LEAF CHROMATOGRAPHY

Purpose: identify the different pigment types in plant leaves

Materials:

Chromatography (filter) paper
Spinach or other soft leaves
Solvent (1:1 acetone:ethanol) or rubbing alcohol
Beaker/cup
Mortar & pestle
Sand
Glass pipet
Tape
Pencil
Ruler
Transfer pipet

Procedure

1. Finely cut up some leaves and fill a mortar to about 2 cm depth.
2. Add a pinch of sand and about a dropperful of solvent from the transfer pipette.
3. Grind the mixture with a pestle for at least three minutes.
4. On a strip of chromatography (filter) paper, draw a pencil line 2 cm from the bottom.
5. Use a fine glass tube (glass pipet) to put liquid from the leaf extract onto the center of the line. Keep the spot as small as possible.
6. Allow the spot to dry, then add another spot on top. Add five more drops of solution, letting each one dry before putting on the next. The idea is to build up a very concentrated small spot on the paper.
7. Attach the paper to the pencil using tape so that when placed in the beaker/cup, the paper is just clear of its base.
8. Place enough solvent in the beaker/cup so it will just contact the chromatography paper and hang the paper so it dips in the solvent. Ensure the solvent level is below the spot.
9. Avoid moving the beaker in any way once the chromatography has started.
10. Leave the experiment until the solvent has soaked near to the top, and then remove the paper from the beaker.
11. You can make this a quantitative activity by calculating the R_f value: Using a ruler and the following formula, measure the R_f values of each pigment. Since the fastest molecules will travel the greatest distance, or to the highest point along the strip, the relative distances can be measured, and the flow rate (migration) of the molecules (R_f) can be calculated by using the following formula:

$$R_f = \frac{\text{Distance pigment traveled}}{\text{Distance solvent traveled}}$$

12. Complete the table below:

Plant type:_____

Color of Pigment	Distance Traveled (mm)	R_f value

13. Identify the pigments in your plant using the information below.
14. How would you utilize this activity?

(over)

Pigment Identification:
Chlorophyll a= blue-green
Chlorophyll b = Olive green
Xanthophyll = yellow
Carotene = orange yellow

Chlorophyll A and **B** in leaves are the main pigments that absorb the energy of sunlight.

Carotenes and **xanthophylls** are pigments that absorb the energy of sunlight and transfer it to chlorophyll. The red, orange, and yellow colors of tomatoes and peppers are due to these pigments. These typically are camouflaged by the chorophylls until the plant is preparing to shed its leaves and quit producing chlorophyll a and b.

Anthocyanins are the pigments in pink, red, and purple flowers. Anthocyanins attract pollinating insects and may also act as a sunscreen to leaves and flowers.