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Pharmaceutical Education & Research  
Rajkot**

**B.Pharm  
Semester III**

**Subject Name: Pharmacognosy and Phytochemistry I  
Subject code:BP305TP**

### **Chapter 1. Introduction to Pharmacognosy:**

**(a) Definition, history, scope and development of Pharmacognosy**

**(b) Sources of Drugs – Plants, Animals, Marine & Tissue culture**

**Organized drugs, unorganized drugs (dried latex, dried juices, dried extracts, gums and mucilages, oleoresins and oleo- gum -resins).**

**Classification of drugs:**

**Alphabetical, morphological, taxonomical, chemical, pharmacological, chemo and sero taxonomical classification of drugs**

**Quality control of Drugs of Natural Origin:**

**Adulteration of drugs of natural origin. Evaluation by organoleptic, microscopic, physical, chemical and biological methods and properties.**

**Quantitative microscopy of crude drugs including lycopodium spore method, leaf constants, camera lucida and diagrams of microscopic objects to scale with camera lucida.**

### **Definition**

Pharmacognosy is **defined** as the scientific study of the structural, physical, chemical and biological characters of crude drugs of animal, vegetable and mineral origin and includes also their history, cultivation, collection and other particulars relating to the treatment they receive during their passage from the producer to the distributor or pharmacist. Pharmacognosy may be **defined** as a branch of bioscience which treats in detail medicinal and related products of crude drug which obtained from plant, animal and mineral origins. In brief, pharmacognosy is also **defined** as the objective study of crude drugs of animal, vegetable and mineral origin, treated scientifically.

The practice of traditional medicine is widespread in China, India, Japan, Pakistan, Sri Lanka and Thailand. The Indian traditional medicines can be classified into two groups. In the first group are the medicinal preparations belonging to the Ayurvedic, Siddha, and the Unani systems while the folk medicines belong to the second group. The word Ayurveda is composed of Ayu (life) and Veda (knowledge) means Ayurveda is science of life. Ayurveda takes a holistic view of human body his/her health and illness. Ayurveda evolved over 5000 years ago, in the far reaches of the Himalayas, presumably from the deep wisdom of spiritually enlightened prophets (Rishis-muni). It is derived from Athurveda. The Unani system of medicine is based on ancient principle and developed by Unani Tibb. Unani medicine is an ancient form of medicine first developed by the

Greeks in 460 BC. In Europe, it is known as Arab medicine. The art of practicing Chinese herbal medicine stretches back over more than 5000 years in China.

### History

The name of pharmacognosy is formed from 2 Greek words, *pharmakon* meaning drug and *gnosis* meaning to acquire the knowledge. The word 'Pharmacognosy' was coined in 1815 by **C.A. Seydler**, a medical student at Halle Germany, in his doctoral thesis. It was necessary to make distinct the material medica as taught to medical students and this was achieved from the beginning by a distinguished physician named **Jonathan Pereira**, recognised as the first British Pharmacognosist. The Greek physician **Hippocrates** (460-370 B.C.) is known as father of medicine. He dealt with anatomy and physiology. **Aristotle** (384-322 B.C.), a Greek philosopher and students of Pluto, is known for his on the animal kingdom. **Theophrates** (370- 287 B.C.) a student of Aristotle, described scientific study of plant kingdom. **Dioscorides**, a Greek physician in 78 B.C. wrote De Materia Medica in which he described several thousand plants having medicinal properties; some of them like opium, ergot, hyosyamus and cinnamon are still used today. Pliny (23-70 B.C.) compiled 37 volumes of natural history. **Galen**, a Greek physician and pharmacist (131-200 A.D.) described the method of preparation of plant and animal drugs, is known as Galenical pharmacy. **Paracelsus** (1493-1541) is to develop mineral salts which might had the potential universal curative agent. The importance of the extraction method and alcohol as an extractant was reported by **Le'mery** (1645-1715). **Swede Linnaeus** (1707-1778) the great systematist classified the plants and introduced the system of naming the plants known as the binomial system which is still followed. Gradually all the natural product prescribed by the physicians and compounded by pharmacists constituted the old '**Materia medica**' in nineteenth century, is known as pharmacognosy. Material medica may be defined as the scientific study of those substances which are used or have been used in medicine and pharmacy. In these details about the action of drugs, their preparation from them and arrangement of drugs of vegetable and animal origin based on accepted botanical and zoological classification were described.

### Development of pharmacognosy

World Health Organization (WHO) has estimated that at least 80% of the world population relies on traditional system of medicine for their primary health care. Pharmacognosy is closely related to botany and plant chemistry and, indeed, both originated from the earlier scientific studies on medicinal plants. As late as the beginning of the 20<sup>th</sup> century, the subject had developed mainly on the botanical side, being concerned with the description and identification of drugs, both in the whole state and in powder and with their history, commerce, collection, preparation and storage. Such branches of pharmacognosy are still of fundamental importance, particularly for pharmacopoeial identification and quality control purposes, but rapid developments in other areas have enormously expanded the subject.

Preparation is usually confined to one or a few companies who process all the raw material. Plant-chemistry (Phytochemistry) has undergone significant development in recent years as a distinct discipline. A pharmacognosist should possess a sound knowledge of the terms used to describe the vegetable and animal drugs as covered under botany and zoology, respectively. The knowledge of plant taxonomy, plant breeding and plant pathology and plant genetics is helpful in the development of cultivation technology for medicinal and aromatic plants. The knowledge of technology involving extraction, purification, characterization of phytopharmaceuticals is a significant development to the pharmacy. The modern developments in the instrumental techniques of analysis and chromatographical methodologies have added numerous complex and natural products to the armory of phytomedicine.

### Scope of Pharmacognosy

- 1) Pharmacognosy is an important bridge between the pharmaceutical and basic sciences.
- 2) Pharmacognosy is the simultaneous application of various scientific disciplines with the object of acquiring knowledge of drugs from every point of view.
- 3) Pharmacognosist should have a sound knowledge of the terms used to describe the vegetable and animal drugs as covered under botany and zoology respectively.
- 4) Pharmacognosy is an important link between pharmacology and medicinal chemistry as a result development of phytochemistry and pharmacological testing methods in recent years.
- 5) The crude drugs have less side effects and less toxicity as compared to the synthetic drugs.
- 6) The use of modern isolation techniques and pharmacological testing procedures means that new plant drugs usually find their way into medicine as purified substances rather than in the form of galenical preparations.
- 7) The knowledge of pharmacognosy can be used of
  1. Cultivation of medicinal plants
  2. Evaluation of new hybrids having more and good quality of active constituents by hybridization and tissue culture, collection, processing, preservation
  3. Study of sensory, physical, chemical and structural characters
  4. Standardization of drugs
  5. Extraction of active constituents
  6. Chemical analysis of phytoconstituents
  7. Uses of crude drugs.

