. Somatic and germinal mutations.
4. Forward, back and suppressor mutation.
5. Chromosomal, genomic and point mutations.

Mutations can be artificially produced by certain agents called mutagens or mutagenic agent. They are two types:

a. Physical mutagens:

(i) Ionizing radiations: X-rays, gamma radiation and cosmic rays.

(ii) Non-ionizing radiation: U.V. radiation,

b. Chemical mutagens:

(i) Alkylating and hydroxylating agents: Nitrogen and sulpher mustard; methyl and ethylsulphonate, ethylethane sulphonates.

(ii) Nitrous acid:

(iii) Acridines: Acridines and proflavins. Ionizing radiation cause breaks in the chromosome. These cells then show abnormal cell divisions. If these include gametes, they may be abnormal and even die prematurely. Non-ionizing radiation like Ultra Violet rays are easily absorbed by purine and pyrimidines. The changed bases are known as photoproducts. U.V. rays cause two changes in pyrimidine to produce pyrimidine hydrate and pyrimidine dimmers. Thymine dimer is a major mutagenic effect of U.V. rays that disturbs DNA double helix and thus DNA replication.

Hybridization:

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

Hybridization particularly between homozygous strains, which have been inbred for a number of generations, introduces a degree of heterozygosis with resultant hybrid vigour often manifest in the dimensions and other characteristic of the plants. A hybrid is an organism which results from crossing of two species or varieties differing at least in one set of characters.

The following steps are involved in hybridization of plant:

1. Choice of parents: The two parents to be selected, at least one should be as well adopted and proven variety in the area. The other variety should have the characters that are absent in the first chosen variety.

2. Emasculation: Removal of stamens or anthers or killing the pollen grains of a flower without affecting the female reproductive organs is known as emasculation. Emasculation is essential in bisexual flowers.

3. Bagging: Immediately after emasculation, the flowers or inflorescences are enclosed in bags of suitable sizes to prevent random cross-pollination.

4. Pollination: In pollination, mature, fertile and viable pollens are placed on a receptive stigma. The procedure consists of collecting pollens from freshly dehisced anthers and dusting them on the stigmas of emasculated flowers.

PLANT TISSUE CULTURE AND ITS APPLICATIONS

Contents:

History

- Haberlandt, Father of plant tissue culture.
  cultured single cells on Knop's salt solution.
- Hanning(1904) Embryo culture of selected crucifers.
- Robbins(1922), in vitro culture of root tips.
- Muir(1953) isolation and culture of single plant cells.
- Skoog and Miller(1957) hormonal control in tissue culture.
- Reinert and Steward(1958) report somatic embryogenesis.






History

- E C Cocking (1960) Isolation of protoplasts by enzymatic degradation method.

- Murashige and Skoog (1962) Development of MS media.

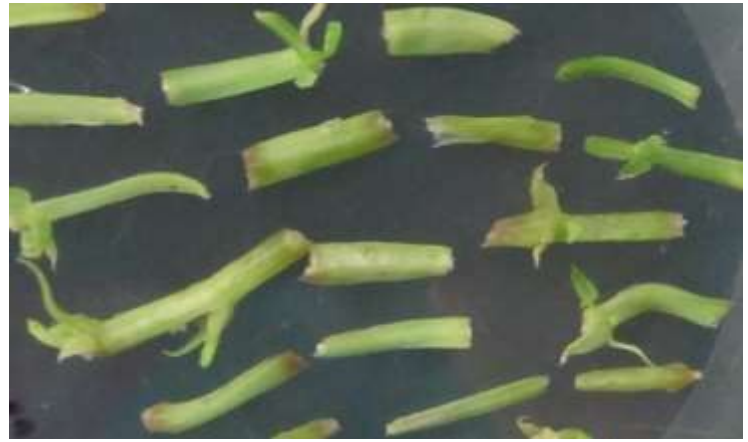
- Guha and Maheshwari (1964) Production of First haploid plant by anther culture

INTRODUCTION:

Plant Tissue Culture

- The growth or regeneration of plant cells, tissues, organs or whole plants in artificial medium under aseptic conditions.

- In this technique use plant parts or cells called Explant.



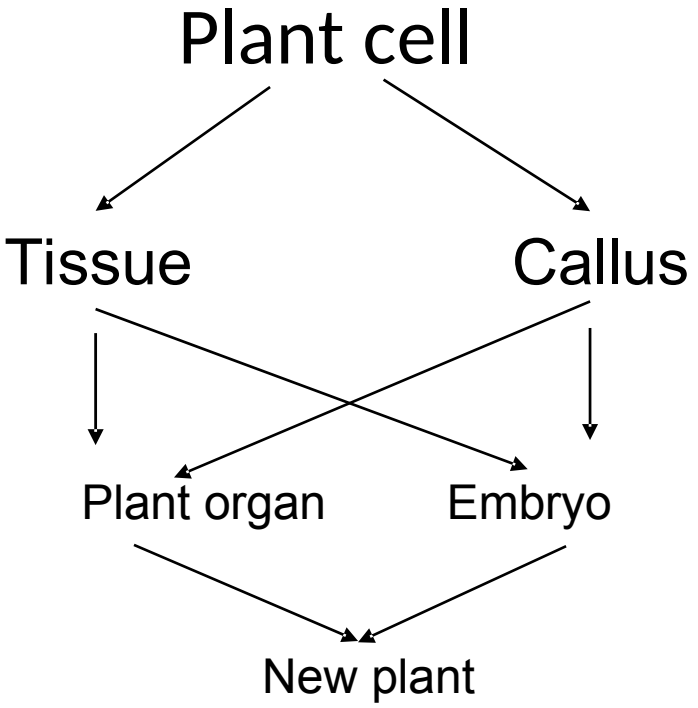
- The most widely used artificial nutrient medium is MS medium.

Fundamental Principles of Plant Tissue Culture

Plant tissue culture depends upon;

- **Totipotency**, ability of plant cells to regenerate into whole new plant

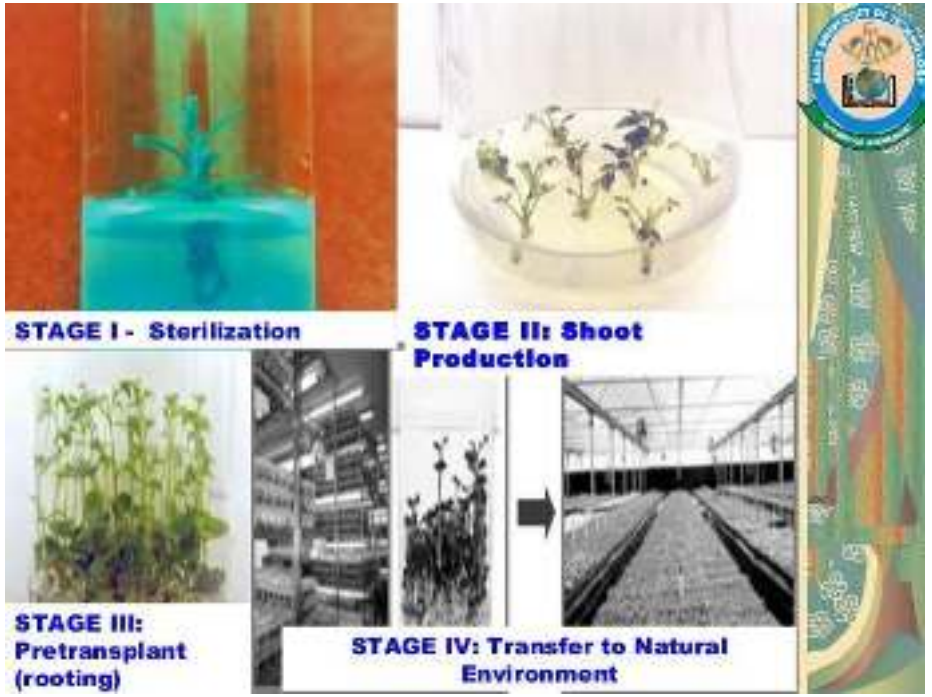
- **Plasticity,** ability of plants to alter their metabolism, growth and development to best suit their environment



Plant Tissue Culture Techniques:

1. Micropropagation:

- Developing high- quality clonal plants.
- Provide rapid and large scale propagation of new genotypes.
- **Steps:**
  - Sterilization and introduction
  - Shoot production
  - Root production
  - Transfer to soil



2. Somatic cell genetics:

- It is used mostly in terms of haploid production and somatic hybridization.

- **Steps:**

  - Isolation of protoplast
  - Fusion by using chemicals
  - Plating of fused protoplast
  - Selection

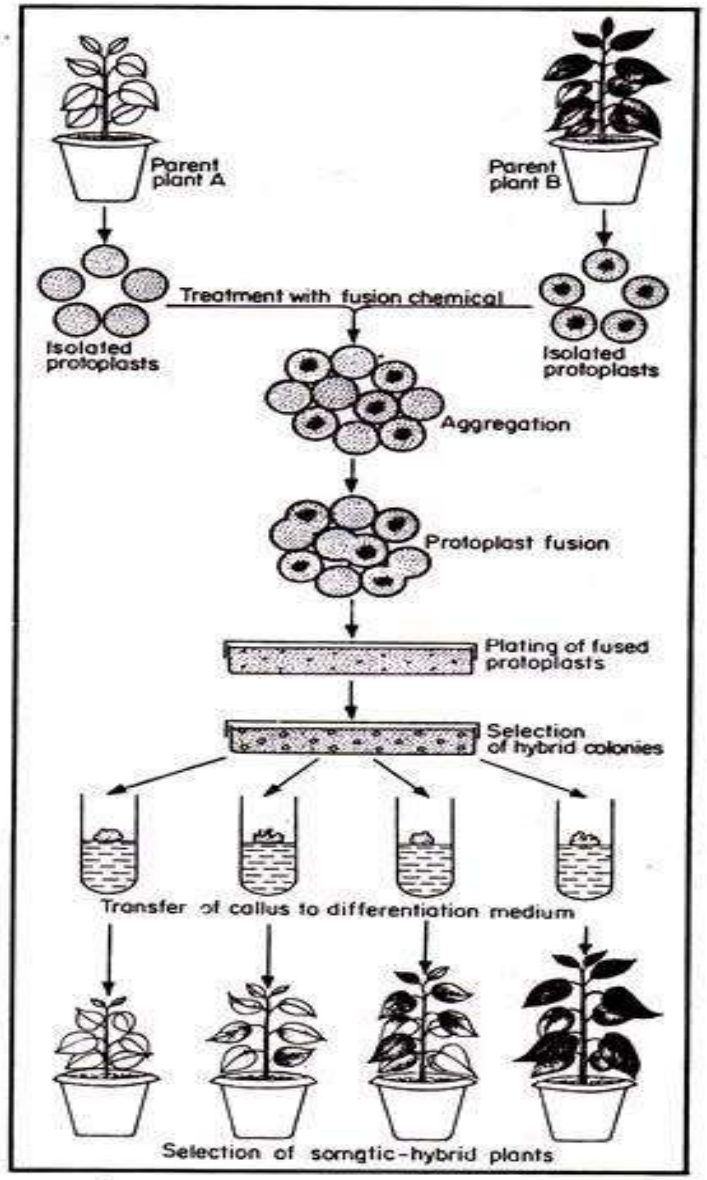
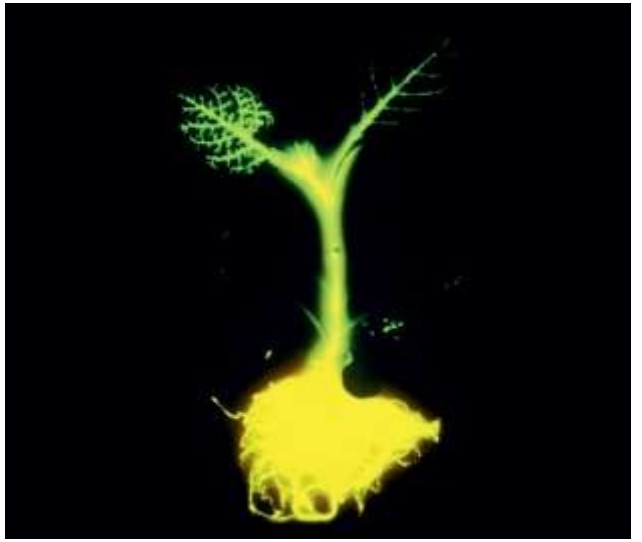


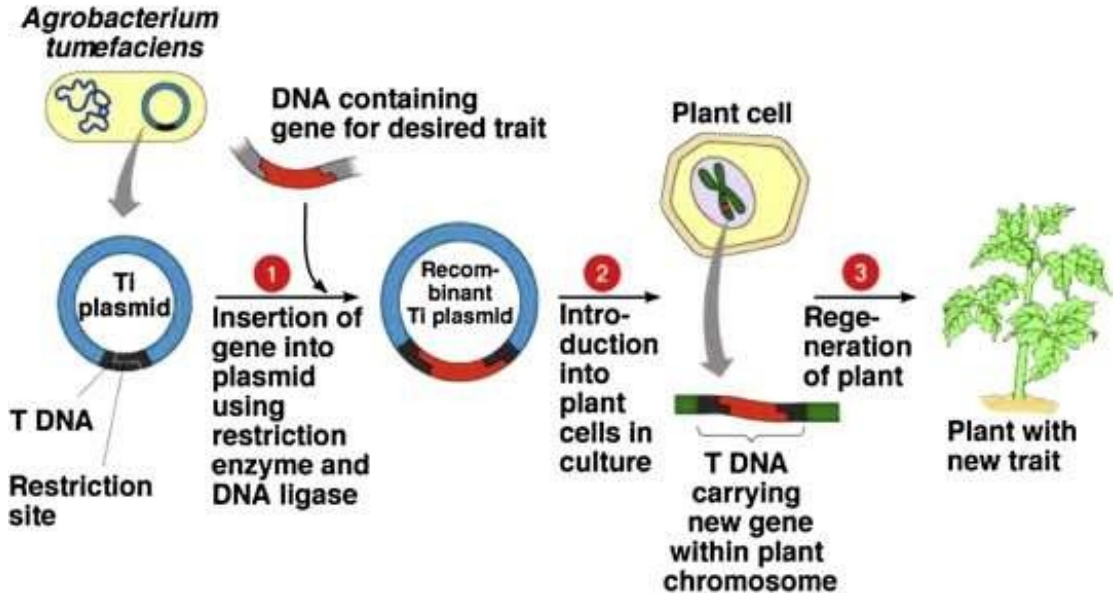
Fig 13.1

Diagrammatic illustration for the fusion of protoplasts from two different plant species and later platting and selection of hybrid clonies and the regeneration of "Somatic-Hybrids" (after Reinert and Bajaj 1977)

3. Transgenic plants:

- Transgenic plants are plants that have been genetically engineered, uses recombinant DNA techniques to create plants with new characteristics.
- They are identified as a class of genetically modified organism (GMO).
- The complete process is described as:

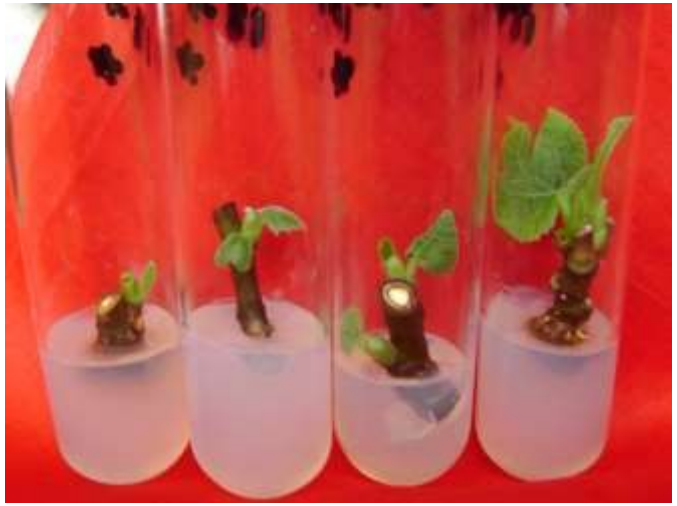




Major Steps of Tissue Culture:

- **Initiation Phase (Stage 1)**
  - The tissue of interest is obtained, sterilized and introduced.

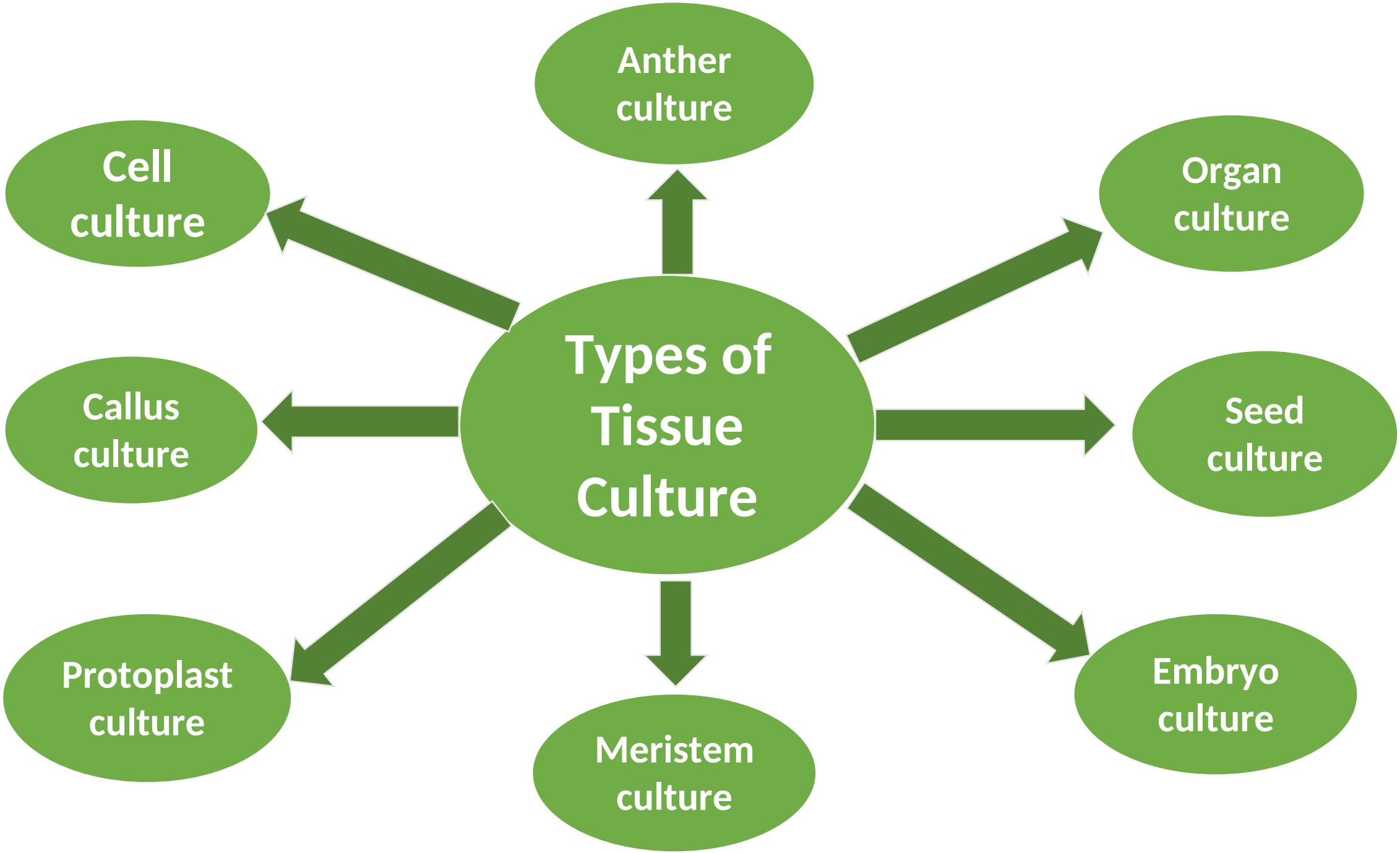
- **Multiplication Phase (Stage 2)**
  - The in vitro plant material is introduced in to the medium.
  - Proliferation of the tissue and the production of multiple shoots.



Major Steps of Tissue Culture:

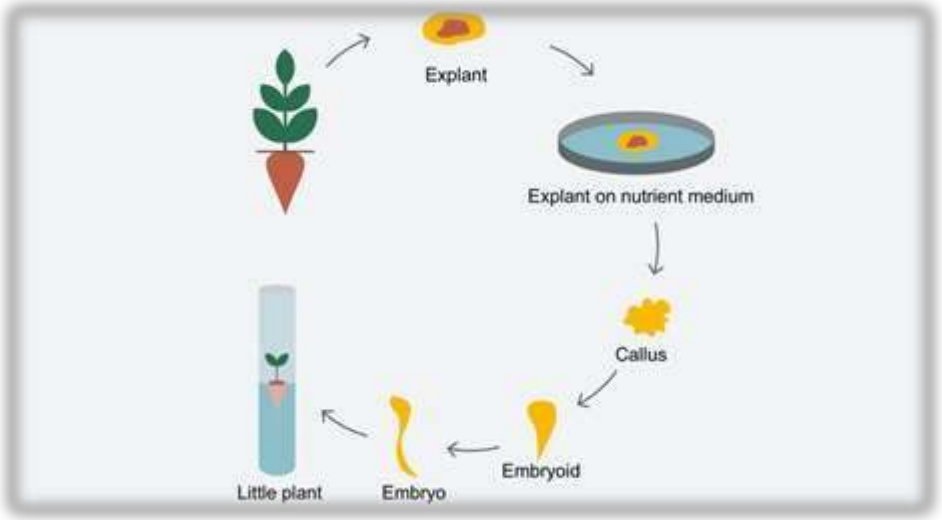
- **Root formation (Stage 3)**

  - Roots are formed.
  - Here, hormones are required in order to induce rooting such as “Auxin”.
  - Consequently complete plantlets.


Types of Tissue Culture
Anther culture
Cell culture
Organ culture
Callus culture
Seed culture
Protoplast culture
Meristem culture
Embryo culture

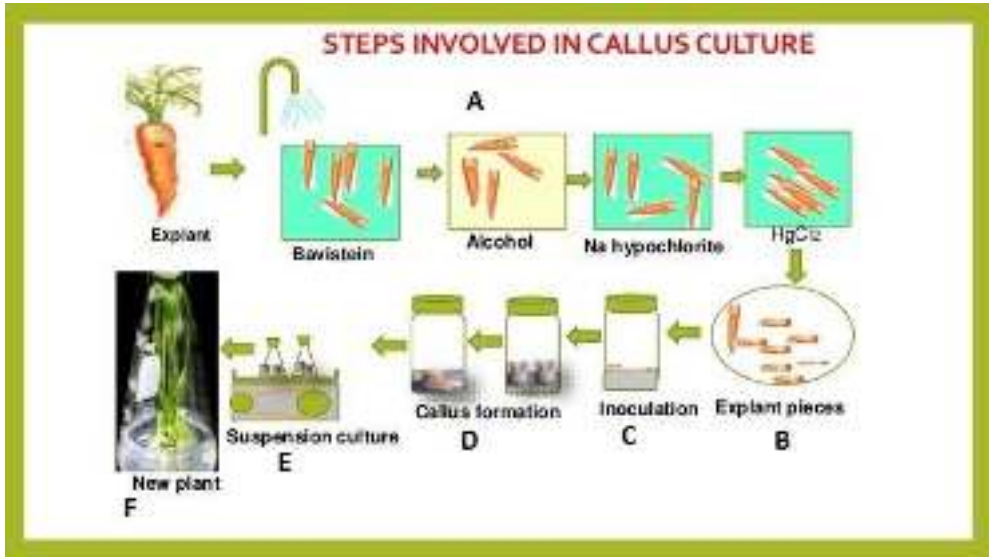
Cell culture:

- The culture of isolated individual cells.
- Carried out in dispersion medium.



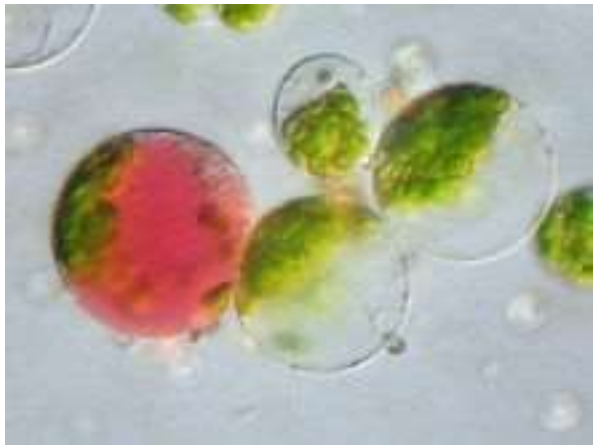
Callus culture:

- The growth of callus from explant by providing appropriate conditions.
- Darkness and solid medium gelled by agar stimulate callus formation.



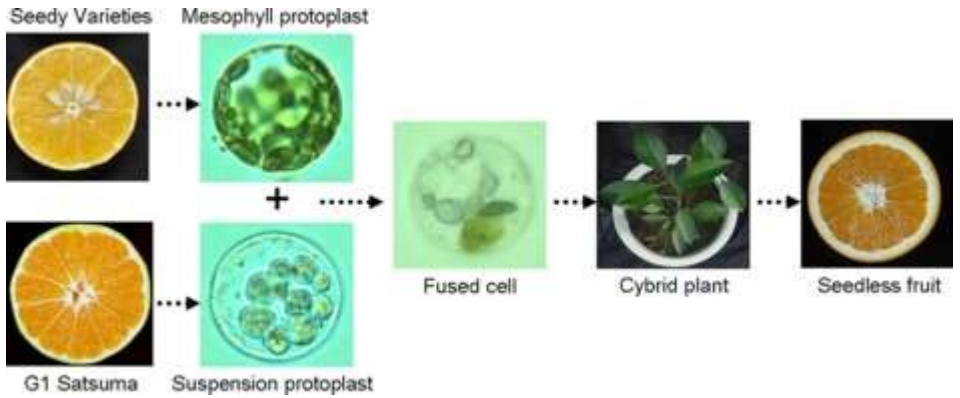
Protoplast culture:

- The cell in which the cell wall has been removed.



- In this, isolated protoplasts are cultured on a suitable medium under the aseptic condition.

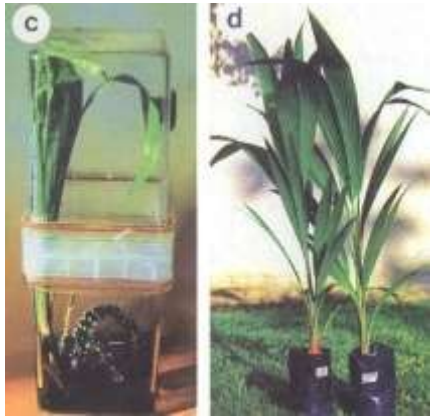
- The protoplast culture is aimed to develop genetically transformed plant.



Organ culture:

- Isolated plant organs (Shoot, root, leaf, and flower) are used as explant.
- The organ culture may be organized or unorganized.

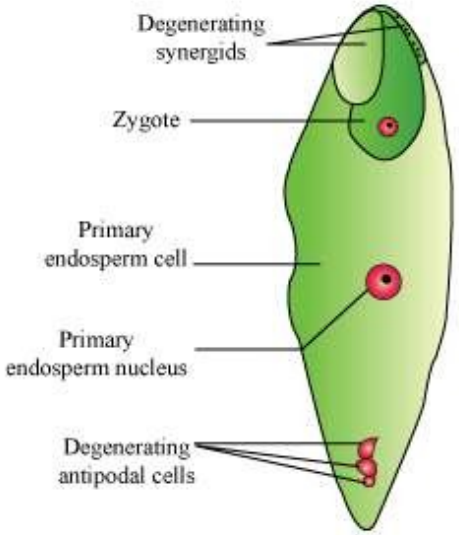
- New growth (differentiated structures) continues given that the organ retains its:
- Physiological features,
- Provide information on patterns of growth,
- As well as development.



Seed culture:

- For this method, explants are obtained from an in-vitro derived plant.
- Introduced into an artificial environment.
- Where they get to proliferate.
- Seed culture is primarily used for plants such as orchids

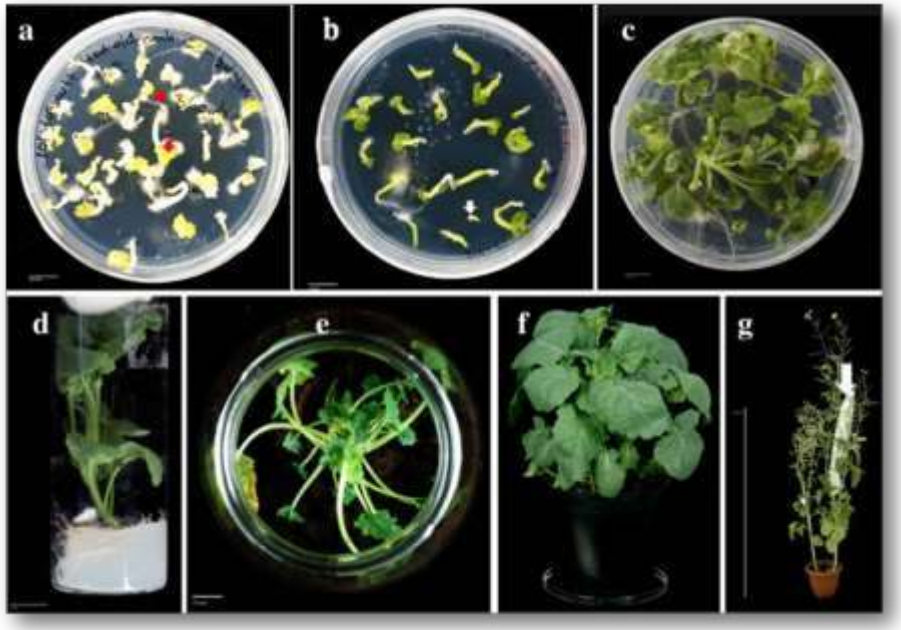
Embryo Culture:



- Embryo culture is the isolation and growth of mature or immature embryo in-vitro.

