First Edition, 2024

A Guide to Lab Practices

Pharmacognosy-I (Basic)

Pharm-D 2nd Professional

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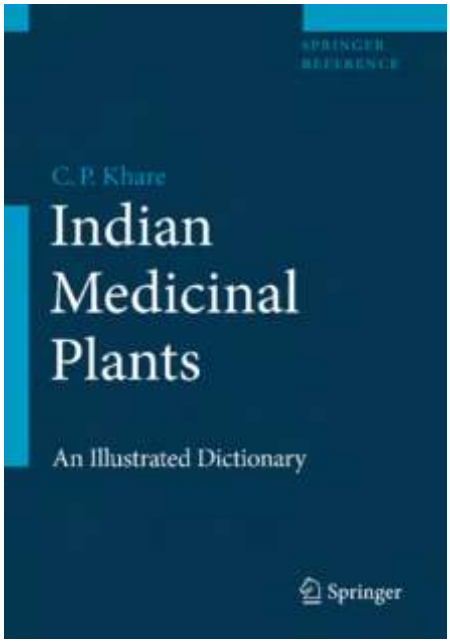
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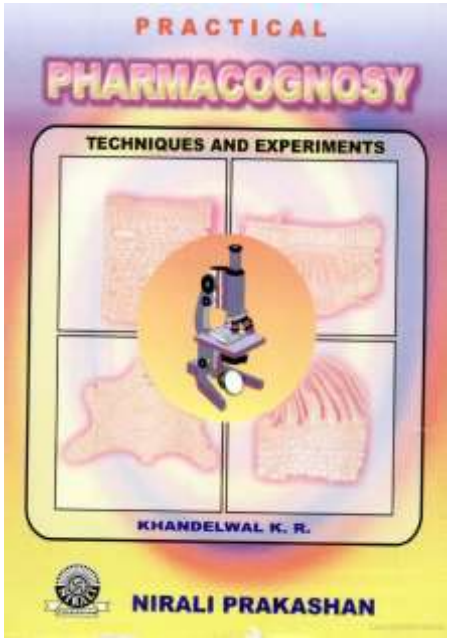
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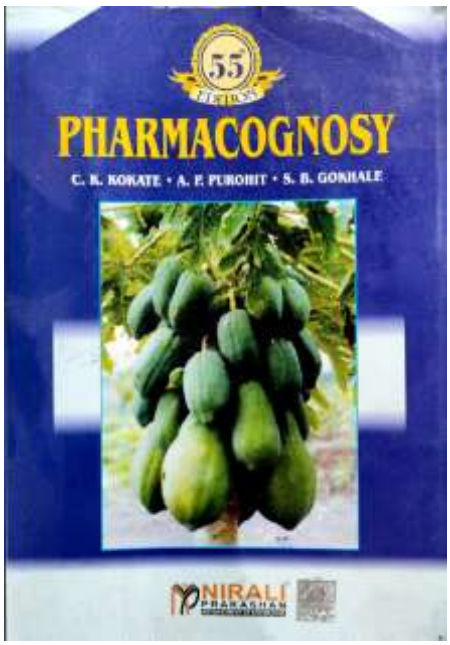
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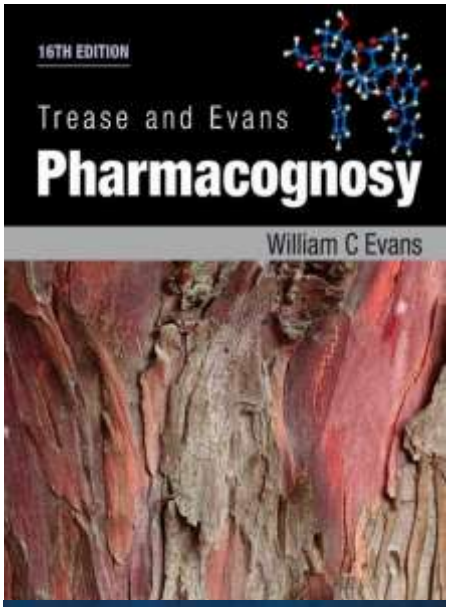
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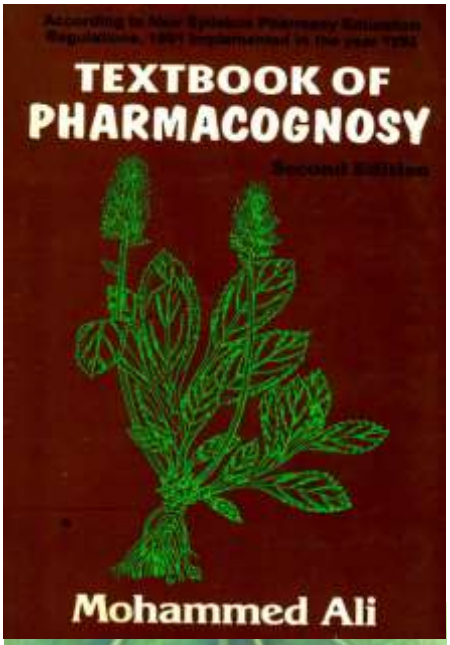
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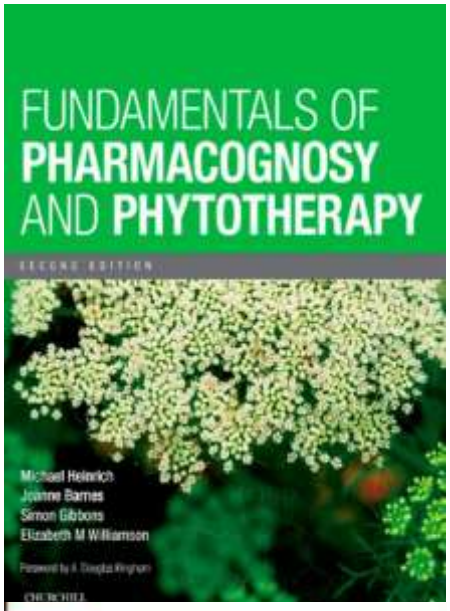
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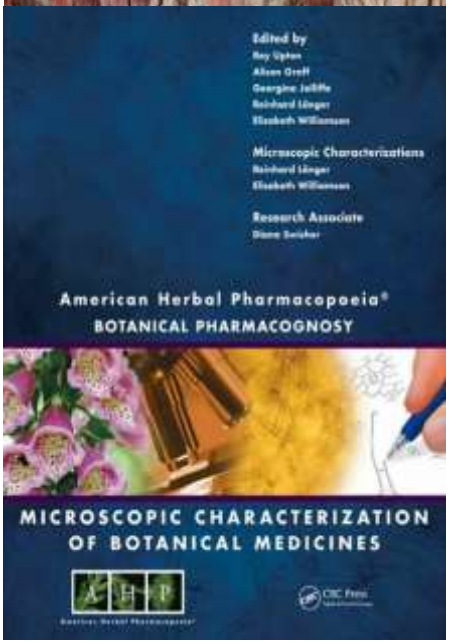
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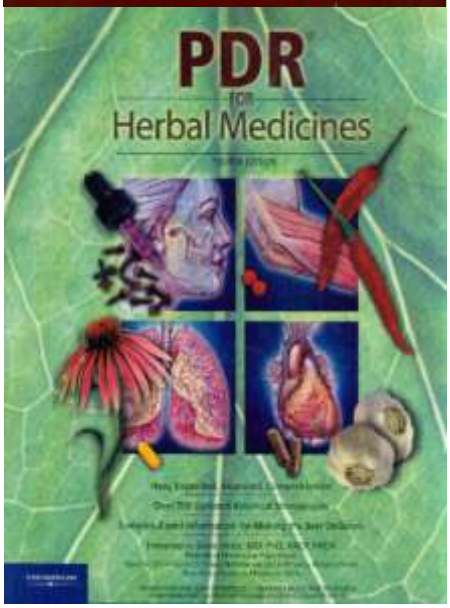
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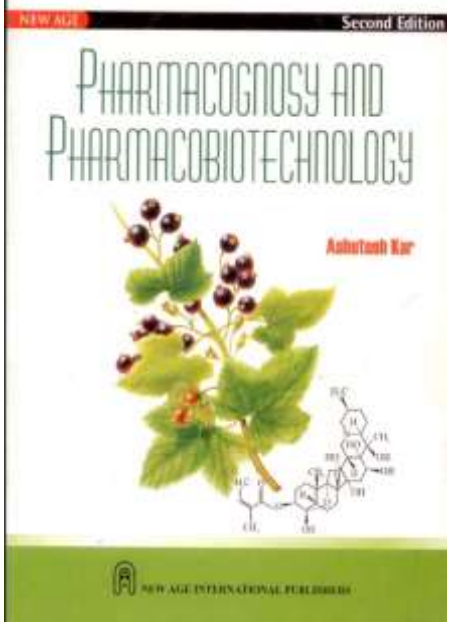
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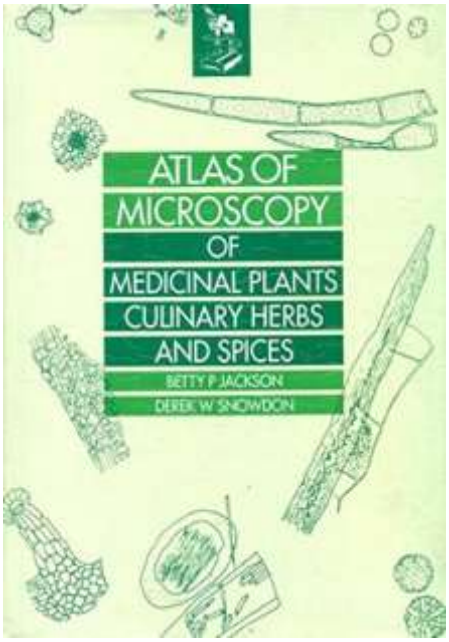
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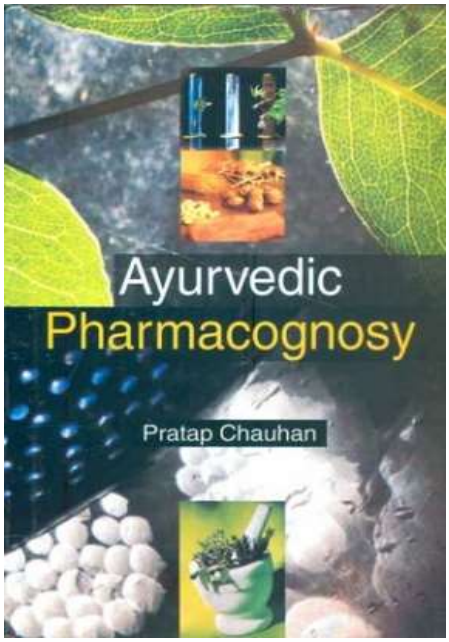
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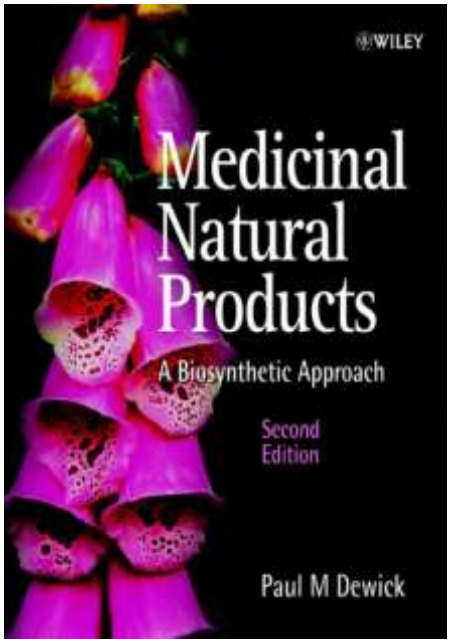
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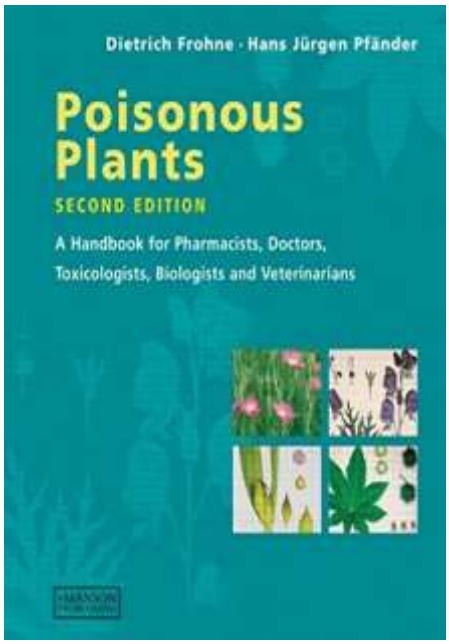
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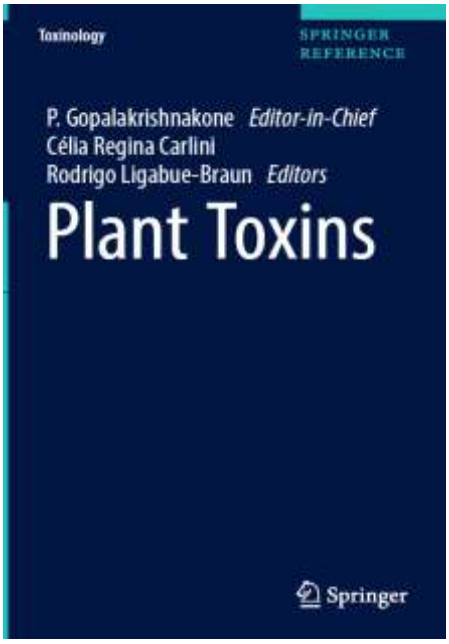
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Pharmacognosy

