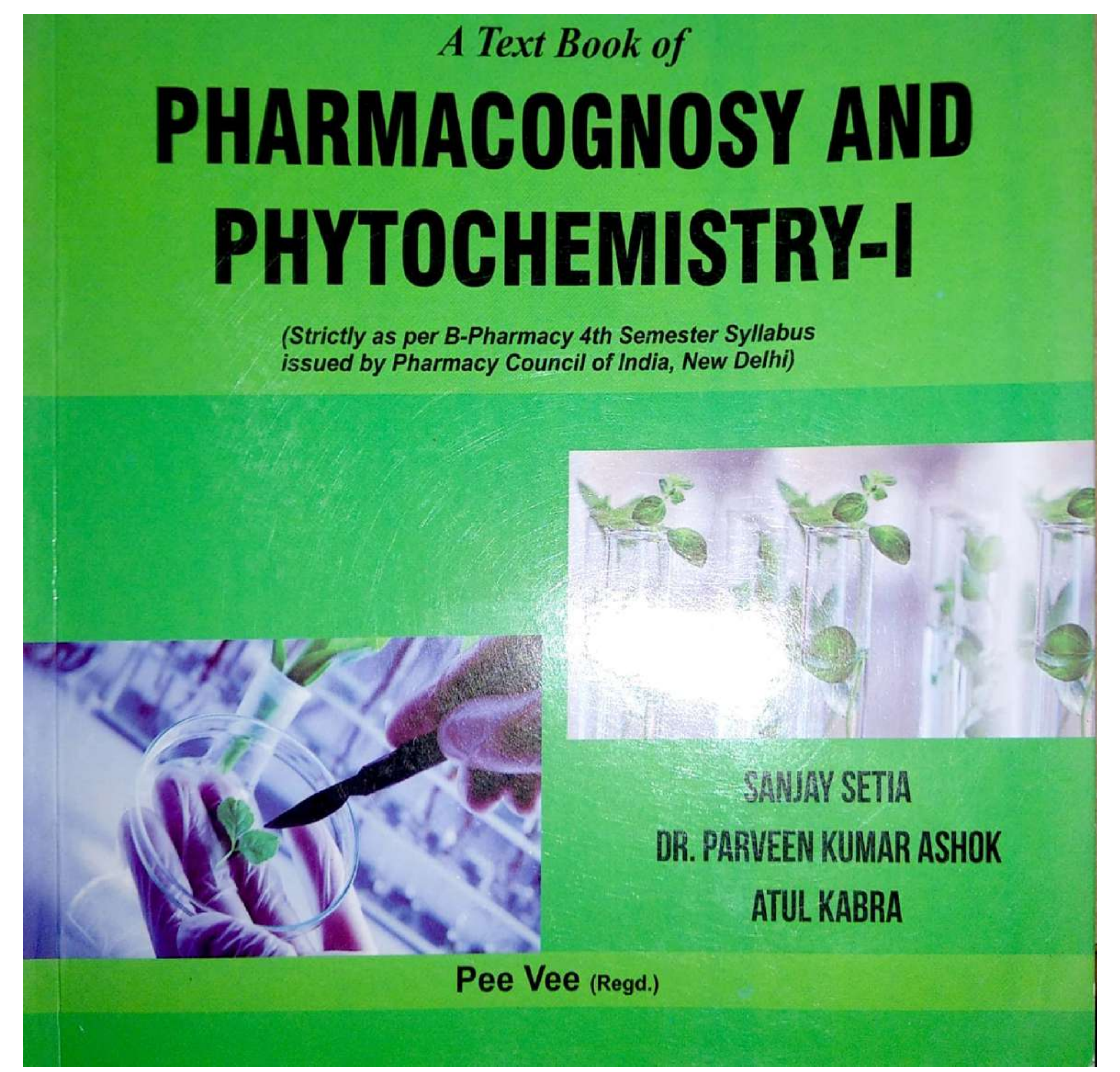

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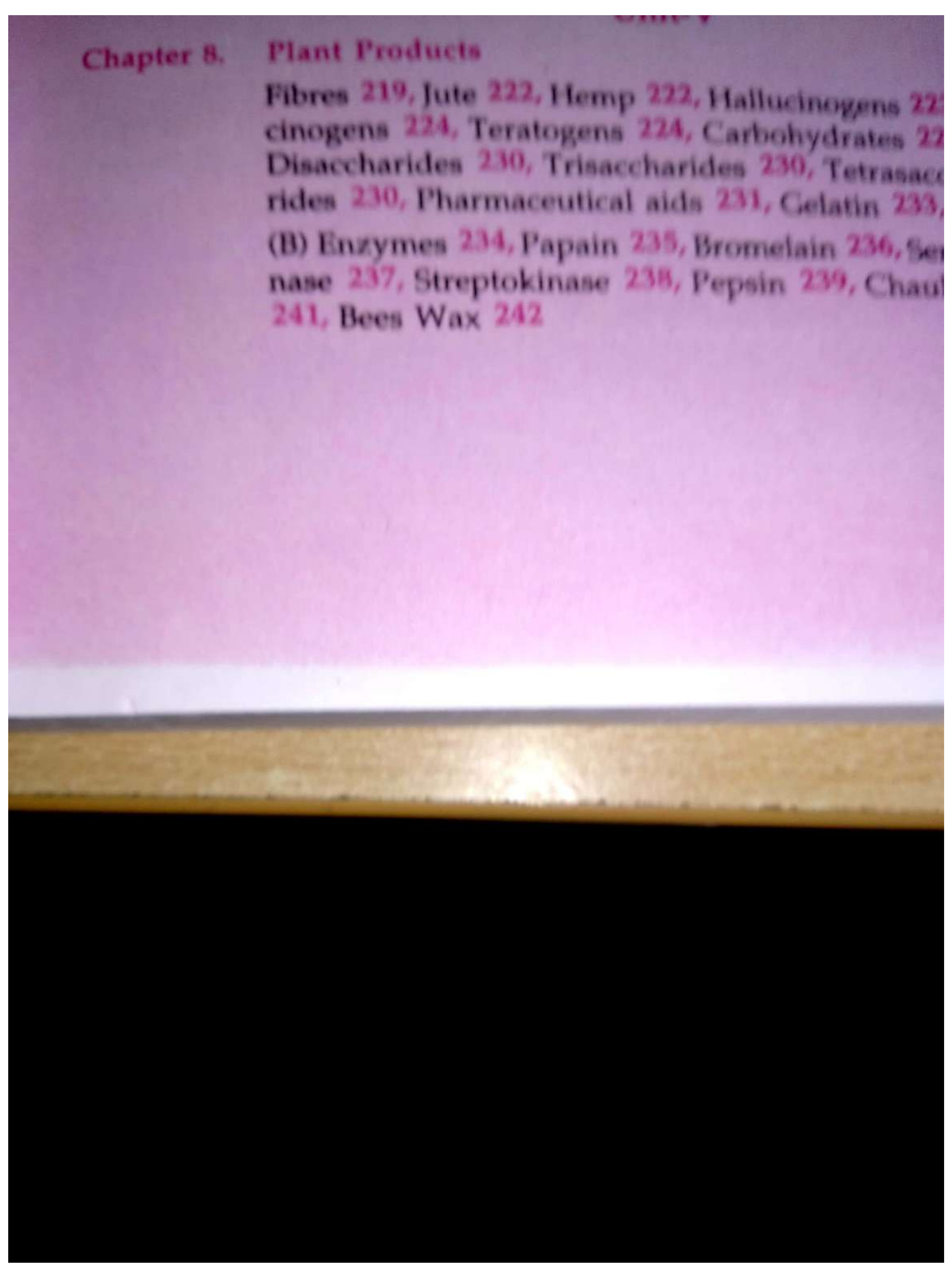

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Chapter 1

INTRODUCTION TO PHARMACOGNOSY

(A) HISTORY OF PHARMACOGNOSY

Man in initial days used the substances obtained from various plants and animal sources as food stuffs. Slowly these substances were also used as drugs to treat the disease because disease were born with man. The use of plants as source of drugs lies in the deep roots of antiquity. No one will ever know what led primitive man to select specific plant materials to treat the disease but it can be attributed to the inquisitive nature of man. The plants are absolutely necessary for the life of man and today we have a vast knowledge of chemical and therapeutic properties of different plants.The large number of drugs are derived from plant kingdom.

The history of herbal drugs dates back perhaps to the origin of human race.

The documents of the ancient era reveals that the plants were used as drugs in Egypt, China, Persia, Arab, Greece and India before the begining of Christian era. Initially man passed his knowledge to others through oral communication. Slowly as the different civilization developed man was able to communicate his knowledge first by carving into stones or clay and later by writing on parchment or on paper so that his knowledge should be known to coming generation. References may be made to the clay writings from the library of Assyrian King to the Egyptian *Papyrus Ebers* (1600B.C). *Papyrus Ebers* is an oldest document containing 700 medicinal herbs and more than 870 formulae. **Shen Nung** an emperor of china wrote *Pen-t'Sao* in 3000 B.C which contains 365 different drugs one for each day of the year. Ayurveda means 'Science of life' an Indian system of medicine is the very foundation stone of the ancient medical science of India. Ayurveda was evolved between 4000 and 600 B.C and objective of Ayurveda is not merely to cure the disease but to preserve the health also. The treatises dealing with Ayurveda are **Sushruta Samhita** and **Charak Samhita** both were compiled between 500-300 B.C. Charak Samhita deals mostly with plants and Sushruta Samhita deals with surgery.

Hippocrates "Father of medicine" (460-360 B.C) gave his contribution on anatomy and physiology of human beings. **Aristotle** "Father of natural history" (384-322B.C) was a philosopher and he wrote on animal kingdom which is considered authoritative even in twenty first century. **Theophrastus** (370-287 B.C) is known for his studies on plant kingdom. The Greek physician **Dioscorides** (40-80A.D) described about medicinal plants like opium, belladonna, colchicum, ergot and these are used even now days. **Galen** (131-200 A.D) known as first pharmacist described the different methods of preparation containing active

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constituents of crude drugs. The branch dealing with extraction of plant and animal drugs is still known as Galenical Pharmacy. After this there was a period of about one thousand years in which a very little progress was made in the field of medical science. No major attempts were made to change the formulation of medicaments but still herbal drugs were used to treat the disease in this period.

The French apothecary **N. Le'mary** (1645-1715) reported the importance of extraction method and alcohol to be used as an *ideal solvent*.* The advent of modern techniques of isolation and characterization led to the chemical screening of thousands of plants and therefore various active constituents were isolated. In 1806 the German chemist **Serturner** isolated morphine from opium. In 1811 the portugese chemist **Gomeriz** isolated cinchonine from cinchona bark. The French chemist **Pelletier** and **Caventou** isolated strychnine (1817) and brucine (1819) from nux vomica seeds. Similarly in the consecutive years quinine (**Pelletier** 1820), veratramine (**Meissner** 1820), nicotine (**Posselt** and **Reiman** 1828), amygdalin (1830), pilocarpine (**Hardy** and **Gerrad** 1875), ephedrine (**Nagai** 1887) and emetine (1894) were isolated. **Stass** and **Otto** in 1852 developed a new process of extraction for alkaloids. Some of the important constituents like reserpine, digoxin, ergometrine, quinidine etc. were isolated in twentieth century.

The developments in the field of botany during 19th century had a direct effect on pharmacognosy. The great Swedish biologist **Linnaeus** (1707-1778) classified the plants and introduced the binomial system of plants which is still followed. Plant classification was further developed by **Bentham** and **Hooker** (1862-1883), **Eichler** (1883), **Engler** and **Prandtle** (1887-1889). The microscopical and chemical studies of crude drugs helped to publish a number of atlases of powdered vegetable drugs. **Berg** in 1865 published anatomical atlas of crude drugs. **Voehl** and **Tschirch** published the anatomical atlas of various powdered drugs which became helpful in that period when adulteration in food articles and drugs were common. In 1904 **Greenish** and **Collin** compiled "An Anatomical Atlas of Powdered Vegetable Drugs".

Thus, up to the beginning of 20th century pharmacognosy was more a descriptive subject mainly of botanical science and consisted of identification of drugs in entire and powdered condition and with their history, commerce, collection, preparation and storage. The development of modern pharmacognosy took place later during the period of 1934 -1960 by simultaneous advancements in the areas of biochemistry, organic chemistry, biosynthesis, pharmacology and modern methods and techniques of analysis like Thin layer, Paper, Gas and High performance liquid chromatography and spectrophotometry. The substances from plants were isolated, their structures were elucidated and their pharmacological actions were studied. Therefore by application of several disciplines pharmacognosy from a descriptive subject has developed into an integral, important discipline of pharmaceutical sciences.

Undoubtedly, the plant kingdom still holds many species of plants containing substances of medicinal value which have yet to be discovered. The large number of plants are constantly being screened for their pharmacological actions. Thus pharmacognosists with a multidisciplinary background are able to make valuable contribution to these rapidly developing fields of study.

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INTRODUCTION TO PHARMACOGNOSY

• DEFINITION

Initially in 19th century the term **'Materia Medica'** was used for the subject now known as pharmacognosy. The word "Pharmacognosy" was coined by German scientist **C. A. Seydler** in 1815 in his work entitled ***Analecta Pharmacognostica.*** The name pharmacognosy is derived from two Greek words viz. Pharmakon (a drug) and Gignosco (to acquire the knowledge of). Broadly, Pharmacogonosy is defined as **the scientific study of the structural, physical, chemical and biological characters of crude drugs along with their history, cultivation, collection, preparation for the market and preservation.**

In short, pharmacognosy is the objective study of crude drugs derived from plant, animal and mineral sources, treated scientifically.

PRESENT STATUS AND SCOPE OF PHARMACOGNOSY

Now a days the medicinal plants are widely used throughout the world. After the second world war almost every country have established its medicinal plant research institutes and laboratories. New plants are constantly being screened and the plants and crude drugs which were investigated and rejected earlier are re-examined using all the modern techniques. Liquorice, valerian, veratrum, podophyllum, senna, digitalis, opium, colchicum, belladonna etc. are some of the examples of older drugs which are re-examined. The medicinal plants used in herbal drugs are also studied on the basis of folklore. As there is continous increase in the demand of herbal products many countries including India have introduced Herbal Pharmacopoeia's which contains regulatory requirements of medicinal herbs, so that the quality of these products can be maintained. Monographs are now available on large number of herbal drugs giving description, tests for identity and purity and assays of active constituents. In this respect recognition should be given to the pioneering production of **British Herbal Pharmacopoeia** first produced in 1974.

Pharmacognosy as an applied science has played a important role in development of different disciplines of science. Pharmacognosy is principally concerned with plant materials therefore pharmacognosist should posses the basic knowledge of botany and zoology. The knowledge of plant taxonomy, plant breeding and plant genetics is helpful in the development of cultivation technology for medicinal plants. Phytochemistry has significantly developed and contributed a chemical knowledge due to elucidation of structure of isolated constituents. Chemotaxonomy is a growing science and has led to many important developments regarding evolution of plant kingdom upon which modern ideas of classification are based. Plant tissue culture, biogentic pathways for formation of primary and secondary metabolites and other related fields like chemical engineering and biochemistry are essential to understand the pharmacognosy.

Pharmacognosy also includes the study of animal products such as bees wax, gelatin, wool fat, vitamins etc. and other natural products like hormones and antibiotics. The materials having no pharmacological action but are significant for the pharmacognosists are like natural fibres, suspending and flavouring agent, disintegrants, stabilizers and colourants. Other areas which are naturally associated with the subject are poisonous and hallucinogenic plants, allergens, herbicides, insecticides and molluscicides.

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The use of herbal drugs is increasing day by day and new plant drugs are finding their way into medicine as purified phytochemicals. Thus, pharmacological and chemical screening of these is essential so that the information regarding their uses and side effects can be disseminated to the people. Hence, pharmacognosy is an vital link between pharmacology and medicinal chemistry. Basically, Pharmacognosy is the infrastructure on which depends evolution of novel medicine and it provides a system where the active principles of crude drugs derived from natural sources are formulated and dispensed into various dosage forms used in ayurvedic and allopathic system of medicine. So, pharmacognosy is also an important link between ayurvedic and allopathic system of medicine.

Briefly, the natural sources of drugs are required to be exploited more and more. The popularity of natural drugs throughout the world clearly indicates the significant contribution of pharmacognosy in modern medicine.

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QUESTION BANK

OBJECTIVE PART

MULTIPLE CHOICE QUESTIONS

1. *Papyrus Ebers* which contains 700 medicinal herbs and more than 870 formulae is an :-
   (A) Indian document (B) Egyptian document
   (C) American document (D) Pakistani document
2. Who wrote *Pen-t'Sao* ?
   (A) Hippocrates (B) Dioscorides
   (C) Shen Nung (D) Aristotle
3. Who is known as Father of Medicine ?
   (A) Hippocrates(460-360B.C) (B) Aristotle(384-322B.C)
   (C) Dioscorides(40-80A.D) (D) Theophrastus(370-287B.C)
4. Dioscorides(40-80A.D) was a :-
   (A) American Physician (B) Greek Physician
   (C) Indian Physician (D) None of the above
5. Who is known as first Pharmacist ?
   (A) Dioscorides (B) N. Le'mary
   (C) Serturner (D) Galen
6. Who isolated morphine from opium ?
   (A) Serturner (B) Pelletier
   (C) Meissner (D) Reiman

(B) SOURCES OF DRUGS

A drug may be defined as an intended for use in diagnoses, cure, mitigation, prevention or treatment of disease in man or other animal, or indented to alter a body function or structure of man or other animals.

Classify sources of Drugs

1. Biological source
   a) Higher plants
   b) Microbes
   c) Animals
2. Marine sources
3. Mineral source
4. Plant tissue culture

1. BIOLOGICAL SOURCE

Higher plants a source of drugs : Plants have been used in the treatment of various diseases from time immemorial. The traditional Indian systems of medicine.

Ayurveda, Siddha, Unani systems are based on the use of plants & other natural substances. There are 200,000 to 250,000 species of flowering plants growing on earth, which belong to 10,500 general and about 300 families.

These genera are source of drugs and are distributed among plant families like :

- **Solanaceae :** Datura, Belladona, Hyocyamus etc.
- **Cruciferae :** Mustard etc.
- **Scrophulariaceae :** Digitalis
- **Leguminaceae :** Senna
- **Labitae :** Tulsi, Pudina etc.
- **Rutaceae :** Lemon
- **Rubiaceae :** Cinchona
- **Umbelliferae :** Fennel, Coriandes, caraway etc.
- **Apocynaceae :** Rauwolfia, Vinca
- **Liliaceae :** Scilla
- **Graminae :** Wheat rice and maize starch
- **Papaveraceae :** Opium
- **Dioscoreaceae :** Dioscorea

– **Spermatophytes :**

- **Angiosperms (Flowering plants) :** They are useful sources of glycoside, voltaile oil and alkaloids like cinchona, belladonna, Ipecacunha, etc.

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- **Gymnosperms (Non flowering plants)** : They are useful source of oil, resin and alkaloids such as Ephedra.

Drugs consisting of entire plant or some plant of it are often designated as crude drugs. Generally only that part of the plant, which contains the maximum amount off aejive constituents is collected and marked. Thus a crude drug may consist of seeds, fruits and leaves, flowers, roots and barks of stem or root. Many of the plant products are important therapeutic agents like alkaloids cardiac glycosides, anthraquinones, flavonoids, mucilage and enzymes. Plant product like steroid sapogenins is important raw material for the synthesis of steroidal hormones and related drugs.

MICROBES AS A SOURCE OF DRUGS

The microbes are microscopic organism which include viruses, bacteria and reckettsiae. These micro-organisms are source of many immunizing biological.

A. Viral Vaccine :

a) **Small pox vaccine :** Contains living virus of vaccinia (cow pox) which has been grown in the skin of a vaccinated bovine calf.

It is used as immunizing agent and prophylactic against small pox infection as well.

b) **Rabies vaccine,** is a sterile preparation of killed, fixed virus of rabies, obtained from duck embryos, which have been infected with fixed rabies virus. It is available in dried forms.

c) **Influenza virus vaccine,** is a sterile aq. solution of suitably inactivated influenza virus.

d) **Poliomyelitis vaccine,** is of two types, poliovirus vaccine inactivated and poliovirus vaccine live oral.

Later the preparation of one or a combination of strains of live, attenuated polioviruses, these are used as active immunizing agent poliovirus.

e) **Measles virus** contains live attenuated rubeola and rubella viruses. The viruses are grown on cultures of other birds embryo tissue or human diploid cell tissue.

f) **Yellow fever vaccine,** yellow fever vaccine is an attenuated strain of living yellow virus as well selected for high antigenic activity and safety. It is prepared by culturing the virus in the living embryo of the domestic fowl.

g) **Hepatitis virus vaccine,** it is composed of chemically inactivated **hepatitis B** surface antigen (HBsAg) particles obtained from the plasma of healthy chronic HBsAg carriers by plasmapheresis, separated from the infectious Dane particle by density gradient, centrifugation and absorbed on aluminium hydroxide.

B. Rickettsial vaccine:

These are a group of very small gram negative microorganism, intermdiate in size between the average bacteria and the large virus. Rickettsia can't be grown in artificial media and like virus require chick embryo or monkey kidney tissue as well, for their growth.

Rickettsial vaccine is exemplified by only one preparation that is typhus vaccine produced in America. It is used for producing active immunity, against typhus fever.

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C. Bacterial Vaccine :

(i) **Typhoid vaccine,** it is a sterile suspension containing killed selected strain of typhoid bacilli, salmonella typhi. It is used for producing immunizing agent typhoid fever.

(ii) **BCG vaccine,** is a dried, living culture of the bacilus calmette Guerin strain of **Mycobacterium tuberculosis** var. bovis. This vaccine is an active immunizing agent against T.B.

(iii) **Pertusis or whooping cough,** is caused by the organism bordetella pertusis. Pertusis vaccine is used as an immunizing agent against this disease. This vaccine is sterile suspension of killed bordetella pertusis of a strains or strains selected for high antigenic efficiency.

(iv) **Plague vaccine :** Which is used to produce immunity against the disease, is a sterile suspension of killed selected strain of plague bacillis, yarsinia pestis.

(v) **Cholera vaccine;** is a sterile suspension of killed **cholera, vibrio.** In saline or other suitable diluents. It is an active immunizing agent for producing immunity against cholera.

D. Toxoids : Tetanus toxoid and diptheria toxoid.

ANIMAL AS SOURCE OF DRUG

Certain animal parts and animal products are used as drug in therapeutic. The major group of animal products used in medicine is hormone, enzymes, animal, extractives organs and bile acids as well.

A) Hormones :

i) **Thyroid :** It is a modified preparation of thyroid gland of sheep and pigs. It is given orally to treat patients suffering from thyroid insufficiency. It contains the hormone thyroxine and liothyronine.

ii) **Conjugated oestrogens** are an amorphous preparation containing water soluble conjugated forms of mixed oestrogens obtained from urine of pregnant mares.

iii) **Insulin,** is a polypeptide hormone secreted by the beta cells of the islets of langerhans, situated in the pancreas of all vertebrates.

iv) **Gonadotropins,** are mucoid hormones secreted by the anterior lobe of the pituitary gland. These hormones are prepared commercially from either horse serum or from the urine of pregnant woman.

v) **Vasopressin,** is also a peptide hormone obtained from the posterior lobe of pituitary hormonal gland of healthy pigs and cattles. It is used in the treatment of intestinal paralysis.

vi) **Oxytocin,** is a polypeptide hormone secreted by posterior pituitary gland. It causes constraction of uterine muscles and also stimulate the ejection of milk in lactating mothers as well. It also can be prepared by synthesis. Oxytocin is used to induce labour in full term pregnant women and to stop hemorrhage after child birth.

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vii) **Epinephrine,** is a hormone produced adrenal medulla in man. It is found in other animals also, because of its simple structures as well, all of epinephrine medicines are used in medicine today and is prepared by synthetic means as well.

B) Enzymes :

i) **Pancreatin,** is a preparation which contains enzymes of the pancreas and is prepared commercially from pig pancreas. It is used in the treatment of pancreatitis condition resulting from a deficient production of these enzymes by the body.

ii) **Pepsin,** is the main proteolytic enzyme of gastic juice, it is produced commercially by grandular layer of fresh pig stomach.

ii) **Fibrinolysin,** is prepared from profibrynogen, which is isolated from human plasma. It is activated to fibrinolysin by streptokinase. It is employed in the treatment of venous thrombosis.

iv) **Trypsin,** is a proteolytic enzyme prepared commercially from an extract of ox pancreas. It is used by topical application for the treatment of wounds, ulcers, fistulas etc.

v) **Chrymotrypsin,** is also proteolytic enzyme produced by the pancreas in the form of inactivate chryrmotrypsinogen. It is obtained commercially from the pancreas of Ox.

C) **Bile,** is a natural secretion of the liver which passes into the intestinal tract and aid in the digestion of fats by emulsifying them and promoting their absorption.

D) **Animal extractives** and **organs,** liver, stomach preparations and bile are examples of this group. Liver and stomach derived from healthy and domesticated animals and converted into suitable preparations, which are used as replacement therapy in pernicious anemia.

2) Marines as a source of drugs

It is a sub-branch of pharmacognosy, which is mainly concerned with the naturally occuring substances of medicinal value from marine.

During the last 30-40 years numerous levels of novel compounds have been isolated from marine organisms having biological activities such as antiviral, antibacterial, antiparasitic, anticoagulants, antimicrobial, anti-inflammatory and cardiovascular active products.

Classification

1. Antimicrobial agents & antibiotics
2. Antiviral compounds
3. Antiparasitic compounds
4. Cardiovascular agents
5. Anticancer agents
6. Anticoagulant agents
7. Antiinflammatory & antispasmodic agents

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ANTIMICROBIAL AGENTS

a) **Cephalosporin :** It is obtained from the marine fungus, ***Cephalosporium acrimonium,*** Cephelothin sodium, used as antibiotic against microbes insensitive to penicillin and ampicillins.

b) **Ircinin,** is obtained from *Iricin oros.*

c) **Variabilin,** is obtained from ***Iricinia variabilis.***

d) **Eunicin** is obtained from the ***Eunicia mammosa.***

e) **Halotoxin A, B, C** is obtained from the **stichopus japonirus** (sea cucumber).

f) Thelpin, is obtained from the **Thelepsus setosul** (annelide).

Antiviral Compounds

1. **Ara-A :** It is obtained from the sponge, tethya crypta.
2. **Avaral & Avarone :** It is obtained from the sponge, *Disidea avara,* have high therapeutic activity of crossing BBB (blood brain barrier) used in the treatment of AIDS.
3. **Eudostomin-A** obtained from the ***Eudostoma olivaceum.***
4. **Patellazole-B** is obtained from ***ascidian lissocilium*** **patella.**
5. **Oppositol :** It is obtained from the ***laurencia*** **suboppostia.**

ANTIPARASITIC COMPOUNDS:

1. **Domoic acid :** It is obtained from red algae ***chondria armata,*** is used as antihelmintic.
2. **α-Kainic acid :** It is obtained from the red algae, *digenia simplex*, is used broad spectrum anthelmintic, it is effective against parasitic round worm, whip worm and tape worm.
3. **Cucumme chinoside-F,** is obtained from sea cucumber used as antiprotozoal activity.
4. **Bengamide-F :** It is obtained from the sponger nudibranch and a zoanthid.
5. **Laminine,** is obtained from the ***Laminaria angustata,*** is used as an anthelmintic as well as smooth muscles relaxants.

ANTI-CANCER AGENTS

1. **Sinularin,** is obtained from ***Sinularia flexibilis.***
2. **Tocotrienal,** is obtained from the brown algae; ***Sargassum tortile.***
3. **Aplidine,** is obtained from a marine organism, mediterranean tunicate ***Aplidium albicans,*** used in medullary thyroid carcinoma.
4. **Asperidol,** is non lactonic cembranoids obtained from gorgonian coral as well.
5. **Aplysistatin,** is obtained from the sea hare ***Aplysia angasia.***
6. **Halitoxins,** is obtained from ***helieloma viridis.***

"ANTICOAGULANTS AGENT"

1. **Carrageenan** is obtained from the *chondrus, Euchauma, Gigrtin a.*
2. **Fucoidan,** is obtained from the ***Fucus vesiculosus*** and ***Polyides rotundus.***

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3. **Galaxtan sulphuric acid,** is obtained from the *Iridaea laminariodes.*

CARDIOVASCULAR & NEUROVASCULAR AGENTS

1. **Eledoisin** is obtained from the *Eledone moschata* (Cephalapod), it is a powerful hypotensive compound.
2. **Laminine,** is obtained from the *Laminaria, angustata.* It is used as hypotensive agent.
3. **Saxitoxin,** is obtained from the *Saxidomus gigantens, Mytilus californionus,* and *Gonyaulax catenella;* used as hypotensive agent.
4. **Tetramine,** is obtained from the *Naptunea antique.* It is show curare like effect.

ANTI-INFLAMMATORY & ANTISPASMODIC AGENTS

1. **Manocalide.** It is obtained from the *Luffariella variabilis.* It is act by direct inactivation of phospholipase A2, which is present in some neurotoxins, also having analgesic and selective anti-inflammatory activity.
2. **Tetradoxins** is obtained from the puffer fishes *Spherides rubripes* (liver & ovaries) used as strong antispasmodic.
3. **Dendalone-3-Hydroxy butyrate :** is obtained from the *Phyllospongia dendy* used as anti-inflammatory agent.
4. **Flaseibilide,** it is a diterpenoid obtained from the sinularia flexibilis.

MINERAL SOURCES OF DRUGS

1. Kaolin
2. Talc
3. Diatomite
4. Bentonite
5. Fullers earth
6. Shilajit
7. Asbestos

PLANT TISSUE CULTURE AS SOURCE OF DRUGS

Culture is term generally used for artificial growth. This refers to growth of the plants, cells, tissue and organ on artificial nutrient media. Tissue culture is an experimental technique through which a mass of cells is produced from explant tissue.

Requirement for tissue culture laboratory :

1. Washing & storage facilities.
2. Media preparation & storage room.
3. Transfer area for aseptic manipulations.

4. Culture room or inoculators for maintanance of cultures under controlled conditions of temperature, light and humidity.
5. Observation or data collection area

1. **Washing & Storage facilities;**

a) **Cleaning glassware :** The glassware is soakeed in a detergent solution for 16 hrs and then rinsed first in tap water followed by a second rinse in distilled water, further cleansing involved a 5 minutes rinse in hot water, 3 minutes rinse in deionized water and a final hand rinse in distilled water.

b) **Plastic Lab Ware :** Presterilised disposable polystyrene culture container (falcon, corning) are available and used in place of glass ware in order to dispense with washing.

- Plastic ware may be washed with a mild non-abrasive detergent and rinse with tap water & distilled water.

2. **Transfer area :**

a) **Plastic box :** This can be sterilized with an ultraviolet (UV) light and by swabbing the floar surface with 95% ethyl alcohol when in operation.

b) Wooden hood

c) Laminar airflow cabinet : A small motor blows air into the units first through a coarse filter, where large dust particles are separated and subsequently passes through a 0.3mm HEPA filter as well, the air is directed either downward/(vertical flow unit) or outward (horizontal flow unit) over the working surface.

3. **Media preparation room :** The media preparation room should be separate and away from the working laboratory.

This area to be utilized for the preparation of culture media. The room should be equipped with

1. Glassware
2. Culture vessels
3. pH meter
4. Hot plates
5. Balance
6. Water bath
7. Bunsen burner
8. Autoclave
9. Refrigerator
10. Microwave oven

4. **Characteristics of incubators and growth chambers;**

- Temperature range 2-40oC
- Temperature control ±0.5oC
- Safety high & low temperature limits
- Continuous temperature recorder

- 24 hours temperature and light programming
- Adjustable fluorescent lighting up to 10,000 use
- Relative humidity range 20-98%
- Relative humidity control : ± 3%
- Uniform forced air distribution
- Capacity upto 0.7m3 of 0.5m2 shelf space.

5. **Data collection area :** The growth of development of tissues cultured invitro are generally monitored by observing culture at regular intervals in the culture room or incubators where they have been maintained under controlled environmental conditions. Data based observations under aseptic conditions may be collected using a laminar airflow cabinet.

PLANT TISSUE CULTURE AS SOURCE OF DRUGS

S.No.	"Secondary Metabolites	Plant Source	Types of Culture
1.	Reserpine	Rawolifa serpentina	Suspension culture
2.	Artimisinine	Artemisia scoparia	Suspension culture
3.	Luteolin	Datura pinnata	Callus culture
4.	Vinblastin	Catharanthus roseus	Cell culture
5.	Quercetin	A.weightii	Callus culture
6.	Nicotine	Nicotina tobacum	Suspension culture
7.	Atropine	Atropa belladona	Hairy root culture
8.	Quinine & Quinidine	C.Ledgeriana	Root culture
9.	Digoxin	Digitalis lanata	Suspension culture
10.	Caffeine	Coffee arabica	Cell culture
11.	Morphine	Papaver Sominferum	Suspension culture
12.	Hyoscyamine	Hyocyamus niger	Suspension culture
13.	Xanthotoxin	Ruta graveolens	Suspension culture
14.	Diosgenin	Dioscorea compositae	Cell culture
15.	Tropane alkaloids	Datura innoxia	Suspension culture
16.	Cardenolides	Digitalis Purpurea	Suspension & cell culture
17.	Rhein	Cassia angustifolia	Callus culture
18.	Salasonine	Solanum xanthocarpum	Callus culture

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(C) ORGANISED DRUGS, & UNORGANISED DRUGS

ORGANISED DRUGS (CELLULAR DRUGS)

These are the drugs which represents a part of plant & possess cellular structure. Examples are :

Leaves- Digitalis, Senna, Datura, Belladonna, Vasaka, Coca, etc.

Fruits- Fennel,Coriander,Carraway Bael, Dill, Gokhru,etc.

Seeds- Nux-vomica, Isapghula, Almond, Mutmeg, Bavchi, Castor, Mustard etc.

Bark- Cinchona, Cinnamon, Arjuna, Cascara, Kurchi, Quillaia etc.

Root- Ipeca, Azonite, Rauwolfia, Senega, Gentian etc.

Rhizomes- Rhubarb, Valerian, Liquorice, Ginger, Podophyllum, Acorus etc.

Flowers- Clove, Saffron, Rose etc.

Hairs & Fibres- Hemp, Cotton & Jute etc.

Entire Plant- Ephedra, Lobelia, Shankhpuspi, Ergot, Chirata, Benatsha etc.

UNORGANISED DRUGS

Unorganised drugs are materials having a structure that is fairly uniform throughout and are not composed of cells.They are usually derived from parts of plant or animals by various process of Extraction, Decoction, Expression or are natural secretions such as Bees wax & Myrrh.

Unorganised drugs can be classified under headings based upon their origin and nature, giving well characterised groups such as dried latex e.g. opium, and dried juice e.g. Aloes; extracts e.g. Catechu, Gum e.g. Acacia, Resins e.g. Colophony; Gum resins e.g. Myrrh; Oleo-resins e.g. copaiba, Waxes e.g. Bees wax; Saccharine substances e.g., Honey : Oils & Fats e.g., Castor oil, Lard; Volatile oil e.g., Clove oil.

Dried Latex : Latex is an emulsion or a suspension the continuous phase of which is a aqueous solution of mineral salts, proteins, sugars, tannins, alkaloids etc. and the suspended particles are oil droplets, resin, gum, proteins, starch, eaoutchoue etc. This turbid fluid is often white in color as in opium but may be red as in rhizo of *Sanguinaria canadensis* or yellow as in *Chelidonium majus*. It occurs in the plants in special structures named as Latiuferous tissues. Latiuferous tissues are of three types viz. Latiuferous cells, Latiuferous tubes & Latiuferous vessels.

OPIUM

Synonyms – Raw opium; Afim (Hindi)

Biological Source – Opium is the latex obtained by incision from the unripe capsules of *Papaver somniferum* Linn, family Papavaeraceae, dried or partly dried by heat or spontaneous evaporation and worked into irregularly shaped masses (natural opium) or moulded into masses of more uniform size and shape (manipulated opium). It contains not less than 9.5% of morphine calculated as anhydrous morphine.

Geographical Source – The main opium producing countries are Turkey, Iran, USSR, Tasmania, Yugoslavia and India. In India opium is collected from Madhya Pradesh, Uttar

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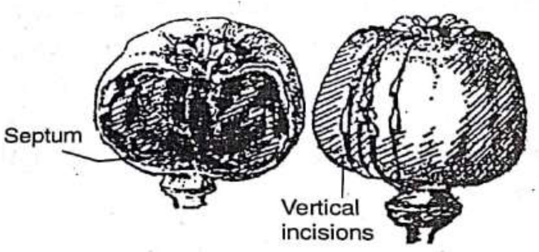
Pradesh and Rajasthan.

Cultivation – The cultivation and other aspects of opium are under the control of government of the respective countries. In India the cultivation, production etc of opium is done under the control of Narcotic Drugs and Psychotropic Substances Act. 1985.

Opium is obtained from poppy plant. Poppy plant is an annual herb about 50 cm to 1.5 meters in height. It bears bluish white or purple colored flowers. Leaves are linear, oblong or ovate oblong with dentate or serrate margin. The different types of varieties viz *P. somniferum* var *album*, *P. somniferum* var *glabrum* and *P. somniferum* var *nigrum* are described here. The plants are cultivated by sowing the seeds in the month of November. Seeds are sown by mixing them with 3 or 4 parts of sand. The distance maintained between two plants is about 25cm. Soil required for opium poppy should be fertile, well drained loamy with fine sand and the pH should be around 7. The thinning of the plants is done periodically and are kept free from weeds and insects. Farmyard manures and fertilizers are added for better growth and high quality yield.

Collection and Preparation – After 3-4 months of sowing the plant bears the flowers and these are converted into capsules. Each plant bears about 5-8 capsules. When the capsules are green or just show a tint of yellow incisions are made by knives which vary in shape in different countries. In India the incisions are made vertically in afternoon by a instrument known as "nustur". It penetrates about 2mm into the capsules. By this latex exudes out and is partly dried which is scrapped and collected in next morning by "charpala". This incising operation is repeated on each capsule three or four times at interval of two or three days. The latex is collected in plastic containers. For next propagation capsules are dried in sun and seeds are collected by beating. The average yield of opium varies from 20-30 kg per hectare.

After collection of opium by the cultivators it is brought to the weightment centers and from there it is transferred to the factory at Ghazipur (U.P) where the opium is further processed.



Poppy Capsules

Macroscopic Characters –

Odour – Strong and Characteristic

Taste – Bitter

Indian opium – It occurs in cubical pieces, weighing about 900gms.and is dark brown in colour. It is enclosed in tissue paper and is plastic in nature. It is also exported in 5 kg to 10 kg of blocks. Powder form of the drug is also available. This opium contains 9-12% of morphine.

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Natural Turkish or European opium – It occurs in more or less rounded or conical, frequently some what flattened masses weighing between 250gms. to 1kg. and is brown or dark brown in colour. It is covered with poppy leaves. It is soft when fresh but on keeping it becomes hard and brittle.

Manipulated Turkish Opium – It occurs in oval masses with flattened upper and lower surface weighing usually about 2000gms. and is chocolate-brown in color. It is covered with broken poppy leaves and is moderately plastic when fresh but it become brittle after some time. This opium contains 10 to 15% of morphine.

Manipulated European opium – It occurs in elongated masses with rounded ends weighing about 160 to 500gms. and internally dark brown in colour. It is hard and brittle.

Persian or Iranian opium – It occurs in brick shaped masses and is dark reddish brown in colour. It is covered with red paper. This opium contain 10 to 12.5% of morphine and is brittle in nature. It is available in 400- 500 gms of masses.

Chemical Constituents – Opium contains more then 25 alkaloids which belongs either to phenanthrene ring system or of benzylisoquinoline ring system. Morphine (10 to 20%), codeine (0.3 to 4%) and thebaine (0.2 to 0.5%) belongs to phenanthrene system and are strong bases where as papaverine, narcotine and narceine belongs to isoquinoline ring system and are weak bases.

Morphine ($C_{17}H_{19}NO_3, H_2O$) is in colourless crystals and is slightly soluble in cold water but readily soluble in caustic alkalies or alkaline earths. It is insoluble in cold ether, chloroform or benzene. Morphine is a powerful hypnotic.

Codeine ($C_{18}H_{21}NO_3$) or methyl morphine is in rhombic crystals soluble in 80 parts of water and readily soluble in chloroform.

Narcotine ($C_{22}H_{23}NO_7$) is in rhombic prisms or needles. It is soluble in 160 parts of ether.

The alkaloids of opium are in combination with meconic acid and sulphuric acid.

O
OH
HOOC O COOH

Meconic Acid

Other constituents like mucilage, wax, sugar and salts of calcium and magnesium are present in small quantities. Starch, oxalic acid and tannins are not present in opium.

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Morphine

Codeine

Thebaine

Papaverine

Narcotine

Chemical Tests –

(1) Opium is dissolved in water and filtered. To the filtrate ferric chloride solution is added. A deep reddish purple colour is obtained which persist even after the addition of few drops of dil Hcl. This confirms the presence of meconić acid in opium.

(2) Morphine is added to concentrated sulphuric acid and formaldehyde. A dark violet colour is produced.

(3) Morphine solution is treated with ferric chloride and potassium ferricyanide solution. Bluish green colour is produced.

Uses – Opium acts on central nervous system causing its depression. It is used as hypnotic,

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analgesic and sedative. It is useful in diarrhoea. Morphine is an analgesic and it is given in severe pains when patient does not respond to the other analgesics. Morphine also causes respiratory depression. Codeine is widely used in cough syrups. It relieves local irritation in bronchial tract and posses mild analgesic effects. Narcotine has non-narcotic property, is useful in respiratory disease and is central antitussive. Several synthetic derivatives have been prepared from opium alkaloids. Heroin* (Diacetyl morphine) has more narcotic analgesic property than morphine. Heroin is more dangerous than morphine and is famous as an addiction causing drug.

Dose- Morphine sulphate-10 mg, 6 times a day, parenterally

Codeine phosphate/sulphate -10-20 mg. every 4-6 hrs., orally

Papaverine hydrochloride - 150mg orally and 30mg parenterally

Narcotine - 15 mg., 4 times a day, orally

Storage – Opium is stored in well closed containers to prevent loss of morphine.

Adulteration and Substitutes – The production of opium is strictly under the control of government, hence adulteration is rarely found. Adulterated form shows the presence of opium capsules in powdered form, gum and sugary fruit.

Dried Juices : The plants which exudes liquid on incission that is present in certain specialized cells are called Dried Juices.

ALOES

Synonyms – Aloe; Ghritkumari, Musabbar(Hindi).

Biological Source – Aloe is the dried juice of the leaves of *Aloe barbadensis* Miller known in commerce as Curacao aloes or of *Aloe perryi* Baker known in commerce as Socotrine aloes or of *Aloe ferox* Miller and hybrids of this species with *Aloe africana* Miller and *Aloe spicata* Baker known in commerce as Cape aloes, family Liliaceae.

Geographical Source – Aloe is cultivated in southern and eastern Africa. It is also found in Europe and in various parts of India.

Cultivation and Collection – The plants yielding aloes bear rosettes of leaves which are thick, fleshy, sessile and spiny. Flowers are red or yellow.

Root suckers are used for propagation. The plant grows in dry climatic conditions as it is a xerophyte plant. Root suckers are planted in the rows about 60 cm apart in the rainy seasons. Water logging near the plant must be avoided. The leaves are cut in second year and the drug is obtained from leaves for twelve years. After twelve years the plants are completely harvested by uprooting and again the land can be used for replantation. During the collection of leaves a cut is given near the base of leaves by which the juice located in parenchymatous cells of pericycle exudes out, due to the pressure exerted by mucilage cells. A single cut is enough for drawing out the entire juice.

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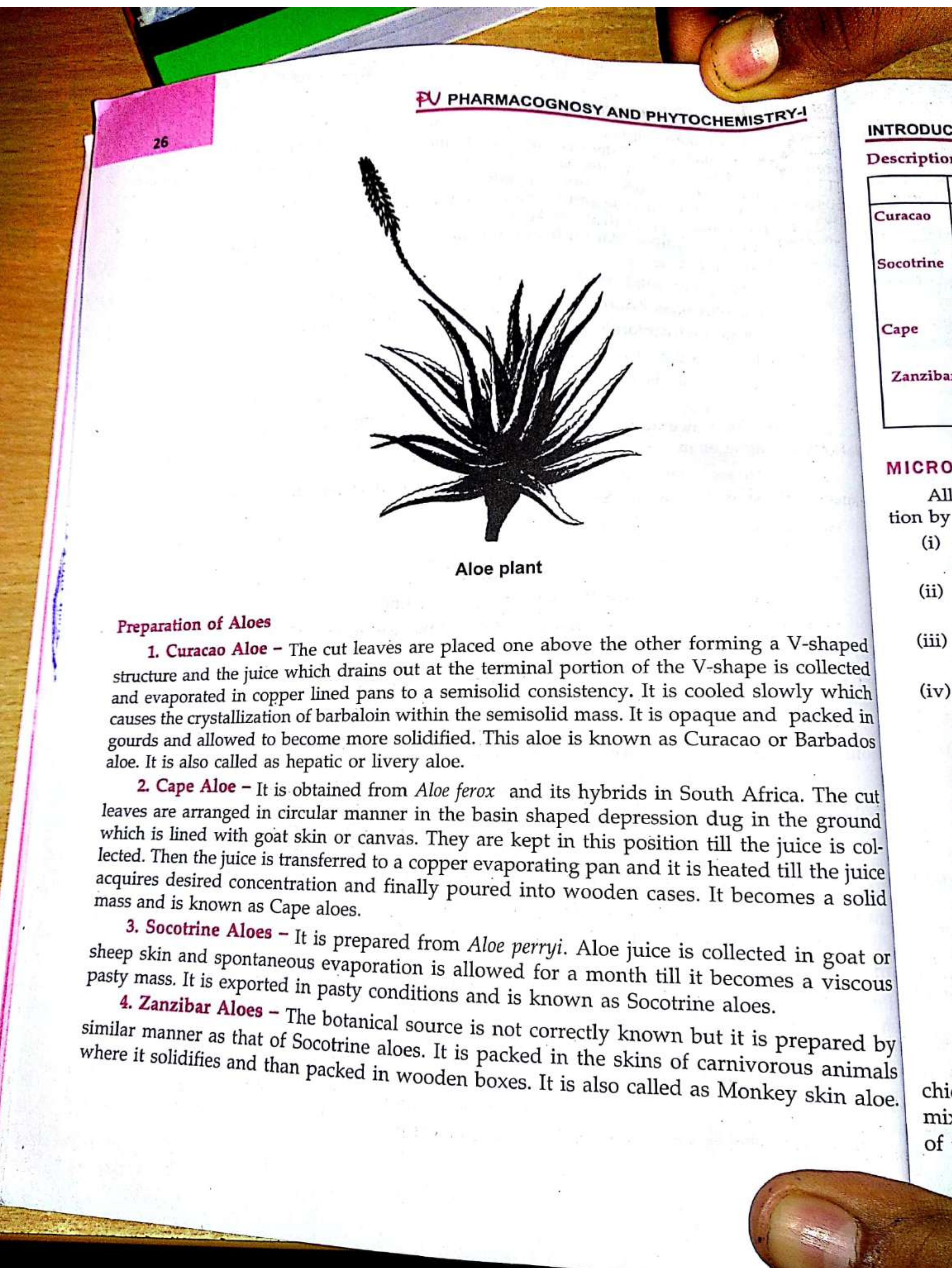

Aloe plant

Preparation of Aloes

1. Curacao Aloe – The cut leaves are placed one above the other forming a V-shaped structure and the juice which drains out at the terminal portion of the V-shape is collected and evaporated in copper lined pans to a semisolid consistency. It is cooled slowly which causes the crystallization of barbaloin within the semisolid mass. It is opaque and packed in gourds and allowed to become more solidified. This aloe is known as Curacao or Barbados aloe. It is also called as hepatic or livery aloe.

2. Cape Aloe – It is obtained from *Aloe ferox* and its hybrids in South Africa. The cut leaves are arranged in circular manner in the basin shaped depression dug in the ground which is lined with goat skin or canvas. They are kept in this position till the juice is collected. Then the juice is transferred to a copper evaporating pan and it is heated till the juice acquires desired concentration and finally poured into wooden cases. It becomes a solid mass and is known as Cape aloes.

3. Socotrine Aloes – It is prepared from *Aloe perryi*. Aloe juice is collected in goat or sheep skin and spontaneous evaporation is allowed for a month till it becomes a viscous pasty mass. It is exported in pasty conditions and is known as Socotrine aloes.

4. Zanzibar Aloes – The botanical source is not correctly known but it is prepared by similar manner as that of Socotrine aloes. It is packed in the skins of carnivorous animals where it solidifies and than packed in wooden boxes. It is also called as Monkey skin aloe.

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Description –

	Form	Colour	Odour	Taste	Fracture
Curacao	Opaque masses	Brownish black	Strong and pleasant	Disagreeable bitter taste	Uneven and wax like
Socotrine	Opaque	Reddish-black to brownish black	Slight and disagreeable	Disagreeable bitter taste	Conchoidal
Cape	Transparent and glassy	Dark brown or greenish brown	Sour but distinct odour	Nauseating and bitter taste	Smooth even and glassy
Zanzibar	Opaque	Liver brown colour	Pleasant like myrrh	Bitter	Smooth and even fracture

MICROSCOPY

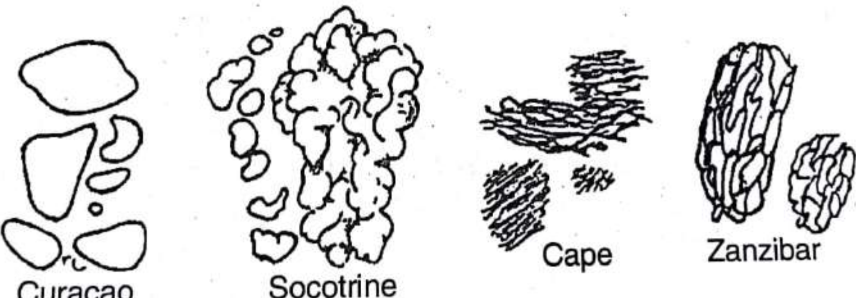
All the four aloes can be identified in powdered form through microscopical examination by mounting the sample in lactophenol.

(i) Curacao aloes – These aloes shows the fragments composed of large number of slender prisms or needles.

(ii) Socotrine aloes – It shows the fragments composed of large prisms grouped irregularly.

(iii) Cape aloes – These are characterized as transparent, brown, angular or irregular fragments.

(iv) Zanzibar aloes – These are characterized as irregular lumps with embedded nodular masses.



Microscopic characters of aloe

Chemical Constituents – Aloes are the major sources of anthraquinone glycosides. The chief constituent of the aloe is aloin and it is present up to an extent of 30%. Aloin is a mixture of three isomers namely barbaloin, b-barbaloin and iso-barbaloin. The proportion of these isomers varies in different commercial varieties of aloes.

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Barbaloin ($C_{21}H_{20}O_9$) is a crystalline water soluble glycoside and is present in all varieties of aloes. It does not get hydrolyzed by heating with dil. acids or alkalies. However it can be decomposed by heating with acid in presence of $FeCl_3$ to yield aloe-emodin and arabinose.

β-barbaloin is amorphous and is produced from barbaloin by heating at 165°C. It is present in cape aloes to the extent of 8%.

Iso – barbaloin is a crystalline isomeric glycoside present in curacao aloe and in traces in cape aloe where as it is completely absent in socotrine aloe.

Aloe also contains aloesone, aloetic acid, chrysophanic acid, chrysamminic acid, glactouronic acid, choline, saponins, and coniferyl alcohol. Aloe also contains a resin ester formed from p-coumaric acid with aloe resinotanol which is known as aloe-resin.

Barbaloin

Aloe-emodin

Aloe resin

Standards

Loss on drying- Losses not more than 10% of its weight when dried to constant weight at 105°

Ash – Not more than 5%

Chemical Test

1. Modified Borntrager's test – To 0.1gm of the drug add 5ml of 5% solution of ferric chloride and 5ml of dil Hcl and heat it on water bath for 5 minutes. Cool the solution and filter it. Filtrate is shaken with an organic solvent like benzene. Separate the benzene layer and add equal volume of dil. ammonia. A pinkish red colour is formed in ammonical layer. This confirms the anthraquinone glycosides.

2. Borax Test – 0.5gm of the drug is boiled with 50ml of water. Add 0.5gm of kiesel-

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guhr. Stir it well and filter it through filter paper. Filtrate is divided into two parts. For one part of the filtrate borax test is done and the second part is kept for bromine test-

To the filtrate add 0.2gm of borax and heat. Form this solution take about 10 drops and dilute it to 10ml in test tube and see against ordinary day light. Green fluorescence is observed due to presence of aloe - emodin.

3. Bromine test – Add equal volume of bromine solution to solution of aloe. Bulky yellow precipitate of tetrabromaloin is formed.

4. Nitrous acid test – Prepare the aqueous solution of aloes and crystals of sodium nitrite along with small quantity of acetic acid is added. The observations are under mentioned -

(i) Curacao aloes – Sharp pink to carmine colour.

(ii) Cape aloes - Faint pink colour.

(iii) Socotrine and Zanzibar aloes – Less change in colour This test is due to isobarbaloin.

5. Nitric Acid Test – To 5ml of solution of aloes 2ml of conc. nitric acid is added. Following observations are there –

(i) Curacao aloes – Deep brownish red colour

(ii) Socotrine aloes – Pale brownish yellow colour

(iii) Cape aloes – First brown changing to green

(iv) Zanzibar aloes – Yellowish brown colour.

6. Cupraloin test – To dilute aqueous solution of aloes a drop of saturated copper sulphate solution is added followed by small quantity of sodium chloride and excess of 90% alcohol. Following observations are there-

(i) Curacao aloes – Wine red colour

(ii) Socotrine aloes – No colour

(iii) Cape aloes – Faint coloration rapidly changes to yellow.

(iv) Zanzibar aloes – No colour

Uses – Aloe and aloin are used as purgative because of its intensely irritating effects on delicate mucosal lining. Rarely aloe is administered alone. If used alone it causes griping therefore it is usually combined with carminatives or antispasmodics. Ointment of aloe gel is used in sun burns, thermal burns, radiation burns, abrasions and skin irritations.

Aloe is one of the ingredient of compound benzoic tincture in which it is pharmaceutical adjunct.

Aloo vera

Dose -

Aloes powder - 0.1 to 0.3 gm

Aloin - 15 to 60 mg.

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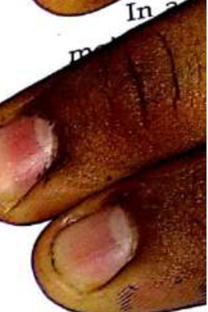

Indian aloe – Indian aloe is obtained from *Aloe vera* var. *officinalis*. This is probably the same species as *Aloe barbadensis* and is found on the coasts of Bombay, Gujarat and Madras. Indian aloe is darker in colour and harder and it resembles to socotrine or cape aloes. Iso-barbaloin is absent in Indian aloe and percentage of aloin in it is about 4%.

During the last few years *Aloe vera* has attained such a reputation that its use is confined to natural cosmetics and health food industries. Many of the cosmetic companies have incorporated *Aloe vera* in there products. *Aloe vera* gel is well known for its ability to relieve pain due to sun burn or thermal burns and is also used to treat wounds, skin irritations with cut and bruises.

Adulterants ad Substitutes –

(i) Natal Aloes – It resembles to cape aloes in microscopic characters therefore it is used as substitute. It is a weak purgative.

(ii) Mocha Aloes – It is brittle, black and glassy aloe with strong odour.

KINO EAST INDIAN, MALABAR, MADRAS, OR COCHIN KINO

Sources. Malabar kino is the juice obtained from incisions in the trunk *Pterocarpus marsupium* Roxburgh, family Leguminose, evaporated to dryness. The tree grows in Southern india and Ceylon.

Collection and Preparation. The phloem of the tree contains, according to v. Hohnel, numerous comparatively wide and short tubular arranged in axial rows; these cells are filled with a red astringent which flowfrom them when they are wounded. Vertical incisions, oblique lateral ones running into them, are accordingly made in the juice that flows is collected in small cups made of leaves, or in other convenient receptacles, and soon dries in the sun to a dark mass that readily breaks up into small angular grains. It is sometimes boiled before it is evaporated, an operation that modifies the subsequent behaviour. The drug. Kino has been imported as a trendy liquid which can easily dried.

Description. Kino occurs in small, glistening, angular grains that appear quite black and are remarkably free from dust; the grains are about 5 mm. in diameter or sometimes as much as 10 mm. When thin laminae or the edges of the grains are examined they are seen to be transparent and of a dark ruby-red coLour. They are hard and brittle, breaking with vitreous fracture and yielding a brownish-red powder. The drug is odourless, but has, when chewed, an astringent taste, and adheres to teeth, colouring the saliva red.

In cold water kino is only partially (from 80 to 90 per cent.) soluble it dissolves to a greater extent in hot water, and is almost entirely soluble in alcohol, 90 per cent. The aqueous solution turns green on the addition of a ferrous salt, violet with an alkali, and throws down a precipitate (kinotannic acid) when acidified with a mineral acid.

Constituents, The principal constituent of kino is kinotannic acid of which it is regarded as containing from 70 to 80 per cent. The reported assays vary within very wide limits (24 to 96 per cent.) ; Hooper found 24.9 per cent. by the cinchonine method and 28.8 per cent, by hide-powder method. A change from kinotannic acid to kino-red commences immediately the juice is exposed to the air, as evinced by darkening in colour, and may proceed rapidly in solutions of the which may gelatinise owing to the formation of insoluble kino-red. It is caused by the presence of an oxydase enzyme, and may be prevented destroying the activity

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of the enzyme by boiling the juice or the solution of the drug. Hence the boiling of the juice before evaporation is a rational procedure. When fused with caustic potash kinotannic acid yields phloroglucin and protocatechuic acid; it appears, therefore, to be allied to quercitannic acid.

In addition to kinotannic acid and kino-red the drug contains about 10-15 percent of moisture, and small quantities of pyrocatechin (catechol gallic acid, and mineral constituents (ash 1.5 percent).

Uses. Kino is a powerful astringent; it is given internally for diarrhoea and dysentery and is also used externally.

Substitutes

1. *Botany Bay kino* from various species of *Eucalyptus* (Australia), the most suitable being *E. calophylla* R. Brown, family Myrtaceae, the tannin of which does not gelatinise. The drug occurs in irregular dark red piece.
2. *African kino* from *Pteroccarpus erinaceus* Poiret, family Leguminosae in West Africa. It contains about 60 per cent, of kinotannic acid closely resembles Malabar kino.
3. *Jamaica kino* is an extract obtained by evaporating a decoction of the leaves, wood and bark of *Coccoloba uvifera* Linn., family Polygonaceae.

RED GUM

RED GUM EUCALYPTUS KINO, GUMNI EUCALYPTI

Sources. Eucalyptus Kino, or as it is commonly termed "red gum",. variety of Australian kino obtained from *Eucalyptus rostrata* Schiech sandal and other species (*E. marginata Smith, E. amygdalina Labillardier)* family Myrtaceae. They are all Australian trees, *E.rostrata* forming forests on the banks of the Murray River in New South Wales and yielding a valuable timber.

Collection and Preparation. *E.rostrata* is usually preferred as the source of red gum for medicinal use, because the tree is gregarious, cannot easily be mistaken for others, and yields freely a drug of good quality. The gum, which is secreted in cavities in the wood, or sometimes between the bark and the trunk of the tree, forming carbuncles, is obtained by making an incision and inserting a trough-shaped piece of tin by which the treacly liquid as it drains from the cut is carried into buckets or tins. In a few days it dries into a solid mass which soon becomes friable, breaking up into very dark fragments; or it may be evaporated by boiling, and much of thee drug is probably prepared by this method. The yield of each tree is very variable, the average being about a litre, some yielding none, others as much as 18 litres.

Description. Red gum occurs in conmerce in small irregular pieces, about 5 to 10 mm in diameter. They are dark reddish-brown, opaque, hard and brittle, and more or less dusty, but thin laminae are transparent and ruby-red, the powder being pale reddish in colour. When chewed it is somewhat tough, and has an astringent taste, colouring the saliva red and adhering to the teeth. Cold water dissolves from 80 to 90 percent. According to Brownscombe (1899) good qualities should yield not less than the latter percentage.

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Constituents. Red gum contains about 47 per cent, of kinotannic acid, which is its principal constituent. Hooper (1925) found (6.3 percent, by the cinchonine method and 20.4 per cent, by the hide powder method. There is also present kino-red, a gelatinisable tannin glycoside, catechin, pyrocatechin, and about 15 per cent, of moisture, the remainder consisting of substances not at present exactly known. According to Smith (1904), eucalyptus kinos contain two tannins giving with Ferric chloride a violet and a green reaction respectively; the former gelatinises readily but the latter does not.

Uses. Red gum is not so powerful an astringent as kino, but its action is said to be slower and more prolonged.

BUTEA GUM. BENGAL KINO, BUTEAE GUMMI

Sources. Butea gum is the juice obtained by incising the stem of *Butea monosperma* (Lam.) o. Kuntze, family Leguminosae, and subsequently dried.

Description. The drug usually occurs in small, irregular, angular fragments to one side of which dull, buff-coloured portions of the cortex and cork of the stem sometimes adhere. When fresh it is ruby-red, transparent in small fragments, and brittle; but on keeping it becomes dull, nearly black, opaque and tough. It is readily reduced to a reddish powder and has an astringent taste. It is partially soluble in water and in alcohol.

Constituents. The chief constituent is kinotannic acid (15 to 62 percent.) ; the insoluble matter may vary from 10 to 46 per cent.

Uses. Butea Gum is powerful astringent, it is given internally for diarrhoea & dysentery and is also used externally.

Dried Extracts : The drugs that are prepared by evaporating aqueous decoctions of parts of certain plants or animals are termed as dried extracts.

PALE CATECHU

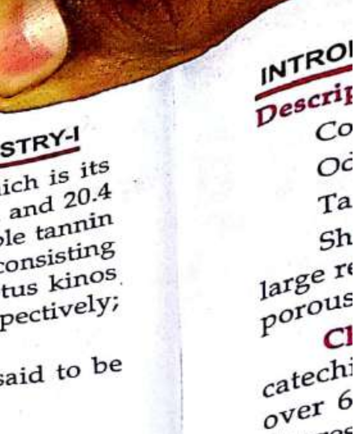
Synonyms – Gambier, Catechu, Terra japonica.

Biological Source – Pale Catechu or Gambier is a dried aqueous extract prepared from the leaves and young twigs of *Uncaria gambier* Roxburgh, family Rubiaceae.

