Module Title	**Quality Control of Drugs of Natural Origin (WHO Guidelines)**
Subject /Course	Introduction to Pharmacognosy
Subject/Course ID	BP105T
Semester	I
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Learning Outcome of Module 03

LO	**Learning Outcome**	**Course Outcome**
LO1	Explain the importance of quality control and WHO guidelines in ensuring the authenticity, purity, and safety of drugs of natural origin.	BP 105.3
LO2	Identify different types of adulteration and evaluate crude drugs using organoleptic, microscopic, physical, chemical, and biological methods.	BP 105.3
LO3	Determine and interpret physicochemical parameters such as extractive values, moisture content, ash values, foreign organic matter, bitterness value, foaming index, haemolytic potential, swelling index, viscosity, optical rotation, refractive index, acid value, and saponification value.	BP 105.3
LO4	Apply qualitative and quantitative microscopic techniques for the authentication and standardization of crude drugs.	BP 105.3
LO5	Explain the principle and significance of DNA barcoding in the identification and quality evaluation of medicinal plants and natural products.	BP 105.4

Content Table

- Quality Control of Drugs of Natural Origin (WHO Guidelines)
- Adulteration of drugs of natural origin. Evaluation of drugs using organoleptic,
- microscopic (qualitative and quantitative), physical, chemical and biological
- methods. Physicochemical parameters: extractive values, moisture content, foreign
- organic matter, ash values, bitterness value, foaming index, haemolytic potential,
- swelling index, viscosity, optical rotation, refractive index, acid value and saponification value. DNA barcoding.

ADULTERATION OF DRUGS OF NATURAL ORIGIN

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

ADULTERATION

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

Adulteration refers to the debasement of a crude drug by mixing, substituting, or adding inferior, spurious, exhausted, or foreign materials that reduce its quality, purity, safety, and therapeutic efficacy. Adulteration may occur either unintentionally or intentionally. It is one of the major problems encountered in the trade of medicinal plants and herbal products.

Types of Adulteration in Pharmacognosy:

I. Unintentional Adulteration

Unintentional adulteration occurs accidentally without any deliberate intention to deceive. It usually results from ignorance, confusion, lack of botanical knowledge, scarcity of authentic drugs, careless collection practices, or improper handling of crude drugs. Such adulteration is common in herbal markets where collectors and traders may not possess adequate taxonomic knowledge.

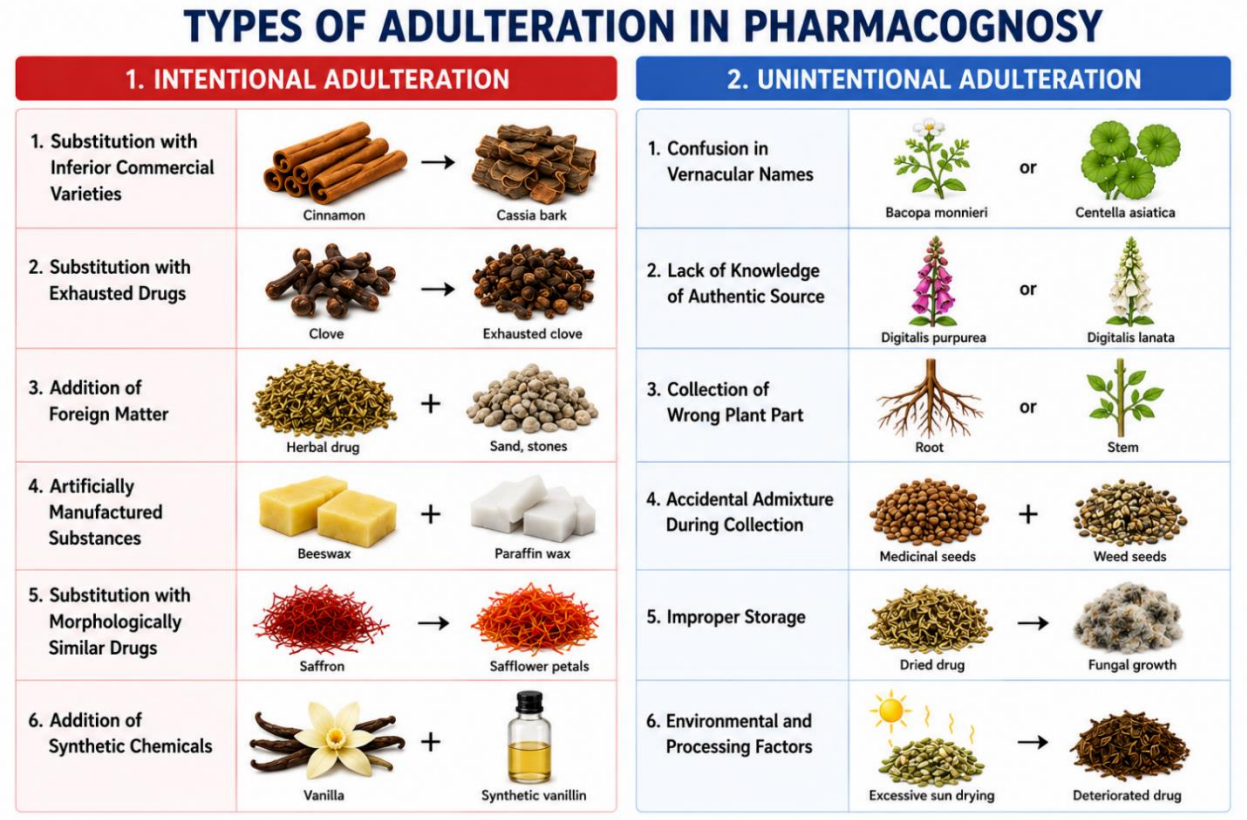

Figure1: Types of Adulteration in Crude drugs

1. Confusion in Vernacular Names

Many medicinal plants possess different local names in different regions, while a single vernacular name may refer to more than one plant species. This creates confusion during collection, marketing, and utilization of crude drugs, leading to inadvertent substitution.

Example

- Parpatta in Ayurveda refers to Fumaria parviflora.
- Parpadagam in Siddha medicine refers to Mollugo pentaphylla.

Because of the similarity in names, both species are frequently interchanged in commercial markets.

2. Lack of Knowledge of Authentic Source

Collectors and traders may not know the genuine botanical source of a drug and therefore collect or market a similar species. This type of adulteration is common when medicinal plants are obtained from different geographical regions.

Example

- The authentic source of Nagakesar is *Mesua ferrea*.
- Flowers of *Calophyllum inophyllum* are frequently sold as Nagakesar due to lack of awareness regarding the authentic source.

3. Non-availability of Authentic Plant

When the genuine medicinal plant becomes scarce because of overexploitation, habitat destruction, seasonal unavailability, or conservation restrictions, collectors often substitute it with related species that are more readily available.

Example

- *Hypericum perforatum* is scarce in India.
- *Hypericum patulum* is frequently substituted and sold under the same name.

4. Similarity in Morphology, Aroma, or Colour

Closely related species often possess similar morphological features, colour, odour, or taste. Such resemblance can lead to accidental substitution during collection and processing.

Example

- *Mucuna pruriens* seeds are frequently mixed with seeds of *Mucuna utilis* and *Mucuna deeringiana* because of their similar appearance.
- *Arnebia euchroma* has replaced *Ventilago madraspatana* as the source of Ratanjot because both yield a similar red dye.

5. Careless Collection

During harvesting, collection, drying, and transportation, unwanted plant parts, weeds, lichens, and other foreign materials may accidentally become mixed with the crude drug. Such contamination occurs due to negligence and poor collection practices.

Example

- *Parmelia perlata* is often mixed with other lichen species such as *Parmelia perforata* and *Parmelia cirrhata*.

6. Other Unknown Reasons

In some cases, adulteration occurs without any clearly identifiable reason. Historical trade practices, local customs, and undocumented substitutions may contribute to such adulteration.

Example

- Vidari is authentically obtained from *Pueraria tuberosa*.
- However, *Cycas circinalis* is frequently sold as Vidari in some regions despite being botanically unrelated.

II. Intentional Adulteration

Intentional adulteration is a deliberate and fraudulent practice performed primarily for economic gain. It involves substitution, addition, or mixing of inferior materials with genuine crude drugs to increase profit margins, compensate for scarcity, or increase the weight and volume of the product.

1. Adulteration Using Manufactured Substances

Artificially manufactured materials are prepared to resemble genuine crude drugs in shape, size, colour, or appearance. Such materials are intentionally added to deceive consumers and traders.

Examples

- Paraffin wax substituted for beeswax.
- Artificial invert sugar substituted for honey.
- Flour dough moulded and coloured to resemble ergot.

This form of adulteration is particularly difficult to detect without careful examination.

2. Substitution Using Inferior Commercial Varieties

In this practice, cheaper and inferior varieties of the same drug are substituted for the authentic variety. Although they may appear similar, their chemical composition and therapeutic value are generally lower.

Examples

- Cassia bark substituted for cinnamon.
- Japanese ginger substituted for Jamaica ginger.
- Arabian senna substituted for true senna.

The main objective is to reduce production costs and increase profit.

3. Substitution Using Exhausted Drugs

Exhausted drugs are materials from which active constituents have already been extracted. These exhausted materials are mixed with genuine drugs and often recoloured or flavoured to resemble the original product.

Examples

- Exhausted cloves mixed with genuine cloves.
- Exhausted fennel fruits mixed with fresh fennel.
- Exhausted gentian made bitter again by adding aloes.

Such drugs possess little or no therapeutic activity.

4. Substitution with Superficially Similar Inferior Natural Substances

This type involves replacement of a drug with another natural material that closely resembles it in appearance but lacks the desired medicinal properties.

Examples

- Safflower petals mixed with saffron.
- Clove stalks mixed with cloves.
- Peach kernels substituted for almonds.

The adulterant may be difficult to identify without detailed pharmacognostic examination.

5. Adulteration Using Vegetative Parts of the Same Plant

The medicinal part of a plant is mixed with excessive quantities of non-medicinal parts from the same plant to increase bulk and weight.

Examples

- Excess stems mixed with lobelia leaves.
- Excess stems present in stramonium leaves.
- Mosses and lichens mixed with cinchona bark.

Although originating from the same plant, these additional parts dilute the quality of the crude drug.

6. Addition of Toxic Materials

Toxic or harmful materials may be intentionally added to increase weight or improve appearance. Such adulteration poses serious health risks to consumers.

Examples

- Lead shot added to opium.

- Limestone pieces mixed with asafoetida.
- Glass fragments added to colophony.

This is considered one of the most dangerous forms of adulteration.

7. Adulteration of Powdered Drugs

Powdered crude drugs are highly susceptible to adulteration because the original morphology is lost during grinding. Cheap powdered substances having similar colour and texture are commonly mixed with genuine powders.

Examples

- Brick powder added to bark powders.
- Red sandalwood powder mixed with chilli powder.
- Powdered olive stones mixed with gentian powder.

Detection often requires microscopic and chemical evaluation.

8. Addition of Synthetic Principles

Synthetic chemicals or pharmaceutical substances are added to enhance the potency, colour, aroma, or market value of herbal products.

Examples

- Citral added to lemon oil.
- Benzyl benzoate added to balsam of Peru.
- Sildenafil added to certain herbal aphrodisiac preparations.

Such adulteration can cause serious adverse effects and safety concerns.

EVALUATION OF PLANT DRUGS

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

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

Methods currently employed in evaluating herbs are organoleptic, microscopic, physical,

chemical, and biological parameters.