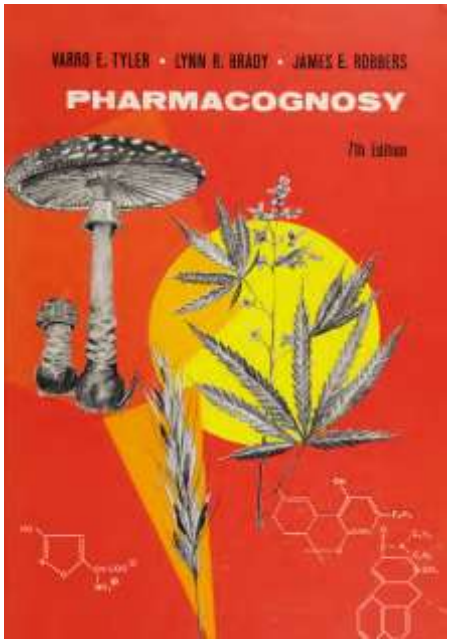
- The term pharmacognosy was introduced by C. A. Seydler, a medical student in Halle/Saale, Germany, in 1815
- The word pharmacognosy literally means knowledge of drugs or pharmaceuticals and is derived from two Greek words, *pharmakon* (drug) and *gnosis* (knowledge)
- Pharmacognosy may be defined as "an applied science that deals with the biologic, biochemical, and economic features of natural drugs and their constituents"
  - It is a study of drugs that originate in the plant and animal kingdoms
  - Modern aspects of the science include not only the crude drugs but also their natural derivatives
    - Digitalis leaf and its isolated glycoside 'digitoxin'
    - Rauwolfia root and its purified alkaloid 'reserpine'
    - Thyroid gland with its extracted hormone 'thyroxine'... are all part of the subject matter of pharmacognosy
  - In a broad sense, pharmacognosy embraces a knowledge of the history, distribution, cultivation, collection, selection, preparation, commerce, identification, evaluation, preservation, and use of drugs and economic substances that affect the health of humans and other animals
  - Such economic substances extend beyond the category of crude drugs and their derivatives to include a variety of commercial and medicinal products often requiring complicated methods of preparation: allergens, allergenic extracts, antibiotics, immunizing biologics, flavoring agents, and condiments
  - As a part of the pharmaceutic curriculum, pharmacognosy forms an important link between pharmacology and medicinal chemistry on one hand and between pharmaceutics and clinical pharmacy on the other
- 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"

References

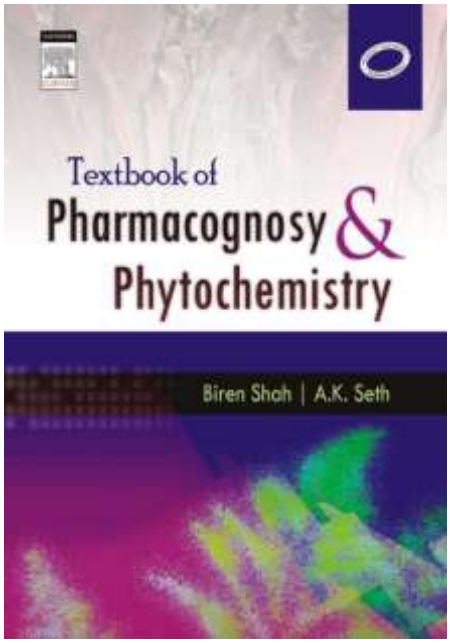
- Tyler VE, Brady LR, Robbers JE. Pharmacognosy. Lea & Febiger, 9th edition, 2003.
- Upton R *et al*. American herbal pharmacopoeia: botanical pharmacognosy – microscopic characterization of botanical medicines. CRC Press, 2016.

Experiment No. 01

Introduction to the books of Pharmacognosy



Book 01: Pharmacognosy



Book 02: Textbook of Pharmacognosy and Phytochemistry

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Introduction to the books of Pharmacognosy

Book 01
Title of book: Pharmacognosy
Name of author(s): Varro E. Tyler, Lynn R Brady, and James E. Robbers
Number of pages:
Published year(s):
Edition(s):
Availability in the library: Available
Recommendation in PharmD curriculum: Recommended

Book 02
Title of book: Textbook of Pharmacognosy and Phytochemistry
Name of author(s): Biren N. Shah and A.K. Seth
Number of pages:
Published year(s):
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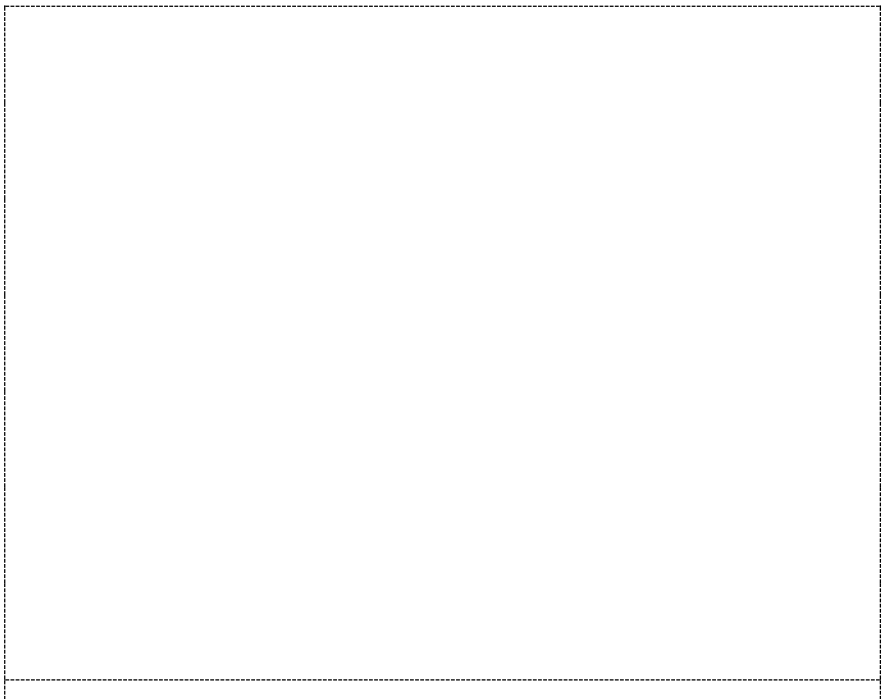

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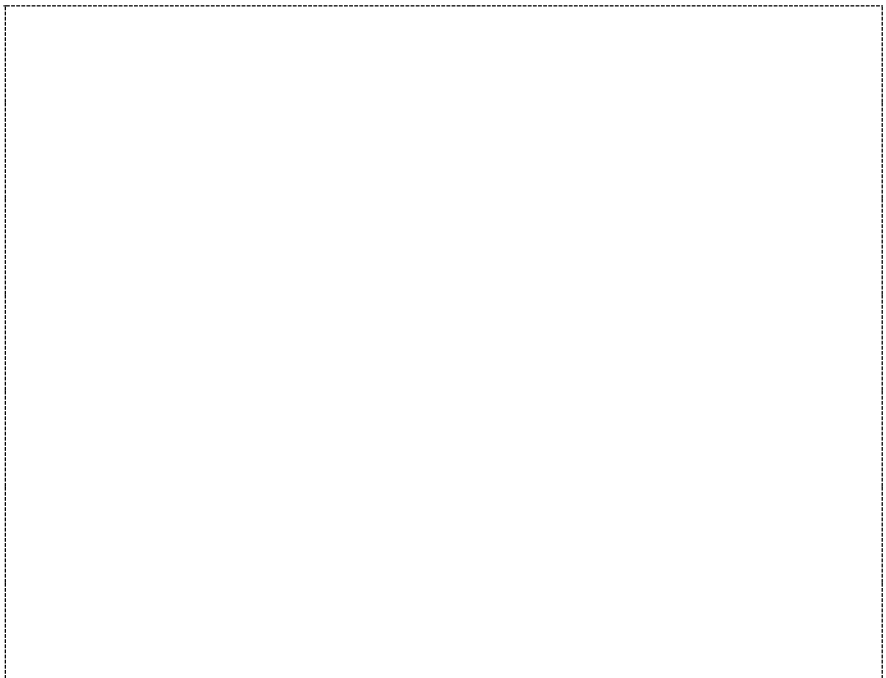

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SECTION A

Study and Organoleptic Evaluation of Crude Drugs

Drug

- The word *drug* is a derivation of the Dutch word *droog* or the French word *drogue*, both of which refer to the *drying plants hanging from the rafters of old-world apothecaries (pharmacies)*
  - The pharmacies of early centuries predominantly consisted of dried plants, crude herbal drugs, and preparations prepared from them
- A drug can be defined as "any substance (other than a food or device) intended for use in the diagnosis, cure, relief, treatment, or prevention of disease or intended to affect the structure or function of the body"
  - For example, antibacterial drugs cure bacterial infections
  - Oral contraceptives are an example of drugs that do not cure any disease, rather they affect the function of the body
- A simpler definition of a drug is "any chemical or biologic substance that affects the body and its processes"
- Drugs can also affect how the brain and the rest of the body work and cause changes in mood, awareness, thoughts, feelings, or behavior
- Some types of drugs, such as opioids, may be abused or lead to addiction

References

- Upton R *et al*. American herbal pharmacopoeia: botanical pharmacognosy – microscopic characterization of botanical medicines. CRC Press, 2016.
- www.msdmanuals.com/home/drugs/overview-of-drugs/overview-of-drugs [Accessed: 29.11.2023].

