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"Designing from Scratch": A Dynamic Interdisciplinary Approach to Engage Upper Level Students in Plant Science Courses

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Plant Science is quite often not the first choice in upper level courses for the undergraduate population. Laboratory sessions are usually restricted to taxonomical identification, the study of physiological processes, as well as microscopy to recognize plant cells and tissues. However, in upper level classes those activities should transcend the pre-digested information and pre-set up experiments with expected outcomes. A mixture of basics and "design from scratch" laboratories would challenge the students to approach plant sciences from a whole different perspective. At Coker College, plant science has joined forces with chemistry to provide students with a full semester research activity. This activity introduces students to the notion of plants as potential antimicrobial agents to treat, and control, human pathogens. Students are responsible for the planning and execution of their experiment. The benefits of the "design from scratch" type of experimentation definitely exceed the perceived downsides of the process (time, additional extracurricular work for professors, use of additional research supplies, negative results after testing, seasonal availability of plant material, etc.) while providing a unique research experience in which ownership of the research by students is successfully achieved.

Keywords: natural products, plant science, inquiry-based learning

Introduction

Plant Science is quite often not the first choice in upper level courses for the undergraduate population. This widespread phenomenon has been termed as the **"plant blindness theory"** - a public's inattention and disinterest in understanding and acknowledging plants as a group (Ebert-May and Holt, 2014; Wandersee and Schussler, 2001). Plants are usually summarized by students as non-motile photosynthetic eukaryotic organisms. Even though the reach of plants as a group goes far beyond that reductionist definition, lecture classes and laboratory sessions do not always fully contribute to extend this limited notion about plants. This occurs in introductory classes as well as in upper level classes. Experiments are restricted to taxonomical identification of plant groups and families, reviewing physiological processes such as photosynthesis, transport and cellular respiration, as well as microscopy tools to recognize plant cells and tissues.

The portion of "hands-on" activities in laboratory sessions is usually limited to a pre-set protocol, data collection and analysis of expected results. This might be sufficient for introductory classes. However, in upper level and major classes, those activities should transcend the pre-digested information and encourage students to perform independent thinking and critical analysis. Fortunately, ideas out there about topics such as GMO (genetically modified organisms), resistance to antibiotics, world hunger, wellness, food security, climate change, organic farming and sustainable living, among others, have contributed to increase the level of curiosity about the relevance of plants as a group.

The mixture of traditional and "design from scratch" laboratories would challenge the students to approach plant science from a different perspective. Using media triggers and interdisciplinary elements in laboratory sessions, students could finally discover and embrace the connection of plants to "real life" science and the impact of this group in every day's human activities.

At Coker College, the plant science course at Coker College (Elective Biology Major course) joined forces with chemistry to provide students with a full semester research activity. This activity introduced the notion of plants as potential antimicrobial agents to treat and control human pathogens. The class was intended for juniors and senior undergraduate students with completed core classes in biology and chemistry.

The benefits of the "design from scratch" type of research definitely exceed the perceived downsides of the process (time, extracurricular work for professors, use of research supplies, negative results after testing, seasonal

The purpose of this activity was:

- To promote an independent student-led research activity to prep them for "real life" scientific problems and experiments.

- To raise awareness of plants by introducing engaging research in plant science and natural product chemistry.

Student Outline

Objectives

1. To familiarize with the antimicrobial properties of medicinal plants
2. To familiarize with common human pathogens and antibiotic resistance
3. To review and apply concepts and techniques learned in chemistry core classes (General Chemistry, Organic Chemistry and Biochemistry) to obtain active extracts from the plant material selected
4. To perform an *'in vitro'* bioassay to identify the active plant extract/s against a selected microbial human pathogen

Introduction

Long before we discovered the existence of microbes, the idea that certain plants had healing potential was well known. However, it was not until the more recent times that we could extract, isolate and characterize the actual compounds reported earlier with antimicrobial properties. Since ancient times, man has used plants to treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment of some medical conditions. Many plants have been reported as potential new sources of antimicrobial activity. For example, the use of bearberry (*Arctostaphylos uva-ursi*) and cranberry juice (*Vaccinium macrocarpon*) has been widely reported to treat urinary tract infections, while species such as lemon balm (*Melissa officinalis*), garlic (*Allium sativum*) and tea tree (*Melaleuca alternifolia*) are described as broad-spectrum antimicrobial agents (Heinrich et al., 2004; Rios and Recio, 2005). New plant based medicines seem to be great contributors of the future of Western Medicine. As more pathogens such as *Candida albicans, Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Aspergillus fumigatus*, *Candida albicans* among others, become resistant to known antifungals and antibiotics, it will become more important to look for alternative methods in treating patients infected with these pathogens.

