FUTURELAB+

AG/ENVIRONMENTAL

Plant to Pharmaceutical

Drug Discovery Using Plant Extracts

Laboratory Investigation

Developed in partnership with:

Bay Area Bioscience Education Community

In this Lesson Plan:

Print the **Teacher Section** →

Print the **Student Section** →

This document is separated into two sections, For Teachers [T] *and* Student Resources [S]*, which can be printed independently.*

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Cover Image
The Solanaceae plant family is rich in bioactive metabolites and has played an essential role in traditional medicine.

AG/ENVIRONMENTAL / PLANT TO PHARMACEUTICAL

Lab: Drug Discovery Using Plant Extracts

DRIVING QUESTION

How can we find bioactive compounds in nature?

OVERVIEW

Plants and plant extracts have played a crucial role in medicine for as long as humans have existed. Archaeologists have found evidence of medicinal plants being used in many ancient cultures, including in China 8,000 years ago and Iraq 60,000 years ago (Pan et al., 2014). The pharmaceuticals we use today are also often derived from bioactive compounds that occur naturally in plants. For example, 85 out of the 175 small molecule drugs (49%) approved to treat cancer by the US Food and Drug Administration (FDA) between 1940–2014 were either natural products or compounds derived from natural products (Newman and Cragg, 2016).

In this lab, students will perform a Kirby-Bauer disk diffusion test, which is often used in drug discovery labs to determine if a particular plant extract or drug candidate has antibacterial activity. Students first collect a plant or plant product of interest and extract possible bioactive compounds from it. This plant extract is then soaked onto disks that are placed on LB agar plates spread with *E. coli* bacteria. Students then look for the presence or absence of bacterial growth around the disks and use these data to make a claim about whether or not the tested plant has antibiotic properties. Finally, students complete further independent research about the medicinal properties of their plant and consider whether it is viable for continued research for use as a potential pharmaceutical.

ACTIVITY DURATION

Five class sessions
(45 minutes each)

ESSENTIAL QUESTIONS

How do we test for antibiotic properties of plants?

What gives plants their beneficial properties?

How have plants been used to treat and prevent disease?

BACKGROUND INFORMATION

Prior experience with micropipetting and bacterial culture is suggested for this lab, which students have been introduced to in an earlier unit. It is also helpful for students to have a basic understanding of bacteria as a useful model organism in science and the basic role of antibiotics, which are additionally covered in an earlier unit.

Sources:

Historical Perspective of Traditional Indigenous Medical Practices: The Current Renaissance and Conservation of Herbal Resources

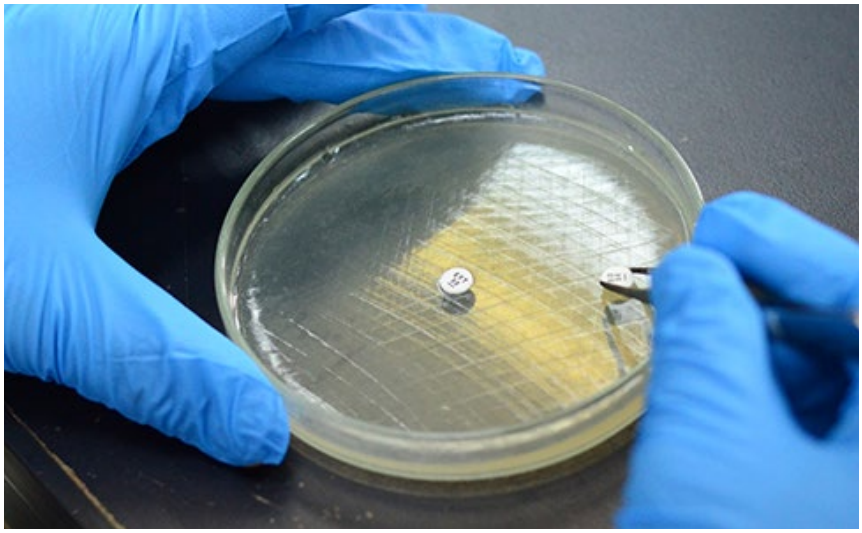
Extraction, Isolation and Characterization of Bioactive Compounds from Plants' Extracts

Natural Products as Sources of New Drugs from 1981–2014

Have you ever wondered…

How new drugs are discovered?

One method used to discover new pharmaceuticals is to first collect biological samples from different biodiversity hotspots around the world. These samples can then be tested for different medicinal properties in a lab; for example, they can be tested for antibacterial properties using a Kirby-Bauer assay. Further research can then be conducted to identify, isolate, and manufacture the bioactive compound in the sample that is responsible for the desired property and distribute it as a pharmaceutical.



MAKE CONNECTIONS!

How does this connect to the larger unit storyline?

This lab provides a hands-on example of how scientists can test plants for beneficial properties such as being antimicrobial. Students will also engage in research about their plant of choice to develop a fuller understanding of its bioactive compounds and historical and cultural uses.

How does this connect to careers?

Microbiologists study microscopic life forms like bacteria. Clinical microbiologists use the disk diffusion method for determining susceptibility of bacteria to antimicrobials.

Pharmaceutical scientists spend most of their time in a laboratory discovering and learning how different compounds interact with disease-associated molecules and organisms. In addition, they investigate how these compounds interact with the human body to ultimately determine if they can become new drugs.

How does this connect to our world?

In this lesson, students explore the relationship between plants and medicine. They will synthesize data from their Kirby-Bauer assay along with research to consider whether the plant they chose is viable for continued research for use as a potential pharmaceutical.

Pedagogical Framing

Instructional materials are designed to meet national education and industry standards to focus on in-demand skills needed across the full product development life cycle—from molecule to medicine—which will also expose students and educators to the breadth of education and career pathways across biotechnology.

Through this collection, educators are equipped with strategies to engage students from diverse racial, ethnic, and cultural groups, providing them with quality, equitable, and liberating educational experiences that validate and affirm student identity.

Units are designed to be problem-based and focus on workforce skill development to empower students with the knowledge and tools to be the change in reducing health disparities in communities.

SOCIAL-EMOTIONAL LEARNING

Students will practice self-management skills throughout this lab, as they are responsible for selecting the plant of focus for their experiment and their independent research. During the lab, they will work with a partner to share resources; however, each student is responsible for their own results. Thus, they must utilize both responsible decision making and cooperative relationship skills.

CULTURALLY AND LINGUISTICALLY RESPONSIVE INSTRUCTION

This lesson centers on students choosing their own plant for the experiment and draws on cultural or familial knowledge around home remedies for illnesses. The background information provided along with the opportunities for further research bring into focus the inextricable relationship between humankind and the natural environment.

COMPUTATIONAL THINKING PRACTICES

Students engage in multiple computational thinking strategies throughout this lab, including collecting and analyzing data. Students collect qualitative data from their Kirby-Bauer assay and analyze it to determine if their plant extract displays antibiotic properties.

OBJECTIVES

Students will be able to:

Explain how bioactive compounds from plants can be used as pharmaceuticals using scientific text.

Perform a Kirby-Bauer assay to determine whether a plant sample has antibacterial properties using scientific protocols.

Analyze results from a Kirby-Bauer assay to determine if a plant extract exhibits antibacterial properties using data collected from the lab.

Create a testable question about a plant and discuss whether it is viable for continued research for use as a potential pharmaceutical using scientific text and data from the lab.

Materials

Documents

Lab Preparation (for teacher)

Background Reading: Bioactive Compounds from Plants (1 per student)

Background Reading: Kirby-Bauer Assay (1 per student)

Vocabulary Tool (1 per student)

Student Protocol, Part 1: Extract Bioactive Compounds (1 per pair)

Student Protocol, Part 2: Kirby-Bauer Assay (1 per pair)

Student Guide (1 per student)

Reagents

Lab Part 1: Extract Bioactive Compounds

- *Plant sample (100 µL crushed per student))*
- *Ampicillin (100 µL per pair)*
- *Extraction buffer (600 µL per pair)Organic cornmeal (non-Bt-corn) (1 tube)*

Lab Part 2: Kirby-Bauer Assay

- *LB nutrient broth, sterilized (250 µL per pair)*
- E. coli *culture plates (5 per class)*
- *LB agar plates (1 per pair)*

Equipment and Consumables

Lab Part 1: Extract Bioactive Compounds

- *1.5 mL tubes (8 per pair)*
- *P200 micropipettes and tips (1 per pair)*
- *P1000 micropipettes and tips (1 per pair)*
- *Microtube rack (1 per pair)*
- *Waste container (1 per pair)*
- *Permanent marker (1 per pair)*
- *Pencil (1 per pair)*
- *Filter paper (4 discs per pair)*
- *Hole punch (1 per teacher)*
- *Micropestles (2 per pair)*
- *Scissors or mortar and pestle (optional)*

Lab Part 2: Kirby-Bauer Assay

- *1.5 mL tubes (1 per pair)*
- *P200 micropipettes and tips (1 per pair)*
- *P1000 micropipettes and tips (1 per pair)*
- *Microtube rack (1 per pair)*
- *Waste container with lysol or 10% bleach (1 per pair)*
- *Permanent marker (1 per pair)*
- *Inoculation loops (1 per pair)*
- *Sterile glass beads (~10 per pair)*
- *Incubator (1 per class)*
- *Strips of parafilm (optional)*

Day 1

Procedure

LEARNING OUTCOMES

Students will be able to:

Predict whether the plant they chose will exhibit antibiotic properties using the Kirby-Bauer assay.

