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Plant-derived drug discovery in an introductory biology laboratory course

Tatiana Kuzmenko
Loyola Marymount University, Tatiana.Kuzmenko@lmu.edu

Ashwarya Sharma
Loyola Marymount University

Demian A. Willette
Loyola Marymount University, demian.willette@lmu.edu

FEATURE ARTICLE

Plant-Derived Drug Discovery in an Introductory Biology Laboratory Course

Tatiana Kuzmenko, Ashwarya Sharma, Demian A. Willette

Abstract

Hands-on, inquiry-based laboratory activities are excellent opportunities to introduce first-year undergraduate students to the lab environment and to catalyze new interest in topics they may not yet know or be as enthusiastic about studying. We describe a multisession introductory laboratory activity that couples the research areas of medicinal drug discovery and plant biology. Selecting from a diversity of native California plants and broadly recognized medicinal plants, students learn and apply an assortment of basic phytochemical assays, analyze preliminary data, and then formulate hypothesis-driven follow-up experiments. Working in small groups, students develop shared project management and collaboration skills, and present activity results to peers in multiple modalities. Furthermore, we summarize findings from 163 student experiments using 29 plant species into an Instructor's Resource Table to facilitate guiding students through their preliminary and follow-up experiments. Lastly, we include student responses from pre- and post-activity surveys on their changing attitudes toward plant biology.

Key Words: *drug development; hypothesis testing; inquiry-based lab; medicinal plants; small group.*

○ Introduction

Student retention and examination scores are higher in science courses that implement active learning rather than traditional lecturing (Gasiewski et al., 2012; Freeman et al., 2014), and hence there is both a critical need and an advantage to guiding college students through hands-on, inquiry-based laboratory courses in their first year. Indeed, first-year undergraduate lab courses serve as an introduction to scientific learning for students who have not had any other laboratory experience, regardless of their prior education or background (DeHaan, 2005; Campbell & Bohn, 2008). Furthermore, students' perceptions and exposure to science prior to college may influence their eagerness to learn a broad diversity of topics in a biological laboratory course. For example, students may be keen to learn more about medicine and less excited to learn about plant biology (Hershey, 1996; Pany, 2014). Lab activities that cut across concepts and disciplines yield opportunities for students to recognize the interrelatedness of the biological sciences.

> *"Lab activities that cut across concepts and disciplines yield opportunities for students to recognize the interrelatedness of the biological sciences."*

Here, we describe a multiweek laboratory activity in which students screened California native and common herbs for medicinal properties. This "drug-discovery" module taught students how to (1) perform an organic solvent extraction of dried plant material; (2) assess the extract's toxicity, its antimicrobial properties, and its stimulatory or sedative activity; and (3) analyze and interpret assay results to craft an original follow-up experiment to promote student-led inquiry learning. The module cumulates in a 12-minute student-led group oral scientific presentation and an individual scientific term paper.

Gleaning from active-learning pedagogies, we selected an inquiry-based approach because of its typical adherence to the steps of the scientific method and increasingly common use in undergraduate biology education (Weaver et al., 2008; Cattaneo, 2017). Banchi and Bell (2008) categorize inquiry-based learning into four stages (Confirmation Inquiry, Structured Inquiry, Guided Inquiry, and Open Inquiry) that afford the student increasing responsibility over the activity's design and scope. The activity described here utilizes two of Banchi and Bell's (2008) stages: Structured Inquiry – instructor provides prescribed procedures for students to answer a question; and Open Inquiry – students design or select procedures to investigate questions they formulate. We created a tiered activity that would first teach basic concepts and methods that the students could then expand upon with more autonomy by generating and testing evidence-driven follow-up questions. Benefits of this approach include (1) a shifted emphasis on the process of learning and discovery rather than reaching a particular result and (2) greater

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student ownership and responsibility of their laboratory experience. These skills are beneficial in any lab environment, especially in independent research later in their academic tenure (Weaver et al., 2008; Spronken-Smith, 2012). Downsides we observed to this approach included some initial caution from the faculty in limiting explicit procedures for the follow-up experiments, and occasional frustration or discomfort experienced by students due to not having a "correct answer" to seek and getting used to the general messiness of empirical research.

