UNIT-3

Quality control of crude drugs

Points to be covered in this topic

- ❑ DRUG ADULTERATION
  - ❖ The intentional type of adulteration
- ❑ EVALUATION OF CRUDE DRUGS
  - ❖ Organoleptic / morphological evaluation
  - ❖ Microscopical evaluation
  - ❖ Chemical evaluation
  - ❖ Physical evaluation
  - ❖ Biological evaluation

3.1. DRUG 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. It is also known as debasement of an article.

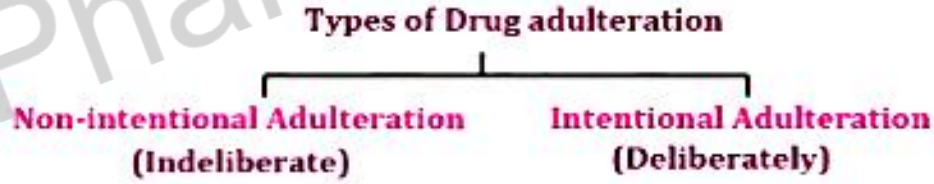
Types of adulterants

- Adulteration involves different conditions such as **deterioration, admixture, sophistication, substitution, inferiority and spoilage.**

Table 3.1 : Types of adulteration

TERMS	DESCRIPTION
Deterioration	Impairment or decrease in the quality of the drug.
Admixture	Addition of one substance to another substance due to ignorance or carelessness or by accident.
Sophistication	Intentional or deliberate type of adulteration.
Substitution	The original drug gets replaced with totally different substance.
Inferiority	Refers to substandard drug.
Spoilage	Due to the attack of microorganisms.

Adulteration can be divided into two types, Unintentional and Intentional adulteration



Nonintentional adulteration may be due to the following reasons:

1. Confusion in the names of local dialects and indigenous medical systems.
2. Lack of knowledge.
3. No availability of the authentic plant.
4. Morphological similarity.

Intentional adulteration may be due to the following reasons:

1. Adulteration by artificially manufactured substances.
2. Substitution by inferior varieties.
3. Substitution using exhausted drugs.
4. Substitution of superficially similar substance.
5. Adulteration by other vegetative part.

6. Addition of toxic(harmful) materials.
7. Adulteration of powders.
8. Addition of synthetic principles.

3.1.1. The intentional type of adulteration

1. Adulteration using artificially manufactured substances:-

The original substances are adulterated by the materials that are artificially manufactured. The materials are prepared in a way that their general form and appearance resemble with various drugs.

Examples:-

a) Bass-wood is cut exactly the required shape of nutmegs and used to adulterate nutmegs.
b) Compressed chicory is used in place of coffee.


Compressed chicory Coffee

2. Substitution using inferior commercial varieties:-

The original drugs are substituted using inferior quality drugs that may be similar in morphological characters, chemical constituents or therapeutic activity.

Example:-

(a) Hog gum or Hog tragacanth to adulterate tragacanth gum.
(b) Mangosteen fruits to adulterate bael fruits.


Hog gum Tragacanth gum

3. Substitution using exhausted drugs:-

The active medicaments of the main drugs are extracted out and are used again. This technique is frequently adopted for the drugs containing volatile oils, such as clove, fennel etc.

Example:-

a) After extraction, saffron and red rose petals are recoloured by artificial dyes.
b) Another example is balsam of tolu that does not contain cinnamic acid.


Saffron

4. Substitution of superficially similar inferior natural substances:-

The substituents used may be morphologically similar but will not be having any relation to the genuine article in their constituents or therapeutic activity.

Example:-

a) Peach kernels and apricot kernels adulterate with almonds.

b) Clove stalks and mother cloves adulterate with cloves.

Apricot kernels Almonds

5. Adulteration using the vegetative part of the same plant:-

The presence of vegetative parts of the same plant with the drug in excessive amount is also an adulteration.

Example:-

a) There may also be unusually high levels of epiphytes, such as mosses, liverworts, and lichens that cover the bark., e.g. cascara or cinchona.

b) Excessive amount of stems in drugs like lobelia, stramonium, hamamelis leaves etc.