Crude drug

- Crude drugs are vegetable or animal drugs that consist of natural substances that have undergone only the processes of collection and drying
  - For example
    - Coriander دھنیہ [coriander fruits, collected and dried]
    - Glycyrrhiza/liquorice ملٹھی [glycyrrhiza roots, collected and dried]
    - Musk کستوری [collected from the preputial follicles of the musk deer and dried]
    - Cantharides [flies, collected and dried]
  - Any process or treatment that is essential for proper packing and to the prevention of decay or deterioration of the crude drug can be performed
    - For example, long branches of a tree, which are collected and dried (it is a crude drug)
    - These branches are very long and for packing we need to cut them in short piece
    - So, such cutting process can be performed because it is required for proper packing of the crude drug
- If we perform any process, other than collection and drying, and if that process is not essential for proper packing and to the prevention of decay or deterioration
  - The drug will not be called "crude drug"
  - For example, dried leaves of peppermint (leaves of peppermint collected and dried) are an example of crude drug
    - If we grind these dried leaves to powder, then this powder will be called "advanced drug", not crude drug
    - Because grinding process is neither essential for proper packing nor required for the prevention of decay or deterioration

Reference

- Tyler VE, Brady LR, Robbers JE. Pharmacognosy. Lea & Febiger, 9th edition, 2003.

Advanced drug

- A natural product that has been advanced in value or improved in condition by shredding, grinding, chipping, crushing, distilling, evaporating, extracting, artificial mixing with other substances or by any other process or treatment beyond what is essential to its proper packing and to the prevention of decay or deterioration pending manufacture
  - For example
    - Coriander powder [coriander fruits; collected and ground]; It is a crude drug
    - Coriander powder [coriander fruits; collected, dried, and ground]; It is an advanced drug
    - In this example, grinding process is neither essential for proper packing nor required for the prevention of decay or deterioration

Reference

- Tyler VE, Brady LR, Robbers JE. Pharmacognosy. Lea & Febiger, 9th edition, 2003.

Natural substances

- The term natural substances refers to those substances found in nature that comprise whole plants and herbs and anatomic parts thereof; vegetable saps, extracts, secretions, and other constituents thereof; whole animals and anatomic parts thereof; glands or other animal organs, extracts, secretions, and other constituents thereof; and substances that have not had changes made in their molecular structure as found in nature
- Examples
  - Digitalis leaf and its glycoside 'digitoxin'
  - Rauwolfia root and its purified alkaloid 'reserpine'
  - Thyroid gland and its extracted hormone 'thyroxine'

Reference

- Tyler VE, Brady LR, Robbers JE. Pharmacognosy. Lea & Febiger, 9th edition, 2003.

Organized crude drugs

- Organized crude drugs are direct parts of plants (e.g., stem, leaf, bark, fruit, etc.) and consist of cellular tissues
- Microscopic characters are used for identification, i.e., cellular structures can be observed under microscope
- Example
  - Coriander (coriander fruits; collected and dried)
  - Senna (senna leaves; collected and dried)

Reference

Shah BN, Seth AK. Textbook of pharmacognosy and phytochemistry, Elsevier, 1st edition, 2010.

Un-organized crude drugs

- Unorganized crude drugs, even though prepared from plants, are not the direct parts of plants
- These are prepared by some intermediary physical processes, such as incision
- Unorganized crude drugs do not show cellular structures under microscope
- Example
  - Aloe
  - Acacia
  - Tragacanth

  Reference
- Shah BN, Seth AK. Textbook of pharmacognosy and phytochemistry, Elsevier, 1st edition, 2010.

Official drug

- A drug included in pharmacopoeia or in national formulary or in recognized books is called an official drug
  - For example
    - Quinine, morphine, and codeine are included in British pharmacopoeia (BP)

Reference

- Alamgir ANM (2017). Drugs: their natural, synthetic, and biosynthetic sources. *Therapeutic Use of Medicinal Plants and Their Extracts*. DOI: 10.1007/978-3-319-63862-1_4.

Unofficial drug

- An unofficial drug is a drug that was recognized earlier in the pharmacopoeia but deleted from the current issue due to severe toxic effects on humans
  - For example
    - Mercurial compounds (used as diuretics)

Reference

- Alamgir ANM (2017). Drugs: their natural, synthetic, and biosynthetic sources. *Therapeutic Use of Medicinal Plants and Their Extracts*. DOI: 10.1007/978-3-319-63862-1_4.

Non-official drug

- A non-official drug is a drug that have never appeared in either of the official books
  - Non-official drug compounds may be published in current journals having proven clinical value, but information about their adverse effects is not yet known
  - For example
    - Curcumin (used in sinusitis)

Reference

- Alamgir ANM (2017). Drugs: their natural, synthetic, and biosynthetic sources. *Therapeutic Use of Medicinal Plants and Their Extracts*. DOI: 10.1007/978-3-319-63862-1_4.

Curing

- Curing is a special process of drying where processes of fermentation or sweating are necessary to bring about changes in the constituents
- For example, vanilla and tobacco are dried by curing process
  - Vanilla beans are wrapped in wool blankets and stored inside a container to provide favorable environment for the production of vanillin, along with other aroma producing components, by enzymatic actions.
  - Tobacco leaves can be cured by digging pits in the ground and keeping the levees inside these pits, covered by soil, for certain number of days

Reference

- Tyler VE, Brady LR, Robbers JE. Pharmacognosy. Lea & Febiger, 7th edition, 1976.

Primary metabolites

- Primary metabolites are the compounds that are directly involved in performing primary functions (growth, development, and reproduction) of living organisms
- Examples of primary metabolites: Proteins, lipids, carbohydrates, etc.
- Primary metabolites are widely distributed in all living organism and in all parts of living organism
- Primary metabolites do not show pharmacological effects in our body, i.e., they do not have medicinal effect and are therapeutically inactive

- The metabolic pathway through which primary metabolites are formed is called “primary metabolic pathway” (**Figure 1**)

Reference

- Tyler VE, Brady LR, Robbers JE. Pharmacognosy. Lea & Febiger, 9th edition, 2003.

Secondary metabolites

- Secondary metabolites are the compounds that are not directly involved in performing primary functions (growth, development, and reproduction) of living organisms
- Examples of secondary metabolites: Alkaloids, glycosides, tannins, flavonoids, terpenes, etc.
- Secondary metabolites protect the plants from environmental stress conditions
- Secondary metabolites act as poisons; protect the plant from insect, animal, and microbial attack
- Secondary metabolites are not widely distributed in all living organism; are mostly found in plants
- Secondary metabolites occur in certain parts of plants and are specific to certain plant species
- Secondary metabolites show pharmacological effects in our body, i.e., they have medicinal effect and are therapeutically active
- The metabolic pathway through which secondary metabolites are formed is called “secondary metabolic pathway” (**Figure 1**)
- Primary and secondary metabolites are called ‘phytochemicals’ (*phyto*: related to plants)

Reference

- Tyler VE, Brady LR, Robbers JE. Pharmacognosy. Lea & Febiger, 9th edition, 2003.

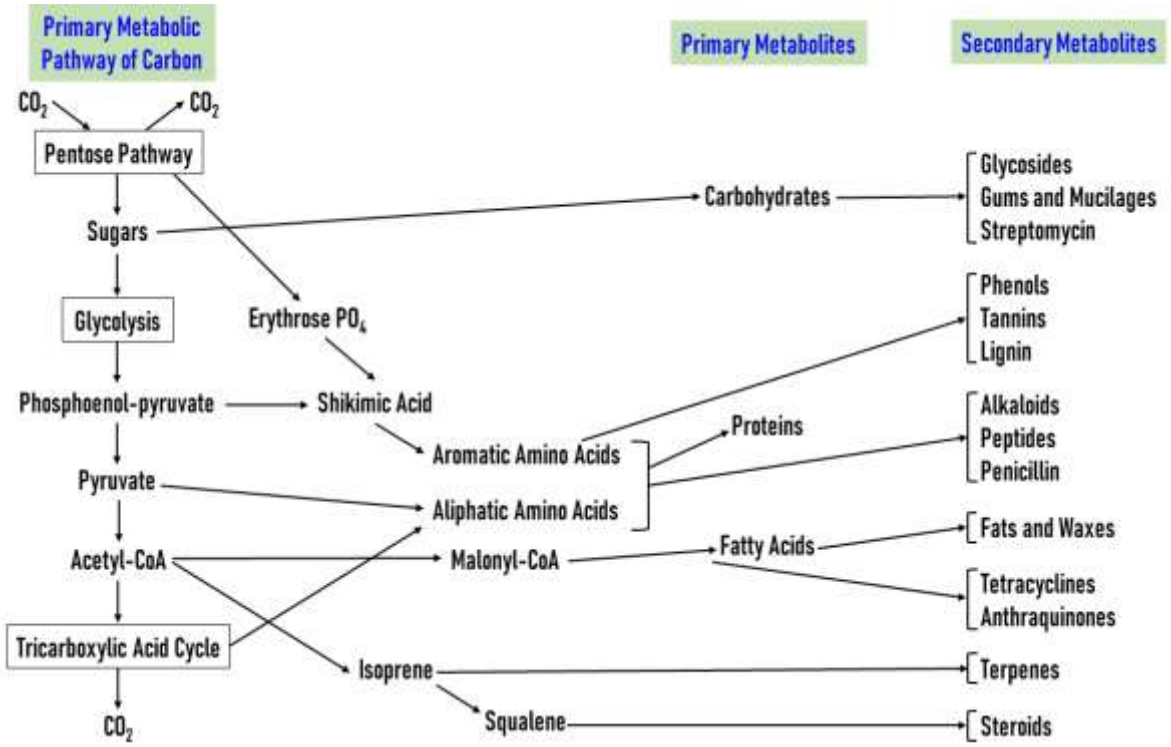
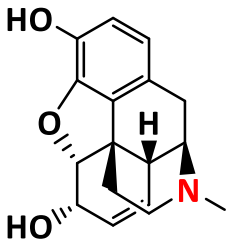


Figure 1. Interrelationships of biosynthetic pathways leading to secondary metabolites in plants.