We humans have long treated our ailments with medicinal plants. The earliest written records of using plants to prepare medicine date back 5000 years ago to a Sumerian clay slab with a dozen drug recipes (Hassan, 2015). The seeds, fruits, barks, roots, and other parts of plants were tested against illnesses by our ancestors through trial and error, and over time they accumulated knowledge that continues to shape modern pharmacotherapy. Our understanding of medicinal plants took a leap forward in the early 19th century. Chemical methods and quantitative testing helped discover, substantiate, and isolate alkaloids and glycosides, followed by other compounds, including tannins, vitamins, and hormones (Kong et al., 2003). Tomorrow's drugs will continue to be derived from plant sources (Koehn & Carter, 2005). In the past five years, 39 clinical trials of "plant-derived" drugs were under way or completed in testing for cancer, cardiovascular, respiratory, inflammation, muscular, skeletal, and gastrointestinal therapeutic use (NIH ClinicalTrials.gov, 2020). Although many drugs are derived from plants, developing new drugs is time-consuming, complex, and expensive. Recent estimates place the preapproval cost of new drug development in the range of $1–2 billion and more than five years (DiMasi et al., 2016). Another hurdle: with >250,000 species of higher plants (gymnosperms and angiosperms) on planet Earth, it is difficult to know which ones to test. Upwards of 20% of known plants have been screened for phytochemical activity (Altemimi et al., 2017).

To help narrow in on promising plants, researchers have developed multiple selection approaches (Katiyar et al., 2012). These include (1) the ***random approach*** – applying selected bioassays to randomly selected plant species in hopes of finding an effective candidate (example: camptothecin and taxol for anticancer activity; Oberlies & Kroll, 2004); (2) the ***ethnopharmacology/ethnobotanical approach*** – extracting and studying compounds from medicinal plants used by ethnic groups to treat various ailments, including diseases (examples: *Andrographis paniculata* for dysentery, *Rauvolfia serpentina* for blood pressure, *Cinchona officinalis* for malaria; Cox & Balick, 1994; Mishra et al., 2007; Roumy et al., 2007); and (3) the ***zoopharmacognosy approach*** – taking our cues from animals by observing what plants they use (example: cattle eating *Cestrum diurnum* for its vitamin D derivatives; Prema & Raghuramulu, 1994). The interest of people in exploring and reporting the properties of medicinal plants has greatly benefited society, and each successive generation's discoveries have advanced us further in health and in realizing our bond with nature.

This multiweek, hands-on lab activity was successfully conducted in a first-year undergraduate general biology lab course at Loyola Marymount University in 2017, 2018, and 2019. In the three years, >600 students from a wide range of life-science and non-life-science majors and multiple academic levels participated (Table 1). The activity was carried out over four laboratory sessions, each lasting four hours, but can be streamlined to fewer sessions. Furthermore, the activity is highly flexible, with components that could be omitted to simplify for a nonmajors biology course or added to increase complexity for biology majors (Table 2).

This activity both ***broadens the scope*** (types of assays, catalogue of tested/testable plants with expected effects, etc.) and ***improves the instructional scaffolding*** (structure for hypothesis-driven follow-up experiments, pre- and post-assessment tools and results) of an earlier iteration of a drug-discovery lab exercise (Shelley, 2009). The activity is designed for student groups of three or four to promote shared project management, entice scientific debate, and train students in the critical skill of collaboration. Lastly, to develop writing and presentation skills, the activity's findings are structured to be presented using multiple communication modalities, including an individually written scientific paper and a group oral presentation (for rubrics, see the Supplemental Material available with the online version of this article). This multimodal approach assesses students' proficiency in the learning objectives while also providing opportunities for the different strengths of students in each group to emerge. The objectives of this activity are for students to

1) realize the value and utility of education in plant science;
2) learn and apply an assortment of basic phytochemical assays (bacterial disk diffusion, brine shrimp toxicity test, and *Daphnia* heart-rate stimulus test);

Table 1. Class enrollment demographics for the activity (BIOL 111 course) across the three years of experiments at Loyola Marymount University.

