Unit 1

1.1 Introduction to Pharmacognosy

The term ‘pharmacognosy’ (combination of two Greek words i.e. pharmakon means drug and gnosis means knowledge) means “acquiring knowledge of drugs” was coined in 1815 by C. A. Seydler, German medical student in his thesis title “AnalyeticaPharmacognostica”. Pharmacognosy is defined as “scientific and systematic study of structural, physical, chemical and biological characters of crude drugs along with history, method of cultivation, collection and preparation for the market”.

The American Society of Pharmacognosy defines pharmacognosy as "the study of the physical, chemical, biochemical and biological properties of drugs, drug substances or potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources’. It is also called as study of crude drugs.

Thus pharmacognostical studies of plant drugs involves study of synonyms, vernacular names, Biological sources, distribution, morphology, histology, chemistry, qualitative test, various physicochemical tests, pharmacological actions along with commercial varieties, substitutes, adulterants and any other quality control parameters of the drugs.

Scope

- The pharmacognosy has played an important role in the transformation of ***various basic science subjects.*** Pharmacognosy has a vital contribution to the advancement of natural and physical science due to advances in technologies of cultivation, purification, identification, characterization of natural drugs.
- Pharmacognosy has enabled to establish a sound knowledge of the vegetable drugs under ***botany and animal drugs*** under zoology through taxonomy, breeding, pathology and genetics concepts.
- This knowledge used to improve and ***modernise cultivation*** methods of medicinal and aromatic plants to fulfill demand from quality raw material to genetic transformations in plants to get desired characters.
- World Health Organization (WHO) has estimated that 80% of world population depends on herbal medicines for their primary health care. Some of the very ***famous crude drugs*** are senna as a purgative, digitalis as a cardiotonic and rauwolfia as an antihypertensive drug. Pharmacognosy explains thorough knowledge of the history, cultivation, collection, quality control, transport, storage and even economic impact of all these crude drugs.
- Pharmacognosy is ***vital link*** between pharmacology and medicinal chemistry because it enables isolation of purified natural drugs, converts into medicine and evaluates its therapeutic effects.
- Pharmacognosy links basic science, pharmaceuticals, ayurvedic and allopathic system of medicines to each other.

- Pharmacognosy helped to improve plant chemistry (***phytochemistry***) significantly with the knowledge of extraction. Varieties of chemical constituents that are accumulated and synthesized by plants have revolutionized the process of natural drug discovery.
- ***Novel techniques*** like "Bioassay Guided Fractionation" helps in the isolation of phytochemicals based on therapeutic potency. This has led to specific use of medicinal constituents or plant parts and its utilization in disease treatment.
- Recently started studies on natural drug-drug, drug-food ***interactions*** are avoiding the untoward effects of severe interactions and hence helping in obtaining the optimal therapeutic outcomes especially for classes like Blood thinners, Protease inhibitors, Cardiac Glycosides, Imuuno Suppressants.
- In the pharmaceutical ***industry***, various drugs of botanical origin are used in drug manufacturing process. Knowledge of pharmacognosy surely helps as a research tools in the new drug/dosage form development.
- Recent guidelines for quality control of crude drugs are to assure the identity, purity and consistency of drug substances, efficacy to determine the therapeutic responses, indications, ***clinical aspects*** and pharmacological effects, safety to avoid untoward toxic reactions, interactions and contraindications.
- However, this subject is as old as pharmacy and humankind evolution; recently it is evolved as a ***multidisciplinary subject*** focusing many modern disciplines like ethanobotany, ethanopharmcology, phytotherapy, phytochemistry, chemo-taxanomy, biotechnology, clinical trials, herbal drug interaction and even novel drug delivery systems like phytosomes rather only botanical and taxanomical descriptions. Recent advances in extraction methods, analytical hyphe-nated techniques, screening methods continues to hasten major changes in this subject. Modernization of conventional and/or traditional dosage forms is opening doors to "***Industrial Pharmacognosy***".
- Due to most recent technologies and innovative chemical concepts, many new drugs or drug candidates still originated from natural products or derivatives thereof. Even in this era of nanotechnology, natural drugs are important part of primary health care which is giving pharmacognosy professionals new possibilities to exploit the huge diversity designed and generated by nature.
- Due to rapid growth in demand and popularity of natural products, research has been directed towards patentable drug discovery and development in the field of pharmacognosy.
- There is a shortage of established scientists engaged in pharmacognosy research; hence detail knowledge of this subject is till need to be studied by conventional scientists. Thus, actual secret of opportunities in pharmacognosy research is that only the tip of the iceberg seems to have been discovered yet.

History

History of Medicines ranged from folklore, evidence-based medicine to antibiotics, nanotechnology, and gene therapy. Few of most notable historical medicinal texts are as follows:

Region	Medicinal Texts
Egypt	Imhotep, Edwin Smith Papyrus, Ebers Papyrus, Kahun Gynecological Papyrus
Mesopotamia	Diagnostic Handbook, Alkindus, De Gradibus
India	Ayurveda, Sushruta Samhita, Charaka Samhita
China	Yellow Emperor, Huangdi Neijing
Greece	Iliad and Odyssey are the earliest sources of Greek medical practise; Hippocratic medicine
Persia	Rhazes, Avicenna, The Canon of Medicine, The Book of Healing
Spain	Abulcasis, Kitab al-Tasrif
Syria	Ibn al-Nafis, Commentary on Anatomy in Avicenna's Canon, Comprehensive Book on Medicine

Through above literatures, it can be concluded that History of pharmacognosy is as old as mankind. Human being came to know medicines from nature itself. Following table is explaining various historical developments, which together contributed to the progress of Pharmacognosy. Various traditional systems of medicines from different corners of world also played vital role in development of pharmacognosy.

Scientists and their work in the development of Pharmacognosy			
Name	**Profession**	**Work**	**Period**
Ebers Papyrus or Papyrus Ebers	Egyptian medical papyrus	Herbal knowledge	1550 BC
Pedanius Dioscorides	Greek physician	De Materia Medica book is compilation of several plants	78 A.D.
Gaius Plinius Secundus or Pliny the Elder	Roman naturalist	Encyclopedic work entitled Naturalis Historia	25-70 A.D.
Aelius Galenus or Claudius Galenus or Galen	Greek pharmacist	Galenical Pharmacy	131 – 200 A.D.
Hippocrates *Father of Medicine*	Greek scientist	Studied human anatomy and Physiology	460 - 360 B.C
Aristotle *Father of Biology*	Greek Philosopher	Animal kingdom	384-322 B.C.
Theophrastus *Father of Botany*	Greek Philosopher	Plant kingdom	370- 287 B.C.
Carl Linnaeus *Father of Taxonomy*	Swedish botanist	Binomial classification	1753
C A Seydler	German	Coined word Pharmacognosy	1815
Sir Joseph D. Hooker	British botanist	Plant nomenclature	1817 - 1911.
George Bentham	English botanist	Plant nomenclature	1800 - 1884
Charles Darwin	English naturalist	Evolutionary theory	1809 - 1882.
Friedrich Sertürner	German chemist	isolated first alkaloid morphine from opium	1804
Mikhail Tsvet	Russian scientist	Separation of plant pigments by chromatography	1900

1.2 Sources of Crude Drug

Crude Drugs

Crude drugs are the drugs, which are obtained from natural sources like plant, animals or minerals and used as such as they occur in nature without any processing except collection, drying and size reduction. It also defined as the drugs that have not been advanced in value or improved in condition by shredding, grinding, chipping, crushing, distilling, evaporating, extracting, artificial mixing with other substances or any other process beyond that which is essential to its proper packing and to prevention of decay or deterioration during manufacturing. Crude drugs and their constituents are commonly used as therapeutic agents. Source of crude drugs are plant (senna, opium, digitalis and Clove), Animal (Musk, Honey, Shark liver Oil) and Mineral (Shilajit, Talc, Bentonite).

Plant	Plant source is the oldest source of drugs. More than 25% of the drugs prescribed worldwide are obtained from plants and more than 150 active chemical compounds from plants are prescribed. Many synthetic drugs obtained from natural precursors. More than 10 % plant formulations of total are considered as basic and essential by the World Health Organisation (WHO). Plants are very rich source of simple as well as complex and extremely diverse structures. Even many of such chemicals cannot be synthesized in laboratory. ***Examples***: digoxin from Digitalis, quinine and quinidine from Cinchona. vincristrine and vinblastine from vinca, atropine from belladonna and morphine and codeine from opium.
Animal	Different animal derived products are always being part of treatment of human ailments or nutritional diet. Example: Honey from honeybee, beeswax from bees, cod liver oil from shark, Bufalin from toad, Insulin from animal pancreas, musk oil from musk, spermaceti wax from sperm whale, woolfat from sheep, carminic acid from colchineal, venoms from snake
Mineral	A mineral is a naturally occurring solid crystalline inorganic substance made up of one element or more elements combined together. Many minerals derived from calcium, sulfur, sodium, iron, zinc, silver, gold, diamond, quartz are practiced as medicine in a highly purified form in traditional Ayurveda, Unanai, Siddha and Chinese systems of medicines . Example: sulfur is a key ingredient in certain bacteriostatic drugs, shilajit is used as tonic, calamine is used as anti-itching agent
Marine	Since thousands of years, marine flora and fauna has provided many compounds which are useful in their natural form or as templates for synthetic modification of bioactives for treatment of many diseases. Marine microorganisms, plants, algae, fungi, invertebrates, and vertebrates are used to isolate more than 10,000 chemical entities. For instance, about more than 1000 patents on bioactive marine natural product have been issued since 1970. Many marine drugs are useful in food, confectionary, textile, pharmaceutical industry as gelling, stabilizing and thickening agents.. Till only 10% of marine flora and fauna have been investigated for therapeutic efficacy. Example: Agar- a jelly like substance from red algae, Carrageenans or carrageenins from red seaweeds, sodium alginate from brown seaweed
Plant tissue culture	Plant tissue culture refers to growing and multiplication of single cell, tissues and organs under aseptic and controlled environment on specific media. This source is very useful for large scale production of plants in limited area, conserve rare and endangered plants, produce genetically varied plants like seedless fruit bearing plants, production of therapeutically important secondary metabolites (example: antihypertensive ajmalicine from callus culture of *Catharanthus roseus*, anti-inflammatory berberine from suspension culture of *Thalictrum minus*, immunomodulatory ginsenoside from callus culture of ginseng)

1.3 Classification of Crude Drug

In Pharmacognosy crude drugs are classified in the following category.

Alphabetical classification: In this classification drugs are classified in alphabetical order using either their Greek name or Latin name. Though pharmaco-poeias, formulary, encyclopedias of various countries follow this classification, but due to lack of scientific value now-a-days this classification is not preferred. **Example:-** Acacia, Bael, Cinchona, Dill, Ergot, Fennel, Ginger, Henbane, Ipecac, Jalap, Kurchi, Licorice, Myrrh, Nux-Vomica, Opium, Podophyllum, Quassia, Rauwolfia, Senna, Tea, Urgenia, Vasaka, Wool Fat, Yam, Zedoary etc. Major Advantage of this method is that it provides quick reference.

Morphological classification: This is most simple classification method where crude drugs are grouped into organized drug (parts of plant like root, rhizome, flower, leaf, fruit, bark, seed, wood etc) and unorganized drug (dried lattices, dried juice, gum, wax, oil etc). But many crude drugs are very similar morphologically and hence difficult to distinguish. Many times crude drug available in powder form that time morphological classification is not so suitable and acceptable.

Difference between organized and unorganized drugs			
Organised crude drugs		**Un-organised crude drugs**	
Parts of plants or animals		Obtained from parts of plants	
Well defined structure		Not well defined structures	
Solid in nature		Semisolid, solid, liquid in nature	
Microscopic studies are useful in quality control		Chemical tests are more useful in quality control	
Examples		***Example***	
Parts	**Example**	**Class**	**Example**
Leaves	Senna, Digitalis, Vasaka, Eucalyptus	Resins	Balsam of tolu, Myrrh, Asafoetida, Benzoin
Barks	Cinchona, Kurchi, Cinnamom, Quaillia	Gums and mucilages	Acacia, Tragacanth, Guar Gum
Woods	Quassia, Sandalwood	Dried latices	Opium
Roots	Rauwolfia, Ipecacuanha, Aconite	Dried juices	Aloes, kino
Rhizomes	Turmeric, Ginger, Valerian, Podophyllum	Volatile oils	Cinnamon oil
Seeds	Nux-vomica, Strophanthus	Fixed Oil	Castor oil and lard
Flowers	Clove, Saffron	Waxes	Beeswax
Fruits	Coriander, Colocynth, Fennel, Bael	Extracts	Catechu
Entire plant	Vinca, Belladonna	Saccharine substances	Honey

Examples of crude drugs based on plant parts	
Plant Part	**Example**
Leaves	Senna, Digitalis, Vasaka, Eucalyptus
Barks	Cinchona, Kurchi, Cinnamom, Quaillia
Woods	Quassia, Sandalwood
Roots	Rauwolfia, Ipecacuanha, Aconite
Rhizomes	Turmeric, Ginger, Valerian, Podophyllum
Seeds	Nux-vomica, Strophanthus
Flowers	Clove, Saffron
Fruits	Coriander, Colocynth, Fennel, Bael
Entire plant	Vinca, Belladonna
Resins	Balsam of tolu, Myrrh, Asafoetida, Benzoin
Gums and Mucilages	Acacia, Tragacanth, Guar Gum
Dried latices	Opium
Dried juices	Aloes, Kino

Taxonomic classification: In this classification crude drugs are arranged according to taxonomic order i.e. phylum, division, class, sub-class, orders, families, genus and species (See chapter 2 for more details).Precise and orderly arrangement of drugs has no ambiguity in this classification. But again this type of classification lacks scientific value and unorganized crude drugs are difficult to classify.

