Enhancing Interdisciplinary and Systems Thinking with an Integrative Plant Chemistry Module Applied in Diverse Undergraduate Course Settings

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ABSTRACT: To meet the complex global challenges that workers in STEM fields face, it is critical that today’s students develop integrative technical skills and cognitive competencies. As a highly interdisciplinary field, medicinal plant chemistry provides an exceptionally rich environment for such training. Here, we describe a hands-on medicinal plant chemistry laboratory module (Phytochemical Laboratory Activities for nTegrative Thinking and Enhanced Competencies; PLANTEC) for undergraduates that targets the development of core competencies in (i) logical thinking and analysis of text and data, (ii) interdisciplinary and systems thinking, (iii) oral and written communication of science, and (iv) teamwork and collaboration. Each student determines the natural product profile of a plant species using thin layer chromatography and gas chromatography—mass spectrometry. Students work in pairs and small groups to analyze their data and interpret their findings in chemical, biochemical, and biological contexts. PLANTEC is scalable and so can be offered in laboratory or lecture courses, and even partially or entirely online. We implemented this module in an undergraduate biology lecture course over six 50 min lessons in the fall semesters of both 2018 and 2019. We also experimented with modifications of PLANTEC to tailor learning objectives and thereby emphasize different disciplines during data interpretation (e.g., plant chemistry, ecology, evolution). Students consistently responded that PLANTEC increased not only their performance, but also their interest in medicinal plant chemistry. Interdisciplinary laboratory modules of this type will be particularly useful in developing an innovative and versatile STEM workforce of the future.

KEYWORDS: Upper-Division Undergraduate, Analytical Chemistry, Biochemistry, Curriculum, Communication/Writing, Hands-On Learning/Manipulatives, Plant Chemistry, Bioanalytical Chemistry

INTRODUCTION

To meet the complex challenges facing the world today, it is critical that educators provide the highest-quality training to tomorrow’s STEM workforce. To accomplish this, national scientific organizations (e.g., the American Association for the Advancement of Science, the National Research Council) recommend that undergraduate educators use scientific teaching approaches in which students develop core cognitive and interpersonal competencies required for future innovation and decision making.1−3 These competencies include (i) logical thinking and analysis of text and data, (ii) interdisciplinary and systems thinking, (iii) oral and written communication of science, and (iv) teamwork and collaboration (Figure 1A). It is also recommended that students develop these competencies in the context of the scientific process,5 making course-based research experiences ideal training venues. Indeed, research has shown that such active learning approaches and research experiences increase student performance,6−8 are generally perceived positively by students as facilitating their learning,9 and can provide a basic research experience to students that may not otherwise have such an opportunity, thus enhancing the performance of under-represented groups of students.10 However, while many courses in STEM fields include laboratory sections, instructors often wish to include authentic scientific experiences in lecture courses lacking a specific laboratory section.

Medicinal plant chemistry is a field of study at the intersection of chemistry, biochemistry, ecology, evolution, physiology, and human medicine. It has been recognized repeatedly as an excellent real-world context for lessons on chemical principles and techniques as well as chemical diversity,11−14 and as an appropriate mini research project topic for nonscience majors.15 It is highly interdisciplinary and requires researchers to exercise core cognitive and inter-
personal competencies to make novel inferences. As such, it is also well-suited to facilitate systems thinking, that is, the consideration of a set of interacting components that are integrated with the environment and that may produce emergent, system-level properties. Developing systems thinking has been identified in the literature as an important component of equipping students to understand and address real-world problems. Accordingly, our aim was to leverage the interdisciplinary nature of medicinal plant chemistry in developing a scalable laboratory module that can be incorporated in undergraduate laboratory or lecture courses and that can be tailored to emphasize different disciplines, according to the focus of the course. The module reported here (Phytochemical Laboratory Activities for iNegrative Thinking and Enhanced Competencies; PLANTEC) uses the scientific teaching framework to develop learning goals that address the above core competencies. We implemented PLANTEC in Plants in Human Medicine, an undergraduate medicinal plant chemistry lecture course at the University of Nebraska Lincoln in the fall semesters of 2018 and 2019. PLANTEC provides a versatile laboratory activity offering students scientific experiences that promote the development of skills that the next generation of scientists must master to tackle emerging global challenges in a wide array of professional fields.