Academic Year	Number of Students by Academic Year (%)	Major/Program	Number of Students by Major/Program (%)
Freshmen	411 (64%)	Biology	201 (31%)
Sophomores	142 (22%)	Biochemistry	59 (9%)
Juniors	44 (7%)	Chemistry	33 (5%)
Seniors	15 (2%)	Environmental Science	26 (4%)
Postbaccalaureate	30 (5%)	Health/Human Science	148 (23%)
		Science, undeclared	39 (6%)
		Psychology	39 (6%)
		Postbaccalaureate	30 (5%)
		Other majors (28)	67 (10%)
Total	**642**	**Total**	**642**

Table 2. Activity components matched with desired level of complexity and investigation difficulty (basic, intermediate, and advanced; IE = independent experiments).

Activity Component	Activity Level		
	Basic	Intermediate	Advanced
	One 4-hr Session	Two 4-hr Sessions	Four 4-hr Sessions
Purchase commercial extracts	X		X
Prepare methanol plant extracts		X	X
Brine shrimp toxicity assay	Select one	X	X
Bacterial sensitivity assay		X	X
Daphnia stimulus assay		X	X
IE: Modify extract concentrations			X
IE: Modify extraction method			X
IE: Source extract from different plant parts			X
IE: Test commercial vs. prepared extracts			X

3) assess preliminary assay results and design a hypothesis-driven follow-up experiment;
4) synthesize all data and present findings in two modalities; and
5) appreciate the challenges and promise of developing new drugs from plants.

○ Methods

Collection & Preservation of Plant Species

Each group of students was assigned one of 29 available plants with known medicinal properties, some of which are California native species (Table 3). Plant material was either sourced from an on-campus medicinal plant garden established for this activity or purchased from local grocery stores. Commercial extracts and essential oils were purchased online. To introduce students to classic plant-preservation methods, they were taught how to create voucher specimens using fresh plant material and a herbarium press as described in Alexiades (1996).

Preparation of Plant Extracts

To prepare plant extracts, supervised students collected and weighed approximately 5–10 g of fresh plant material, cut the material into small pieces, and placed it in a foil pouch. The pouches were partially closed, with a corner left open for ventilation, and placed in a drying oven at 50°C until a consistent dry weight was achieved (approximately seven days). The part of the plant (stem, leaves, root, or fruit) used varied by species, but when possible was informed by published ethnobotanical knowledge. This step was not conducted on plant material that was purchased predried (e.g., teas, herbs, beans). Dried plant material was ground into a consistent fine powder using an electric coffee grinder.

One gram of dry, ground plant material was transferred to individually labeled 1.7 mL microcentrifuge tubes, and then 1000 µL of laboratory-grade methanol was added. For voluminous plant material, a larger microcentrifuge tube may be used. Tubes were closed, vortexed for one minute to mix, steeped for 15 minutes, vortexed for an additional 15 seconds, and steeped again for 15 minutes. Tubes were centrifuged at maximum speed (13,400 rpm) for one minute, and supernatant was carefully transferred to a new 1.7 mL labeled tube. This supernatant served as the students' plant extract, which could then be used in the selected assays. Students typically selected two of the three experimental assays described below to test the putative medicinal properties of their plant species.

Assay 1: Antimicrobial Properties of Plant Extracts

Plant extracts were tested for potential antimicrobial properties by conducting a disk diffusion assay (Bauer et al., 1966). Students had the ability to choose at least three among 12 different bacterial strains available with various properties and natural environments (Table 4).

Fresh bacterial strains were swabbed from a single colony to create bacterial lawns on TSA (Tryptic Soy Agar) plates using sterile cotton swabs. Sterile paper disks were saturated with 10 µL of either a plant extract or controls, allowed to air dry for one minute to remove solvent residue, and applied on the plates (Figure 1); 10 µL of 100% methanol was used as a negative control, and 5 µL of a commercial thyme essential oil was used as a positive control due to its strong, consistent antimicrobial properties against multiple bacteria (Juven et al., 1994). Plates were incubated at 30°C for 24–48 hours or until halos were clearly visible and stored at 4°C until the next lab period. After incubation, the halo size was measured around each disk (three measures transecting the center of the disk to margins of halo) to quantify the known bacterial strains' sensitivity to the plant extract. Halo diameters were calculated for descriptive statistics (mean, standard deviation) and contrasted among strains using a one-way analysis of variance (ANOVA) and, when appropriate, a Tukey post hoc test.