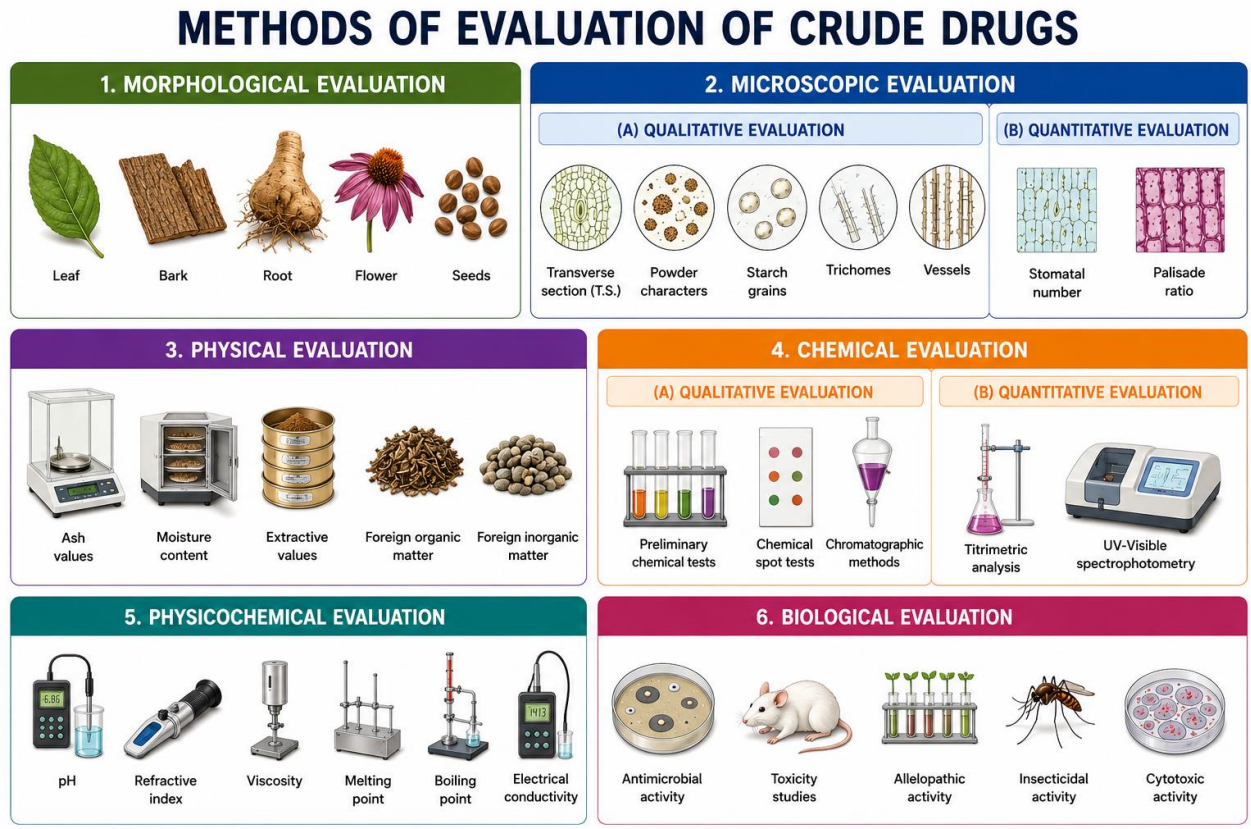

Figure 2: Methods of evaluation of Crude Drugs

ORGANOLEPTIC EVALUATION

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

- Talka gum, which is used as a substitute for acacia gum could be identified by its colour and form. Talka gum is usually broken and also some tears are brown in colour and other colourless, whereas acacia is white to yellow in colour.
- Mangosteen fruits are a substitute for bael fruits and can be identified by darker rind and the wedge shaped radiate stigmas.

- Cuprea Bark *(Remijia pedupiculata)* differs in its morphological character with cinchona.
- Blood Root used as an adulterant for hydrastis is dark reddishbrown in colour, whereas hydrastis is yellow in colour.
- *Rheum rhaponticum* are much smaller than those of the Chinese rhubarb and are easily distinguished.
- Ginger and capsicum have pungent taste, whereas gentian and chirata have bitter taste.
- Morphological differentiation of leaves and pods of Indian senna and Alexandrian senna, sweet taste of liquorice, odours of umbelliferous fruits, disc shaped structure of nux vomica, conical shape of aconite, quills of cinnamon, etc. are few examples of this organoleptic evaluation.

MICROSCOPICAL EVALUATION

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

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

Quantitative Microscopy:

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

Linear measurements

It include size of starch grains, length and width of fibres, trichomes, etc. The diameter of starch grains present in ipecacuanha assists in distinguishing its varieties. The diameter of starch grains in cassia bark distinguishes from cinnamon and detects senna stalk in powdered senna leaf.

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

Stomatal index

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

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

where, S = number of stomata per unit area and

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

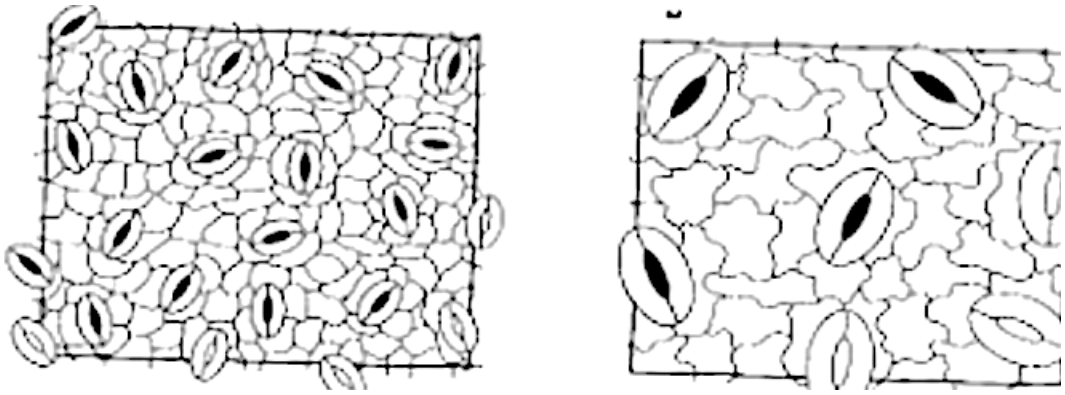

Figure 3: Stomatal no and Stomatal index

Table 1: Stomatal index of various drugs

Crude Drug	Botanical Name	Stomatal Index (%)	Leaf Surface
Belladonna	*Atropa belladonna*	20.2–23.0	Lower
Belladonna (Indian)	*Atropa acuminata*	16.2–18.3	Lower
Indian Senna	*Cassia angustifolia*	17.0–20.0	Lower
Alexandrian Senna	*Cassia acutifolia*	10.8–12.6	Lower

Vein-islet number

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

Table 2: Vein islet number of various drugs

Crude Drug	Botanical Name	Vein Islet Number (per mm²)
Coca	*Erythroxylum coca*	8–12
Truxillo Coca	*Erythroxylum truxillense*	15–26
Digitalis	*Digitalis purpurea*	2–5.5
Indian Senna	*Cassia angustifolia*	19–23
Alexandrian Senna	*Cassia acutifolia*	25–30

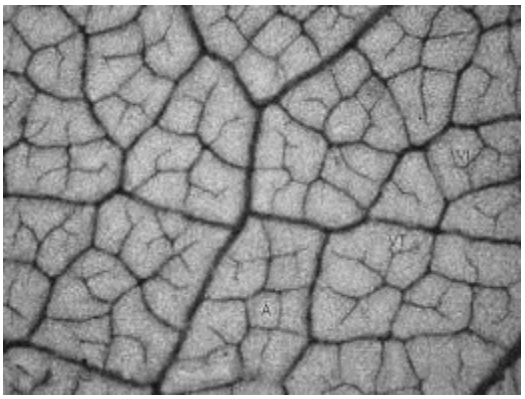

Figure 4: Vein islet and Vein terminations

Veinlet termination number

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

Palisade ratio

It is defined as the average number of palisade cells beneath each epidermal cell. The technique of palisade ratio determination was introduced by Zorning and Weiss (1925) in their studies on Compositae.

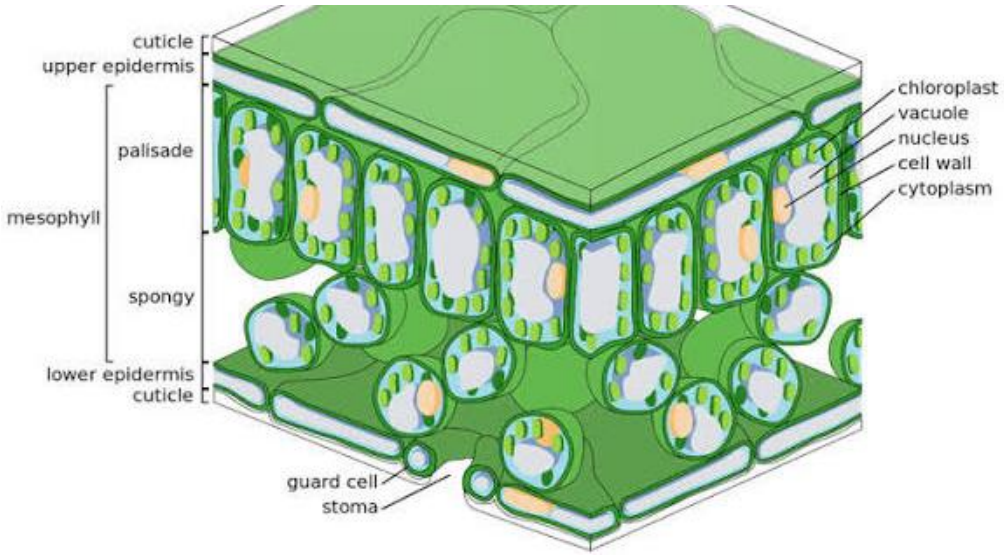

Figure 5: Palisade ratio

Table 3: Palisade tio of various drugs

Crude Drug	**Botanical Name**	**Palisade Ratio**
Belladonna	*Atropa belladonna*	6–10
Datura	*Datura stramonium*	4–7
Digitalis	*Digitalis purpurea*	2.5–6
Indian Senna	*Cassia angustifolia*	5–6

Quantitative Microscopy (Lycopodium Spore Method)

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

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

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

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

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

where,

N = number of characteristic structures (e.g. starch grains) in 25 fields; W = weight in mg of lycopodium taken;

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

M = weight in mg of the sample, calculated on basis of sample dried at 105°C; and P = 2,86,000 in case of ginger starch grains powder.

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

PHYSICAL EVALUATION

1. Solubility

Solubility is the ability of a drug or its constituents to dissolve in a particular solvent under specified conditions. It is determined by observing the behaviour of a drug in different solvents such as water, alcohol, ether, chloroform, and petroleum ether.

Method

A known quantity of drug is mixed with a solvent and the extent of dissolution is observed.

Significance

- Helps in identification of crude drugs.
- Detects adulteration.
- Assists in selecting extraction solvents.

Examples

- Colophony is soluble in light petroleum.
- Castor oil is soluble in half its volume of light petroleum.
- Alkaloidal bases are soluble in organic solvents while alkaloidal salts are soluble in water.

2. Specific Gravity

Specific gravity (relative density) is the ratio of the mass of a substance to the mass of an equal volume of water at a specified temperature. It is determined using a **pycnometer** or specific gravity bottle.

Method/Instrument

Instrument: Pycnometer/Specific Gravity Bottle

Significance

- Identification of oils and liquid drugs.
- Purity testing.
- Detection of adulteration.

Examples: Cottonseed oil: 0.88–0.93, Coconut oil: 0.925

3. Melting Point

Melting point is the temperature at which a solid changes from solid state to liquid state. It is determined using a **melting point apparatus**.

Method/Instrument

Instrument: Digital Melting Point Apparatus

Significance

- Identification of pure compounds.
- Detection of impurities.
- Quality control of isolated phytoconstituents.