- For embryo culture, the ovule, seed or fruit from which the embryo is to be obtained is sterilized.

- Culturing them in nutrient media, help in developing viable seedlings.

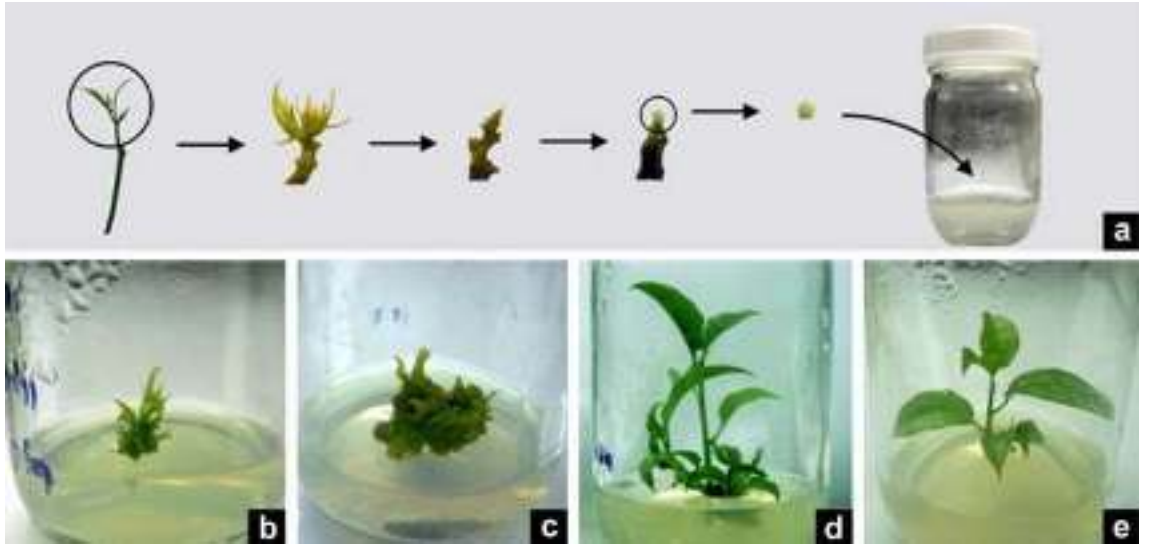


Meristem culture:

- A meristem is a localized group of cells, which are actively dividing and give rise to permanent tissue.

- The apical meristem of shoots is cultured.

- To get the disease-free plants.



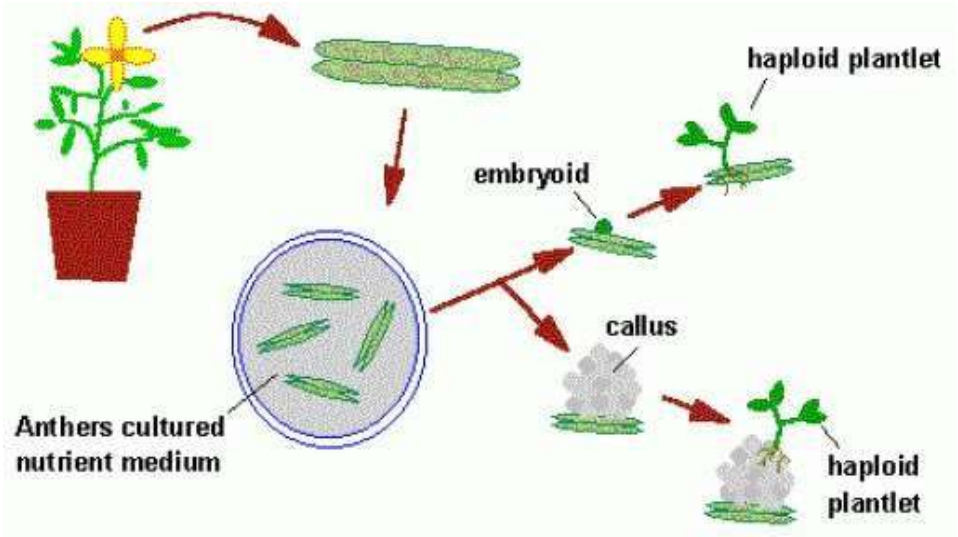
Anther Culture:

- Anthers of some plants are cultured on a suitable medium to produce haploid plants, it is called anther culture.

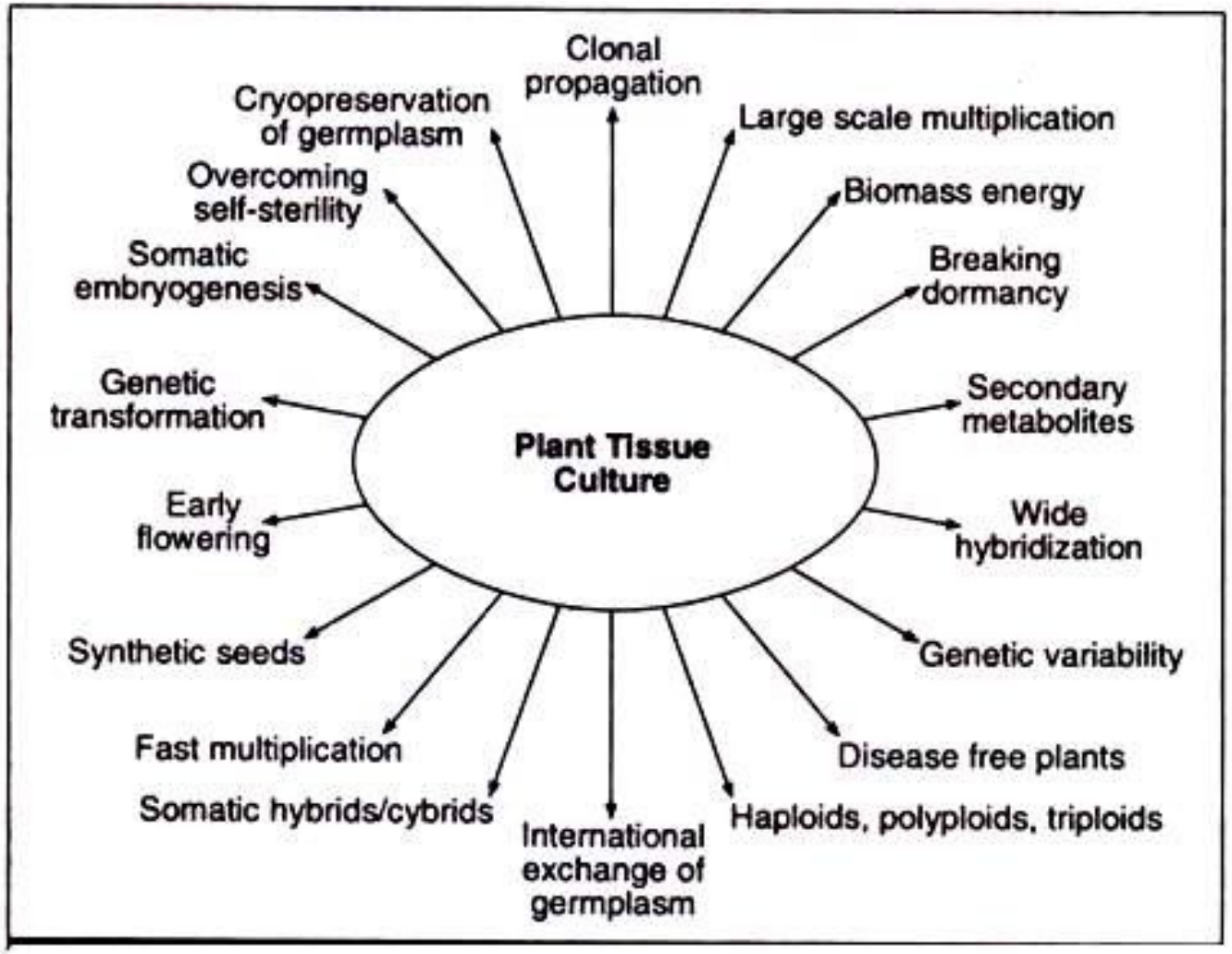
- Separated anthers from flowers.

- Cultured on a suitable medium.

- This technique was first used in India to produce haploids of Datura.



Applications of Plant Tissue Culture:



Clonal Propagation:

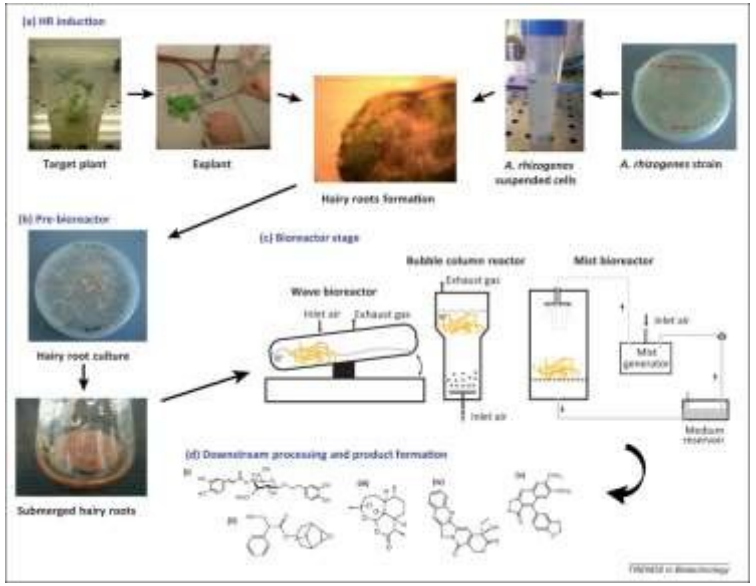
- Useful tool to get a large population of plant species having desirable traits.

- This technique is very much used in horticulture and silviculture—orchids and many fruit plants.

- Large scale production of plants of same genetic stock.

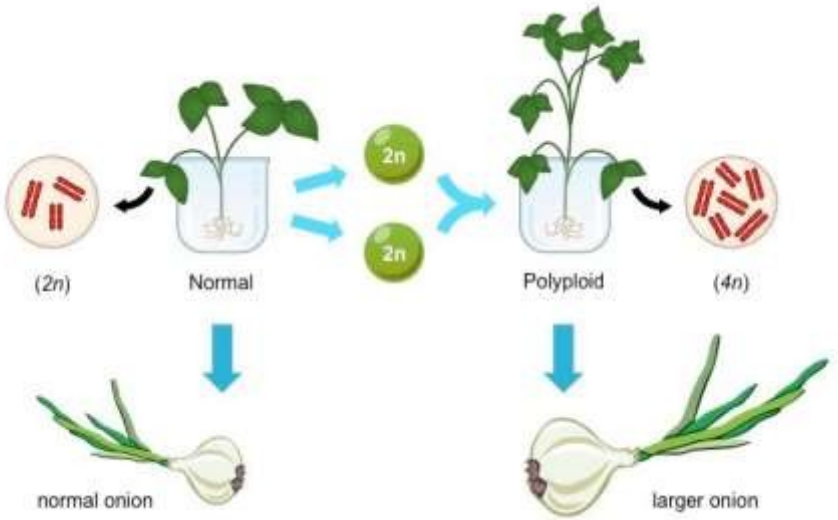
Secondary Metabolites:

- Secondary metabolites produced by plant cell cultures are rather small in amount.
- By clonal selection the particular high yielding clone of cells can be isolated.
- Production of many useful compounds can be done by plant cell culture.



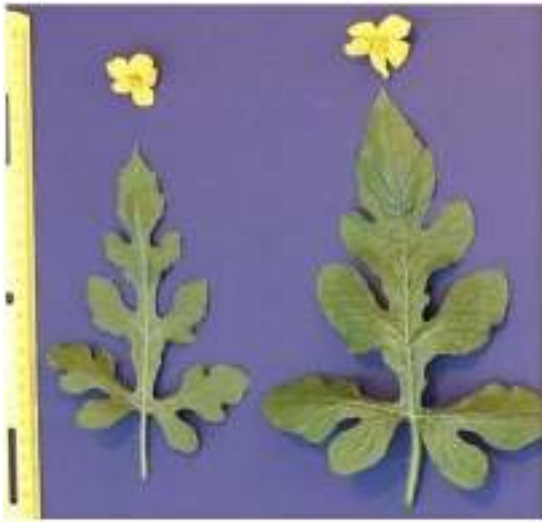
Genetic Variability:

- The chromosomal instability in the cultured cells play an important role in polyploidization of cells and genetically variable plants can be raised.



- Such kind of variations may show some useful characters such as:

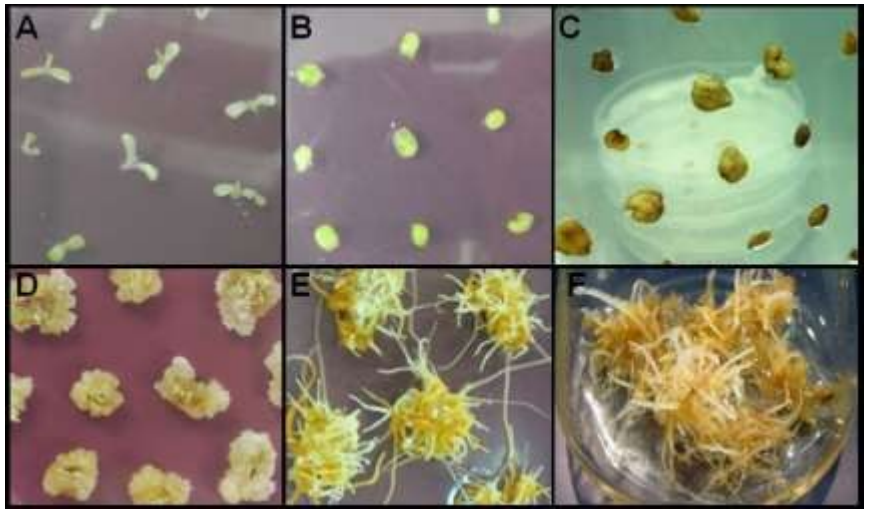
- Different kinds of morphological variations in leaf and flowering.



Somatic Embryogenesis and Synthetic Seed:

- Direct or indirect somatic embryogenesis may be achieved from:
- Pro-embryonic cell of the direct explant
- The embryoids developed within the callus tissue.

- The application of this technique is:
  - the mass production of adventitious embryos which ultimately develop into complete plantlet.

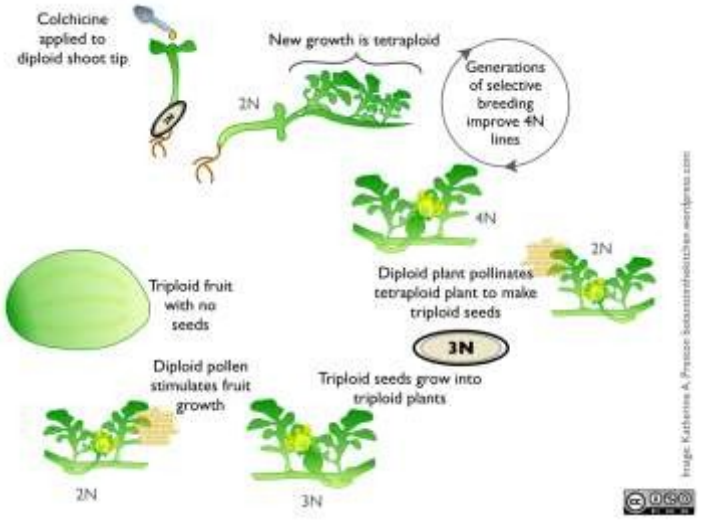


Haploid Plants:

- Haploid plants can be obtained through anther or pollen culture (androgenesis).

- Colchicine treatment within a very short period.

- These haploids produced homozygous diploid or polyploid lines.



Somatic Hybrids:

- Somatic hybridization, using isolated somatic protoplasts is a new tool to make the wide hybridization successful.

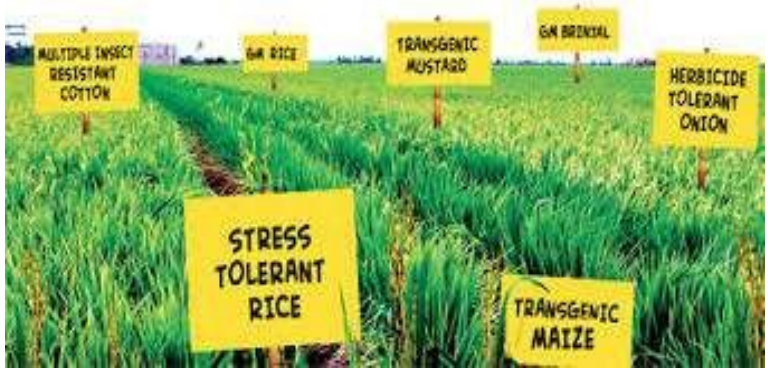
- Obtain somatic hybrid plants between sexually compatible and incompatible plants.

- Production of cybrid is also of immense importance in the plant breeding program.

Transgenic Plants:

- The genetically modified (GM) plants, in which a functional foreign gene has been incorporated by biotechnological method.

- A number of transgenic plants have been produced carrying genes for different traits.



- The direct DNA uptake through protoplast is the most ideal method.

Germplasm Conservation:

- Many of the important crop species produce recalcitrant seeds.

- Mainly the plant species which are endangered, needed to be conserved by ex-situ method of germplasm conservation.

- Plant tissue culture may be applied for this purpose.

EDIBLE VACCINES

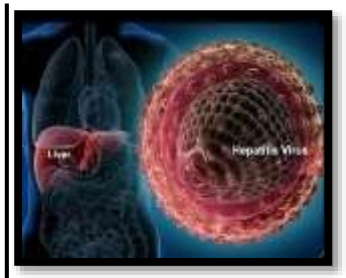
VACCINE

- A biological preparation that improves immunity to a particular disease.
- contains an agent that resembles a disease-causing microorganism and is often made from weakened or killed forms of the microbe
- stimulate the body's immune system to recognize the agent, destroy it, and keep a record of it for later encounters
- reduced mortality rate caused by various organisms.
- one of the safe and effective measure to control various infectious diseases.
- A protein which acts as the vaccine, present in food and consumed as the internal composition of food is known as EDIBLE VACCINE

EDIBLE VACCINE- WHY?

- Immunization through DNA vaccines is an alternative but is an expensive approach
- Edible vaccine gives cost-effective, easy-to-administer, easy-to-store and socio-culturally readily acceptable vaccines for their delivery systems.
- Oral vaccines provide “mucosal immunity” at various sites by secreting antibodies.
- Don’t need to worry about re-use, misuse and lack of sterilization. Thus, low risk of infection.

History of Edible vaccines

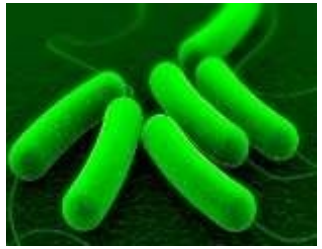


Hepatitis

Hepatitis B surface antigen (HBsAg)

Tobacco/leaf

Mason et al 1992



E. Coli

Diarrhea

Heat labile toxin B subunit (LTB)

Potato/tuber, tobacco/ leaf

Haq et al 1995

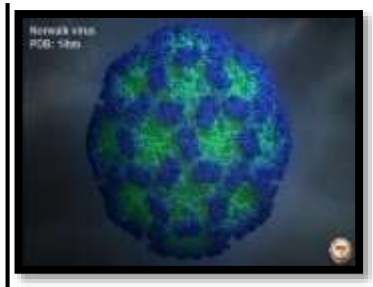
Rabies virus

Rabies

Rabies virus glycoprotein (RVG)

Tomato/leaf, fruit

McGarvey et al 1995



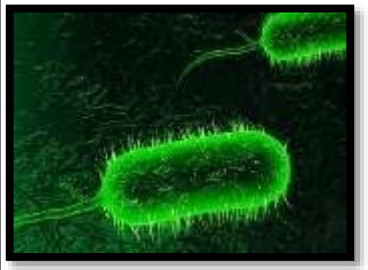
Norwalk virus (NV)

Gastroenteritis

Norwalk virus capsid protein (NVCP)

Potato/tuber tobacco/leaf

Mason et al 1996



V. Cholerae

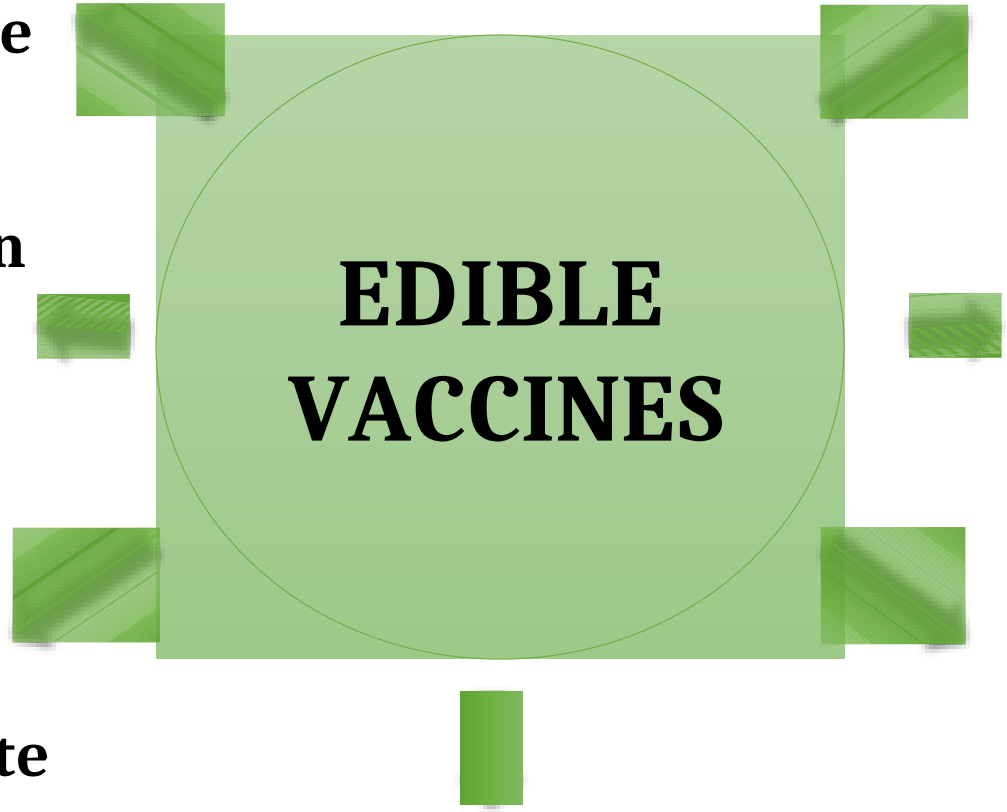
Cholera

Cholera toxin B subunit (CTB)

Tobacco/leaf

Hein et al 1996 ….

IDEAL PROPERTIES



Sould be effective in affordable

Nontoxic or Nonpathogenic

Vaccination should be Simple

very low levels of side effects

Not contaminate the Environment

Not cause problems in individuals with impaired immune system

Long lasting humoral and cellular immunities

Plants used for edible vaccine

- Tobacco
- Potato
- Banana
- Tomato
- Rice
- Lettuce
- Soybean
- Alfalfa
- Carrot
- Peanuts
- Wheat
- corn

Plant species

Potato :

Advantage

Easily transformed.
Easily propagated.
Stored for long periods without refrigeration.

Disadvantage

Need cooking which denature antigen.

Banana

Advantages

Do not need cooking.
Protein not destroyed even after cooking.
Inexpensive .
Grown widely in developing countries.

Disadvantages

Trees take 2-3 to mature years.
Spoils rapidly after ripening.

Rice

Advantages

Commonly used in baby food.
High expression of antigen.

Disadvantages

Grows slowly.
Requires glasshouse condition.

Tomato

Advantage

Grow quickly.
Cultivate broadly.
High content Vitamin-A may boost immune response.

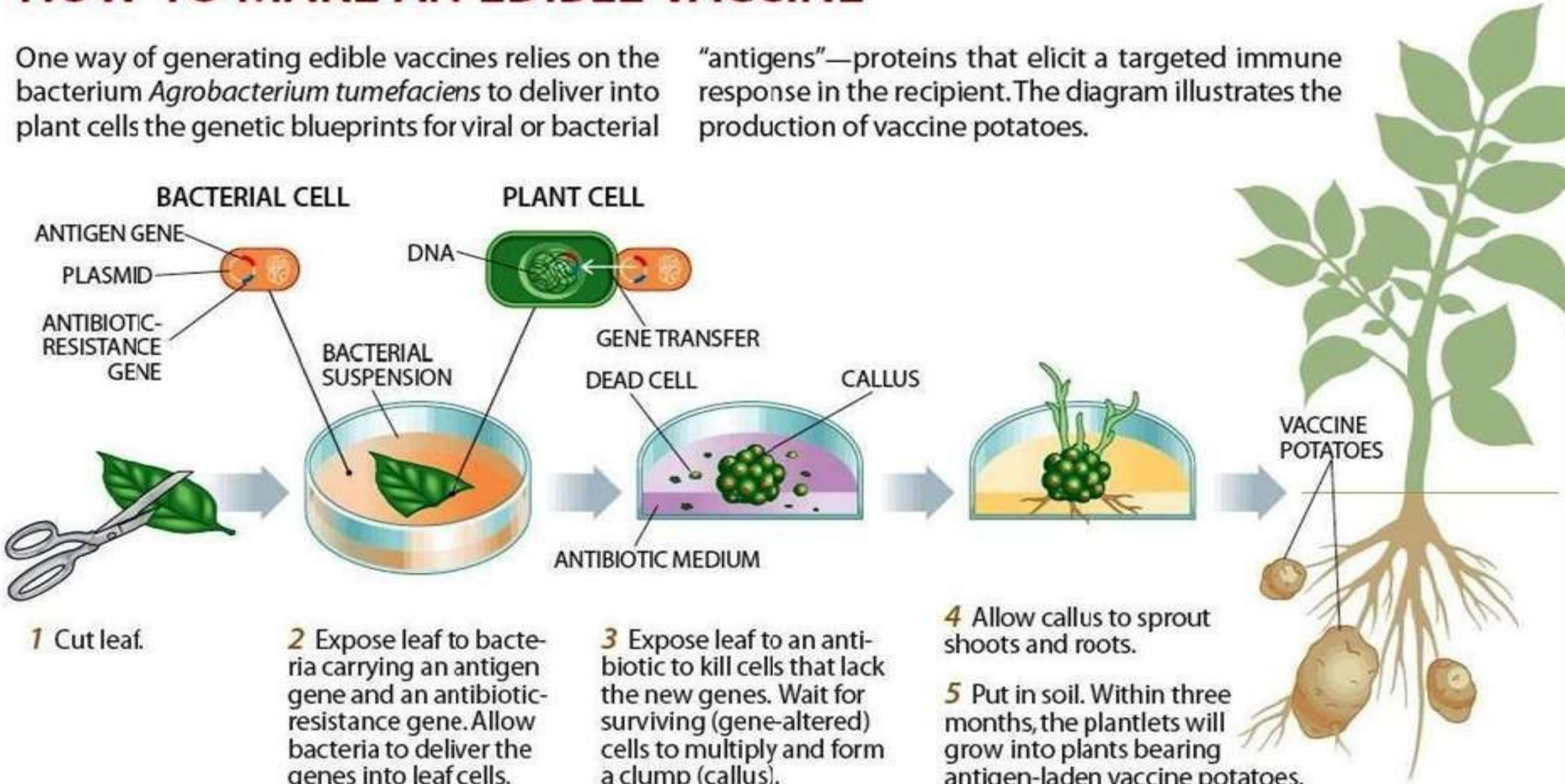
Disadvantages

Spoils readily.

TARGET PATHOGENS	EXPRESSED IN	MODE OF ADMINESTRATION
Enterotoxigenic Ecoli (humans)	Potato, tobacco	Immunogenic and protective when administered orally.
Vibrio cholera(humans)	Potato	Immunogenic and protective when administered orally.
Hepatitis B virus (humans)	Tobacco	Extracted proteins is Immunogenic when administered by injection
Hepatitis B virus (humans)	Potato	Immunogenic and protective when administered orally.
Norwalk virus(humans)	Potato	Virus like particles form and Immunogenic when administered orally.
Rabies virus (humans)	Tomato	Intact glycoproteins
Foot and mouth disease (agricultural domestic animals)	Arabidopsis	Immunogenic and protective when administered orally
Foot and mouth disease (agricultural domestic animals)	Alfalfa	Immunogenic and protective when administered by injection or orally
Transmissible gastroenteritis corona virus	Maize	Protective when administered oral

HOW TO MAKE AN EDIBLE VACCINE

One way of generating edible vaccines relies on the bacterium *Agrobacterium tumefaciens* to deliver into plant cells the genetic blueprints for viral or bacterial "antigens"—proteins that elicit a targeted immune response in the recipient. The diagram illustrates the production of vaccine potatoes.