Assay 2: *Daphnia* Stimulatory or Sedative Properties

Daphnia magna is a freshwater crustacean commonly found in ponds and is a versatile organism to use in laboratory experiments; its transparent body allows its heart rate to be easily visualized through a microscope (Baylor, 1942). Live *Daphnia* were purchased from a laboratory supplier and cultured throughout the semester in 10 L freshwater tanks to maintain an ample supply of animals for the experiments.

Table 3. Instructor's Resource Table. Activity results of 163 student experiments conducted over three years. The numbers indicate how many groups have independently obtained this result. While accessing this data, please consider that there was an expected range in quality of work performed by students, occasionally resulting in a diversity of experimental outcomes. Antimicrobial Properties: "Yes" indicates that extract produced halos with at least of one of the bacterial strains tested, "No" indicates that extract did not produce halos with any bacterial strain tested. Brine Shrimp Toxicity: "Yes" indicates that extract presence caused statistically significant inhibition or death of the brine shrimp, "No" indicates that extract presence did not affect brine shrimp movement or cause death; Stimulus/Sedative Assay: ↑ indicates that extract caused a statistically significant stimulatory effect (increase of heart rate) on *Daphnia*, ↓ indicates that the extract caused a statistically significant sedative effect (decrease of heart rate) on *Daphnia*, "No" indicates that extract presence did not have a stimulatory or sedative effect on *Daphnia* (no change in heart beat). "–" indicates that this test has not been conducted. Plants with limited effectiveness/no effect have only shown a mild/no effect in any of our assays by multiple groups of students.

Effectiveness	Medicinal Plant	Scientific Name	Number of Groups That Tested	Antimicrobial Properties	Brine Shrimp Toxicity	Stimulant↑/ Sedative↓	Source		Extraction Method (Other Than MeOH)
							Field	Commercial	
Effective	Black sage	*Salvia mellifera*	6	Yes 5 (MeOH); No 1 (EtOH, hot H_2O)	No 1	↓3	✔	✔ (Essential oil)	EtOH, hot H_2O
Shows some effect	Borage	*Borago officinalis*	2	Yes 1 (MeOH and acetate)	–	–	✔	–	EtOH, acetate, hot H_2O, salt H_2O
Effective	California sagebush	*Artemisia californica*	5	Yes 4	–	↓3	✔	✔ (Essential oil)	EtOH, acetate
Effective	Chinese motherwort	*Leonurus japonicus*	2	–	Yes 1 (MeOH)	↓2 (EO, MeOH)	✔	–	–
Effective	Cinnamon	*Cinnamomum verum*	4	Yes 3 (EO)	Yes 1 (MeOH, EO)	↓1	–	✔ (Essential oil)	EtOH
Effective	Clove	*Syzygium aromaticum*	8	Yes 5 (EO, MeOH)	Yes 3	–	–	✔ (Essential oil)	EtOH, hot H_2O, cold H_2O
Effective	Coffee	*Coffea arabica*	10	No 2	Yes 1	↑6 (MeOH, Cold, room temp. H_2O)	✔	✔ (Essential oil)	Hot H_2O, cold H_2O, room temp. H_2O
Effective	Common sage	*Salvia officinalis*	6	Yes 4 (MeOH, EtOH)	–	↑1 (MeOH); ↓3	✔	✔ (Essential oil)	EtOH
Effective	Danshen	*Salvia miltiorrhiza*	3	Yes (MeOH)	Yes 1	↓3 (MeOH, hot, cold H_2O)	✔	–	Cold, room temp., and hot H_2O
Effective	Garlic	*Allium sativum*	7	Yes 3	Yes 1; No 1	No 2	✔	✔ (Essential oil, pills)	Fresh juice, hot H_2O
Effective	Ginger	*Zingiber officinale*	8	Yes 3 (EO, MeOH)	Yes 1	–	✔	✔ (Essential oil)	EtOH
Effective	Green tea	*Camellia sinensis*	9	Yes 5 (MeOH, EtOH)	–	↑2; ↓1	–	✔ (Dry, extract)	EtOH, hot H_2O, cold H_2O
Effective	Guayusa	*Ilex guayusa*	9	Yes 1 (EtOH)	Yes 1	↑4; ↓1	–	✔ (Dry, extract)	EtOH, hot H_2O, cold H_2O