Examples: Beeswax: 62–65°C, Wool fat: 34–44°C, Agar: 85°C

4. Fluorescence Analysis (Ultraviolet Light)

Certain crude drugs exhibit characteristic fluorescence when exposed to ultraviolet radiation. This property is examined using a **UV chamber**.

Method/Instrument

Instrument: UV Chamber (254 nm and 366 nm)

Significance

- Identification of crude drugs.
- Detection of adulteration.
- Useful for powdered drugs.

Example: Different varieties of rhubarb can be distinguished by their fluorescence under UV light.

PHYSICOCHEMICAL PARAMETERS

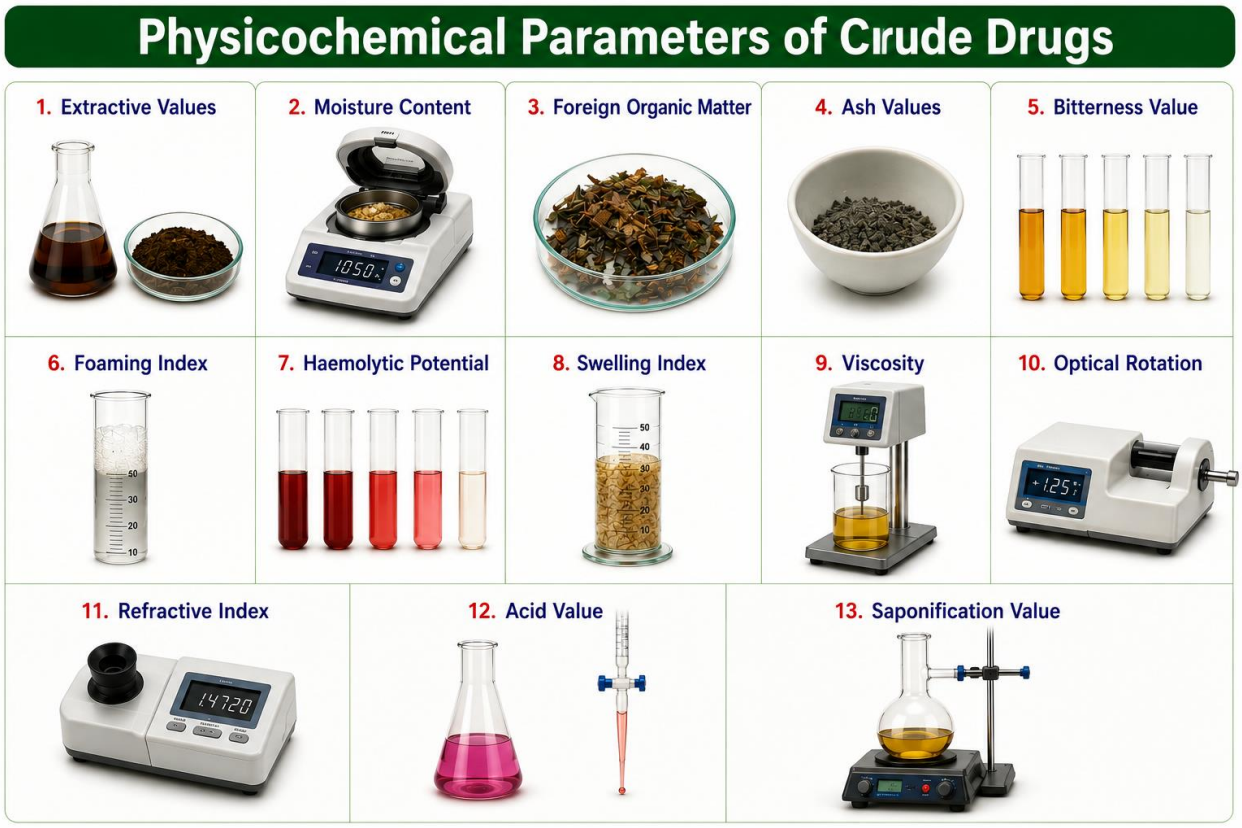

Figure 5: Physicochemical Parameters for evaluation of Crude Drugs

1. Extractive Values

Extractive values represent the percentage of active constituents extracted from a crude drug using a specified solvent. They provide an approximate measure of the amount of chemical constituents present in the drug and are particularly useful for evaluating drugs whose active principles are not easily estimated by other methods.

Principle

The crude drug is exhausted with a suitable solvent, and the soluble constituents are extracted. The solvent is evaporated and the residue obtained is weighed. The percentage of extractive matter is then calculated with reference to the air-dried drug.

Procedure

A known quantity of air-dried powdered drug (usually 5 g) is macerated with 100 mL of solvent in a closed flask for 24 hours with frequent shaking during the first 6 hours. The mixture is filtered and a measured volume of filtrate is evaporated to dryness in a tarred dish. The residue is dried at 105°C and weighed.

Types

Water-Soluble Extractive: Used for drugs containing glycosides, tannins, sugars, gums, and mucilage.

Alcohol-Soluble Extractive: Used for drugs containing alkaloids, glycosides, resins, and tannins.

Ether-Soluble Extractive: Used for drugs containing fixed oils, volatile oils, fats, and resins.

Significance

- Detects exhausted drugs.
- Evaluates active constituent content.
- Helps in quality control and standardization.

Table 4: Alcohol and water soluble extractive value of various crude drugs

Crude Drug	**Botanical Name**	**Alcohol-Soluble Extractive (% w/w)**	**Water-Soluble Extractive (% w/w)**
Senna	*Cassia angustifolia*	Not less than 15.0	Not less than 40.0
Ashwagandha	*Withania somnifera*	Not less than 15.0	Not less than 22.0
Liquorice	*Glycyrrhiza glabra*	Not less than 20.0	Not less than 30.0
Amla	*Emblica officinalis*	Not less than 30.0	Not less than 50.0

2. Moisture Content (Loss on Drying)

Moisture content is the amount of water and volatile matter present in a crude drug. Excess moisture can promote microbial growth and chemical degradation.

Principle

The sample is heated at a specified temperature until a constant weight is obtained. The loss in weight represents moisture and volatile substances.

Procedure

About 2–5 g of powdered drug is accurately weighed and placed in a drying oven at 105°C. Drying is continued until two consecutive weighings differ by not more than 0.25%.

Significance

- Prevents fungal and bacterial growth.
- Prevents hydrolysis of active constituents.
- Improves storage stability and shelf life.

Table5: Moisture content of various crude drugs

Crude Drug	**Botanical Name**	**Moisture Content / Loss on Drying (% w/w)**
Senna	*Cassia angustifolia*	Not more than 8.0
Digitalis	*Digitalis purpurea*	Not more than 5.0
Ginger	*Zingiber officinale*	Not more than 10.0
Liquorice	*Glycyrrhiza glabra*	Not more than 10.0
Turmeric	*Curcuma longa*	Not more than 10.0

3. Foreign Organic Matter

Foreign organic matter includes any material other than the specified plant part of the crude drug. The sample is visually examined and foreign matter is separated and weighed.

Procedure

A known quantity of the drug is spread on a tray. Foreign matter such as stems, insects, moulds, stones, weeds, and soil particles are manually separated and weighed. The percentage is calculated.

Significance

- Indicates purity of the drug.
- Detects adulteration and poor collection practices.
- Reflects quality of harvesting and storage.

Table 6: Foreign Organic Matter of various crude drgs

Crude Drug	Botanical Name	Foreign Organic Matter (% w/w)
Ashwagandha	*Withania somnifera*	Not more than 2.0
Amla	*Emblica officinalis*	Not more than 2.0
Fennel	*Foeniculum vulgare*	Not more than 1.0
Coriander	*Coriandrum sativum*	Not more than 2.0
Isabgol	*Plantago ovata*	Not more than 2.0

4. Ash Values

Ash value is an important physicochemical parameter used in the evaluation and standardization of crude drugs. It determines the amount of inorganic residue remaining after complete incineration of the plant material. Ash consists of naturally occurring mineral salts (physiological ash) and extraneous inorganic matter such as sand, soil, silica, and other contaminants (non-physiological ash). Determination of ash values helps assess the purity, identity, quality, and adulteration of crude drugs.

a. Total Ash

Total ash is the total amount of inorganic residue remaining after complete incineration of a crude drug. It includes both physiological ash (naturally occurring mineral content of the plant) and non-physiological ash (extraneous matter such as sand and soil). Determination of total ash is used for quality control, standardization, detection of adulteration, and assessment of the overall mineral content of crude drugs.

Principle

When a crude drug is heated at about **450–600°C**, all organic matter is completely burnt off, leaving behind inorganic mineral residues. The weight of this residue, expressed as a percentage of the air-dried drug, represents the total ash value.

Procedure

About **2–3 g** of accurately weighed air-dried crude drug is placed in a previously ignited and tarred silica crucible. The sample is gradually incinerated by increasing the temperature until it becomes free from carbon and a white or grey ash is obtained. The crucible is cooled in a desiccator and weighed. The total ash is calculated as **percentage w/w** of the air-dried sample.

b. Acid-Insoluble Ash

Acid-insoluble ash is the portion of the total ash that remains insoluble after boiling with dilute hydrochloric acid. It mainly consists of silica, sand, siliceous earth, and other insoluble inorganic impurities. This parameter is used to **detect contamination with soil, sand, and earthy materials** during collection, handling, or storage.

Principle

Total ash is treated with dilute hydrochloric acid, which dissolves most inorganic salts except silica and other acid-insoluble materials. The remaining insoluble residue is collected, ignited, weighed, and expressed as acid-insoluble ash.

Procedure

The total ash is boiled with **25 mL of dilute hydrochloric acid** for about **5 minutes**. The insoluble matter is filtered through ashless filter paper, washed with hot water until free from acid, dried, ignited in a silica crucible, cooled in a desiccator, and weighed. The acid-insoluble ash is calculated as **percentage w/w** of the air-dried crude drug.

c. Water-Soluble Ash

Water-soluble ash is the portion of the total ash that is soluble in water. It provides an estimate of the amount of water-soluble inorganic salts present in a crude drug and helps detect **exhausted, adulterated, or improperly processed plant materials**.

Total ash is treated with water, and the insoluble portion is separated by filtration. The difference between the weight of the total ash and the weight of the water-insoluble ash gives the water-soluble ash.

Procedure

The total ash is boiled with **25 mL of distilled water** for about **5 minutes**. The insoluble matter is filtered through ashless filter paper, washed with hot water, dried, ignited, cooled, and weighed. The weight of the insoluble ash is subtracted from the weight of the total ash to obtain the water-soluble ash, which is expressed as **percentage w/w** of the air-dried drug.

Table 7: Ash values of various crude drugs

Crude Drug	**Botanical Name**	**Total Ash (% w/w)**	**Acid-Insoluble Ash (% w/w)**	**Water-Soluble Ash (% w/w)**
Senna	*Cassia angustifolia*	NMT 12.0	NMT 2.0	NLT 3.0
Ashwagandha	*Withania somnifera*	NMT 6.0	NMT 1.0	NLT 2.0
Liquorice	*Glycyrrhiza glabra*	NMT 8.0	NMT 2.0	NLT 2.5
Tulsi	*Ocimum tenuiflorum* (*O. sanctum*)	NMT 14.0	NMT 2.5	NLT 4.0
Turmeric	*Curcuma longa*	NMT 9.0	NMT 1.5	NLT 3.0

Significance of Ash Values

- Determines the purity and quality of crude drugs.
- Detects contamination with sand, soil, and other inorganic impurities.
- Helps identify adulteration and substitution.
- Serves as an official pharmacopoeial standard for quality control.
- Assists in comparing different batches of crude drugs.
- Ensures compliance with pharmacopoeial specifications during standardization.

5. Bitterness Value

Bitterness value is the measure of bitterness of a drug compared with quinine hydrochloride.

Principle

The bitterness of a drug solution is compared with that of a standard quinine solution.