### Sources of crude drugs

A **drug may be defined** as intended for use in diagnosis, cure, mitigation, prevention or treatment of disease in man or other animals, or intended to alter a bodily function or structure of man or other animals. There are different sources of drugs. Mainly two categories

1) Synthetic

2) Natural

Many drugs used in medicine today are developed by chemical synthesis. A recognized number of drugs are obtained from natural sources. The most important natural sources are plants, biological, mineral, marine organisms & today also tissue culture as good source of drugs.

### Plants

Plants have been used in the treatment of various diseases from time immemorial. The traditional Indian systems of medicine, Ayurveda, Siddha and Unani systems are based on the use of plants and other natural substances. There are 2, 00,000 to 2, 50,000 species of flowering plants growing on earth which belong to 10,500 genera and about 300 families. These genera are distributed among plant families like *Solanaceae*, *Compositae*, *Papaveraceae*, *Scrophulariaceae*, *Leguminosae*, *Rutaceae*, *Rubiaceae*, *Umbelliferae*, *Dioscoriaceae*, *Apocyanaceae*, *Rhamnaceae*, *Plantaginaceae*, *Liliaceae*, *Sterculiaceae* and *Gramineae*. Majority of the drugs are derived from seed bearing plants (spermatophytes). Among the spermatophytes the angiosperms (flowering plants) have yielded a good number of useful medicinal plants than the gymnosperms (nonflowering plants). The gymnosperms are useful source for oils, resins and the alkaloids such as ephedrine. Within the angiosperms both monocotyledons and dicotyledons provide many useful drugs. Tulsi & lemon grass is important drugs obtained from monocotyledons plants. Among the dicotyledons cinchona, ipecac, Rauwolfia, belladonna, vinca, vasaka, Punarnava, senna, Nux-vomica, ginseng, ashwagnadha, datura, Rasna, Brahmi, Shankhpushpi, Black catechu, cassia, Clove, Fennel, Nutmeg, Cardamom, Kanchnar, colocynth, Harde, Baheda Amla, gymnema, nagod, tylophara, Bhilama, palash, karanj and liquorice is some of the important drugs from higher plants. Drugs consisting of entire plant or some part of it are often designated as crude drugs. Generally only that part of the plant which contains the maximum amount of active constituents is collected and marketed. The simple medicines prepared from these drugs are herbal teas, extracts, tinctures etc.

### Animal sources

Certain animal parts and animal products are used as drugs in therapeutics. The major groups of animal products used in medicine are hormones, enzymes, animal extractives, organ and bile acids. These include carmine, a colouring principle obtained from cochineal insects; cod liver oil; cantharidin, an irritant constituent of cantharides insects and heparin. Wool fat and lanolin are used in certain formulations and in cosmetic industry. Hirudin, extracted from leeches is used in thrombosis and inflammation. Epibatidine from skin extracts of Equadorian has analgesic activity. Venoms and toxins from snakes, spiders, scorpions, insects and microorganisms are extremely potent and so are used as a tool to study receptors, ion channels and enzymes. Thyroid gland, parathyroid gland, pituitary gland, ox gall, pepsin, pancreas, musk etc. are the glands or glandular secretions obtained from the animal kingdom.

### Microbes

The microbes are microscopic organisms which include viruses, bacteria, fungi and rickettsiae. These microorganisms are source of many immunizing biological. Vaccines are suspensions of living, dead or attenuated (less virulent) microbes. They are used as inoculations to stimulate the production of antibodies against pathogenic microorganisms. Toxoids are also microbial products used to produce active immunity against disease. Various antibiotics are obtained from microorganisms, especially fungus. E.g. penicillin, tetracyclines, aminoglycosides, chloramphenicol and cyclosporine. Fungal metabolite such as lovastatin used as lipid lowering drug, huperzine active against Alzheimer disease and Andriamycin and cerubidine have antitumor activity.

### Mineral sources

Shilajit, prepared chalk, kieselguhr etc. are the drugs from mineral sources. Use of shilajit as a general tonic is reported in ancient Indian literature. Shilajit is produced naturally in the mountainous areas of Himalayas, Vindhya and other mountains of Indian and Nepal. Purified kieselguhr is used for the filtration of oils, fats, syrups etc. The chalk is used as an adsorbent and antacid. Other drugs of mineral origin are asbestos, bentonite, calamine, kaolin, talc, mica, fueller's earth etc. In Ayurveda, bhasma are prescribed for treating various diseases. Bhasma are unique metallic-herbal Ayurvedic high potency medicinal preparations recommended for the treatment of chronic ailments. They are herbo-mineral formulations. E.g. Loh bhasma, jasat bhasma etc.

### Marine source

Crude drugs are obtained from sea or marine organisms are known as marine drugs. Marine Pharmacognosy is a sub-branch of Pharmacognosy, which is mainly concerned with the naturally occurring substances of medicinal value from marine. The 139 million square miles of sea water that covers 71% of our earth area and contain over 200,000 invertebrates and algal species and giving us important elements, food, raw materials and some useful drugs. Marine organisms has tremendous source of new molecular entity. This has led to the isolation of substances possessing antimicrobial, antiviral, anticancer, cardioactive, anti-inflammatory, anthelmintic and anticoagulant, neurophysiological and insecticidal activities. Marine invertebrates (particularly sponges, bryozoans, tunicates and ascidians) and marine actinomycetes are the sources of novel, bioactive secondary metabolites.

