Natural Antibiotics: A Hands-on Activity on Garlic’s Antibiotic Properties

Maria João Fonseca, Fernando Tavares

Abstract
This work details a science experiment on garlic’s antibiotic properties designed to acquaint high school and introductory-level undergraduate students with concepts such as natural antibiotics, bioactive substances, and biosafety. This activity is optimized to be implemented by teachers with limited experience in laboratory activities and/or in poorly equipped schools. A list of materials is provided, along with safety and procedural instructions, discussion topics, and assessment suggestions.

Key Words: Allicin; Allium sativum L.; science experiment; natural antibiotics.

Garlic (Allium sativum L.) is widely used for its culinary and pharmacological properties, which include antimicrobial traits. Garlic extracts are known to inhibit the growth of fungi, protozoa, viruses, and numerous bacteria, namely Salmonella spp., Staphylococcus aureus, and Escherichia coli (Rahman, 2007). Garlic’s antimicrobial activity is mainly ascribed to allicin, a bioactive compound present in injured garlic cloves (Harris et al., 2001). Allicin is considered a promising substitute or co-adjuvant for commercial antibiotics (Cutler & Wilson, 2004). In addition to their scientific interest, natural antibiotics like allicin can be used in classroom activities to introduce students to concepts like antibiotics and biosafety as well as basic microbiology techniques (Shimabukuro & Haberman, 2006). Furthermore, assessing bacterial susceptibility to natural antibiotics fosters the discussion of antibiotic resistance, a major public health issue worth addressing in school (Lawson, 2008). This work describes a hands-on activity on the antibiotic effect of garlic shoot juice (GSJ), an allicin-containing aqueous extract. Bacillus cereus, a rod-shaped, spore-forming, Gram-positive food-borne bacterium, is used to encourage students to investigate how aromatic herbs traditionally used in cooking can inhibit microbial flora. Its short generation time provides visual results in approximately one day, and its culturability can be preserved over long periods by freezing spore suspensions. This activity can be conducted in unequipped schools using inexpensive materials available in domestic kitchens.

Learning Objectives
Students will
- demonstrate the existence of phyto-antimicrobials;
- understand the concepts of antibiotics, antibiotic susceptibility, and biosafety;
- perform basic microbiology procedures;
- interpret and discuss experimental outcomes resulting from qualitative observations; and
- develop creativity skills related with motivation and imagination to devise alternative problem-solving strategies.

Materials
Bacillus cereus LMG 6923T (strain for teaching purposes, BCCM™/LMG Bacteria Collection); fresh garlic bulbs; garlic press; 10-mL plastic syringe; gauze; 250-mL glass containers; glass rods; gram scale; microwave oven; petri dishes; Pasteur pipettes and 1-mL pipettes; microbiological loops; agar (bacteriological or available in supermarkets and health food stores); meat (pork or beef); table sugar; kitchen salt; distilled water; 1-L growth-medium flasks (or equivalent microwave glass containers); glass burners; discard container with bleach (20%); ethanol (70%); paper towels; and Falcon and Eppendorf tubes (or equivalent).

Safety Concerns
This activity requires handling of bacteria. Therefore, students must act responsibly. They must wash their hands before and after the exercise, and they must not eat or drink in the lab. Work surfaces must be disinfected with ethanol (70%), and the materials used must be previously sterilized. Liquids, plastics, and glassware can be sterilized using a microwave oven. Metallic materials can be sterilized using boiling water. All materials in contact with bacteria must be sterilized prior to disposal.
**Methods**

This activity is based on antibiotic susceptibility testing using the agar-diffusion method (Figure 1). Students should prepare the growth medium, the bacterial inoculum, and the GSJ extract. Bacterial susceptibility to GSJ on meat agar plates is assessed by observing inhibitory halos surrounding GSJ drops.

