STANDARDISATION OF HERBAL DRUGS

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HERBS

- ARE - crude plant material such as leaves, flowers, fruit, seed, stems, wood, bark, roots, rhizomes or other plant parts, which may be entire, fragmented or powdered.

HERBAL DRUGS/ HERBAL FORMULATION

- ARE – 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.
Phytomedicines or Phytopharmaceuticals sold as Over The Counter (OTC) products in modern dosage forms such as Tablets, Capsules & Liquids for oral use.

Dietary Supplements containing Herbal Products, also called Neutraceuticals available in modern dosage forms.

Herbal Medicines consisting of either Crude, Semi Processed or Processed Medicinal Plants.
STANDARDISATION

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, microscopical, physical, chemical and biological observations.”
Standardization & Quality Evaluation of Herbal Drugs

- Colour
- Odour
- Taste
- Texture
- Fracture

Macroscopic

- Shape
- External
- Mar

Microscopic

• Qualitative
• Quantitative
• SEM Studies
• Powder Studies

1. BOTANICAL

2. ORGANOLEPTIC

3. PHYSICAL

4. CHEMICAL

5. BIOLOGICAL

STANDARDISATION OF HERBAL DRUGS

Microbial Contamination
A) Total viable aerobic count
B) Determination of pathogens
C) Aflatoxins content

Pharmacological
- Bitterness value
- Haemolytic activity
- Foaming index
- Swelling index

Toxicological
- Determination of pesticide residues
- Determination of heavy metals

Antagonistic
- Bacterial
- Fungal

HPTLC
- Qualitative
- Quantitative
- Chromatography
- Radioactive contamination

GC
- Sec. Metabolites

HPLC
- DNA Finger printing

Finger printing

HPTLC Finger printing
Macrosopic study

- Visual inspection provides the simplest and quickest means by which to establish identity, purity and quality.

- Macrosopic identity of medicinal plant materials is based on shape, size, colour, surface characteristics, texture, fracture characteristics and appearance of the cut surface.
Microscopic study

- Detail of cell structure and arrangement of the cells useful for differentiating similar species.

- Select a representative sample of the material & If it is dried parts of a plant than it may require softening before preparation for microscopy, preferably by being placed in a moist atmosphere, or by soaking in water.

- Any water-soluble contents can be removed from the cells by soaking in water. Starch grains can be gelatinized by heating in water.
Histochemical detection

- Starch grains
- Aleurone grains
- Fats, fatty oils, volatile oils and resins
- Calcium oxalate/carbonate crystals
- Lignified cell wall
- Cellulose cell wall
- Mucilage
- Tannin
• **Measurement of specimen**

• Stomatal number
• Stomatal index
• Palisade ratio
• Vein-islet number
• Vein termination number
• Lycopodium spore method
Foreign organic matter

- Parts of the medicinal plant material or materials other than those named with the limits specified for the plant material concerned;

- Any organism, part or product of an organism, other than that named in the specification and description of the plant material concerned;

- Mineral admixtures that is adhering to the medicinal plant materials, such as soil, stones, sand, and dust.

Foreign matter: NMT 2%w/w
Ash value

- It involves non-volatile inorganic components.

- High ash value is the indicative of contamination, substitution, adulteration or carelessness in preparing the crude drugs.
Total ash

- Total ash is designed to measure the total amount of material produced after complete incineration of the drug material at as low temperature as possible (about 450°C) to remove all the carbons.

- Total ash usually consists of carbonates, phosphates, silicates and silica.
- IP and USP: 675±25°C
- BP: 600±25°C
- WHO: 500-600°C
Acid insoluble ash

- Ash insoluble in HCl is the residue obtained after extracting the total ash with HCl. It gives idea about the earthy matter

- IP method: 25mL 2M HCL solution
- USP method: 25mL 3N HCL solution
- BP method: 15mL water and 10mL HCL
- WHO method: 25 ml of hydrochloric acid (~70g/l)
Water soluble ash

- Total ash content which is soluble in water. It’s good indicator of presence of previous extraction of water soluble salts in the drug or incorrect preparation or amount of inorg. matter

- Carbonated ash: Ash is treated with ammonium carbonate.

- Nitrated ash: Ash is treated with dilute nitric acid.
Extractive value

- Amount of the active constituents present in crude drug material when extracted with specific solvent.
- There are following Methods for determination of Extractive value.
  
a) Cold method  
b) Hot method  
c) Soxhlet method
COLD EXTRACTION METHOD:
• Water soluble extractive value

• Alcohol soluble extractive value: Solvent strength: 20-95% v/v

• Solvent Hexane soluble extractive value: Continuous extraction for 20 hours  
  eg. *Phyllanthus amarus*: NLT 3%

• Volatile ether soluble extractive value:  
  Anhydrous ether-continuous extraction for 20 hours

• Nonvolatile ether soluble extractive value:  
  Drugs having lipid content, fixed oils eg. *Colocynthis* fruits: NMT 3% (Pulp-medicinal value)
**Insoluble matter:**

- Presence of woody matter or vegetable debris or pieces of bark materials.

