1. Stomata & Stomatal index

Several types of stomata are present in plant leaves, distinguished by the form and arrangement of the surrounding cells. (Figure 1)

1.The anomocytic (irregular - celled) type: the stomata is surrounded by a varying number of cells in no way differing from those of the epidermis generally.
2. The anisocytic (unequal -celled) type: the stomata is usually surrounded by 3 subsidiary cells, of which one is markedly smaller than the others,
3. The diacytic (cross- celled) type: the stomata is accompanied by 2 subsidiary cells, whose common wall is at right angles to the angles to the guard cells.
4. The paracytic (parallel - celled) type: the stomata has on each side on one or more subsidiary cells parallel to the long axis of the pore and guard cells.

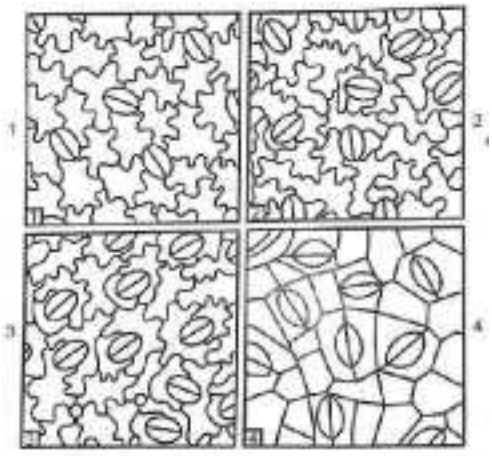

Figure 1

$$\text{Stomatal Index} = \frac{100 \times S}{E + S}$$

S = the number of stomata in a given area of leaf,
E = the number of epidermal cells (including trichomes) in the same area of leaf.

For each sample of leaf, make not fewer than 10 determinations and calculate mean.

2. Swelling Index

The swelling index is the volume (expressed in milliliters) occupied by 1 g of an herbal drug and the adhering mucilage, after it has swollen in an aqueous liquid for 4 hours. Place 1.0 g of the drug, whole or of the degree comminution prescribed in a monograph in a 25 ml ground-glass stoppered cylinder graduated over a height of 125 ± 5 mm in 0.5ml divisions. Unless otherwise prescribed moisten the drug with 1.0ml of *ethanol* and 25ml of *water* and close the cylinder. Shake vigorously every 10 min for 1 hour. Allow to stand for 3 hours. After the beginning of the test, at 90 min, release any large volumes of liquid retained in the layer of the drug and any particles of the drug floating at the surface of the liquid by rotating the cylinder about a vertical axis. Measure the volume occupied by the drug, including any adhering mucilage. Perform the test 3 times simultaneously.

The swelling index is given by the mean of the 3 tests.

3. Essential oils in Herbal drugs

Essential oils in herbal drugs are determined by the steam distillation apparatus. The distillate is collected in the graduated tube, using *xylene* to take up the essential oil, and the aqueous phase is automatically returned to the distillation flask.