Geographical Source – The plant is a climbing shrub and native of Malaya. It is cultivated in Singapore, Indonesia and Borneo.

Cultivation and Collection = The cultivation is done up to an altitude of 170-180meters. The propagation is made by sowing the seeds in nursery. After nine months seedlings are transplanted in the fields at distance of 3 meters. When the plants are of two years the first harvesting is done by cutting the leaves and young twigs. The plant provides the best drug after seven years and continues up to twenty years.

Preparation – The leaves and young twigs are collected and put into "Cauldron" which consists of water. The bottom of cauldron is made up of iron and sides are of hard wood. The contents are boiled for about 3hrs and decoction obtained is concentrated till it acquires a pasty consistency of yellowish green colour. Then it is cut into cubes and dried in sun.



Description

Colour – Externally redd y pale brown

Odour – None

Taste – First bitter and astringen weet.

Shape – Gambier occurs in cubes, de of which is about 2 to 3cm or sometimes in large rectangular blocks about 4cm long or in irregular broken pieces. Internally gambier is porous.

Chemical Constituents – Gambier contains condensed tannins. It contains 7 to 33% of catechin, and 22 to 50% of catechutannic acid. These two substances constitute together over 60% of the drug. Other constituents of the drug are catechu red, quercetin and a fluorescent substance called as gambier-fluorescin. Brown substances rubinic acid and japonic acid of unknown chemical nature are also present.

Catechin

Chemical Test

1. **Gambier-fluorescin test** – The drug is extracted with alcohol and filtered. To the filtrate add sodium hydroxide and few drops of light petroleum. It is shaken and kept aside for sometime. Petroleum layer shows green fluorescence. Black catechu does not show this test.
2. 0.5g of the drug is heated with 5ml of chloroform on water bath and filtered in porcelain dish. The filtrate is evaporated to dryness. A greenish yellow residue because of chlorophyll is present.
3. Match stick test – Wooden match stick is dipped in decoction of pale catechu and dried. Dip it in hydrochloric acid and warm near the flame. A purple or magenta colour is produced due to conversion of catechin into phloroglucinol.
4. Vanillin hydrochloric acid test – Prepare a solution containing vanillin 1ml, alcohol 10ml and dil hydrochloric acid 10ml. To the drug add small quantity of this solution. Gambier shows pink or red colour due to formation of phloroglucinol.
5. To the drug add ferric chloride solution, a green colour is produced due to catechutannic acid.

Uses – Gambier is used as local astringent in the form of lozenges and as an astringent in treatment of diarrhoea. It is also used in dyeing and tanning industries.

Adulterants – Pale Catechu is adulterated with mineral matter (ferric hydroxide, clay etc), astringent extracts and starch.

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BLACK CATECHU

Synonyms – Cutch, Catechu nigrum, Catechu; Katha (Hindi).

Biological Source – Black catechu consists of the dried aqueous extract prepared from the heart-wood of *Acacia catechu* Willd and *Acacia chundra* Willd, family Leguminosae.

Geographical Source – *A. catechu* and *A. chundra* are found wild and cultivated in India and Myanmar.

Preparation of Black Catechu – Katha and cutch are two different products of the same plant. Katha is rich in catechin and cutch is rich in catechutannic acid.

The tree is felled and bark and sapwood are removed from the trunk. The heartwood is cut into small pieces and boiled in water in earthen pots. The decoction is filtered and concentrated in iron vessels until it acquires syrupy consistency. It is cooled by refrigeration and then centrifuged to isolate the cake of katha. The cake is cut into various sizes and then dried. The mother liquor left during centrifugation is concentrated and cooled which finally gives cutch.

Description –

Colour – Dark brown to almost black

Odour – Odourless

Taste – First bitter then sweet and astringent

Shape – Cubes or irregular masses

Size – Each side of cube is 2 to 3cm long.

Feature – Externally it is rough and dull or rarely glossy, frequently having pieces of brownish buft leaves attached to them.Internally they are soft and porous.

Chemical Constituents – Black catechu contains 25 to 35% of catechu tannic acid and 10 to 12% of acacatechin. Acacatechin is also called as acacia catechin. The other constituents of the drug are catechu-red, quercetin and 20 to 30% of gum. The drug does not contain chlorophyll and a fluorescent substance as in pale catechu.

Catechin

Standards

Ash – Not more than 6%

Loss on drying – Losses not more than 12% of its weight when dried to constant weight at 105°

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Alcohol insoluble residue – Leaves not more than 40% of residue when exhausted with alcohol (90%) and dried to constant weight at 105°

Chemical Test –

1. Match stick test and vanillin hydrochloric acid test are positive in black catechu which are descried under pale catechu.
2. Add a few drops of fresh aqueous extract to 10ml of lime water. A brown colour is produced and on standing for three minutes a red ppt. is formed.
3. To 5ml of a 1% w/v solution, add 1ml of a 0.1% w/v solution of ferric ammonium sulphate; a dark green colour is produced. Add solution of sodium hydroxide, the colour changes to purple.
4. Warm 0.3g with 2ml of 90% alcohol; cool and filter it. To the filtrate add 2ml of solution of sodium hydroxide and few drops of light petroleum. Shake and allow to separate. A brilliant green fluorescence is not produced in upper layer. (This test distinguishes black catechu from gambier).

Uses – Katha posses an astringent, cooling and digestive properties. It is beneficial in cough and diarrhoea. It is applied externally to ulcers, boils and eruptions of the skin.

Cutch is mainly used in dyeing and tanning industries. It is much used for tanning fishing nets.

Dose - (*Acacia catechu*) - Crude- 3 to 10 gms.

Dried extract - 2.5 to 5 ml

CURARE

Synonyms – Qurari, Urari

Biological Source – The term curare is used for the poisonous extract obtained from the plants found in Amazon region. They are all arrow poisons prepared by the tribes. The plants which yield curare are viz *Strychnous toxifera, S.gubleri, S.castelnoei, S.crevauxii* (family Loganiaceae) and *Chondodendron tomentosum* (family Menispermaceae).

Geographical Source – All the plants are found in Brazil, Peru, Venezuela, Columbia and Guiana.

Description – Curare appears to be a dark brown or nearly black extract and contains small cavities. It is odourless and bitter in taste. Curare is soluble in dil alcohol and cold water.

Chemical Constituents – Curare consist of alkaloids like (+) tubocurarine, chondrocurine, isochondradendrine, curine, curarine cycleanine and tomentocurine.

(+) Tubocurarine is most potent in activity and it is a bisbenzylisoquinoline alkaloid which is derived form dopamine.

(+) Tubocurarine chloride ($C_{38}H_{44}Cl_2N_2O_6 . 5H_2O$) is a quaternary base and it is extracted from the curare extract. It is soluble in water and insoluble in organic solvents.

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(+) Tubocurarine chloride

Chemical Tests-

(1) To the saturated solution of (+) tubocurarine, ferric chloride is added. It gives green colour.

(2) To the (+) tubocurarine, mercuric nirate solution is added. It produces cherry red colour.

Uses- Curare is used to extract the alkaloids. (+) Tubocurarine chloride is used in surgical operations to secure muscular relaxation and in certain neurological conditions.

Dose - 15-30mg. intramuscularly as initial dose.

AGAR

Synonyms – Agar- agar, Japanese isinglass.

Biological Source – Agar is a dried gelatinous substance obtained from *Gelidium amansi* (Gelidaceae) and several other species of red algae like *Gracilaria* (Gracilariaceae) and *Pterocladia* (Gelidaceae).

Geographical Source – Agar is produced in Japan, Australia, U.S.A, New Zealand, South Africa, Korea and India. In India it is produced in the coastal regions of Bay of Bengal.

Collection and Preparation – In Japan the red algae is collected in May and in October. Red algae grows on rocks of ocean and is collected by diving or by rakes with long handles. Sometime the poles are also planted in the sea to encourage the growth of the algae upon them. These poles are removed and the algae is stripped off. The algae is spread upon the beach to dry. They are beaten and shaken to remove sand and shells etc and are taken to factories. Here they are washed in water, bleached by exposure to the sun and then boiled in open boilers for 5 or 6 hrs with acidulated water (about 1 part of dry algae to 55 or 60 parts of water). The liquor is filtered through cloth and transferred to wooden troughs where it is allowed to cool in the open air. On cooling a jelly is produced which is cut into bars. These bars are forced through wire netting to form the strips. The moisture is removed by successively freezing, thawing and drying at about 35°C and for this reason the manufacture is conducted in winter (November to February).

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Description -

Colour – It depends upon the shape and form. It is yellowish grey or white or nearly colourless

Odour – Odourless

Taste – Mucilaginous

Shape – It occurs in strips, sheets, flakes or coarse powder.

Size – Strips are 4mm wide, sheets are about 45 to 60cm long and 10 to 15cm wide. Strips are slender, translucent and nearly colourless where as flakes are greyish- white in colour.

Solubility – Agar is practically insoluble in cold water but swells to a gelatinous mass. It is also insoluble in organic solvents. It is soluble in boiling water.

Standards

Acid insoluble ash	– Not more than 1%
Foreign organic matter	– Not more than1%
Sulphated Ash	– Not more than 5%
Loss on drying	– Looses not more than 18% of its weight when dried to constant weight at 105°

Chemical Constituents – Agar is a heterogeneous polysaccharide and contains two principal constituents, agarose and agaropectin.

Agarose is a neutral galactose polymer (free from sulphate) and is responsible for the gel strength of agar. It consists of alternate residues of 3,6- anhydro-L-galactose and D-galactose. It contains about 3.5% of cellulose and 6% of nitrogen containing substance. Agaropectin is responsible for the viscosity of agar solutions and it appears to be a sulphonated polysaccharide in which galactose and uronic acid units are partly esterified with sulphuric acid.

Identification Test-

(i) Boil 1.5g of the drug with 100ml of water and cool it to room temperature. A stiff gel is produced.

(ii) To 0.2% solution of agar in water add 1ml of hot solution of tannic acid. No precipitate is produced.

(iii) Mount a small quantity of powder in solution of ruthenium red and examine microscopically. The particles acquire a pink colour.

Uses – Agar is largely used for the preparation of bacteriological culture media. It is used as an emulsifying agent and in the treatment of chronic constipation. It is employed in the preparation of jellies and confectionery items. Both agar and agarose find extensive use in affinity chromatography.

SODIUM ALGINATE

Synonyms – Algin, Sodium polymannuronate.

Biological Source – Sodium alginate is the sodium salt of alginic acid. It is a polysaccharide extracted from giant brown seaweed of *Macrocystis pyrifera* (L) (Lessoniaceae)

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or from horsetail kelp *Laminaria digitata* (L) (Laminariaceae) or from sugar kelp *Laminaria saccharina* (L).

Alginic acid is a polyuronic acid composed of reduced mannuronic acid and glucoronic acids.

Geographical Source – The sea-weeds are found in Atlantic and Pacific oceans especially on the coastal lines of U.S.A, Australia, U.K, Scotland, and Canada. In India they are found on the coasts of Saurashtra.

Preparation of Sodium Alginate – The sea weeds are dried and washed with faintly acidulated water. They are chopped and bruised in a hammer mill. The sea weeds are macerated with dilute sodium carbonate solution which results in a pasty mass. It is then diluted with soft water to separate the insoluble matter and filtered. Solution of calcium chloride is added to the filtrate which precipitates out calcium alginate and is removed. It is treated with hydrochloric acid which precipitates the alginic acid. This alginic acid is neutralized with sodium carbonate which produces sodium alginate.

Description

Colour – White or slightly yellowish powder

Odour – Odourless

Taste – Tasteless

Solubility – It is soluble in water and forms a viscous, colloidal solution. Insoluble in alcohol, chloroform and ether.

Sodium alginate occurs as coarse or fine powder. It is incompatible with calcium salts, phenyl mercuric acetate and nitrate. It looses about 20% of its weight on drying.

Alginic acid is a linear polymer of b-(1®4)-D- mannosyluronic acid and a-(1®4)-L-gulosyluronic acid residues. It is tasteless and very slightly soluble in water. It is insoluble in alcohol, ether and chloroform. It is capable of absorbing 200-300 times its weight of water.

Identification Test -

1% solution in water forms heavy gelatinous precipitate with dil sulphuric acid.

Uses – It is used as suspending, thickening and emulsifying agent. It is employed as binding and disintegrating agent in tablets and lozenges. It is used in manufacture of jellies and ice-creams. It is used for the flocculation of solids in water treatment. Externally it is used as haemostatic.

GELATIN

Synonym – Gelatinum

Biological Source – Gelatin is a protein obtained by the partial hydrolysis of collagenous tissue derived from the skin, bones, tendons and ligaments of animals.

Preparation – The raw materials like skin and tendons are treated with soda lime for 10 to 40 days so that the fatty material attached gets saponified and is removed by washing with water. The bones are defatted with an organic solvent like benzene and sometime decalcified by treatment with hydrochloric acid. This preliminary treatment gives the collagen material. The treated material from skin, tendons and bones is heated with water at 85°C in

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open pans. This converts the collagen to gelatin which dissolves. An electrolyte solution is added to remove the impurities by sedimentation. The crude gelatin solution is decolorized with charcoal or kieselguhr. The clear liquid obtained is concentrated under reduced pressure to a gelatin content of about 45% and allowed to set in shallow trays. These trays are passed through a series of drying rooms at temperature of about 30,40,50, and 60°C. This drying process takes 3 to 4 weeks and removes the moisture content.

Description

Colour – Colourless or pale yellowish translucent sheets, flakes, shreds or coarse to fine powder.

Odour – Slight

Taste – Slight

Solubility – It is practically insoluble in cold water but swells and softens when immersed in it, gradually absorbing from 5 to 10 times its own weight. It is soluble in hot water and practically insoluble in alcohol, chloroform and solvent ether. It is soluble in hot mixture of glycerine and water and in acetic acid.

Gelatin is stable in air when dry but is subjected to microbial decomposition when moist or in solution. The quality of gelatin is judged by its 'Bloom strength' or 'Jelly strength' which is determined by a Bloom gelometer as mentioned in British pharmacopoeia. Gelatin is hard and brittle. If broken it first bends and then breaks suddenly with a short fracture.

Constituents – Gelatin mainly consists of protein glutin. The jelly formation is due to the presence of this nitrogenous substance. It contains different amino acids of which major is lysine but does not contain tryptophan. Gelatin should be free from chondrin.

Standards –

Ash – Not more than 3.25%

Loss on drying – Not more than 16%

Heavy metals – Not more than 50ppm

Identification Tests –

(1) When gelatin is heated with soda lime it evolves ammonia vapours which confirms the presence of nitrogen.

(2) If gelatin dissolves in acetic acid then it confirms the absence of chondrin.

(3) A dilute aqueous solution of gelatin yields a precipitate with picric acid and with tannic acid solution but not with other dilute solution of lead acetate, solution of alum or solution of ferric chloride.

Uses – Gelatin is used to prepare hard and soft capsule shells. In the manufacture of tablets it is used as a coating material and as binding agent. It is also used for preparing pastes, pessaries, suppositories and pastiles. Specially purified and pyrogen free gelatins are available for intravenous injections. Gelatin is also employed in the preparation of bacteriological culture media. It is used as haemostatic in the form of absorbable gelatin sponge.

Storage – Preserve gelatin in well-closed container in a dry place.

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LITMUS, LACMUM

Litmus is a colouring matter obtained from various lichens of the subclass Ascolichenes, chiefly *Roccella tinctoria de Candolle* (*Cape Verde R. montagnei Bel. (Mudagascar), Ochrolechia leucophaea Linn.*, etc.

Preparation. The method of preparation is guarded as a trade secret but it appears to depend mainly upon the slow fermentation of the soaked and ground lichen in the presence of ammonium and potassium carbonates. A red colour is first produced which gradually changes to blue. The blue liquid is drawn off and evaporated, with the addition of chalk and gypsum, the mass is then cut into small rectnauglar cakes and dried. The cakes have an edge of about 6 mm; they are dark blue to bluish-violet,finely granular and friable. Litmus used as an indicator in acidimetry has a pH range of 5 to 8.

Constituents. The chief constituents are erythrolitmin and azolitmin together with eryhtrolein and spaniolitmin. The lichens themselves contain lecanoric acid, erythrin and orcin; by the action of alkalies, these yield orsellinic acid; orsellinic acid by further change yields orsin, from which, by oxidation in the presence of ammonia, the colouring matters are produced.

Cudbear (Persio) is a reddish colouring matter prepared by an analogous method from similar lichens, one of which is *Leconora tartarea Ach.*

Gums & Muciloges :

Gums – Gums are amorphous, translucent substances yielded by the trees and shrubs. They are the abnormal or pathological products produced as a result of injury or unfavourable conditions of growth and are usually formed by changes in cell walls, presumably by means of enzymes and bacterias. The change in cell walls and the exudation of gum is called gummosis.

Chemically, gums consist of calcium, potassium and magnesium salts of complex substance known as polyuronides. On prolonged boiling with dil. acids they yield mixture of sugars (pentoses or hexoses) and organic acids. Gums are either soluble in water to yield viscous adhesive solution or by absorbing water to form jelly like mass. However they are insoluble in alcohol and in most of the organic solvents. They are produced by plants belonging to the families such as Rutaceae, Leguminosae, Combretaceae, and Sterculiaceae etc.

Classification - Gums are classified on the basis of occurrence as mentioned below-

Natural Gums – It includes like acacia, tragacanth, guar gum, algin, pectin and chitin.

Prepared Gums- It includes like starch and its derivatives, cellulose derivatives and dextran etc.

Mucilages – Mucilages are the normal products of the cell and are produced without injury to plant. They are related to the gums and are normally sulphuric acid esters; the ester group is a complex polysaccharide. With water they swell and due to this swelling property they are utilized for their assay like swelling factor. Mucilages may be neutral or acidic or mixture of both. Mucilages and gums are related to hemicelluloses in composition.

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INDIAN GUM

Synonyms – Acacia, Gum acacia, Gum arabic, Babul or Kikar gond (Hindi).

Biological Source – Indian gum is the dried gummy exudation obtained from the stem and branches of *Acacia arabica* (Lam.) Willd, family Leguminosae.

Geographical Source – It is a medium size tree with a short trunk usually attaining a height up to 15meters and found throughout drier regions of India like Rajasthan, Gujarat, Punjab, Andhra Pradesh etc. The tree is also found in Srilanka.

Cultivation and Collection – The tree is not cultivated on commercial scale. Gum is collected from wild grown plants. The procedure for collection of gum is commenced as the rainy season is over. The bark of wild growing trees is tapped and transverse cuts are given to the stem and branches to expose cambium. Due to this injury tears of gum are collected on the cambium and newly formed phloem. After 20-30 days the tears of gum which have formed on the surface may be picked off. They are made free from bark pieces and other foreign organic matter. The tears are dried in the sun and due to drying numerous cracks develop on the surface of lumps of tears and the gum is bleached.

Description –

Colour- Tears are cream-brown or red in colour. Powder is light brown in colour

Odour – Odourless

Taste – Bland and mucilaginous

Shape and Size – Irregular and broken tears of varying size

Solubility – It is almost entirely soluble in twice its weight of water.

Practically insoluble in alcohol.

Acacia Arabic

Standards

Total Ash – Not more than 5%

Acid insoluble ash – Not more than 1%

Loss on drying – It should loose not more than 15% of its weight when dried to constant weight at 105°C

Moisture – Not more than 15%

Chemical Constituents – Acacia contains mainly arabin which is a complex mixture of calcium, magnesium and potassium salts of arabic acid. Arabic acid on hydrolysis with dil. sulphuric acid yields D-galactose, D-glucuronic acid, L-arabinose and L-rhamnose. Acacia also contains an enzyme oxidase and peroxidase.

Identification Test –

1. An aqueous solution of gum is gelatinsed by the addition of solution of lead sub acetate.
2. Acacia does not produce a pink colour with the solution of ruthenium red. *

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3. Dissolve 0.25g of drug in 5ml of water. Add 0.5ml of solution of hydrogen peroxide and 0.5ml of a 1% w/v solution of benzidine in alcohol (90%). Shake it and allow to stand for sometime. A blue colour is produced due to oxidase enzyme.

Uses – Acacia is a demulcent. It is used as an emulsifying agent for fixed oils and volatile oils. It is employed as a suspending agent especially in mixture with resinous substance. Acacia is a good binding agent and is used in the preparation of compressed tablets, pastillies and lozenges. It's demulcent properties are employed in cough, diarrhoea and throat preparations. Because of the compatibility of acacia with all plant hydrocolloids, starches and carbohydrates it is widely used in food, drinks and other industries.

Storage – Acacia or powdered acacia should be stored in cool dry place in air tight containers.

Substitutes and Adulterants -

1. *Acacia senegal* - The plant grows in Africa. The tears are round or ovoid, 0.5 to 6cm in diameter, often white or sometime yellow in colour and opaque. It is substituted for Indian gum.
2. Indian gum is also adulterated with gum ghati, starch, dextrin, tragacanth and sterculia gum.

TRAGACANTH

Synonym – Gum tragacanth

Biological Source – Tragacanth is the dried gummy exudation obtained by making incisions to stems and branches of *Astragalus gummifer* Labillardiere and other species of *Astragalus*, family Leguminosae.

Geographical Source – The plants of tragacanth are thorny shrubs about 1meter in height and found in mountainous regions of Syria, Iran, Iraq Antolia, USSR and India. In India central Punjab, Garhwal and Kumaon are the regions where these plants can be found. Iran and North Syria supply Persian tragacanth and Smyrna port supply Smyrna tragacanth.

Collection – The plants from which tragacanth is collected grow at an altitude of 1000-3000meters. Gum is collected from two years old plants. The earth is removed from the base to a depth of 5cm and the exposed part is incised with a sharp knife. Normally a wedge-shaped piece of wood is used and it is left in the cut for 12-24hrs to widen the incision. Tragacanth gum is formed as a result of transformation of the cells of pith and medullary rays into gummy substance. The shape of gum depends upon the type of incision. If the incision is straight, gum is flat and ribbon shaped and if round then gum is vermiform. Gum is collected after two days of the incision.

Description

Colour – White or pale yellowish white
Odour – Odourless
Taste – Mucilaginous
Shape – It occurs in thin, flattened, curved, ribbon shaped flakes of a translucent, horny appearance.

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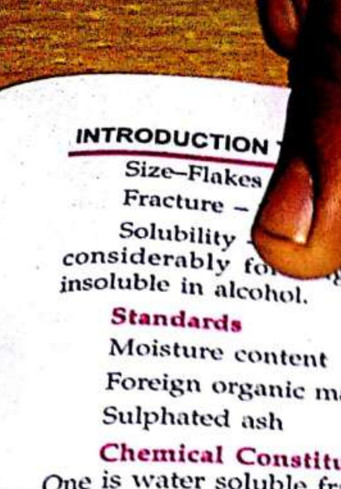

Tragacanth

Size–Flakes ... g, 1cm ... thick

Fracture – ...

Solubility – ... ly soluble in water in which it swells considerably fo... g a tena... atinous mass. It is insoluble in alcohol.

Standards

Moisture content	– Not more than 15%
Foreign organic matter	– Not more than 1%
Sulphated ash	– Not more than 4%

Chemical Constituents – Tragacanth consists of two fractions when added to water. One is water soluble fraction known as tragacanthin (8-10%) and second is water insoluble fraction called as bassorin (60-70%). Tragacanthin is a complex mixture of polysaccharides containing D-galacturonic acid, other sugars, and traces of starch and cellulose. Bassorin contains methoxy group and is responsible for the swelling and gelatinizing properties of the drug.

Identification Test –

1. Mount a small quantity of tragacanth powder in ruthenium red and examine microscopically. Tragacanth particles do not acquire pink colour where as sterculia gum stains pink.
2. Tragacanth powder when boiled with solution of potash it gives canary yellow colour. (Sterculia gum gives brown colour).
3. When solution of tragacanth is boiled with few drops of 10% aqueous ferric chloride solution, a deep yellow precipitate is formed.
4. To 0.1g of powder, add N/50 iodine. It acquires an olive green colour.

Uses – Tragacanth is used as an emollient in cosmetics. It is used as thickening, emulsifying* and suspending agent. Tragacanth is used along with acacia as a suspending agent. It is employed as binding agent in tablets and pills. It is used as an emulsifying agent for fixed oils, volatile oils and resins. Tragacanth is also used in cosmetic formulations, confectionary and food industries.

Substitutes –

1. Indian tragacanth (Sterculia gum or Karaya gum) – It is obtained from *Sterculia urens* Roxburgh, family Sterculiaceae and possibly other species. It occurs in irregular, striated, often vermiform, whitish or pale brownish or pinkish brown pieces. It has acetous odour.

GHATTI GUM. GUMMI INDICUM

Sources. Ghatti gum is obtained from *Anogeissus latifolia* Wallich, family Combretaceae, a large tree indigenous to India and Ceylon.

Description. The gum occurs in verrniform or rounded tears, the best qualities being almost colourless, the inferior yellow to dark brown. The surface is dull and somewhat rough, not exhibiting cracks, the fracture is uniform and glassy. Its aqueous solution gives only a slight precipitate with solution of lead subacetate (that of acacia gum gives a copious

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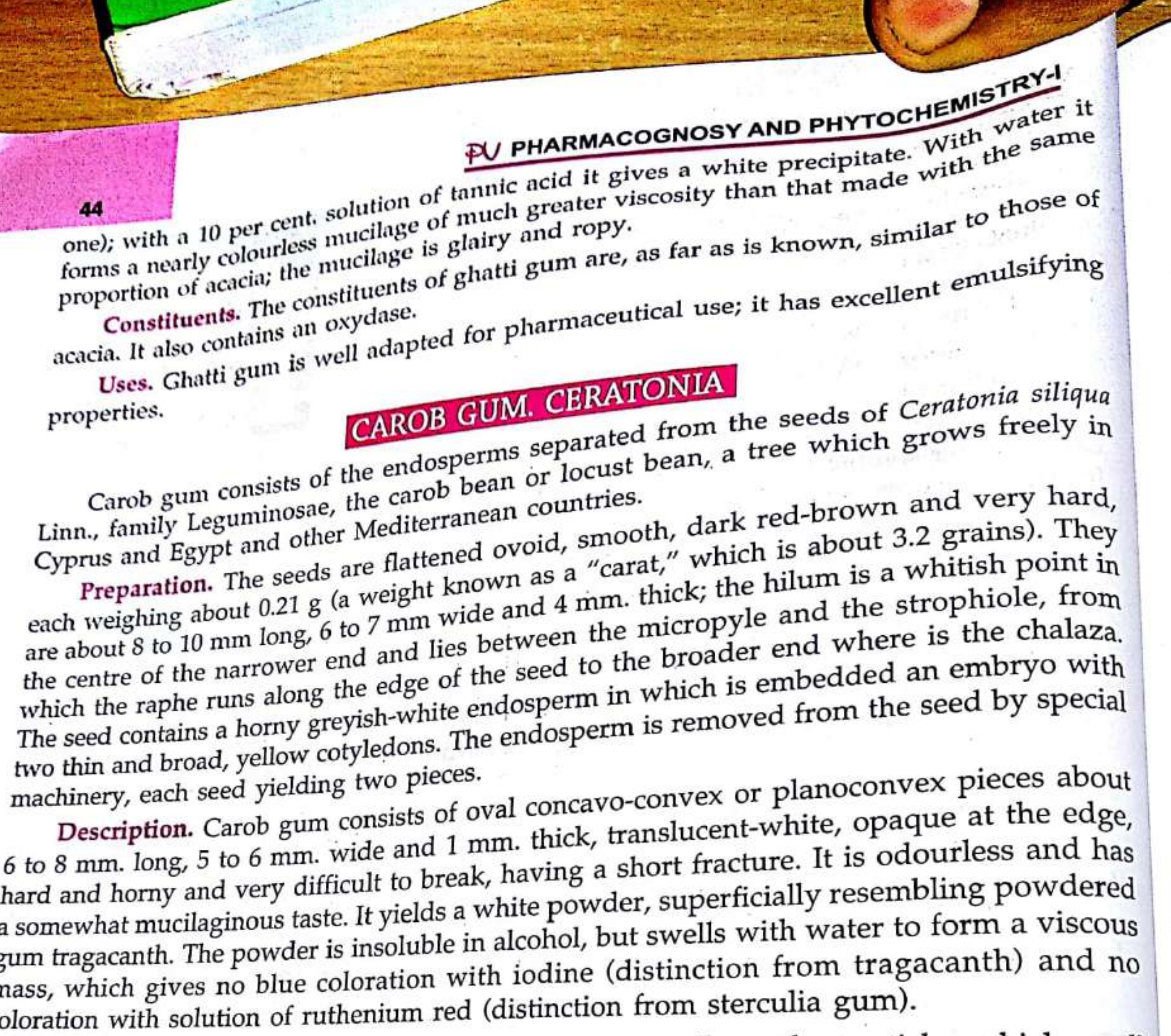

one); with a 10 per cent. solution of tannic acid it gives a white precipitate. With water it forms a nearly colourless mucilage of much greater viscosity than that made with the same proportion of acacia; the mucilage is glairy and ropy.

Constituents. The constituents of ghatti gum are, as far as is known, similar to those of acacia. It also contains an oxydase.

Uses. Ghatti gum is well adapted for pharmaceutical use; it has excellent emulsifying properties.

CAROB GUM. CERATONIA

Carob gum consists of the endosperms separated from the seeds of *Ceratonia siliqua* Linn., family Leguminosae, the carob bean or locust bean, a tree which grows freely in Cyprus and Egypt and other Mediterranean countries.

Preparation. The seeds are flattened ovoid, smooth, dark red-brown and very hard, each weighing about 0.21 g (a weight known as a "carat," which is about 3.2 grains). They are about 8 to 10 mm long, 6 to 7 mm wide and 4 mm. thick; the hilum is a whitish point in the centre of the narrower end and lies between the micropyle and the strophiole, from which the raphe runs along the edge of the seed to the broader end where is the chalaza. The seed contains a horny greyish-white endosperm in which is embedded an embryo with two thin and broad, yellow cotyledons. The endosperm is removed from the seed by special machinery, each seed yielding two pieces.

Description. Carob gum consists of oval concavo-convex or planoconvex pieces about 6 to 8 mm. long, 5 to 6 mm. wide and 1 mm. thick, translucent-white, opaque at the edge, hard and horny and very difficult to break, having a short fracture. It is odourless and has a somewhat mucilaginous taste. It yields a white powder, superficially resembling powdered gum tragacanth. The powder is insoluble in alcohol, but swells with water to form a viscous mass, which gives no blue coloration with iodine (distinction from tragacanth) and no coloration with solution of ruthenium red (distinction from sterculia gum).

Powdered carob gum mounted in alcohol appears as small angular particles which swell rapidly when water is added. If mounted in iodine water, the granular cell contents stain deep yellow, showing the presence of protein, the cell-walls remain colourless. When mounted in solution of chioral hydrate, the swollen cell-walls are evident.

Constituents. Carob gum contains mannan, about 58 per cent., galactan, about 29 percent, pentosans, about 3 per cent., proteins, about 5 per cent., cellulose, about 4 per cent., and yields about 0.8 per cent, of ash; an oxydase is present and also an enzyme named ceratoniase. Starch and calcium oxalate are absent.

(B) RESINS AND RESIN COMBINATIONS

Resins are defined as **"the amorphous non nitrogenous products of complex chemical nature"**. Resins are the mixture of essential oil, oxygenated products of terpenes and carboxylic acids. They are the exudation products from the trunk of various trees. Resins are formed in schizogenous or schizolysigenous ducts or cavities of the plant. When the resins are produced as a normal product of metabolism without injury to the plant they are termed as normal or physiological resin like resins of pinus. If the resins are produced by

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injury or wound to the plant they are called as abnormal or pathological resin like benzoin and tolu balsam. Resins are present in different parts of the plant such as roots, rhizomes, fruits, seeds, trunk, flowers and fruiting tops etc. Chemically resins contain resin acids, resin phenol, resin alcohol, esters and inert substances. They are normally used as antiseptics, carminative, purgative, expectorant and analgesic etc. Resins are also obtained from animals e.g. shellac.

Properties

(i) Resins are transparent or translucent solids, semisolid or liquid substances.

(ii) They are insoluble in water but soluble in organic solvents like alcohol, fixed oil, volatile oil and chloral hydrate solution.

(iii) They burn with smoky flame as they contain large number of carbon atoms.

(iv) On heating they soften and finally melt.

(v) Resins have specific gravity more than one and are heavier than water.

(vi) On storage, they darken in colour.

Classification – Resins are classified into two categories as mentioned below:-

1. Chemical classification – The resins are classified on the basis of chemical constituents such as-

(i) Acid resin – These contain a large portion of carboxylic acid and phenols. They combine with alkali and their metallic salts are termed as resinates. With aqueous solution of alkali they form soap-like solution or colloidal suspension. Various examples of resin acids are abietic acid (colophony), copaivic acid and oxycopaivic acid (copiba), primaric acid (fankicense) and commiphoric acid (myrrh) etc.

(ii) Resin alcohol – Resin alcohols are also called as ressinols. They have high molecular weight and occur in both i.e. free form and combined form. Ressinols are tetracyclic or pentacyclic alcohols and are normally a-amyrine and b-amyrine derivatives. They do not give positive test with iron salts. Examples are like benzoresinol from benzoin, gurjuresinol from gurjun balsam and storesinol from storax.

(iii) Resin phenol – Resin phenols are also called as resinotannols. They also have high molecular weight and occur in both i.e. free form and combined form. The phenolic group of tannins is combined with resins acid. They give positive test with iron salts. Examples are like peruresinotannol from balsam of peru, toluressinotannols from balsam of tolu and siaressinotannol from sumatra benzoin.

(iv) Ester Resins – These are the esters of resin alcohol or resinotannol combined with resin acid or balsamic acid. Examples are cinnamyl cinnamate from storax and benzyl benzoate from benzoin.

(v) Resenes – These are the neutral and inert substances as they do not contain characteristic functional group. They do not show any specific chemical properties. They do not form salts or esters and are not hydrolyzed by alkalies. They have high molecular weight. The drugs which contain resenes are asafoetida, gutta purcha and colophony.

(vi) Glycoresins – These contain the glycosidal resins. Glycoresins on hydrolysis yields

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sugar and complex acids, e.g. is jalap resin from jalap.

2. Constituents of Resins – Resins are also classified on the basis of major constituents present either in resin or resin combination. The homogenous combination of resins with other plant products is called as resin combinations.

(i) Acid resin – This is discussed under chemical classification.

(ii) Oleo-resin – When there is a homogenous mixture of resin and volatile oil it is termed as oleo-resin like capsaicin, ginger and copaiba.

(iii) Oleo-gum-resin – These are the homogenous mixture of resin, gum and volatile oil like asafoetida, myrrh, and turmeric.

(iv) Gum resins – These are the homogenous mixture of gum and resin, e.g. gamboge.

(v) Balsams – Balsams contain benzoic acid or cinnamic acid or both. Examples are benzoin, storax and tolu balsam.

Extraction and Isolation – Resins can be extracted from plants and animals by any one method of the following:-

(i) By extraction with alcohol and then precipitating with water, e.g. ipomoea, and jalap.

(ii) As plant exudates by injury or incisions, e.g. asafoetida, myrrh etc.

(iii) By heating the plant part e.g. guaiacum.

(iv) By distillation method e.g. colophony

(v) By various treatment of the excretions obtained from animal e.g. shellac.

Identification Test – Resins can be identified by physical test and specific chemical test which are mentioned in individual drugs.

COLOPHONY

Synonyms – Colophonium, Resin, Resina, Amber resin.

Biological Source – Colophony is the residue left after the distillation of the oil of turpentine from the crude oleo resin of various species of *Pinus* like *Pinus roxburghii* or *Pinus palustris*, family Pinaceae.

Geographical Source – Colophony is prepared in Portugal, China, Morocco, Spain, France, Greece, Russia, India and U.S.A.

Method of preparation – It is discussed in turpentine oil.

Description

Colour – Pale yellow or brownish yellow

Odour – Slight turpentine

Taste – Similar to turpentine and bitter

Shape – It occurs in irregularly shaped pieces of different size.

Solubility – It is insoluble in water and freely soluble in alcohol, ether, benzene, glacial acetic acid, carbondisulphide and oils.

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Standards

Melting point – 75° to 85°C

Acid value – Not less than 150

Saponification value – 188 to 192

Ash value – Not more than 0.125%

Chemical Constituents – Colophony contains 90% of abietic acid (resin acid), 5 to 6% of resene, 0.5% of volatile oil and traces of bitter substance. The abietic acid is found in its three isomeric modifications a, b and g abietic acids. Other acids present in colophony are primaric and sapinic acids.

Abietic acid

Chemical Test

(i) 0.1g of colophony is dissolved in 10ml of acetic anhydride by gentle heating. Cool it and add 1 drop of sulphuric acid. It produces bright red colour which changes to violet.

(ii) Colophony is dissolved in light petroleum and filtered. To this two times of dil cupric acetate solution is added. Petroleum layer shows emerald green colour. Adulterations of colophony can be detected by this test.

Uses – Colophony posses stimulant and diuretic properties. It is commonly used as an ingredient of plasters and ointments. Industrially it is used in manufacturing of varnishes, paint driers, printing ink, soaps, wood polishes, cements, paper, plastics, and fire works.

Storage – Colophony should be stored in large pieces in well closed containers away from light.

SANDARAC, GUM JUNIPER, SANDARACA

Sources. Sandarac is a resin obtained from *Tetraclinis articulata* (Vahl.) Masters, family Cupressaceae, a small tree about 7 metres high, growing on the mountains in the north-west of Africa. It is usually obtained by incision, the tears when sufficiently hard being collected and exported chiefly from Mogadore.

Description, Sandarac occurs in small tears about 5 to 20 mm, long and 2 to 5 mm. thick, more or less clindrical or stalactitic in form, two or more of which are sometimes united into a small, flattened mass ; globular or pear-shaped tears are few in number. The tears have a dull, dusty surface and a pale yellowish colour; they are brittle, breaking with a glassy conchoidal fracture, and exhibiting a clear, transparent interior, in which small

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insects are occasionally embedded. The resin has a slight terebinthinate odour and a terebinthinate, slightly bitter taste; when chewed it breaks up between the teeth into a sandy powder which, unlike mastich, shows no disposition to agglomerate into a plastic mass.

It is completely soluble in ethyl and in amyl alcohol and in ether, partially only in chloroform, carbon disulphide, oil of turpentine and light petroleum.

Constituents. Sandarac consists of resin associated with traces of volatile oil, bitter principle, etc. The chief constituent of the resin is (optically) inactive pimaric acid (85 per cent.), obtainable in acicular crystals melting at 170°; other constituents are sandaracinic acid (2 percent.), amorphous callitrolic acid (10 per cent,), and sandaracoresene. Callitrolic acid is easily converted into the actone which is insoluble in alcohol.

Uses. Sandarac is chiefly used in the manufacture of varnishes; it is paler in colour than shellac, and is therefore more suitable for light woods. It is good resin for making permanent microscopical preparations.

Substitute. *Australian sandarac*, from *Callitris verrucoa* Robert Brown, is occasionally imported. The tears are softer, larger, and more aromatic than those Of African sandarac, which it otherwise resembles. Its composition is similar, but it contains more volatile oil and more inactive pimaric acid.

GUAIACUM RESIN. RESINA GUAIACI

Source, etc. Guaiacum resin is the resin obtained from the stem of *Guaiacum officinale* Linn., or *Guaiacum sanctum* Linn., family Zygophyllaceae.

Preparation. The bulk of the resin of commerce is produced in the following rather crude way from the trunk of the tree, the heartwood of which contains from 20 to 25 per cent, of resin : A log of the wood is supported in a horizontal position above the ground by two upright bars Each end of the log is then set on fire, and, a large incision having being previously made in the middle, the melted resin runs out therefrom in considerable abundance or one end of a log of wood is raised, and the fire applied to it, when the melted resin will run out of a groove cut in the other end, and may be received in potsherds (block resin). The resin may also be obtained in the form of tears by incisions made into the trunk, but the tear resin of commerce is certainly not so produced; probably it consists of the last runnings of the melted resin which solidify in the form of tears. The resin is also prepared by extracting the wood with alcohol.

Description. Guaiacum resin is usually seen in large masses of dark colour, often more or less covered with a greenish powder. The resin breaks easily with a clean, glassy fracture, thin splinters viewed by transmitted light being transparent, and varying in colour from yellowish green to reddish-brown. The powder is greyish, but becomes green by exposure to light and air. It has a slightly acrid taste, and, especially when warmed, a somewhat balsamic odour. It is freely soluble in alcohol, chlorofonn, and solution of caustic potash, incompletely in ether, and only slightly soluble in petroleum spirit, carbon disulphide, or benzene.

The resin in tears occurs in rounded masses, 2 to 3 cm. in diameter, usually covered with a greenish powder, and exhibiting the characters already detailed.

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The commercial drug is never completely soluble in alcohol. The residue, left by tear resin is about 1.5 percent., and in good samples of the lump averages about 7.5 percent. and should not exceed 10 percent., in exceptional samples, however, it may amount to as much as 25 per cent. It consists chiefly of vegetable debris, gum, etc.

Constituents. Guaiacum resin consists chiefly of a- and b-guaiaconic acids, about 70 percent., guaiaretic acid, about 11.25 percent., and a small amount of guaiacic acid. Other constituents are guaiac-b-resin, about 15 per cent., guaiac yellow, vanillin and guaiac-saponin.

α-Guaiaconic acid is a colourless amorphous substance, probably a mixture, one constituent of which is changed by oxidising agents to deep blue guaiac-blue. b-Guaiaconic acid is colourless and crystalline. Guaiaretic acid is light brown, amorphous, and insoluble in ether. Guaiac-b-resin is brown and amorphous, and appears to be chiefly a decomposition product of the guaiaconic acids; it contains the substance that yields guaiac-blue by oxidation.

Guaiacum resin is easily identified by its reaction with cxidising agents. This is best seen by dissolving a little of the resin in alcohol and adding a drop of dilute solution of ferric chloride; the liquid instantly assumes a deep blue colour which is destroyed by reducing agents, but restored by oxidising agents.

Uses. The action of guaiaeum is that of a local stimulant or, in large doses, irritant. It has been employed locally in the form of a lozenge, and has also been given in chronic gout and rheumatism. In the form of a tincture, it is used for the detection of oxidases.

BENZOIN

There are two commercial varieties of Benzoin – Sumatra Benzoin and Siam Benzoin. Benzoin is a pathological product of the tree and its formation is induced by injury to the tree.

SUMATRA BENZOIN

Synonym – Gum benjamin

Biological Source – Sumatra benzoin is a balsamic resin obtained from *Styrax benzoin* Dryand or *Styrax paralleloneurus* Perkins, family Styraceae.

Geographical Source – It is a deciduous tree of 8-9 meters in height and is cultivated in Sumatra, Java and Borneo.

Collection and Preparation – Sumatra benzoin is collected from 6-20 years old trees. The trees are tapped for resin near the base of the tree. After a week yellowish sap oozes out which is not utilized and it is removed. The subsequent flow which is white and viscous is collected; it is the finest quality and known as "almond" of the benzoin. It is used for medicinal purpose. Then the third and fourth flow which oozes out is also collected but they are darker in colour and inferior in quality. In this way whole stem is tapped by making incisions at the gap of 4cm. These three varieties are sent to the processing centers where they are mixed in definite ratios, softened in the sun and solidified into masses. A single tree yields about 10kg of material in a year.

Description

Colour – Reddish brown or greyish brown

Odour – Agreeable and balsamic

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Taste – First sweet and then slightly acrid

Shape – It occurs in masses of varying size, made up of tears.

Standards

Acid insoluble ash – Not more than – 1%

Benzoic acid content – Not less than 6%

Alcohol soluble extractive – Not less than 75%

Loss on drying – Not more than 10%

Chemical Constituents – Sumatra benzoin mainly consists of balsamic acids i.e. cinnamic and benzoic acid and esters derived from them. Triterpenoid acid like summaresinolic acid is also present. The drug contains in addition, traces of vanillin (1%), styrol, styracin, benzaldehyde and phenyl propyl cinnamate.

Benzoic acid   Cinnamic acid   Summaresinolic acid

Chemical Test –

(i) Sumatra benzoin powder (0.5g) is heated with 10ml of potassium permanganate solution. A strong odour of benzaldehyde is produced.

(ii) To 0.25g of the drug, 5ml of solvent ether is added and from it 1ml of ether solution is decanted into porcelain dish. Add 2 to 3 drops of sulphuric acid in dish; a deep reddish brown colour is produced.

Uses – Sumatra benzoin internally acts as expectorant, diuretic and carminative. Externally it acts as antiseptic, astringent and stimulant. It can be used on wounds and ulcers to tighten and disinfect the affected tissue. In pharmaceutical preparations only sumatra benzoin is used.

Storage – Preserve sumatra benzoin in well closed container.

Adulterants – Sumatra benzoin is adulterated with pieces of bark and other debris, which can be detected by determining matter insoluble in alcohol.

SIAM BENZOIN

Biological Source – Siam benzoin is a balsamic resin obtained from *Styrax tonkinensis* Craib, family Styraceae.

Geographical Source – It is a deciduous tree which grows at an altitude of 1200 to 1600 meters and is cultivated in Vietnam, Laos and Thailand.

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Collection and Preparation – Fungus play an important role in formation of resin. Incisions are made on the trunk of trees of 6-20 years old at an interval of about 5cm. First exudation is not collected but subsequent exudations are collected and sent to processing centers where they are processed and solidified. It is used in perfumeries.

Description

Colour – Yellowish brown to rusty brown

Odour – Agreeable, balsamic vanilla like

Taste – First sweet then slightly acrid

Shape – It occurs as separate tears or in form of masses composed of tears of variable size.

Standards

Acid insoluble ash – Not more than 0.5%

Benzoic acid content – Not less than 12%

Alcohol soluble extractive – Not less than 90%

Loss on drying – Not more than 10%

Foreign organic matter – Not more than 1%

Chemical Constituents – Siam benzoin mainly contains 75% of coniferyl benzoate. The drug also contains siaresinol benzoate, d- siaresinolic acid and an amorphous benzoate. Small quantities of vanillin and esters of benzoic acid are also present.

(The main difference between the siam benzoin and sumatra benzoin is that siam benzoin contains insufficient cinnamic acid and it gives insufficient odour of benzaldehyde when heated with potassium permanganate solution.)

CH = CH—CH$_2$O—OC—(phenyl), ring with OCH$_3$ and OH

Coniferyl Benzoate

Chemical Test – To 0.25g of the drug, 5ml of solvent ether is added and from it 1ml of ether solution is decanted into porcelain dish. Add 2 to 3 drops of sulphuric acid in dish; a deep purplish red colour is produced.

Uses – Externally it is used as mild disinfectant. It is also used in perfumeries and cosmetics as fixative. Internally it is used as expectorant and carminative.

Storage – Preserve siam benzoin in well closed container.

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DRAGON'S BLOOD. SANGUIS DRACONIS

Sources. Dragon's blood is a resinous secretion from the fruits of *Daemonorops propinquus* Beccari, *D. ruber* Martius, and probably other species. The two species named were formerly included in *Calamus draco* Willdenow, family Palmae; they are climbing palms with long, flexible stems, and are indigenous to Sumatra and Borneo.

Collection. The plant produces numerous small fruits about the sizeof a cherry, covered with hard, yellowish, imbricated scales, which overlap one another from apex to bsse. From between these scales a red resin, probably produced in the pulp of the fruit, exudes and more or less completely encrusts the fruit. The fruits are beaten and shaken together in sacks or baskets, and the separated resin mixed with water, pressed into moulds, and then melted ; or it is made into a cake which is wrapped in a cloth, steeped in hot water and pressed to form a solid block. It is said to be nearly always mixed with the milky juice of *Garcinia parviflora* Miquel family Guttiferae.

Description. Dragon's blood occurs in lumps of very varying size and shape. They are often large rounded masses, sometimes weighing several kilograms, bearing the impress of sacking or reed-matting, or they may be rounded, flattened cakes 10 cm. or more in diameter and about 5 cm. in thickness. Occasionally it is imported in sticks about 20 to 25 cm. long and 2 to 3 cm. thick or 30 cm. long and 1.5 cm thick, each carefully wrapped in the leaf of a species of Licuala family Palmae. These varieties are known as "lump,""saucer,""reed," etc., dragon's blood.

Good samples of the drug usually have a dull, dark red colour, and are more or less covered, where the pieces have rubbed against one another, with a crimson powder. They are brittle and friable, breaking with a glossy but irregular, uneven fracture, minute fragments being translucent and of a deep garnet-red colour.

The drug yields when crushed a bright crimson powder, has no odour and is practically tasteless, breaking up when chewed into a fine gritty powder.

Tears, in which form the drug is now seldom seen, give a glassy, conchoidal fracture, thin flakes being of a clear garnet-red colour.

Constituents. Dragon's blood consists principally of a red resin (57 per cent), a compound of dracoresinotannol (a resin-alcohol) with benzoic and benzoylacetic acids. Other constituents are white, amorphous dracoalban (2.5 per cent.), yellow dracoresene (14 per cent.), vegetable debris (18.4 per cent.), and ash (8-3 per cent.).

Uses. Dragon's blood is chiefly used for colouring varnishes, etc.

Adulterants and Substitutes. Dragon's blood is frequently adulterated both with earthy matter and with fragments of the scales of the fruits, the amount of residue insoluble in alcohol amounting sothetimes to as much as 40 per cent. of the drug. The term" dragon's blood "has also been applied to several other resins resembling Sumatra dragon's blood in appearance. They may be distinguished by their insolubility in benzene and carbon disulphide. The only one of these that appears in commerce is Socotrine dragon's blood, which is occasionally imported from Bombay and Zanzibar and is technically termed "*Zanzibar drop*" dragon's blood. It is obtained from Dracoena ombet Kotschy, family Liliaceae. It occurs in small tears or fragments seldom exceeding 2 cm. in length with a vitreous fracture, thin splinters being of a ruby-red colour. It does not evolve an odour of benzoic acid when heated, and contains no scales similar to those found in Sumatra dragon's blood.

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SBELLAC. LAC. LACCA

Sources. Shellac is a resinous substance prepared from an excretion from the bodies of scale insects of the species *Laccifer lacca* Kari, family Coccidae (Lacciferidae) of the order Hemiptera. The lac insect obtains its nourishment by piercing, with its proboscis, the outer tissues of the twigs of certain trees, including *Butea frondosa* Roxb. and *Acacia catechu* Willd., both family Leguminosae, *Schteichera trijuga* Willd., family Sapindae, *Zizyphus jujuba* Lam. and *Z. xylopyrus* Willd., family Rhamnaceae, and other trees. Lac is produced chiefly in the provinces of Orissa and Bihar.

Formation. The lac insects are orange-red and about 0.5 mm. long; the females are wingless, but the males have membranous wings and, soon after pairing with the females, they die. The fecundated females, in large numbers, become permanently attached to the twigs by their proboscides ; they rapidly increase in size and secrete a resinous matter from glands found on all parts of their bodies. Larva, in large numbers, about 1,000, develop inside the body of each female insect and the abundant resinous secretion of the closely packed insects coalesces to form a continuous mass surrounding the twigs to a thickness of about 7 mm. and embedding the insects. The larvae escape from the body of the dead parent and swarm over the branches; many are carried by the breeze or by animal agencies (bees, birds, squirrels, etc.) to other plants. Artificial infection of trees is accomplished by removing twigs with gravid females and attaching them to suitable trees.

Collection and Preparation. The encrusted twigs are taken from the trees chiefly during May to July and a second crop in October and November; they are dried in the sun, which often leads to shrinkage of the twig which falls out, leaving a tubular mass of resin; this product is known as stick-lac. The resinous crust is broken from the twigs; it is then soaked in water for twenty four hours and is thoroughly extracted by treading under foot in the troughs containing the water. The coloured water is run off, evaporated down and the residue pressed into cakes, known as lac-dye. The resin is further extracted with water or dilute solution of sodium carbonate and is finally spread out on floors to dry and bleach, thus forming the brownish product known as "*seed-lac*." The seed-lac is put into long narrow bags made of special cloth and these are heated in front of a fire and twisted so as to force the molten resin through the cloth on to tiles where it forms a flat cake which is stretched out while hot to form thin sheets of 3 mm. or less in thickness; these sheets when broken up form shellac.

Description. Shellac consists of flakes of various sizes; they are thin, brittle, translucent, often slightly curved and of a reddish-orange to a reddish-brown colour. The paler coloured kinds are considered the best and are known as T.N. shellac. Shellac is odourless and tasteless, but has a slight characteristic odour when melted. It is insoluble in water and in fixed oils and yields not more than 5 to 6 per cent, to light petroleum. It is soluble in cold alcohol, leaving not more than about 2 percent, of residue which is a measure of the waxy constituent present in the shellac; it is soluble also in caustic alkali and in solution of borax. Digested with solution of ammonia in a closed vessel, it swells to a gelatinous mass.

Shellac for pharmaceutical use should be the variety described as " Wax and Arsenic Free" which contains less than 3 parts per million of arsenic and not more than 10 parts per million of lead. Iodine value at 22° C. is 10 to 18 and the acid value 55 to 70.

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Constituents. Shellac contains from 70 to 85 per cent, of resinous matter, of which 65 per cent. is insoluble in ether and is a resinotannol ester of aleuritic acid, and 35 per cent. soluble in ether, including a yellow colouring matter named erythrolaccin. In addition shellac contains about 6 per cent, of wax soluble in light petroleum and up to 6 per cent, of a pigment, allied to that of cochineal, named laccinic or laccaic acid.

Uses. Shellac is chiefly used for making French polish, varnishes and lacquers. It is an important ingredient of cements such as sealing-wax and cements for ringing microscopical preparations. An important pharmaceutical use is for making enteric coatings for pills and tablets for which purpose it is combined with cetyl alcohol, 10 parts of each ingredient being made up to 100 fluid parts by dissolving them in acetone.

Substitutes and Adulterants. Button lac consists of rounded mssses flattened on one side, prepared by dropping portions of molten lac onto a flat surface. Garnet lac consists of broken sheets having a deep reddish colour. Both these forms are made from seed-lac.

Bleached Shellac is made by dissolving shellac in alkali, usually solution of sodium carbonate, and bleaching with sodium hypochlorite. The liquid is then acidified with sulphuric acid and the precipitate collected, and washed by kneading and pulling under hot water till free from acid. The finished product is made into sticks, which have a yellowish white colour and a silky sheen and are kept immersed in cold water. It is soluble in alcohol when freshly prepared, but becomes insoluble on exposure to the air or by long storage under water.

Colophony is sometimes added to shellac, chiefly with the object of lowering the melting point; the amounts found in adulterated samples vary from 2 to 20 per cent. It is best detected by dissolving the sample in alcohol, pouring the solution into water and collecting the precipitate on a filter paper. The dried precipitate is rubbed down with light petroleum and filtered. The filtrate is shaken with a 01 per cent, solution of copper acetate and allowed to separate when the light petroleum showN a green colour if colophony is present.

GUM-RESINS

The gum-resins consist, as their name indicates, chiefly at least of resin and gum. With these constituents, however, these are always associated small quantities of other substances such as volatile oil, bitter principle, enzyme, etc. They are secreted either in schizogenous or schizolysigenous ducts or in secretion cells ; in the former case they are formed in the epithelial cells, and discharged into the ducts in the form of milky liquids which exude when the ducts are punctured.

The gum of most of the gum-resins resembles, but is not identical with, acacia gum; very possibly it may consist of two or more glycosidal acids in varying proportions. It is always accompanied by an enzyme from which it has never yet been freed; it therefore always contains traces of nitrogen.

GAMBOGE. CAMBOGIA

Sources. Gamboge is a gum-resin obtained from *Garcinia hanburii* Hooker filius, family Guttiferae, a tree of moderate size found in Cambodia, Siam, and the southern parts of Cochin China.

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Collection. The bark of the tree contains in the cortex, as well as in the phloem, secretory ducts filled with a yellow, resinous emulsion, the two systems of ducts being connected by transverse canals at the nodes.

The gamboge is obtained by making, in the rainy season, a spiral cut in the bark from the height of about 3 metres down to the ground. Tho emulsion wells out and trickles down the incision into a hollow bamboo placed to receive it. From this it is transferred to smaller bamboos; these are set aside until, in about a month, the gamboge has solidified. It is removed from the bamboo by drying over a fire until the bamboo cracks and can be stripped off. The drug is sold to local collectors, who convey it to Bangkok or Saigon, whence it is exported to Europe.

It is occasionally formed whilst soft into cakes of various shapes or into thick sausage-like masses, which are wrapped in leaves, the impression of which they bear on their surface (Saigon gamboge).

Description. The finest qualities of gamboge occur in rolls, 3 to 5 cm. in thickness, and from 10 to 20 cm. in length, nearly cylindrical, solid or hollow in the centre, and marked externally with longitudinal striations derived from the inner surface of the bamboos in which they have been dried. The drug breaks easily, with a smooth, uniform, conchoidal fracture the freshly fractured surface having a dull gloss and being of a rich reddish-yellow or brownish-orange colour. It is easily reduced to a bright yellow powder, with little odour, but with an acrid taste.

Microscopy. Thin splinters mounted in oil exhibit a ground-mass of gum in which numerous minute granules of resin are scattered accompanied by occasional crystals of calcium oxalate and starch grains derived from the incised tissues.

Constituents. Gamboge consists essentially of a mixture of 70 to 80 percent, of resin with 15 to 25 per cent, of gum.

The resin, formerly known as cambogic acid, is soluble in alcohol, ether, chloroform, benzene, petroleum spirit, etc., as well as in solutions of alkaline hydroxides and carbonates; from its alkaline solutions it is precipitated by acids. From it three acids have Been separated, viz., α-, β– and γ-garcinolic acids, the last named being characterised by the red colour of even a very dilute alkaline solution. The gum is analogous to acacia gum; it is laevorotatory and contains an oxydase enzyme.

Rubbed with the wet finger gamboge instantly forms a yellow emulsion. It is almost completely dissolved by the successive action of alcohol and water. The yellow emulsion yielded with water becomes nearly clear and deep orange-red on the addition of ammonia.

Varieties. Pipe gamboge, as above described, is the best variety. Inferior gamboge breaks with a dull, rough, granular fracture, and the fractured surface, which often exhibits small cavities, is of a dark brownish colour.

Lump or cake gamboge consists of pipe gamboge bent and pressed whilst soft so as to form a cake; or it may occur in irregular lumps which are frequently soft in the interior and often contain abundant visible impurity in the shape of sand, small stones, etc.

Saigon gamboge is occasionally exported from Saigon in short, thick, cylindrical cakes wrapped in palm leaves.

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Uses. Gamboge produces purging and in large doses vomiting. It has been employed as a hydragogue cathartic.

Adulterants. The chief adulterants are starch, inorganic matter (such as sand, etc.), and vegetable debris. These are all easily detected by their insolubility in alcohol and water used successively or in dilute ammonia.

Indian gamboge is obtained in India from *G. morella Desrousseaux*, and resembles Siam gamboge in its essential qualities; it is used as an equivalent of gamboge in India and the neighbouring countries.

MYRRH

Synonyms – Myrrha, Gum myrrh; Bol (Hindi).

Biological Source – Myrrh is an oleo-gum resin obtained from the stem of *Commiphora molmol* Engler and from other species of *Commiphora*, family Burseraceae.

Geographical Source – It is a small tree indigenous to north-east Africa especially Somalia island. It is also found in Saudi Arabia, Iran, Abyssinia and Thailand.

Collection – The schizogenous ducts and lysigenous cavities are present in phloem and these are filled with granular oleo-gum resin. The bark of the tree is wounded and the secretion oozes out. This secretion is of yellowish white fluid which changes to reddish brown hard mass in the presence of air. The gum resin is collected in the bags made up of goat skin.

Description

Colour – Reddish brown or reddish yellow

Odour – Aromatic

Taste – Aromatic and bitter

Shape – It occurs in irregular rounded tears or in masses of agglutinated tears.

Size – Irregular rounded tears are about 3cm in diameter.

Chemical Constituents – Myrrh contains 25 to 40% of resin, 3 to 7% of volatile oil, 57 to 61% of gum, 3 to 4% of impurities and moisture. Resin contains ether insoluble and ether soluble fractions. Ether insoluble fraction contains a and b-heerabomyrrholic acids and the ether soluble fraction contains a,b and g commiphoric acid, commiphorinic acids, esters of resin acid and two phenolic resin a and b-heerabomyrrhol.

The volatile oil present is yellow in colour and contains terpenes, sesquiterpenes, cuminic aldehyde, eugenol and esters.

Myrrh should yield not more than 70% of substance insoluble in alcohol and not more than 5% of ash.

Chemical Test –

(i) Myrrh forms yellowish white emulsion when it is triturated with water.

(ii) Powder myrrh is extracted with ether and it is evaporated in dish in such a manner that it leaves thin film on the dish. Pass bromine vapours over the film; a violet colour is produced.

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Uses – Myrrh is used as stomachic, digestive, stimulant, diuretic, anthelmintic, antinflammatory and carminative. It is also used to treat amenorrhoea, dysmenorrhoea and other menstrual disorders, bronchitis, asthma, wounds, ulcers and rheumatoid arthritis. It also posses antiseptic and astringent properties and it is widely used in mouth washes.

Substitutes and Adulterants –

(i) Yemen myrrh – It is dark brown in colour, taste is bitter and odour is less aromatic then genuine myrrh.

(ii) Arabian myrrh – It occurs in small pieces and is free from white marking. Its odour is less fragrant and taste is less bitter than genuine myrrh.

(iii) Bissabol or Perfumed bdellium – It closely resembles to myrrh, yellowish in colour and exhibit white markings. It's odour and taste is different from myrrh and it does not show violet reaction with bromine vapours.

(iv) Indian bdellium – It is dark reddish brown in colour and occurs in large masses. It's odour is feeble and cedar like and taste is slightly acrid and devoid of bitterness.

(v) Gum hotai – It occurs in opaque masses and is commonly used for washing the hairs.

ASAFOETIDA

Synonyms – Gum asafoetida, Devil's dung; Heeng (Hindi)

Biological Source – Asafoetida is an oleo-gum resin obtained from rhizomes and roots of *Ferula foetida* Regel, *Ferula rubricaulis* Boiss and other species of *Ferula*, family Umbelliferae.

Geographical Source – It is a small tree of 3 meters in height and mainly cultivated in Iran, Afghanistan and Pakistan.

Collection and Preparation – A whitish, gum resinous emulsion is filled in schizogenous ducts which are present in cortex of the stem and root. After five years when the plant is about to flower in March, the stem is cut off near the crown and upper part of root is laid bare. The juice exudes from the cut surface and the cut surface is covered by dome shaped covering made up of leaves. After few days the hardened gum resin is scrapped off and again the fresh cuts are made and juice is collected as described above. This process is repeated with interval of about 8-10 days till the plant cease to produce juice. Oleo gum resin is collected and packed in containers.