Phylum - Spermatophyta

Division - Angiospermae

Class - Dicotyledons

Sub-class - Sympetalae

Order - Tubiflorae

Family - Solanaceae

Genus - *Atropa*

Species - *belladonna*

Biological or pharmacological classification: In this classification, Crude drugs having similar therapeutic effects or pharmacological activity are grouped together but drugs having more than one therapeutic effect are difficult to classify. It also don't give any idea about chemistry or taxonomy.

Examples of pharmacological classification of crude drugs	
Pharmacological Action	**Drug**
Carminatives	Fennel, Dill, Coriander, Clove.
Purgatives	Cascara, Aloe, Senna, And Rhubarb.
Cardio tonics	Digitalis, Squill, Strophanthus
Anti- cancer	Taxaol, Vinca, Podophyllum
CNS Stimulant	Nuxvomica
Expectorant	Vasaka, Liquorice
Bitter tonic	Gentian, Chirata

Chemical classification: This classification is purely based on chemistry of constituents. Different crude drugs are classified according to the presence of major active constituents. This is most preferred method of classification.

Examples of chemical classification of crude drugs	
Chemical class	**Drugs**
Alkaloid	Cinchona Rauwolfia, Datura.
Volatile oil	Clove, Fennel oil, Coriander
Glycoside	Senna, Digitalis, Licorice.
Resin	Jalap, Ginger, Tolu Balsam
Carbohydrates	Acacia, Honey, Starch, Isapgol
Tannins	Arjuna, Ashoka,
Lipid	Castor oil, Peanut Oil, Mustard,
Proteins Enzymes	Casein, Gelatin
	Papain, Trypsin

Chemotaxonomic classification: Chemo-taxonomy is a technique which establishes relation between chemistry and taxonomy. It is also called as chemosystematics. Morphological characters and chemical constituents are interrelated and have a lot significant for the plant taxonomy. Examples: In case of eucalyptus, feather-veined leaves have high Pinene content in their essential oil, while intermediate veined leaves contain both pinene and Cineole. Chemotaxonomic study starts with exact choice of group, then sound sampling, analysis of chemical content, inter-pretation, comparison and finally classification.

Serotaxonomical classification:

Serology deals with studies of antigen-antibody reaction to provide knowledge of origin and properties of antisera. Serotaxonomic classification involves phytoserology which carries in-vitro immunochemical reaction of plant proteins (antigens or agglutinogens) to detect taxonomic homology based on antibodies (agglutinins) produced in animals. Desipite significant contribution made in the serotaxonomy, it has so far not gained much importance in the plant classification. The most common approach in serotaxonomic classification of plants is “precipitin reaction”. Precipitin is antibody which causes precipitation.

Precipitin reaction: After injecting a crude plant protein extract into the blood stream of an experimental animal like rabbit or a rat results in the production of specific antibodies. When animal serum containing antibodies also called antiserum reacts in-vitro with the antigenic proteins as well as proteins from other related taxa, of which the affinities are in question, leads to formation of a precipitate. This is called precipitin reaction. The degree of protein homology is determined by the amount of precipitation and hence it is taken as a phylogenetic marker and taxonomic character. If no precipitation is observed then there is no relation and if high precipitate then close relationship among examined taxas.

Crude protein extracts contain a large number of proteins, which stimulates the production of a vast range of antibodies, which differ in their specificity and reactivity. Some are produced in abundance while others are hardly detectable. But advanced serologic techniques allows to deals with single antigen and antibody. The "antisystematic" reactions have recently been shown to result from variation in the systematic ranges of determinants; and the absorption (pre-

saturation) technique for removing common determinants increases the accuracy of serological placements. Immunodiffusion in Agarose Gels, Rocket Immuno-electrophoresis and Enzyme-Linked Immuno-sorbent Assay (ELISA) are commonly used techniques in serotaxonomy.

Following are Parameters to be analysed in Pharmacognostic study of crude drug

Parameters	Description
Common names	Names in various languages
Biological source	Genus, species and family
Geographical source	Location
History	Discovery of crude drug
Cultivation , collection and preparation for market	Time and method of cultivation, irrigation, climate, fertilizers, collection time, processing etc.
Morphological description	Color, odor, taste, size, shape, extra features
Microscopical description	Cell, tissue type and arrangement, cell inclusions, special characters etc
Chemical constituents	major and minor chemical constituents present
Chemical tests	To Identify crude drug and its chemistry
Uses and pharmacological actions	Various therapeutic applications
Adulterants and Commercial varieties	Useful for quality control
Formulations available in Market	To understand market potential
Quality control and standardization	To establish qualitative and quantitative standards with the help of sophisticated instruments.

1.4 Quality Control of Drugs of Natural Origin

Evaluation of crude drugs involves the process of identification of adulteration and determination of quality of crude drugs. Or Evaluation means "*confirmation of its identity and determination of its quality and purity.* This can be organoleptic or morphological, microscopic, biological, physical and chemical evaluation. Thus to determine impurities is also part of evaluation and one of the major reasons of impurity in crude drugs is **drug adulteration. Adulteration means** sub standardisation of drug with respect to therapeutic and chemical properties by replacing wholly or partially original drug. Types and terminologies related to adulteration are given as follows:

Types of adulteration on the basis of reasons	
Unintentional	
Misidentification	**Due to confusion**: for herb Lakshmana different species are used *Arlia quinquefolia*, *Ipomea sepiaria* **Due to lack of knowledge of authentic plant**: All plants like *Cressacretica, Selaginella bryopteris, Desmotrichum, fimbriatum, Malaxis acuminata (M. wallichii, Microstyliswallichii), Trichopuszeylanicus*and *Terminalia chebula* are consistently and repeatedly referred as Sanjeevani
Carelessness	Root of *Sida cordifolia* are replaced with the whole plant of *Sida cordifolia*
Geographical Unavaibility	*Plucia lanceolata* is used as Rasna in northern India while *Alpinia galanga* is used as Rasna in southern India.

Table 1.7 *Contd….*

Morphological similarity	*Cassia angustifolia* replaced with *Cassiaacutifolia* and *Euphorbia dracunculoides* Lam. (Euphorbiaceae) with *R. graveolens*
Intentional	
Adulteration with substandard commercial varieties	Rhubarb replaced with Chinese rhubarb or raphnotic rhubarb
Adulteration with superficially similar inferior drugs	Pimpalii *(Piper nigrum*) adulterated by papaya seeds
Adulteration with artificially manufactured substances	Artificial invert sugar are mixed with or replaced with pure Honey
Adulteration of exhausted drugs	Ginger is sold after extraction of its volatile oil
Adulteration with synthetic materials	Addition of synthetic Citral to oil of lime.
Adulteration with harmful substances	Pieces of limestone in asafoetida and of lead in opium.
Intentional	
Adulteration of the species belonging to same family	Mixing or replacement of *Datura metal* with *Datura stramonium*
Adulteration of different species	Mixing of *Tribulus terrestris*(zygophylaceae and *Pedalium murex* (Pedaliaceae)
Adulteration with totally different drugs	Bharangi (*Clerodendron indicum*) is totally replaced with Kantakari (*Solanum xanthocarpam*)
Adulteration with low cost drug	Kumkuma (saffron) being costly herb is substituted by Kusumbha (dried flowers of American saffron -*Carthamustinctorius*)

Terminologies related to adulteration	
Inferiority	Impairment of quality with naturally substandard drug Example: The dried ripe seeds of *Strychnosnuxvomica* contain 1.15% of strychnine. Seeds containing less than 1.15% of strychnine, considered as inferior substandard drug.
Spoilage	Impairment of quality due to addition of Spoiled drug by the action of microorganism and thus renders the crude drug unfit for human consumption.
Deterioration	Impairment of the quality by destruction of any valuable constituent by extraction, moisture attack, heat treatment, microbial attack or by any other means. Example: Coffee that has largely lost its caffeine through over roasting is an example of deterioration.
Admixture	Impairment of the quality by addition of one product to another through accident, ignorance or carelessness. Example: *Senna* containing a few stems
Sophistication	Impairment of the quality by sophistication means the intentional addition of inferior material to any substance. Example: The addition of yellow soil to powdered turmeric powder
Substitution	Impairment of the quality by substitution means entirely different material is used instead of original drug. Example: Cotton seed oil is sold in the place of olive oil

Following are few Examples of Adulterants of various crude drugs	
Crude drug name	**Adulterant**
Aconite	Japanese aconite (*A. unicinatum)* and Indian aconite (*A. chasmanthum)*
Alexandrian senna (Cassia acutifolia)	Dog senna, Palthe senna, Bombay, Mecca or Arabian senna.
Aloes (Aloe barbadensis)	Natal aloes which contain natalion, homonatalion and resin with nataloresinotannol; Mocha aloes, black catechu, pieces of iron and stones.
Arachis oil	Cotton seed oil or sesame oil.
Arjuna	*Terminalia tomentosa* (Etheral extract of arjuna gives pinkish fluorescence, while *T. Tomentisa* gives pale blue)
Artemisia	*Artemisia vulgaris* Linn (Compositae)
Asafoetida	Gum Arabic, rosin, gypsum, red clay, chalk and barley or wheat flour.
Belladonna herb	Leaves of *Phytolacca americana* (Idioblast present), *Solanum nigrum*, and *Ailanthus glandulosa* (needle shaped crystals of calcium oxalate present).
Black pepper	*Piper attenuatum , Piper brachystachyum* , *Piper longum*
Caraway	Indian dill fruits. *Cuminum cyminum* (contains cuminic aldehyde)
Cardamom	Orange seeds and unroasted coffee grains, *Elettaria cardamom*, Korarima cardamom, Cardamom husk
Chenopodium oil	*Chenopodium ambrosoides, Chenopodium album*
Chirata	*S. Densifolia , S. Ciliate , S. Paniculata.*
Cinchona	Cuprea bark (Remijia pedunculata, a coppery red coloured drug, contain quinine, quinidine and other alkaloid which resemble to those from cinchona bark. The bark contains numerous stone cells. Along with cinchona alkaloids, it also contains cupreine. False cupre bark (*R.purdiena*) contains alkaloids called cusconidine, traces of cinchonine, cinchonamine, but no quinine.
Cinnamon	Jungle cinnamom , Cinnamom chips, Saigolcinnamom, *Cinnamomum loureirii* (Lauraceae), Java cinnamom, *Cinaamomumburmanii* (Lauraceae).
Clove	Mother clove, Blown clove, Clove stalks
Digitalis	Leaves of *Verbascum thapsus* (Schophulariaceae) contain large woolly branched candelabra trichomes. The primrose leaves from *Primula vulgaris* (Primulaceae) contains uniseriate covering trichomes, which are 8 to 9 celled long. Comfrey leaves from *Symphytum officinale* (Boraginaceae) contains multicellular trichomes forming hook at the top.
Dill	*Anethum sowa*
Dioscorea	*Dioscoreaflouribunda* and *D.villosa*Linne
Ephedra	*E. eduisetina* and *E. sinica* (Both Chinese). *E. intermedia*, *E. major, E. helryetica* and *E. alata,Aconitum napelles* (Ranunculaceae): *Sida cordifolia;* and *S. rhombifolia*(Malvaceae); *Roemeriarefracta* (Papaveraceae); and *Taxus baccata* (Taxaceae)
Fennel	Exhausted fennel fruits
Ginger	Exhausted ginger
Guggul	Resins of various *commiphora* species like C. abyssinica, *C. roxburghii, C. molmol* and *Boswellia serrata.*
Honey	Artificial invert sugar contains furfural which is detected by Fieh's test and by resorcinol in hydrochloric acid.
Liquorice	Manchurian liquorice from *Glycyrrhiza uralensis*
Male fern	Lady fern Athyrium filix-foemina.
Musk	Beaver (Castor fiber), civet (Viuerrazibetha) and America musk (Fiber zibeythicus), Musk mallow (*Abelmoschus moschantus*)
Myrrh	Arabian myrrh, Yemen myrrh, Indian bdellium (Balsamodendronmukul)

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Crude drug name	Adulterant
Opium	*Papaver argemone, P. dubium, P.orientate, P. psendoorientale*and *P. bracteatum* which does not contain morphine and hence new source of opiate.
Peppermint oil	Mentha oil
Psyllium seed (Flea seed)	Seeds of *Plantago psyllium*, Seeds of *Plantago lanceolata*
Punarnava	*Trianthemaportulacastrum, Trianthemaobcordata*and *T. decandra*
Rasna	*Apinia galangal* (Java galangal or Greater galangal)
Rauwolfia	*Reserpine containing*: African rauwolfia species(Rauwolfia vomitoria, caffra, R. cumminsfi, R.mombasiana, R. oreogiton, R. obscura, R. rosea and R. volkensii), R.tetraphylla and R. nitida, Alstoniavenenata and A. constricta; *Ajmalicine containing*: Catharanthus roses; *Yohimbine containing*: Pausinystaliayohimba,
Rhubarb (Indian rhubarb)	Rhaphontic rhubarb obtained from rhizome of *R. rhaphonticum.* It lacks rhein , emodin or aloe-emodin butit contains rhaphonticin.
Senega	Indian senega is *Polygala chinesis* Linn which does not contain Spurious Indian senega is *Glinusoppositifolia* family Molluginaceae. It contains a Saponins and starch. It shows several rings of vascular bundles. White senega is root of *Polygala alba* does not show keel
Shankhpushpi	*Canscoradiffusa*
Spermaceti	Mixtures of esters of saturated fatty alcohol and saturated fatty acids
Starch (Maize, Rice, Wheat, Potato)	Tapioca starch or cassava or Brazilian arrowroot obtained from Manihot esculenta (Euphorbiaceae)
Storax	Rosin, olive oil, Red Gum or Sweet Gum or American storax from *Liquidamabarstyraciflia*, Stramonium; *Xanthium strumarium* (Compositae) and *Solanum nigrum* (Solanaceae). Later contain no calcium oxalate crystals.
Crude drug name	**Adulterant**
Thyme	Wild thyme: *Thymus serpyllum* (Labiateae)
Tolu Balsam	Exhausted balsam of tolu, Fictitious tolu Balsam, Colophony
Turmeric	*Curcuma amda*
Turpentine oil	Resin oil, wood turpentine and petroleum jelly
Yellow Bees wax	Colophony, hard paraffin, stearic acid, japan wax, spearmaceti, carnauba wax