Describe how plants have been used as medicine and can be used to create pharmaceuticals using scientific text.

Teacher Note > *At least one week before the lab, refer to Lab Preparation document for instructions on aliquoting reagents, preparing agar plates and* E.coli *culture plates, and setting up lab stations. Be sure to streak at least five* E.coli *starter plates per class section 24 hours before Day 3.*

Teacher Note > *Before the lab, each student needs to choose and collect a plant or plant product to test using the Kirby-Bauer assay (they need ~500 µL when crushed). This might be the same plant they have already collected for their botanical sample, but it can be a different plant or plant product that they find in their home. Because students will be asked to provide a rationale for the item they chose and why they believe it may have antibiotic properties, it is helpful if they have a personal connection or a family tradition of using the plant or plant product.*

Whole Group (10 minutes)	
1	For the warm up, ask students: What are some ways you have used plants or plant products to benefit your health? (Answers might include traditional herbal remedies, herbal teas, vitamin supplements, essential oils, aromatherapy, etc.)
2	Have student pairs discuss their ideas and use popsicle sticks or another equitable participation strategy to choose a few students to share with the whole class. This is a great opportunity to celebrate students' diverse cultural backgrounds.
3	Give each student one copy of the *Student Guide* and one copy of *Background Reading: Bioactive Compounds from Plants*.

Small Group (30 minutes)	
1	Ask students to take turns discussing with their elbow partner why they chose their plant or plant product to use in this lab.
2	Prompt students to make a prediction about whether or not the plant they chose will have antibiotic properties. They should explain their reasoning in *Student Guide, Part 1: Pre-Lab*.
3	Ask students to read *Background Reading: Bioactive Compounds from Plants* and answer the questions. You may wish to read the first few sentences as a class and model how to annotate to increase comprehension.

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Day 1

Continued

Procedure

Individual (5 minutes)	
1	Hand out the *Vocabulary Tool* and ask students to write a sentence for each term for homework.
2	Exit ticket: Describe what a "bioactive compound" is in your own words and give an example of one you have heard of, seen, or eaten before.

Teacher Note > *An alternative option to the exit ticket above is to have students add to their Student Guide by completing the first question for Lesson 5.*

Day 2

Procedure

LEARNING OUTCOMES

Students will be able to:

Extract bioactive compounds from plants using protocols.

Explain the purpose and process of a Kirby-Bauer assay using scientific text.

Teacher Note > *Before class, set up lab stations using Lab Preparation. We suggest bringing a few common medicinal plant samples for students who did not bring one (cinnamon, garlic, dried hibiscus, etc.),*

Whole Group (10 minutes)	
1	For the warm up, ask students to describe a process they might use to determine if their plant has the ability to kill bacteria.
2	Ask students to share with a partner and come up with a process together that combines their ideas or improves upon one.
3	Call on a few students at random to share the idea they brainstormed with their partner.
4	Break students into lab pairs and give each pair a copy of *Student Protocol, Part 1: Extract Bioactive Compounds* and each student a copy of *Background Reading: Kirby-Bauer Assay.*
5	Share with students that today they will complete Part 1 of the lab in which they will extract bioactive compounds from their plant to determine if it has antibacterial activity. Ask them to complete the questions in the Background Reading during wait times.

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Day 2

Continued

Procedure

Small Group (30 minutes)

1. Ask students to take out their plant sample and go to their lab station with their partner. Share these safety guidelines before they begin:
   - **a.** Wash your hands before and after the lab.
   - **b.** Do not eat or drink during the lab.
   - **c.** Keep your lab station clean and clear of clutter.
2. Ask students to complete the "Sample Key" table in *Student Guide, Part 2: Lab* question #1, then follow the steps in *Student Protocol, Part 1: Extract Bioactive Compounds* with their partner.
3. When students have finished, collect their four 1.5 mL tubes containing their filter paper discs and store at room temperature until the next class.

Individual (5 minutes)

1. As an Exit Ticket, ask students: If you were to repeat this protocol, what is one thing you would change to increase the concentration of bioactive compounds from your plant on your filter paper disc? Why do you think this might be an important improvement on the protocol?
2. Ask students to complete the *Background Reading: Kirby-Bauer Assay* for homework if they did not finish.

Teacher Note > *An alternative option to the exit ticket above is to have students add to their Student Guide by completing the second question for Lesson 5.*

Day 3

Procedure

LEARNING OUTCOMES

Students will be able to:

Perform a Kirby-Bauer assay to determine whether their plant sample has antibacterial properties using protocols.

Teacher Note > *Before beginning the lab today, ensure the* E.coli *culture plates you streaked 24 hours prior have grown and the incubator is set to 37˚C. Take out the tubes of filter paper discs from Part 1 and set up lab stations using Lab Preparation.*

Whole Group (10 minutes)	
1	Warm-up: Ask students to look at their drawings in question #3 of *Background Reading: Kirby-Bauer Assay*. Ask them to add to their drawings to make them more specific to their experiment with their own plant sample.
2	Ask students to share one addition to their drawing and why they added it with a partner.
3	Call on a few students at random to share their additions.
4	Break students into their lab pairs and give each pair a copy of *Student Protocol, Part 2: Kirby-Bauer Assay*.
5	Remind students that today they will complete Part 2 of the lab in which they will use their plant extracts from *Student Protocol, Part 1* to perform a Kirby-Bauer assay.

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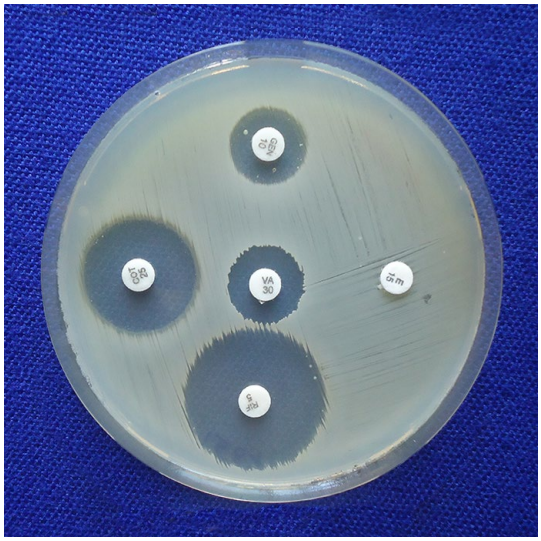

Day 3

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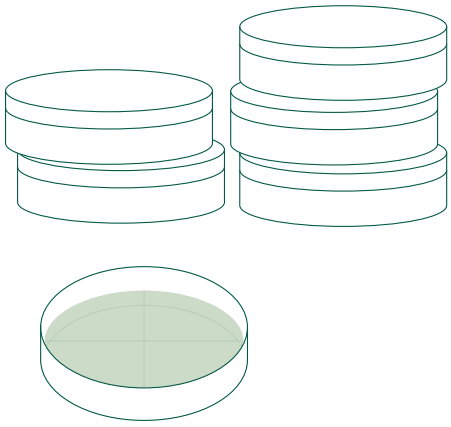
Procedure



Small Group (30 minutes)

1 Ask students to retrieve their filter paper discs and go to their lab station with their partner. Share that students will be working with bacteria (a non pathogenic strain of *E. coli*) and that it is important to follow these safety guidelines:

- **a.** Wash your hands before and after the lab.
- **b.** Do not eat or drink during the lab.
- **c.** Keep your lab station clean and clear of clutter.

2 Ask students to follow the steps in *Student Protocol, Part 2: Kirby-Bauer Assay* with their partner.

3 When students have finished, place their plates upside down in the incubator and incubate at 37˚C for 24 hours. Store plates in the refrigerator until ready for analysis. If possible, have students seal their plates with a strip of parfilm to prevent media dehydration and microbial contamination.

Individual (5 minutes)

1 For the Exit Ticket, ask students: In a few sentences, describe what a Kirby-Bauer assay is as if you were explaining it to a younger sibling.

Teacher Note > *An alternative option to the exit ticket above is to have students add to their Student Guide by completing the third question for Lesson 5.*

Day 4

Procedure

LEARNING OUTCOMES

Students will be able to:

Analyze results from a Kirby-Bauer assay to determine if their plant extract exhibits antibacterial properties using data collected from the lab.