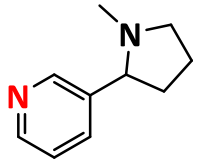
Some classes of phytochemicals are very briefly explained below to facilitate comprehension of the chemical constituents of the crude drugs included in this lab manual

Alkaloids

- Alkaloids are basic nitrogenous organic compounds
- Alkaloids usually contain 1 nitrogen atom (N)
- Some alkaloids may contain up to 5 nitrogen atoms
- Alkaloids are derived from amino acids
- The names of alkaloids end in 'ine', e.g., nicotine (present in tobacco), caffeine (present in tea and coffee), atropine, (present in belladonna), morphine (present in opium), etc.
- Mostly (not all) alkaloids are white or colorless



Morphine

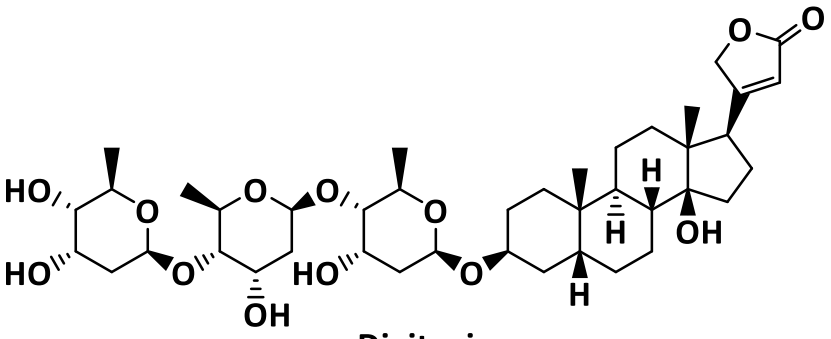


Nicotine

Glycosides

- A glycoside is a compound formed by linking sugar(s) with a non-sugar component
- The non-sugar component is called 'aglycon' and the sugar component is called 'glycon'
  - So, glycosides are the compounds that yield one or more sugars on hydrolysis
- When the sugar component is glucose, the substance may be called a 'glucoside'
- For example, digitoxin is a glycoside
  - Digitoxin consists of three sugars 'digitoxose' and one non-sugar component 'digitoxigenin'

Digitoxin: Digitoxose-*O*-digitoxose-*O*-digitoxose-*O*-digitoxigenin

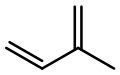


Digitoxin

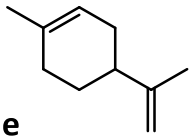
Volatile oils and terpenes

- Volatile oils are odorous principles found in various plant parts
- These evaporate when exposed to air at ordinary temperature, so are called 'volatile oils'
- Volatile oils represent the 'essences' of plants, so are called 'essential oils'
- Examples: Clove oil, peppermint oil, orange peel oil, lemon oil, cinnamon oil, etc.
- Volatile oils are immiscible with water
- Soluble in ether, alcohol, and most organic solvents
- Almost any type of organic compound may be found in volatile oils (hydrocarbons, alcohols, ketones, aldehydes, ethers, oxides, esters, and others)
- Chemical constituents of most of the volatile oils are terpene derivatives (derived from terpenes)
- Terpenes are natural products which are composed of isoprene units (C_5)
- Monoterpenes are composed of 2 isoprene units (C_{10})
- Sesquiterpenes are composed of 3 isoprene units (C_{15})
- Diterpenes are composed of 4 isoprene units (C_{20})

- Triterpenes are composed of 6 isoprene units (C_{30})
- In volatile oils, mostly monoterpenes are found, a few are sesquiterpenes
- These monoterpenes can occur in acyclic, monocyclic, and bicyclic forms
- Volatile oils are usually obtained by distillation process
- Terpenes are simple hydrocarbons while if terpenes are modified and contain various functional groups then are called 'terpenoids'



Isoprene unit



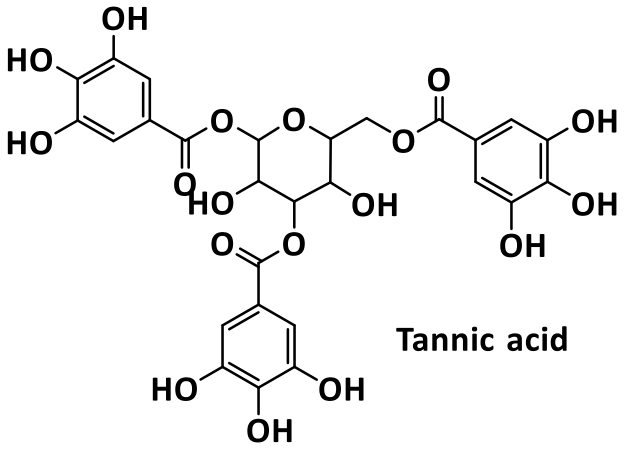
Limonene
(a monocyclic monoterpene)

Fixed oils

- Fixed oils are triglycerides of long chain unsaturated fatty acids
- Examples: Peanut oil, coconut oil, castor oil, olive oil, etc.
- Fixed oils are usually obtained by expression i.e., pressing the plant material under pressure

Tannins

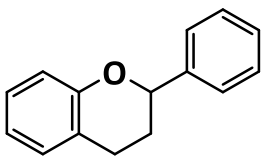
- Tannins are polyphenolic compounds
- Solutions of tannins precipitate heavy metals, alkaloids, and proteins
- They form colored compounds with iron salts like ferric chloride
- Examples: Tannic acid



Tannic acid

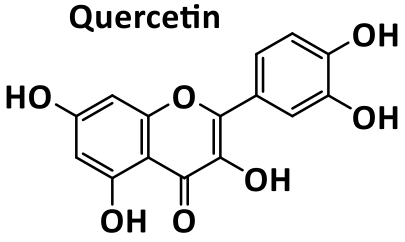
Flavonoids

- Flavonoids are polyphenolic compounds
- Their general structure, comprising fifteen carbons, consists of two aromatic rings connected by a three-carbon heterocyclic ring
- Examples: Quercetin, rutin, kaempferol, etc.



Basic skeleton of flavonoids

Quercetin



Resins

- Resins are solid or highly viscous substances of plant origin
- Plants secrete resins for their protective benefits in response to injury
  - Resins protect plants from insects and pathogens
- Physically resins are usually hard, transparent, or translucent
  - On heating, they soften and finally melt
- These are insoluble in water but dissolve more or less completely in alcohol, chloroform, and ether
- 'Oleoresins' are homogenous mixtures of resins and volatile oils
- 'Oleogum resins' are homogenous mixtures of resin, gum, and volatile oil

Evaluation of crude drugs

- The identification of crude drugs and determination of their quality and purity is called evaluation of crude drugs
- Different methods of the evaluation of crude drugs are;
  - Organoleptic evaluation
  - Microscopic evaluation
  - Biologic evaluation
  - Chemical evaluation
  - Physical evaluation

Organoleptic evaluation

- Organoleptic evaluation is the evaluation of macroscopic characters of crude drugs by means of sense organs
- In simple world, identify something and/or determine its quality by using our sense organs is called organoleptic evaluation
- Examples to understand the concept of organoleptic evaluation;
  - From the samples of sugar, washing powder, and salt; you can identify sugar
    - This is done based on the organoleptic evaluation which is performed using the sense organs (eyes and tongue) by checking its color and taste
  - When you go home, your parents identify you, by performing your organoleptic evaluation
    - They identify you, by using their sense organs, i.e., by hearing your voice, by looking at your face, etc.
  - You can evaluate the quality of food by checking its taste and aroma
    - This is also an example of organoleptic evaluation
- Organoleptic evaluation is a very basic type of evaluation, but is very important because it can be performed easily by using just sense organs
- In the monograph of a crude drug, we can find its organoleptic character, e.g., shape, size, taste, color, etc.
  - These characters can help us identify that crude drug and evaluate its quality
  - For example, we ordered a crude drug named 'fenugreek seed'
  - After receiving the order from the supplier (**Figure 2**), we evaluated the sample of 'fenugreek seed' by organoleptic evaluation
  - In the monograph of the 'fenugreek seed', it is written that their size is 3-5 mm, shape is rectangular shaped, and color is brownish yellow
  - But the sample we received does not match these characters
  - It means the supplier supplied us some other drug, not 'fenugreek seed'

Figure 2. Sample of 'fenugreek seed' received form the supplier

- Dear students, pursuing PharmD and engaged in the study of Pharmacognosy, it is expected that you acquire proficiency in the following aspects as part of your academic curriculum;
  - Mastery in conducting organoleptic evaluations of given samples of crude drugs
  - Proficiency in articulating these organoleptic characteristics using precise and appropriate terminologies

Organoleptic characters of crude drugs can be described by following parameters

Shape

- Evaluate the shape of the given sample of crude drug by comparing with the following possible shapes
  - Cylindrical
  - Sub-cylindrical
  - Conical
  - Fusiform
  - Ovoid
  - Pyriform
  - Terete
  - Disk-shaped
  - Globular
  - Ellipsoidal
  - Simple or branched (especially underground parts)
- Barks occur in different shapes (**Figure 3**)
  - Flat
  - Curved
  - Channelled
  - Single quill
  - Double quill
  - Compound quill
  - Single quills
  - Double quills
  -
- Barks have two surfaces (an outer and an inner)
- Different shapes of leaves are explained in **Figure 4**

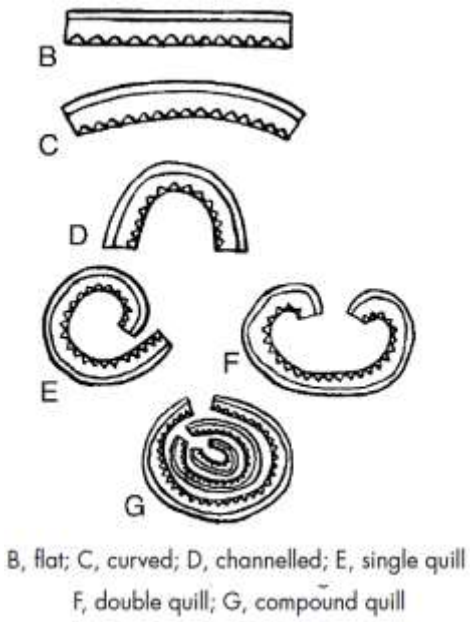


Figure 3. Different shapes of barks

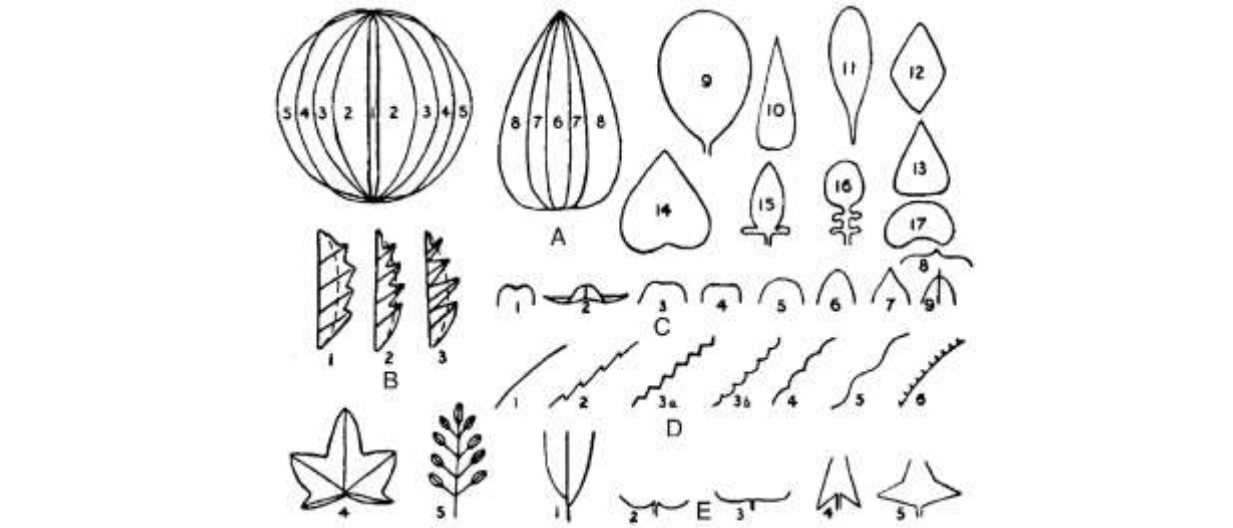
Texture/feel

- Touch the surface of the crude drug and write its texture/feel by using following terminologies
  - Soft
  - Hard
  - Smooth
  - Rough

External markings

- Identify the markings on the surface of the crude drug with the help of the following terminologies
- If the crude drug is too small to observe the markings, or
  In case of unorganized crude drug, when there are no characteristic external markings, you can write N/A
  - Furrows: Alternating ridges and valleys which are more or less parallel and well defined
  - Wrinkles: Fine or delicate furrows
  - Annulations: Transverse ring-like markings
  - Fissures: Splits extending into the tissues

- Nodules: Rounded outgrowths on the surface
- Projections: Such as roots, stem bases, and buds
- Scars: Such as leaf scars, stem-base scars, root scars, bud scars, and bud-scale scars



Terms applied to leaves. A, Shape: 1, acicular; 2, elliptical; 3, oval; 4, oblong; 5, round; 6, linear; 7, lanceolate; 8, ovate; 9, obovate; 10, subulate; 11, spatulate; 12, diamond-shaped; 13, cuneate; 14, cordate; 15, auriculate; 16, lyrate; 17, reniform. B, Composition and incision: 1, pinnatifid; 2, pinnatipartite; 3, pinnatisect; 4, palmatifid; 5, imparipinnate. C, Apex: 1, emarginate; 2, recurved; 3, retuse; 4, truncate; 5, obtuse; 6, acute; 7, acuminate; 8, mucronate; 9, apiculate. D, Margin: 1, entire; 2, serrate; 3a and 3b, dentate; 4, crenate; 5, sinuate; 6, ciliate; E, Base: 1, asymmetric; 2, cordate; 3, reniform; 4, sagittate; 5, hastate.

