ADULTERATION & EVALUATION

DEFINITION :

- It is a practice of substituting the original crude drug partially or fully with other substances which is either free from or inferior in therapeutic and chemical properties or addition of low grade or spoiled drugs or entirely different drug similar to that of original drug substituted with an intention of enhancement of profits.
- Adulteration involves different conditions such as
  - Deterioration
  - Admixture
  - Sophistication
  - Substitution
  - Inferiority and
  - Spoilage

- DETERIORATION is the impairment in the quality of a drug.
- ADMIXTURE is the addition of one article to another due to ignorance or carelessness, or by accident.
- SOPHISTICATON is the intentional or deliberate type of adulteration.
- SUBSTITUTION occurs when totally different substance is added in place of original drug.
- INFERIORITY refers to any substandard drug.
- SPOILAGE deterioration due to the attack of microorganisms.

- Adulteration may take place by two ways:
  - Direct or intentional adulteration
  - Indirect or unintentional adulteration

Indirect or unintentional adulteration

Unintentional adulteration which sometimes occurs without bad intention of the manufacturer or supplier.

- Sometimes in the absence of proper means of evaluation, an authentic drug partially or fully devoid of the active ingredients may enter the market.
- Factors such as geographical sources, growing conditions, processing, and storage are all factors that influence the quality of the drug

Direct or intentional adulteration

- Direct or intentional adulteration is done intentionally which usually includes practices in which an herbal drug is substituted partially or fully with other inferior products.
- Due to morphological resemblance to the authentic herb, many different inferior commercial varieties are used as adulterants.
- These may or may not have any chemical or therapeutic potential.
- Substitution by “exhausted” drugs entails adulteration of the plant material with the same plant material devoid of the active constituents

- This practice is most common in the case of volatile oil-containing materials, where the dried exhausted material resembles the original drug but is free of the essential oils.

- Foreign matter such as other parts of the same plant with no active ingredients, sand and stones, manufactured artifacts, and synthetic inferior principles are used as substitutes

Types of adulteration

- Generally the drugs are adulterated by substitution with sub-standard commercial varieties, inferior drugs, or artificially manufactured commodities.

- The following types of adulteration are common

1. Substitution With Sub-standard Commercial Varieties
2. Substitution With Superficially Similar Inferior Drugs
3. Substitution With Artificially Manufactured Substances
4. Substitution With Exhausted Drug
5. Use Of Synthetic Chemicals
6. Presence Of Vegetative Matter From The Same Plant
7. Harmful Adulterants
8. Adulteration Of Powders

1. Substitution With Substandard Commercial Varieties

- The adulterants here may resemble the original crude drug in morphological, chemical, or therapeutic characters, but are sub-standard in nature and hence are cheaper in cost. This is a rather most common practice of adulteration

Examples

- *Strychnous nux-blanda* or *S.potatorum* in place of S.*nux-vomica*,
- *Capsicum annuum* in place of *C.minimum*,
- Indian senna is substituted with arabian senna or dog senna,
- Medicinal ginger is substituted with inferior varieties of African, Japanese, or Cochin ginger.

2. Substitution With Superficially Similar Inferior Drugs

- These inferior drugs used may or may not be having any chemical or therapeutic value as that of original drug.
- Due to their morphological resemblance to the authentic drug, they are used as adulterants.
- **Examples**
  - Belladonna leaves are substituted with Ailanthus leaves,
  - Saffron is admixed with dried flowers of *Carthamus tinctorious*
  - Bees wax is substituted with Japan wax

3. Substitution With Artificially Manufactured Substances

- It has been also observed that substances artificially prepared to resemble the original drug are used as substitutes. This practice is followed for much costlier drugs.

  e.g.,

  - Compressed Chicory in place of coffee,
  - Yellow coloured paraffin wax for bees wax,
  - Properly cut and shaved basswood for nutmeg

4. Substitution With Exhausted Drug

- The same drug is admixed but is devoid of any medicinally active constituents as they are already extracted out.
- This practice is more common in case of volatile oil containing drugs like fennel, clove, coriander, caraway etc.
- Sometimes natural characters of exhausted drugs like colour and taste are manipulated by adding other additives and then it is substituted.

e.g

- Exhausted gentian made bitter with aloes,
- Artificial colouring of exhausted saffron, etc.