- Eg. In catechu
  Water insoluble matter: NMT 33%
  Alcohol insoluble matter: NMT 30%
Total solid content

- The residue obtained when prescribed amount of preparation is dried to constant weight under the specified condition (Residue on evaporation)
  - Powdered extract: NLT 95%
  - Semisolid extract: NLT 70%
Water Content

- Loss on drying (Gravimetric determination)

- Volumetric Azeotropic distillation (toluene distillation) method

- Titrimetric Karl fisher method

- Gas chromatographic method
Volatile oil content

- Volatile oils are the liquid components of the plant cells, immiscible with water, volatile at ordinary temperature and can be steam distilled at ordinary pressure.

- Many herbal drugs contain volatile oil which is used as flavouring agent.

- E.g. Clove: NLT 15%v/w
Bitterness value

• Medicinal plant materials that have a strong bitter taste ("bitters") are employed therapeutically, mostly as appetizing agents. Their bitterness stimulates secretions in the gastrointestinal tract, especially of gastric juice.

• The bitter properties of plant material are determined by comparing the threshold bitter concentration of an extract of the materials with that of a dilute solution of quinine hydrochloride.

• The bitterness value is expressed in units equivalent to the bitterness of a solution containing 1g of quinine hydrochloride R in 2000 ml.
• Bitterness value calculated in units per g using the following formula:

\[
\frac{2000 \times c}{a \times b}
\]

Where,

- \(a\) = the concentration of the stock test solution (\(S_T\)) (mg/ml),
- \(b\) = the volume of test solution \(S_T\) (in ml) in the tube with the threshold bitter concentration,
- \(c\) = the volume of quinine hydrochloride R (in mg) in the tube with the threshold bitter concentration.
Haemolytic activity

- Many medicinal plant materials, of the families Caryophyllaceae, Araliaceae, Sapindaceae, Primulaceae, and Dioscoreaceae contain saponins.

- The most characteristic property of saponins is their ability to cause haemolysis; when added to a suspension of blood, saponins produce changes in erythrocyte membranes, causing haemoglobin to diffuse into the surrounding medium.

- The haemolytic activity of plant materials, or a preparation containing saponins, is determined by comparison with that of a reference material, saponin R, which has a haemolytic activity of 1000 units per g.
Serial dilution for the preliminary test

<table>
<thead>
<tr>
<th>Tube no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant material extract (ml)</td>
<td>0.10</td>
<td>0.20</td>
<td>0.50</td>
<td>1.00</td>
</tr>
<tr>
<td>Phosphate buffer pH 7.4 TS (ml)</td>
<td>0.90</td>
<td>0.80</td>
<td>0.50</td>
<td>-</td>
</tr>
<tr>
<td>Blood suspension (2%) (ml)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>
• Calculate the haemolytic activity of the medicinal plant material using the following formula:

\[ 1000 \times \frac{a}{b} \]

Where,

1000 = the defined haemolytic activity of saponin R in relation to ox blood,

\( a \) = quantity of saponin R that produces total haemolysis (g)

\( b \) = quantity of plant material that produces total haemolysis (g)
Determination of tannins

• Tannins (or tanning substances) are substances capable of turning animal hides into leather by binding proteins to form water-insoluble substances that are resistant to proteolytic enzymes.

• This process, when applied to living tissue, is known as an "astringent" action and is the reason for the therapeutic application of tannins.

• Chemically, tannins are complex substances; usually occur as mixtures of polyphenols that are difficult to separate and
• Calculate the quantity of tannins as a percentage using the following formula:

\[
\frac{[T_1 - (T_2 - T_0)] \times 500}{w}
\]

where:
- \(w\) = the weight of the plant material in grams
- \(T_1\) = Weight of material extracted in water
- \(T_2\) = Weight of material not bound to hide powder
- \(T_0\) = Weight of hide powder material soluble in water
Determination of swelling index

- The swelling index is the volume in ml taken up by the swelling of 1 g of plant material under specified conditions.
- Its determination is based on the addition of water or a swelling agent as specified in the test procedure for each individual plant material (either whole, cut or pulverized).
**Determination of foaming index**

- Many medicinal plant materials contain saponins that can cause a persistent foam when an aqueous decoction is shaken.