Following are WHO parameters for standardization of herbal raw material, extracts and their products	
Preliminary evaluation	Sampling, Foreign matter determination, Determination of total fiber
Morphological evaluation	Qualitative evaluation of color, odor and taste, size, shape, extra features ~~etc~~
Microscopical evaluation:	➢ Qualitative microscopy: histological evaluation of types and arrangements of tissues ➢ Quantitative microscopy: o *Leaf constant*: assessment of palisade ratio, vein-islet, vein termination, stomatal index, stomatal number o *Lycopodium spore method* ➢ Powder microscopy
Physical Qualitative evaluation	Solubility, refractive index, optical rotation, melting point, boiling point, density, viscosity, chromatographic and spectroscopic evaluation
Physical quantitative or Physicochemical evaluation	Ash value, extractive value, moisture content, volatile oil determination
Chemical evaluation	➢ *Qualitative chemical evaluation*: to detect different classes of phytochemicals ➢ *Quantitative chemical evaluation*: determination of phytochemicals, assay

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WHO Specific Parameters	Swelling index Foam index Hemolytic index Bitterness value Total tannin value
Biological evaluation	
Toxicological evaluation	Microbial load determination Aflatoxin detection Pesticide residue determination Radioactive contamination Heavy metal detection
Pharmacological evaluation	In-vivo, ex-vivo evaluation (Animal, animal organ or tissue activities)
Analytical evaluation	Chromatographic (TLC, Paper, HPTLC, HPLC and GC data) and spectroscopic evaluation
Along with above parameters there is need to evaluate herbal formulation for specific pharmaceutical parameters, such as: tablet: weight variation, friability, disintegration, and dissolution.	

Morphological Evaluation

Morphological or organoleptic evaluation is preliminary examination and considered as a first step towards establishment of identity and degree of purity. This is the evaluation by means of organs of senses to evaluate appearance of the drug, its odour and taste, occasionally the sound or snap of its fracture and feel of the drug to the touch. In the case of whole crude drugs, the macroscopic and sensory characters are usually sufficient to enable the drug to be identified. It provides simplest and quickest mean to establish the identity and purity and thereby ensure quality of a particular sample. Judgment may vary from person to person and time to time based on individual's nature. Description of these features are very difficult so that often the characteristic like odour and taste can only described as "characteristic" and reference made to the analyst's memory. The organoleptic characterization is based on the color, odor, shape, size, surface feel, texture of whole crude drug, fracture and appearance of the cut surface.

Leaf venations			
 Arcuate -Secondary arching toward the apex	 Dichotomous- Veins splitting in two	 Parallel -All veins parallel	 Longitudinal -All veins aligned mostly with the midvein
 Reticulate - repeated branching, net veined	 Rotate - radiating veins from the center of the leaf	 Transverse -Tertiary veins perpendicular main vein connecting secondary veins	 Pinnate -Secondary veins borne from midrib

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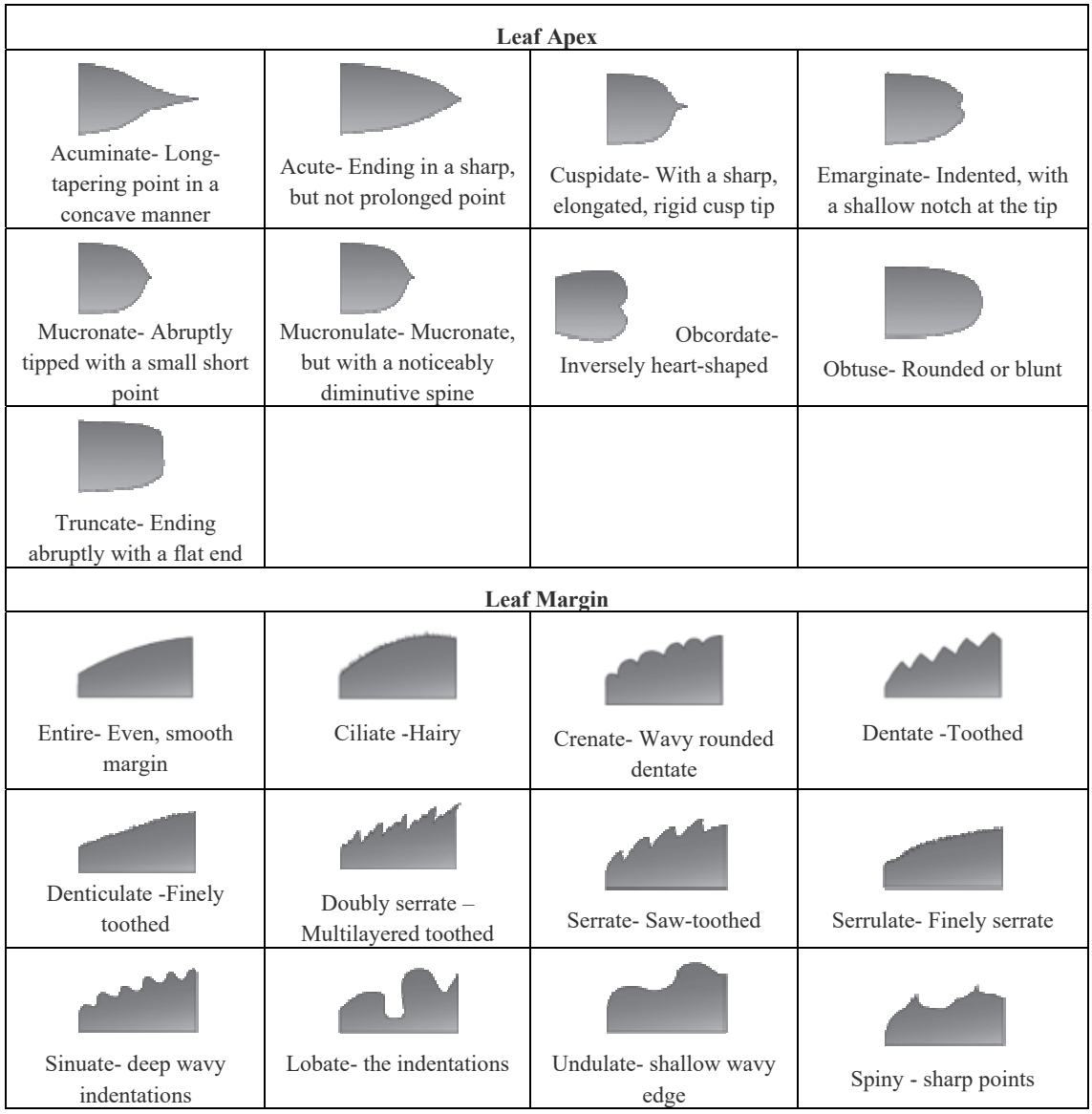

Fig. 1.1 Types of leaf venentaions, apex and margin

- **Color:** It is to be examine under an artificial light source and or day light. The color of the sample should be compared with that of a reference material. Example: Senna leaves are fresh green in color while Digitalis leaves are dark green in color.
- **Odour**: Slow and repeated inhalation of the material provides necessary information of its odour. Where no distinct odour is perceptible, crude drug is to be crushed using gentle pressure to verify exact odour. If the material is known to be toxic or dangerous, then determine its odour by other suitable means such as pouring a small quantity of boiling water

on to the crushed sample placed in a beaker. Determine the strength of the odour like weak, distinct, strong, characteristic, and then the sensation like musty, moldy, rancid, fruity, aromatic etc. Example: Essential oil containing crude drugs have aromatic odour while ergot, vinca like crude drugs have disagreeable odour and many of crude drugs are odourless.

- **Taste**: Non-toxic crude drugs can be tasted while toxic crude drugs like Nux-vomica, Aconite should not be tasted. Example: Most of alkaloid containing drugs are bitter in taste; senna leaves have mucilaginous taste while digitalis leaves are bitter in taste; Licorice is sweet in taste and cinnamon is in sweet-pungent in taste.
- **Size and shape:** The length, width and thickness of the crude materials are of great importance while evaluating a crude drug. Example: Width of Indian senna is smaller than Tinnevelly senna leaves. Length of *Digitalis lanata* leaves is more than *Digitalis purpurea* leaves. Rauwolfia root is wavy (snake like) in shape. Following are shapes of barks:
  - Flat: Arjuna bark
  - Curved: Cassia bark
  - Re-curved: Kurchi bark
  - Channeled: Cinchona bark
  - Quill: Casacara bark
  - Double quill: Cinnamon bark

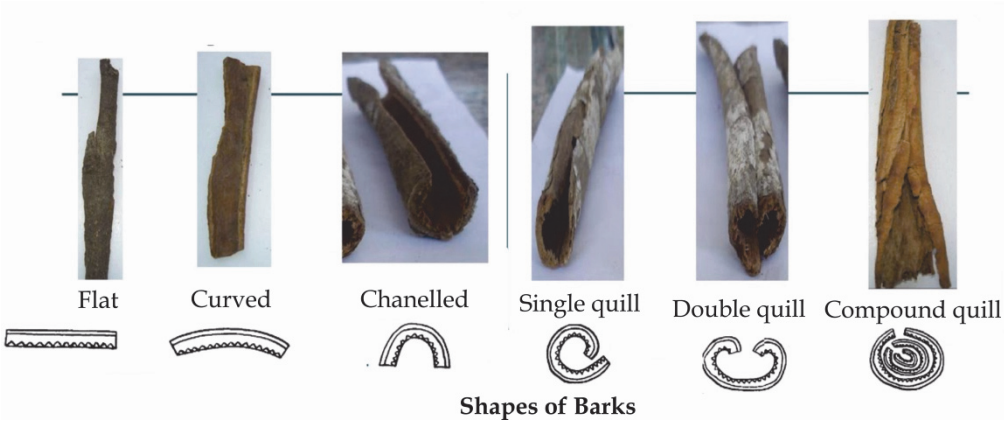

Fig. 1.2 Various shapes of bark

- **Extra features:** The texture is best examined by taking a small quantity of material and rubbing it between the thumb and forefinger, it is usually described as 'smooth', 'rough', 'gritty'. Touch of the material describes its softness or hardness. Bend and rupture caused to the sample, provides information of the brittleness.

  Following are important morphological characters of bark crude drugs:
  - Lenticle: Pores on stem of woody plant for gaseous exchange
  - Lichens: composite organism from symbiosis association of algae, cyanobacteria and fungi

- Fissures: Deep cracks
- Striations: Parallel or longitudinal linings
- Furrows: Troughs between wrinkles
- Corrugations: Inner surface wrinkles
- Fracture: Appearance of broken bark surface

Appearance of the fractured plane as fibrous, smooth, rough, granular, etc. helps in distinguishing bark, stem and root like crude drugs. All these characteristics are valuable in indicating the general type of material and the presence of more than one adulterant components. Example: Nux-vomica seed exhibits smooth silky feel due to presence of trichomes. Outer surface of Rhubarb rhizome shows presence of star spots and cascara bark shows presence of lenticles and lichen.

Microscopical Evaluation

Qualitative microscopical evaluation: It involves histological study of type and arrangement of tissues, presence of characteristic features such as stomata, starch grains, calcium oxalate crystals by using magnification power of microscopes. It utilizes stains to distinguish and identify different microscopical characters. Example: iodine for starch detection, phloroglucinol and HCl for lignin detection, Sudan red –III for oil detection etc.

Starch grains

Starch is composed of amylose and amylopectin, with the level of amylose ranging from 20% to 30% for most cereal starches. Starch grains are typically microscopically identified with either optical or electron microscopy. Starch grains can become clearer if they are stained a darker color with Iodine Stains. Logol's Iodine is one, used for staining starch because iodine reagents easily bind to starch but less easily to other materials. Features that allow identification of starch grains include: presence of hilum (core of the grain), lamellae (or growth layers), birefringence, and extinction cross (a cross shape, visible on grains under revolving polarized light) which are visible with a microscope and shape and size.

Structure and Amylose Content of Some Whole Granular Cereal Starches

Source	Granule Shape	Granule Size (nm)	Amylose Content (%)
Wheat	Lenticular or round	20–25	22
Maize	Round or polyhedral	15	28
Waxy maize	Round	15 (5–15)	1
High-amylose	Round or irregular sausage-shaped	25	52
Barley	Round or elliptical	20–25	22
Rice	Polygonal	3–8	17–19[a] 21–23[b]
Oats	Polyhedral	3–10	23–24

a-Japonica, b-Indica

Adapted from Lineback (1984).

Calcium Crystals

There are two types' of calcium crystals forms due to excess carbonic acid or oxalic acid.