**MODULE DESIGN**

Here, we describe PLANTEC, a medicinal plant chemistry laboratory module in which students analyze the natural products present in plant species and develop technical and cognitive core competencies. Prior to administering PLANTEC in the course, we identified sets of plant species or tissues of a plant that would yield chemically or biologically interesting comparisons by screening plant extracts using gas chromatography–mass spectrometry (GC–MS). Since students work in pairs in PLANTEC, we sought to identify pairs of closely related plant species or pairs of tissues from a single plant that yielded chromatograms with a manageable number (1–10) of identifiable peaks (i.e., chemical compounds). We anticipate that a skilled teaching assistant or technician could perform a screening for a course of 20 students in 2 days. Although GC–MS instruments are nearly ubiquitous in research universities, instructors may not always have cost-effective access to them. We therefore provide several alternative ways to implement PLANTEC when the GC–MS data cannot be generated in the course itself, which is also useful for administering the module in online teaching settings (see the Supporting Information). In fact, this activity is ideally suited for completely remote execution, since GC–MS data can be provided electronically to students from online databases, e.g., datadryad.org/stash, and publications, e.g., refs 24 and 25; videos can be used to illustrate the extraction and sample preparation procedures, and video conferences can be used to enable group data analysis and writing sessions.

The workflow of activities in PLANTEC is centered around the scientific process (Figure 1B). The module thus includes several phases: (i) introduction and background research, (ii) the development of research objectives, (iii) collecting observational data, (iv) analyzing and interpreting data in pairs and small groups, and (v) communicating research in a student-focused scientific symposium and a mini-scientific manuscript. Below, we describe each phase. A student handout (see the Supporting Information) serves as a detailed guide to the module and includes grading rubrics as well as descriptions of assignments and example frameworks for data analysis. We also provide a document for the instructor (see the Supporting Information).

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**Figure 1.** Core competencies, PLANTEC module outline, and illustrated activities. (A) List of student core competencies targeted for development. Each of the four core competencies is designated with its own color and font face (i.e., italic, bold, small caps, etc.). (B) Module outline (based on the scientific method). Each box represents a step in the module and corresponds to a step in the scientific process. Steps move from one to the next as indicated by solid arrows. The dotted arrow closes the loop that defines the scientific process, though it is not a step in the module reported here. (C) Activities in each module segment are color and font face coded according to the core competency that they target. (D) Pictures illustrating the (i) data gathering process, extraction, gas chromatography–mass spectrometry sample preparation, and thin layer chromatography, and (ii) data analysis, laptop-based gas chromatography–mass spectrometry data analysis, as well as analysis and interpretation of partner and small group data sets.
Introduction, Background Research, and Research Objectives

At the beginning of the module, we use the workflow diagram as a visual aid to introduce students to the specific learning goals and how they are achieved through each activity (Figure 1C). We also explain how the learning goals and module activities contribute to the development of students’ core competencies and professional skills as a way to increase student buy-in, which has been shown to improve exam and course grades.1 To orient students to the module activities, we explain (i) that each student will apply the scientific method to the study of a plant species’ natural products to meet a research objective of their design, (ii) that the methods used will be thin layer chromatography (TLC) and GC−MS, and (iii) that the students will communicate their results in a presentation and mini manuscript. Each student then finds a partner, and each pair chooses one of the pairs of plant species that we had preselected. As partners, students perform background research on their species and the methods they will use, guided by a set of thought questions (see the Supporting Information). Students also read and summarize a scientific article that serves as a model for what they will do in PLANTEC, namely, in which TLC and GC−MS are used to study medicinal plant chemicals (for article suggestions, see refs 26−29 and the Supporting Information). Students are guided in this assignment by a set of key questions to answer in their article summaries, which includes several questions about research objectives and their scope (see below).