#### Various drugs obtained from marine sources

<b>(1)Anti-cancer drugs</b> <ol style="list-style-type: none"><li>1. Bryostatin</li><li>2. Dolastatins</li><li>3. Ara-C</li><li>4. Ara-A</li></ol>	<b>(6)Antiviral compounds</b> <ol style="list-style-type: none"><li>1. Ara-A</li><li>2. Avarol and Avarone</li><li>1. Didemnins</li></ol>
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<b>(2)Anti-microbial drugs</b> <ol style="list-style-type: none"><li>1. Cephalosporins</li><li>2. Istamycins</li></ol>	<b>(7)Anti-inflammatory agents</b> <ol style="list-style-type: none"><li>1. Pseudopterosins</li><li>2. Marine bi-indole</li></ol>
<b>(3)Anticoagulants</b> <ol style="list-style-type: none"><li>1. Carrageenan</li><li>2. Laminarin</li></ol>	<b>(8)Antiparasitic compound or Anthelmintic</b> <ol style="list-style-type: none"><li>1. Kainic acid</li><li>2. Domoic acid</li></ol>
<b>(4)Miscellaneous agent</b> <ol style="list-style-type: none"><li>1. Protamine</li><li>2. Pralidoxime</li><li>3. Cod liver oil and shark liver oil</li></ol>	<b>(9)Pharmaceutical aids</b> <ol style="list-style-type: none"><li>1. Sodium alginate</li><li>2. Agar</li><li>3. Spermaceti</li><li>4. Chitin</li></ol>
<b>(5)Proteins</b> Lectins	

Biochemicals produced by marine organisms, are very different than those from related terrestrial organisms and thus offer great potential as new classes of medicines. In western medicine agar, alginic acid, carrageenan, protamine sulphate, spermaceti and cod and halibut liver oils are the marine medicinal established products. Therefore, marine natural products will play a major role in drug discovery in the future. When compared to land plants and animals, the use of marine organisms in traditional medicine is very low. Last 40 years, marine organisms as sources of biologically active compounds have been confined. The chapter deals with the bioactive metabolites of marine algae, bacteria and fungi.

### Biotechnology OR Plant tissue culture

Biotechnology is rapidly growing branch of science. Biotechnology means that is the interaction between biology and technology. Biotechnology is defined as “the integration of natural sciences in order to achieve the applications of organisms, cells, parts thereof and molecular analogues for products and services.” Field of biotechnology divided into eight major areas namely,

1. Recombinant DNA technology
2. Hybridoma technology
3. Enzyme and biocatalyst technology
4. Plant cell culture
5. Animal cell culture
6. Fermentation technology
7. Waste treatment and utilization
8. Process engineering

### Application of biotechnology

1. Manufacturing of several substances such as steroid hormones, human insulin, human growth hormone, somatostatin are very efficiently done by DNA recombinant technology.
2. Genetically engineered microorganism's guarantees reliable, expandable and constant supply of such as human insulin.
3. Production of vaccines, tissue plasminogen activator, urokinase by biotechnology.

### 4. Plant tissue culture

Plant tissue culture is **defined** as in vitro cultivation of environmental plant cell or tissue under aseptic and controlled environmental conditions, in liquid or on semisolid well-defined nutrient media for the production of primary and secondary metabolites or to regenerate a plant.

### Advantages of plant tissue culture

1. Availability of raw material
2. Fluctuation in supplies and quality
3. Novel methods for isolation
4. Biotransformation
5. Disease free
6. Biosynthetic pathway

With the help of plant tissue culture agronomically rapid multiplication of selected plants identical to original plants can be done. New plant obtained is different from original and more efficient according to certain defined criteria. Production of high-yielding, herbicide, drought, insect, salt resistant crops are obtained. On industrial scale production of known molecule, using biosynthetic capacities of plant cells breed in a bioreactor. It is an innovative aspect, employing the new source of variability accessible *in-vitro* to obtain new molecules. Secondary plant metabolites like alkaloids, terpenoids, flavonoids, lipids, oils, tannins, anthraquinones, flavones, naphthoquinones, vitamins, proteins, anticancer agents, antiviral agents etc. are isolated from plant tissue culture. Plant tissue culture technology has been used in almost all the field of biosciences. Its **applications** include:

1. Production of phytopharmaceuticals and secondary metabolites by
  - a. Biotransformation (Biochemical conversion)
  - b. Plant cell immobilization
  - c. Genetic transformation (Transgenic plant)
  - d. Elicitors
2. Micropropagation (Clonal propagation)
3. Synthetic seed
4. Protoplast culture and somatic hybridization
5. Hairy root culture
6. Cryopreservation
7. Tracing the biosynthetic pathways of secondary metabolites
8. Generate novel compound(s) from the plant
9. Respiration, organ function and metabolism in plant tissue culture can be studied.
10. Plant improvement by studying diseases of plant and their elimination.
11. Mutant cell selection is done by addition of toxic substance to cells followed by isolation of resistant cells.
12. Production of economical valuable chemicals/phytoconstituents which are not possible by other chemical methods.



### Crude drug

Crude drug means to the products from plant and animal origin found in a raw form. Crude drugs are further grouped as organized (cellular) or unorganized (acellular) according to whether they contain a regular organized cellular structure or not.

### Organized drug

The drugs obtained from the direct parts of the plants are called as **organized drugs (cellular)**. They are made up of whole plants or any parts derived from them. E.g. Vinca, Vasaka, Datura, Rasna, Bael. These can be used directly or can be used by modifying or by extracting the active ingredient from it.

### Unorganized drug

The drugs which are prepared from plants by some intermediate physical process such as incision, drying or extraction with a solvent are called **unorganized drugs**, e.g., Dried juice (Aloe juice), Dried extract (agar), Dried latex (Opium latex), Honey, Beeswax etc. these products may be solid, semisolid or liquid. The physical, chemical and analytical standards may be applied for testing their quality and purity.

The crude drugs may be classified according to their alphabetical status or the taxonomy of plants and animals from which they are derived or their morphology or the chemical nature of their active constituents or their pharmacological actions and therapeutic applications or chemotaxonomical status.

