Chapter IV

STANDARDIZATION
INTRODUCTION

Standardization of drug includes scientific documentation, pharmacognosy and phytochemistry of drugs undertaken in order to establish standards for the manufacture. The program of drug standardization is mainly concerned with evolving standards of drugs of proven efficacy. It is important that drugs should be uniform in quality, both as regard to origin and cleanliness and also with respect to the content to therapeutically active constituents. Such uniformity necessary to ensure an expected effect when a particular dose is prescribed. World health organization currently recommends, encourages and promotes traditional/herbal remedies in national health care programme because (i) such drugs are easily available at low cost (ii) are comparatively safe and (iii) the people have faith in such remedies. Plant materials and herbal remedies derived from the represent a substantial promotion of the global drug market and in this respect adoption of internationally recognized guidelines for their quality assessment are necessary. The WHO Assembly in a number of resolutions emphasized the need to ensure quality control of medicinal plant products by using modern techniques and applying suitable standards.

Adulterations and substitution have become a major problem in the availability of standards relating to genuineness of drugs, skill hand sand cost factors etc. Several pharmacopoeia including Indian Pharmacopeia, British Pharmacopeia, Pharmacopeia of Republic of China, Japanese pharmacopeia and united States Pharmacopeia do cover monographs and quality control tests for few of medicinal plants used in those countries but basically these pharmacopoeia are designed to cater the chemical based medicines and pharmaceutical necessities by giving their standards test methods etc. (Kholi, 1996).

The quality of a vegetable product depends on the geographical origin, time and stage of growth when collections have been done and post harvest handling. The raw material presently available to the industry is procured from more than one geographical region over different time and stage of growth when collections are done and post harvest
handling. In most cases, villagers or tribals residing in the vicinity of forest collect the material in their spare time. The plant is collected without paying attends to the stage of maturity, dried haphazardly and stored for long period under unsuitable conditions. The quality of crude drug is more often degraded.

The method used for standardization help in identification and collection of genuine drugs and discard of the exhausted, adulterated and spurious drugs. Otherwise by the drug. Besides economic loss, the use of standard drug can cause serious adverse effects in the body, for example if drug is affected with aflatoxins even in small amount it can cause hepatotoxicity and hepatic cancer. Therefore it is the need of the hour to standardize every drug to provide the best drugs.

Parameters of Standardization- (Organoletic parameters, Physical Parameters & Chemical parameters)

Morphological characters
1. Loss on Drying
2. pH of crude drug
3. Ash Values
   - Total Ash
   - Acid insoluble ash
   - Water soluble ash
4. Extractive Values
   - Cold Extraction
   - Successive Extraction

1. Morphological Characters:

In some cases, general appearance of the herb is related to similar species. Detailed study of the morphological characters can be helpful in differentiating them. The macroscopy of a drug includes its visual appearance to the naked eye. It depends to a large extent on the part of the plant from which the drug is obtained (Ali 1998).
2. **Loss of Weight on Drying:**

The herbal drugs contain variable limit of water. When crude drugs are sold in the market with the guaranteed assay for active constituents must be calculated taking into consideration the percentage of water there in. The amount of moisture present in the drug is known as moisture content. This is important parameter as excess moisture if present affects the quality and constituents of drug.

3. **pH of Crude Drug:**

By determination of pH of a drug solution, we can have information whether the drug is acidic or basic in nature and to what extent.

4. **Extractive Values:**

The amount of an extract that a drug yields in a particular solvent is often an approximate measure of the amount of certain types of constituents that the drug contains. Generally petroleum ether, alcohol and water extractive values are taken into consideration for fixing the standards of any drug. The should be extracted with different solvents in order of their increasing polarity to get the correct and dependable values. The petroleum ether extract contains fixed oil. Resins and volatile substances are volatized leaving only resin, coloring matter and fixed oil. Alcohol can dissolve almost all the substances, but is generally used for determining the extractive index for those drugs which contain glycosides, resins, alkaloids etc. Water is used for the drugs containing water soluble substances as chief constituents.

5. **Ash Values:**

This parameter can be used for the determination of inorganic materials such as carbonates, silicates, oxalates and phosphates. Heating causes the loss of organic material in the form of CO2 leaving behind the inorganic components. Ash value is an important characteristic of a drug and with the help of this parameter we can detect the extent of adulteration, exhausted drugs and excess of sandy or earthy material. We can establish the quality and purity of drug by calculating ash values. It is more applicable to the
powered drugs. Different types of ash figures are used as total ash, acid insoluble ash and water soluble ash. A total ash figure is useful to exclude drugs which have been coated with chalk, lime or calcium sulphate to improve their appearance, as is done with nutmegs and ginger.