Description

Colour – Dull yellow changing to reddish brown

Odour – Intense, penetrating, alliaceous odour

Taste – Bitter, acrid and alliaceous taste

Shape – Asafoetida occurs in two forms viz tears and masses. Tears are rounded or flattened more or less agglutinated together. Mass consist of agglutinated tears with foreign mass like stone, earth, pieces of roots, calcium sulphate etc and it is of inferior quality as compared to tears.

Size – Tears are 0.5 to 4cm in diameter.

Chemical Constituents – Asafoetida contains 40 to 63% of resin, 8 to 17% of volatile oil and 25% of gum. The resin contains 1.3% of free ferulic acid* and about 15% of unstable ester of ferulic acid with asaresinol. Ferulic acid on treatment with Hcl produces umbellic

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acid which losses water to form umbelliferone. The volatile oil contains secondary butyl propanyl disulphide, disulphide, trisulphides and other terpenes. The oil posses evil smell which is due to sulphur compounds of the formulae $C_7H_{14}S_2$, $C_8H_{16}S_2$, $C_7H_{14}S_3$ and $C_8H_{16}S_3$.

OH, OCH_3, CH = CHCOOH
Ferulic acid

OH, O, CH = CHC = O
Umbelliferone

HO, OH, CH = CH—COOH
Umbellic Acid

Chemical Test –

(i) When asafoetida is triturated with water it forms yellow orange emulsion.

(ii) Boil 0.5g of the drug with dil HCl and filter it into ammonia solution. A blue fluorescence is produced due to presence of umbelliferone.

(iii) When fractured surface of asafoetida is treated with sulphuric acid; a reddish brown colour is produced.

(iv) To the fractured surface add a drop of 50% nitric acid; a green colour is produced.

Uses – Asafoetida is used as carminative, antispasmodic, anthelmintic, laxative, nervine tonic and digestive. It is used to treat flatulence colic, constipation, asthma, bronchitis, whooping cough and epilepsy. It is also used as flavouring agent in sauces, pickles and curries.

AMMONIACUM. AMMONIACUM

Sources. Anunoniacum is a gum-resin exuded from the flowering and fruiting stem of *Dorema ammoniacum*, D. Don, family Umbelliferae, and probably other species, distributed throughout Persia and extending into southern Siberia. The drug is collected chiefly in central Persia.

Collection. The stems of the ammoniacum plants contain, especially in the cortex, numerous, large, schizogenous ducts full of a milky secretion. In the summer, when the plant is fruiting, it is visited by numbers of beetles, which puncture the stem and cause an abundant exudation of the secretion in the form of milky drops, some of which harden on the stem, whilst others drop on to the ground. It is collected, sorted, and exported from the Persian Gulf ports.

Description. Ammoniacum occurs in commerce in two forms—viz, *tear ammoniacum and lump ammoniacum.*

The tears are small, rounded or nodular masses varying usually from 0.5 to 3 cm. in diameter. When fresh they are of a pale, dull yellow colour, which, however, darkens by keeping. They are hard and brittle when cold, but soften when warmed. Internally the tears are opaque, and vary in colour from milky-white to pale brownish.yellow, the freshly fractured surface having a waxy lustre. The drug has a characteristic but not alliaceous odour, and a bitter, acrid taste. Triturated with water it forms a white emulsion, which is coloured deep orange-red by a solution of chlorinated soda, yellow by solution of potash,

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and, transiently, faintly violet by ferric chloride, owing to the presence of traces of free salicylic acid.

Lump ammoniacum consists of agglutinated, whitish, yellowish-grey or bluish-grey tears, mixed with varying quantities of extraneous substances, such as stores, dirt, stems and other debris of the plant and occasionally the broad, flat mericarps of the fruit, the presence of which indicates the time at which the drug was collected. The substance of the tears agrees with the description of the tears already given. Good qualities consist of tears varying in size from a pea to a hazel-nut or even larger, with a little intervening dark-coloured ground substance, and but few pieces of stem, fruits, etc. Intermediate forms composed of more or less agglutinated tears also occur.

Constituents. Ammoniacum consists of volatile oil (0.1 to 1.0 per cent.), resin (about 65 to 70 per cent.), gum (about 20 percent, moisture (2 to 12 per cent.), ash (1 per cent.), and insoluble residue (3.5 percent.)

The main constituent of the resin is a phenolic substance, ammoresinol, which was obtained in colourless crystals, m.p. 110°, and is the cause of the orange-red colour given with chlorinated soda. The gum is allied to gum acacia. Both free and combined umbeiliferone are absent from ammoniacum. The drug contains also traces of free salicylic acid Good qualities yield about 3 per cent. of ash and 65 per cent. of resin.

Uses. Ammoniacum is a stimulant, and, being excreted by the bronchial mucous surfaces, stimulates and disinfects the secretion. It is used as disinfectant expectorant in chronic bronchitis with profuse discharge, and in plasters as a stimulant to the skin.

Substitute. Persian ammoniacum is distinguished from African ammoniacum, said to be obtained in Africa from Ferula communis Lirin,. var. brevifolia, by the orange-red colour it yields with solution of chlorinated." l soda, and also by yielding a negative result with the tests for umbelliferone.

BALSAM OF TOLU

Synonyms – Tolu balsam, Balsamum tolutanum.

Biological Source – Tolu balsam is a solid or semi-solid balsam obtained by incision from the trunk of *Myroxylon balsamum* (Linn) Harms, family Leguminosae. It contains not less than 35% and not more than 50% of total balsamic acids.

Geographical Source – It is a tall tree and native of Colombia. It is cultivated in Venezuela, Cuba and West Indies.

Collection – Balsam of tolu is a pathological product. The drug is collected by making V-shaped incisions in the bark. A small gourd or similar vessel is attached under the point of V in which the transparent fluid is collected which exudes from the wound. Many incisions are made in the trunk and drug is collected. The collected liquid balsam is put into tins.

Description

Colour – Brownish yellow to brown.

Odour – Aromatic and vanilla like

Taste – Aromatic

Solubility – It is easily soluble in alcohol (90%), chloroform and acetone but partially soluble in carbondisulphide.

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Feature – When fresh it is soft and tenacious but subsequently becomes harder and brittle.

Standards

Acid value – 97 to 160
Alcohol (90%) insoluble matter – Not more than 5%
Ester value – 47 to 95
Saponification value – 170 to 230
Loss on drying – Not more than 4%

Chemical Constituents – Tolu balsam contain 80% of resin derived from resin alcohol i.e. toluresinotannol, combined with cinnamic and benzoic acids. The drug contains 13-15% of free cinnamic acid, about 8% of free benzoic acid, 7.5% of an oily liquid cinnamicin (consisting of benzyl benzoate and benzyl cinnamate) and traces of vanillin. Tolu yields 1.6 to 3% of yellow coloured fragrant volatile oil which contains styrol, toluene and other mono and sesquiterpene hydrocarbons.

Chemical Test –

(i) Dissolve 1gm of the drug in 5ml of water and heat it. Filter it and add 30mg of potassium permanganate and again heat it; the odour of benzaldehyde is produced.

(ii) Add few drops of ferric chloride to alcoholic solution of tolu balsam. Green colour is produced because of toluresinotannol.

(iii) Dissolve 1gm of drug in 10ml of alcohol by heating. It shows acidic reaction with litmus.

Uses – Tolu balsam is used as an expectorant and antiseptic. It is a common ingredient of cough mixtures. It is added as a flavouring agent in medicinal syrups, confectionaries and chewing gums.

Adulterants

(i) Colophony – Tolu balsam is adulterated by colophony. Colophony can be detected by following test:-

Dissolve tolu balsam in petroleum ether and add double volume of cupric acetate (0.1%). The petroleum ether layer acquires bright green colour, if colophony is present.

(ii) Exhausted tolu balsam – The drug is also adulterated by adding balsams from which cinnamic acid has been removed. These are known as exhausted tolu balsams.

BALSAM OF PERU

Synonyms – Peru balsam, Peruvian balsam.

Biological Source – Peru balsam is obtained from the trunk of *Myroxylon balsamum* var Pereirae, family Leguminosae, after it has been beaten and scorched.

Geographical Source – It is a tall tree about 12-30 meters in height and grows wild in tropical forests. It is native of Central America (Guatemala, San Salvador and Honduras).

Collection and Preparation – Peru balsam is a pathological product. The bark from the trees is removed in the form of strips of about 30×15cm which are beaten with back of an axe so as to remove the corky layer and wound the inner tissue. As a result ducts are formed and secretion of balsam takes place which is soaked up with rags. By this method balsam is collected from the other strips of bark and soaked up by the rags. These rags are

collected and put into strong rope bags and are pressed in such a manner that balsam is allowed to fall into hot water. The balsam sinks into hot water where as impurities float which is decanted. Finally balsam is strained and packed in tins.

Description

Colour – A viscid liquid of dark brown in colour when seen in bulk, but in thin layer it is reddish brown and transparent.

Odour – Vanilla like

Taste – Slightly bitter taste

Density – 1.140 to 1.171

Solubility – It is soluble in chloroform, glacial acetic acid and equal volume of 90% alcohol. It is insoluble in water.

Chemical Constituents – Peru balsam consists of 50-65% of oily fluid called as cinnamein which is mixed with the resin. Cinnamein consists of balsamic esters like benzyl benzoate, benzyl cinnamate and cinnamyl cinnamate. The resin which constitutes about 28% consists of peruresinotannol associated with benzoic and cinnamic acid, alcohols (nerolidol, and farnesol), traces of vanillin and free cinnamic acid.

Uses – Peru balsam is a strong antiseptic and stimulates repair of damaged tissue. It is taken internally as an expectorant and anticatarrhal remedy to treat bronchitis and bronchial asthma. It is also taken to treat sore throats and diarrhoea. Externally peru balsam is used as antiseptic and parasiticide especially in scabies.

Adulteration – Peru balsam is adulterated with liquids such as fixed oils, alcohol, copaiba, turpentine, and gurjun balsam. This adulteration lowers the specific gravity of the drug which can be detected by various tests.

STORAX

Synonyms – Purified or Prepared storax, Styrax, Levant storax; Silaras (Hindi).

Biological Source – Storax is a balsam obtained from the wounded trunk of *Liquidambar orientalis* Miller, family Hamamelidaceae and subsequently purified by solution in alcohol, filtration and evaporation of the solvent. It contains not less than 30% of total balsamic acids, calculated with reference to the substance dried on water bath for one hour.

Geographical Source – *L. orientalis* is a medium sized tree of 6-15 meters in height and found in forests of south west Turkey.

Collection and Preparation – In early summer the bark is injured by bruising or by making incisions. This bark is collected in autumn and it is put into horse-hair bags and pressed in water. Sometime the bark is boiled in water and pressed. The liquid storax obtained is poured in cans or casks and exported.

Description

Colour – A brown viscous substance and transparent in thin layers.

Odour – Agreeable and balsamic

Taste – Agreeable and balsamic

Solubility – It is soluble in alcohol (90%), ether, chloroform and carbondisulphide and practically insoluble in water.

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Standards

Acid value – 50 to 80

Ester value – 100 to 133

Saponification value – 170 to 200

Loss on drying – Not more than 5%

Chemical Constituents – Storax consist of alcoholic resin (32 to 50%) known as storesin and oily fluid. Storesin is present in both free and combined form with cinnamic acid. The oily fluid contains phenyl propyl cinnamate (10%), cinnamyl cinnamate (styracin), phenylethylene, ethyl cinnamate, vanillin and free cinnamic acid (5-15%).

Chemical Test –

(i) Warm 1g of storax with 5g of sand and 5ml of potassium permanganate solution. An odour of benzaldehyde is produced due to presence of cinnamic acid.

(ii) Shake 1g of drug with 10% of solution of potassium chromate and 1ml of sulphuric acid. An odour of benzaldehyde is produced.

Uses- Storax is used as antiseptic, antibacterial, expectorant, emmenagogue, stimulant and febrifuge. It is used to treat foul ulcers, wounds, leprosy, skin diseases, chronic cough and diarrhoea. It is used in fumigating pastilles and powders and in preparations of balsam and benzoin inhalations. It is also used as a microscopical mountant for diatoms.

Adulterants and Substitutes

(i) American storax – It is a balsam obtained from wounded trunk of *Liquidambar styraciflua* Linn. It is also called as sweet gum. It is transparent, yellowish viscous liquid and suggested as main substitute.

COPAIBA. COPAIVA, BALSAM OF COPAIBA

Sources. Copaiba is an oleo-resin obtained from the trunk of *Copaifera lansdorfii* Desfontaines, family Leguminosae, and other species of *Copaifera*, The trees from which the oleo-resin is obtained are large trees indigenous to Brazil and the north of South America. The drug, which was highly esteemed by the natives of Brazil, and had probably long been used by them as a medicine, was introduced into Europe about the beginning of the seventeenth century.

Collection. The oleo-resin is contained in anastomosing, schizogenous secretion ducts that form an extensive network in each zone of tho secondary wood of both stem and root, extending throughout the entire length of the zone. These ducts are formed in the young wood and rapidly attain their normal diameter, which is often very considerable ; at the level of the insertion of the branches a number of lateral ducts connect zone with zone. In addition to these schizogenous ducts lysigenous cavities also appear to be formed by the breaking down of the cell walls and their probable transformation into resinous or oleo-resinous substances.

The oleo-resin is collected by cutting in the trunk of the tree near the base a cavity sloping inwards and downwards, and penetrating to the centre of the trunk, resembling the box made in the trunk of the turpentine trees. Into this cavity the oleo-resin is discharged it is transferred to barrels and other vessels for exportation.

The large size of the secretion ducts and lysigenous cavities, and their extensive distribution in each zone of wood throughout the entire length of the tree, render the amount of oleo resin that may be secreted by each tree very considerable. Even as much as 48 litres are said to have been obtained from a single tree, others again yielding but little.

The drug is exported from Para, Maranharn, Maracaibo, Bahia & Cartagena.

Description. *Maracaibo copaiba* is a clear, viscous, brownish-yellow fluid with a slight but distinct green fluorescence, It possesses a characteristic aromatic odour and an unpleasant, acrid and rather bitter taste. It is miscible in all proportions with chloroform, carbon disulphide, and benzene,. and also with an equal volume of petroleum spirit, but with larger proportions of the latter a slight precipitation takes place with absolute alcohol it behaves similarly. The specific gravity varies from 0.980 to 0.999, or even slightly higher. The proportion of volatile oil varies from about 35 to 50 per cent.

Para copaiba closely resembles the Maracaibo variety. The specific gravity is lower and varies from 0.917 to 0.980. The percentage of volatile oil is high, viz, from about 55 to 90 per cent. It does not fluoresce.

African copaiba, the botanical source of which is not known, is imported from the Niger basin in West Africa. It is a rather dark yellow, slightly fluorescent oleo-resin possessing an aromatic, piperaceous odour and frequently depositing crystals on standing. The specific gravity varies from 0.985 to 1.000. It contains about 40 per cent, of volatile oil and 60 per cent, of resin (including the crystalline substance).

Constituents. *Maracaibo copaiba* consists of a mixture of resin and volatile oil with which traces of a bitter principle and fluorescent substance are associated. Para copaiba is a similar mixture of volatile oil and resin, but the resin differs from that of the Maracaibo variety. African copaiba, the oil boils at 260° to 275° and differs essentially from the oil of South American copaiba in being dextrorotatory, the rotation in 100 mm. tube being about 10° 21'.

The crystalline deposit consists of illurinic acid identical with that obtained from Maracaibo copaiba. The remainder of the resin consists of amorphous resin-acids, fluorescent substance, etc.

Uses. The active principles of copaiba are absorbed into the blood, the volatile oil, at least, being excreted by the kidneys. bronchi, and skin; hence copiba produces along the whole genito-urinary tract, as well as in the brohchi, a stimulant and disinfectant action, increasing the mucous secretion and exciting expectoration. It is now chiefly employed in inflammatory affections of the bladder and urethra, and occasionally in chronic bronchitis.

Aduiterants. Fixed vegetable oils, volatile oil of turpentine, colophony and paraffin oil have been reported as adulterants.

Gurjun balsam also occurs as an adulterant; it is an oleo-resin obtained by incision from the tnmk of *Dipterocarpus turbinatus Gaertner*, family Dipterocarpaceae and other species, large trees indigenous to eastern India and Burma. It is dark in colour and is fluorescent. Its presence in copaiba may be recognised by adding 4 drops to a mixture of 5 mils of glacial acetic acid and 4 drops of nitric acid; a purple or reddish coloration indicates gurjun balsam. It may also be detected by adding 4 drops of the volatile oil, obtained by distillation in steam or under reduced pressure, to a mixture of 1 drop of nitric acid and 3 millilitres of glacial acetic acid when a red or purple colour indicates the presence of gurjun balsam.

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Chapter 2

CLASSIFICATION OF DRUGS

Crude drugs are the natural products obtained from plants, animals and minerals. Most of the crude drugs are derived from plants and only a small number comes from animal and mineral kingdom. Crude drugs are referred to **the natural products that has not been improved in condition or advanced in value by any treatment or process beyond that which is necessary for its packing and prevention from deterioration.** In simple words **the crude drugs are plant, animal or mineral drugs that have undergone no other process than collection and drying.** Crude drugs are further classified as :-

(i) Organised drugs (Cellular drugs) - These are the drugs which represents a part of the plant and posses cellular structure. Examples are -

Leaves- Digitalis, Senna, Datura

Fruit- Fennel, Coriander

Seed- Nux-Vomica, Isapghula

Bark- Cinchona, Cinnamon

Root- Ipecac, Aconite

(ii) Unorganised drugs (Acellular drugs) – These are the drugs which do not contain any part of the plant but contain solid and liquid material obtained from natural sources by adopting extraction procedures. Therefore they do not posses cellular structure. Examples are –

Gums- Tragacanth, Acacia

Resins- Colophony, Jalap

Dried Juices- Aloe, Kino

Fats- Lard

Waxes- Beeswax, Spermaceti

There are large number of crude drugs therefore it becomes necessary to study them in particular sequence of arrangement. In Pharmacognosy the crude drugs are classified as follows –

1. Alphabetical Classification
2. Taxonomical Classification
3. Morphological Classification
4. Pharmacological or Therapeutical Classification
5. Chemical Classification
6. Chemotaxonomical Classification

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Each of the above mentioned classification has its own limitations because none of these provides a full profile of natural drugs.

1. Alphabetical Classification : The crude drugs are classified on the basis of alphabetical order of their latin or English names. This classification is used by various pharmacopoeias, dictionaries, reference books etc. Alphabetical classification is found in –

1. Indian Pharmacopoeia
2. British Pharmacopoeia
3. British Pharmaceutical Codex
4. British Herbal Pharmacopoeia
5. British Herbal Compendium
6. European Pharmacopoeia
7. Extra Pharmacopoeia
8. United States Pharmacopoeia and National Formulary

The disadvantage of this classification is that it is unable in distinguishing the drugs of plant, animal or mineral sources.

2. Taxonomical or Biological Classification : **Pyrame de Candole** introduced the term *taxonomy* which is defined as the principle of classifying plants and animals. In this classification the drugs are classified according to the plants or animals from which they are obtained in

Division-	**Angiospermae**	**Division-**	**Angiospermae**
Class-	Dicotyledons	Class-	Dicotyledons
Order-	Tubiflorae	Order-	Umbellflorae
Family-	Labiatae	Family-	Umbellifeare
Genus-	Mentha	Genus-	Foeniculum
Species-	*Mentha piperita*	Species-	*Foeniculum vulgare*
Division-	**Angiospermae**	**Division-**	**Magnoliophyta**
Class-	Dicotyledons	Class-	Liliopsida
Order-	Tubiflorae	Order-	Arecales
Family-	Solanaceae	Family-	Arecaceae
Genus-	Atropa	Genus-	Areca
Species-	*Atropa belladonna*	Species-	*Areca catechu*
Division-	**Magnoliophyta**	**Division-**	**Traecheophyta**
Class-	Liliopsida	Class-	Magnoliopsida
Order-	Liliales	Order-	Gentianales
Family-	Smilacaceae	Family-	Apocynaceae
Genus-	Smilax	Genus-	Nerium
Species-	*Smilax regelii*	Species-	*Nerium indicum*

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phyla, orders, families, genera, species etc. This classification is based on the principle of natural relationship or phylogenetic similarities of plants or animals. Examples are –

The disadvantage of this classification is that it does not provide the information regarding chemical constituents and therapeutic uses of crude drugs. Moreover it is also unable to recognize the organised or unorganised nature of crude drugs.

3. Morphological Classification : The crude drugs are classified according to the part of the plant used as drug and are represented as organised (Leaves, Fruit, Seed, Bark, Root etc.) and unorganised drugs (Gums, Resins, Dried Juices, Fats, Waxes etc). This classification is helpful in the practical study of pharmacognosy. Examples of Morphological classification –

A. Organised drugs :

Leaves – Vasaka, Digitalis, Senna, Datura, Hyoscyamus
Fruits – Fennel, Coriander, Cardamom, Dill, Bael
Seeds – Nux vomica, Isapgula, Castor, Mustard, Linseed
Bark – Cinchona, Cinnamon, Kurchi, Cassia, Cascara
Roots – Ipecac, Aconite, Rauwolfia, Jalap, Senega
Rhizomes – Ginger, Rhubarb, Turmeric, Podophyllum, Valerian
Woods – Sandal wood, Red scanders, Quassia
Flowers – Rose, Pyrethrum, Clove, Artemisia
Entire Plant – Belladonna, Ergot, Ephedra, Tulsi

B. Unorganised drugs :

Gums – Tragacanth, Acacia, Guar gum
Resins & resin combinations – Colophony, Jalap, Balsam of Tolu, Benzoin
Dried juices – Aloe, Kino
Dried latex – Opium, Papain
Dried extracts – Catechu, Gelatin, Agar
Fats – Lard
Waxes – Bees wax, Spermaceti

The drawback of this classification is that it does not provide any information regarding the chemical composition and therapeutic activity of the drug.

4. Pharmacological or Therapeutical Classification : In this classification the drugs are classified according to the pharmacological action of their chief constituent or their therapeutic uses. All those drugs which posses same pharmacological actions are grouped together regardless of their taxonomical status or morphology or chemical constituents. Hence vinca, taxus and podophyllum are grouped together as anticancer because all exhibit similar pharmacological action. The advantage of this classification is that if the nature of chemical constituents of any drug is not known then it can be grouped according to its therapeutic uses. Some of the examples of crude drugs under pharmacological classification are as follows –

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DRUGS ACTING O... ESPIRATORY SYSTEM

Expectorants – Liquorice, Balsam of Tolu, Ipecac
Antitussives – Opium (Codeine)
Bronchodilators – Tea, Ephedra

DRUGS ACTING ON GASTRO – INTESTINAL TRACT

Bitters – Nux vomica, Cinchona, Picrorrhiza
Laxatives – Isapghula, Agar
Purgatives – Castor oil, Cascara, Senna, Aloe
Carminatives – Coriander, Dill, Fennel, Asafoetida
Antiamoebic – Ipecac, Kurchi
Emetics – Ipecac

DRUGS ACTING ON CARDIOVASCULAR SYSTEM

Cardiotonic – Digitalis, Strophanthus, Squill
Cardiac depressants – Veratrum, Cinchona
Antihypertensive – Rauwolfia.

DRUGS ACTING ON CENTRAL NERVOUS SYSTEM

CNS stimulants – Coffee, Tea
CNS depressants – Hyoscyamus, Belladonna, Opium
Central analgesics – Opium (morphine)
Hallucinogen - Cannabis, Coca (Cocaine)

DRUGS ACTING ON AUTONOMIC NERVOUS SYSTEM

Adrenergics – Ephedra
Cholinergics – Pilocarpus
Anticholinergics – Datura, Belladonna
Antirheumatics – Guggul, Aconite
Antispasmodics – Datura, Belladonna, Hyoscyamus
Antimalarials – Cinchona, Artemisia
Anthelmintics – Male fern, Quassia wood
Anticancer – Vinca, Taxus, Podophyllum
Astringents – Black catechu, Ashoka bark, Myrobalan
Anti inflammatory – Colchicum corm and seed
Local anaesthetics – Coca
Immunizing agents – Sera, Vaccine

The disadvantage of this classification is that the drugs having different pharmacological actions are classified separately in more than one group; for example cinchona is an antimalarial drug due to quinine but due to the presence of quinidine it can also be classified under cardiac depressant.

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5. Chemical Classification : The crude drugs are classified on the basis of the chemical nature of their chief constituents. The pharmacological action and therapeutic uses of the crude drugs are also due to these chemical constituents. Therefore much importance is given to this classification. Examples of the crude drugs under this classification are as follows –

Alkaloids – Vinca, Cinchona, Belladona, Opium, Ipecac, Tea , Coffee, Vasaka.

Glycosides – Aloe, Senna, Digitalis, Mustard, Rhubarb, Brahmi, Ginseng, Senega.

Tannins - Amla, Bahera, Myrobalan, Black Catechu, Pale catechu, Pterocarpus.

Resins & resin combinations- Asafoetida, Myrrh, Colophony, Balsam of Tolu, Balsam of Peru, Podophyllum, Jalap

Carbohydrates & related products- Honey, Tragacanth, Sterculia gum, Guar gum, Starch, Isapghula

Lipids(Fixed oil, fats & waxes)- Arachis oil, Chaulmoogra oil, Shark liver oil, Hydrous wool fat, Spermaceti, Lard, Suet

Volatile oils- Cassia, Cinnamon, Dill, Carraway, Clove, Tulsi, Ajowan, Black pepper, Fennel

Proteins- Casein,Gelatin, Yeast, Collagen

Enjymes- Diastase, Pepsin, Pancreatin, Streptokinase

Hormones- Oxytocin, Insulin

6. Chemotaxonomical Classification : Recently much attention is paid on this subject by phytochemist and this subject has brought the phytochemist to systematic botany. The concept that plants can be classified on the basis of their chemical constituents is not new and it has a long been of practical value for e.g. aroma from the crushed leaves and fruits (due to presence of characteristic essential oil) of the Apiaceae (Umbelliferae) and Lamiaceae are the characteristic points for the identification of the members of these two families and the two sub families of Asteraceae ; the Tubiflorae (latex vessels are absent) and Liguliflorae (latex vessels are present) are distinguished on the basis of absence or presence of latex vessels respectively. When compared with morphological characters, chemical constituents are easily definable and are more fundamentally significant for classification purpose.

Chemotaxonomy is based on the fact that there are certain compounds which have keen, found to be characterizing certain groups. Chemotaxonomy establishes the relationship between position of the plant in taxonomy and chemical nature of drugs . The characters which are studied in chemotaxonomy are secondary metabolites like alkaloids, glycosides, flavonoids, carbohydrates (rare sugars), amino acids, glucosinolates, terpenoids and waxes. Therefore, the knowledge of chemotaxonomy could serve as the basis of classification of crude drugs. Serotaxonomy, DNA hybridization and amino acid sequencing techniques are also gaining importance in this method of classification.

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SERO[illegible]XONOM[illegible] CLASSIFICATION

Serotaxonomy developed & beaca[illegible]pular in Germany which has been an active center since the beginning of this cent[illegible].

Serology is defined as that portion of biology which is concerned with the nature & interactions of antigenic material & antibodies. When foreign cells or particles (antigen) are introduced into an organism, antibodies are produced in the blood (antiserum). The substance capable of stimulating the formation of an antibody is called antigen and the highly specific protein molecule produced by plasma cells in the immune system in response to antigen is called antibody.

Proteins most widely used as antigen in serotaxonomy are those which carry useful taxonomic information & are easy to handle. Both structural and reserve proteins can be used in the field of systematics as long as they belong to the same group and the same organs are always compared. Generally storage proteins are most amenable for taxonomic studies followed by Pollen Proteins. Stem tubers, algal cells, fern spores, fruits and leaves can also be employed as satisfactory antigenic material for systematic investigations.

In this method acrude protein extract of a particular plant taxa (antigen) is injected into the blood stream of an experimental animal, usually a rabbit or a rat to develop antibodies. In response to the specific antigen injected a specific antibody is produced in the blood of animal. The serum (termed the antiserum) containing the antibody is then collected and made to react invitro while the antigenic proteins as well as proteins from other related taxa of which the affinities are in question serological reactions between antibodies & antigenic materials results information of prepitate. This is called precipitation reaction. Kraws showed that this reaction ends scabs similarity of antigens. The degree of protein homology is determined by the amount of precipitation & hence is taken as a phylogenetic marker and taxonomic character.

Serological studies using crude plant protein extracts have been widely used in elucidatug the taxonomy of a wide variety of higher-level taxa and in estimating phylogenetic relationships. For example a close relationship among the magrdivae, Hamamelididae and Comiflorae of the angiosperms has been found bared on comparative serological studies of their major seed proteins.This has refuted the idea of their independent evolution. Another example based on phytoserological studies is that pickering and Fair brothers (1970) have proposed the classification of family umbellifrae into Hydrocotyloideae, Saniculoidae and Apioideae was found to the more closely related to Saniculoideae than to Hydrocotyloideae.

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Chapter 3

QUALITY CONTROL OF DRUGS OF NATURAL ORIGIN

DRUG ADULTERATION

Adulteration means the debasement of an article. Adulteration can be defined as **"the substitution of original crude drug with inferior, spurious, defective or harmfull substances"**. The adulteration is done deliberately but in some cases it may also occur accidently. Adulteration is done when the cost of drug is high or there is a scarcity of drug. The motive of adulteration is to increase the profit.The various conditions involved in adulteration are :-

Substitution – It is done when totally different substances are added in place of original drug.

Sophistication – It is the deliberate or intentional type of adulteration.

Deterioration – The impairment in the quality of drug is called as deterioration.

Admixture – It is the addition of one article into another due to carelessness or by an accident.

Inferiority – The substandard drug is called as inferiority.

TYPES OF ADULTERANTS

The various types of adulterants are found in natural drugs which can be detected during quality control by performing various tests. The different types of adulterants are described below :-

Substitution with inferior drugs – The inferior drugs used have similar morphological characters to the genuine drug but they may or may not have any chemical or therapeutic value as that of genuine drugs. For example, mother clove and clove stalks are adulterated with clove ; saffron is admixed with dried flowers of *Carthamus tinctorsus*.

Substitution with substandard commercial varieties – The adulterant used have similar morphological, chemical and therapeutic characters as that of original crude drug but are substandard in nature and cheaper in cost. For example, Indian senna adulterated with Arabian senna ; Medicinal ginger adulterated with Japanese and African ginger ; *Capsicum minimum* substituted with *capsicum annum*.

Substitution with exhausted drugs- In this type of adulteration the same type of drug is admixed but it does not contain any chemical constituents because they are already extracted out.This type of adulteration is done mainly with volatile oil containing drugs such as caraway, fennel, clove, cinnamon etc. because volatile oil extracted by steam distillation does not in any way change external physical characters of these drugs.

Sometime the natural characters of exhausted drugs like colour, taste etc.are manipulated by adding various additives and then they are mixed with original drugs. For e.g. used tea leaves are collected, dried, sometime dyed and mixed with fresh tea leaves.

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Substitution with artificially manufactured substances- The substances which are artificially manufactured resembles to the original crude drugs, are also substituted. This type of adulteration is common in costly drugs. For e.g. bees wax substituted with paraffin wax; chicory powder is used as an adulterant in coffee.

Substitution by synthetic material- The various types of synthetic materials are added to the original drugs which enhances the natural characters. For e.g. addition of citral to orange oil and lemon oil ; benzyl benzoate to balsam of peru.

Harmful adulterants- In this type of adulteration the waste material is admixed with genuine drugs which may become harmful. Generally it can be seen in unorganised drugs. For e.g. white oil added to coconut oil; pieces of amber coloured glass in colophony ; addition of rodent fecal material to cardamom seeds ; limestone to asafoetida.

Substitution by the vegetative parts- The various types of vegetative which grow along with medicinal plants are mixed with genuine drugs because they have similar colour, odour and sometime chemical constituents also. For e.g. liver worts, moss etc. which grow on the bark portion are mixed with cinchona ; stem portions are mixed with the leaves of senna, datura and hyoscyamus.

Adulteration of powders- The drugs which are in powdered form are also frequently adulterated. For e.g. powdered bark adulterated with brick powder ; powdered liquorice or gentian adulterated with powdered olive stones ; nuxvomica adulterated with powdered guaiacum wood.

DRUG EVALUATION

Drug evaluation means confirmation of its identity, determination of its purity & quality and detection of nature of adulteration. The evaluation of crude drugs is essential due to several reasons (i) there may be substitution or adulteration because of carelessness or intentional (ii) biochemical variations in the crude drug (iii) deterioration due to treatment or storage of crude drugs.

The methods of evaluation have undergone systematic changes from the last few decades. Due to increase in the chemical knowledge of crude drugs and with the advent of separation techniques and instrumental analysis it is possible to have qualitative and quantitative evaluation of the drugs. To confirm any drug which is listed in pharmacopoeia it must agree in all the points with the monograph written in pharmacopoeia. The different methods used in the **standardization** of crude drugs are mentioned below:-

- Morphological or Organoleptic Evaluation
- Microscopic Evaluation
- Chemical Evaluation
- Physical Evaluation
- Biological Evaluation

MORPHOLOGICAL OR ORGANOLEPTIC EVALUATION

It is a technique of qualitative evaluation in which drugs are evaluated by means of our organs of sense. Organoleptic evaluation refers to the evaluation of drug through **gross morphology** and other **sensory characters** such as colour, odour, taste, touch and texture.

Study of gross morphology- The drugs are arranged in various morphological groups

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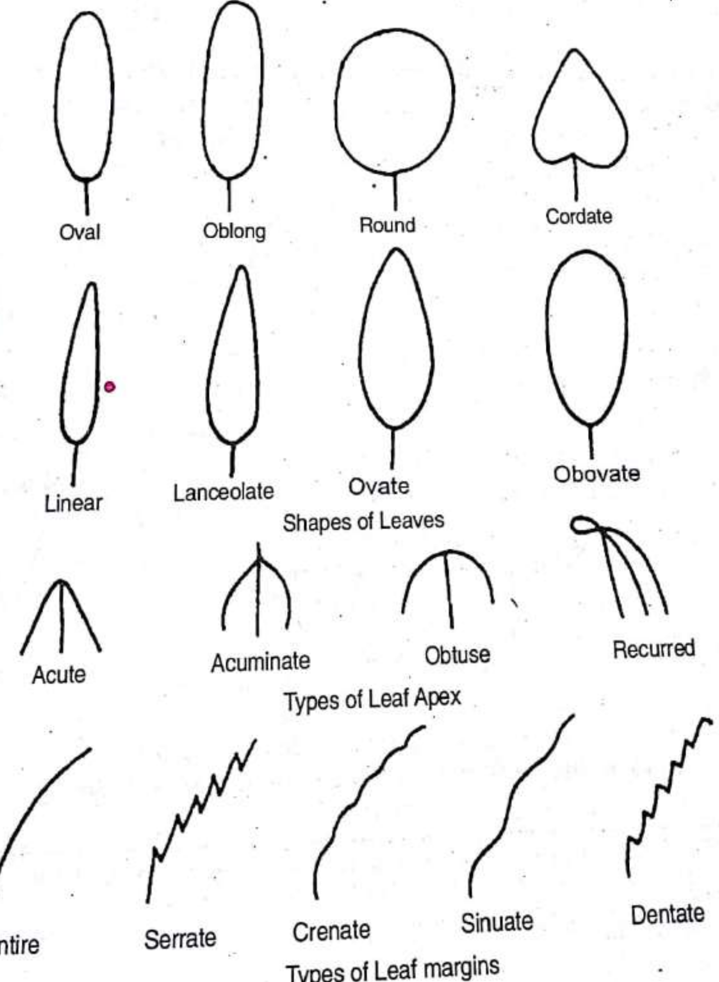
such as leaves, flowers, barks, seeds, fruits, woods etc. For every morphological group a systematic evaluation can be carried out.

Leaves- Leaves are the flattened lateral outgrowth of stem. Leaves are of two types viz simple and compound leaves. A simple leaf bears bud in its axil and it is generally without incisions except in the basal regions in some plants whereas compound leaf has many leaflets in the axils of which buds do not arise and the whole leaf is divided by incisions in many segments which arise on a common rachis.

Simple leaf Compound leaf

Types of Leaves

There are different shapes and sizes of the leaves. The different shapes of leaves, their apex, margin, base, and venation are helpful in identification of drugs. These are shown in following figures:-



Shapes of Leaves

Types of Leaf Apex

Types of Leaf margins

Symmetrical Asymmetrical Cordate Decurrent

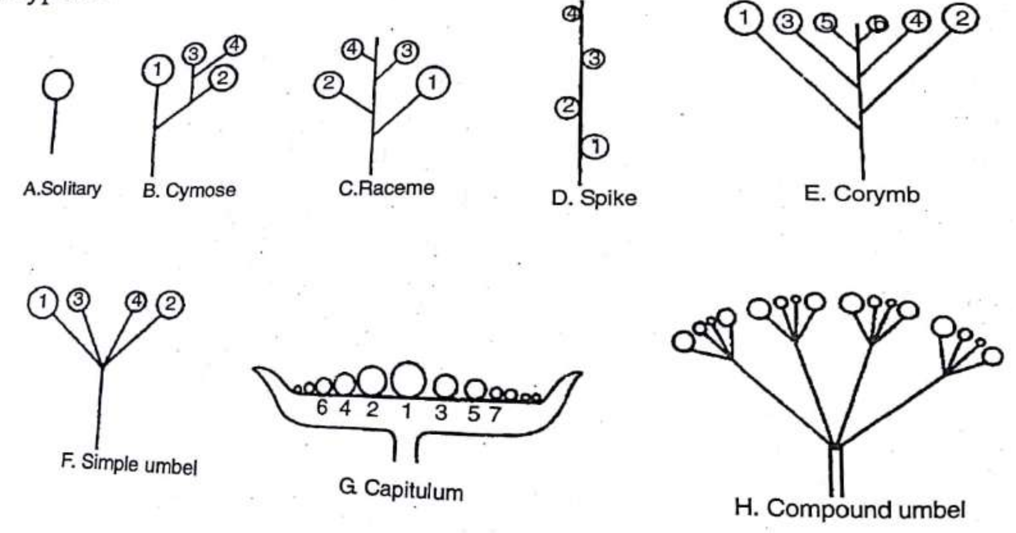
Types of Leaf Bases

Parallel

Reticulate palmate Reticulate pinnate

Fig. Types of venation of leafy drugs

Flowers- A flower is a modified shoot meant for production of seeds and it is built up on the enlarged end of stem called as thalamus. It consist of four basic parts i.e the calyx*, corolla**, androecium and gynoecium. The bunch of flowers is called inflorescence. The different types of inflorescence is shown in following figures:-



A to H : Types of Inflorescence
(Numbers refer to the sequence to flower opening

* A collective name of for the outer whorl of the flower formed by leaf like parts known as sepals and which are usually green

** Collective name of the petals of a flower.

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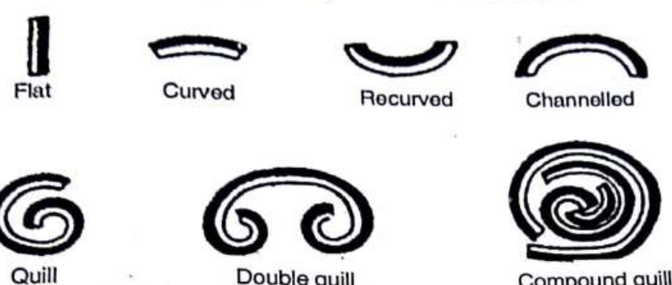
Barks- Due to the continuous formation of cork, cork cambium etc. and production of secondary tissue, the cork cambium layers are pressurized and move towards outside. In such condition these cells do not get nutrients and become dead and the layers formed by these cells is known as bark. Barks are collected from branches and trunks of the trees and is obtained in the form of strips. The shape of bark varies and it depends upon the type of incision given at the time of collection. During the drying process due to unequal shrinkage of various layers, bark assumes different shapes as shown below:-

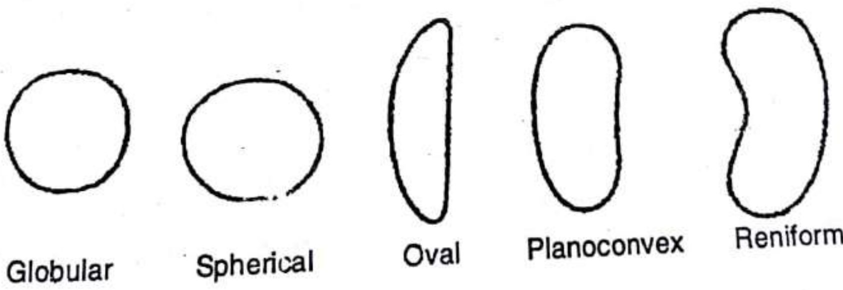


Shapes of bark

Fruits- Fruits are defined as the matured *ovary with or without associated parts. Fruits are classified into three main groups viz (i) Simple fruits (ii) Aggregate fruits (iii) Multiple or Composite fruits.

A simple fruit is that which develops from a single ovary of single flower with or without other parts. They are categorized into two main groups viz dehiscent and indehiscent. Aggregate fruits are developed from polycarpellory apocarpus ovary. Each carpel forms a single fruitlet. All the fruitlets arise from a single flower and are attached on the same axis therefore termed as aggregate fruits. Composite fruits are developed from the inflorescence. The peduncle; perianth or calyx and corolla as well as ovular parts after maturity and ripening forms a fleshy fruit. The shape of fruits may be oblong, ellipsoidal and globular. The example of fruit drugs are fennel, coriander, cardamom, dill, bael etc.

Seeds- A seed is a fertilized ovule. It consist of three parts viz. seed coat, embryo and endosperm. Seeds are characterized by the **hilum,** a point of attachment of seed to stalk, the **micropyle,** a minute opening for the absorption of water and the **raphe,** a longitudinal marking of adherent stalk. The examples of seed drugs are nuxvomica, isapghula, castor, mustard, linseed etc. The various shapes of the seeds are shown below:-



Shapes of seeds

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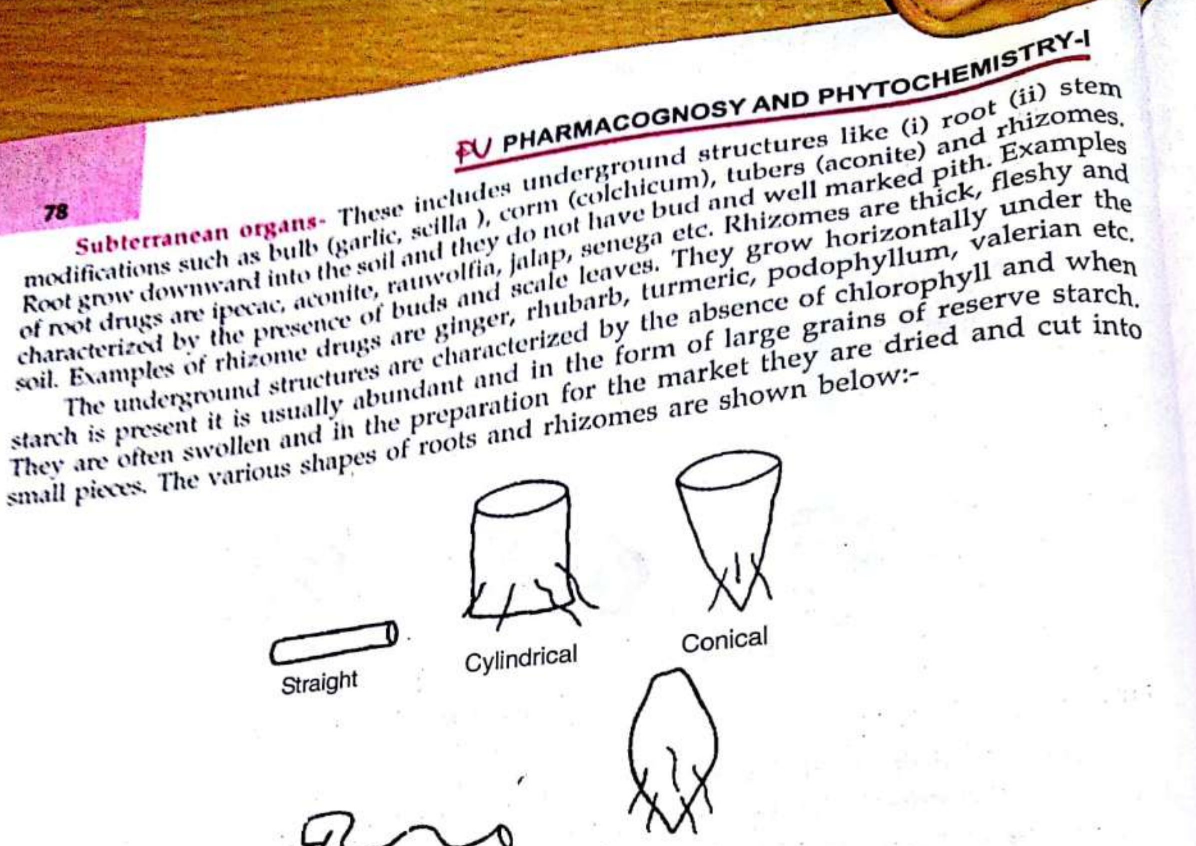
Subterranean organs- These includes underground structures like (i) root (ii) stem modifications such as bulb (garlic, scilla), corm (colchicum), tubers (aconite) and rhizomes. Root grow downward into the soil and they do not have bud and well marked pith. Examples of root drugs are ipecac, aconite, rauwolfia, jalap, senega etc. Rhizomes are thick, fleshy and characterized by the presence of buds and scale leaves. They grow horizontally under the soil. Examples of rhizome drugs are ginger, rhubarb, turmeric, podophyllum, valerian etc.

The underground structures are characterized by the absence of chlorophyll and when starch is present it is usually abundant and in the form of large grains of reserve starch. They are often swollen and in the preparation for the market they are dried and cut into small pieces. The various shapes of roots and rhizomes are shown below:-



Shapes of underground drugs

Herb- Herb consist of aerial parts of the plant composed of leaves, flowers, stem and fruits, so each part should be explained.

Study of sensory characters- It refers to the evaluation of colour, odour, taste and texture of the drug. Every drug has a specific colour and if they are improperly dried the colour of the drug may change. The volatile oil containing drugs such as caraway, fennel, ajowan etc.have a characteristic odour and if these drugs are devoid of their volatile oil content the appropriate aroma will not be observed. Similarly if some drugs are not stored properly they may deteriorate and emit bad odour. Drugs like liquorice have a sweet taste, cinchona and gentian have a bitter taste and ginger and capsicum have pungent taste, are some examples of evaluation.The texture of drug can be evaluated by breaking a piece of drug under examination. The fractured surfaces of cascara and cinchona barks are important diagnostic characters.

MICROSCOPIC EVALUATION

This method enables a detailed microscopic examination of organized crude drugs in their entire and powdered forms. A very thin sections of the drugs are prepared and histological studies are performed. The various characteristics of cell walls, cell contents, starch grains, lignin, calcium oxalate crystals, fibres, vessels and trichomes etc. can be studied.

* A part of flower that bears the ovules.

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The various stains and reagents are used to study the different cellular structure. This method is also helpful in studying the constituents by application of chemical methods to histological sections of drugs or to the drugs in powdered form. For example, mucilage is stained pink with ruthenium red and starch and hemicellulose is stained blue with N\50 iodine solution. Quantitative microscopy is also studied under this method.

Trichomes- Trichomes are the tubular or glandular out-growth of the epidermal cell and are known as plant hairs. They are present on the aerial parts of the plant but are absent on the roots. Trichomes are present in various parts of plant such as leaves(Datura, Tulsi), fruits(Ladies finger), seeds(Strophanthus) etc. Trichomes performs various functions. They excrete water and in some plants like *Mentha piperita* they excrete volatile oil. The hairs of tobacco and plumbago plants produce a kind of gummy material. Therefore trichomes are important diagnostic characters for the identification of drugs. Trichomes are classified below on the basis of structure and number of cells present in them:-

NON GLANDULAR OR CLOTHING TRICHOMES

Glandular trichomes

Hydathodes

Non glandular or Clothing trichomes- Clothing trichomes are of two types :-

1- Unicellular trichomes :- These trichomes vary from small papillose outgrowth to large robust structure.

Linear, thick walled and warty trichomes - Damiana

Linear, strongly waved, thick walled trichomes- *Yerba santa*

Large, conical, longitudinally striated trichomes - Lobelia

Long, tubular, flattened, and twisted trichomes - Cotton

Lignified trichomes - Nuxvomica, Strophanthus

Short, conical trichomes - Tea

Short, conical, warty trichomes - Senna

Short, sharp, pointed, curved, conical trichomes - Cannabis

2- Multicellular trichomes :- These trichomes are of two types :-

(A) Multicellular unbranched trichomes :-

(i) Uniserate-

Bi-cellular, conical - Datura

Three celled long - Stramonium

Three to four celled long - Digitalis

Four to five celled long - Belladonna

(ii) Biserate - These type of trichomes are found in *Calendula officinalis.*

(iii) Multiserate - Multiserate trichomes are found in *Euphorbia pilulifera* and male fern.

(B) Multicellular branched trichomes :- It is of four types-

Stellate :- These are found in hamamelis and altheae leaves.

Peltate (Shield like structure) :- These are found on leaves of Eleagnus and on the leaves and young twigs of *Croton eleuteria.*

Candelabra (Uniserate branched axis) :- These can be found in *Verbascum thapsus* and

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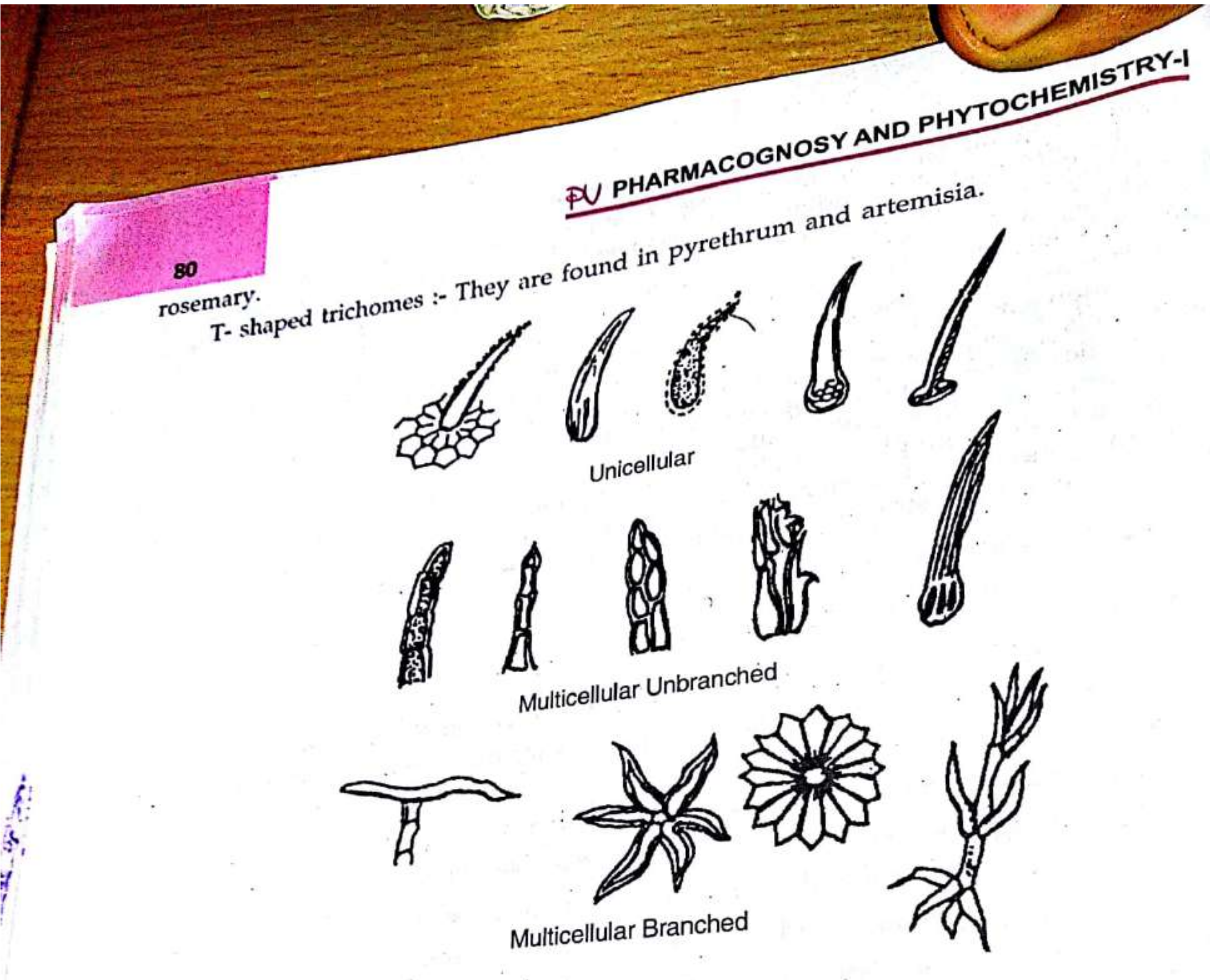
rosemary.

T- shaped trichomes :- They are found in pyrethrum and artemisia.



Non-glandular Trichomes

Glandular trichomes- These trichomes have glandular cell at the apex. They are classified as :-

1- **Unicellular glandular trichomes :-** They do not posses stalk for eg *Piper betel* and vasaka.

2- **Multicellular glandular trichomes :-** Most of the glandular trichomes are multicellular.

Uniseriate stalk with single spherical secreting cell at the apex - *Digitalis purpurea.*

Uniseriate multicellular stalk with single spherical cell at the apex - *Digitalis thapsi* and belladonna

Unicellular stalk and a bicellular head - *Digitalis purpurea*

Uniseriate stalk and multicellular head - Hyoscyamus

Biseriate stalk and biseriate secreting head - Santonica and plants of Compositae

Short Stalk and secreting head formed of a rosette of club -shaped cells - *Mentha* species

Multiseriate cylindrical stalk and a capitate rosette of secreting cells - Cannabis

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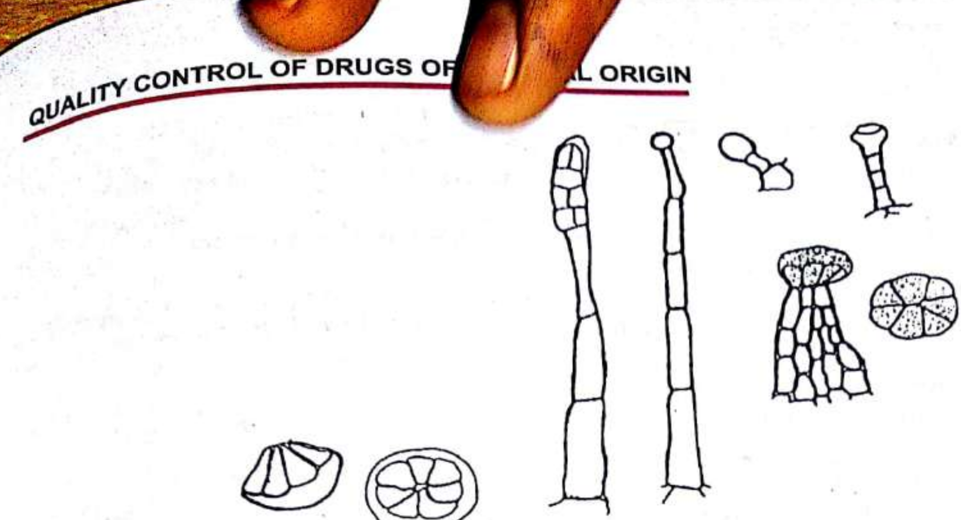

Glandular Trichomes

Hydathodes- Hydathodes are the glands of secretion or absorption developed in certain plants. They may consist of unicellular or multicellular hairs. They are most commonly found on the leaves of aquatic plants or herbaceous plants growing in moist places. They occur either at the tip or on the margins of the leaves. Each hydathode is found in very intimate relation of a vein. Example is *Piper betle.*

Stomata- Stomata is a epidermal structure which has a central pore and two kidney shaped similar cells called as guard cells and different numbers of subsidiary cells (epidermal cells) covering the guard cells. The main function of stomata is to exchange the gaseous and it also helps in transpiration. Generally stomata are present in the epidermis of leaves. They are also present in fruits, flowers and stems. It is not necessary that each plant should contain stomata. For example, stomata are completely absent in the leaves of bryophytes and submerged leaves of aquatic plants such as *Elodea canadensis*. The distribution of stomata between upper and lower epidermis shows a great variation. The stomata may be entirely confined to the lower epidermis as in the leaves of coca, boldo and bearberry. They may be present in exceptional cases on the upper surface only such as in floating leaves of aquatic plants (water-lily). But sometime stomata is evenly distributed on upper and lower surfaces as in senna and mistletoe. However stomata are more numerous on the lower surface then the upper surface.

Types of stomata- Depending upon the characters of guard cells stomata are of four types:

(i) Moss type :- This type is found on the apophysis of the theca and when mature posses guard cells which are united by the breaking down of the dividing wall during growth. For example as in *Funaria.*

(ii) Gramineous type :- This type is the characteristic of Graminaceae and Cyperaceae and has guard cells which in surface view are more or less dumb-bell shaped and the out line sub-rectangular.

(iii) Gymnospermous type :- This type have guard cells which are oval in transverse section and are placed at an angle of about 45^0 with the outer surface and have walls which are in part lignified. For example as in savin.

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(iv) Dicotyledonous type :- This type is oval or circular in outline in surface view with arcuate guard cells. This type of stomata have a diagnostic importance.They are classified into five types depending upon the form and arrangement of the subsidiary cells-

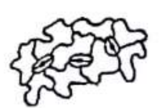
Caryophyllaceous or Diacytic (Cross celled) type – The stoma is accompanied by two subsidiary cells, the long axes of which are at right angles to that of stoma. Examples are thyme, spearmint and peppermint.

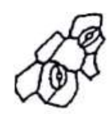
Cruciferous or Anisocytic (Unequal- celled) type- The stoma is surrounded by usually three subsidiary cells of which one is markedly smaller than the others. Examples are henbane, belladonna and datura.

Ranunculaceous or Anmocytic (Irregular celled) type- The stoma is surrounded by a varying number of subsidiary cells resembling other epidermal cells. Examples are bearberry, lobelia and foxglove.

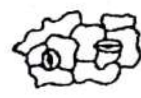
Rubiaceous or Paracytic (Parallel-celled) type- The stoma has two subsidiary cells, the long axes of which are parallel to that of stoma. Examples are senna, boldo, and coca.

Actinocytic (Radiate-celled) type- The stoma is surrounded by a circle of radiating cells.


Anomocytic (Ranuculaceous)


Paracytic (Rublaceous)

Diacytic (Caryophyllaceous)


Anisocytic (Cruciferous)

Types of dicotyledonous stomata.

Quantitative Microscopy- The parameters to be studied under this topic are:-

Leaf measurements and Lycopodium spore method.

Leaf measurements- The various types of leaf measurements are mentioned below-

Stomatal number- It is the average number of stomata per square millimeter of epidermis of the leaf. Stomatal number is constant for a particular species of the same age and it can be a important diagnostic character for evaluation. Some of the examples are as follows:-

Species	Lower surface
Atropa belladonna	77.5 to 176
Datura stramonium	145 to 254
Cassia angustifolia	195 to 256
Prunus laurocerasus	140 to 180

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

$$S.I. = \frac{S \times 100}{(E+S)}$$

where S.I = stomatal index

S = number of stomata per unit area
E = number of epidermal cells in the same unit area

Stomatal index is relatively constant for a particular species and it can be a important diagnostic character. Some of the examples are as follows:

Species	Lower surface
Atropa belladonna	19.5 to 23.9
Datura stramonium	24.1 to 26.2
Cassia angustifolia	17 to 19.3
Digitalis purpurea	17.9 to 19.5

Vein islet number- It is the number of vein islets per square millimeter of the leaf surface midway between the midrib and margin. Vein islet number is constant for a particular species and it does not changes with the age of plant. Some of the examples are as follows-

Species	Vein – islet number
Cassia angustifolia	19 to23
Digitalis purpurea	2 to 5.5
Digitalis lutea	1 to 1.5
Erythroxylum coca	8 to12

Veinlet termination number- It is the number of veinlet termination per square millimeter of the leaf surface midway between the midrib and margin . Some of the examples are listed below:-

Species	Veinlet termination number
Atropa belladonna	6.3 to 10.3
Datura stramonium	12.6 to 20.1
Cassia angustifolia	25.9 to 32.8
Digitalis purpurea	2.6 to 4.2

Palisade ratio- It is the average number of palisade cells beneath each upper epidermal cell. Palisade ratio is constant for a particular species and it can be a important diagnostic character for evaluation. Some of the examples are as follows:-

Species	Palisade ratio
Atropa belladonna	6 to 10
Datura stramonium	4 to 7
Digitalis purpurea	3.7 to 4.2
Solanum nigrum	2 to 4

Lycopodium spore method:- This method was developed by Wallis. It is an analytical technique for powdered drugs and used when physical and chemical methods of evaluation of drug fails to measure the accurate quality. Lycopodium spores are uniform in size (25um) and 1 mg of powdered lycopodium contains 94000 spores. This method is used for those powdered drug sample which contains-

(i) well defined particles which can be counted (e.g. pollen grains or starch grains) ; or

(ii) single layered tissues or cells, the area of which may be traced at a suitable magnification and the actual area calculated ; or

(iii) particles of uniform thickness, the length of which can be measured at a suitable magnification and the actual length calculated.

Lycopodium spore method is used for the evaluation of powdered drugs such as ginger, clove, nutmeg, umbelliferous fruits etc.

In the following method it is described how to determine the number of starch grains per mg of ginger by lycopodium spore method.

Determine the loss on drying of the powdered material at 105°C. Mix 100mg of powdered drug and 50mg of Lycopodium on a glass plate, with a little of suspending fluid.

Add sufficient quantity of a suspending fluid (glycerine : mucilage of tragacanth : water in the ratio of 2:1:2 or enoil) in the above mixture until a smooth thin part is formed. Transfer this to a stoppered tube by washing with excess of suspending fluid. Adjust the final volume so that about 15 to 20 spores are observed in a field using a 4mm objective (Approximately 4ml of suspending agent is adequate for 50mg of Lycopodium). Rotate the stoppered container to obtain uniform suspension. Place one drop of the suspension on each of two slides & spread it with a thin glass rod & add the cover slip & leave it aside for few minutes to settle the fluid mixture evenly.