1 Cut leaf.

2 Expose leaf to bacteria carrying an antigen gene and an antibiotic-resistance gene. Allow bacteria to deliver the genes into leaf cells.

3 Expose leaf to an antibiotic to kill cells that lack the new genes. Wait for surviving (gene-altered) cells to multiply and form a clump (callus).

4 Allow callus to sprout shoots and roots.

5 Put in soil. Within three months, the plantlets will grow into plants bearing antigen-laden vaccine potatoes.

Plasmid as a transfer vector

Gene of interest

Agrobacterium tumefaciens

Agrobacterium tumefaciens

Plant cell

Seeds can be easily distributed worldwide without requiring complex systems (e.g., cooling systems)

Vaccine's oral entrance (fruits/leaves)

Antigenic protein delivered by an edible vaccine

Antibodies discharged by activated B cells

Cells from intestinal epithelium

M cell

Dendritic cell in charge of preseting antigen

B cells activated by cytokines

Defferentiation into CD4+ T helper and CD8+ cytotoxic T cell populations in addition to low numbers of memory T cells

Gene (red) that encodes the antigenic protein of interest for a specific antigen	Leaf or growing tissue secction of the plant that was previously ruptured is exposed to *A. tumefaciens*, which bears both the gene of resistance gene	*In vitro* callus proliferation from plant cells resistant to antibiotics and carrying the genes of interest after cultivation on agar medium supplemented with antibiotics and hormones to guide plant growth	Cultivation and multiplication of plants with stem leaves, roots or fruits will fulfill the roles of edible vaccines

CONCEPT OF EDIBLE VACCINE

Developed by Arntzen in the 1990s.

Introduce genes of interest into plants (Transformation)

↓

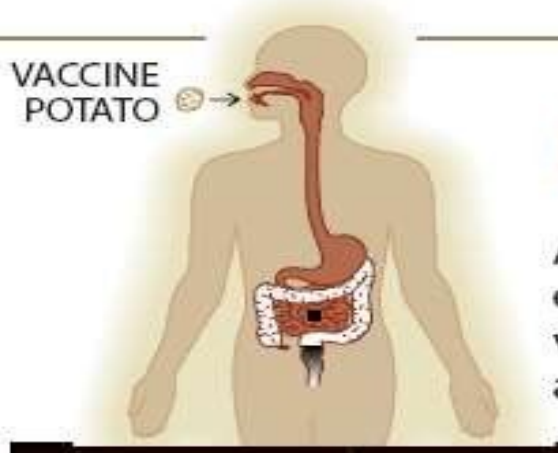
Genes expressed in the plant tissues edible parts (Transgenic plants)

↓

Genes encode putatively protective vaccine antigens from viral, bacterial, and parasitic pathogens that cause disease in humans and animals

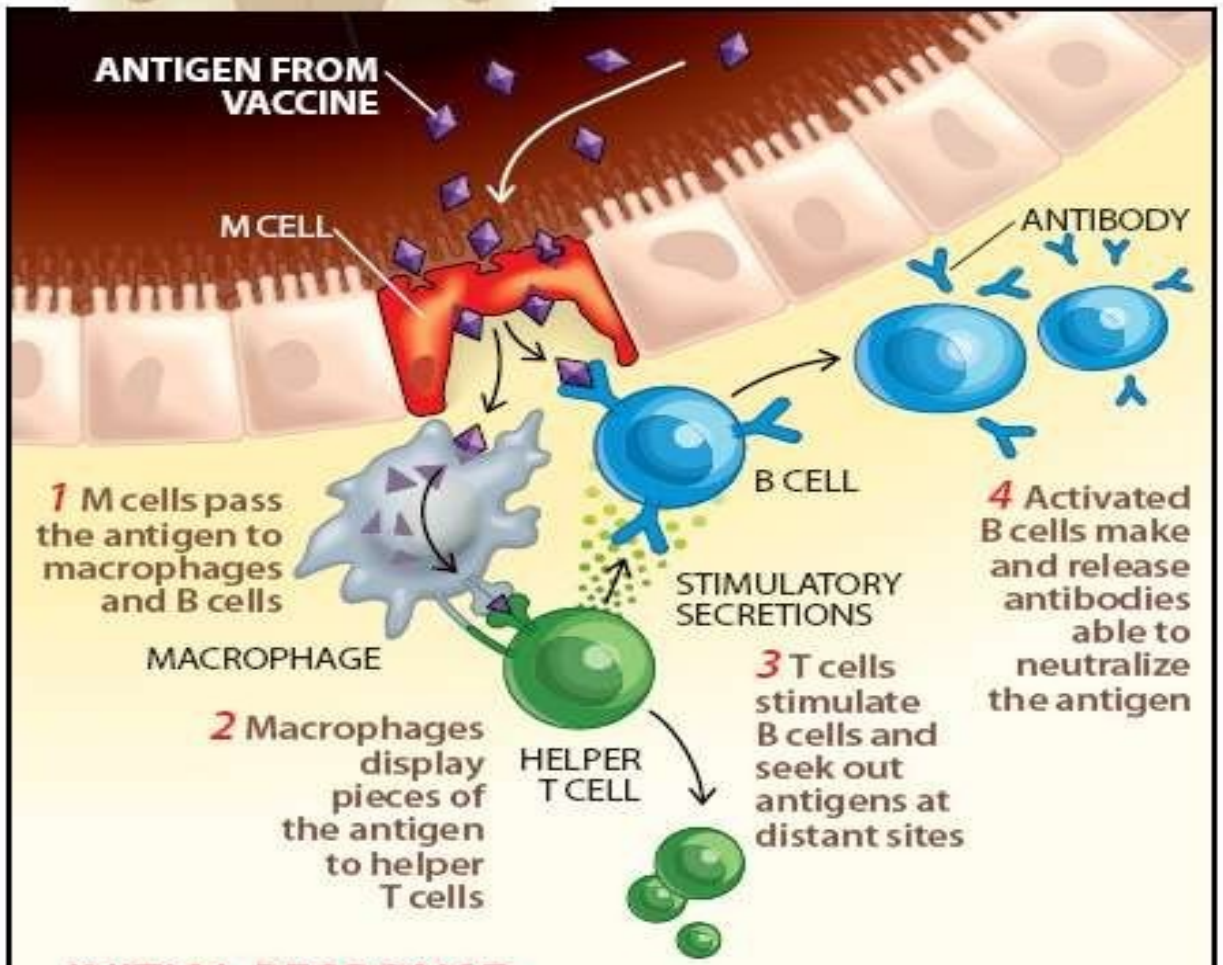
↓

Ingestion of the edible part of the transgenic plant
(Oral delivery of vaccine)

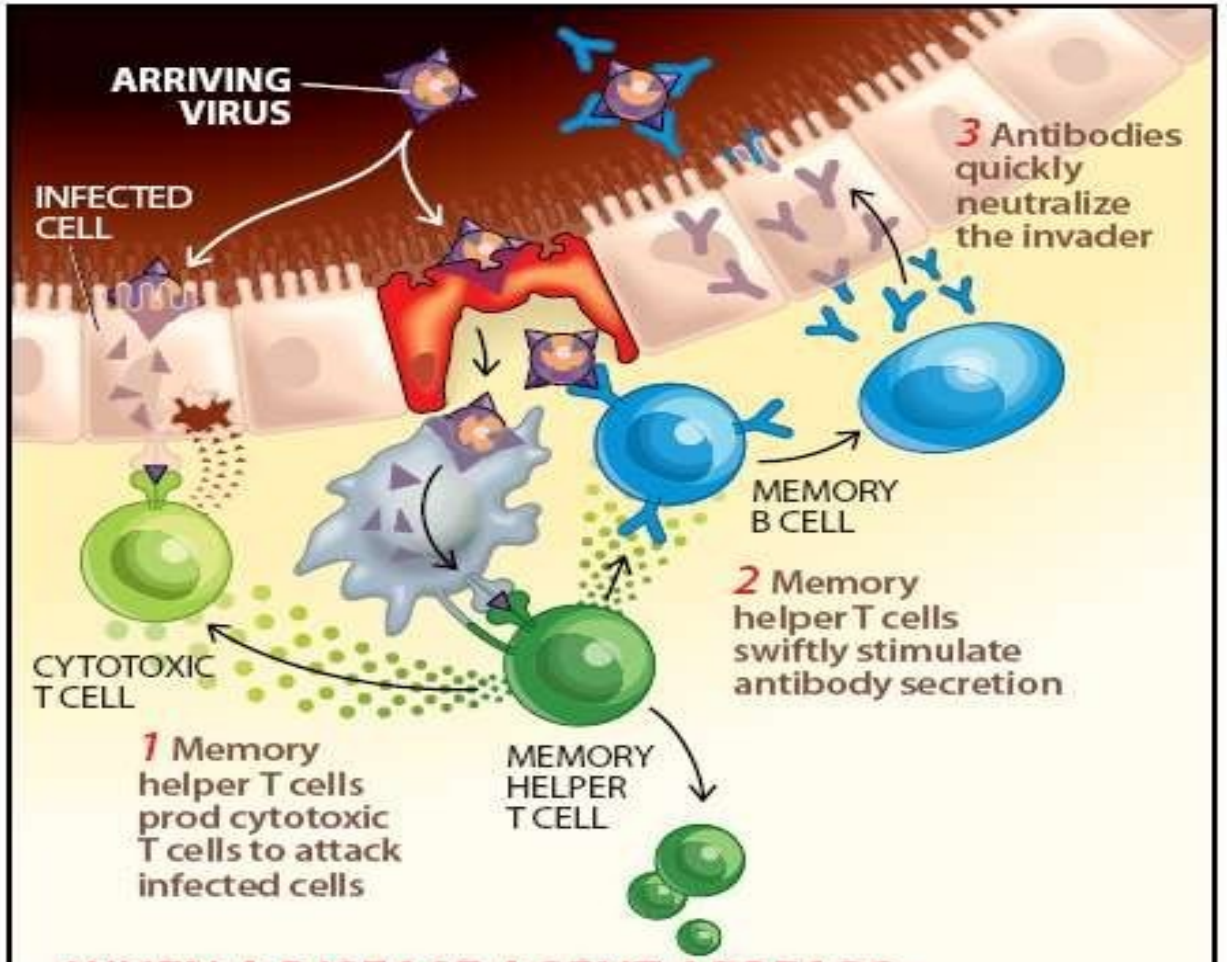


HOW EDIBLE VACCINES PROVIDE PROTECTION

An antigen in a food vaccine gets taken up by M cells in the intestine (*below, left*) and passed to various immune-system cells, which then launch a defensive attack—as if the antigen were a true infectious agent, not just part of one. That response leaves long-lasting "memory" cells able to promptly neutralize the real infectious agent if it attempts an invasion (*right*).

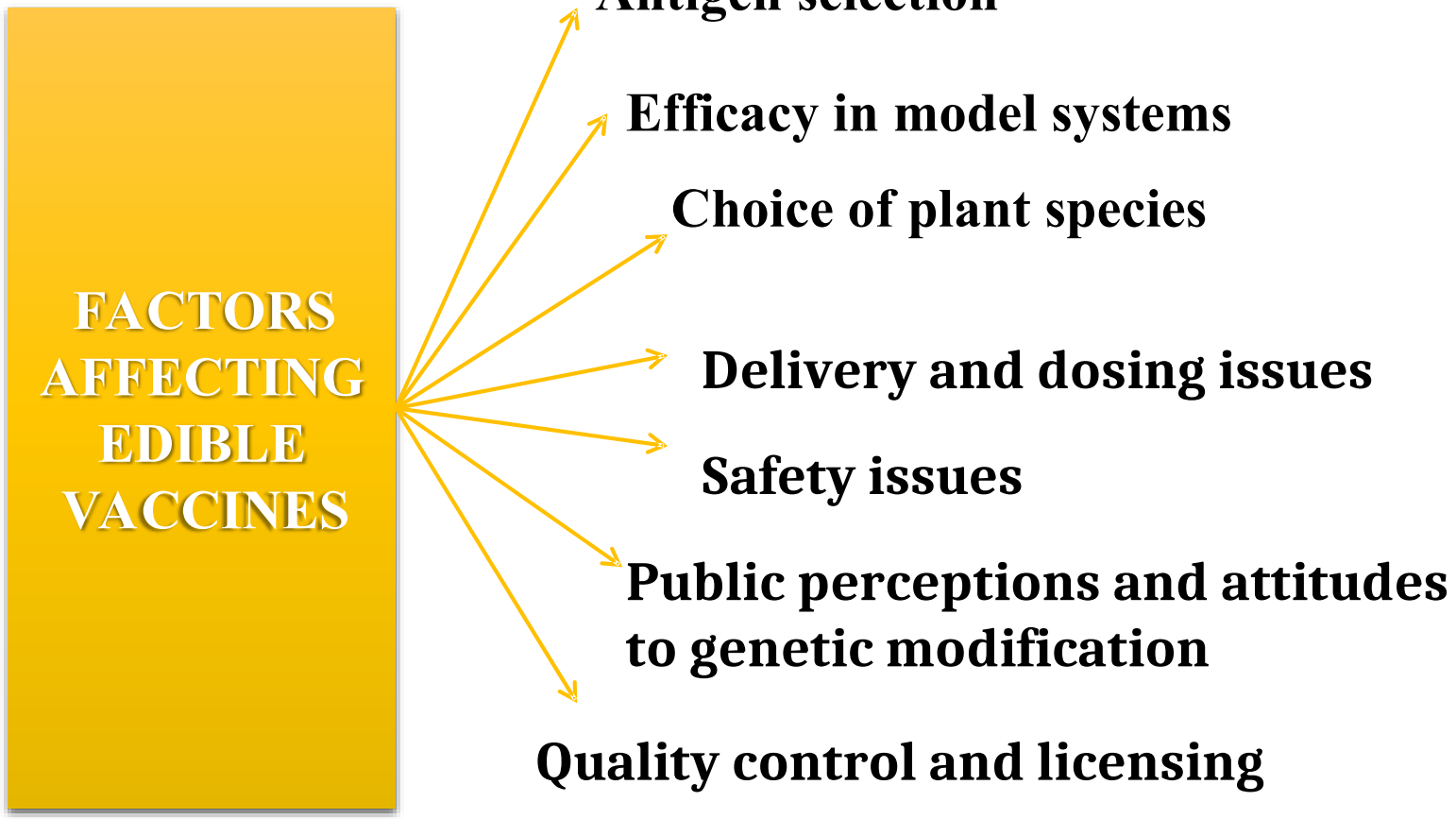


INITIAL RESPONSE



WHEN A DISEASE AGENT APPEARS

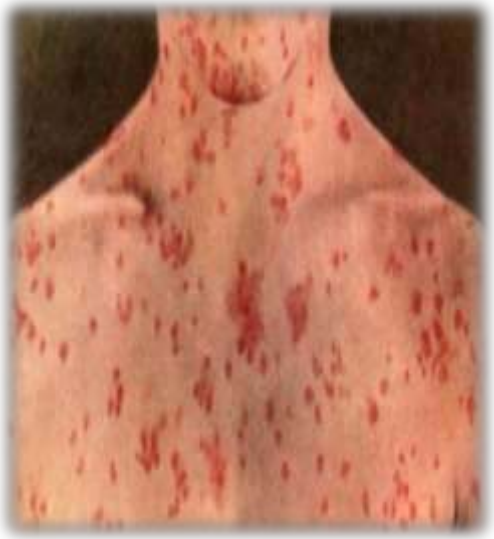
FACTORS AFFECTING EFFICACY OF EDIBLE VACCINES



APPLICATIONS

1. MALARIA

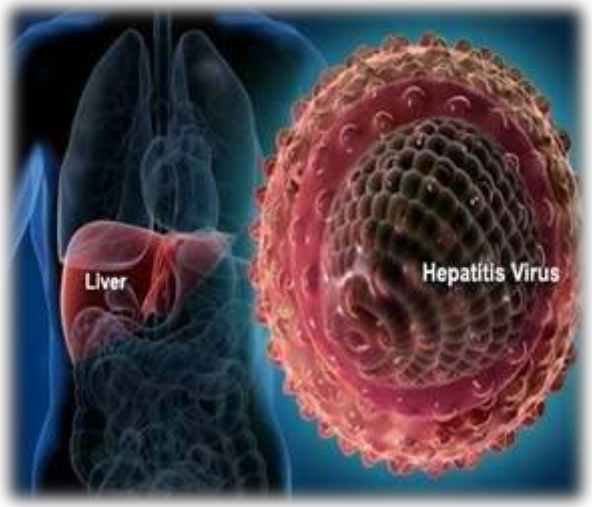
Three antigens are currently being investigated for the development of a plant-based malaria vaccine, merozoite surface protein (MSP) 4 and MSP 5 from *Plasmodium falciparum*, and MSP 4/5 from *P. yoelli*. Wang *et al* have demonstrated that oral immunization of mice with recombinant MSP 4, MSP 4/5 and MSP1, co-administered with CTB as a mucosal adjuvant, induced antibody responses effective against blood-stage parasite.



2. MEASLES

Mice fed with tobacco expressing MV-H (measles virus haemagglutinin from Edmonston strain) could attain antibody titers five times the level considered protective for humans and they also demonstrated secretory IgA in their faeces.

Carrot, banana and rice are the potential candidates



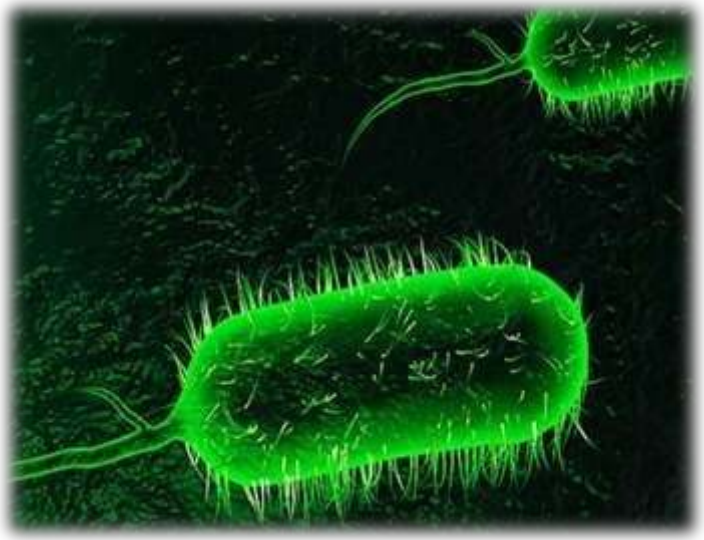
3. HEPATITIS B

significantly exceeded the protective level of 10 mIU/mL in humans..

potato-based vaccine against hepatitis B have reported The amount of HBsAg needed for one dose could be achieved in a single potato.

4. STOPPING AUTOIMMUNITY

The transgenic potato and tobacco plants when fed to nonobese diabetic mice showed increased levels of IgG, an antibody associated with cytokines that suppress harmful immune response.



5. CHOLERA

plants were transformed with the gene encoding B subunit of the *E. coli* heat liable enterotoxin (LT-B). Transgenic potatoes expressing LT-B were found to induce both serum and secretory antibodies when fed to mice; these antibodies were protective in bacterial toxin assay *in vitro*. This is the first "proof of concept" for the edible vaccine.

Advantages of Edible vaccines

- DO not require administration by injection.
- Possible production of vaccines with low costs.
- Do not require separation and purification of vaccines from plant materials.
- Necessary syringe & needles not required.
- Economical in mass production and transportation.
- Heat stable, eliminating the need for refrigeration.

DISADVANTAGE OF EDIBLE VACCINE

- Development of immunotolerance to vaccine peptide or protein.
- Consistency of dosage form fruit to fruit, plant-to-plant, and generation-to-generation is not similar.
- Stability of vaccine in fruit is not known.
- Dosage of vaccines would be variable.
- Selection of best plant is difficult.
- Certain foods like potato are not eaten raw, and cooking the food might weakens the medicine present in it.
- Not convenient for infants.

Safety aspects

- contamination through cross pollination.
- vaccine antigen may affect browsing animals.
- vaccine contamination via plant debris spreading on surfaces and ground waters.
- Affect on humans living in the area drinking vaccine polluted water or breathing vaccine polluted dust.
- cultivation and production of pharmaceutical crops should be limited to control the production facilities like greenhouse, or in plant tissue culture, that prevent the environmental release of biopharmaceuticals.

Edible Vaccines

DEFINITION

Edible vaccines are nothing but transgenic plant and animal based production of or those that contain agents that trigger an animal's immune response. In simple terms, edible vaccines are plant or animal made pharmaceuticals. This essay highlights the importance of edible vaccines produced in plants.

INITIAL DEVELOPMENTS IN DESIGNING THE EDIBLE VACCINES

The concept of edible vaccines was developed by Arntzen (www.genomenewsnetwork.org) in the 1990s. He currently heads the department of plant biology at the Arizona State University. He fell upon the idea after he attended a conference in New York, organized by the WHO. Although the idea seemed quite simple in the beginning, making it into a reality has required sophisticated science. The earliest demonstration of an edible vaccine was the expression of a surface antigen from the bacterium Streptococcus mutans in tobacco. As this bacterium causes dental caries, it was envisaged that the stimulation of a mucosal immune response would prevent the bacteria from colonizing the teeth and therefore protect against tooth decay.

CURRENT STATUS

Several plant derived vaccines for human use are approaching the market but it is likely that the first commercial Plant derived vaccine will be a veterinary vaccine. At least 30 such products have been expressed in plants, some providing protection against challenges with disease causing agents.

HOW DO EDIBLE VACCINES WORK?

Edible vaccines contain DNA fragments from the original pathogen. These fragments code for a protein that is usually a surface protein of the pathogen. This is responsible for eliciting the body's immune response.

SOME EXAMPLES OF EDIBLE VACCINES

•Transgenic Potatoes For Diarrhea

The first human trial for an edible vaccine took place in 1997. Volunteers ate transgenic potatoes that contained the b-subunit of the E. coli heat-labile toxin, which causes diarrhea. Ten of the 11 volunteers showed a 4-fold increase in serum antibodies.

Researchers at the Boyce Thompson Institute at Cornell University conducted another clinical trial of an edible vaccine in 1999. Potatoes containing the Norwalk virus (which causes vomiting and diarrhea) fed to volunteers elicited an immune response in 19 out of 20 volunteers.

The disadvantage of using potato-based edible vaccine is that it has to be consumed raw; when cooked the protein may get denatured or in some cases less effective. Research has shown that by partial boiling at least half the vaccine remained alive.

- Transgenic Tomatoes Against Diarrhea

In the US at the Cornell University, researchers have developed transgenic tomatoes against the Norwalk virus, which causes severe diarrhea. The tomatoes produced a surface protein specific to the virus. Mice that ate these tomatoes developed an immune response to the virus. Recently, banana has been explored as an alternative source because not only does it eliminate the need for cooking but also it's a locally grown plant. The expression of a protein in banana will depend on the identification of a tissue specific promoter. Other examples include rabies

glycoprotein being expressed in viral vectors in spinach and hepatitis B surface antigen in lettuce and potato.

ADVANTAGES OF EDIBLE VACCINES

1. They are cheap; therefore they can be mass-produced.

2. They can be ingested by eating the plant/part of the plant. So, the need to process and purify does not arise.

3. Extensive storage facilities like cold storage are not required.

4. If the local/native crop of a particular area is engineered to produce the vaccine, then the need for transportation and distribution can be eliminated.

5. Most importantly, they trigger the immunity at the mucosal surfaces such as those that line the mouth (mucosal immunity) which is the body's first line of defense.

DISADVANTAGES OF EDIBLE VACCINES

1. Will the antigens be able to survive the hostile, acidic conditions of the stomach and even if they did, will they be able to trigger the immune system in the right way? Although initial trials have shown promising results in human subjects, it is not clear what will happen when the person comes in contact with the actual virus.

2. How can the vaccine dose be controlled? This remains the most difficult task. There seems to be a danger that too high a dose could provoke oral tolerance of an invading bacteria or virus, instead of an immune response. Also, the dosage requirements for children and adults will be different. So, research is on its way to find a solution to these problems.

3. Plants are living organisms that change, so the continuity of the vaccine production might not be guaranteed.

4. Glycosylation patterns in plants differ from those in humans and could affect the functionality of the vaccines.

5. People may develop an allergy to the fruit or vegetable expressing the foreign antigen

Summary

The first trial on humans in 1997 (using the heat labile B- toxin from E. coli) is a milestone on the road to creating inexpensive vaccines that might be particularly useful in immunizing people in developing countries, where high cost and logistical issues, such as transportation and the need for certain vaccines to be refrigerated, can thwart effective vaccination programs. The hope is that edible vaccines could be grown in many of the developing countries where they would actually be used. Whatever may be the current situation, a day is not far off when we will be able to pluck a fruit from the garden, eat it and be protected from diseases...making needles needless.

Alkaloids

- A precise definition of the term 'alkaloid' (alkali-like) is somewhat difficult because there is no clear-cut boundary between alkaloids and naturally occurring complex amines.
- ***Typical alkaloids*** are derived from plant sources, they are basic, they contain one or more nitrogen atoms (usually in a heterocyclic ring) and they usually have a marked physiological action on man or other animals.
- The name ***'proto-alkaloid'*** or 'amino-alkaloid' is sometimes applied to compounds such as hordenine, ephedrine and colchicine which lack one or more of the properties of typical alkaloids.

Physico-chemical properties

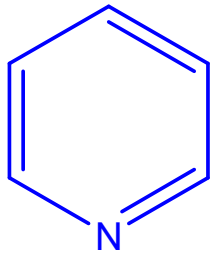
- Most alkaloids are well-defined crystalline substances which unite with acids to form salts.
- In the plant they may exist in the free state, as salts or as *N*-oxides.
- In addition to the elements carbon, hydrogen and nitrogen, most alkaloids contain oxygen.
- A few, such as coniine from hemlock and nicotine from tobacco, are *oxygen-free and are liquids*.
- Although colored alkaloids are relatively rare, berberine, for example, is yellow and the salts of sanguinarine are copper-red.

- As a general rule, alkaloids as bases are not soluble or are sparingly soluble in water, soluble in apolar or only slightly polar organic solvents, and are soluble in concentrated hydroalcoholic solutions.

- The basicity of alkaloids varies greatly, since this property depends entirely on the availability of the lone pair of electrons on the nitrogen atom:

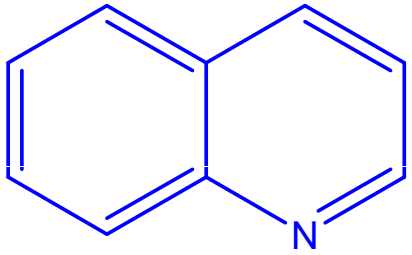
1. ***Electron-withdrawing*** groups in close proximity to the nitrogen atom decrease the basicity, whereas
2. ***Electron-donating*** groups enhance the basicity.

- *The basic character of the heterocyclic ring itself varies:*

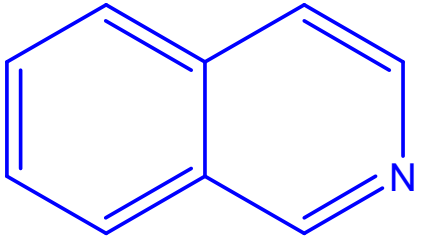


Pyridine

- in the molecule of ***pyridine***, with 6 π electrons, and in the case of *quinoline* and *isoquinoline*, the lone pair of electrons on the nitrogen atom is available and the basicity is clear.



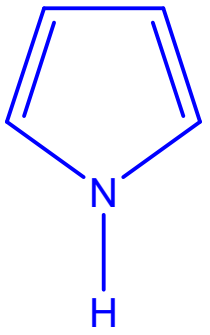
Quinoline



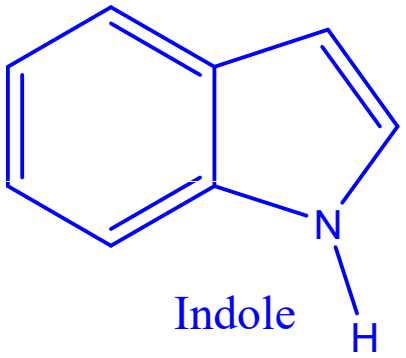
Isoquinoline

- In the case of ***pyrrole*** or *indole*, the lone pair of electrons on the nitrogen atom plays a role in the aromatic character, and the compounds are not basic (they are acidic).

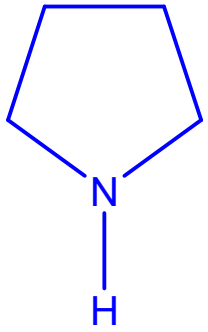
- Another example is ***pyrrolidine***, which is saturated, and is a strong base.



