

# PART - C

## PHYSIOLOGY

### ACTIVITY 11

## EXTRACTION AND CHROMATOGRAPHY OF LEAF CHLOROPLAST PIGMENTS

#### Requirements:

- (i) Fresh or Frozen leaves
- (ii) Acetone (80%)
- (iii) Pestle and mortar
- (iv) Chromatography paper (Whatman No. 1)
- (v) Funnel
- (vi) Dropper
- (vii) Beaker
- (viii) Acetone and Petroleum ether mixture (1:9)

#### Theory:

The leaf contains different pigments. Chromatography is a technique for the separation and identification of these pigments, such as carotene, xanthophyll, chlorophyll-a and chlorophyll-b.

#### Procedure:

##### 1. Extraction of chlorophyll of leaves:

- (i) Take the dry spinach leaves, and grind to fine powder.
- (ii) Soak this leaf powder in 80% Acetone.
- (iii) Filter this extract. It can be used for chromatography.

##### 2. Separation of pigments by chromatography:

- (i) Take a strip of chromatography paper (Whatman No. 1). Draw a pencil line across the strip about 3cm from the end.
- (ii) Place the chlorophyll extract in the centre of pencil line by the help of capillary tube. The spot should be placed many times until it becomes darker. This process is known as **loading**.
- (iii) Take mixture of Acetone-Petroleum ether (1:9) about 1.5cm in a test tube and placed the chromatography paper strip in the solvent. The spot should not be dipped in solvent.
- (iv) Close the mouth of test tube.
- (v) After 15 to 20 minutes take out the strip, mark the position of solvent and mark the different spots of pigments in the form of circle.