Mosses & liverworts

6. Addition of toxic materials:-

In this type of adulteration the materials used for adulteratio would be toxic in nature.

Example:-

(a) Stone mass was found in the centre of a bale of liquorice root.

(b) Limestone pieces with asafoetida, lead shot in opium, amber-coloured glass pieces in colophony, barium sulphate to silvergrain cochineal and

7. Adulteration of powders:-

Adulterants used are generally powdered waste products of a suitable colour and density.

Brick powder Bark

Example:-

a) Powdered olive stones adulterate with powdered gentian, liquorice or pepper.
b) Brick powder adulterate with barks.
c) Red sanders wood adulterate with chillies.
d) Dextrin adulterate with powdered ipecacuanha.

8. Addition of synthetic principles:-

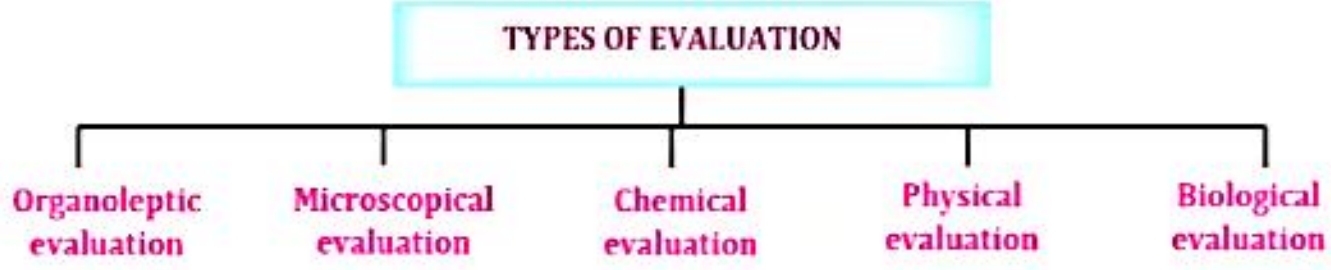
Synthetic pharmaceutical principles are used for market and therapeutic value.

Example:-

a) Citral is adulterate with lemon oil.
b) Benzyl benzoate is adulterate with balsam of Peru.
c) Diabetes Angel containing glyburide and phenformin.

3.2 EVALUATION OF CRUDE DRUGS

Drug evaluation establishes a drug's identity, as well as its quality and purity. The biochemical variations in the drug, the effects of handling and storing the drug, and adulterations and substitutions are the main causes for the requirement for evaluation of crude pharmaceuticals.



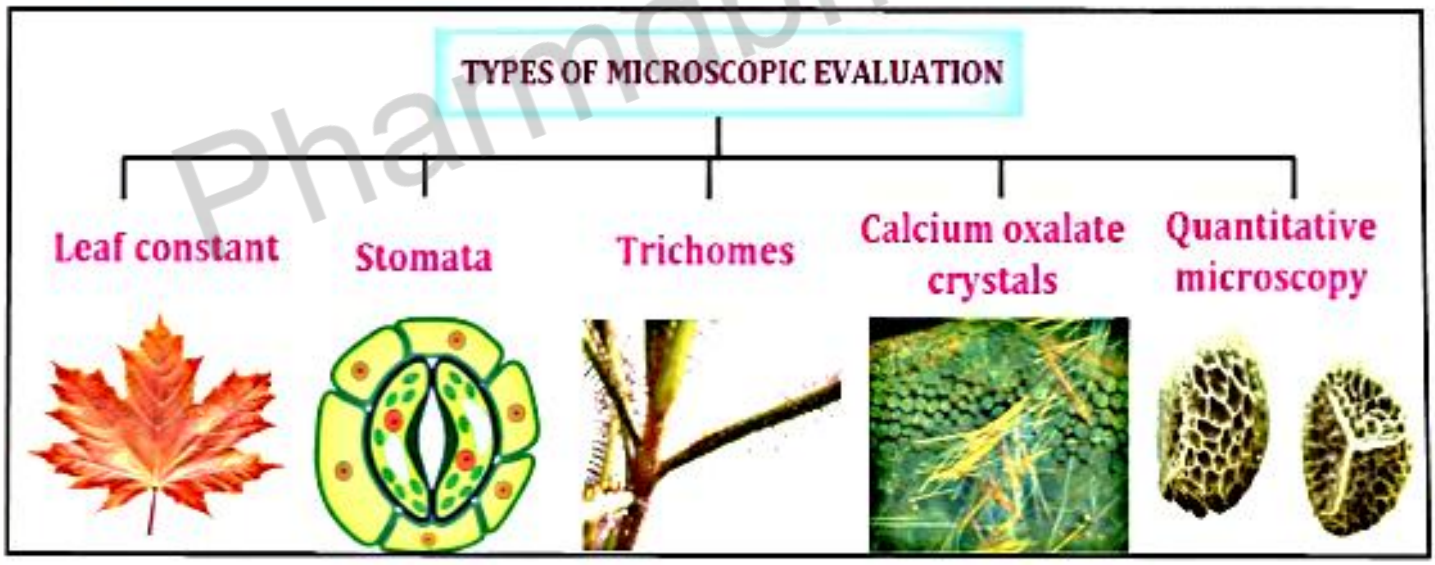
3.2.1. ORGANOLEPTIC / MORPHOLOGICAL EVALUATION