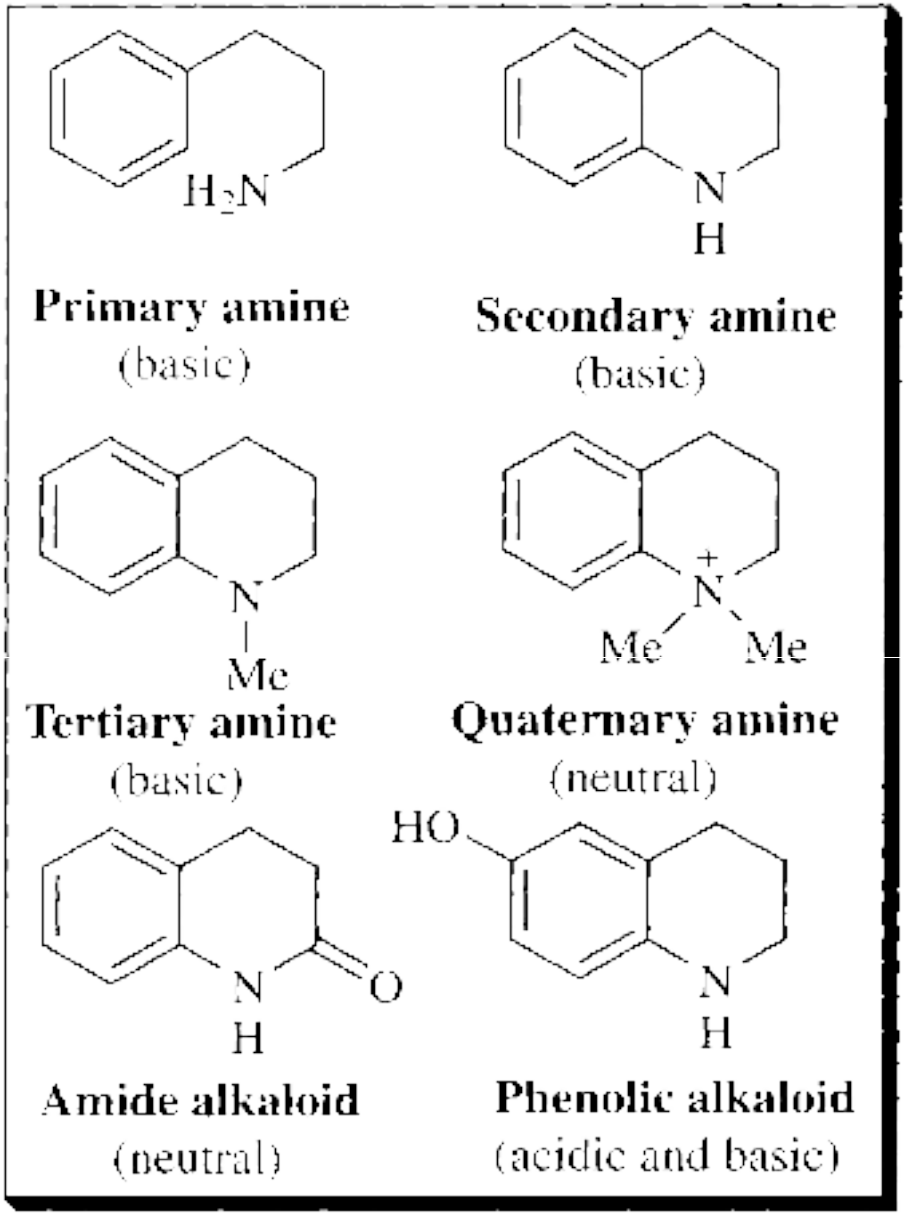
Pyrrole



Indole



Pyrrolidine

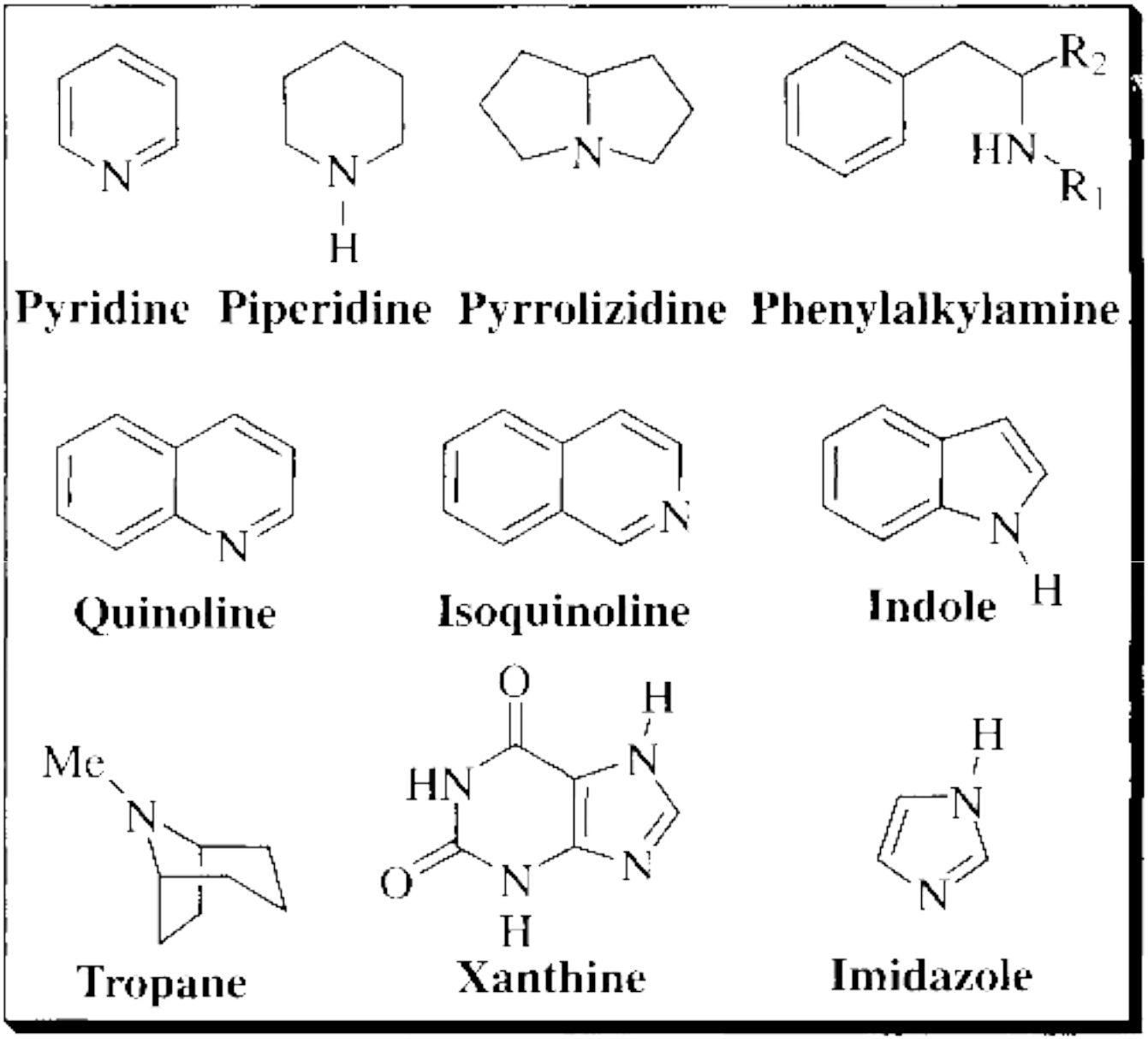

H2N
Primary amine
(basic)
N
H
Secondary amine
(basic)
N
Me
Tertiary amine
(basic)
N+
Me
Me
Quaternary amine
(neutral)
N
H
O
Amide alkaloid
(neutral)
HO
N
H
Phenolic alkaloid
(acidic and basic)

Structure and classification

- Generally, there are two broad divisions:

1. Heterocyclic or typical alkaloids, divided into ***14 groups*** according to their ring structure.
2. Nonheterocyclic or atypical alkaloids, sometimes called ‘protoalkaloids’.

- *Alkaloids are usually classified according to the nature of the basic chemical structures from which they derive*.


Pyridine
Piperidine
Pyrrolizidine
Phenylalkylamine
Quinoline
Isoquinoline
Indole
Tropane
Xanthine
Imidazole

Nomenclature

- The name of all alkaloids should end with the suffix '-***ine***'.
- The names of the alkaloids are obtained in various ways:

1. From the generic name of the plant yielding them, e.g. *atropine*.
2. From the specific name of the plant yielding them, e.g. *cocaine*.
3. From the common name of the drug yielding them, e.g. *ergotamine*.
4. From their physiologic activity, e.g. *emetine*.
5. From the discoverer, e.g. *pelletierine*.

Functions of alkaloids in plants

- There are several speculations about the advantages of their presence in plants, including:

1. Poisonous agents protecting the plant against insects and herbivores "Animals that feed chiefly on plants".
2. End products of detoxification reactions.
3. Regulatory growth factors.
4. Reserve substances capable of supplying nitrogen or other elements necessary to the plant's economy.

Biosynthetic origin

- **Alkaloids** are formed from amino acids, but other precursors, e.g. terpenes or steroids, are often also built into the final alkaloidal skeleton.
- The amino acids that most often serve as alkaloidal precursors include:

  phenylalanine, tyrosine, tryptophan, histidine, anthranilic acid, lysine and ornithine.

Tests for alkaloids

- There are several general reagents, which may be used to test the presence of alkaloids or to help their identification. This includes the alkaloidal precipitating reagents and the alkaloidal coloring reagents. In addition, there are some special reagent that can be used for recognizing and confirming the identity of each alkaloid.
- ***Alkaloidal precipitating reagents*:**

1. **Mayer's reagent** (potassiomercuric iodide solution)
2. **Wagner's reagent** (solution of iodine in potassium iodide)
3. **Dragendorff's reagent** (potassium bismuth iodide)

- ***Alkaloidal coloring reagents*:**

1. **Marqui's reagent** (Formaldehyde-sulfuric acid)
2. **Mandalin's reagent** (sulphovanadic acid)
3. **Erdmann's reagent** (Nitric acid-sulfuric acid)

Extraction of alkaloids

- There are several methods that can be used for the extraction of the alkaloids from plant materials. However, the common procedures are largely based on: **(1)** the basic nature of most alkaloids; **(2)** the subsequent ability to form salts with acids; **(3)** the ease by which the free bases can be liberated from their salts by alkalinization and finally **(4)** the relative solubility of the alkaloids and their salts in water and various organic solvents.
- The conventional process involved in the alkaloids separation and isolation may be divided as follows:

1. Preparation of the sample.
2. Liberation of the free alkaloidal base, by treating the dried material with suitable alkali.
3. Extraction of the alkaloidal base with an organic solvent.
4. Purification of the alkaloidal extract.

Pharmacological activity and uses

- *Alkaloids are particularly interesting substances because of their multiple pharmacological activities*:

1. on the CNS, whether they are *depressants* (**morphine**) or *stimulants* (**caffeine**);
2. on the autonomic nervous system: *sympathomimetics* (**ephedrine**) or *sympatholytics* (**yohimbine**, certain ergot alkaloids), *parasympathomimetic* (pilocarpine), *anticholinergics* (**atropine**, hyoscyamine), or *ganglioplegics* (nicotine).

- In addition, alkaloids include *local anesthetics* (**cocaine**), *agents to treat fibrillation* (**quinidine**), *antitumor agents* (**vinblastine**), *antimalarials* (**quinine**), *antibacterials* (**berberine**), and *amebicides* (**emetine**).

Essential oils

or

Volatile oils

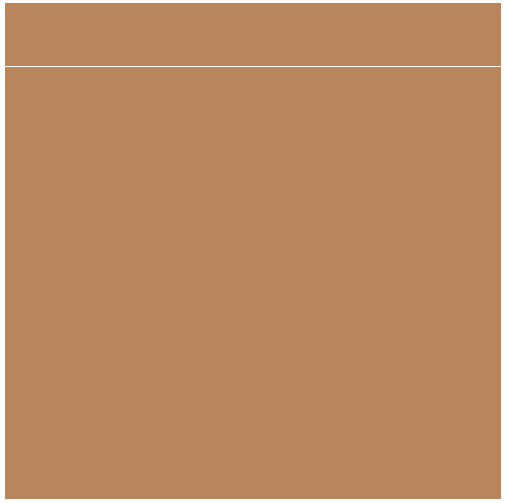
Volatile Oils / Essential oils / Ethereal oils

Volatile oils are complex mixtures of odoriferous (having odour) principles of varying chemical composition, which easily evaporate when exposed to air at ordinary temperature, and which are used for either their specific therapeutic activity or aroma.

- Oily liquids, which are entirely or almost entirely volatile without decomposition
- Plant products, giving the odors and tastes characteristic of the particular plant, thus possessing the essence.
- Ether like in their volatility.

In some cases the essential oils don't pre-exist but formed by decomposition of a glycoside

-Bezaldehyde (amygdalin) in bitter almond



-Allyl isothiocyanate (sinigrin) in black mustard

Physical properties of Essential Oils

1. Liquid at ambient temperature
2. Volatile, (c.f. fixed oils)
3. Colourless liquids with the exception of chamomile oil (violet in colour)
4. Their density is lower than water, with the exception clove or cinnamon heavier than water)
5. They have a high refractive index and most of them rotate the plane polarized light.
6. They are soluble in common organic solvent and lipo-soluble (lipophilic)
7. Steam distilled and sparingly soluble in water; they are soluble enough, however, to impart a distinct fragrance to water /aromatic water)

Volatile Oil Composition

- An essential oil is a mixture, often containing hundreds of compounds, and reflects the characteristics of a mixture.

- Individual compounds are covalently bonded volatile liquids that contribute their individual properties to the oil.

- An individual component may be harmful on its own but, when diluted and with other ingredients, it may have an additive and beneficial effect; this is an example of ***synergy***.

- ***As with all mixtures, the*** composition of oils may vary and thus the overall characteristics of an oil may vary. The composition may also vary depending on the plant species and subspecies and such variations are genetically determined.

Composition of Volatile oils

Essential oils are complex and highly variable mixtures of constituents which belong to two groups

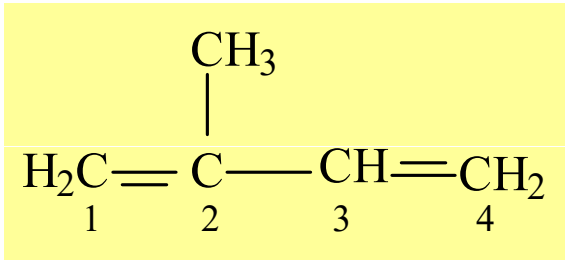
1. The group of **terpenoids**
2. The group, far less common, of aromatic compounds derived from **phenylpropane**

Some essential oils contain

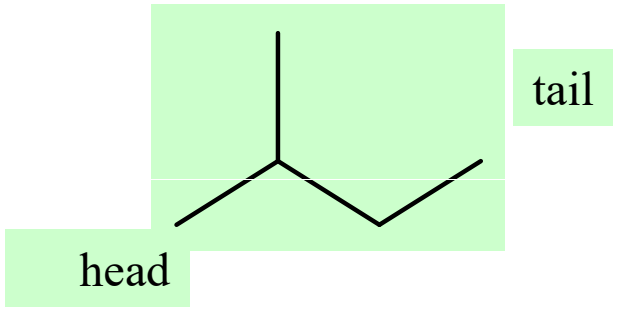
- Degradation products of non-volatile constituents (results of enzymatic hydrolysis)
- Nitrogen containing compounds
- Sulphur containing compounds
- Polyacetylenes

Terpenoids

Form a large and structurally diverse family of natural products derived from C_5 isoprene units joined in a head to tail fashion



Isoprene



Isoprene unit
C_5

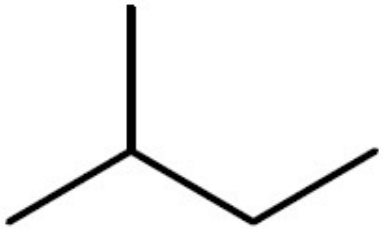
The biologically active isoprene units were identified as the pyrophosphate esters IPP (Isopentenyl pyrophosphate) And DMAPP (Dimethylallyl pyrophosphate)

What are terpenes ?

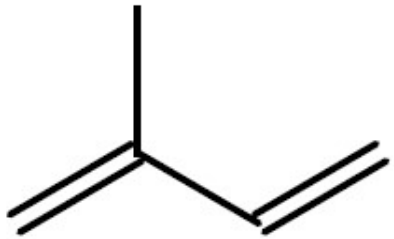
Terpenes are defined as natural products whose structures may be divided into **isoprene units.**

Isoprene units:

- arise from **acetate** via **mevalonic acid**
- branched-chain, 5-carbon units containing 2 unsaturated bonds



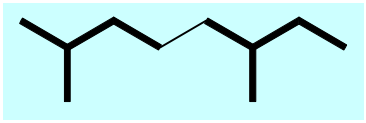
Isoprene unit



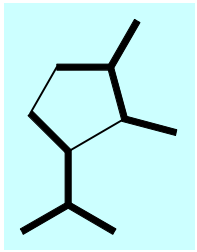
Isoprene

Terpenoids

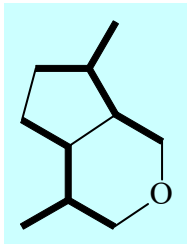
- C10-Monoterpenes
  - Regular monoterpenes (essential oils, oleoresins, iridoids)
  - Irregular monoterpenes (pyrethrins)
- C15-Sesquiterpenes (essential oils, sesquiterpenoid lactons)
- C20-Diterpens (e.g. retinol)
- C30-Triterpens & steroids (saponins, cardiac glycosides)
- C40-tetraterpenes (e.g. β-carotenes)



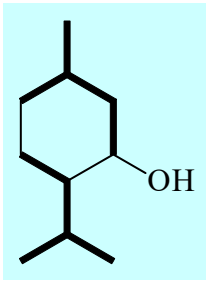
Regular monoterpene skeleton



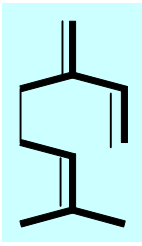
Iridane



Iridoid

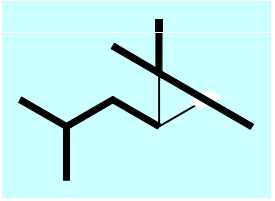


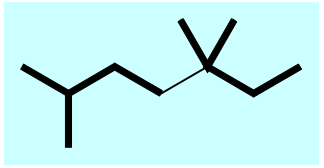
Menthol



myrcene

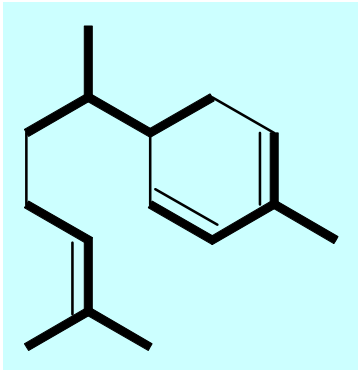
The iridan skeleton found in iridoids is monoterpenoid in origin and contains a cyclopentane ring which is usually fused To a six-membered oxygen heterocycle



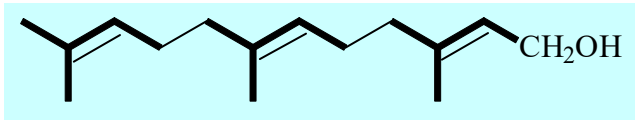


Irregular monoterpene skeletons
Pyrethrins

Zingiberine



Sesquiterpenes (C15)



Farnesol

Comparison between fixed oils and essential oils

- Their volatility
- When smeared on paper
- Oxidation (resinified, fixed oil rancid).
- Chemical structure
- Saponification by KOH (NOT saponify)

➢ Function

- Attracting (help polination) or repelling insects
- Protection from heat or cold
- As antibacterial agents

➢ Uses

- Pharmacy, aromatherapy
- Perfumery
- Food technology

Chemical Tests- Qualitative

- **Menthol**

- 0.5 ml of oil solution in a clean test tube + 2 drops of (1%) aq. $FeCl_3$ –Ve (Yellow color).
- 0.5 ml of oil solution in a clean porcelain dish W.B residue, ~~dissolve~~ in drops of Conc. H_2SO4 + drops of vanillin/H_2SO4 orange yellow dps of H_2O Reddish violet color.
- Special test for thymol gives ~~–ve result~~

Chemical Tests- Qualitative…

- **Thymol**

➢ 0.5 ml of oil solution in a clean test tube + 2 drops of (1%) aq. $FeCl_3$ ⟶ + Ve (Green color).

➢ 0.5 ml of oil solution in a clean porcelain dish $\xrightarrow{W.B}$ residue, dissolve in 0.5 ml of glacial acetic acid + dps of Conc. H_2SO4 + dps of Conc. HNO_3 ⟶ Bluish green color.

N.B. This test is –ve for menthol.

Chemical Tests- Qualitative

- **Eugenol**

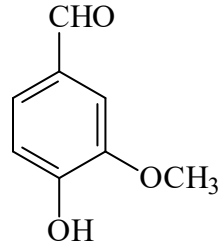
➢ 0.5 ml of oil solution in a clean test tube

+ 2drops of (1%) aq. $FeCl_3$ → + Ve (Green color).

➢ 0.5 ml of oil solution in a clean test tube + 2 drops of (2%) alc. $FeCl_3$ → Green to bluish green color.

➢ 0.5 ml of oil solution in a clean test tube + 2 drops of aq. saturated $FeCl_3$ → Green color + 2 ml H_2O → turbid brown.

Chemical Tests- Qualitative



❑Vanillin

- 0.5 ml of oil solution in a clean test tube + 2 drops of (1%) aq. $FeCl_3$ → + Ve (Blue to dark blue color).

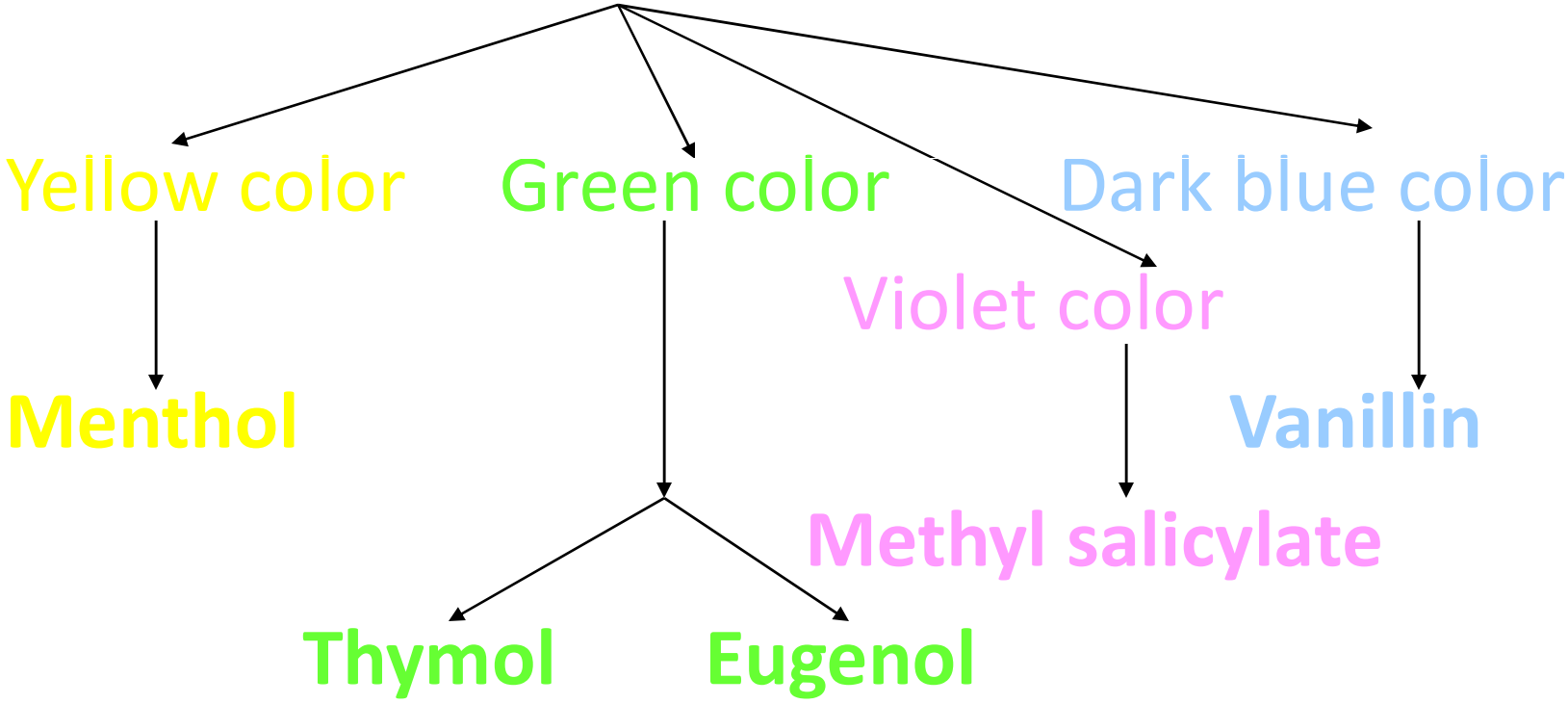
❑Methyl salicylate

- 0.5 ml of oil solution in a clean test tube + 2 drops of (1%) aq. $FeCl_3$ → + Ve (Violet color).

N.B. DON'T leave the oil on W.B too much, when making a residue.

Scheme for Volatile oils identification

- 0.5 ml of the oil unknown in a clean test tube + 2 drops of (1%) aq. FeCl3



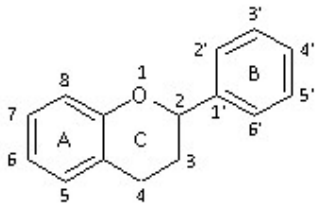
Flavonoids

Flavonoids are the most abundant polyphenols in human diet, representing about 2/3 of all those ones ingested. Like other phytochemicals, they are the products of secondary metabolism of plants and, currently, over 4000 have been identified. In fruits and vegetables, they are usually found in the form of glycosides and sometimes as acylglycosides, while acylated, methylated and sulfate molecules are less frequent and in lower concentrations. They are water-soluble and accumulate in cell vacuoles.

Chemically flavonoids are based upon a fifteen-carbon skeleton consisting of two benzene rings (A and B as shown in Figure linked via a heterocyclic pyrane ring (C).

Chemical structure of flavonoids

Their basic structure is a skeleton of **diphenylpropane**, namely, two benzene rings (ring A and B, see figure) linked by a three carbon chain that forms a closed pyran ring (heterocyclic ring containing oxygen, the C ring) with benzenic A ring. Therefore, their structure is also referred to as C6-C3-C6.



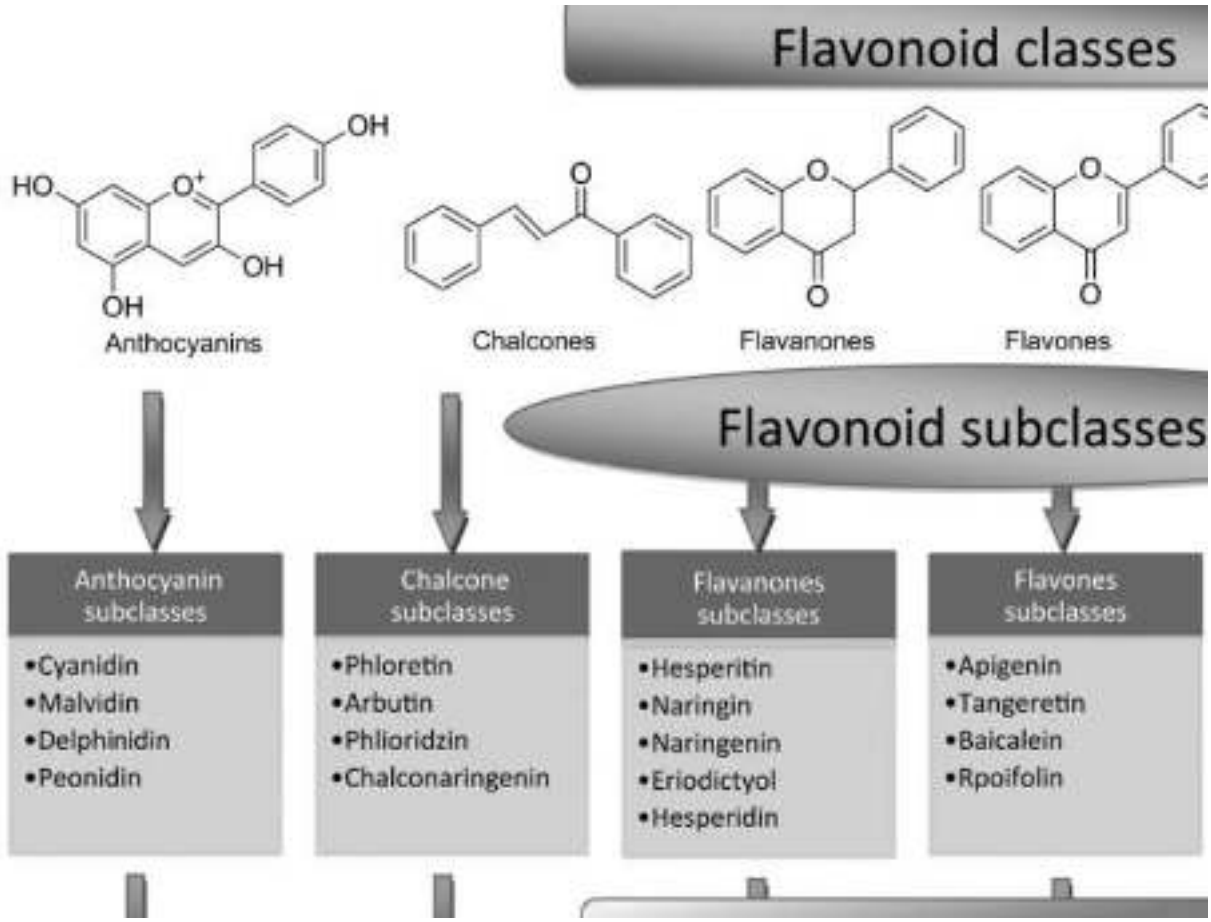
In most cases, B ring is attached to position 2 of C ring, but it can also bind in position 3 or 4; this, together with the structural features of the ring B and the patterns of glycosylation and hydroxylation of the three rings, makes the flavonoids one of the larger and more diversified groups of phytochemicals, so not only of polyphenols, in nature.
Their biological activities, for example they are potent antioxidants, depend both on the structural characteristics and the pattern of glycosylation.