Effectiveness	Medicinal Plant	Scientific Name	Number of Groups That Tested	Antimicrobial Properties	Brine Shrimp Toxicity	Stimulant↑/ Sedative↓	Source		Extraction Method (Other Than MeOH)
							Field	Commercial	
Effective	Lavender	*Lavandula angustifolia*	13	Yes 5 (EO)	Yes 1	↓6 (EO, MeOH)	✔	✔ (Essential oil)	–
Effective	Mugwort	*Artemisia douglasiana*	7	Yes 3	No 2 (MeOH)	↓2 (MeOH)	✔	✔ (Essential oil)	–
Effective	Oregano	*Origanum vulgare*	6	Yes 3 (EO, MeOH)	–	↓3 (EO, MeOH, EtOH)	✔	✔ (Essential oil)	EtOH, hexane
Shows some effect	Passion fruit	*Passiflora edulis*	5	–	No 1	↑1; ↓2	✔	✔ (Extract)	–
Shows some effect	Peppermint	*Mentha × piperita*	5	Yes 2 (EO)	No 1	–	✔	✔ (Essential oil)	Hot H_2O
Effective	Purple coneflower	*Echinacea purpurea*	6	Yes 3 (MeOH, EtOH)	–	No 1	✔	✔ (Extract)	EtOH
Effective	Rosemary	*Rosmarinus officinalis*	10	Yes 5 (EO, MeOH)	–	No 1	✔	✔ (Essential oil)	–
Effective	Rue	*Ruta graveolens*	2	Yes 1 (EO)	Yes 1 (MeOH, EO)	↓2 (EO, MeOH)	✔	✔ (Essential oil)	–
Effective	Russian comfrey	*Symphytum officinale*	3	No 1	Yes 1 (MeOH, EtOH, Acetic Acid)	↑1 (MeOH)	✔	–	EtOH, acetic acid, hot H_2O
Shows some effect	Sea onion	*Bowiea volubilis*	2	–	–	↓1 (Hexane)	✔	–	Hexane, hot H_2O, DI H_2O
Effective	Australian tea tree	*Melaleuca alternifolia*	4	Yes 2 (EO)	Yes 1 (EO)	–	–	✔ (Essential oil)	–
Effective	Garden thyme	*Thymus vulgaris*	3	Yes (EO); No (MeOH)	No 1	↓3 (Hexane, MeOH, EtOH)	✔	✔ (Essential oil)	Hexane, EtOH
Effective	Valerian	*Valeriana officinalis*	6	–	–	↑1 (MeOH); ↓2 (Glycerine extract)	✔	✔ (Glycerine extract)	EtOH, hot H_2O
Effective	White sage	*Salvia apiana*	10	Yes 7 (MeOH)	Yes 1 (Hexane)	↓3 (MeOH)	✔	✔ (Essential oil)	EtOH, hexane
Effective	Wormwood	*Artemisia absinthium*	2	Yes 2 (MeOH, EtOH)	–	↓1 (Hexane)	✔	–	–
Effective	Yerba mansa	*Anemopsis californica*	6	Yes 4 (MeOH, EtOH)	Yes 1	↓1, ↑1, No 1	✔	✔ (Extract)	–
Plants Tested with Limited Effectiveness	Chamomile (*Matricaria chamomilla*); Giant horsetail (*Equisetum myriochaetum*); Mule fat (*Baccharis salicifolia*); Toyon (*Heteromeles arbutifolia*); Yarrow (*Achillea millefolium*); Yerba buena (*Clinopodium douglasii*); Doveweed (*Croton setiger*); Hummingbird sage (*Salvia spathacea*); Basil 'Queen of Sheba' (*Ocimum basilicum*); Camphor basil (*Ocimum kilimanscharicum*); Cayenne pepper (*Capsicum annuum*); Cuban oregano (*Plectranthus amboinicus*); Mustard seed (*Brassica nigra*); Onion (*Allium cepa*); St. John wort (*Hypericum perforatum*); Turmeric root (*Rhizomatous herbaceous*)								
Plants Tested with No Effect	Black walnut (*Juglans nigra*); California coffeeberry (*Frangula californica*); *Gingko biloba*; Jimsonweed (*Datura stramonium*); Marjoram (*Origanum majorana*); Coastal plantain (*Plantago subnuda*); Wood strawberry (*Fragaria vesca*)								

Table 4. Bacterial strains used in the disk diffusion assay.