- Using the sense organs to evaluate medications is known as organoleptic evaluation. It alludes to analytical techniques such as color, smell, taste, size, form, and unique traits like touch and texture.

- **Organoleptic Characters:** - It includes an examination of visual appearance.

  - **Colour** - Some drugs are green in colour when they are dried in shade but they become pale and bleached when dried in direct sunlight.
  - **Smell** - A number of crude drugs have characteristic smell, for instance drugs contains volatile oil.
  - **Taste** - Drugs can be evaluated by taste also. drugs like Gentian, Gelatin, Chirata (Bitter), Glycyrrhiza (Sweet), Ginger (Pungent) can be distinguished by their taste.
  - **Texture** - Leaves that are dried in the sun become flexible whereas those that are dried in the shadow become brittle.

3.2.2. MICROSCOPICAL EVALUATION



❖ Leaf Constants or Diagnostic Characters of Leaves

(i) Palisade ratio: - Average number of palisade cells beneath each epidermal cell. Quite fine powders can be used for the determination.

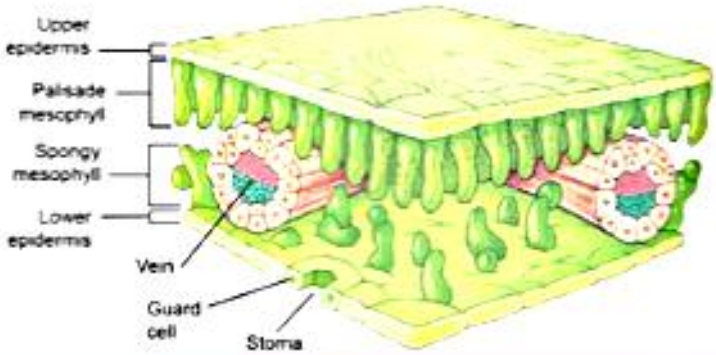


Table 3.2: Palisade ratio of drugs

DRUG	PALISADE RATIO
Adhatoda vasica	5.5-6.5
Bacopa monniera	1.5-2.25
Cassia acutifolia	4.5-9.5 (upper) 3.5-7.0 (lower)
Datura metel	5-6.5
Eucalyptus globules	5.5-6.5 (upper) 3.5-5 (lower)

(ii) Vein-islet Number: - Defined as the number of vein-islets per sq. mm of the leaf surface midway between the midrib and the margin.

Table 3.3: Vein-islet Number of drugs

DRUG	VEIN-ISLET NUMBER
Adhatoda vasica	6-8
Bacopa monniera	6-13
Cannabis sativa	20-30
Cassia angustifollia	18-24
Datura metel	19-22

(iii) Vein-termination Number: - Number of veinlet terminations per sq. mm of the leaf surface midway between midrib and margin.

