Supplementary Material

for

Integrating disciplines in a plant chemistry laboratory module to enhance interdisciplinary and scientific thinking in undergraduate students

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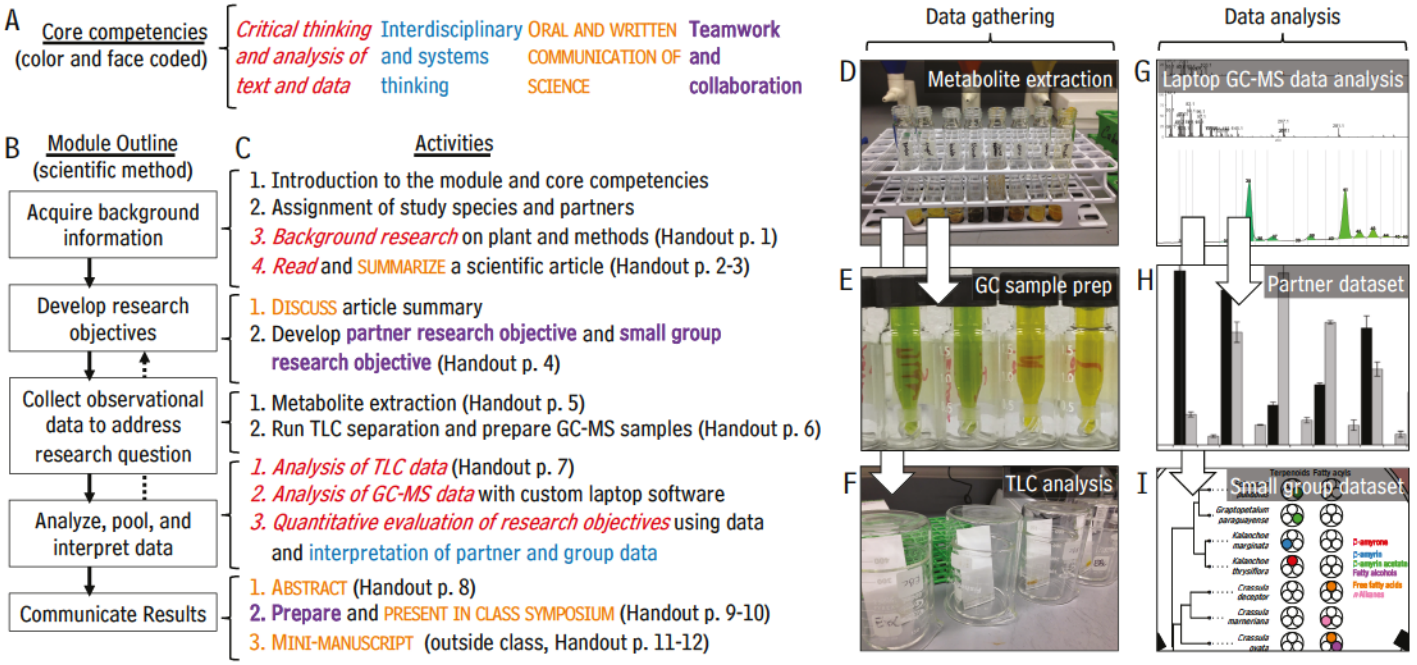
Contents:

Supplementary Student Material

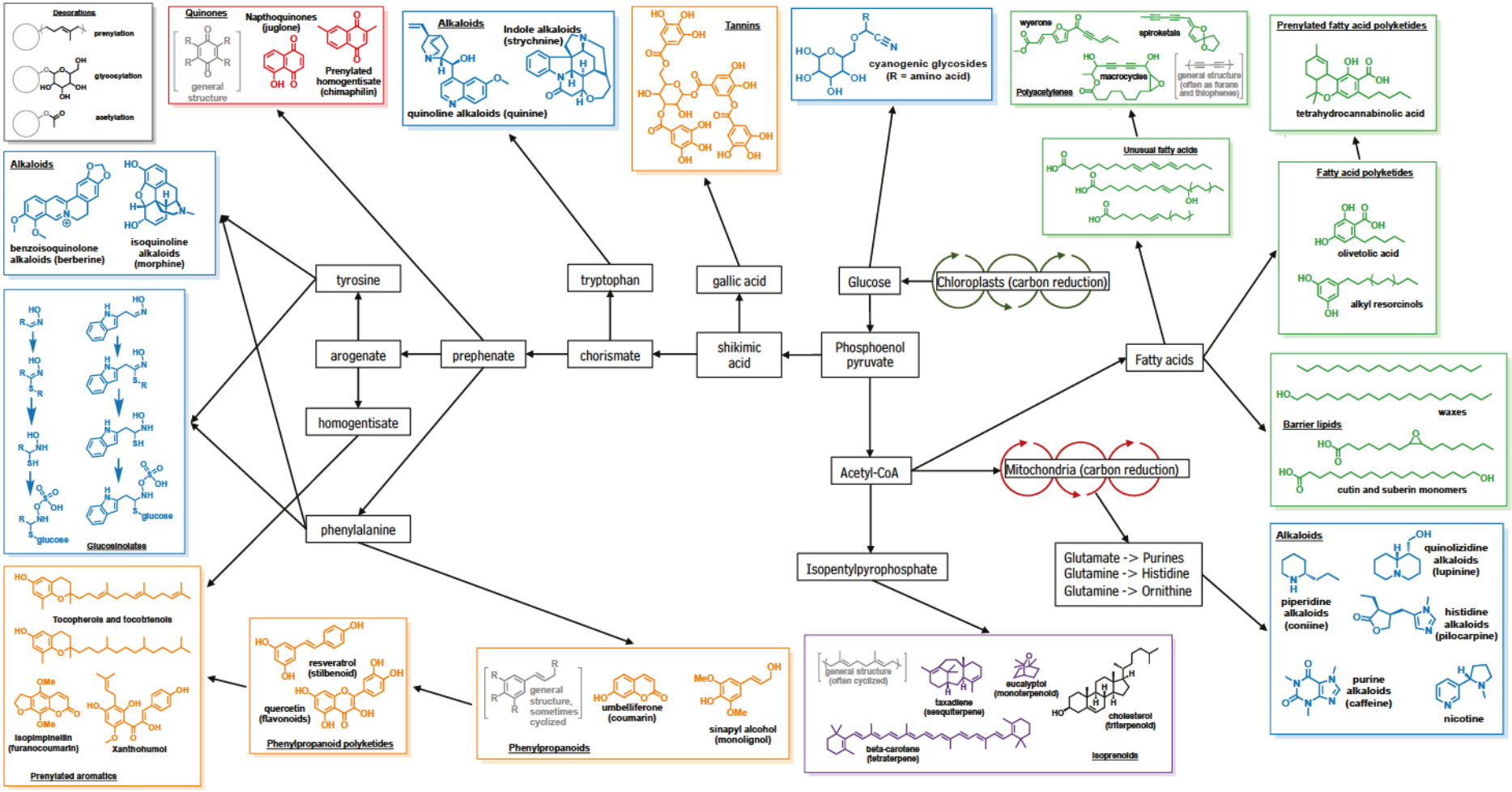
Contents:

Core Competencies, Module Outline, and Illustration of Data Gathering and Data Analysis Procedures



Core competencies, PLANTEC module outline, and illustrated activities. A List of student core competencies targeted for development. **B** Module outline (based on the scientific method). **C** Activities in each module segment are color and font face coded according to the core competency with which they are related. Pictures illustrating the data gathering process of **D** extraction, **E** gas chromatography-mass spectrometry sample preparation, and **F** thin layer chromatography, and data analysis, including **G** laptop-based gas chromatography-mass spectrometry data analysis, as well as analysis and interpretation of **H** partner and **I** small group datasets.

Schematic of plant specialized metabolism



Background Research Guide

Partners: Write down your and your partner's name and the species each of you will be working on:

Name:_____, Species, plant structure:_____

Name:_____, Species, plant structure:_____

Background Research: perform background research on your species and answer the following questions:

Describe visual appearance of your plant and the plant structure you will analyze:

_____

Is this a wild or domesticated plant?

_____

To what organ does the structure belong?

_____

What is the function of that organ for the plant?

_____

What kind of stressors could this organ encounter in its environment?

_____

Is this plant used in traditional medicine? Does it have other documented human uses?

_____

_____

_____

Additional observations:

_____

_____

_____

Instructions for Scientific Article Summary [graded]

Research articles use a standard format to clearly communicate information about a scientific study. A research article usually has seven major sections: Title, Abstract, Introduction, Methods, Results, Discussion, and References. **The length of your Article Summary should be 500-1000 words.**

Reading the Article: Allow enough time. Before you can write about the research, you have to understand it. This can often take a lot longer than you think! Only when you can clearly explain the study in your own words to someone who has not read the article are you ready to write about it. Scan the article first. If you try to read a new article from start to finish, you may get bogged down in detail. Instead, first try to find the main points.

1. Briefly look at each section to identify:
   - the objective of the study: a hypothesis, research question, or reason for the study (usually stated in the Introduction)
   - how the objective was met (Methods)
   - the findings (Results, including tables and figures)
   - how the findings were interpreted in light of the objective (Discussion)

2. Read actively: underline key sentences or write the key point (e.g., objective, finding 1, finding 2, etc.) of each paragraph in the margin. Although the abstract can help you to identify the main points, you cannot rely on it exclusively because it contains very condensed information. Take notes in your own words. Forcing yourself to summarize points in your own words will reveal whether you understand the article. If you find yourself sticking closely to the original language in the article and making only minor changes to the wording, then you probably do not understand the article.

3. After you have highlighted the main points, re-read each section. As you read, ask yourself these questions:
   - How does the design of the study address the objective?
   - How convincing are the results? Are any of the results surprising?
   - What does this study contribute toward meeting the original objective?
   - What aspects of the original objective remain unmet?

Writing the Summary: Like an abstract in a published research article, the purpose of an article summary is to give the reader a brief overview of the study. To write a good summary, identify what information is important and condense that information for your reader. Address these topics in your 500 - 1000 word summary:

1. Look in the introduction section and find the objective of the paper. Restate it in your own words.
2. Why is this objective important? What will we learn from this study?
3. Briefly describe the methods: experimental design, materials, procedure, what was manipulated, what was measured, and how data were analyzed.
4. Describe the main results. What do you think were the most important things the study found?
5. Explain the key implications of the results. Did they meet their objective? What new insights do the authors say are contributed by this study?

