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A Theme Based Experimental Methods Course Provides Research Experience for Biology Majors

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In order to provide our upper level biology students with a more comprehensive research experience, a course was designed that enabled students to investigate a problem from literature to the bench, within the limits of a fifteen week semester. The objective of the course was to provide students with direct, hands-on experience that would be relevant in most biological career fields. Although biological disciplines vary widely, all require the mastery of a basic set of skills that are essential to scientific inquiry. By using an experimental approach, this course allowed students to follow their own scientific inquiry from conception to "publication," giving them an integrated understanding of how basic research is performed. By use of their own collected data, students more readily understood data analysis and the conclusions to which it leads. This course was taught as a project-focused course with the instructors selecting a research theme as a scaffold for the assignments and activities involved in developing student research, analytical, and presentation skills. As an example of this methodology, we are reporting on the module created for students to investigate anti-microbial compounds found in plants.

Keywords: inquiry-based experiment, natural antibiotics, natural antimicrobials

Introduction

At Georgia Gwinnett College, senior biology majors are required to complete a disciplinary research project with a faculty member or to complete an internship in an appropriate biological or biomedical setting. As the demand for student research opportunities has increased dramatically in recent years due to large increases in student enrollment, another capstone research option for students was needed. To provide our students with an equivalent rigorous and comprehensive research experience, a theme-based research course was developed. The resulting course, *BIOL4570: Experimental Methods in Biology* was offered for the first time in the spring of 2013.

One of the two research themes for the initial sections of the course was the investigation of antibacterial compounds found in plants. This is currently an area of intense investigation due to both the clinical need for new antibiotics and the realization that many plant antimicrobial substances are active against a broad spectrum of microbes (Cowan, 1999, Kasagana *et al.* 2011, Tegos *et al.* 2002,and Voon *et al*. 2012). This theme allowed students to follow their own line of inquiry in an area of active research in the scientific community.

To begin their research project, students were directed to select a plant that they wished to investigate for antimicrobial activity. The class was instructed that they could choose any plant from the local environment. A native plant expert assisted with identification of their plant specimen. Students were then instructed on how to perform a comprehensive literature search to determine if the species of plant they selected had previously documented antimicrobial activity. If there was no previous literature demonstrating that the selected plant has been investigated for production of antimicrobial substances, the student could proceed with planning their research protocol. If previous research on the plant had been published, the student was required to select another plant species.

The next phase of the project was experimental design of the protocol to extract the antimicrobial activity. Time and resource constraints limited the options for extraction methods to the most common methods of using water or simple alcohols for extraction from plant roots, stems, leafs, fruits, or seeds (Cowan,1999). Students were given several papers that provide detailed methods for preparation of plant materials and basic extraction methods for antimicrobial materials (Cowan, 1999, Tegos *et al*. 2002, and Voon *et al*. 2012). They were instructed to design their experiments based on these protocols.

Before beginning their experiments, students presented their research protocol to their classmates for review and discussion. After receiving feedback from their classmates,

student wrote a detailed research protocol that was submitted to the instructor for approval before they began to prepare for their lab work. This research prospectus involved selecting the best approach for preparation of their chosen plant species and writing basic protocols for the extraction procedure. Students were also instructed on how to maintain a laboratory research notebook and how to document their laboratory activities. They would later turn in their laboratory notebooks as part of the course evaluation.

To test materials extracted from their plants, students performed standard disk diffusion techniques (Cowan, 1999 and Mbah *et al.* 2012). Students tested all materials obtained during their extractions on the following representative organisms: *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Bacillus subtilis,* and *Mycobacterium smegmatis* (Fig. 1). All disk diffusion experiments employed appropriate antibiotic discs for each species to be used as positive controls and solvent and blank discs were utilized as negative controls. Students were taught to perform appropriate statistical analysis on their data, and relate the significance to their findings. All data collected was presented to the class for review and discussion in a lab meeting format.

When students had concluded their experiments and data analysis, they were required to write a scientific "publication" giving a brief overview of the current literature, materials and methods utilized in their experiments, results, and a discussion of their findings. Their paper was required to include appropriate graphs, tables, and charts demonstrating their results (Table 1). They were also required to include a discussion of potential problems with their research and suggestions for future work on their project. The paper was submitted in draft form first so that they could receive substantial feedback and guidance from the instructor on how scientific publications are composed. Students discussed the challenges they experienced with the composition of this paper in class.

Table 1. Representative student data from disk diffusion assay. Diameters of zones of inhibition are shown in millimeters.