In this study, the identification of the active plant extracts showing antimicrobial activity against a selected pathogen will be researched. Diffusion agar assays and the presence of inhibition halos will be used to test the potential activity of different extracts on microbe's growth and mortality '*in vitro*'.

This laboratory exercise will be conducted throughout the semester as a full research activity. The selection of the plant material as well as the pathogen will be picked by the student groups after revision and agreement with the professor. At the end of this activity, the students will be asked to submit a full research paper including novel results and a thorough discussion. Limitations, pitfalls and future directions will be included as part of the critical analysis.

Methods and Data Collection

Part A: Plant material and Microbial Strain Selection

The plant material and the microbial strain/s to be used in this study will be selected after carefully reading the provided reference material and the individual/groups search for already known antimicrobial properties of their plant. The final decision on the plant/pathogen selection will be discussed in class and approved by your instructor.

Part B: Chemistry Section: Extraction of Plant Material

Collect 600-1000 g of the plant sample, remove all excess material (stems, insects, etc.) combine and weigh sample and place into a 1-L beaker. The plant sample is submerged in ethanol, the amount of ethanol will vary sample to sample just make sure no plant material is above the solvent line. Stir the mixture and allow to sit overnight. After ~24 hours of soaking remove the ethanol solution via filtration. This is a bulk filtration so either a 1-L Buchner funnel, or a kitchen strainer with a coffee filter will work, excess solvent should be pressed out of the bulk plant material. Put the plant material back into its container and perform a second extraction with ethanol. Isolate the second ethanol extraction and combine it with the first. Place the ethanol solution into a pre-weighed 1 or 2-L round bottomed flask. Remove solvent via rotavap. Once all the solvent is removed reweigh the flask with the concentrated sample and determine the total mass of the extracted material. Add ~250 mL of DI water to the flask and get as much of the sample back into solution as possible. All material will not dissolve, this will be addressed latter.

Transfer the aqueous solution to a 500-mL separatory funnel. The aqueous sample will now be extracted with various solvents in order of increasing polarity. The aqueous solution will be extracted first with hexane, then chloroform, and finally ethyl acetate. You may substitute petroleum ether for hexane and methylene chloride for chloroform if desired. Each solvent extraction should be performed with 3 x 50 mL of the solvent. Before placing the solvent in the separatory funnel wash the round bottomed flask with the neat solvent to help remove any of the undissolved plant material, by the final solvent wash, ethyl acetate, no material should be left in the round bottomed flask. Once ~150 mL of the three solvents has been collected

dry each collection with magnesium (II) sulfate, $MgSO_4$. Remove the $MgSO_4$ via filtration using a Buchner funnel and place each solvent into a pre-weighed round bottomed flask. Remove solvent via rotovap and record mass of concentrated samples. Remove enough of the sample to create a 1g/10 ml solution of plant material/DMSO. The remaining material should be re-dissolved in ethyl acetate for long term storage in a 0 to -20℃ freezer. The DMSO solution is now ready for biological testing.

Part C: Biological Assays and Antimicrobial Activity

Bactericidal and/or antifungal effect of the plant extracts, depending on the nature of the pathogens selected, will be determined by an agar well diffusion assay (Murray *et al*., 1995).

Pour 15-20 ml of sterile TSB-g agar medium into 90 mm sterile Petri dishes and allow to solidify. Once plates are ready to use, punch 3 wells in the agar surface of each Petri dish using a using a sterile core borer (4 mm diameter). Pour 100 µl of each extract (1g/10ml) into the plate's wells followed by the streaking (inoculation) of the reference bacterial, or fungal strain, using a sterile cotton swab. A positive and negative control will be used. The extract-free control (water) will represent the negative control while reference, antibiotics or antifungals, will be used as positive controls for the selected bacterial or fungal pathogen, respectively. Label the plates clearly. Incubate the Petri dishes at 37 ^{o}C for a period of 24-48 hours. For each plant extract tested, evaluate antimicrobial activity by measuring the diameter of the zones of inhibition (mm) against the tested microbe (bacteria and/or fungi) using a flexible plastic ruler. Use the bottom of the Petri dishes to properly read the inhibition zones. For each of the replicates the readings will be taken in three different fixed directions per well and the average values will be recorded. Each of the plant extracts will be tested in triplicates and the whole experiment should be repeated twice, if time permits.