Teacher Note > *Before class, retrieve the Kirby-Bauer agar plates from the incubator or refrigerator. If they have been in the refrigerator, give them time to come to room temperature before students view the results; otherwise, condensation on the lid will obscure the plate.*

Whole Group (10 minutes)	
1	For the warm up, tell students: Draw what you would expect to see on your Kirby-Bauer agar plate if a sample has strong antibiotic properties. Draw what you would expect to see on your Kirby-Bauer agar plate if a sample has no antibiotic properties.
2	Ask students to explain their drawing to a partner.
3	Ask a student with the correct answer to share with the class, making sure they use the phrase “zone of inhibition.” This is a great opportunity to highlight a student who rarely shares.
4	As an optional activity, search for a “Kirby Bauer assay” video and show it to students as a preview of the lab.

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Day 4

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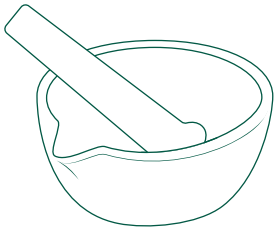

Procedure

Small Group (20 minutes)

1 Ask students to retrieve their Kirby-Bauer agar plate and go to their lab station with their partner. Share that students will be working with bacteria (a non pathogenic strain of *E. coli*) and that it is important to follow these safety guidelines:

- **a.** Wash your hands before and after the lab.
- **b.** Do not eat or drink during the lab.
- **c.** Keep your lab station clean and clear of clutter.

2 Ask students to label an image of their plate. They should collect, rate, and record the zone of inhibition for their positive control, negative control, and experimental samples in the table provided in *Student Guide, Part 2: Lab* questions #2–3. It is easiest to see the results with the lid on and the plate upside down or the lid off and the plate right side up.

3 Prompt students to complete questions on the *Student Guide, Part 3: Data Analysis* questions #1–2 with their lab partner.

Individual (15 minutes)

1 Direct students to return to their instructions for the Collecting and Preserving Plant Specimens Capture Sheet from Lesson 3 and complete Step 6.

Day 5

Procedure

LEARNING OUTCOMES

Students will be able to:

Research beneficial properties and historical use of their plant to determine whether this information supports their results using scientific text and data from the lab.

Create a testable question about their plant and discuss whether it should be researched further for use as a pharmaceutical using scientific text and data from the lab.

Teacher Note > *Students will need Internet access to complete the research portion of Student Guide, Part 4: Obtaining Information.*

Whole Group (5 minutes)	
1	For the warm up, ask students: What other plants or compounds might you want to test using the Kirby-Bauer assay and why?
2	Ask students to share their answers with a partner.
3	Do a whip-around the whole class and ask each student to share their top choice of plant to test.

Individual (40 minutes)	
1	Ask students to complete *Student Guide, Part 4: Obtaining Information*.
2	If time permits, break the class into groups of three to five and ask students to present their answers to questions #4 and #5 to the rest of their group. Then, ask each group to come to a consensus on which plant they would recommend for further research as a pharmaceutical. Ask one spokesperson from each group to share their recommendation with the class.

Teacher Note > *An alternative option to the exit ticket above is to have students add to their Student Guide by completing the final question for Lesson 5.*

National Standards

Next Generation Science Standards	**LS4.D: Biodiversity and Humans** Humans depend on the living world for the resources and other benefits provided by biodiversity. Thus sustaining biodiversity so that ecosystem functioning and productivity are maintained is essential to supporting and enhancing life on Earth.
	Obtaining, Evaluating, and Communicating Information Gather, read, and evaluate scientific and/or technical information from multiple authoritative sources, assessing the evidence and usefulness of each source. Communicate scientific and/or technical information or ideas (e.g. about phenomena and/or the process of development and the design and performance of a proposed process or system).
	Stability and Change A system can be stable on a small time scale, but on a larger time scale it may be seen to be changing.
Math	**MP2 Reason abstractly and quantitatively** Students analyze qualitative and quantitative data collected from a scientific investigation to identify patterns and construct explanations.
Career and Technical Education (CTE)	**A3.3** Employ standard techniques of DNA extraction, purification, restriction digests, bacterial cell culture, and agarose gel electrophoresis and document and evaluate results.
	A4.2 Describe conditions that promote cell growth under aseptic conditions in the laboratory and workplace.
	A4.3 Use various methods to monitor the growth of cell cultures.
	A8.1 Follow written protocols and oral directions to perform a variety of laboratory and technical tasks.

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National Standards

CTE *Continued*	**A8.6** Properly and safely use and monitor a variety of scientific equipment, including pH meters, microscopes, spectrophotometers, pipettes, micropipettes, and balances.
	A8.7 Determine which equipment is appropriate to use for a given task and the units of measurement used.
	A8.8 Perform specimen collection, label samples, and prepare samples for testing.
	A9.4 Cite examples of plant parts or extracts used as pharmaceuticals. A9.5 Use the Internet to find information about traditional pharmaceuticals, herbal remedies, and recombinant pharmaceuticals.

Lab

Preparation

KEY

When the preparation task should take place in relationship to the lab

The amount of time necessary to complete the preparation task

Quick Tips	
1	Before continuing, check the *Materials List* to make sure you have all the necessary equipment and reagents for the lab.
2	Before the lab, every student needs to collect a small amount (~500 µL when crushed) of a plant or plant extract of their choice. This could be a piece of leaf, root, ground spice, etc.
3	We recommend having students complete this lab in pairs, with each student collecting and preparing their own plant sample. For easier set-up, we suggest having two pairs share some of the reagents described below.
4	*Virtual Learning Options* for this lab, including digital-only resources, are provided.

Preparation

1 — Up to 4 weeks before lab — 1 hr

Part 1: Extract Bioactive Compounds

☐ Use a hole punch to prepare four filter paper discs per pair plus a few extra.

☐ Aliquot the following into 1.5 mL tubes for two student pairs to share:

Reagent	Exact volume per pair	Aliquot volume per two pairs (includes overage)
Extraction buffer	600 µL	1.25 mL
Antibiotic (ampicillin)	100 µL	225 µL

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Lab

Continued

Preparation

2 | Up to 4 weeks before lab | 2+ hrs

Part 2: Kirby-Bauer Assay

☐ Pour one LB agar plate per pair plus 10 LB plates per class section (five for *E. coli* culture and five extra) and store upside down in the refrigerator. If storing for more than one week, place the plates in plastic bags to prevent drying out.

— View *BABEC How-To Videos: How to Pour Plates*

— See instructions *Preparing LB Agar Plates*

☐ Aliquot the following for two student pairs to share:

Reagent/Material	Exact volume per pair	Aliquot volume per two pairs *includes overage*	Container
LB	250 µL	600 µL	1.5 mL tube
Sterile glass beads	~10 beads	~1 mL of beads	1.5 mL tube
Lysol or 10% bleach	N/A	25–50 mL	Waste beaker

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Lab

Continued

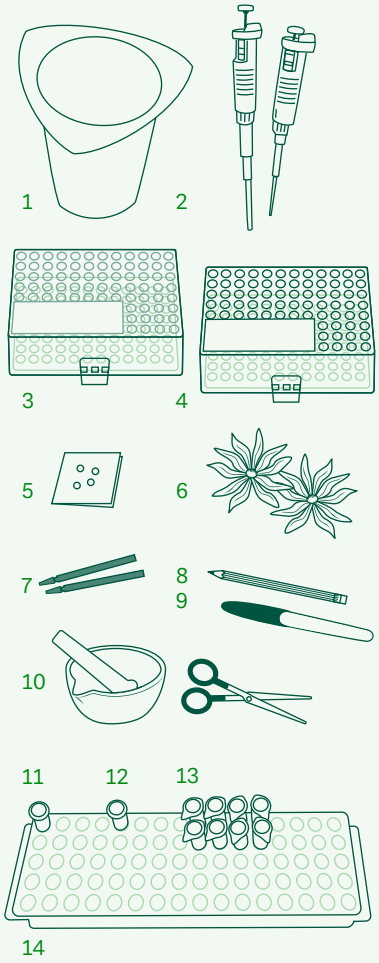
Preparation

3 | 2 days before lab | 30 min

Part 1: Extract Bioactive Compounds

☐ Set up lab stations (1 per pair).