No.	Organized drugs	Unorganized drugs
1	The drugs obtained from the direct parts of the plants	The drugs which are prepared from plants by some intermediate physical process
2	These drugs are named as root, stem, flower, seed, fruit, leaf, bark and wood	These drugs are named as gums, mucilages, resins, juices, lattices and extracts.
3	These are solid in nature	These are solids, semisolids or liquids in nature, e.g., oils, gums and balsams
4	Microscopical characters are important criteria for identification of drug.	Microscopical characters are not important criteria for identification of drug.
5	e.g. Vinca, Vasaka, Datura, Rasna, Bael	e.g. Honey, Beeswax, aloe, opium

**Oleoresins:** Oleoresins are the homogenous mixture of resin with volatile oils. **E.g. Turpentine, Ginger, Copaiba, Canada resin**

**Gum resin:** Gum resins are the naturally occurring mixture of resins with gums. **E.g.**

**Ammioniacum, gamboges**

**Oleo gum resin:** Oleo gum resins are the naturally occurring mixtures of resin, volatile oil, gum.

**E.g. Gum myrrh, Asafetida**

### Classification of crude drugs

#### Alphabetical classification

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The crude drugs are arranged according to the alphabetical order of their Latin and English names e.g. Acacia, Benzoin, Cinchona, Dill, Ergot, Fennel, Gentian, Hyoscyamus, Ipecac, Jalap, Kurchi, Liquorice, Myrrh, Nux-vomica, Opium, Podophyllum, Quassia, Rauwolfia, senna, Turmeric, Uncaria gambier, Vasaka, Wool fat, Yellow bee-wax, Zedoary. This arrangement is employed for dictionaries, reference books, Pharmacopoeias such as Indian Pharmacopoeia, British Herbal Pharmacopoeia, British Pharmaceutical Codex, British Pharmacopoeia, United States Pharmacopoeia and National formulary, European Pharmacopoeia etc.

### Advantages

1. Suitable for quick reference
2. Location, tracing and addition of drug are easy.

### Disadvantages

1. Does not provide any information about drug like the source of drugs whether it is from plant, animal or mineral.
2. Does not indicate whether drugs are organized or unorganized.

### Taxonomical classification

The drugs are classified according to plants or animals from which they are obtained in phyla, orders, families, genera, species, subspecies etc. this method of classification is based on the consideration of natural relationship or phylogeny among plants or animals. In this system crude drugs are arranged according to the natural groups (e.g. Families) of their source. The taxonomical classification for crude drug is as follows. E.g. belladonna plant

Phylum	Spermatophyta
Division	Angiosperms
Class	Dicotyledons
Sub-class	Sympetalae
Order	Tubiflorae
Family	Solanaceae
Genus	Atropa
Species	Atropa belladonna

Family	Drugs
<b>Thallophytes</b>	
Bacteria	Vaccines produced from pathogenic bacteria
Algae	Agar, carrageenan
Fungi	Penicillin, Cephalosporin, Ergot, Lactobacillus, Gibberellins
Lichens	Oak mass, Manna, Lichen dyes
<b>Pteridophytes</b>	Male Fern, Equisetum, Lycopodium
Gymnosperms	Ginkgo, Ephedra, Colophony, Turpentine, Taxol
<b>Angiosperms</b>	
<b>Monocotyledons</b>	Only few important families of crude drugs
Liliaceae	Squill, Aloe, Colchicum
Graminae	Maize, starch, Lemon grass, Vetiver
Zingiberaceae	Ginger, Turmeric, Cardamom

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Dioscoreaceae	Dioscorea
<b>Dicotyledons</b>	Important families of crude drugs
Leguminosae	Liquorice, Senna, Acacia, Psoralea, Black catechu
Rutaceae	Bael, Jaborandi, lemon, Orange oil
Myrtaceae	Eucalyptus, Clove
Apocynaceae	Vinca, Rauwolfia
Solanaceae	Solanaceous drugs. eg. Tropane alkaloids
Umbelliferae	Umbelliferous drugs. eg., volatile oils

### Merits

1. This system helps in classification in terms of botanical characters.
2. Precise and ordered arrangement

### Demerits

1. Fails to recognize the organized and unorganized nature of the drug.
2. Fails to take in to account chemical nature of active constituent and therapeutic significant of crude drugs.
3. Many drugs are not entire plants but plant parts or exudates that have been processed systemically.

### Morphological classification

In this system, the drugs are grouped according to the part of the plants, such as roots, leaves, stems, barks, flowers, seeds etc.

No.	Morphological part	Examples
1	Barks	Cinchona, Kurchi, Cinnamon, Quillaia
2	Woods	Quassia, Sandalwood
3	Leaves	Senna, Digitalis, Vasaka, Datura, Vinca, Eucalyptus, Nagod
4	Flowers	Clove, Saffron, Pyrethrum, Dhatakipushpa,
5	Fruits	Coriander, colocynth, Fennel, Bael, Dill, Caraway
6	Seeds	Nux-vomica, Strophanthus, Isabgol, Castor, Mustard
7	Roots	Rauwolfia, Ipecac, Aconite, Jalap, Liquorice, valerian, Withania
8	Rhizome	Rhubarb, Gentian, Ginger, Turmeric, Dioscorea, Podophyllum
9	Dried lattices	Opium, papain
10	Resins	Myrrh, Benzoin, Asafoetida, Balsam of Tolu
11	Dried juices	Aloe, Kino
12	Gums	Acacia, Tragacanth, Guar gum
13	Dried extract	Gelatin, Catechu, Agar, Curare

### Merit

1. This system of classification is more convenient for practical study especially when the chemical nature of the drug is not clearly understood.
2. Practical application to the study of plant drugs

### Demerit

1. There is no co-relation of chemical constituents with the therapeutic actions.

2. Microscopical studies are needed to identify powdered herbs

### Chemical classification

The biological activity of a drug is due to the presence of certain chemical constituents in the crude drug. Plants and animals synthesize chemical compounds such as carbohydrates, protein, fat, volatile oils, alkaloids, resin etc. The chemical classification of drugs is dependent upon the grouping of drugs with identical chemical constituents. The crude drugs belonging to different morphological or taxonomical categories may be brought together, provided there is some similarity in the chemical nature of active principles. It would appear that chemical classification of crude drugs is the preferred method of study.