Calcium carbonate Rare and generally associated with cell wall They are also called as cystoliths as they appear in the form of grapes in the tissues. Example: *Ficus elastica*

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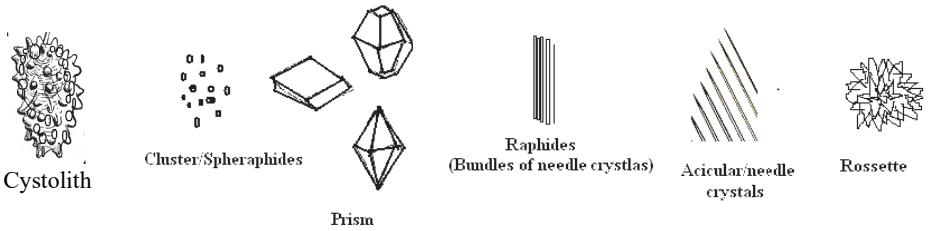


Calcium oxalate

Very common and present in almost each part of plant.

Prisms/single crystals: they are large, single or small groups and well developed.

Cluster crystals/ spheraphides: they are group of numerous prisms/pyramids. The crystal are projecting, pointed, acute angled, and more or less spherical.

Rosette crystals: they are large number of crystals in spherical mass (in the centre of which is an organic substance). Components of crystals radiate from the centre to the periphery and form a toothed circumference.

Acicular crystals/ raphids: they re needle like, slender, long pointed at the ends. They may be single or in bundles.

Microcrystal/ crystal sand / micro sphenoid: occur like an amorphous mass in cell. They are very minute and are present in large number in a single cell which is usually enlarged than other cells and is called idioblast.

Table 1.1 Common crude drugs and their Calcium crystals

Crude drugs	Type of calcium crystals
Asparagus, Aloe, Centella, Clove Flower Bud, Digitallis, Ephedra, Ginger, Isapgol, Nuxvomica	Absent
Azadircata, Senna, Clove, Rhubarb, Wild cherry bark, Tinosperma	Prism and cluster
Bacopa, Rauwolfia, Vasaka, Liquorice, Clove stalk, Senna, Kurchi, Cocca, Qaussia, Cascara	Prism
Cinchona	Microprism
Caraway	Rosette
Coriander, Dill, Fennel	Microrosette
Cassia, Cinnamon, Gentian	Acicular
Ipecac, Squill	Raphides
Cinnamon	Tubular
Datura	Spherophide crystals
Eucalyptus, Podophyllum	Clusters
Kurchi	Rhomboidal
Vinca	Microtubular, tactoid or needle shaped
Withania, Cinchona, Belladonna	Microsphenoid

Stomata

It is a pore, found in the upper epidermis of leaves, stems, and other organs, that controls the rate of gas exchange. The pore is bordered by a pair of specialized parenchyma cells known as guard cells that are responsible for regulating the size of the stomatal opening.There are different types of stomata and they are mainly classified based on their number and characteristics of the surrounding subsidiary cells. Listed below are the different types of stomata.

Types of stomatas present in Dicots:

- ***Paracytic(meaning parallel celled) or rubiaceous type:*** stomata have one or more subsidiary cells parallel to the opening between the guard cells. These subsidiary cells may reach beyond the guard cells or not. Examples families like Rubiaceae, Convolvulaceae and Fabaceae.

Contd….

- ***Diacytic(meaning cross-celled) or caryophyllaceous type***: stomata have guard cells surrounded by two subsidiary cells, that each encircle one end of the opening and contact each other opposite to the middle of the opening. Examples families like Caryophyllaceae and Acanthaceae.
- ***Anomocytic(meaning irregular celled) or ranunculaceous type:*** stomata have guard cells that are surrounded by cells that have the same size, shape and arrangement as the rest of the epidermis cells. Examples families like Apocynaceae, Boraginaceae, Chenopodiaceae, and Cucurbitaceae.
- ***Anisocytic(meaning unequal celled) or cruciferous type:*** stomata have guard cells between two larger subsidiary cells and one distinctly smaller one. Examples families like Brassicaceae, Solanaceae, and Crassulaceae.
- ***Actinocytic(meaning star-celled)*** stomata have guard cells that are surrounded by at least five radiating cells forming a star-like circle. Examples families like Ebenaceae.
- ***Hemiparacyticstomata*** are bordered by just one subsidiary cell that differs from the surrounding epidermis cells, its length parallel to the stoma opening. Examples families like Molluginaceae and Aizoaceae.

Types of stomatas present in Monocots:

- ***Gramineous (meaning grass-like) stomata*** have two guard cells surrounded by two lens-shaped subsidiary cells. The guard cells are narrower in the middle and bulbous on each end. This middle section is strongly thickened. The axis of the subsidiary cells are parallel stoma opening. Examples families like Poaceae and Cyperaceae.
- ***Hexacytic(meaning six-celled) stomata*** have six subsidiary cells around both guard cells, one at either end of the opening of the stoma, one adjoining each guard cell, and one between that last subsidiary cell and the standard epidermis cells.
- ***Tetracytic(meaning four-celled) stomata*** have four subsidiary cells, one on either end of the opening, and one next to each guard cell. This type occurs in many monocot families, but also can be found in some dicots. Examples families like Tilia and several Asclepiadaceae.

Types of stomatas present in ferns:

- ***Hypocyticstomata*** have two guard cells in one layer with only ordinary epidermis cells, but with two subsidiary cells on the outer surface of the epidermis, arranged parallel to the guard cells, with a pore between them, overlying the stoma opening.
- ***Pericyticstomata*** have two guard cells that are entirely encircled by one continuous subsidiary cell (like a donut).
- ***Desmocyticstomata*** have two guard cells that are entirely encircled by one subsidiary cell that has not merged its ends (like a sausage).
- ***Polocyticstomata*** have two guard cells that are largely encircled by one subsidiary cell, but also contact ordinary epidermis cells (like a U or horseshoe).

Table 1.2 Common crude drugs and their stomata

Crude drugs	Type of Stomata
Senna, Coca	Paracytic (Rubiaceous)
Centella,Vasaka, mentha, peppermint, spearmint	Diacycytic (Caryophyllaceous)
Belladonna, stramonium, Datura, henbane, Vinca	Anisocytic(Cruciferous or unequal celled)
Digitallis, azadircata, bacopa, eucalyptus	Anomocyctic (Ranunculaceous)

Trichomes Trichomes on plants are epidermal outgrowths of various kinds.These are fine outgrowths or appendages on plants, algae, lichens, and certain protists.Trichomes can protect the plant from a large range of detriments, such as UV light, insects, transpiration, and freeze intolerance. Glandular trichomes found to store secondary metabolites like volatile oil, flavonoids etc.

Contd….

Trichome type may assess the number of cells per trichome. A unicellular trichome consists of a single cell and is usually quite small. A multicellular trichome contains two or more cells. Multicellular trichomes can be either uniseriate, having a single vertical row of cells, or multiseriate, having more than one vertical row of cells. The number of cell layers in a trichome can also be diagnostic.

Many trichomes are diagnosed based on their general shape and morphology. Tapering trichomes are those ending in a sharp apex. Malpighian or dolabriform (also termed "two-armed" or "T-shaped") trichomes are those with two arms arising from a common base. (Malpighian is named after the family Malpighiaceae, where this trichome type is common.) Glandular trichomes are secretory or excretory trichomes, usually having an apical glandular cell. Glandular trichomes can be pilate-glandular, with a glandular cell atop an elongate basal stalk, or capitate-glandular, with a glandular cell having a very short or no basal stalk. Branched trichomes include two types: stellate, which are star-shaped trichomes having several arms arising from a common base (either stalked or sessile); and dendritic, which are treelike trichomes with multiple lateral branches. Peltate trichomes are those with a disk-shaped apical portion atop a peltately attached stalk.

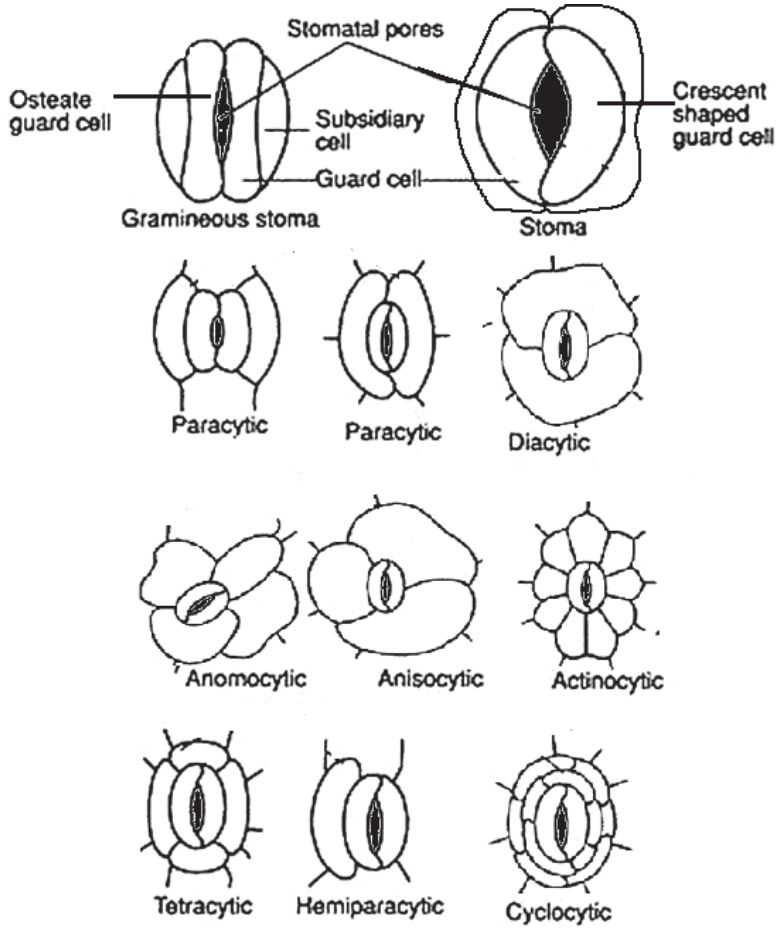


Fig. 1.3 Various types of stomata

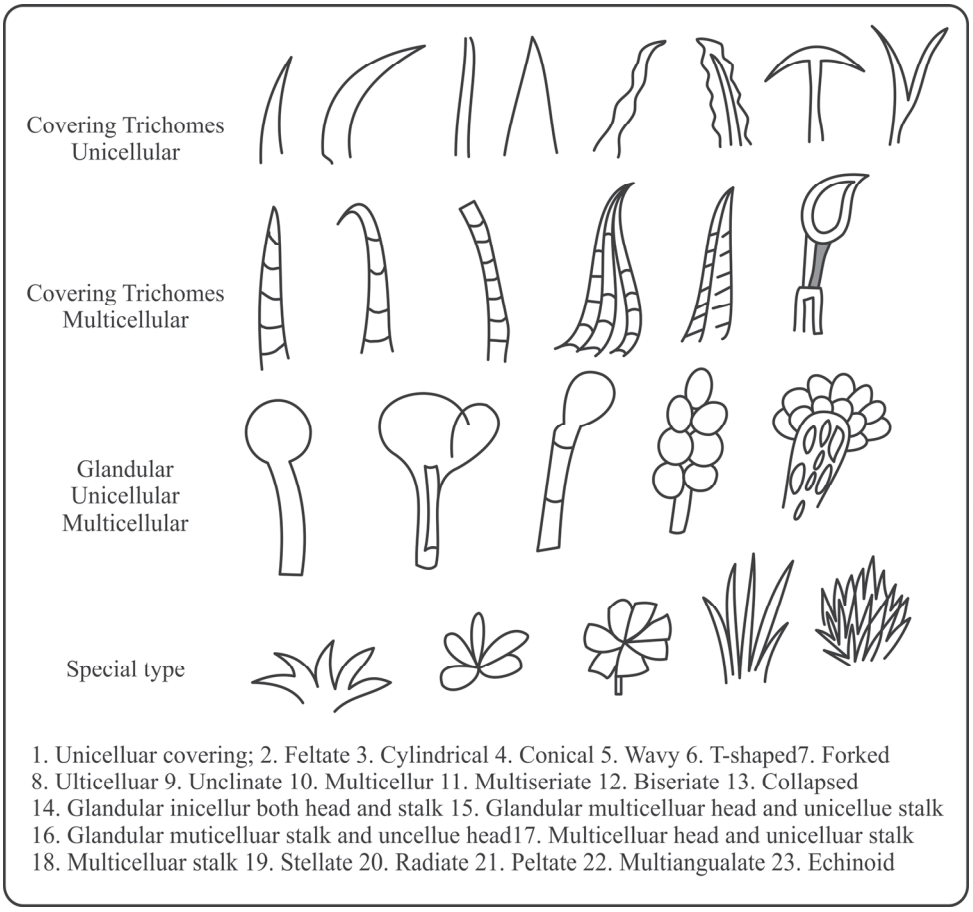
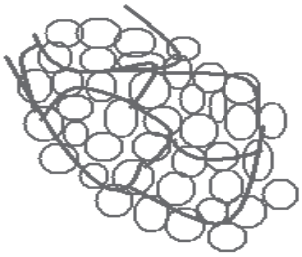


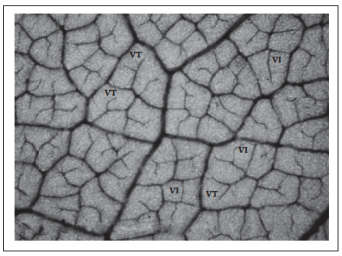
Fig. 1.4 Various types of trichomes

Quantitative microscopical evaluation: It involves determination of quantity of microscopical characters such as

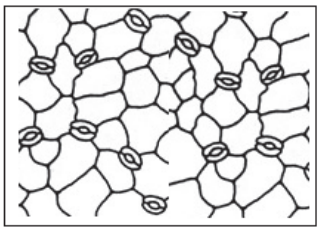
- ***Leaf constant***: Palisade ratio, Vein islet number, Vein termination number, Stomatal number, Stomatal index
- ***Lycopodium spore method***: Newer technique which can be applied to determine percentage purity of any plant part crude drug unlike leaf constants which are applicable only to leaf crude drugs. Additional significance of this method is that it requires powder form of crude drugs unlike leaf constants which requires fresh or dry whole crude drug. Whole fresh or drug crude drugs cannot available throughout year while powder form of crude drug can be available and stored easily.



