Antimicrobial Activity of Plants

In our lab section we have been using a convenience product – our lab manual - which provides us with instructions, background information, pre-made tables to fill in and questions to ponder. It has its' advantages, but in other lab classes across the nation, and through the reaches of time all students follow the time honored practice of doing everything themselves by writing lab reports.

Overview

Plants produce carbohydrates via photosynthesis. These carbohydrates can be used for energy (sugars), used to store energy for later use (starch), or can be used for structural support (cellulose). In addition, plants can (and have to) synthesize all of their other macromolecules necessary for the survival and reproduction, including nucleic acids, proteins and lipids. Metabolically plants are photoautotrophs. However, when plant biochemistry was first studied it was noticed that some plants also synthesized other classes of molecules that did not have any apparent function; these were termed plant secondary compounds. Now it is realized that the role of these compounds is to defend plants from predators and parasites.

Objective

The objective of this lab is to assess and quantify the antimicrobial properties of common plants that can be purchased from a typical supermarket. This includes:

- raw plants, fruit, vegetables, flowers
- products made only from raw plants (dried herbs, tea, coffee, spices)

Processed products (such as pickles are more difficult to evaluate because antimicrobial activity could be due to vinegar, salt, or preservatives added during processing.

Research and Preparation

Which plants have antimicrobial properties? To answer this question will require some research in order to identify likely candidates before the class lab to assess any antimicrobial affects. WebAccess Assignment #5 will provide instructions how to use the Cañada College Library Article Search Database. Google Scholar is also a useful starting place to browse for topics "Antimicrobial properties of……"

Example

Evaluation of Garlic and Ginger Extracts
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3609356/

Lab Procedure

The time constraints of the lab mean that we cannot have each individual student pick their own plant to test for antimicrobial activity- **so each table will decide upon one plant** to use. However, each student will be assessed upon their lab write up as individuals.

Sample Preparation Day 1

We will essentially perform the Disk Diffusion technique to quantify the inhibition of bacterial growth by the chemicals extracted from your plant.

Each table must bring their plant with them. In lab we will have mortars and pestles, which will be used to grind your plant into a pulp (no chewing please).

Because plants synthesize a range of different molecules. Polar molecules are soluble in water, so grinding the plant with water will capture these molecules, but many herbs and spices owe their properties to essential oils. These non-polar molecules are insoluble in water, and we therefore need to use a nonpolar solvent to obtain these molecules.

Water Extract

Each groups will weigh out 1.0 g of leaves/seeds and
Start grinding your leaves/seeds dry. After a couple of minutes you can add water in increments of 1000 ul using a P-1000 micropipette. Keep note of how many ml of water you have added. It will take approximately 10 minutes of grinding. If you add too much water the extract might be too dilute to inhibit microbial growth.

Methanol Extract

Each groups will weigh out 1.0 g of leaves/seeds and
Start grinding your leaves/seeds dry. After a couple of minutes you can add methanol in increments of 1000 ul using a P-1000 micropipette. Keep note of how many ml of solvent you have added. As methanol will evaporate readily to might need to add more methanol than water.

Preparing your Filter paper Disks

Once you have obtained a liquid extract you will need to soak some filter paper disks in the extract to transfer the extract to the inoculated plates. Sterilize your forceps by dipping them in ethanol and passing them through a Bunsen flame. The alcohol will burn off sterilizing the forceps. Pick up one filter paper disk and dip it into the extract so that it can soak up the extract. Then transfer the wet disks to an empty, sterile Petri dish, to dry. You will have separate Petri dishes for the water extract disks and the methanol extract disks, and 4 disks soaked in Lysol.

Drying Disks

We wish to determine the antimicrobial properties of chemicals synthesized by your plant, not the antimicrobial properties of the solvent used for the extract. Once you have soaked 8 disks for each extract place the Petri dish in the fume hood to allow the disks to

dry. It will take 10 minutes. You can open the lid a fraction to allow air to circulate to speed up the process.

Placing Disks on Plates
Sterilize your forceps and carefully place the following disks on each of your inoculated plates:

2 disks soaked in aqueous extract
2 disks soaked in methanol extract
1 control disk soaked in Lysol (control)

Controls
What is the purpose of controls? You want to prove that any inhibition of bacterial growth is due to the plant chemicals, not to any other procedure used in the experiment. What would be appropriate positive and negative controls?