Chemical constituents	Drugs
<b>1. Carbohydrates</b> a) Monosaccharide b) Disaccharide c) Polysaccharide Gum Mucilages Cellulose	- Dextrose, fructose, galactose - Sucrose, Lactose, Maltose - Starch - Acacia, Tragacanth - Plantago seed - Cotton
<b>2. Glycosides</b> a) Cardiac b) Anthraquinone c) Saponins d) Cyanophore	- Digitalis, strophanthus - Aloe, Cascara, senna - Arjuna - Wild cherry bark
<b>3. Tannins</b>	Amla, Bahera, Ashoka bark
<b>4. Volatile oil</b>	Clove oil, rose oil, peppermint oil, tulsi etc
<b>5. Resins</b>	ginger, capsicum etc.
<b>6. Lipids</b> a) Fixed oils & fats b) Waxes	- Olive oil, castor oil, coconut oil etc - Bees wax
<b>7. Alkaloids</b> a) Pyridine & piperidien b) Tropane c) Quinoline d) Isoquinoline e) Indole f) Steroidal g) Purine	a) Nicotiana, areca nut b) Coca, Belladonna, Datura c) Cinchona d) Opium, Ipecac e) Ergot, Nuxvomica, Rauwolfia, vinca, Physostigma f) Kurchi g) Tea, Coffee
<b>8. Protein</b>	Gelatin, gluten etc

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9. <b>Vitamins</b>	Thiamine (B <sub>1</sub> ), Riboflavin (B <sub>2</sub> ), Ascorbic acid etc
10. <b>Antibiotics</b>	Penicillin, streptomycin, tetracycline etc
11. <b>Hormones</b>	Adrenaline, thyroxine etc

### Merits

1. It is preferred method of study because the pharmacological activity and therapeutics significance of crude drugs are based on the nature of their chemical constituents.
2. It given logical reasoning for the biological activity.

### Demerits

1. Sometimes it becomes difficult to classify the drugs, if it has two different types of chemical constituents e.g. Nutmeg has both fixed oils and volatile oils. Cinchona contains both glycoside and alkaloid.

### Pharmacological classification

In pharmacological classification the drugs are grouped according to their therapeutic use. For example cardiotonic drug include digitalis; purgative drugs include castor oil. Regardless morphology, taxonomical status or chemical relationship, the drugs are grouped together, provided they exhibit similar pharmacological action. Thus, cascara, castor oil, senna, jalap, colocynth grouped together as purgatives or laxatives because of their common pharmacological action.

No.	Pharmacological action	Drugs
1	Carminative	Dill, 13rtemi, cardamom
2	Purgatives	Senna, Aloe, Castor oil, Plantago husk
3	Emetics	Ipecac
4	Anti-amoebic	Kurchi, ipecac
5	Anthelmintics	Quassia, Vidang, Bhilama
6	Antimalarial	Cinchona, 13rtemisia
7	Anticancer	Vinca, Podophyllum
8	Expectorants	Liquorice, vasaka
9	Antiexpectorants	Stramonium
10	Antitussive	Opium
11	Bronchodilators	Ephedra, Tea
12	Cardiotonic	Digitalis, Strophanthus
13	Antihypertensive	Rauwolfia
14	Vasoconstrictors	Ergot
15	Anticholinergic	Datura, belladonna
16	CNS stimulant	Coffee, tea
17	Cholinergics	Phytostigma, Pilocarpus
18	Tranquillizer	Rauwolfia root
19	CNS depressant	Hyoscyamus, Belladonna
20	Analeptics	Nux-vomica, lobelia

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21	Anti-inflammatory	Turmeric, colchicum
22	Analgesic	Opium, cannabis
23	Skeletal muscle relaxants	Curare
24	Immunomodulatory agent	Ashwagandha, Tulsi, ginseng
25	Astringent	Catechu, Myrobalan
26	Skin and mucous membrane	Olive oil, wool fat, Arachis oil
27	Local anaesthetics	Coca
28	Bitter	Chirata, kalmegh
29	Immunizing agents	Vaccines, sera, toxoids antitoxins
30	Chemotherapy	Antibiotics – penicillins, cephalosporines

### Merits

1. The drug whose chemical nature is not known easily be grouped in this system.
2. Drugs differing in mechanism of action, but with the same pharmacological effects are grouped together. E.g., bulk purgatives, irritant purgatives, emollient purgatives etc.

### Demerits

1. The main drawback of this classification is that a drug can be placed in various classes according to its therapeutic use. For example: Cinchona (quinine) can be grouped in antimalarial and antiarrhythmic categories.
2. This system does not give any indication about morphological and chemical nature of drugs.
3. The constituents of one drug may have more than one therapeutic action (fall into numerous groups e.g. flavonoids)

### Role of Chemotaxonomy in classification

Chemotaxonomy is an interdisciplinary field in which chemical constituents of plants are used as characters to determine inter- and infra specific relationships of plant taxonomy. i.e. on the basis of chemical constituents and morphological characters of plants. The phytochemical observations has revealed the existence of close relationship between constituents of plants and their taxonomical status. Certain chemical compounds have been found to characterize certain botanical groups of plants. The characters studied in chemotaxonomy are secondary metabolites of pharmaceutical significance such as alkaloids, glycosides, flavonoids etc. the knowledge of chemotaxonomy could serve as the classification of crude drugs.

Examples are like carbohydrates derivatives such as Polysaccharides compounds like inulin are characteristic of the *Compositae* family while the fructans is present in *Graminae*. Cyanogenetic compounds more commonly found in *Rosaceae*, *Passifloraceae*, *Leguminosae*, *Sapindaceae* and *Graminae* families. Isothiocyanates compounds are the characteristic flavours of various plants of the *Cruciferae*, *Brassicaceae* families. In dicotyledons the polyprenylated benzoquinones derivative embelin are good taxonomic markers for *Myrsinaceae* while primitin compound for Primulaceae family. Anthraquinones occur in various genera of higher plants and mainly in dicotyledons, such as Cassia (*Caesalpinaceae*, *Leguminosae*), Rhamnus (*Rhamnaceae*), Rheum (*Polygonaceae*) and Aloe (*Liliaceae*). Xanthones have been applied as chemosystematic markers in the *Gentianaceae* family. The steroidal saponins are present mainly to the monocotyledons families like *Liliaceae*, *Dioscoreaceae* and in dicotyledons families *Solanaceae* and *Scrophulariaceae*.



### Serotaxonomical Classification

Serology is defined as that portion of biology, which is concerned with the nature and interactions of antigenic material and antibodies. Smith (1976) defined it as “**the study of the origins and properties of antisera.**” When foreign cells or particles (antigens) 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 the antigen is called antibody. Proteins most widely used as antigens in serotaxonomy are those, which carry useful taxonomic information and 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. Phytoserology, which deals with immunochemical reactions, between serum antibodies and antigens, has also established itself as a valid method in systematics, because it helps to detect homologous proteins. It uses the specific properties of antisera produced by animals against plant proteins as characters to assess plant relationships. Serotaxonomy developed and became popular in Germany, which has been an active center since the beginning of this century.