Palisade ratio



Vein islet (VI) and vein termination (VT) number

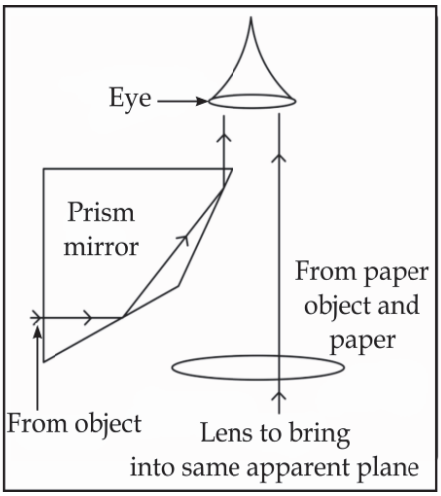


Stomatal number and stomatal index

Fig. 1.5 Leaf constants

Camera Lucida

The name "camera lucida" (Latin for "light chamber") is an optical device used to draw microscopic images. It facilitates accurate sketching of minute objects. Originally the camera lucida performs an optical superimposition of the subject being viewed upon the surface upon which the artist is drawing. The principle is very simple. By looking into the prism from just the right angle, two images will enter the eye; one, of the object to be sketched, the other of the pencil and paper with which you intend to work with. The resulting effect is that your eye perceives illusion of seeing objects in front of the instrument on the drawing surface beneath. This is possible only after proper angle adjustment between mirror or prism and drawing board.



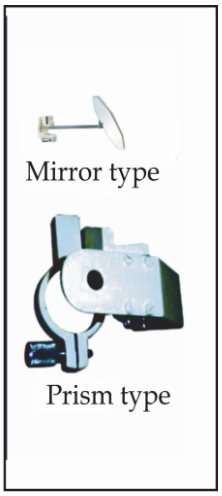


Fig. 1.6 (a) Principle of working camera lucida **Fig. 1.6 (b)** Types of camera lucida

Two types of camera lucida are available in market as given in figure 1.6 (b). There are three main parts of camera lucida – attachment ring, prism and mirror. Attachment ring attaches camera lucida around the body tube and prism over eyepiece. Prism reflects the image on mirror and then mirror reflect it on paper. Thus it allows passing light from prism to paper and from paper to prism as shown in figure 1.6(a). Thus this is a unique tool to aid perspective drawing and the recording of fine detail.

Micrometer

Micrometer is scale which is used to measure microscopically magnified images like cells, fibers, trichomes, starch grains and calcium oxalate crystals.

***Stage micrometers**:* Stage Micrometer is simply a microscope slide with a finely divided scale of 1 mm marked on the surface. 1 mm is divided into 100 divisions so 1 division is 1/100 i.e. 10 um. This micrometer is just useful to calibrate eyepiece micrometer reticle. Stage Micrometer is removed after calibration and slide of sample is placed.

***Eyepiece micrometer**:* An ocular micrometer is a glass disk with etched scale that fits in place of a eyepiece and this micrometer is actually used to measure the size of magnified objects. In this micrometer scale of 1 mm is also divided into 100 divisions so 1 division is 1/100 i.e. 10 um. But the physical length of the marks on the scale depends on the degree of magnification which varies due to objective/eyepiece combination and mechanical tube length of the microscope. Hence it is very necessary to calibrate eyepiece micrometer divisions by stage micrometer and arbitrary units of the transfer scale (reticle) must be converted to absolute units, such as millimeters or micrometers.

Calibration of eyepiece micrometer: Calibration of the scale is commonly performed by imaging a stage micrometer with the objective to be used for specimen measurements. Put stage and eyepiece micrometer in appropriate place. Superimpose the scale of stage and eyepiece micrometer. Observe any one line of stage micrometer which is coinciding with eyepiece micrometer and then see the next line of stage micrometer which is coinciding with eyepiece micrometer. Count the divisions between these two lines.

Example:

3 divisions of stage micrometer = 29 divisions of eyepiece micrometer

But 1 division of stage micrometer = 10 um

Hence 30 um = 29 divisions of eyepiece micrometer

So 1 divisions of eyepiece micrometer = 30/29 = 1.03 um

This value is often referred to as the micrometer value, or calibration factor, for that particular objective. Once the value has been determined, the size of any specimen feature may be calculated by multiplying the number of eyepiece reticle divisions spanned by the feature with the calibration factor for the objective in use.

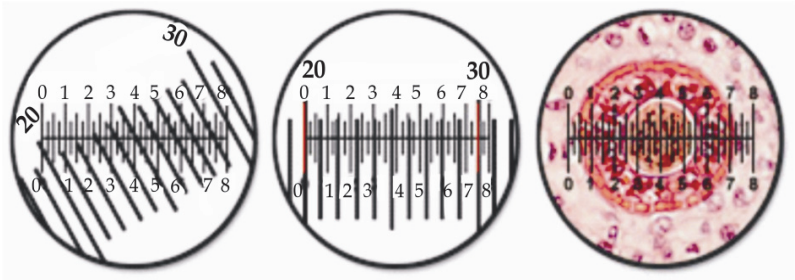


Fig. 1.7 Calibration and measurment by micrometer

Leaf constant determination

Palisade ratio: This is average number of palisade cell beneath each epidermal cell. It can be also determined with powdered drugs.

Procedure: Clear a piece of the leaf by boiling in choral hydrate solution for about thirty minutes. Arrange camera lucida and drawing board for making drawings to scale. Place stage micrometer on the microscope and using 16 mm objectives, draw a line equivalent to 1 mm as seen through the microscope. Construct a square on this line. Move the paper so that the square is seen in the eye piece, in the centre of the field. Place the slide with the cleared leaf (epidermis on the stage). Trace off at least four epidermal cells and draw palisade cells beneath each epidermal cell. Calculate average palisade cell beneath each epidermal cell.

Vein-islet number: This is number of vein-islets per square mm of the leaf surface midway between midrib and margin.

Procedure: Clear a piece of the leaf by boiling in choral hydrate solution for about thirty minutes. Arrange camera lucida and drawing board for making drawings to scale. Place stage micrometer on the microscope and using 16 mm objectives, draw a line equivalent to 1 mm as seen through the microscope. Construct a square on this line. Move the paper so that the square is seen in the eye piece, in the centre of the field. Place the slide with the cleared leaf (epidermis on the stage). Trace off the veins which are included within the square, completing the outlines of those islets which overlap two adjacent sides of the square. Count the number of vein islets in the square millimeter. Where the islets are intersected by the sides of the square, include those on two adjacent sides and exclude those islets on the other sides. (To obtain a critical result for a leaf, 4 sq mm should be used, preferably in one large area of 4 sq mm). Find the average number of vein islets from the four adjoining squares, to get the values for one sq mm.

Examples of Vein-islet numbers of few leaf crude drugs

Vein-islet numbers	Species	Range of vein-islet numbers	Average
Senna	*Cassia senna*	15–29.5	26
	Cassia angustifolia	19.5–22.5	21
Coca	*Erythroxylum coca*	8–12	11
	Erythroxylum truxillense	15–26	20
Digitalis	*Digitalis purpurea*	2–5.5	3.5
	Digitalis lanata	2–3.5	2.7
		3–8	4.4
	Digitalis lutea	1–1.5	1.2
	Digitalis thapsi	8.5–16	

Vein–termination number: This is number of vein termination per square mm of the leaf surface midway between midrib and margin.

Procedure: Clear a piece of the leaf by boiling in choral hydrate solution for about thirty minutes. Arrange camera lucida and drawing board for making drawings to scale. Place stage micrometer on the microscope and using 16 mm objectives, draw a line equivalent to 1 mm as seen through the microscope. Construct a square on this line. Move the paper so that the square is seen in the eye piece, in the centre of the field. Place the slide with the cleared leaf (epidermis on the stage). Trace off the veins which are included within the square, completing the outlines of those islets which overlap two adjacent sides of the square. Count the number of veinlet terminations present within the square. Find the average number of veinlet termination number from the four adjoining squares, to get the values for one sq. mm.

Contd….

Examples of Vein- termination numbers of few leaf crude drugs	
Veinlet termination numbers	
Atropa acuminata	1.4–3.5
Atropa belladonna	6.3–10.3
Cassia angustifolia	25.9–32.8
Cassia senna	32.7–40.2
Datura stramonium	12.6–20.1
Digitalis purpurea	2.5–4.2
Erythroxylum coca	16.8–21.0
Erythroxylum truxillense	23.1–32.3
Hyoscyamus niger	12.4–19.0

Stomatal number: This is average number of stomata per square mm of epidermis of the leaf.

Procedure: Clear the piece of the leaf (middle part) by boiling with chloral hydrate solution or alternatively with chlorinated soda. Peel out upper and lower epidermis separately by means of forceps. Keep it on slide and mount in glycerin water. Arrange a camera lucida and drawing board for making the drawings to scale. Draw a square of 1 mm by means of stage micrometer. Place the slide with cleared leaf (epidermis) on the stage. Trace the epidermis cell and stomata. Count the number of stomata present in the area of 1 sq. mm. Include the cell if at least half of its area lies within the square. Record the result for each of the ten fields and calculate the average number of stomata per sq mm.

Stomatal index: This is percentage of the number of stomata forms to the total number of epidermal cells each stoma being counted as one cell.

Procedure: Clear the piece of the leaf (middle part) by boiling with chloral hydrate solution or alternatively with chlorinated soda. Peel out upper and lower epidermis separately by means of forceps. Keep it on slide and mount in glycerin water. Arrange a camera lucida and drawing board for making the drawings to scale. Draw a square of 1 mm by means of stage micrometer. Place the slide with cleared leaf (epidermis) on the stage. Trace the epidermis cell and stomata. Count the number of stomata, also the number of epidermal cells in each field. Calculate the stomatal index using the above formula. Determine the values for upper and lower surface (epidermis) separately.

$$\text{Stomatal index} = \frac{\text{Stomatal number}}{\text{Toal number of stomata + Total number of epidemol cells}} \times 100$$

Examples of stomatal index of few leaf crude drugs		
Stomatal index	**Upper surface**	**Lower surface**
Atropa acuminata	1.7 to 4.8 to 12.2	16.2 to 17.5 to 1.83
Atropa belladonna	2.3 to 3.9 to 10.5	20.2 to 21.7 to 23.0
Cassia senna	11.4 to 12.4 to 13.3	10.8 to 11.8 to 12.6
Cassia angustifolia	17.1 to 19.0 to 20.7	17.0 to 18.3 to 19.3
Datura inermis	18.1 to 18.3 to 18.7	24.5 to 24.9 to 25.3
Datura metel	12.7 to 17.4 to 19.4	21.2 to 22.3 to 23.9
Datura stramonium	16.4 to 18.1 to 20.4	24.1 to 24.9 to 26.3
Datura tatula	15.6 to 20.2 to 22.3	28.3 to 29.8 to 31.0
Digitalis lanata	13.9 to 14.4 to 14.7	14.9 to 16.1 to 17.6
Digitalis lutea	2.5 to 5.5 to 8.4	21.6 to 22.9 to 25.2
Digitalis purpurea	1.6 to 2.7 to 4.0	17.9 to 19.2 to 19.5
Digitalis thapsi	5.9 to 7.0 to 7.8	11.9 to 12.4 to 13.5
Erythroxylum coca	Nil	12.2 to 13.2 to 14.0
Erythroxylum truxillense	Nil	8.9 to 10.1 to 10.7
Phytolacca acinosa	Nil	15.0
Phytolacca americana	2.9 to 4.2 to 5.7	13.0 to 13.2 to 13.4

Lycopodium spore method:

Lycopodium is composed of the spores of *Lycopodium clavatum L.* each spore is tetrahedral in shape, the base is rounded and the three flat sides meet to form three well-marked covering ridges, which join one another at the apex. The whole surface of the spore is covered with minute reticulations and the interior is filled with fixed oil. The spores are exceptionally uniform in size (25 μm), so that one can always know that a definite number of spores represent a particular weight of lycopodium. The whole process can be simplified as 1 mg of spores contains averagely 94000 spores. By this figure one can calculate the weight of any number of spores under any condition under the microscope. If the lycopodium has been fixed with a definite proportion of another substance, one can find immediately how much of the second substance has been added, when examined microsopically. If it is admixed with any fine particles like pollen grains, starch etc. with characteristic countable particles it is possible to calculate the number of such characteristic particles per mg. In this way it is possible to have a standard figure that represents any such material. The number of characteristic particles per unit weight is often constant and is useful in assessing the quality of a sample. To use this method the number of particles in a good quality sample must either be known or first determined.

$$\%\ \text{Purity}: \frac{N \times W \times 940000}{S \times M \times P} \times 100$$

where

N = Number of Characteristic particlesof sample in 25 fields

W = Weight of Lycopodium spores taken in mg

S = Number of Lycopodium spores in 25 fields

M = Weight of sample in mg

P = Standard value of number of characteristic samples per mg in taken sample material (Example: 1 mg ginger powder contains 2, 86, 000 starch grains)

Powder Microscopical Evaluation

Powder microscopical evaluation is done using powders of crude drugs unlike to histological studies where whole crude drug is used. Every time it is impossible to obtain fresh or to store whole dried crude drug so powder microscopy is most feasible. All microscopical characters can be observed in disperse form without intact information like exact arrangement of cells, tissues.