Table 3.4: Vein--termination Number of drugs

PLANT NAME	VEINLET TERMINATION NUMBER
Digitalis purpurea	2.6-4.2
Atropa belladonna	6.3-10.3
Datura stramonium	12.6-20.1
Cassia angustifolia	25.9-32.8
Erythroxylum coca	16.8–21

(iv) Stomata Number: - Number of stomata per sq. mm of epidermis of the leaf.

Table 3.5: Stomata Number of drugs

PLANT NAME	UPPER SURFACE	LOWER SURFACE
Atropa acuminata	05-14	78-95
Cassia angustifolia	220-260	240-265
Datura stromonium	98-150	200-207
Datura metel	147-160	200-209
Hyoscyamus niger	165-195	190-230

(v) Stomata Index: - Percentage which the numbers of stomata form to the total number of epidermal cells; each stoma being counted as one cell.

$$SI = \frac{S}{E+S} \times 100$$

Where,

SI= Stomata Index

S = Number of stomata per unit area

E = Number of epidermal cells in the same unit area

Table 3.6: Stomata Index of drugs

PLANT NAME	UPPER SURFACE	LOWER SURFACE
Atropa acuminata	2.5-4.7	15.5-17.9
Cassia angustifolia	17-21.4	16.5 - 21
Bacopa monniera	12.9-17.8	12.4-16.4
Hyoscyamus niger	18.6 - 20	25.4 – 28.1
Datura stramonium	13.6 - 20	14.4 -15.4

❖ Stomata

Stomata is a epidermal structure which has a central pore and two kidney shaped similar cells called as guard cells and different numbers of subsidiary cells (epidermal cells) covering the guard cells.

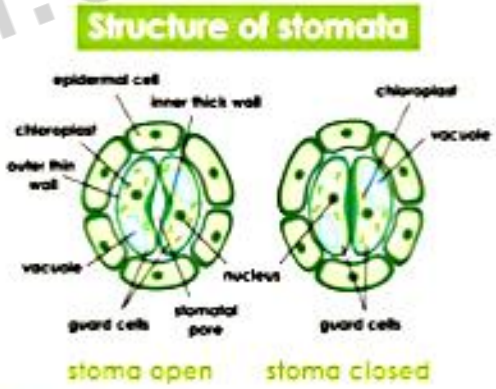


Fig 3.1: Stomata structure

Types of stomata:-

- Depending upon the type of the guard cells and arrangement of subsidiary cells, stomata are divided into four types: Moss type, Gymnospermous type, Gramineous type, Dicotyledonous type.
- Dicotyledonous stomata are classified into following types-

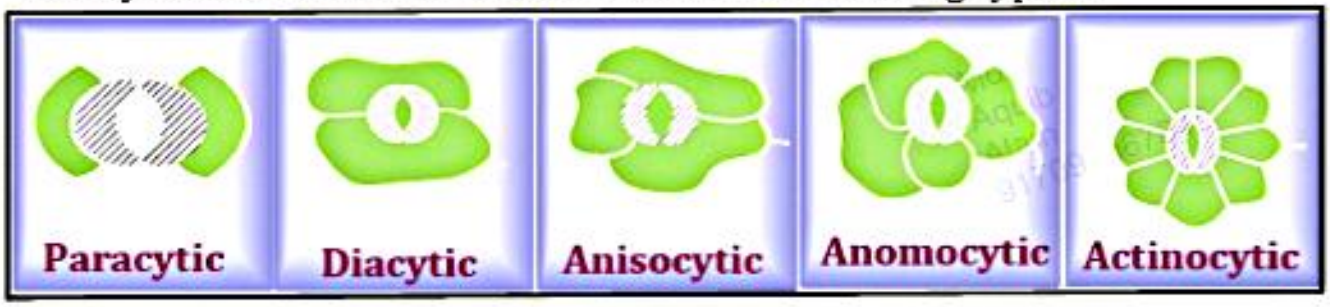


Fig 3.2: Type of Stomata

A. **Paracytic /Rubiaceous/ Parallel-celled stomata:-**These type of stomata comprise two guard cells covered by two subsidiary cells, the long axes of which are parallel to that of stoma.
e.g. Coca and Senna leaves.

