Race to Find the Cure
Isolation of Chemicals from Plant Leaves

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A modification of "Isolation of Synthetic Chemicals from Plant Leaves"
(From Chemicals in the Environment Activities, Project LABS – Learning About Basic Science)
(Original Developers: Andrea Martin, Abington Friends School, Jenkintown, PA; Dr. Paul Reibach & Dr. Diana Bender, Rohm & Haas Company, Spring House, PA)

Summary: This activity simulates the extraction, identification, and separation of chemicals in or on plants using chromatography. Students work in groups representing different pharmaceutical companies racing to find the miracle cure for cancer.

Application: Using chemicals in plants for pharmacuetical drugs; qualitative analysis techniques; Isolation of synthetic chemicals such as pesticides from plants.

Materials (per class):

- Whatman #1 filter paper
- Colored Markers &/or food coloring
- Water
- Scissors
- 6 Rulers
- Mortar & pestle or blender
- Pencils
- Plants (spinach & fresh beets)
- 18 small vials w/ lids
- Gloves
- Plastic pasteur pipettes
- 18 Weigh boats
- Tape
- Gauze

<u>Teacher Preparation</u>

1. Place spinach & a small amount of water in blender and blend (you want the liquid to be very concentrated – dark green). Repeat for the beets – use the leafy part. You do not need a lot of liquid since the students only use a very small amount.
2. Put mixture into gauze and squeeze liquid into a container. Keep beet mixture separate.
3. Distribute spinach liquid into 16 of the vials, and beet liquid into 2 vials.
4. Number the vials according to the table below.
5. Add 2 drops of food coloring per vial according to the table below.

(variation – grow the plants with food coloring in the water or spray the coloring onto the plant to simulate pesticides)

Sample Number	Identity	Standard ID
1	Red	C
2	Green	D
3	Blue	A
4	Yellow	B
5	Unknown (beet)	
6	Red/Green	
7	Yellow/Red	
8	Blue/Green	
9	Blue/Yellow	

Groups	Samples	Answers
1	5,1,8	Unknown, C, AD
2	2,6,9	D, CD, AB
3	3,4,7	A, B, BC
4	5,4,9	Unknown, B, AB
5	2,6,8	D, CD, AD
6	1,7,3	C, BC, A
		*note: some blue food coloring will have blue & red lines & green may have blue & yellow lines which will affect answers.

6. Make chromotography paper: cut 6 cm long strips of Whatman #1 filter paper & mark lines with a pencil 2 cm from bottom, 1 cm from top.

7. Make the standards: Label 4 pieces of chromatography paper with A, B, C, & D. Place samples with the primary colors (A= 3 = blue, B = 4 = yellow, C = 1 = red, D = 2 = green) on the bottom line.

8. Set up weigh boats with water and hang tape from the lab bench shelves. The chromotography paper will dip into the water and be held in place by the tape. Allow the water to absorb until it reaches the mark at the top of the paper.

    - This is better than a jar because the paper falls into the jar & touches the sides, which messes up the chromotography.
    - Otherwise you have to hold the paper, which takes too long.

<u>Student Activity</u>

9. Break up students into groups each containing 4-5 students. Assign pharmaceutical company names to each group and tell the students that they are teams working at competing companies to find the plant containing the potential "miracle cure" for cancer.

Ideas for company names (or use real companies): BLTC (Better Living Through Chemistry) Co.; We Save U Pharmaceuticals; Mighty Meds Company; Mortar & Pestle. Inc.; FixU-Up Pharmaceutical Company.

10. Have students make their own chromatography paper by cutting strips of Whatman #1 filter paper.

    - Doing this demonstrates how simple science can be (yet it likes to use “big” words like chromotography)

11. Have each group mark the blank chromotography paper (2 cm from bottom, 1 cm from top).

12. Apply the unknown extracts using pipettes. Be sure to use a different pipette for each vial so there is no cross contamination.

13. Place the bottom of the chromotography paper with the unknowns in separate weighboats filled with distilled water, tape the top to hold the paper upright.

14. Remove the chromatography paper from the water when the water reaches the top mark (10 – 15 minutes).

15. Answer the following questions while you are waiting for your chromatograph to finish.

    Discussion Questions: Discuss the answers to these questions among your group and write down your answers.

