

Practical-4

Date: 27/03/2025

Objective: To separate and identify different pigments present in spinach leaves using **paper chromatography**.

Materials Required: Fresh spinach leaves, Chromatography paper, Solvent (Mobile Phase): A mixture of acetone, petroleum ether, and ethanol, Mortar and pestle, Acetone (for extraction), Capillary tube or dropper, Beaker with lid, Pencil & ruler

Procedure

1. Preparation of Extract

- Grind fresh spinach leaves with acetone using a mortar and pestle.
- Filter the extract to get a concentrated green pigment solution.

2. Setting Up Chromatography

- Take a **chromatography paper**.
- Draw a light **pencil line** (origin) about 2 cm from the bottom.
- Using a capillary tube, apply **pigment extract** as a small spot on the origin line.

3. Developing the Chromatogram

- Pour the solvent mixture into a beaker (about 1 cm deep).
- Place the chromatography paper **vertically** inside the beaker, ensuring the pigment spot is **above** the solvent level.
- Cover the beaker and let the solvent move **upward** by capillary action.

4. Observations

- As the solvent rises, different pigments separate based on **solubility** and **molecular interactions**.
- When the solvent reaches near the top, remove the paper and let it dry.

5. Analysis of Pigments

Different pigments appear as distinct bands at various positions:

- **Carotenoids (Yellow to Orange)** – Move the farthest (non-polar, high solubility)
- **Chlorophyll a (Blue-Green)** – Moves intermediate distance
- **Chlorophyll b (Yellow-Green)** – Less mobile than chlorophyll a
- **Xanthophylls (Yellow)** – Least mobile (more polar)

Results & Conclusion

- The experiment demonstrates **pigment separation** based on polarity and solubility.
- Chlorophyll absorbs light for **photosynthesis**, while carotenoids and xanthophylls protect against excess light.