Count the starch grains of ginger & the lycopodium spore in each of 25 different fields selected for observation.

Prepare another similar suspension & repeat the exercise. From the mean of 4 sets of the counts & percentage of moisture present, calculate the number of starch grains per mg of the powder with reference to the powder dried at 105°C. Pure Jamaica ginger contains 28600 starch grains per mg.

Calculate the percentage purity of ginger powder usingthe following formula.

$$= \frac{N \times W \times 94000}{S \times M \times P} \times 100$$

where N – number of characteristic structure (starch grains) in 25 fields.

W – weight in mg. of lycopodium taken.

S – number of lycopodium spores in 25 fields.

M – weight in mg. of sample .

P – it is a constant (for ginger P = 286000).

The above method can also be used to determine the stalk (pedicel) in clove by counting the sclereides characteristic of stalk. An authentic sample of clove stalk has been found to contain 1100 scl/mg. Thus clove mixed with 10% of its weight of stalk would give a count of 100 slcereids/mg of the mixture.

CAMERA LUCIDA

Different types of apparatus are available in which a magnified image of the object under the microscope can be traced on paper. The Swift-Ives camera lucida & the Abbe darawing apparatus are examples.

The Swift-Ives camera lucida (Fig. A) should be fit over the eyepice & the light from

the object passes direct tothe observer's eye through on opening in the silvered surface of the left hand prison (Fig. B). At the same time light from the drawing paper & pencil is reflected by the right hand prism & by the silvered surface so that the pencil appears superimposed on the object which can be traced. While using the instrument the illumination of both object & paper must be tilted at the correct angle to avoid distortion. The correct position of the drawing board to which the paper is pinned is found as follows : Place a stage micrometer on the microscope stage & trace its division on paper. Measure the distance between lines drawn & if they are unequal then tilt the board & repeat the tracing & measuring until all the lines are equally spaced.

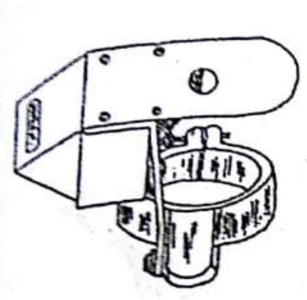


Fig. A. Camera lucida

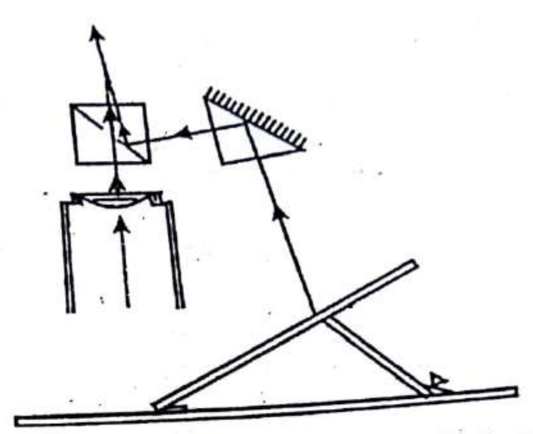


Fig. B. The path of light rays through the instrument

The Able drawing apparatus is another form of apparatus which can be used to trace the image of an object without any inclination in the board. It utilizes a plone mirror carried on a side-arm, instead of the adjustable prism, with the mirror at 45° to the bench surface.

CHEMICAL EVALUATION

The estimation of active constituents by chemical process is termed as chemical evaluation. Chemical evaluation of drugs is done by following two methods :-

Chemical Tests

Chemical Assay

Chemical Tests – It comprises of qualitative and quantitative tests. In qualitative tests the drugs are identified by performing several general and specific chemical tests. For examples drugs containing alkaloids such as belladonna, cinchona, nuxvomica, rauwolfia etc. can be identified by performing the tests with alkaloidal reagents such as Mayer's reagent (cream precipitate), Wagner's reagent (reddish brown precipitate), Dragendorff's reagent (orange red precipitate) and Hager's reagent (yellow precipitate). Specific tests for various alkaloids such as Vitali's morin test for tropane alkaloids, Van Urk's test for ergot and Thalleoquin test for cinchona can also be performed. Borntrager's test is employed for detecting anthraquinone glycosides in purgative drugs such as aloe, cascara, rhubarb, senna and Keller- Killani's test for cardiac glycosides such as digitalis. Similarly, tests can also be

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performed for tannins, proteins, fixed oils etc.

Quantitative test includes acid value, ester value, iodine value, saponification value, acetyl value, peroxide value and hydroxyl value etc. These tests are helpful in evaluation of drugs like volatile oils (acetyl and ester value), balsams (acid, ester and saponification value), resins (acid value and sulphated ash value) and gums (volatile acidity and methoxy determination).

Chemical Assay - The crude drugs can be assayed for a particular group of constituents. The techniques which are used commonly for chemical assay are titrimetric and gravimetric methods. By titrimetric methods the alkaloids can be estimated from alkaloidal drugs; for example quinine from cinchona, reserpine from rauwolfia, atropine from belladonna, emetine from ipecac and strychnine from nuxvomica etc. Similarly anthraquinone and cardiac glycosides can also be estimated by chemical assay. The chemical assay method can also be used for the estimation of carvone in dill oil and caraway oil, cineole in eucalyptus oil, aldehydes in lemon oil etc.

The chemical evaluation also includes the phytochemical investigation carried out for maintaining the chemical profile of crude drug. The phytochemical investigation of plant involves the following stages :-

(i) The procurement of raw material and quality control

(ii) Extraction of plant material

(iii) Separation and isolation of the constituents

(iv) Characterization of the isolated compounds

(v) Investigation of biosynthetic pathways to a particular compound

(vi) Quantitative evaluation

The most commonly employed technique for the separation of active constituents from crude drug is extraction in which different solvents are used. The plant material used for extraction should be properly authenticated. The choice of extraction depends on the nature of plant material and components to be isolated. Dried materials are generally powdered before extraction. When fresh plant parts are used they are homogenized or macerated with a solvent like alcohol. Alcohol is a common solvent for many plant constituents but it may cause problem in the subsequent elimination of pigments, resins etc. Water immiscible solvents like light petroleum is employed for the extraction of essential and fixed oil and steroids. Ether and chloroform are generally used for the extraction of alkaloids, quinones etc. The extraction of oragnic bases such as alkaloids usually necessiates basification of the plant material if a water- immiscible solvent is to be used whereas for phenols and aromatic acids acidification may be required. Glycosides are soluble in water and alcohol but insoluble in chloroform and ether. Tannins are soluble in water, alcohol, dil alkalies, glycerine and are insoluble in organic solvents such as benzene, ether and chloroform. Extraction can be performed by repeated maceration with agitation, percolation or by continous extraction by soxhlet apparatus.

Preliminary phytochemical screening - The plant contain different types of constituents such as alkaloids, glycosides, tannins, resins, essential oil, lipids, carbohydrates etc that exert physiological and therapeutic effects. The compounds which are responsible for the therapeutic property of the drug are usually secondary metabolites. A systematic study of crude drug involves the thorough consideration of primary and secondary metabolites.

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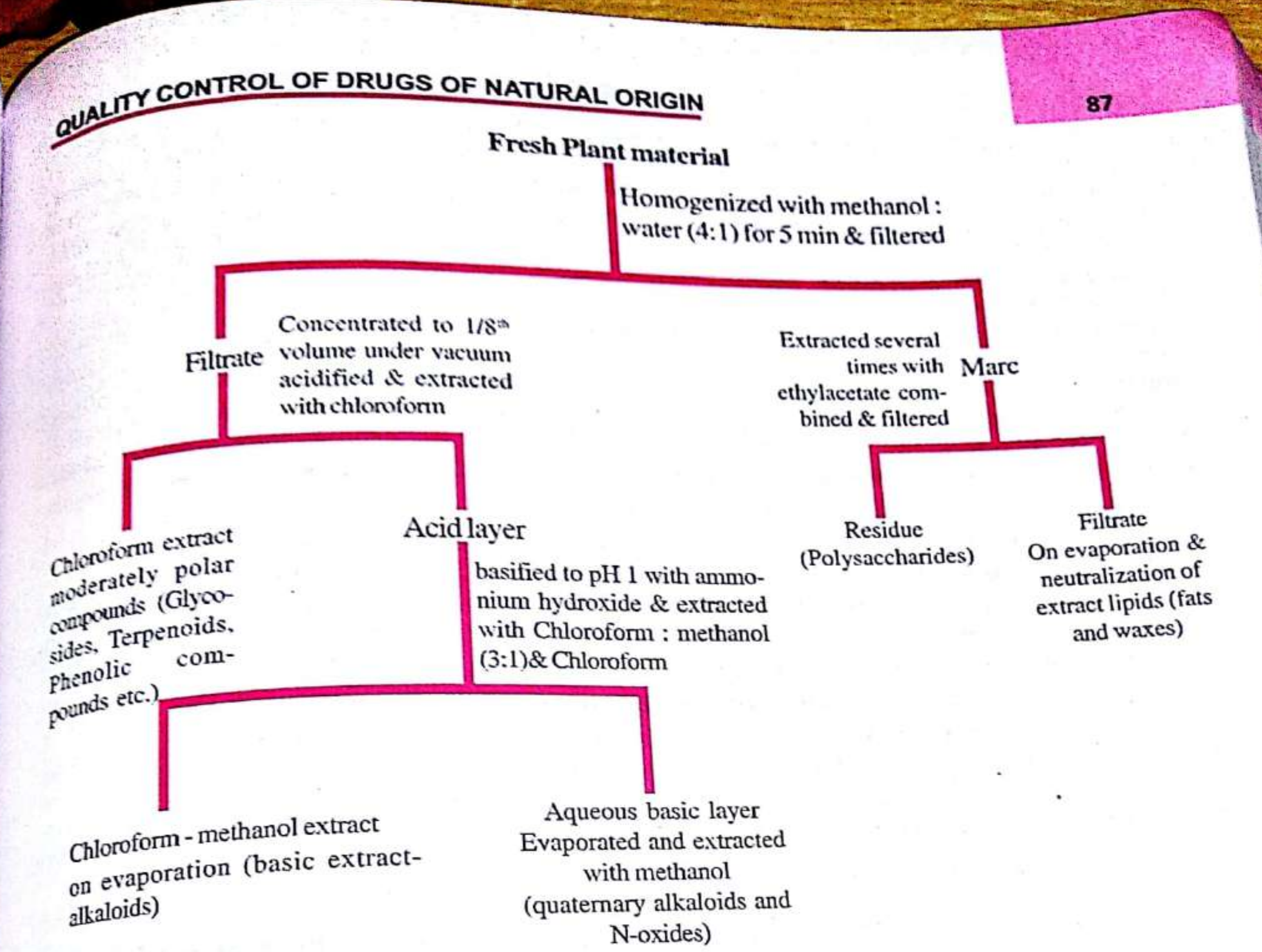

The plant is subjected to preliminary phytochemical screening for the detection of different phytoconstituents as per the following guidelines :-

Extract about 50 gms of the air dried powdered plant material successively with polar and non polar solvents like petroleum ether, benzene, chloroform, acetone, ethanol and methanol in soxhlet assembly .

Each time before extracting with the next solvent dry the powdered material in hot air oven below 50^0 C.

Finally macerate the marc with chloroform water for 24 hrs. to obtain the aqueous extract. .

Concentrate each extract by distilling off the solvent and then evaporating to dryness on water bath.

Weigh the extract obtained with each solvent and calculate its percentage in terms of air - dried weight of the plant material. Also note the colour and consistency of the extract.

A general approach to extract the different phytoconstituents from fresh plant is shown in following chart :-

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It is clear that extract obtained by adopting extraction method should be pure to get the accurate test reaction. If it is not pure then test reaction may not be accurate. Hence some purification procedures are usually adopted before the characterization of individual component. The extract obtained may contain along with desired compound some other substances such as chlorophyll, organic and inorganic acids, fatty substances, resins, other pigments etc. So depending upon the impurities present in the extract the method of purification procedure is adopted. However the separation of constituents by partitioning between two immiscible solvents in which compound dissolves preferentially or precipitation of either the desired medicinal compound or impurity by certain reagent are some of the method commonly used . The extract obtained by the above method is partially purified and it contains closely related constituents in traces. Therefore , the purification of extract is performed by adopting various techniques such as sublimation, distillation, fractional liberation, fractional crystallization, chromatography etc. Out of these the most commonly employed modern technique is chromatography.

Sublimation is sometime possible on the whole crude drug. **Fractional distillation** is used for separation of components of volatile oils. **Steam distillation** is used to isolate volatile oil and hydrocyanic acid from plant material. Some groups of compounds lend themselves to **fractional liberation** from a mixture for e.g. a mixture of alkaloid salts in aqueous solution when treated with aliquots of alkali will give first the weakst base in the free state followed by base liberation in ascending order of basicity . If the mixture is shaken with an organic solvent after each addition then a fractionated series of bases will be obtained. **Fractional crystallization** method exploits the differences in solubility of the components of mixture in a solvent. Frequently derivatives of particular components such as picrates of alkaloid, osazone of sugars are employed.

QUALITATIVE CHEMICAL TESTS

The extracts obtained by the above method are subjected to qualitative test for the identification of different phytoconstituents.

1. Test for Alkaloids - Evaporate the aqueous, alcoholic and chloroform extracts separately. To residue add dil Hcl and filter it. With filtrate perform the following tests -

- Mayer's reagent test - To the filtrate add Mayer's reagent (potassium-mercuric iodide solution); it gives cream coloured precipitate.
- Wagner's reagent test - To the filtrate add Wagner's reagent (potassium-tri iodide solution); it gives reddish brown precipitate.
- Dragendorff 's reagent test - To the filtrate add Dragendorff 's reagent (potassium-bismuth iodide solution) ; it gives reddish brown or orange red precipitate.
- Hager's reagent test - To the filtrate add Hager's reagent (saturated solution of picric acid); it gives yellow coloured precipitate.

2.Test for Glycosides

Test for Anthraquinone glycosides

Modified Borntrager's test :- To 5 ml of extract add 5 ml of 5% solution of ferric chloride and 5 ml of dil Hcl and heat it on water bath for 5 minutes. Cool the solution and filter. Filtrate is shaken with an organic solvent like benzene. Separate the benzene layer and equal volume of dil ammonia is added. A pinkish red colour is formed in ammonical layer. This confirms the anthraquinone glycosides.

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Test for Cardiac glycosides

Keller - Killani test :- 2 ml of extract is boiled with 10 ml of 70% alcohol for 2 minutes. Extract is filtered. To the filtrate 5ml of water and 0.5ml of strong solution of lead acetate is added. Shake it and separate the filtrate. The filtrate is mixed with equal volume of chloroform and evaporated to get the extractive.This extractive is dissolved in glacial acetic acid and cooled and then 2 drops of ferric chloride solution are added. These contents are transferred to test tube containing 2ml of concentrated sulphuric acid. A reddish brown colour is seen at the junction of two liquids which changes to bluish green colour on standing due to presence of deoxy sugars.

Legal test :- The extract is dissolved in pyridine ; sodium nitroprusside solution is added and made alkaline. A pink of red colour is produced.

Baljet test :- To the thick section of drug, sodium picrate solution is added. Yellow to orange colour is seen which confirms the presence of cardiac glycosides.

Liebermann's reaction :- To the 3 ml of extract add 3 ml of acetic anhydride and heat it. After cooling add few drops of conc. H_2SO_4 .Blue colour appears which confirms the presence of cardiac glycosides.

Test for Saponins

Foam test :- Shake the drug extract or dry powder vigorously with water. Persistant foam is observed. This confirms the presence of saponin glycosides.

Haemolytic test :- Add the drug extract to one drop of blood placed on glass slide. Haemolytic zone appears which confirms the saponin glycosides.

TEST FOR CYANOGENETIC GLYCOSIDES

Sodium picrate test :- Soak a filter paper strip in sodium picrate and dry it. Add the moistened powdered drug into conical flask and suspend the filter paper at the neck of flask. After sometime the yellow colour of filter paper changes to brick red due to liberation of hydrocyanic acid which confirms the presence of cyanogenetic glycosides.

A paper is dipped in guaicum resin and it is moistened with dil copper sulphate. This is exposed to fresh drug ; a blue stain is produced.

TEST FOR COUMARIN GLYCOSIDES

Transfer the moistened drug powder in test tube and cover it with filter paper soaked in dil NaoH. Keep in water bath and after sometime expose the filter paper to UV light. A yellowish- green fluorescence is seen.

3. Test for Tannins and Phenolic compounds

To 3 ml of aqueous or alcoholic extract add few drops of following reagents-

5% $FeCl_3$ solution	- Deep blue - black colour
1% gelatin solution containing 10% sodium chloride	- White ppt.
10% lead acetate solution	- White ppt.
Bromine water	- Decoloration of bromine water
Potassium dichromate solution	- Red ppt.
Dil. iodine solution	- Transient red colour

4. Test for Carbohydrates

Molisch's test :- To the 2-3ml of aqueous extract add few drops of Molisch's reagent (5% a napthol in alcohol) and shake it. Slowly add conc. H_2SO_4 along the side of test tube. A violet coloured ring at the junction of two liquids confirms the presence of carbohydrates.

Fehling's test :- To the aqueous extract add equal quantity of Fehling's solution Aand B and boil it. A yellow or brick red ppt. confirms the presence of carbohydrates.

Benedict's test :- To the aqueous extract add equal quantity of Benedict's reagent and heat it for 2-3 minutes. The yellow, red or green colour precipitate confirms the presence of reducing sugars.

Barfoed' s test :- To the aqueous extract add equal quantity of Barfoed' s reagent and heat it on water bath for 2-3 minutes and cool it. A red precipitate confirms the presence of monosaccharide.

Iodine test :- To the 3 ml of extract add few drops of dil iodine solution. Blue colour appears which disappears on boiling and reappears on cooling. Polysaccharide (for e.g. starch) are present.

5. Test for Proteins

Small quantity of alcoholic and aqueous extract is dissolved in few ml of water and subjected to following tests-

Millon' s test :- To 3 ml of extract add 5ml of Millon's reagent. A white precipitate is produced which on warming turns to red colour.

Biuret test :- To 3 ml of extract add 10% NaOH and few drops of 0.5% of copper sulphate solution. Violet or pink colour confirms the presence of proteins.

6. Test for Amino acids

Small quantity of alcoholic and aqueous extract is dissolved in few ml of water and subjected to following test:-

Ninhydrin test :- To 3 ml of extract add 3 drops of 5% Ninhydrin solution and heat on water bath for 10 min. Purple or bluish colour confirms the presence of amino acids.

7. Test for Fixed oil and Fats

A small quantity of extract is pressed between two filter papers. Oil stained on the filter paper indicates the presence of fixed oil.

To the extract add few drops of 0.5 N alcoholic potassium hydroxide and a drop of phenolphthalein and heat it on water bath for 1-2 hours. Formation of soap or partial neutralisation of alkali indicates the presence of fixed oil and fats.

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8. Test for Volatile oils

About 50 gms. of powdered material is subjected to hydro distillation. If volatile oil is present it will be collected in graduated tube of assembly. Separate it and perform the following test -

Stain the filter paper with oil. It will not be permanently stained. This confirms the presence of volatile oil.

The volatile oil posses a characteristic odour.

9.Test for Phytosterols

The petroleum ether, acetone and alcoholic extracts are refluxed separately with alcoholic potassium hydroxide solution till complete saponification takes place. The saponification mixture is diluted with distilled water and extracted with ether. The etheral extract is evaporated and the residue is subjected to Liebermann's Burchard reaction-

Liebermann' s Burchard reaction :- To the residue add chloroform and 2 ml of acetic anhydride and 2 drops of conc.sulphuric acid along the side of test tube. First red then blue and finally bluish green colour confirms the presence of phytosterols.

PHYSICAL EVALUATION

Physical standards are helpful in evaluation of crude drugs. Some of them are mentioned below:-

Foreign organic matter- I.P describes, foreign organic matter is the material consisting of any or all of the following-(i) parts of the organ from which the drug is derived other than the parts named in definition and description or for which a limit is prescribed in the individual monograph

(ii) any organs other than those named in the definition and description

(iii) moulds, insects or other animal contamination.

Pharmacopoeias prescribes the maximum limit of foreign organic matter for vegetable crude drugs. If it exceeds the limit than the drug is declared substandard and deterioration in the quality of drug can occur. Examples are mentioned below:-

Drugs	Limit of foreign organic matter
Fennel	Not more than 2%
Caraway	Not more than 2%
Cardamom fruit	Not more than 1%
Dill	Not more than 2%

Moisture content- The moisture present in the crude drug may cause deterioration due to microbial growth or chemical changes. Therefore the moisture content of crude drugs should be minimized and it should be within the prescribed limits of pharmacopoeias. Moisture content can be determined by heating the drug at 105°C to constant weight and calculating the loss of weight . The moisture content of drugs containing volatile oil can be determined by toluene distillation method. Some of the examples are listed below:-

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Drugs	Moisture content (% w/w)
Digitalis	Not more than 5
Balsam of tolu	Not more than 4
Storax	Not more than 5
Tragacanth	Not more than 15

Melting point- The crude drugs obtained from plants and animals have different types of chemicals so they are described with the particular range of melting point. Hence the melting point range of standard drugs should comply with the prescribed range of pharmacopoeias. The melting point of pure chemicals or phytochemicals is sharp and constant. Some of the examples are as follows:-

Drugs	Melting point
Hydrous wool fat	34 – 40° C
Spermaceti	45 – 50° C
Lard	36 – 42° C
Lac	115 – 120° C

Viscosity- Viscosity is a property of a liquid which is closely related to the resistance to flow. Viscosity of a liquid is constant at a given temperature. If any type of adulterant is present it will change the viscosity of the drug. The viscosity of Newtonian liquids can be determined by capillary viscometer and for Non- Newtonian liquids rotating viscometer is used. Some of the examples are mentioned below –

Liquid paraffin – Kinematic viscosity not less than 64 centistokes at 37.8°

Light liquid paraffin – Kinematic viscosity not greater then 30 centistokes at 37.8°.

Solubility - The study of solubility is also helpful in evaluation of drugs. Alkaloidal salts are freely soluble in water, whereas its bases are soluble in organic solvents. Fixed oils and fats are soluble in ether, chloroform and benzene whereas they are insoluble in alcohol except castor oil whose solubility is due to presence of hydroxyl group in ricinoleic acid. Volatile oils are soluble in alcohol, chloroform, ether and acetone etc. whereas they are insoluble in water. Similarly Indian gum is entirely soluble in twice its weight of water. Therefore if any type of adulterant is present it will alter the solubility of drug.

Refractive index – The refractive index of a substance is the ratio of the velocity of light in vacuum to its velocity in the substance. It can also be defined as the ratio of sine of the angle of incidence to the sine of angle of refraction. The refractive index of liquid drugs like fixed oils and volatile oils is measured and it is constant. If any type of adulterant is present the refractive index of drug will alter. The refractive index of any substance generally varies with the wavelength of refracted light and with temperature. Some of the examples of refractive index are given for sodium light at 25° C (± 0.5): –

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Drugs	Refractive index
Mustard oil	1.4758 to 1.4798
Orange oil	1.472 to 1.476
Cod liver oil	1.471 to 1.477
Lemon grass oil	1.4808 to 1.4868

Optical rotation – Certain substances in a pure state or in solution posses the property of rotating the plane of polarized light. These substances are said to be optically active and the property of rotating the plane of polarized light is known as optical rotation. This property may be utilized for identifying a substance. The extent of rotation is expressed in degrees; plus (+) indicating rotation to right (dextrorotatory) and minus (-) indicating rotation to left (levorotatory). Optical rotation is measured by polarimeter using sodium lamp as a source of light at a temperature of 25°C. Some of the examples are listed below –

Drugs	Angle of optical rotation
Fennel oil	+12° to + 24°
Pepermint oil	- 18° to - 33°
Dill oil	+ 70° to + 80°
Ajowan oil	0° to + 2°

Volatile oil content – Aromatic drugs have a pharmaceutical importance due to presence of volatile oil content in them. These drugs can be evaluated on the basis of volatile oil content. Some of the examples are listed below –

Drugs	Volatile oil content (% v/w)
Cassia	Not less than 1
Bitter orange peel	Not less than 2.5
Coriander	Not less than 0.3
Caraway	Not less than 3.5
Ajowan	Not less than 2
Calamus	Not less than 1.5

Ash values – When a crude drug is incinerated it leaves a residue behind it which is called as ash content. This residue contains inorganic salts such as carbonates, phosphates, silicates and silica which may adhere to the drug naturally or deliberately added to it for adulteration purpose. Sometimes the crude drugs are admixed with various substances such as sand, calcium oxalate, chalk powder or other drugs with various inorganic contents. Different types of ash figures such as Total ash, acid insoluble ash, water soluble ash and sulphated ash are used and these may be helpful in evaluation of crude drugs.

Total ash consist of carbonates, phosphates, silicates and silica. The acid insoluble ash is helpful in determining the excessive sand mixed with the drug. The water soluble ash is used to detect the presence of material exhausted by water. Some of the examples are mentioned below–

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Drugs	Total ash (% w/w)	Acid insoluble ash (%w/w)	Water soluble ash (%w/w)
Senna	-	Not more than 2	-
Ginger	Not more than 6	-	Not less than 1.7
Clove	Not more than 7	Not more than 0.75	-
Ipecacuanha	Not more than 5	Not more than 2	-
Digitalis	-	Not more than 5	-
Cinchona	Not more than 4	-	-
Cardamom seed	Not more than 6	Not more than 3.5	-
Cinnmon	-	Not more than 2	-
Fennel	-	Not more than 1.5	-
Rauwolfia	Not more than 8	Not more than 2	-
Aconite	Not more than 5	Not more than 1	-
Nutmeg	Not more than 3	Not more than 0.5	-

Extractive values- The determination of extractive values is used as a means of evaluating crude drugs, the chemical constituents of which are not readily estimated by other means. Extractive values indicates the approximate measures of chemical constituents. The drug contain different types of chemical constituents therefore the solvent selected for extraction process should be capable to dissolve the appreciable quantities of desired substances. The extractive values are classified below on the basis of solvent used:-

Water-soluble extractives – This method is used for those drugs which contains water soluble active constituents such as sugar, glycosides, tannins, mucilage, plant acids etc. Some of the examples are given below –

Drugs	Water-soluble extractive (%w/w)
Aloe	Not less than 25
Ashoka	Not less than 11.4
Liquorice	Not less than 20
Bael	Not less than 30
Senna leaves	Not less than 30
Ginger	Not less than 10

Alcohol soluble extractive – Alcohol is an ideal solvent for extraction of various constituents such as resins, tannins etc. Normally 95% ethyl alcohol is used in this method. Sometimes depending upon the solubility of various constituents of drugs the diluted alcohol is also used. Examples are as follows:-

Drugs	Alcohol soluble extractive (%w/w)
Aloe	Not less than 10
Sumatra benzoin	Not less than 75
Siam benzoin	Not less than 90
Bael (90% alcohol)	Not less than 40
Ginger (90% alcohol)	Not less than 4.5
Quillaia (45% alcohol)	Not less than 28
Valerian (60 % alcohol)	Not more than 30

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Ether soluble extractives- There are two types of ether soluble extractive values viz. volatile ether soluble extractives and non- volatile ether soluble extractives. The volatile ether soluble extractive represents the volatile oil content of drug whereas the non- volatile ether soluble extractives represents fixed oils, resins and colouring matter present in the drug. Some of the examples are listed below:-

Drugs	Non- volatile Ether soluble extractive (%w/w)
Nutmeg	Not less than 25
Capsicum	Not less than 12
Linseed	Not less than 25

CHROMATOGRAPHY

Chromatography technique has become popular for both the qualitative and quantitative evaluation of herbal drugs. Chromatography is the separation of a mixture into individual components using a stationary phase and a mobile phase. The stationary phase may be porous or finely divided solid or liquid which is coated as a thin layer on an inert support material. The mobile phase may be liquid or mixture of liquids or gas or mixture of gases and it moves through or over the stationary phase.

Thin layer chromatography (TLC)- In 1958 Stahl demonstrated the application of TLC in analysis. The principle of separation is **adsorption.** One or more compounds are spotted on thin layer of adsorbent coated on a chromatographic plate. The mobile phase solvent flows through it because of capillary action. The components move according to their affinities towards the adsorbent in such a manner that the component with lesser affinity towards the stationary phase travels faster and the component with more affinity towards the stationary phase travels slower. In this way the components are separated on thin layer chromatographic plate.

The TLC plates are prepared by coating adsorbent such as silica gel* H or G or GF to a thickness of 0.25 mm by a spreader(air drying) so that cracks do not develop on the surface of adsorbent. After setting plates are activated by keeping in an oven at 100°C to 120°C for 1hr and used. The samples are spotted by using capillary tube at least 2cm above the base of plate and chromatogram is developed by keeping the plates in development tank containing mobile phase. The spots are detected by spraying the specific reagents. The qualitative analysis can be done by calculating the Rf (Retardation factor) value by using the following formula :-

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance traveled by solvent front}}$$

The R_f value is specific and constant for every compound in a particular combination of stationary and mobile phase.The Quantitative analysis is done by using densitometric method. The TLC technique is useful in analysis of vitamins, proteins, carbohydrates, glycosides, alkaloids and other plant extracts. There is no limitation to the compounds that can be analysed by TLC. The advantages of TLC are mentioned below:-

It is a simple method and cost of the equipment is also low.

Any type of compound can be analyzed.

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Even the microgram of substance can be separated.

TLC is a rapid technique and it does not consumes time like column chromatography.

The capacity of thin layer can be altered. So analytical and preparative separation can be made.

It has a efficiency of separation . Very small particle size can be used which increases the efficiency of separation.

Detection is easy.

The following table consist of some specific examples of application of thin layer chromatography to drugs.

Constituents	Thin layer	Developing solvents	Detection of separated components
Alkaloids			
Cinchona	Silica gel	Isopropanol - benzene-diethylamine (2:4:1)	UV light
Opium	Alumina	Chloroform - ether- water (3:1:1)	Iodine in carbon tetrachloride
	Silica gel	Methanol - Chloroform (1:9)	Iodine in carbon tetrachloride
	Alumina	Benzene - Methanol (8:2)	Dragendorff's reagent
		Benzene - acetic acid (9:1)	Potassium iodoplatinate reagent
		Benzene - Chloroform -acetone (70:15:15)	
Rauwolfia	Alumina	Chloroform- ethanol-acetone (90:5:5)	Dragendorff"s reagent
Vinca	Silica gel	Ethyl acetate - absolute ethanol (3:1)	Cerric ammonium sulphate reagent
Glycosides			
Aloes	Polyamide	Ethylacetate-formic acid-water (10:2:3) upper phase Ethylacetate - chloroform -ethanol (9:5:0.5) Ethylacetate -pyridine - water (5:1:4) upper phase	Ammonia vapour ; 2.5% potassium hydroxide solution
Cardiac glycosides	Silica gel	Dichoromethane - methonal-tormamital (80:19:1)	Antimony tricholridin chloroform
Sapogenins discover	Silica gel	Choloform- ethenol (95:5) Choloform - outove (3:1)	Antimony chloride in chloroform
Valatile oil	Silica gel	Benzone- Chloform (1:1) Benzone Ethyloatato (95:5)	Antimony tricholorarte in chloroform

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High performance thin layer chromatography (HPTLC)- HPTLC is a sophisticated and automated form of TLC. It is useful in qualitative and quantitative analysis of natural products. The principle of separation is **adsorption** (Same as that of TLC). In HPTLC the precoated plates are used and the particle size of stationary phase is less than 10u in diameter. There is a wide choice of stationary phases like silica gel for normal phase and C18, C8 etc. for reverse phase mode. HPTLC provides a higher efficiency than TLC because adsorbents used are small and uniform in size.

A very less amount of sample is spotted on the plate so the sample prepared should be highly concentrated. The size of the sample spot should not be more than 1mm in diameter. The samples are spotted by various techniques and commonly used method is by self loading capillaries in which sample is spotted to the HPTLC plate surface using platinum –irridium tubing fused into the end of a length of glass tubing. The other methods used for spotting the samples are like chemical focusing, contact spotting and programmed multiple development.

New types of development chambers are used in HPTLC which requires less amount of solvents for developing. A linear development technique is commonly used. The plate is placed vertically in development chambers containing solvent and chromatogram can be developed from the sides. The plates can be developed by other methods such as circular development, anti-circular device and multiple development. In HPTLC, UV/ Vis / Fluorescence scanner is used therefore it scans the entire chromatogram qualitatively and quantitatively. The scanner is an advanced type of densitometer.

HPTLC is used for the standardization of herbal extracts and other formulations. By using this technique the analytical profiles of alkaloids, cardenoloids, anthracene glycosides, flavonoids, lipids, steroidal compounds etc. have been developed.HPTLC is also employed to obtain finger print patterns of various herbal formulations and quantification of active ingredients. The HPTLC methods used for estimation / detection of some herbal constituents are mentioned below :-

Aloin

Source - Tincture of *Aloe vera* var officinalis

Stationary phase- Silica gel

Mobile phase - Ethylacetate -formic acid - water (17:2:3)

Quantification :- UV absorbance in densitometry at 350 nm.

Carvone

Source - Extract of Cuminum cyminum

Stationary phase - Silica gel

Mobile phase - Chloroform - acetone (100:2)

Detection - By dipping in anisaldehyde sulphuric acid reagent and heating at 80^0 C for 10 minutes

Quantification - UV absorbance in densitometry at 410 nm.

Cholesterol

Source - Bear gall bladder powder

Stationary phase- Silica gel

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Mobile phase- Ethyl acetate - acetone - petroleum ether (2:1:11)

Detection - Spraying with 10% sulphuric acid in alcohol and heating at 100^0 C for 5 minutes

Quantification - UV absorbance in densitometry at 400 nm.

Panaxadiol and Panaxatriol

Source - Market formulation of ginseng

Stationary phase - Silica gel

Mobile phase - Chloroform - ether (1:1)

Detection - Spraying with 10% sulphuric acid in methanol and heating at 105^0 C for 10 minutes

Quantification - UV absorbance in densitometry at 544nm and 52 nm.

Column chromatography- When a column of stationary phase is used the technique is called as column chromatography and when stationary phase is solid it is called as **column adsorption chromatography**. The principle of separation of column adsorption chromatography is **adsorption**. When a mixture of component dissolved in the mobile phase is introduced into the column the individual components move with different rates depending upon their relative affinity. The compounds with lesser affinity towards the stationary phase (adsorbent) moves faster and it is eluted out first from the column. The one with greater affinity towards stationary phase (adsorbent) moves slower and is eluted out later. Hence the compounds are separated.

In column chromatography the various adsorbents used are like silica gel (activated magnesium silicate), activated alumina, activated magnesia, calcium carbonate, magnesium carbonate, fuller's earth, talc, starch and inulin.The different mobile phase used either singly or in combination are like petroleum ether, carbon disulphide, ether, benzene, toluene, water, organic acids, carbon tetrachloride etc. .

Column chromatography is used for the separation of constituents such as glycosides, alkaloids, amino acids and plant extracts. The impurities present in compounds can be removed by using appropriate stationary phase. The active constituents present in crude drugs or plant extracts can be separated by using this technique.

Column partition chromatography- When a stationary phase is liquid it is called as Column partition chromatography. This type of chromatography is not used widely.

High performance liquid chromatography (HPLC)- High performance liquid chromatography has a improved performance when compared to classical column chromatography. It is also known as high pressure liquid chromatography as high pressure is used when compared to column chromatography.

The various instruments used in HPLC are pump, mixing unit, injector, guard column, analytical column, detectors and recorders. The solvents or mobile phases used must be passed through the column at a high pressure of about 1000 to 3000 psi. This is achieved by using either mechanical pumps or pneumatic pumps. Mixing unit is employed to mix solvents in different proportions and pass through the column. The sample is injected either manually or by auto injection through injectors. The various injectors used are Rheodyne injector and Septum injectors. Guard column is used to improve the life of analytical column. It acts as a

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prefilter to [illegible]ired matter and the guard column does not contribute to separation. The [illegible] column is the most important part of HPLC technique which decides the efficien[illegible] separation. The columns are made up of either glass or stainless steel, or poly ether [illegible] ketone (PEEK). The length of column varies from 5cm to 30 cm and diameter ranges from 2mm to 50mm. This apparatus is suitable for all types of liquid chromatography columns such as adsorption, partition by use of bonded liquid phases, reversed phase, gel filtration, ion exchange and affinity. The detectors used in HPLC depends upon the property of compounds to be separated. The various types of detectors available are like Photodiode array detector, UV- detector, Flourimetric detector and Amperometric detector etc. The recorders are used to record the responses obtained from detectors after amplification. Now a days computers and printers are used for recording and processing the obtained data and for controlling several operations.

HPLC is a versatile and sensitive technique by which the qualitative and quantitative analysis of various alkaloids, glycosides, flavonoids, terpenes, plant pigments, steroids and antibiotics can be done. Apart from its use in pharmaceutical field it is also used in chemical and petrochemical industries, forensic laboratories, environmental applications, biotechnology, food analysis etc. Infact there is no field where HPLC is not being used.

Gas chromatography- Gas chromatography is of two types viz. Gas solid chromatography (GSC) and Gas liquid chromatography (GLC). In both types gas is used as mobile phase and either solid or liquid is used as stationary phase. GSC is not used widely because of limited number of stationary phases available. Gas liquid chromatography (GLC) is widely used and all the discussion in this topic refers to GLC technique only. GLC was introduced by James and Martin in 1952.

The principle of separation in GLC is **partition**. Gas is used as mobile phase. Liquid which is coated on to a solid support is used as stationary phase. The mixture of components to be separated is converted to vapour and mixed with gaseous mobile phase. The component which is less soluble in stationary phase travels faster and eluted out first and the component which is more soluble in stationary phase travels slower and eluted out later. Hence components are separated according to their partition co-efficients.

The compounds to be analysed by gas chromatography should be volatile and thermostable. The practical requirements of gas chromatography are carrier gas, flow regulators, flow meters, injection devices, columns, temperature control devices, detectors and recorders. The choice of carrier gas determines the efficiency of chromatography separation. The commonly used gases are helium, hydrogen, nitrogen and argon. As carrier gases are stored under high pressure flow regulators are used to deliver the gas with uniform pressure.Flow meters are used to measure the flow rates of carrier gas. The different types of injection devices are used to inject the samples (gas, liquid or solid) into the column. Column is one of the most important part of gas chromatography which decides the efficiency of separation. Columns are made up of glass or stainless steel. Preheaters are used to control the temperature and they convert the sample into its vapour form and mix them with the carrier gas or mobile phase. Detectors are the most important part of gas chromatographic instruments. The various detectors used are like flame ionization detectors, thermal conductivity detectors (katharometer), electron capture detector and argon ionization detector. Recorders are used to record the responses obtained from detectors after amplification.

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Many drugs which contain sugars, phenols, carboxylic acids and alcohol etc. produce badly tailed peaks due to interaction of functional groups with stationary phase.To overcome this problem derivatisation technique is adopted which is of two types viz. pre-column derivatisation and post column derivatisation. In pre-column derivatisation the components are converted to more volatile and thermolabile derivatives. In post column derivatisation the components are converted in such a way their ionization or affinity towards electrons is increased. The commonly used derivatising agent is BSA reagent (Bis trimethyl acetamide reagent).

Gas chromatography is helpful in qualitative and quantitative analysis of alkaloids, glycosides, resins, plant acids, steroidal compounds, amino acids, sugars etc.

Gel permeation chromatography or Size exclusion chromatography- In this chromatography the mixture of components with different molecular sizes are separated by using gels. The gel used acts as a molecular sieve and hence mixture of substances with different molecular sizes are separated. Soft gels like dextran, agarose or polyacrylamide are used. Semi rigid gels like alkyl dextran, polystyrene in non- aqueous medium are also used.

The mechanism of separation is by steric and diffusion effects. For the separation purpose the stationary phases used are cross-linked polymers which provide an open network with large number of pores of uniform size. During the flow of mobile phase through this stationary phase the large sized molecules are unable to enter into the pores and hence get excluded and travel along with mobile phase where as the low sized molecules enter freely into different pores and hence find a longer path through the column. Therefore the molecules of larger size are eluted out first with mobile phase followed by molecules with smaller size.

Gel permeation chromatography is used for desalting protein solution, studies of plasma-binding of drugs and determination of molecular size of drugs.

Affinity Chromatography – Affinity chromatography uses the affinity of the sample with specific stationary phases. The adsorbent used is a biological substance (called as receptor) and it has a specific affinity for other substance. These two substances are biologically interacting pairs. Such type of adsorbent is attached to porous stationary phase and placed in column. When a mixture containing the other complement of the adsorbent (interacting pairs) is passed through stationary phase, selective separation occurs. During elution, the complementary part absorbed is collected in pure form by dissociating the interacting pair with the help of changing the ionic strength or pH of the column buffer.

This technique is employed for the separation of enzymes, proteins, ribosomes, nucleic acid, peptides, antibodies, antigens etc. This technique is mostly used in the field of biotechnology, biochemistry and microbiology.

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SPECTROPHOTOMETERY

Crude drugs contain specfic group of phytoconstituents and assay of these group of phytoconstituents, for example alkaloids of solanaceous drugs or glycosides of cardiotonic drugs, helps in evaluation of drugs. In spectroscopic analysis, the basis of drug evaluation is the capacity of certain molecules to absorb vibration at specific wavelength. The techniques frequently employed in pharmaceutical analysis includes ultra- violet, visible, infra- red, nuclear magnetic resonance (NMR), mass spectroscopy etc. The wavelength range available for these measurements extends from the short wavelength of ultra-violet through infra-

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red. The spectral range is roughly divided into the ultra violet (185 -380 nm), the visible (380-780 nm) , the near-infra red (14000-4000 cm-1) and the mid- infra red (4000-400 cm^{-1})

Ultraviolet and Visible Spectroscopy :- Ultraviolet - Visible spectroscopy involves the sepctroscopy of photons in the UV - Visible region. It uses light in the UV (185-380 nm) and visible (380-780nm) region. In this region of electromagnetic spectrum molecule undergoes transition from ground state to the excited state . The absorption in the visible region directly affects the colour of the chemical involved .

The instrument used in the ultraviolet - visible spectroscopy is called UV/Vis spectrophotometer. It measures the intensity of light passing through a sample (I) and compares it to the intensity of light before it passes through the sample (Io).The ratio I / Io is called transmittance. The basic parts of a spectrophotometer are a light source, a holder for the sample, a diffraction grating or monochromator to separate the different wavelengths of light and a detector . A spectrophotometer can be either single beam or double beam. In a single beam instrument all of the light passes through the sample cell. Io is measured by removing the sample. In double beam instrument the light is split into two beams before it reaches the sample. One beam is used as the reference ; the other beam passes through the sample. Some double beam instruments have two detectors (photodiodes) and the sample and reference beam are measured at the same time. In other instruments the two beams pass through a beam chopper which blocks one beam at a time. The detector alternates between measuring the sample beam and the reference beam. Samples of UV/Vis spectrophotometery are most often liquids although the absorbance of gases and even of solids can also be measured. Samples are typically placed in a transparent cell know as cuvette. An ultraviolet - visible spectrum is essentially a graph of light absorbance versus wavelength in a range of ultraviolet or visible regions.

UV - Visible spectroscopy is now a days most widely used technique in pharmaceutical analysis. The variety of natural products of pharmaceutical importance can be analyzed by this technique. Following table comprises of some examples of the phytoconstituents that can be analyzed by UV - Visible spectroscopy.

Infra Red Spectroscopy - Infra red spectroscopy is the subset of spectroscopy that deals with infra red region of electromagnetic spectrum. It covers a range of technique the most common being a form of absorption spectroscopy. The infra red portion of the electromagnetic spectrum is divided into three regions namely the near-, mid - and far- infra red named for their relation to the visible spectrum. The far- infra red (400-10 cm^{-1}) lying adjacent to the microwave region has low energy and may be used for rotational spectroscopy. The mid- infra red (4000-400 cm^{-1}) may be used to study the fundamental vibrations and associated rotational - vibrational structure. The near -infra red (14000-4000 cm^{-1}) has higher energy and can excite overtone or harmonic vibrations. Of these regions, only the mid-infra red region is commonly used in the analysis of drugs and pharmaceuticals.

The technique is based upon the simple fact that a chemical substance shows marked selective absorption in the infra red region. After absorption of I.R radiations the molecules of a chemical substance vibrate at many rates of vibration giving rise to close - packed absorption bands called as I.R absorption spectrum which may extend over a wide wavelength range. Various bands present in I.R spectrum correspond to the characteristic functional groups and bonds present in a chemical substance. Thus, an I.R spectrum of a chemical substance is a finger print for its identification.

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Region of spectrum	Constituents	Wavelength
Ultraviolet	Colchicine Lobeline Morphine Reserpine Vincristine Vinblastine Cassia oil (aldehyde content) Vitamin A (cod liver oil)	350NM 249NM 286NM 268Nm 297Nm 267Nm 286Nm 328Nm
Visible	Ergot	550Nm
	Morphine	442 NM (By Nitroso reaction)
	Reserpine	390 nm (By treatment of alkaloid with sodium nitrate in dil acid)
	Capsaicin in capsicum	730 nm (after reaction with phospho-molybdic acid and sodium hydroxide solution)
	Menthol from peppermint oil	500- 570 nm (green filter) by use of p- dimethylaminobenzaldehyde reagent.

I.R spectrophotometers may be single or double beam instruments.The main parts of I.R spectrophotometers are radiation source, monochromators, sample cells and detectors. Fourier transform spectrophotometer is the latest advancement in the field of I.R. spectroscopy. Fourier transform spectroscopy is a measurement technique whereby spectra are collected based on measurement of the temporal coherence of a radioactive source using time domain measurements of the electromagnetic radiation or other type of radiation.

Infra red spectroscopy is generally used in the identification of the functional group of biomolecules thus aiding in their structure elucidation. This identification has been extended to such a diverse application as the determination of hormones, steroids and pharmaceutical chemicals is easily possible. The lipids, carbohydrates, amino acids, proteins, nucelic acid , enzymes and many other biochemical compunds have been extensively studied. The quantitative determination of various compunds by I.R spectroscopy is based on determination of the concentration of one of the functional group of the compound being estimated. The quantitative analysis of steroidal sapogenins, antibiotics, alkaloids (strychnine and quinine) is feasible.

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Fluorimetry - The phenomenon of emission of light radiations by substances due to excitation in any form is know as Luminescence. The basic principle of fluorimetry is that the absorption of UV / Visible radiations causes transition of electrons from singlet ground state to singlet excited state . As this state is not stable it emits the energy in the form of UV /Visible radition and returns to singlet ground state. The study or measurement of the emitted radiations when electrons undergo transition from singlet excited state to singlet ground state is the principle of Fluorimetry . Fluorescence is the phenomenon of emission of radiation when there is transition from singlet excited state to singlet ground state.

The instruments used for the measurement of fluorescence are known as fluorimeters. The most commonly used flourimeters are (i) Single beam (filter) fluorimeter (ii) Double beam (filter) fluorimeter

(iii) Spectrofluorimeter (Double beam). They consist of source of light (lamp), filter and monochromators, sample cells and detectors. In single beam filter fluorimeter the primary filter absorbs visible radiation and transmits UV radiation which excites the molecules present in sample cell. The emitted radiations are measured at 90° by using a secondary filter and a detector. Double beam filter fluorimeter is similar to single beam except that the two incident beams from a single light source pass through primary filters separately and fall on either sample or reference solution. The emitted radiations from sample or reference pass separately through secondary filter and produces response combinedly on a detector. In spectrofluorimeter (double beam) the primary filter is replaced by excitation monochromator and the secondary filter is replaced by emission monochromator. The incident beam is split into sample and reference beam by using beam splitter. The advantage of spectrofluorimeter is that it is sensitive and provides accuracy and there is rapid scanning.

Light rich in short wavelength is very active in producing fluorescence and for this reason strong ultraviolet light (which can be obtained from mercury vapour lamp or tungusten arc) produces fluorescence in many substances which do not visibly fluoresce in day light. Fluorescence lamps are usually fitted with suitable filter which eliminates visible radiation from lamp and trasmits ultraviolet radiation of desired wavelength. Under fluorescent light cinchona bark shows yellow patches and few light blue ones. Many alkaloids evaluated qualitatively shows distinct colour for eg aconitine (light blue), berberine (yellow) and emetine (orange). Ipecac root produces a brightly luminous appearance whereas the hydrastis rhizome shines golden yellow. Slices of calumba appear intensely yellow with the cambium and phloem distinguished by their dark green colour. In general fixed oil and fat fluoresce least, waxes more strongly and mineral oils (paraffins) most of all.

The quantitative fluorescence analysis is also possible. This technique utilizes the fluorescence produced by a compound in UV light. The instrument used is a fluorimeter or spectrofluorimeter. With plant extracts it is important to note that (i) the substance being determined is the only one in the solution producing a fluorescence at the measured wavelength. (ii) there are no substances in the solution which absorb light at the wavelength of the fluorescence. Quinine can be assayed by the measurement of the fluorescence (366 nm) produced by irradiation of the alkaloid in dilute sulphuric acid solution of about 450 nm. Alexandrian senna has been assayed by the measurement of the fluorescence produced in Borntrager's reaction. The hydrastine content of hydrastis root may be determined by oxidizing an extract of drug with nitric acid. Emetine and papaverine may be determined fluorimetrically after oxidation with acid permanganate and noscapine after oxidation with

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per- sulphate.

Nuclear Magnetic Resonance Spectroscopy - NMR spectroscopy is the study of spin changes at the nuclear level when a radiofrequency energy is absorbed in the presence of magnetic field. When a proton (Hydrogen) is studied it is called as proton magnetic resonance (PMR). When other nuclei 13C, 19 F, 35 Cl etc are studied then it is called as NMR. Commonly, in practice the study of hydrogen (proton) itself is called as NMR spectra. Nuclei with odd mass number only give NMR spectra for e.g., 1H, 13C, 19F, 35 Cl etc because they have assymetrical charge distribution. Other nuclei like 12C, 16O, 14 N, 2H etc do not give NMR spectra because of symmetrical charge distribution. The principle of NMR spectroscopy is that any proton or nucleus with odd mass number spins on its own axis. By the application of an external magnetic field (Ho), the nucleus spins on its own axis and a magnetic moment is created resulting in precessional orbit with a frequency called as precessional frequency. This state is known as ground state. In this state the magnetic field caused by the spin of nuclei is aligned with the externally applied magnetic field. When energy in the form of radiofrequency is applied and when applied frequency is equal to precessional frequency, absorption of energy occurs and a NMR signal is recorded.

In any NMR instrument the main components are RF transmitter, RF receiver / detector, sweep generator, recorder and sample cell. In practice the radiofrequency is kept constant and the length of magnetic field is varied since vice versa is difficult to achieve. In NMR spectroscopy as we analyze the organic compounds for the nature, type, number and environment of protons (Hydrogen), therefore the solvent used should not contain hydrogen atoms. Hence the solvents used are like Deuterated water (D_2O), carbon tetrachloride (Ccl4), Deuterated methanol (CD_3OD), Deuterated chloroform ($CDCl_3$) etc.

NMR spectroscopy is extensively used in the elucidation of molecular structure especially the stereochemistry and configuration. It also have several applications in the determination of impurities and minor components in mixtures because of specificity of the analysis. It is employed for identification test in pharmaceutical analysis. The use of NMR best known to general public is magnetic resonance imaging (MRI) for medical diagnosis and MR microscopy in research settings. NMR can also be coupled with mass spectrometer . The quantitative analysis can also be performed by NMR spectroscopy. The assay of components for e.g. single component or multicomponent without separation of components can be quantitatively measured. Specific peak for each component is identified and the peak area / height ratio given by integral value is found using standard and sample and the quantity can be estimated. Surfactant chain length determination can be done from the proportion of hydrogen atom in poly - oxethylene chain. The percentage of hydrogen in compound can also be determined. Iodine value which is measure of double / triple bond can be known from the proportion of olefinic protons.

Mass Spectrometry - The mass spectrometry principle consist of ionizing chemical compounds to generate charged molecules or molecule fragments and measurement of their mass to charge ratio (m / e). In the technique of mass spectrometry the compounds under investigation is bombarded with a beam of electrons which produce an ionic molecule or ionic fragments of the original species. The resulting arrangement of charged particles is then separated according to their masses. The spectrum produced known as mass spectrum is a record of information regarding various masses produced and their relative abundances. MS instrument consist of three modules ; an ion source which splits the sample

molecule into ions ; a mass analyzer which sorts the ions by their masses by applying electromagnetic field and a detector which measures the value of an indicator quantity and thus provides data for calculating the abundances of each ion present.

Mass spectrum is an analytical technique which provides information regarding the structure of molecule. It is also used to determine directly the moelcular weight of compounds. A mass spectrometer is also useful to investigate reaction mixture and in tracer work. An important enhancement to the mass resolving and mass determining capabilities of mass spectrometry is using it in tandem with chromatographic separation technique. A common combination is gas chromatography - mass spectrometry (GC-MS). Another commonly used technique is liquid chromatography - mass spectrometry (LC-MS). LC-MS differs from GC-MS in that the mobile phase is liquid usually a mixture of water and organic solvents instead of gas. Another employed technique with MS is the ion mobility spectrometry /mass spectrometry (IMS/MS).

X - Ray Diffraction- This method is based on scattering of X-rays by crystals. X-ray crystallography is used to determine the arrangements of atoms within a crystal in which a beam of X-rays strikes a crystal and scatters into many different directions. From the angles and intensities of these scattered beams a three dimensional picture of density of electrons within the crystal is produced. From this electron density the mean positions of atoms in the crystal as well as their chemical bonds can be determined.

Since many materials can form crystals such as salts, metals, minerals, semiconductors as well as various inorganic, organic and biological molecules. This method is commonly used to determine the size of atoms, length and type of chemical bonds and the atomic scale differences among various materials especially minerals and alloys. It is also employed to reveal the structure and functioning of many biological molecules including vitamins, drugs, proteins and nucleic acid such as DNA. X-ray crystal structure can also account for unusual or elastic properties of material, shed light on chemical interactions and processes or serve as the basis for a designing pharmaceuticals against diseases.

Immunoassays- These assays are highly sensitive and very specific and now developed as a powerful analytical tool for the quantitative determination of many compounds in biological fluids.

(i) Radioimmunoassay (RIA) :- It is a sensitive method and depends upon the highly specific reactions of antibodies to certain antigens. This technique was introduced in 1960 by Berson and Yalow as an assay for the concentration of insulin in plasma. Owing to the work of Weiler, Zenk and colleagues in 1976, this method has been successfully applied to plant medicinals. There are various modifications of the technique and the saturation method has been developed for phytoanalysis. Usually the small molecules (below MW 1000) constituting the secondary plant metabolites are not involved in such immuno responses but when bound covalently to protein carriers or haptens they do become immunogenic. If such a hapten is prepared in the labelled condition (eg ^{3}H- or ^{125}I- labelled) with a known specific activity, mixed with an unknown amount of unlabelled hapten and added to a limited amount of antibody in the form of a serum then there will be competition between the labelled and unlabelled antigen for the restricted number of binding sites available. This results in some bound and unbound hapten. These can be separated and a determination of radioactivity in either fraction with reference to a standard curve enables the amount of unlabelled antigen to be calculated. The antiserum is raised in suitable animals.

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The advantage of Radio immunoassay is that small amount of plant materials are required. Relatively crude unprocessed plant extracts can be used and the process can be mechanized. It is an efficient tool for analyzing the large number of plants and hundred of specimens can be assayed in a single day. With herbarium material assays can be performed on the samples ranging from 0.5 mg to few milligrams and in the examination of individual plants, structures as small as anther filaments can be accommodated. The clinical applications of RIA are like drug detection, blood bank screening for hepatitis virus, early cancer detection, measurement of growth hormone levels, diagnosis and treatment of peptic ulcers etc.

(ii) Enzyme- linked immunosorbent assays (ELISA) - In the enzyme linked immunosorbent assay, competition for an immobilized antibody takes place with a modified form of compound under analysis that has an enzyme bound to it. The release of compound - enzyme complex from binding site and determination of enzyme activity enables the original solution to be quantified . As with RIA ,this method is very sensitive for e.g. the pyrolizidine alkaloid retronecine can be measured in the parts per billion range and one sclerotium of ergot can be identified in 20 kg. of wheat.

BIOLOGICAL EVALUATION

The drugs which cannot be evaluated by physical or chemical means are subjected for biological evaluation. Biological evaluation is also done for the confirmation of therapeutic activity of raw material and finished products. When the potency of crude drug or its preparation is estimated by measuring its effect on living organisms like bacteria, animal tissue or entire animal it is termed as bioassay. The bioassay methods are of three types – (i) toxic (ii) symptomatic and (iii) tissue method. In toxic and symptomatic methods the animals are used whereas in tissue method the isolated organ or tissue are used.

The biological activity is represented in units called as International Units (I.U). Some of the examples of biological activity contained in each I.U of few drugs are listed below:-

Digitalis – 1 I.U is contained in 76 mg of standard preparation.
Vitamin A- 1 I.U is present in 0.344 ug of standard preparation.
Vitamin D- 1 I.U is contained in 0.025 ug of standard preparation.

Some of the drugs which are subjected to bioassay are like cardiac glycosides, antibiotics and natural pesticides. In evaluation / standardization of herbal drugs assessment of biological efficacy is found to be most assuming method. The following methods are used for biological evaluation :-

(i) Antipyretic activity
(ii) Anti- inflammatory activity
(iii) Hypoglycaemic activity
(iv) Antiulcer activity
(v) Analgesic activity
(vi) Microbiological assays
(vii) Cardiac activity :- Drugs containing cardiac glycosides like digitalis are bioassayed on frog, cat or pigeon.
(viii) Anthelmintic activity :- Anthelmintic drugs such as male fern are bioassayed on earthworms.

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(ix) The bitlter drugs like gentian, chirata and quassia can be evaluated by bitter value whereas capsicum and ginger can be evaluated for pungency value.

(i) Antipyretic activity :- For testing the antipyretic activity rabbits are used. Rabbits of both sexes and of different strains with a body weight between 3 to 5 kg. are used. Rabbits are placed in cages and thermocouples connected with an automatic recorder are inserted into the rectum. Rabbits are allowed to adopt to the cages for one hour. Then 0.2 ml / kg. containing 0.2 ug of lipopolysaccharide is injected intravenously into the ear of rabbit. After one hour the test compound is injected into the rabbit either orally or subcutaneously. Body temperature is monitored for at least 3 hours. A decrease of body temperature for at least 0.5^0 C for more than half an hour as compared with the temperature value before administration of the test compound is considerd to be the positive response.

(ii) Anti-inflammatory activity :-

Paw oedema method :- The principle underlying the testing of anti-inflammatory activity is the reduction of local oedema induced in rat paw by injecting irritant and inflammatory substances. Most common inducer is carrageenan which is a sulphated polysaccharide extracted from the red seaweed *Chondrus crispus*. For testing the anti- inflammatory activity male or female rats with a body weight from 100 to 150 gms are used. Rats are starved overnight. To insure uniform hydration the rats are given 5 ml of water by stomach tubes (control) or the test drug dissolved in the same volume. After thirty minutes rats are challenged by subcutaneous injection of 0.1 ml. of 1% w/v solution of carrageenan into the plantar side of the left hind paw. The paw is marked with ink at the level of lateral malleolus and immersed in mercury up to this mark. The paw volume is measured by Plethysmometer just after the injection and then every after an hour. The increase of paw volume after 3 or 6 hours is calculated as percentage compared with the volume measured immediately after injection of irritant for each rat. Effectively treated animals show much less oedema. The difference of average values between treated animals and control groups is calculated for each time interval and statistically evaluated.

(iii) Hypoglycaemic activity :- Generally the plant extracts that possess hypoglycaemic activity are like Karela (*Momordica charantia*), Fenugreek (*Trigonella foenum - gracecum*), Jamun (*Syzigium cumini*) and Gudmar (*Gymnema sylvestre*). For testing the hypoglycaemic activity experimental diabetes is induced in animals by streptozocin or alloxan. Alloxan is an urea derivative and its suitable dose induces moderate diabetes (fasting- blood sugar levels 180-250 mg/ml) and then herbal drug extracts are tested. Streptozocin is an nitrourcido glucopyranose derivative which also induces diabetes.

Before treatment with plant extract glucose loading is given or animals are fasted and then glucose tolerance test is carried out. Animals employed for this activity are rabbits, rats or mice. Alloxan in a single injectable dose (140-180 mg/kg.) is given to all types of animals in marginal ear vein of rabbit or intraperitoneally in mice or rats. It causes diabetes in 7 days in rabbits and 2 days in mice or rats. Streptozocin in a single oral dose (mice -150 mg./kg. and rats- 80 mg./kg.) causes diabetes within 4to 7 days . The blood glucose levels are measured by two classical methods using glucose oxidase or ortho-toluidine. Glucose autoanalysers are also employed to measure the glucose level. Sometime the insulin level is measured in glucose tolerance test to find out whether glucose itself or plant extracts are stimulating insulin secretion to produce hypoglycaemic effect. Radio - immunoassay (RIA)

and Enzyme linked immunosorbent assays (ELISA) can also be employed for measurement of insulin levels.

(iv) Anti-ulcer activity:- The factors which are responsible for the acidity are diet, stress (physical and mental), alcohol and non steroidal anti-inflammatory drugs. Plant drugs that are used as anti-ulcerogenic are liquorice, hyoscine, atropine and gefarnate (obtained from white cabbage juice). For testing the anti-ulcer activity of a plant drug , the ulcer is induced by anyone of the following agents-

Chemicals :- The ulcer can be induced by alcohol (1ml/kg. body weight, orally), aspirin(200 mg./kg., body weight, orally), prednisolone, endomethacin and serotonin(5-HT).

Stress :- The stimuli such as immobilisation and cold are used . Animal is immobilised in a cage and temperature is maintained between 4-5⁰C. Animal used is male wistar rat or guinea pig. The animals are grouped as following :-

Those treated with normal saline.

Those treated with ulcerogen in saline or ulcerogenic stress procedure.

Test groups- Along with ulcerogen (chemical or stress) plant extracts in suitable dose.

Standard reference group- Along with ulcerogen (chemical or stress) a known anti-ulcer drug such as liquorice or ranitidine.

The ulcers are induced by ulcerogens .After one hour the animals are sacrificed and the stomach or duodenum (for histamine induced ulcer) are given a slit along the curvature to asses the damage caused due to ulcer. Sometimes gastric acid is also measured so for this purpose before slitting along the curvature the stomach is ligated and contents are drained and collected . The damage caused by ulcer and protection given by anti- ulcer agent is measured by ulcer index. Ulcer index is expressed on simpler scale as-

0	=	No damage
1	=	Redness of mucosa
2	=	Erosion of mucosa
3	=	Ulceration

In elaborate ways the ulcer index is expressed as :-

0	=	Absence of ulcer
1	=	Slightly dispersed and haemorrhagic ulcers (less than 2 mm in length)
2	=	One ulcer upto 5 mm in length
3	=	More than one ulcer
4	=	One ulcer above 5 mm in length
10	=	Total ulceration and haemorrhage

The further ulcer healing activity can be tested by performing the experiments such as estimation of sialic acid content and DNA determination.