As a class, we discuss the scientific article, emphasizing the objective(s) of the study and how the methods were used to meet those aims. With this example fresh in mind, the students confer with their partners and develop a “partner research objective” based on their plant species. We guide students toward feasible research objectives (i.e., objectives that can be met using the methods in PLANTEC), and then ask several pairs to share their objectives aloud with the class. Partner objectives are commonly descriptive, for example, “Our partner research objective is to identify and quantify chemical constituents in roots and leaves of a carrot plant”. Data generated by meeting this objective enable comparisons of organ-specific chemistry (i.e., biochemical processes that are restricted to specific plant organs, which are leaves, stems, roots, and reproductive organs), predictions of the functions of organ-specific chemistry in the context of plant ecology, and thus opportunities for interdisciplinary and systems thinking.

Depending on how PLANTEC is deployed in the classroom, the instructor can also aggregate student pairs into small groups that will eventually pool their results to create larger data sets documenting chemical profiles from a set of multiple species pairs. For example, the plant species represented in the small groups could be from a single genus or family, which would enable questions related to the evolution of plant chemical properties to be addressed. In these small groups (we used groups of four to eight), students develop a “small group research objective”. This objective should not be achievable using a single pairs’ data alone and instead require the larger, group data set (for example data sets, see the Supporting Information). Group objectives often relate to comparative biochemistry or chemical evolution, for example, “Our small group research objective is to determine if more closely related species have more closely related chemical profiles by studying root chemistry across species in the order Apiales: carrot, parsnip, celeriac, and ginseng”. Having both a “partner research objective” and a “small group research objective” provides an opportunity for students to gain experience distinguishing between primary and secondary research objectives in the context of a single study, as well as the close correspondence between research objectives and the data that can address them.

Data Collection and Analysis

Next, we meet with the students in a teaching laboratory space. Each student is instructed on safety protocols (see the Hazards section) and then given tissue from their plant species that they then grind or muddle in an organic solvent using a glass rod (for detailed protocols, see the Supporting Information). The released metabolites are partitioned between the solvent and an aqueous phase, and the extract is obtained as the top, organic layer in the extraction tube (Figure 1D). One aliquot of the extract is transferred to a gas chromatography vial using a Pasteur pipet (Figure 1E), and another is loaded onto a TLC plate that is then developed in a fumehood (Figure 1D). Students visualize TLC bands with a UV-active stain. The instructors collect the students’ GC vials and, before the next class period, analyze them with a GC−MS system using a method designed to detect a wide range of compounds (for details and alternatives, see the Supporting Information; we used a previously reported GC−MS method30). We then export each student’s raw data as a .cfd file (standard export format of both Shimadzu and Agilent systems) and electronically send each student their raw data file.

We then guide students through data analysis and interpretation, starting with their own data, then progressing to their partner data set, and finally to their small group’s data set. Students record TLC band data (Rf values, band size, and band color) in a table provided in their handout. Using R Shiny,31,32 we have built an application (part of the R package phylochemistry33) that students then use to analyze their GC−MS data. Students load their .cfd file and can extract mass spectra from any peak, perform spectral matching using the MassBank of North America34 (>18,000 spectra), integrate peaks to estimate relative abundances of different compounds, adjust baseline parameters, and more (Figure 1G). Although the R Shiny app is straightforward to use and requires only a basic knowledge of R, some alternatives are to provide GC−MS results as printouts or to analyze GC−MS data on the instrument’s native software, class size permitting. In analyzing their GC−MS data, students determine the sum of the area under all peaks in their chromatogram, the number of peaks that can be identified as a particular compound, and the sum of the area of those identifiable peaks. They tabulate this information and calculate the relative abundance of each identifiable chemical in their extract. Finally, students meet in their small groups and create their small group data sets by aggregating the data from each pair that comprises the group. Ultimately, their data are entered into a spreadsheet in which to visualize their partner data set (grouped bar chart, Figure 1H) and their group data set (Figure 1I). If the plant species in the small group were chosen based on shared evolutionary history, then the spreadsheet can also include an annotated phylogenetic tree with phylogenetic distances between species pairs obtained from the literature or databases (opentreeofflora.github.io, treebase.org; example template provided in the
Since PLANTEC is based on scientific teaching principles, it is critical to allow students time and liberty to be creative about data analysis and interpretation, while being guided by the instructor to make scientifically correct inferences. We allow several class meetings for data analysis and interpretation so that students become familiar with their data and how to use it to address their research objectives. The science communication component of PLANTEC provides an opportunity for each pair of students to showcase their interpretations as they prepare a scientific abstract, give a presentation, and write a miniature scientific manuscript. Guidelines in the handout describe how the activities in the module align with the segments of their abstracts, presentations, and manuscripts, thereby providing a framework within which students can initiate their writing. Grading rubrics for each assignment are also included in the handout. While running PLANTEC in 2018 and 2019, we used several sessions for in-class writing.