Apparatus

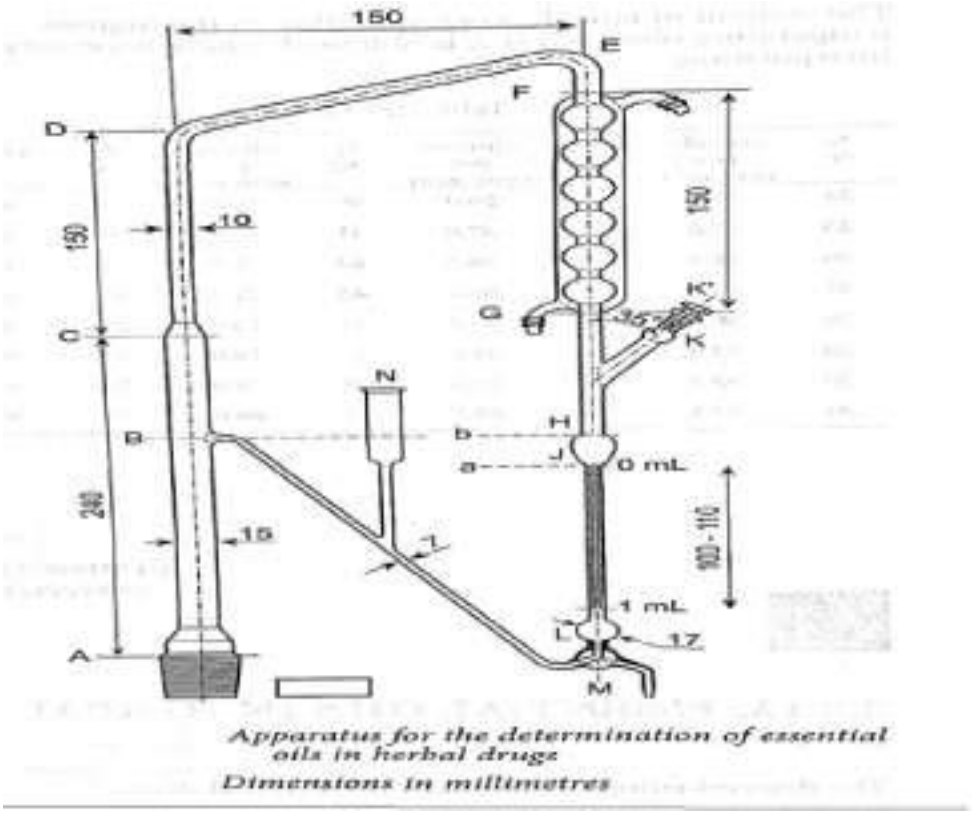

Figure 2

Method

Add prescribed volume of distillation liquid in the flask, add pieces of porous porcelain and attach the condenser assembly. Add *water* by using filling funnel until it reaches to the level B. Remove the stopper and add the prescribed quantity of *xylene,* using a pipette. Replace the stopper and ensure that the orifice coincides with the vent. Heat the liquid in the flask to boiling and adjust the distillation rate to 2-3 ml/min, unless otherwise prescribed.

To determine the rate of distillation, during distillation lower the level of the water by means of the three-way tap until the meniscus is at the level of the lower mark (a). Close the tap and measure the time taken for the liquid to reach the upper mark (b). Open the tap and continue the distillation, distillation rate is regulating by modifying the heat. Continue the distillation process for 30 min., stop the heating and after at least 10 min read off the volume of *xylene* in the graduated tube.

Place the drug in flask and continue the distillation as described above. Stop the heating and after 10 min read the volume of liquid collected in the graduated tube and subtract the volume of previous ml of *xylene.* The difference represents the quantity of essential oil in the mass of the drug taken. Calculate the result as milliliters per kilogram of drug.

When the essential oil is to be used for other analytical purposes, the *water* - free mixture of *xylene* and *essential oil* may be recovered as follows: remove the stopper and introduce 0.1ml of 1 g/l solution of *sodium fluoresceinate* and 0.5 ml of *water*. Lower the mixture of *xylene* and *essential oil* into the bulb - shaped swelling L by means of the three-way tap, allow standing for 5 min and lowering the mixture slowly until it just reaches the level of the tap. Open the tap anti-clockwise so that the *water* flows out of the connecting tube. Wash the tube with *acetone* and with a little *toluene* introduced through the filling funnel. Turn the tap anticlockwise in order to recover the mixture of *xylene* and *essential oil* in an appropriate flask.

4. Tannins in Herbal drugs

Take a herbal drug or extract in 250.0 ml round-bottomed flask with 150 ml of water. Heat on water bath for 30 min. Cool under running water and transfer in 250.0 ml of volumetric flask. Rinse the round-bottomed flask and collect the washing in the volumetric flask and make up the volume up to 250.0 ml with *water*. Allow the solids to settle and filter using filter paper 125 mm in diameter. Discard the 50.0 ml of the filtrate.

In case of a liquid extract, dilute the stated amount of sample to 250.0 ml with *water*. Filter the mixture using filter paper 125 mm in diameter. Discard the first 50 ml of the filtrate.

Total Polyphenols: Dilute 5.0 ml of the filtrate with 25.0 ml of water. Mix 2.0 ml of this solution with 1.0 ml of *phosphomolybdotungstic* reagent and 10.0 ml of water and dilute to 25.0 ml with a 290 g per liter solution of *sodium carbonate*. After 30 min measure the absorbance (2.4.7) at 760nm (A_1) using *water* as blank solution.

Polyphenols not adsorbed by hide powder: Take 10.0 ml of the filtrate with 0.10 g of hide powder reference standard and shake for 60 min. Filter and dilute 5.0 ml of the filtrate to 25.0 ml of water. Mix 2.0 ml of this solution with 1.0ml of *phosphomolybdotungstic* reagent and 10.0ml of *water* and dilute to 25.0ml with 290 g per liter solution of carbonate R. After 30 min measure the absorbance (2.4.7) at 760 nm (A_2), using *water* as blank solution.

Standard.
Dissolve immediately before use 50.0 mg of *pyrogallol* in *water* and dilute to 100.0 ml with the *Water*. Dilute 5.0 ml of the solution to 100.0 ml with *water*. Mix 2.0 ml of this solution with 1.0 ml of *phosphomolybdotungstic* reagent and 10.0 ml of *water* and dilute to 25.0 ml with a 290 g per liter solution of *sodium carbonate*. After 30 min measure the absorbance (2.4.7) at 760 nm (A_3), using *water* as blank solution.

Calculate the percentage content of tannins expressed as *pyrogallol* from the expression:

$$\frac{62.5\ (A_1 X\ A_2)\ m_2}{A_3\ X\ m_1}$$

Where,

m_1 = mass of the sample to be examined, in grams

m_2 = mass of pyrogallol, in grams.