With in narrow limits in case of the same drug. The acid insoluble ash consists mainly of silica and high acid insoluble ash thereby indicates the contamination with earthy materials. The water soluble ash is used to estimate the amount of inorganic elements. The water soluble ash is used to detect the presence of material exhausted by water and is used more especially for tea leaves and ginger rhizome (Wallis, 2001).

METHODS

1. Morphological Characters:

In some cases, general appearance of the herb is similar to related species. Detailed study of the morphological characters can be helpful in differentiating them. The macroscopy of a drug includes its visual appearance to the naked eye. It depends to a large extent on the part of the plant from which the drug is obtained. For each particular morphological group, a particular systemic examination can be carried out. Size, colour, odour, and taste are important parts of morphology of a particular drug. The characters of the drug were observed and recorded (Ali, 1998).

2. Loss of Weight or Drying:

The drug sample was taken in clean petridish of known weight and petridish was weighted along the drug sample and weight of the drug was noted again. The drug containing petridish was kept in the oven at 105°C for 2 hr and then the weight was noted. Then again the drug with petridish was kept in an oven at 105°C for next 2 hr. Then the weight of the drug was taken. The process was repeated till constant weight was obtained.
3. pH of Crude Drug:

**pH of 1% solution**

Five gm of crude drug was weighted and dissolved in 100 ml of distilled water. The resulting solution/mixture was filtered and pH was measured with a standard glass electrode.

**pH of 10% solution**

The experiment was performed in the same manner as above by taking 10 gm of drug instead of 5 gm.

4. **EXTRACTIVE VALUES:**

(A) **Cold Extraction:**

The air-dried coarse drug powder (20 gm) was macerated with solvent (Petroleum, ether, chloroform, alcohol and water) of volume 100 ml in a closed flask for 24 hrs., shaking frequently during six hrs. and allowed standing for 24 hrs. It was filtered rapidly, taking precaution against loss of solvent, the filtrate was evaporated to dryness in a flat bottom dish and dried at 105°C, to constant weight and weighted.

(C) **Successive Extraction:**

The dried and coarsely powdered material (20 gm) was subjected to successive extraction in a soxhlet apparatus with different solvents like petroleum ether, chloroform and alcohol. The extracts were evaporated to dryness and their constant weights were recorded.

5. **ASH VALUES**

(A) **Total Ash:**

The ground drug (1 gm) was incinerated in a silica crucible in furnace at a temperature not exceeding 450°C until free from carbon. It was then cooled and weighted to get the total ash content.
(B) Acid Insoluble Ash:
Ash was boiled with 25 ml dilute HCl (6 N) for 5 minutes. The insoluble matter collected on an ashless filter paper was washed with hot water and ignited at a temperature not exceeding 450°C to a constant weight.

(C) Water Soluble Ash:
Ash was dissolved in distilled water and the insoluble part was collected on an ashless filter paper and ignited at 450°C to constant weight. By subtracting the weight of insoluble part from that of the ash, the weight of soluble part of ash is obtained.

STANDARDIZATION OF CODED UNANI FORMULATION, (UNIM-104)

1. Morphological characters:
The Unani formulation UNIM-104 is in the form of majoon and taste is sweet with dark brown color (coffee color) was provided by Central Council for Research in Unani Medicine, New Delhi.

2. Loss on drying / Moisture content:
This parameter is used to determine the amount of moisture present in the sample.
The crude drug sample 5g was placed on a dry Petri dish. The Petri dish along with drug was dried at 105 °C for 2 hrs. in oven and weighed. The drying was continued until two successive reading matched each other.
Calculations and results are as follows:

Weight of dry Petri dish, P1 = 33.25 g.
Weight of Petri dish + drug, P2 = 38.25 g.
Weight of Petri dish + drug after drying for 2 hrs, P3 = 37.88 g.
Weight of Petri dish + drug after drying for 1 hrs again, P4 = 37.68 g.
Weight of Petri dish + drug after drying for 1 hrs again, P5 = 37.68 g.
Weight of drug $W_1 = (P_2 - P_1) = (38.25 - 33.25) = 5$g
Weight of properly dried drug $W_2 = (P_5 - P_1) = (37.68 - 33.25) = 4.43$ g.
Loss on drying (in Grams) = $(W_1 - W_2) = (5.0 - 4.43) = 0.57$g
Loss on drying (in percent) = $(0.57/5 \times 100) = 11.4\%$

**Result**: Loss on drying/moisture content in the sample was found 11.4%.

**Ash Value**

The ash of any organic material is composed of their non-volatile inorganic components. Controlled incineration of crude drug results in ash residue consisting of inorganic material (metallic salts and silica). This value varies within fairly wide limits and is, therefore, an important parameter for the purpose of evaluation of crude drugs. The ash value determined is the total ash, the acid insoluble ash and the water-soluble ash.