1	Waste bucket
2	P200, P1000
3	P200 yellow tips
4	P1000 blue tips
5	4 filter paper disks
6	Student plant samples
7	2 micropestles
8	Pencil
9	Permanent marker
10	Mortar and pestle or scissors
11	Ampicillin (for two pairs)
12	Extraction buffer (for two pairs)
13	8 1.5 mL tubes
14	Microtube rack



☐ **Note** > *Micropestles are reusable. Clean with soap and water and soak in ethanol to sterilize between uses. Filter paper discs are shown on a paper towel for reference. You can put them all into a microtube for your students.*

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Lab

Continued

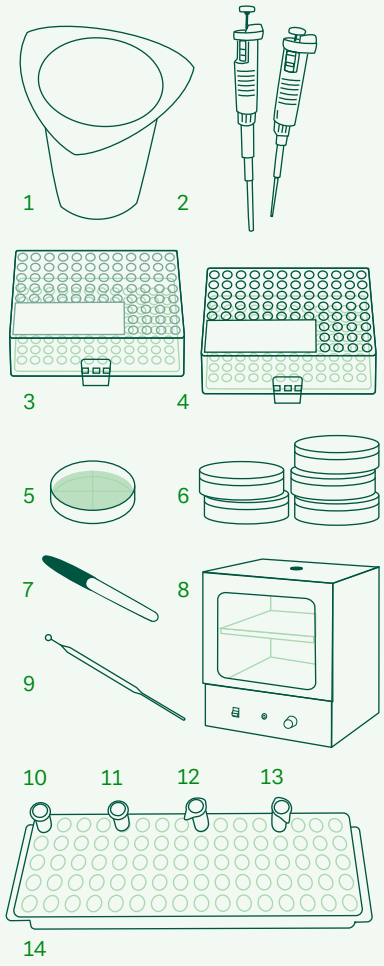
Preparation

4 | 2 days before lab | 30 min

Part 2: Kirby-Bauer Assay

☐ Set up lab stations (1 per pair).

1	Waste bucket with lysol
2	P200, P1000
3	P200 yellow tips
4	P1000 blue tips
5	LB agar plate
6	*E. coli* culture (5 plates per class)
7	Permanent marker
8	Incubator
9	Inoculation loop
10	1.5 mL tube
11	LB (for two pairs)
12	Filter discs #1–4 from Student Protocol Part 1
13	Glass beads (for two pairs)
14	Microtube rack



☐ **Note** > *Suggest having two groups of four share one* E. coli *culture plate or setting up two to three stations around the room with one to two plates at each.*

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Lab

Continued

Preparation

5	24 hours before lab	30 min
	Prepare E. coli *starter plates.*	
☐	Set the incubator to 37˚C.	
☐	Take LB agar plates out of the refridgerator to bring them to room temperature.	
☐	Streak five starter plates per class section on LB agar plates and place at 37˚C. Bacteria should be 24 hours old and warm for the lab. **Note >** *See Streaking Starter Plates resource.*	
6	After the lab	30 min
☐	Properly dispose of lab supplies. **Note >** *See Lab Safety.*	

Lab

Continued

Virtual Learning Options

1	Anytime	30 min
☐	Ask students to complete this *Kirby-Bauer Assay Simulation* from Michigan State University.	
☐	Students are presented with one of three randomized Kirby-Bauer assay plates, containing *Staphylococcus aureus*, *Escherichia coli*, or *Pseudomonas aeruginosa* that have been tested for susceptibility to 13 different antibiotics.	
☐	Students will measure the zones of inhibition for each and determine if the bacteria is susceptible, intermediate, or resistant to each antibiotic.	

Skills

Lab Safety

Your school's specific safety and disposal policies should always take precedence.

Make sure you are **familiar** with the procedures at your site.

To disinfect consumables and used plates

1 Select your cleaning agent: You can use the following easily available solutions for killing bacteria on classroom surfaces, agar plates, plastic consumables:

- 10% bleach solution
- Lysol or similar disinfectant spray
- Rubbing alcohol (isopropanol or ethanol, at least 70% concentration)

Note > *If using bleach solution, make sure it does not mix with ammonia, acids, or other cleaners and wear gloves when removing the plates.*

2 Disinfect agar plates with live bacteria:

- Use a pail, bucket, or other large liquid storage unit and fill with your cleaning agent of choice.
- Put all the bacterial plates into the cleaning solution, making sure that each plate has contact with the solution.
- Let soak for 10–20 minutes.
- Remove plates from the bleach solution and put into a trash bag.
- Trash can be disposed as usual and the cleaning solution can be disposed of in the sink with running water.

3 Disinfect plastic consumables:
For inoculating loops, transfer pipettes, pipette tips, and any other disposable materials that have come into contact with live bacteria, follow the same procedures as listed above.

Note > ***If you have an autoclave****, you can sterilize any used LB agar plates and plastic consumables following the manufacturer's directions. Autoclaved waste can then be disposed of in the regular waste.*

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Skills

Continued

Lab Safety

The *E.coli* used in this lab	
1	*E. coli* is a bacteria found everywhere in our environment. The strain we use for this lab and in many research scientific labs are harmless to humans and are NOT pathogenic. They have been specially engineered to help scientists with their work.
2	If you touch the bacteria with your hands, simply wash with soap and water. If you get some bacteria in your eyes, simply flush with water. As always, use safety precautions when working in the laboratory.

Aseptic technique	
1	When growing bacteria in culture, it is important to prevent the growth of unwanted microorganisms in the nutrient-rich media.
2	Aseptic technique is a series of methods that are used to minimize the chances of contamination.
3	Examples include use of sterile tubes and pipettes, sterilized solutions, cleaning the work area with disinfectants, use of Bunsen burners, and keeping the caps of tubes, plates, and pipette boxes closed.

UV safety	
1	Ultraviolet (UV) radiation can cause damage to eyes and skin.
2	Use UV rated safety glasses or goggles if looking directly at UV light.

Skills

Preparing LB Agar Plates

Prepare in Advance	
1	Set up the following materials: • LB Agar, 300 mL bottles • Microwave (to heat LB agar before pouring) • Oven mitt (to handle heated LB agar) • Permanent markers (black, red, green) • 40–120 of 60 x 15 mm Petri dishes (small plates)

Procedure	
1	Loosen the cap of a bottle of 300 mL LB agar. *A single bottle will pour 25–35 plates (or 1+ sleeves).*
2	Microwave the bottle for 60–90 seconds.
3	Use caution and oven mitts as you swirl the mixture. *Caution: solid agar may superheat so agitate the bottle by pushing on it gently in the microwave so it can bubble and release pressure before you pick it up.*
4	Repeat Steps 2–3 until the LB agar is liquefied.
5	Cool each bottle to approximately 50–55˚C. *Do not use a thermometer to check.* *It should be comfortable to the touch, but has not yet solidified.* While waiting for it to cool, continue to the next step.

Continues next page >

Skills

Continued

Preparing LB Agar Plates

6	Stack plates 3–5 high (depending on how many you can comfortably pick up with one hand).
7	Holding the whole stack in your hand, start by lifting up the lid of the plate on the bottom of the stack. *Hold the lid open just enough for you to pour the LB Agar from the bottle.*
8	Fill the plate about 1/3 full of liquid agar and replace the lid. • If you notice air bubbles or that the liquid agar has not evenly spread throughout the plate, gently swirl the plate, avoiding splashing agar on the lid of the dish. • You may also use a sterile pipette tip to get rid of air bubbles.
9	Continue pouring from the bottom plate up. *Work quickly as the agar may solidify.*

Note > *Once you start pouring a bottle of LB agar, the whole bottle should be poured. Extra plates are useful for student practice.*

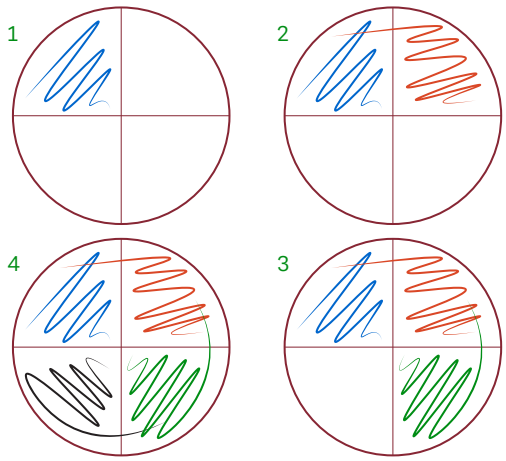
10	Plates should be left out (cured) at room temperature for one to two days after pouring. Curing evaporates off the excess moisture from the condensation accumulated on the plates.
11	Tape sleeves closed to prevent accidental opening.
12	For storage, refrigerate as a stack in their original plastic sleeves with the plates *upside down (the lid on the bottom)*.

Skills

Streaking Starter Plates

Teacher Note > *Starter plates are needed to produce bacterial colonies of* E. coli *on agar plates. LB agar plates should be streaked to produce single colonies and incubated at 37°C for 24 hours before the transformation begins. Under favorable conditions, one cell multiplies to become millions of genetically identical cells in just 24 hours. There will be millions of individual bacteria in a single millimeter of a bacterial colony. Depending on time, you may prefer your students to learn how to streak their own plates for individual colonies.*

Procedure	
1	Draw quadrants on the underside of the Petri dish. Using a sterile inoculation loop or sterile pipette tip, pick up one bacterial colony from a live *E. coli* culture plate.
2	Using a back and forth motion, gently spread the colony into one quadrant of the LB starter plate. Keep the lid slightly tilted open—only as much as necessary. Be careful not to puncture the agar.
3	Rotate the plate one-quarter of a turn. Go into the previous streak about two times and then back and forth as shown for a total of about five to ten times.
4	Again, rotate the plate one-quarter of a turn and pass over a previous streak from the previous quadrant several times with the loop.
5	Repeat Step 3, but this time, drag out the loop to form a tail not touching any previous streaks. Close your plate to avoid further contamination.



