# A Classroom Demonstration of Garlic Extract and Conventional Antibiotics' Antimicrobial Activity

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**Abstract:** The Kirby-Bauer method is regularly used to test bacterial susceptibility to antibiotics, and is often employed in the classroom for teaching this concept. In this exercise, additional materials and instructions were given to students for the preparation of garlic extract and loading on blank BBL paper discs. They were further instructed to test the efficacy of the extract as compared with conventional antibiotics against *Escherichia coli*, *Staphylococcus aureus and Klebsiella pneumoniae.* The conventional antibiotics used were kanamycin, novobiocin, neomycin, erythromycin and tetracycline on BBL paper discs.After 48 hours of incubation, students measured the diameters of inhibition zones. Garlic was rated as being equal to, or less in inhibition than the antibiotics. A survey administered after the exercise showed that students gained a better awareness of garlic extract's potency as an antimicrobial agent. Students also became enthusiastic about searching for additional sources of antimicrobial substances to in light of antibiotic resistance.

**Key Words:** garlic extract, antibiotics, inhibition, allicin

**Introduction:**

The concept of antibiotic sensitivity is very important in the study of microbiology, especially to those in the health-related disciplines. Various modifications of the Kirby-Bauer diffusion technique have commonly been used to demonstrate bacterial sensitivity to antibiotics (Madigan *et al*., 2006). Other demonstration techniques include the well method and tube dilution techniques (Alderson, 2004; Kleyn & Bicknell 2004). However, these methods have usually been applied only to testing conventional antibiotics and disinfectants or antiseptics for classroom demonstrations (Alderson, 2004).

Students, just like other members of the scientific and general community, are being re-introduced to the potentials of the use of phytochemicals for various purposes. Although, many claims about the medicinal properties of phytochemicals are not supported by reproducible scientific experiments, much of the literature provides convincing evidences for some of them. One such phytochemical is allicin, which is released when garlic (*Allium sativum)* is crushed (Rees *et al.,* 1993; Sivam, 2001; Iwallkum *et al.,* 2004,).

Garlic has been used as a medicinal herb for centuries (Donkers *et al.*, 1999), and Louis Pasteur was reputed to be the first to demonstrate the antibacterial effects of garlic and onion extracts (Sivam, 2001). Garlic extract's reported activities include reducing cholesterol, tumor suppressing, and acting as an antifungal, antibacterial, antiviral and anti-parasitic agent (Reuter et al., 1996).

Garlic is widely consumed and is known to be safe. It has been reported that a human would need to eat 20 grams of garlic per kilogram body weight to reach toxic levels. Additionally, there is no known report in the literature regarding resistance of bacteria to allicin, the main active ingredient in garlic, whenever it has been found to be effective (Paszewski & Jarosz, 1978; Singh & Shukla,1984).

One problem with the practical use of allicin as a commercial antimicrobial agent is its instability. Its rate of deterioration increases with increasing temperature. In addition to allicin, other products of garlic that are more stable than allicin have been found to have antifungal properties (Harris *et al.*, 2001). As a wide spectrum antimicrobial agent, one concern is that garlic could be detrimental to beneficial gut bacteria. However, no reports of negative effects on gut bacteria occur in the literature (Dankert *et al.*, 1979).

In this laboratory exercise, students received additional materials and instructions to include garlic extract as one of the antimicrobial substances being tested as outlined below under "*Materials and Methods*". This is in addition to the instructions in their lab manual (Kleyn & Bicknell, 2004). The goal of this exercise was to supplement standard antibiotic testing by including a natural product whose source is familiar to students.

This exercise may help students to develop an appreciation for the potential for new antibiotic discovery in the future and from diverse sources. Furthermore, this exercise may aid botany students to realize the importance of plant products to humans, as well as the concepts and uses of plant secondary metabolites.

## Materials and Methods

### *Preliminary Class Discussion*

Prior to this exercise, students were assigned readings and class discussions on antimicrobial agents, including antibiotics. In addition, a class discussion was generated by supplying students with additional reading materials about Fleming's discovery of penicillin and other materials related to the early discovery of phytochemicals (Prescott *et al.*, 2002; Madigan *et al.*, 2006)

### *Materials for the whole class*

*(Estimated class size of 24 students)*

- Kitchen blender
- Sterile paper discs ¼'' BBL (about 100)
- Balance
- Five average-sized garlic bulbs

### *Materials per students group*

Students worked in groups of three or four. Each group was provided with the following:

- Ampoules of antibiotic discs of kanamycin, novobiocin, neomycin, erythromycin, tetracycline.
- Balance
- Forceps (one per student)
- Filter paper and funnel (previously sterilized by autoclaving)
- Agar plates of Mueller-Hinton medium (*5 per group*)
- Sterile swabs (one per student)
- Sterile Erlenmeyer flasks (250ml)
- Twenty four hour old cultures (in Tryptic Soy Broth) of *Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumonia* (One set per group)
- Bunsen burner and lighter.