Grading Rubric for Scientific Article Summary

Level of Achievement	Objective	Methods	Results and Implications
Exemplary	• Effectively identifies the objective from the article • Accurately restates the objective in own words. • Identifies importance of the objective using other information from the introduction as context. • Clearly states what will be learned if the objective is met. • Uses excellent writing style and grammar.	• Accurately and concisely describes methods in own words. • Identifies key manipulations or experimental treatments • Accurately describes how data were analyzed using clear language.	• Accurately and concisely describes major results and distinguishes from minor results. • Identifies if research objective was met. • Describes major new insights the authors claim in own words. • Uses excellent writing style and grammar.
Adequate	• Somewhat effectively identifies the objective from the article • Reasonably accurately restates the objective in own words. • Touches on importance of the objective • Clearly states what will be learned if the objective is met. • Uses acceptable writing style and grammar.	• Somewhat accurately and concisely describes methods in own words. • Identifies some key manipulations or experimental treatments • Accurately describes how data were analyzed using moderately clear language.	• Somewhat accurately and concisely describes major results and distinguishes from minor results. • Identifies if research objective was met. • Somewhat reasonably describes major new insights the authors claim in own words. • Uses acceptable writing style and grammar.
Needs Improvement	• Does not identify the objective from the article • Does not restate the objective in own words. • Does not identify importance of the objective using other information from the introduction as context. • Is unclear about what will be learned if the objective is met. •Fails to use acceptable writing style and grammar.	• Describes methods poorly or not in own words. • Fails to identify key manipulations or experimental treatments • Fails to accurately describes how data were analyzed.	• Accurately and concisely describes major results and distinguishes from minor results. • Identifies if research objective was met. • Describes major new insights the authors claim in own words. • Uses poor writing style and grammar.
No Answer (0 pts)			

Template for Research Objectives

Based on your answers to the questions above, formulate a research objective for you and your partner:

*** After collecting and analyzing your data. Based on your results, did you meet your research objective? Explain.

Based on your answers to the questions above, formulate a research objective for your small group:

*** After obtaining the small-group dataset. Based on your results, did the class meet their research objective? Explain.

Example Protocols for Plant Metabolite Extraction and TLC Separation

Example 1: Leaf Terpenoids:

1. Place a small amount of your structure in an extraction tube.
2. With a Pasteur pipette, add 1 ml heptane to the tube, grind with a glass rod or with the electric homogenizer
3. With a Pasteur pipette, add 1 ml water to the tube, cap, vortex, then centrifuge for 4 min. Top layer is the extract.
4. Obtain a GC-MS vial and write your name on it. Cover your writing with a small piece of scotch tape. CAREFULLY fill the vial with a small amount of your extract using a Pasteur pipette.
5. Prepare a TLC plate by GENTLY making lines 1 cm across its width from its the top and bottom with a pencil. Write group name and species name above the top line.
6. Perform the following steps in the hood:
   6.1. Load your extract onto the bottom line of the thin-layer chromatography plate using a capillary tube
   6.2. Prepare a TLC development chamber with about 10 ml 2:1 heptane:ethyl acetate
   6.3. Develop plates using the using a 2:1 mixture of heptane:ethyl acetate until the solvent reaches the top line.
   6.4. Remove the plate from the chamber and set it on the pencil in the hood to dry.
   6.5. Spray the plate with primuline mixture and set it on the pencil in the hood to dry.

Example 2: Root Metabolites:

1. Place a small amount of your structure in an extraction tube.
2. In the hood, use a Pasteur pipette to add 1 ml of ethyl acetate to the tube, then grind with the electric homogenizer
3. Add 1 ml saturated sodium chloride, cap, vortex, then centrifuge for 4 min. The top layer is your extract.
4. Obtain a GC-MS vial and write your name on it. Cover your writing with a small piece of scotch tape. CAREFULLY fill the vial with a small amount of your extract using a Pasteur pipette.
5. Prepare a TLC plate by GENTLY making lines 1 cm across its width from its the top and bottom with a pencil. Write group name and species name above the top line.
6. Perform the following steps in the hood:
   6.1. Load your extract onto the bottom line of the thin-layer chromatography plate using a capillary tube
   6.2. Prepare a TLC development chamber with about 10 ml 2:1 heptane:ethyl acetate
   6.3. Develop plates using the using a 2:1 mixture of heptane:ethyl acetate until the solvent reaches the top line.
   6.4. Remove the plate from the chamber and set it on the pencil in the hood to dry.
   6.5. Spray the plate with primuline mixture and set it on the pencil in the hood to dry.

Example 3: Epicuticular Wax Blooms:

1. Go with the instructor to the greenhouse, find the species you have been assigned
2. Use the provided qtip to gather five 2-inch streaks-worth of epicuticular wax bloom and place the qtip in the provided extraction tube.
3. In the fume hood, add 1 ml chloroform to the tube and spin the qip in the chloroform. Discard the qtip in the waste beaker. The tube contains your extract.
4. Obtain a GC-MS vial and write your name on it. Cover your writing with a small piece of scotch tape. CAREFULLY fill the vial with a small amount of your extract using a Pasteur pipette.
5. Prepare a TLC plate by GENTLY making lines 1 cm across its width from its the top and bottom with a pencil. Write group name and species name above the top line.
6. Perform the following steps in the hood:
   6.1. Load your extract onto the bottom line of the thin-layer chromatography plate using a capillary tube
   6.2. Prepare a TLC development chamber with about 10 ml 2:1 heptane:ethyl acetate
   6.3. Develop plates using the using a 2:1 mixture of heptane:ethyl acetate until the solvent reaches the top line.
   6.4. Remove the plate from the chamber and set it on the pencil in the hood to dry.
   6.5. Spray the plate with primuline mixture and set it on the pencil in the hood to dry.

Data Table for TLC Results

In the table below, record the characteristics of all the bands (or at least the most prominent 10) on your TLC plate that you outlined in pencil.