General Procedure

A known quantity of the powdered crude drug is extracted with purified water by boiling, cooled, filtered, and the filtrate is made up to a definite volume. Serial dilutions of the test extract are then prepared and compared with corresponding dilutions of a standard **quinine hydrochloride** solution. Approximately **10 mL** of each dilution is held in the mouth for about **30 seconds** and then expelled without swallowing. The highest dilution at which bitterness is still perceptible is recorded and compared with the bitterness threshold of the standard solution. The bitterness value is then calculated using the pharmacopoeial formula and expressed as **bitterness units**.

$$\text{Bitterness Value} = 2000 \times C / (A \times B)$$

where:

- **A** = Mass of the crude drug used (g)
- **B** = Volume (mL) of the most dilute test solution in which bitterness is still detectable
- **C** = Dilution factor corresponding to the quinine hydrochloride solution at the bitterness threshold

The result is expressed as the **number of units equivalent to the bitterness of 1 g of quinine hydrochloride**.

Significance

- Standardization of bitter drugs.
- Correlates with pharmacological activity.

Table 7: Bitterness value of crude drugs

Crude Drug	**Botanical Name**	**Bitterness Value**
Chirata	*Swertia chirayita*	Not less than 2,000
Gentian	*Gentiana lutea*	Not less than 10,000
Quassia	*Quassia amara*	Not less than 40,000
Andrographis	*Andrographis paniculata*	Not less than 6,000
Neem	*Azadirachta indica*	Not less than 3,000

6. Foaming Index

Foaming index measures the ability of a drug to produce stable foam due to the presence of saponins.

Principle

Saponins reduce surface tension and produce persistent froth when shaken with water.

Procedure

About **1 g** of the coarsely powdered crude drug is boiled with **100 mL** of water for **30 minutes**, cooled, filtered, and the volume is adjusted to **100 mL**. Successive volumes (1–10 mL) of the decoction are transferred into ten stoppered test tubes and the volume in each tube is made up to **10 mL** with distilled water. Each test tube is shaken vigorously for **15 seconds** and allowed to stand undisturbed for **15 minutes**. The height of the foam is measured, and the tube producing a stable foam of **1 cm** is noted. The foaming index is then calculated according to the pharmacopoeial method.

If **1 cm** of foam is produced in tube containing **a mL** of the decoction:

$$\text{Foaming Index} = 1000/a$$

where **a** = volume (mL) of the decoction producing **1 cm** of stable foam after 15 minutes.

Table 8: Foaming index of various crude drugs

Crude Drug	**Botanical Name**	**Foaming Index**
Liquorice	*Glycyrrhiza glabra*	>100
Shatavari	*Asparagus racemosus*	>100
Soapnut	*Sapindus mukorossi*	>1000
Quillaja	*Quillaja saponaria*	>1000
Dioscorea	*Dioscorea floribunda*	>100

7. Haemolytic Potential (Haemolytic Value)

Haemolytic value is the quantity of drug required to cause haemolysis of red blood cells.

Principle

The haemolytic value is based on the ability of saponins to interact with cholesterol present in the erythrocyte (red blood cell) membrane, resulting in membrane disruption and haemolysis. The degree of haemolysis is proportional to the concentration of saponins present in the crude drug. The haemolytic activity is compared with a standard, and the haemolytic value is calculated accordingly.

General Procedure

A known quantity of the powdered crude drug is extracted with a suitable solvent, and serial dilutions of the extract are prepared. A standard suspension of defibrinated or citrated blood is added to each dilution and incubated under controlled conditions, usually at **37°C** for a specified period. The tubes are then examined for complete haemolysis, indicated by a clear red solution without intact erythrocytes. The lowest concentration of the extract producing complete haemolysis is recorded, and the haemolytic value is calculated according to the pharmacopoeial method.

Significance

- Evaluates saponin-containing drugs.
- Indicates biological activity.

Table 9: Examples of Haemolytic Value

Crude Drug	Botanical Name	Relative Haemolytic Activity
Quillaja	*Quillaja saponaria*	Very high
Soapnut	*Sapindus mukorossi*	High
Liquorice	*Glycyrrhiza glabra*	Moderate
Shatavari	*Asparagus racemosus*	Moderate
Dioscorea	*Dioscorea floribunda*	Moderate

8. Swelling Index

Swelling index is the volume occupied by one gram of plant material after swelling in water.

Principle

The swelling index is based on the ability of mucilage and other hydrophilic constituents present in crude drugs to absorb water and swell, thereby increasing in volume. The extent of swelling is directly proportional to the amount of mucilage present. The swelling index is expressed as the volume in **millilitres (mL)** occupied by **1 g** of plant material after swelling under specified conditions.

General Procedure

Accurately weigh **1 g** of the coarsely powdered crude drug and transfer it into a **25 mL glass-stoppered graduated cylinder**. Add **25 mL of water**, shake thoroughly every **10 minutes for 1 hour**, and then allow the mixture to stand undisturbed for **3 hours** at room temperature. After the swelling period, measure the final volume occupied by the swollen plant material, including any mucilage. The swelling index is expressed as the number of **millilitres occupied by 1 g of the plant material**.

Table: Examples of Swelling Index

Crude Drug	Botanical Name	Swelling Index (mL/g)
Isabgol	*Plantago ovata*	Not less than 10
Linseed	*Linum usitatissimum*	Not less than 4
Fenugreek	*Trigonella foenum-graecum*	Not less than 6
Quince Seed	*Cydonia oblonga*	Not less than 8
Basil Seed	*Ocimum basilicum*	Not less than 12

Significance

- Used for the standardization of mucilage-containing crude drugs.
- Helps estimate the mucilage content of medicinal plants.
- Detects adulteration and substitution of crude drugs.

9. Viscosity

Viscosity is a **physical evaluation parameter** that measures the internal resistance of a liquid or semisolid crude drug, extract, gum, mucilage, or fixed oil to flow under an applied force. It reflects the friction between adjacent layers of a fluid during movement. It is particularly useful for evaluating drugs such as **Acacia, Tragacanth, Isabgol, Aloe vera gel, and Guar gum**.

Procedure:

The test sample is prepared according to the specified concentration and maintained at a constant temperature, usually **25°C**. The sample is transferred into a suitable viscometer, such as an **Ostwald, Ubbelohde, or Brookfield viscometer**. The time required for the liquid to flow between two marked points (capillary viscometer) or the resistance to spindle rotation (rotational viscometer) is measured. The viscosity is then calculated using the instrument constant or read directly from the instrument and expressed in **millipascal-second (mPa·s)** or **centipoise (cP)**.

Table 11: Examples of Viscosity

Crude Drug/Formulation	**Botanical Name**	**Approximate Viscosity**
Acacia mucilage (10%)	*Acacia senegal*	40–60 cP
Tragacanth mucilage (1%)	*Astragalus gummifer*	300–500 cP
Guar gum solution (1%)	*Cyamopsis tetragonoloba*	2,000–4,000 cP
Aloe vera gel	*Aloe barbadensis*	500–2,000 cP
Isabgol mucilage (1%)	*Plantago ovata*	800–1,500 cP

Significance

- Standardizes herbal extracts, gums, and mucilages.
- Assesses the consistency and flow behavior of herbal formulations.
- Detects adulteration or degradation of natural gums and mucilages.
- Ensures batch-to-batch uniformity and product quality.

- Helps in formulation development by evaluating the rheological properties of liquid and semisolid herbal products.

10. Optical Rotation

Optical rotation is a **physical evaluation parameter** that measures the ability of an optically active substance to rotate the plane of plane-polarized light. Optical rotation is widely used for the detection of adulteration, and quality control of crude drugs, volatile oils, fixed oils, sugars, alkaloids, glycosides, and other optically active phytoconstituents. It is particularly useful for evaluating essential oils such as peppermint, eucalyptus, turpentine, and fennel oils.

Principle

When plane-polarized light passes through an optically active substance, the plane of polarization is rotated either to the **right (clockwise), known as dextrorotatory (+)**, or to the **left (anticlockwise), known as levorotatory (−)**. The angle through which the light is rotated is measured using a **polarimeter**.

General Procedure

The sample, either in the pure state or as a solution of known concentration, is placed in a clean polarimeter tube of known path length, ensuring that no air bubbles are present. The tube is inserted into the polarimeter, and monochromatic light (usually the sodium D-line at **589 nm**) is passed through the sample. The analyzer is rotated until the field appears uniformly illuminated, and the angle of rotation is recorded. The optical rotation or specific optical rotation is then calculated according to pharmacopoeial requirements.

Table 12: Examples of Optical Rotation

Crude Drug/Oil	**Botanical Name**	**Optical Rotation**
Peppermint Oil	*Mentha × piperita*	**−10° to −30°**
Eucalyptus Oil	*Eucalyptus globulus*	**0° to +10°**
Turpentine Oil	*Pinus* spp.	**+10° to +25°**
Fennel Oil	*Foeniculum vulgare*	**+6° to +24°**
Clove Oil	*Syzygium aromaticum*	**−3° to 0°**

Significance

- Identifies optically active phytoconstituents.

- Detects adulteration and substitution of volatile and fixed oils.
- Assesses the purity and authenticity of crude drugs.

11. Refractive Index

Refractive index is the ratio of the velocity of light in vacuum to its velocity in a substance. Refractive index is an important quality control parameter of volatile oils, fixed oils, balsams, and liquid herbal preparations. It is widely used in the evaluation of essential oils such as clove oil, eucalyptus oil, peppermint oil, fennel oil, and cinnamon oil.

Principle

The determination of refractive index is based on the change in the direction of light as it passes from one medium to another with a different optical density. The refractive index of a substance is the ratio of the sine of the angle of incidence to the sine of the angle of refraction (Snell's law), or equivalently, the ratio of the speed of light in air (or vacuum) to its speed in the test substance.

Procedure

A few drops of the liquid sample are placed on the clean prism of an **Abbe refractometer** (or digital refractometer), ensuring that the prism surface is completely covered and free from air bubbles. The prisms are closed, and the instrument is maintained at the prescribed temperature, usually **20°C or 25°C**. Monochromatic light, generally the **sodium D-line (589 nm)**, is passed through the sample. The refractive index is then read directly from the instrument scale or digital display and reported as the refractive index at the specified temperature (e.g., **$n^{20}D$** or **$n^{25}D$**).

Significance

- Evaluation of volatile oils.
- Detection of adulteration.

Table 13: Example of refractive index of various crude drugs

Crude Drug/Oil	**Botanical Name**	**Refractive Index (20°C)**
Clove Oil	*Syzygium aromaticum*	**1.527–1.535**
Cinnamon Oil	*Cinnamomum verum*	**1.573–1.591**
Peppermint Oil	*Mentha × piperita*	**1.456–1.466**
Eucalyptus Oil	*Eucalyptus globulus*	**1.458–1.470**
Fennel Oil	*Foeniculum vulgare*	**1.528–1.538**

12. Acid Value

Acid value is the number of milligrams of potassium hydroxide (KOH) required to neutralize the free acids present in **1 gram** of a substance.

Acid Value= (VxNx56.1)/W

Where:

- **N** = Normality of KOH
- **V** = Volume of KOH used (mL)
- **W** = Weight of sample (g)
- 56.1= Mol. Wt. of KOH

Significance

- Indicates the amount of free acids present.
- Helps detect decomposition, rancidity, or deterioration.
- Useful for evaluation of resins, balsams, fats, and oils.

Examples: Benzoin, Tolu balsam, Peru balsam

13. Saponification Value

Saponification value is the number of milligrams of potassium hydroxide (KOH) required to saponify the esters and neutralize the free acids present in **1 gram** of a substance.

Formula

Saponification Value= (56.1x N x (B-S)/W

Where:

- **B** = Volume of blank titration
- **S** = Volume of sample titration
- **N** = Normality of KOH
- **W** = Weight of sample

Significance

- Indicates average molecular weight of fatty acids.