**HAZARDS**

PLANTEC involves only a few hazards, including organic solvents, applying gentle pressure to glass rods in glass test tubes, and, potentially, bioactive plant natural products (allergens, etc.). Students wear safety glasses, safety gloves, long pants, and close-toed shoes; work in fume hoods; and are warned about hazards and instructed about safety protocols before the extraction and TLC separations (see the Supplementary Instructor Material in the Supporting Information for a point-by-point list).

**RESULTS AND DISCUSSION**

Our primary objective was to develop a laboratory module that leverages the interdisciplinary nature of medicinal plant chemistry to help students develop core competencies. Here, we discuss learning goals associated with the module’s activities and how they address these competencies (illustrated in Figure 1). The integrative nature of the data collected makes it possible for the instructor to encourage students to focus on particular relationships for detailed analysis and thus tailor the module to the themes of the course in which it is deployed.

**Logical Thinking and Analysis of Text and Data**

One of the first learning goals in the module is for students to conduct background research on their plant species so that they can describe several ecological, biochemical, or medicinal functions of its natural products. This exercise requires students to conduct independent research and relate that to other material taught in the course (i.e., lessons prior to the module). In “Plants in Human Medicine”, the medicinal plant chemistry course in which we ran PLANTEC, students often describe how a plant’s chemicals might function in plant defense or a system of traditional medicine. In PLANTEC, students also analyze an assigned scientific article describing a study that is similar to the one they carry out. They should be able to, for example, identify and articulate the functions of key sections of the article—the research objective, methods used, as well as the main results and their key implications. Students should also be able to think logically about their partner data, for example, be able to calculate and explain the meanings of the Rf values and retention times of the compounds on their TLC plates and in their GC–MS chromatograms in relation to the resolved chemical structures and properties. Building on this, students should be able to connect the concepts of polarity and boiling point such that they can make reasonable predictions about correspondence between bands on their TLC plate and peaks in their GC–MS chromatograms (Figure 2, upper portion of "Chemistry").

**Interdisciplinary and Systems Thinking**

Medicinal plant chemistry is a highly integrative field requiring multifaceted thinking about biological systems and thus provides an excellent framework for learning goals involving interdisciplinary thinking and integration of information into a systems context. In analyzing their data, students must use logical thinking to discover the connections between the information they found in their background research and their results concerning their species’ natural products. For example, a student pair found that the carrot root epidermis, but not root cortex, contains known antifungal compounds (Figure 2, upper portion of "Biology"). With guidance, students explained this pattern in terms of the processes operating the plant root–soil system, namely, natural selection favoring defense of resources stored in roots in the soil environment where there may be pathogenic soil fungi. Moreover, students should be able to connect this concept with plant life histories, namely, that wild carrot plants are biennials with tap roots that must survive for two seasons if the plant is to reproduce, further underscoring the importance of protecting the root from fungal pathogens. By using the Socratic method during the multiday data analysis and interpretation working sessions, instructors can help students think independently and critically about their data by guiding them as they identify novel insights and connections between their chemical measurements and the potential biological functions of those chemicals.

PLANTEC provides many possibilities for connecting concepts in chemistry, biochemistry, and/or biology (including ecology, evolution, or physiology). Consider the example of a small group identifying that one set of plant species had terpenoid chemicals on its leaf surfaces, whereas a very closely related set of species bore fatty acid derivatives on its leaf surfaces (Figure 2, central annotated phylogeny). The group should be able to describe, for example, differences in
biochemical systems that might underlie this difference: that the relative activities of fatty acid and triterpenoid biosynthetic pathways in the epidermal cells of these two groups of species may have diverged (Figure 2, “Biochemistry”). With instructor guidance, they should also be able to place these findings in an evolutionary context, for example, by quantitatively determining whether more closely related species have more similar chemistries by regressing chemical similarity against the phylogenetic distance between each pair of species (Figure 2, “Biology”). Students could also place their findings in ecological and biogeographic contexts by considering the habitats, climates, and geographic ranges that these plant species occupy. Students could also highlight their findings in a chemical context, comparing fragmentation patterns of compounds from different chemical classes (Figure 2, “Chemistry”). Though the amount of instructor guidance needed for students to appreciate the multifaceted nature of their data sets is initially high, interpreting a single data set in the light of multiple disciplines is an excellent means by which to develop student competencies in interdisciplinary and systems thinking.