Gram-Positive Bacteria	Gram-Negative Bacteria
Lysinibacillus sphaericus (prev. Bacillus sphaericus)	*Escherichia coli*
Micrococcus luteus	*Enterobacter aerogenes*
Bacillus megaterium	*Shewanella oneidensis*
Bacillus cereus	*Proteus vulgaris*
Staphylococcus epidermis	*Pseudomonas spp.*
Staphylococcus aureus	
Corynebacterium pseudodiphtheriticum	

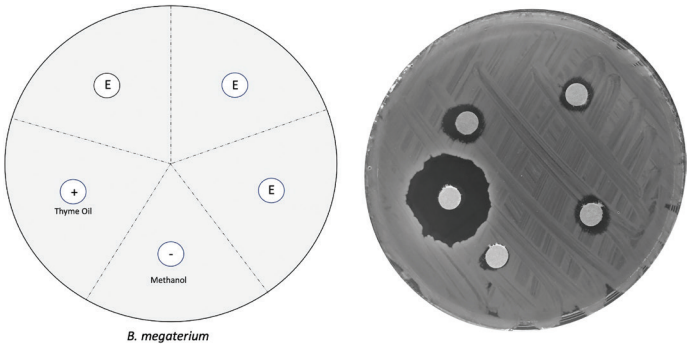

Figure 1. Example assay testing *Origanum vulgare* methanol extract against *Bacillus megaterium*.

Daphnia heart rate was observed by placing an individual crustacean on concave microscope slides under 40× magnification. *Daphnia* are sensitive to temperature and intense light, so students were shown how to work quickly with the animals to minimize stress. First, baseline heart rate was measured for each *Daphnia* by placing the animal in a 1.7 mL tube. To set up this control, 20 µL MeOH was added to the tube and evaporated out prior to adding 200 µL fresh, room temperature water. The Daphnia was kept in the tube for a three-minute acclimation period and then moving it to a concave slide (Figure 2). Resting heart rate was calculated through the microscope by counting the beats over a 15-second period and then multiplying by 4. This was repeated three times and averaged for the baseline heart rate. *Daphnia* were then moved to a new 1.7 µL tube containing 20 µL of plant extract and 180 µL of fresh water allowed to acclimate to the extract for three minutes, and then returned to the concave slide and the heart rate measured again as above. The measurements were repeated with three different, similarly sized *Daphnia*. Descriptive statistics were calculated, and a paired Student's *t*-test was used to determine whether there was a change in heart rate after exposure to the plant extract.

Assay 3: Brine Shrimp Plant Toxicity

Brine shrimp (*Artemia salina*) are small aquatic crustaceans that are commonly used in benchtop bioassays for chemical toxicity of plant compounds (Campbell et al., 1994), in part due to a positive correlation between compound toxicity to brine shrimp and human cancer cells (Anderson et al., 1991). Brine shrimp eggs are available from laboratory suppliers and are easy to set up in 1 L saltwater containers with aeration. We would set up hatcheries 48 hours