Classification

They can be subdivided into different subgroups depending on the carbon of the C ring on which B ring is attached, and the degree of unsaturation and oxidation of the C ring.

- **Flavones:** They have a double bond between positions 2 and 3 and a ketone in position 4 of the C ring.
- **Flavonols:** Compared to flavones, they have a hydroxyl group in position 3 of the C ring, which may also be glycosylated.
- **Flavanones:** Flavanones, also called dihydroflavones, have the C ring saturated; therefore, unlike flavones, the double bond between positions 2 and 3 is saturated and this is the only structural difference between the two subgroups of flavonoids.
- **Flavanonols:** Flavanonols, also called dihydroflavonols, are the 3-hydroxy derivatives of flavanones.

- **Isoflavones:** Isoflavones are a subgroup of flavonoids in which the B ring is attached to position 3 of the C ring. They have structural similarities to estrogens, such as estradiol, and for this reason they are also called phytoestrogens.
- **Neoflavonoids:** They have the B ring attached to position 4 of the C ring.
- **Anthocyanidins:** Chemically, anthocyanidins are flavylium cations and are generally present as chloride salts. They are the only group of flavonoids that gives plants colors (all other flavonoids are colorless). Anthocyanins are glycosides of anthocyanidins.
- **Chalcones:** Chalcones and dihydrochalcones are flavonoids with open structure; they are classified as flavonoids because they have similar synthetic pathways.



Shinoda test for flavonoids

In this test, four pieces of magnesium fillings (ribbon) are added to the ethanolic extract followed by a few drops of concentrated hydrochloric acid. A reddish colour indicates the presence of flavonoid.

GLYCOSIDES

A glycoside is an organic compound, usually of plant or animal origin,which on enzymatic or acid hydrolysis gives one or more sugar moieties along with non-sugar moiety.

Sugar portion ……. Glycone
Non-sugar portion…... Aglycone / Genin

Linkage between sugar and non-sugar portion is usually called as "Glycosidic linkage"

Classification & Types: Based on linkage between glycone and aglycone portion

1. **C- glycosides:**

   Glycone -OH+ CH- Aglycone ⟶ Glycone –C-aglycone + H_2O

   Some of the anthraquinone glycoside like cascaroside in cascara, aloin in aloes shows the particular linkage.
   C-glycosides are called aloin type glycoside present in aloes. They do not hydrolyzed by heating with dil. acid or alkalis but by oxidative hydrolysis with Fecl3. cochical contains c-glycoside in the form of coloring matter called carminoic acid

2. **O- glycosides:**

   Glycone -OH+ OH- Aglycone ⟶ Glycone –O-Aglycone + H_2O

   They are common in higher in plants. Ex: senna, rhubarb
   They are hydrolyzed by treatment with acid or alkali into glycone and aglycone portion.

3. **S- glycosides:**

   Glycone -OH+ SH- Aglycone ⟶ Glycone –S-Aglycone + H_2O

   They occurrence of this glycoside is restricted to isothiacyanate glycoside like sinigirin in black mustard formed by the condensation of sulphohydryl group aglycon to OH group of glycone.

4. **N- glycosides:**

   Glycone- OH+ HN- Aglycone⟶ Glycone –N-Aglycone + H_2O

They most typical representation of this is nucleoside where the amino group reacts with OH group of ribose or deoxyribose resulting into N-glycoside.

Based on Chemical Nature:

(a) Cardioactive glycosides: Digitalis, Strophanthus and white squill

(b) Anthraquinone glycosides: Cascara, Aloe, Rhubarb, Cochineal and Senna

(c) Saponin glycosides: Glycyrrhiza, Sarsaparilla

(d) Cyanophore glycosides: Wild cherry

(e) Isothiocyanate glycosides: Black Mustard

(f) Lactone glycosides: Cantharide

(g) Aldehyde glycosides: Vanilla

(h) Miscellaneous glycosides: Gentian, Quassia, Dioscorea

Properties: -

1) Glycoside contains sugar but still the physical, chemical and therapeutic activity is based on aglycon portion. Sugar facilitates the absorption of the glycoside .helping it to reach the site of action

2) Glycoside are crystalline, amorphous substance which are soluble in water, and dilute alcohol but in soluble in the Chloroform and ether. The aglycon moiety is insoluble in non polar solvent like Benzene.

3) Glycosides are easily hydrolyzed by mineral acids, water and enzyme. They show optical activity normally they are levorotatory.

4) Glycoside cannot reduce Fehling's solution until they are hydrolyzed.

Functions: -

From biological point of view glycosides play an important role in the life of plants involving its regulatory, transperatory and protective functions.

Hydrolysis: -

Glycosides are hydrolyzed by acid or alkali and by enzymes resulting in cleavage of glycosidic linkage. However certain glycosides are broken down or modified.

Isolation of glycosides:

The method by which glycoside are isolated is called stas-otto method. The drug containing glycoside is finely powdered and subjected to successive extraction in a soxhlet apparatus with alcohol or suitable solvent. The non-glycosidal impurities which get extracted along with glycosides are removed by precipitating them with lead acetate solution. The excess of lead acetate is then removed by passing hydrogen sulphide gas through the extract. Lead gets precipitated as lead sulphide, which is filtered out. The filtrate contains the glycosides. The glycoside can be obtained by removal of the solvent under reduced pressure or any other suitable procedure. Further purification of the isolated glycosides is done by column chromatography.

CHEMICAL TESTS FOR GLYCOSIDES

a. sterol and triterpenoid glycoside:

• General tests :

(1) Antimony trichloride test: An alcoholic extract of drug →evaporate → dry → make extract with chloroform + saturated solution of antimony trichloride in chloroform containing 20% acetic anhydride → appearance of pink colour on heating → presence of steroids and triterpinoids.

(2) Tetranitro methane test : Alcoholic extract of drug + tetranitro methane solution →formation of yellow colour → presence of .sterol and triterpenoid.

• Specific tests for sterol :

(1) Libermann burchard test : Alcoholic extract of drug → evaporated → dry →extracted with CHCl3 + few drops of acetic anhydride + conc.salphuric acid (from the side wall of test tube) → appearance of violet ring → blue colour → presence of sterol group in drug.

(2) Salkowaski test : Alcoholic extract of drug→evaporated →dry → extracted with CHCl3 + conc. H2SO4(from side wall of test tube) →appearance of yellow colored ring (at the junction of two liquids) → turn to red → presence of sterol group in drug

• Specific test for triterpenes :

Trichloro acetic acid test: Drug + saturated trichloro acetic acid → coloured precipitate → presence of triterpenes .

2. Anthraquinone glycosides :

Specific tests: For c- types of anthraquinone glycosides

(i) Modified brontrager's test : 1 gm of drug sample + 5ml of dilute HCl + 5 ml of ferric chloride →Boil for 10 min. on waterbath → cool (on room temperature) →filter → extract of filterate with carbon tetrachloride or benzene + equal amount of ammonia solution →appearance of pink to red colour → presence of anthraquinone moiety.

(ii) Borntrager's test :
1gm of drug sample + 5-10 ml of dilute HCl + 10 min. boil on waterbath and filter + extract of filterate with CCl4 or benzene + equal amount of ammonia solution to filterate + shake → appearance of pink to red colour → indicate presence of anthraquinone moiety.

3. Cyanogenetic and cyanophoric glycoside :

• Specific tests for cyanogenetic glycoside:

(i) Cuprocyanate test : Saturate the filter paper in freshly prepared solution of guaic resin + dissolved in ethanol → dry . → make contact of that filter paper with dilute solution of CuSO4 →place it with contact of drug sample → Generation of HCN gas with the appearance of stain → the presence of cynogenetic glycoside.

(ii) Feeriferrocyanide test: 1 gm of drug sample + 5 ml of alcoholic KOH → transfer it to aqueous solution of $FeSO_4$ and $FeCl_3$ → keep it on room temperature for10 minutes. → transfer whole solution to 20 % HCl →appearance of Prussian blue colour → the presence of cynogenetic glycosides .

(iii) Precipitation of mercury from $HgNO_3$: Generation of HCN gas by→reduction of aqueous mercurous nitrite solution → metallic Hg → the presence of cyanogenetic glycosides.

• Specific tests for cynophoric glycosides:

(i)Sodium picrate test : small amount of drug sample → humidification with water in a conical flask + few drops of conc. H_2SO_4 → appearance of brick colour → presence of cynophoric glycoside.

4. Saponin glycosides:

(i) Haemolysis test : A drop of blood on slide + few drops of aq. saponin solution → appearance of ruptured red blood cells →the presence of sapnonin glycoside.

(ii) Foam test : 1 gm of sample drug + 10 to 20 ml of water →well shaked →generation of froths → the presence of saponins.

5. Flavonoid glycosides :

General test:

(i) Shinoda test : alcoholic extract of 1 gm of drug + magnesium turning+ dilute HCl → appearance of red colour → the presence of flavonoids.
• alcoholic extract of 1 gm of drug sample + zinc turning + dil. HCl →appearance of deep red colour → turns to magenta colour → the presence of dihydro flavonoids(other type of flavonoid glycoside).

(ii) Ammonia test : To the alcoholic solution of 1 gm of drug sample , when filter paper dipped and after that exposed to ammonia vapor, appearance of yellow spot on the filter paper indicates the presence of flavonoid.

(iii) Vanilin HCl test :
Alcoholic solution of drug sample + vanillin HCl → appearance of pink colour→ presence of flavonoid

6. Coumarin glycoside :

specific tests:

(i) Fluorescence test :

Alcoholic extract of drug sample + NaOH solution (1N) → generation of blue – green fluorescence → indicates presence of coumarins.

(ii) Ferric chloride test :

Concentrate alcoholic extract of drug sample + few drops of alcoholic $FeCl_3$ solution →appearance of dark green colour → turned to yellow after some time on addition of conc. HNO_3 → indicates the presence of coumarins.

7. Cardiac glycosides :

Specific test:

(i)3,5-dinitro benzoic acid test :
Alcoholic solution of drug sample + few drops of NaOH + 2 % solution of 3,5- dinitro benzoic acid →appearance of pink colour → indicates the presence of cardiac glycosides.

General tests :

(i) Legal test :
Alcoholic extract of drug + equal volume of water + 0.5 ml of strong lead acetate solution → well shake →filter → filtrate extracted with same volume of $CHCl_3$→$CHCl_3$ extract evaporate → dryness → remainder dissolve with 2 ml of pyridine and sodium nitropruside + NaOH solution to make it alkaline → appearance of pink colour → indicates the presence of aglycon or glycoside moiety.

(ii) Baljet test :
Section of drug containing cardiac glycoside (thick section of leaf of digitalis) →dipped into sodium picrate solution → appearance of yellow to orange colour → indicate the presence of aglycon moiety.

(iii)Keller – kiliani test :
Alcoholic extract of drug + equal volume of water + 0.5 ml of strong lead acetate solution → shake → filter→ filtrate extracted with same amount of $CHCl_3$ → $CHCl_3$ extract evaporated to dry → remainder dissolved in 3 ml of glacial acetic acid + few drops of $FeCl_3$ solution + 2ml of conc. H_2SO_4→ reddish brown layer → turns to bluish green → indicate the presence of aglycon (digitoxose).

Resins

Resins, in general, are amorphous solid or semisolid substances that are invariably water insoluble but mostly soluble in alcohol or other organic solvents. However, physically they are found to be hard, translucent or transparent and fusible *i.e.*, upon heating they first get softened and ultimately melt. But chemically, they are complex mixtures of allied substances, such as: **resin acids, resin alcohols** (or **resinols**), **resinotannols, resin esters, glucoresins** and the like.

Another school of thought considers **Resins** as amorphous products having an inherent complex chemical entity. These are normally produced *either* in schizogenous or in sehizolysigenous ducts or in carities and are regarded as the end products of metabolism. The physical general characteristic features of resins are namely: hard, transparent, or translucent and, when heated they yield usually complex mixtures that comprise of resin acids, resin alcoholds, resinotannols, esters and resenes. Some researehers do believe that the resins are nothing but the oxidation products of the **terpenes.** They are found to be mostly insoluble in water, but soluble in ethanol and organic solvents. They are electrically non-conductive and combustible in nature.

Physical Properties of Resins

The various physical properties of **resins** can be generalized as detailed below:

1. **Resins,** as a class, are hard, transparent or translucent brittle materials.
2. They are invariably heavier than water having the specific gravity ranging from 0.9-1.25.
3. **Resins** are more or less amorphous materials but rarely crystallisable in nature.
4. On being heated at a relatively low temperature **resins** first get softened and ultimately melt down thereby forming either an adhesive or a sticky massive fluid, without undergoing any sort of decomposition or volatilization.
5. On being heated in the air *i.e.*, in the presence of oxygen, resins usually burn readily with a smoky flame by virtue of the presence of a large number of C-atoms in their structure.
6. On being heated in a closed container *i.e.*, in the absence of oxygen, they undergo decomposition and very often give rise to **empyreumatic products** *i.e.*, products chiefly comprising of hydrocarbons.
7. Resins are bad conductors of electricity, but when rubbed usually become negatively charged.
8. They are practically insoluble in water, but frequently soluble in ethanol, volatile oils, fixed oils, chloral hydrate and non-polar organic solvents *e.g.*, benzene, n-hexane and petroleum ether.

Chemical Properties of Resins

The various chemical properties of **resins** may be summarized as stated below:

1. Resins, in general, are enriched with carbon, deprived of nitrogen and contain a few oxygen in their respective molecules.
2. Majority of them undergo slow atmospheric oxidation whereby their colour get darkened with impaired solubility.
3. Resins are found to be a mixture of numerous compounds rather than a single pure chemical entity.
4. Their chemical properties are exclusively based upon the functional groups present in these substances.
5. Consequently, the resins are broadly divided into **resin alcohols, resin acids, resin esters, glycosidal resins** and **resenes** (*i.e.*, inert neutral compounds).
6. Resins are regarded as complex mixtures of a variety of substances, such as: **resinotannols, resin acids, resin esters, resin alcohols and resenes.**
7. One school of thought believes that resins are nothing but **oxidative products of terpenes.**

8. They may also be regarded as the end-products of *destructive metabolism.*
9. The acidic resins when treated with alkaline solutions they yield soaps (or **resin-soaps**).

Solubility

The solubility of various types of **resins** are as follows:
1. Majority of **resins** are water-insoluble and hence they have practically little taste.
2. They are usually insoluble in petroleum ether (a non-polar solvent) but with a few exceptions, such as: colophory (freshly powdered) and mastic.
3. They are also freely soluble in many other organic solvents, namely: acetone, carbon disulphide, as well as in fixed oils and volatile oils.

Chemical Composition of Resins

The copious volume of information with regard to the **'chemistry of resins'** is mainly attributed by the meaningful research carried out by Tschirch and Stock, who advocated that the proximate constituents of resins may be classified under the following heads, namely:

(*i*) *Resin Acids*

(*ii*) *Resin Esters and their Decomposition Products i.e.*, *Resin Alcohols* (*Resinols*) and *Resin Phenols* (*Resinotannols*).

(*iii*) *Resenes i.e.*, the chemical inert compounds.

However, it has been observed that in majority of the known **resins** these *three* aforesaid categories evidently predominates and thus the resulting product consequently falls into one of these groups. It is worth mentioning here that representatives of all the three said groups are rarely present in the same product.

Given below are some typical examples of resin substances that predominates the *three* classes suggested by Tschirch and Stock, namely:

A. **Resin-Esters** : *Examples:* Ammoniacum; Asafoetida; Benzoin; Balsam of Peru and Tolu; Galbanum; Storax;

B. **Resin-Acids** : *Examples:* Colophony; Copaiba; and

C. **Resenes** : *Examples:* Bdellium; Dammar; Mastic; Myrrh; Olibanum.

A few important and typical chemical constituents that have been duly isolated and characterized from various **naturally occurring resins** are discussed below:

(*iii*) *Resenes i.e.*, the chemical inert compounds.

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C. **Resenes** : *Examples:* Bdellium; Dammar; Mastic; Myrrh; Olibanum.

A few important and typical chemical constituents that have been duly isolated and characterized from various **naturally occurring resins** are discussed below:

1. Resin Acids

The **resin acids** essentially contain a large portion of carboxylic acids and phenols. However, they occur both in the *free state* and as their *respective esters*. They are usually found to be soluble in aqueous solutions of the alkalies, thereby forming either soap like solutions or colloidal suspensions. A typical Resin acid is **Abietic Acid (Synonym Sylvic Acid)**

2. Resin Alcohols

In general, resin alcohols are complex alcohols having higher molecular weight. These are of *two* types, namely:

(2*a*) *Resinotannols:* The resin alcohols which give a specific tannin reaction with iron salts are termed as **resinotannols.**

A number of **resinotannols** have been isolated from the plant kingdom. It is an usual practice to name them according to the resins in which they are found, such as:

- **Peruresinotannol** – From Balsam of Peru *i.e.*, the balsam obtained from *Myroxylon balsamum* var Pereirae (Royle) Harms (Family: *Fabaceae*);
- **Siaresinotannol** – From Sumatra Benzoin (Benzoin, Styrax) *i.e.*, the gum exuded from *Styrax benzoin* Dryander (Family: *Styracaceae*).
- **Toluresinotallol** – From Balsam of Tolu *i.e.*, the Balsam obtained from *Myroxylon balsamum* (Linn.) Harms. (belonging to the family. *Leguminosae*).

(2*b*) *Resinols:* The resin alcohols that fail to give a positive reaction with tannin and iron salts are known as *resinols. The following* are some typical examples of *resinols*, for instance:

- **Benzoresinol** – From Benzoin which is purely a pathological product obtained either from *Styrax benzoin* Dryander and *Styrax paralleloneurus* Brans. (*Sumatra Benzoin*) or from *Styrax tonkinensis* Craib. (*Siam Benzoin*) belonging to family *Styraceae*.
- **Storesinol** – From storax which is the balsamic resin usually obtained from the trunk of *Liquidamber orientalis* Mill. family *Hamamelidaceae*.
- **Gurjuresinol** – From Gurjun Balsam that is the aleo-resin obtained from *Dipterocarpus turbinatus* Gaertn. F. belonging to family: *Dipterocarpaceae*.
- **Guaiaresinol** – From Guaiacum Resin obtained from the heartwood of *Guaiacum officinale* Linn. and *Guaiacum sanctum* Linn. belonging to family: *Zygophyllaceae*.

3. Resenes

These are oxygenated compounds, but are not affected either by alkalies or acids. In fact, they are more or less neutral substances being devoid of characteristic functional groups, and, therefore, do not exhibit any characteristic chemical properties. Interestingly, they are immune to oxidizing agents and variant climatic conditions, a fact which essentially attributes the resins containing them one of their major plus points for the manufacture of **varnishes.** A few important examples of *resenes are* as follows:

Dracoresene – Derived from the scales of the fruit of Dragon's Blood *i.e.*, *Daemonorops draco* Bl. (and other species) belonging to the natural order (*Arecaceae*).

Masticoresene – Derived from Mastic-an oleo-resin obtained from *Pistacia lentiscus* Linn belonging to family: *Anacardiaceae.*

Chemical Tests:

A) Benzoin (Styrax benzoin Styraceae.)

1. Heat a small amount of benzoin slowly in a dry test tube -----> melts and white fumes are produced.
2. Benzoin when heated with potassium permanganate solution -----> odor of benzaldehyde. (Sumatra benzoin)
3. Benzoin is extracted with alcohol and to the extract add water ------>milky white solution is formed.4. Digest benzoin with few drops of petroleum ether for 5

B) Asafoetida: (Ferula foetida Umbelliferae.)

1. Powdered drug triturated with water –--> yellowish emulsion is produced.
2. Combined umbelliferone test –The drug is boiled with HCL for 5 minutes, it is filtered and ammonia is added to the filtrate –-----> A blue fluorescence is observed.
3. The drug is treated with few drops of 50% HNO3 –----> green color is produced.
4. The drug is treated with few drops of sulphuric acid – red color is produced which changes to violet on washing with water.

C) Colophony (Rosin):

1. 0.1 g of powdered colophony is dissolved in 2-3 ml of acetic anhydride in a test tube and a drop of conc. H_2S0_4 is added whereby purple to violet colour is observed.
2. An alcoholic solution of colophony is acidic to litmus.
3. Colophony is dissolved in light petroleum and filtered. To the filtrate 2 to 3 times its volume, copper acetate solution is added whereby emerald green colour is seen in the petroleum layer (Upper layer).

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 taste

State:

Non-crystalline

Solubility:

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

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

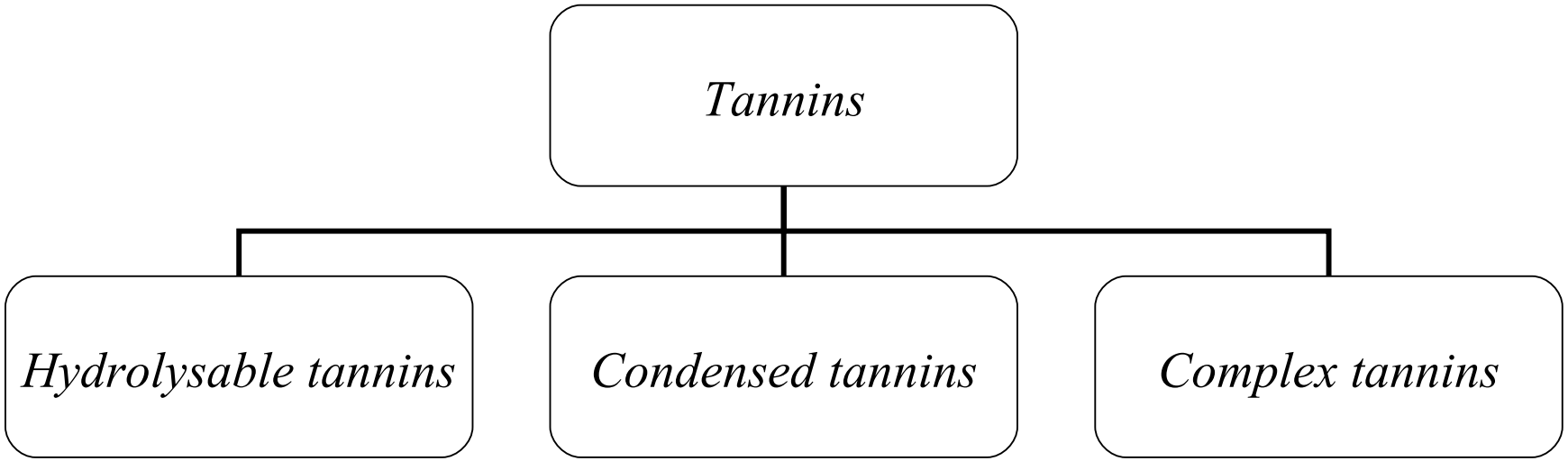
Decrease in blood urea nitrogen content

Inhibition of plasmin

Lipolysis in fat cells

Chemical Classification

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



(i) Hydrolysable tannins

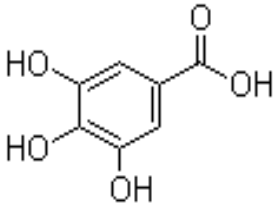
These tannins are hydrolyzed by enzymes or acids.

Precursors:

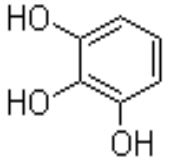
Phenolic acid (Gallic acid, Ellagic acid)

Glucose residue

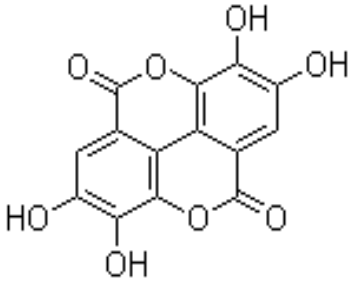
Between phenolic acids and glucose sugar, there is ester linkage



Gallic acid



Pyrogallol



Ellagic acid

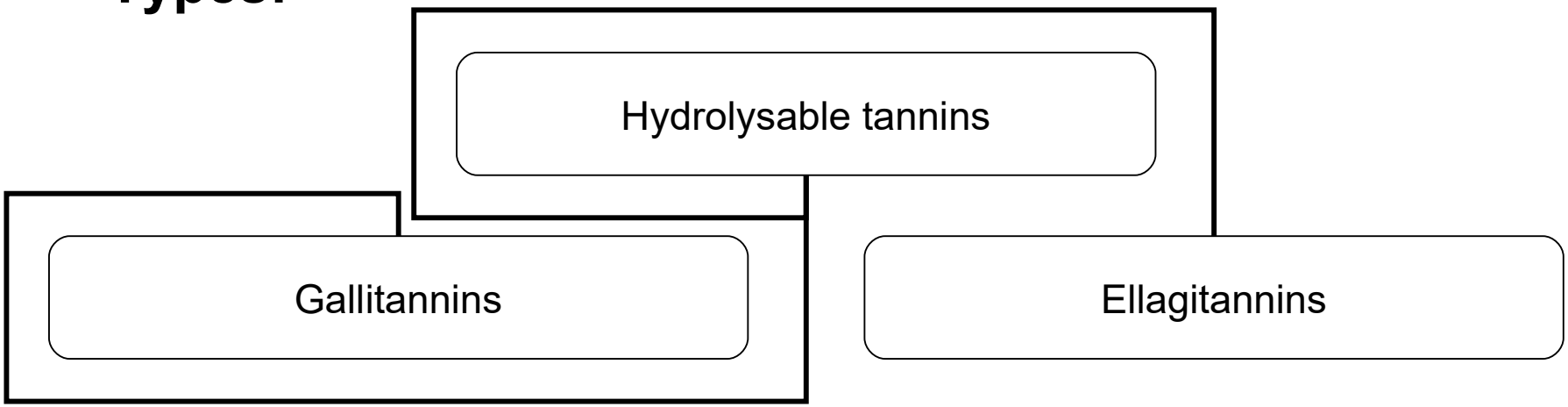
Properties:

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* *Hamamelis*	*Pomegranate* *Eucalyptus*
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

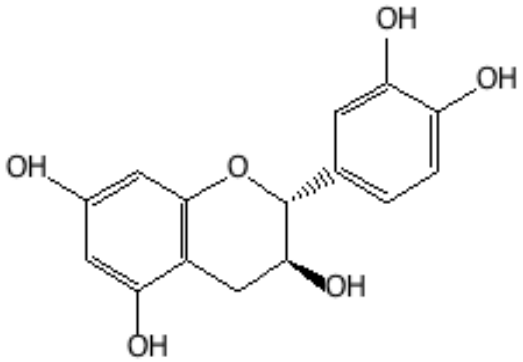
These tannins are derivatives of Flavonoid, catechin, flavonol-3-4-diol.

Precursors:

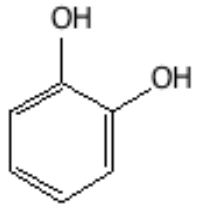
Flavonoid

Catechin

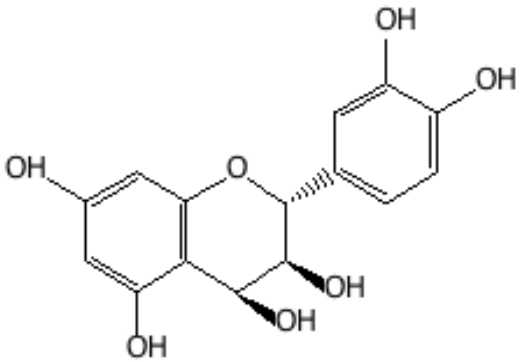
Flavonol-3-4-diol



catechin



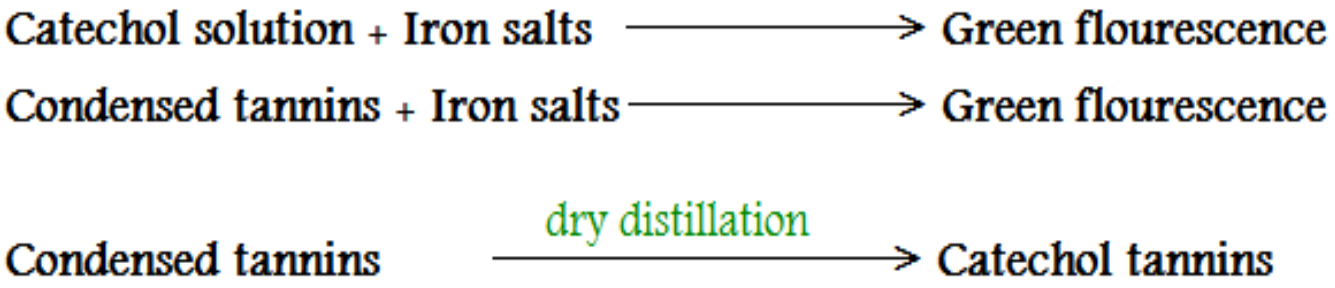
catechol



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

Cinchona

Cinnamon

(iii) Complex tannins

These tannins are mixtures of both, hydrolysable and condensed tannins

Examples:

Tea

Quercus

Castanea

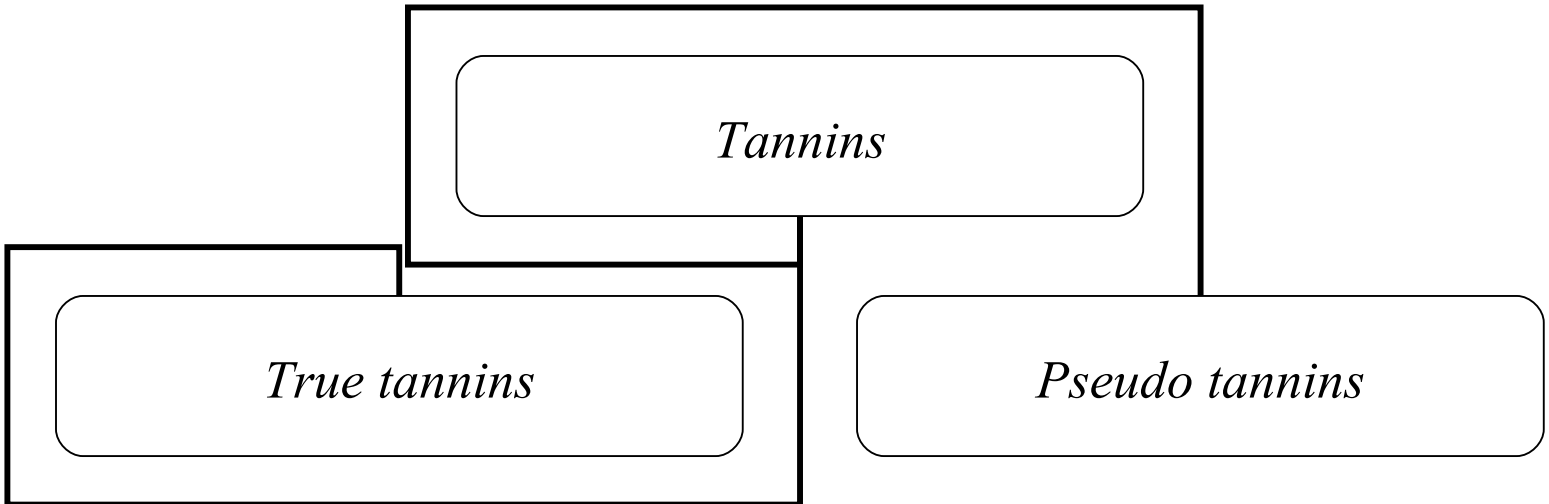
2nd Classification

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.*

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.