TLC Data Table

Band #	R_f (see formula)	Color (naked eye)	Color (with stain)	Compound ID*	Rationale for ID*

* To be filled out once GC-MS analysis is complete

R_f = (distance from origin to band) / (distance from origin to solvent front)

Tape your TLC plate here!

Data Table for GC-MS Results

Once you have completed your analysis, transfer the peak data to the table below and fill out the summary statistics at the bottom of the page:

Genus: _____, species: _____, structure: _____

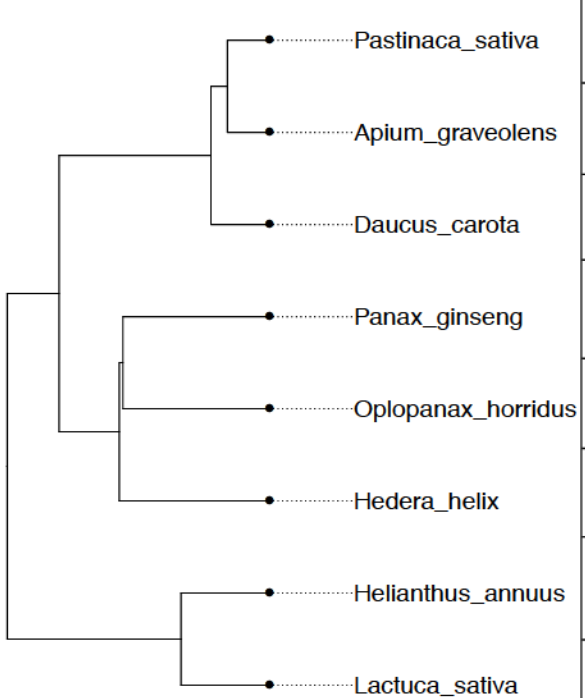
Peak Table (first line is an example based on the previous page's analysis; you can use one line for each of your peaks)

Retention Time (peak start)	Compound ID	area
311.587	(not identified)	1301332
331.659	(not identified)	1158949
356.19	(not identified)	2839577
377.005	Caryophyllene	28039250
410.457	(not identified)	16101018
440.193	Abietic acid	177092628
473.645	(not identified)	7203188
508.584	(not identified)	2047128
594.816	(not identified)	3865352

Total peak area: _____ (in example, 239648422)
Percent peak area identified: _____ (in example, 85%)

Example Quantitative Data Table for Small Group GC-MS Results

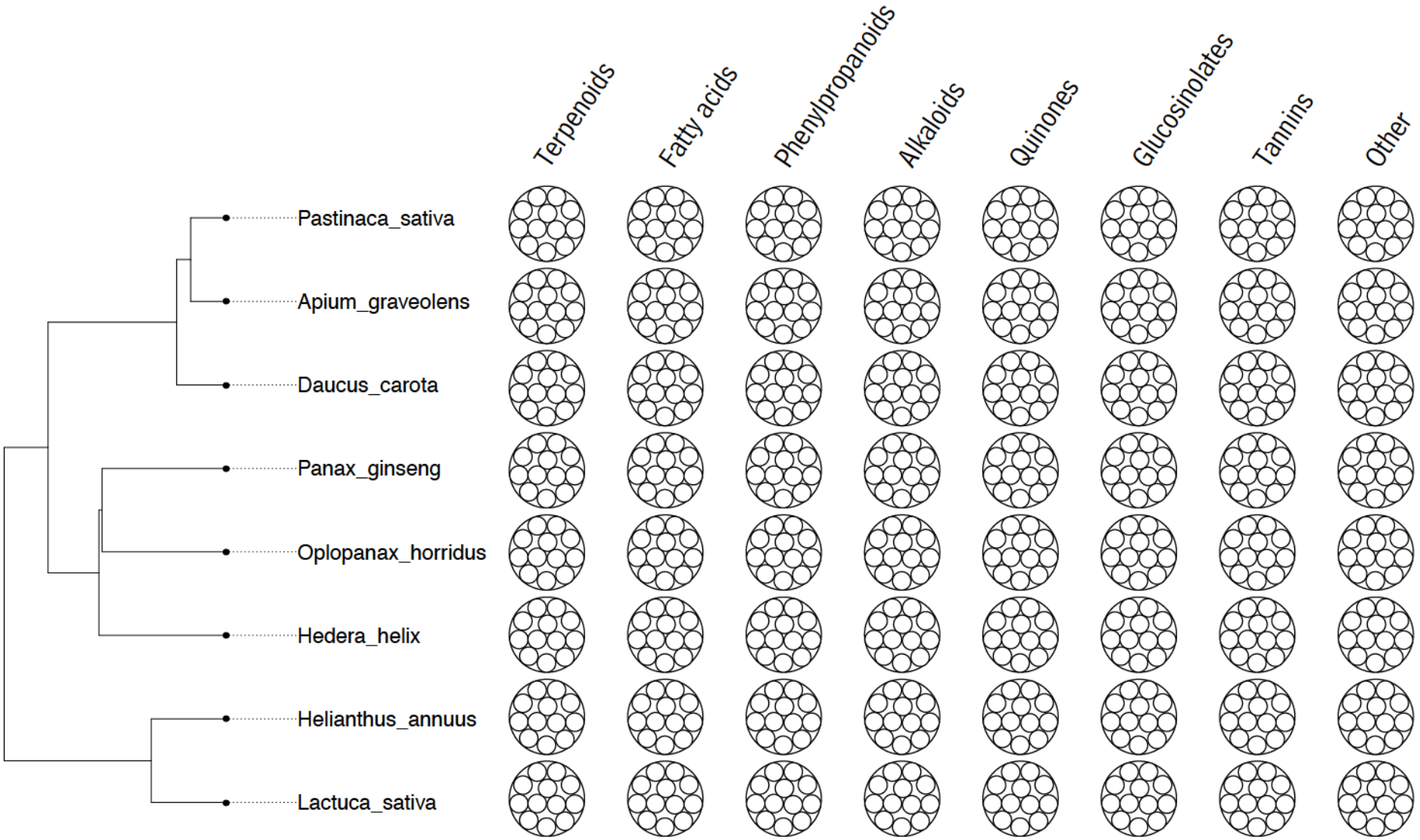
Get together in your small groups and share your GC-MS data with one another. You could all enter your data into a shared spreadsheet, or use the template below. In your data table, each row should correspond to a unique plant species or tissue, and each column to a unique plant chemical. Each cell should indicate the relative abundance of each chemical in each plant species or tissue. Row 1 in the table below contains an example.