Cultures:

A selection of different bacteria will be present in lab for you to choose from. Each group will inoculate 4 plates using the four different cultures provided. Plates will be inoculated using the spread plate technique to ensure an even distribution of bacteria. 100 ul of broth culture will be spread.

Determining Antimicrobial Activity (Day 2)

Following 48 hours of incubation antimicrobial activity will be determined my measuring the diameter of the zone of inhibition around each of the disks in mm.

Class Data
The eight tables which performed eight independent experiments will compile their data in a table. Your results section will contain results for your experiment and for the class results. Use the class data to draw a chart displaying the results of the class experiments.

Example Table of Results of Previous Classes

Escherichia coli

Table #	Table 1	Table 2	Table 3	Table 4	Table 5	Table 6	Table 7	Table 8
Plant	Broccoli	Mint	Aloe	Honey	Tomato	Onion	Oregano	Ginger
Zone of inhibition (mm)	0mm	0mm	0mm	0mm	0mm	0mm	0mm	0mm
Zone of inhibition (mm) (+ Control)	1mm	3mm	8mm	10mm	5mm	10mm	9mm	43.5mm
Zone of inhibition (mm) (-Control)	0mm	0mm	0	0mm	0mm	0mm	0mm	0 mm

Staphylococcus aureus

Table #	Table 1	Table 2	Table 3	Table 4	Table 5	Table 6	Table 7	Table 8
Plant	Broccoli	Mint	Aloe	Honey	Tomato	Onion	Oregano	Ginger
Zone of inhibition (mm)	0mm	0mm	0mm	0mm	0mm	0mm	20mm	0mm
Zone of inhibition (mm) (+ Control)	6.5mm	5mm	15mm	15mm	7mm	7mm	18mm	43.5mm
Zone of inhibition (mm) (-Control)	0mm	0mm	0mm	0mm	0mm	0mm	0mm	0mm

How to do a Formal Lab Write Up

The Formal Lab write up is worth 30 points- the points for each section are given in parenthesis. Each Lab Write up must contain the following sections.

Introduction: Based upon your research you must explain to the reader the background of the experiment. Set out the purpose of the experiment. Describe the characteristics organisms (plants and bacteria) selected, and why these (compared to other organisms) were selected for this experiment. Describe how we will evaluate the antimicrobial properties of plant extracts (how the disk diffusion technique works). Also, for our experiment, provide some information about the antimicrobial properties of plants. Reading the introduction sections in the articles you retrieved should help. You should cite sources in this section and include full references in the Reference Section. (6)

Hypothesis: State your hypothesis here. A hypothesis is a statement of prediction that must relate to the experiment in question. It must be focused. It does not need to include statement such as "I think that…' or "because' explanations will be considered after the results of the experiment have been analyzed. (2)

Materials and Methods: Briefly list any reagents used (materials), then describe the techniques used to perform the experiment (methods). The objective of this section is to explain to the reader what you did to get your results. Approach it as though another student could follow your instructions and perform the same experiment as you. Include incubation time and temperatures. (6)

Results: A brief section featuring tables presenting the results of your experiment, and a table presenting the class data for comparison. Each table requires a title, table number, and columns should be labeled. You may also include a chart of the class results. Your chart must have a title, and labeled axes Leave any interpretation of the results to the next section. It might also be a good idea to take some digital photos of your results to include. (6)

Include the results of your controls in your results section and your discussion. Explain how the controls contribute to the experiment.

Discussion: Interpretation of results. Explain the meaning of the results. Also you can discuss anything that may have affected your results (if results are not what you expected). Compare your results with the other tables. You can also refer to results from the articles you used in your research. (6)

References: List in alphabetical order the works cited in the Introduction, Materials and Methods, and Discussion Sections. Aim for at least 4 references. At this level you should

not be relying on encyclopedia type sources (textbooks or wikipedia) retrieve the primary sources (research articles). References listed must be used in the text. APA format. Websites must be correctly formatted (4)

(Length: 1000-1500 words +/- 250 words)

Summary of the Scientific Method

After the report identify the following:

Variable in Experimental Design	In Our Lab
Independent Variable	
Dependent Variable	
Standardized variables	1. 2. 3.
Control(s)	

Due Date 2/27/14