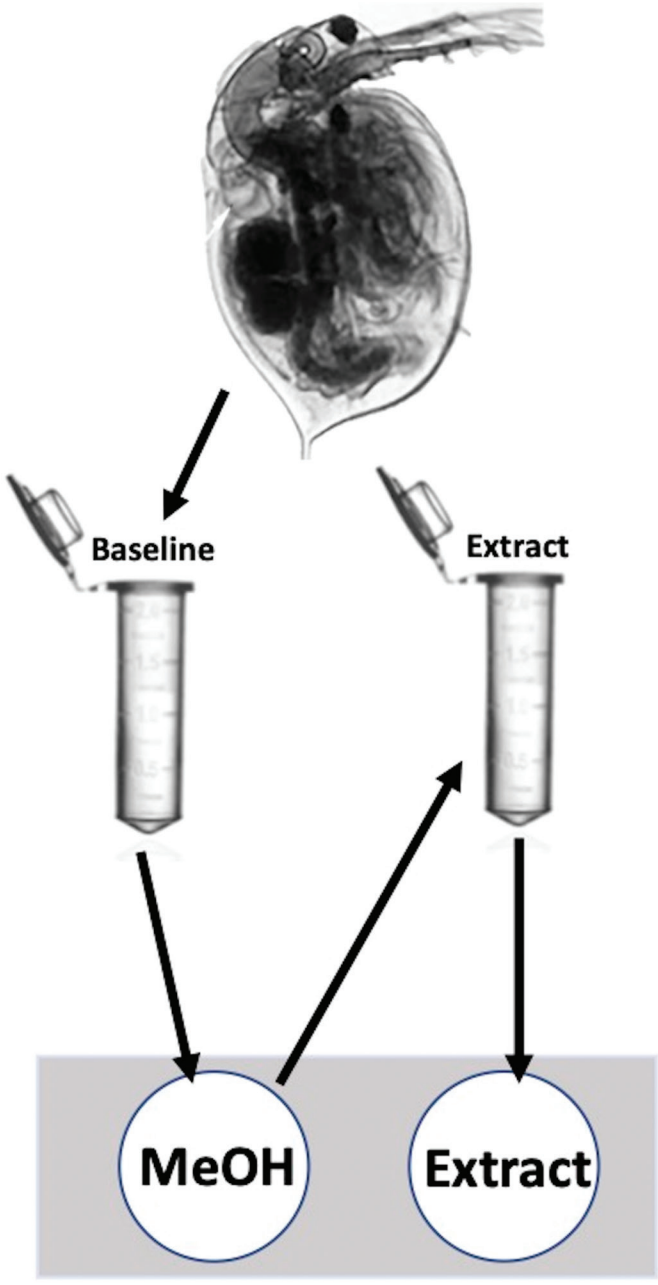

Figure 2. Experimental setup for the *Daphnia* stimulus test.

before the brine shrimp assay, with new cohorts hatched as needed for subsequent experiments. Hatched brine shrimp have positive phototaxis and can easily be collected by shining a bright lamp at one corner of the container and pipetting out for use.

Students set up this assay by labeling four 50 mm Petri dishes (20 µL, 100 µL, Positive Control, and Negative Control) to test different doses of the extract. First, 20 µL and 100 µL of extract, 100 µL of methanol (Negative Control), and 10 µL of neem oil (Positive Control; Abdullah-Al-Emran et al., 2011) were added to the respective dishes; the methanol was allowed to evaporate away; and then 4 mL of seawater was added to each dish. Ten brine shrimp were added to each dish and observed under a stereoscope every 20 minutes for ≥60 minutes. Observations of inhibition of movement or mortality were recorded. Students graphed the results of all three tests to determine the LD_{50}, or the lethal dose at which 50% of the animals die, and the IC_{50}, or the concentration at which 50% of the

animals show inhibition. To visualize inhibition, students also used a combination of time-lapse videos and swim speed across a grid to quantify movement in each sample. Results were explored using descriptive statistics and a one-way ANOVA and, when appropriate, a Tukey post hoc test.

Follow-Up Experimentation

Following two weeks of guided experimentation, students were given an additional two weeks for independent experimentation. Through analyzing their initial results and comparing them with previous literature, groups determined other experiments that could be conducted to expand upon their initial results and wrote a research proposal, prior to beginning, for teaching assistants to approve.

There was a large emphasis on determining, through a literature review, what chemical compound(s) could be causing the effect that they were interested in and whether they were present in their original plant extracts. At the beginning of the module, 100% methanol was used as a general solvent, because it is known to extract a variety of nonpolar and polar components from plants. To look at other compounds, students tested other solvents, including 95% and 75% ethanol, acetone, acetic acid, and different temperatures of water. These various extraction methods were compared against the original methanol extract. Groups also used different parts of the plant to compare properties across the entire spectrum of the plant (example: leaf vs. fruit). To compare the different extraction methods, students viewed the extract tubes under a blue light to visualize differences in flavonoid presence (Bilger et al., 2001).

