Chapter-10

Evaluation of Drugs

WHO & ICH Guidelines for the Assessment of Herbal Drugs:

Assessment/Evaluation/Standardization of drug means confirmation of its identity and determination of its quality and purity and detection of nature of adulterant by various parameters like Morphological, Microscopic, Physical, Chemical and Biological observations. The Evaluation of herbal drugs is necessary because of three main reasons -

1. Biochemical variation in drug.
2. Deterioration due to improper processing and storage.
3. Adulteration and Substitution.

Crude Drugs:

Crude drugs are plant, animal or their parts which after collection are subjected to only drying or making them into transverse/ longitudinal slice piece or peeling them in some cases.

Crude Drug Occurrence:

Crude drugs are generally obtained by plant, animal and mineral origin.

1. **Plant Origin:** Whole plant or part of plant like leaves flowers, seed and barks or vegetable saps, extracts and secretions.
2. **Animal Origin:** Whole animals, glands or organs, extracts and secretions.
3. **Mineral Origin:** Ferrous sulphate, Magnesium, Zinc, Gold etc.,

Herbal Drug/Formulation:

According to WHO, a herbal drug or formulation is regarded as finished labelled products that contain active ingredients such as aerial or underground parts of plant or other plant material or combinations thereof, whether in the crude state or as plant preparations.

Guidelines for Quality Control of Herbal formulations:

WHO (World Health Organization) has given certain guidelines for assessment of herbal drugs and most of the countries have adopted these guidelines. The following are the aims and objectives of WHO guidelines in standardizing the herbal drugs -

- Quality Control of crude drugs material, plant preparations and finished products.
- Stability assessment and shelf life.
- Safety assessment: documentation of safety based on experience or toxicological studies.
- Assessment of efficacy and evaluating their biological activity.

Definition of Drug Evaluation

Drug Evaluation may be defined as the determination of identity, purity and quality of a drug.

- **Identity:** Identification of biological Source of the drug.
- **Quality:** The Quantity of the active constituent present.
- **Purity:** The extent of foreign organic material present in a crude drug.

Importance of Evaluation of Crude Drugs:

Determination of biochemical variation in the drugs. Identification of deterioration due treatment and Storage.

Reporting substitution and adulteration, as result of carelessness, ignorance and fraud.

Methods of Standardization and Herbal Drug Evaluation:

The evaluation of a drug is done by following methods

1. Organoleptic evaluation
2. Morphological evaluation
3. Microscopic evaluation
4. Physical evaluation
5. Chemical evaluation
6. Biological evaluation

Authentication of Herbal Drugs:

The existence of numerous plant species and subspecies make it difficult to properly identify them, hence it is essential that before starting any processes on herbs, they need to be properly identified and authenticated from a reputed institution or organization. The following institutes are involved in the authentication of herbs.

Name of Institutes:

- Central Council for Research in Ayurveda and Siddha (CCRAS)
- Central Council for Research in Unani Medicine (CCRUM)
- Central Council for Research in Homeopathy (CCRH)
- Central Council for Research in Yoga and Naturopathy (CCRYN)
- Central Council for Indian Medicine (CCIM)
- Central Council for Homeopathy (CCH) etc.

Laboratories:

- Pharmacopoeial Laboratory for Indian Medicine (PLIM)
- Homeopathy Pharmacopoeia laboratory (HPL) etc.

National Institutes:

- National Institute of Homeopathy (NIH)
- National Institute of Ayurveda (NIA)
- National Institute of Unani Medicine (NIUM)
- National Institute of Naturopathy (NIN)
- National Institute of Siddha (NIS)
- Institute of Post-Graduate Training and Research in Ayurveda (IPGTRA)
- Rastriya Ayurveda Vidyapeeth (RAV)
- Morarji Desai National Institute of Yoga (MDNIY) etc.

1. Organoleptic Evaluation:

This refers to drug evaluation by means of organs of sense and includes other sensory organs like colour, odour, taste, size, shape and texture. It includes the study of morphology and other sensory characters.