Figure 4. Different shapes of leaves

Size

- Estimate the length, width, diameter, etc. of the crude drug
- Size can also be compared with some well-known object/drug
  - For example, it can be said “the size of the given crude drug is almost equal to ‘pea’ size”

External color

- Note the external color of the crude drug
- In case of barks or leaves, note down the color of both sides

Fracture

- Break the crude drug with hands and observe its type of fracture
  - Complete: Breaking clean across
  - Incomplete: Breaking only part way across
  - Short: A clean smooth break with a quick snap
  - Fibrous: A break accompanied by resistance and characterized by the projection of fibers from the broken surfaces
  - Splintery: Breaking irregularly across into pieces with larger and smaller projecting edges and splinters
  - Brittle: Easily broken, usually into many pieces when dropped onto a hard surface
  - Tough: Breaking with difficulty
  - Weak: Breaking with little effort
  - Unbreakable with hands

Fractured surface

- After fracture of the crude drug, observe the fractured surfaces and write the characters of the fractured surfaces
- If the crude drug cannot be fractured by hand, then the 'fractured surface' will be N/A
  - Even: A smooth surface
  - Uneven: An irregularly broken surface
  - Granular: Having a grain like appearance
  - Hard: A compact surface
  - Horny: A hornlike surface
  - Mealy: A surface characterized by powdering, usually due to an abundance of starch (starchy)
  - Resinous: A smooth glossy surface
  - Conchoidal: A resinous surface characterized by having the surfaces curved in convex and concave fashion
  - Waxy: Exhibiting a dull wax-like surface
  - Dull
  - Smooth
  - Rough

Internal color and internal structures

- After fracture of the crude drug, observe and write the
  - Color of fractured surfaces (internal color)
  - Internal structures, e.g., seeds
- If the crude drug cannot be fractured by hand, then the 'internal color and internal structures' will be N/A

Odor

- Observe the odor of the crude drug, which can be
  - Distinct
  - Indistinct
  - Aromatic
  - Balsamic
  - Spicy
  - Alliaceous
  - Camphoraceous
  - Terebinthinate
  - Corelated to some natural substance

Taste

- If the crude drug is not toxic, taste it, which can be
  - Acid (sour)
  - Saline (salty)
  - Saccharine (sweet)
  - Bitter
  - Alkaline
  - Metallic
  - Spicy
  - Tasteless
  - Oily
  - Astringent
  - Pungent
  - Acrid
  - Nauseous
- A drug frequently gives more than one taste
  The first taste noted is produced by the most soluble chemical constituent of the crude drug
- Do not taste the poisonous crude drug

Un-organized crude drugs

- Un-organized crude drugs may occur in
  - Tears or small rounded masses formed naturally as the exudation hardens
  - Cylindrical pieces
  - Rounded or flattened masses

- Angular masses, broken up materials that has hardened in the container
- Agglutinated masses, harder portions massed with soft material
- May occur in a state varying from a liquid to a semi-plastic mass

- The size is of importance in the case of tear shaped crude drugs
- Significant external markings are usually absent

References

- Evans WC. Trease and Evans Pharmacognosy. Elsevier, 16th edition, 2009.
- Tyler VE, Brady LR, Robbers JE. Pharmacognosy. Lea & Febiger, 7th edition, 1976.

#	Crude drug	Local name	Biological origin	Main chemical constituents	Major uses
1	**Coriander** (Fruit)	دھنیہ	Coriandrum sativum Umbelliferae	Volatile oil [containing linalool (also called coriandrol), limonene, *p*-cymene, and α-pinene); Fatty oil.	Flavoring agent; Carminative; Coriander is used for dyspeptic complaints, loss of appetite, and complaints of the upper abdomen.
2	**Fennel** (Fruit)	سونف	Foeniculum vulgare Umbelliferae	Volatile oil [containing anethole, fenchone, estragole, anisaldehyde, and α-pinene].	Flavoring agent; Carminative; Antispasmodic.
3	**Cassia / Golden shower / Amaltas** (Fruit)	املتاس	Cassia fistula Leguminosae	Anthraquinone glycosides [rhein]; Sugars; Coloring matter; Volatile oil (in traces); Fatty oil; Citric acid.	Laxative; Senna pods are used for flatulence, constipation, fever, anorexia, gout, and jaundice; The drug is contraindicated in acute-inflammatory diseases of the intestine and appendicitis; It is also contraindicated for children under 12 years of age and for women during pregnancy or while nursing.
4	**Cardamom** (Fruit)	الائچی	Elettaria cardamomum Zingiberaceae	Volatile oil; Starch; Fixed oil; Calcium oxalate.	Antimicrobial; Anti-inflammatory; Analgesic; Antispasmodic; Gastroprotective; Flavoring agent in curries and cake; In the manufacture of liqueurs; Cardamom is known as “queen of spices”.
5	**Asgand / Winter cherry / Ashwagandha** (Fruit)	اسگند / اشوگندھا	Withania somnifera Solanaceae	Fatty acids; Withanolides; Alkaloids; Steroidal lactones; Saponins.	Diuretic; Antibacterial; Cough; Asthma; Epilepsy; Eye infections; Reproductive system problems.
6	**Colocynth** (Fruit)	کوڑ تمہ	Citrullus colocynthis Cucurbitaceae	Cucurbitacins (responsible for the bitter taste of colocynth); Glycosides; Flavonoids; Phenolic acids; Alkaloids; Fatty acids.	To treat diabetes, constipation, intestinal disorders, asthma, jaundice, joint pain, and bacterial infections. Formulations of this fruit are also used in traditional veterinary medicines.
7	**Tribulus / Puncture vine** (Fruit)	بھکھڑا / گوکھڑو	Tribulus terrestris Zygophyllaceae	Steroidal; Saponins; Flavonoids; Alkaloids.	Aphrodisiac; To tonify kidneys; Diuretic; Cough expectorant; To treat mammary duct blockage; Cardiotonic; Anti-inflammatory.
8	**Reetha / Indian soapberry /**	ریٹھا	Sapindus mukorossi	Saponins; Sugars; Mucilage.	Expectorant; Natural surfactant; To treat pimples, epilepsy, eczema, dental caries, and

	Chinese soapberry (Fruit)		Sapindaceae		constipation; To remove tan and freckles from the skin; As a cleanser for washing hair.
9	**Nigella seeds / Black cumin** (Seed)	کلونجی	Nigella sativa Ranunculaceae	Fixed oil; Terpenes; Saponins; Flavonoids; Minerals.	To treat back pain, asthma, fever, bronchitis, cough, chest congestion, chronic headache, inflammation, infertility, GIT disorders; Seed oil is used for the remedy of an abscess, nasal ulcer, swollen joint, and eczema; Analgesic; Liver tonic; Diuretic.
10	**Fenugreek seeds** (Seed)	میتھی دانہ	Trigonella foenum-graecum Leguminosae	Alkaloids [trigonelline]; Flavonoids; A minor amount of volatile oil [neryl acetate, camphor, and β-Pinene] and fixed oils [rich in linoleic acid, linolenic acid, and oleic acid]; Diosgenin; Mucilage; Amino acids; The alkaloid and volatile compound present are the two main chemical constituents that cause the bitter taste and the odor of the seeds.	To treat loss of appetite, flatulence, dyspepsia, colic, diarrhea, dysentery, enlargement of liver and spleen, and hypercholesterolemia; Tonic; Antiseptic; Anti-diabetic; Anti-inflammatory.
11	**Moringa** (Leaf)	سوہانجنا	Moringa oleifera Moringaceae	Vitamins [carotenoids (with pro-vitamin A potential) and vitamin C]; Minerals; Tannins; Flavonoids [myrecytin, quercetin, and kaempferol]; Phenolic acids [chlorogenic acid and gallic acid].	To treat diabetes, constipation, and asthma; To increase breast milk production; To overcome the deficiency of vitamins and minerals; To help in fat loss.
12	**Senna** (Leaf)	سنا مکی	Cassia acutifolia / C. angustifolia Leguminosae	Anthraquinone glycosides [sennoside A, sennoside B, sennoside C, and sennoside D]	Cathartic (The cathartic effect is realized by inhibition of water and electrolyte absorption from the large intestine, which increases the volume and pressure of the intestinal contents. This will stimulate colon motility resulting in propulsive contractions. The cathartic action of senna is partially mediated via stimulation of colonic fluid and electrolyte secretion, and this secretion is mediated by stimulation of endogenous prostaglandin E_2 formation); Senna is contraindicated in the presence of intestinal obstruction, acute inflammatory intestinal diseases, or appendicitis.
13	**Neem**	نیم	Azadirachta indica	Terpenoids [azadirachtin, nimbolinin, and nimbin];	Used for eye disorders, intestinal worms, stomach upset, loss of