Some students chose to compare the effectiveness and potency of their plant extracts through comparisons with commercial essential oils, infusions, or pills of the same plants (example: fresh garlic vs. garlic pills). Other groups focused on comparing plants with similar properties (examples: the stimulatory effect of green tea vs. coffee or the antimicrobial effect of sage vs. lavender). Additional tests used less frequently but successfully included usage of fresh mice hepatocytes to display extract toxicity and testing plant extract effectiveness against yeast and fungal pathogens like *Fusarium oxysporum* and *Aspergillus niger*.

○ Changing Attitudes about Plant Biology

Plants are often considered a challenging topic to discuss by biology instructors and described as less interesting than animals by students (Hershey, 1996). Although plant studies have played essential roles in core biological concepts – such as pea plants in Mendelian genetics – there is a tendency to use animals rather than plants in explaining ecological and evolutionary processes. This "plant blindness" (Wandersee & Schussler, 2001) is not, however, insurmountable in teaching. People can distinguish and accurately identify plants when they live in societies in which plants are valued and valuable for survival (Berlin, 1972), and students who participate in botany educational programs form a greater appreciation for plants (Lindemann-Matthies, 2005). Furthermore, Pany (2014) found that students have a particularly high interest in medicinal plants and stimulant herbal drugs, and focusing on these may catalyze students' enthusiasm for plant biology in general.

As part of the development of this hands-on, inquiry-based lab, we included a voluntary pre- and post-activity survey of students' attitudes toward plants. Although we did not observe any statistical difference between pre- and post-activity responses to questions

Figure 3. Word cloud showing the diversity of terms students use to describe the value of plants. The size of each word indicates its relative frequency.

regarding students' interest in learning about plants (*t*-test for two independent groups: $t = 1.18$, df = 311, $p = 0.23$), their interest in plant use in medicine ($t = 1.80$, df = 311, $p = 0.07$), or their feeling that learning about plants is important ($t = 0.75$, df = 305, $p = 0.45$), students' rating of the effectiveness of the activity was high (3.6 of possible 5.0), and (anecdotally) words students associated with the value of plants included more scientific terms post-activity than pre-activity (Figure 3).

○ Conclusions

We found this hands-on, inquiry-based lab activity to be an effective strategy for engaging first-year life-science and non-life-science students by leveraging a topic that is exciting or more familiar to them (medicine) to dive deeper into a field they may be less enthusiastic about (plant biology). The activity navigated the enrolled first-year biology, chemistry, human science, and environmental science majors through key components of experimental design, including replicates, controls, and hypothesis testing, while teaching them to appreciate the sometimes inherent challenges of doing science to understand the unknown.

This activity has been refined over its three-year development and implementation by >600 undergraduate students and 44 teaching assistants at Loyola Marymount University, yet it is quite malleable. It is designed to be conducted over four weeks by first-year college students but can be used with different levels of learners by choosing the basic, intermediate, or advanced design (Table 2). Further, students can contribute additional insight on species of local origin or interest by adding to the findings from 163 student experiments using 29 plants in the Instructor's Resource Table (Table 3). We also see this activity's strong potential for a collaborative crossover with first-year general chemistry lab courses. We are exploring ways for students to probe deeper into the chemistry principles that underpin the extraction methods as well as the chemical properties and identification of the plant-extract compounds.

○ Supplemental Material

The following are available with the online version of this article:

- Scientific Oral Presentation Rubric
- Scientific Paper Rubric

○ Acknowledgments

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TATIANA KUZMENKO (tatiana.kuzmenko@lmu.edu) is an Instructor and Laboratory Associate, ASHWARYA SHARMA (ashwarya.sharma1@gmail.com) is a recent biology graduate, and DEMIAN A. WILLETTE (demian.willette@lmu.edu) is an Assistant Professor, all in the Biology Department, Loyola Marymount University, Los Angeles, CA 90045.