### *Preparation of garlic extract paper discs*

Instructions on the preparation of garlic extract discs were provided for each student. Student volunteers from the groups prepared the extract based on the following procedure:

- Blend 10 grams of garlic cloves in 100ml of sterile de-ionized water at high speed for one minute.
- Strain the slurry through sterile cheesecloth, and then filter through sterile filter paper into a sterile 250ml Erlenmeyer flask.
- Pipette about 15ml into a sterile Petri-dish
- Use a sterile forceps to dip paper discs into the extract and allow for brief drain age on sterile filter paper.

### *Plate inoculation, antibiotics and garlic extract application*

Each group member was given the opportunity to inoculate a different bacterial species to a Mueller-Hinton agar plate. Plates were seeded using sterile BBL cotton swabs. The tips of swabs were dipped into 24 - hour-old cultures of each species and spread over the surface of the agar plate. After a demonstration, each group member used the BBL multi-disc dispenser to apply discs of the different antibiotics from the ampoules onto the seeded plates. (Where a dispenser is not available, discs may either be ejected directly from each ampoule, or may be pushed out using a dissecting needle or any other pointed instrument). The plates were placed in a refrigerator for about 10 minutes after the application of the antibiotic discs to facilitate the diffusion of the antibiotics into the medium while bacterial growth is delayed.

The students then prepared the garlic extract disc as described above and placed it at the center of the plate with the conventional antibiotic discs. The plates were then incubated at 35$^{o}$C until the following class meeting (approximately 48-hours). Thereafter, students measured the zones of inhibition with a ruler and recorded their measurements. For each antibiotic or garlic extract discs, class averages of inhibition diameter were calculated for each species. They then compared the inhibition zones of the garlic extract with those of the other antibiotics used.

## Results

The table on the following page represents the results of student measurements of the inhibition zones.

| CLASS AVERAGES OF DIAMETER OF INHIBITION ZONES BY GARLIC EXTRACTS AND CONVENTIONAL ANTIBIOTICS. (millimeters) | | | | | | |
|---|---|---|---|---|---|---|
| | Erythromycin | Kanamycin | Neomycin | Novobiocin | Tetracycline | Garlic Extract |
| *Escherichia coli* | 11.0 | 19.0 | 19.0 | 14.0 | 23.5 | 17.0 |
| *Klebsiella pneumoniae* | 8.6 | 20.0 | 16.6 | 13.0 | 17.6 | 12.6 |
| *Staphylococcus aureus* | 21.6 | 21.3 | 21.3 | 27.5 | 29.0 | 21.6 |

**Table 1.** Summary of class report for comparative zones of inhibition of bacterial growth by conventional antibiotics and garlic extract.

The photographs in **Figure 1. (a-c)** show sample plates of garlic extract inhibition (center) as compared to the inhibition of conventional antibiotics.


**Figure 1.** (a) *Escherichia coli*

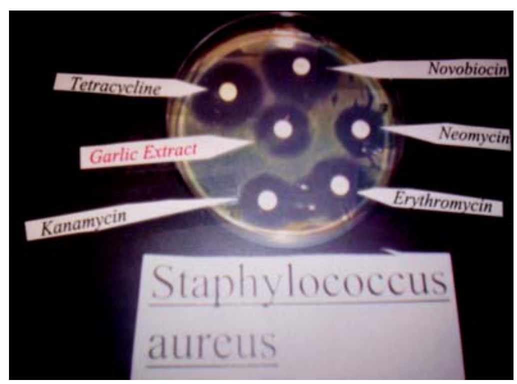


**Figure 1.** (b) *Staphylococcus aureus*

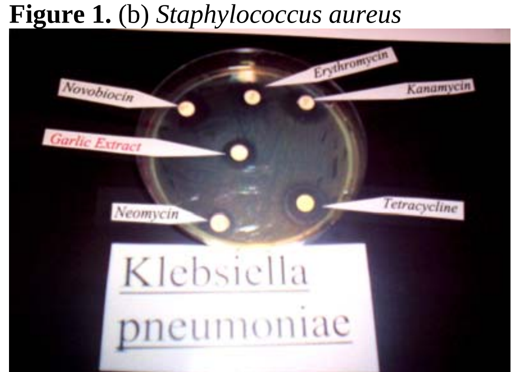


**Figure 1.** (c) *Klebsiella pneumoniae*

## Evaluation Tool

The tool for evaluation was designed to test the students' general awareness of antimicrobial phytochemicals before and after the exercise, making simple water extraction from plants, and concrete awareness of possibilities of using plant extracts for therapeutic purposes. An open ended question about their experience with the exercise allowed them to make comparison between garlic extract and the antibiotics and to freely make other comments about the exercise.