### Adulteration

**Definition:** Debasement of an article is known as adulteration. **OR** Adulteration of crude drugs means a drug which resembles to original drug with its morphological characters but is quite inferior or less effective and may contain less or may not contain active chemical constituents and contain more foreign matter. Adulteration means admixture, sophistication, substitution, deterioration, inferiority and spoilage. **Deterioration** is impairment in the quality of drug while admixture is addition of one article to another due to ignorance or carelessness or by accident. **Sophistication** is the intentional or deliberate type of adulteration. **Substitution** occurs when some totally different substance is added in place of original drug. **Inferiority** refers to any substandard drug and **spoilage** is due to the attack of microorganisms. The different types of adulterants found in market are given here.

### Methods of adulteration

- 1. Artificial substitutes:** The substitutes of standard drug are manufactured artificially which resemble the general form and appearance of original drug. Generally, the practice is followed for much costlier drugs. E.g. pieces of bass wood are cut or mixture of clay and exhausted powder is moulded as the shape of nutmeg to substitute for nutmeg; paraffin wax is yellow coloured and used in place of beeswax, compressed chicory in place of coffee.
- 2. Exhausted drugs:** Such crude drug contains high grade active constituents which is extracted or isolated. These exhausted drugs are used entirely or partly in place of standard drug. This practice is more common in volatile oil containing drugs.

## Chapter 1 Introduction to Pharmacognosy

Drug	Constituents removed
Clove	Volatile oil
Umbelliferous fruits	Volatile oil
Ginger	Gingerol, volatile oil and resin
Liquorice	Glycyrrhizin and other water soluble substances
Balsam of tolu	Balsamic acid
Ginger and colocynth	Exhausted ginger powder

3. **Inferior commercial variety:** The drug which may be similar appearance but less quality are sometimes added into authentic drug. This is rather a most common practice of adulteration because of inferior variety is cheaper compare to authentic drug.

Drug	Inferior drug
Leaves and pods of senna	Dog senna and palthe senna
Seed of <i>Strychnos nux vomica</i>	<i>S. nuxblanda</i> and <i>S. potatarum</i>
Leaves of belladonna	Phytolacca and scopolia
Pale catechu	Black catechu

#### 4. Addition of foreign matter

Drug	Foreign matter
Myrrh	Quartz and other mineral matter
Asafoetida	Lime and stone

5. **Addition of improperly dried and stored drug:** Sometimes drugs are not properly dried and stored and it mix with official drugs and consider to be adulteration eg, Digitalis purpurea and colchicum if dried higher temperature degraded active chemical constituents, cod liver oil on heating at higher temperature reduce vitamin D content and change the colour, odour and taste of fixed oil. Digitalis, Belladonna, and stramonium if not stored in moisture proof containers, decompose the active constituents. Volatile oils containing drugs if not protected from temperature, light and air, may be evaporated volatile oils.
6. **Addition of synthetic principle:** Authentic drug is replaced wholly or partly by some synthetic principle which has enhanced the natural character of official drug.

Drug	Synthetic
Oil of lemon	Citral
Balsam of peru	Benzyl benzoate

7. **Addition of excessive quantity of other part of plant:** If the part of plant other than official plant part is mixed with main part of plant.

Drug	Excess of part of plant
Leaves of lobelia and stramonium	Stem
Leaves of senna and buds of clove	stalks

**8. Addition of some waste product:** Many times some of waste products are added into authentic drug.

Drug	Waste product
Ipecac	Dextrin
Capsicum	Red sander's wood

**9. Addition of improperly collected drug:** The drug contains optimum active constituents at a particular time, season, and stage of development and age of plant. Sometimes the drugs are not collected during this period and may contain less or no active constituents at all. Such drugs added into authentic drug and considered to be adulteration.

### Evaluation of crude drugs

It means that identification and determination of the quality and purity of drug.

#### Identification

The identification can be established by careful observational study of the collected drug, and then compared with authentic specimen by the collector. Therefore, for proper identification of a drug from plant or animal sources, a collector must be educated about plant taxonomy and very much experienced with his/her job. Therefore, drugs from plants/animals are identified by

1. A qualified, specialized & experienced personnel
2. Comparison with the authentic sample specimen.

In every country, there is a national herbarium where most of plants specimen are preserved. A number of specialists are working on plant identification there.

#### Quality

The word "quality" refers to the intrinsic value of the drug, i.e., the amount of medicinal principles or active constituents present. These principles are classified as carbohydrate, alkaloid, glycoside, volatile oil, lipid, antibiotics and steroids etc. A high grade of quality in a drug is of primary importance. An effort should be made to obtain and maintain high quality. To maintain high quality products one should do the following:

1. Select proper source (wild or cultivated)
2. Appropriate time of collection
3. Collection of required parts of plants (bark, leaf, stem, rhizome, root)
4. Preparation of the collected drug by proper cleaning, drying.
5. Proper preservation to avoid contamination by microorganisms and moisture, heat, air and light.

#### Purity

The purity of drug can be achieved by

1. Estimation of active constituents
2. Quality assurance.

### Methods of evaluation

1. Morphological / Macroscopical / Organoleptic
2. Microscopical
3. Physical
4. Chemical
5. Biological
6. Chromatographic

### Morphological / Macroscopical / Organoleptic evaluation

Morphological evaluation means the study of a drug with the help of organs of sense. It is a technique of qualitative evaluation based on the study of morphological and sensory profiles of whole drugs. It includes any drug's macroscopic or external appearance of color, odor, taste & sounds of its fracture etc. Organoleptic evaluation means conclusions drawn from impressions on organs of senses. The study form of a crude drug is morphology and description of the form is **morphography**. The general appearance of the lot of a crude drug often indicates whether it is likely to comply with prescribed standards. The macroscopic or external characteristic of a drug may be divided into 7 headings

1. Shape
2. Size
3. Colour
4. Odor
5. Taste
6. Surface
7. Extra features

### Microscopical evaluation

- ❖ Microscopic evaluation of drug can be done in the laboratory by the use of microscopes and utilizes various microscopic characters of the drugs. The study of plant cells, tissues, measurement cells and cell contents with use of microscope to helps identification of crude drugs. The various methods is used such as
  1. Transverse section (structure of different cells and tissues system)
  2. longitudinal section
  3. Surface preparation & types of stomata:- e.g. paracytic stomata
  4. Powder microscopical characters like epidermal cells, phloem fibres etc.
  5. Chemo-microscopy= e.g. lignified cells
  6. Types of vascular bundles
  7. Types of trichomes
  8. Measurement of diameter of different cells like phloem fibres, xylem fibres
  9. Different types of xylem vessels and measurement of diameter of its.
  10. Leaf constants measurement like stomatal index, palisade ratio etc.

