

### **Ex. No. 5: Extraction of Photosynthetic Pigments by Solvent Wash method.**

**Aim:** To separate photosynthetic pigments by solvent wash method.

**Principle:** The separation of photosynthetic pigments is based on solubility of different pigments with specific solvents.

**Materials Required:** Mulberry leaves, pestle and mortar, 80% acetone, calcium carbonate, separating funnel, beaker, measuring cylinder, petroleum ether, acetone, diethyl ether, 30% methanolic KOH, 95% methanol, distilled water *etc.*,

#### **Procedure:**

1. Take 10 g of fresh leaves in a pestle and mortar. Crush them with 40 ml of 80% acetone. Add a pinch of calcium carbonate, crush again and filter the extract on a Buchner filter or double layered muslin cloth.
2. Fill the homogenate in to separating funnel, add equal amount of petroleum ether, shake the contents gently and leave it for separation of acetone and petroleum ether layers. The upper petroleum ether layer contains all the pigments. Care should be taken to release the pressure built up in the separating funnel by opening the top lid.
3. Discard the lower acetone layer by opening the tap of separating funnel. Wash the petroleum ether layer with 20-30 ml of distilled water, discard lower water layer.
4. Now to the petroleum ether layer add 20 ml of 95% methanol mix the contents by shaking and leave it for separation of upper petroleum ether and lower methanol layers.
5. Collect the lower methanol layer and store it separately.
6. To the upper petroleum ether layer add 16 ml of 30 % methanolic KOH and 4 ml of distilled water shake gently; leave the contents for the separation of two layers. Collect the upper blue green petroleum ether layer and lower yellowish methanolic KOH layers separately as chlorophyll a and carotenes.
7. Now take methanol fraction (collected and stored at step 5) in separating funnel and add equal amount of diethyl ether (16 ml) as well as distilled water (4 ml). Mix the contents and discard lower methanol layer as it contains no pigments.
8. To the upper diethyl ether fraction add equal amount of methanolic KOH and distilled water (8+8 ml), mix gently and collect upper diethyl ether layer contains greenish chlorophyll b and lower methanolic KOH layer contains orange red xanthophylls.

**Observation:** After isolation identify the different pigments with the help of their specific colours as shown in the previous experiment.

**FLOW CHART**