    - Why do you think the chemicals separate? (*different molecular weights; solubility in solvent use*)

    - How do you think this technique can be useful? (*can be used to physically separate/isolate chemicals; can be used to identify chemicals*)

    - Why would we want to separate chemicals? (*application of a specific chemical for its properties – e.g. pharmaceuticals, pesticides; its behavior can be affected in the presence of other chemicals;*)

    - * Advanced Question: Based on what you know about molecules, what are some other potential chromatographic or separation techniques? (SIZE: ***gel-filtration chromatography*** *– uses a carbohydrate polymerbeads in a column, smaller molecules diffuse into the beads and are retained in the column longer than larger molecules; CHARGE:* ***ion-exchange chromatography & electrophoresis*** *– separate molecules based on the charge they carry. Electrophoresis uses electrodes & is a very powerful technique. More than 1,000 different proteins have been extracted from one species of bacterium in just one experiment. BONDING AFFINITY:* ***affinity chromatography*** *– separates molecules based on hydrogen bonds & other attractive forces.)* [1]

16. Try to identify chemicals by referring to the standards (see table above for the answers)

    - The students who received the beet extract will not find a match for it in their standards. This is a good opportunity to discuss that there are thousands of unidentified chemicals in this world, perhaps this chemical is the one that cures cancer.

- Extension: discuss process for testing chemicals used in pharmaceutical drugs.

17. Extract the separated chemicals from the chromatography paper. Cut out the "individual" colors/chemicals from the chromatography paper. Place the fraction into a weigh boat containing water. Observe the chemical dissolve into the water.

    - Discussion Questions: What are some challenges you can see from trying to extract/isolate a chemical? (*still may not have isolated a specific chemical i.e. could be a complex mixture; get a very small amount of the chemical per plant)*

    - Plants can vary in the amounts of compounds they contain within a species, or even within a given plant, what are some factors you can think of that can affect the amount of a chemical a plant produces? (*growth conditions; genetics; time of year)*

    - How can this variation affect you as a consumer? (*in unregulated industries such as herbal products, you do not know how much of the "active" chemical you are getting within a single brand or between brands; In regulated industries where there are standards, such as pharmaceuticals, this variation can affect prices. This is why plants are bred for maximum yield and consistancy).*

If time &/or desire – each group can find the Rf for each standard, enter onto a data compilation sheet, find average Rf.

Rf = dye distance/solvent distance

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[1] Vollhardt, K.P.C. and Schore, N.E. Organic Chemistry: 2nd Edition. W.H. Freeman and Company, New York. 1997.

8/2000

GROUP MEMBERS: ____

Race to Find the Cure
Student Instructions & Notes

1. Break up into groups of 4-5 students each.

2. Make your own chromatography paper by cutting 6cm strips of Whatman #1 filter paper.

3. Mark the blank chromotography paper (2 cm from bottom, 1 cm from top).

4. Apply the unknown extracts (using pipettes) on the bottom line (2 cm line) on the chromotagraphy paper. Be sure to use a different pipette for each vial so there is no cross contamination.

5. Place the bottom of the chromotography paper in separate weighboats filled with distilled water, tape the top to hold the paper upright. (Only the tip of the paper needs to touch the water – don't put it in past the 2 cm mark where you put your sample).

6. Remove the chromotography paper from the water when the water reaches the top mark (10-15 minutes).

7. Answer the following questions while you are waiting for your chromatograph to fininish.

    - Discussion Questions: Discuss the answers to these questions among your group and write down your answers.

        - Why do you think the chemicals separate?

        - How do you think this technique can be useful?

        - Why would we want to separate chemicals?

        - * Advanced Question: Based on what you know about molecules, what are some other potential chromatographic or separation techniques?

8. Identify chemicals by referring to the standards.

What chemicals did you find in your plants? (A, B, C, D, & chlorophyll)

Plant/vial # ______:

Plant/vial # ______:

Plant/vial # ______:

18. Extract the separated chemicals from the chromatography paper. Cut out the "individual" colors/chemicals from the chromatography paper. Place the fraction into a weigh boat containing water. Observe the chemical dissolve into the water. **You have now isolated a chemical!**

- Discussion Questions: Discuss the answers to these questions among your group and write down your answers.
    - What are some challenges you can see from trying to extract/isolate a chemical?
    - Plants can vary in the amounts of compounds they contain within a species, or even within a given plant, what are some factors you can think of that can affect the amount of a chemical a plant produces?
    - How can this variation affect you as a consumer?

The Company Signs

We Save U Pharmaceuticals

BLTC Co.

(Better Living Through Chemistry)

Mighty Meds Co.

Mortar & Pestle Inc.

Fix U Up Company