"Tissue" extracted	Caryophyllene	Abietic acid																			
Example	13.6	86.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Example Qualitative Data Table for Small Group GC-MS Results

Your Quantitative Data Table for Small Group GC-MS Results (above) documents results at the compound level and in a quantitative way. In this table, you should document your results in a qualitative way (i.e. presence/absence) and at both the compound and class level. Your instructor will show you an example.



Instructions for Scientific Abstract [graded]

The abstract of a scientific paper or a scientific presentation is a short summary of the entire study. It includes the motivation or reason for conducting the study, what the hypothesis was, how the hypothesis was tested, what the results were, how the results are interpreted in light of the hypothesis (does it support or refute the hypothesis?), and a concluding sentence as to the general contribution or importance of the study. See *How to do Ecology* page 94. Since abstracts are short (yours should be no more than 400 words long), they must be concise and use language efficiently! The abstract, a title for your presentation, and the list of authors (i.e., your and your partner's name), is **due by the date specified on the course schedule (emailed to Dr. Russo)**.

Your abstract should be about your and your partner's work/measurements, not the group's results.

Purposes of an Abstract

(1) Informs readers about the article's content
(2) Summarizes complex information in a clear, concise manner
(3) Helps readers decide whether or not to read the article
(4) Used in conferences to summarize what the speaker will say during his/her presentation

Characteristics of an Effective Abstract (and grading criteria)

(1) Uses one or more well-developed paragraphs, which are unified, coherent, concise, and able to stand alone
(2) Usually about 200-500 words (yours should be no more than 400 words)
(3) Uses an **introduction-body-conclusion structure** in which the parts of the paper are discussed in order:
    a. **Introduction (1-2 sentences)**: Describe the topic, the motivation, and overall purpose of the research (Why is this research interesting and important? What gap in our knowledge does it fill?)
    b. **Research Objective (1-2 sentences)**: Specific research objective, and potentially hypotheses/predictions
    c. **Methods (1-2 sentences)**: Very concise overview of the methods used to address the research questions
    d. **Results (1-3 sentences):** Describe the major results (what you found) and interpretation of the results (what the results mean)
    e. **Conclusions (1-2 sentences):** Synthesizes the major contributions of the study in light of the new information provided by the results of the study – what does it all mean? Why is the result important, What did we learn in the bigger picture from doing this study?)
(4) Can stand-alone - the abstract can be understood without reading the paper
(5) Can be understood by a wide audience.

Grading Rubric for Scientific Abstract

Level of Achievement	Introduction and / Hypothesis	Results and Interpretation
Exemplary	•Addresses the question explicitly and comprehensively. •States a relevant and justifiable answer. •Presents arguments in a logical order and provides a synthetic, coherent answer. •Uses good writing style and grammar.	•Demonstrates an accurate and complete understanding of the question. •Provides interpretations and conclusions that are supported by relevant evidence and information. •Uses multiple ideas, examples, or arguments that support the answer.
Adequate	•Does not address the question explicitly, but does so tangentially. •States a reasonably relevant and justifiable answer. •Presents arguments in a logical order and is reasonably coherent. •Uses acceptable writing style and grammar.	•Demonstrates accurate but only adequate understanding of question •Provides interpretations and conclusions that are only weakly supported by relevant evidence or information. •Uses few ideas, examples, or arguments to support the answer.
Needs Improvement	•Does not adequately address the question. •States little or no relevant answers. •Indicates misconceptions. •Is not coherently or logically organized. •Fails to use acceptable writing style and grammar.	•Does not demonstrate accurate understanding of the question. •Does not provide interpretations or conclusions. •Does not provide evidence to support their answer to the question.
No Answer (0 pts)		

Instructions for Medicinal Plant Chemistry Student Symposium Presentations [graded]

Each student pair will give a 7-minute presentation on their results in the Medicinal Plant Chemistry Student Symposium, followed by 2 minutes of questions and discussion by class members. Each pair will work together to collaboratively write the presentation. The structure of your presentation should be similar to the structure of your Scientific Abstract, but with verbal elaboration as well as figures and diagrams. A good guideline for presentation length is to plan to spend an average of about one minute per slide (some slides with require more time, some less). Each group will send one Powerpoint or PDF file to the instructor prior to their presentation date. Check the Class Schedule for the due date.

Presentation Contents

The primary focus of your manuscript should be your and your partner's work/measurements, not the group's results. However, you should include the Quantitative Data Table for Small Group GC-MS Results and Qualitative Data Table for Small Group GC-MS Results in your results section and interpret your and your partner's results in the context of the group's results.