B. **Diacytic /Caryophyllaceous/ Cross-celled stomata:-:-**The guard cells are covered by two subsidiary cells but the arrangement of subsidiary cells on the guard cell is at right angle to that of stoma.
e.g. Peppermint, Spearmint and Vasaka.

C. **Anisocytic/Cruciferous/ Unequal-celled stomata:-**The number of guard cells is two, as in all other cases, but, the guard cells are covered by three subsidiary cells, of which one is markedly smaller than the other two.
e.g. Belladonna, Datura and Stramonium.

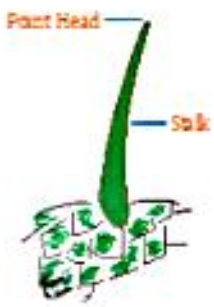
D. **Anomocytic/Ranunculaceous/ Irregular-celled stomata:-**In this type, stoma is surrounded by varying number of subsidiary cells resembling other epidermal cells.e.g. Buchu, Digitalis and Lobelia.

❖ Trichomes

- Trichomes are also known as plant hairs.
- Trichomes consist of two parts namely root and body.
- Trichomes are present in most of the parts of the plant such as, seeds, fruits etc.

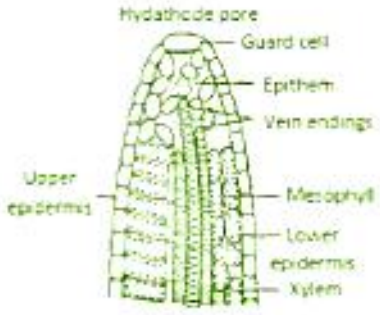
Types of Trichomes

Covering trichomes



Glandular trichomes

Hydathodes



Vertical section of a leaf showing hydathode

Table 3.7: Types of trichomes

<table>
<tr><th colspan="3">COVERING TRICHOMES</th></tr>
<tr><th>Unicellular trichomes</th><th colspan="2">Multicellular trichomes</th></tr>
<tr><td rowspan="2">1. Lignified trichomes – Nux vomica, Strophanthus
1. Short, sharply pointed, curved - Cannabis
2. Large, conical, strongly shrunken - Lobelia
3. Short, conical, unicellular- Tea, Buchu
4. Strongly waved, thick walled - Yerba, Santa</td><td>Unbrached</td><td>Branched</td></tr>
<tr><td>(i) Uniseriate
• Bi-cellular, conical- Datura
• Three celled long- Stramonium
• Three to four celled long- Digitalis
• Four to five celled long - Belladonna
(ii) Biseriate: Calendula officinalis
(iii) Multiseriate: Male fern</td><td>1. Stellate- Hammamelis, Helicteris isora
2. Peltate- Humulus
3. Candelabra - Verbascum Thapsus
4. T shaped trichomes Artemisia, Pyrethrum</td></tr>
<tr><th colspan="3">GLANDULAR TRICHOMES</th></tr>
<tr><th>Unicellular trichomes</th><th colspan="2">Multicellular trichomes</th></tr>
<tr><td>The stalk is absent e.g. Piper Betel, Vasaka.</td><td colspan="2">1. Trichomes with unicellular head and unicellular stalk, e.g. Digitalis purpurea.
2. Unicellular head and uniseriate multicellular stalk, e.g. Digitalis thapsi, Belladonna etc.
3. Multicellular head, multicellular, biseriate stalk, e.g. Santonica and Sunflower, etc.
4. Unicellular stalk and biseriate head, e.g. Digitalis purpurea.
5. Short stalk with secreting head formed of rosette or club shaped cells, e.g. Mentha species.
6. Trichomes with multicellular, multiseriate cylindrical stalk and a rosette of secretory cells, e.g. Cannabis sativa.
7. Multicellular multisteriate head and multicellular uniseriate stalk, e.g. Indian hemp, Tobacco.</td></tr>
<tr><th colspan="3">HYDATHODES</th></tr>
<tr><td colspan="3">These are organs of absorption or secretion of water developed in certain plants e.g. Piper betal, London pride, etc.</td></tr>
</table>

❖ Quantitative Microscopy

Lycopodium spore method for percentage purity

- It is an important analytical technique for powdered drugs.
- It is inexpensive technique with official status.