	(Leaf)		Meliaceae	Flavonoids [quercetin]; Minerals.	appetite, skin ulcers, diabetes, gum disease (gingivitis), and liver problems. Wound healing; Anti-inflammatory.
14	**Glycyrrhiza / Licorice** (Root)	ملٹھی	Glycyrrhiza glabra Leguminosae	Saponin glycosides [glycyrrhizin (also called glycyrrhizic acid), liquiritin, isoliquiritin, liquiritoside, isoliquiritoside]; Glycyrrhizin is a saponin glycoside, 50 times as sweet as sugar; Glucose; Mannitol; Starch.	Demulcent; Expectorant; It is used in cough syrups; To treat peptic ulcer; Flavoring agent to mask the bitter taste of drugs; Surfactant (this property facilitates the absorption of poorly absorbed drugs); Anti-inflammatory (It is used in dermatological practice); Glycyrrhizin increases sodium and fluid retention and promotes potassium depletion (Persons with cardiac problems and hypertension should avoid the consumption of significant quantities of licorice); Commercially, licorice is added to chewing gums, chocolate candy, cigarettes, smoking mixtures, chewing tobacco, and snuff (When it is added to beer, it increases the foaminess)
15	**Rauwolfia** (Root)	چھوٹی چندن	Rauvolfia serpentina Apocynaceae	Alkaloids [reserpine, rescinnamine, deserpidine, ajmaline, isoajmaline, rauwolfinine, serpentine, serpentinine, alstonine]; Phytosterols; Fatty acids; Sugars.	Rauwolfia preparations and reserpine are used in the management of essential hypertension and in certain neuropsychiatric disorders; The drug has sedative activity.
16	**Aaqarqarhaa** (Root)	عقر قرحا	Anacyclus pyrethrum Asteraceae	Alkaloids [pellitorine]; Alkylamides; Pyrethrins.	To treat toothache, angina, digestive problems, lethargy, female infertility, paralysis of the tongue and limbs; Aphrodisiac; Immunostimulant; Antidepressant; Anti-inflammatory.
17	**Turmeric** (Rhizome)	ہلدی	Curcuma longa Zingiberaceae	Curcumin (a bright yellow phenolic compound); Volatile oil.	Curcumin is a broad-spectrum antimicrobial; Curcuma is used as a coloring agent and condiment in curry powders and pickles; It also has been employed to stimulate biliary secretions and treat gallstone; Turmeric possesses anti-oxidant and anti-inflammatory properties and is used to treat inflammatory conditions; It is also used, orally and topically, to heal fractured bones
18	**Ginger** (Rhizome)	سونٹھ	Zingiber officinale Zingiberaceae	Volatile oil [containing zingiberene, zingiberol, bisabolene]; This volatile oil is responsible for the	Ginger is used in loss of appetite and in dyspeptic complaints; Flavoring agent; Carminative; Anti-inflammatory; It is used to

				characteristic aroma of ginger; Oleoresin [containing zingerone and shogaol (aromatic ketones)]; This oleoresin is responsible for the characteristic pungency of ginger; Starch (more than 50%).	control motion sickness vomiting; Anti-emetic; Condiment; To manufacture ginger ale (ginger-flavored soft drinks).
19	**Rhubarb** (Rhizome)	ریوند چینی	Rheum officinale Polygonaceae	Anthroquinone glycosides; Stilbenes; Tannins; polysaccharides.	Cathartic; Anti-inflammatory; To treat cholestatic hepatitis.
20	**Clove** (Flower bud)	لونگ	Syzygium aromaticum (also called Eugenia caryophyllus) Myrtaceae	Volatile oil (containing eugenol and eugenol acetate); Tannins.	Flavoring agent; Toothache remedy (applied topically); Used in tooth pastes; Antiseptic; Counter irritant; Carminative.
21	**Ephedra** (Aerial branches)	سوم کلپا	Ephedra sinica Ephedraceae	Alkaloids [ephedrine and pseudoephedrine]	To treat and relieve the symptoms of asthma, bronchitis, hay fever, cold, and flu.
22	**Cinnamon bark** (Stem bark)	دار چینی	Cinnamomum loureirii Lauraceae	Volatile oil [containing cinnamic aldehyde]; Cinnamaldehyde decomposes to styrene because of oxidation as a result of bad storage or transport conditions; Catechins; Mucilage, Starch.	Flavoring agent (very common in cold coffee, shakes, etc., in Europe); Carminative; Antiseptic.
23	**Tragacanth** (Dried gum)	گوند کتیرا	Astragalus gummifer Leguminosae	Sugars; Uronic acid units; Tragacanthic acid; Steroidal glycoside.	To treat GIT disorders; An ingredient in toothpastes, hand lotions, and vaginal creams and jellies; In foods, tragacanth is important for stabilizing and thickening ingredients.
24	**Asafoetida** (Dried solid resin)	ہینگ	Ferula asafoetida Umbelliferae	Volatile oil [containing isobutylpropanyl disulfide, that is accompanied by a number of related organic sulfides]; Resins; Gum; Some terpenes; Umbelliferone (in combined form).	Carminative; Expectorant; Laxative; Antispasmodic; The drug has a mild intestinal disinfectant effect; The drug is contraindicated in pregnancy.

Instruction about the Experiments of Section A

- Section A is focused on the 'study and organoleptic evaluation of crude drugs'
- The students will be provided with the samples of crude drugs
- The students have to perform organoleptic evaluation of the given samples and write the organoleptic characters in the given format / space of the lab manual
- Moreover, the students are supposed to search online, the crude drug(s) and/or available product(s) / brand(s) / formulation(s) containing the given crude drug(s) and write the details in the relevant section of the lab manual
- The details might include

1. The crude drug(s) itself
2. The name of the available product(s) / brand(s) / formulation(s) containing the given crude drug(s)
3. Composition of the formulation(s)
4. Uses of the crude drug(s) and/or available product(s) / brand(s) / formulation(s) containing the given crude drug(s)
5. Manufacturer / supplier of the crude drug(s) and/or available product(s) / brand(s) / formulation(s) containing the given crude drug

Experiment No. A01

Study and organoleptic evaluation of coriander

	Organoleptic characteristics
	**Shape:**
	Texture/feel:
	External markings:
	Size:
	External color:
	Fracture:
	Fractured surface:
	Internal color:
Coriander	**Odor:**
	Taste:

Available brands / formulations

Experiment No. A02

Study and organoleptic evaluation of fennel

	Organoleptic characteristics
	**Shape:**
	Texture/feel:
	External markings:
	Size:
	External color:
	Fracture:
	Fractured surface:
	Internal color:
Fennel	**Odor:**
	Taste:

Available brands / formulations

Experiment No. A03

Study and organoleptic evaluation of cassia and cardamom

	Organoleptic characteristics
	**Shape:**
	Texture/feel:
	External markings:
	Size:
	External color:
	Fracture:
	Fractured surface:
	Internal color:
Cassia	**Odor:**
	Taste:

Available brands / formulations

Experiment No. A03

Study and organoleptic evaluation of cassia and cardamom

	Organoleptic characteristics
	**Shape:**
	Texture/feel:
	External markings:
	Size:
	External color:
	Fracture:
	Fractured surface:
	Internal color:
Cardamom	**Odor:**
	Taste:

Available brands / formulations

Experiment No. A04

Study and organoleptic evaluation of ashwagandha and colocynth

	Organoleptic characteristics
	**Shape:**
	Texture/feel:
	External markings:
	Size:
	External color:
	Fracture:
	Fractured surface:
	Internal color:
Ashwagandha	**Odor:**
	Taste:

Available brands / formulations

Experiment No. A04

Study and organoleptic evaluation of ashwagandha and colocynth

	Organoleptic characteristics
	**Shape:**
	Texture/feel:
	External markings:
	Size:
	External color:
	Fracture:
	Fractured surface:
	Internal color:
Colocynth	**Odor:**
	Taste:

Available brands / formulations

Experiment No. A05

Study and organoleptic evaluation of tribulus and reetha

	Organoleptic characteristics
	**Shape:**
	Texture/feel:
	External markings:
	Size:
	External color:
	Fracture:
	Fractured surface:
	Internal color:
Tribulus	**Odor:**
	Taste:

Available brands / formulations

Experiment No. A05

Study and organoleptic evaluation of tribulus and reetha

	Organoleptic characteristics
	**Shape:**
	Texture/feel:
	External markings:
	Size:
	External color:
	Fracture:
	Fractured surface:
	Internal color:
Reetha	**Odor:**
	Taste:

Available brands / formulations

Experiment No. A06

Study and organoleptic evaluation of nigella seeds and fenugreek seeds

	Organoleptic characteristics
	**Shape:**
	Texture/feel:
	External markings:
	Size:
	External color:
	Fracture:
	Fractured surface:
	Internal color:
Nigella seeds	**Odor:**
	Taste:

Available brands / formulations

Experiment No. A06

Study and organoleptic evaluation of nigella seeds and fenugreek seeds

	Organoleptic characteristics
	**Shape:**
	Texture/feel:
	External markings:
	Size:
	External color:
	Fracture:
	Fractured surface:
	Internal color:
Fenugreek seeds	**Odor:**
	Taste:

Available brands / formulations

Experiment No. A07

Study and organoleptic evaluation of moringa and senna

	Organoleptic characteristics
	**Shape:**
	Texture/feel:
	External markings:
	Size:
	External color:
	Fracture:
	Fractured surface:
	Internal color:
Moringa	**Odor:**
	Taste:

Available brands / formulations

Experiment No. A07

Study and organoleptic evaluation of moringa and senna

	Organoleptic characteristics
	**Shape:**
	Texture/feel:
	External markings:
	Size:
	External color:
	Fracture:
	Fractured surface:
	Internal color:
Senna	**Odor:**
	Taste:

Available brands / formulations

Experiment No. A08

Study and organoleptic evaluation of neem and licorice

	Organoleptic characteristics
	**Shape:**
	Texture/feel:
	External markings:
	Size:
	External color:
	Fracture:
	Fractured surface:
	Internal color:
Neem	**Odor:**
	Taste:

Available brands / formulations

Experiment No. A08

Study and organoleptic evaluation of neem and licorice

	Organoleptic characteristics
	**Shape:**
	Texture/feel:
	External markings:
	Size:
	External color:
	Fracture:
	Fractured surface:
	Internal color:
Licorice	**Odor:**
	Taste:

Available brands / formulations

Experiment No. A09

Study and organoleptic evaluation of rauwolfia and aaqarqarhaa

	Organoleptic characteristics
	**Shape:**
	Texture/feel:
	External markings:
	Size:
	External color:
	Fracture:
	Fractured surface:
	Internal color:
Rauwolfia	**Odor:**
	Taste:

Available brands / formulations

Experiment No. A09

Study and organoleptic evaluation of rauwolfia and aaqarqarhaa

	Organoleptic characteristics
	**Shape:**
	Texture/feel:
	External markings:
	Size:
	External color:
	Fracture:
	Fractured surface:
	Internal color:
Aaqarqarhaa	**Odor:**
	Taste:

Available brands / formulations

Experiment No. A10

Study and organoleptic evaluation of turmeric and ginger

	Organoleptic characteristics
	**Shape:**
	Texture/feel:
	External markings:
	Size:
	External color:
	Fracture:
	Fractured surface:
	Internal color:
Turmeric	**Odor:**
	Taste:

Available brands / formulations

Experiment No. A10

Study and organoleptic evaluation of turmeric and ginger

	Organoleptic characteristics
	**Shape:**
	Texture/feel:
	External markings:
	Size:
	External color:
	Fracture:
	Fractured surface:
	Internal color:
Ginger	**Odor:**
	Taste:

Available brands / formulations

Experiment No. A11

Study and organoleptic evaluation of rhubarb and clove

	Organoleptic characteristics
	**Shape:**
	Texture/feel:
	External markings:
	Size:
	External color:
	Fracture:
	Fractured surface:
	Internal color:
Rhubarb	**Odor:**
	Taste:

Available brands / formulations

Experiment No. A11

Study and organoleptic evaluation of rhubarb and clove

	Organoleptic characteristics
	**Shape:**
	Texture/feel:
	External markings:
	Size:
	External color:
	Fracture:
	Fractured surface:
	Internal color:
Clove	**Odor:**
	Taste:

Available brands / formulations

Experiment No. A12

Study and organoleptic evaluation of ephedra and cinnamon bark

	Organoleptic characteristics
	**Shape:**
	Texture/feel:
	External markings:
	Size:
	External color:
	Fracture:
	Fractured surface:
	Internal color:
Ephedra	**Odor:**
	Taste:

Available brands / formulations

Experiment No. A12

Study and organoleptic evaluation of ephedra and cinnamon bark

	Organoleptic characteristics
	**Shape:**
	Texture/feel:
	External markings:
	Size:
	External color:
	Fracture:
	Fractured surface:
	Internal color:
Cinnamon bark	**Odor:**
	Taste:

Available brands / formulations

Experiment No. A13

Study and organoleptic evaluation of tragacanth and asafoetida

	Organoleptic characteristics
	**Shape:**
	Texture/feel:
	External markings:
	Size:
	External color:
	Fracture:
	Fractured surface:
	Internal color:
Tragacanth	**Odor:**
(un-organized crude drug)	**Taste:**

Available brands / formulations

Experiment No. A13

Study and organoleptic evaluation of tragacanth and asafoetida

	Organoleptic characteristics
	**Shape:**
	Texture/feel:
	External markings:
	Size:
	External color:
	Fracture:
	Fractured surface:
	Internal color:
Asafoetida	**Odor:**
(un-organized crude drug)	**Taste:**

Available brands / formulations

SECTION B

Physical Evaluation of Crude Drugs

Physical evaluation of crude drugs

- Physical evaluation refers to the evaluation of physical contents of crude drugs
- Physical parameters are rarely constant for crude drugs, but may help in evaluation
- Physical evaluation parameters include moisture content (loss on drying), swelling index, foaming index, ash values, specific gravity, density, optical rotation, refractive index, melting point, viscosity, solubility in different solvents, etc.