11. Types of starch grains and measurement of diameter of starch grains
12. Different types of calcium oxalate crystals & measurement of diameter of its.

- ❖ Microscopical character of crude drugs such as stomata, trichome, epidermis, palisade cells, and cork cells, volatile oils containing cells, ground tissue or cortex cells, stone cells, idioblast cells, arrangement of vascular bundle, phloem fibres, xylem vessels, calcium oxalate and starch grains helps identification of drugs.

Drug	Character
<i>Rauwolfia serpentina</i>	Stratified cork is present and stone cells are absent
<i>Rauwolfia canescens</i>	Stratified cork is absent and stone cells are present
Indian rhubarb	Star pots are absent
Rhapontic rhubarb	Star pots are present

- ❖ **Chemo-microscopy or Microchemistry** means that study of the constituents by application of chemical methods to small quantities of drugs in powdered form or to transvers section of drug. E.g. lignified tissues system identified by treatment with Phloroglucinol and HCl.

### Physical evaluation

The physical evaluation of crude drugs is accomplished by the determination of various physical parameters and characteristics using various **physico-chemical techniques** like solubility, moisture content, extractive values (H<sub>2</sub>O soluble extractive value, alcohol soluble extractive value, ether soluble extractive value), ash values (total ash, water soluble ash & acid insoluble ash) and **physical constants** such as viscosity, specific gravity, optical rotation, melting point, boiling point, and refractive index is used for evaluation of crude drugs.

1. **Optical rotation:** Certain substance is found to have the property of rotating the plane of polarised light in the pure state or in the solution. Thus, they are described to be optically active and this property is known as optical rotation. Plane of polarised light may be rotated towards right (dextrorotary) or towards left (levorotary). Normally, the optical rotation is determined at 25°C using sodium lamp as the source of light.

Drugs	Angles of optical rotation
Caraway oil	+75° to +80°
Castor oil	+3.5° to +6°
Clove oil	+0° to -1.5°
Honey	+3° to -1.5°
Eucalyptus oil	+0° to +10°

2. **Viscosity:** Viscosity of a liquid is constant at a given temperature and is an index of its composition. Hence it can be standardizing liquid drugs. E.g. liquid paraffin has kinematic viscosity not less than 64 centistokes at 37.8°.

3. **Refractive index:** When a ray of light passes from one medium to another of different density, it is bent from original path. Thus, the ratio of the velocity of light in vacuum to its velocity in the substance is termed as refractive index of the second medium. So it is one of the parameter for standardizing the crude drug. Refractive indices of the following compounds are for sodium light and at a temperature of 25°C.

Drug	Refractive Index
Arachis oil	1.4678 to 1.47
Caraway oil	1.4838 to 1.4858
Castor oil	1.4758 to 1.527
Clove oil	1.527 1.535

4. **Melting point:** It is one of the parameters to check the purity of crude drugs. Crude drugs contain mixer of phytochemical which has certain range of melting point.

Drugs	Melting point (°C)
Colophony	75-85
Kokum butter	39-42
Cocoa butter	30-33
Bees wax	62-65
Wool fat	34-44

**Moisture content:** The percentage of active chemical constituents in crude drugs is mentioned on air-dried basis. The moisture content of a drug should be minimized in order to prevent decomposition of crude drugs either due to chemical change or microbial contamination. The moisture content is determined by heating a drug at 105°C in an oven to a constant weight.

Drugs	Moisture content (%)	w/w
Aloes	Not more than	10
Digitalis	Not more than	05
Ergot	Not more than	08
Acacia	Not more than	15
Starch	Not more than	15

**Solubility:** Castor oil is soluble only in 3 volumes 90% alcohol, while the adulterated form may show good solubility in alcohol while the adulterated form may show good solubility in alcohol. Alkaloidal base is soluble in chloroform while alkaloidal salts are soluble in polar solvent. The glycosides are soluble in alcohol and water but their aglycone part is soluble in non-polar organic solvent such as ether.



**Extractive values:** The extracts are obtained by exhausting crude drugs due to contain of chemical constituents. Due to presence of variety of chemical constituents are present hence various solvents are used for determination of extractives.

- a. **Water soluble extractive**
- b. **Alcohol soluble extractive**
- c. **Ether soluble extractive**

**Ash values:** The residue is remaining after incineration of crude drug is known as ash content of drug which simply represents inorganic salts. Various types of ash values such as

- I. **Total ash**
- II. **Acid insoluble ash** and
- III. **Water soluble ash** values are calculated.

**Foreign Organic Matter:** The parts of organ or organs other than those named in definition and description of the drug are defined as foreign organic matter.

### Chemical evaluation

Chemical evaluation of drugs involves both qualitative and quantitative determination of their active principles. In this method characteristic qualitative chemical tests are employed to identify crude drugs and their constituents.

1. **Chemical tests:** Certain crude drugs are identified by performing chemical test with particular chemical reagents. If negative result is obtained, it may chance to adulteration. E.g. Potassium chlorate and HCl are used to estimate emetine in Ipecac. Strychnine in Nux-vomica is detected with ammonium vanadate and sulphuric acid. Quantitative chemical tests such as acid value, saponification value, ester value, acid value etc. halphen's test for cotton seed oil, Van Urk's reagent for ergot, murexide test for purine bases etc. are examples of specific test.

<b>Drug constituents</b>	<b>Test/reagent used for identification</b>
Alkaloids	Dragendroff's, Mayer's, Wagner reagents
Trophane alkaloids	Vitali - morin test
Anthraquinone glycosides	Borntrager's test
Saponin glycosides	Foam, haemolytic tests
Cardiac glycosides	Kedde's, Raymond's reagents
Cyanogenetic glycosides	Sodium picrate test
Carbohydrates	Molisch's, Fehling's tests
Lipids	Sodium hydroxide test
Amino acids	Ninhydrin's test

2. **Chemical constants:** Sometimes chemical constants such as acid value, iodine value, ester value, acetyl value, Saponification value, non-saponifiable matter etc. are determined especially for fixed oils, fats and waxes if variations in these constants indicate may be some adulteration.