- Lycopodium spores are very characteristic in shape and uniform in **size (25 µm).**
- On an average, **94,000 spores per mg** of powdered Lycopodium.
- A powdered drug is evaluated by this technique, if it contains

Fig 3.3: Lycopodium

(a) Well defined particles which may becounted.
(b) Single layered cells or tissues.
(c) The objects of uniform thickness, the length of which can be measured & actual area calculated.

The percentage purity of an authentic powdered ginger is calculated using the following equation,

$$\%\text{Purity of drug} = \frac{N \times W \times 94,000}{S \times M \times P} \times 100$$

Where,

N = Number of characteristic structures (e.g. starch grains) in 26 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

P = 2, 86,000 in case of ginger starch grains powder.

Lycopodium spore

3.2.3 CHEMICAL EVALUATION

- Chemical assays, Quantitative chemical testing, Qualitative chemical tests, and Instrumental analysis are all part of the chemical evaluation. The isolation, purification, and identification of active constituents are chemical methods of evaluation.

Chemical Evaluation

Quantitative chemical tests
- Acid value
- Saponification value
- Ester value
- Iodine value
- Hydroxyl value
- Unsaponifiable matter

Qualitative chemical tests
- Mayer's reagent
- Hager's reagent
- Wagner's reagent
- Borntrager's test
- Salkowaski test
- Shinoda test

Instrumental analyses

Chromatographic methods
Paper chromatography, TLC, GC, HPLC and HPTLC

Spectroscopic methods
UV, IR , Mass spectroscopy, NMR

❖ Quantitative chemical tests

Quantitative Chemical Analysis is to accurately determine the concentration, amount or percentage of one or more elements in a test sample. For example:-

❖ Qualitative chemical tests

Identification tests for numerous phytoconstituents such alkaloids, glycosides, tannins, etc. are included in qualitative chemical assays. For example:-

Table 3.8: Chemical tests

DETECTION OF ALKALOIDS	• **Mayer's reagent** (Cream precipitate), • **Dragendorff's reagent** (Orange brown precipitate), • **Hager's reagent** (Yellow precipitate), • **Wagner's reagent** (Reddish-brown precipitate).
DETECTION OF GLYCOSIDES	• **Anthraquinone Glycosides** -Borntrager's test, Modified borntrager's test • **Saponin Glycosides-** Haemolysis test, Foam test • **Steroid and Triterpenoid Glycosides-** Libermann burchard test, Salkowaski test, Antimony trichloride test. • **Cardiac Glycosides-** Keller-kiliani test, Legal test, Baljet test • **Chemical Tests for Coumarin Glycosides-** $FeCl_3$ test, Fluorescence test • **Cynophoric Glycoside-** Sodium picrate test. • **Flavonoid Glycosides-** Shinoda test, Vanillin HCl test
DETECTION OF CARBOHYDRATES	Fehling's reagents, Barfoed's reagents and Benedict's reagents, Selivenoff,s test, Osazone formation test
DETECTION OF FLAVONOIDS	Shinoda test, Alkaline reagent test, Zinc hydrochloride test.
DETECTION OF TANNINS	Goldbeater's skin test, Ferric chloride test, Phenazone test, Gelatin test

❖ Instrumental analysis

Instrumental analyses are used to analyse the chemical groups of phytoconstituents using chromatographic and spectroscopic methods.

- **Chromatographic methods include** (Paper chromatography, Thin-layer chromatography, Gas chromatography etc.).
- **Spectroscopic methods include** (UV spectroscopy, IR spectroscopy etc.).

3.2.4. PHYSICAL EVALUATION

Physical methods are frequently used in crude plant evaluation to ascertain the **solubility, specific gravity, optical rotation, viscosity, refractive index, melting point, water content, level of fiber elasticity,** and other physical properties of the herb material.

- **Moisture content:** To avoid crude pharmaceuticals decomposing owing to microbial contamination, it is important to assess and manage a drug's moisture content. By heating a medicine to a constant weight at 105°C in an oven, the moisture content can be calculated.