- The foaming ability of an aqueous decoction of plant materials and their extracts is measured in terms of a foaming index. Calculate the foaming index using the following formula:

\[
\text{foaming index} = \frac{1000}{a}
\]

where \( a \) = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1 cm is observed.
Determination of pesticide residues

- Not more than 1%
- An ARL (in mg of pesticide per kg of plant material) can be calculated on the basis of the maximum acceptable daily intake of the pesticide for humans (ADI), as recommended WHO, and the mean daily intake (MDI) of the medicinal plant material.

$$\text{ARL} = \frac{\text{ADI} \times E \times 60}{\text{MDI} \times 100}$$

ADI = maximum acceptable daily intake of pesticide (mg/kg of body weight);
E = extraction factor, which determines the transition rate of the pesticide from the plant material into the dosage form;
MDI = mean daily intake of medicinal plant product.
Determination of arsenic and heavy metals

- Contamination of medicinal plant materials with arsenic and heavy metals can be attributed to many causes including environmental pollution and traces of pesticides.
- Limit test for arsenic
- Limit test for cadmium and lead
- The contents of lead and cadmium may be determined by inverse voltametry or by atomic emission spectrophotometry.
- The following maximum amounts in dried plant materials, which are based on the ADI values, are proposed:
  - lead, 10 mg/kg;
  - cadmium, 0.3 mg/kg.
Determination of microorganisms

Test strains and culture media for use in validating the tests for specific microorganisms

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Strain number</th>
<th>Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>e.g. NCIMB 8545 (ATCC 8739, CIP 53.126)</td>
<td>lactose broth</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>e.g. NCIMB 8626 (ATCC 9027, CIP 82.118)</td>
<td>soybean-casein digest medium</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>No strain number is recommended. Species not pathogenic for humans, such as <em>Salmonella abony</em> (NCTC 6017, CIP 80.39), may be used</td>
<td>lactose broth</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>e.g. NCIMB 8625 (ATCC 6538 P, CIP 53.156) or NCIMB 9518 (ATCC 6538, CIP 4.83)</td>
<td>soybean-casein digest medium</td>
</tr>
</tbody>
</table>
Table 1. Limits for microbial contaminants in finished products & Raw materials

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Finished product</th>
<th>Raw materials</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>$10^1$</td>
<td>$10^4$</td>
</tr>
<tr>
<td>Salmonella</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total aerobic bacteria</td>
<td>$10^5$</td>
<td>-</td>
</tr>
<tr>
<td>Enterobacteria</td>
<td>$10^3$</td>
<td>-</td>
</tr>
</tbody>
</table>
Aflatoxins Content

- Aflatoxins are naturally occurring mycotoxins produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus*.

- The presence of aflatoxins can be determined by chromatographic methods using standard aflatoxins B1, B2, G1, G2 mixtures.

- IP method: NMT 2 µg/kg of aflatoxins B1 & Total aflatoxins 4 µg/kg
- USP method: NMT 5ppb of aflatoxins B1 & Total aflatoxins 20ppb
Radioactive contamination

• The range of radionuclides that may be released into the environment as the result of a nuclear accident might include long-lived and short-lived fission products, actinides, and activation products.

• Microbial growth in herbals is usually avoided by irradiation. This process may sterilize the plant material but the radioactivity hazard should be taken into account.

• The nature and the intensity of radionuclides released may differ markedly and depend on the source (reactor, reprocessing plant, fuel fabrication plant, isotope production unit, etc.).
• The radioactivity of the plant samples should be checked accordingly to the guidelines of International Atomic research centre.
CHROMATOGRAPHY OF HERBAL DRUG

• Separation, identification, impurity detection and assay of herbal drug in the formulation or in the extract are carried out by following methods :-

  a) TLC
  b) HPTLC
  c) HPLC/Densitometric chromatography
  d) GLC
a) T.L.C. Finger-print profile of Methanolic extract of *Coscinium fenestratum*

Detection:
- (a) Under UV 366nm
- (b) Under UV 254 nm
- (c) Under visible light after
b) Comparative HPTLC profile of Berberine in different market samples
c) Densitometric scan of different samples of Berberis spp. at UV 266 nm
ANALYTICAL SPECIFICATIONS OF VATI/GUTIKA (TABLET/PILLS)

1. Description
   Colour
   Odour
2. Weight variation
3. Disintegration time - Not more than 15 min
4. Identification TLC/HPTLC/GLC
5. Assay
6. Test for heavy/Toxic metals
   Lead
   Cadmium
   Mercury
   Arsenic
7. Microbial contamination
   Total bacterial count
   Total fungal count
8. Test for specific Pathogen
   E. coli
   Salmonella spp.
   S. aureus
   Pseudomonas aeruginosa
9. Pesticide residue
   Organochlorine pesticides
   Organophosphorus pesticides
   Pyrethroids
10. Test for Aflatoxins (B1,B2,G1,G2)
ANALYTICAL SPECIFICATIONS OF SYRUP (LIQUID ORAL)

1. Description, Colour
2. Odour
3. Total – ash
4. Acid – insoluble ash
5. Water-soluble extractive
6. Alcohol – soluble extractive
7. PH
8. Total sugar content
9. Viscosity
10. Identification TLC/HPTLC/HPLC
11. Test for heavy metals
   - Lead
   - Cadmium
   - Mercury
   - Arsenic
12. Microbial contamination
   - Total bacterial count
   - Total fungal count
13. Test for specific Pathogen
   - E. coli
   - Salmonella spp.
   - S. aureus
   - Pseudomonas aeruginosa
14. Pesticide residue
   - Organochlorine pesticides
   - Organophosphorus pesticides
   - Pyrethroids
References