5. **Use of Synthetic Chemicals**

- Besides these common practices, sometimes other methods are also employed like **use of synthetic chemicals** to enhance the natural character as in case of

  - Addition of benzyl benzoate to balsam of peru,
  - Citral to citrus oils like oil of lemon and orange oil, etc.

6. Presence of vegetative matter from the same plant

- Sometimes, the other miniature plants growing along with medicinal plants are admixed with the authentic drug, due to their resembling colour, odour, and in some cases constituents.

Egs.,

- The lower plants like moss, liverworts, and epiphytes growing on bark portion are mixed with cascara or cinchona.

- The stem portions are mixed along with leaf drugs like stramonium , lobelia and senna

7. Harmful Adulterants

- Sometimes the waste from the market are collected and admixed with the authentic drug. This is particularly noticed for liquids or unorganized drugs.
- Examples

- Pieces of amber coloured glass in colophony,

- Limestones in asafoetida, lead shot in opium, white oil in coconut oil, cocoa butter mixed with stearin or paraffin.

- The addition of rodent feacal matter to cardamom seed is a very harmful adulteration

8. Adulteration of Powders

- Besides the entire drug, the powdered forms are frequently found to be adulterated.

- **Examples**
  - Dextrin in ipecacuanha
  - Powered liquorice or gentian admixed with powdered olive stones
  - Exhausted ginger powder in powdered colocynth or ginger

Evaluation of Crude Drugs

Definition:

Evaluation of drug means

- Confirmation of its identity
- Determination of its quality and purity and
- Detection of nature of adulteration.

- **Types of Evaluation**
  1. Organoleptic evaluation
  2. Microscopic evaluation
  3. Physical evaluation
  4. Chemical evaluation
  5. Biological evaluation

Organoleptic evaluation

- It means evaluation of drug by the organs of sense (skin, eye, tongue, nose and ear) or macroscopic evaluation and it includes evaluation of drugs by
  - Color
  - Odour
  - Taste
  - Size
  - Shape and
  - Special feature, like touch, texture etc

- It is the technique of **qualitative evaluation** based on the study of morphological and sensory profile of whole drugs.

eg. The **fractured surfaces** in cinchona, quillia, cascara barks and quassia wood are important characteristics.

Aromatic odour of umbelliferous fruits and sweet taste of liquorice are the examples of this type of evaluation where **odor** of drug depends upon the **type** and **quality** of odourous principles (volatile oils) present

- **Shape** of drug may be cylindrical (sarsapilla), subcylindrical (podophyllum), conical (aconite), fusiform (jalap) etc, size represent length, breadth, thickness, diameter etc.
- **Color** means external color which varies from white to brownish black are important diagnostic characters.
- **Taste** is specific type of sensation felt by epithelial layer of tongue. It may be acidic (sour), saline (salt like), saccharic (sweetish), bitter or tasteless (possessing no taste).

- The **general appearance** (external marking) of the crude drug often indicates whether it is likely to comply with prescribed standard like

  - furrows (alternate depression or valleys)
  - wrinkles (fine delicate furrows)
  - annulations (transverse rings)
  - fissures (splits)
  - nodules (rounded outgrowth)
  - scars (spot left after fall of leaves, stems or roots)

Microscopic evaluation

- It involves detailed examination of the drug and it can be used to identify the organized drugs by their known histological characters
- It is mostly used for qualitative evaluation of organized crude drugs in entire and powder forms with help of microscope
- **Qualitative microscopy**

Using microscope detecting various cellular tissues, trichomes, stomata, starch granules, calcium oxalate crystals and aleurone grains are some of important parameters which play important role in identification of certain crude drug.

- **Quantitative microscopy**

Quantitative aspects of microscopy includes study of stomatal number and index, palisade ratio, vein-islet number, size of starch grains, length of fibers etc which play important role in the identification of drug

Qualitative Microscopy

Stomata

- There are several types of stomata, distinguished by the forms and arrangement of the Surrounding cells (Subsidary cells), e.g.