Procedure: Clear powder with clearing agents. Spread thin layer of powder on glass slide and observe under microscope. To differentiate cells (lignified and non-lignified, starch grains, oil glands) use staining reagents. Following characters can be observed according to plant parts:

Common Powder characteristics of different plant parts are as follows:

Leaves	Epidermal cells, palisade cells, stomata, trichomes, calcium crystals, starch grains,
Roots/Rhizomes	Cork cell, parenchyma cells, phloem fibers, xylem, calcium crystals, starch grains, stone cells
Bark/wood	Cork cell, parenchyma cells, phloem fibers, xylem, calcium crystals, starch grains, stone cells, pericyclic fibers, sclerides, fibers
Flowers	Epidermal cells, anthers, pollen grains, oil globules, pigments,
Seeds	Endosperm, oil glands, aleurone grains, starch grains, pigment
Fruits	Epidermal cells, pericarp, mesocarp, oil glands (vittae), sclerenchymatous cells

Physical Evaluation

Qualitative Physical Evaluation

This evaluation gives idea about quality of crude drug either pure or impure. This involves determination of following parameters:

- *Solubility*: Solubility is the property of a solid, liquid, or gaseous chemical substance called solute to dissolve in a solid, liquid, or gaseous solvent to form a homogeneous solution of the solute in the solvent.
  - Fats and oils: soluble in non polar solvents like petroleum, ether, benzene, and hexane
  - Carbohydrates, glycosides, tannins, flavonoids: soluble in different types of alcohols or water
  - Aglycone part of glycosides, bases of alkaloids: soluble in non-polar solvents

Descriptive Term	Parts of Solvent for 1 part of solute
Very Soluble	Less than 1
Freely Soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly Soluble	From 30 to 100
Slightly Soluble	From 100 to 1000
Very Slightly Soluble	From 1000 to 10,000
Practically Insoluble, or Insoluble	More than 10,000

- *Optical rotation* of a liquid is the angle through which the plane of polarization of light is rotated when the polarized light is passed through a sample of the liquid, rotation clockwise or anticlockwise. Clove oil 0 to -1.5 while eucalyptus oil having 0 to +10.
- *Melting point*: All solid pure phytochemicals should be evaluated for its melting point. Difference in melting point indicates presence of impurities.
- *Boiling point*: This parameter is applicable to all liquid phytochemicals like essential oils or few alkaloids. Shift in boiling point range helps in determining purity of phytochemicals.
- *Refractive index (RI)*: The refractive index of a substance is the ratio between the velocity of light in air and the velocity in the substance under test. The refractive index of the material is given by the sine of the angle of incidence divided by the since of the angle of refraction. The RI varies with the temperature; Pharmacopoeial determinations are made at 20°C.
- *Viscosity*: Viscous natural drugs like gums, mucilages or pectin like compounds should be evaluated for its viscosity.
- *Density*, *specific gravity determination*: All liquid phytochemicals have to be evaluated for its density and specific gravity. This parameter is very essential for volatile oil standardization.
- *Spectroscopic evaluation*: λmax values in UV, wave number values in FTIR, delta values in NMR and m/e values in mass spectroscopy useful to identify impurities and thus to determine purity of samples.

Spectroscopy is the study of the interaction between matter and radiation. It measures radiation intensity as a function of wavelength. Spectroscopic analyses are based on measuring

the amount of radiation produced or absorbed by molecular or atomic species of interest. Spectroscopy is a common technique used in analytical chemistry for the identification of substances through the spectrum emitted from or absorbed by them. Spectroscopic methods can be classified according to the region of the electromagnetic spectrum involved in the measurement. The regions that have been used include gamma-ray, X-ray, Ultraviolet (UV), Visible, Infrared (IR), Microwave and Radio frequency (RF). Spectroscopic methods are mostly classified as atomic, molecular or ionic based on whether or not they apply to atoms, molecules or ions. The nature of their interactions can also be used to classify spectroscopic methods. These can be divided in three categories;

- Absorption spectroscopy which uses the range of the electromagnetic spectra in which substance absorbs photons. Example: Infrared, ultraviolet, visible and microwave spectroscopy are molecular techniques of absorption spectroscopy.
- Emission spectroscopy that uses the range of electromagnetic spectra in which photons are emitted by the substance. Example: Fluorescence spectroscopy, flame photometry
- Scattering spectrometry where the amount of light that a substance scatters depends on polarization angles and wavelength. Example: Raman spectroscopy.
- *Chromatographic evaluation*: Presence of extra band after development of chromatogram in paper or TLC, HPTLC indicates presence of impuri-ties.

Chromatography is based on the principle where molecules in mixture applied onto the surface or into the solid, and fluid stationary phase (stable phase) is separating from each other while moving with the aid of a mobile phase.

The factors effective on this separation process include molecular characteristics related to adsorption (liquid-solid), partition (liquid-solid), and affinity or differences among their molecular weights. Because of these differences, some components of the mixture stay longer in the stationary phase, and they move slowly in the chromatography system, while others pass rapidly into mobile phase, and leave the system faster.

Based on this approach three components form the basis of the chromatography technique.

- Stationary phase: This phase is always composed of a "solid" phase or "a layer of a liquid adsorbed on the surface a solid support".
- Mobile phase: This phase is always composed of "liquid" or a "gaseous component."
- Separated molecules

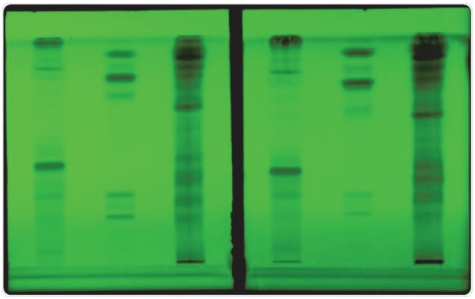
The type of interaction between stationary phase, mobile phase, and substances contained in the mixture is the basic component effective on separation of molecules from each other.

Chromatography methods based on partition are very effective on separation, and identification of small molecules as amino acids, carbohydrates, and fatty acids. However,

- Affinity chromatographies (ie. ion-exchange chromatography) are more effective in the separation of macromolecules as nucleic acids, and proteins.
- Paper chromatography is used in the separation of proteins, and in studies related to protein synthesis;
- gas-liquid chromatography is utilized in the separation of alcohol, esther, lipid, and amino groups, and observation of enzymatic interactions,

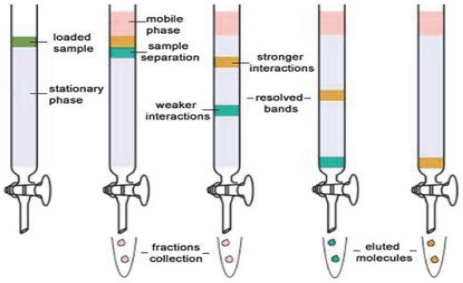
- molecular-sieve chromatography is employed especially for the determination of molecular weights of proteins.
- Agarose-gel chromatography is used for the purification of RNA, DNA particles, and viruses.

Types of chromatography



PlanarChromatography

- Paper chromatography
- Thin-layer chromatography
- High Perfomrmace Thin-layer chromatography (HPTLC)



Column Chromatography

- Column chromatography
- Ion-exchange chromatography
- Gel-permeation (molecular sieve) chromatography
- Affinity chromatography
- Gas chromatography
- High-pressure liquid chromatography (HPLC)

Fig. 1.8 Types of chromatography

Quantitative physical Evaluation or Physicochemical evaluation

Ash value determination:

The residue remaining left after incineration of the crude drug is designated as ash. The residue obtained usually represents the inorganic salts naturally occurring in the drug and adhering to it. It varies with in definite limits according to the soils. It may also include inorganic matter deliberately added for the purpose of adulteration. Hence, an ash value determination furnishes the basis for judging the identity and cleanliness of any drug and gives information relative to its adulteration/contamination with inorganic matter, thus ash values are helpful in determining the quality and purity of drug.

Muffle furnace Muffle furnace is an oven type instrument which can reach high temperatures. The furnace achieves the high-temperature on the basis of the insulating material which is fitted inside the chamber. The insulating material which is provided in the chambers acts as a muffle and stops the heat from escaping out of the chamber.

Crucible: There are a number of different types of crucible available for ashingcrude drug samples, including quartz, pyrex, porcelain, steel and platinum. Selection of an appropriate crucible depends on the sample being analyzed and the furnace temperature used. The most widely used crucibles are made from porcelain because it is relatively inexpensive to purchase, can be used up to high temperatures (< 1200^{o}C) and are easy to clean. Porcelain crucibles are resistent to acids but can be corroded by alkaline samples, and therefore different types of

crucible should be used to analyze this type of sample. In addition, porcelain crucibles are prone to cracking if they experience rapid temperature changes.

Types of ash values

***Total ash*:** The determination of ash is useful for detecting low grade products, exhausted drugs & excess of sandy and earthy material. Total ash is useful to exclude drugs which have been coated with chalk, lime or calcium sulphate to improve their appearance. Example: Ginger and Nutmeg. This test is designed to measure the amount of material remaining after ignition. Physiological ash is derived from the plant tissue. Nonphysiological ash is the residue after ignition of the extraneous matter (Example: sand and soil) adhering to the surface.

Procedure: weigh accurately into previously ignited and tared crucible, usually platinum, silica about 2 to 3 g of the ground material. Spread the material in an even layer in the crucible. Ignite the material by gradually increasing the heat to 450 ^{o}C until free from carbon, cool in desiccator and weigh. If carbon-free ash can't be obtained in this manner, cool the crucible and moisten the residue with about 2 ml of water or a saturated solution of ammonium nitrate, dry on a water bath. Then on hot plate and ignite to constant weight without delay. Calculate the content of total ash in mg/g of air-dried material.

Acid-insoluble ash: Acid-insoluble ash is the residue obtained after boiling the ash with dilute Hcl and igniting the washed insoluble matter left on the filter. This determination measures the presence of silica especially sand and siliceous earth. Acid-insoluble ash is useful for detecting the presence of excessive earthy material.

Procedure: To the crucible containing the total ash, add 25 ml of Hcl(70 g/l) TS, cover with a watch-glass and boil gently for 5 mins. Rinse the watch-glass with 5 ml of hot water and add this liquid to the crucible. Collect the insoluble matter on an ash less filter paper and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on hot plate and ignite to the constant weight. Allow the residue to cool in a suitable desiccator for 10 min and weigh without delay. Calculate the content of acid insoluble ash in mg/g of air dried material.

Water-soluble ash*:* Water soluble ash is the calculated difference in wt between the total ash and the residue remaining after treatment of total ash with water. Water-soluble ash is useful to detect the presence of material exhausted by water. Example: Tea leaves and Ginger. For the ginger the value of total ash is 2.5-6% and water-soluble ash is 1.9-3.0%.While for the exhausted ginger the value of total ash is 2-4% and water-soluble ash is 0.2-0.5%.

Procedure: To the crucible containing the total ash, add 25 ml of water and boil for 5 min. Collect the insoluble matter in a sintered glass crucible or on an ash less filter paper. Wash with hot water and ignite for 5 min at a temperature not exceeding 450 ^{o}C. Subtract the weight of this residue in mg obtained from the weight of total ash. Calculate the content of water soluble ash in mg/g of air dried material.

- ***Dry Ashing***: Dry ashing procedures use a high temperature muffle furnace capable of maintaining temperatures of between 500 and 600 ^{o}C. Water and other volatile materials are

vaporized and organic substances are burned in the presence of the oxygen in air to CO_2, H_2O and N_2. Most minerals are converted to oxides, sulfates, phosphates, chlorides or silicates. Although most minerals have fairly low volatility at these high temperatures, some are volatile and may be partially lost. Example: iron, lead and mercury. If an analysis is being carried out to determine the concentration of one of these substances then it is advisable to use an alternative ashing method that uses lower temperatures. The food sample is weighed before and after ashing to determine the concentration of ash present. The ash content can be expressed on either a dry or wet basis:

$$\%\ \text{As(dry basis)} = \frac{M_{ASH}}{M_{DRY}} \times 100$$

$$\%\ \text{As(wet basis)} = \frac{M_{ASH}}{M_{DRY}} \times 100$$

where M_{ASH} refers to the mass of the ashed sample, and M_{DRY} and M_{ASH} refer to the original masses of the dried and wet samples.

A number of dry ashing methods have been officially recognized for the determination of the ash content of various foods (AOAC Official Methods of Analysis). Typically, a sample is held at 500-600^{o}C for 24 hours.

Advantages: Safe, few reagents are required, many samples can be analyzed simultaneously, not labor intensive, and ash can be analyzed for specific mineral content.

Disadvantages: Long time required (12-24 hours), muffle furnaces are quite costly to run due to electrical costs, loss of volatile minerals at high temperatures, Example: Cu, Fe, Pb, Hg, Ni, Zn.

Recently, analytical instruments have been developed to dry ash samples based on microwave heating. These devices can be programmed to initially remove most of the moisture (using a relatively low heat) and then convert the sample to ash (using a relatively high heat). Microwave instruments greatly reduce the time required to carry out an ash analysis, with the analysis time often being less than an hour. The major disadvantage is that it is not possible to simultaneously analyze as many samples as in a muffle furnace.