Data Analysis

After completion of the semester research experiment, students will submit their findings as a full research article. Students will use their own collected data to develop the results section of the research article. Students should be able to calculate the mean values and standard deviation for each of the plant extracts tested. Students could present the information as a descriptive table or a bar graph figure (Fig. 2). The use of statistical analysis is highly recommended. Due to the nature of the information collected, a one-way analysis of variance followed by a lsd (Fisher's least significant difference) mean comparison or Dunnet's test would be an appropriate statistical analysis. However, the limited number of replicates or negative results might prevent the use of statistics.

Cited References

Heinrich M, Barnes J, Gibbons S, Williamson EM. 2004. Fundamentals of Pharmacognosy and Phytotherapy: Medicinal Plants and Antimicrobial Activity. Churchill Livingstone, Edinburgh, pp. 245–252.

Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken HR. 1995. Manual of Clinical Microbiology, 6th Ed. ASM Press, Washington DC, 1995; 15-18.

Rios JL, Recio MC. 2005. Medicinal plants and antimicrobial activity. Journal of Ethnopharmacology. 100: 80-84

Materials

The extraction process is for ~600-1000 g of material. If larger amounts of plant material are collected it may be necessary to scale the amounts of solvents used. If time permits further purification experiments can be performed using column chromatography and thin layer chromatography techniques

Notes for the Instructor

This experiment was successfully reproduced with two different plant specimens during 2015 and 2017: *Kalmia latifolia*, commonly known as 'mountain laurel' and *Metasequoia glyptostroboides*, an ancient gymnosperm known as 'dawn redwood'.

Over a full semester period, students have the opportunity to be exposed to a fully 'hands-on' research activity that they personally designed and proposed. Instructors initiate the discussion introducing students to notions such as medicinal plants, natural products, the antibiotics and antifungal crisis, microbial resistance to antibiotics, mortality rate in the United States due to resistant microbial strains, new avenues and alternatives to western medicine: true or myth?, among other ideas. This would contribute to the start of their own search for reading material on plants and antimicrobial properties. After that, the instructors can suggest certain plant-pathogen systems to work on to facilitate, and speed up, the start of the project but still allowing them the final decision of plant and pathogen selection and the full independence on the experimental design. The instructors simply modulate and advise their activities in the laboratory.

The benefits of the "design from scratch" approach resides in many aspects of this activity: 1) it includes an interdisciplinary approach that allow the students to use and connect previously learned information in a single project, 2) it promotes ownership and critical analysis of one's own work, 3) there are no 'expected' results. The research represents a unique project with unique problems and conclusions every semester, 4) students are faced with authentic science troubleshooting and pitfalls problems, 5) it is a versatile system. Any combination of plant and pathogen selected can be chosen, 6) it can be done with minimum extra cost to laboratory supplies, and 7) it represents an engaging and unique biological system for plant research and plant awareness.

For the 'cons' aspect of this activity can be cited: 1) it works best with a reduced class/number of students. It has the potential to be modified to accommodate a bigger population. However, it would probably lose the independent research style and certain steps, such as plant material collection, and extraction of the plant material would have to be done in advance by the instructor, 2) extracurricular time for the instructors as well as class time coordination, 3) availability of plant material, quantity and location, 4) it demands a tight scheduling to be able to finish on time. This project is part of BIO350-Plant Science laboratory session which includes other basic labs that have to be completed during the semester as well, and 5) negative results after a semester of extracting and testing, among others.

The marking rubric was carefully designed to emphasize the importance of creativity and inquiry, as opposed to a nonselective listing of information. Students were informed well in advance of their presentations that they would be marked for their creativity and the quality of their presentation, as well as for the scientific accuracy and completeness of information. As a result, students needed to master basic concepts and apply them in a meaningful way to prepare a successful presentation.

Cited References

Ebert-May, D. Holt, E. 2014 Seeing the Forest and the Trees: Research on Plant Science. Life Sciences Education. 13:361-362.

Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken HR. 1995. Manual of Clinical Microbiology, 6th Ed. ASM Press, Washington DC, 1995; 15-18.