(a) **Anomocytic** (Ranunculaceous) irregular celled: Digitalis

(b) **Anisoytic** (Cruciferous) unequal celled: Datura

(c) **Diacytic** (Caryophyllaceous) perpendicular celled: Mentha

(d) **Paracytic** (Rubiaceous) parallel celled: Senna

(e) **Actioncytic:** 4 or more subsidary cells,elongated radially to stomata- Mucaceae

(f) **Cyclocytic:** 4 or more subsidary cells,arranged in narrow ring around stomata-Palmae

Trichomes

Trichomes are divided and subdivided as follows-

(i) Covering Trichomes

(a) Unicellular Trichomes: Nux vomica, Cannabis

(b) Uniseriate Multicellular Unbranched Trichomes: Datura

(c) Biseriate Multicellular unbranched Trichomes: *Calendula officinalis*

(d) Multiseriate Multicellular unbranched Trichomes: Male fern

(e) Multicellular branched Trichomes: *Verbascum Thapsus*

(ii) **Glandular Trichomes**

(a) Unicellular Glandular Trichomes: Vasaka

(b) Multicellular Glandular Trichomes: *Digitalis purpurea*

(iii) **Hydathode Trichomes:** *Piper betal*

Quantitative Microscopy

- It involves different parameters like

Palisade Ratio: It is defined as average number of palisade cells beneath each epidermal cell.

e.g. *Atropa belladonna (6-10)*

Digitalis Lanata (2.5-6.5)

- **Stomatal Number :** It is defined as average number of stomata per square millimeter area of epidermis.

e.g.: *Atropa belladonna: {6.0-14-37.5 (Upper* Surface), 62.5-93-174 (lower Surface)}.

- **Stomatal Index:** It is the percentage which the number of stomata forms to the total number of epidermal cells. It is calculated by,

$$SI = S / S + E \times 100$$

Where, S.I = Stomatal Index;

S = Number of stomata per unit area;

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

- **e.g.** *Atropa belladonna:- 2.3-3.9-10.5 (Upper Surface),* 20.2 - 21.7 - 23.0 (Lower Surface)

*Digitalis Purpurea - 1.6-2.7-4.0 (Upper Surface);*19.2- 25.2 (lower Surface)

- **Vein Islet Number:** It is defined as average number of Vein Islet per square millimeter of the leaf surface midway between midrib and the margin.
  - Note : **Vein Islet:** The small areas of green tissue outlined by the veinlets are termed as vein islets

    i. *Digitalis Lanata — 2.0-8.0*

    ii. *Digitalis Purpurea — 2.0-5.5*

- **Vein Termination Number:** It is defined as average number of Vein terminations per square millimeter of the leaf surface midway between midrib and the margin.
  - Note : Vein terminations are the ultimate free terminations of a veinlet or branch of a veinlet

    Atropa belladonna — 6.3-10.3

    Atropa acuminate — 1.4-3.5

Physical evaluation

- Physical constants are sometimes taken into consideration to evaluate certain drugs.
- These include
  - Moisture content
  - Specific gravity
  - Optical rotation
  - Refractive index
  - Melting point
  - Viscosity and
  - Solubility in different solvents
- All these physical properties are useful in identification and detection of constituents present in plant.
- Ash values
  - Total ash
  - Water soluble ash
  - Acid insoluble ash
  - Sulphated ash
- Fluorescence analysis
- Foreign matter

- **Determination of Total Ash**
- Incinerate about 2 to 3 g accurately weighed, of the ground drug in a tarred platinum or silica dish at a temperature not exceeding 450ºC until free from carbon, cool and weigh. Calculate the percentage of ash with reference to the air-dried drug.
- Total ash value = Wt. of total ash /Wt. of crude drug taken x 100
- **Acid insouble ash**
- Test measures the amount of silica present, especially as sand siliceous earth.
- The obtained total ash was boiled with 25 ml of 2N HCl for 5 min.
- The insoluble ash was collected on ash less filter and washed with hot water. The insoluble ash was transferred into pre-weighed silica crucible, ignited, cooled and weighed.

- The procedure was repeated till the constant weight was obtained. The percentage of acid insoluble ash was calculated with reference to the air dried drugs.

- % Acid insoluble ash value = Wt. of acid insoluble ash / Wt. of crude drug taken x 100

Drugs	**Acid insoluble ash (%w/w)**
Agar	Not more than 1.0
Amla	Not more than 2.0
Bael	Not more than 1.0

Determination of water soluble ash value

- The total ash obtained was boiled with 25 ml of chloroform water for five min. The insoluble matter was collected on a ash less filter paper & and washed with hot water.
- The insoluble ash was transferred into pre-weighed silica crucible, ignited for 15 min at a temperature not exceeding 450°C, cooled and weighed.
- The weight of the insoluble matter was subtracted from the weight of total ash. The percentage of water soluble ash was calculated with reference to the air-dried sample drug.
- % Water soluble ash value = Wt. of total ash - Wt. of water insoluble ash / Wt. of crude drug taken x 100