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Skills

Continued

Streaking Starter Plates

6	Place the used loop (or tip) in a disinfectant solution waste cup. Follow this procedure for the remaining starter plates. Once starter plates are inoculated, *incubate them upside down (lid on the bottom)* in a 37˚C incubator for 24 hours.
7	The next day you should see individual bacterial colonies in Quadrant 4, and very dense bacterial growth in Quadrant 1. Quadrants 2 and 3 will have bacterial density somewhere in between, similar to what is seen below:

Bioactive Compounds from Plants Questions

ANSWER KEY **Do not share with students**

Directions
Answer the questions below after closely reading the Bioactive Compounds from Plants background material.

1. Describe why maintaining biodiversity of plants is important for finding new medicines.

Plants have extensive diversity in chemical compounds, many of which may have medicinal properties. Therefore, maintaining biodiversity is essential for preserving compounds that have not yet been identified but may be discovered in the future as pharmaceuticals.

2. What is a bioactive compound? Provide an example.

A bioactive compound is a type of chemical found in small amounts in plants that have actions in the body that may promote good health. An example of a bioactive compound is curcumin.

3. What steps are involved in creating a pharmaceutical derived from a plant compound? For each step, write a synonymous word or short phrase that helps you understand the purpose and definition of that step.

— Extraction—take out the compound of interest.
— Screening using an assay to determine pharmacological properties—evaluate what beneficial properties it has for use as medicine.
— Toxicological evaluation—make sure it is safe.
— Clinical evaluation—test to understand how it will affect the body.

Kirby-Bauer Assay Questions

ANSWER KEY — **Do not share with students**

Directions
Answer the questions after closely reading the Kirby-Bauer Assay background material.

1. Briefly describe the two main uses of Kirby-Bauer assays.

a. Used in drug discovery to identify new antibiotics in extracts from things like plants and microorganisms

b Used in diagnostics to determine the appropriate antibiotic to treat a patient's bacterial infection

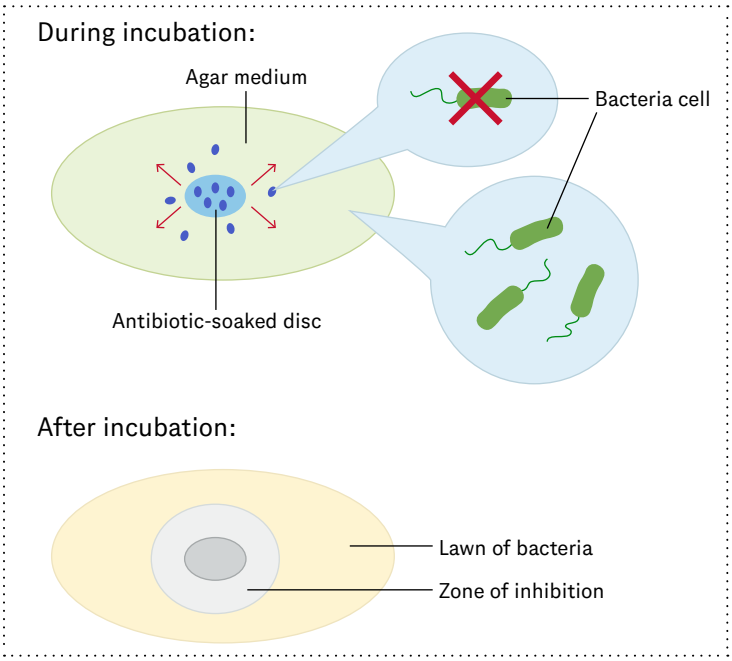
2. Name three characteristics of the *E. coli* bacteria used in research.

— Non-pathogenic—won't harm humans
— Duplicates every 20 min when grown in nutrient-rich media at 37˚C
— Looks white and sticky on a plate of agar media
— It is the most commonly cultured bacteria in labs.

3. Why do you think a Kirby-Bauer assay is also called a "disc diffusion test"?

The antibiotic that the paper disc is soaked in diffuses out of the disc and into the agar medium. This inhibits the growth of the bacteria growing on the medium during incubation, resulting in a circular zone around the disc with no visible bacteria.

4. Draw a picture to describe what happens during the assay.



Student Guide, Part 1: Pre-Lab

ANSWER KEY — **Do not share with students**

Directions

In this lab, you will play the role of a pharmaceutical scientist looking to discover plants with antimicrobial properties that can be further explored for developing new drugs.

1. Choose a plant you would like to test and make a prediction for whether or not it will have antibiotic properties.

2. Explain why you selected the plant you did for the Kirby-Bauer test. This may include traditional or familial knowledge, Internet research, a personal experience, or an anecdote.

Example:

		Reasoning
Your plant	Garlic	My family uses garlic oil whenever someone has an ear, nose, or throat infection and it always seems to work in helping to reduce symptoms or clearing up the illness altogether. I think this means that there are antibiotic properties that are helping to kill the bacteria that is causing the infection.
Will it have antibiotic properties?	Yes	

Student Guide, Part 2: Lab

ANSWER KEY **Do not share with students**

Directions
Answer the questions below to prepare for the lab and record your data after completing the lab.

1. Complete the table below with each partner's name and the contents of their sample.

Example:

Sample Key

ID	Name	Contents
1	Negative Control	Extraction Buffer
2	Positive Control	Antibiotic (ampicillin)
3	Student 1 CL	Garlic
4	Student 2 ED	Cinnamon

Continues on next page >

Student Guide, Part 2: Lab

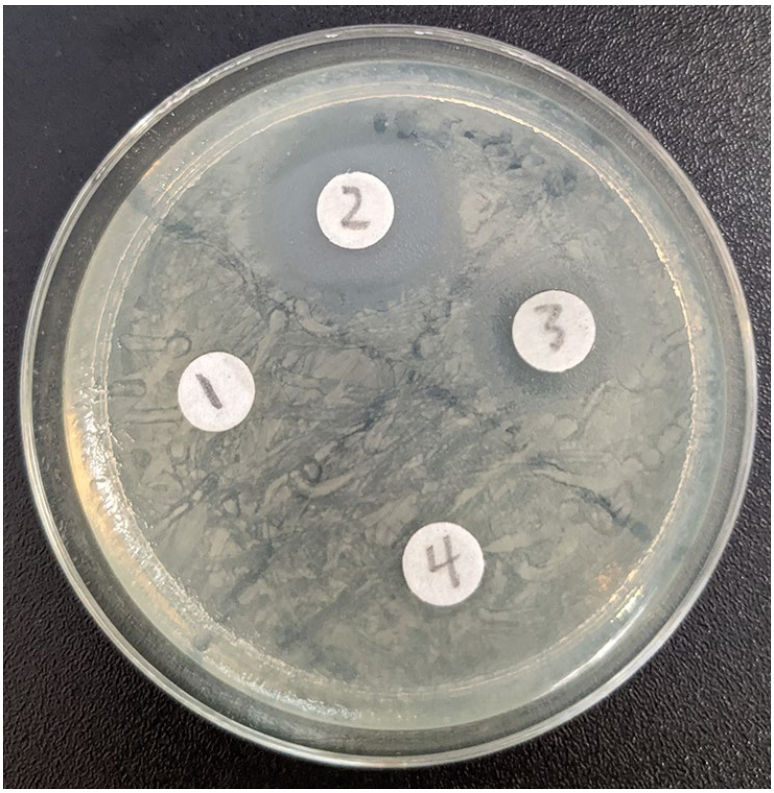
ANSWER KEY **Do not share with students**

Continued

2. Draw or paste the picture of your petri dish below.
   Label each disc (without writing directly on the picture).

Example results:

1 = extraction buffer (negative control)
2 = ampicillin (positive control)
3 = garlic
4 = cinnamon



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Student Guide, Part 2: Lab

ANSWER KEY **Do not share with students**

Continued

3. Rate each sample for the size of its zone of inhibition on a scale of 0 to 5 (0 = no inhibition of growth, 5 = largest zone of inhibition).

Example data:

ID	Sample Name	Zone of Inhibition (0–5)
1	Negative Control	0
2	Positive Control	5
3	Student 1 Garlic	3
4	Student 2 Cinnamon	1

Student Guide, Part 3: Data Analysis

ANSWER KEY **Do not share with students**

Directions

Analyze your results from the lab by answering the questions below.

1. Based on the results of the Kirby-Bauer test, does your plant have antibiotic properties? Explain how you know. Does this support or refute your prediction? How does it compare to the other samples on the plate?

Sample answer:

According to our Kirby-Bauer test, garlic does have some antibiotic properties. There was a medium-sized zone of inhibition surrounding the disc soaked in garlic extract (3 out of 5), meaning the *E. coli* bacteria were not able to grow in the agar medium in which the extract had diffused. This supports my prediction because garlic is often taken as a health supplement so it seemed likely that it also has antibiotic properties.