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(v) Analgesic activity

Hot plate method :- Animals used for this method are mice. Groups of 10 mice of either sex with an initial weight of 18 to 22 gms. are selected for each dose. The hot plate consists of electrically heated surface and the temperature is maintained at 55 to 56°C. The mice are placed on the hot plate and the time untill either flicking or jumping occurs is recorded by a stopwatch. The latency is recorded before and after 20, 60 and 90 minutes following oral or subcutaneous administration of the test or standard drug.

Tail immersion test :- Animals employed for this test are young female wister rats having body weight from 170-210 gms. Rats are placed into individual restraining cages in such a condition that tail hangs out freely. They are allowed to adapt to the cages for half an hour. The lower 5 cm part of tail is marked and is immersed in a cup of freshly filled water having a temperature of 55°C. Within few seconds rat reacts by withdrawing the tail. This reaction time is recorded in 0.5s units by stopwatch and after each determination tail is dried carefully. The reaction time is recorded before and periodically after either oral or subcutaneous administration of the test substance for e.g. 0.5, 1, 2, 3, 4, and 6 hours. The cut off time of the immersion is 15 s. The withdrawl time of untreated animals is between 1 to 5.5s . A withdrawl time of more than 6 s is considered as a positive response.

Heffner's tail clip method :- Animals employed for this method are male mice. The control group and test group consists of 7-10 mice. The test compounds are administered subcutaneously or given orally to fasted animals. The drug is administered 15, 30 or 60 minutes prior testing. An artery clip is applied on the root of tail about 1 cm. from the body to induce pain. The mice quickly responds to this noxious stimulus by biting the clip or tail near the location of clip. The time between stimulation onset and response is measured by stopwatch.

(vi) Microbiological assays:- In this method the bacteria, yeast and moulds are employed for assaying antibiotics and few vitamins. The procedure employed in microbial assay of antibiotics is divided into two broad categories:-

(A) Cylinder - Plate method or Cup Plate method :- The most commonly used method for the assay of majority of important antibiotics is the cylinder plate method. This method depends upon the diffusion of an antibiotic through a solidified agar layer to such an extent as to give a 'Zone' of inhibition around the cylinder (cups) containing the antibiotic solution. Thus the potency of antibiotic is determined by comparing the dose of the sample with the dose of standard preparation necessary for producing a clear zone of inhibition of the same size.

(B) Turbidimetric method or Serial dilution method :- The turbidimetric assay of drug potency is based on inhibition of microbial growth as indicated by the measurement of the turbidity (transmittance) of a suspension of a suitable microorganism in a fluid medium to which has been added a graded amount of the test compounds. Changes in transmittance produced by the tested compound are compared with those produced by known concentrations of standard.

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Chapter 4

CULTIVATION, COLLECTION, PROCESSING AND STORAGE OF DRUGS OF NATURAL ORIGIN

CULTIVATION

There are certain drugs which are obtained only from cultivated plants such as Indian hemp, isapghula, cardamom, saffron, peppermint, linseed, ginger etc. But there are few drugs which are derived from both wild and cultivated plants. Majority of the medicinal plants are now a days grown because supplies from wild plants are insufficient to meet the demands. Cultivation of medicinal plants is gaining importance in India and other countries. The emergent of new scientific techniques have contributed a lot and these techniques have subjected a systematic cultivation of various medicinal plants. Even though there are some drugs which are obtained from wild or natural sources but cultivation of medicinal plants provides various advantages over their wild sources. The various advantages of cultivation are listed below-

It provides the medicinal plants or crude drugs of better quality and purity. The crude drugs obtained posses a good quality with regard to colour, odour, taste, shape, and size. The drugs contain appropriate quantities of chemical constituents and therefore exerts a better therapeutic action.

It gives higher yield of crude drugs. However it depends upon the collection of crude drugs which is a skilled operation . If the collection of drugs is performed by skilled labour then higher yield can be maintained. For example the higher yield of latex* from poppy capsules can only be maintained when it is collected by skilled labour.

It ensures a regular supply of crude drugs to the market and industries. Therefore it also helps in the development of various types of industries. The cultivation of tea in Assam, Tamilnadu and West Bengal has given rise to cottage and small scale industries.

Cultivation also helps in application of new scientific techniques like hybridization, mutation** and polyploidy which ultimately provides a improved quality of crop.

METHODS OF CULTIVATION

There are two methods of cultivation viz. Sexual and Asexual method.

SEXUAL METHOD (PROPAGATION FROM SEEDS)

In sexual method the plants are raised by propagating the seeds. These plants are known as seedlings. Seeds which are to be used for propagation should be of standard quality and they should be free from disease, insects and extraneous material. They should posses high germination rate. If the seeds are not to be germinated in near future they should be stored

* A viscid, milky juice secreted by some plants.

** A permanent transmissible change in the genetic material

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in cool and dry place and must not be kiln dried. Long storage of all seeds decreases the percentage of germination. If the seeds have slow germination rate then special treatments can be given to the seeds which increases the germination rate. For example soaking the seeds in water for 24 hrs. or 0.2% solution of gibberellic acid for 48 hrs. A more drastic method can be used in which seeds are soaked in sulphuric acid e.g. henbane seeds. Another method recommended is the partial removal of the testa by means of grindstone. Seeds which do not germinate in higher temperature or in dark, normally thiourea is employed for germination.

ADVANTAGES OF SEXUAL METHOD

Seedlings are cheap and can be raised easily.

Seedlings are strong and have a longer life.

These plants produce heavily (in fruits).

It is a method of choice when any other vegetative method cannot be employed.

DISADVANTAGES

These plants takes a longer time to bear as compared to the grafted plants.

These plants are not uniform in the growth and they yield less as compared to grafted plants.

It is not possible to avail the modifying influence of root stocks on scion.

These plants are less resistant against the disease as compared to grafted plants.

ASEXUAL METHOD

In the asexual method of vegetative propagation a vegetative part is detached from the body of mother plant and this detached part grows up into a new independent plant under suitable conditions. These method are –

Underground stems – The underground stems are modified and buds are produced on them which gradually grows up into new plants.

(a) Rhizome – Turmeric, Ginger

(b) Tuber – Potato

(c) Bulb – Onion

(d) Corm – Colchicum

Sub-aerial stems – The runner, stolon and sucker, sub-arieal stems give rise to new plants e.g. mints, strawberry, valerian etc.

Cuttings – Stem and root cuttings are taken from plants and put into moist soil where they strike root at the base and develop adventitious roots which grow into new plants. Examples are –

(a) Stem cuttings – Sugarcane, Duranta etc.

(b) Root cuttings – Lemon, Citrus etc.

Layering – In this method, the stem-branch, which is to form a new plant, remain attached to the parent plant. It is pegged down so that part of it lies along the ground and from this horizontal pieces, the leaves are removed. This injury prevents the sugar made in leaves from passing down to the roots that is towards the growing point of the plant. The horizontal portion of the stem is covered with soil and when it is well rooted, the branch can be removed and planted elsewhere. It is practiced in plants like rose, lemon, jasmines etc.

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Gootee – Gootee is slight modific[illegible] of layering. It differs from layering only in so far that the injured branch is not burie[illegible]he ground but is bound up with wet earth, rags etc. which are kept moist with the help of an earthen pot containing water. After about a month or two when roots develop at this portion the branch is severed from the parent plant and planted in soil. This method is practiced in plants like orange, litchi, pomegranate etc.

Grafting – In grafting two cut surfaces of different but closely related plants are placed so as to unite and grow together. The rooted plant is known as **stock** and the portion cut off is called as **scion** or **graft.**

Budding – Budding is essential similar to grafting. It differs in so far that instead of a branch with many buds, a single bud which is to be propagated is removed. This portion is nearly diamond shaped and is inserted in T- shaped incision made in stock. After about 15-20 days it is observed that the cambium of the bud and the stock grows together and that the bud has become a part of the new plant. This method is practiced in citrus species, roses etc.

Aseptic method of micropropagation – It is a new method for propagating the medicinal plants. In this method plants are raised in an artificial medium under aseptic conditions from pieces of plants like single cells, embryo, seeds, shoot tips, root tips etc.

ADVANTAGES OF VEGETATIVE PROPAGATION

The plant acquire quick mastery over their surroundings by producing large number of plants.

It is far more certain method of producing new plants than that of seed propagation.

There is no variation between plant grown and the parent plant.

Plants bear early as compared to seedling plants.

It is possible to avail the modifying influence of root stocks on scion.

These plants are more resistant against the disease as compared to seedling plants.

The seedless varieties of the fruits can be propagated e.g. lemon, grapes etc.

The plants are uniform in size and yield more as compared to seedling plants

DISADVANTAGES

Due to over crowding of large number of plants near the parent plant, there is a severe competition between the members of same species. Thus many plants become stunted and weak.

There may be degeneration of the species due to the lack of sexual stimulus.

The organs used in vegetative reproduction are very poor means of propagation.

FACTORS AFFECTING CULTIVATION

There are several factors which affects the cultivation of medicinal plants. These are briefly discussed here –

Altitude – Altitude is an important factor which affects the cultivation of medicinal plants. With an increase in altitude above sea level there are changes in values of temperature, humidity, solar radiation etc. Due to these changes vegetation at different altitudes differ much. Tea and coffee are cultivated at an altitude of 1000-2000 meters. Rhubarb, cinchona, tragacanth also requires elevation for cultivation. Altitude also affects the chemical

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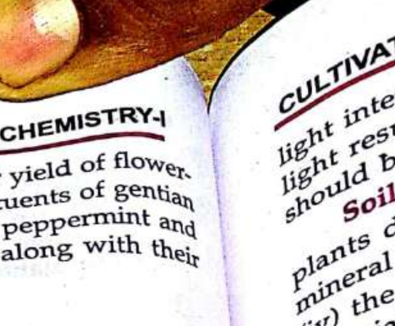

composition of medicinal plants. For example pyrethrum provides the better yield of flower-heads and pyrethrins near the equator or at high altitudes. The bitter constituents of gentian increases with altitude whereas the alkaloids of aconite and oil content of peppermint and thyme decreases. The examples of few medicinal plants are given below along with their altitudes-

Plant	Altitude(Meters)
Rhubarb	2500-4000
Digitalis	1600-3000
Cinchona	1000-3500
Cinnamon	800-1200
Rauwolfia	up to 1300
Vinca	up to 600
Clove	up to 900

Temperature – Temperature profoundly affects the plant growth and metabolism. It also affects the rate of transpiration and regulates the activity of enzymes which inturn regulates the physiological processes. Extremes of temperature both on cold and hot sides affects the quality of medicinal plants. Although each species has become adapted to its own natural environment, plants are frequently able to exist in considerable range of temperature. Some of the examples of medicinal plants are mentioned below along with their temperature range required for their optimum growth –

Plant	Temperature range (°C)
Digitalis	20-30
Ipecac	22-40
Cinchona	10-30
Rauwolfia	10-40

Rainfall – Different regions of earth receive different quantities of rainfall depending upon geographical features. The quantity, duration and intensity of rainfall regulates the plant life. The effects of rainfall on vegetation must be considered in relation to the annual rainfall, its distribution throughout the year and its effects related to the water holding capacity of the soil. Majority of the plants need sufficient amount of rainfall for the growth. Excessive or less rainfall affects the plant life and constituents of the plant. For example in *Cassia angustifolia* it has been proved that short term drought increases the concentration of sennosides A and B but in longer term it causes loss of biomass.

Day- length and radiation characteristics – Light plays an important role in photosynthesis. Light also regulates carbondioxide and oxygen exchange between plants and atmosphere. Plants vary in both the amount and intensity of light which they require. Light in some plants determines the content of constituents. For example in cinchona and belladonna a full sunshine gives a higher content of alkaloids as compared to shade. There are many plants which initiate flowering only in certain day- lengths therefore light factor should be considered carefully before planting the plant. Light also controls the daily variation in the proportion of secondary metabolites. The type of radiation received by the plants is also an important factor as it influences the various chemical contents. The reduced

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light intensity and variation in temperature affects the metabolic activity where as excess light results in overheating of plants which is again injurious. Therefore more attention should be given to light factor.

Soil – Soil is the medium in which root grows, anchor the plants and from which the plants derive water and nutrients. All the soils have the following components; (i) the mineral matter (ii) the soil organism and the organic matter (iii) soil water and soil solution (iv) the soil atmosphere. The mineral particles such as sand, silt or clay are the primary material that constitute the soil. Depending upon the size of mineral matter the **International society of soil science** has given different names to these mineral particles which are as follows –

Name of particle	Diameter range
Clay	Less then 0.002mm
Silt	0.002 to 0.02mm
Fine sand	0.02 to 0.2mm
Coarse sand	0.2 to 2mm
Stones and Gravel	Above 2mm

Clay is formed as the final product of weathering and through precipitation of aluminuim and silicon salts present in dissolved state in the soil moisture. It provides adhesive and cohesive properties to the soil. The relative percentage of coarse sand, fine sand, silt and clay determines soil texture. On the basis of the proportion of different sized particles soils are classified into different textural groups as follows –

Textural group	Relative proportion of different sized mineral particles
Sandy soil	85% sand + 15% clay or silt or both
Loamy sand	70% sand + 30% clay or silt or both
Loam soil	50% sand + 50% clay or silt or both
Silt	90% silt + 10% sand

The organic matter in soil is received from the dead bodies of plants and animals of all types and sizes. Organic matter is the chief source of mineral's return to soil. The quantity and availability of soil water to the plants is a great determining factor of the nature and composition of vegetation of any place. Rain is the principal source of water for the soil. An ideal soil required for the plant growth should have half of the pores filled with water and rest with air as good aeration stimulates the root development.

The pH values of soil shows much correlations with the soil type, vegetation type thus affecting plants growth, lime requirement and mineral nutrition. The pH of the soil strongly affects the microbial activities. The maximum nutrients are available to the plants in between the pH range of 6.5 to 7.5.

Soil fertility is defined as the capacity of the soil to provide nutrition to the plants in balanced and adequate amount.

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Fertilizers- Plants need 16 nutrient elements for their growth and metabolism. Depending upon the quantity needed the nutrients are classified into macronutrients and micronutrients.Macronutrients are needed in large quantities and micronutrients in traces. Carbon, hydrogen, nitrogen, oxygen, calcium, potassium, phosphorous, sulphur, and magnesium are the macronutrients and copper, zinc, boron, molybdenum, iron, manganese, chlorine are the micronutrients (Trace elements). Carbon, hydrogen and oxygen are obtained from air and water.

These elements are supplied to the plants through the soil. These elements can also be supplied to the plants through animal manures and chemical fertilizers because soil is unable to cater the needs of plants. Each element has its own role in growth and development of plants and their deficiency may cause disease. For example fertilizers containing nitrogen increases the size of plants and also influence the chemical constituents like alkaloids, glycosides, volatile oils etc.Commonly used chemical fertilizers are Urea, DAP(Di ammonium phosphate), NPK, and SSP(Single super phosphate) etc.

Pests and pest control – Pest is an undesired animal or plant species. The various types of pest which infests the plants are like virus, fungi, weeds, insects and non inect pests. These pests directly affects the plant growth and development and produce disease which ultimately influences the quality and yield of crude drugs. Hence, control of pest is essential and it should be given importance.

Types of Pests – The various types of pests which infests the plants are like virus, fungi, weeds, insects and non insect pests.

Virus – Various types of virus causes disease in medicinal plants. Strains of *Cucumber mosaic virus* causes disease in hyoscyamus whereas *Tobacco mosaic virus, Tobacco ring spot virus* and *Cucumber mosaic virus* are known to cause infection in digitalis. Potato virus -X causes mosaic disease in potato in which inter-veinal mottling of leaves is common and it is followed by necrosis. The affected leaves droop and wither. *Banana virus-* I causes bunchy top in banana which results in marginal chlorosis and curling of leaves and ultimately plant remains stunted. The other commonly known viruses are *Rugose leaf curl, Yellow vein mosaic, Distortion mosaic*, Graft transmissible virus etc.

Fungi – The various types of fungi are known to cause disease in medicinal plants. *Phytophthora erythrosceptica* causes damping off in young seedlings and wilt in matured plants. *Cerscospora atropae* causes leaf spot in which round brown spots are produced on the both sides of leaves. *Cerscospora dioscorea* produces leaf-spot on dioscorea and *Alternaria tennussima* produces leaf spot on datura. *Uromyces hobosonii* produces rust and *Cerscospora jasminicola* causes leaf blight on *Jasminum* species. *Uromyces ciceris-arietini* and *Uromyces fabae* produces rust on gram and pea respectively. *Cerscospora personata* and *Cerscospora arachidicola* causes tikka disease in groundnut. *Fusarium oxysporum f. udum*, *Fusarium oxysporum f. vasinfectum* and *Fusarium oxysporum f. cubense* causes wilt in cotton and banana. *Phythium aphanidermatum* causes stem rot in papaya and *Phytophthora infestans* causes potato blight. Similary several other pathogenic fungi infest the medicinal plants and causes disease.

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Weeds – A weed is an undesired plant growing in crop field. Weeds causes drastic damages to the plants and this problem is common in agriculture. Majorly it causes loss of nutrients and water in all the plants. It also causes loss of space, increases the attacks of fungi, bacteria, virus and insects which causes disease and ultimately it influences the quality and price of crude drugs.

There are few weeds which causes allergies like medican tea and ragweed causes hay fever. Varnish tree and western poison oak causes dermatitis. Therefore weeds should be controlled properly.

Insects – Different types of insect pests are reported which attacks on plants and causes severe problems. The plants should be protected from them. The various examples of insects pest are like *Ephestia elutella* (Moth)* attacks on tobacco, rose petals and cocoa. *Tinea pellionella* and *Ephestia kuehniella* (Moths) attacks on almond, tobacco and capsicum. *Ptinus fur* and *Ptinus hirtellus* (Beetles) attacks on ginger, capsicum, nutmeg and cereals. *Plantia virdicolis* and *Diaphania nilgirica* attacks on rauwolfia.

The other types of insect pests which causes damage to medicinal plants are like aphids, caterpillar, termites, grass-hoppers, spiders, mites and locusts.

Non – insect pests – Non insect pests are classified into two groups viz. vertebrates** and invertebrates.*** Vertebrates includes rabbits, monkey, rat, squirrel, pigs, hares and deer etc. Invertebrates are like snails, crabs, mites and nematodes etc. The rodents have sharp and gnawing incisor with which they causes severe damage to stored crude drugs. The fecal material of these animals causes contamination of crude drugs.

Methods of pest control – The different types of methods used to control the pest are discussed briefly –

Agricultural method – It involves various types of methods. One of the method is that in which fields are deeply ploughed which removes the weeds and insects. Crop rotation can also be followed. In this method crops are grown alternatively.

Another method which is common now a days is **crop improvement.** It is achieved by a technique called as plant breeding. By this technique hybrid varieties of the plants are produced which are resistant to disease and pest.

Biological method – Biological control brings about reduction in activity of pest mostly insect, by another organism. This may be biocidal or biostatic. In biocidal biological control one organism kills the other while in biostatic the organism only inhibits the other. Biological control is defined by Garrett as "any condition under which or practice whereby survival or activity of a pathogen is reduced through the agency of any other living organism (except man) with the result that there is a reduction in the incidence of the disease caused by pathogen".

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* It is a winged insect

** Animals having a spinal column

*** Animals having no spinal column

Mechanism of Biological control – " Antagonism" which plays key role in biological control operates in three ways –

Antibiosis – where one species secretes some chemicals which inhibits the growth of the other.

Exploitation – where one is parasitic or predatory on other.

Competition – the organism competes for a substances which is in short supply.

The real value of the biological control does not lie in natural methods but in abstracting their underlying mechanism and applying them.

Chemical control- Pests can be controlled by using pesticides. Pesticides are the chemicals derived from natural or chemical sources effective against pest in small concentrations. Pesticides posses toxic effects so their use is governed by Insecticide Act in India. Pesticides are classified according to the type of organism against which they are effective viz. fungicides, nematocides, molluscicides, rodenticides, bactericide etc.

Fungicides- These are the agents that destroy fungi . Examples are Chlorophenols, Bordeaux mixture, Azoxystrobin, Benomyl, Benzalkonium chloride, Allyl alcohol etc.

Insecticides- Agents which kill the insects are termed as insecticides. Examples are Parathion, Malathion, *D.D.T, D.D.E, Sodium arsenate, Benzene hexachloride (BHC), Heptachlor, Phorate, Demeton, Carbaryl, Dieldrin, Aldrin, Methoxychlor etc.

Herbicides- The weed killers are termed as herbicides. Examples are Sulphuric acid, Calcium arsenate, 2,4-dichlorophenoxy acetic acid etc.

Rodenticides- These agents are destructive to rodents. Examples are Strychnine, Red squill, Warfarin, Bromodiolone, Brodifacoum, Zinc phosphide, Yellow phosphorous etc.

Azoxystrobin

Benomyl

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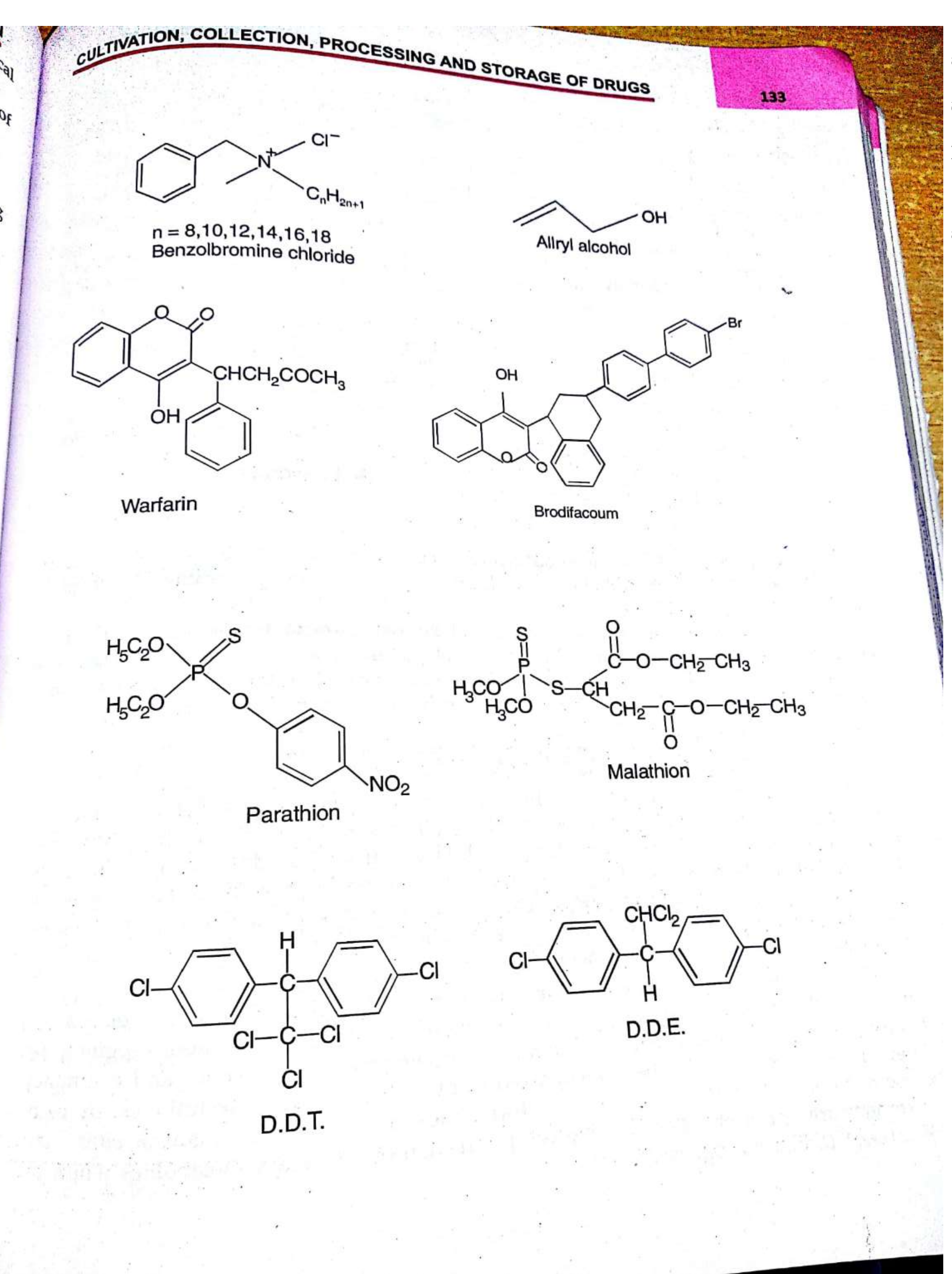
* Dichlorodiphenyl trichloroethane is a contact insecticide and its use is now a days generally discouraged.



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Carbaryl

Dieldrin

Methoxychlor

Aldrin

Mechanical control- This method involves the destruction of pests by manual labour using different devices. The techniques used are hand-picking, pruning, burning and trapping of pests.

Flying insects can be trapped by placing flavoured attractants (flavoured with anise oil, rose oil etc.) mixed with saw-dust in funnel shaped container. These containers are designed in such a way that entry into these containers is easy but it is very difficult to come out. Rodents like rats, rabbits etc. can be trapped by using rat traps.

PLANT GROWTH REGULATORS

The growth of plants is regulated by certain organic compounds which are present in very small quantities. These are called growth regulators in the sense that they either promote, inhibit or in some way modify the growth, development and differentiation in plants. The term plant hormones or phytohormones is applied for the growth regulators which is synthesized in one part of plant but which is responsible for a particular response at some other part (site) in that plant. It is transported or channelized through the plant body from its site of production to its site of action.

Plant hormones belong to five groups viz. (i) auxins (ii) gibberellins (iii) cytokinins (iv) abscisic acid and its derivatives (v) ethylene. In general, the plant hormones regulate cell enlargement, cell division, cell differentiation, organogenesis, senescence and dormancy. Their importance have also been recognized in plant tissue culture techniques. By using these hormones it is now possible to culture almost any part of the plant *in vitro*. Plant hormones are also useful in enhancing cell production of secondary metabolites which are of interest to Pharmacognosist.

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Auxins- Auxins derive their name from Greek word "auxano" which means 'to increase'. Auxins are the most thoroughly studied group of plant hormones and are a major coordinating signal in plant development. There are two types of auxins viz natural auxins and synthetic auxins.

Natural auxins- These are produced by plant themselves and include like indole- 3-acetic acid (IAA), 4-chloro- indoleacetic acid, indole -3- acetonitrile (IAN) and phenyl acetic acid. Of these IAA is the principle auxin.

Synthetic auxins- These have same actions as that of natural auxins and include like indole -3- butyric acid (IBA), 1- naphthalene acetic acid (NAA), 1-napthyl acetamide (NAD), 2- napthyloxyacetic acid (NOA), 5-carboxymethyl-N, N-dimethyl dithiocarbamate, 2,4-dichlorophenoxy acetic acid (2,4-D) and trichlorophenoxyacetic acid (2,4, 5-T).

Auxins play an essential role in coordination of many growth and behavioural processes in plant life cycle. Some of the important roles of auxins are enumerated below-

It stimulates considerable cell enlargement and cell elongation due to its effect on the cell wall plasticity.

It promotes growth of the root at very low concentration and that of the shoot at higher concentration.

It stimulates seed germination.

Auxins are responsible for morphogenetic effect and causes tissue differentiation.

Auxins help in production of parthenocarpic fruits.

Auxins have a strong stimulating effect on reproductive processes like flowering, pollen grain germination, fertilization and fruit development.

Auxins prevent preharvest fruit drop in apples, pear etc.

It exerts inhibitory effect on growth of the lateral buds which are present in the axil of the leaves present at the lower nodes. This inhibition of growth due to the influence of auxin produced by the main growing stem apex is called apical dominance.

Synthetic auxins like IBA and NAA are used to accelerate the rooting of woody and herbaceous cuttings. The promising results were obtained in *Cinchona, Pinus, Coffea, Carica* and others species. 2,4-D and 2,4,5-T in higher concentrations are used as selective herbicides or weed-killers in horticulture and agriculture. 2,4-D is particularly toxic to dicotyledonous plants while in suitable concentration have little effect on monocotyledonous also. It is therefore widely used to destroy dicotyledonous weeds such as dandelion and plantain from grass lawns. The addition of IAA, NAA and 2,4-D in tissue culture techniques has a considerable effect on production of secondary metabolites. The treatment of seedlings and young plants of *Mentha piperita* with NAA has shown about 30-50% increase in volatile oil content. The addition of 2,4-D stimulates the production of ubiquinone and scopolatin in tobacco cultures and solasodine content in *Solanum eleagnifolium*. There are also examples available when auxins inhibited the production of secondary metabolites for e.g NAA and IAA inhibited similar to 2,4-D, the synthesis of anthocyanin in cell suspension cultures of carrot.

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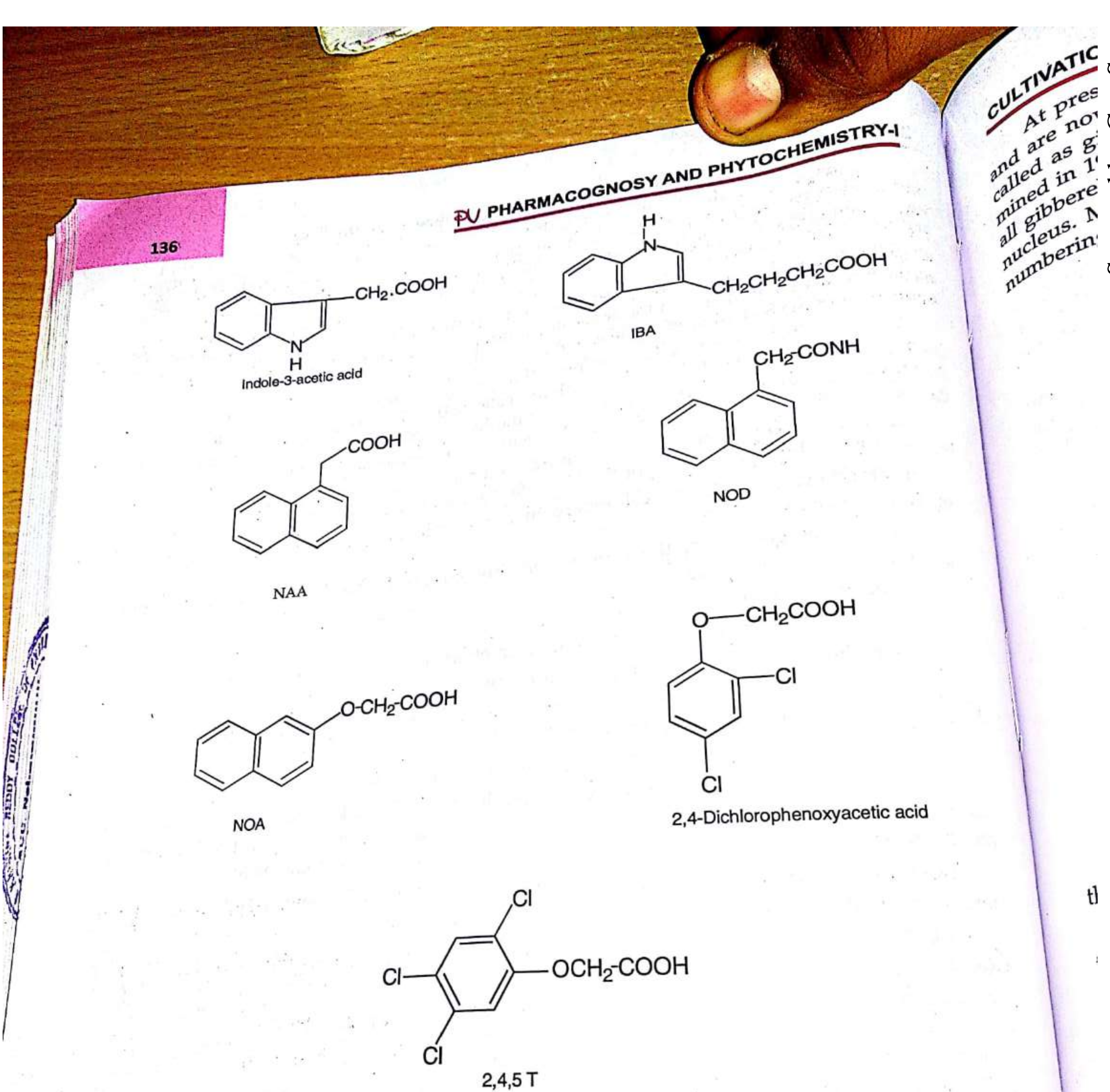

Gibberellins- Gibberellin (GA) was first recognized in 1926 by a Japanese scientist Erichi Kurosawa when he observed that in rice field certain seedlings grew very tall as compared to the others. It was found by him that such extremely tall rice plants were infected by the fungus *Gibberella fujikuroi* which resulted in "Bakanae" (foolish seedlings) disease of rice. In 1935, Yabuta and Hayashi isolated a substance in crystalline form from the fungal strains (*Gibberella fujikoroi*) which they called "gibberellin". Gibberellins are present in different organs like leaves, shoots, roots, buds, root nodules, fruits, floral apices and callus tissues. Gibberellins has not been synthesized but can be produced by large scale fermentation on commercial scale.

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At present more than 120 gibberellins are identified from plants, fungi and bacterias and are now distinguished as GA1, GA2, GA3, GA4, GA7 etc. Of these GA3 (commonly called as gibberellic acid) is almost exclusively used and its structure was finally determined in 1959. GA3 and mixtures of GA4 and GA7 are commercially available. Chemically, all gibberellins are diterpeniod acids based on gibberellane skelton containing the gibbane nucleus. Mazor structural differences lie in the substituents at positions 4a, 7, 8 (gibbane numbering) and the presence or absence of a Y -lactone ring.

Gibbane

Gibberellane

Gibberellic acid

Functions of gibberellins-

The most characteristic action of gibberellin is that it causes cell elongation of stem in the intact plants and not in isolated sections.

GA has little effect on the growth of the root, it rather inhibits the initiation of adventitious roots.

It stimulates the development of seedless or parthenocarpic fruits.

It stimulates the cell division especially the division of cambial cells.

It helps in breaking the dormancy.

It promotes the growth of leaf.

It counteracts the phenomenon of apical dominance which results in the stimulated growth of lateral buds which are about ready to grow.

It is responsible for *bolting* in the rosette plants i.e it helps in the emergence of stem from the rosette of leaves which ends in a flowering axis e.g. in *Hyoscyamus niger* (henbane), *Daucas corota* (carrot) and *Brassica oleracea* var. Capitata (cabbage). In henbane it is responsible for flowering even under non - inductive conditions.

It gives a positive results in 'dwarf maize test' and 'dwarf pea test'. Brian (1955) in

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England and Phinney (1956) in USA discovered that application of GA causes the stem of dwarf pea plants and single gene 'dwarf mutants' to grow into tall plants.

The gibberellins have been used to treat many medicinal plants which contain secodnary metabolites. With *Anethum graveolens* specific doses of gibberellin, increased the volatile oil content by up to 50% and with *Anethum sowa* by up to 30%. The daily treatment of *Digitalis purpurea* with gibberellin has shown increase in the content of cardioactive glycosides. Similar results were obtained in *Digitalis lanata* (weekly treatment). Application of gibberellin to *Cassia angustifolia* (Tinnevelly senna) resulted in the reduction of sennoside content of leaves but there was slight increase in the dry weight of the shoot. The application of gibberellin to datura, vinca, rauwolfia, hyoscyamus and tobacco resulted in the reduction of alkaloid content.

Cytokinins- Cytokinins are the compounds with a structure resembling to adenine and promotes cell division (Cytokinesis). Cytokinins are also involved in other plant processes like shoot and root morphogenesis, chloroplast maturation, cell enlargement, auxillary bud release and senescence. These may be either natural or synthetic compounds.

Natural Cytokinins- Naturally occuring cytokinins are zeatin, N6 dimethyl amino purine and N6 -2-isopentenyl aminopurine. Zeatin is the most common form of natural occuring cytokinin in plants today and it was isolated from corn (*Zea mays*).

Synthetic Cytokinins- These include like kinetin, adenine, 6-benzyl adenine benzimidazole and N.N1 -diphenyl urea.

Kinetin was the first cytokinin discovered and so named because of the compounds ability to promote cytokinesis (cell division). It was isolated by Miller, Folke Skoog and other members of the team in 1955 at Wisconsin University USA, from old stock of nucleic acid. It has also been obtained from coconut milk, apple fruit extract and many other plant extracts. Chemically, the substance was identified as 6- furfurylaminopurine (6-furfuryladenine). The important functions of kinetin are-

It promotes cell division.

It supports and enhances the growth of callus for long period.

It helps in expansion of foliage leaves and also cotyledonary leaves.

It enhances protein synthesis and at the same time decreases the degradation of proteins. If amino acids are added to water containing the treated leaves then amino acids tend to collect in the treated portion of the leaf.

It counteracts the root- formation at the basal cut end of the cuttings treated with IAA but stimulates callus formation at the basal end.

It overcomes ageing or senescence in leaves i.e it inhibits breakdown of the leaf pigments, proteins and nucleic acids which are degraded as the leaf gets old. Kinetin thus play a double role in the life of the leaf viz in young stage it stimulates the growth while at maturity it prevents ageing by keeping the leaves green and fresh.

Kinetin has no effect on parthenocarpy, abscission and flowering of rosetted plants.

Cytokinins are also employed in tissue culture work. In cell culture they have been shown to promote the biosynthesis of berberine (*Thalictrum minus*) and condensed tannins (*Onobrychis viccifolia*). The low concentration of this hormone in *Cassia angustifolia* was found to increase slightly the sennoside content of leaves and also enhances the dry weight of

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shoots. In opium they help in the formation of elongated capsules and reduce the alkaloid content. Leaves of the coffee plant after kinetin treatment developed a transient increase of up to 10% in their caffeine content but this effect was transitory and passed after 6-12 days.

Kinetin

Zeatin

Abscisic acid (ABA) - Abscisic acid is a single compound unlike auxins, gibberellins and cytokinins. In 1963, abscisic acid was first identified and characterized by Frederich Addicott and his associates. They were studying the compounds responsible for the abscission of fruits in cotton plants and two compounds were isolated namely abscisin I and abscisin II. Abscisin II is presently called as Abscisic acid (ABA). Abscisic acid was earlier known as Dormin by Robinson et al in 1963 because it had a major role in bud dormancy. Though ABA generally is thought to play a mostly inhibitory roles but it also has many promoting functions.

ABA is a naturally occuring compound in plants. It is a sesquiterpenoid (15-carbon) which is partially produced via the mevalonic pathway in chloroplasts and other plastids. Because it is synthesized partially in the chloroplast, it makes sense that biosynthesis primarily occurs in leaves. The production of ABA is enhanced in stress conditions like water loss, mineral deficiency, flooding and injury. It is believed that biosynthesis occurs indirectly through the production of carotenoids.

Functions of Abscisic acid- The following are some of the physiological responses known to be associated with abscisic acid-

It stimulates the closure of stomata .

It inhibits shoot growth but do not have much affect on roots or may even promote growth of roots.

It inhibits the gibberellin induced synthesis of a-amylase and other hydrolytic enzymes.

During maturation abscisic acid accumulates in many seeds and helps in seed dormancy.

It induces gene transcription especially for proteinase inhibitors in response to wounding which may explain an apparent role in pathogen defense.

The other synthetic growth inhibitors reported are such as glyphosine, ancymidol, piproctanyl bromide, maleic hydrazide, chlorophonium choride,chromequat chloride, S, S, S-tributyl phosphorotrithioate and daminozide. A group of synthetic substances known as morphactins is also a potent inhibitor and it includes like chloroflurecol methyl, flurecol-butyl and 2,3,5- tri-iodobenzoic acid (TIBA).

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Abscisic acid (Abscisin II)

Chloroflurecol methyl

Flurecol-butyl

(2, 3, 5-tri-iodobeonzoic acid) TIBA

Ethylene- Ethylene is a gaseous hormone and is the only member of its class. It is present in ripening fruits, flowers, seeds, stems, roots and tubers. It is present in very less quantity in plant normally about 0.1ppm. It is produced in all higher plants and is usually associated with fruit ripening and tripple response.

Ethylene has been used in practice since the ancient Chinese would burn incense in closed rooms to enhance the ripening of pears. **Doubt** discovered that ethylene stimulated abscission in 1917. In 1934, **Gane** reported that plants synthesize ethylene. In 1935, **Crocker** proposed that ethylene was the plant hormone responsible for fruit ripening as well as inhibition of vegetative tissues.

Ethylene is produced in all higher plants and is produced from methionine in essentially all tissues. Production of ethylene varies with the type of tissue, the plant species and also the stage of development. The mechanism by which ethylene is produced from methionine is a 3 step process.

ATP is an essential component in the synthesis of ethylene from methionine . ATP and water are added to methionine resulting in loss of the three phosphates and S-adenosyl methionine (SAM).

1-amino - cyclopropane-1- carboxylic acid synthase (ACC-synthase) facilitates the production of ACC from SAM.

Oxygen is then needed in order to oxidize ACC and produces ethylene.

Functions- Ethylene is known to affect the following plant processes-

It stimulates fruit ripening.

It stimulates shoot and root gowth and differentiation (tripple response).

It stimulates the release of dormancy.

It may have role in adventitious root formation.

It stimulates leaf an[illegible] [illegible]ission.
It enhances the late[illegible] [illegible]ubber t[illegible]

POLYPLOIDY

In some orgnasims the chromosomes can be grouped not in pairs but in three, four or higher numbers. These are polypoloid inividuals- triploid, tetraploid, octoploid etc. These type of polyploids can be derived by multiplication of the chromosomes of a single species (autoploids) or as a result of the multiplication of the chromsomes following hybridization between two species (alloploids). The later case furnishes a mechanisms whereby a hybrid of two species, itself infertile, may give rise to constant fertile type by polyploid formation. In the new polyploid form, pairing of the chromosomes at meiosis is possible which was probably not so in original hybrid. ***Primula Kewensis*** formed as above from the infertile hybrid ***Primula verticillata & Primula Floribunda*** was first reocorded in 1912. Since than many new species have been synthesized of the (fertile). F_1 hybrid of **Datura Ferox** × ***Datura stramonium*** by polyploid formation. In some species the somatic chromosomes number varies irregularly withen wide limits. Such plants are called as aneuploids & these do not breed true & often exhibit apogametic (asexual) reproduction.

Polyploidy can be artificially induced in many plants by suitable treatment with the alkaloid colchiane. The cytological effect of colchicine or dividing cells was reported by Dusten, Havas & Lits in 1937. In the presence of colchicine, chromosomes in a undergoing mitosis will continue to divide without the formation of a mitotic spindle figure. Sister cells therefore are not formed and in growing root tips of onion (2n = 16), a 72-h treatment with colchicine solution has given rise to cells, containing as many as 256 chromosomes. This C-mitotic activity of colchicine may arise from its interaction with disulphide bonds of the spindle protein & by inhibitor of the conversion of globular protein to fibrous proteins. On cessation of treatment the spindle figure agains forms in the normal way. C-mitotic activity is highly influenced by modification of colchiceine molecule. Colchicine is 100 times more active than its isomer isocolchicine & colchicine is virtually inactive.

Plant materials can be treated with colchicine in a number of ways. Seeds are frequently soaked in an aqueous solution of colchicine (0.2-2% solution 1 to 40ays), before planting & seedlings are inverted on filter paper soaked in the solution so that the growing points are not damaged. Alternatively the nut around the roots of young seedlings can be moistened with alkaloid solution. Young buds & roots can be treated by immersion & lanolin partes & agar gels are useful for general application to tissues.

Typical afffects of polyploidy compared with the diploid state are larger flowers, pollengrains & stomata. Generally the effects of polyploidy are not predictable & each species must be examined individually. Care must be taken that the method used to express the

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results does not give a deceptive effect. In some species polyploidy does not affect the relative portions of the individual constituents for example Solaneceous herbs produce increased quantities of tropane alkaloids in the 4th state & reduced amounts as haploids, but the proportion of hyosine to hyosccyamine remains altered. The proprotion of caruone in oil of carvone derived from 4n plants is also unchanged.

Extrachromosomal types : Sometimes plant occur with one or more chromosomes extra to the somatic number & these are called as extrachromosomal types. These were first noticed by Blakeslee's group in 1915. Although their genetic constitution was not immediately apparent when they sporadically appeared in pure line cultures of *Datura stramonium*. Such plants were later shown to posses 25 choromosomes in somatic cells & with datura (n=12), Twelve 2n+1 types are possible, each one containing a different extra chromosomes. The chromosomes were designated by numbering their halves (or ends), so that the largest chromosomes is 1.2 & the smallest is 23.24. All twelve types eventually appeared in Blakeslee's cultures & were originally named according to some characteristics of plant (e.g. Globe, Rolled, Ilex etc.) although the end numbering system can also be used to identify them; thus Globe 2n+21.22. Other 2n+1 types are also produced & are termed as secondaries, tertiaries & compensating. Secondary types have the extra chromosomes made up of two identified halves of a chromosomes (e.g. 2n+1) & in tertiary types it is composed two halves of different chromosomes compensating types lock one of the normal chromosomes which is compensated for by two others each carrying a different half of the missing one (e.g. 2n-1.2 + 1.9 + 2.5). At meiosis 2n+1 types produce a mixture of n & n+1 gametes & do not breed true.

MUTATIONS

Mutation is the permanent alteration of the nucleotide sequence of the genome of an organism, virus or extrachromosomal DNA or other genetic elements. Mutations results from error during DNA replication (especially during meiosis) or other type of damage to DNA (such as may be caused by exposure to radiation or carcinogens) which then may undergo error-prone repair (especially microhomologymediated end joining) or cause an error during replication (translesion synthesis). Mutations may also result from insertion or deletion of segments of DNA due to mobile genetic elements.

Radiation induces mutation to develop new variety of crops. Now with newer & more powerful sources of radiations (UV shortwave, Xray, Alpha, Beta & Gamma waves) & many chemicals (mutagens) e.g. caesium, ethyl methane sulfonate, nitromethyl (urea) we can increase the rates of mutations. Collectively ionizing radiation & chemicals will produce a mutation spectrum. The former, however, produce in the chromosomes, aberrations of a more random nature than chemicals which often act principally at certain Loci-particularly at those areas of the Chromosomes which stain differently at mitosis (heterochromatin). Also, the distribution of effects between nuclei is mere random with x-rays than with chemicals.

Mutagenic agents act as various stages of nuclear organisation. Thus at the stage of interphase (nondividing) nucleus when DNA synthesis is taking place, aberration involving chromated exchanges & isochromatid breaks occur. These effects do not become immediately (0-8 hours) manifest in the call but appear as delayed effects (8-48 hours) after treatment. Ionizing radiation & most chemicals produce aberration of this type. Clearly breaks which occur in the interphase nucleus chromosomes before DNA synthesis occurs (chromo-

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somes unsplit) would be of the chromosomes type & these are induced by X-ray treatment & by the chemicals (ethoxycaffeine & streptongrin). Other mutations may be induced during DNA post-synthetic stage of the interphase nucleus & during mitosis itself- as in the production of polyploids by colchicine & in the inducement of binucleate or polynucleate conditions due to inhibition of cell plate formation by cyclic organic compounds (e.g. halogenated derivatives of benzene & toulene, hydrazinotropone compounds, aminopyrine).

Factors which may influence the effect of mutagenic treatment includes oxygen tension within the tissues, temperature & pH. Chemical mutagens can be applied in a similar way to colchicine. Seeds, whole plants, isolated organs, growing points etc. are suitable for direct irradiation. Inorder to obtain single mutation in plant irradiation of pollen which is subsequently used to fertilise normal flower is often advantageous. It is unlikely that a pollen grain will retain its viability if it undergoes more than one mutational change.

Among plants of medicinal interest the Blakesledee radiation work *Datura Stramonium* resulted in the production of vary single gene mutation type (e.g. Zigzag, Ouercina, Banchy, Equisetum-names derived from some characteristic aspect of plant). These mutants are not isolated individually but are produced regularly by radiation treatment. Some forms such as pale (chlorophyll-deficient) are more frequent than others. In many cases Blakeslee was able to map the formation of genes responsible for these effects. Other mutants obtained in these studies were of the extra-chromosomal type.

Hybridization : In plant breeding, hybridization forms a possible means of combining in a single variety the desirable characters of two or more lines, varieties or species & occasionally of producing new & desirable characters not found in either parents. Hybridization particularly between homozygous strains which have been inbreed for a number of generations, introduced a degree of heterozygosity with resultant hybrid vigour (heterosis) often manifest in the dimensions & other characteristic of plant. There are several methods of breeding crops by sexual hybridization but in this topic more emphasis is given on chemical variants of a particular species in addition to intervarietal hybridization, interspecific hybridization in which hybrid vigour is also apparent.

The hybrid nature of number of drugs is well known. In the genus *Datura* the effect of hybridization on chemical constituents is illustrated by the cross *D.Ferrox* x *D.stramonium*. The aerial organs of the later normally contains hyoscyamiae & hyoscine (2:1 ratio) at the lowering period; and those of the former hyoscine with some metelodine. The F_1 of the cross consists of plants larger than either of the parents & containing hyoscine as the principal of alkaloid with only small amounts of other basis. In the F_2, segregation occurs as regards with morphological characters & alkaloid constituents with *D.leichhardin and D. innoxia* the former plant produces hyocyamine & hyoscine (2:1) & the later species usually mainly hyoscine but sometimes according to conditions of growth appreciable quantities of hyoscyamine. In this instance the F_1 hybrid contains a hyoscyamine : hyoscine ratio intermediate between that of two parents.

Nicotiana tobacum as now cultivated must have been derived from at least two different plant species & synthetic tobacoos can be prepared by using suspected species as parents. Although it has not been possible to produce in this way exactly comparable to *N.tobaccum*, such synthetic plants are most useful for the study of alkaloid inheritance characteristic. This is important in commercial production of tobacco, in which both the quantity & nature of

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alkaloid produced are important. Demethylation of nicotine may take place in the leaves of some species & by hybrid studies this reaction has been shown to be due in the group of plants studied to either one pair of dominant factors or two pairs of dominant & indegendent factors.

The preliminary studies carried by Cornish et al in 1983 showed that foenugreek seed, a potential source of diosgenin is capable of genetic improvement regarding the monohydroxysopogenin yield by hybridization of various races of *Trigonella foenum-graecum.*

The inheritance of opium alkaloids (morphine, codeine, thebaine, narcotine & papaverine) has been studied in the cross *Papaver somniferum x P.setigerum*. A heterotic increase in Iodeine & thebaine was found in different F_1 plants & in the F_2 plants, with the exception of codeine, some increase in alkaloid content was noted. As absence of narcotine was generally dominant over the presence. A continution of this work to the F_8 generation resultant in population that was completely diploid but which showed considerably diverting with regard to opium content of morphine, narcotine & papeverine. The patterns of alkaloids was closer to that of *P.somniferum* than to that of *P.setigerum* with morphine contents ranging from 8 to 30%. It was envisaged that a suitable breeding programme could result in opium with higher level of morphine that was normally encountered. F_1 hybrids of *P.bracteatum* and *P.orientale* contained a lower thebaine content & higher oripavine content than in either parent, a result which provided genetic evidence for the biosynthetic linkage between these alkaloids.

COLLECTION OF CRUDE DRUGS

Crude drugs may be collected from cultivated or wild plants. The factors like season, time, age of plants etc. affects the collection of crude drugs. Season is an important factor which should be considered during collection because it influences the nature and amount of active constituents. There are certain drugs like wild cherry, podophyllum, aconite, rhubarb etc.in which active constituents is not constant through out the year.For example rhubarb does not contain anthraquinone derivatives in winter but in summer the anthranol is converted into anthraquinone by oxidation; the contents of C-glycosides, O-glycosides and free anthraquinones in the developing shoots and leaves of *Rhamnus purshiana* fluctuate throughout the year.

Day and night affects the daily variation of proportion of secondary metabolites in some plants. For example there is daily variation in the alkaloid content of hemlock, ergot, broom and poppy. Daily variation is also observed in glycosidal content of *Digitalis lanata* and *Digitalis purpurea*, the simple phenolic glycosides of *Salix* and the volatile oil content of *Salvia* and *Pinus*.

The age of plant is an another factor which influences the collection of crude drugs.Firstly, it controls the total quantity of active constituents produced and secondly it also controls the relative proportion of components of the active mixture. For example in *Digitalis lanta* highest level of total glycoside is observed in first year leaves but glycosides which are medicinally important (e.g. Lanatosides C) reaches to their highest level in second year plants.

The **Leaves** are collected when flowers are just beginning to expand or the flowering is just arriving at its height.The collection should be done in dry weather since leaves collected in wet weather deteriorate in quality and may become discolored during drying. The time

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of collection is sometimes varied for special reasons ; for e.g. coca leaves are collected when they are nearly ready to fall from stem whereas bearberry leaves may be collected at any time of the year. **Flowers** are collected just before they are full expanded in dry weather because petals which are damp when gathered become badly discolored during drying. For e.g. red rose and clove are collected when in bud and kousso is collected after pollination and fertilization. The **fruits** are collected when they are fully grown but they may be either ripe or half ripe. For e.g. fennel, dill and ajowan are collected when they are fully ripe where as cardamom is collected just before they dehiscence.

The **Barks** are collected in spring or early summer when the cambium is active as it is easy to separate them from wood in this season. Sometimes the bark like cinnamon is collected in rainy season and wild cherry bark is collected in autum because during this season the content of active constituents is highest. Barks are collected by three methods namely (i) felling (ii) uprooting (iii) coppicing . In felling method the tree is cut down near the ground level and bark is removed from branches and stem. This method is rarely used now a days. In uprooting method the trees from ten to fifteen years are cut down and root is dug up; the bark is then removed from trunk and branches and from root also. Cinchona bark may be collected by this method. In coppicing method trees are allowed to grow for a definite period of time and then the stems are cut down at a short distance from the ground. The bark is removed from trunk and branches. The stumps which remain in ground are allowed to send out a certain number of shoots which develop further independetly yielding aerial parts. These new parts are again cut down and bark is collected. Commercially this method is widely used because it is more economical and less time consuming. Cinnamon, cinchona and oak are collected by this method.

The **Underground Organs** such as roots and rhizomes are generally collected in autum as their tissues are fully stored with reserve foods; it is being assumed that chemical constituents will be most abundant in this season. Underground organs should be freed from soil and this can be achieved by shaking or brushing the drug. The drugs like valerian which contain clay are thoroughly washed. Large roots and rhizomes are generally sliced transversely or longitudinally or in both directions to facilitate drying.

The **Unorganised drugs** such as latex, juices, gums, resins etc. are collected as soon as they exudes out of the plant. The juice of aloe is collected as it oozes out after giving incision to leaves. Tragacanth gum is collected after two days of the incision. Myrrh is collected from the wounded bark as soon as it oozes out.

DRYING

Drying is a process of removing the moisture content of crude drugs. Drying includes various treatments/operations which depends upon the chemical constituents and sources of crude drugs. Drying increases the resistance against the growth of microorganism, enhances the quality and helps in size reduction of drugs. After drying it is easy to store the drugs for a longer time. The method of drying is selected on the basis of chemical constituents present in the crude drugs. There are two methods of drying viz. **Natural drying** (Sun drying) and **Artificial drying**. Artificial drying can be accomplished by (i) Tray dryers (Hot air oven) (ii) Spray dryers (iii) Vacuum dryers.

After collection of drugs they are subjected to drying. The drugs like gentian root, vanilla pods and cocoa seeds where enjyme action is to encouraged should be subjected to slow drying at moderate temperature. The drugs where enjyme action is not desired should be dried as soon as possible. The drying process can be completed from few hours to few

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weeks. The drugs like cardamom, clove, cinnamon etc. which are subjected to open air drying largely depends upon weather. They require suitable weather for drying. The drugs which are dried in hot or warm weather should be covered under sheds. The drugs which are cultivated in countries containing high humidity are subjected to an artificial drying.

The most important factor in the process of drying is the temperature. The temperature should be controlled on the basis of chemical constituents and physical nature of crude drugs. For example Digitalis leaves should be dried at a temperature below 60°C. Colchicum corm are dried at a temperature not exceeding 65°C. Generally speaking the flowers, leaves, herbs should be dried between 20 to 40°C and roots, rhizomes and barks between 30 to 60°C.

GARBLING

Garbling refers to the dressing of crude drugs. After drying, garbling is the next step in the preparation of crude drugs for the market. This is required when dirt, sand, foreign organic matter of the same plant which is not required is adhered to the crude drugs. This extraneous material can be removed by several methods which are practically possible at the site of preparation of crude drugs. If the extraneous material is not removed from the crude drugs then it alters the quality of drugs and at the same time they does not pass the Pharmacopoeial standards . Hence it is an important step which should be carefully performed. For e.g. drugs that are in rhizomes form need to be separated from roots and rootlets and stem bases. Excessive stems in the case of stramonium and lobelia should be removed. Cloves should be freed from their peduncles (stalks) . Pieces of iron in senna and vinca is removed by shifting whereas in castor seeds the iron pieces are removed by the help of magnet. The pieces of bark in Indian gum (acacia) and sterculia gum is removed by peeling method. The dirt and sand from fennel, dill, coriander, carraway etc is removed by winnowing.

PACKING

During packing of crude drugs various factors such as morphological and chemical nature of drug, effect of climatic conditions during transportation and storage and their uses should be taken into consideration. Turkish opium is imported in rounded or conical cakes covered with poppy leaves. Persian opium occurs in brick shaped masses and is covered with red paper. Indian opium is in cubical pieces enclosed in tissue paper and its weight is also kept constant. Zanzibar aloe is packed in the skin of carnivorous animals and pieces of skin in the drug indicates its source. Colophony is packed in large pieces in kerosene tins to avoid auto -oxidation. The drugs which are sensitive to moisutre and are costly such as digitalis, squill, ergot etc also needs special care during packing. If the moisture content of digitalis increases beyond 5% then it looses its potency due to decomposition of glycosides. Ergot becomes susceptible to microbial growth and squill becomes flexible if brought in contact with moisture during storage. Therefore these types of drugs should be packed along with suitable dehydrating agent.

The packing of drugs containing volatile oil also requires special attention. Cinnamon bark which is available in the form of quills is packed one inside the other quill to facilitate the loss of volatile oil. Similarly fennel, clove, coriander, cumin, caraway, dill etc are also packed in well closed containers. Fixed oils such as cod liver oil, sesame oil, shark liver oil are sensitive to light therefore such drugs should be stored in those containers which are

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not affected by sunlight. Leafy drugs like vinca, senna, vasaka etc are pressed and baled. Crude drugs like seeds, roots, rhizomes etc do not require attention and are packed in gunny bags. Sometimes these bags may be coated with polythene, internally.

STORAGE

Drugs can be maintained in good conditions for a longer time by adopting proper methods of storage. The factors which affects the storage of crude drugs or drug deterioration are moisture content, temperature, light and oxygen of the air.

The **Moisture content** is the important factor which should be considered during storage of drugs. It is clear that living organisms cannot develop or survive without the moisture. A number of drugs absorb moisture during storage which increase the bulk of drugs and are liable to the attack of microorganisms. For e.g. the moisture content more than 9% enhances the growth of fungi and bacteria in cotton wool. The drugs like digitalis and wild cherry bark when absorbs excessive moisture activates enjymatic reactions and it leads to decomposition of glycosides. These drugs should be stored in sealed containers with dehydrating agent. The powdered squill which contains mucilage if not properly stored absorb moisture and is converted into a sticky mass. Various types of bacteria, insects, mites , nematodes, worms, moths etc. are reported to attack the crude drugs if not stored properly. The effects produced by bacteria are not always very visible but in case of the chromogenic species their presence is recognized . For e.g. *Bacillus* (Chromobacterium) *Prodigiosus* produces red patches on potatoes, bread, paste and other starchy materials. For other bacteria the effect of their presence cannot be seen immediately. This happens with cotton fibres which are eventually rendered brittle by bacterial attack thus causing the trichomes to break into short lengths which make the cotton- wool objectionably dusty. The mites can develop in flour if moisture content is more than 11%. Other mites that infest the drugs and foodstuffs are like *Glycyphagus spinipes, Aleurobius farinae, Tyroglyphus siro* etc. Nematodes such as *Anguina tritia* and *Anguillula aceti* attack on wheat flour. Another nematode worm Tylenchus devastatrix is found in the stems of foxglove and belladonna. The moths can easily attack an cocoa, tobacco, almond, groundnut, rose petals etc. Moths such as *Ephestia sericarium, Plodia interpunctella* lay their eggs in the dried vegetable material and the grubs which hatch out feed upon the drug and rapidly reduce it to powder. The beetles like *Stegobium paniceum, Ptinus hirtellus, Niptus hololeucus, Trigonogenius globulus* , *Lyctus brunneus, Lasioderma serricorne* etc bore holes into all kind of materials.The damage is done mainly by larvae which as they feed bore tunnels into the drugs and produces quantities of fine powder commonly called as "Pore dust". Other types of insects that can also be present are like cockroaches and ants. The attack of various types of insects, moths, mites , moulds etc can be prevented by drying the crude drugs properly and also by some form of fumigation. The well known fumigants which are used for disinfecting the crude drugs during storage is methyl bromide and mixture of ethylene oxide and carbondioxide.

An increase in **temperature** along with moisture enhances the enzymatic activity. High temperature causes loss of volatile oil from the drugs like ginger, buchu, asafoetida, chamomile flowers etc. Therefore majority of drugs should be stored in cool as well as dry place. **Direct sunlight** also influences the crude drugs. It causes bleaching of flowers and leaves. For example the rose petals and flowers of henbane changes their colour in direct sunlight. It also decomposes the chemical constituents of few drugs like cod liver oil and ergot. The ill-effects produced by direct sunlight can be prevented by using opaque or amber glass containers. **Oxygen of the air** causes rancidification of fixed oils and can deteriorate the

drugs containing volatile oil. Hence oxygen of the container can be replaced by an inert gas like nitrogen.

Therefore the crude drugs should be stored in well closed, moisture proof and light resistant containers such as cans, tins, opaque or amber glass containers in cool and dry place. Crude drugs should not be stored in paper bags and wooden boxes.