- Higher value indicates shorter-chain fatty acids.
- Used for identification and purity testing.

Examples: Peru balsam, Tolu balsam, Fixed oils and fats

CHEMICAL EVALUATION

The chemical evaluation includes qualitative chemical tests, quantitative chemical tests, and instrumental analysis. The isolation, purification, and identification of active constituents are chemical methods of evaluation.

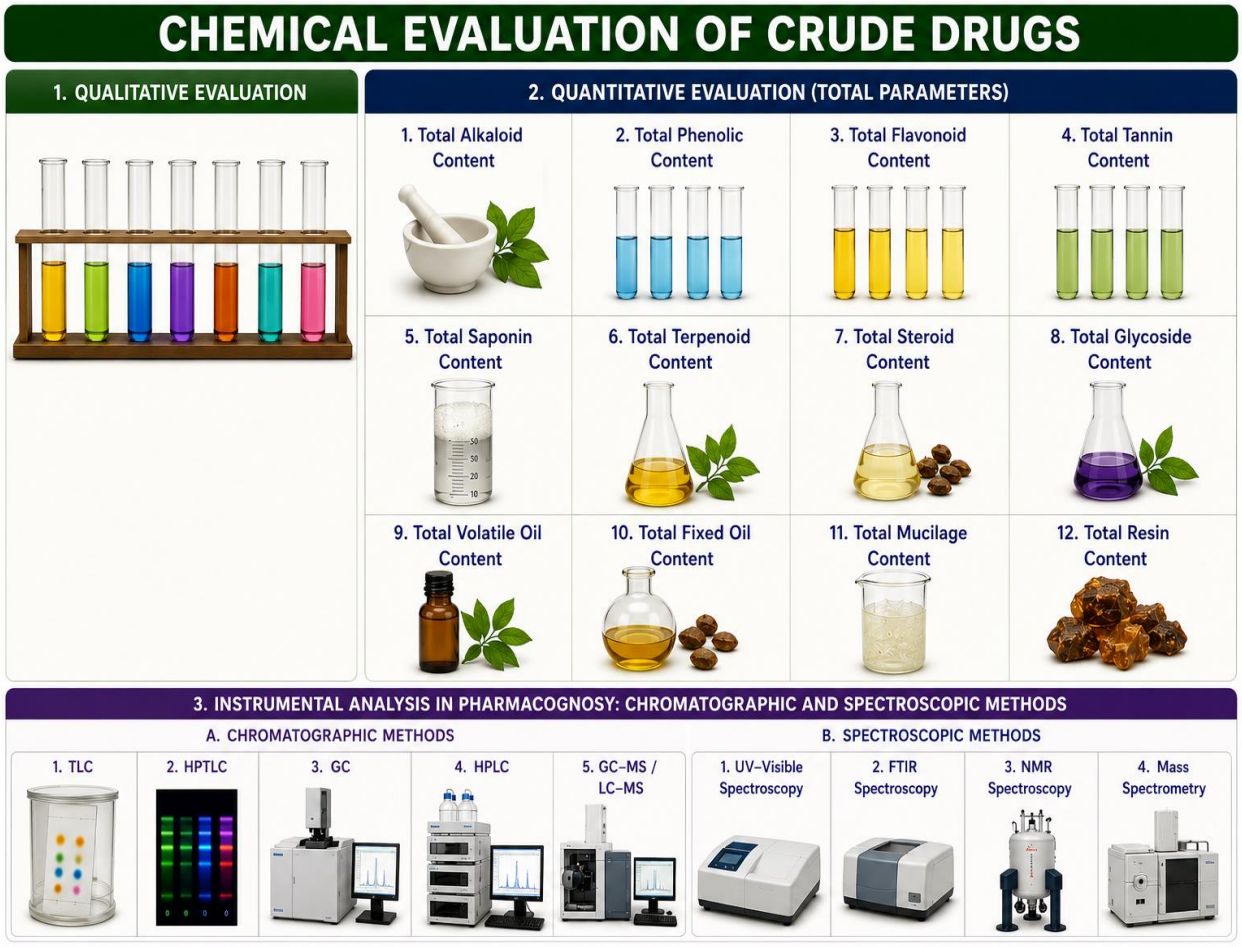

Figure 6: Chemical evaluation of Crude Drugs

Qualitative Chemical Evaluation

Qualitative chemical tests include identification tests for various phytoconstituents like alkaloids,

glycosides, tannins, etc.

Table 14: Qualitative evaluation of various phytoconstituents In Crude Drugs

Phytoconstituent	**Test**	**Reagent Used**	**Positive Observation**
Alkaloids	Dragendorff's Test	Dragendorff's reagent (Potassium bismuth iodide)	Orange or reddish-brown precipitate
	Mayer's Test	Mayer's reagent (Potassium mercuric iodide)	Cream or white precipitate
	Wagner's Test	Wagner's reagent (Iodine in KI)	Reddish-brown precipitate
	Hager's Test	Saturated picric acid	Yellow precipitate
Glycosides (Cardiac)	Keller-Killiani Test	Glacial acetic acid, $FeCl_3$, H_2SO_4	Brown ring at interface and bluish-green layer
	Legal's Test	Sodium nitroprusside + Pyridine + NaOH	Pink to blood-red colour
	Baljet's Test	Sodium picrate solution	Orange to red colour
Anthraquinone Glycosides	Borntrager's Test	Benzene + Ammonia	Pink or red colour in ammoniacal layer
	Modified Borntrager's Test	$FeCl_3$ + HCl + Ammonia	Pink to red colour
Saponin Glycosides	Foam Test	Water	Persistent honeycomb froth for 10–15 min
	Haemolysis Test	Blood agar	Clear haemolytic zone
Flavonoids	Shinoda Test	Magnesium turnings + Conc. HCl	Pink, red or orange colour
	Alkaline Reagent Test	NaOH	Intense yellow colour turning colourless with acid
	Lead Acetate Test	Lead acetate solution	Yellow precipitate

Tannins & Phenolics	Ferric Chloride Test	5% $FeCl_3$	Blue-black or greenish-black colour
	Gelatin Test	Gelatin solution + NaCl	White precipitate
	Lead Acetate Test	Lead acetate solution	White precipitate
Proteins	Biuret Test	$CuSO_4$ + NaOH	Violet colour
	Xanthoproteic Test	Conc. HNO_3	Yellow colour
	Millon's Test	Millon's reagent	Brick-red precipitate
Amino Acids	Ninhydrin Test	Ninhydrin reagent	Purple or blue colour
Carbohydrates	Molisch's Test	α-Naphthol + H_2SO_4	Violet ring at junction
	Fehling's Test	Fehling A & B	Brick-red precipitate
	Benedict's Test	Benedict's reagent	Green, yellow, orange or red precipitate
Starch	Iodine Test	Iodine solution	Blue-black colour
Fixed Oils & Fats	Spot Test	Filter paper	Permanent translucent oily spot
	Sudan III Test	Sudan III reagent	Orange-red staining
Steroids	Salkowski Test	Chloroform + Conc. H_2SO_4	Red colour in chloroform layer
	Liebermann-Burchard Test	Acetic anhydride + H_2SO_4	Bluish-green colour
Terpenoids	Salkowski Test	Chloroform + H_2SO_4	Reddish-brown interface
Resins	Acetone-Water Test	Acetone followed by water	Turbidity or precipitate
Gums & Mucilage	Ruthenium Red Test	Ruthenium red solution	Pink or red colour

Quantitative Chemical evaluation

Quantitative methods used to determine the amount of active constituents present in crude drugs. They provide accurate information regarding the strength, purity, potency, and quality of a drug. These assays are particularly important for drugs whose therapeutic activity depends on specific chemical constituents such as alkaloids, glycosides, resins, volatile oils, vitamins, and other secondary metabolites.

The results obtained from chemical assays help in:

- Standardization of crude drugs.
- Detection of inferior quality drugs.
- Identification of exhausted drugs from which active constituents have already been removed.
- Detection of adulteration and substitution.
- Determination of therapeutic potency.

If the assayed constituent is present below the pharmacopoeial limit, it may indicate deterioration, poor quality, or exhaustion of the drug. Complete absence of the constituent generally suggests substitution with a worthless material.

1. Total Alkaloid Content (TAC)

Total alkaloid content is the quantitative estimation of the total amount of alkaloids present in a crude drug or plant extract. Alkaloids are naturally occurring nitrogen-containing secondary metabolites that exhibit diverse pharmacological activities such as analgesic, antimalarial, antihypertensive, anticancer, and antimicrobial effects. Estimation of total alkaloid content is an important quality control parameter for standardizing medicinal plants.

The estimation is based on the formation of a colored ion-pair complex between alkaloids and bromocresol green (BCG) under acidic conditions. The resulting complex is extracted into chloroform and its absorbance is measured using a UV-Visible spectrophotometer, usually at 470 nm. The intensity of the color is directly proportional to the concentration of alkaloids present in the sample.

The plant extract is dissolved in dilute hydrochloric acid and filtered to remove insoluble matter. Bromocresol green reagent and phosphate buffer are added to the filtrate to form the alkaloid-BCG

complex. The complex is extracted with chloroform, and the absorbance of the chloroform layer is measured at 470 nm using a UV-Visible spectrophotometer. A standard calibration curve is prepared using atropine or berberine, and the total alkaloid content is expressed as mg Atropine Equivalents (AE)/g or mg Berberine Equivalents (BE)/g of extract.

2. Total Phenolic Content (TPC)

Total phenolic content is the estimation of the total concentration of phenolic compounds present in a plant extract. Phenolics are one of the most abundant groups of plant secondary metabolites and contribute significantly to antioxidant, anti-inflammatory, antimicrobial, and anticancer activities.

The assay is based on the reduction of Folin–Ciocalteu reagent by phenolic compounds under alkaline conditions, resulting in the formation of a blue-colored molybdenum-tungsten complex. The absorbance of the colored solution is measured at 765 nm, and the intensity of the color is proportional to the total phenolic content.

A known volume of plant extract is mixed with Folin–Ciocalteu reagent and allowed to react for a few minutes. Sodium carbonate solution is then added to provide alkaline conditions, and the mixture is incubated at room temperature for 30–60 minutes. The absorbance is measured at 765 nm using a UV-Visible spectrophotometer. A calibration curve is prepared using gallic acid, and the results are expressed as mg Gallic Acid Equivalents (GAE)/g of extract.

3. Total Flavonoid Content (TFC)

Total flavonoid content is the quantitative estimation of flavonoids present in a plant extract. Flavonoids are polyphenolic compounds known for their antioxidant, anti-inflammatory, cardioprotective, hepatoprotective, and antimicrobial properties.

Flavonoids react with aluminium chloride ($AlCl_3$) to form stable yellow-colored complexes. The intensity of the color produced is directly proportional to the flavonoid concentration and is measured spectrophotometrically at 415 nm.

The plant extract is mixed with aluminium chloride solution and potassium acetate or sodium nitrite, depending on the method used. After incubation for about 30 minutes at room temperature, the absorbance is measured at 415 nm. Quercetin or rutin is used to prepare the standard calibration

curve, and the results are expressed as mg Quercetin Equivalents (QE)/g or mg Rutin Equivalents (RE)/g of extract.

4. Total Tannin Content (TTC)

Total tannin content is the quantitative estimation of tannins present in a plant material or extract. Tannins are high molecular weight polyphenolic compounds possessing astringent, antioxidant, antimicrobial, and wound-healing properties.

The estimation is commonly based on the Folin–Ciocalteu reaction, in which tannins reduce the reagent to produce a blue-colored complex under alkaline conditions. The absorbance is measured spectrophotometrically at 760–765 nm, and the color intensity is proportional to the tannin concentration.