Oral and Written Communication of Science

While it is imperative that scientists develop finely honed communication skills so they may both share information with one another and engage in public discussions about the real-life problems that science endeavors to solve, effective communication is an important life skill, regardless of profession. Accordingly, PLANTEC includes several learning goals related to oral and written communication. The first two relate to the comprehension of information in scientific articles. Students should be able to concise and accurately describe the key features of the article in a written summary, and then describe and evaluate these key features in a classroom discussion. When communicating about partner and group data sets, each student should be able to compose a scientific abstract that accurately describes the key elements in their own study. As partners, they should be able to deliver a short, conference-style oral presentation that communicates to the class the conceptual framework and objectives of their research, how they addressed these objectives, and their findings and interpretations in a way that sparks questions from the audience because it creates a “story” with a clear, logical flow and scientifically valid, well-justified conclusions. To help create an atmosphere resembling professional scientific meetings, we run the class presentations as a student-focused Medicinal Plant Chemistry Symposium featuring abstract booklets and invite course alumni and other department members to attend. Using feedback from the instructors and the questions posed during the symposium to enhance their interpretations, each student should be able to prepare a miniature scientific manuscript that describes their study and elaborates their findings and conclusions in clear and concise language. In this way, these science communication assignments build on each other and provide students an opportunity to develop their understanding of the logical development of an argument, clear presentation of ideas, and how to draw justifiable conclusions. Some students initially found this assignment difficult since it was different from the more formulaic lab reports they had written for traditional “canned” laboratory experiences. However, they were also excited and motivated by the notion that they could focus on putting their creative and independently developed ideas and interpretations into their own words in the format of a scientific article. Using the article in the prereading assignment as a guide during this stage helped create a feeling of “coming full circle” to this last assignment in the module.

Teamwork and Collaboration

Nearly all professions involve teamwork and collaboration, and so it is important that higher education help develop these skills. This is especially true in STEM fields, as research becomes more interdisciplinary, and different types of advances are facilitated by collaborative teams of various sizes. Several PLANTEC learning goals address this core competency. First, students work in pairs to develop research objectives and then deliberate and identify which of the objectives are feasible given the measurement tools at their disposal. Working in small groups, students should be able to describe to one another their ideas for how an expanded data set may enable new objectives to be addressed and then evaluate which objectives can be met by the single pair’s data alone versus require the aggregated data from the small group. When interpreting data, partners should compare the chemical profiles from their two species, explain to each other how the profiles differ or are similar, and discuss possible ways in which the comparison could be interpreted in different contexts (e.g., chemical, biochemical, biological, etc.). We have watched PLANTEC foster many dynamic interactions among students

Figure 2. Schematic illustrating how plant chemical data sets can be interpreted in diverse contexts. The annotated phylogenetic tree in the center of the wheel uses colored circles to indicate the distribution of six chemical compounds or classes across eight species. It is an example of a small group data set that was generated during one implementation of this module. The color and position of each circle indicate the species in which that particular chemical was found. These data can be interpreted from chemical, biochemical, and biological perspectives, indicated by the colored, truncated rings. For example, a student could discuss correlations between phylogenetic relatedness and chemical relatedness (biological perspective), relationships between TLC bands and particular GC–MS peaks (chemical perspective), and/or how the distribution of chemicals across the phylogeny may relate to divergent underlying biochemicals (biochemical perspective).
in the small groups, including peer instruction, independent online research, considerable deliberation of group findings, and discussions with arguments and counterarguments as to their interpretations and conclusions of what their findings mean. These interactions reinforce student ownership of their own learning and promote the metacognitive skills that are a hallmark of lifelong learners. Finally, when presenting as partners in the student-focused Medicinal Plant Chemistry Symposium, students develop skills to logically organize and clearly present their research, and their dynamic small group interactions give them the knowledge and confidence to effectively answer impromptu questions about their work.