You should include the following sections:

Introduction
Research Objective
Methods
Results
Interpretation
Conclusion

For information about each section, see the descriptions below in the Instructions for Miniature Scientific Manuscripts section. Addressing each point of those instructions in your presentation will probably take more than 7 minutes, so you should choose which points are the most important and focus on those.

Delivery

One portion of your grade will be determined by the quality of the delivery of your presentation. You should hold the attention of entire audience with the use of direct eye contact, seldom looking at notes. You should speak with fluctuation in volume and inflection to maintain audience interest and emphasize key points. When showing figures that contain data, you should explain them clearly: what do the axes mean? What does each point, line, or bar mean? What do colors correspond to? Etc.

Formatting and Figures

A checklist for your presentation's format is below. Presentations not conforming to this checklist lose 1/3 of a grade (for example, a paper receiving a B+ grade would drop to a B grade).

☐ 7 minutes long + 2 minutes for questions
☐ Numbered slides
☐ Maximum two figures containing data per slide
☐ Text and figures should be LARGE, filling the entire slide so people in the back of the room can clearly see them.
☐ There should be at least one picture on every slide (though it does not necessarily need to contain data)

Grading Rubric for Medicinal Plant Chemistry Student Symposium Presentations

Level	Intro	Research Objective	Methods	Results	Interpretation	Delivery
Exemplary	Begins with relevant attention-grabbing statement/image/information of general appeal Justifies importance of the research topic in accessible terms Introduces the study species in the context of the introduction	The research objective is obviously relevant to the topic in the introduction Research objective is clear and achievable given the tools at hand The research objective encompasses all the experiments/data that follow	The methods used are presented efficiently and in enough detail to understand and evaluate the results.	All TLC and GC-MS results (bands and their properties, chromatograms, compounds and their structures) from both plant species are presented in a concise format Results are verbally described accurately and succinctly	Results are accurately interpreted from multiple points of view – chemistry, biochemistry, and biology. The research objective is evaluated in the discussion/interpretation The presentation ends with a very short description of the meaning of the results/interpretation in the context of the introduction	Holds attention of entire audience with the use of direct eye contact, seldom looking at notes Speaks with fluctuation in volume and inflection to maintain audience interest and emphasize key points
Adequate	Initial attention-grabbing statement/image/information is irrelevant, difficult to understand, or esoteric Importance of the research topic is mentioned but unclear Study species are mentioned, but not in the context of the introduction	The research objective is only slightly relevant to the topic in the introduction Research objective is somewhat clear and partially achievable given the tools at hand The research objective encompasses only some of the experiments/data that follow	The methods are either presented in far too much detail, or so briefly that the audience is unable to evaluate the quality or validity of the results	Only some TLC and GC-MS results (bands and their properties, chromatograms, compounds and their structures) from both plant species are presented Results are verbally described but not accurately or succinctly	Results are accurately interpreted from multiple points of view – two of chemistry, biochemistry, and biology. The research objective is poorly evaluated in the discussion/interpretation The bearing of the results/interpretation on the introduction material is only tangentially mentioned	Consistent use of direct eye contact with audience, but still returns to notes Speaks with satisfactory variation of volume and inflection
Poor	No initial effort to grab audience attention Importance of the research topic is not mentioned Study species are not mentioned	The research objective is not relevant to the topic in the introduction Research objective is not clear and not achievable given the tools at hand The research objective is not entirely relevant to the experiments/data that follow	Methods are not presented.	TLC and GC-MS results (bands and their properties, chromatograms, compounds and their structures) are unclear or incomplete Results are poorly verbally described	Results are interpreted from only one points of view – two of chemistry, biochemistry, and biology. The research objective is not evaluated in the discussion/interpretation The presentation ends without returning to the information in the intro	Displays minimal eye contact with audience, while reading mostly from the notes Speaks in uneven volume with little or no inflection

Instructions for Miniature Scientific Manuscripts

You will write a scientific paper based on your data and your small groups data. Your paper will include the following sections: Abstract (you already wrote this), Introduction, Methods, Results, Discussion, and Conclusion. See for a description of these sections. We will also discuss these sections in class as well as other information you can include in your manuscript. You will first write a draft that will not be graded. We will return some comments to you on your Draft so that you can improve it for the final version. Check the course schedule for the due dates. Each student in the pair will include the same data in his/her papers (that is, the data that the pair collected), but each student must write his or her own draft and his or her own final paper in his or her own words. Although the draft paper is not graded, if it does not represent a reasonable effort, then one letter grade will be deducted from the final paper grade.

Miniature Manuscript Contents

The primary focus of your manuscript should be your and your partner's work/measurements, not the group's results. However, you should include the Quantitative Data Table for Small Group GC-MS Results and Qualitative Data Table for Small Group GC-MS Results in your results section and interpret your and your partner's results in the context of the group's results.

Abstract: see Instructions for Presentation Abstract above

Introduction: The job of the Introduction is to prepare the reader by giving the reader sufficient background to understand the study as a whole. It therefore should only contain information pertinent to understanding the study and its broader significance. It should include:

- General background information about the topic that is necessary to understand the study's goals
- Introduction to your plant
- Clear, concise statement of the goals of the study ("The goal of this study was to...")

Materials and Methods: The job of the Materials and Methods is to describe how the study was performed and how the goals of the study were accomplished. Refer to the lab activity protocols that were handed out in class as needed.