- ***Wet Ashing:*** Wet ashing is primarily used in the preparation of samples for subsequent analysis of specific minerals (see later). It breaks down and removes the organic matrix surrounding the minerals so that they are left in an aqueous solution. A dried ground food sample is usually weighed into a flask containing strong acids and oxidizing agents (Example: nitric, perchloric and/or sulfuric acids) and then heated. Heating is continued until the organic matter is completely digested, leaving only the mineral oxides in solution. The temperature and time used depends on the type of acids and oxidizing agents used. Typically, a digestion takes from 10 minutes to a few hours at temperatures of about 350^{o}C. The resulting solution can then be analyzed for specific minerals.

  Advantages: Little loss of volatile minerals occurs because of the lower temperatures used, more rapid than dry ashing.

Disadvantages: Labor intensive, requires a special fume-cupboard if perchloric acid is used because of its hazardous nature, low sample throughput.

Ash values of popular crude drugs as per pharmacopoeias			
Ash values	**Name of pharmacopeia**		
	Indian Pharmacopeia Vol-III, [2018]	**Ayurvedic Pharmacopeia Vol-I, [1986]**	**Indian Herbal Pharmacopeia [2002]**
Ashwagandha			
Ash value [NMT %]	7	4	7
Acid insoluble value [NMT %]	2	1	1.2
Turmeric			
Ash value [NMT %]	10	9	9
Acid insoluble value [NMT %]	2	1	1
Sarpagandha (Rauwolfia)			
Ash value [NMT %]	8	8	8
Acid insoluble value [NMT %]	2	1	2
Sunthi (Ginger)			
Ash value [NMT %]	8	6	6
Acid insoluble value [NMT %]	1.5	1.5	1.5

Extractive value determination

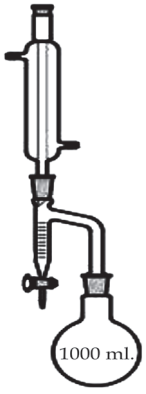
Extractive value gives idea about soluble chemical constituents in particular solvents. According to IP, alcohol, Pet. Ether and water soluble extractive values should be determined.

Procedure: Weigh accurately quantity of crude drug and macerate it for 24 hr. With intermittent shaking for first 6 hr and allow to stand for further 18 hr. After 24 hr filter and evaporate filtrate. Residue remains after evaporation is value of extractive value of that particular solvent. While determining water extractive value, add 5% chloroform as a microbial growth inhibitor.

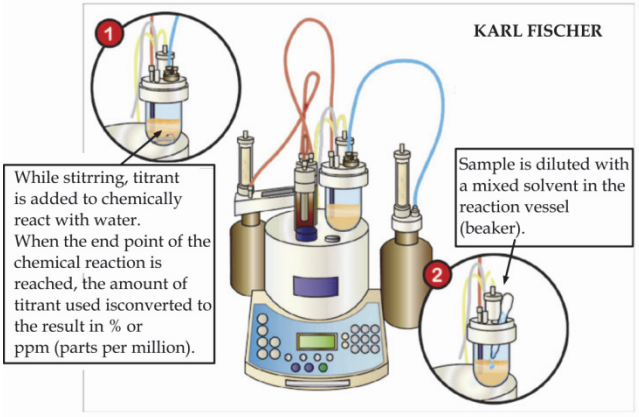
Extractive values of popular crude drugs as per pharmacopoeias			
Extractive values	**Name of pharmacopeia**		
	Indian Pharmacopeia Vol-III, [2018]	**Ayurvedic Pharmacopeia Vol-I, [1986]**	**Indian Herbal Pharmacopeia [2002]**
Ashwagandha			
Ethanol soluble extractive value [NLT %]	10	2	20
Water soluble extractive value [NLT %]	15	8	16
Turmeric			
Ethanol soluble extractive value [NLT %]	6	8	8
Water soluble extractive value [NLT %]	12	12	12
Sarpagandha (Rauwolfia)			
Ethanol soluble extractive value [NLT %]	2	4	9
Water soluble extractive value [NLT %]	5	10	8
Sunthi (Ginger)			
Ethanol soluble extractive value [NLT %]	2	3	2
Water soluble extractive value [NLT %]	10	10	10

Determination of moisture content:

An excess of water in medicinal plant material will lead to deterioration through microbial growth or enzyme mediated hydrolysis in glycoside containing plants. Therefore limits for the amount of water should be set for every plant material. Methods of determination of moisture content include:

Loss on Drying: (Gravimetric method):	This test determines loss of both water and volatile matter by drying thermostabel substance at 100-105 °C in oven or thermo labile substances in a desiccators over phosphorus Pentoxide R under atmospheric or reduced pressure and temperature for a specified period of time. In an LOD test, the sample is weighed, dried, and weighed again. The difference in the two weights (Loss on Drying) is then compared with either the original weight (Wet-base test) or final weight (Dry-base test) and the moisture content calculated. Tests can be manually conducted (weigh, oven dry, weigh) or automated (integrated weight and heating unit) with systems called Moisture Determination Balances. Depending on the balance and heating mechanism, a wide array of precision and accuracy is available. Today there are even micromoistureanalyzers, using microbalances that can provide moisture measurement to the PPM level, consistent with the limits of KF testing.LOD Moisture Measurement can be done by: • Forced air ovens. • Convection ovens. • Vacuum ovens. • Infrared moisture balances. • Microwave (drying) ovens. *Procedure:* Take accurately weighed quantity of (about 2-5 g) of the material to be tested in silica crucible and dry the sample by one of the following techniques until constant weight is obtained. Calculate the loss of weight in mg/gof air dried material. ➢ Dry in an oven at 100-105 °C for 4 hr ➢ Dry in desiccator over phos-phorouspentaoxide R under
Azeotropic Method (Toluene distillation method):  **Fig. 1.9** Water content determination apparatus (Dean Stark apparatus)	This method gives a direct measurement of the water present in the material being examined. When the sample is distilled together with an immiscible solvent, such as toluene R or xylene R. The water present in the sample is absorbed by the solvent, they are distilled together and separated in the receiving tube on cooling. *Procedure:* Take accurately weighed quantity of the material expected to give about 2-3 ml of water into the flask. Heat the flask gently. When boiling begins, distill at a rate of 2 drops/sec until most of the water has distilled over, and then increase the rate of distillation to about 4 drops/sec. After complete distillation, rinse the inside of condenser tube with toluene R. Continue the distillation for 5 more min and again wash any droplets of water adhere to the walls of the receiving tube with toluene. Allow the water and toluene layers to separate and measure volume of the water. Calculate the content of water in % using the formula: % of Water = 100 (n'- n) / w where w = the weight in g of the material being examined. n = the number of ml of water obtained in the first distillation n'= the total number of ml of water obtained in both distillations.

Contd….

Karl Fischer Method	In this methodcolored solution of Karl Fischer reagent (pyridine, sulfur dioxide, iodine, and anhydrous methanol) reacts quantitatively with water to form a colorless solution. This is coulometric or electrochemical titration method to determine water only unlike other methods of moisture content determination where volatile components are also estimated. *Principle:* The working electrode is an iodine electrode, while the reference electrode is a platinum electrode. The reaction involves converting solid iodine into hydrogen iodide in the presence of sulfur dioxide and water. Pyridine, which is a base, is often used to counteract the formation of sulfuric acid. All reagents must be anhydrous for the analysis to be quantitative. Procedure: The main compartment of the titration cell contains the anode solution plus the analyte. The anode solution consists of an alcohol (methanol or diethylene glycol monoethyl ether), a base Pyridine, Sulfur dioxide ($SO_{2)}$ and iodine ($I_{2)}$. The titration cell also consists of a smaller compartment with a cathode immersed in the anode solution of this main compartment. A dehydrating solvent suitable for the sample is placed in this flask. The sample is then added. Titration is carried out using a titrant. According to Faraday's laws, the iodine is produced in proportion to the quantity of electricity. This means that the water content can be determined immediately from the coulombs required for electrolytic oxidation. **1 mg of water = 10.71 Coulombs** Water reaction with Karl-Fischer reagent: $H_2O + I_2 + SO_2 + 3\ C_5H_5N \rightarrow 2(C_5H_5N{+}H)\ I + C_5H_5N \cdot SO_3$ $C_5H_5N \cdot SO_3 + CH_3OH \rightarrow (C_5H_5N{+}H)\ O\text{-}O_2 \cdot OCH_3$  **Fig. 1.20** Karl Fischer apparatus
Modern Methods of moisture content determination	Electrical Methods, Microwave, Nuclear and Near-Infrared methods

➢ Determination of volatile oils

This determination is important standardization parameter for volatile oils containing plant materials. Initially in round bottom flask, add sufficient quantity of xylene R or the solvent given for the material, attach condenser and graduated collector tube. Heat the liquid in the

flask until it begins to boil and adjust the distillation rate to 2-3 ml/min. Stop the heating after 30 min, turn off the heater and at least 10 min later, record the volume of solvent xylene collected in the graduated tube. Take the accurately weighed crude plant material and introduce into the flask and continue the distillation. After further 10 min, record the volume of the oil collected in the graduated tube and subtract the volume of the solvent (xylene) previously noted. The difference represents the volume of the volatile oil in the weight of plant material taken. Calculate the content in ml of oil per 100 g of plant material.

Chemical Evaluation

Qualitative Chemical Evaluation: This involves various chemical tests to identify different phytochemicals. For example, Alkaloids can be detected by Mayer's reagent, Dragendroff's reagent, Wagner's reagent; cardiac glycosides by killer killani tests etc.Specific chemical tests can also be helpful in chemical evaluation. For example, Thalleoquin test for quinine.

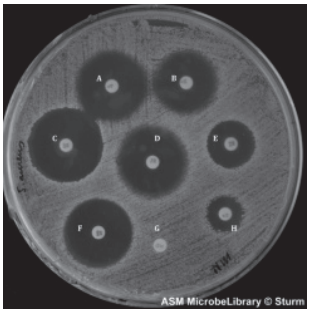
Quantitative Chemical Evaluation:Quantitative Chemical Assay procedure (Radio immuno assay, Enzyme-linked immunosorbent assay), values (Acid value, iodine value, saponification value for fats and oils whileester value, acetyl value, aldehyde content for volatile oil) and quantitative estimation of individual phytochemicals by using chromatography, spectroscopy etc. are parameters of quantitative chemical evaluation.

Biological Evaluation

When potency of crude drug or its preparations is measured by its effect on microorganisms, organs or tissues of animal then it is known as biological evaluation and if on whole animals like rat, mice, monkey, guinea pig then it is known as pharmacological evaluation. When strength of drug in its preparation is to be evaluated then it is named as bioassays. This is preferred method when chemical or physical evaluation is not satisfactory for material.

In vivo methods	In vivo means "within the living. In this method drug effects are tested on whole, living organisms or cells, usually animals, including humans and plants. Animal testing and clinical trials are major elements of in vivo research. **Anti- inflammatory activity determination** Method/Model : Carrageenan rat paw edema model Animal : Rats Standard drug : Diclofenac Disease inducing agent Carrageenan Duration : 3 hr Method of evaluation : Measurement of paw edema by using Plethysmometeror vernier caliper Calculations : Percentage of inhibition of
In vitro methods	In vitro studies are performed with microorganisms, cells, or biological molecules outside their normal biological context in labware such as test tubes, flasks, Petri dishes, and microtiter plates **In vitro anticancer activity on determination using animal cells or tissues:** In metabolic assays in which, the cellular reduction of a colorless tetrazolium salt (MTT or XTT) yields a colored formazan in proportion to viable cell number. The formazans could be measured con-veniently in an automated colorimeter.

Contd….

	Procedure: Take cancer cells of 3 x 105 cells/well concentration in a 96 well plate except at least three wells without cells to serve as a control for the minimum absorbance. Incubate plate overnight at 37°C in a humidified incubator, 5% CO2 for the cancer cells to grow and adhere to the surface. Add Test compounds in to the plate. Include replicates for a range of concentrations. Include negative controls (including vehicle control) and a positive control. The final volume will be 100µl per well. Incubate plate for overnight (or for some other appropriate time) at 37°C in a humidified incubator, 5% CO2. Then add 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent (20µl/100µl per well of the 96 well plate). Incubate at 37°C for 3 hours then add 2.0 ml of 10% Trichloroacetic acid to stop further reaction and shake plate at room temperature for a minimum of 1 hour. After the 1 hour incubation, ensure the formazan precipitate is dissolved by pippeting each well up and down until no precipitate is visible. Read the plate on a plate reader using wavelength at 572 nm. Tabulate results and calculate the % viability.
	Antimicrobial activity Three methods can be useful to determine antimicrobial activity i.e. agar well method, turbidometric method and bioautographic method. In agar well method, select media specific to microorganisms and prepare plates of solid media Add microbial strains of optimum dilutions and then prepare well in plate by bore, add sample and standard solution in wells, incubate it for 24 hr for bacteria and 72 hr for Fungi. Measure zone of inhibition in mm by scale and compare with standard. In turbidometric method, prepare tubes of liquid media; add strains and sample solutions, incubate it 24 hr for bacteria and 72 hr for Fungi. Measure turbidity of liquid solutions by spectrophotometer and compare with standard. Bioautographic method involves use of TLC plates where media and microbial strain is applied over pre developed plates and allowed to incubate for 24 to 72 hr depending upon microorganism. Sepa-rated fractions on TLC having antimicro-bial activity would show zone of inhibition in visible light or after spraying with reagents like MTT which are able to differentiate between live or dead cells.  **Fig. 1.21** Zone of inhibition area indicates antimicrobial potency
Ex vivo methods	Ex vivo refers to experimentation or measurements done in or on tissue from an organism in an external environment with minimal alteration of natural conditions. Ex vivo conditions allow experimentation on an organism's cells or tissues under more controlled conditions than is possible in in vivo experiments (in the intact organism), at the expense of altering the "natural" environment. Tissues may be removed in many ways, including in part, as whole organs, or as larger organ systems.
In silico methods	Experiments are performed on computer or via computer simulation.Virtual screening (VS) is a computational technique used in drug discovery to search libraries of small molecules in order to identify those structures which are most likely to bind to a drug target, typically a protein receptor or enzyme. There methods can be ligand-based, structure-based or hybrid techniques.