Table 3.9: Moisture content of drugs

Drugs	Moisture content (w/w)
Digitalis	NMT 5
Ergot	NMT 8
Aloe	NMT 10
Acacia	NMT 15
Starch	NMT 15

- **Viscosity:** Viscosity of a liquid is constant at a given temperature and is an index of its composition. Since it is constant at a given temperature, it is used as an evaluation parameter.

Table 3.10: Viscosity of drugs

Drugs	Kinematic viscosity
Liquid paraffin	NLT 64 centistokes at 37.8°
Pyroxylin	1100-2450 centistokes

- **Melting Point:** The melting points of components of plants are extremely precise. Because of the combination of ingredients, the melting point range for crude pharmaceuticals has been defined.

Table 3.11: Melting point of drugs

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

❖ **Solubility:** The presence of adulterant in a drug could be indicated by solubility studies, useful for the examination of many oils, oleoresins, etc.

1. Castor oil is only soluble in three volumes of 90% alcohol. whereas adulterated versions of the substance may show good alcohol solubility.
2. Balsam of Peru is soluble in chloral hydrate solution.
3. Colophony is freely soluble in light petroleum.
4. Asafoetida is soluble in carbon disulphide.
5. Alkaloidal bases are soluble in chloroform. while alkaloidal salts are soluble in polar solvent.
6. The glycosides are extractable with alcohol and water. while their aglycones moieties are soluble in non-polar solvents.

Fig 3.4: Solubility of drugs

❖ **Optical Rotation:** It has been discovered that several chemicals, whether in their pure form or dissolved, have the ability to rotate planar polarized light.

Table 3.12: Optical Rotation of drugs

Drugs	Angle of Optical Rotation
Eucalyptus oil	(0° to +10°)
Honey	(+3° to -15°)
Chenopodium oil	(-30° to -8°)

❖ **Foreign Organic Matter:** Organ components that are not those of the medications stated in the definition and description of the drug are referred to as "foreign organic materials."

❖ **Ash Values and Extractives:** Drug evaluation essentially requires drug identification, which can be accomplished using microscopic or morphological characteristics. Even when a medication is discovered, it is typically of low quality since it was incorrectly gathered or preserved. The following tests can be used to show its acceptability as a drug, thus it should whenever possible.

a) Ash content

The medication's ash content, which is the byproduct of incineration, simply indicates inorganic salts that are either naturally present in the drug.

Total Ash

Carbon and organic matter present in a drug are converted to ash at 450°C or above. It mostly contains carbonates, phosphates, silicates and silica. The total ash value can be used further to study water soluble and acid insoluble ash contents.

a) Extractives

The extracts made from exhausted crude pharmaceuticals provide an approximation of the amounts of their chemical components. The determination of extractives uses a variety of solvents due to the variability in the chemical composition and characteristics of drug contents.

Ether-soluble extractives

- Determined for evaluation of crude drugs are volatile (volatile oil content) and non-volatile (resin, fixed oils or colouring matter) ether soluble extractives.

Alcohol-soluble extractives

- It is frequently employed to determine resin content of a drug. 95% ethyl alcohol is used for determination of alcohol-soluble extractive.

Water-soluble extractives

- This method is applied to drugs which contain active constituent. e.g. tannins, mucilage, sugar.

3.2.5. BIOLOGICAL EVALUATION

- The plant or extract can be evaluated by various biological methods to determine pharmacological activity, potency, and toxicity.
- The bioassay methods are Estimation of potency of crude drug which are of three types they are, toxic, symptomatic and tissue or organ methods.
- While animals are utilized in the toxic and symptomatic procedures, the tissue method allows for the observation of a drug's effects on a single organ or tissue.

- For the purpose of determining the lethal dose and effective dose of crude drags, toxicology studies are carried out on suitable animal models. Mice are used to test the effects of various vaccines.

In other words, a bioassay tests if a sample can have the same biological impact as a reference preparation. International Unit (IU) units serve as a representation of this activity

Table 3.12: Biological activity contained in each IU

The specific biological activity contained in each IU of the few drugs	
Drug	**Biological activity contained in each IU**
Vit. A	0.344 mg of standard preparation
Vit. D	0.025 mg of standard preparation
Heparin	0.025 mg of standard preparation
Digitalis	76 mg of standard preparation