

1. ORGANOLEPTIC (MORPHOLOGICAL) EVALUATION

- It refers to evaluation of drugs by colour, odour, taste, size, shape and special features, like touch, texture, sound etc.
- Example: Aromatic odour of umbelliferous fruits and sweet taste of liquorice.
- The study of form of a crude drug is Morphology, while description of the form is Morphography.
- However, it should be noted that colour, shape and size of crude drugs as described in official books should only be considered as guidelines and may vary depending upon several factors. For example, colour of the crude drug may fade if it gets exposed to sunlight for very long duration or if the drug is not stored properly.
- Depending upon the condition under which the drug is growing or cultivated, i.e., availability of proper irrigation, fertilizers or even, high temperature may influence the size of the drug. If it gets favorable conditions, leaf, seed and fruit drugs of maximum size may be available and the crude drugs if grown in adverse conditions, size may get reduced.
- The adulteration of seed of *Strychnos nux-vomica* with the seed of *Strychnos nux-blanda* or *Strychnos potatorum*, caraway with Indian dill, Alexandrian senna with dog senna or palthe senna are identified by morphological technique.

2. MICROSCOPIC EVALUATION

- This method allows more detailed examination of a drug and it can be used to identify organised drugs by their known histological characters.
- Microscope, by virtue of its property to magnify, permits the minute structure under study to be enlarged and can be used to confirm the structural details of the drugs from plant origin.
- For the effective results, various reagents or stains can be used to distinguish the cellular structure.
- The microscopic evaluation also covers study of constituents by application of chemical tests to small quantities of drugs in powdered form or to histological sections of the drug (Microchemistry).

- Histological studies are made from very thin sections of the drugs. The characteristics of cell wall, cell contents, trichomes, fibres, vessels etc., can be studied in details, eg. Signified trichomes in nux-vomica.
- The microscopic linear measurement and quantitative microscopy are also covered under this technique of evaluation. The following few constants illustrate the importance of microscopic measurements.

(a) Stomatal number: It is the average number of stomata present per square mm of the epidermis.

The actual number of stomata per sq. mm of leaf preparation may vary for leaves of the same plant grown in different environmental conditions. Stomatal number is relatively a constant for particular species of same age and hence, it is taken into consideration as a diagnostic character for identification of a leaf drug. The adulteration can also be detected by stomatal number. It can be further illustrated by following examples

Table 1: Stomatal number of few leaf drugs

Species	Stomatal number
Datura stramonium	087 (Upper epidermis)
Datura innoxia	141 (Upper epidermis)
Hyoscyamus niger	125 (Upper epidermis)

(b) Stomatal index: It is the percentage which the number of stomata forms to the total number of epidermal cells, each stoma being counted as one cell. It can be calculated by a formula:

$$I = \frac{S \times 100}{(E + S)}$$

where I - Stomatal index;

S. Number of stoma per unit area;

E - Epidermal cells in the same area.

The stomatal number varies considerably with the age of leaf and due to changes in climatic conditions, stomatal index is relatively constant and therefore, it is significance for a given

species. It is useful in differentiation of closely related species and also for detection of adulterants (Table 2).

Table 2: Stomatal index of few leaf drug

Species	Stomatal index (lower surface)
Atropa belladonna	20.2 to 23
Atropa acuminata	16.2 to 18.3
Indian senna	17 to 20
Alexandrian senna	10.8 to 12.6

(c) **Vein islet number:** It is the number of vein islets per sq. mm of leaf surface. It is a constant for a given species of the plant. It usually does not alter with the age of plant and is independent of the size of the leaf (Table 3).

Table 3: Vein-islet number of few leaf drugs

Species	Vein islet number
Erythroxyton coca	08-12
Erythroxyton truxillence	15-26
Digitalis purpurea	2-5.5
Digitalis thapsi	8.5-16
Cassia angustifolia	19-23
Cassia acutifolia	25-30

(d) **Palisade Ratio:** It is the average number of palisade cells, beneath one epidermal cell, using four continuous epidermal cells for the count.

Since the palisade cells in the mesophyll of the leaves bear a definite relation to the epidermal cell, the palisade ratio as defined above, is constant for a species of a genus (Table 4)

Table 4: Palisade ratio of some leaf drug

Species	Palisade ratio
Atropa belladonna	6-10
Datura stramonium	4-7
Digitalis purpurea	3.7-4.2

(e) Quantitative Microscopy (Lycopodium Spore Method):

- It is an important analytical technique for powdered drugs, especially when chemical and other methods of evaluation of crude drugs fail as accurate measures of quality.
- Lycopodium spores are very characteristic in shape and appearance and exceptionally uniform in size (25 µm). On an average, 94000 spores per mg of powdered lycopodium are present.
- A powdered drug is evaluated by this technique, if it contains:
 - i) Well defined particles which may be counted, e.g. starch grains or pollen grains,
 - ii) Single layered cells or tissues, the area of which may be traced under suitable magnification and actual area calculated or
- The objects of uniform thickness, the length of which can be measured under suitable magnification and actual area calculated.
- The percentage purity of an authentic powdered ginger is calculated using the following equation:

$$\text{Percentage purity} = \frac{N \times W \times 94000 \times 100}{S \times M \times P}$$

Where, N: Number of characteristic structures (e.g., starch grains) in 25 fields.

W: Weight in mg of lycopodium taken

S: Number of lycopodium spores in the same 25 field

M: Weight in mg of the sample calculated on the basis of sample dried at 105 °C.

P: 2, 86, 000 in case of ginger starch grain powder

- Lycopodium spore method can be used for evaluation of powdered clove, ginger, cardamom, nutmeg and umbelliferous fruits.

The study of microscopical or histological characteristics is useful in detection of adulterants in both entire and powdered forms of crude drugs. Apart from variations in cellular arrangement, many a times, the type of cuticle of epidermis and cell inclusion also help in detection of the adulterants.

- The common adulterant of *Digitalis purpurea* is *Verbascum thapsus*, containing candelabra trichomes, while *Digitalis* contains either multicellular uniseriate trichomes or glandular trichomes.
- The powdered cloves contain neither prisms of calcium oxalate nor the sclereids, but in case of powdered clove stalks both are present.
- Starch is absent in clove, but is present in the powdered clove fruits.
- The leaves of *Digitalis purpurea* do not contain calcium oxalate crystals, while they are present in all other varieties of *Digitalis*.
- Surinam quassia does not contain calcium oxalate, but Jamaica quassia contains prismatic crystals of calcium oxalate.
- The size of a starch grain is also important in detection of adulterants. In case of *Cinnamomum cassia*, the diameter of starch grains is usually more than 10 microns.
- The dimension of fibres also helps in detecting adulteration in case of cinnamon.

The number of sclerenchymatous cells per square cm in cardamom is one of the criteria for detection of varieties of cardamom seed in powdered form.