Wandersee, JH, Schussler, EE. 2001. Toward a Theory of Plant Blindness. Plant Science Bulletin. 47: 2-9

Heinrich M, Barnes J, Gibbons S, Williamson EM. 2004. Fundamentals of Pharmacognosy and Phytotherapy. Medicinal plants and antimicrobial activity. Churchill Livingstone, Edinburgh, pp. 245–252.

Rios JL, Recio MC. 2005. Medicinal plants and antimicrobial activity. Journal of Ethnopharmacology. 100: 80-84

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Appendix A
Sample Results

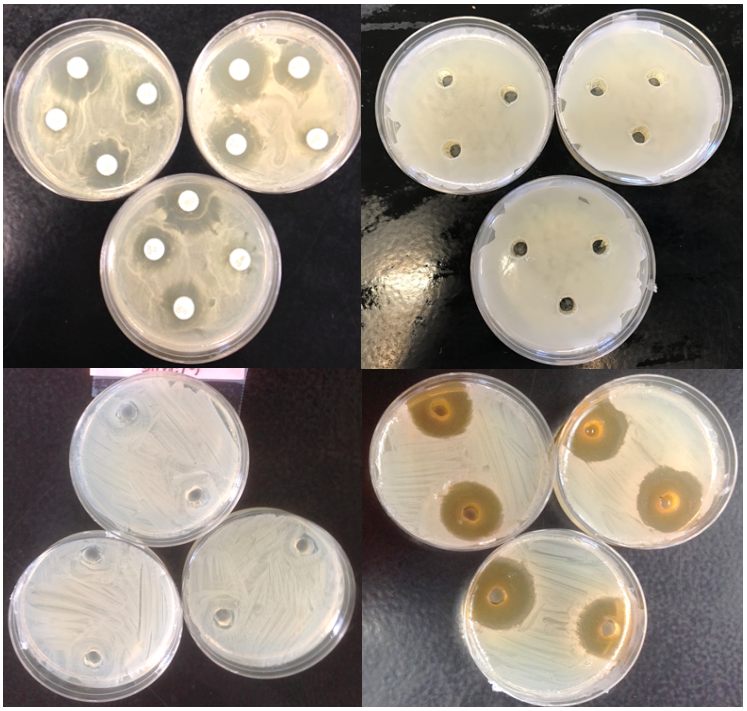

Figure 1. Inhibition of Growth of *S. aureus* by "Dawn redwood" extracts. **A**: Control (+) - commercial antibiotics, **B**: Control (-) DMSO/Water, **C**: Negative- Chloroform extract, **D:** Positive- Ethyl Acetate extract/

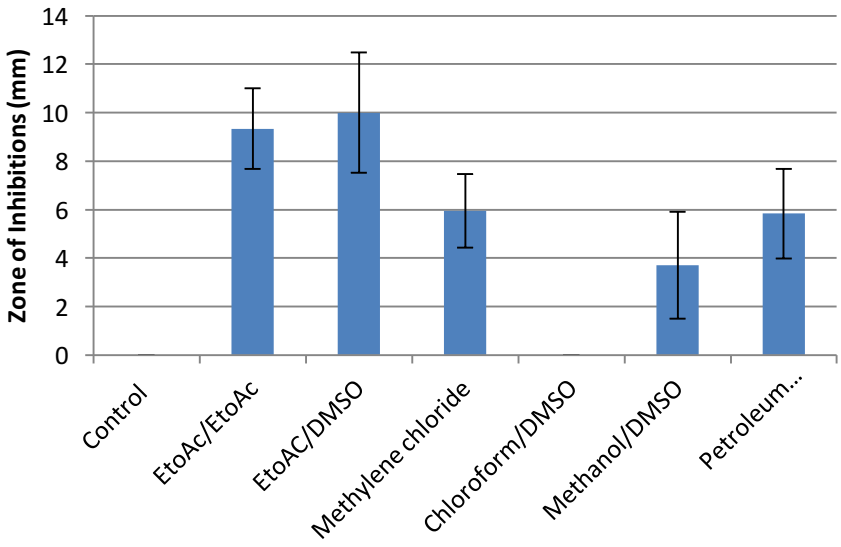

Figure 2. Antimicrobial properties of *Kalmia latifolia* against *Staphylococcus aureus in vitro*

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