# What Color Are the Leaves Really Turning?

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## Experimental Procedure

1. Go on a nice walk with an adult and collect some fresh leaves from different stages of color change during fall. It is best for all of your leaves to come from the same tree, so look for a tree with a variety of leaves at different stages. Figure 2 shows an example of some collected leaves:

**Figure 2.** Red, green, and yellow leaves collected from a tree in the neighborhood.

2. Separate and group the leaves into color groups, with ten good leaves in each group (unless you are using a tree with small leaves, like aspen or birch, then you should use a higher number of leaves). Try to form groups from colors that are as different as possible. For example, make a green group, a yellow group, and a red group, as shown in Figure 3. In each group, chose leaves in the deepest colors possible:

**Figure 3.** All leaves are separated into a pile of yellow, red, and green leaves.

3. Cut the leaves into very small pieces with your scissors (all pieces should be smaller than about ¾ inch long) and put each group into the bottom of a heat resistant, strong glass (Figure 4):

**Figure 4.** Each pile of leaves is cut into small pieces and placed into a different heat resistant strong glass.

4. Add 1 Tbsp. of 90% isopropyl alcohol to each glass.
5. Using the blunt end of a wooden spoon, macerate (soften) the chopped leaves by squashing them into the isopropyl alcohol at the bottom of the cup.
6. As you squish the leaves, you will notice that the alcohol will start to turn the color of the leaves. This is called **extraction**, and the isopropyl alcohol is called the **solvent**.
7. Continue until the liquid turns a deep shade of the color of the leaves, about 5 minutes per glass.
8. Let the macerated suspensions sit for 30 minutes in a dark, room-temperature place to allow the color molecules to fully extract.
9. Using a fork, lift out the bits and pieces of leaf material and set them aside. Take care to remove any liquid by gently pressing the leafy bits against the glass before you remove them. You should be left with a dark suspension of leafy color in isopropyl alcohol at the bottom of your cup.
10. Pour some water into a pot and heat it up on the stove. Then switch off the stove and place the three heat resistant glasses with your extracts into the hot water. Let them sit in the hot water for about 20–30 minutes, or until the isopropyl alcohol has mostly evaporated. As the alcohol evaporates, your extracts should become thicker when stirred with a fork.
    a. Concentrating the extracts should not require more than 30 minutes of evaporating off the isopropyl alcohol, although the extracts will become more concentrated the longer the alcohol is allowed to evaporate off.
    b. Stir each color thoroughly to blend and loosen any bits of dried up pigment from the side of the bowl. Be sure to use a clean fork for each color so you do not mix them!
11. In the meantime, prepare your chromatography paper. Cut the chromatography paper into strips approximately 2 centimeters (cm) wide by 6.5 cm long. Prepare a total of 6 chromatography strips this way.
    a. *Science Buddies Kit*: The kit comes with 20 long strips of chromatography paper; two 6.5 cm strips can be cut from each long strip.
12. Take one of the chromatography strips and use a ruler and pencil to draw a line across it horizontally 1 cm from the bottom. This is the origin line, see Figure 5 for details. Repeat this step for all 6 of the chromatography strips.

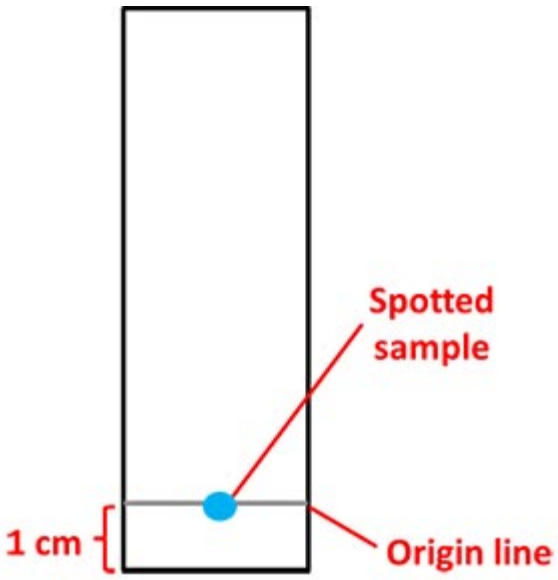


**Figure 5.** Draw an origin line on the chromatography strip. The leaf extract to be tested will be spotted in the middle of the origin line.

13. When your color extracts are concentrated enough, use the pipette to place a small dot of extract at the center of the origin line of one chromatography strip as shown in Figure 5. The drop size should be about the size of a pencil eraser. The more extract you have on your paper towel strip the darker the chromatography will be. Some plant pigments can stain, so you should do this on a plate so that the color won't seep through and stain your work surface. Try to apply the extract as smoothly and evenly as you can along the line. Repeat with 2-3 more strips, using the same color extract, so that you have triplicates for each color pigment.
    a. Use a *pencil* to label the chromatography strip "red extract, yellow extract, or green extract". Do not use a pen to label the strips: the ink might run when the alcohol passes through the strips.
    b. Allow the spot to dry. If the spot is too faint, you will need to thicken your extract and repeat step 13 on a new strip.
14. Repeat step 13 with the other colored extracts so that you have three paper strips for each color extract. Be sure to rinse the pipette between each color extract! Allow the strips to dry.
15. Using your binder clips, clip two of the prepared chromatography strips to a wooden splint, as shown in Figure 6. Make sure the two strips do not touch each other and that the bottoms align.
16. Rest the wooden splint on top of the 100 mL beaker so that the strips hang into the beaker and do not touch the sides of the beaker, nor the bottom of the beaker. Estimate the distance between the bottom of the beaker and the end of the chromatography strips.
17. Remove the wooden splint with the chromatography strips and add 90% isopropyl alcohol to the beaker so the alcohol would just reach the end of the chromatography strips if they are placed back.
18. Place the wooden splint back on top of the beaker. Do both strips touch the alcohol? The goal is to have the strip just touching the surface of the alcohol, as shown in Figure 6. As long as the alcohol touches the strips and the alcohol level is below the extract dots, you are good to go!
    a. If necessary, remove the paper strips from the beaker and add more 90% isopropyl alcohol to reach the desired alcohol level.

**Figure 6.** Your setup should look similar to this example. The end of the chromatography strip should just touch the alcohol. *Note*: This picture does not show chromatography strips with plant extract. The colors on your paper strips should look different.

19. Set the beaker aside for about 30 to 60 minutes, and watch as the pigments separate along the length of the strip. As soon as one of the colors reaches the top of a strip, remove the strips and allow them to dry.
20. Repeat steps 15 to 19 with all your remaining paper strips.
21. Compare the colors found in the different strips. What happened to the colors? Did the different groups of leaves have unique colors, or shared colors, or both? Is each color found in the same place along each strip, or in different places? Are the colors in the same order, or in a different order of separation along the strip?
22. *Tip*: If you have a pale chromatography, next time try using more leaves, cutting them up into smaller pieces (in step 3), and/or adding more of your colored extract solution onto the pencil line on the chromatography paper (step 13). Alternatively, you can also try to use the extracts themselves as your solvent. Replace the isopropyl alcohol in your beaker with one of the extracts. Then hang the paper strips directly into the extract. Repeat with the other leaf extracts.