Subjective Questions

1. Define Pharmacognosy. Write in brief about scope and developments of Pharmacognosy.
2. What are different sources of crude drugs?
3. How to classify crude drugs? Explain with suitable examples.
4. What is difference between chemotaxonmy and serotaxonomy?
5. Define adulteration and explain how crude drugs are adulterated?
6. What is oraganoleptic evaluation? Explain with suitable examples.
7. What is stomata? What are different types of stomata. Explain with examples.
8. What is trichome? What are different types of trichomes. Explain with examples.
9. What is cystoliths?
10. What is quantitative microscopy? Explain with suitable examples.
11. Write note on leaf constants.
12. What is formula for stomatal index determination?
13. What is formula for determination of percentage purity by Lycopodium spore method?
14. What is difference between vein islets and vein termination?
15. Write a note on Lycopodium spore method.
16. Why spores of Lycopodium are useful for quantitative microscopy.
17. What is camera lucida and how it is useful in Pharmacognosy?
18. What is micrometer? How it is useful in pharmacognosy?.

Multiple Choice Questions (MCQs)

1. ……. is one of the oldest as well as famous surviving document belonging to 1600 B. C
   a. Papyrus Ebers
   b. Materia medica
   c. Samhita
   d. Analecta pharmacognostica
2. ……is the oldest chinese herbal doccument written by emperor Shen Nungarround 3000 B. C
   a. Huang Di Nei Jing
   b. Pen-t' sao
   c. Internal medicine
   d. None of the above

3. *Charak Samhita* and *Sushrutha Samhita* are well known treaties in
   a. Homeopathy
   b. Sidha
   c. Unani
   d. Ayurveda
4. Which one of the following is known as “father of Medicine”?
   a. Hippocrates
   b. Aristotle
   c. Galen
   d. None of the above
5. Which one of the following is known as first pharmacist to introduce opium as a pain relieving material in his apothecary?
   a. Hippocrates
   b. Galen
   c. Aristotle
   d. Dioscorides
6. Paracelsus (1493 - 1541) is doccumented in history for
   a. Development of mineral salts as drug
   b. Development of sea salts as drugs
   c. Development of rock salts as drugs
   d. Both a and b
7. The importance of extraction method and alcohol as an extractant was reported by…….
   a. William Withering
   b. Serturner
   c. Le’mery
   d. None of the above
8. Who isolated narcotine from opium in 1803?
   a. Serturner
   b. Derosne
   c. both a and b
   d. None of the above
9. In 1806, morphine was isolated from opium by…….
   a. Derosne
   b. Serturner
   c. Pelletier
   d. None of the above

10. In 1852 extraction process for alkaloids was first introduced by……
    a. Stass and Otto
    b. Posslet and Reimann
    c. Gerrard and Hardy
    d. Neumann
11. Nicotine was first time isolated in 1828 by…….
    a. Stass and Otto
    b. Posslet and Reimann
    c. Gerrard and Hardy
    d. Neumann
12. The term "Pharmacognosy" was first coined by German scientist……..
    a. Seydler in 1815
    b. Seydler in 1820
    c. Pelletier in 1815
    d. Pelletier in 1820
13. Coccaine was first discovered by……..
    a. Reimann
    b. Neumann
    c. Neuton
    d. Neimann
14. Pharmacognosy deals with
    a. Crude drugs from natural origin
    b. Crude drugs from synthetic origin
    c. Both a and b
    d. None of the above
15. Pharmacognosy is important link between……..
    a. Pharmacology
    b. Medicinal chemistry
    c. Both a and b
    d. None of the above
16. Crude drug materials which represent a part of the plant are classified as,
    a. Organized crude drugs
    b. Unorganized crude drugs
    c. Both a and b
    d. None of the above

17. Crude drugs representing a diverse group of solid and liquid materials which do not consists of parts of plants and obtained from natural sources are classified as,
    a. Organized crude drugs
    b. Unorganized crude drugs
    c. Both a and b
    d. None of the above
18. Dried latex, gums, mucillages comes under category of
    a. Organized crude drugs
    b. Unorganized crude drugs
    c. Both a and b
    d. None of the above
19. Quinine was first isolated in
    a. 1818
    b. 1819
    c. 1820
    d. 1821
20. Ephedrine was first discovered by ……..
    a. Neumann
    b. Nagai
    c. Kuersten
    d. Gerrard
21. In pharmacopoeias, crude drugs are arranged in alphabetical order of their ..... and ..... names
    a. Latin and English.
    b. German and English.
    c. French and English
    d. Italian and English
22. Which one of the classification is based on phylogeny among plants or animals?
    a. Alphabetical
    b. Taxonomic
    c. Chemotaxonomic
    d. Therapeutic
23. In the drug name, *Glycerrhiza glabra, Glycerrhiza* represents
    a. Species.
    b. Genus.
    c. Family
    d. Class

24. Which one of the following drug belongs to solanaceae family?
    a. *Hyoscyamus Niger.*
    b. *Atropa belladonna.*
    c. *Glycyrhhiza glabra*
    d. Both a and b
25. *Myroxylonbalsamum* belongs to family
    a. Solanaceae.
    b. Apocynsceae.
    c. Scrophulariaceae
    d. Leguminosae
26. The drug isabgol according to morphology, classified as
    a. Seeds.
    b. Husk.
    c. Leaves
    d. Bark
27. Cinnamon drug represents …….. part of plant
    a. Wood.
    b. Roots.
    c. Rhizomes
    d. Bark
28. Gelatin comes under the category of
    a. Dried juices.
    b. Dried latices.
    c. Dried extracts
    d. None of the above
29. Choose correct pair from following

a.	Glycosides	1. Eucalyptus
b.	Alkaloids.	2. Vinca
c.	Tannins.	3. Cinchona
d.	Lipids	4. Ashoka

    a. A-2
    b. C-4
    c. B-1
    d. C-3

30. Papain chemically classified as
    a. Vitamins and hormones.
    b. Proteins and enzymes.
    c. Resins
    d. Glycosides
31. Which drugs acts as purgative?
    a. Cascara.
    b. Colocynth.
    c. Senna
    d. All of the above
32. Which one of the following drug classified as both anti-malarial as well as bitter tonic?
    a. Cascara
    b. Kurchi.
    c. Cinnamon
    d. Cinchona
33. Choose the correct pair from following

A. Emetic.	A. Ipecacunha
B. Expectorant.	B. Morphine
C. Antitussive.	C. Pilocarpus
D. Cholinergic.	D. Liquorice

    a. AA
    b. BB.
    c. CC.
    d. DD
34. Which one of the following acts as skeletal muscle relaxant?
    a. Opium.
    b. Coffee
    c. Datura.
    d. Curare
35. Which one of the following acts as smooth muscle relaxant?
    a. Coffee.
    b. Curare.
    c. Opium
    d. Ipecacunha

36. Coca is therapeutically characterized as
    a. Antidepressant.
    b. Analgesic.
    c. Local anaesthetic
    d. CNS stimulant
37. ..... is therapeutically classified as anti-expectorant
    a. Vasaka.
    b. Ipecacunha.
    c. Liquorice
    d. Stramonium leaves
38. Which one of the following is therapeutically catagorized as analeptic?
    a. Camphor.
    b. Lobelia.
    c. Both a and b
    d. None of the above
39. Chemotaxonomic classification mainly deals with
    a. Understanding of biological evolution of plant and their chemistry
    b. Understanding of therapeutic activities
    c. Understanding of morphology
    d. None of the above
40. DNA hybridisation and taxonomy are involved in
    a. Pharmacology.
    b. Serotaxonomy.
    c. Chemotaxonomy
    d. None of the above
41. Adulteration is
    a. Debasement of natural drug
    b. Substitution of original crude drug partially or wholly with spurious substances
    c. Performed deliberately for commercial benefits
    d. All of the above
42. Impairment in quality of drug is known as
    a. Admixture.
    b. Sophistication.
    c. Substitution
    d. Deterioration

43. Admixture is the practice in which one article is added to another due to
    a. Carelessness.
    b. Ignorance.
    c. Accidental
    d. All of the above
44. The term used for deliberate or intentional adulteration is called
    a. Substitution
    b. Sophistication
    c. Both a and b
    d. None of the above
45. .....occurs when some totally different substance is added in place of original drug
    a. Substitution.
    b. Sophistication.
    c. Deterioration
    d. d. Both b and c
46. Spoilage is due to
    a. Microorganisms.
    b. Minerals.
    c. Heat
    d. Cold
47. In terms of adulteration, inferiority means
    a. High standard drug
    b. Low standard drug
    c. Substandard drug
    d. Both a and b
48. Strychnusnux-vomica is commercially adulterated with
    a. Strychnusnuxblanda
    b. Strychnuspotatorum
    c. Both a and b
    d. None of the above
49. Alianthus leaves are inferior quality substitution for
    a. Catharanthus rosus.
    b. Atropa belladonna.
    c. Azadirecta indica
    d. CentellaAsiatics

50. Saffron is usually adulterated with inferior quality
    a. Carthamus victorious.
    b. Carthamus tinctorius.
    c. Carthamus tinctona
    d. Carthamus roseus
51. Evaluation of crude drug is necessary because
    a. Biochemical variation of a drug
    b. Deterioration due to treatment and storage
    c. Substitution and adulteration
    d. All of the above
52. Morphological or organoleptic evaluation is technique of
    a. Qualitative investigation
    b. Quantitative investigation
    c. Both
    d. None
53. Microscopy is
    a. Study of form of crude drug
    b. Description of form of crude drug
    c. Both
    d. None
54. Morphology is
    a. Study of form of crude drug
    b. Description of form of drug
    c. Both
    d. None
55. Chemo-microscopy involves
    a. Application action of chemicals to micro constituents of drug before study under microscope
    b. Application of chemicals to micro constituents of drug after study under microscope
    c. Application of chemicals to microscope
    d. None of the above
56. A drop of phlroglucinol and concentrated hydrochloric acid gives .....stain in the presence of lignin
    a. Yellow.
    b. Green.
    c. Violet
    d. Red

57. In powdered microscopic of aloe, different varieties of aloe can be identified based on
    a. Number of stomata
    b. Number of calcium oxalate crystals
    c. Presence or absence of raphide crystals
    d. Presence or absence of chloroplast pigments
58. The diameter of starch grains in *Cinnamomum cassia* is
    a. 9 micron.
    b. 11 micron.
    c. 10 microns
    d. 12 microns
59. Palisade ratio of leaf drug *Digitalis purpura*
    a. 3.1 – 4.2.
    b. 3.2 – 4.2.
    c. 3.7 – 4.2
    d. 3.8 – 4.2
60. In microscopic evaluation, which one of the following represents quantitative analysis?
    a. Lycopodium Assay
    b. Vein islet number
    c. Stomatal index.
    d. All of the above
61. Which types of stomata are present in coca and senna leaves?
    a. Paracyclic
    b. Rubiaceous.
    c. Both a and b
    d. Anisocytic
62. Which type of trichome is present in *Calendula officinalis*?
    a. Unbranched triseriate.
    b. Unbranched biserriate.
    c. Unbranched uniserriate
    d. d. None of the above
63. Diameter of Lycopodium spores is
    a. 20 micrometer.
    b. 22 micrometer.
    c. 23 micrometer
    d. 25 micrometer

64. 1 milligram of powdered Lycopodium contains on an average
    a. 95000 spores.
    b. 94000 spores.
    c. 10000 spores
    d. 99000 spores
65. Which one of the following technique is used for separation of components of volatile oils?
    a. Extraction.
    b. Distillation.
    c. Sublimation
    d. Fractional crystallization
66. Which one of the following method is used for purification of alkaloids from extracts?
    a. Sublimation.
    b. Steam distillation.
    c. Fractional crystallization
    d. d. Fractional liberation
67. Foam test is specifically used for identification of
    a. Tannins.
    b. Saponins.
    c. Alkaloids
    d. Glycosides
68. Melting point range of cocoa butter
    a. 30 – 33.
    b. 25 – 30.
    c. 35 - 40
    d. 40 – 45
69. Camera Lucida is specifically used for determination of leaf constants like
    a. Stomatal number.
    b. Vein islet number.
    c. Palisade ratio
    d. All of the above
70. Volatile oil content in clove is……..
    a. Not less than 15.0 (%w/w).
    b. More than 15% w/w.
    c. Not less than 10% w/w
    d. More than 10% w/w

Answer Key

1.a	2. b	3. d	4. a	5. b	6. a	7. c	8. b	9. b	10. a
11.c	12. a	13. b	14. a	15. c	16. a	17. b	18.b	19. c	20. b
21.a	22.b	23. c	24. d	25. d	26. a	27. d	28. c	29. b	30. b
31. d	32. d	33.a	34. d	35. c	36. c	37. d	38. c	39. a	40. b
41. d	42. d	43. d	44. b	45. a	46. a	47. b	48. c	49. b	50. b
51. d	52. a	53. b	54. a	55. a	56. d	57. c	58. a	59. c	60. d
61. c	62. b	63. d	64. b	65. b	66. d	67. b	68. a	69. d	70. a