**SETTING, ASSESSMENT, AND CHALLENGES**

We administered PLANTEC over six 50 min class sessions during each fall semester of 2018 and 2019 in “BIOS 368: Plants in Human Medicine”, a midlevel undergraduate lecture course on medicinal plant chemistry with average annual enrollment of 20 students at the University of Nebraska—Lincoln, USA. We assessed student achievement of core competencies and learning goals, as well as the effectiveness of the module, in two ways. First, anonymous student surveys showed (i) that the learning goals associated with each section of the module were clear to the students, (ii) that PLANTEC increased student confidence in analyzing, interpreting, discussing, and writing about new kinds of data and complex ideas, and (iii) that the module increased student interest in medicinal plant chemistry (Figure 3). Second, we assembled excerpts of student writing from two assignments, the scientific article summary and the mini scientific manuscript, and analyzed these with respect to their corresponding grading rubrics, which align with the core competencies in Figure 1A (Supplementary Instructor Material, “Assessment”, in the Supporting Information). In summary, we found that most students could clearly communicate their science, achieved competency in basic analyses of text and data, and were able to collaborate to achieve new insights. Interdisciplinary and systems thinking skills were the most challenging core competencies to achieve, but many students displayed these skills by the end of the course, for example, by integrating plant chemical profiles with knowledge of plant biology to interpret their small group data sets with respect to potential functions of the molecules they detected.

Students consistently found several components of the module challenging, in part because many students seem accustomed to undergraduate lab activities with predetermined outcomes of which they are often aware, whereas PLANTEC’s formative and summative assessments are more open-ended and engage students’ creative scientific reasoning and capacity to draw on diverse knowledge. First, students initially found it challenging to use observational data to address research objectives. It was therefore difficult for them to understand that, depending on the particular emphasis of the research objective, there could be multiple “correct answers”, and that the “most correct answer” is determined by the evidence in the data and their own scientific rationale and justification. For research questions defined by specific testable hypotheses, students also struggled to figure out ways to analyze or present data so as to test specific hypotheses. This was not simply a matter of statistical knowledge (which was not expected of students), but rather identifying the type of graph or depiction of the data that would represent predictions from the hypothesis. We focused on the scientific reasoning and linkage between hypotheses, predictions, and data, guiding them in how to produce that data depiction, and, after that, instructed them how to test it statistically. A related challenge was encountered with what students considered to be open-ended formats for assignments. Students overcame this challenge when guided with concrete examples of how they could present different kinds of information and what constitutes a scientifically logical flow of ideas in the context of professional scientific processes and papers, which are the main assessment formats used in PLANTEC.

The above examples highlight how PLANTEC challenges students to independently engage in creative and logical thinking and analysis in order to discover unknown outcomes and interpretations on their own. In overcoming all these challenges, we consistently found that using the Socratic method was highly effective because it encourages students to learn to take responsibility for their own learning and develop the skills to reason through problems independently. After students overcome the initial intellectual hurdle of understanding, they (not the instructor or the lab activity) are in control of discovering what messages the data convey. We consistently observed that students were enthusiastic and eager to embrace the challenge of exploring their data in new ways. This maturation in the way students approach knowledge and its creation is one of the key mechanisms for enhancing the core competencies that PLANTEC seeks to develop.

**CONCLUSIONS**

PLANTEC provides a framework for teaching undergraduates to think outside of the confines of narrow disciplines, which is fundamental to developing core competencies that provide translational skills for diverse career paths. A key strength of PLANTEC lies in having an overarching conceptual framework that simultaneously accommodates and integrates across a wide range of disciplines, from plant biochemistry, to herbal medicine, to ecology and evolution. Based on its success in the
classroom, PLANTEC will help educators meet the needs for an innovative and versatile STEM workforce of the future and may also serve as a model for the development of similar scalable laboratory modules that can be flexibly implemented in online and in-person classroom settings.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available at https://pubs.acs.org/10.1021/acs.jchemed.0c00395.

Supplementary Student Material and Supplementary Instructor Material (PDF, DOCX)

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Notes
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