Antimicrobial Effects of Plants

Overview:
This lab will test the antimicrobial power of certain plant derived compounds. You will need to make tree extracts, and nutrient agar. The lab will use the extracts as well as some every day plant materials like lemon juice to see how well they prevent bacterial growth.

Rational:
This lesson is intended to promote inquiry, data collection, and an interest in the medicinal properties of plants. It does align with the Maine Learning Results and the Parameters for Essential Instruction. Without listing all of the details for these, I have provided the codes B1, C1, and E3. The lesson does require attention to detail since even innocuous bacteria could be hazardous.

Student Objectives:
1. The students will be able to formulate /identify a variable and a control in an experiment.
2. The students will be able to make a prediction and justify it.
3. The students will be able to discuss the limiting factors in a Petri dish.
4. The students will be able to interpret their results.

Time Commitment: The extracts and nutrient agar need to be made a day ahead and refrigerate. These should not be made too far ahead as they will spoil. You will need a source of bacteria that is safe like E. coli (the venders sell a safe version). Once the experiment is set up, the dishes will need time to incubate. If you have an incubator, a couple of days are all you need to see results. If you have to leave the dishes at room temperature, than a week will be needed to see results.(Check the dishes after to 4 days just to be sure).

Making the tree or plant extracts: See Tree Extracts

Making Nutrient agar: Follow the directions on the bottle. To save money, I have 4 students work with one plate. One liter of water and 13 grams of agar powder usually makes enough for 100 students(25 plates). Place the dishes in the fridge the night before. Another option is to make the agar in the morning before the students arrive. I find the agar will set and be ready after 30 minutes.

Materials:

1. 25 sterile Petri dishes
2. Nutrient agar
3. Water
4. Hot plate or another source of heat

5. 1 Liter beaker/pan for boiling water
6. Loop
7. Extracts
8. Garlic
9. Onion
10. Hot sauce (Tabasco)
11. Lemon juice
12. Blueberries

Obviously you can pick and choose the plant items you would like to use or have the students pick from the options.

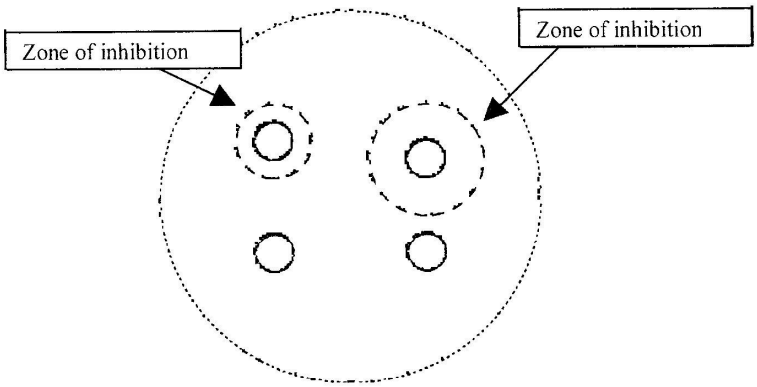
13. Blank paper filter disks
14. Forceps
15. Dishes/bowls
16. Mortal and pestles for each food item or anyway device to grind them.
17. Sharpie
18. A centimeter/millimeter stick

After you as the teacher have read this, you may decide that this procedure is very involved. Do you want your students using the alcohol burners etc? If you do not want the students involved in the sterile techniques, you could still do this lab. You can set it up, model the sterile technique for the students, and then have the students make predictions as to which substance will have the best antimicrobial effect.

Procedure:

1. Have students choose the 4 substances they want to test and assign a number to each substance. Then use a sharpie and quarter the dish and number each quadrant. You want the numbers to be small so they will not effect your view.
2. Light the alcohol burner and flame the loop briefly.
3. Let the loop cool for 20 seconds and dip it into the bacteria.
4. Open a dish and gently spread the loop over the dish. (like you were painting it). You do need a soft touch. The agar is the consistency of jello and you do not want to tear it.
5. Flame the loop and place it on a paper towel.
6. Flame the forceps quickly and let cool.
7. Pick up a paper disk and dip it in the substance of choice and place it on the agar in its spot that corresponds with its number.
8. Flame the loop and repeat steps 5-7 for each substance you want to us.
9. Create a control plate with 4 disks all dipped in water.
10. Tape the dishes shut and remind students that the dishes must remain shut throughout. Do not open ever again.
11. Everyone should wash their hands with soap and water.
12. Incubate for 4 days at 35degrees C. If leaving at room temperature, place the dishes in a dark warm spot and check after 5 and 7 days.

13. REMEMBER to keep the dishes taped shut. Remove them from the incubator and look for zones of inhibition or areas of no growth around the disk. Measure the zone in millimeters and record results in a data table.



Results

Antimicrobial	Size of the Zone of Inhibition (mm)

Extensions:

1. Have students research foxglove, the pacific yew tree, and capsaicin oils. All of these have medicinal properties and all are being used in mainstream medicine.
2. Have students do the experiment again with different bacteria and predict whether the same agent will work the best.
3. Have students try to make different concentrations of the extracts to see if that affects the antimicrobial power.