1. Determination of loss on drying (LOD)

- The loss on drying (LOD) is determined by heating a drug at 105°C in an oven or putting in a desiccator to a constant weight
- To perform the experiment, place about 2-5 g, or the quantity specified, of the crude drug (raw or powdered), in a previously dried and tared flat weighing bottle or China dish
- Dry the sample by one of the following techniques;
  - In an oven at 105 °C, or
  - In a desiccator over phosphorus pentoxide, under atmospheric pressure at room temperature
- Dry until two consecutive weighings do not differ by more than 5 mg, or dry for 2 hours, unless otherwise specified in the test procedure
- Calculate the 'loss in weight' by subtracting the 'final weight' from the 'initial weight'
- Calculate LOD in percentage by using the following formula
  - LOD (%) = [loss in weight ÷ Initial weight] × 100

2. Determination of swelling index

- The swelling index is the "volume in mL taken up by the swelling of 1 g of herbal material under specified conditions"
- To perform the experiment, convert the crude drug into a coarse powder
- Take a sample of 1 g
- Introduce the sample into a 25 mL glass-stoppered measuring cylinder
- Unless otherwise indicated in the test procedure, add 25 mL of water and shake the mixture thoroughly every 10 minutes for 1 hour
- Allow to stand for 2 hours at room temperature, or as specified
- Measure the volume in mL occupied by the herbal material, including any sticky mucilage
  - This volume, in mL, is the swelling index of the crude drug
- For substances characterized by significant swelling tendencies (e.g., ispaghula husk)
  - It is advisable to use a reduced quantity of sample, potentially around 0.1 g
  - This precautionary measure is essential to prevent the material from overflowing the cylinder during the swelling process
  - After measuring the volume of this 0.1 g drug, adjust the result (swelling index value) relevant to 1 g
  - This adjustment is crucial as the swelling index represents the volume in mL occupied by 1 g of herbal material after swelling

3. Determination of ash

Total ash values

- The total ash method is designed to measure the total amount of material remaining after ignition
- This includes both 'physiological ash' and 'non-physiological ash'
  - Physiological ash is derived from the plant tissue itself
  - Non-physiological ash is the residue of the extraneous matter (e.g., sand and soil) adhering to the plant surface

- To perform the experiment, place about 2-4 g of the ground air-dried material, accurately weighed, in a previously ignited and tared crucible (usually of platinum or silica)
- Spread the material in an even layer and ignite it by gradually increasing the heat to 500-600 °C until it is white, indicating the absence of carbon
- Cool in a desiccator and weigh
- If carbon-free ash cannot 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 a hot-plate and ignite to constant weight
- Allow the residue to cool in a suitable desiccator for 30 minutes, then weigh without delay
- Calculate the content of total ash in mg per g of air-dried material

Acid-insoluble ash

- Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter
- This measures the amount of silica present, especially as sand and siliceous earth
- To perform the experiment, add 25 mL of hydrochloric acid (~70 g/L) TS to the crucible containing the total ash
- Cover with a watch-glass and boil gently for 5 minutes
- Rinse the watch-glass with 5 mL of hot water and add this liquid to the crucible
- Collect the insoluble matter on an ashless 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 a hotplate and ignite to constant weight
- Allow the residue to cool in a suitable desiccator for 30 minutes, then weigh without delay
- Calculate the content of acid-insoluble ash in mg per g of air-dried (original sample of the crude drug taken) material

Water-soluble ash

- Water-soluble ash is the difference in weight between the total ash and the residue after treatment of the total ash with water
- To perform the experiment, add 25 mL of water to the crucible containing the total ash
- Boil for 5 minutes
- Collect the insoluble matter in a sintered-glass crucible or on an ashless filter paper
- Wash with hot water and ignite in a crucible for 15 minutes at a temperature not exceeding 450 °C
- Subtract the weight of this residue in mg from the weight of total ash
- Calculate the content of water-soluble ash in mg per g of air-dried (original sample of the crude drug taken) material

4. Determination of foaming index

- Many herbal materials contain saponins that can cause a persistent foam when an aqueous decoction is shaken
- The foaming ability of an aqueous decoction of herbal materials and their extracts is measured in terms of a foaming index
- To perform the experiment, reduce about 1 g of the herbal material to a coarse powder (sieve size number 1250)
- Weigh accurately and transfer to a 500 mL conical flask containing 100 mL of boiling water
- Maintain at moderate boiling for 30 minutes

- Cool and filter into a 100 mL volumetric flask
- Add sufficient water through the filter to dilute to volume 100 mL
- Pour the decoction into 10 stoppered test-tubes (height 16 cm, diameter 16 mm) in successive portions of 1 mL, 2 mL, 3 mL, etc. up to 10 mL
- Adjust the volume of the liquid in each tube with water to 10 mL
- Stopper the tubes and shake them in a lengthwise motion for 15 seconds, two shakes per second
- Allow to stand for 15 minutes and measure the height of the foam
- The results are assessed as follows;
  - If the height of the foam in every tube is less than 1 cm, the foaming index is less than 100
  - If a height of foam of 1 cm is measured in any tube, the volume of the herbal material decoction in this tube (*a*) is used to determine the index
    - If this tube is the first or second tube in a series, prepare an intermediate dilution in a similar manner to obtain a more precise result
  - If the height of the foam is more than 1 cm in every tube, the foaming index is over 1000
    - In this case repeat the determination using a new series of dilutions of the decoction in order to obtain a result
- Calculate the foaming index using the following formula;
  $1000 \div a$
  a = the volume in mL of the decoction used for preparing the dilution in the tube where foaming to a height of 1 cm is observed

Reference

- WHO. Quality control methods for herbal materials. World Health Organization, 2011.

Instruction about the Experiments of Section B

- Section B is focused on the 'physical evaluation of crude drugs'
- In each experiment, two samples (sample A and sample B) of the same crude drug will be evaluated
- Sample A: Consists of the actual good quality crude drug
- Sample B: Consists of the same crude drug as sample A, but is
  - Adulterated, and/or not properly stored, and/or aged, and/or of superior quality, etc.
- After performing the experiment, the students will have to write 'Discussion'
  - In discussion, the students will speculate the possible reasons about the difference in the results/values of physical evaluation parameters of sample A and sample B
  - The students will also write the physical evaluation results/values of that crude drug from official books, e.g., the values of loss on drying, total ash, and swelling index of fenugreek seeds mentioned in British Pharmacopoeia are maximum 12 %, minimum 5 %, and minimum 6, respectively
  - The students will also speculate the possible reasons about the difference in the results/values of physical evaluation parameters, they obtained, and the results/values of physical evaluation mentioned in official books
  - Moreover, the students will also speculate the possible reasons about the difference in the results/values of physical evaluation, they obtained, of two different drugs (e.g., that of experiment B01 and experiment B02)
    - For example, if the students evaluate swelling index of fenugreek seeds in one experiment and swelling index of ispaghula husk in next experiment, then
      The students can discuss why the swelling index of fenugreek seeds is lower than swelling index of ispaghula husk
- Any crude drug can be evaluated in section B 'physical evaluation of crude drugs'

Experiment No. B1

Determination of loss on drying (LOD) of fenugreek seeds

Sample A of fenugreek seeds:
Sample B of fenugreek seeds:
Discussion

Experiment No. B2

Determination of loss on drying (LOD) of _____

Discussion

Experiment No. B3

Determination of swelling index of ispaghula husk

Discussion

Experiment No. B4

Determination of swelling index of _____

Sample A of fenugreek seeds:
Sample B of fenugreek seeds:
Discussion

Experiment No. B5

Determination of foaming index of licorice

Sample A of fenugreek seeds:
Sample B of fenugreek seeds:
Discussion

Experiment No. B6

Determination of foaming index of _____

Discussion

Experiment No. B7

Determination of ash values of _____

Discussion

Experiment No. B8

Determination of ash values of _____

Discussion

SECTION C

Organoleptic and Microscopic Evaluation of Powdered Drugs

Microscopic evaluation

- Microscopic evaluation involves a detailed examination of microscopic characters of a crude drug
- A microscope is used to detect various cellular tissues, such as trichomes, stomata, starch granules, calcium oxalate crystals, and aleurone grains
- Crude drugs can be identified microscopically by making their powders and/or cutting the thin TS (transverse) or LS (longitudinal) sections and by staining them with staining reagents
- Microscopic evaluation is performed on organized crude drugs

Methodology for microscopic evaluation of powdered drugs

- Place 1 or 2 drops of chloral hydrate TS on a glass slide
- Moisten the tip of a needle with water and dip into the drug powder
- Transfer a small quantity of the drug powder that adheres to the needle tip into the drop of chloral hydrate TS on the slide
- Stir thoroughly, but carefully
- Place a precleaned cover glass, held with tweezers, on the slide in such a manner that its one edge first makes contact with the mounting medium (chloral hydrate TS) and then is lowered into the place
  - In this method, large air bubbles are escaped instead of remaining in the sample
- Press lightly on the cover-glass (e.g., with the handle of the needle) and remove excess fluid from the margins of the cover-glass with a strip of filter-paper
- If necessary, heat very gently by passing the slide *to and fro* over a very low flame
  - The use of a micro burner is recommended
- As soon as bubbles start to appear, stop the heating and, if necessary, run more chloral hydrate TS under the cover glass
- If the material contains considerable amounts of starch or mucilage it will probably be necessary to repeat the heating several times
  - It is important to ensure that sufficient liquid is always present to prevent the preparation from drying out
- When a satisfactory preparation has been made, observe under the microscope
- If necessary, add one or two drops of glycerol, before examination under the microscope
  - To inhibit the formation of crystals of chloral hydrate during the examination

Books to see the monographs of powdered drugs

- Jackson BP, Snowdon DW. Atlas of microscopy of medicinal plants, culinary herbs and spices. Belhaven Press, 1990.
- Evans WC. Trease and Evans Pharmacognosy. Elsevier, 16th edition, 2009.
- Upton R *et al*. American herbal pharmacopoeia: botanical pharmacognosy-microscopic characterization of botanical medicines. CRC Press, 2016.