SUGGESTED READINGS

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QUESTION BANK

OBJECTIVE PART

MULTIPLE CHOICE QUESTIONS

1. **The advantage of cultivation of medicinal plants is -**
   (A) It provides the medicinal plants or crude drugs of better quality and purity
   (B) The crude drugs are of good quality with regard to colour, odour, taste, shape and size.
   (C) It gives a higher yield of crude drugs.
   (D) All of the above
2. **Which is not the asexual method of propagation?**
   (A) Cutting (B) Grafting
   (C) Budding (D) By seeds
3. **Which is not the advantage of vegetative method of propagation?**
   (A) There is no variation between plants grown and the parent plant.
   (B) It is possible to avail the modifying influence of root stocks on scion.
   (C) The plants are less resistant against the disease as compared to seedling plants
   (D) Plants bear early as compared to seedling plants.
4. **Which of the following is the factor affecting cultivation of medicinal plants?**
   (A) Altitude and temperature
   (B) Rainfall and soil
   (C) Day length and radiation characteristics
   (D) All of the above
5. **Digitalis grows at an altitude of –**
   (A) 4000 – 5000 mts. (B) 3700 – 4000 mts.
   (C) 1600 – 3000 mts. (D) 200 – 600 mts.

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Chapter 5

PLANT TISSUE CULTURE

INTRODUCTION

In developing countries the most important challenges are to produce sufficient food, fibre and fuel for the continously increasing population from inelastic land area. Plant tissue culture offers excellent opportunities of mass propagation of plants in test tubes. The idea of totipotency has been the foundation for tissue culture techniques. Tissue culture is the process whereby small pieces of living tissue (explants) are isolated from plant and grown aseptically for indefinite periods on a semi-defined or defined nutrient medium. Explants range from large seedlings and organs (as in ovule and embryo culture) to small single cells and protoplasts. **In short, tissue culture is *in vitro* cultivation of plant cell or tissue under aseptic and controlled environmental conditions in a defined nutrient medium for the production of primary and secondary metabolites or to regenerate the plant.**

HISTORY OF PLANT TISSUE CULTURE

The technique of plant tissue culture is about 100 years old but its importance have been realised in the last two decades in various fields including pharmacy also. The principles of plant tissue culture can be traced in the cell theory proposed by ***Schleiden*** and ***Schwann*** in 1839. They proposed that each living cell of an organism, if provided with proper environment is capable of independent development. This theory gave birth to the concept of totipotency predicted by Haberblandt. In 1902, the German botanist **G. Haberblandt** reported culture of isolated single palisade cells from leaves in Knop's salt solution enriched with sucrose. The cells remained alive up to one month, increased in size, accumulated starch but failed to divide. Efforts to demonstrate totipotency led to the development of techniques for cultivation of plant cells under defined conditions. This was made possible by brilliant contribution from R.J Gautheret in France and P.R. White in U.S.A and by others.

The first **Embryo culture,** although crude was carried out by **Hanning** in 1904. He cultured nearly mature embryos of certain Crucifers (*Cochleria donica*, *Raphanus landra*, *R. sativus and R. caudatus*) and grew them up to maturity. This became an important area of investigation using an *in vitro* technique. In 1908, ***Simon*** achieved success in regenerating the bulky callus, buds and roots from popular stem segments and thus he succeeded in establishing the basis for callus culture and to some extent also micropropagation. **Kotte** in 1922, cultured small excised root tips of pea and grew it for two weeks by using a variety of nutrients containing salts of Knop's solution, glucose and various nitrogenous compounds.

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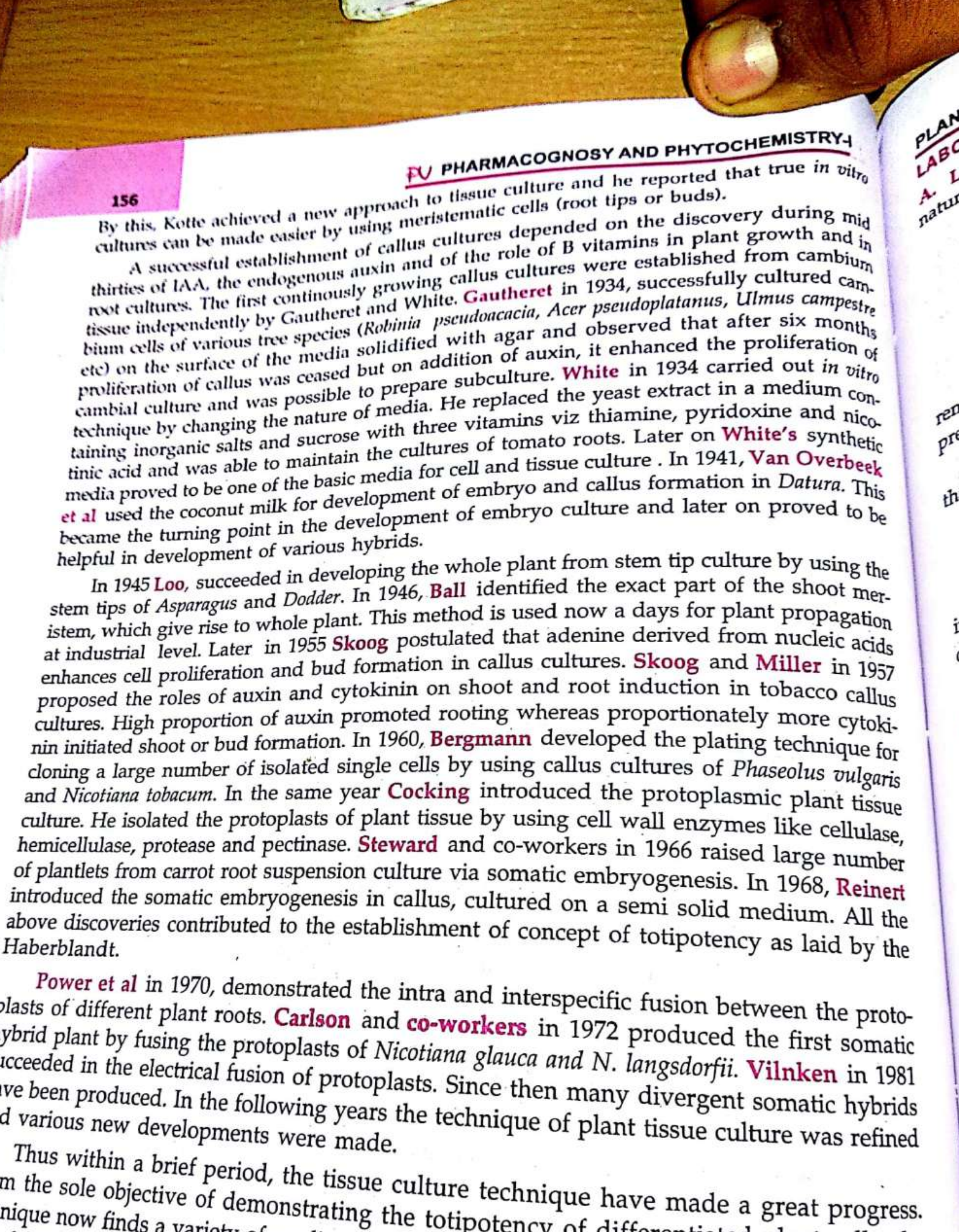

By this, Kotte achieved a new approach to tissue culture and he reported that true *in vitro* cultures can be made easier by using meristematic cells (root tips or buds).

A successful establishment of callus cultures depended on the discovery during mid thirties of IAA, the endogenous auxin and of the role of B vitamins in plant growth and in root cultures. The first continously growing callus cultures were established from cambium tissue independently by Gautheret and White. **Gautheret** in 1934, successfully cultured cambium cells of various tree species (*Robinia pseudoacacia*, *Acer pseudoplatanus*, *Ulmus campestre* etc) on the surface of the media solidified with agar and observed that after six months proliferation of callus was ceased but on addition of auxin, it enhanced the proliferation of cambial culture and was possible to prepare subculture. **White** in 1934 carried out *in vitro* technique by changing the nature of media. He replaced the yeast extract in a medium containing inorganic salts and sucrose with three vitamins viz thiamine, pyridoxine and nicotinic acid and was able to maintain the cultures of tomato roots. Later on **White's** synthetic media proved to be one of the basic media for cell and tissue culture . In 1941, **Van Overbeek et al** used the coconut milk for development of embryo and callus formation in *Datura*. This became the turning point in the development of embryo culture and later on proved to be helpful in development of various hybrids.

In 1945 **Loo**, succeeded in developing the whole plant from stem tip culture by using the stem tips of *Asparagus* and *Dodder*. In 1946, **Ball** identified the exact part of the shoot meristem, which give rise to whole plant. This method is used now a days for plant propagation at industrial level. Later in 1955 **Skoog** postulated that adenine derived from nucleic acids enhances cell proliferation and bud formation in callus cultures. **Skoog** and **Miller** in 1957 proposed the roles of auxin and cytokinin on shoot and root induction in tobacco callus cultures. High proportion of auxin promoted rooting whereas proportionately more cytokinin initiated shoot or bud formation. In 1960, **Bergmann** developed the plating technique for cloning a large number of isolated single cells by using callus cultures of *Phaseolus vulgaris* and *Nicotiana tobacum*. In the same year **Cocking** introduced the protoplasmic plant tissue culture. He isolated the protoplasts of plant tissue by using cell wall enzymes like cellulase, hemicellulase, protease and pectinase. **Steward** and co-workers in 1966 raised large number of plantlets from carrot root suspension culture via somatic embryogenesis. In 1968, **Reinert** introduced the somatic embryogenesis in callus, cultured on a semi solid medium. All the above discoveries contributed to the establishment of concept of totipotency as laid by the Haberblandt.

Power et al in 1970, demonstrated the intra and interspecific fusion between the protoplasts of different plant roots. **Carlson** and **co-workers** in 1972 produced the first somatic hybrid plant by fusing the protoplasts of *Nicotiana glauca and N. langsdorfii*. **Vilnken** in 1981 succeeded in the electrical fusion of protoplasts. Since then many divergent somatic hybrids have been produced. In the following years the technique of plant tissue culture was refined and various new developments were made.

Thus within a brief period, the tissue culture technique have made a great progress. From the sole objective of demonstrating the totipotency of differentiated plant cells, the technique now finds a variety of applications in both basic and applied researches in a number of fields.

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LABORATORY REQUIREMENTS FOR PLANT TISSUE CULTURE

A. Laboratory space- The organization of tissue culture laboratory depends mainly on the nature and the scale of activity. In general space for the following is needed :

1-Washing, drying and storage of vessels
2-Preparation, sterilization and storage of media
3-Aseptic handling of explant and cultures
4-Maintenance of cultures, and
5-Observation of cultures

In the modern laboratories the activities 1 and 2 are done in **Media room** whereas the remaining activities 3 to 5 are performed in **Culture room.** In such a situation the following precautions should be taken :

The working area should be physically separated, even by a temporary partition from that used for medium preparations.

The weighing balances should be kept in a separate space.

Refrigerator, Deep freeze, Incubators and Autoclave may be kept in corridor.

For aseptic manipulations, laminar flow hoods are commonly used which can be housed in culture room. A small table having a stereoscopic microscope may be adequate for culture observation.

B. Culture room- The culture room should have the following facilities-

Controlled temperature (usally at $25^0 \pm 2$ degree C with the help of airconditioners and room heaters; higher or lower temperature may be desirable in some cases)

Culture racks fitted with light (generally 1000 lux or lower light generated by fluorescent tubes)

A shaker for agitation of liquid cultures. It is desirable to have a generator set for providing electricity to the culture room when there is electricity failure or cuts.

C. Culture vessels and their washing- Generally glass culture vessels are used as they are cheaper, reusable and autoclavable. It is desirable to use only borosilicate or pyrex glass ware as ordinary soda glass may be toxic to some tissues. Culture vessels of plastic are available for a variety of purpose; these vessels are generally presterilized and disposable, but certain types are autoclavable and therefore reusable. In general , plastic vessels in the long run are costly than glass vessels.

Tissues are cultured in culture tubes (rimless tubes of 25 X 150 mm or larger), flasks (long neck or even ordinary conical flasks) and petriplates; but mainly especially designed dishes are also used. Wide mouth bottles, including milk bottles are often employed especially for micropropagation work. Suspension cultures of necessity are maintained in long neck culture flasks.

Culture tubes and flasks are usually stoppered with cotton plugs, which are often wrapped

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in cheese cloth, but preparing such plugs on large scale may be time consuming and inconvenient. Many workers use caps made up of either polypropylene or a metal for this purpose. These caps are effective but their design may affect the performance.

Culture vessel and other labware are generally soaked in a suitable nontoxic detergent solution overnight, washed with a suitable brush, thoroughly rinsed, clean with tap water, followed by rinse with distilled water and dried in hot air oven (75 to 80°C). Culture vessels having contamination are first autoclaved. Washed culture vessels should be stored preferably in a dust proof cabinet.

D. Sterilization- All the materials like instruments, vessels, plant materials, medium etc. used in culture work should be free from microorganisms. This is achieved by one of the following method-

(i) Dry Heat- Glassware and Teflon plastic ware (empty vessels) and instruments can be sterilized by dry heat in hot air oven at 160-180°C for 3 hours. But most of the workers prefer to autoclave glass ware and plastic ware and flame sterilize instruments like forceps etc. More recently glass bead sterilizers (300°C) are being employed for the sterilization of forceps, scalpels etc.

(ii) Flame Sterilization- The instruments like scalpels, needles, forceps etc. are ordinarily flame sterilized by dipping them in 95% alcohol followed by flaming. These instruments are repeatedly sterilized during the operation to avoid contamination. The mouths of culture vessels are flammed prior to inoculation / subculture.

(iii) Autoclaving- Culture vessels etc. (both empty and containing media) are generally sterilized by heating in an autoclave to 121°C at 15 p.s.i (Pounds per square inch) for 15 (20-50 ml of medium) to 40 (2 L medium) minutes, the time being longer for larger medium volumes. Sterilization during autoclaving depends mainly on temperature. Certain types of plasticware and instruments like micropipettes etc are also autoclavable. All the vessels should be stoppered during autoclaving.

(iv) Filter Sterilization- Certain vitamins, enzymes and growth regulators like Zeatin, GA3, Abscisic acid (ABA) and Urea are heat liable, so these compounds are filter sterilized by passing their solution through a membrane filter of 0.45 u or lower pore size. The membrane filter is held in a suitable assembly; the assembly together with the filter is sterilized by autoclaving before use.

Laminar air flow cabinets are used to create an aseptic working area by blowing filter-sterilized air through an enclosed (on all sides except one) space. The air is first filtered through a coarser prefilter to remove larger particles; it is then passed through High efficiency particulate air (HEPA) filter, which filters out all particles larger than 0.3um. This sterilized air blows through the cabinet at 1.8 km/hr which is sufficient to keep the enclosed working area aseptic. Inside the cabinet there is an arrangement of bunsen burner and UV tube fitted on the ceiling of the cabinet which helps the area to be free from live contamination.

Laminar Air Flow

(v) Wiping with 70% Ethanol- The surfaces that cannot be sterilized by other techniques e.g platform of the laminar flow cabinet, hands of the operator etc. are sterilized by wiping them thoroughly with 70% ethyl alcohol and alcohol is allowed to dry.

(vi) Surface Sterilization- In this method all plant materials used for culture is treated with an appropriate sterilizing agent to inactivate the microbes present on their surface. The sterilizing agents used for surface disinfection are sodium hypochlorite (2%), calcium hypochlorite (9-10%), mercuric chloride (0.1-1%), H_2O_2 (10-12%), bromine water(1-2%) and antibiotics (4-50mg/litre). Among these sodium or calcium hypochlorite gives very good results and mercuric chloride gives satisfactory results and these are most commonly used. The duration of treatment varies from 15-30 minutes. As these agents are toxic to plant tissues the duration and concentration used should be such as to cause minimum tissue death.

Surface sterilization protocol depends mainly on the source and the type of tissue of the explant, which determines the contamination load and tolerance to the sterilizing agent. An **explant** is the excised piece of tissue or organ used for culture. Explant can be taken from any part of the plant like root, stem, leaf, meristematic tissue like cambium and floral parts like stamens, anthers etc. **A general protocol for disinfection of respective explant is mentioned below :**

Seeds- Dip the seeds into 70% ethyl alcohol for 30 seconds and than treat with 0.2% mercuric chloride (acidified with few drops of 1N Hcl) for 10-15 minutes. Again rinse with 70% ethyl alcohol and finally rinse 4-6 times with sterilized distilled water. The entire protocol should be carried out in an aseptic area generally created by laminar air flow.

Leaves- Wash the explant thoroughly by purified water to remove the dirt and rub the surface with ethyl alcohol. Dip the explant in 0.1% mercuric cholride solution, wash with sterile water and finally dry the surface with sterilize tissue paper.

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Fruits- Rinse the fruit with absolute alcohol and then dip into 2% sodium hypochlorite solution for 10 minutes. Finally wash thoroughly with sterile water and remove seeds of interior tissue.

Stem- Wash the explant thoroughly with running tap water and rinse with pure alcohol. Dip into 2% sodium hypochlorite solution for 15-20 minutes and wash 2-3 times with sterile water.

PRODUCTION OF CALLUS FROM EXPLANT

The sterilized explant is transferred aseptically to a defined medium in the flasks. These flasks are incubated in BOD incubator for maintenance of culture at the temperature of 25 ± 2^{0}C. Little amount of light is also essential for the production of callus (unorganized mass of cells). After 3 to 8 days of incubation, sufficient amount of callus is produced.

Proliferation of callus

When callus is well developed it should be cut into pieces and transferred to another fresh medium. This medium contains an altered composition of hormones which supports the growth. The medium used for production of more amount of callus is known as proliferation medium.

Subculturing of callus

After a period of time, it becomes neccessary to transfer the callus to fresh media chiefly due to nutrient depletion and medium drying. In general, callus cultures are subcultured every 4 to 6 weeks.

Suspension culture

Tissue and cells cultured in a liquid medium produce a suspension of single cells called as suspension cultures. For the preparation of suspension culture, callus is transferred to the liquid medium, which is constantly agiated by a rotary shaker at 50-150 rpm. This facilitates aeration and keeps the cells separate. After the production of sufficient number of cells subculturing can be done. In general, suspension cultures are subcultured every 3 to 14 days.

CULTURE MEDIA

The plant tissues or organs growing *in vitro* have different nutritional requirements for their satisfactory growth. But there is no single medium which is entirely sufficient for the satisfactory growth of all types of plant tissues and organs. Hence details of culture medium need to be worked out by hit and trial method for each plant material separately. The various culture media developed during last few decades are Gautheret(1942), White(1943), Haller(1953), Murashige and Skoog (MS) (1962), Erikson(ER) (1965) and Gamberg et al (B5) (1968). Out of these MS and B5 are most commonly used. The pH of the medium is usually adjusted between 5.0 to 6.0 with 1N Hcl or 1N NaOH. The composition of some plant tissue culture media is listed in Table no:-1

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TABLE NO. 1

Ingredient	White's medium	Haller's medium	MS medium	ER medium	B5 medium
Micronutrients					
NH_4NO_3	-	-	1650	1200	-
KNO_3	80	-	1900	1900	2527.5
$NaNO_3$	-	600	-	-	-
$Ca(NO_3)_2.4H_2O$	300	-	-	-	-
$MgSO_4.7H_2O$	750	250	370	370	246.5
KH_2PO_4	-	-	170	340	-
$CaCl_2.2H_2O$	-	75	440	440	150
$NaH_2PO_4.H_2O$	19	125	-	-	150
$(NH_4)_2SO_4$	-	-	-	-	134
Micronutrients					
$MnSO_4.H_2O$	-	-	-	-	10
$MnSO_4.4H_2O$	5	0.1	22.3	2.23	-
$CuSO_4.5H_2O$	0.01	0.03	0.025	-	0.025
$CoCl_2.6H_2O$	-	-	0.025	0.0025	0.025
$ZnSO_4.7H_2O$	3	1	8.6	-	2
$Fe_2(SO_4)_3$	2.5	-	-	-	-
$FeSO_4.7H_2O$	-	-	27.8	27.8	-
$NaMoO_4.2H_2O$	-	-	0.25	0.025	0.25
KI	0.75	0.01	0.83	-	0.75
KCl	65	750	-	-	-
MoO_3	0.001	-	-	-	-
$FeCl_3.6H_2O$	-	1	-	-	-
$AlCl_3$	-	0.03	-	-	-
H_3BO_3	1.5	1.0	6.2	0.63	3
$NiCl_2.6H_2O$	-	0.03	-	-	-
EDTA					
$Zn.Na_2EDTA$	-	-	-	15	-
$Na_2EDTA.2H_2O$	-	-	37.3	37.3	-
Organic nutrients					
Vitamins					
Pyridoxine HCl	0.01	-	0.5	0.5	1
Nicotinic acid	0.05	-	0.5	0.5	1
Thiamine HCl	0.01	-	0.1	0.5	10

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Inositol	-	-	100	-	100
Amino acids					
Glycine	3.0	-	2.0	2.0	-
Carbon Source					
Sucrose	2%	-	3%	4%	2%
Growth regulators					
IAA	-	-	1.0	30	-
2-4 Dichlorophenoxyacetic acid	-	-	0.1	1.0	-
NAA	-	-	-	1.0	-
Kinetin	-	-	0.04	10.0	0.02
Ph	5.5	-	5.7	5.8	5.5

MS-Murashige & skoog

ER- Erikson

B5- Gamberg et al

Media constituents- The major constituents of medium that are essential to maintain the vital functions of culture are-

1- **Inorganic nutrients**

2-**Organic nutrients**

3-**Growth regulators (Hormones)**

4-**Gelling agent (Agar)**

1-Inorganic nutrients- In addition to C, H and O all culture media requires 12 elements for the growth of plant tissues. Out of these, six elements viz Nitrogen(N), Phosphorous(P), Potassium(K), Calcium(Ca), Sulphur(S) and Magnesium(Mg) are needed in the concentration greater than 0.5 m mol l^{-1} and are known as **macronutrients**. The remaining six elements viz Iron(Fe), Zinc(Zn), Manganese(Mn), Copper(Cu), Boron(B) and Molybdenum(Mo) are required in the concentration less than 0.5 m mol l^{-1} and are known as **micronutrients**.

The active factor in the culture medium is the ions of different types rather than salt. A single ion can be contributed by more than one salt. For e.g in Murashige and Skoog's medium K^+ ions are contributed by KNO_3 and KH_2PO_4 whereas NO^{3-} ions are contributed by NH_4NO_3 and KNO_3. The various culture media differ mainly in quantity rather than in quality of these elements. Therefore the various culture media provide different concentration of the inorganic nutrients for e.g. in White's medium the concentration of K and N is very less as compared to MS and B5 medium. The White's medium though widely used earlier was later found inadequate by various investigators because the inorganic nutrients which provides the good callus growth were very less in quantity. Hence most of the plant tissue culture media that are now being used widely (Table no-1) are richer in mineral salts as compared to White's medium.

In most of the medium, iron is now used as FeEDTA and in this form iron remains available at higher pH (>5.8). FeEDTA may be prepared by using $Na_2EDTA.2H_2O$ and $Fe_2(SO_4)_3.7H_2O$.

The inorganic nitrogen is supplied in the medium in the forms of nitrates and ammonium compounds. When nitrate is used alone, the pH of the medium shifts towards alkalinity. So, to check this drift small amount of ammonium compound is added along with nitrate.

In addition to the above mentioned elements , the various media are also enriched with sodium (Na), Cobalt (Co) and Iodine (I) but their necessity has not been established.

2-Organic nutrients- The organic nutrients can be broadly calssified into nitrogen sources and carbon sources.

Nitrogen sources- For the optimum callus growth it is necessary to supplement the tissue culture media with one or more vitamins and amino acids. The vitamins required are pyridoxine, thiamine, nicotinic acid and inositol. Of these thiamine is essential and the rest are promotory. Pantothenic acid is also known to be promotory but is not included in most of the recipes.

Other complex nutrients of undefined composition such as casein hydrolysate, coconut milk, corn milk, malt extract, tomato juice and yeast extract have also been used to promote the growth of tissue culture. However it is recommended to avoid their use and replace each by a single amino acid, as these substances may affect the reproducibility of results because of variation in the quantity and quality of growth promoting constituents in these substances.

Carbon sources- The most commonly used carbon source for all cultured plant materials including even green shoots is sucrose. It is used in the concentration of 2-5%. **Ball** demonstrated that autoclaved sucrose is better than filtered sterilized sucrose because autoclaving causes the hydrolysis of sucrose which enhances its availability to plant cells. Generally, monocots grow better with glucose whereas dicotyledonous roots do best with sucrose. Plant tissues can utilize other sugars also like galactose, lactose, mannose and even starch, but these are rarely used.

3-Growth regulators(Hormones) - The growth hormones included in culture media are auxins, cytokinins and gibberellins.

Auxins- Auxins are mainly used to facilitate cell division and root differentiation. Commonly used auxins are IAA (indole-3-acetic acid), IBA (indole-3-butyric acid), NAA (naphthalene acetic acid), NOA (naphthoxy acetic acid), p-CPA (Para-chlorophenoxyacetic acid), 2, 4-D (2,4 dichlorophenoxy acetic acid) and 2,4, 5-T (trichlorophenoxyacetic acid). IBA and NAA are widely used for rooting and (in combination with cytokinin) for shoot proliferation. 2,4-D and 2,4,5-T are very effective for the induction and growth of callus. Auxin generally dissolve in ethanol or dil NaoH.

Cytokinins- Chemically, cytokinins are adenine derivatives and are employed to promote cell division, regeneration of shoots, often somatic embryo induction, to enhance proliferation and growth of auxillary buds. Commonly used cytokinins are Kinetin (furfurylamino purine), BAP (6-benzylamino purine), 2-ip (isopentenyl adenine), Zeatin and TDZ (thiadiazuron). 2-ip and Zeatin are naturally occuring cytokinins while Kinetin and BAP are

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derived synthetically. Cytokinins generally dissolve in dil Hcl or NaoH.

Gibberellins- Of the over 120 gibberellins known, GA_3 is almost exclusively used. It promotes shoot elongation and somatic embryo germination. Gibberellins is soluble in cold water up to 1000 mg^{-1}.

4-Gelling agent- Another component of culture medium is the gelling agent which makes the medium solid because in liquid medium the tissue submerges and die due to lack of availability of oxygen.In solid medium there is improved oxygen supply and provides the support to the culture growth (agar is not a nutrient) as compared to liquid medium.For this purpose the most commonly used gelling agent is agar-agar obtained from red algae like *Gracilaria*. Agar is used at a concentration of 0.8-1.0%. If the concentration is increased than it makes the medium very hard and than diffusion of nutrients into the tissue medium is not possible. Agar(Agarose) has the resistance to enzymatic hydrolysis at incubation temperature and due to this characteristic it is commonly used in culture medium. Moreover it is neutral to the media constituents and thus do not react with them.

However, agar is not an essential constituent of the nutrient medium.Single cell and cell aggregates can also be grown in suspension culture,devoid of agar,but such cultures should be aerated regularly either by bubbling sterile air or by gentle agitation. Another gelling agents used to solidify liquid media are like alginate, carrageenan, starch, gelatin, polyacrylamide, silica gel and hydroxyethylcellulose.

MEDIA PREPARATION

Glass distilled water and chemicals of highest purity should be used.A convenient approach to prepare a medium is to have stock solutions of all the nutrients (macronutrients, micronutrients, iron solution and organic components) and store them in refrigerator. The preparation of **Murashige** and **Skoog's** medium is discussed below-

TABLE NO. 2

Ingredients	Amount (mg/litre)
Group 1	
NH_4NO_3	1650
KNO_3	1900
$MgSO_4.7H_2O$	370
KH2PO4	170
$CaCl_2.2H_2O$	440
Group 2	
$MnSO_4.4H_2O$	22.3
$CuSO_4.5H_2O$	0.025
$CoCl_2.6H_2O$	0.025
$ZnSO_4.7H_2O$	8.6
$Na_2MoO_4.2H_2O$	0.25
KI	0.83
H_3BO_3	6.2
Group 3	
$FeSO_4.7H_2O$	27.8
$Na_2EDTA.2H_2O$	37.3
Group 4	
Pyridoxine HCl	0.5
Nicotinic acid	0.5
Thiamine HCl	0.1
Inositol	100
Glycine	2.0

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All the ingredients of Murashige & Skoog's medium as listed in table no-2 is categorised into four groups-

(1) Concentration of ingredients- The stock solution of group I is prepared 20x concentrated solution and of Group 2, 200x concentrated solution. Group 3 , iron salts is prepared 200x concentrated and group 4 organic ingredients(except sucrose) 200x concentrated.

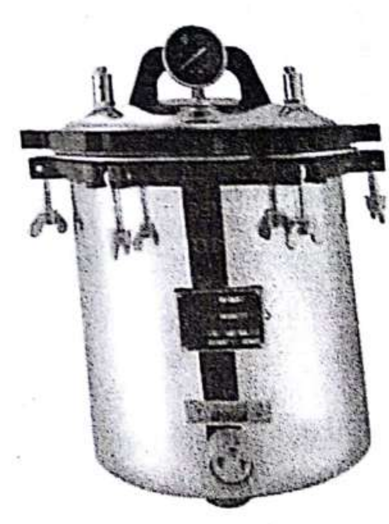
(2) Solution preparation- Stock solutions are prepared in the strength of 1m mol l^{-1} or 10m mol l^{-1}. In the preparation of stock solution each component should be weighed and dissolved separately in glass distilled water and than mix them together.

IAA, 2,4-D and similar compounds are dissolved in small amount of ethanol and made to desired volume with water. The cytokinins are dissolved in a small amount of 0.5 NHcl with slight heat and then made to volume with water. The iron solution is prepared by dissolving $Na_2EDTA.2H_2O$ and $FeSO_4.7H_2O$ separately in 450 ml of distilled water by gentle heating and continous stirring . Mix the two solutions and make up the volume to 1L with ditilled water.

(3) Semisolid media preparation- Agar and sucrose are weighed as per requirement and dissolved in 3/4 th volume of the distilled water by heating on water bath. The adequate quantities of stock solution (for 1L media, 50ml of stock solution of Group I and 5 ml of stock solution of Group 2,3 and 4) are added . Other desired supplements are also added and final volume is made up with distilled water. The pH of the medium is adjusted to 5.7 using 1N Hcl or 1N NaoH and medium is poured in the culture vessels.

(Note- A large variety of prepared media are now available in the market in the powdered form from Sigma and Himedia companies.The powdered media is disolved in 3/4 th volume of distilled water and after adding sucrose, agar and other desired chemicals, final volume is made up with distilled water. pH is adjusted and finally sterilized by autoclave. However, media prepared in the laboratory cost less as compared to ready- made media purchased from market.)

(4) Sterilization of media- All the culture vessels containing media are plugged with non-absorbent cotton, covered with aluminium foil and are sterilized by autoclaving at 121^{0}C for 15-40 minutes (time depends on the volume of liquid to be sterilized). These vessels may be stored at 4^{0}C and used whenever needed .



Autoclave

TYPES OF PLANT TISSUE CULTURE

The present knowledge permits the use of any plant part as a source of material to initiate cultures. The plant part used for this purpose is known as an explant. Nodal and

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inter nodal segments of stem, apical and axillary bud, leaf, leaf disc, petiole, anther, pollen, flower bud, petal, ovule, orary root, and even isolated epidermal peel, gland and trichome have been used as an explant. A suitable explant of a species is desirable for successful regeneration. Various types of cultures are discussed below:-

(i) Stem cultures- When stem segments are used to initiate the cultures, the cut ends are sealed with molten wax and then sterilized with any disinfectant and washed thoroughly with sterilized distilled water. Sterilized stem pieces are transferred in a pre-sterilized petridish or sterilized filter paper and ends are removed with the help of scalpel. The explants of suitable size consisting of node/nodes are prepared and transferred to the medium.

(ii) Anther cultures- The anthers may be taken from plants grown in the field or in pots but ideally these plants should be grown under controlled temperature, light and humidity.

Flower buds of the appropriate developmental stage are collected, surface sterilized and their anthers are excised and placed horizontally on culture medium. Flower buds with small anthers may themselves be cultured and in some cases the entire inflorescence has been cultured. Care should be taken to avoid injury to anthers since it may induce callus formation from anther walls.

(iii) Pollen cultures- Pollen cultures may be isolated either by squeezing or float-culturing the anthers. About 50 anthers may be placed in 20 ml of medium and squeezed with glass rod ; the solution is filtered through a nylon mesh of suitable pore size and centrifuged. The pollen pellet is collected, washed twice and suspended at a final density of 10^3 - 10^4 pollen\ml.

In float culture, excised anthers are floated on a shallow liquid medium in a petridish; the anthers dehisce in a few days releasing their pollen grains into the medium.

(iv) Embryo culture- For embryo culture, embryos are excised from immature seeds under laminar air flow cabinet. Sometimes the immature seeds are surface sterilized and soaked in water for few hours before the embryos are excised. The excised embryos are directly transferred to culture media.

(v) Ovule culture- Ovules after fertilization have been successfully cultured to obtain mature embryo / seeds. Depending upon when the embryo aborts, the ovules have to be excised any time soon after fertilization to almost developed fruits, which may sometimes be lost due to premature abscission. However, ovule culture is mainly tried only in those cases where embryo aborts very early and embryo culture is not possible due to difficulty of its excission at a very early stage. In some cases the medium may need to be supplemented with fruit/vegetable juice to accelerate initial growth.

(vi) Ovary culture- Ovary culture is often used when embryo culture and ovule culture either fail or are not feasible due to very small ovules. The ovaries are excised at the zygote stage or at the two celled proembryo stage and normal development is completed *in vitro*.

(vii) Leaves or leaf primordia culture- Leaves of 800 um are separated from shoots, surface sterilized and are transferred to medium. Growth rate in culture depends on their stage of maturity of excission. Young leaves have more rate of growth as compared to mature leaves.

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(viii) Shoot tip culture- The excised shoot tips of 100-1000 um long of various plant species are cultured on nutrient media. It forms adventitious roots and regenerate into entire plant.

Selected examples of regeneration from different explants and cultures-

A- Stem culture

Urginea indica
Tamarindus indica
Rose hybrida
Tecomella undulata
Camellia sinensis
Dalbergia latifolia
Ziziphus mauritiana

B- Inflorescence culture

Brassica oleracea var botrytis
Musa species
Pennisetum americanum
Sorghum almum
Triticum aestivum
Zea mays

C- Flower culture

Arachis hypogaea
Phlox drumondii
Rannunculus scleratus
Tagetes erecta
Ultricularia inflexa

D-Embryo culture

Arachis hypogaea
Allium cepa
Costus speciosus
Eucalyptus citriodora
Hordeum vulgare
Podophyllum hexandrum

E- Leaf culture

Artemisia annua
Azadirachta indica
Cicer arietinum
Curculigo orchioides
Dioscorea floribunda
Lycopersicon esculentum
Oryza sativa
Rauwolfia serpentina
Saccharum officinarum
Triticum aestivum
Zea mays

F-Shoot tip culture

Atropa belladonna
Acacia auriculiformis
Chrysanthemum monifolium
Gladiolus species
Morus indica
Phoenix dactilifera
Piper nigrum
Picrorhiza kurroa
Terminalia bellerica
Zinziber officinale

G- Root culture

Albizzia lebbeck
Aegle marmelos
Dalbergia sissoo
Vigna aconitifolia

H-Seed and seedling callus

Acacia auriculiformis
Albizzia lebbeck
Dalbergia latifolia
Comniphora wightii
Carthamus tinctorium
Helianthus annuus
Prosopis tamarugo
Tecomella undulata
Sesbania grandiflora
Vigna mungo
Ziziphus mauritiana

I- Endosperm culture

Dendrophthoe falcata
Oryza sativa
Taxillus vestitus

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Apart from the above mentioned cultures the other methods that are commonly used for culturing of plant cells/tissue are:-

Protoplast culture

Hairy root culture

Protoplast culture

Protoplasts are the naked plant cells which do not contain cell walls. The real start of plant protoplast research was made by **E.C.Cocking** in 1960 when he demonstrated that naked cells called as protoplasts can be obtained through enzymatic degradation of cell walls. In view of this the isolation and culture of protoplasts has become a very important area of research within the realm of plant biotechnology. Protoplasts are isolated by two methods namely, mechanical and enzymatic.

Mechanical method- In this method the plasmolysed cells (infact cell walls) are cut with sharp knife to release the protoplasts. This method gives poor yield of protoplasts thus it is no more practically used. It is only a historical method.

Enzymatic method- The enzymatic method is almost invariably used now for the isolation of protoplasts where cells are not broken and osmotic shrinkage is minimum. The protoplasts can be isolated from a variety of tissues including leaves, roots , *in vitro* shoot cultures, callus, cell suspension and pollen. However, the most commonly used part are leaves which can be employed for isolation of protoplasts using the following steps -

Fully expanded leaves are obtained from about 10 weeks old plants and are surface sterilized by first dipping them into 70% ethyl alcohol for one minute and then treating them with 2% solution of sodium hypochlorite for 20-30 minutes. The leaves are then rinsed three times with sterile distilled water and subsequent operations are carried out under laminar air flow. The lower epidermis of the sterilized leaves is carefully peeled off and the stripped leaves are cut into small pieces. Mesophyll protoplasts can be obtained from these peeled leaf segments while those for epidermis are obtained from peeled epidermis. From the peeled leaf segments the protoplasts can be isolated using any one of the two methods:

(i)- direct (one step) method, in which treatment with macerozyme (or pectinase) and cellulase is done simultaneously, or

(ii)- sequential (two step) method, in which cells are first isolated using macerozyme and then cells are treated with cellulase to isolate protoplasts.

The isolated protoplasts are cleaned by centrifugation and decantation method. The cleaned protoplast solution of known density (1x105 protoplast/ml) is poured on sterile culture media in the petridishes and mix them gently by rotating each petridish. Allow the medium to set, seal the petridishes with paraffin film and incubate the petridishes in inverted position in BOD incubator. The protoplasts which are capable of dividing, undergo first division within 2-7 days and form callus after 2-3 weeks. The callus is then transferred to fresh medium (subculturing of callus) containing appropriate proportions of auxin and cytokinin. Embryogenesis begins and the embryo develops into plantlets. Subsequently, the plantlets may be transferred to pots.

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Protoplasts isolation procedure

HAIRY ROOT CULTURE

A relatively new type of plant culture which consists of highly branched roots covered with a mass of tiny root hairs originated directly from the explant in response to *Agrobacterium rhizogenes* infection. This bacteria is able to induce hairy root symptoms. These cultures can even grow on simple media of salts and sugars (devoid of hormones or vitamins). These hairy roots can be excised and cultivated indefinitely under sterile conditions. A feature of hairy root systems of paramount importance for their commercial exploitation is their stable, high level production of secondary metabolites.

In the production of hairy root cultures, the explant material is inoculated with a suspension of *Agrobacterium rhizogenes*. The bacteria contains root inducing (Ri) plasmid. This culture is generated by growing bacteria in yeast maltose broth (YMB) medium for 48 hours at 25^{0}C with rotary shaking, pelleting by centrifugation (5x10^3 rpm, 20 min) and resuspending the bacteria in YMB medium to form a thick suspension. Transformation may be induced on aseptic plants grown from seed or on detached leaves, leaf disc, petioles or stem segments from green house plants followed by sterilization of the excised tissues. In some

species a profusion of root may appear directly at the site of inoculation, but in others a callus will form initially and roots emerge subsequently from it. In either case, hairy roots appear within one to four weeks. The susceptibility of species to infection is very variable. Addition of acetylsyrigone, the compound produced during the wounding response of plants, activates the Vir(Virulence) genes of *Agrobacterium* adding plasmid T-DNA transfer.

Cultures may be cleared of bacteria by several passage in media containing 200 mg/L cephalosporin and 500 mg/L ampicillin. The infection of plants with *Agrobacterium rhizogenes* causes one or both of two pieces of T-DNA (Tt and Tg) of Ri plasmid to be inserted into the plant genome. Integration alters the auxin metabolism of transformed tissues in such a way that the hairy root phenotype is expressed and amino acid metabolism is modified in such a way that specific metabolites such as opines are produced.

Hairy Roots

Establishment and Maintenance of various cultures-

The growth establishment and maintenance of various plant tissue cultures can be done by three main culture systems which are selected on the basis of the objective-

1- Callus culture (also called as Static culture)

2-Suspension cultures

3-Protoplast culture- The protoplast culture can be grown as-

Callus culture

Suspension culture

CALLUS CULTURE

The unorganised mass of cells which proliferates from the cells of an explant is termed as **callus.** The cultivation of callus on an agar-gelled medium under aseptic conditions is called as **callus culture.** This technique is described below-

INITIATION OF CALLUS CULTURE

(i) Selection of an explant- Callus cultures can be obtained from any organ or culture such as seedlings, young shoots or buds, root tips or developing embryos: fruits, floral parts, tubers and bulbs.

(ii) Preparation of an explant- After selection , the explant is taken and surface sterilized

It is washed with tap water and sterilized with sodium hypochlorite (2%) or mercuric chloride (0.1- 1%) solution for 15-30 minutes. Finally it is washed with sterile glass distilled water and cut into small segments of 2-5 mm. (For detail, please refer the surface sterilization).

(iii) Culture media- The culture of the medium depends upon the species of plant and objective of study. The nutrient media required should be well defined and it should contain inorganic nutrients, organic nutrients and growth hormones. The growth hormones like auxins, cytokinins and gibberelins are added to media according to the objective of culture. Auxins like IBA and NAA are widely used for rooting and in combination with cytokinins for shoot proliferation. 2,4-D and 2,4,5-T are very effective for induction and growth of callus. Cytokinins are employed for the promotion of cell division , regeneration of shoots and growth of auxillary buds.

The well defined semi solid nutrient media is prepared and pH of the medium is adjusted between 5 to 6. It is poured into culture vessels, plugged with non- absorbent cotton, covered with aluminium foil and are sterilized by autoclaving.

(iv) Transfer of an explant- Surface sterilized explant is transferred aseptically to the vessels containing semi solid nutrient media.

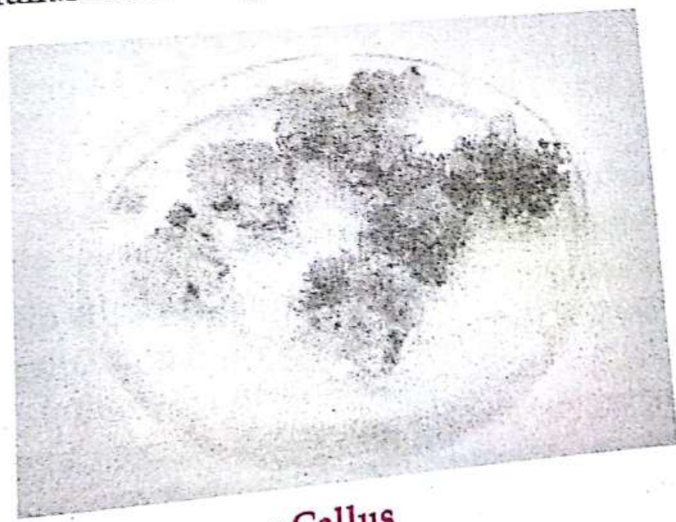
(v) Incubation- These inoculated vessels are incubated in BOD incubator at the temperature of $25\pm2^{\circ}C$ using light and dark cycles of each 12 hours duration. After 3 to 8 days of incubation sufficient amount of callus is produced and after 3 to 4 weeks, callus should be 4 to 5 times, the size of an explant. Callus is formed through three stages of development viz-

(A) Induction - In this stage, metabolic activities of the cell increases therefore it accumulates the organic contents and finally divide into a number of cells.

(B) Cell division- In this stage the active cell division takes place as the explant cells revert to meristematic state.

(C) Cell differentiation- In this stage the cellular differentiation takes place i.e. the morphological and physiological differentiation occurs resulting in the formation of secondary metabolites.

Maintenance- After a period of time it becomes neccessary to transfer the callus to fresh media (subculturing of callus) chiefly due to nutrient depletion and meduum drying. In general, callus tissue of 5-10mm in diameter and 20- 100mg in weight are transferred aseptically to fresh medium.Subculturing of callus is done after every 4 to 6 weeks.



Callus

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Callus cultures are slow growing systems. Cells grow as clumps or masses in callus cultures and only lower cells are in contact with the medium whereas cells in upper layers get their nutrients from cells in lower layers. The main feature of callus is its capability to develop into normal root and shoot and ultimately forming a plant. Secondary plant metabolites can also be produced from callus cultures but on the whole it is good source for establishment of suspension cultures.

SUSPENSION CULTURE

Tissue and cells cultured in a liquid medium (without agar) produce a suspension of single cells and cells clumps of few to many cells; these are called as suspension cultures.

Initiation of suspension culture

Cell suspension cultures are initiated by transferring the friable callus to liquid nutrient medium (without agar). In liquid nutrient medium plant tissue remains submerged which leads to anaerobic conditions and ultimately there is death of cells. Therefore such cultures are agitated by a rotary shaker at 50-150 rpm. Agitation serves both to aerate the cultures and to disperse the cells. After the production of sufficient number of cells, subculturing can be done in fresh liquid medium.

It is common observation that if relatively small number of cells are transferred (low inoculum density) to a new medium (either static or liquid), they may fail to divide whereas a larger quantity of tissue transferred from the same culture may proliferate rapidly on the same medium. This observation has led to the concept of 'critical initial cell density' . This is defined as the smallest inoculum per volume of medium, from which a new culture can be reproducibly grown. There are few conditions which determine the critical initial density of cells. They are :

(i)-The cultures physiological characteristics.

(ii)-The length of time and conditions under which the culture was previously maintained.

(iii)-The composition of fresh medium.

The third point is of interest. As the isolated cells failed to grow on fresh medium, 'conditioned medium' or 'nurse tissue' conditions are used to grow isolated cells or protoplasts. A 'conditioned medium' is the medium on which some tissues were previously grown. Conditioning makes the minor adjustment in the nutrients and chemical substances released in the medium by the callus , promotes the growth of isolated cells of protoplasts. In suspension cultures, cells grow as isolated single cells and cell aggregates of a few cells to a few hundred cells. Cell aggregation vary from species to species.

MAINTENANCE OF SUSPENSION CULTURE

The suspension culture can be maintained by the following ways:

(a) Batch culture- In the batch culture technique the cells are allowed to multiply in liquid medium which is continously agitated. Except for circulation of air, the system is 'closed' with respect to addition or substraction from the culture. To get the growth again on the stationary phase either the cells are transferred to fresh medium or more amount of liquid medium is added to the original culture. Each fresh medium containing culture (suspension) constitutes a batch. Such cultures are grown again and again in batches for the purpose of experiment.

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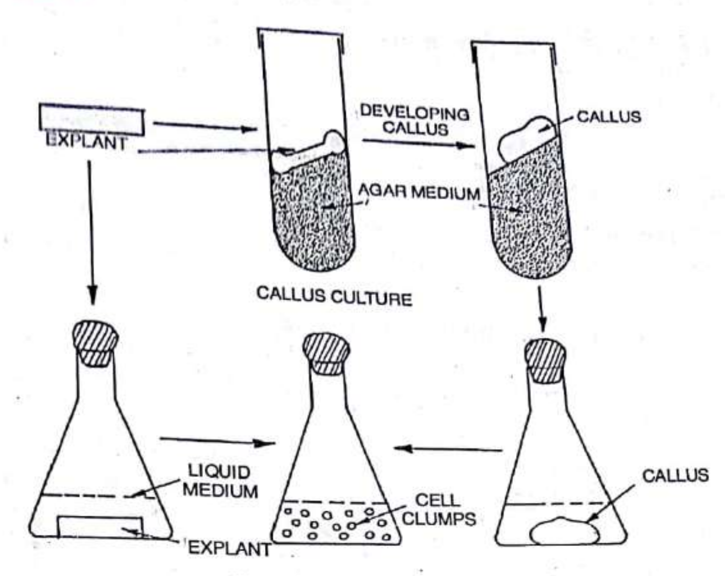
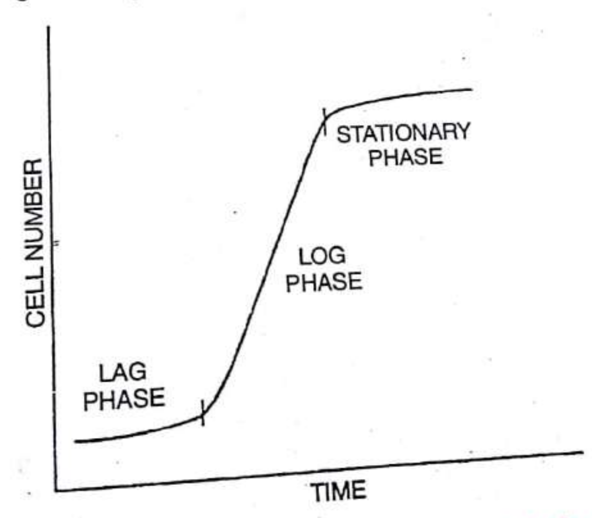


Initiation of callus and suspension cultures

In batch culture there is no steady state of growth. The cell number or biomass of a batch culture exhibits a typical **sigmoidal** curve having a lag phase during which the cell number of biomass remains unchanged, followed by a logarithmic (log) phase (Exponential phase) when there is rapid increase in cell number and finally ending in a stationary phase during which cell number gradually declines.



A model curve for cell number in a batch culture.

The lag phase duration depends mainly on inoculum size and growth phase of culture from which inoculum is taken. The log phase lasts about 3-4 cell generations (a cell generation is the time taken for doubling of cell number) and duration of a cell generation may vary from 22-48 hours, depending mainly on the plant species. The stationary phase is forced

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on the culture by a depletion of the nutrients and possibly due to an accumulation of cellular wastes. If the culture is kept in stationary phase for a prolonged period, the cells may die. Therefore subculturing should be done.

(b) Continous culture- In this tecnique the cell population is maintained in a steady state for a long period by draining out the used medium and adding fresh medium. Such culture systems are of two types-

(i) Closed type- In closed continous culture, cells are separated from the used medium taken out for replacement and added back to the culture so that cell biomass keeps on increasing.

(ii) Open type- In open continous culture, both cells and the used medium are taken out and replaced by equal volume of fresh medium. The replacement volume is so adjusted that cultures remain at submaximal growth indefinitely. Further open continous culture are of two types viz. **turbidostat** and **chemostat** types.

Turbidostat type- In turbidostat, cells are allowed to grow up to a preselected turbidity (usually measured as OD) when a predetermined volume of the culture is replaced by fresh normal culture medium.

Chemostat type- In this a chosen nutrient is kept in a concentration so that it is depleted very rapidly to become growth limiting while other nutrients are still in concentration higher then required. In such a situation any addition of the growth- limiting nutrient is reflected in cell growth.

SUBCULTURE

The growth of cell suspension culture is always higher than callus culture therefore they should be subcultured every 3-14 days. The inoculum volume should be 20-25% of the fresh medium volume; in any case the initial cell density of the fresh culture (just after inoculation) should be around $5x10^4$ cells ml^{-1} or higher otherwise the cells may fail to divide.

Estimation of growth

The various parameters used for estimating the growth of cultured cells are like fresh weight, dry weight, cell number and packed cell volume.

Fresh weight- This parameter is employed to measure the growth of both suspension and callus cultures. In case of callus cultures, the cell mass is placed on a pre- weighed dry filter paper or nylon filter and weighed to determine fresh weight.

In case of suspension cultures, the cells from suspension cultures are filtered on to a filter paper or nylon filter and washed with ditilled water. The excess of water is removed under vacuum and weighed along with the filter (filter is pre weighed in wet conditions).

Dry weight- This parameter is also employed to measure the growth of both suspension and callus cultures. Dry weights are determined by drying the cells and filter in an oven at 60^0C for 12 hrs and weighed ; the filter is pre- weighed in dry conditions.

Cell number- Cell number is the most informative measure of cell growth and is applicable to only suspension cultures. Cell aggregates are treated with pectinase or 5-15% chromic acid. To the 1 volume of cell suspension culture, 2 volumes of 8% chromic acid and trioxide solution is added and it is heated at 70^0C for 5-15 minutes . The mixture is cooled

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and agitated for 10 minutes. The suspension so obtained is certrifuged, chromic acid is removed and the pellet is suspended in 8% saline solution. After few minutes, free cells are counted by haemocytometer.

Packed cell volume- This is determined by pipetting a known volume of suspension culture (4-7ml) into a 15 ml graduated centrifuge tube, spinning at 200 x g for 5 min and reading the volume of cell pellet which is expressed as ml cells/ L of culture.

APPLICATIONS OF PLANT TISSUE CULTURE IN PHARMACOGNOSY

Now a days the plant tissue culture technique is widely used in all the fields of biosciences including pharmacognosy also. It's applications are-

1- Production of secondary metabolites

2- Biotransformation

3- Clonal propagation or Micropropagation

4- Somaclonal variation

5- Cell Immobilization

1-Production of secondary metabolites-

It is well known that plants are an important source for a variety of chemicals used in pharmacy, medicine and industry.

In recent years, plant cell suspension cultures, callus cultures and immobilized cells are being utilized for the production of these chemicals on commercial scale due to following advantages over extraction from plants-

1. The yield and quality of the product is more consistent in cell cultures because it is not influenced by the environment.
2. The production schedule can be predicted and controlled in the laboratory or industry.

The most important chemicals produced using cell cultures are secondary metabolites which are defined as 'those cell constituents which are not essential for survival'. These secondary metabolites include alkaloids, glycosides, terpenoids, steroids and a variety of flavours, perfumes,colours etc. The yield of these chemicals in cell culture is though generally lower than in whole plants, it can be substantially increased by manipulating physiological and biochemical conditions. In some cases cell cultures accumulate these secondary metabolites at levels higher (2-10 times) than those found in whole mother plants, from which cell culture has been prepared. Automation in cell cultures can be used for industrial production of secondary metabolites. However, sometime immobilized plant cells are used instead of suspension cultures to increase the efficiency of production system. Some of the important secondary metabolites obtained from plants are listed in following tables.

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TABLE NO.3

Alkaloids produced in culture and their pharmacological activity

Plant species	Product	Culture type	Activity
Atropa belladonna	Atropine	S	Anticholinergic
Catharanthus roseus	Vincristine	Shoot culture	Anticancer
	Vinblastine	Shoot culture	Anticancer
	Ajmalicine	S	Hypotensive
Cinchona officinalis	Quinine	S	Antimalarial
Colchicum autumnale	Colchicine	C	Antimitotic
Coffea Arabica	Caffiene	C	Stimulant
Cephaelis ipecacuanha	Emetine	Root culture	Emetic
Datura stramonium	Scopolamine	Hairy root culture	Antihypertension
Ephedra gerardiana	Ephedrine	S	Spasmolytic
Nicotiana tabacum	Nicotine	S	Stimulant
Ochrosia elliptica	Ellipticine	S	Antitumour
Papaver somniferum	Morphine	S	Analgesic
	Papaverine	S	Spasmolytic
	Codeine	S	Sedative, Analgesic
Rauwolfia serpentine	Reserpine	S	Antihypertensive

S- Suspension culture

C- Callus culture

FACTORS AFFECTING THE PRODUCTION OF SECONDARY METABOLITES

The factors that affects the production of secondary metabolites are :-

(1) Physical factors

(2) Effect of nutrients

(3) Selection of cells

(1) Physical factors- The effect of **light** on growth and metabolite production has been extensively studied. Light is involved in light mediated enzyme metabolism and photomorphogenesis which indirectly affects the secondary metabolites. Phytochemical responses are affected by both irradiance and light quality. Blue light induced maximum anthocyanin formation in *Haplopappus gracilis* cell suspension. White light induced the anthocyanin synthesis in *Catharanthus roseus* and *Populus* species. In contrast to these, white or blue light completely inhibited naphthoquinone biosynthesis in callus culture of *Lithospermum erythrorhizon*. The production of chlorogenic acid in *Haplopappus gracilis* was stimulated by white, blue and red light; of which blue light was the most effective. Anthocyanin synthesis in cultures of *Daucus carota, Linum usitatissimum, Vitis vinifera* and *Helianthus tuberosus* required white light. Callus cultures of *Ephedra gerardiana, Scopolia acutangula* and *Peganum harmala* produce more alkaloid in light than in dark.

Effect of **temperature** on secondary metabolites production is little studied. Work on *Catharanthus roseus* cell culture is widely cited for demonstrating effect of temperature.Indole alkaloid production increased two fold when cells of *C.roseus* were incubated at 16^{0}C instead of 27^{0}C. However at lower temperature (16^{0}C) growth was three fold slower. Thus produc-

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TABLE NO. 4

Saponine& steroids produced through tissue culture

Plant species	Product formation
Saponins	
Aesculus hippocastanum	Aescin
Agave insalna	Hecogenin
Dioscorea deltoidea	Diosgenin
Glycyrrhiza glabra	Glycyrrihizin
Ponax ginseng	Ginseng saponins
Cardiac glycosides	
Digitalis lanta, D.purpurea	Digoxin, Digitoxin
Strophantus species	Quabain
Urginea maritime	Proscilariddin
Other steroids	
Holarrhenna antidysenterica	Sitosterol, stigma sterol, cholesterol
Solanum xanthocarpum	Solasodine
Withania somnifera	Withanolides

TABLE NO. 5

Food additives produced by tissue culture

Plant species	Product
Colour	
Daucus carota	Anthocyanin
Euphorbia milli	Anthocyanin
Vitis vinifera	Anthocyanin
Beta vulgaris	Betalaines
Crocus sativus	Crocin, crocetin
Flavours	
Allium cepa	Onion flavor
Capsicum annuum	Capsicum, capsaicin
Capsicum frutesceus	Capsicum, capsaicin
Crocus sativus	Safranal
Vanilla planifolia	Vanilla,vanillin
Sweetner	
Stevia rebaudiana	Stevioside
Thaumatococcus danielli	Thaumatin

tivity of cultures remained same. Change in incubation temperature of C.*sinensis* or N. *tobacum* resulted in decreased synthesis of caffeine and nictotine respectively.

Plant cells are usually cultured on media having a pH range of 5 to 6. There are several reports which clearly demonstrate that the **pH** of the growth medium can drastically influence the production of phytochemicals by cultured cells, e.g. anthocyanins, anthraquinones and alkaloids etc. Cultures of *Daucus carota* produced less anthocyanin when grown at pH 5.5 than when grown at pH 4.5 . It was suggested that it was because of increased degradation of anthocyanin at higher pH. Anthocyanin contents decreased by 90% at pH 5.5 compared to tissues grown at pH 4.5.

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(2) Effect of nutrients- Cultured plant cells are usually grown on medium containing all the elements required for their sustained growth. Plant cell cultures are totipotent and possess all the capabilities of the intact plant to synthesize primary and secondary metabolites. Therefore it is imperative that medium ingredients such as carbohydrate, nitrogen, phosphorous and plant growth regulators affect the growth and metabolism of cultured cells and the production of secondary metabolites.

(a) Effect of carbon source- Carbohydrates are incorporated at 2-5% concentration in the medium and are known to influence the production of phytochemicals. In *Catharanthus roseus* cultures alkaloid content fluctuated with sucrose concentration in the medium; it increased as the sucrose concentration was increased (4-10%). Similarly the nature and concentration of the carbohydrate source had a significant effect on diosgenin production by *Dioscorea deltoidea* cell suspension cultures. It was recorded that on 1.5% sucrose supplemented medium, tissues yielded a higher amount of diosgenin in *D.deltoidea* compared to tissues grown on media with same amount of fructose, galactose lactose or starch. Cells of *D.deltoidea* with the greatest diosgenin productivity were those grown on medium containing 3% sucrose.

(b) Effect of nitrogen source- A mixture of nitrate and ammonium compounds is used in all the standard media as a source of nitrogen. The nitrogen source also affects the production of secondary metabolites. However, different types of results in relation to secondary metabolites by varying the nitrogen in the medium are obtained. It is reported that synthesis of 1,4- naphthaquinones in callus cultures of *Lithospermum erythrorhizon* increased with increase in total nitrogen from 67mM to 104 mM, while further increase in nitrogen in the medium suppressed yield. Zenk and co-workers reported that anthraquinone production by *Morindra citrifolia* cells decreased when KNO_3 levels were varied either above or below the range 2 to 4.5 g/L. Changes in total ubiquinone production in *Nicotiana tobacum* suspension cultures were recorded with changed ammonium to nitrate ratio in the medium from 3:1 to 1:3 but keeping the total nitrogen level constant. The biosynthesis of indole alkaloids in *Peganum harmala* decreased when ammonia or glutamine were substitued for nitrate.

(c) Effect of plant growth regulators- Effect of growth regulators on cultured plant cell is manifested in growth, metabolism and differentiation. The production of all secondary metabolites is affected by growth regulators. There are several reports in literature stating that by reducing the concentration of 2,4-D in the medium or replacing it with another auxin, the accumulation of secondary metabolites can be enhanced e.g. alkaloids in the cultures of tobacco, ephedra and pigment (shikonin) in the cultures of *Lithospermum erythrorhizon*. But the inhibitory effect of 2,4-D is not universal since there are many instances of an increase in metabolite content e.g. 2,4-D stimulates the production of ubiquinone and scopolatin in tobacco cultures and solasodine content in *Solanum eleagnifolium*. There are also examples available where in other auxins inhibited the production of secondary metabolites e.g. NAA and IAA inhibited, similar to 2,4-D the synthesis of anthocyanin in cell suspension cultures of carrot. It may be generalised that to a certain extent increase in concentration of an auxin, the medium has adverse effects on alkaloid content of the tissues.

The effect of cytokinins is similar to that of auxins as far as secondary metabolites are concerned, e.g. (i) activation of production of metabolites : DOPA in the tissues of Stizolabium, scopolin and scopoletin in the tissues of tobacco and carotenes in the cells of *Ricinus*, ajmalicine in *Catharanthus roseus* or (ii) inhibition of metabolites : anthraquinones in the tissues of *Morinda citrifolia*, shikonin of cells of *Lithospermum erythrorhizon* and nicotine of cells of tobacco etc. It

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is worth mentioning that the concentration and combination of plant growth regulators modulate growth of the tissues and production of secondary metabolites.

(d) Precursors- Precursors are molecules which are directly incorporated into secondary metabolites but perhaps with some structural changes. When such precursors are fed to culture medium they affect the growth and concentration of secondary metabolites. For e.g. addition of phenylalanine to the cultures of *Ephedra gerardiana* increases the ephedrine production. Vanillylanine and isocarpic acid precursors increases the production of capsaicine in the cultures of *Capsicum frutescens*. Addition of phenyl propane to the cultures of *Podophyllum hexandrum* increases the production of podophyllotoxin by 128 folds. Similarly addition of tryptamine and secologanin to the cultures of *Catharanthus roseus* improves the production of ajmalicine.