The plant extract is mixed with Folin–Ciocalteu reagent followed by sodium carbonate solution. After incubation for approximately 30 minutes, the absorbance is recorded at 760–765 nm. A calibration curve is prepared using tannic acid, and the tannin content is expressed as mg Tannic Acid Equivalents (TAE)/g of extract.

5. Total Saponin Content (TSC)

Total saponin content refers to the quantitative estimation of saponins present in medicinal plants. Saponins are glycosidic compounds characterized by their foaming property and possess expectorant, hypocholesterolemic, immunomodulatory, and anticancer activities.

In the spectrophotometric method, saponins react with vanillin and sulfuric acid to produce a colored complex. Alternatively, they may be isolated gravimetrically after solvent extraction. The intensity of the color is proportional to the saponin concentration.

The plant extract is reacted with vanillin reagent followed by concentrated sulfuric acid. The mixture is heated in a water bath, cooled, and the absorbance is measured at 544–560 nm. Diosgenin is used as the reference standard, and the results are expressed as mg Diosgenin Equivalents (DE)/g of extract.

6. Total Terpenoid Content (TTC)

Total terpenoid content is the quantitative estimation of terpenoids present in medicinal plants. Terpenoids are important secondary metabolites responsible for various biological activities, including antimicrobial, antioxidant, anticancer, and anti-inflammatory effects.

Terpenoids react with concentrated sulfuric acid to produce colored derivatives that can be quantified spectrophotometrically. The absorbance of the resulting solution is proportional to the terpenoid concentration.

The extract is treated with chloroform followed by concentrated sulfuric acid. After incubation, the absorbance is measured using a UV-Visible spectrophotometer. A suitable terpenoid standard, such as linalool or ursolic acid, is used to prepare the calibration curve, and the results are expressed as mg equivalent/g of extract.

7. Total Steroid Content

Total steroid content is the quantitative estimation of phytosterols and steroidal compounds present in plant extracts. These compounds exhibit anti-inflammatory, cholesterol-lowering, and immunomodulatory activities.

Steroids react with Liebermann–Burchard reagent (acetic anhydride and concentrated sulfuric acid) to produce a green-colored complex. The intensity of the color is directly proportional to the steroid concentration.

The extract is mixed with Liebermann–Burchard reagent and allowed to stand for color development. The absorbance is measured at approximately 620–650 nm. Cholesterol is used as the standard, and the steroid content is expressed as mg Cholesterol Equivalents (CE)/g of extract.

8. Total Glycoside Content

Total glycoside content is the quantitative estimation of glycosides present in medicinal plants. Glycosides consist of a sugar moiety linked to a non-sugar component (aglycone) and possess important pharmacological activities depending on the aglycone structure.

The assay is based on hydrolysis of glycosides followed by spectrophotometric or chromatographic estimation of the released aglycone or intact glycosides. The measured absorbance is proportional to the glycoside concentration.

The extract is hydrolysed using dilute acid when required, neutralized, and analyzed spectrophotometrically or by HPLC using a suitable standard. The glycoside content is expressed as mg/g or % w/w of extract.

Table 15: Quantitative evaluation of Crude Drugs

Parameter	**Crude Drug**	**Official/Reported Standard Value**
Total Alkaloid Content	*Rauwolfia serpentina*	Not less than 1.2% w/w
Total Phenolic Content	*Camellia sinensis* (Green tea)	120–180 mg GAE/g extract
Total Flavonoid Content	*Ginkgo biloba*	Not less than 24% w/w
Total Tannin Content	Catechu (*Acacia catechu*)	Not less than 12% catechin
Total Saponin Content	*Glycyrrhiza glabra*	Not less than 2.5% w/w
Total Terpenoid Content	*Andrographis paniculata*	Not less than 2.0% w/w
Total Steroid Content	*Withania somnifera*	Not less than 1.5% w/w
Total Glycoside Content	Senna (*Cassia angustifolia*)	Not less than 2.5% w/w

Instrumental Analysis in Pharmacognosy

Instrumental analysis involves the use of sophisticated analytical instruments to identify, separate, characterize, and quantify phytoconstituents present in crude drugs. These techniques provide highly accurate, sensitive, and reproducible results and are extensively used for standardization, quality control, authentication, and detection of adulteration in herbal drugs.

Instrumental methods are broadly classified into:

1. Chromatographic Methods
2. Spectroscopic Methods

A. Chromatographic Methods

Chromatography is a separation technique in which the components of a mixture are separated based on their differential distribution between a stationary phase and a mobile phase.

Table 16: Types of Chromatographic Methods

Method	**Principle**	**Application**
Paper Chromatography (PC)	Separation based on partition between water adsorbed on paper and solvent	Amino acids, sugars, alkaloids

Thin Layer Chromatography (TLC)	Separation on adsorbent-coated plate	Identification and fingerprinting of crude drugs
Gas Chromatography (GC)	Separation of volatile compounds based on boiling point and partition coefficient	Volatile oils, terpenoids
High Performance Liquid Chromatography (HPLC)	Separation under high pressure using liquid mobile phase	Alkaloids, glycosides, flavonoids
High Performance Thin Layer Chromatography (HPTLC)	Advanced form of TLC with higher resolution and sensitivity	Herbal fingerprinting and quantitative analysis

B. Spectroscopic Methods

Spectroscopy involves interaction of electromagnetic radiation with matter to identify and characterize compounds.

Table 17: Types of Spectroscopic Methods

Method	**Radiation Used**	**Information Obtained**
UV-Visible Spectroscopy	UV and Visible Light	Concentration and chromophores
Infrared Spectroscopy (IR)	Infrared Radiation	Functional groups
Mass Spectroscopy (MS)	Ionized particles	Molecular weight and structure
Nuclear Magnetic Resonance (NMR) Spectroscopy	Radiofrequency radiation	Detailed molecular structure

BIOLOGICAL EVALUATION OF CRUDE DRUGS

Biological evaluation, also known as **bioassay**, is the assessment of the **biological activity, potency, efficacy, and safety** of a crude drug or its phytoconstituents using suitable biological systems such as microorganisms, isolated cells, tissues, organs, or experimental animals. It is carried out to determine the pharmacological effects of medicinal plants and to establish their therapeutic potential. Biological evaluation plays an important role in the **standardization, quality control, authentication, detection of adulteration, pharmacological screening, and**

safety assessment of crude drugs, particularly when chemical evaluation alone cannot accurately predict biological efficacy.

Principle

Biological evaluation is based on the ability of the active constituents present in a crude drug to produce a measurable biological response in a suitable experimental model. The biological response produced by the test sample is compared with that of a standard or reference drug under controlled experimental conditions. The magnitude of the response is directly related to the biological activity or potency of the crude drug.

Types of Biological Evaluation

1. In vitro Bioassays

These assays are performed outside the living organism using isolated enzymes, cultured cells, tissues, isolated organs, or microorganisms. They are rapid, economical, reproducible, and suitable for preliminary screening of biological activities.

2. In vivo Bioassays

These assays are carried out in whole experimental animals such as mice, rats, rabbits, guinea pigs, or zebrafish. They provide information on the pharmacological activity, pharmacokinetics, efficacy, toxicity, and safety of crude drugs under physiological conditions.

Biological Activities Determined

- Biological evaluation is used to determine a wide range of pharmacological activities, including:
- Antioxidant activity
- Antimicrobial activity (antibacterial, antifungal, antiviral)
- Anti-inflammatory activity
- Analgesic activity
- Antipyretic activity
- Antidiabetic activity
- Anticancer and cytotoxic activity
- Hepatoprotective activity

- Nephroprotective activity
- Cardioprotective activity
- Neuroprotective activity
- Immunomodulatory activity
- Antiulcer activity
- Wound healing activity
- Anthelmintic activity
- Antimalarial activity
- Anticonvulsant activity
- Antihyperlipidemic activity
- Antihypertensive activity
- Enzyme inhibitory activity (α-amylase, α-glucosidase, acetylcholinesterase, etc.)
- Acute, subacute, chronic, and genotoxicity studies

General Procedure

The crude drug is first authenticated, dried, powdered, and extracted using a suitable solvent. The extract is administered to an appropriate biological model at different dose levels along with control and standard groups. Depending on the study, the model may be a microorganism, isolated organ, cultured cell line, enzyme system, or experimental animal. The biological response is observed and recorded using suitable biochemical, physiological, microbiological, or behavioral parameters. The results are statistically analyzed and compared with those of the standard drug to determine the biological activity, potency, and therapeutic efficacy of the crude drug.

Applications

- Standardization of crude drugs and herbal formulations.
- Screening of medicinal plants for pharmacological activities.
- Discovery of new bioactive phytoconstituents.
- Evaluation of therapeutic efficacy.
- Determination of biological potency.
- Detection of adulteration and substitution.
- Toxicological and safety assessment.

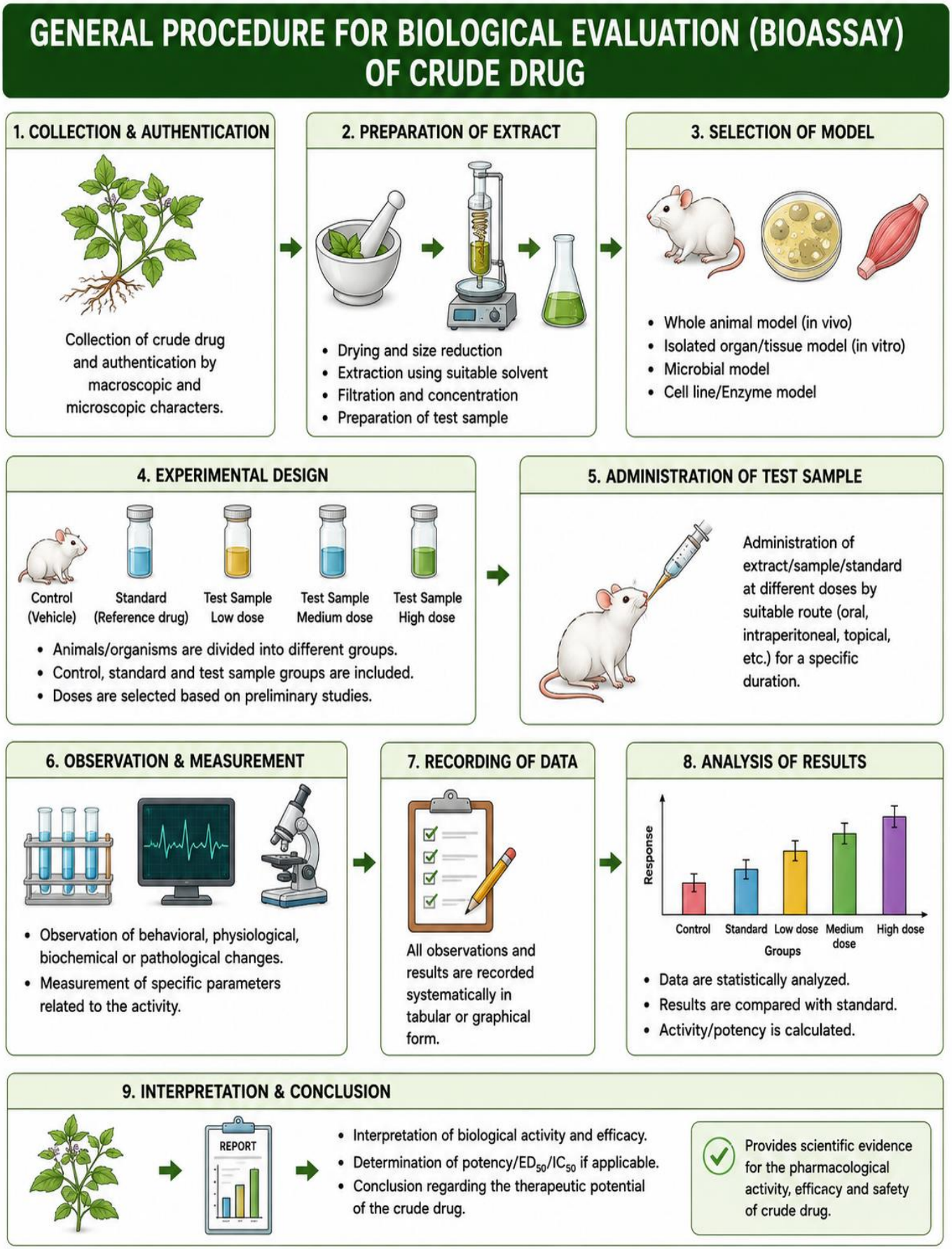

Figure 7: General Procdeure for Biological evaluation of Crude drugs

Advantages

- Evaluates the actual biological effect of crude drugs.
- Measures pharmacological activity directly.
- Useful when active constituents are unknown or difficult to quantify chemically.
- Detects synergistic effects among phytoconstituents.
- Provides information on efficacy as well as safety.
- Essential for drug discovery and pharmacological research.