Carbohydrates and Derived Products

INTRODUCTION

Carbohydrates, as the name suggest, were defined as a group of compounds composed of carbon, hydrogen and oxygen in which the latter two elements are in the same proportion as in water and were expressed by a formula $(CH_2O)_n$, that is, hydrates of carbon.

The term 'carbohydrates' arose from the mistaken belief that substances of this kind were hydrates of carbon, because the molecular formula of many substances could be expressed in the form $C_X(H_2O)_Y$, for example, glucose (C_6 H_{12} O_6), sucrose (C_{12} H_{22} O_{11}), etc. In these examples, the hydrogen and oxygen are present in the same ratio

as in water. But this definition has certain drawbacks as given below:

- It should be kept in mind that all organic compounds containing hydrogen and oxygen in the proportion found in water are not carbohydrates. For example, formaldehyde HCHO for the present purpose written as $C(H_2O)$; acetic acid CH_3COOH written as $C_3(H_2O)_2$; and lactic acid $CH_3CHOHCOOH$ written as $C_3(H_2O)_3$ are not carbohydrates.
- Also, a large number of carbohydrates such as rhamnose ($C_6H_{12}O_5$), cymarose ($C_7H_{14}O_4$), digitoxose ($C_6H_{12}O_4$), etc., are known which do not contain the usual propor-tions of hydrogen to oxygen.
- Finally, certain carbohydrates are also known which contain nitrogen or sulphur in addition to carbon, hydro-gen and oxygen.

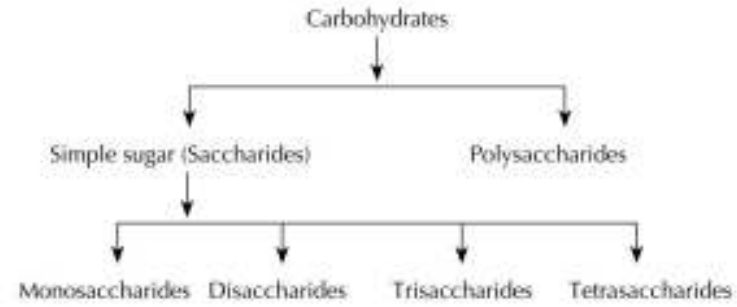
From the above discussion, it can be concluded that the definitions described above are not correct; however, carbohydrates are now defined chemically as polyhydroxy aldehyde or polyhydroxy ketones or compound that on hydrolyses produce either of the above.

Carbohydrates are among the first products to arise as a result of photosynthesis. They constitute a large proportion of the plant biomass and are responsible, as cellulose, for the rigid cellular framework and, as starch, for providing an important food reserve. Of special pharmacognostical importance is the fact that sugars unites with a wide variety of other compounds to form glycosides and secondary metabolites. Mucilage, as found in marshmallow root and psyllium seeds, act as water-retaining vehicles, where as gums and mucilage, which are similar in composition and properties, are formed in the plant by injury or stress and usually appear as solidified exudates; both are typically composed of uronic acid and sugar units. The cell walls of the brown seaweeds and the middle lamellae of higher plant tissues contain polysaccharides consisting almost entirely of uronic acid components.

Low molecular weight carbohydrates are crystalline, soluble in water and sweet in taste, for example, glucose, fructose, sucrose, etc. The high molecular weight carbohydrates (polymers) are amorphous, tasteless and relatively less soluble in water, for example, starch, cellulose, inulin, etc.

Classification of Carbohydrates

The term ‘monosaccharides’ is employed for such sugars that on hydrolysis yield no further, lower sugars. The general formula of monosaccharides is Cn H2n On. The monosaccharides are subdivided as bioses, trioses, tetroses, pentoses, hexoses, heptoses, depending upon the number of carbon atoms they possess.



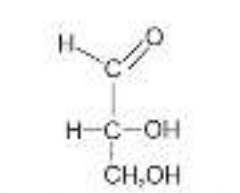
Monosaccharides

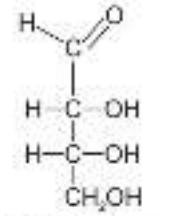
The term ‘monosaccharides’ is employed for such sugars that on hydrolysis yield no further, lower sugars. The general formula of monosaccharides is C_n H_{2n} O_n. The monosaccharides are subdivided as bioses, trioses, tetroses, pentoses, hexoses, heptoses, depending upon the number of carbon atoms they possess.

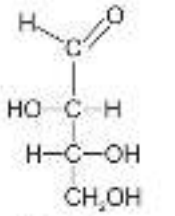
Bioses: They contain two carbon atoms. They do not occur free in nature.

Trioses: They contain three carbon atoms, but in the form of phosphoric esters, for example, glyceraldehydes.

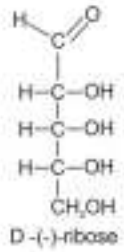
Tetroses: They contain four carbon atoms, for example, erythrose, threose, etc.

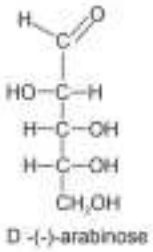


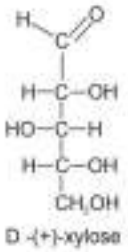




Pentoses: They are very common in plants and are the products of hydrolysis of polysaccharides like hamicelluloses, mucilages and gums, for example, ribose, arabinose and xylose.







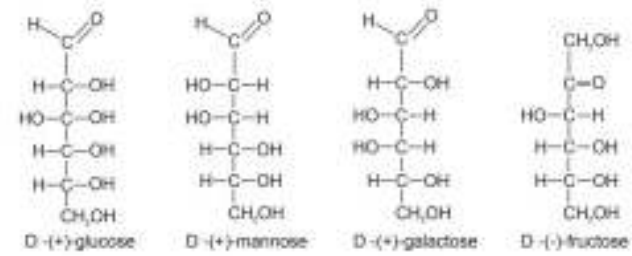
Hexoses: They are monosaccharides containing six carbon atoms and are abundantly available carbohydrates of plant kingdom. They are further divided into two types: aldoses and ketoses. They may be obtained by hydrolysis of polysaccharides like starch, insulin, etc.

Depending upon the type of product of hydrolysis these are further classified as Pentosans and Hexosans. Xylan is pentosan, whereas starch, insulin and cellulose are the examples of hexosans.

Cellulose is composed of glucose units joined by β-1, 4 linkages, whereas starch contains glucose units connected with α- 1, 4 and α- 1, 6 units. Polyuronides, gums and

Aldoses : Glucose, mannose, galactose

Ketoses : Fructose and sorbose



Heptoses: They contain seven carbon atoms, vitally important in the photosynthesis of plant and glucose metabolism of animals and are rarely found accumulated in plants, for example, glucoheptose and manoheptose.

Disaccharides: Carbohydrates, which upon hydrolysis yield two molecules of monosaccharides, are called as disaccharides.

Sucrose	Hydrolysis →	Glucose + fructose (sugarcane)
Maltose	Hydrolysis →	Glucose + Glucose (malt sugar)
Lactose	Hydrolysis →	Glucose + Galactose (cow's milk)

Trisaccharides: As the name indicates, these liberate three molecules of monosaccharides on hydrolysis.

Raffinose	Hydrolysis →	Glucose + fructose + galactose (in beet) (sugarcane)
Gentianose	Hydrolysis →	Glucose + Glucose + fructose (gentian roots)

Tetrasaccharides: Stachyose, a tetrasaccharide, yields on hydrolysis, four molecules of monosaccharide, found in manna.

Polysaccharides: On hydrolysis they give an indefinite number of monosaccharides. By condensation, with the elimination of water, polysaccharides are produced from monosaccharides.

Depending upon the type of product of hydrolysis these are further classified as Pentosans and Hexosans. Xylan is pentosan, whereas starch, insulin and cellulose are the examples of hexosans.

Cellulose is composed of glucose units joined by β-1, 4 linkages, whereas starch contains glucose units connected with α- 1, 4 and α- 1, 6 units. Polyuronides, gums and mucilages are the other pharmaceutically important poly-saccharide derivatives.

TESTS FOR CARBOHYDRATES

The following are some of the more useful tests for sugars and other carbohydrates.

Reduction of Fehling's Solution

To the solution of carbohydrate, equal quantity of Fehling's solutions A and B is added. After heating, brick red pre-cipitate is obtained.

Molisch Test

The test is positive with soluble as well as insoluble car-bohydrates. It consists of treating the compounds with α-naphthol and concentrated sulphuric acid which givespurple colour. With a soluble carbohydrate this appears as a ring if the sulphuric acid is gently poured in to form a layer below the aqueous solution. With an insoluble, carbohydrate such as cotton wool (cellulose), the colour will not appear until the acid layer is shaken to bring it in contact with the material.

Osazone Formation

Osazones are sugar derivatives formed by heating a sugar solution with phenylhydrazine hydrochloride, sodium acetate and acetic acid. If the yellow crystals which form are examined under the microscope they are sufficiently characteristic for certain sugars to be identified. It should be noted that glucose and fructose form the same osazone (glucosazone, m.p. 205°C). Before melting points are taken, osazones should be purified by recrystalization from alcohol. Sucrose does not form an osazone, but under the condi-tions of the above test sufficient hydrolysis takes place for the production of glucosazone.

Resorcinol Test for Ketones (Selivanoff's Test)

A crystal of resorcinol is added to the solution and warmed on a water bath with an equal volume of concentrated hydrochloric acid. A rose colour is produced if a ketone is present (e.g. fructose, honey or hydrolysed inulin).

Test for Pentoses

Heat a solution of the substance in a test tube with an equal volume of hydrochloric acid containing a little phloroglu-cinol. Formation of a red colour indicates pentoses.

Keller-Kiliani Test for Deoxysugars

A Deoxysugar (found in cardiac glycosides) is dissolved in acetic acid containing a trace of ferric chloride and trans-ferred to the surface of concentrated sulphuric acid. At the junction of the liquids a reddish-brown colour is produced which gradually becomes blue.

Furfural Test

A carbohydrate sample is heated in a test tube with a drop of syrupy phosphoric acid to convert it into furfural. A disk of filter paper moistened with a drop of 10% solution of aniline in 10% acetic acid is placed over the mouth of the test tube. The bottom of the test tube is heated for 30–60s.

A pink or red stain appears on the reagent paper.

ACACIA GUM

Synonyms

Acacia gum, Acacia vera, Egyptian thorn, Gummi africanum, Gum Senegal, Gummae mimosae, Kher, Sudan gum arabic, Somali gum, Yellow thorn, Indian Gum and Gum Arabic.

Biological Source

According to the USP, acacia is the dried gummy exuda-tion obtained from the stems and branches of *Acacia senegal* (L.) Willd or other African species of Acacia. In India, it is found as dried gummy exudation obtained from the stems and branches of *Acacia arabica* Willd, belonging to family Leguminosae

Geographical Source

Acacia senegal is the characteristic species in the drier partsof Anglo-Egyptian Sudan and the northern Sahara, and is to be found throughout the vast area from Senegal to the Red Sea and to eastern India. It extends southwards to northern Nigeria, Uganda, Kenya, Tanzania and southern Africa. The plant is extensively found in Arabia, Kordofan (North-East Africa), Sri Lanka and Morocco. In India it is found chiefly in Punjab, Rajasthan and Western Ghats. Sudan is the major producer of this gum and caters for about 85% of the world supply.

Cultivation and Collection

Acacia is a thorny tree up to 6 m in height. In Sudan, gum is tapped from specially cultivated trees while in Senegam-bia, because of extremes of climate; cracks are produced on the tree and the gum exudes and is collected from the wild plants. Acacia trees can be cultivated by sowing the seeds in the poor, exhausted soil containing no minerals. The trees also grow as such by seed-dispersal.

Gum is collected by natives from 6 to 8 years old trees, twice a year in dry weather in November or in February— March. Natives cut the lower thorny branches to facilitate the working and by means of an axe make 2–3 ft long and 2–3 inches broad incision on the stem and branches, loosen the bark by axe and remove it, taking care not to injure the cambium and xylem. Usually they leave a thin layer of bark on xylem. If xylem is exposed, white ant enters the plant and gum is not produced. After injury in winter gum exudes after 6–8 weeks while in summer after 3–4 weeks. It is believed that bacteria finding their way through the incision are more active in summer and gum is produced quickly. The exuded gum is scraped off, collected in leather bags and then is cleaned by separating debris of bark and wood and separating sand, etc., by sieving.

Gum is dried in the sun by keeping it in trays in thin layers for about 3 weeks when bleaching takes place and it becomes whiter. This result in uneven contraction and cracks and fissures are formed on its outer surface and as a result original transparent gum becomes opaque. This process is called ripening of the gum.

Morphology

Colour	Tears are usually white, pale-yellow and sometimes creamish-brown to red in colour. The powder has off-white, pale-yellow or light-brown in appearance.
Odour	Odourless
Taste	Bland and mucilaginous
Shape and Size	Tears are mostly spheroidal or ovoid in shape and having a diameter of about 2.5–3.0 cm
Appearance	Tears are invariably opaque either due to the presence of cracks or fissures produced on the outer surface during the process or ripening. The fracture is usually very brittle in nature and the exposed surface appears to be glossy.

Chemical constituents

Acacia consists principally of arabin, which is a complex mixture of calcium, magnesium and potassium salts of arabic acid. Arabic acid is a branched polysaccharide that yields L-arabinose, D-galactose, D-glucuronic acid and L-rhamnose on hydrolysis. 1, 3-Linked D-galactopyranose units form the backbone chain of the molecule and the terminal residues of the 1, 6-linked side chains are primarily uronic acids. Acacia contains 12–15% of water and several occluded enzymes such as oxidases, peroxidases and pectinases. The total ash content should be in the range of 2.7–4.0%.

Chemical Tests

1. **Lead acetate test:** An aqueous solution of acaciawhen treated with lead acetate solution yields a heavy white precipitate.
2. **Reducing sugars test:** Hydrolysis of an aqueoussolution of acacia with dilute HC1 yields reducing sugars whose presence are ascertained by boiling with Fehling's solution to give a brick-red precipitate of cuprous oxide.
3. **Blue colouration due to enzyme:** When theaqueous solution of acacia is treated with benzidine in alcohol together with a few drops of hydrogen peroxide (H_2O_2), it gives rise to a distinct blue colour due to the presence of oxidases enzyme.
4. **Borax test:** An aqueous solution of acacia affords astiff translucent mass on treatment with borax.
5. **Specific test:** A 10% aqueous solution of acacia failsto produce any precipitate with dilute solution of lead acetate (a clear distinction from Agar and Tragacanth); it does not give any colour change with Iodine solution (a marked distinction from starch and dextrin); and it never produces a bluish-black colour with $FeCl_3$ solution (an apparent distinction from tannins).

Uses

The mucilage of acacia is employed as a demulcent. It is used extensively as a vital pharmaceutical aid for emulsification and to serve as a thickening agent. It finds its enormous application as a binding agent for tablets, for example, cough lozenges. It is used in the process of 'granulation' for

the manufacturing of tablets. It is considered to be the gum of choice by virtue of the fact that it is quite com-patible with other plant hydrocolloids as well as starches, carbohydrates and proteins. It is used in combination with gelatin to form conservates for micro-encapsulation of drugs. It is employed as colloidal stabilizer. It is used extensively in making of candy and other food products. Gum acacia solution has consistency similar to blood and is administered intravenously in haemodialysis. It is used in the manufacture of adhesives and ink, and as a binding medium for marbling colours.

Allied Drugs

Talka gum is usually much broken and of very variablecomposition, some of the tears being almost colourless and others brown.

Ghatti or *Indian gum* is derived from *Anogeissus latifolia* (Combretaceae). It is produced in much the same localities as sterculia gum, and is harvested and prepared in a similar manner. It resembles talka in possessing tears of various colours. Some of the tears are vermiform in shape and their surface shows fewer cracks than even the natural acacia. Aqueous dispersions of the gum have a viscosity intermedi-ate between those of acacia and sterculia gums.

West African Gum Combretum, obtained from *Com-bretum nigricans,* is not permitted as a food additive but isexploited as an adulterant of gum arabic. Unlike the latter in which the rhamnose and uronic acid units are chain terminal, in gum combretum these moieties are located within the polysaccharides chain.

Many other gums of the acacia type are occasionally met with in commerce, and many gum exudates of the large genus *Acacia* have been given chemotaxonomic con-sideration.

Toxicology

Acacia is essentially nontoxic when ingested. Allergic reac-tions to the gum and powdered forms of acacia have been reported and include respiratory problems and skin lesions.

Acacia contains a peroxidase enzyme, which is typically destroyed by brief exposure to heat. If not inactivated, this enzyme forms coloured complexes with certain amines and phenols and enhances the destruction of many pharmaceutical products including alkaloids and readily oxidizable compounds, such as some vitamins. Acacia gum reduces the antibacterial effectiveness of the preservative methyl-p-hydroxybenzoate against *Pseudomonas aeruginosa* presumably by offering physical barrier protection to the microbial cells from the action of the preservative. A trypsin inhibitor also has been identified, but the clinical significance of the presence of this enzyme is not known.

TRAGACANTH

Synonyms: Goat's thorn, gum dragon, gum tragacanth, hog gum.

Biological Source

It is the air dried gummy exudates, flowing naturally or obtained by incision, from the stems and branches of *Astra-galus gummifer* Labill and certain other species of *Astragalus*,belonging to family Leguminosae.

Geographical Source

Various species of *Astragalus* which yield gum are abundantly found in the mountainous region of Turkey, Syria, Iran, Iraq and the former U.S.S.R. at an altitude of about 1,000–3,000 m. Two important varieties of tragacanth, that is, Persian tragacanth and Smyrana or Anatolian tragacanth come from Iran and turkey respectively. In India it is found wild in Kumaon and Garhwal region.

The approximate distribution of a number of gum-producing species found in the areas where tragacanth is collected is shown in Table below.

Cultivation, Collection and Preparation

Most of the plants from which tragacanth is collected grow at an altitude of 1,000–3,000 m. The shrubs are very thorny; each of their compound leaves has a stout, sharply pointed rachis which persists after the fall of the leaflets. The mode of collection varies somewhat in different districts, but the following details of collection in the province of Far are typical.

Gums can be obtained from the plants in their first year but is then said to be of poor quality and unfit for commercial use. The plants are therefore tapped in the second year. The earth is taken away from the base to depth of 5 cm, and the exposed part is incised with a sharp knife having a thin cutting edge. A wedge-shaped piece of wood is used by the collector to force open the incision so that the gum exudes more freely. The wedge is generally left in the cut for some 12–24 h before being withdrawn. The gum exudes and is collected 2 days after the incision.

Some of the plants are burned at the top after having had the incision made. The plant then sickens and gives off a greater quantity of gum. However, this practice is not universal, as many plants can not recover their strength and are killed by the burning. The gum obtained after burning is of lower quality than that obtained by incision only, and is reddish and dirty looking. The crop becomes available in August–September.

After collection, the gum is graded as ribbons and flakes which are further categorized into various sub-grades on the basis of shape, size and colour (Table below). The best grades form the official drug, while the lower grades are used in the food, textile and other industries.

Morphology

Colour	The flakes are white or pale yellowish-white
Odour	Odourless
Taste	Mucilaginous
Shape and Size	Tragacanth occurs in the form of ribbon or flakes. Flakes are approximately 25 x 12 x 2 mm in size
Appearance	The gum is horny, translucent with transverse and longitudinal ridges Fracture is short

Chemical Constituents

Interestingly, tragacanth comprises two vital fractions: first, being water soluble and is termed as ‘tragacanthin’ and the second, being water insoluble and is known as ‘bassorin’. Both are not soluble in alcohol. The said two components may be separated by carrying out the simple filtration

of very dilute mucilage of tragacanth and are found to be present in concentrations ranging from 60% to 70% for bassorin and 30–40% for tragacanthin. Bassorin actually gets swelled up in water to form a gel, whereas tragacanthin forms an instant colloidal solution. It has been established that no methoxyl groups are present in the tragacanthin fraction, whereas the bassorin fraction comprised approximately 5.38% methoxyl moieties. Rowson (1937) suggested that the gums having higher methoxyl content, that is, possessing higher bassorin contents yielded the most viscous mucilage.

Tragacanth gum is composed mainly of sugars and uronic acid units and can be divided into three types of constituents. The acidic constituents tragacanthic acid on hydrolysis yields galactose, xylose and galacturonic acid. A neutral polysaccharide affords galactose and arabinose after its hydrolysis while a third type is believed to be steroidal glycoside.

Chemical Tests

1. An aqueous solution of tragacanth on boiling with conc. HCl does not develop a red colour.
2. It does not produce red colour with ruthenium red solution.
3. When a solution of tragacanth is boiled with few drops of $FeCl_3$ [aqueous 10% (w/v)], it produces a deep-yellow precipitate.
4. It gives a heavy precipitate with lead acetate.
5. When tragacanth and precipitated copper oxide are made to dissolve in conc. NH_4OH, it yields a meager precipitate.

Uses

It is used as a demulcent in cough and cold preparations and to manage diarrhoea. It is used as an emollient in cosmetics. Tragacanth is used as a thickening, suspending and as an emulsifying agent. It is used along with acacia as a suspending agent. Mucilage of tragacanth is used as a binding agent in the tablets and also as an excipient in the pills. Tragacanth powder is used as an adhesive. It is also used in lotions for external use and also in spermicidal jellies. It is also used as a stabilizer for ice cream in

0.2–0.3% concentration and also in sauces. Tragacanth has been reported to inhibit the growth of cancer cells in vitro and in vivo.

Adulterant and Substitutes

Tragacanth gum of lower grades known as hog tragacanth is used in textile industry and in the manufacture of pickles. The gum varies from yellowish brown to almost black. Citral gum obtained from *A. strobiliferus* is also used as an adulterant.

Karaya gum which is sometimes known as sterculia gum or Indian tragacanth is invariably used as a substitute for gum tragacanth.

AGAR

Synonym: Agaragar, Japanese Isinglass, Vegetable gelatin.

Botanical Source

It is the dried gelatinous substance obtained by extraction with water from Gelidium amansii or various species of red algae like Gracilaria and Pterocladia, belonging to family Gelidaceae (Gelidium and Pterocladia), Gracilariaceae (Gracilaria).

Geographical Source

Japan was the only country producing agar before the World War II, but it is now produced in several countries like, Japan: Gelidium amnasii and other Gelidium species, Australia; Gracilaria confervoldes, New Zealand; Pterocladia lucida and other allied species, Korea, South Africa, United States, Chile, Spain, and Portugal.

Collection

The red algae are grown in rocks in shallow water or on the bamboos by placing them in the ocean. Collection of the algae is usually made in summer (May and October). The bamboos are taken out and the seaweeds are stripped off. Algae are dried, beaten with sticks and shaken to remove the sand and shell attached to them. Then the entire material is taken to high altitude, washed with water and bleached by keeping them in trays in the sunlight, sprinkling water and rotating them periodically. The agar is then boiled; one part of algae with 50 parts of water acidified with acetic acid or dilute sulphuric acid. The hot extract is subjected for coarse and fine filtration using cloth to remove the large and small impurities present in them. The filtered extract is then transferred into wooden trough which on cooling forms a jelly like mass. The mass thus obtained is then passed through screw press to obtain strips of agar. These strips contain water and to remove the water present in them, the agar strips are placed in open air to get the benefits of the Japanese climate. During this season, Japan has a very warm day and the nights are very cold with a temperature less than 0°C. As a result of this climate the water present on top of the strips are converted into ice at night, and during day they are reconverted to water and the excess water present in them are removed. Then, these strips are again dried in the sunlight in trays.

Modern method of deep freezing is being utilized in the preparation of agar in recent development of technology. The algae which is collected is washed in running water for a day and then extracted firstly with dilute acid in steam heated digester and then with water for 30 min, the hot solution so obtained is cooled and deep freezed in an ice machine. The water present in the agar is converted to ice and these masses are powdered, melted and filtered in rotary vacuum filter. The moist agar is dried using dry air and powdered agar is obtained.

Chemical Constituents

Agar is a complex heterosaccharide and contains two dif-ferent polysaccharides known as agarose and agaropectin. Agarose is neutral galactose polymer and is responsible for the gel property of agar. It consists of D-galactose and L-galactose unit. The structure of agaropectin is not completely known, but it is believed that it consists of sulphonated polysaccharide in which galactose and uronic acid are partly esterified with sulphuric acid. Agaropectin is responsible for the viscosity of agar solution.

Chemical Tests

1. Agar responds positively to Fehling's solution test.

2. Agar gives positive test with Molisch reagent.

3. Aqueous solution of agar (1%) is hydrolysed with con-centrated HCl by heating for 5–10 min. On addition of barium chloride solution to the reaction mixture, a white precipitate of barium sulphate is formed due to the presence of sulphate ions. This test is absent in case of starch, acacia gum and tragacanth.

4. To agar powder a solution of ruthenium red is added. Red colour is formed indicating mucilage.

5. Agar is warmed in a solution of KOH. A canary yellow colour is formed.

6. An aqueous solution of agar (1%) is prepared in boiling water. On cooling it sets into a jelly.

7. To agar solution an N/20 solution of iodine is added. A deep crimson to brown colour is obtained (distinctive from acacia gum and tragacanth).

8. To a 0.2% solution of agar an aqueous solution of tannic acid is added. No precipitation is formed indicating absence of gelatin.

9. Agar is required to comply with tests for the absence of E. coli and Salmonella, and general microbial contamination should not exceed a level of 103 microorganisms per gram as determined by a plate count. It has a swelling index of not less than 10.

Uses

Agar is used to treat chronic constipation, as a laxative, sus-pending agent, an emulsifier, a gelating agent for suppositories, as surgical lubricant, as a tablet excipient, disintegrant, in production of medicinal encapsulation and ointment and as dental impression mold base. It is extensively used as a gel in nutrient media for bacterial cultures, as a substitute for gelatin and isinglass, in making emulsions including photographic, gel in cosmetic, as thickening agent in food especially confectionaries and dairy products, in meet canning; sizing for silk and paper; in dying and printing of fabrics and textiles; and in adhesive.

Substitutes and Adulterants

Some of the common adulterants present in agar are gelatin and Danish agar. The presence of gelatin can be detected by addition of equal volume of 1% trinitrophenol and 1% of agar solution; the solution produces turbidity or precipitation. Danish agar has an ash of 16.5–18.5%, it is formed from rhodophyceae indigenous to the Denmark costal region. The Danish agar has a gel strength which is half of its gel strength of Japanese agar.

HONEY

Synonyms: Madhu, Madh, Mel, Purified Honey.

Biological Source

Honey is a viscid and sweet secretion stored in the honey comb by various species of bees, such as Apis mellifera, Apis dorsata, Apis florea, Apis indica and other species of Apis, belonging to family Apideae (Order: Hymenotera).

Geographical Source

Honey is available in abundance in Africa, India, Jamaica, Australia, California, Chili, Great Britain and New Zealand.