(i) **Total Ash**:

Crucible is weighed ($C_1$) and then 5g of drug is kept in it and weighed again ($C_1 + D$). Crucible with drug was kept in Muffle furnace at not more than 450 degree centigrade for 4 hrs. Crucible with resulting ash was cooled and weighed. Now, again crucible with ash was kept in Muffle furnace for two hours at same temperature and weighed again. The weight was found constant ($C_1 + A$). The process was repeated for tree times and all the values were noted as represented below in table form.

<table>
<thead>
<tr>
<th>Wt, in g-&gt;</th>
<th>$C_1$</th>
<th>$C_1 + D$</th>
<th>$C_1 + A$</th>
<th>Total Ash</th>
<th>Ash %</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>33.16</td>
<td>38.16</td>
<td>33.57</td>
<td>0.41</td>
<td>8.2%</td>
</tr>
<tr>
<td>II</td>
<td>33.16</td>
<td>38.16</td>
<td>33.47</td>
<td>0.32</td>
<td>6.4%</td>
</tr>
<tr>
<td>III</td>
<td>33.16</td>
<td>38.16</td>
<td>33.49</td>
<td>0.33</td>
<td>6.6%</td>
</tr>
</tbody>
</table>

Thus, Average Total ash (in %) = $(8.2+6.4+6.6)/3 = 7.06%$

**Result**: Total Ash == 7.06%
(ii) Water Soluble Ash:

Total Ash was calculated by the above mentioned method and 20 ml of water was put in the crucible and heated over waterbath. The resultant was filtered through ashless filter paper and again the crucible was kept in Muffle furnace for 2 hrs. at 450°C. Crucible was cooled and weighed again.

Calculations and results are as follows;

Weight of the crucible = Cl = 33.16 g
Weight of the crucible + Drug = Cl+D = 38.16 g
Weight of the crucible + Ash = Cl+A = 33.57 g
Total Ash = 33.57 − 33.16 = 0.41 g
Weight of crucible + Insoluble Ash = Cl+A' = 33.36
Total insoluble ash = 33.36 − 33.16 = 0.20
Total soluble ash = 0.41 − 0.20 = 0.21
Thus, Water soluble Ash % = (0.21/5 X100) = 4.2%

Result: water Soluble Ash percent = 4.2%

(iii) Acid Insoluble Ash:

Total ash was calculated as mentioned. The total ash was boiled with 25 ml of 2N HCl for 5 min. The insoluble matter was collected on an ash less filter paper after filtering. The crucible with ash less filter paper and insoluble matters was again kept in Muffle furnace for 2 hrs at same temperature. The percentage of acid insoluble ash with reference to the air-dried drug was calculated.

Calculations and result are as follows:

Weight of the crucible = Cl = 33.16 g
Weight of the crucible + Drug = Cl+D = 38.16 g
Weight of the crucible + Ash = Cl+A = 33.57 g
Total Ash = 33.57 − 33.16 = 0.41 g
Weight of crucible + Acid Insoluble Ash = C1+A' = 33.26g
Total Acid insoluble ash = 33.26 - 33.16 = 0.10 g
Thus, Acid Insoluble Ash % = (0.10/5 X100) = 2%

Result: Acid Insoluble Ash = 2 %

(iv) Sulphated Ash:

To the sample of 5g drug, 10ml of 25% Sulphuric acid is added in a weighed crucible. Muffle furnace was set at 600°C, and the crucible was kept in it for 5 hours. Crucible was cooled and weighed. It was again kept in furnace for 2hr and constant weight was found.

Calculation and results are as follows:
Weight of the crucible, C1 = 33.16 g
Weight of the crucible + Drug = C1+D = 38.16 g
Total Ash = 33.57 - 33.16 = 0.41 g
Weight of crucible + Sulphated ash = 33.50 g
Sulphated ash = 33.50-33.16 = 0.34

Thus, percentage of Sulphated ash = (0.34/5 X100) = 6.8%

Result: Percentage of Sulphated ash is 6.8%

Successive Extraction:

The drug UNIM-104 (100 gm) was subjected to successive extraction in a Soxhlet apparatus with different solvents like petroleum ether, chloroform, methanol and water. The extracts were evaporated to dryness and their constant weights were recorded.
Table: 2

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Wt.of Empty Beaker</th>
<th>Wt. of beaker + extract</th>
<th>Wt. of extract</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>126 g</td>
<td>126.65 g</td>
<td>0.65 g</td>
<td>0.65%</td>
</tr>
<tr>
<td>Chloroform</td>
<td>126g</td>
<td>126.24 g</td>
<td>0.24 g</td>
<td>0.24 %</td>
</tr>
<tr>
<td>Methanol CH3OH</td>
<td>126g</td>
<td>186 g</td>
<td>60 g</td>
<td>60 %</td>
</tr>
<tr>
<td>Water</td>
<td>126g</td>
<td>165 g</td>
<td>39 g</td>
<td>39 %</td>
</tr>
</tbody>
</table>