The zone of inhibition around the positive control was much larger than around the garlic (5 out of 5), meaning the bacteria are not as susceptible to garlic extract as they are to the antibiotic ampicillin used as a positive control. However, the zone of inhibition around the other plant extract tested, cinnamon, was smaller (a 1 out of 5), indicating garlic has stronger antibiotic properties than cinnamon.

2. Describe two potential sources of error in this lab and how they may affect the results. Types of error include systematic, procedural, random, and human error.

Examples:

Source of error	How it may affect results
Plant extract was not concentrated enough (systematic/procedural).	False negative result—zone of inhibition may be smaller than if more of the bioactive compounds in the plant had been present on the disc.
Alcohol used did not extract bioactive compounds from the plant (systematic/procedural).	False negative result—if alcohol was not the appropriate chemical to extract the bioactive compounds in the plant, the compounds would never be on the disc.
Alcohol used to extract bioactive compound remained on disc (systematic/procedural).	False positive result—zone of inhibition may be larger than if all the alcohol evaporated from the disc. This would mean the alcohol was responsible for inhibiting growth, not the plant extract.
Filter paper or forceps were contaminated (human).	False positive result—zone of inhibition may be larger than expected if the materials were contaminated with the positive control antibiotic or another plant extract with antibiotic properties.
Bacteria were incubated for too long or too much bacteria was plated (human).	False negative result—the longer the plate incubates, the more diffuse the plant extract becomes in the agar, leading to more bacterial growth.

Student Guide, Part 4: Obtaining Information

ANSWER KEY — **Do not share with students**

Directions

Conduct further research about your plant using multiple scientific sources, including at least one peer-reviewed journal article. Organize your findings into the tables below.

1. Provide information on the beneficial properties of your plant and the bioactive compounds that may be responsible for these properties.

Example:

Your plant	Garlic
Scientific name	Allium sativum

Three beneficial properties of your plant	**1**	Antioxidant activity
	2	Anti-Inflammatory activity
	3	Antimicrobial activity

For each property, briefly describe the bioactive compounds in the plant that confer those benefits, if this information is available.	Antioxidant Activity: Both raw and cooked garlic exhibit antioxidant effects due to its active ingredients such as phenols and saponins. The mechanism of antioxidative action of garlic might be involved with the enhancement of antioxidant enzyme activities and the regulation of the Nrf2-ARE pathway.
	Anti-Inflammatory Activity: Garlic can inhibit inflammation mainly by inhibiting inflammatory mediators such as nitric oxide (NO). Studies show that some of the active compounds found in garlic responsible for this are ethyl linoleate and 14-kDa protein.
	Antimicrobial Activity: Garlic has a broad spectrum of antibacterial and antifungal properties, suppressing growth of bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis*. One specific bioactive compound found in garlic is called allicin which has strong bactericide power.
Sources	*Bioactive Compounds and Biological Functions of Garlic (Allium sativum L.)*

Continues next page >

Student Guide, Part 4: Obtaining Information

ANSWER KEY **Do not share with students**

Continued

2. Provide a brief history of your plant including but not limited to where it originates and at least three examples of how it has been used by various civilizations or Indigenous cultures throughout time.

Garlic originates in Middle Asia in the region now known as West China to Kazakhstan. Sumerians and ancient Chinese peoples were using garlic as early as 2700 BC for its "heating and stimulating effects". Therefore, it was recommended to those who suffered from depression. In ancient Indian medicine, garlic was used almost as a cure-all for ailments such as cough, rheumatism, weakness, skin diseases, etc. In Egypt, slaves were fed garlic because it was believed to make them strong and capable of doing more work. In ancient documents, garlic is touted as efficient in healing 32 illnesses and garlic bulbs have been found in the pyramids.

Sources	*Extracts from the history and medical properties of garlic*

Continues next page >

Student Guide, Part 4: Obtaining Information

ANSWER KEY **Do not share with students**

Continued

3. Does your research support your Kirby-Bauer results? Explain.

Yes, the research supports the Kirby-Bauer results. There are many bioactive compounds found in garlic that have antibacterial properties such as allicin. The results showed a medium zone of inhibition around the garlic extract indicating that the *E. coli* bacteria were not able to grow, demonstrating antibacterial properties.

4. Based on your research and results, create a testable question about the properties of your plant or plant extract that interests you for further experimentation.

Sample answer: In what form does garlic confer the strongest antibacterial properties? Garlic oil, aged garlic, young garlic?

5. Would you recommend your plant for further research as a pharmaceutical? Provide rationale for your answer.

Example: Yes, I would definitely recommend my plant for further research as a pharmaceutical. Both my research and Kirby Bauer results show that garlic has antibacterial properties which can be helpful for treatment of bacterial infections. Additionally, my research shows that garlic has many useful bioactive compounds that reduce inflammation and have antioxidant properties.

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Background Reading: Bioactive Compounds from Plants

As new illnesses emerge and the pathogens that make us sick evolve, there is more and more need to discover new medicines. When looking for new pharmaceuticals, naturally-derived products, such as plant extracts provide nearly unlimited opportunities because of the extensive diversity in their chemical compounds. Numerous medicinal plants have already been identified, particularly in biodiverse areas such as rainforests, and are used regularly by many cultures. The World Health Organization (WHO) estimates that there are nearly 20,000 medicinal plants in 91 countries, 12 of which have been labeled 'mega biodiversity' countries (Duraipandiyan et al., 2006).

When exploring a particular plant for potential medicinal use, the first step is to extract its bioactive compound. Bioactive compounds are plant chemicals that treat disease and/or promote good health. For example, the bioactive compound curcumin, which is found in turmeric, has been shown to have beneficial anti-cancer and anti-inflammatory properties (Sharifi-Rad et al., 2020).

Continues next page >

Medicinal plants and plant extract

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Background Reading: Bioactive Compounds from Plants

Continued

After a bioactive compound has been extracted from a plant, it can be tested using a variety of assays to determine if it has pharmacological properties, such as being antibacterial. Once a particular compound has been identified as having the desired characteristics, it can then continue through the product development life cycle by being evaluated for toxicity and tested through clinical trials. These trials help scientists understand how compounds move within the body, their bioavailability (the proportion that enters the bloodstream), and efficacy (ability to produce an intended result).

An example of a pharmaceutical that was developed directly from a plant is metformin, a drug widely-used to treat type 2 diabetes. Metformin is produced from galegine, a blood-sugar lowering bioactive compound found in goat's rue or French lilac (*Galega officinalis*). In medieval Europe, this plant was used to relieve symptoms of the disease, such as frequent urination, a symptom we now know to be associated with type 2 diabetes (Bailey, 2017).

To read more about the history of metformin and its herbal lineage in French lilac, see the article *Metformin: Historical Overview.*

Sources

Extraction, Isolation and Characterization of Bioactive Compounds from Plants' Extracts

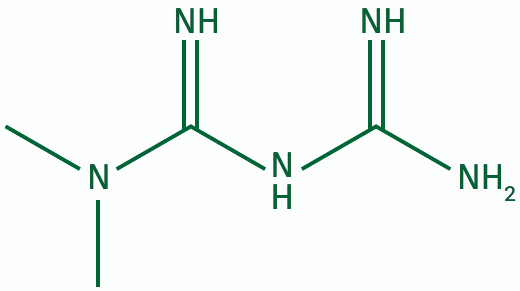
The Blooming of the French Lilac

Metformin: Historical Overview

Turmeric and Its Major Compound Curcumin on Health

Metformin as found in French lilac

French lilac (*Galega officinalis*)



Metformin ($C_4H_{11}N_5$)

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Bioactive Compounds from Plants Questions

Directions
Answer the questions below after closely reading the Bioactive Compounds from Plants background material.

1. Describe why maintaining biodiversity of plants is important for finding new medicines.

2. What is a bioactive compound? Provide an example.

3. What steps are involved in creating a pharmaceutical derived from a plant compound? For each step, write a synonymous word or short phrase that helps you understand the purpose and definition of that step.

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Background Reading: Kirby-Bauer Assay

To safely study infection-causing bacteria and the antibiotics that kill them, scientists grow them in a controlled setting in a lab and perform many different types of tests. One of these tests is called the Kirby-Bauer assay, also known as a disk diffusion test. It is named for two scientists that helped develop it in the 1950s and 60s: W.M.M. Kirby and A.W. Bauer. This assay is used both in drug discovery to identify new antibiotics, and in diagnostics to determine if a patient's bacterial infection can be treated with a certain antibiotic. There are two main components of a Kirby-Bauer assay: 1) an agar plate spread with bacteria and 2) paper discs soaked in an antibiotic (or compound being tested as an antibiotic).