But sometimes the precursor may cause toxicity in the medium for the cells or may be degraded by extra-cellular enzymes. Positive influence of ornithine, phenylalanine, tyrosine and Na-Phenylpyruvate or alkaloid biosynthesis in *Datura* cell cultures was recorded with growth inhibition by these precursor amino acids. Once entered in the cell, the pecursor is stored in the cellular compartments and thus may not be available for incorporation. Therefore, the incorporation of precursors in the medium may not be encouraging.

(e) Production medium- It has been concluded from the results obtained from the various studies on optimization of secondary product formation in cultured cells that-

(i) Higher concentration of auxin in the medium particularly 2,4-D suppresses secondary metabolites.

(ii) Lower carbohydrate level (sucrose) favours cell proliferation while higher concentration arrests cell growth and increases secondary product formation.

(iii) Higher concentration of phosphate in the medium causes cell growth and lower concentration enhances secondary metabolite levels.

(iv) In certain cases higher nitrogen level in the medium enhances cell proliferation while low concentration increases secondary product formation.

(v) Increased synthesis of secondary products occurs during the stationary phase of cultures when primary metabolism and cell proliferation comes to halt.

On the basis of above conclusions, a secondary metabolite production or induction medium was devised by Zenk et al in 1977 in which the above conditions were combined. Cells grown on maintenance medium proliferate rapidly and such cultures are then transferred to induction or production medium (optimal for secondary metabolites) in which growth is arrested or cells enter in a stationary phase of growth. Such induction medium contains the same constituents but with low levels of phosphate, nitrogen (not always) and auxin (2,4-D) and very high sucrose concentration (6-10%).

Therefore, if during exponential phase of growth, cells in maintenance medium are transferred into production medium, growth comes to halt and a carbohydrate and other nutrients are available. So primary metabolites are rapidly diverted to synthesis of secondary metabolites instead of cell growth, thereby enhancing the secondary product synthesis.

(3)-Selection of cells- In this topic we will discuss how selection procedures are helpful in increasing the yield of cultures. Before producing secondary metabolites at the industrial

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or commercial level it is a prerequisite to achieve optimal yield of secondary metabolites through optimization and select the cells for maximal yield. Though the production of secondary metabolite is a genetically controlled phenomenon, cells can be selected for high yield of secondary metabolites from a heterogeneous population to improve the overall quality of the cultures, in relation to the production of active principle. Before starting the selection procedures it is necessary that heterogeneous nature of the cultures is established and a sensitive method of analysis is available to analyze a large number of clones. The 'stability' of such selected clones is of paramount importance for developing further method to achieve industrial production.

(a) Variability in field grown materials- Field grown plants particularly the cross polinated plants are heterozygous and express different phenotypic and physiological characteristics . It is because of the heterozygocity that the difference is also expressed in terms of secondary metabolite content. Variation in the alkaloid yeld is well documented in such plants as *Catharanthus roseus* (Apocynaceae, cross polinated crop) and *Lupinus polyphyllus* (Leguminosae, self pollinated crop).

In a population of field grown *C.roseus* plants, a complete spectrum from very low to very high alkaloid (ajmalicine and serpentine) producing plants was recorded and very high alkaloid producing plants were found scattered in the population. Similary in lupin fields animals avoid grazing plants with high alkaloid contents. From these two examples it is clear that there are plants with high alkaloid (secondary metabolites) levels and if cultures are initiated from such plants, optimization and selection procedures can generate very high alkaloid -yielding clones. Similar variation in alkaloid yield has also been observed in the fields of *Nicotiana* and *Hyoscyamus*.

(b) Producer/Non-Producer cells- In the plant itself certain cells, tissues or organs accumulate more secondary products than others. This is evident from the presence of a high level of alkaloid containing idolblasts distributed in leaf epidermis of *Catharanthus roseus* or gland cells in citrus or glandular trichomes or hairs present in many species. When an explant is transferred on to a medium it grows by division of cells producing an undifferentiated mass of cells called as callus. Callus is derived mostly from parenchyma cells present in the explant. When sufficient callus is produced by an explant it is separated and subcultured on to a fresh medium. In this growing system certain cells divide rapidly while others are slow in division. In cell suspension cultures plant cells grow as free cells or cell aggregates. The size of the cell aggregate depends upon the inherent genetic make up of the species as well as the growth cycle. We know that rapidly dividing cells accumulate less compared to stationary phase cultures. In fast growing cultures certain cells accumulate more secondary metabolite compared to others because cells differ in growth. Therefore all the cultures (cell populations) are mixture of cells containing high secondary metabolites (Producer) and low secondary metabolites (Non-producers) .This has been observed in cultures of several species. In case of pigmented cells, demarcation between high and low pigment containing cells can be made very easily by the naked eye e.g. anthocyanin production in carrot and grape cells. In species in which secondary metabolites constitute a fluroscent compound, it is very common to visualize and differentiate between the producer and non- producer cells ; the producer cells are highly fluroscent under ultraviolet (UV) light. This way cells plated on petridish can be marked, selected and separated mechanically to get high alkaloid -yielding cell lines. Thus, all the cultures are a mixture of producer and non -producer cells and analy-

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sis of such cultures gives an average value of secondary metabolites. From such cultures if producer or high product forming cells are separated from non- producer cells and grown separately, they give rise to high product yielding clones.

2- Biotransformation

A biotransformation or bioconversion can be defined as the conversion of one chemical into another i.e. of a precursor (or substrate) into a final product using a cell suspension acting as a biocatalyst. The biocatalyst can be microorganism, plant or animal cells, either growing or in a quiescent state or an extract from such cells or a purified enzyme. The biocatalyst may be free, in solution, immobilized or on solid support or entrapped in a matrix. Following are some of the examples of the use of cell cultures in biotransformation-

(i) Suspension culture of *Digitalis lanata* can convert digitoxin or methyl digitoxin into medicinally important digoxin or methyl digoxin which is used for the treatment of heart disease. The conversion rate has been estimated to be as high as 15% in 24 hrs and 70% in 7 days.

(ii) Datura cell culture possess ability to convert hydroquinone into arbutin (used as diuretic and urinary antiseptic) through glycosylation.

(iii) Cell cultures of *Stevia rebaudiana* and *Digitalis purpurea* can convert steviol into steviobiocide and stevioside which are 100 times sweeter than cane sugar.

3- Clonal Propagation or Micropropagation

The variety of plant species that can be conveniently propagated through techniques of cell, tissue or organ culture is popularly described as micropropagation.The basic concept is to achieve rapid multiplication without creating unwanted somaclonal variation. Therefore, axillary, adventitious budding and somatic embryogenesis are most frequently used methods of micropropagation. The major benefits of this method includes the following-

(i) rapid multiplication of superior clones and maintenance of uniformity

(ii) multiplication of disease free plants

(iii) multiplication of sexually derived sterile hybrids

The various stages involved in the method of micropropagation are described in short-

Stage I involves establishment of tissue *in vitro*

Stage II involves multiplication of shoots (often media is not changed between stage I and stage II

Stage III concerns root formations and conditioning of propagules prior to transfer to the green house, this stage requires high intensity and alteration of media for promotion of root formation

Stage IV involves growth in pots followed by field trials

There are now many commercial companies in India and developed countries producing millions of plantlets through micropropagation. A few selected genera micropropagated commercially are enlisted in following table -

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TABLE NO. 6

Plant genera micropropagated at large scale

Vegetables, crop & other species	Ornamentals	Woody species
Actidinia	Anthurium	Araucaria
Allium	Bromeliads	Betula
Arachis	Chrysanthemum	Coffea
Asparagus	Chrysanthemum	Eucalyptus
Brassica	Ferns	Malus
Cardamom	Freezia	Pinus
Cicer	Gerbera	Populus
Festuca	Hyacinth	Prunus
Glycine	Iris	Ribes
Musa	Narcissus	Rose
Rheum	Phlox	Salix
Solanum	Saintpaulia	Santalum
	Saxifraga	Tectona
	Syngonium	Vitis
	Tulipa	

4- Somaclonal Variation

Clonal propagation or Micropropagation has been established as most widely applied application of plant tissue culture almost 30 years back.With this application large number of plants were regenerated from explants, callus and cell cultures and lastly from protoplast cultures. In clonal propagation clones are produced from tissue culture with uniform characters but few clones may show variations among the population of clones which were not present in parent cells. This formation of variant clones from cultured tissue is called as **Somaclonal variation.** In 1981, **Larkin** and **Scowcroft** named the phenomenon of variation found in plants regenerated from cell cultures as Somaclonal variation.

It may be necessary to remember that the variation may be transient (epigenetic) or genetic; only the later is transmitted to the next generation and is thus important for crop improvement. Although the details of the genetic basis of somaclonal variation in most crops are still unknown; variation in structure and number of chromosomes has been suggested to be one possible basis. Polyploidy, aneuploidy, translocations, inversions and deletions have been reported in several cases. Meiotic crossing over involving symmetric and asymmetric recombination could also be responsible for a part of the variation observed in the regenerated plants. A number of plant species where useful somaclonal variation has been reported are listed in following table-

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TABLE NO. 7

A list of crop species where desirable and heritable somaclonal variation has been reported

Species	Characters which were modified
A.Monocotyledons	
Allium sativa	Bulb size & shape; clove numbers; aerial bulbil
Avena sativa	Plant height; heading date; awns
Lolium hybrids	Leaf size; flower, vigour, survival
Oryza sativa	Plant height; heading date; seed fertility; grain number & weight
Saccharum officinarum	Disease (eye spot, Fiji virus, leaf scald)
Triticum aestivum	Plant & ear morphology; awns gliadins, grain weight; yield
Zea mays	Ttoxkin resistance; male fertility; mt DNA
B.Dicotyledons	
Lactuca sativa	Leaf weight, length, width, flatness & color
Lycopersicon esculentum	Leaf morphology; branching habit; fruit color; pedicel; male fertility; growth
Solanum tuberosum	Tuber shape; maturity date; plant morphology; resistance for early & late blight; photoperiod; leaf colour; vigour; height; skin colour

5-Cell Immobilization

Immobilization of plant cells and organs is a relatively new development in the techniques of plant tissue culture used for the production of secondary metabolites and development of synthetic seeds. Immobilization of plant cells, protoplasts or embryos (also enzymes and mucilages) is achieved by binding these materials on to or within a solid support. The plant cells can be immobilized by using matrices such as alginates, polyacrylamides, agarose and polyurethane fibres. The most widely used technique for the immobilization of cells with preserved viability has been their entrapment in alginate or carrageenan. Advantage in using these polymers is the simplicity with which spherical particles can be obtained by dripping a polymer cell (or embryo) supension into a medium containing appropriate cation. The main applications of cell immobilization are mentioned below-

(i) Enhanced production of secondary metabolites- Experimental evidence indicates that immobilization can have a dramatic impact on cellular physiology and secondary metabolism. Lindsey (1985) demonstrated that the process of immobilization reduces the rate of cell division, protein synthesis and these effects are conducive for increasing the yield of secondary metabolites. The another consequence of plant cell immobilization is to reduce the production of cell wall material which contain a substantial amount of bound phenolic compounds. This caused increased availability of precursors for secondary metabolism. The production of secondary metabolites in immobilized cells are listed in following table-

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TABLE NO. 8

Production of secondary metabolites in immobilized cells

Species	Product	Increase (X folds)
Capsicum frutescens	Capsaicin	>100
Capsicum annuum	Capsaicin	>100
Coffea Arabica	Methyl xanthenes	13
Catharanthus roseus	Ajmalicine	35

(ii)Biotransformation- Hydroxylation of cardiac glycosides has proved to be an interesting application of immobilized plant cells. Bioconversions of b-methyl digitoxin into b-methyl digoxin has been achieved using *Digitalis lanata* immobilized cell cultures up to 70 days.

TABLE NO. 9

Selected one-step bioconversion by immobilized cells

Cell-culture species	Reaction type	Precursor	Product	Matrix
Digitalis lanata	Hydroxylation	β-methyl digitoxin	β-methyl digoxin	Alginate
Daucus carota	Hydroxylation	Digitoxigeni	Periplogenin	Alginate
Mentha species	Reduction	(-)-menthone	(+)-necomenthol	PAAH
Papaver somniferum	Reduction	Codinone	Codeine	Alginate & PUR

EDIBLE VACCINES

Edible vaccines are transgenic plant and animal based production of or those that contain agents that trigger an animals immune response. In simple terms edible vaccines are plant or animal made pharmaceuticals.

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.

The concept of edible vaccines was developed by Arntzen in 1990s (Head of department of plant biology at Arizona State University). Although the idea seemed quite simple in the beginning but 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 *Steptococcus mutans* in tobacco.

There are several advantages of edible vaccines :

i) They are cheap so they can be produced in large.

ii) They can be ingested by eating the plant/part of the plant. So the need to process & purify does not arise.

iii) Extensive storage facilities like cold storage are not required.

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iv) 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.

Despite the advantages there are various disadvantages of edible vaccines.

i) There is a question mark in the survival of antigen in the acidic conditions of the stomach & if they did will they be able to trigger the immune system in right way. Although initial trials have shown promising results in human subjects but it is not clear what will happen when the person comes in contactwith actual virus.

ii) To control the dose of vaccine is the most difficult task. There seems to be danger that too high dose could provoke oral tolerance of an invading bacteria or virus instead of an immune response. Also the dosage requirements for children & adults will be different.

iii) Plants are living organism that change, so the continuity of the vaccine production might not be guaranteed.

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

v) Glycosylation patterns in plants differ from those in humans & could affect the functionality of vaccines.

So the research is on its way to find the solution of above problems.

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UNIT-IV

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Chapter 6

PHARMACOGNOSY IN VARIOUS SYSTEMS OF MEDIC

TRADITIONAL AND ALTERNATIVE SYSTEM OF MEDICINES

AYURVEDA

The word Ayurveda is composed of two components viz. 'Ayush' means life and 'Veda' means science hence Ayurveda is the 'Science Of Life'. The origin of this ancient science dates back to vedic period about 5000 years ago, Brahma, the creator, was the originator of this system who passed it on to the Ashwini kumars (Physician of God) who in turn imparted it to the Rishis from where it was promoted among the people through generation. The main objective of Ayurveda is maintenance and promotion of positive health and cure of disease through medicine, dietary restrictions and regulated life style

The basic principles of Ayurveda involves two theories, one is *Panchamahabhuta* theory and the other is the *Tridosha* theory. According to Ayurvedic philosophy all the living and non living matters are made up of five basic elements in various proportions, they are Prithvi (Earth), Jala (Water), Teja (Fire), Vayu (Air) and Aakash (Ether). Even the human body is made up of these elements known collectively as the Panchamahabhutas. According to Ayurveda again all the physiological functions of the body are governed by three biological units viz. Vata, Pitta and Kafa each of which in turn is made up of the Mahabhutas. Physiologically these three doshas are responsible for various specific functions.

VATA (Air), transmits sense impression to the mind and responses to various places of the body, maintains the integrity of body and proper functioning of its various constituent elements. The sensory organs of touch and sound depends upon vata. It stimulates agni and produces joy.

PITA (Bile), is responsible for all digestive and metabolic activities.

KAFA (Phlegm), provides the static energy (strength) for holding body tissues together. It also provides lubricants at various point of friction.

When these doshas are in normal state of functioning it is health and when they lose their equilibrium and get vitiated by various internal and external factors they produce various types of diseases (Vyaadhi) in human body. Hence Ayurvedic treatment of any disease is aimed at restoring the equilibrium of the doshas. Ayurveda is mainly classified into eight branches which specialize in different fields of medicine viz. Kaya chikitsa (Internal medicine), Shalya Tantra (Surgery), Shalakya Tantra (Otorhinolaryngology), Kaumarbhrtya (Paediatrics), Rasayana (Rejuvenating therapy), Vajikarana (Aphrodisiac therapy), A-gada Tantra (Toxicology) and Bhut-Vidya (Psychiatry). Of these Rasayana and Vajikarana deals with preservation and promotion of health and vigour. The remaining branches deals with disease. (The detailed study of Ayurveda can be done from the chapter Ayurvedic Dosage Forms).

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CHINESE SYSTEM OF MEDICINE

Traditional Chinese system of medicine was developed from the ideas recorded between 200 B.C and A.D 100 from the *Yellow Emperor's Classic of Internal medicine (Huang Di Nei Jung)*. This text is based on detailed observations of nature and a deep understanding of the way that all life is subjected to natural laws. In traditional Chinese medicine living in harmony with these principles is the key to good health and longevity.

Traditional Chinese medicine has two quite different systems – the *Yin* and *Yang* theory and the five elements similar to Indian tradition. They developed quite separately in China and the five elements system was only accepted and fully incorporated into Chinese medicine during the Song dynasty (A.D 960-1279). According to traditional Chinese system of medicine everything in universe is composed of *Yin* and *Yang*–words that were first used to denote the dark and light side of valley. Everything has *Yin* and *Yang* aspects or complementary opposites–such as day and night, up and down, wet and dry. Every *Yin* or *Yang* category can itself also be subdivided–so that while the front of body is *Yin* relative to the back which is *Yang*, the abdomen is *Yin* relative to the chest which is *Yang*. The five elements theory associates constituents of the natural world-wood, fire, earth, metal and water. Each element gives rise to the next in a perpetual fashion. The five elements have a central role in Chinese herbal medicine in the grouping of taste of herbs and parts of the body.

In traditional Chinese medicine causes of illness depends upon the patterns of disharmony which are expression of imbalance between *Yin* and *Yang*. The health results depends upon a deficiency or excess of either *Yin* or *Yang*. For example cold is not just the result of virus but a sign that the body is not adapting to external factors such as wind-heat, wind-cold or heat. Similarly a high temperature indicates too much *Yang* and shivering is the result of an excess of *Yin*. Therefore according to this theory a harmony is to be restored between *Yin* and *Yang* both within the patient's body and between the patient and the world at large.

Influence of Traditional Chinese medicine in Japan and Korea — Japan and Korea have been strongly influenced by ideas of traditional Chinese medicine practices. Kampoh, traditional Japanese medicine traces its origin back to the 5th century A.D when Buddish monks from Korea introduced their healing arts largely derived from Chinese medicine into Japan. Direct Chinese influence on Japanese medicine which was practiced for the most parts by monks continued for 1000 years. The concepts of Kampoh is currently taught at Toyama University in Honsu. Korean herbal medicine is very similar to Chinese medicine and almost all the Chinese herbs are used in Korea.

Even today traditional Chinese medicine is the valid medical system in China and available to the Chinese on an equal footing with conventional western medicine.

UNANI SYSTEM OF MEDICINE

Unani system of medicine is also known as Islamic medicine, Loniah medicine, Oriental medicine and Arab medicine. This system was originated in Greece and has been influenced by African, Persian and Egyptian medicine. It was introduced in India by the Arabs around 10th century A.D with the spread of Islamic civilization. Now unanipathy has become a part of Indian system of medicine and India is one of the leading countries so far as its practice is concerned. It is very much similar to Ayurveda. Hippocrates and Aristotle made a valuable contribution for this system.

Unani system of medicine is based on two theories namely the Hippocratic theory of four humours and the Phythagorian theory of four proximate qualities. The four humours or fluids which exists within the body are Dam (blood), Balgham (phlegm), Safra (yellow bile or choler) and Sauda (black bile or melancholy). Each humour has its own temperament - blood is hot and moist, phlegm is cold and moist, yellow bile is hot and dry and black bile is cold and dry. The ideal person bears all four in equal proportions. However in most of the people one or more humours predominate giving rise to a particular character. For instance excess choler produces choleric -type person who is likely to be short tempered, sallow, ambitious and vengeful. The four proximate qualities are the states of living human body like hot, cold, dry and moist. They are represented as earth, water, air and fire. According to Unani if the four main humours and four proximate qualities are in state of mutual equilibrium, one is considered healthy. This system was influenced by Arabian physicians. They laid down seven working principles (Umur-e-Tabia) and included elements like organs, spirits, temperaments, life, energy, action and humours. According to them these seven principles are responsible for health and disease.

Unani system of medicine treats the cause of disease rather than its symptoms. The thorough history of patient is noted and he is subjected for pulse, stool and urine examination. This system observes the influence of surroundings and ecological conditions such as air, food, drinks, body movement and repose, psychic movement and repose, sleep and wake fulness and excretion and retention on the sate of health. This influence causes a dominance of one of the four humours in every human body. Unani believes that it is the dominance which gives a man his individual habit and complexion i.e his temperament. In this system the diseases are teated as follows-

(i) Hajbil Tadbeer (Regimental therapy)- It includes venesection, diaphoresis , diuresis, turkish bath, massage, cauterisation, purging, emesis and exercise.

(ii) Hajbil Ghiza (Dietotherapy)- It deals to treat certain ailments by administration of specific diets or by regulating the quantity and quality of food.

(iii) Hajbil Dava (Pharmacotherapy)- It deals with the use of naturally occuring drugs mostly herbal drugs.

Some drugs of animal and mineral origin are also used. Single drugs or their combination in raw form are preferred over compound formulations.

The traditional healer who practices the Unani system is called as *Hakim*. *Hakims* not only cures bodily disease but also acts as an ethical instructor. Unanipathy has shown remarkable results in curing diseases like Arthritis, Leucoderma, Jaundice, Bronchial asthma, Filariasis and several other acute and chronic disease where other systems do not give the desired level of positive response. The Unani system is a secular system in character and is popular among the masses.

SIDDHA SYSTEM OF MEDICINE

Siddha system of medicine is one of the oldest system of medicines in India. It owes its origin to the Dravidian culture which is of pre- vedic period. The Siddha system of medicine is prevalent in the Southern parts of India, Srilanka, Malaysia and Singapore where Dravidian civilization flourished. According to tradition the origin of Siddha system of medicine is attributed to the great Siddha **Agasthya**. The Tamils who are inhabiting the

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Southern Peninsula of the sub-continent of India have an impressive and venerable past. They undertook a systematic study of nature and its elements and from what they were able to grasp, they had developed a highly systematized medicine which is now known as Siddha system. It is well founded on basic principles of nature and its elements offer a careful and thorough study of human system. The term Siddha means achievements and Siddhars (Tamil word) is derived from its root 'chit' means perfection in life or heavenly bliss. It generally refers to eight kinds of supernatural powers attainable to man. The person who had achieved such miraculous powers in life were known as Siddhars. In the annals of the ancient Siddha system of medicine, the first medicinal plant mentioned as well as found a place in ancient Tamil literature is Margosa or Neem. This has been used by Tamils from time immemorial as a deterrent for Small pox and other infectious disease.

The principles and doctrine of this system have a close similarity to Ayurveda. Like ayurveda, this system believes that all objects in the universe including human body are composed of five basic elements namely earth, water, fire , air and ether (sky). The food which the human body takes and drugs it uses are all made of these five elements. As in Ayurveda, this system also considers the human body as a conglomeration of three humours, seven basic tissues and the waste products of the body such as faeces, urine and sweat. The three humours are *Vatham*, *Pitham* and *Karpam*.

Vatham- It's characteristics are lightness, dryness, coldness and motility. It is formed by sky and air and controls the nervous action that constitute movement, activity, sensation etc. It predominates in first one third of life.

Pitham- It is formed by fire and controls the metabolic activity of the body, digestion, warmth, lusture, intellect etc. It predominates in the second one third of life.

Karpam- It's characteristics are firmness, smoothness, heaviness and viscidity. It is formed by earth and water and controls the stability of the body such as strength, potency and smooth working of joints. It predominates in the last one third of life.

The seven basic tissues (called as dhatus) are *Rasa* (lymph), *Kurvdhi* (blood), *Tasai* (muscle), *Kozhuppu* (adipose tissue), *Elumbu* (bone), *Majjai* (marrow) and *Sukkilam and Artavam* (male and female hormones). The food is considered to be basic building material of human body which gets processed into humours, body tissues and waste products. The equilibrium of humours is considered as a health and disturbance or imbalance leads to disease.

The Siddha system has developed rich and unique treasure of drug knowledge in which use of metals and minerals is very much advocated. The drug classification is briefly discussed below-

There are 25 varities of water soluble inorganic compounds called as UPPU. These are different types of alkalies and salts.

There are 64 varities of mineral drugs that do not dissolve in water but emit vapours when put in fire. Thirty two of these are natural and remaining are artificial.

There are seven drugs that do not dissolve in water but emit vapour on heating.

The system has classified separately classes of metals and alloys which melt when heated and solidifies on cooling. These include items like gold, silver, copper, tin, lead and iron. These are incinerated by special processes and used in medicine

There is a group of drug that exhibit sublimation on heating and includes mercury and

its different forms like mercury metal, red sulphide of mercury, mercury chloride, mercury subchloride and red oxide of mercury.

Sulphur, which is insoluble in water finds a crucial place in Siddha materia medica along with mercury for use in therapeutics and in maintenance of health.

In addition to these there are drugs obtained from animal sources.

The diagnosis of disease involves identifying it's causes. Identification of causative factors is through the examination of pulse, urine, eyes, study of voice, colour of body, tongue and the status of the digestive system. The system has worked out detail procedures of urine examination which includes study of its colour, smell , density, quantity and oil drop spreading pattern. It is holistic in approach and the diagnosis involves the study of person as a whole as well as his disease. The Siddha system is capable of treating all types of disease other than emergency cases. Practitioners have claimed that Siddha medicines are effective in reducing the highly debilitating problems that manifest themselves among patients of AIDS. More research into the efficacy of these medicines is presently in progress.

HOMOEOPATHY

Homoeopathic system of medicine was developed by the German physician and chemist Samuel Hahnemann (1755-1843) in eighteenth century. He proposed that the cause of disease may also be its remedy and above all it does not produce any harmful effects. The word Homoeopathy is derived from Greek words *homoios* meaning like and *pathos* meaning treatment. Hahnemann forwarded the laws of similars i.e. like can be cured by like (similae similibus curentur). This is the fundamental principle of Homoeopathy and with this concept he began to experiment on himself and he started with cinchona. He observed, infact, that cinchona produced a fever similar to that of malaria although it was well known that the drug was used to combat the disease. With the help of colleagues and friends he succeeded in getting relevant results from the wide range of plant, animal and mineral extracts and he published all these results in the text of homoeopathy called as 'The Organon of Medicine'.

In homoeopathy the drug treatment depends upon the symptoms as described by the patient. This is based on the concept of Proving and Prover. The healthy person is called as Prover who takes the different dose of drug extract and the symptoms produced are noted which is called as Proving. The Prover maintains a precise and accurate record of physical, mental and emotional changes produced due to drug extract. In this way the same drug extract is induced to the patient and symptoms are recorded. Consequently the symptoms of Prover and patient are compared.

The drugs used in homoeopathy are extracted in the form of mother tincture which is further diluted in terms of *centesimal* or *decimal* potencies. If one drop of mother tincture is added to 99 drops of inert solvent such as alcohol or water then it is denoted by the symbol of 1c. If one drop from the 1c is added to further 99 drops of solvent then it is denoted as 2c. Similarly, typically potencies of 6c, 12c, 30c, 200c and 1000c can be prepared. Alternatively *decimal* potencies in the dilution series of 1 in 10 are prepared by adding 1 part of mother tincture to 9 parts of diluent. These are denoted by the symbol D2, D30 etc.However in homoeopathic system of medicine each dilution is claimed to increase the healing power of drug.

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AROMATHERAPY

Aromatherapy is regarded as specialized branch of phytotherapy, concerns the use of essential oils for their healing properties. It is an ancient healing art which was used by our ancestors. Egyptians used the essential oils for embalming and from the evidence of paintings it is clear that they were also seen as vital offerings to the gods. In vedic literature; Rigveda in India dating before 2000B.C list of about 700 substances are mentioned such as camphor, sandal wood, cinnamom, myrrh etc. Similar literature is available in African and Asian countries.

The term Aromatherapy was coined in 1928 by Rene-maurice Gattefosse, a French chemist working in his family's perfumery business. He became fascinated with the therapeutic possibilities of the oils after discovering by accident that Lavender oil was able to heal and prevent scarring of his severe burn. Valnet developed the ideas of Gattefosse and he used these techniques in the treatment and he published his work in the book entitled 'Aromatherapie'. However the extension of these techniques to a wider concept of well being is credited to Maury, through her book 'The Secret of Life and Youth'.

Aromatherapy provides treatment through the stimulation of the sense of smell using pungent materials. The various types of essential oils are extracted from plant sources and topically applied both in local and whole body massage. This stimulates the healing process of the body by increased blood flow in the skin and at the same time the pungent aromas stimulate the 'limbic' system or emotional centre of brain. In addition to massage, aromatherapy can also be effected by using essential oils in aromatic baths and through inhalation. Aromatherapy is used to treat the skin problems, rheumatism, acne, poor circulation of blood and nervine disorders like stress, insomnia, headache etc. It is also used to heal the wounds. The different types of essential oils used are Lavender, Sandal wood, Fennel, Rosemary, Ginger, Jasmine, Clove, Citronella and Calamus oils etc.

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Chapter 7

INTRODUCTION TO SECONDARY METABOLITES

ALKALOIDS

The term "Alkaloid" was proposed by W. Meissner in 1819. The term is derived from the word 'alkali like', so they have some character similar to naturally occurring complex amines. It is difficult to define alkaloids precisely because there is diversity in chemical and physiological activity. So various studies and researches were done on the alkaloids and now a days the alkaloids are defined as **"Alkaloids are the organic products of plant origin, basic in nature and contain one or more nitrogen atoms normally of heterocyclic in nature, and have marked physiological action when administered internally."**

Alkaloids are present in plants- both in free form and salts of organic acid such as quinic, maleic muconic, oxalic acid etc. They are present in different parts of the plant like leaves, fruits, barks, seeds, roots & rhizomes and stems etc. and can be easily extracted. Alkaloids are poisonous in nature but when used in small quantities exert useful physiological action.

As far as nomenclature of alkaloids is concerned there is a lack of any agreed systematic prevailing system. Hence by general agreement the chemical rules suggests that the name of alkaloids must end with the suffix (- ine). For example belladonine and atropine from *Atropa belladonna*, morphine and narcotine from *Papaver somniferum* and ergotamine form *Claviceps purpurea* etc.

GENERAL PROPERTIES OF THE ALKALOIDS

The properties of alkaloids are discussed under two headings –

Physical properties – Almost all the alkaloids are colourless, crystalline solids and posses a sharp melting point. Some alkaloids like nicotine and coniine are liquid and volatile in nature. Some alkaloids are coloured like berberine is yellow and bentanidin is red.

The solubility of various alkaloids and their salts exhibit considerable variation. The free alkaloidal bases are fairly soluble in organic solvents, non polar solvent, and lower alcohols but they are either practically insoluble or very sparingly soluble in water. The alkaloidal salts are freely soluble in water, relatively less soluble in alcohol and very sparingly soluble in organic solvents. For example atropine sulphate and morphine hydrochloride are freely soluble in water than their corresponding bases ie atropine and morphine. The differences of solubilities of alkaloids is utilized for extraction, isolation, purification and assay of alkaloids.

Chemical properties – The normal elements present in the alkaloids are carbon, hydrogen and oxygen but every alkaloid should essentially contain at least one nitrogen atom. The nitrogen present in the alkaloid imparts basic properties. The nitrogen in the alkaloids may be primary amine (RNH_2) e.g. mescaline, as secondary amine (R_2NH) e.g ephedrine, as tertiary amine (R_3N) e.g. morphine and quaternary ammonium compounds (R_4N^+X) e.g tubocurarine chloride. Quaternary ammonium compounds are not alkaloids in the true sense

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en atom does not posses a n... n atom and their chemical properties are but as a matter of convenie... hey are legitimately grouped along with degree of basicity of alkaloids mostly depends upon the influence caused ...tatic status of the nitrogen atom present in alkaloids. There are certain alkaloids due ... n oxygen atom. These type of alkaloids are found in solid state but there are which few exceptions where oxygenated alkaloids usually occur as non- volatile liquids for e.g. pilocarpine.

Many alkaloids are optically active. Amongst dextra and levo isomers, the levo isomers are pharmacologically more active.

Precipitation by specific reagents (Chemical tests of Alkaloids) – Most of the alkaloids are precipitated with specific reagent. They show characteristic coloured precipitate with specific reagents as mentioned below –

Mayer's reagent (Potassium – Mercuric iodide solution) gives cream colored precipitate.

Wagner's reagent (Potassium Tri iodide solution) gives reddish brown precipitate.

Dragendorff's reagent(Potassium bismuth iodide solution) gives reddish brown or orange red precipitate.

Hager's reagent (Saturated solution of Picric acid) gives yellow colored precipitate.

An utmost care must be taken while performing the above chemical test with alkaloids because proteins, coumarins and a-pyrones also yield precipitate with the above mentioned reagents. Hence the test with heavy metals in some cases may be false. So the specific test of individual alkaloid should be performed which are mentioned under individual drug.

CLASSIFICATION OF ALKALOIDS

There are various methods of classification of alkaloids which are discussed below: –

Biosynthetic Classification – In this classification the importance is given to the precursor from which the alkaloids are produced in plant biosynthetically. So all the alkaloids which are derived from the same precursor can be brought under same group even they have different taxonomic distribution and pharmacological activity. For e.g. piperidine alkaloids derived from *lysine*, pyrrolidine alkaloids derived from *ornithine* and indole alkaloids derived from *tryptophan*.

Pharmacological classification – The alkaloids exhibit a wide range of pharmacological actions. In this classification alkaloids are classified on the basis of their pharmacological action for e.g. analgesic,. CNS stimulant or depressant and anti malarials etc. Hence individual alkaloid may exhibit different action within the same drug for e.g. in cinchona, quinine is an anti malarial where as quinidine is a cardiac depressant, in opium morphine is a narcotic analgesic where as codeine is antitussive. However this classification is not commonly used.

Taxonomic classification – This classification deals with the 'Taxon' i.e taxonomic category. Common taxa are like genus, subgenus, species and subspecies etc. In this classification the large number of alkaloids are classified on their distribution in various plant families like rubiaceous alkaloids and solanaceous alkaloids.

Some phytochemists have stepped further and classified alkaloids based on chemotaxonomic classification.

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Chemical classification – This is the most widely accepted classification of alkaloids. The basis of the classification is the ring structure (normally heterocyclic ring) present in the alkaloids. The alkaloids are divided into two categories viz.:-

Non heterocyclic or Prota alkaloids

Heterocyclic or Typical alkaloids

Heterocyclic alkaloids – There are large number of alkaloids which posses heterocyclic ring structure as mentioned below :-

S.No.	Type	Basic ring structure	Examples
1.	Pyrrolidine	N—H	Hygrine, Stachydrine
2.	Pyridine	N	Ricinine, Arecoline
3.	Piperidine	NH	Lobeline, Connine
4.	Tropane [Piperidine-Pyrrolidine (N-Methyl)]	N—CH_3	Atropine, cocaine
5.	Quinoline	N	Quinine, Quinidine, Cinchonidine, Cinchonine
6.	Isoquinoline	N	Papaverine, Morphine, Emetine, Berberine
7.	Aporphine (reduced isoquinoline/ naphthalene)	CH_3 N	Boldine

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S.No.	Type	Basic ring structure	Examples
8.	Norlupinane	N	Sparteine, Lupanine, Cytisine
9.	Indole or Benzopyrrole	N H	Ergotamine, Ergometrine, Reserpine, Brucine, Vinblastine, Vincristine
10.	Imidazole	N N H	Pilocarpine, Pilosine
11.	Purine (Pyrimidine/ Imidazole)	N N N N H	Caffeine, Theophylline, Theobromine
12.	Steroidal (Cyclopentan operhydrophe nanthrene)		Connesine, Solanidine, Veratramine, Funtumine
13.	Diterpene	$C_{20}H_{32}$	Aconine, Aconitine, Lyctonine
14.	Pyrrolizidine	N	Senneciphylline, Sennecionine
15.	Amino alkaloids	CHOH CHCH3 NHCH3	Ephedrine, Pseudo-ephedrine

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GENERAL METHODS OF EXTRACTION AND ISOLATION OF ALKALOIDS

The extraction of alkaloids depends upon the following factors:-

the basic nature of alkaloids

the ability of formation of alkaloidal salts with acids

the solubility of the alkaloid either in aqueous medium or in polar organic solvents like alcohol, chloroform, acetone etc.

The extraction of alkaloids is done by following methods. However any one of the following can be used.

Method 1 :- The drug is powdered with the help of grinders. It is moistened with water and treated with lime. Then the drug is extracted with organic solvent like petroleum spirit or ether. Filter it and collect the filtrate. To the filtrate add water and separate the organic layer. The organic layer is shaken with aqueous acid and allowed to separate. Reject the organic layer. The aqueous layer obtained contains the alkaloidal salts.

Method 2 :- The drug is powdered with the help of grinders. It is moistened with water and treated with acid. Then the drug is extracted with alcohol or water. Filter it and to the filtrate add acetone. Reject the organic layer which contain several impurities. The aqueous layer so obtained contains alkaloids. It is treated with ammonia or sodium bicarbonate and organic solvent is added. Separate the layers. Reject the aqueous layer. The organic layer obtained contains the alkaloids.

From the above methods we will get the crude mixture of alkaloids. So the separation and purification of individual alkaloids can be done by following methods -

Fractional crystallization – It is a easy method but it does not give better results in complex mixture.

Steam distillation – This method is used for volatile liquid alkaloids such as nicotine and coniine.

Chromatographic techniques – This is the latest and widely accepted method employed for the separation of individual alkaloids from complex mixtures. The various chromatography techniques used are like thin layer chromatography, high performance thin layer chromatography (HPTLC) high performance liquid chromatography (HPLC), column chromatography, gas chromatography and ion exchange chromatography etc.

FUNCTIONS OF ALKALOIDS IN PLANTS

Alkaloids play a vital role in the plants. There different types of functions are listed below –

They may have a vital role in growth regulatory factors.

The alkaloids are poisonous in nature thus they protect the plants from grazing animals or insects.

They act as a reserve substances in plant and supply nitrogen or other elements.

They might be the by-products of various detoxification reaction in plants and by this way they cease the formation of harmful substances in plants.

They are present in association with plant acids like quinic acid, cinchotannic acid etc. Hence they may provide the means of storing or transportation of such acids.

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[illegible]sides are the complex [illegible]nic substances widely distributed in plant kingdom. [illegible]e substances are prese[illegible] in combination with sugar moieties. Glycosides exhibit a wi[illegible]trum of pharmacological actions for e.g. like analgesic, purgative, cardiotonic, demulcent, anti- rheumatic etc. In plants glycosides occur in different parts like leaf, fruit, root, rhizome, bark, seeds, wood etc.

Glycosides can be defined as **"The organic compounds mainly of plant origin and rarely of animal which on enzymatic or acidic hydrolysis yields one or more sugar moieties (Glycone) and a non sugar moiety (Aglycone or Genin)."** Glycosides are considered to be sugar ethers or acetals, and they are formed by condensation of hydroxyl group of non sugar and hemiacetal hydroxyl group of sugar. The sugar (glycone) present in glycosides are monosaccharides like glucose and rhamnose or more rarely deoxysugars such as cymarose found in cardiac glycosides. The aglycone part may be alcohol, phenol or amines. The linkage between glycone and aglycone is known as glycosidic linkage and on this basis of linkage a and b stereo isomers are assigned. Practically all natural glycosides, however are of b-type. The simplest glycosides are a- methyl glycosides and b-methyl glycosides which can be synthesized from union of methyl alcohol and glucose.

α -Methyl glycoside

Bβ. -Methyl glycoside

PROPERTIES OF GLYCOSIDES

Glycosides are colourless compounds but some of them are coloured like flavonoids are yellow and anthracene glycosides are red .

They are crystalline or amorphous solid compound.

Glycosides are optically active and normally levo form is more active.

Glycosides are soluble in water and alcohol but insoluble in chloroform and ether.

Glycosides can be hydrolyzed by mineral acids, water and enzymes.

ISOLATION – (STAS-OTTO METHOD)

The glycosides are extracted by using Stas-Otto Method. The drug is powdered by grinders. The powdered drug is extracted with alcohol by continous hot percolation method.

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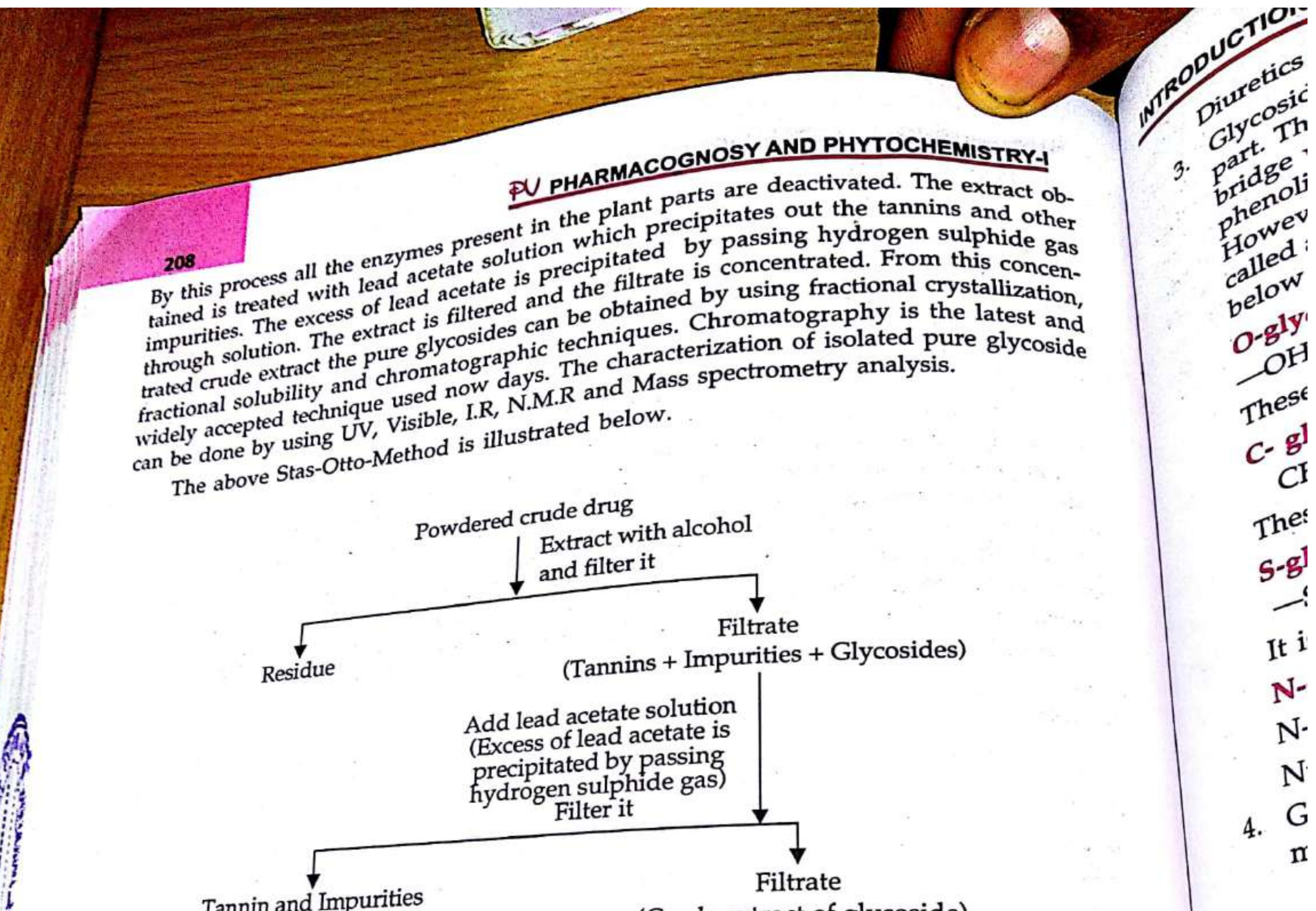

By this process all the enzymes present in the plant parts are deactivated. The extract obtained is treated with lead acetate solution which precipitates out the tannins and other impurities. The excess of lead acetate is precipitated by passing hydrogen sulphide gas through solution. The extract is filtered and the filtrate is concentrated. From this concentrated crude extract the pure glycosides can be obtained by using fractional crystallization, fractional solubility and chromatographic techniques. Chromatography is the latest and widely accepted technique used now days. The characterization of isolated pure glycoside can be done by using UV, Visible, I.R, N.M.R and Mass spectrometry analysis.

The above Stas-Otto-Method is illustrated below.

Identification test – These are no simple identification test for glycosides. Depending upon the nature of glycone and aglycone moiety specific chemical test of the drugs are performed which are mentioned in individual drugs.

CLASSIFICATION OF GLYCOSIDES

The glycosides are classified in the following four ways-

(1) On the basis of the type of the sugar or the glycone part for e.g. glucosides with glucose, fructoside with fructose and pentosides with pentose etc.

(2) Glycosides are classified on the basis of the pharmacological action exhibited by them. For e.g.

Purgative glycosides – Aloe, Senna

Cardiac glycosides- Digitalis, Thevetia

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3. Glycosides are also classified on the basis of linkage between glycone and aglycone part. The point of linkage [illegible] between glycone and aglycone portion is an oxygen [illegible] phenolic group present in the aglycone [illegible] alcoholic or [illegible]. However, if 'O' is replaced by 'C' [illegible]. Such glycosides are called as O-glycosides [illegible] called as S-glycosides and if replaced by 'N' it is called as C-glycosides, if replaced by 'S' it is called as N-glycosides as mentioned below :-

O-glycosides – In these glycosides sugar is connected to OH or phenol group.

$—OH + HO—C_6H_{11}O_5 \rightarrow —O—C_6H_{11}O_5 + H_2O$

These glycosides are found in higher plants like rhubarb and senna etc.

C-glycosides – In this type of glycosides sugar is connected to carbon atom.

$CH + HO—C_6H_{11}O_5 \rightarrow —C—C_6H_{11}O_5 + H_2O$

These are present in aloe and cascara.

S-glycosides – In these glycosides Sulphur of SH group is attached to the sugar.

$—SH + OH—C_6H_{11}O_5 \rightarrow S—C_6H_{11}O_5 + H_2O$

It is found only in isothiocynate glycosides like sinigrin from black mustard.

N-glycosides – In these glycosides N of NH (amino group) is attached to the sugar.

$N\text{-}H + HO—C_6H_{11}O_5 \rightarrow N—C_6H_{11}O_5 + H_2O$

Nucleosides is the example of N-glycosides.

4. Glycosides are also classified on the basis of the chemical nature of the aglycone moiety. This is the most widely accepted classification. They are grouped as –

   1. Anthracene or Anthraquinone glycosides
   2. Saponin glycosides
   3. Cardiac glycosides
   4. Cyanogenetic or Cyanophoric glycosides
   5. Isothiocynate glycosides
   6. Coumarin and Furanocoumarin glycosides
   7. Aldehyde glycosides
   8. Steroidal glyco-alkaloids
   9. Phenol glycosides
   10. Flavonoid glycosides
   11. Bitter glycosides and Miscellaneous glycosides

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TANNINS

Tannins are widely distributed in plant kingdom. As the name indicates they posses the property to 'tan' i.e. to convert hide and skin into leather. So tannin is a substance which is detected qualitatively by tannins test i.e. the Goldbeater's skin test. These are secondary metabolites and are present in cell sap and vacuoles. They act as astringent as they have the capacity to combine with proteins and precipitate them. Tannins can be defined as **"the complex, organic, non-nitrogenous, polyphenolic substances of higher molecular weight."** They are used as antiseptics and in gastro-intestinal diseases like diarrhoea. They are also used in leather industries.

PROPERTIES

Tannins are soluble in water, alcohol, dil alkalies, glycerine and acetone but are insoluble in organic solvent such as benzene, ether and chloroform.

They should posses tanning properties.

Tannins with ferric salts give blue, black, violet or green colour.

Tannins give precipitate with alkaloids and heavy metals therefore they are used as antidotes in alkaloidal and heavy metal poisoning.

In aqueous solution tannins produce acidic reaction and have astringent taste.

Classification – Tannins are classified in two classes on the basis of chemical nature as follows –

1. Hydrolysable tannins
2. Condensed tannins.

1. Hydrolysable tannins – These tannins are hydrolyzed by acids or enzymes and produce gallic acid or ellagic acid. Chemically they are esters of sugar usually glucose with one or more trihydroxybenzene carboxylic acid. With ferric chloride they produce blue colour, hence they are used in manufacture of ink. When these tannins are heated, pyrogallol is produced. The examples of hydrolysable tannins are gallotannin from rhubarb, chestnut, nutgall and clove and ellagitannin from myrobalans and oak.

COOH, HO, OH, OH
Gallic acid

OH, OH, OH
Pyrogallal

CO, O, OH, HO, O, CO, OH, OH
Ellagic Acid

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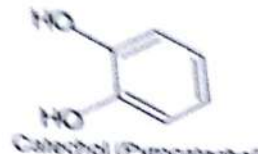
Condensed [illegible] – These tannins are also called as phlobatannins or [illegible] related to flavonoid pigments and have the polymeric formula [illegible]. On treatment with acids or enzymes these tannins are decomposed into red insoluble compounds called as phlobaphenes. With ferric chloride they produce green colour. On heating these tannins produce catechol, therefore they are also called as catechol tannins. The drugs which contain condensed tannins are cinchona bark, cinnamon bark, pale and black catechu, cocoa, kola seeds etc.



Catechol (Pyrocatechol)

Pseudotannins – Pseudotannins are low molecular weight compounds and do not respond to Goldbeater's skin test. Examples of pseudotannins are catechins from cocoa and chlorogenic acid form nuxvomica and coffee.

Extraction and Isolation of tannins – The various types of the methods of extraction depending upon the source of tannins are employed. As the tannins are high molecular weight compounds so it becomes difficult to isolate the tannins in pure form. Thus the solvents used are the mixture of polar, non-polar and semi polar solvent like alcohol, ether, water, acetone etc.

IDENTIFICATION TESTS

1. **Goldbeater's skin test** – The Goldbeater's skin* (a membrane prepared from the intestine of ox) is soaked in hydrochloric acid. Then it is rinsed with distilled water and is added to the tannin solution (sample) for 5 minutes. It is washed with distilled water and transferred to 1% ferrous sulphate solution. A brown or black colour on the skin confirms the presence of tannins.
2. **Phenazone test** – 10ml of aqueous extract of tannins is prepared and 1g of sodium acid phosphate is added. Warm it, cool and filter it. To the filtrate 2% phenazone solution is added. All the tannins present are precipitated.
3. **Gelatin test** – To the solution of tannins add 1% gelatin solution containing 10% sodium chloride. The precipitate obtained confirms the presence of true tannins and pseudotannins.
4. **Test with ferric chloride** – To the solution of tannins add ferric chloride solution. A blue, black, violet or green precipitate or colour confirms the presence of tannins.
5. **Match-Stick test** – Dip a match stick in plant extract and dry it. Moisten it with conc. Hcl and warm near the flame. The wood of match stick turns to pink or red in colour which confirms the presence of tannins. (On heating tannins with conc. Hcl produce phloroglucinol. Further phloroglucinol reacts with the lignin of wood and produce pink colour.)

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VOLATILE OILS

Volatile oils are defined as **"the odorous and volatile constituents of plant and animal species"**. Volatile oils are also termed as 'etheral oils' because they evaporate when exposed to air at an ordinary temperature. They are also called as 'essential oils' as they are the essences or active constituents of the plant. Chemically they are derived from hydrocarbons and their oxygenated derivatives. They are composed of terpenes, monoterpenes ($C_{10}H_{16}$), sesquiterpenes ($C_{15}H_{24}$), diterpenes ($C_{20}H_{32}$), polyterpenes (C_5H_8)n and their derivatives.

Volatile oils are present in the entire plant or any part of the plant such as bark, fruit, leaf, root, rhizome, wood and seed etc. They are secreted in the schizogenous or lysigenous glands, ducts and glandular trichomes. Volatile oils are formed by hydrolysis of some glycosides and by the protoplasm directly. They are present in plants belonging to family like Umbelliferae, Rutaceae, Lauraceae, Zingiberaceae, Piperaceae and Labiatae etc. Volatile oils are widely used as spices and flavouring agent. They are used in perfumery and cosmetic industries. They are also used as carminative, antiseptic, antispasmodic and antimicrobial.

Terpeneless volatile oil – When terpenes are removed from volatile oils they are termed as terpeneless volatile oils. They posses good flavouring properties so they are used in cosmetics and perfumeries.

PROPERTIES

(i) Majority of volatile oils posses a characteristic odour which differs from one specimen to another.

(ii) Volatile oils evaporate completely at room temperature and do not leave spot on paper.

(iii) The specific gravity of volatile oils is less than 1 and are lighter than water. But there are few exceptions whose specific gravity is more than one such as oil of cinnamon, oil of garlic, oil of clove and oil of cherry laurel.

(iv) They posses high refractive indices.

(v) Volatile oils are optically active

(vi) Volatile oils are insoluble in water but soluble in alcohol, chloroform, ether, acetone and carbon disulphide etc.

(vii) On storage, due to oxidation and resinification of volatile oils they become dark in colour.

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Classification – The volatile oils and volatile oil containing drugs are classified as follows –

Class	Examples of drug
Hydrocarbon volatile oils	Turpentine, Black pepper.
Aldehyde volatile oils	Cinnamon, Cassia, Lemon grass, Lemon peel, Bitter almond, Bitter orange peel.
Alcohol volatile oils	Peppermint, Coriander, Sandalwood, Citronella oil.
Ketone volatile oils	Dill, Caraway, Cumin, Camphor, Jatamansi, Buchu, Musk, Spearmint.
Phenol volatile oils	Clove, Tulsi, Thyme, Ajowan.
Phenolic ether volatile oils	Fennel, Anise, Calamus, Nutmeg.
Oxide volatile oils	Eucalyptus, Chenopoduim, Cardamom.
Ester volatile oils	Valerian, Garlic, Lavender.

Extraction – The volatile oils are extracted by the following methods –

1. Distillation 2. Expression 3. Extraction

1. Distillation – Three different techniques of distillation are used –

(i) Water distillation – It is a common method in which water is used to extract the volatile oils from herbal drugs. It is employed for those drugs whose constituents do not degrade by boiling up to 100°C.

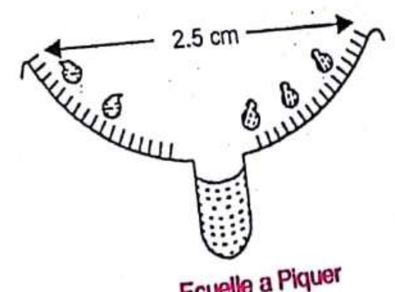
(ii) Water and Steam distillation – It is generally employed to those drugs whose constituents undergo degradation by direct boiling.

(iii) Steam distillation – It is generally used for the fresh drugs which contains moisture and do not require maceration.

2. Expression – There are various drugs in which the volatile oil present decomposes when they are subjected to distillation. Therefore the volatile oil present in the rind of fruits like lemon peel and orange peel can be obtained by method of expression (i.e. by application of pressure). The major advantage of this method is that the natural fragrance of the drug is preserved. The various expression methods used are –

(i) Sponge method – The rind of the citrus fruits such as orange, bergamot and lemon is separated and squeezed so that the secretory glands rupture. The volatile oil which oozes out is collected by the sponge and subsequently the sponge is squeezed in a vessel. Further the oil is separated.

(ii) Ecuelle a Piquer *– Ecuelle a Piquer is a bowl like apparatus and its inner layer consists of pointed metal needles which are long enough to penetrate the epidermis of the fruits. The fruits such as lemon are placed in the bowl and rotated continuously until oil glands are punctured and discharge the oil. The oil is collected and further decanted and filtered.



Ecuelle a Piquer

(iii) Mechanical method – Now days the various volatile oils are extracted by different mechanical methods which work on the above principles. No doubt the output of the oil has been increased by using these methods.

3. Extraction – This method is employed for those drugs which contain very less amount of volatile oil or the constituents of oil may decompose due to exposure to steam e.g. volatile oil obtained from jasmine flowers, narcissus flowers and gardenia flowers etc. The extraction can be done by following two methods –

(i) Extraction with volatile solvents – The drug is extracted with low boiling volatile solvent like benzene, ether, n-hexane etc either by hot continous percolation or by percolation. The advantage of this method is that the uniform temperature can be maintained during extraction which helps in preserving the natural fragrance.

(ii) Extraction with non-volatile solvents – Generally this procedure is used to prepare high quality of perfume oil. The volatile oil present in the flower petals is extracted by this method as it is not feasible to remove the volatile oil by any other method. Commonly three methods are employed:-

(A) Enfleurage method – In this method a layer of fat is applied on the glass plates which are arranged in wooden frame. The drug (fresh flower petals) is spread on the glass plate and allowed to imbibe in the fat for 24hrs after which the exhausted petals are removed and replaced by fresh flower petals. This process is carried out till the fatty material is saturated with essential oil. The saturated fatty material (known as pomade) is than extracted with alcohol to separate the volatile oil.

(B) Pneumatic method – In this, the warm air is passed through the flowers which help in loading of volatile oil particles in the air. This loaded air is passed through a fine spray of melted fat in a closed chamber wherein the volatile oils gets absorbed.

(C) Maceration – The fresh flower petals are gently heated with melted fat with continous stirring. The flowers are strained and squeezed and the fat is allowed to cool. The fat is extracted continuously three times with alcohol to separate the volatile oil.

IDENTIFICATION TEST

Volatile oils can be identified by physical tests (colour, odour, boiling point, optical rotation and refractive index) and specific chemical tests which are mentioned in individual drugs.

Storage – Volatile oils should be stored in well closed, well filled containers away from light and in cool place.

(B) RESINS AND RESIN COMBINATIONS

Resins are defined as **"the amorphous non nitrogenous products of complex chemical nature"**. Resins are the mixture of essential oil, oxygenated products of terpenes and carboxylic acids. They are the exudation products from the trunk of various trees. Resins are formed in schizogenous or schizolysigenous ducts or cavities of the plant. When the resins are produced as a normal product of metabolism without injury to the plant they are termed as normal or physiological resin like resins of pinus. If the resins are produced by injury or wound to the plant they are called as abnormal or pathological resin like benzoin and tolu balsam. Resins are present in different parts of the plant such as roots, rhizomes,

fruits, seeds, trunk, flowers and fruiting tops etc. Chemically resins contain resin acids, resin phenol, resin alcohol, esters and inert substances. They are normally used as antiseptics, carminative, purgative, expectorant and analgesic etc. Resins are also obtained from animals e.g. shellac.

PROPERTIES

(i) Resins are transparent or translucent solids, semisolid or liquid substances.

(ii) They are insoluble in water but soluble in organic solvents like alcohol, fixed oil, volatile oil and chloral hydrate solution.

(iii) They burn with smoky flame as they contain large number of carbon atoms.

(iv) On heating they soften and finally melt.

(v) Resins have specific gravity more than one and are heavier than water.

(vi) On storage, they darken in colour.

Classification – Resins are classified into two categories as mentioned below:-

1. Chemical classification – The resins are classified on the basis of chemical constituents such as-

(i) Acid resin – These contain a large portion of carboxylic acid and phenols. They combine with alkali and their metallic salts are termed as resinates. With aqueous solution of alkali they form soap-like solution or colloidal suspension. Various examples of resin acids are abietic acid (colophony), copaivic acid and oxycopaivic acid (copiba), primaric acid (fankicense) and commiphoric acid (myrrh) etc.

(ii) Resin alcohol – Resin alcohols are also called as ressinols. They have high molecular weight and occur in both i.e. free form and combined form. Ressinols are tetracyclic or pentacyclic alcohols and are normally a-amyrine and b-amyrine derivatives. They do not give positive test with iron salts. Examples are like benzoresinol from benzoin, gurjuresinol from gurjun balsam and storesinol from storax.

(iii) Resin phenol – Resin phenols are also called as resinotannols. They also have high molecular weight and occur in both i.e. free form and combined form. The phenolic group of tannins is combined with resins acid. They give positive test with iron salts. Examples are like peruresinotannol from balsam of peru, toluressinotannols from balsam of tolu and siaressinotannol from sumatra benzoin.

(iv) Ester Resins – These are the esters of resin alcohol or resinotannol combined with resin acid or balsamic acid. Examples are cinnamyl cinnamate from storax and benzyl benzoate from benzoin.

(v) Resenes – These are the neutral and inert substances as they do not contain characteristic functional group. They do not show any specific chemical properties. They do not form salts or esters and are not hydrolyzed by alkalies. They have high molecular weight. The drugs which contain resenes are asafoetida, gutta purcha and colophony.

(vi) Glycoresins – These contain the glycosidal resins. Glycoresins on hydrolysis yields sugar and complex acids, e.g. is jalap resin from jalap.

2. Constituents of Resins – Resins are also classified on the basis of major constituents present either in resin or resin combination. The homogenous combination of resins with other plant products is called as resin combinations.

(i) Acid resin – This is discussed under chemical classification.

(ii) Oleo-resin – When there is a homogenous mixture of resin and volatile oil it is termed as oleo-resin like capsaicin, ginger and copaiba.

(iii) Oleo-gum-resin – These are the homogenous mixture of resin, gum and volatile oil like asafoetida, myrrh, and turmeric.

(iv) Gum resins – These are the homogenous mixture of gum and resin, e.g. gamboge.

(v) Balsams – Balsams contain benzoic acid or cinnamic acid or both. Examples are benzoin, storax and tolu balsam.

Extraction and Isolation – Resins can be extracted from plants and animals by any one method of the following:-

(i) By extraction with alcohol and then precipitating with water, e.g. ipomoea, and jalap.

(ii) As plant exudates by injury or incisions, e.g. asafoetida, myrrh etc.

(iii) By heating the plant part e.g. guaiacum.

(iv) By distillation method e.g. colophony

(v) By various treatment of the excretions obtained from animal e.g. shellac.

Identification Test – Resins can be identified by physical test and specific chemical test which are mentioned in individual drugs.

SUGGESTED READINGS

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Sim S.K. "Medicinal Plant Glycosides" University of Toronto Publications.
Harborne J.B. "Biochemistry of Phanolic Compounds" Academic Press, New ork.
Pridham J.B., "Terpenoids in Plants" Academic Press New York.

QUESTION BANK

SHORT ANSWER QUESTIONS

Q.1. Define Alkaloids. Explain their properties & functions.
Q.2. Discuss the classification of Alkaloids.
Q.3. Define Glycosides. Explain their properties.
Q.4. Discuss the isolation of Glycosides.
Q.5. Define Resins. Explain their properties.
Q.6. Discuss the classification of Resins.
Q.7. Define Volatile oils. Discuss their properties.
Q.8. Explain the various extraction procedure of volatile oils.
Q.9. Define Tannins & Explain their properties.
Q.10. Discuss the classification of Tannins.
Q.11. Explain the identification tests of Tannins.