A. Odour:

- Distinct
- Indistinct
- Aromatic

B. Taste:

- Acidic (sour)
- Saccharine(sweet) indicates sugar or sugar like substance Ex. liquorice
- Saline (salty)
- Alkaline

- Bitter: indicates presence of substance such as bitter principle Ex. alkaloids, Glycosides.
- Tasteless
- Distinctive sensation to the tongue
  - ✓ Mucilaginous and Oily (soft feeling) Ex. linseed
  - ✓ Astringent indicates presence of tannin.
  - ✓ Pungent (warm biting sensation) Ex. Ginger
  - ✓ Acrid (irritant sensation) Ex. Aconite, coca
  - ✓ Nauseous (tending to excite vomiting) Ex. Ipecac

C. Colour:

- White: Ex. Starch
- Pale yellow: Ex. Ginger, quill, White Pepper
- Deep yellow: Ex. Peeled Liquorice
- Light pale brown: Ex. Nux-vomica, Fennel
- Dark brown: Ex. Cloves bud
- Dark reddish brown: Ex. Cinchona
- Red (brick red): Ex. Cinnamon bark inner portion
- Pale green: Ex. Lobelia
- Greenish brown: most of the leaves herb

2. Morphological Evaluation:

Study of morphology includes visual examination of drug like study of shape & size of various parts of crude drug.

A. Flower:

- Floral parts, corollas, anther, Ovary and receptable

B. Leaves and leaflet:

- Length, width, apex, margin, venation, the texture of the leaf and the hairs in upper and lower surface.
- The feel of the surface described as soft, hairy smooth etc.,

C. Bark:

The barks occur in three shapes

- Flat or curved pieces
- Single quill
- Double quills

Barks have two surfaces, an outer and an inner. The inner surface is usually lighter in colour, than the outer surface.

D. Roots and Rhizome:

A general scheme of examination of subterranean parts includes the size, shape, colour, surface, direction of growth, fracture, transverse surface, fractured surface, odour and taste, food reserves, chemical tests and special features etc.,

E. Fruit:

A general method of macroscopical examination of fruit drug includes

- Exocarp
- Mesocarp
- Endocarp
- Seed

3. Microscopical Evaluation:

Helps in the study of the presence of adulterants & correct identification of the medicinal plants. Drug is soaked in water if it is not fresh, then fine T.S is taken and stained for study of the arrangement of the cells important staining liquids used are phloroglucinol and HCl for lignified tissues, Chlor-zinc iodide for cellulose tissues, Ruthenium Red for gums & mucilage containing cells.

The slides of this test drug are compared with the slides of the authentic crude drugs. This helps in the study of substances like

starch, fixed oils, aleurone grains, calcium oxalate, mucilage etc., Ex. P. amarous shows wavy walled epidermal parenchyma whereas P. madraspatensis shows straight walled epidermal parenchyma.

A. Palisade Ratio:

It represents the average number of palisade cells beneath one epidermal cell, using four continuous epidermal cells for the count. It is determined from powdered drug with the help of camera lucida.

Examples

- Adhatoda vasica:5.5-6.5
- Cassia angustifolia:5.5-10.0

B. Stomata:

A minute epidermal opening present on arial parts of plants, stomata consist of central pore, two kidney shaped similar cells (guard cells) and varying number of subsidiary cells. Epidermal of leaf shows different characteristics Ex. Cuticle, stomata, trichome

Types of Stomata- 4 types-

- Moss type
- Gymnospermous type
- Gramineous type
- Dicotyledonous - It is having diagnostic significance and classified based on form of arrangement of subsidiary cells.

Dicotyledons types - 5 types -

a) Paracytic or rubiaceous or parallel stomata: In these stomata two guard cells covered by two subsidiary cells Ex. Senna

b) Diacytic or caryophyllaceous or cross celled stomata: In these stomata the guard cells are covered by two subsidiary cells on right angle to that of stomata. Ex. Peppermint

c) Anisocytic or cruciferous or unequal celled stomata: In these stomata number of guard cells is two but covered by three

subsidiary cells and in that one is small in size with other two Ex. Datura

d) Anomocytic or ranunculaceous or irregular celled stomata: In these type stomata is surrounded by varying number of subsidiary cells Ex. Digitalis

e) Actinocytic or radiate celled stomata: Two guard cells are surrounded by radiating subsidiary cells.

C. Stomatal number:

The average number of stomata present per square per square millimetre of the epidermis is known as stomatal number.

Stomatal index:

It is the percentage proportion of the number of stomata to the total number of epidermal cells.

Stomatal number varies considerably with the age of the leaf but stomatal index is relatively constant for a given species.

Stomatal index calculated by:

$$S.\ I = S/E+S$$

Where,

- S.I - Stomatal index
- S - Number of Stomata per unit area
- E - Number of epidermal cells in the same unit area

D. Vein-islet Number:

Vein-islet number is defined as the number of vein-islets per sq.mm of leaf surface.