Preparation of chloral hydrate TS

- Chloral hydrate TS is prepared by dissolving 50 g of chloral hydrate in 20 mL of water with heating
- Chloral hydrate is a colorless hygroscopic crystalline substance
- It is effectively used as a clearing and bleaching agent to dissolve starch, protein, chlorophyll, resins, and other materials
- It also causes shrunken cells to expand
- It does not dissolve calcium oxalate, so it can be used effectively to detect these crystals
- It clears and expands tissues without producing marked distortion

- For all kinds of crude drugs of herbal origin, whether fresh or dried, chloral hydrate can be used most effectively as a clearing reagent

References

- World Health Organization. Quality control methods for herbal materials. World Health Organization, 2011.
- Jackson BP, Snowdon DW. Atlas of microscopy of medicinal plants, culinary herbs and spices. Belhaven Press, 1990.
- Evans WC. Trease and Evans Pharmacognosy. Elsevier, 16th edition, 2009.
- Upton R *et al*. American herbal pharmacopoeia: botanical pharmacognosy-microscopic characterization of botanical medicines. CRC Press, 2016.
- Wheeler BP, Wilson L. Practical forensic microscopy-A laboratory manual. Wiley-Blackwell, 2008. Page xiii-xvii. [just few pages at the start of the book to understand the basics of microscopy].

Instruction about the Experiments of Section C

- Before starting the experiments of section C, learn the handling of microscope
  - Pages at the start of the book "Practical forensic microscopy-A laboratory manual" can be helpful for this purpose
- Evaluate the organoleptic characters (e.g., color, taste, odor, fineness, etc.) of the given sample of powdered drug
  - After that, perform the microscopic evaluation
- On left side of the notebook (blank side)
  - Write organoleptic characters of the powdered drug
  - Draw the microscopic structures observed under the microscope
- On right side of the notebook (with lines)
  - Write the details of these microscopic structures, e.g., as written in the book "Atlas of microscopy of medicinal plants, culinary herbs and spices"

Experiment No. C1

Organoleptic and microscopic evaluation of _____

Organoleptic characters:
Microscopic observations

Experiment No. C1

Organoleptic and microscopic evaluation of _____

Experiment No. C2

Organoleptic and microscopic evaluation of _____

Organoleptic characters:
Microscopic observations

Experiment No. C2

Organoleptic and microscopic evaluation of _____

Experiment No. C3

Organoleptic and microscopic evaluation of _____

Organoleptic characters:
Microscopic observations

Experiment No. C3

Organoleptic and microscopic evaluation of _____

Experiment No. C4

Organoleptic and microscopic evaluation of _____

Organoleptic characters:
Microscopic observations

Experiment No. C4

Organoleptic and microscopic evaluation of _____

SECTION D

Section Cutting and Microscopic Evaluation of Crude Drugs

Section cutting of crude drugs

- Cutting the thin TS (transverse) or LS (longitudinal) sections and observe them under microscope, after staining them with staining reagents, is also an integral part of microscopic evaluation

Methodology for section cutting of crude drugs

- Soak the crude drug in water to make it soft
- Cut the sections of the given crude drug by sharp razor blade
- Thin crude drugs (e.g., leaves) can be put in a cube of potato and cut
- For each cut the blade should be cleaned and moistened with water
  - To avoid contamination of sections and to cut smoothly
- These sections are dehydrated by treating them with alcohol in increasing concentration
  - Treat, step by step, for two minutes in each concentration of ethanol i.e., 10% → 20% → 30% → 40% → 50% → 60% → 70% → 80% → 90% → 95%, → absolute ethanol
  - Petri dishes ca be used for this purpose
- Staining (e.g., with safranin and fast green) can also be done during this dehydration process
  - Treat, step by step, for two minutes in each concentration of ethanol i.e., 10% → 20% → 30% → 40% → 50% → *50% ethanol containing two drops of safranin solution* → 50% ethanol → *50% ethanol containing two drops of fast green solution* → 60% → 70% → 80% → 90% → 95% → absolute ethanol
- Sections are treated with clove oil for one minute
  - To clear the sections and to counter the shrinking and hardening of tissues caused by ethanol
- The sections are then washed with xylol
  - To remove all traces of clove oil (to avoid color fading and brittleness of tissues)
- Then these sections are transferred on a clean glass slide, fixed with Canada balsam, covered with a clean cover glass, and observed under microscope
- Literature can be searched to find the histological monographs of the crude drugs being evaluated

Preparation of safranin TS and fast green TS

- Dissolve 1 g of safranin in 100 mL of distilled water
- Dissolve 0.5 g of fast green in 100 mL of 95% ethanol
- Safranin and fast green are among the extensively used staining agents for histochemical studies of plant materials
- Safranin gives pink to red color to lignified tissues while fast green (counter stain) gives green color to all non-lignified tissues

Books to see the monographs of section cutting

- Evans WC. Trease and Evans Pharmacognosy. Elsevier, 16th edition, 2009.
- Upton R *et al*. American herbal pharmacopoeia: botanical pharmacognosy-microscopic characterization of botanical medicines. CRC Press, 2016.
- Research Papers

References

- World Health Organization. Quality control methods for herbal materials. World Health Organization, 2011.
- Johansen DA. Plant microtechnique. McGraw-Hill, 1st edition, 1940.
- Mukherjee PK. Quality control and evaluation of herbal drugs: Evaluating natural products and traditional medicine. Elsevier, 2019. Chapter 1 (Optimal preparation of tissue sections for light-microscopic analysis of phloem anatomy).

- Khandelwal KR. Practical pharmacognosy: Techniques and experiments. Nirali Prakashan, 19th edition, 2008.

Instruction about the Experiments of Section D

- Before starting the experiments of section D, the students can be trained for section cutting on fresh stems of herbaceous plants
- Cut the thin TS (transverse) or LS (longitudinal) section of the given drug, stain it, and observe under the microscope
- On left side of the notebook (blank side)
  - Draw the microscopic structures observed under the microscope
- On right side of the notebook (with lines)
  - Write the details of these microscopic structures

Experiment No. D1

Section cutting and microscopic evaluation of _____

Experiment No. D1

Section cutting and microscopic evaluation of _____

Experiment No. D2

Section cutting and microscopic evaluation of _____

Experiment No. D2

Section cutting and microscopic evaluation of _____

Experiment No. D3

Section cutting and microscopic evaluation of _____

Experiment No. D3

Section cutting and microscopic evaluation of _____

Experiment No. D4

Section cutting and microscopic evaluation of _____

Experiment No. D4

Section cutting and microscopic evaluation of _____

SECTION E

Projects

Experiment E1

- Each student has to search one traditional formulation / home remedy
- In the notebook, the student has to explain it scientifically, as explained below
  - The example explained below is a of a hypothetical formulation
- The student can speculate the possible mechanism of action

Experiment E1

A traditional formulation to treat superficial burns

Ingredients:
Honey, peppermint, and camphor.

Preparation:
In 10 g of honey, add 20 mg of fresh peppermint paste and 25 mg of camphor, and mix thoroughly.

Method of use:
Apply topically on burn surface.

Chemical constituents:
Peppermint is rich in menthol. Camphor is a monoterpene ketone. Honey contains invert sugars.

Proposed mechanism of action:
The honey has emollient, skin protective, and antibacterial properties while camphor has cooling properties. Peppermint contains menthol which is also well known for having cooling effect. These properties make this formulation suitable for superficial burn healing.

Source of the formulation:
I took the recipe this formulation from my grandmother.

References:
Cite the references.

Experiment E2

- Each student has to search one drug which is obtained from natural sources and is used in modern medicine
  - The drug can be of plant source (e.g., phytochemical), of animal source (e.g., enzyme), or of microbial source (e.g., vaccine)
- The student will write one page on this drug
  - Below is the example of hyoscine
  - For animal and microbial source drugs, the scheme (explained below) can be modified accordingly

Experiment E2

Hyoscine

Botanical source:
Dried leaf and flowering or fruiting tops with branches of *Datura stramonium*, dried leaf and flowering or fruiting top of *Atropa belladonna*, and dried leaf, with or without stem and flowering or fruiting tops of *Hyoscyamus niger* (Fam. Solanaceae).

Mechanism of action:
XXXXXXXXXXXXXXXXXXXXXXXXXXX.

Therapeutic uses:
XXXXXXXXXXXXXXXXXXXXXXXXXXX.

Contraindications:
XXXXXXXXXXXXXXXXXXXXXXXXXXX.

Adverse effects:
XXXXXXXXXXXXXXXXXXXXXXXXXXX.

Brands available in Pakistan:
Buscopan®

Experiment No. E1

Experiment No. E2

SECTION F

Preparation of Herbarium Sheets

Preparation of Herbarium Sheets

- Creating a herbarium involves preserving plant specimens by mounting them on paper and documenting relevant information

Here's a step-by-step guide to prepare a herbarium

- Choose healthy and representative samples of the plant
- Collect parts that include leaves, flowers, stems, and if possible, roots
- Place the plant specimen between two sheets of blotting paper
  - If blotting paper is not available, newspaper can be used
  - The plant specimen will be sandwiched between layers of blotting paper
- Place the 'blotting paper with the plant specimen' between two pieces of cardboard
  - If cardboard is not available, place the 'blotting paper with the plant specimen' over a smooth surface and put some flat piece of wood, board, or marble tile, etc. over it
- Apply weight on top of the cardboard
- Ensure even pressure to avoid warping
- Change the blotting paper every 2-3 days to prevent mold and ensure thorough drying
- This process may take about 1-2 weeks
- Once dried, carefully remove the specimens
- Arrange the specimen on the given space of practical notebook
- Ensure that the arrangement shows important features like leaf shape, flower structure, etc.
- Use glue or adhesive tape to attach the specimen to the paper
- Dried plant specimen can be pasted directly on the page, or
  - Can be covered by a plastic film to avoid damage or falling off and then pasted on the page

Tips

- Handle dried specimens with care as they are fragile
- Ensure all parts are securely attached to prevent them from falling off

Experiment No. F1

Common Name:
Scientific Name:
Family:
Date of Collection:
Location:
Collector's Name:
Additional Notes:

Experiment No. F2

Common Name:
Scientific Name:
Family:
Date of Collection:
Location:
Collector's Name:
Additional Notes:

SECTION G

Preparation of Crude Drugs

Preparation of crude drugs

Here's a step-by-step guide to prepare a crude drug

Collection

- The sample should be collected at the most appropriate time, e.g., flowering season, spring, fall, bud opening, etc.
- Collect the sample from cultivated plants or from wild plants

Harvesting

- Harvest the sample with care by hand, or
  - By some tool, e.g., plant cutter

Drying

- Dry the collected samples under the shade, or
  - If specified for that particular crude drug, dry in oven or by curing

Garbling

- Remove the extraneous matter, such as other parts of the plant, dirt, etc.

Packaging and storage

- Pack in a plastic pouch and affix to the designated location in your practical notebook

Reference

- Tyler VE, Brady LR, Robbers JE. Pharmacognosy. Lea & Febiger, 7th edition, 1976, page 8-13.

Experiment No. G1

Crude drug:	**Local name:**
Biological origin:	
Main chemical constituents:	
Major uses:	
Organoleptic characteristics	
Shape:	**Fracture:**
Texture/feel:	**Fractured surface:**
External markings:	**Odor:**
Size:	**Taste:**
External color:	

Date of Collection:	**Location:**
Collection time:	
Harvesting method:	
Drying method:	
Garbling method:	
*Affix **here** the plastic pouch containing the crude drug*	

Experiment No. G2

Crude drug:	**Local name:**
Biological origin:	
Main chemical constituents:	
Major uses:	
Organoleptic characteristics	
Shape:	**Fracture:**
Texture/feel:	**Fractured surface:**
External markings:	**Odor:**
Size:	**Taste:**
External color:	

Date of Collection:	**Location:**
Collection time:	
Harvesting method:	
Drying method:	
Garbling method:	
*Affix **here** the plastic pouch containing the crude drug*	