3. **Chemical assays:** The active constituent present in drug is estimated quantitatively by methods such as volumetric, gravimetric, titrimetric, colorimetric, and chromatographic techniques. Spectroscopic (UV & fluorescence) methods using the specific absorption of the constituents in UV, IR, and fluorimetric analysis are examples of physical assay methods.

### Chromatographic evaluation

- ❖ Chromatography (in Greek: Khromatos = colour and graphos = written) is a series of techniques that are used to separate, analyze, purify and isolate from complex mixtures.
- ❖ It depends on the differential migration of the solutes in two immiscible phases.
  1. **Stationary phase:** A fixed bed of large surface area
  2. **Mobile phase:** A fluid or gas which moves through surface
- ❖ Chromatography is also useful for the fractionation of complex mixture, separation of closely related compound such as isomers and in the isolation of unstable substances.
- ❖ The identification, separation, purification, isolation and estimation of plant constituents is mainly carried out using one or other or a combination of chromatographic techniques.
- ❖ The choice of technique depends largely on the solubility properties and volatilities of the compounds to be separated.
- ❖ Small volumes needed (1-50 $\mu$ l for TLC; 1-5 $\mu$ l for GC and 5-100 $\mu$ l for HPLC) to analysis of sample.
- ❖ Various techniques are such as TLC & HPTLC, Column, HPLC, and GC, size exclusion, ion-exchange, affinity chromatography, electrophoresis, flash, VLC, Sorbent countercurrent chromatography and supercritical fluid chromatography.

### Biological evaluation

The crude drugs can not satisfactory assayed by chemical or physical methods; they are assayed by biological methods. The biological evaluation of crude drugs is very useful in determining the pharmacological activity of crude drugs extract or constituents *in vivo* and *in vitro* and compare with standard preparation. Since living organism or their isolated living tissues are used, this method is also called the biological method or bioassay. Many drugs, particularly the antibiotics, toxins and toxoids and also vitamins are assayed by this method. Biological evaluation procedures are generally less precise, more time consuming and more expensive to conduct than chemical evaluation.

### WHO guidelines for evaluation of Herbal drugs.

The pharmacopoeias of different countries include monographs indicating quality parameters and standards for various herbal drugs and also for some of their products. To ensure standards and to validate traditional medicines, WHO has laid down certain guidelines and recommended procedures as following.

1. General notices
2. Powder fineness and sieve size
3. General advice on sampling

4. Determination of foreign matter
5. Macroscopic and microscopic examination
6. Thin-layer chromatography
7. Determination of ash
8. Determination of extractable matter
9. Determination of water and volatile matter
10. Determination of volatile oils
11. Determination of bitterness value
12. Determination of haemolytic activity
13. Determination of tannins
14. Determination of swelling index
15. Determination of foaming index
16. Determination of pesticide residues
17. Determination of arsenic and heavy metals
18. Determination of microorganisms
19. Radioactive contamination
20. Culture media and strains of microorganisms
21. Specifications for adsorbents for use in thin-layer chromatography

### Quantitative microscopy

- ❖ **Quantitative microscopy** means measurement of cells such as stomatal number and index, vein islet number and termination number, palisade ratio, phloem fibre and cell contents such as calcium oxalate and starch grains helps identification of drugs.
- ❖ **Lycopodium spore method:** Whenever other microscopical methods, chemical and physical methods not give satisfactory results or not applicable then this method is used. In this method, the proportion of cell or cell content such as starch grains, calcium oxalate, sclerides etc. are determined. The Lycopodium spore which have characteristic shape, appearance and particular size and suitable suspending, staining and mounting agents are used for preparing slide. The number of Lycopodium spore and particular character are counted, and percentage of purity of powdered drug is calculated using following formula.

$$\text{Particular Character} = \frac{n \times w \times 94,000 \times 100}{s \times m \times p}$$

Where,

n = number of particles in given area

s = number of Lycopodium spores in the same area

w = weight in mg of Lycopodium

m = weight in mg of samples

p = number of particles per mg in pure foreign matter (e.g., p = 286000 for ginger starch grains powder)

### Camera Lucida Theory

**Working of Camera lucida:** Various forms of apparatus have been designed so that magnified image of the object under the microscope may be traced on a paper. The most widely used is the Swift Ives camera lucida which fits over the eyepiece.

**Principle:** The light from the object passes direct to observer's eye through an opening in the silver surfaces of the left hand prism. At the same time light from the drawing paper and pencil is reflected by the right hand prism and by the silvered surface, so that the pencil appears superimposes on the object, which may thus be traced.

Camera lucida is useful for the determination of Leaf constants like stomatal index, Vein islets and veinlet termination number and palisade ratio.

### Leaf constants

1. **Stomatal Number** is defined as the average number of stomata per sq. mm. of the epidermis of the leaf.
2. **Stomata index** is the percentage, which the number of stomata form to the total number of 0000epidermal cells, each being counted as one cell. Stomatal index can be calculated by using the following equation.

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

Where, S.I.=Stomata index

S=Number of stomata per unit area

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

Stomatal number varies considerably with the age of the leaf and due to changes in the environmental conditions, stomatal index is relatively constant and therefore of diagnostic significance for a given species. It is employed for the differentiation of allied or closely related species of the same genus in air-dried, as well as, fresh conditions.

3. **Palisade ratio:** It is the average number of palisade cells beneath each epidermal cell. It is a useful parameter for identification in powdered leaf drugs.
4. **Vein-islet and vein let termination number:** The mesophyll of the leaf in dicot leaves is divided into small portions by branching of the veins throughout the tissues. The small areas of the green tissue outlined by the vein lets are termed as vein-islets. Vein islet number is defined as the number of vein-islets per sq mm of the leaf surface midway between midrib and the margin. It is a constant for a given species of the plant and it used as a characteristic for the identification of allied species. Vein-let termination number is defined as the number of vein let termination per sq mm of the leaf surface mid way between midrib and margin. A vein termination is the ultimate free termination of vein let.