Determination of Sulphated ash value

- Silica crucible was heated to redness for 10 minute, allowed to cool in a desiccator and weighed.
- The total ash obtained was taken in the crucible and weighed accurately. It was ignited gently at first, until the substance is thoroughly charred. the residue was cooled, moistened with 1 ml of conc. Sulphuric acid, heated gently until white fumes are no longer evolved and ignited at 450°C temperature until all black particles have disappeared.
- The crucible was allowed to cool, a few drops of conc. sulphuric acid was added and heated. Ignited as before, allowed to cool and weighed. The percentage of Sulphated ash was calculated.
- % Sulphated ash value = Wt. of Sulphated ash / Wt. of crude drug taken x 100

DETERMINATION OF FOREIGN MATTER

- Weigh 100 –500 g of the drug sample to be examined or the minimum quantity prescribed in the monograph, and spread it out in a thin layer.

- The foreign matter should be detected by inspection with the unaided eye or by the use of a lens (6x). Separate and weigh it and calculate the percentage present.

Chemical evaluation

Most of drugs have definite chemical constituents to which their biological or pharmacological activity is attributed.

- Qualitative chemical test are used to identify certain drug or to test their purity.
- The isolation, purification, identification of active constituents is based on chemical methods of evaluation
- Some of these are useful in evaluation of
    - resins (acid value, sulphated ash),
    - balsams (acid value, saponification value and bester values),
    - volatile oils (acetyl and ester values) and
    - gums (methoxy determination and volatile acidity).
- Preliminary phytochemical screening is a part of chemical evaluation.
- These qualitative chemical tests are useful in identification of chemical constituents and detection of adulteration

Chemical evaluation scheme

Extraction of crude drug powder (successive solvent extraction)

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Preliminary phytochemical screening

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Chromatography & Spectroscopy

Step 1 – Preliminary Phytochemical screening

Major phytochemicals

- Alkaloids
- Flavonoids
- Phenols
- Resins
- Carbohydrates
- Volatile/essential oils
- Terpenoids
- Glycosides
- Steroids
- Tannins
- Amino acids
- Gums
- Fixed oils
- Saponins

Test for Alkaloids

i) **Mayer's test** (Potassium Mercuric Iodide): The acid layer with few drops of Mayer's reagent gives a **creamy white precipitate**.

ii) **Wagner's Tests** (Solution of Iodine in Potassium Iodide): The acid layer with few drops of Wagner’s reagent gives **reddish brown coloured precipitate**.

iii) **Hager's Test** (Saturated solution of picric acid): The acid layer with Hager's reagent gives **yellow precipitate**.

iv) **Dragendroff's test** (Solution of Potassium Bismuth Iodide): Acid layer with few drops of Dragendroff’s reagent gives **reddish brown precipitate**

Tests for Steroids

i) Salkowski Tests: Chloroform solution of the extract when shaken with concentrated sulphuric acid and on standing yields red colour.

ii) Lieberman Burchard tests: Chloroform solution of the extract with few drops of acetic anhydride and one ml of concentrated sulphuric acid from the sides gives reddish ring at the junction of 2 layers

Tests for Triterpenes

i) Salkowski test: Chloroform solution of the extract when shaken with concentrated sulphuric acid, lower layer turns to yellow on standing.

ii) Lieberman Burchard test: Chloroform solution of the extract with few drops of acetic acid and one ml concentrated sulphuric acid gives deep red at the junction of 2 layers

Test of Tannins

1. Goldbeater's skin test : Goldbeater's skin is a prototype of untanned fresh skin of an animal & is obtained as a membrane from the intestine of Ox. This membrane is treated with HCl; rinsed with distilled water and place in the tannin solution 5 min. Washed with distilled water and transferred into a solution of $FeSO_4$. A brown or black colour on the skin denotes the presence of tannins.

- Goldbeater's skin is a membrane prepared from the intestine of the ox and behaves similarly to an untanned hide.

2- Phenazone Test:

- To 5 ml of aqueous solution of tannin containing drug, add 0.5 g of sodium acid phosphate. Warm the solution, cool and filter. Add 2 % phenazone solution to the filtrate. All tannins are precipitated.
- **3- Gelatin Test:** To a 1 % gelatin solution, add little 10 % sodium chloride. If a 1 % solution of tannin is added to the above gelatin solution, tannins cause precipitation of gelatin from solution.