Collection and Preparation

The nectar of the flowers is a watery solution containing 25% sucrose and 75% water. The worker bee sucks this nectar through its hollow tube of mouth (proboscis) and deposits in honey-sac located in abdomen. The enzyme invertase present in saliva of the bee converts nectar into invert sugar, which is partially utilized by the bee and the remaining is deposited into honey comb. Honey comb is smoked to remove the bees and honey is obtained by applyng the pressure to it or allowing it to drain naturally. The honey of commerce is heated to 80°C and allowed to stand. The impurities which float over the surface are skimmed off and the liquid is diluted with water to produce honey of 1.35 density. Natural honey has the density of 1.47. Many-a-time, honey is extracted from the comb by centrifugation. It must be free from foreign substances. Honey is liable to fermentation, unless it is suitably processed. Honey is heated to 80°C before it is sent to the market, so as to avoid fermentation. It should be cooled rapidly or else it darkens in colour on keeping. If necessary (and if not prepared by centrifugation method), honey is required to be filtered through wet cloth or funnel.

Chemical Constituents

The average composition of honey is as follows: Moisture 14–24%, Dextrose 23–36%, Levulose (Fructose) 30–47%, Sucrose 0.4–6%, Dextrin and Gums 0–7% and Ash 0.1–0.8%. Besides, it is found to contain small amounts of essential oil, beeswax, pollen grains, formic acid, acetic acid, succinic acid, maltose, dextrin, colouring pigments, vitamins and an admixture of enzymes, for example, diastase, invertase and inulase. Interestingly, the sugar contents in honey varies widely from one country to another as it is exclusively governed by the source of the nectar (availability of frag-ment flowers in the region) and also the enzymatic activity solely controlling the conversion into honey.

Chemical Tests

Adulteration in honey is determined by the following tests:

1. Fiehe's Test for Artificial Invert Sugar: Honey (10 ml) is shaken with petroleum or solvent ether (5 ml) for 5–10 min. The upper ethereal layer is separated and evaporated in a china dish. On addition of 1% solution of resorcinol in hydrochloric acid (1 ml) a transient red colour is formed in natural honey while in artificial honey the colour persists for sometime.

2. Reduction of Fehling's Solution: To an aqueous solution of honey (2 ml) Fehling's solutions A and B are added and the reaction mixture is heated on a steam bath for 5–10 min. A brick red colour is produced due to the presence of reducing sugars.

3. Limit Tests: The limit tests of chloride, sulphate and ash (0.5%) are compared with the pharmacopoeial specifications.

Uses

Honey shows mild laxative, bactericidal, sedative, antiseptic and alkaline characters. It is used for cold, cough, fever, sore eye and throat, tongue and duodenal ulcers, liver disorders, constipation, diarrhoea, kidney and other urinary disorders, pulmonary tuberculosis, marasmus, rickets, scurvy

and insomnia. It is applied as a remedy on open wounds after surgery. It prevents infection and promotes healing. Honey works quicker than many antibiotics because it is easily absorbed into the blood stream. It is also useful in healing of carbuncles, chaps, scalds, whitlows and skin inflammation; as vermicide; locally as an excipient, in the treatment of aphthae and other infection of the oral mucous membrane. It is recommended in the treatment of preoperative cancer. Honey, mixed with onion juice, is a good remedy for arteriosclerosis in brain. Diet rich in honey is recommended for infants, convalescents, diabetic patients and invalids.

Honey is an important ingredient of certain lotions, cosmetics, soaps, creams, balms, toilet waters and inhalations. It is used as a medium in preservation of cornea.

Today, as in earlier times, honey is used as an ingredient in various cough preparations. It is also used to induce sleep, cure diarrhoea, and treat asthma. A review of literature found at least 25 scientific articles verifying honey's wound and topical ulcer healing powers.

Interestingly, potent antibacterial peptides (apidaecins and abaecin) have been isolated and characterized in the honeybee (Apis mellifera) itself and a new potent antibacterial protein named royalisin has been found in the royal jelly of the honeybee.

Adulterant and Substitutes

Due to the relatively high price of pure honey, it is invari-ably adulterated ether with artificial invert sugar or simply with cane-sugar syrup. These adulterants or cheaper sub-stituents not only alter the optical property of honey but also its natural aroma and fragrance.

FIBRES

INTRODUCTION

Fibres may be defined as any hair-like raw material directly obtainable from an animal, vegetable, or mineral source and convertible into nonwoven fabrics such as felt or paper or, after spinning into yarns, into woven cloth. A natural fibre may be further defined as an agglomeration of cells in which the diameter is negligible in comparison with the length. Although nature abounds in fibrous materials, especially cellulosic types such as cotton, wood, grains, and straw, only a small number can be used for textile products or other industrial purposes. Apart from economic considerations, the usefulness of a fibre for commercial purposes is determined by such properties as length, strength, pliability, elasticity, abrasion resistance, absorbency, and various surface properties. Most textile fibres are slender, flexible, and relatively strong. They are elastic in that they stretch when put under tension and then partially or completely return to their original length when the tension is removed.

Natural fibres can be classified according to their origin.

1. The vegetable, or cellulose-base, class includes such important fibres as cotton, flax, and jute.
2. The animal, or protein-base, fibres include wool, mohair, and silk.
3. Regenerated and synthetic fibres include Nylon, Terylene, Orlon, Viscose, Alginate fibres, etc.
4. An important fibre in the mineral class is asbestos.

The vegetable fibres can be divided into smaller groups, based on their origin within the plant. Cotton, kapok, and coir are examples of fibres originating as hairs borne on the seeds or inner walls of the fruit, where each fibre consists of a single, long, narrow cell. Flax, hemp, jute, and ramie are bast fibres, occurring in the inner bast tissue of certain plant stems and made up of overlapping cells. Abaca, henequen, and sisal are fibres occurring as part of the fibrovascular system of the leaves.

Chemically, all vegetable fibres consist mainly of cellu-lose, although they also contain varying amounts of such substances as hemicellulose, lignin, pectins, and waxes that must be removed or reduced by processing. The animal fibres consist exclusively of proteins and, with the exception of silk, constitute the fur or hair that serves as the protective epidermal covering of animals. Silk filaments are extruded by the larvae of moths and are used to spin their cocoons.

COTTON

Synonyms: Raw cotton, purified cotton, absorbent cotton.

Biological Source

Epidermal trichomes of the seeds of cultivated species of the *Gossypium herbaceum* and other species of *Gossypium* (*G. hirsutum, G. barbadense*) freed from impurities, fats and sterilized, belonging to family Malvaceae.

Geographical Source

United States, Egypt, some parts of Africa, and India.

Gossypium herbaceum or the African-West Asian cotton: *Gossypium herbaceum* is the indigenous species in India. It isnative to semidesert conditions like in sub-Saharan Africa and in Arabia. It is a perennial shrub. It is widely cultivated in Ethiopia and also in Persia, Afghanistan. Turkey, North Africa, Spain, Ukraine, Turkestan, and China (first cultivation in China reported was in about A.D. 600). It reaches a height of 2–6 feet, with palmate hairy leaves, lobes lanceolate, acute yellow petals and a purple spot in centre, capsule when ripe splits itself and exposes the loose white clump surrounding the seeds and strongly adhering to the outer coating. *G*. *herbaceum* requires warm weather to ripen its seeds.

Gossypium arboreum or the Pakistani-Indian cotton: Itis native to Northwest India and Pakistan. The use and production of cotton dates back to 2000 BC, by the Harappan civilization of the Indus Valley. Some of them are tall perennial while others are short annuals. People of Nubia are considered to be the first cotton weavers of Africa. This cotton variety extended into other parts of Africa (Nigeria) that became a cotton-manufacturing centre from the 9th century onwards.

Gossypium barbadense or South American cotton: *G. bar-badense* gives the Sea Island, or long-stapled cotton. Theoldest cotton textiles recorded from South America date to 3600 B.C. The first sign of domestication of cotton species comes from Peruvian coast where cotton bolls dating to 2500 B.C. were found. Cotton became a commercial slave plantation crop in the West Indies and as a result of it Barbados in 1650s became the first British West Indian colony to export cotton. Later on around 1670, planting of *G. barbadense* also began in the British North American colonies.

Gossypium hirsutum or Mexican cotton: *G. hirsutum*arefound in coastal vegetation of Central and Southern North America and also in the West Indies. There are evidences of cotton remains dating back to 3500 B.C. in the Tehuacan Caves in Mexico and even the Spanish explorers have found cotton cultivation in the 1500s.

Cultivation, Collection, and Preparation

Cotton is cultivated by means of seed sowing method. The seeds are sown in rows of about 4–5 ft in distance. Proper fertilizers are provided timely. The cotton plants are shrubs or small trees that bare fruits (capsules) after flowering. The capsule consists of three to five seeds and is covered with hairs. The bolls are collected when ripe, separated from the capsule, dried, and subjected to the ginning press for processing. In ginning process, hairs and seeds are put before the roller with a small space, which separates the trichomes from the seeds. The short and long hair separated by delinter. Short hairs are known as ‘linters’, which are used in the manufacturing inferior grade cotton wool, whereas long hairs are used for preparation of cloth. The seeds remain after the removal of hair is used for the preparation of cotton seed oil and oil cake for domestic animal feed. The raw cotton so obtained is full of impurities like the colouring matter and fatty material. It is then subjected to further purification by treating it with dilute soda ash solution under pressure for about 15 hours. It is then bleached and washed properly, dried, and packed. The packed cotton is then sterilized using radiations.

Description

Colour	White
Odour	Odourless
Taste	Tasteless
Shape	These are fine filaments like that of hair, which are soft and unicellular.
Size	2.2–4.6 cm in length and 20–35 micron in diameter

Chemical Constituents

It consists of 90% of cellulose, 7–8% of moisture, wax, fat and oil 0.5% and cell content about 0.5%. Purified cotton has almost cellulose and 6–7% of moisture.

Chemical Tests

1. On ignition, cotton burns with a flame, gives very little odour or fumes, does not produce a bead, and leaves a small white ash; distinction from acetate rayon, alginate yarn, wool, silk, and nylon.
2. Dried cotton is moistened with N/50 iodine and 80% w/w sulphuric acid is added. A blue colour is produced; distinction from acetate rayon, alginate yarn, jute, hemp, wool, silk, and nylon.
3. With ammoniacal copper oxide solution, raw cotton dissolves with ballooning, leaving a few fragments of cuticle. Absorbent cotton dissolves completely with uniform swelling, distinction from acetate rayon, jute, wool, and nylon.
4. In cold sulphuric acid (80% w/w) cotton dissolves; distinction from oxidized cellulose, jute, hemp, and wool.
5. In cold sulphuric acid (60% w/w) cotton, is insoluble; distinction from cellulose wadding and rayons.
6. In warm (40°C) hydrochloric acid it is insoluble; distinction from acetate rayon (also silk, nylon).
7. It is insoluble in 5% potassium hydroxide solution; distinction from oxidized cellulose, wool, and silk.
8. Treat it with cold Shirla stain A for 1 min and wash out. It shows shades of blue, Tilac or purple; distinction from viscose, acetate rayons, alginate yarn, wool, silk, and nylon.
9. Treat it with cold Shirla stain C for 5 min and wash out; raw cotton gives a mauve to reddish-brown colour and absorbent cotton a pink one; distinction from flax, jute, hemp. The Shirla stains may be usefully applied to a small piece of the whole fabric under investigation to indicate the distribution of more than one type of yarn.
10. It does not give red stain with phloroglucinol and hydrochloric acid; distinction from jute, hemp, and kapok.

Uses

Cotton is used as a filtering medium and in surgical dressings. Absorbent cotton absorbs blood, pus, mucus, and prevents infections in wounds.

JUTE

Synonym: Gunny.

Biological Source

It consists of phloem fibres from the stem of various species of the *Corchorus; C. capsularis* Linn, *C. olitorius* Linn, and other species like *C. cunninghamii, C. junodi* etc., belonging to family Tiliaceae.

Geographical Source

West Bengal and Assam.

Description

They are tall, usually annual herbs, reaching to a height of 2–4 m, unbranched and if branched it has only a few side branches. The leaves are alternate, simple, lanceolate, 5–15 cm long and a finely serrated or lobed margin. The flowers are small (1.5–3 cm in diameter) and yellow, with five petals; the fruit encloses many seeds in the capsule.

Preparation

Retting is the process for the preparation of bast fibres. This process is done by three methods, that is, microbial (or water), steam, and mechanical process. The microbial or water retting process is the oldest and the popular method employed for the breaking of lignin bond present between parenchyma and sclerenchyma. The breaking of this bond facilitates the easy procurement of skin from its core. Then the material is washed dried to release pectin bond which makes the hard skin to fine thread like fibres. The jute fibres are graded according to its colour, strength and fibre length. The fibres are of white to brown and 1–4 m. long.

Microscopy

A thin transverse section of the strand when treated with phuloroglucinol and HCl, stains the strands deep red, indicating the presence of lignin. Each strand is a collection of polygonal cells which are surrounded by lumen with various sizes. These strands can be separated by treating it with mixture of potassium chloride and nitric acid.

Chemical Constituents

Jute fibres are composed primarily of the plant materials cellulose and lignin. Jute is composed of about 50–53% cellulose, nearly 20% of hemicellulose and 10–11% of lignin along with other constituents like moisture not more than 12–13%, fats, wax, and ash contributing to 1% each.

Uses

It has a large range of use (about 1,000 uses). It is listed as the second most important vegetable fibre after cotton. Jute is used chiefly to make cloth for wrapping bales of raw cotton, in the preparation of sacks and coarse cloth. They are also woven into curtains, chair coverings, carpets, Hessian cloth very fine threads of jute can be made into imitation silk and also in the making of paper. It is even used in the manufacture of tows, padding splints, filtering, and straining medium. Jute is used for the preparation of coarse bags.

HEMP

Biological Source

Hemp is the pericyclic fibre obtained from *Cannabis sativa* Linn., belonging to family Cannabinaceae.

Geographical Source

Hemp is grown at any altitude from Norway to the Equator. The raw materials are imported from China, Hungary, America, Germany, Switzerland, Australia, Canada, France, and Norway.

Chemical Constituents

Hemp mainly consist of cellulose and lignin.

Uses

Hemp is mentioned historically to have more than 25,000 diverse uses. The historically mentioned uses are printing inks, paints, varnishes, paper, bibles, bank notes, food, textiles (the original Levi's jeans were made from Hemp cloth), canvas and building materials. Due to its high tensile strength, bast fibres are ideal for such specialized paper products as: tea bags, industrial filters, currency paper, or cigarette paper.

Drugs Containing Lipids

INTRODUCTION

The lipids are a large and diverse group of naturally occur-ring organic compounds that are related by their solubility in non-polar organic solvents (e.g. ether, chloroform, acetone, and benzene) and are generally insoluble in water. There is great structural variety among the lipids and comprise of fixed oils, fats, and waxes. The lipids of physiological importance for humans have the following major functions:

1. They serve as structural components of biological membranes.
2. They provide energy reserves, predominantly in the form of triacylglycerols.
3. Both lipids and lipid derivatives serve as vitamins and hormones.
4. Lipophilic bile acids aid in lipid solubilization.

FIXED OILS AND FATS

Fixed oils and fats are obtained from plants or animal. They are rich in calories and in plant source, they are present mostly in the seeds, as reserve substances and in animals they are present in subcutaneous and retroperitoneal tissues. They differ only according to their melting point and chemically they belong to the same group. If a substance is liquid at 15.5–16.5°C it is called fixed oil and solid or semisolid at the above temperature, it is called fat. They are made from two kinds of molecules: glycerol (a type of alcohol with a hydroxyl group on each of its three carbons) and three fatty acids joined by dehydration synthesis. Since there are three fatty acids attached, these are known as triglycerides. These fatty acids may be saturated, monounsaturated or polyunsaturated. The terms saturated, mono-unsaturated, and poly-unsaturated refer to the number of hydrogens attached to the hydrocarbon tails of the fatty acids as compared to the number of double bonds between carbon atoms in the tail. Fats, which are mostly from animal sources, have all single bonds between the carbons in their fatty acid tails, thus all the carbons are also bonded to the maximum number of hydrogens pos-sible. Since the fatty acids in these triglycerides contain the maximum possible amount of hydrogens, these would be called saturated fats. The hydrocarbon chains in these fatty acids are, thus, fairly straight and can pack closely together, making these fats solid at room temperature. Oils, mostly from plant sources, have some double bonds between some of the carbons in the hydrocarbon tail, causing bends or ‘kinks’ in the shape of the molecules. Because some of the carbons share double bonds, they are not bonded to as many hydrogens as they could if they weren’t double bonded to each other. Therefore these oils are called unsaturated fats. Because of the kinks in the hydrocarbon tails, unsaturated fats can’t pack as closely together, making them liquid at room temperature.

Fixed oils and fats are insoluble in water and alcohol and are soluble in lipid solvents like light petroleum, ether, chloroform, and benzene. Only exception in this solubility is castor oil that is soluble in alcohol because of its hydroxy group of ricinoleic acid. They float in water since their specific gravity is less than one. They produce a permanent translucent stain on the paper and are called fixed oils. Fixed oils and fats cannot be distilled without their decomposition.

Examples of saturated and unsaturated fatty acids are given in table below.

Table : Examples of saturated and unsaturated fatty acids

Fatty acid	Source
Saturated fatty acids	
Butyric acid	Butter fat
Lauric acid	Coconut oil
Myristic acid	Palm oil
Palmitic acid	Arachis oil, sesame oil
Stearic acid	Arachis oil
Arachidic acid	Mustard oil
Unsaturated fatty acids	
Linolenic acid	Linseed oil
Linoleic acid	Sesame oil, sunflower oil
Arachidonic acid	Arachis oil
Oleic acid	Safflower oil, corn oil

Analytical Parameters for Fats and Oils

Following are the parameters used to analyse the fats and oils.

1)**Iodine value:** The iodine value is the mass of iodinein grams that is consumed by 100 g of fats or oil. It is a measure of the extent of unsaturation and higher the iodine value, the more chance for rancidity.

2)**Saponification value:** The saponification value is thenumber of milligrams of potassium hydroxide required to saponify 1 g of fat under the conditions specified. It is a measure of the average molecular weight of all the fatty acids present.

3)**Hydroxyl value:** The hydroxyl value is the numberof mg of potassium hydroxide (KOH) required to neutralize acetic acid combined to hydroxyl groups, when 1 g of a sample is acetylated.

4)**Ester value**: The ester value is the number of mg of potassium hydroxide (KOH) required to saponify the ester contained in 1 g of a sample.

5)**Unsaponifiable matter:** The principle is the saponification of the fat or oil by boiling under reflux with an ethanolic potassium hydroxide solution. Unsaponifiable matter is then extracted from the soap solution by diethyl ether. The solvent is evaporated and then the residue is dried and weighed.

6)**Acid value:** It is the amount of free acid presentin fat as measured by the milligrams of potassium hydroxide needed to neutralize it. As the glycerides in fat slowly-decompose the acid value increases.

7)**Peroxide value:** One of the most widely used tests for oxidative rancidity; peroxide value is a measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation. Milliequivalents of peroxide per kg of fat are measured by titration with

iodide ion. High peroxide values are a definite indication of a rancid fat, but moderate values may be the result of depletion of peroxides after reaching high concentrations.

WAXES

Waxes are esters of long-chain fatty acids and alcohols. The fatty acids are same in wax and fats, but the difference being saponification. Waxes are saponified only by alcoholic alkali but the fats may be saponified either by alcoholic alkali or by aqueous alkali. Along with fatty acids it also contains monohydroxy alcohols of high molecular weight especially cetyl alcohol, melissyl alcohol, and myricyl alcohol. Some-times cholesterol or phytosterols are also present.

As such they are not suitable as food because hydrolysing enzymes of wax are not present in system. Waxes are widely distributed in nature. The leaves and fruits of many plants have waxy coatings, which may protect them from dehydration and small predators. The feathers of birds and the fur of some animals have similar coatings which serve as a water repellent.

Spermaceti, beeswax, carnuba wax, etc. are the examples of waxes.

CASTOR OIL

Synonyms: Castor bean oil, castor oil seed, oleum ricini, ricinus oil, cold-drawn castor oil.

Biological Source

Castor oil is the fixed oil obtained by cold expression of the seeds of *Ricinus communis* Linn., belonging to family Euphorbiaceae.

Geographical Source

It is mainly found in India, Brazil, America, China, Thai-land; in India it is cultivated in Gujarat, Andhra Pradesh, and Karnataka.

Preparation

Castor oil is obtained from castor seeds. The oil is obtained by two ways; either after the removal of the seed coat or with the seed coat. Seed coats are removed by crushing the seeds under the grooved rollers and then they are subjected to a current of air to blow the testas. The kernels are fed in oil expellers and at room temperature they are expressed with 1 to 2 tons pressure per square inch till about 30% oil is obtained. The oil is filtered, steamed 80–100°C to facilitate the coagulation and precipitation of poisonous principle ricin, proteins and enzyme lipase present in it. Oil is then filtered and this oil with 1% acidity is used for medical purpose.

The oil cake which remains contains of ricin, lipase and about 20% oil. The cake is grounded, steamed to 40° to 80°C, and a pressure of 3 tons pressure per sq. inch is applied. This yields the second quality of oil with 5% acidity and is used for industrial purpose.

The residual cake which remains after the expression of the second quality oil still contains about 8 to 10% oil. This oil is obtained by subjecting it to extraction in soxhlet with lipid solvents. This oil obtained is also used in industry. The residual cake is used as manure and not fed to animal due to the presence of ricin. The cake is also used for the production of lipase.

Characteristics

Medicinal or the first grade or Pale pressed castor oil is colourless or slightly yellow coloured. It is a viscid liquid which has slight odour with slightly acrid taste. Castor oil is soluble in absolute alcohol in all proportions; Specific gravity is 0.958 to 0.969, refractive index at 40°C is 1.4695 to 1.4730, acid value not more than 2, saponification value 177 to 187, and acetyl value is about 150.

Chemical Constituents

Castor oil consists of glyceride of ricinoleic acid, isoricinoleic, stearic, and dihydroxy stearic acids. Ricinoleic acid is responsible for laxative property. Castor oil also contains vitamin F. 90% of the fatty acid content is ricinoleic acid. The ricinoleic acid is an 18-carbon acid having a double bond in the 9–10 position and a hydroxyl group on the 12th carbon. This combination of hydroxyl group and unsaturation occurs only in castor oil.

Identification Tests

About 5 ml of light petroleum (50° to 60°) when mixed with 10 ml of castor oil at 15.5° shows a clear solution, but if the amount of light petroleum is increased to 15 ml, the mixture becomes turbid. This test is not shown by other oils.

Uses

Castor oil is mild purgative, fungistatic, used as an ointment base, as plasticizer, wetting agents, as a lubricating agent. Ricinoleic acid is used in contraceptive creams and jellies; it is also used as an emollient in the preparation of lipsticks, in tooth formulation, as an ingredient in hair oil. The dehydrated oil is used in the manufacture of linoleum and alkyl resin. The main use of castor oil is the industrial production of coatings, also employed to make pharmaceuticals and cosmetics in the textile and leather industries and for manufacturing plastics and fibres.

CHAULMOOGRA OIL

Synonyms: Hydnocarpus oil; gynocardia oil.

Biological Source

Chaulmoogra oil is the fixed oil obtained by cold expression from ripe seeds of *Taraktogenos kurzii* King, (syn. *Hydnocarpus kurzii* (King) Warb.), *Hydnocarpus wightiana* Blume, *H. anthelminticta* Pierre, *H. heterophylla*, and other species of Hydnocarpus, belonging to family Flacourtiaceae.

Geographical Source

The plants are tall trees, up to 17 m high, with narrow crown of hanging branches; native to Burma, Thailand, eastern India, and Indo-China.

Characteristics

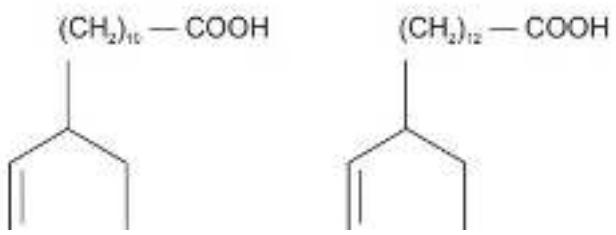
The oil is yellow or brownish yellow. Below 25°C it is a soft solid. It has peculiar odour and sharp taste. It is soluble in benzene, chloroform, ether, petrol; slightly soluble in cold alcohol; almost entirely soluble in hot alcohol and carbon disulphide.

Chemical Constituents

Chaulmoogra oil contains glycerides of cyclopentenyl fatty acids like hydnocarpic acid (48%), chaulmoogric acid (27%), gorlic acid with small amounts of glycerides of palmitic acid (6%), and

oleic acid (12%). The cyclic acids are formed during last 3–4 months of maturation of the fruit and are strongly bactericidal towards the Micrococcus of leprosy.

The seeds of H. wightiana contain a flavonolignan hydnocarpin; isohydnocarpin, methoxy hydnocarpin, apigenin, luteolin, chrysoeriol, hydnowightin, epivolkenin, and cyclopentenoid cyanohydrin glycosides.



Uses

The oil is useful in leprosy and many other skin diseases. The cyclopentenyl fatty acids of the oil exhibit specific toxicity for Mycobaeterium leprae and M. tuberculosis. The oil has now been replaced by the ethyl esters and salts of hydnocarpic and chlumoogric acids. At present organic sulphones have replaced Chaulmoogra oil in therapeutic use.

BEESWAX

Synonyms: White beeswax, yellow beeswax, cera alba, and cera flava.

Biological Source

Beeswax is the purified wax obtained from honeycomb of hive bee, *Apis mellifera* Linn and other species of Apis, belonging to family Apidae.

Geographical Source

It is mainly found in Jamaica, Egypt, Africa, India, France, Italy, California etc.

Preparation

The worker bee secretes the wax due to the ability of maintaining a high temperature and the wax is secreted in the last four segments of abdomen on the ventral surface. Just below the sterna it has a smooth layer of cells form the chitinous area that secretes the wax. The chitinous area has small pores through, which the wax exudes out. The wax is passed to the front leg and later to the mouth; in the mouth it gets mixed with the saliva, which is then built on the comb. This wax forms a capping on the honey cells. Wax forms about 1/8th part of the honeycomb. After removal of honey, honeycomb or the capping is melted in boiling water. On cooling the melted wax gets solidified and floats on the surface of water while the impurities settle below and honey leftovers get dissolved in water. The pure wax is then poured into earthen vessels wiped with damp cloth and the wax so obtained is yellow beeswax.

White beeswax is obtained from yellow beeswax. The yellow beeswax is runned on a thin stream of spinning wet drum, from which long ribbon like strips are scrapped off. The ribbon strips are placed on cloth in thin layers, rotated from time to time and bleached in sunlight till the outer layer

becomes white. White beeswax is obtained by treating yellow beeswax chemically with potassium permanganate, chromic acid or chlorine or charcoal.

Characterisitics

Yellow wax or Cera flava is yellowish to greyish brown coloured solid, with agreeable, honey-like odour and a faint, characteristic taste. When cold, it is somewhat brittle and when broken, shows presence of a dull, granular, noncrystalline fracture. Yellow wax is insoluble in water and sparingly soluble in cold alcohol. It is completely soluble in chloroform, ether, and in fixed or volatile oils, partly soluble in cold benzene or in carbon disulphide and completely soluble in these liquids at about 30°C.

White wax is less unctuous to the touch; it is yellow, soft, and ductile at 35°C and fusible at 65°C. A yellowish-white solid, somewhat translucent in thin layers. It has a faint, characteristic odour which is free from rancidity and tasteless. It is insoluble in water, soluble in chloroform, ether, fixed oil, and volatile oils (hot turpentine oil) and sparingly soluble in alcohol. It is not affected by the acids at ordinary temperatures, but is converted into a black mass when boiled with concentrated sulphuric acid.

Chemical Constituents

Beeswax contains myricin, which is melissyl palmitate; melting point 64°C, free cerotic acid ($C_{26}H_{52}O_2$), myricyl alcohol ($C_{30}H_{61}OH$) is liberated when myricyl palmitate is saponified. Melissic acid, some unsaturated acids of the oleic series, ceryl alcohol, and 12 to 13% higher hydrocarbons are present.

Uses

Beeswax is used in the preparation of ointments, plaster, and polishes.

Adulterants

Beeswax is adulterated by solid paraffin, ceresin, carnauba wax, or other fats and waxes of animal or mineral origin. Spermaceti and lard render wax softer and less cohesive, of a smoother and less granular fracture and different odour when heated. The melting point and specific gravity are lowered by tallow, suet, lard, and especially by paraffin. Ceresin, a principle obtained from ozokerite is also employed as an adulterant. In yellow wax the iodine value is also of use as a test for detection of adulterants but in white wax the bleaching process has altered the bodies which absorb the iodine.

WOOLFAT (ANHYDROUS LANOLIN)

Synonyms: Wool fat; Oesipos; Agnin; Alapurin; Anhydrous lanolin; Adeps lanae; Laniol.

Biological Source

Lanolin is the fat-like purified secretion of the sebaceous glands which is deposited into the wool fibres of sheep, *Ovis aries* Linn., belonging to family Bovidae.

Preparation

Wool is cut and washed with a soap or alkali. An emulsion of wool fat, called as wool grease, takes place in water. Raw lanolin is separated by cracking the emulsion with sulphuric acid. Wool grease floats on the upper layer and fatty acids are dissolved in the lower layer. Lanolin is purified by treating with sodium peroxide and bleaching with reagents.