Limitations

- Time-consuming and relatively expensive.
- Requires ethical approval for animal experiments.
- Biological responses may vary among species and individuals.
- Experimental conditions must be carefully standardized.
- Requires specialized laboratory facilities and trained personnel.

DNA BARCODING IN PHARMACOGNOSY

DNA barcoding is a molecular identification technique that uses a short, standardized DNA sequence from a specific region of the genome as a "barcode" to identify plant, animal, or microbial species. In pharmacognosy, DNA barcoding is employed for the authentication, identification, and quality control of crude drugs and medicinal plants.

Unlike morphological and chemical methods, DNA barcoding is not affected by environmental conditions, plant age, harvesting season, or storage conditions, making it a highly reliable tool for species identification.

Principle

Every species possesses a unique DNA sequence. DNA barcoding works by amplifying and sequencing a specific DNA region (barcode region) and comparing it with reference sequences available in DNA databases such as GenBank and BOLD (Barcode of Life Data System).

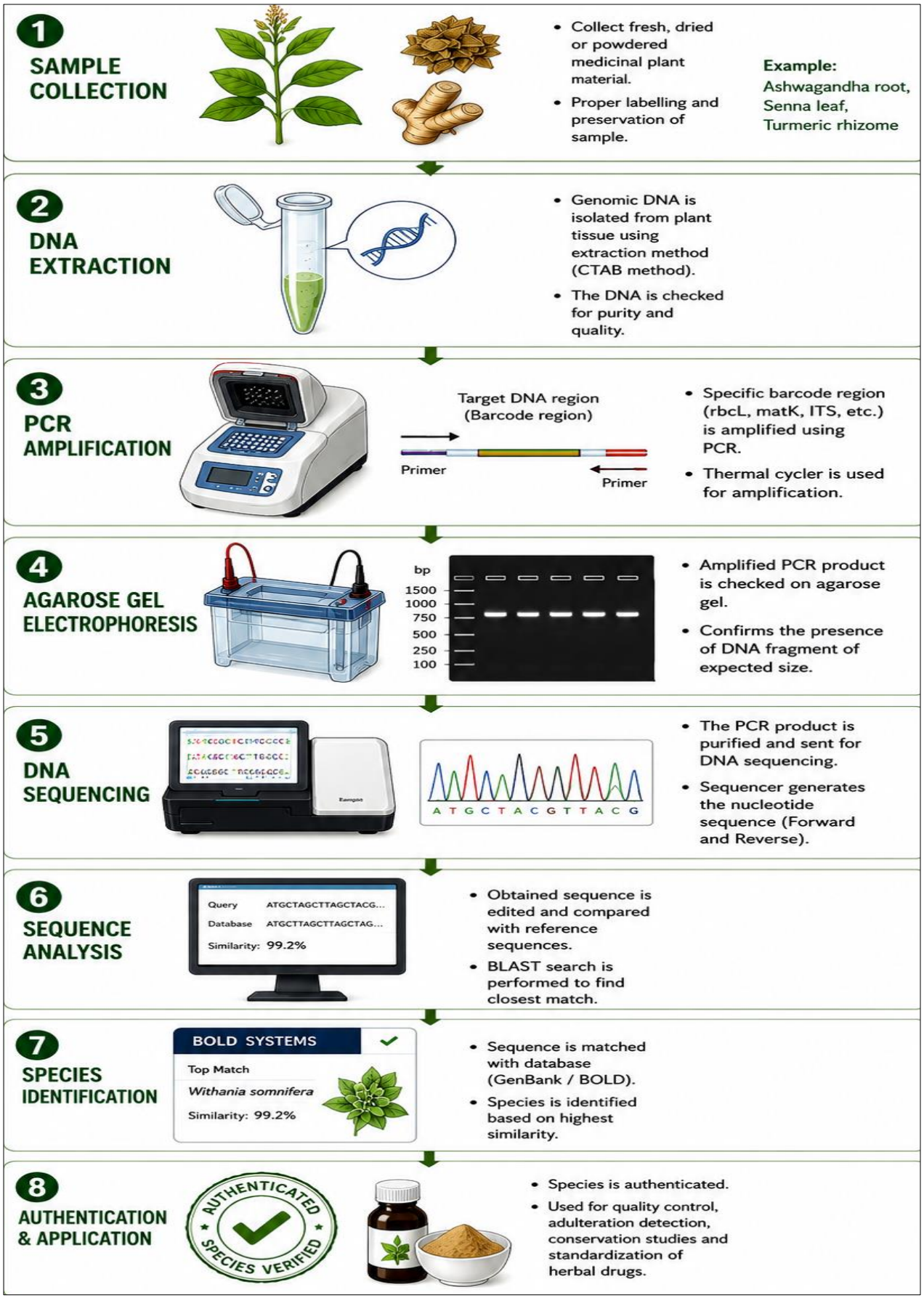

Figure 8: General Procedure for DNA barcoding

The unknown sample is identified based on sequence similarity with authenticated reference species.

Procedure of DNA Barcoding

1. Sample Collection

Fresh, dried, powdered, or processed medicinal plant material is collected.

Examples: Senna leaves, Ashwagandha roots, Turmeric powder

2. DNA Extraction

Genomic DNA is isolated from plant tissues using extraction methods such as CTAB (Cetyl Trimethyl Ammonium Bromide) method.

Purpose: To obtain pure DNA suitable for amplification.

3. PCR Amplification

The selected barcode region is amplified using Polymerase Chain Reaction (PCR) by using Thermal Cycler (PCR Machine)

Purpose: To produce millions of copies of the barcode DNA region.

4. DNA Sequencing

The amplified DNA fragment is sequenced using automated DNA sequencers.

Purpose: To determine the exact nucleotide sequence.

5. Sequence Analysis

The obtained sequence is compared with authenticated sequences in databases.

Databases Used: GenBank, BOLD System

Purpose: Species identification and authentication.

Table 18: Common DNA Barcode Regions in Medicinal Plants

Barcode Region	**Genome Source**	**Characteristics**
rbcL	Chloroplast DNA	Highly conserved, easy amplification
matK	Chloroplast DNA	High discriminatory power
ITS	Nuclear DNA	Most widely used for medicinal plants
ITS2	Nuclear DNA	Excellent species differentiation
psbA-trnH	Chloroplast DNA	Useful supplementary marker

Most Common Plant DNA Barcodes

1. rbcL Gene

- Located in chloroplast DNA.
- Encodes large subunit of RuBisCO enzyme.
- Easy to amplify and sequence.

Example: Used in identification of medicinal herbs.

2. matK Gene

- Located in chloroplast genome.
- Shows higher sequence variation than rbcL.

Example: Differentiation of closely related medicinal species.

3. ITS Region

- Located in nuclear ribosomal DNA.
- Highly variable among species.

Example: Authentication of herbal drugs and powdered samples.

Applications of DNA Barcoding in Pharmacognosy

1. Authentication of Medicinal Plants

DNA barcoding is widely used to authenticate medicinal plants by confirming their botanical identity at the species level. It helps distinguish genuine medicinal species from morphologically similar plants. **Example:** Authentication of *Withania somnifera* (Ashwagandha) and differentiation from other *Withania* species using ITS and **matK** barcode regions.

2. Detection of Adulteration and Substitution

DNA barcoding identifies intentional or accidental substitution of medicinal plants with inferior, cheaper, or unrelated species, thereby ensuring the quality and safety of herbal drugs. **Example:** Detection of adulteration of *Bacopa monnieri* (Brahmi) with *Centella asiatica* and differentiation of true cinnamon (*Cinnamomum verum*) from cassia (*Cinnamomum cassia*).

3. Identification of Powdered and Processed Herbal Drugs

Since morphological characteristics are often lost during drying or powdering, DNA barcoding enables accurate identification of medicinal plants in processed forms. **Example:** Authentication of powdered turmeric (*Curcuma longa*), ginger (*Zingiber officinale*), and senna (*Cassia angustifolia*).

4. Quality Control of Herbal Medicines

DNA barcoding is employed as a quality control tool to verify the identity of raw materials used in herbal formulations and to ensure product consistency. **Example:** Authentication of ingredients in Ashwagandha capsules, Triphala, and Chyawanprash.

5. Identification of Closely Related Species

It differentiates closely related plant species that are difficult to distinguish based on morphology or microscopy alone. **Example:** Identification of *Panax ginseng*, *Panax quinquefolius*, and *Panax notoginseng*.

6. Conservation of Endangered Medicinal Plants

DNA barcoding assists in documenting and conserving rare and endangered medicinal plants by enabling accurate species identification. **Example:** Conservation studies involving *Nardostachys jatamansi*, *Saussurea costus*, and *Taxus wallichiana*.

7. Biodiversity Assessment

DNA barcoding facilitates the cataloguing and monitoring of medicinal plant diversity in forests, botanical gardens, and protected areas. **Example:** Preparation of DNA barcode libraries for medicinal plants of the Himalayan and Western Ghats regions.

8. Detection of Illegal Trade

It helps regulatory agencies identify protected medicinal plants involved in illegal harvesting, smuggling, and trade. **Example:** Identification of illegally traded *Taxus wallichiana*, *Aquilaria malaccensis* (agarwood), and *Pterocarpus santalinus* (red sandalwood).

9. Herbal Product Traceability

DNA barcoding enables traceability of medicinal plants throughout the supply chain, from cultivation to the finished herbal product. **Example:** Verification of the botanical source of commercial Ashwagandha and Ginseng products.

10. Discovery of New Species

DNA barcode analysis aids taxonomists in identifying cryptic or previously undescribed medicinal plant species. **Example:** Discovery of new species of orchids and medicinal herbs using DNA sequence analysis.

11. Phylogenetic and Evolutionary Studies

DNA barcoding is used to study genetic relationships and evolutionary history among medicinal plant species. **Example:** Phylogenetic analysis of *Ocimum* (Tulsi) and *Curcuma* species.

12. Forensic Botany

DNA barcoding provides reliable molecular evidence for legal investigations involving medicinal or poisonous plants.
Example: Identification of *Datura stramonium* in poisoning cases and authentication of protected medicinal species in wildlife crime investigations.

13. Standardization of Herbal Formulations

DNA barcoding ensures that the correct botanical ingredients are used during the manufacture of polyherbal formulations, improving product safety and efficacy. **Example:** Standardization of polyherbal antidiabetic, hepatoprotective, and immunomodulatory formulations.