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**Growth Media Preparation**

A meat agar medium replaces commonly used growth media such as nutrient agar. To prepare 250 mL of meat agar (enough for approximately 10 plates), boil 150 g of meat and filter the solution through gauze into a 1-L medium flask. Dissolve 6 g of sugar and 1.25 g of kitchen salt, and add 3.75 g of agar. Sterilize the medium

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**Figure 1.** Schematic protocol for assessing antibiotic effect of garlic shoot juice (GSJ).
using a microwave oven (900 W) for 5 minutes (or an autoclave for 15 minutes). To avoid spillage due to overheating, heat the medium in 1-minute increments interspersed with 15-second cooling periods. Because agar solidifies at ~42°C, pour it into the sterile Petri dishes (~25 mL per 9-cm-diameter dish) as soon as the medium temperature allows handling. Plating must be performed quickly in a recently disinfected bench. Allow the medium to solidify before using the plates.

**Bacterial Inoculum Preparation**

To prepare *B. cereus* spore suspensions, pour 5 mL of sterile distilled water onto a four- to five-day-old culture plate, suspend the spores with a sterile glass rod, and transfer the suspension to a sterile Falcon tube containing 20 mL of sterile distilled water. Dilute the suspension 10-fold (1.9 mL of sterile distilled water). Place the used pipettes in a container with bleach (20%). The suspensions can be stored in a freezer (–20°C) for at least 2 months.

**GSJ Extraction**

Peel and wash three or four medium-sized garlic cloves with running water. Using a sterile garlic press, obtain ~5 g of smashed garlic, and then add sterile distilled water in the same proportion (1:1). Mix and filter the homogenate through a 10-mL sterile syringe containing sterile gauze into sterile Eppendorf tubes. The extracts can be stored at 4°C for at least 3 months.

**Bioassay**

Transfer 1 mL of spore suspension into each meat agar plate, and distribute it evenly by carefully tilting the closed plates. Position the plates at a slant for 1 minute, and then remove and discard the extra amount of suspension using a sterile pipette. Leave the plates to rest for 10 minutes to allow the bacterial cells to adhere to the medium. Using a Pasteur pipette, apply 1 drop of sterile distilled water (~20 μL) onto one side of each plate (control) and 1 drop of GSJ on the other. Leave the plates to rest for a few minutes, allowing the drops to diffuse into the medium, and then incubate the plates in an inverted position at room temperature for 24 hours (or at 37°C for ~12 hours).

Sterilize all biological waste in the microwave oven prior to disposal.

**Results & Discussion**

In this activity students witness the antibacterial effect of GSJ on *B. cereus* (Figure 2). Given garlic’s pervasive culinary use, students are led to reflect on the health benefits of using it in food processing and preservation. This can lead them to engage in further investigations regarding, for example, the effects of different cooking techniques, times, and temperatures on garlic’s antimicrobial activity. Because garlic is most commonly used in cooking, it was decided to prepare an aqueous extract. Students may be interested in testing other solvents used with garlic to cook or season foods, such as wine or vinegar.

**Extensions & Discussion Topics**

**Test the susceptibility of different bacteria to GSJ**

Students can compare the inhibitory activity of GSJ on different bacteria by obtaining foodborne isolates from retail meat, for instance. They can distinguish different isolates on the basis of colony shape, size, texture, and coloring. So far, only bacteria naturally thriving on garlic are reported to resist allicin (Shim & Kyung, 1999). Searching for other resistant bacteria is a way to enhance students’ motivation and creativity.

![Figure 2. Bacillus cereus susceptibility to the antibiotic effect of GSJ (20 μL; 1:1) registered after incubation at room temperature for 24 hours.](image)

**Explore how allicin is produced**

Allicin is produced when raw garlic is injured and the vacuolar enzyme allinase makes contact with allin’s precursor in the cytoplasm, allin (Harris et al., 2001; Rahman, 2007). A simple experiment assessing the effects of intact and sectioned garlic on bacterial growth demonstrates that allicin production requires cell damage (Figure 3). This experiment can lead to discussion of concepts related to enzymatic reactions, cell structure, and host defense mechanisms.