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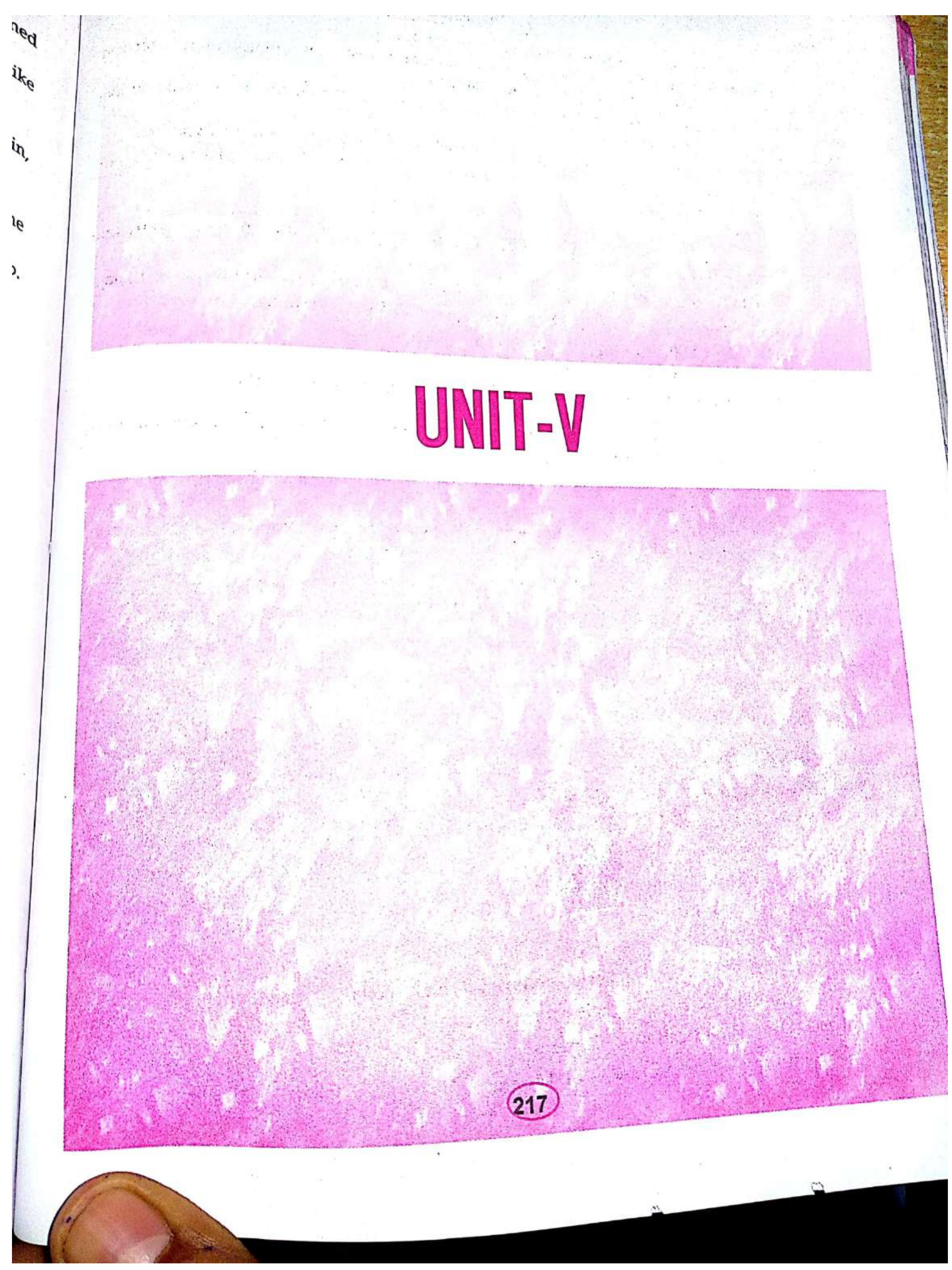

UNIT-V

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Chapter

PLANT PRODUCTS

INTRODUCTION

As well as food and medicines. Plants provide other useful products. Many plant cells form natural fibres that strengthen and support the plant. The same properties make them perfect for textiles and paper. Timber from trees is used to build boats, houses and furniture. Palm leaves are woven into baskets, hats and mats. People also extract perfumed oils and natural dyes from the flowers and leaves of certain plants.

Plants produce long groups of cells, called fibres. These can be used to make textiles, such as cotton, as well as other materials such as paper or felt. All plant fibres are strong because their cell walls contains tough molecule called cellulose, but to be useful fibres also need other properties, such as flexibility and length.

FIBRES

Natural fibre, 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 spining 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.

The fibres as refferred to in pharmacognosy, "are elongated thick walled cells with pointed ends, cell walls of which consists of cellulose and may or may not contain lignin".

The term "Fibre" as used with reference to surgical dressings includes both natural and artificial fibres.

Fibres originating from biological material are made up of long chain molecules. Similarly the synthetic fibres are made up of man-made long-chain molecules.

Fibres obtained from various sources can be categorised as follows :

(a)	Plant fibres	Jute, flax, banana, cotton, hemp
(b)	Animal fibres	Silk, wool
(c)	Regenerated and Synthetic fibres	
(1)	Fibres regenerated from carbohydrate material	Alginate yarn, artificial silk or rayon or regenerated cellulose

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(2) Fibres regenerated from Protein materials — Aridil from groundnut protein and fibrolin from milk casein.

(3) Synthetic — Nylon, terylene, orlon

(d) Mineral fibres — Glass, asbestos

Various chemical tests can be applied for the identification of fibres. The microscopical examination is the main criterion to confirm the identity of fibres.

SURGICAL FIBRES

S.No.	Name of Synonym of fibre	Biological Source	Active Constituents	Uses
1	Cotton (Absorbent cotton, Surgical cotton, Medicinal cotton)	Epidermal trichomes of seeds of Gassipium species (Malvaceae)	93 to 94% cellulose and moisture 5-7%	Surgical dressing Filerating media and Insulation
2	Jute (Gunny-bag fibres).	Phoem fibres of corchorus SP Family : Tiliaceae	Cellulose hemicellulose and lignin	Manufacture of tows and gunny bags straining filtration media
3	Flax	Pericyclic fibres of stem of Linum-Usitatissimum Family : Linaceae	Pecto-cellulose	Straining and filtering media,
4	Silk	Fibres obtained from silk worm cocoons of *Bombyx mori* Family : Bombycidae	Protein known as fibroin	Sutures, Ligatures
5	Waal	Fibres from flees of Sheep **Quisaries** Family : Bouidae	Protein known as Keratin	In the manufacture of surgical dressings like domette, crepe bandage

A PLANT FIBRES

COTTON

Synonyms : Raw cotton, cotton wool, Absorbent cotton, Surgical cotton

Biological source : Cotton consists of the epidermal trichomes or hairs of the seeds of cultivated species of the Gossiium (*Gossypium herbaceurre, Gossypium barbadense*), Other species of *Gossypium*, Belonging to family Malvaceae.

Purified cotton or absorbent cotton consists of the trichomes as mentioned above, but freed from fatty matter and adhering impurities. It is also bleached and sterilized.

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Geographical source : Cotton is produced commercially in U.S.A., Egypt and India. It is also cultivated in various parts of Africa and South America. In India, seven million hectares of land is under cultivation of cotton, of which 30% is irrigated and 70% rainfed.

Description

Colour - White (due to bleaching)

Odour- Odourless

Taste- Tasteless

Size - Cotton fibres are 2.5 to 4.5 cm in length and 25 to 35 micron in diameter

It is free from pieces of leaves, seed coat, foreign matter and dust. It may be slightly off-white in colour, if sterilized.

Standards :

Absorbent cotton wool I.P. has the following standards :

1. Length of Staples - Not less than 15mm
2. Water soluble extractive - Not more than 0.5%
3. Sulphated ash- Not more than 0.5%

Chemical constituents : Raw cotton contains about 90% of cellulose, 7 to 8% of moisture, wax, fat and remains of protoplasm. Purified cotton on absorbent cotton is entirely cellulose, with 6 to 7% of moisture.

CH₂OH O H H OH H HO H OH H -O- H OH H OH H H CH₂OH O -O- CH₂OH O H H OH H H H OH -O- H OH H OH H H, OH H CH₂OH O H

Cellulose Chain

Chemical Tests :

1. Soak cotton fibres in N/50 iodine water and dry. Add few ml. of 80% w/w H_2SO_4. Trichomes assume blue or bluish colour (distinction from jute, hemp, wool, silk, nylon, alginate yarn and acetate rayon).
2. Ammonical copper oxide solution (cuoxam reagent) dissolves raw cotton fibres with the formation of balloons, while absorbent cotton dissolves completely with uniform swelling.
3. Cotton is insoluble in 5% potassium hydroxide solution and hydrochloric acid (distinction from silk).
4. On ignition, cotton burns with a flame give 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.

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5. In cold sulphuric acid (80% w/w) cotton dissolves; distinction from oxidized cellulose, jute, hemp and wool.
6. In cold sulphuric acid (60% w/w) cotton, is insoluble/distinction from cellulose wadding and rayons.
7. 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. It is also used as an insulating material. Absorbent cotton absorbs blood, mucus, pus and prevents the wounds from injections.

JUTE

Synonym : Gunny

Biological Source : It consists of phloem fibres of the stem of various species of the Corchorus (*Corchorus olitorius* and *Corchorus capsularis Linn*). Family : Tiliaceae.

Geographical source : The plants producing jute are cultivated in West Bengal, in the basins of Ganges and in Assam. The jute plants grow successfully in areas having loamy alluvial soil with pH values of 6 to 8.

Preparation : The plants grow well in alluvial soil and requires damp and warn climate. Jute fibres are prepared in the month of july when the plants are in flowering stage. The stems are cut, leaves are removed and stem are tied into bundles. These stem bundles are submerged into a water tank or pool for ten to twenty one day and are covered with straw to protect from direct sun rays. This process is called retting. The retting process facilitates the separationn of the bark from the wood and the strands of phloemm fibres from the surrounding softer tissue. The fibres are separated from the wood by beating the ends of stems. The separated fibres are cleaned by jerking them backward and forward on the surface of water. The fibres are dried and bleached by hanging them in sun. The jute fibres are graded according to its colour, strength and length. The fibres are of white to brown and 1-4mm long.

Chemical Constituents : The fibres are yellowish brown in colour and contain cellulose (53%), hemicellulose (20%) and lignin (10%).

Chemical Test : The middle lamella is highly liquified and gives red colour with phloroglucinol and hydrochloric acid. Indicating the presence of lignin.

Uses : It is used in the manufacture of tows (Stupa), Padding splints, Filtering and Straining medium.

Jute fibres are used for the preparation of coarse bags (Gunny bags).

HEMP

Synonyms : Cannabis Indica, Indian hemp, Ganja, Charas, Marihuana.

Biological source : Cannabis consists of dried flowering and fruiting tops of the pistillate plants of *Cannabis sativa* Linn.

Family : Cannabinaceae

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Geographical source : Tropical parts of India as Maharashtra, North India, Bengal, also Africa and America

Chemical constituents :

1. Resin
   (i) Cannabidiol
   (ii) Cannabiodolic acid (Sedative and antibiotic)
   (iii) Cannabinol
   (iv) Cannabigerol
   (v) Cannabichromene and
   (vi) Tetrahydro cannabinol (THC)
2. Volatile oils
3. Trigonelline
4. Choline

Uses : Hemp is used to make a variety of commercial and industrial products including rope, clothes, food, paper, textiles, plastics, insulation and biofuel.

The bast fibers can be used to make textiles that are 100% hemp, but they are commonly blended with other organic fibers such as flax, cotton or silk, to make woven fabrics for apparel and furnishings.

Due to its high tensile strength, bast fibres are ideal for such specialized paper products as : tea bags, industrial filters, currency paper or cigarrette paper and textiles (the original Levi's jeans were made from Hemp cloth).

HALLUCINOGENS

Halluconiogens are natural and synthetic (synthesized) substances that, when ingested (taken into the body), significantly alter one's state of consciousness. Hallucinogenic compounds often cause people to see (or think they see) random colours, patterns, events and objects that do not exist. People sometimes have a different perception of time and spare, hold imaginary conversations, believe they hear music and experience smells, tastes and other sensation that are not real. The other names of hallucinogens are cartoon acid, Microdot, and magic mushrooms.

Many types of substances are classified as hallucinogens, solely because of their capacity to produce such hallucinations. These substances are sometimes called psychedelic or mind expanding drugs. They are generally illegal to use in the United States, but are sometimes sold on the street by drug dealers. A few hallucinogens have been used in medicine to treat certain disorders, but they must be given under controlled circumstances. Hallucinogens found in plants and mushrooms were used by humans for many centuries in spiritual practice worldwide. Unlike such drugs as barbiturates and amphetamines (which depress or speed up the central nervous system (CNS) respectively), hallucinogens are not physically addictive (habit forming). The real danger of hallucinogens is not their toxicity (poison level), but their unpredictability. The actual causes of such hallucinations are chemical substancces in the plants. These substances are true narcotics. Contrary to popular opinion, not all narcotics

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are dangerous and addictive. A narcotic is any substances that has a depressive effect, whether slight or great on the CNS. People have had such varied reactions to thes substances, especially to lysergic acid diethylamide (LSD) that it is virtually impossible to predict the effect of a hallucinogen that will have on any given individual. Effects depend upon the person's mood, surroundings, personality and expectation while taking the drug.

Natural hallucinogens are formed in dozens of psychoactive plants, including the peyotecactus, various species of mushrooms and the bark and seeds of several trees and plants. Marijuana and hashish- two substances derived from the hemp plant (cannabis sativa). are also considered natural hallucinogens although their potency (power) is very low when compared to others. Marijuana a green herb from the flower of the hemp plant is considered a mild hallucinogen. Hashish is marijuana in a more potent, concentrated form. Both drugs are usually smoked. Their effects include a feeling of relaxation, faster heart rate the sensation that time is passing more slowly, and a greater sense of hearing, taste, touch and smell.

MEDICAL USES OF HALLUCINOGENS

Hallucinogens have been studied for possible medical uses, including the treatment of some forms of mental illness alcoholism and addiction to the drug opium. They have also been given to dying patients. Most of these uses have been abandoned, however. A synthetic form of the active chemical in marijuana, tetrahydro cannabinol (THC) has been approved for prescription use by cancer patients, who suffer from severe nausea after receiving chemotherapy (treatment cancer with drugs). THC is also used to reduce eye pressure in treating severe cases of glaucoma. Phencyclidine (PCP) is occasionally used by veterinarians as an anaesthetic and sedative for animals.

Some of the important plant hallucinogens are as follows : Belladonna (*Atropa belladonna*) California poppy (*Eschscholzia californica*), Daturas (*Datura sp.*), Fennel (*Foeniculum vulgare*), Henbane (*Hyoscyamus niger*), Lobelia (*Lobelia inflata*), Nutmeg (*Myristica fragrans*), Tobacco (*Nicotiana tobacum*), worm wood (*Artemisia absinthium*), etc.

TERATOGENS

These agents can cause a birth defect by permanently altering the structure and/or function of organs exposed to them during development.

There was reportedly 510,000 deaths in 2010 due to congenital defects of all the birth defects, teratogens constitute to about 10% and other factors include genetic defects, poor maternal nutrition, infection and environmental toxins.

If a plant teratogenic toxin has to exert it effect, it has to be present in a high enough dose, have the ability to cross the placenta and manifest it's effect during a specific time of gestation. These toxins can even cause fetal death or gross abnormalities. Based on their mehcanisms, they can cause vascular disruption, oxidative stress and can target specific receptors and enzymatic sites and cause endocrine and central nervous system (CNS) disruption and may affect a single anatomical feature or an entire system.

Teratogens are compounds that induce cogenital defects through insult to a developing conceptus. Plant teratogens affecting livestock has not moved forward in a systematic way nor has it been an overby " crowded" field of investigation even through teratoglogy itself is a burgeoning field. Practical consideration require that attention be directed to the

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...owing. The plants responsible for the deformities must be identified as when this ... is coupled with a consideration of the general principles that relate to introduction of congenital deformities by teratogens, then progress can be made on the practical level of reducing incidence.

- **Principles Governing Introduction of Congenital Defects by Teratogens :** It is new recognized that certain plants ingested by livestocks during pregnancy are responsible for some of the common congenital defects of livestock.
- **Principle no. 1 :** Genotype determines susceptible genetic inheritance is not responsible for teratogen-induced defects, but there is nontheless considerable variation to teratogen susceptibility among genotypes.
- **Principle no. 2 :** Teratogen must reach the conceptus or produce an influence which does because virally are unbound chemicals in maternal plasma have access to the conceptus across the placenta, the important consideration is wheather they or their metabolites reach the conceptus.
- **Principle no. 3 :** Deformities induced by Teratogens are Dose Dependent. Factors that determine dose of plant teratogens to that determine dose of plant teratogens to the conceptus in livestock include the following- amount of the plant eaten, amount liberated from the ingesta, amount surviving degradation in the rumen and elsewhere in the gut, amount absorbed into the maternal circulation, amount surviving metabolism in the dam, amount passing the plaecnta and reaching the circulation of the conceptus and finally, amount reaching the site of insult at the susceptible gestational period.
- **Principle no. 4 :** A teratogen can produce death ratbar than deformities at high doses, many teratogens either will the conceptus or the dam, so in livestock, a higher incidence of abortions or resorptions may accompany or signal a problem with plant.
- **Principle no. 5 :** The conceptus must be exposed at the susceptible development period during development of a conceptus or the dam, so in liverstock gastrointestinal period but particularly during first the rod, for a teratogen to induce a specific deformity, it must exert its influence at exactly the right moment in gestation.
- **Principle no. 6 :** Teratogens exert their effects by specific mecbanismms, structurally dissimilar teratogens may influence the same mechanism and give rise to similar deformities as weu.

Luupinus species could produce the disease, in fact severity of deformities was directly related to the concentration of anagyrine present in the preparation fed, with about 30mg/kg producing a severe effect.

Conium, *Conium maculatum*, both conine and coniceien, two piperidine alkaloids of the plant are the teratogens responsible for the condition, Livestock classes varry in the susceptibility, to both the toxic & teratogenic effects of coniins.

Conc. of the teratogens in the plant is highly variable, thus there is little hope to lower dose by selective grazzingg during a low hazard periods, such as can be done with lupin.

Known teratogenicc plants with unidentified teratogens;

Astragalus, some of the Astragalus plants knwon to cause classical locoismm for example *Astragalus lentigeneus* and *Astragalus pubentisimus*, also induce deformities and abortions in offspring from dams that ingested these plants during gestation.

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Nicotiana plants, an interesting teratogenic effect occurs in offspring from sows allowed to graze waste stauss of *Nicotiana taleacum* during gestation.

SUSPECTED TERATOGENIC PLANTS

Datura, Alipald et al (1973) spaculated that an outbreak of arthorogryposis in new born pigs in kansas was due to maternal ingestion of the plant datura stromonium during the second & third month of pregnancy, further more after the plant was eradicated no cases turned up the following year.

Cyanogenic glycoside containing plants two otherwise unrelated plants *Sorghum sudanence* and *Prunus sarotine* which are believed to cause livestock deformities. If the plants prove teratogenic by seeding trials, perhaps the cyanide could be responsible for the deformities in view of the lenew teratogenic propensity of hypoxia & the ability of cyanide to induce the hypoxic state and reported, teratogenicity of amygladin, cyanogenic glycoside in hamsters.

Factors that influence teratogenecity include :

The nature of the teratogenic agent the dosage and the rouge of delivery into the embryo fetus duration & frequency of exposure.

1. **Lupimus :** Food & health related uses.
2. **Senecio :** Contains biocides in the form of alkaloids.
3. **Veratrum :** Used in cancer treatment but contains cyclopamine.
4. **Vinca rosea :** Contains vinblastine & vincristine used for chemotherapy.
5. **Sorghum :** Used as food, biofuel.
6. **Indigofera spicata :** Used as an analgesic & anti-inflammatory drug.
7. **Astragalus :** Used in herbal medicine in traditional chinese and persian medicine.
8. **Colchieum autumnase :** Used as medicine & cancer treatment.
9. **Datura Stromonium,** used for asthma treatment due to presence of atropine.
10. **Asparagus racemosus,** methanolic extracts can cause gross malformations in fetus, can increase the rate of re-absorption in the fetus and may also intrauterine growth.

NATURAL ALLERGENS

Allergens are inciting agents of allergy i.e. the substances capable of sensitizing the body in such way that an unusual response occurs in hypersensitive person. It may be biologic, chemical or synthetic origin.

Common to speak about the substances such as pollens, danders, dust etc. as natural allergens, although the chemical identity of allergen is unknown, but most common and known allergens are protein or glycoprotein and do not have much difference from other immmunogens except perhaps being somewhat smaller in size as well (mol. wt. 10,000-70,000).

- **Allergy :** The allergy (hypersensitivity) may be defined as specific immunologic reaction to an immunogen- a normally harmless substance (allergen), it was first defined in

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1906 by Von Pirquet who described as changed or altered reaction in the body of an individual in resposne to a substance or condition that is harmless to others.

Sneezing is always considered to be a symptom of a cold but sometimes it is an allergic reaction to something in the air.

Following are predispensing factors which make the person hypersensitive to allergens :

1. Hereditary tendency to allergic response.
2. Dysfunction of the endocrine glands.
3. Increased excitability of sympathetic and parasympathetic nervous system.
4. Absorption of metabolic and catabolic substances.
5. Hepatic dysfunction.
6. Psychic influences.

Types of allergens : On the bais of symptoms, allergens are classified as-

1. Inhalant allergens
2. Ingestant allergens
3. Injectant allergens
4. Contactant allergens
5. Infectant allergens

1. **Inhalant allergens :** These are airborne substances as chemicals, causing disease inflammation in the nose and lungs. Inflammation in the nose is manifested by sneezing, lacrimation, itching and swelling of nose and eyes. This symptom is known by sinusitis or hay fever.

Symptoms :

1. Sneezing often accompanied by a runny or elogged nose.
2. Coughing and postnasal drip.
3. Itching eyes, nose, throat
4. Allergic shiner
5. Watering eyes, conjunctivitis.

The allergens that cause airborne allerrgies include pollens, dust, mites, mould spores and animal allergy (epidermis or dander).

2. **Pollens allergens :** Pollen are tiny, egg-shaped round, angular, square, rectangular or otherwise shaped male cells (organ) of flowering plants. These are necessary for plant fertilization. The average pollen particle size is less than the width of an average human hair.

Pollens are classified into two types :

- **Anemophilous (wind pollinated) :** Anemophilous pollens are usually small 14-45mm in diameter, light, nonadhesive and relatively smooth and are produced by plain looking plants; ex : oak, walnut, grasses : timothy, bermuda.

Most of common allergic reactions are produced by wind pollinated (anemophilous) pollens because of their light weight and the dry nature; these pollen grains are carried for long distances.

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- **Entomophilous :** These are usually larger in size (upto 200mm in diameter), heavier, adhesive and may be somewhat spiny, plants are scented with coloured flowers such as clover, rose; etc.

3. **Injectant allergens :** These are caused symptoms similar to these of antibiotics, ex : penicillin, cephalosporin and semisynthetic penicilin, etc. Itching of palms of the hands and the soles of the feet, erythema and peeling of skin. In severe cases Anaphylactic shock may caused. The natural sources of injectable allergens are produced by stingg of bees, hornets, wasps, the allergens injected by the stiengs of such insects, sometime it is caused death. In addition to penicillin products other injectable that may cause allergies are liver extracts, anti-toxins and the glandular products.

4. **Contactant Allergens :** A number of plants and their products have been identified as the causes of contact allergies, the plant most responsible for contact dermitis in North America belongs to the Ancardiaceae family, includes poison ivy, oak and sumac. The allergen component of these plants called urushiols (a phenolic compound) are found in the oleoresin fraction and are derivatives of penta-decylcatechol or heptadecylcatechol.

5. **Infectant allergens :** Allergy caused by the metabolic product of living micro-organism in the human body, such as the continual presence of certain types of bacteria, protozoa, moulds, helminthes, based on chronic infections, for which patients are not aware. The continuous presence of growth products and metabolic waste of parasitic organism such as hookworms, tape worms, pinworms, thread worms and dermatophytes.

6. **Ingestant allergens :** Allergens which are present in food stuff and swallowed are termed ingestants (food allergy). A food allergy is an immune system response to a food. The G.I. symptoms are mainly affected by the food allergens, but they also caused skin rash, puffed lips; tongue, rhinitis.

Most common food allergens are injected are milk, eggs, tree nut, walnut, cashew nut; etc.

PRIMARY METABOLITES

A primary metabolic is a kind of metabolic that is directly involved in normal growth, development, and reproduction. It usually performs a physilogical function in the organism (i.e. an intrinsic function).

A primary metabolite is typically present in many organism or cells.

These are biomolecules required for basic metabolic processes.

These are produced in generous quantities and can easily be extracted from the plant.

These are found throughout the plant kingdom.

These are part of the basic molecular structure of the cell.

They are highly useful to plant.

They are found from the start of plant life.

Some common examples of primary metabolites include **lactic acid** and certain **amino acids.**

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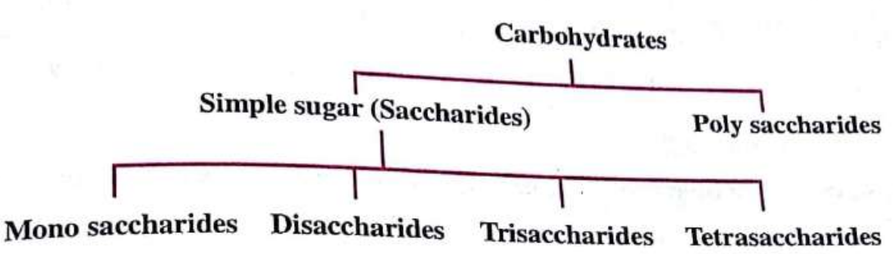
CARBOHYDRATES

Carbohydrates were defined as a group of compounds composed of carbon, hydrogen and oxygen in which the later two elements are in the same proportion as in water and were expressed by a formula (H_2O) i.e. hydrates of carbon.

The carbohydrates are defined as polyhydroxy aldehydes or polyhydroxy ketones or compounds than on hydrolysis produce either of the above.

They are substances of universal occurrence and are much abundant in plants, rather than in animals.



Carbohydrates are grouped into two major classes : Simple sugars (Saccharides) and Polysaccharides. Low molecular weight carbohydrates are crystalline, soulble in water and sweet in taste e.g. glucose, fructose and sucrose. The high molecular weight carbohydrates (polymers) are amorphoic tasteless, and relatively less soluble in water e.g. starch, cellulose, gums, pectins, inulin, etc.

Depending upon the chemical structures, saccharides are subdivided as monosaccharides, disaccharides and trisaccharides.

(A) MONOSACCHARIDES

Monosaccharides are sugars, which cannot be further hydrolysesd to simple sugars. However, they are classified according to the number of carbon atoms in sugar molecules.

1. **Bioses :** They contain two carbon atoms. They do not occur free in nature.
2. **Trioses :** They contain three carbon atoms, but in the form of phospheric esters e.g. glyceraldehyde.
3. **Tetroses ($C_4H_8O_4$) :** They contain four carbon atoms e.g. erythrose and threose.
4. **Pentoses ($C_5H_{10}O_5$) :** They are very common in plants and are the products of hydrolysis of polysaccharides like hemicellulose, mucilage and gums e.g. arabbinose, ribose and xylose.
5. **Hexoses :** They are the monosaccharides containing six carbon atoms and are abundantly suitable carbohydrates of plant kingdom. They are further divided into two types aldoses and ketoses. They are obtained by the hydrolysis of polysaccharides like starch, inulin etc.
6. **Heptoses :** They contain 7 carbon atoms, vitally important in the photosynthesis of plant and glucose metabolism of animals and are rarely found accumulated in plants e.g. glucopeptose and mannoheptose.

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(B) DISACCHARIDES

Carbohydrates, which upon hydrolysis yield two molecules of monosacccharides are called as disaccharides.

Sucrose (Cane-sugar) $\xrightarrow{\text{Hydrolysis}}$ Glucose + Fructose

(C) TRISACCHARIDES

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

Raffinose $\xrightarrow{\text{Hydrolysis}}$ Glucose + Fructose + Galactose (in beet and manna)

Gentianose $\xrightarrow{\text{Hydrolysis}}$ Glucose + Glucose + Fructose (Gentian roots)

(D) TETRASACCHARIDES

Stachyose or manneotetrose is the example of tetrasaccharide. Its products of hydrolysis are as under :

Stachyose (Manneotetrose) $\xrightarrow{\text{Hydrolysis}}$ Glucose + Fructose + Galactose + Galactose

Plant's containing tetrasaccharides are Stachys Japonic and manna Fraxinus-urnus.

(E) POLYSACCHARIDES

On hydrolysis, they give and indefinite number of monosaccharides. By condensation, with the eliminatioon of water, polysaccharides are produced from monosaccharides.

CHEMICAL TEST FOR CARBOHYDRATES

1. **Molisch's Test :** The test is positive with soluble, as well as, insoluble carbohydrates. It consists of treating the compounds with a-hapthol and conc. sulphuric acid which gives purple colour.
2. **Fehling test :** Take Fehling solution (A and B) in test tube add sample solution and boil. Formation of 'A' precipitate of brownish red cupourous oxide. Presence of reducing sugar.
3. **Benedict test :** Take sample solution and add Benedict reagent, mix well, boil and mixture vigoursley for 2 minutes to produce red, yellow or green colour precipitate, presence of reducing sugar.
4. **Iodine test :** Sample solution and Iodine solution to produce blue colour, presence of polysaccharide.
5. **Barfoed's test :** Sample solution and add Barfoed's reagent (copper acetate) 13.3gm and glacial acetic acid 1.8ml), boil for 3 minutes and cool. Red colour produce presence of monosaccharides.
6. **Seliwan off's test :** Sample solution and add Saliwan off's reagent (resorcinol 50 miligram in conc. HCl 33ml, 33%) boil for 2 minutes. Red colour is produced, presence of fructose.

PHARMACEUTICAL AIDS

For the production of drugs various techniques such as purification, filtratioon, adsorption, solubilization, absorption, suspensioon, emulsification etc. are employed. A number of natural products are used in these techniques. Flavouring, colouring, coating and permuming agents are used in drug industries. These agents possess little or no therapeutic value, but they are used in the preparation of many pharmaceutical products. These agents are called as pharmaceutical aids which may be of plant, animal, mineral or synthetic origin.

In Pharmaceutical industry **Starch** and **Guar gum** are used as a disintegrating agent. Sodium alginate acts as Stabilizing, thickening, emulsifying, defloculating, gelling and filming agent. Glucose and sucrose are sweetning and coating products. Agar is used as emulsifying agent and for culture media. Acacia and Tragacanth are employed as binding, suspending and emulsifying agents. Mucilages like Ispaghol and Linseed act as demulcent and soothing agents. Quillaia contains saponins and is used in coal tar emulsion. Most of the volatile oils are flavouring products. Fixed oils like olive, seasame, cotton seed, almond and castar oils act as emollients and vehicles for drugs.

Technical Products :

In perfumery the natural substances lavender, sandalwood, Citronella, Balsam of peru, Balsam of Tolu and Storax are used as technical products.

In food industry Acacia, Agar, Alginates, Starches and Sterculia gum are used in conjection and bakery products. Citrus fruits and ginger are employed in soft drinks. The vegetable oils used as food are coconut, seasame, cottonseed, peanut and mustard.

In Tobacco industry Glycyrrhija and Vanilla are used in Cigarettes, Cigars, Snuffs and other products.

PROTEINS AND ENZYMES DRUGS

A. PROTEINS AND PROTEIN DRUGS

Proteins are complex nitrogenous organic substances of plant and animal origin. Proteins are essential nutrients for the human body. They are one of the building blocks of body tissue and can also serve as a fuel source.

Proteins are polymer chains made of amino acids linked together by peptide bonds. During human digestion, proteins are broken down in stomach to smaller polypeptide chains via hydrochloric acid and protease actions.

They are easily extractable from plant sources and are generally stored in the form of aleurone grains in plants.

In animals they are present as structural material in the form of collagen (connective tissue), Keratin (hair, wool, nail, feathers and horns), elastin (epithelial connective tissue), casein (milk) and plasma proteins.

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Casein, gelatin, heparin and haemoglobins are pharmaceutically important proteins of animal origin.

Proteins contain carbon, hydrogen, oxygen, nitrogen and rarely sulphur. The ultimate products of complete hydrolysis of proteins, either by chemical reagents or enzymes, are amino acids.

Proteins are broady classified as under :

I. Simple Proteins : They yield only amino acids on hydrolysis.

1. **Albumins** are soluble in water and are coagulated by heat. Examples are egg albumin and lactalbumin.
2. **Globulins** are insoluble in water but are soluble in dilute salt solution. They are coagulated by heat. Examples are ovoglobulin, myosin, arachin, amandin and serum globulin.
3. **Glutelins** are soluble in dilute acids and alkalies, and insoluble in neutral solvents. Examples are glutenin of wheat and oryzenin of rice.
4. **Prolamines** are soluble in 70-80% alcohol, and insoluble in water, dilute salt solution, or absolute alcohol.

   Examples : Zein of corn, gliadin of wheat.
5. **Scleroproteins** are insoluble in water or salt solution, but are soluble in strong acids or alkalis. Examples are Kertains of hair, horns and hoofs, elastin of connective tissues, collagen of bones.
6. **Histones** are soluble in water and insoluble in dilute ammonia. They are readily soluble in dilute acids and alkalies.

   Example : Globin and gadus histone of codfish sperm.

II. Conjugated Proteins : They are composed of a simple protein combined with a non-protein group known as the prosthetic group.

1. **Chromoproteins** are proteins united with coloured prosthetic groups such as haemoglobin or chlorophyll.
2. **Lipoproteins** are the combination of proteins with lipids such as lecithin of fatty acids. They are found in blood, milk, egg yolk, and the chloroplasts.
3. **Metalloproteins** are proteins which contain heavy metals such as Fe, Co, Mn, Zn, Cu, Mg, etc.
4. **Mucoproteins** are proteins and mucopolysaccharide. They are found in serum, human urine and albumin.
5. **Nucleoproteins** as the name indicate or proteins and nucleic acids. The tobacco mosaic virus is best known as nucleoproteins.
6. **Phosphoproteins** contain phosphoric acid. They are available in casein and egg yolk.

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GELATIN

Synonyms : Gelatin, Gel foam.

Biological Source : Gelatin is a protein extracted by partial hydrolysis of animal collagenous tissue like skins, tendons, ligaments and bones with boiling water.

Description : This protein product is available in the form of flakes, sheets, shreds or a coarse or fine powder. It has a characteristic odour and faintly yellow to amber colour.

PREPARATION OF GELATIN

For the manufacture of gelatin, the bones are to be defatted and decalcified with organic solvent and mineral acid respectively. The material obtained by this treatment is treated with water at 85°C in successive quantities, due to which collagen dissolves into gelatin. It is further bleached and concentrated under reduced pressure to specific gelatin content and allowed to set in shallow trays. Such moulded gelatin is dried in drying room to eliminate moisture.

Chemical Constituents

As a protein, chemically, it contains different amino acids out of which major is lysine, an essential amino acid, but does not contain tryptophan. Gelatin is composed by glutin proteins.

Standards

Ash ≯ 3.2%

Gel strength : 150-250

L.O.D. ≯ 15%

PH (1.0% Solution) : 3.6 to 7.6

Microbial limits : 19 should comply for absence of E-coli and 109 for Salmonella. Total bacterial count less than 1000/g.

Identification

1. It evolves ammonia when heated with soda lime.
2. It is precipitated by trinit rophenol and solution of tannic acid, but not with alum, lead acetate or acids which indicates that it does not contain chondrin.
3. It gives a white precipitate with mercuric nitrate and on warming turns to brick red colour.

Uses :

1. Gelatin is mainly used in manufacture of hard and flexible capsule shells.
2. Used for preparing pessaries, pastes, pastiles and suppositories.
3. Gelatin in the form of absorbable gelatin sponge is used as haemostatic. Sometimes, it is also recommended for treatment of brittle finger nails and non-mycotic defects of the nails.

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4. Gelatin is employed for micro encapsulation of drugs, perfumes, flavours and some industrial materials.
5. Gelatin is also used in preparation of bacteriological cuulture media, absorbable gelatin sponge and gelatin film.

CASEIN

Biological Source : Casein is a principal phospho protein in milk and constitutes 3.0% milk. It comprises about 80% total protein content of milk.

There are two types of casein in the market.

- **Acid casein :** Warm skimmed milk is acidified with dilute acid, the whey is separated, curd is washed several times, dried and pulverised.
- **Rennet casein :** Skimmed milk is treated with an enzyme, rennet extract, product is separated, and purified.

Description : It is white, slightly yellow, tasteless, odourless amorphous solid, hygroscopic, stable when dry but deteriorates rapidly when damp.

It is insoluble in water, soluble in dil. alkalies, concentrated acids, but precipitates from dil. acid solutions.

Chemistry of casein : Casein is a phosphoprotein containing about 0.85% phosphorus and 0.75% sulphur.

It contains about 15 amino acids also rich in essential amino acids. Molecular weight 75000-3,70,000. Isoelectric point- 4.7, Nitrogen content- 15-16%..

Standards :

Loss on drying- Not more than 6.0%

Sulphated ash- Not more than 1.5%

Specific gravity- 1.25 - 1.31

Uses :

Dietary supplement source of protein in pre and post operative care. As a base in the standardisation of proteolytic enzymes and as emulsifying agent.

Industrially, it is used in sizing of textile and paper, as an adhesive, in preparation of casein plastic and casein paints.

(B) ENZYMES

Enzymes are the proteins which act as biological catalysts. They play a vital role in the function of cells and activities of an organism.

The enzymes show maximum activitty between 35^0 to 40^0C. They are practically inactive at 0^0C and beyond 65^0C get denatured. Although, they are soluble in water and dilute alcohol, concentrated alcohol precipitates them.

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The properties of enzymes which make them exceptional catalysts are as under :-

1. They catalyse only a specific range of reactions and in many cases only one reaction is catalyzed by a given enzyme.
2. As a group, they are exceptionally versatile catalysts. They effectively catalyse hydrolytic reactions, dehydrations, oxidation reduction reaction, acyl-transfer reactions.
3. They are exceedingly efficient under optimal conditions. Most of the enzymatic reactions proceeds 8 to 10 times more rapidly than the corresponding non-enzymatic reactions.

The enzymes are classified into following categories.

1. **Hydrolases** for catalysis of hydrolytic reactions.
2. **Transferases** for the transfer of chemical group from one molecule to another.
3. **Oxido-reductases** catalyse the oxidation-reduction reactions.
4. **Lyses** catalyse the addition of groups to double bonds or vice versa.
5. **Isomerases** are responsible for intra molecular rearrangements.
6. **Synthetases** catalyse the condensation of two molecules coupled with the cleavage of pyrophosphate bond of ATP or similar triphosphate.

Further, on the basis of site of action, enzymes can be grouped as under :

a) **Endoenzymes :** Those which act only inside the cell are known as endoenzymes or intracellular enzymes. These involve in the synthesis of cell components, food reserves and bioenergetic i.e. liberation of energy from food stuffs.

   Examples : Syntheases, Isomerases, Phosphorylases

b) **Exoenzymes:** The enzymes which are secreted outside the cell are known as exoenzymes or extracellular enzymes. These are normally digestive in their function.

PAPAIN

Biological Source : It is a mixture of proteolytic enzyme derived from the latex of unripe fruit of tropical melon tree, *Carica papaya,* belonging to family Caricaceae.

Method of Preparation :

For processing of papain, the latex of these fruits is collected in aluminium trays. To the collected latex, potassium metabisulphite (5gm/kg of latex) is added. The extraneous matter is cleared out by passing through sieves and latex is dried in vacuum shelf drier at 55-60°C. It is also processed by Spray-drying method. This dried latex is called papain.

Description :

Papain is available as light brown or white coloured amorphous powder with typical odour and taste. It shows maximum proteolytic activity between pH 5 to 6. It is soluble in water and glycerine.

Chemical Nature :

The different proteolytic enzymes present in papain are the mixture of papain and chymopapain, proteolytic enzymes act on polypeptides and amides.

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Identification :

1. It decolourises aqueous potassium permanganate solutions.
2. It causes curdling of milk.

Uses :

It is used in clarification of beverages and as a meat tenderises. It is employed in cheese manufacture as a substitute of renin. It is also used for degumming of silk fabrics in textile industry and in leather industry for dehairing of skins and hides.

Medicinally, it is used as an anti-inflammatory agent. It has shown relieving symptoms of episiotomy.

BROMELAIN

Biological Sources : Bromelain is a mixture of proteolytic enzymmes from the stem and ripen fruits of pineapple plant *Ananas comosus*, belonging to family Bromeliaceae.

Activity : It is a protein digesting and milk clotting enzyme.

Chemical Constituent : Peptidase, anain, cosmosain etc. fruits is rich in soluble mono and disaccharides, inorganic acids and vitamins.

Description : It is available as odourless to slightly putrid buff coloured powder with irritating taste.

Extraction of Bromelain from fruit

1. Fruits were cut into small pieces, weighed, macerated and juice was obtained.
2. Juice was pressed and filtered through cheese cloth.
3. pH of the juice was adjusted to 6.
4. Ammonium sulphate was added until saturation to precipitate the enzyme.
5. Partial purification was done by redissolving crude enzyme in NaCN and repeatedly precipitating it, firstly with 0.6% ammonium sulphate and then with acetone.
6. The precipitate is thoroughly washed with acetone and ether and dried in vacuum oven at low temperature.

Solubility :

It has slight soluble in water. It is insoluble in organic solvents like ether, chloroform, alcohol etc.

Uses :

It is used in treatment of soft tissue inflammation and oedema due to surgery and injury.

SERRATIOPEPTIDASE

Biological Source : It is a proteolytic enzyme derived from the bacteria belonging to genus Serratia, present in the gut of silk worm.

Serratiopeptidase is considered as very effective bacterial enzyme and it is found to have better effects than trypsin and chymotrypsin, with negligible toxicity and side effects.

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Given orally, it enters systemic circulati[illegible] [illegible]anged form and can penetrate into all [tis]sues especially inflammed areas.

It exerts histamin and bradykinin hydrolysing and proteolytic effects. Hence, it reduces [ca]pillary permeability and also breaks down proteins and exudates and hence support wound healing. Unlike chymotrypsin, Serratiopeptidase, being a bacterial enzyme, shows rarely the allergic reactions.

Preparation :

Serratiopeptidase (Serratia E-15 protease, also known as Serralysin, Serrapeptase, Serratiapeptase, Serratia peptidase, Serratio peptidase or Serrapeptidase is a proteolytic enzyme (Protease produced by enterobacterium Serratia SP E-15. This microorganism was originally isolated in the late 1960s from Silkworm, Bombyx mori L. (intestine), Serratiopeptidase is present in the silkworm intestine annd allows the emerging moth to dissolve is cocoon. Serratiopeptidase is produced by purification from culture of Serratia E-15 bacteria.

Therapeutic Applliccations :

(a) Resolution of inflammation

(b) Sputum liquification due to lysis of various protein in sputum and hence lowering viscosity.

(c) Enhancemment of antibiotic effects due to removal of inflammatory barrier and hence increasing antibiotic transfer to infected areas.

Chemical constituents : Serratiopeptidase is a proteolytic enzyme of protease type XXVI. The preparatio contain 7.1 units/mg solid.

Uses : Serratiopeptidase is the most widely prescribed anti-inflammatory enzyme in developed countries and also in India. It is also used as a fast wound healing agent. It is proving to be a superior alternative to the nonsteroidal anti-inflammatory drug traditionally used to treat rheumatoid arthritis and osteoarthritis.

UROKINASE

Urokinase, also known as urokinase type plasminogen activator (UPA) is a serine protease present in humans and other animals.

Urokinase was originally isolated from human urine, and it also present in the blood and the extracellular matrix of many tissues.

Description : It is a lyophilised white powder, soluble in water. It is an activator of endogenous fibrinolytic system, which converts plasminogen to plasmin and degrades fibrinogen, fibrin clots and other plasma proteins.

Preparation : A highly potent preparation of urokinase has been separated from human urine and has been successfully heat-treated for 10 hours at 60°C. It is free of thromboplastic activity and is non-pyrogenic.

Clinical Applications : Urokinase is used clinically as a thrombolytic agent in the treatment of severe or massive deep venous thrombosis, pulmonary embolism, myocardia infraction, and occluded intravenous or dialysis connulas.

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Used to dissolve fibrin or blood clots in anterior chamber of eye and in acute massive pulmonary emboli.

It is generally administered intravenously in a dose of 4400 units/kg body weight per hour for twelve hours.

Chemical Constituents : Urokinase enzymes are serine proteases that occcur 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.

STREPTOKINASE

It is an enzyme obtained from culture filterate of beta-hemolytic streptococci group C. This enzyme has the property of activating human plasminogen to plasmin.

Description : It is available as a sterile, friable solid or white powder. It is soluble in water with maximum activity at pH 7. The solution at higher concentrations is stable for 6 hours at 4°C, otherwise dilute solutions are unstable.

Uses : It is used in the treatment of thromboembolic disorders for the lysis of pulmonary emboli, arterial thrombus, deep vein thrombus and acute coronary artery thrombosis.

The activity of this enzyme is due to activation of plasminogen to a proteolytic enzyme, viz. Plasmin which degrades fibrin clots, fibrinogen and other plasma proteins.

Production of Streptokinase :

Extraction of streptokinase from streptococcus equisimilis group C, strain H46A culture is done as :

The bacteria were cultured in TSA (Tripticase Say Agar) at 37°C. One of the colonies was grown in 25ml THB (To add Hewitt Broth Media) at 37°C. By increasing the turbidity to the level of OD = 0.6 at 600nm, it was subcultured in 250ml of broth; the activity of secreted streptokinase was determined by solid and liquid calorimetric methods. It was observed that the optimum pH for cell growth and streptokinase activity was at the neutral condition (pH=7). To improve the growth condition, the pH of the culture was maintained at 7 during incubation at 37°C for 8 hours by adding sterile 4% (w/v) glucose and 5.0N NaOH. The culture was centrifuged for 25 minutes at 10,000g. Prior to addition of solid ammonia sulfate to a final concentration of 65% (w/v), the supernatant was filtered through a 0.45μm cellulose acetate filter. After standing at 4°C overnight, the precipitate was harvested by centrifugation at 4°C for 20 minutes at 12,000 gm and dissolved in 1ml of 10mm. Tris buffer, pH = 8.0, and dialyzed against repeated changes of the same buffer.

Storage : Lyophilized streptokinase although stable at room temperature for 3 weeks, should be stored desiccated below 18°C upon reconstitution streptokinase should be stored at 4°C between 2-7 days and for future use below –18°C.

Chemical Constituents : Streptokinase is a bacterial protein with half-life of 23 minutes. Its antisolylated plasminogen activator complex (APSAC) has a higher half-life of six hours.

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is a substance containing proteolytic enzyme [illegible] present in the gastric juice of [illegible]s. It is obtained from the glandular layer (mucous membranes) of fresh stomach of [illegible]s *scrofa*. Vardomesticus, belonging to family suidae.

Description : Pepsin is light buff or white coloured amorphous powder. It also occurs as translucent scales. It has a little acidic or saline taste with slightly meaty odour. It is soluble in water, but insoluble in alcohol, ether and chloroform. If pepsin is heated with alkali or pancreatic enzymes, its biological activity is lost. It shows maximum activity at pH 1.8. Pepsin has the capacity to digest 2500 times its weight of coagulated egg albumin. It is also available in other forms which may digest even upto 10,000 times their weight of coagulated egg albumin.

Preparation : For preparation, the mixed stomach linings are digested with hydrochloric acid followed by clarification, controlled evaporation, dialysis and concentration of the digested solution. When processed, solution is subjected carefully to vacuum evaporation, spongy pepsin is obtained.

Therapeutic uses : Pepsin is used for proper digestion of food when patients lack its secretion. It is mainly used for patients suffering form indigestion.

Pepsin also helps in brekaing the proteins of the food into tiny bits and absorption of nutrients.

Storage : Storage conditions of pepsin solution is stable at 2 to 8°C, at least for one week, at neutral pH under germ free conditions. Frozen aliquots of the enzyme solution are expected to be more stable.

CASTOR OPR

Synonym : Castar OPR, castor bean oil, oleum ricini, ricinus oil, oil of palma christi, cold drawnn castor oil.

Biological source : It 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, Thailand, in India it is cultivated in Gujrat, Andhra Pradesh & Karnataka.

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 slight acrid taste, castor oil is soluble in absolute alcohol in all preparations, specific gravity is 0.958-0.969, refractive index at 40°C is 1.4695-1.4730, acid value is not more than 2, acetyl value is 150.

Chemical constituents : Castor oil consists of glyceride of ricinoleie acid, isoricinoleie, stearic and dihydroxy stearic acids, Ricinoleie is responsible for its luxative property. Castor oil contains also vitamin E, 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 & a hydroxyl group on the 12th carbon, this combination of hydroxyl group and unsaturation occurs only in castor oil.

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Identification test :

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

Uses : It 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 jellyes, it is also used as an emollient in the preparation of lipstics, in tooth formulations, as ingredient in hair oil.

Marketed Products : It is one of the ingredients of the preparations known as lip balm and muscle and joint rub, (Himalaya Drug Company).

CHAULMOOGRA OIL

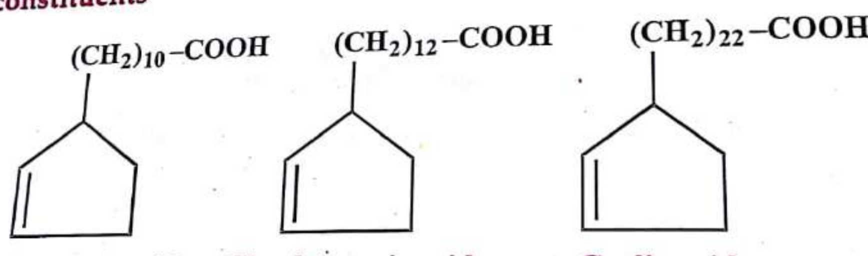
Synonym : Gynocardia oil, Hydnocarpus oil

Biological source : It is a fixed oil, obtained by cold expression from ripe seeds of *Taraktogenos kurzii* king, Hydnocarpus wightiana a Blume, *H.anthelmintica pierre*, and other species belonging to the family Flacourtiaceae.

Geogragphical source : The plants are tall trees upto 17cm high with narrow crown hanging branches, native to Burma, thiland, East India & Indo-China.

Characteristics : The oil is yellow or brownish yellow, below 25°C, it is a semi-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



Hydnocarpic acid **Chaulmoogric acid** **Gorlic acid**

This oil contains glyceries of cyclopentenyl fatty acids like hydrocarpic acid (48%), chaulmoogric acid (27%), garlic 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, are strongly bactericidal towards the micrococcus of leprosy. The seeds of *H.wightinia* contains a flavonolignan, hydrocarpin, isohydrocarpion, methoxy hyrocarpin, apigenin, lutealin, cyclopentenoid cyanohydrin glycosides as well.

Uses : This oil is useful in leprosy & many other skin diseases. The cyclopentanyl fatty acids of the oil exhibit specific toxicity for **Mycobacterium leprae** and **M.tuberculosis,** the oil has now been replaced by the ethyl esters and salts of hydnocarpic & charrlmoogric acids, at present organic sulphones have replaced this oil in therapeutic use.

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WOOL FAT

Synonyms : Lanolin, Adeps Lanae

Biological Source : Wool fat is the purified fat like substance, obtained from the wool of the sheep *Ovis aries* Linn, family : Bovidae, it contains between 25-30% of water, it is the secretion of sebaceous glands of sheep deposited onto the wool fibers as well.

Geographical source : Commercially lanolin is manufactured in Australia, the USA, and to a very less extent in India.

Method of Preparation : Raw wool contains about 31% wool fibers, suinnt or wool sweat about 32% earthy matter and about 25% of wool grease or crudelanolin. Crude lanolin is separated by washing with sulphuric acid or suitable organic solvent or soap solution. It is further purified and bleached.

Solubility : It is practically insoluble in water but soluble in chloroform and solvent ether with separation of water.

Standards : M.P : 34-44°C

Acid value : Not more than 1

Iodine value : 18-36

Saponification value : 90-105

Peroxide value : Not more than 20

Identification test : Dissolve 0.5gm hydrous wool fat in chloroform and to it add 1ml of acetic acid anhydride and few drops of H_2SO_4 acid, a deep green colour is produced, indicating presence of cholesterol.

Chemical Constituents :

CH_3CH_3 H CH_3 H H OH

Cholesterol

It is a complex mixture of esters and polyesters, of 33 high mol. wt. alcohols and 36 fatty acids.

Hydrous wool fat contains mainly esters of cholesteral and isocholesterol with carnaubic, oleic, myristic, palmitic acids.

It also contains 50% of water.

Uses : The lanolin is mainly used as water absorable ointment base.

It is a common ingredient and base for several water soluble creams and cosmetic preparations, it can be allergic also.

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BEESWAX

Synonym : Yellow wax, yellow bees wax, cera-flava.

Biological source : Beeswax is purified wax obtained from the honey comb of the bees. *Apis melifira* and other species of Apis, belonging to the family Apidae.

Geographical source : It is processed and commercially prepared in France, Italy, West Africa, Jamaica & India.

Description : Colour-yellow to yellowish brown

Odour- Agreeable & honey like

Extra features

Yellow beeswax is non-crystalline solid, it is soft to touch and crumbles under the pressure of fingers to plastic mass, under molten condition, it can be given any desired shape, it breaks with a granular fracture.

Solubility, It is insoluble in water, but soluble in hot alcohol, ether, chloroform, carbon tetrachloride & volatile oils.

Standards

M.P.	62-65°C
Specific gravity	0.958-0.967
Acid value	05-10
Sap. value	90-103
Ester value	80-95

Chemical test : Saponification cloud test : Boil 0.5gm of beeswax with 20ml of aq. caustic soda solution for 10 mins, cool it, no turbidity is produced.

Chemical constituents : It consists of esters of straight chain monohydric alcohols with straight chain acids, the cheif constituent of the beeswax is myricin i.e. myricyl palmitate, free cerotic acid, small quantities of melissic acid and aromatic substance cerolein are other constituents, India beeswax is characterized by its low acid value.

Preparation & Processing : The combs and capping of honeycomb are broken and boiled in soft water, these are then enclosed in a porous bag weighted to keep under water, the boiling causing oozing of the wax which gets collected outside the bag and forms a cake after cooling, the debris on outer surface is removed by scrapping; beeswax is purified by heating in boiling water as well.

This process is repeated several times and finally wax is skimmed off.

Uses : Used in the preparation of ointments, plasters, polishes, it is used in ointment for hardening purposes and the manufacturing of candles, moulds and in dental industries.

It is also used in cosmetics for the preparation of lipsticks, face creams, pharmaceutically it is an ingredient for paraffin ointment IP.

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- **Novel Medicinal Agents from Marine sources :** Marine pharmacognosy is a subbranch of pharmacognosy, which is mainly concerned with naturally occuring substances of medicinal value from marine source. In the western medicine agar, alginic, carrageenan, protoamine sulphate, spermaceti and cod and haei but liver oils are the established marine product as well.
- Macroalgae or seaweeds have been used as crude drugs in the treatment of iodine deficiency, stages such as goitre, etc.
- Some seaweeed are used as the rich source of vitamins as well, in the treatment of anaemia during pregnancy.
- It is also used in the treatment of various intestinal disorders as vermifugres, hypocholesterolaemic and hypoglycemic agent.

During the last 30-40 years numerous novel compounds have been isolated from marine organisms having biological activities such as antibacterial, antiviral, antitumour, antiparasitic, anticoagulants, anti-microbial, anti-inflammatory, and cardiovascular active products.

Antiviral agents : *Ara-A,*

It is a semisynthetic antiviral agent based on the arabinosyl nucleoside isolated from the marine sponge ***Tetha erypta.*** The compound shows a prominant therapeutic activity.

Educestomins, These are the β-caboline derivative which are isolated from the sponges and gorgonians ***Eudistoma olviaceum,*** family polycitoridae. These compounds are also found in tunicates. Eudistomin compound can be classified into 4 groups i.e. pyrrolyl substituted, pyrrolinyl substituted, unsubstitutde and tetrahydro β-carboline derivatives with a 1,3,7-oxathiazepine ring.

NH_2 N N N N HOH_2C O OH OH

Ara-A

OH Br N N H NH

Eudistamin A

Didemina : These are the promising antiviral and antitumour agents, isolated from the **Trididemnum** spp. family Didemnidae; a compound Didemnin is found to be a potential antiumour agents during its clinical trials.

Avarol and Avarones : These two sesquiterpene benzenoids are derived from the sponge ***Disidea avara,*** it has exhibited strong anti HIV activity against the human immunodeficiency virus (HIV). It shows the greater promises in the treatment of AIDS.

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Patenazole B : It is a complex derivative isolated from the ascidian, ***Lissoclinum patella***. It has shown the potent activity against herpes simple virus.

Fucodian : Fucodian a sulphated polysaccharide compound extracted from brown algae Laminaria has shown the activity against HIV and herpes simplex virus.

- **Antimicrobial agents :** A large variety of antimicrobial agents are produced by number of marine organisms, such as sponges, algae, gorgonian cerals, annelids; etc. many of them are active against gram (+)ve & gram (–)ve, micro-organisms, protozoal and fungal strains.
- **Antiparasitic agents :** Various compounds isolated from the marine organisms, have demonstrated remarkable antiparasitic activities, some important agents have been listed below :

(i) α-Kainic acid : α-Kainic acid isolated from the red-algae, Digenea simplex shows the broad spectrum antihelminthic activity against parasitic round worms.

[α-Kainic acid]

A japanese pharmaceutical company takes a pharmaceuticals, produced various preparations of this drug.

(ii) Demoic acid : It is a compound chemically related to kainic acid, has been isolated from red algae ***chondria armata*** and ***Alsidium corallinum,*** has shown prominent antihelmintic activity.

[Domoic acid]

(iii) Laminine : It is a methylated lysine derivative found in the marine red algae of the order laminariales as well as in brown algae, laminarine also shows the hypotensive and smooth muscle relaxant activities, along with its potential anti-parasitic activity.

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$H_3C-N^+(CH_3)_2-CH_2-CH_2-CH_2-CH_2-C(NH_2)-COO^-$

Laminarine

(iv) **Bengamide F :** Bengamide F is the recently isolated and characterized compound from marine sponge, it has demonstrated a remarkable antiparasitic activity during invitro studies.

CH3, OH, OMe, O, CH3, CH3, N, N, OH, OH, O

[Bengamide F]

- **Prostaglandins :** It constitute a class of natural products with the variety of therapeutic activities, varieties of these substances are found in marine algae, cerals and soft cerals.

O, COOH, CH3, OH

[15 epi-PGA$_2$]

O, COOH, CH3, OH, OH

[Prostalglandin E$_2$]

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Plexaura homomalla, is regarded such as rich source of these compounds as well. PGE_2 and PGF_{2a} types of prostaglandins have been isolated from the red algae ***Gracilaria lichenoids,*** PGF_2 have also been derived from ***G.verrucosa.***

Halogenated marine prostanoid names as punaglandin have been isolated from ***Teiesta risei,*** it has remarkably inhibited L_{1210} leukamia cell, proliferation demonstrating strong anti-tumour activity.

Prostanoide

- **Anticoagulants :** These are reported from the marine sources are mostly polysaccharide derivatives obtained from marine algae, carrageenans from ***Chondrus crispus*** and galactan sulphuric acid from ***Iridaea laminarioides*** have shown anti-coagulant effect through inactivation of thrombin.

Fucoidin isolated from the brown algae ***Fucus vesiculosus*** has shown a very good anti-coagulant activity, the antithrombin effect of fucoidin is mediated through heparin co-factor II.

Insecticides : Nereistoxin an insecticidal compound has been isolated from the marine annelid ***Lumbriconereis heteropoda*** many semisynthetic and synthetic analogue have been produced on the structural model of nereistoxin. One of the derivative named as cartap is used as an insecticide in Japan.

[Nereistoxin]

Cartap

Antispasmodic agents : A sesquiterpene derivative isolated from Okinawa sea sponge Agelas spp. has demonstrated very good antispasmodic activity in animal models. Agelasidine A is the first marine natural products containing guanine and sulfone units.

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O=S(=O)—CH —CH —NH—C(=NH)NH₂

Agelasidine A

Antiinflammatory agents : Marine organisms have shown the presence of novel anti-inflammatory agents a series of bio-indole derivatives, isolated from marine cyanobacterium *Rirularia firma* has shown potential anti-inflammatory activity in models of carrageenan induced rat paw oedema.

$(CH_2)_{15}-CH_3$

OH

CH_3

Butanolide derivative

[Bio-indole]

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Cardiovascular agents : Several cardiovas ceular agents have been isolated and characterized from marine organisms, their chemical nature rangingg from steroidal compounds to polypeptide of 49 amino acids residues have been isolated from the sea anemone.

Anthopleura Xanthogrammica : It is highly potent heart stimulant with about 5000 times more active than cardiac glycosides.

Fledoisin : It is a peptide compound has been isolated from posterior salivary glands of Cephalopod **Eledone moschata** and other related species.

It has shown potent hypotensive and vasodilatory activity.

It is found to be about 50 times more potent than acetyl choline, histamine or bradikinin.

Octopamine : D(–)-Octopamine a simple phenolic derivative isolated from salivary glands of **Octopus vulgaris, O.macropous** and **Eledone moschata.**

OH
H — C — CH_2–NH_2
(benzene ring)
OH

(Octopamine)

- **Anti-cancer agents :** Several compounds with anticancer and cytotoxic–C, activities have been isolated from various marine organisms, such as marine sponges, gargonian corals, sea algae, sea hores, sea cucumbres,

One of the most important agent is Cystosine arabinoside, also known as Ara-e. It originates from the natural sources, spongothymidine, isolated from caribean sponge **(Cryptoteth a crypta).** It is marketed under the trade name cytosar by upjohn pharmaceutical company for the treatment of myelogenous leukemia and human actue leukemia. Ara-e is a potenti inhibitor of tumour in the cases of sarcoma-180, Erlich carcinoma and L-1210, leukemia in mice.

The other compounds isolated from the above caribbean sponge are spongosine and spongouridine as dell.

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Ara-C

[Spongothymidine]

[Spongowidine]

Bryostatin-I Isolated from **Bugula neritina** a bryozoal marine organism showed highly potent antineoplastic activity in an extremely low dose level.

Conclusion : The greater part of the earth surface is covered by seas and ocean, which contains about 500,000 species of marine organisms, since the natural products chemists diverted their attention to exploit the vast resources of marine flora and animal world.

Although the impact of marine natural products are presently less or on the pharmaceutical industry. It may come forward in a big way to provide new lead compounds for the development of potential therapeutically active compounds.

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