E. Vein-termination Number

It is defined as the number of veinlet termination per sq.mm of the leaf surface between midrib and margin.

F. Trichomes or plant hairs:

These may be referred to as plant hairs. These are warty outgrowth of epidermal cells. A trichome consists of two parts, root which is based in the epidermal lining and body which is outside the epidermal lining.

Trichomes are of three types

- ✓ Covering Trichomes
- ✓ Glandular trichomes
- ✓ Hydathodes

They may be unicellular or multicellular.

G. Calcium oxalate Crystals:

Several cells contents present in vegetable drugs. The inorganic crystalline compound by virtue of their specific shapes can be utilized for the identification of herbal drugs. Due to this reason, they are called Diagnostic Characters of Plants.

a) Cubical (cube shape) Ex. Senna, Glycyrrhiza
b) Rhombic (diamond shape)
c) Tetragonal Ex. Onion
d) Mono Clinic (all three axes are unequal) Ex. Gall
e) Acicular (long Slender, pointed, bundles) Ex. Squill, Cinnamon
f) Rosettes Clusters (aggregation of Crystals) Ex. Clove, Arjuna
g) Micro-sphenoidal (minute in structure) Ex. Henbane

H. Quantitative Microscopy:

Lycopodium spore method: It is used especially chemical and other methods of evaluation of drugs fail to determine quality. Lycopodium spores are much characterized in shape and appearance and uniform in size (25pm) on average, 94000 spores present/mg of Lycopodium powder.

It consists of -

- Well defined particles which may be counted.
- Single layered cells or tissues the area of which may be traced under suitable magnification and actual area calculated.
- The objects of uniform thickness, the length of which can be measured and actual area calculated.

4. Physical Evaluation:

A. Determination of foreign Organic matter:

Drugs should be free from moulds, insects, animal, faecal matter and other contamination such as earth stones and extraneous matters. Foreign organic matter should be not more than 2% W/W.

B. Determination of Ash value:

- **Total Ash:** Total Ash is designed to measure the amount of inorganic impurities present in the crude drug. The drug material is subjected to incineration at a temperature of about 500-600 0C to remove all the carbons. Total ash usually consists of carbonates, phosphates, silicates and silica.
- **Acid Soluble Ash:** Acid insoluble Ash is the residue obtained after extracting the total ash with HCl. It gives an idea about the earthy matter present in the drug.
- **Water Soluble Ash:** The total ash content which is soluble in water is known as water soluble ash. It gives an idea about the presence of water-soluble salts present in the drug.

C. Determination of Extractive value:

It gives an idea about the amount of chemical constituents present in the drug. Extractive values are again sub classified based on the nature of constituents present in the drug as water soluble extractive, alcohol soluble extractive and non-volatile ether soluble extractive value.

D. Determination of Moisture content:

10gm of drug is taken in an evaporating dish. Then it is dried at 1050C for 3 hours and weighed again. Drying and weighing is continued for an hour interval until difference between two successive weighing corresponds to not more than 0.25 percent. The reading is taken after a constant weight is reached and the moisture content is determined.

E. Refractive Index:

When a ray passes from one medium to another of different density, it is bent from original path. Thus, the ratio of velocity of light in vacuum to its velocity in a substance is termed as refractive index of the second medium. Depending upon purity, it is constant for a liquid and can be consider as one of its standardizations.

5. Chemical Evaluation:

It consists of Qualitative and Quantitative methods.

A. Qualitative Chemical Evaluation:

Test for Alkaloids:

- Mayer' s test:

  To a few ml of plant sample extract, two drops of Mayer's reagent are added along the sides of test tube. Appearance of white creamy precipitate indicates the presence of alkaloids.

- Wagner's test:

  A few drops of Wagner's reagent are added to few ml of plant extract along the sides of test tube. A reddish- Brown precipitate confirms the test as positive.

- Test for Amino acids:

  The extract (100 mg) is dissolved in 10 ml of distilled water and filtered through Whatman No. 1 filter paper and the filtrate is subjected to test for Amino acids.

- Ninhydrin test:

  Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) are added to 2 ml of aqueous filtrate. Appearance of purple colour indicates the presence of amino acids.

Test for Carbohydrates:

- Molish's test:

  To 2 ml of plant sample extract, two drops of alcoholic solution of α- naphthol are added. The mixture is shaken well and few drops of concentrated sulphuric acid is added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates.