14. Authentication of Food and Nutraceutical Products

DNA barcoding is used to verify the identity of plant materials used in spices, herbal teas, dietary supplements, and nutraceuticals. **Example:** Authentication of saffron (*Crocus sativus*), turmeric (*Curcuma longa*), black pepper (*Piper nigrum*), oregano, basil, and green tea products.
Table 19: Examples of DNA Barcoding in Pharmacognosy

Medicinal Plant	Barcode Used	Purpose
Ashwagandha	ITS, matK	Authentication
Senna	rbcL, ITS	Detection of adulteration
Turmeric	ITS2	Species identification
Ginseng	ITS	Authentication
Saffron	matK	Detection of substitution
Tulsi	rbcL	Quality control

Advantages of DNA Barcoding

1. Accurate species identification.
2. Detects adulteration and substitution.
3. Applicable to powdered and processed drugs.
4. Not affected by environmental factors.
5. Useful for endangered medicinal plants.
6. Rapid and highly reproducible.
7. Supports pharmacopoeial standardization.

Limitations of DNA Barcoding

1. Requires specialized laboratory facilities.
2. Relatively expensive.
3. DNA degradation may occur in highly processed products.
4. Cannot determine concentration of active constituents.
5. Cannot assess pharmacological activity.
6. Closely related species may require multiple barcode regions.

Table 20: Comparison with Traditional Methods

Parameter	Morphological Evaluation	Chemical Evaluation	DNA Barcoding
Species Identification	Moderate	Moderate	Excellent
Processed Drugs	Difficult	Possible	Excellent
Adulteration Detection	Limited	Moderate	Excellent
Environmental Influence	High	Moderate	Negligible
Reproducibility	Moderate	Good	Very High

Significance in Pharmacognosy

DNA barcoding has emerged as one of the most powerful modern tools in pharmacognosy for authentication and quality control of medicinal plants. It complements traditional morphological, microscopic, chemical, and chromatographic methods by providing molecular-level identification. The technique is particularly valuable for detecting adulteration, authenticating crude drugs, conserving endangered medicinal plants, and ensuring the safety and efficacy of herbal medicines.

Chapter B: Quality Control of Drugs of Natural Origin (WHO Guidelines)

Multiple Choice Questions (MCQs)

The primary objective of quality control of crude drugs is to ensure:

A. High yield

B. Purity, safety, and efficacy

C. Low cost

D. Attractive appearance

2.

WHO guidelines for herbal medicines focus on:

A. Quality assurance

B. Standardization

C. Safety

D. All of the above

3.

Adulteration of crude drugs refers to:

A. Proper storage

B. Addition or substitution of inferior material

C. Standardization

D. Purification

4.

Which type of adulteration involves substitution with a different plant species?

A. Intentional adulteration

B. Natural adulteration

C. Biological adulteration

D. Chemical adulteration

5.

Evaluation based on color, odor, taste, and texture is called:

A. Microscopic evaluation

B. Chemical evaluation

C. Organoleptic evaluation

D. Biological evaluation

6.

Which is an organoleptic characteristic?

A. Refractive index

B. Taste

C. Ash value

D. Optical rotation

7.

Microscopic evaluation helps in:

A. Authentication of crude drugs

B. Determination of viscosity

C. Determination of acid value

D. Estimation of moisture content

8.

Quantitative microscopy includes determination of:

A. Stomatal number

B. Odor

C. Color

D. Taste

9.

Stomatal index is a parameter of:

A. Chemical evaluation

B. Organoleptic evaluation

C. Quantitative microscopy

D. Biological evaluation

10.

Physical evaluation includes determination of:

A. Viscosity

B. Starch grains

C. Trichomes

D. Stomatal number

11.

Ash value is used to determine:

A. Organic matter

B. Inorganic residue

C. Moisture content

D. Volatile oils

12.

Total ash represents:

A. Organic content

B. Total inorganic matter

C. Moisture content

D. Protein content

13.

Acid-insoluble ash indicates contamination with:

A. Sugars

B. Siliceous matter

C. Alkaloids

D. Glycosides

14.

Water-soluble ash is useful in determining:

A. Water-soluble inorganic salts

B. Lipids

C. Proteins

D. Volatile oils

15.

Moisture content is determined to prevent:

A. Adulteration

B. Microbial growth and deterioration

C. Color changes only

D. Odor changes only

16.

Extractive values indicate:

A. Amount of soluble constituents

B. Moisture content

C. Fiber content

D. Protein content

17.

Alcohol-soluble extractive is useful for estimating:

A. Water-soluble compounds

B. Alcohol-soluble constituents

C. Ash content

D. Moisture content

18.

Foreign organic matter refers to:

A. Active constituents

B. Unwanted extraneous material

C. Moisture

D. Volatile oils

19.

Bitterness value is used for evaluation of:

A. Bitter drugs

B. Volatile oils

C. Fixed oils

D. Resins

20.

Foaming index is associated with:

A. Alkaloids

B. Glycosides

C. Saponins

D. Tannins

21.

Swelling index is determined for drugs containing:

A. Mucilage

B. Alkaloids

C. Volatile oils

D. Resins

22.

Haemolytic potential is related to:

A. Destruction of RBCs

B. Formation of RBCs

C. Coagulation

D. Blood pressure

23.

Viscosity is a measure of:

A. Density

B. Flow resistance of a liquid

C. Acidity

D. Solubility

24.

Optical rotation is exhibited by:

A. Optically active substances

B. Proteins only

C. Lipids only

D. Ash

25.

Refractive index is commonly used in evaluation of:

A. Volatile oils

B. Fibers

C. Proteins

D. Starch

26.

Acid value indicates:

A. Free fatty acid content

B. Moisture content

C. Sugar content

D. Protein content

27.

Saponification value measures:

A. Alkaloid content

B. Amount of alkali required to saponify fats

C. Ash content

D. Moisture content

28.

A high acid value indicates:

A. Fresh oil

B. Hydrolysis of fats

C. High moisture

D. Low quality ash

29.

Biological evaluation is based on:

A. Pharmacological activity

B. Color

C. Odor

D. Taste

30.

DNA barcoding is primarily used for:

A. Extraction

B. Authentication

C. Storage

D. Packaging

31.

DNA barcoding is based on:

A. Morphological markers

B. Genetic markers

C. Chemical markers

D. Organoleptic markers

32.

DNA barcoding is especially useful for:

A. Whole plants only

B. Powdered crude drugs

C. Fresh leaves only

D. Flowers only

33.

Which parameter is NOT a physicochemical constant?

A. Ash value

B. Foaming index

C. Moisture content

D. Stomatal index

34.

The presence of excessive foreign organic matter indicates:

A. High quality

B. Adulteration or contamination

C. High potency

D. Standardization

35.

Which method can detect starch grains and fibers?

A. Organoleptic

B. Microscopic

C. Biological

D. Physical

36.

WHO stands for:

A. World Health Organization

B. World Herbal Organization

C. World Health Office

D. World Herbal Office

37.

The first step in crude drug evaluation is often:

A. Organoleptic examination

B. DNA analysis

C. Ash value determination

D. Biological testing

38.

A drug with high moisture content is more susceptible to:

A. Microbial spoilage

B. Improved stability

C. Increased purity

D. Better efficacy

39.

Foaming index is particularly important for:

A. Saponin-containing drugs

B. Alkaloid drugs

C. Resinous drugs

D. Protein drugs

40.

Swelling index is useful for evaluating:

A. Isabgol

B. Clove

C. Cinnamon

D. Nutmeg

41.

The evaluation of a drug based on its pharmacological activity is called:

A. Organoleptic evaluation

B. Chemical evaluation

C. Biological evaluation

D. Physical evaluation

42.

Which of the following is a quantitative microscopic parameter?

A. Stomatal index

B. Odor

C. Taste

D. Color

43.

An increase in ash value may indicate:

A. Inorganic contamination

B. High protein content

C. Presence of lipids

D. Low moisture content

44.

Optical rotation is measured using:

A. Microscope

B. Polarimeter

C. Refractometer

D. Viscometer

45.

Refractive index is measured using:

A. Polarimeter

B. Refractometer

C. Microscope

D. Colorimeter

46.

Viscosity is measured using:

A. Viscometer

B. Refractometer

C. Polarimeter

D. Microscope

47.

Which evaluation method helps detect adulteration in powdered drugs?

A. DNA barcoding

B. Microscopy

C. Chemical tests

D. All of the above

48.

The quality control of herbal drugs aims to ensure:

A. Identity

B. Purity

C. Quality

D. All of the above

49.

A major advantage of DNA barcoding is:

A. Rapid authentication

B. Dependence on morphology

C. Low specificity

D. Limited application

50.

Standardization of crude drugs is essential for:

A. Reproducible therapeutic efficacy

B. Increased adulteration

C. Reduced identification

D. Elimination of evaluation

Answer Key

Q. No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**
Answer	B	D	B	A	C	B	A	A	C	A	B	B	B	A	B	A	B	B	A	C	A	A	B	A	A

Q. No.	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**
Answer	A	B	B	A	B	B	B	D	B	B	A	A	A	A	A	C	A	A	B	B	A	D	D	A	A

Next: Chapter C – **Phyto-Therapeutic Agents (50 MCQs)**.

2 MARKS QUESTIONS

Q. No.	**Question**	**Cognitive Domain**
1	Define adulteration of crude drugs.	Remembering
2	What is organoleptic evaluation?	Remembering
3	Define ash value.	Remembering
4	What is DNA barcoding?	Remembering
5	Define extractive value.	Remembering
6	Apply qualitative and quantitative microscopy for the evaluation of a crude drug sample.	Applying
7	Analyze the significance of moisture content in maintaining the quality and stability of crude drugs.	Analyzing

8	Analyze the importance of determining foreign organic matter during the quality evaluation of crude drugs.	Analyzing
9	Evaluate the quality of a crude drug sample showing a high ash value and justify your conclusion.	Evaluating
10	Design an appropriate authentication protocol for a medicinal plant sample that is difficult to identify morphologically, and justify the methods selected.	Creating

5 MARKS QUESTIONS

Q. No.	**Question**	**Cognitive Domain**
1	Discuss the various types of adulteration in drugs of natural origin.	Remembering
2	Explain organoleptic evaluation of crude drugs with suitable examples.	Understanding
3	Describe microscopic evaluation methods used in quality control of crude drugs.	Understanding
4	Write a note on ash values and their significance.	Remembering
5	Explain DNA barcoding and its applications in pharmacognosy.	Understanding
6	Apply physicochemical parameters to assess the quality and standardization of a crude drug sample.	Applying
7	Analyze the significance of bitterness value, foaming index, and swelling index in the quality evaluation of crude drugs.	Analyzing
8	Analyze the suitability of physical, chemical, and biological evaluation methods for quality control of different crude drugs.	Analyzing
9	Evaluate the impact of excessive foreign organic matter on the quality, safety, and therapeutic efficacy of a crude drug sample.	Evaluating
10	Design a comprehensive evaluation protocol to establish the authenticity of a powdered herbal drug suspected of adulteration.	Creating

10 MARKS QUESTIONS

Q. No.	Question	Cognitive Domain
1	Discuss adulteration of drugs of natural origin and methods for its detection.	Understanding
2	Describe organoleptic, microscopic, physical, chemical, and biological methods used in the evaluation of crude drugs.	Understanding
3	Explain various physicochemical parameters used in the quality control of crude drugs.	Understanding
4	Write a detailed account of ash values, extractive values, and moisture content in crude drug evaluation.	Remembering
5	Explain the principle, methodology, and applications of DNA barcoding in medicinal plant identification.	Understanding
6	Apply WHO guidelines to ensure the quality, safety, and efficacy of drugs of natural origin during quality control.	Applying
7	Analyze the significance of optical rotation, refractive index, viscosity, acid value, and saponification value in the quality evaluation of crude drugs.	Analyzing
8	Analyze the importance of microscopic and physicochemical parameters in the standardization of herbal drugs.	Analyzing
9	Evaluate the quality of a crude drug sample exhibiting abnormal foaming index, ash value, and moisture content, and justify your conclusion.	Evaluating
10	Design a comprehensive quality control strategy for authenticating a medicinal plant before commercial production using morphological, microscopic, physicochemical, and DNA barcoding techniques.	Creating