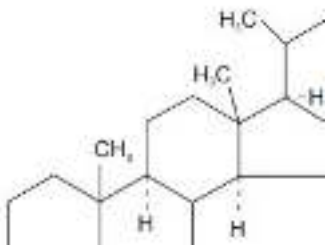
Characteristics

Lanolin is a yellowish white, tenacious, unctuous mass; odour is slight and characteristic. Practically, it is insoluble in water, but soluble in chloroform or ether with the separation of the water. It melts in between 34 and 40°C. On heating it forms two layers in the beginning, continuous heating removes water. Lanolin is not saponified by an aqueous alkali. However, saponification takes place with alcoholic solution of alkali.

Anhydrous lanolin is a yellowish tenacious, semisolid fat with slight odour. Practically it is insoluble in water but mixes with about twice its weight of water without separation. It is sparingly soluble in cold, more in hot alcohol, freely soluble in benzene, chloroform, ether, carbon disulphide, acetone, and petroleum ether.

Chemical Constituents

Lanolin is a complex mixture of esters and polyesters of 33 high molecular weight alcohols, and 36 fatty acids. The alcohols are of three types; aliphatic alcohols, steroid alcohols, and triterpenoid alcohols. The acids are also of three types: saturated nonhydroxylated acids, unsaturated nonhydroxylated acids, and hydroxylated acids. Liquid lanolin is rich in low molecular weight, branched aliphatic acids, and alcohols, whereas waxy lanolin is rich in high molecular weight, straight-chain acids, and alcohols.



The chief constituents of lanolin are cholesterol, iso-cholesterol, unsaturated monohydric alcohols of the formula $C_{27}H_{45}OH$, both free and combined with lanoceric ($C_{30}H_{60}O_4$), lanopalmitic ($C_{16}H_{22}O_3$), carnaubic, and other fatty acids. Lanolin also contains esters of oleic and myristic acids, aliphatic alcohols, such as cetyl, ceryl and carnaubyl alcohols, lanosterol, and agnosterol.

Identification Tests

Dissolve 0.5 g of lanolin in chloroform, and to it add 1 ml of acetic anhydride and two drops of sulphuric acid. A deep green colour is produced, indicating the presence of cholesterol.

Uses

Lanolin is used as an emollient, as water absorbable ointment base in many skin creams and cosmetic and for hoof dressing. Wool fat is readily absorbed through skin and helps in increasing the absorption of active ingredients incorporated in the ointment. However, it may act as an allergenic contactant in hypersensitive persons.

ENZYMES

Enzymes are organic catalysts produced in the body by living organisms. They perform many complex chemical reactions that make up life processes. Enzymes are lifeless and when isolated, they still exert their characteristic catalytic effect. Their chemical composition varies, and they do show several common properties. They are colloids, soluble in water and dilute alcohol but are precipitated by concentrated alcohol. Most enzymes act best at temperatures between 35 and 40°C; temperatures above 65°C, especially in the presence of moisture, destroy them, whereas their activity is negligible at 0°C. Certain heavy metals, formaldehyde, and free iodine retard the enzymes activity. Their activity is markedly affected by the pH of the medium in which they act or by the presence of other substances in this medium. They are highly selective in their action.

The enzymes are proteins having molecular weight from about 13,000 to 8,40,000. At present they are divided according to their action by a complex system established by the Commission on Enzymes of the International Union of Biochemistry. Six major classes are recognized; each has 4 to 13 subclasses, and each enzyme is assigned a systematic code number (B.C.) composed of 4 digits. The major classes are given in table below.

Enzymes are found in combination with inorganic or organic substances that have an important part in the catalytic action. If these are nonprotein organic compounds, they are known as coenzymes. If they are inorganic ions, they are referred to as activators. Coenzymes are integral components of a large number of enzyme systems. Several vitamins (thiamine, riboflavin, nicotinic acid) have a coenzymatic function.

Enzymes are obtained from plant and animal cells and many have been purified. They are used as therapeutic agents and as controlling factors in certain chemical reactions in industry. Pepsin, pancreatin, and papain are used therapeutically as digestants. Hyaluronidase facilitates the diffusion of injected fluids. Streptokinase and streptodornase dissolve clotted blood and purulent accumulations. Zymase and rennin are used in the fermentation and cheese industries; and penicillinase inactivates the various penicillins.

The names used to designate enzymes usually end in -ase or -in. The important enzymes are given hereunder.

Properties of Enzymes

1. Enzymes are sensitive to heat and are denatured by excess heat or cold, i.e. their active site becomes permanently warped, thus the enzyme is unable to form an enzyme substrate complex. This is what happens when you fry an egg, the egg white (augmentin, a type of protein, not an enzyme), is denatured.
2. Enzymes are created in cells but are capable of functioning out side of the cell. This allows the enzymes to be immobilized, without killing them.
3. Enzymes are sensitive to pH, the rate at which they can conduct reaction is dependent upon the pH of where the reaction is taking place, for example, pepsin in the stomach has an optimum pH of about 2, whereas salivary amylase has an optimum pH of about 7.
4. Enzymes are reusable and some enzymes are capable of catalysing many hundreds of thousands of reactions, for example, catalase working on hydrogen peroxide, try putting some liver into hydrogen peroxide.

5. Enzymes will only catalyse one reaction, for example, invertase will only produce glucose and fructose, when a glucose solution is passed over beads of enzyme.

6. Enzymes are capable of working in reverse, this act as a cut off point for the amount of product being produced. If there are excess reactants, the reaction will keep going and be reversed, so that there is no overload or build up of product.

GELATIN

Synonyms

Gelfoam; puragel; gelatinum.

Biological Source

Gelatin is a protein derivative obtained by evaporating an aqueous extract made from bones, skins, and tendons of various domestic animals. Some important sources are: Ox, *Bos taurus*, and Sheep, *Ovis aries* belonging to family Bovidae

Preparation

The process of manufacture of gelatin vary from factory to factory. However, the general outline of the process is given below.

Raw material

Bones, skins, and tendons of Bovideans is collected and subjected to liming operation.

Liming Process

The raw material is first subjected to the treatment known as 'liming'. In this process, the skins and tendons are steeped for fifteen to twenty and sometimes for 40 days in a dilute milk of lime. During this, fleshy matter gets dis-solved, chondroproteins of connective tissues gets removed and fatty matter is saponified. The animal skin is further thoroughly washed in running water.

Defattying

In case of bones, the material is properly ground and defatted in close iron cylinders by treatment with organic solvents such as benzene. The mineral and inorganic part of the bone is removed by treatment with hydrochloric acid.

Extraction

The treated material from bones, skins and tendons is boiled with water in open pans with perforated false bottom. This process can also be carried out under reduced pressure. The clear liquid runs of again and again and is evaporated until it reaches to above 45 per cent gelatin content.

Setting

The concentrated gelatin extract is transferred to shallow metal trays or trays with glass bottom. It is allowed to set as a semisolid jelly.

Drying

The jelly is transferred to trays with a perforated wire netting bottom and passed through series of drying compartments of 30–60°C increasing each time with 10°C. About a month is taken for complete drying.

Bleaching

In case of darker colour, finished product is subjected to bleaching by sulphur dioxide. Bleaching affords a light coloured gelatin.

Characteristics

Gelatin occurs as a colourless or slightly yellow, transparent, brittle, practically odourless, tasteless sheet, flakes or course granular powder. In water it swells and absorbs 5–10 times its weight of water to form a gel in solutions below 35–40°C. It is insoluble in cold water and organic solvents, soluble in hot water, glycerol, acetic acid; and is amphoteric. In dry condition it is stable in air, but when moist or in solution, it is attacked by bacteria. The gelatinizing property of Gelatin is reduced by boiling for long time. The quality of gelatin is determined on the basis of its jelly strength (Bloom strength) with the help of a Bloom gelometer. Jelly strength is used in the preparation of suppositories and pessaries.

Commercially two types of gelatin, A and B, are avail-able. Type A has an isoelectric point between pH 7 and 9. It is incompatible with anionic compounds such as Acacia, Agar and Tragacanth. Type B has an isoelectric point between 4.7 and 5, and it is used with anionic mixtures. Gelatin is coloured with a certified colour for manufacturing capsules or for coating of tablets. It may contain various additives.

Chemical Constituents

Gelatin consists of the protein glutin which on hydrolysis gives a mixture of amino acids. The approximate amino-acid contents are: glycine (25.5%), alanine (8.7%), valine (2.5%), leucine (3.2%), isoleucine (1.4%), cystine and cysteine (0.1%), methionine (1.0%), tyrosine (0.5%), aspartic acid (6.6%), glutamic acid (11.4%), arginine (8.1%), lysine (4.1%), and histidine (0.8%). Nutritionally, gelatin is an incomplete protein lacking tryptophan. The gelatinizing compound is known as chondrin and the adhesive nature of gelatin is due to the presence of glutin.

Chemical Tests

1. ***Biuret reaction:*** To alkaline solution of a protein (2 ml), a dilute solution of copper sulphate is added. A red or violet colour is formed with peptides containing at least two peptide linkages. A dipeptide does not give this test.
2. ***Xanthoproteic reaction:*** Proteins usually form a yellow colour when warmed with concentrated nitric acid. This colour becomes orange when the solution is made alkaline.
3. ***Millon's reaction:*** Millon's reagent (mercuric nitrate in nitric acid containing a trace of nitrous acid) usually yields a white precipitate on addition to a protein solution which turns red on heating.
4. ***Ninhydrin test****:* To an aqueous solution of a protein an alcoholic solution of ninhydrin is added and then heated. Red to violet colour is formed.
5. On heating gelatin (1 g) with soda lime, smell of ammonia is produced.
6. A solution of gelatin (0.5 g) in water (10 ml) is precipitated to white buff coloured precipitate on addition of few drops of tannic acid (10%).
7. With picric acid gelatin forms yellow precipitate.

Uses

Gelatin is used to prepare pastilles, pastes, suppositories, capsules, pill-coatings, gelatin sponge; as suspending agent, tablet binder, coating agent, as stabilizer, thickener and texturizer in food; for manufacturing rubber substitutes, adhesives, cements, lithographic and printing inks, plastic compounds, artificial silk, photographic plates and films, light filters for mercury lamps, clarifying agent, in hectographic matters, sizing paper and textiles, for inhibiting crystallization in bacteriology, for preparing cultures and as a nutrient.

It forms glycerinated gelatin with glycerin which is used as vehicle and for manufacture of suppositories. Combined with zinc, it forms zinc gelatin which is employed as a topical protectant. As a nutrient, Gelatin is used as commercial food products and bacteriologic culture media.

CASEIN

Biological Source

Casein is a proteolytic enzyme obtained from the stomachs of calves. It is extracted from the proteins of the milk; in the milk, casein is structured in voluminous globules. These globules are mainly responsible for the white colour of the milk. According to various species, the casein amount within the total proteins of the milk varies.

The casein content of milk represents about 80% of milk proteins. The principal casein fractions are alpha (s1) and alpha (s2)-caseins, β-casein and κ-casein. The distinguishing property of all casein is their low solubility at pH 4.6. The common compositional factor is that caseins are conjugated proteins, most with phosphate group(s) esterified to serine residues. These phosphate groups are important to the structure of the casein micelle. Calcium binding by the individual caseins is proportional to the phosphate content.

Within the group of caseins, there are several distinguishing features based on their charge distribution and sensitivity to calcium precipitation:

Alpha (s1)-casein: (molecular weight 23,000; 199 residues, 17 proline residues).

Two hydrophobia regions, containing all the proline residues, separated by a polar region, which contains all but one of eight phosphate groups. It can be precipitated at very low levels of calcium.

Alpha (s2)-casein: (molecular weight 25,000; 207 residues, 10 prolines).

Concentrated negative charges near N-terminus and positive charges near C-terminus. It can also be precipitated at very low levels of calcium.

β-*casein:* (molecular weight 24,000; 209 residues, 35 prolines).

Highly charged N-terminal region and a hydrophobia C-terminal region. Very amphiphilic protein acts like a detergent molecule. Self association is temperature-dependent; will form a large polymer at 20°C but not at 4°C. Less sensitive to calcium precipitation.

κ-*casein:* (molecular weight 19,000; 169 residues, 20 prolines).

Very resistant to calcium precipitation, stabilizing other caseins. Rennet cleavage at the Phe l05 – Met l06 bond eliminates the stabilizing ability, leaving a hydrophobia portion, para- κ-casein and a hydrophilic portion called κ-casein glycomacropeptide (GMP), or more accurately, caseinomacropeptide (CMP).

Characteristics

The isoelectric point of casein is 4.6. The purified protein is water insoluble. While it is also insoluble in neutral salt solutions, it is readily dispersible in dilute alkalis and in salt solutions such as sodium oxalate and sodium acetate. Casein does not coagulate on heating. It is precipitated by acids and by a proteolytic enzyme (rennet).

Chemical Constituents

Milk consists of 80% of milk proteins (casein). The major constituents of casein are alpha (s1) and alpha (s2)-caseins, β-casein and kappa-casein. These caseins are conjugated proteins with phosphate group(s) which are esterified into serine residues they have a low solubility at pH 4.6.

Uses

It is used in the manufacture of binders, adhesives, protective coatings, plastics (such as for knife handles and knitting needles), fabrics, food additives, and many other products. It is commonly used by bodybuilders as a slow-digesting source of amino acids. There is growing evidence that casein may be addictive for some individuals, particularly those on the autism spectrum or having schizophrenia.

PAPAIN

Synonyms

Papayotin, vegetable pepsin, tromasin, arbuz.

Biological Source

Papain is the dried and purified latex of the green fruits and leaves of *Carica papaya* L., belonging to family Caricaceae.

The plant is cultivated in Sri Lanka, Tanzania, Hawai, and Florida. The plant is 5–6 m in height bearing fruits of about 30 cm length and a weight up to 5 kg. The epicarp adheres to the orange-coloured, fleshy sarcocarp, which surrounds the central cavity. This cavity contains a mass of nearly black seeds.

Preparation

It is distributed throughout the plant, but mostly concentrated in the latex of the fruit.

The latex is obtained by making two to four longitudinal incisions, about 1/8 inch deep, on the surface on four sides of nearly mature but green fruits while still on the tree. The incisions are made early in the morning, at intervals of three to seven days. The latex flows freely for a few seconds but soon coagulates. The exudate is collected in nonmetallic containers. The latex is dried as soon as possible after collection. Rapid drying or exposure to sun or higher temperature above 38°C produce dark colour product with weak in proteolytic activity. The use of artificial heat yields the better grade of crude papain. The final product should be creamy white and friable. It is sealed in air-tight containers to prevent loss of activity. If 10% common salt or 1% solution of formaldehyde is added before drying, the product retains its activity for many months.

Fully grown fruits give more latex of high enzyme potency than smaller or immature fruits. The yield of Papain varies from 20 to 250 g per tree. The yield of commercial Papain from latex is about 20%.

Characteristics

Papain occurs as white or greyish-white, slightly hygroscopic powder. It is incompletely soluble in water and glycerol. It may digest about 35 times its weight of lean meat. Best grades render digestion of 200–300 times their weight of coagulated egg albumin in alkaline media. A temperature range of 60–90°C is favourable for the digestive process with 65° the optimum point. Best pH is 5.0, but it functions also in neutral or alkaline media. It is activated by reduction (HCN and H_2S) and inactivated by oxidation (H_2O_2, iodoacetate).

Chemical Constituents

Papain contains several enzymes such as proteolytic enzymes peptidase I capable of converting proteins into dipeptides and polypeptides, rennin-like enzyme, clotting enzyme similar to pectase and an enzyme having a feeble activity on fats.

The enzymes, papain, papayaproteinase, and chymopapain, have been isolated in crystalline form from the latex. Papain is atypical protein digesting enzyme with isoelectric point. It contains 15.5% nitrogen and 1.2% sulphur. Crystalline papain is most stable in the pH range 5–7 and is rapidly destroyed at 30°C below pH 2.5 and above pH 12. Papain is a protein of 212 amino acids and having a molecular weight of about 23,000 daltons. It is resistant to heat, inactivated by metal ions, oxidants and reagents which react with thiols, and is an endopeptidase activated by thiols and reducing moieties, for example, cysteine, thiosulphate, and glutathione.

The leaves possess dehydrocarpaines I and II, fatty acids, carpaine, pseudocarpaine, and carotenoids.

The fruits yield lauric, myristoleic, palmitoleic and arachidic acids, malonated benzyl-p-o-glucosides, 2-phenyl ethyl glucoside, and 4-hydroxy-phenyl-2-ethyl glucoside.

Uses

Papain is used to prevent adhesions; in infected wounds; internally as protein digestant, as anathematic (nematode), to relieve the symptoms of episiotomy (incision of vulva), in meat industry for tenderizing beef, for treatment of dyspepsia, intestinal and gastric disorders, and diphtheria, for dissolving diphtheria membrane; in surgery to reduce incidence of blood clots where thromboplasma is undesirable and for local treatment of buccal, pharyngeal, and laryngeal disorders.

It is used in digestive mixtures, liver tonics, for reducing enlarged tonsils, in prevention of postoperative adhesions, curbuncles, and eschar burns. It is an allergic agent causing severe paroxysmal cough, vasomotor rhinitis and dyspnea. It is a powerful poison when injected intravenously. In industry it is used in the manufacture of proteolytic preparations of meat, lever, and casein, with dilute alcohol and lactic acid as meat tenderizer, as a substitute for rennet in cheese manufacture, in brewing industry for making chill-proof bear, for degumming natural milk, in preparation of tooth pastes and cosmetics, in tanning industry for bathing skin and hides, and as an ingredient in cleansing solutions for soft contact lenses.

Test

Papain is reacted with a gelatin solution at 80°C in the presence of an activating cysteine chloral hydrate solution for an hour. The solution is cooled to 4°C for long time. The treated solution must not regel in comparison to a blank solution under identical con-ditions.

Adulteration

Commercial papain is often adulterated with arrowroot starch, dried milk of cactus, gutta percha, rice flour, and pepsin.

BROMELIN

Synonyms

Bromelin, bromelain.

Biological Source

Bromelin is a mixture of proteolytic enzymes isolated from the juice of *Ananas comosus* (pineapple), belonging to family Bromeliaceae.

Geographical Source

Pineapple is a native of tropical America. It is grown in almost all parts of the world including India, China, Thai-land, United States, Brazil, Philippines, Mexico, Hawaii, and Taiwan.

Cultivation, Collection, and Preparation

Bromelin is found in pineapple fruit juice and stem. Pine-apple is perennial, and it does not have a natural period of dormancy. It is propagated through suckers, slips, and crowns. In India it is planted in August, the plant generally flowers in February–March, and the fruit ripens during July–October.

The fruits must be left on the plant to ripen for the full flavour to develop. Dark green unripe fruits gradually change to yellow and finally to deep orange. The fruits are cut off. The enzyme bromelin does not disappear as the fruit ripens. The enzyme from fruit and stem are known as fruit bromelin and stem bromelin, respectively. It is isolated from pineapple juice by precipitation with acetone and also with ammonium sulphide

Characteristics

The optimum pH of bromelain is 5.0–8.0. In solution pH below 3.0 and above 9.5 inactivates the enzyme. The optimum temperature is between 50 and 60°C, still it is effective between 20 and 65°C too. The moisture content should not exceed 6%. It is obtained in light brown-coloured powder.

Chemical Constituents

Bromelain is not a single substance, but rather a collection of enzymes and other compounds. It is a mixture of sulphur-containing protein-digesting enzymes, called proteolytic enzymes or proteases. It also contains several other substances in smaller quantities, including peroxidase, acid phosphatase, protease inhibitors, and calcium.

Uses

Bromelain is an effective fibrinolytic agent; bromelain inhibits platelet aggregation and seems to have both direct as well as indirect actions involving other enzyme systems in one of the primary uses of bromelain in several foreign countries; it can modify the permeability of organs and tissues to different drugs. The potentiation of antibiotics and other medicines by bromelain may be due to enhanced absorption, as well as increased permeability of the diseased tissue which enhances the access of the antibiotic to the site of the infection. It is also thought that the use of bromelain may provide a similar access to specific and nonspecific components of the immune system, therefore, enhancing the body's utilization of its own healing resources. Bro-melain has been used successfully

as a digestive enzyme following pancreatectomy, in cases of exocrine pancreas insufficiency and in other intestinal disorders. Research has indicated that bromelain prevents or minimizes the severity of angina pectoris and transcient ischemic attacks (TIA); it is useful in the prevention and treatment of thrombosis and thrombophlebitis. If administered for prolonged time periods, bromelain also exerts an antihypertensive effect in experimental animals. It may even be useful in the treat-ment of AIDS to stop the spread of HIV. It has no major side effects, except for possible allergic reactions.

SERRATIOPEPTIDASE

Synonym

Serrapeptase, serratiopeptidase.

Biological Source

Serratiopeptidase is a proteolytic enzyme isolated from nonpathogenic enterobacteria *Serratia* E 15. It is also produced by the larval form of the silk moth.

Preparation

Serratiopeptidase is produced by fermentation technology by using nonpathogenic enterobacteria species such as *Serratia* E 15. The larvae of silk moth produce this enzyme in their intestine to break down cocoon walls. It can thus be obtained from the silk moth larvae.

Characteristics

Serratiopeptidase is very much vulnerable to degradation in the acidic pH. When consumed in unprotected tablet or capsule, it is destroyed by acid in stomach. However enteric coated tablets facilitate its absorption through intestine. One unit of the enzyme hydrolyses casein to produce colour equivalent to 1.0 µmol of tyrosine per minute at pH 7.5 and 35°C.

Chemical Constituents

Serratiopeptidase is a proteolytic enzyme of protease type XXVI. The preparation contains 7.1 units/ mg solid.

Uses

Serratiopeptidase is the most widely prescribed antiinflammatory enzyme in developed countries and also in India. It eliminates inflammatory oedema and swelling, accelerate liquefaction of pus and sputum, and enhance the action of antibodies. It is also used as a fast wound healing agent. It is proving to be a superior alternative to the nonsteroidal antiinflammatory drugs traditionally used to treat rheumatoid arthritis and osteoarthritis. It has wide ranging applications in trauma surgery, plastic surgery, respiratory medicine, obstetric and gynaecology.

UROKINASE

Synonym

Uroquinase.

Biological Source

Urokinase is serine protease enzyme isolated from human urine and from human kidney cells by tissue culture or by recombinant DNA technology.

Preparation

Urokinase is a fibrinolytic enzyme produced by recombinant DNA using genetically manipulated *E. coli* cells. It is produced firstly as prourokinase q.v. and then converted to active form by plasmin or kallikrein. Urokinase used medicinally is also purified directly from human urine. It binds to a range of adsorbents such as silica gel or kaolin which can be use to initially concentrate and purify the product. It can be further purified by precipitation with sodium chloride or ethanol or by chromatography. Human urokinase needs sterile filtration, a septic filling and freeze drying.

Characteristics

Urokinase enzyme occurs in two different forms as single and double polypeptide chain forms. It has a half-life of 10–16 minutes after intravenous administration. These enzymes act on an endogenous fibrinolytic system.

Chemical Constituents

Urokinase enzymes are serine proteases that occur as a single low molecular weight (33 kDa) and double, high molecular weight (54 kDa) polypeptide chain forms. They differ in molecular weight considerably. A single chain is produced by recombinant DNA technique and is known as SCUPA.

Uses

Urokinase is used in the treatment of pulmonary embolism, coronary artery thrombosis and for restoring the potency of intravenous catheters. It is generally administered intra-venously in a dose of 4,400 units/kg body weight per hour for twelve hours.

STREPTOKINASE

Synonym

Estreptokinase, plasminokinase.

Biological Source

Estreptokinase, plasminokinase is a purified bacterial protein produced from the strains of group C β-haemolytic *S. griseus.*

Preparation

Streptokinase is a bacterial derived enzyme of serine pro-tease group. The ancestral protease activity lies within the first 230 amino-acid residues at the N-terminal part of the protein that evolves from serine protease due to the replacement of histamine at 57th amino acid by glycine. The amino terminal residue polypeptide chain shows sequence homology to serine protease. Duplication and fusion of gene generate an ancestral streptokinase gene. Streptokinase is produced by fermentation using streptococcal culture and is isolated from the culture filtrate. It is produced in the form of a lyophilized powder in sterile vials containing 2,50,000 to 7,50,000 IUs.

Characteristics

Streptokinase is a bacterial protein with half-life of 23 minutes. Its anisolylated plasminogen activator complex (APSAC) has a higher half-life of six hours.

Chemical Constituents

Streptokinase is the purified bacterial protein with about 484 amino-acid residues.

Uses

Streptokinase is the first available agent for dissolving blood clots. It binds to plasminogen in a 1:1 ratio and changes molecular conformation. Thus, the complex formed becomes an active enzyme and promotes the activity of fibrinolytic enzyme plasmin. Plasmin breaks fibrin clots. Anistreptase or the anisolylated plasminogen streptokinase activator complex (APSAC) can also be used in a similar way for degrading blood clots. Streptokinase and anistreptase are both used in the treatment of pulmonary embolism, venous, and arterial thrombosis and coronary artery thrombosis. It is also sometimes administered along with heparin to counter act a paradoxical increase in local thrombin.

PEPSIN

Biological Source

It is the enzyme prepared from the mucous membrane of the stomach of various animals like pig, sheep, or calf. The commonly used species of pig is *Sus scrofa* Linn, belonging to family Suidae.

Pepsin is the first in a series of enzymes that digest proteins. Pepsin binds with protein chains and breaks it up into small pieces. Pepsin cleaves proteins preferentially at carboxylic groups of aromatic amino acids such as phenylalanine and tyrosine but does not cleave at bonds containing amino acids like valine or alanine. Pepsin mainly cleaves C-terminal to F, L, and E, and it does not cleave at V-, A-, or G-terminals. Structurally, the active site is located in a deep cleft within the molecule. Optimal activity of pepsin is at pH of 1.8 –3.5, depending on the isoform. They are reversibly inactivated at about pH 5 and irreversibly inactivated at pH 7–8.

Preparation

The mucous membrane is separated from the stomach either by the process of stripping or it is scrapped off, and it is placed in acidified water for autolysis at 37°C for 2 hours. The liquid obtained after autolysis consist of both pepsin and peptone. It is then filtered and sodium or ammonium salts are added to the liquid till it is half saturated. At this point only the pepsin separates out, and the peptone remains in the solution. The precipitates are collected and subjected to dialysis for the separation of salts. Remaining amount of pepsin if any in the aqueous solution is precipitated by the addition of alcohol into it. The pepsin is collected and dried at low temperature.

Description

Pepsin occurs in pale yellow colour, they are odourless or with very faint odour, translucent grains and slightly bitter in taste. It is soluble in dilute acids, water, and physiological salt (NaCl) solution. It is best active at a temperature of 40°C with pH 2–4. Pepsin is unstable above pH 6. The enzyme gets denatured at a temperature of 70°C and in the presence of alcohol and sodium chloride. Pepsin can be stored for 1–2 years at 2–8°C.

Uses

It is used in the deficiency of gastric secretion. Pepsin is also used in the laboratory analysis of various proteins; in the preparation of cheese, and other protein-containing foods.

Bioactive (Medicinal) Constituents of Marine Organisms

Because there is great chemical diversity in marine plants, including marine algae and mangroves, products isolated from these plants have been shown to possess antibacterial, antifungal, analgesic, anti-inflammatory, cytotoxic, hypotensive, and spasmogenic activities.

Polyphenols, polysaccharides, and alkaloids are among the highly active, biologically potent and predominant anticancer compounds isolated from marine organisms.

Polyphenols

Polyphenols are categorized into phenolic acids, flavonoids, tannins, catechin, anthocyanidins, epigallocatechin, lignin, epicatechin, epigallate, and gallic acid. Polyphenolic compounds are known for their potential to reduce the mitotic index and decrease the levels of cellular proteins needed for cancer cell proliferation and colony formation.

Polysaccharides

The other potent group of compounds that is abundantly present in several marine organisms is polysaccharides, primarily alginates, agar, and carrageenans.

Alkaloids

Alkaloids derived of marine origin are divided into four groups, namely, indoles, halogenated indoles phenylethylamines, and other alkaloids, most of which belong to phenylethylamines and indoles. Two derivatives, namely, lophocladine A and lophocladine B, have been isolated from the red alga Lophocladia spp. Similarly, the presence of alkaloids, e.g., acanthicifolin, brugine and benzoquinones, in Acanthus illicifolius, Bruguiera sexangula, and Kandelia candel has been reported.

Peptides

Different types of peptides, have been isolated from a wide variety of marine flora. In the last decade, about 2500 new peptides with anti-proliferative activity have been identified. Purified peptides have exhibited cytotoxic effects against various human cell lines, including pancreatic, breast, bladder and lung cell lines.

Apratoxin A, a cyclic depsipeptide, exerted cytotoxic effects against human HeLa cervical carcinoma cells via cell cycle inhibition. A similar mechanistic effect was reported for the cyclic depsipeptide coibamide A, isolated from Leptolyngbya sp., and lyngbyabellin B, isolated from Lyngbya majuscule.

Two novel cyclodepsipeptides, namely, scopularide A and B, isolated from the marine fungus Scopulariopsis brevicaulis, significantly inhibited the growth of pancreatic and colon cancer cell lines.

Sansalvamide A is a structurally unique cyclic depsipeptide isolated from various marine fungi. This compound exhibited cytotoxic activities against different carcinomas, i.e., pancreatic, colon, breast and prostate sarcomas.

Antibiotics

Antitumor antibiotics are among the most important cancer chemotherapeutic agents and include members of the anthracycline, actinomycin, and aureolic acid families. Clinically useful agents from these families include peptolides, dactinomycin. Anthracyclines are among the most widely used antitumor antibiotics in the clinic and exert antitumor activity mainly by inhibiting topoisomerase II.