An agar plate is a Petri dish filled with a gel-like substance (called agar) that contains nutrients for the bacteria (called media). It looks like pale yellow jello and provides a solid surface on which bacteria can grow. Bacteria that are grown under controlled conditions in a lab form a bacterial culture. The most commonly cultured bacteria used in labs, including this lab activity, is *Escherichia coli* (*E. coli*). You may have heard of harmful strains of *E. coli* that cause food poisoning and can even lead to death; however, the strains used in research are non-pathogenic and do not pose a risk to humans.

E. coli are single-celled organisms that reproduce asexually about every 20 minutes when grown at 37˚C in nutrient-rich media. This means that about every 20 minutes, the population of bacteria doubles on an agar plate until they begin to run out of nutrients. After about 24 hours of being spread with *E. coli* and incubated at 37˚C, an agar plate will be covered with a "lawn" of bacteria. This looks like a sticky, white coating on the surface of the agar.

There are four main steps in a Kirby-Bauer assay:

1. Spread an even layer of diluted bacteria onto an agar plate. You will not be able to see the bacteria at this point because there are too few cells on the plate to be seen by the unaided eye.
2. Soak a paper disc in the antibiotic (or in the extract that may contain an antibiotic) and place it on the plate that has been spread with bacteria.

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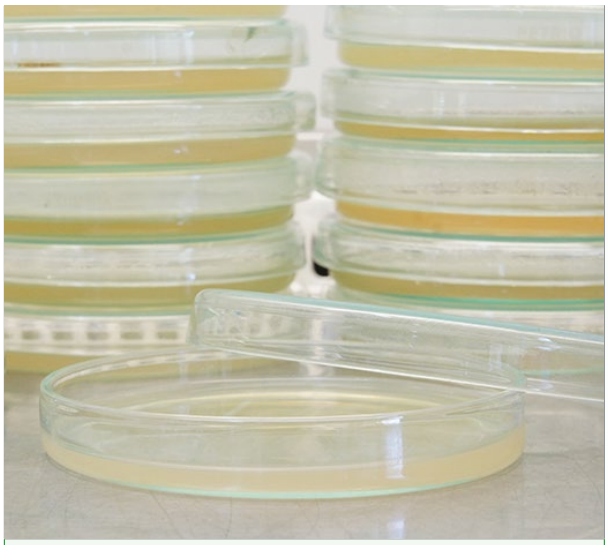
Stacks of agar plates and one plate with *E. coli* growing on the surface after incubation



Agar media for culturing bacteria

E.coli on agar plate (Cross streak technique)

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Background Reading: Kirby-Bauer Assay

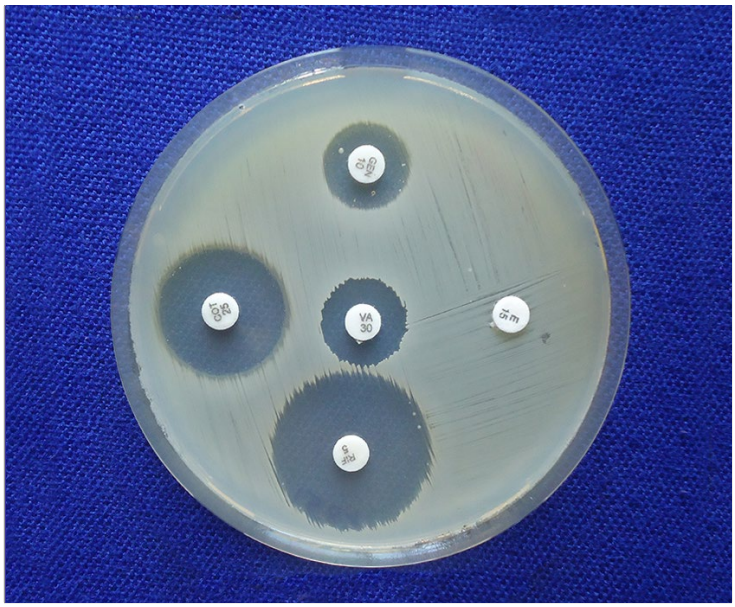
Continued

3. Incubate the plate at 37˚C for 24 hours. This is the same as human body temperature and is the ideal temperature for *E. coli* bacteria to grow.
4. Look for a "zone of inhibition" around the paper disc. A zone of inhibition is a circular region surrounding the disc where bacteria did not grow. A larger zone of inhibition means that the bacteria were more susceptible to (more likely to be killed by) that particular antibiotic.

In drug discovery, the goal of the Kirby-Bauer assay is to determine if an extract from a plant or microorganism can inhibit the growth of bacteria. If the compound successfully prevents the bacteria from growing, then it likely has antibiotic properties and can be further explored as a new antibiotic. In diagnostics, the bacteria grown on the agar plate have first been isolated from a patient's infection. Different antibiotics are then tested to see which most successfully inhibits the growth of the bacteria. Doctors can then choose the most effective antibiotic for the patient's particular infection.

The image below shows a Kirby-Bauer assay in which five different antibiotics were tested. Because the disc at the bottom of the plate is surrounded by the largest zone of inhibition (largest diameter without bacteria), it contains the most effective antibiotic for inhibiting the growth of the bacteria on the plate.

Kirby-Bauer Disc Diffusion Test



Sources

Kirby-Bauer Disk Diffusion Susceptibility Test Protocol

Growth of Bacterial Populations

Kirby Bauer Assay

Kirby-Bauer Disk Susceptibility Test

Zone of Inhibition Test for Antimicrobial Activity

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Kirby-Bauer Assay Questions

Directions
Answer the questions after closely reading the Kirby-Bauer Assay background material.

1. Briefly describe the two main uses of Kirby-Bauer assays.

2. Name three characteristics of the *E. coli* bacteria used in research.

3. Why do you think a Kirby-Bauer assay is also called a "disc diffusion test"?

4. Draw a picture to describe what happens during the assay.

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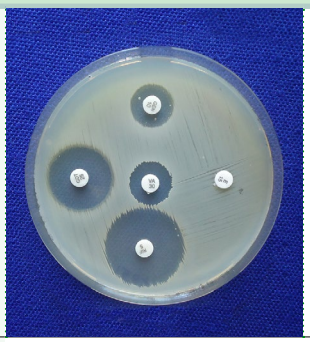
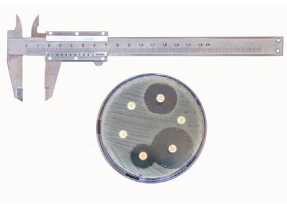
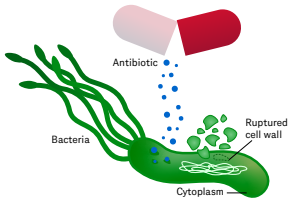
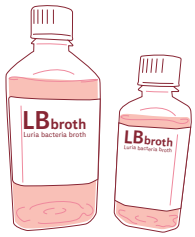
Vocabulary Tool

Directions
For each vocabulary word, write a new sentence that helps you practice using it.

Word	Image	Definition	Example Sentence	My Sentence
Kirby-Bauer assay		A lab technique that uses bacteria culture to test antibiotics	To determine if a plant has antibiotic properties, scientists can perform a *Kirby-Bauer assay.*	
Zone of inhibition		The area surrounding an antibiotic-soaked disc in a Kirby-Bauer assay where bacteria are not growing	The larger the *zone of inhibition*, the more susceptible the bacteria are to the antibiotic.	
Antibiotic		A substance that inhibits the growth of or kills bacteria	Ampicillin is a commonly used *antibiotic* in a lab.	
LB *Luria Broth*		A nutrient-rich liquid used to grow bacteria	*LB* is food for the bacteria.	

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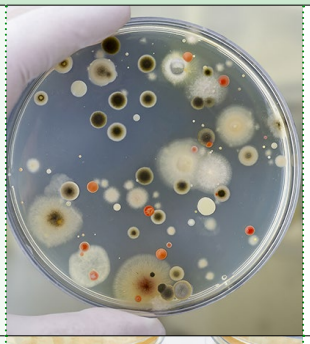
Vocabulary Tool

Continued

Word	Image	Definition	Example Sentence	My Sentence
Bacteria culture		A method where bacteria is grown in a petri dish using a nutrient-rich medium.	*E. coli* is a commonly used *bacteria culture* in a lab.	
Agar plate		A nutrient-rich, jelly-like solid in a petri dish used to grow bacterial cultures	You will grow bacteria on *agar plates*.	
Bioactive compound		A type of chemical found in plants that may have medicinal properties and promote good health	Turmeric contains a *bioactive compound* called curcumin, which has been shown to have anticancer properties.	