- What you did: the steps you took, reagents and equipment used.
- What data you collected and how it was obtained (i.e. written down, obtained via internet or from other papers, etc.)
- How you analyzed the data

Results: The job of the Results section is to report the findings of the study. Do not interpret what the results mean; only report them It should include:

- Photo(s)/drawing(s) of TLC plates
- TLC band data table
- GC-MS data
    - Chromatograms
    - Mass spectra
    - Compound structures

Discussion (will require some literature research): The job of the Discussion section is to interpret the results of the study and explain what they mean in light of the goal of the study. In this way, the Introduction and the Discussion are like reflections of each other.

- What can we learn from the structures of the compounds in your plant? For example:
    - What biosynthetic pathways do your plant's compounds come from?
    - What do these compounds and pathways tell us about the kinds of metabolic investments the plant is making (i.e. defense, photosynthesis, storage of resources)?
    - Are there known functions for these compounds in your plant?
    - Are there known medicinal properties for the compounds your plant?

  - What are the major compounds from your plant that have been studied previously? What have these previous studies found?
  - Are any of these compounds missing from your GC-MS profile? If so, why do you think this might be?

- What can we learn from the compounds you and your partner found in the context of what the whole group found? For example:
  - Were some compounds found in all species studied by the group? What might this mean for the biology of these species?
  - Were some compounds only found in specific species among those studied by the group? What might this mean for the biology of these species?
  - Do the chemical results you obtained exhibit any other patterns across the phylogeny your group studied?
  - What do you think trends like those above might mean?
  - If you can't find any clear trends, how might a future study be designed such that any trends present could be uncovered?

Conclusions: The job of the Conclusions section is to convey a short statement of the take-home messages of your study. What are the most important things that you want the reader to remember from your study?

- What are the important take home messages from your study?
- What would be the next steps for further research?
- What could be improved about the way this study was conducted?

References Cited: See Instructions for the Presentation & Mini Scientific Manuscript for citation format for the in-text citations and the References Cited section.

- In-text citations: When you make a scientific statement or observation (usually in the Introduction, Discussion, or Conclusions) that is not "general knowledge" (ie, the Earth orbits the Sun) or not your own original work (ie, results or interpretation of the data in your paper), you must cite the source of that statement. For example: A person's diet influences the composition of their gut microbiome (David et al., 2014). This information is not general knowledge, and a study had to be performed to demonstrate that it is the case.
- The full citation for very study that you cite in your main text needs to appear in the References Cites section. Conversely, no study should be in the references cited section that is not cited in your main text. For example: David, L.A., Maurice, C.F., Carmody, R.N., Gootenberg, D.B., Button, J.E., Wolfe, B.E., Ling, A.V., Devlin, A.S., Varma, Y., Fischbach, M.A. and Biddinger, S.B. (2014) Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 505:559-561.

Formatting and Figures

You may use figures from other sources, such as molecular structures of chemicals; however, you must state specifically the source from which you obtained the figure and also cite the source appropriately in the references section.

A checklist for your paper's format is below. Papers not conforming to this checklist lose 1/3 of a grade (for example, a paper receiving a B+ grade would drop to a B grade).

☐ Double-spaced
☐ Numbered pages
☐ 12-point Times New Roman
☐ 1-inch margins
☐ 10-12 pages long, excluding references

Grading Rubric for Miniature Scientific Manuscripts

Level	Intro	Research Objective	Methods	Results	Interpretation	Writing
Exemplary	Begins with relevant attention-grabbing statement /information of general appeal Justifies importance of the research topic in accessible terms Introduces the study species in the context of the introduction	The research objective is obviously relevant to the topic in the introduction Research objective is clear and achievable given the tools at hand The research objective encompasses all the experiments/data that follow	The methods used are presented efficiently and in enough detail to understand and evaluate the results.	All TLC and GC-MS results (bands and their properties, chromatograms, compounds and their structures) from both plant species are presented in a concise format Results are verbally described accurately and succinctly	Results are accurately interpreted from multiple points of view – chemistry, biochemistry, and biology. The research objective is evaluated in the discussion/interpretation The presentation ends with a very short description of the meaning of the results/interpretation in the context of the introduction	Uses excellent writing style and/or grammar. Writing is clear in all sections.
Adequate	Initial attention-grabbing statement/information is irrelevant, difficult to understand, or esoteric Importance of the research topic is mentioned but unclear Study species are mentioned, but not in the context of the introduction	The research objective is only slightly relevant to the topic in the introduction Research objective is somewhat clear and partially achievable given the tools at hand The research objective encompasses only some of the experiments/data that follow	The methods are either presented in far too much detail, or so briefly that the audience is unable to evaluate the quality or validity of the results	Only some TLC and GC-MS results (bands and their properties, chromatograms, compounds and their structures) from both plant species are presented Results are verbally described but not accurately or succinctly	Results are accurately interpreted from multiple points of view – two of chemistry, biochemistry, and biology. The research objective is poorly evaluated in the discussion/interpretation The bearing of the results/interpretation on the introduction material is only tangentially mentioned	Uses acceptable writing style and/or grammar. Some sections contain writing that is not perfectly clear.
Poor	No initial effort to grab reader attention Importance of the research topic is not mentioned Study species are not mentioned	The research objective is not relevant to the topic in the introduction Research objective is not clear and not achievable given the tools at hand The research objective is not entirely relevant to the experiments/data that follow	Methods are not presented.	TLC and GC-MS results (bands and their properties, chromatograms, compounds and their structures) are unclear or incomplete Results are poorly verbally described	Results are interpreted from only one points of view – two of chemistry, biochemistry, and biology. The research objective is not evaluated in the discussion/interpretation The presentation ends without returning to the information in the intro	Uses poor writing style and/or grammar. Many sections contain writing that is not clear.