- Benedict's test:

  To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic-coloured precipitate indicates the presence of sugar.

Test for Fixed oils and Fats:

- Spot test:

  A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

- Saponification test:

  A few drops of 0.5 N alcoholic potassium hydroxide solution is added to a small quantity of extract along with a drop of phenolphthalein. The mixture is heated on a water bath for 2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

Test for Glycosides:

For 50 mg of extract is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests.

- Bontrager's test:

  To 2 ml of filtered hydrolysate, 3 ml of chloroform is added and shaken, chloroform layer is separated, and 10% ammonia solution is added to it. Pink colour indicates presence of glycosides.

- Legal's test:

  50 mg of extract is dissolved in pyridine, sodium nitroprusside solution is added and made alkaline using 10% NaOH. Presence of glycoside is indicated by pink colour.

Test for Phenolic compounds and Tannins:

- Ferric Chloride test:

  The extract (50 mg) is dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution are added. A dark green colour indicates the presence of phenolic compound.

- Gelatin test:

  The extract (50 mg) is dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing 10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds.

- Lead acetate test:

  The extract (50 mg) is dissolved in of distilled water and to this 3 ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

Test for phytosterols:

- Liberman-Burchard's test:

  The extract (50 mg) is dissolved in of 2 ml acetic anhydride. To this, 1 or 2 drops of concentrated sulphuric acid are added slowly along the sides of the test tube. An array of colour change shows the presence of phytosterols.

Test for Proteins:

The extract (100 mg) is dissolved in 10 ml of distilled water and filtered through Whatman No. 1 filter paper and the filtrate is subjected to test for proteins.

- Millon's test:

  To 2 ml of filtrate few drops of Millon's reagent are added. A white precipitate indicates the presence of proteins.

- Biuret test:

  2 ml of filtrate is treated with 1 drop of 2% copper sulphate solution. To this 1 ml of ethanol (95%) is added, followed by excess of potassium hydroxide pellets. Pink colour ethanolic layer indicates the presence of protein.

Test for Saponins:

The extract (50 mg) is diluted with distilled water and made up to 20 ml. The suspension is shaken in a graduated cylinder for 15 minutes. A two cm layer of foam indicates the presence of saponins.

Test for gum and Mucilage:

The extract (100 mg) is dissolved in 10 ml of distilled water and to this 2 ml of absolute alcohol is added with constant stirring. White or cloudy precipitate indicates the presence of Gums and Mucilage.

Test for volatile oil:

For volatile oil estimation 50 mg of powdered material (crude drug) is taken and subjected to hydro- distillation. The distillate is collected in graduate tube of the assembly, wherein the aqueous portion automatically separated out from the volatile oil.

B. Quantitative Chemical Evaluation:

It includes chemical assays and chromatographic methods which are used to quantify the chemical compounds present in the crude drug.

- Chromatographic Techniques:

The Chromatographic techniques are the new and most common methods used to separate, identify and quantify the plant constituents. It consists of various methods which are as follows –

- Thin Layer Chromatography (TLC):

  TLC technique has become a most important analytical tool for separation and determination of natural products. It is simple, economical and rapid method to analyse plant extracts and carryout the fingerprinting of samples by using the standard or marker compounds.

- High Performance Thin Layer Chromatography (HPTLC):

  It is one of the very useful methods for the qualitative and quantitative analysis of plant extracts. It is advanced form of TLC with shorter time and precise results.

- Column Chromatography:

  Basically, it is a liquid chromatography in which mobile phase in the form of liquid phases over the stationary phase packed in a column. The column is made of either glass or metal. It is the oldest method and most commonly practiced method for the isolation of pure compounds.

- High Performance Liquid Chromatography (HPLC):

  It is one of the most versatile, safest, dependable, fastest and sensitive chromatographic techniques for the quality control of drugs. The term liquid chromatography refers to those methods where separation takes place in a packed column which act as stationary phase. A mobile phase is used as eluent. In HPLC, the mobile phase is forced through the column under high pressure.

- Gas Liquid Chromatography (GLC):

  It is the most selective and versatile form of gas chromatography. Commonly it is used in the assay and analysis of starting materials and drug substances, quantification of drug substances

in formulations and assay of impurities and solvents in the drug substances.

6. Biological Evaluation:

It consists of following evaluation methods –Bitterness value

- Haemolytic activity
- Swelling Index
- Foaming Index
- Pesticide Residues
- Heavy Metals
- Micro-organism
- Aflatoxins
- Radioactive Substances