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Student Protocol

Part 1: Extract Bioactive Compounds

A		Prepare Materials
☐	1	Label a clean 1.5 mL microtube with your initials and group number on the lid and side of the tube.
☐	2	Record the contents of each sample in the *Sample Key* table in the Student Guide.
☐	3	Get four filter paper discs and label them #1–4 with a pencil.
☐	4	Label four 1.5 mL tubes #1–4 to be used for extraction.
☐	5	Label four 1.5 mL tubes #1–4 to be used to store the filter paper discs. Add a unique symbol or both sets of initials to easily identify these tubes in the next class.
B		Prepare Student Samples
		Note > *Each student prepares their own sample.*
☐	6	If your plant sample is large (like a leaf) break or crush it into smaller pieces with scissors or a mortar and pestle.
☐	7	Get one of the tubes with your Sample ID (#3 or #4) and fill it with your sample up to the 100 µL mark.
☐	8	Add 250 µL of extraction buffer to the tube.
☐	9	Gently mash the sample with a micropestle for one to two minutes.
☐	10	Add the corresponding filter disc to the tube and use a pipette tip to completely submerge it in the liquid.
☐	11	Wait two minutes for the disc to fully soak up the extract.
☐	12	Get the other tube with your Sample ID (#3 or #4).
☐	13	Use a pipette tip to move the disc out of the liquid and into the new tube.
☐	14	Close the lid of the new tube.

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Student Protocol

Part 1: Extract Bioactive Compounds

Continued

C		Prepare Control Samples
		Note > *One student prepares the negative control and one prepares the positive control.*
		Negative Control
☐	15	Add 100 µL of extraction buffer to one of the tubes with Sample ID #1.
☐	16	Add the filter disc labeled #1 to the tube.
☐	17	Close the lid and tap the tube gently on the table to make sure the disc is submerged.
☐	18	Wait one minute for the disc to fully soak up the extraction buffer.
☐	19	Get the other tube with Sample ID #1.
☐	20	Use a pipette tip to move the disc out of the liquid and into the new tube.
☐	21	Close the lid of the new tube.

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Student Protocol

Part 1: Extract Bioactive Compounds

Continued

		Positive Control
☐	22	Add 100 µL of antibiotic (ampicillin) to one of the tubes with Sample ID #2.
☐	23	Add the filter disc labeled #2 to the tube.
☐	24	Close the lid and tap the tube gently on the table to make sure the disc is submerged.
☐	25	Wait one minute for the disc to fully soak up the antibiotic.
☐	26	Get the other tube with Sample ID #2.
☐	27	Use a pipette tip to move the disc out of the liquid and into the new tube.
☐	28	Close the lid of the new tube.
D		Finish
☐	29	Give your set of tubes #1–4 with the filter paper discs to your teacher to store at room temperature until the next class.
☐	30	Clean up: — Keep your extra plant sample in case you want to repeat the experiment. — Return micropestles to your teacher for cleaning. — Return excess controls to your teacher. — Throw used 1.5 mL tubes in the trash.

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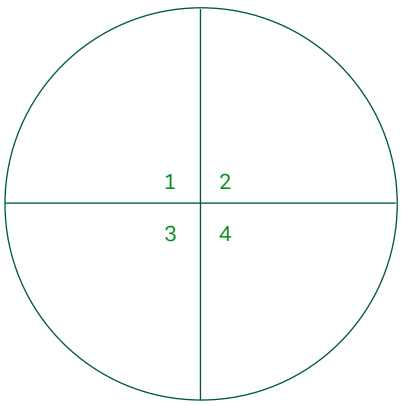
Student Protocol
Part 2: Kirby-Bauer Assay

A		Prepare Liquid Cell Suspension
☐	1	Gather all the materials needed for the lab and clear the working area of clutter.
☐	2	Label a clean 1.5 mL tube with “Cells”.
☐	3	Add 250 µL of LB to the tube.
☐	4	Get a plate of *E. coli* bacteria cells from your teacher.
☐	5	With a loop, pick up a small smear of cells (about the size of a grain of rice) and carefully submerge it in the labeled tube. Twist and tap the loop to get the cells off the loop and into the LB.
☐	6	Close the lid of the tube and “rack” the tube to resuspend the cells (hold the top of the tube firmly and drag it all the way across the wells of a microtube rack). The LB should now be cloudy with no visible clumps of cells.
B		Prepare Agar Plate
☐	7	Get an LB agar plate and permanent marker.
☐	8	Use the marker to divide the bottom side of the plate (not the lid) into four sections. Label each section #1–4 in small writing close to either the center or the outer edge: 
☐	9	Label the side of the plate (not the lid) with your and your partner’s initials.

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Student Protocol

Part 2: Kirby-Bauer Assay

Continued

C		Plate the Cell Suspension
☐	10	Set the agar plate lid-side up, gently lift the lid, and set the lid to the side.
☐	11	Transfer 100 µL of liquid cell suspension to the middle of the plate (not the lid).
☐	12	Pour a small amount of glass beads (~10) onto the plate.
☐	13	Put the lid of the plate back on.
☐	14	Gently swirl the plate back and forth so that the glass beads evenly spread the cell suspension across the plate.
☐	15	Take the lid off and pour the glass beads off the plate into a dedicated beaker filled with Lysol or similar disinfectant.
☐	16	Put the lid of the plate back on.

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Student Protocol

Part 2: Kirby-Bauer Assay

Continued

D		Plate the Filter Paper Discs
☐	17	Get your tubes with the filter paper discs labeled #1–4 from Part 1.
☐	18	Label a paper towel with #1–4 spaced out in a row.
☐	19	Gently tap each disc from each tube onto the paper towel next to the corresponding number.
☐	20	Let the discs air dry for at least one minute. Make sure all the discs are dried before moving on.
☐	21	Gently lift the lid off the plate and set the lid to the side.
☐	22	Place each disc into the center of its labeled section of the plate.
☐	23	Use a pipette tip to gently press the filter paper onto the LB agar surface. Change the tip in between each sample.
☐	24	Put the lid of the plate back on.
☐	25	Flip the plate upside down (agar side up, lid down).
☐	26	Incubate the plate at 37˚C for 24 hours.

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Student Guide, Part 1: Pre-Lab

Directions

In this lab, you will play the role of a pharmaceutical scientist looking to discover plants with antimicrobial properties that can be further explored for developing new drugs.

1. Choose a plant you would like to test and make a prediction for whether or not it will have antibiotic properties.

Your plant	
Will it have antibiotic properties?	

2. Explain why you selected the plant you did for the Kirby-Bauer test. This may include traditional or familial knowledge, Internet research, a personal experience, or an anecdote.

Reasoning

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Student Guide, Part 2: Lab

Directions
Answer the questions below to prepare for the lab and record your data after completing the lab.

1. Complete the table below with each partner's name and the contents of their sample.

Sample Key

ID	Name	Contents
1	Negative Control	Extraction Buffer
2	Positive Control	Antibiotic (ampicillin)
3	Student 1	
4	Student 2	

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Student Guide, Part 2: Lab

Continued

2. Draw or paste the picture of your petri dish below.
   Label each disc (without writing directly on the picture).

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Student Guide, Part 2: Lab

Continued

3. Rate each sample for the size of its zone of inhibition on a scale of 0 to 5 (0 = no inhibition of growth, 5 = largest zone of inhibition).

ID	Sample Name	Zone of Inhibition (0–5)
1	Negative Control	
2	Positive Control	
3	Student 1	
4	Student 2	

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Student Guide, Part 3: Data Analysis

Directions

Analyze your results from the lab by answering the questions below.

1. Based on the results of the Kirby-Bauer test, does your plant have antibiotic properties? Explain how you know. Does this support or refute your prediction? How does it compare to the other samples on the plate?

2. Describe two potential sources of error in this lab and how they may affect the results. Types of error include systematic, procedural, random, and human error.

Source of error	How it may affect results

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Student Guide, Part 4: Obtaining Information

Directions
Conduct further research about your plant using multiple scientific sources, including at least one peer-reviewed journal article. Organize your findings into the tables below.

1. Provide information on the beneficial properties of your plant and the bioactive compounds that may be responsible for these properties.

Your plant	
Scientific name	

Three beneficial properties of your plant	**1**
	2
	3

For each property, briefly describe the bioactive compounds in the plant that confer those benefits, if this information is available.	
Sources	

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Student Guide, Part 4: Obtaining Information

Continued

2. Provide a brief history of your plant including but not limited to where it originates and at least three examples of how it has been used by various civilizations or Indigenous cultures throughout time.

Sources

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Student Guide, Part 4: Obtaining Information

Continued

3. Does your research support your Kirby-Bauer results? Explain.

4. Based on your research and results, create a testable question about the properties of your plant or plant extract that interests you for further experimentation.

5. Would you recommend your plant for further research as a pharmaceutical? Provide rationales for your answer.

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Obtaining and Communicating Information Rubric

Score	4	3	2	1
Sources	At least two relevant, reliable scientific sources are cited, including at least one peer-reviewed journal article.	Two relevant, reliable sources are cited.	One relevant source is cited.	No sources are cited or those cited are not relevant or reliable.
Content and Clarity	Strong, focused supporting examples and details are provided. Information and ideas are communicated thoroughly and clearly.	Specific supporting examples and details are provided. Information and ideas are communicated thoroughly.	Supporting examples are provided in the explanation but may be inconsistent in clarity or focus.	Inadequate or missing supporting examples; lacks clarity and focus.
Final Score				