**Screen different organisms for natural antibiotics**

Most culinary herbs and spices are rich in antimicrobials, such as phenolic compounds (parsley, laurel), aldehydes (cinnamon, cumin), and acids (vanilla, rosemary) (Vigil et al., 2005). By studying garlic’s antibacterial properties, students reflect on the concept of antibiotics and become aware of naturally occurring bioactive substances of pharmaceutical interest. This naturally sparks their curiosity, motivating...
them to propose experiments to screen different antibiotic-producing organisms.

**Address plant defense mechanisms**

Anti-phytopathogenic bioactive compounds, like allicin, participate in chemical plant-defense mechanisms and have promising applications in phytoprotection programs (Slusarenko et al., 2008). Exploring garlic’s antibiotic properties introduces students to ecology concepts, such as predation and parasitism, and to physiology topics, including phytoanticipin, phytoalexin, and other secondary metabolites.

**Curricular Framing & Assessment**

This activity is framed within the *National Science Education Standards* for grades 9–12 (National Research Council, 1996), as summarized in Table 1, and it can be adapted to instructional levels from high school through introductory undergraduate microbiology courses. The exercises presented promote students' abilities to conceptualize topics such as natural antibiotics and antibiotic resistance, to plan and execute experiments, and to develop inquiry-based scientific reasoning. The *National Science Education Assessment Standards* (National Research Council, 1996) recommend that student assessments focus on microbiology and cell biology concepts, performance of laboratory techniques, and awareness about scientific inquiry. Accordingly, students may be asked to produce a report covering basic background information, the hypothesis tested, experimental design, results, discussion, and conclusions. In 10-minute sessions, they can present and discuss their reports and propose alternatives to overcome eventual drawbacks.

**Conclusions**

This activity addresses the concept of natural antibiotics by engaging students in a microbiological procedure used to assess antibiotic susceptibility. The exercises proposed promote students' critical, reflexive, and reasoning competencies. Because it uses affordable and easily available materials, the experiment is accessible regardless of a school's laboratory facilities.

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**References**


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**Table 1. Framing of the proposed experiments and discussion topics within the *National Science Education Standards* for grades 9–12 (National Research Council, 1996).**

<table>
<thead>
<tr>
<th>Content Standards</th>
<th>Activity/Discussion Topic</th>
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<tr>
<td><strong>Life Science</strong></td>
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<td>Content Standard C</td>
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<td>The cell</td>
<td>Allicin synthesis</td>
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<td></td>
<td>Enzymatic reaction (concept of precursor, substrate, and enzyme)</td>
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<td>Cell structure (intracellular location of allicin's precursor and allinase enzyme)</td>
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<td>The interdependence of organisms</td>
<td>Plant defense mechanisms (ecological concepts of parasitism and competition)</td>
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<tr>
<td>The behavior of organisms</td>
<td>Allicin synthesis</td>
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<td>Plant response to injury</td>
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<td><strong>Science &amp; Technology</strong></td>
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<td>Content Standard E</td>
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<tr>
<td>Identify a problem or design an opportunity</td>
<td>Screen different organisms for natural antibiotics</td>
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<td>Test the susceptibility of different bacteria to garlic shoot juice</td>
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<td><strong>Science in Personal &amp; Social Perspectives</strong></td>
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<td>Content Standard F</td>
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<td>Personal and community health</td>
<td>Discussion of antibiotic resistance – natural antibiotics as substitutes or co-adjuvants of commercial antibiotics</td>
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<td>Population growth</td>
<td>Test the susceptibility of different bacteria to garlic shoot juice</td>
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<tr>
<td>Natural resources</td>
<td>Plant defense mechanisms (concepts of parasitism and competition)</td>
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<td>Discussion of antibiotic resistance – antibiotics as natural resources</td>
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