Supplementary Instructor Material

Scientific Article Suggestions for Pre-Reading Assignment

For the prereading article we used Berkov et al. 2008 during both the 2018 and 2019 implementations of PLANTEC. However, we suspect that any of the following articles, which rely on combined TLC and GC-MS analyses, would work well:

Busta, L.; Budke, J. M.; Jetter, R. Identification of β-Hydroxy Fatty Acid Esters and Primary, Secondary-Alkanediol Esters in Cuticular Waxes of the Moss Funaria Hygrometrica. *Phytochemistry* **2016**, *121*, 38–49. https://doi.org/10.1016/j.phytochem.2015.10.007 .

Berkov, S.; Bastida, J.; Nikolova, M.; Viladomat, F.; Codina, C. Rapid TLC/GC-MS Identification of Acetylcholinesterase Inhibitors in Alkaloid Extracts. *Phytochem Analysis* **2008**, *19* (5), 411–419. https://doi.org/10.1002/pca.1066 .

Li, X.; Teitgen, A. M.; Shirani, A.; Ling, J.; Busta, L.; Cahoon, R. E.; Zhang, W.; Li, Z.; Chapman, K. D.; Berman, D.; et al. Discontinuous Fatty Acid Elongation Yields Hydroxylated Seed Oil with Improved Function. *Nat Plants* **2018**, *4* (9), 711–720. https://doi.org/10.1038/s41477-018-0225-7 .

von Wettstein-Knowles, P.; Mikkelsen, J.; Madsen, J. Nonan-2-Ol Esters in Sorghum Leaf Epicuticular Wax and Their Collection by Preparative Gas Chromatography. *Carlsberg Res Commun* **1984**, *49* (7), 611. https://doi.org/10.1007/bf02907493 .

Grading and Student Participation

Consider making student participation and attendance part of their grade.

Selection of Plant Species

It is generally desirable for both students in a pair to study closely related species but, across each small group, that diverse plant species are represented from within the same plant family or order. We have had success with plants purchased at a local grocery store, including roots (carrot, turnip, celeriac, ginseng, ginger, turmeric, etc.) and culinary herb leaves (mint, basil, thyme, rosemary, etc.), as well as surface chemicals from succulents growing in our university's greenhouse. We suspect that commercial essential oils would work particularly well.

Extraction of Plant Metabolites

Though the exact materials required will depend on the type of plant tissue, the following are probably necessary: test tubes and tube racks, Pasteur pipettes and bulbs, organic solvents (hexane, heptane, ethyl acetate, or diethylether), and water or saturated sodium chloride. If working with highly rigid tissues like roots, we recommend the use of a lyophillizer and an electric homogenizer, though we have had success without such equipment. In general, we were successful when (i) extracting with heptane and washing with water or (ii) extracting with ethyl acetate and washing with saturated sodium chloride.

Developing Thin-Layer Chromatography Plates

For the thin-layer-chromatography analysis, the students will require TLC plates, pencils, capillary tubes, development chambers, and solvent for the mobile phase. We use small aluminum-backed plates, nested beakers as development chambers (Fig. 1F), and 2:1 v/v heptane:ethyl acetate as a mobile phase. For visualizing bands, we used a 1% v/v solution of primuline-saturated acetone in acetone and a UV light source. Students applied their extracts to the origin line on the

plate, developed the plates in the solvent chambers, then allow the plantes to dry in a fume hood, apply a UV-active stain, allow the acetone to evaporate in the hood, visualize the bands under UV light and mark their locations with a pencil, then store their TLC plate by taping it to a page in their handouts.

Developing Research Questions

Partner research objectives should be answerable with a single pair's dataset. Some examples for partner research objectives are:

- "Based on chemical profiles, to determine the specialized metabolic pathways that are active in species A and species B."
- "In what ways are the chemical extracts from plant species A and plant species B different?"

Small group research questions should be unanswerable with a single pair's data and instead require the whole small group's dataset. Some examples are:

- "Do species that are more closely related have more similar chemical profiles?"
- "To describe how surface chemistry vary across eight species in plant family A"

Gas Chromatography-Mass Spectrometry

For this module, we had access to a standard resolution, single quadrupole GC-MS system. We configured the machine to run a previously reported method from Guo et al. We recommend silylating or acetylating the samples prior to analysis. Although GC-MS machines are ubiquitous in research universities, we suggest some alternatives if access cannot be obtained: (i) use chemical profiles from common grocery store plant species in lieu of student-generated data (hosted on lucasbusta.github.io/resources) or (ii) use the supplemental data for diverse Lamiaceae species from Boachon et al.

Guo, Y.; Busta, L.; Jetter, R. Cuticular Wax Coverage and Composition Differ among Organs of Taraxacum Officinale. *Plant Physiol Bioch* **2017**, *115*. https://doi.org/10.1016/j.plaphy.2017.04.004 .

Boachon, B.; Buell, R. C.; Crisovan, E.; Dudareva, N.; Garcia, N.; Godden, G.; Henry, L.; Kamileen, M. O.; Kates, H.; Kilgore, M. B.; et al. Phylogenomic Mining of the Mints Reveals Multiple Mechanisms Contributing to the Evolution of Chemical Diversity in Lamiaceae. *Mol Plant* **2018**. https://doi.org/10.1016/j.molp.2018.06.002 .

Gas Chromatography-Mass Spectrometry Data Analysis

Code for the GC-MS data analysis app and for generating phylogenetic trees is hosted at:
https://github.com/LucasBusta/phylochemistry