Other questions tested a variety of reactions from the students about the exercise in general and the concept of plants as source of antimicrobial agents. Students were also asked to indicate their future interest in searching for additional antimicrobial substances of plant origin.

The following evaluation questions were given to the students.

Key:

*5= Strongly agree;*

*4= Agree*

*3= Agree with reservation*

*2= Disagree*

*1= Strongly disagree*

1. After the exercise, I had acquired the skill of making extract from plants [1] [2] [3] [4] [5]

2. The possibility of obtaining medicine from plants became more apparent to me [1] [2] [3] [4] [5]

3. I am now more curious about other uses of plant extracts for medicinal purposes [1] [2] [3] [4] [5]

4. I think this is a useful supplement to the lab on antibiotics [1] [2] [3] [4] [5]

## Result of Students' Responses

This exercise was first given during a previous year without data collection. However, the data below were collected from 30 students, in Introductory Microbiology and Botany courses in the following year. The students were either first or second year nursing, science or liberal arts students.

| Question | Mean score | SD |
|---|---|---|
| Acquired the skill of extraction | 3.1 | 2.4 |
| Possibility of more  plant antimicrobial became more apparent | 4.2 | 0.4 |
| More curious about other medical uses of plant extracts | 4.4 | 0.7 |
| Added exercise a useful supplement to existing one. | 4.4 | 0.2 |

**Table 2.** Summary of students' response to the exercise on antimicrobial action of garlic extract

**Overview of Students' Comments**

Pre-lab, students were asked if they thought it was necessary to search for additional antimicrobial agents. All the students were positive about this. Reasons given include the need to use them to complement existing ones, and the widespread development of resistance to many of the existing antibiotics by pathogenic organisms.

After the results of the experiment had been read, students were asked what they considered their most significant learning experience in the exercise. Most of the responses showed that a practical demonstration of microbial inhibition by plants was their most significant learning experience. For example, one student commented that "*I had no idea you could make antibiotics from plants.*" Other comments conveyed the same general idea. An interesting point of view of one student was that if plant antimicrobial substances were used in medicine and microorganisms acquired resistance to them, plants could "get overrun and killed" Some students also saw the opportunity for obtaining antimicrobial substances from plants that could help combat the prevalence of antibiotic resistance.

**Note to Instructors**

The part of this exercise involving the conventional antibiotics is usually performed in most introductory Microbiology classes. The addition introduced here could enhance students' interest in the subject matter. The exercise could be extended to many other plant extracts, with the precaution that the extraction method, as well as treatment of the extract before loading to the disc may differ considerably. For example, licorice root has been found to be effective only in moderate amounts against only *Staphylococcus aureus*, among the bacteria tested (Not reported as part of this exercise).

Also, fresh samples of garlic should be purchased for each experiment. This does not imply that garlic samples could lose their allicin content, as long as they are not crushed, but this is a precautionary measure to ensure the success of the experiment. Moreover, garlic extract should be loaded on the blank paper discs as soon as possible after blending, since the active ingredient is well known to lose most of its potency with time after extraction, especially at higher temperatures. Commercially prepared blank discs should be used instead of using regular filter paper discs cut with a hole-puncher to carry the garlic extract. Based on preliminary trials, commercially prepared blank BBL discs worked better. Attention should be paid to the garlic to water ratio so that there is an adequate amount of the active ingredient. Sterile BBL discs of the same diameter, soaked in sterile distilled water could be included as a negative control in each plate.

Emphasis should be placed on care in handling microorganisms. This exercise is suitable for students of Introductory Microbiology who have acquired basic skills in handling microorganisms. It has also been used, mostly as a demonstration, for students of

Introductory Botany to reinforce the concepts of secondary plant metabolites and their possible uses.

**Conclusion**

Overall, students' responses were quite favorable. Most students did not participate directly in the extraction. Consequently, they responded to the question on the extraction aspect more negatively. This exercise stimulated the students' interest in the search for antimicrobial substances of plant origin. They were also optimistic about combating antibiotic resistance through such discoveries. The addition of plant extract to the exercise was considered useful by 100% of the students.

There is a strong indication that the added aspect could positively affect students' attitude and interest in screening for phytochemicals for therapeutic, especially antimicrobial purposes. The exercise is recommended as a useful addition to what is currently demonstrated, especially for students in the health-related sciences, as well as all students of Microbiology and Botany.

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