Assay 1	*M. smegmatis*	*B. subtilis*	*P. aeruginosa*	*E. coli*	*S. aureus*
Gentamycin	31	21	15	20	12
Chloramphenicol	39	27	0	22	17
Whole Stem 1	11	7.5	0	0	8
Whole Stem 2	11	8	0	0	0
Whole Stem 3	11	8	0	0	0
Bark 1	10	8	0	0	0
Bark 2	9	8	0	0	0
Bark 3	9	8	0	0	0

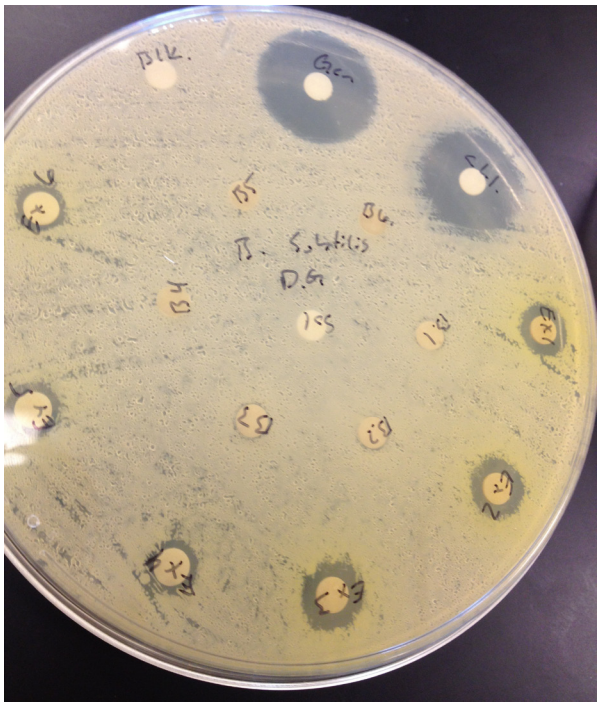

Figure 1. Representative image of agar plate containing *B. subtilis* from a disk diffusion assay performed by a student in the course.

Student Outline

Table 2. Details of Student Activities and Assignments

Week		Activities
1	Introduction to Biological Research	Student will be given journal articles pertinent to the course theme to review and discuss. Experimental protocols will be introduced. Laboratory notebooks and record keeping will be introduced.
2	Use of Scientific Literature	Research an area of interest for investigation
3	Experimental Design Outline	Design experiments – include hypothesis and appropriate controls in the outline. Provide references from the literature supporting the experimental design
4	Experimental Design Presentation	Present experimental design draft in class for class critique
5	Prospectus Writing	Write a proposal for the experiment(s) they plan to perform. The proposal must include materials and methods, and a list of items that must be purchased if not readily available in lab. Appropriate referenced sampling methods must be included.
6	Set Up for Experiments	Collect all reagents required for experiment. Prepare all solutions, cultures and reagents required for the experiment.
7	Begin Experiments	Students will begin data collection for their experiment.
8	Experiments	Lab meeting to discuss progress and obstacles encountered during the experimental process.
9	Repeat Experiments	Data collection
10	Statistical Analysis of Data	Students will use appropriate statistical methods for analysis of their data. Students will first use classroom instruction and practice problems to refresh their knowledge of basic statistical methods.
11	Data Presentation	Students will present their data in appropriate graphical and statistical formats. Critiques will involve suggestions for experimental design revision.
12	Begin Writing Scientific Report of the Experiment	Students begin writing introduction, materials and methods portions of the scientific report.
13	Revised Experiments	Data collection on revised experiments
14	Revised Experiments	Turn in draft of scientific report
15	Revised Experiments	Based on instructor feedback, students revise reports as needed.
16	Revise Scientific Reports Final Scientific Reports Due	Class presentations on completed project

Notes for the Instructor

One of the first observations of students' reactions to the course and the assignments was their initial stunned reaction that they were going to be the ones to design their own research project. Thus far in their education, students participated in laboratory exercises that would give them predictable results if they followed the steps in their lab manuals or exercises.

For students to identify a plant for their research and then conduct an exhaustive literature review to make ensure that similar research had not been published, seemed to be a quite daunting task for most students. To accomplish this task, students required extensive instruction on how to use research publication databases at the beginning of the course. This instruction increased their level of confidence and allowed them to make progress on this assignment. Also, encouragement from the instructor seemed to boost their attempts to identify an appropriate plant for their research.

Several weeks were devoted to plant selection as most students initially picked a plant that was most convenient for them to harvest. They usually found that the plant had been extensively researched and was therefore not a candidate for their project. Often, several attempts were made before finding a suitable candidate for the project. While this situation was sometimes frustrating for the students, it provided valuable insights about the nature of scientific research. It also demonstrated that this class was not going to be another "cookie cutter" laboratory experience for them.

Another area where students were challenged was in writing the research prospectus. Many students did not have the composition skills that I had anticipated at the junior/senior level. As we discussed these problems in class, it became clear that many students had not received substantive feedback on their writing skills since their freshmen composition courses. An obvious challenge was to write a proposal using appropriate scientific format and terminology with correct grammar and punctuation. Due to the challenges with their composition skills, many hours were required to revise their drafts and discuss how to improve their scientific composition skills in class. By the end of the class, students were quite excited about their research and were pleased with what they had learned in the course. Several students also commented on how much they appreciated the assistance they received on improving their scientific composition skills.

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About the Authors

Diane Dorsett joined Georgia Gwinnett College (GGC) faculty as an Associate Professor in the fall of 2006. Prior to joining GGC, she served as the Department Coordinator for Natural Sciences at the Atlanta Campus of Georgia Military College. Her current research interests are emerging infectious diseases and identification of natural antibiotic compounds. She teaches courses in virology, immunology, microbiology, and introductory biology for non-majors at GGC.

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