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Student-Designed Projects that Ascertain Antibiotic Properties of Natural Substances

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For thousands of years, certain foods have been utilized for purposes beyond nutrition. St. Francis College students chose to examine antibiotic properties of natural substances for a General Biology project. Specifically, it has been demonstrated that essential oils, honeys, and vinegars can inhibit the growth of pathogenic bacteria. The students quantified the relative efficacies of tea tree oil, cinnamon oil, garlic extract, apple cider vinegar, and Manuka honey against Staphylococcus aureus and Escherichia coli by measuring the zones of bacterial growth inhibition after a 24-hour period of incubation. The experimental agent was administered either by drop directly onto the inoculated agar surface or on a saturated piece of filter paper. The conventional antibiotics tetracycline and erythromycin were used as positive controls and as baselines for halo size.

The students found that all natural agents tested were comparably or more effective than conventional antibiotics as measured by the diameter of the zone of growth inhibition. The success of these food remedies was encouraging since they are generally inexpensive and widely available. Food products that have been used historically as remedies are still very relevant and could be potent sources of antibiotic activity. This student-designed lab activity yielded much data that the students could analyze and relate to existing data on antibiotics.
Keywords: natural antibiotics, natural antibacterials, inquiry-based experiment

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Introduction

The St. Francis College General Biology lab students are required to do a cooperative group project (with up to four students) that takes about a month to complete. They devise and test a hypothesis, produce a poster, present a PowerPoint presentation on their findings to their peers, and describe their experiment in a poster session open to the entire college community. The hypothesis for this particular project was: There is no difference in the efficacy of natural products (Manuka honey, cinnamon oil, garlic extract, apple cider vinegar, tea tree oil) in inhibiting the growth of *Escherichia coli* and *Staphylococcus aureus* compared to biopharmacological antibiotics tetracycline and erythromycin. We encouraged the students to test the null hypotheses and explained that, if there are any differences in the experimental groups, they would reject their hypothesis.

The students conducted a literature search and discovered that there has been much research on using natural substances as antibiotics. We will list just a few examples that they found here. For example, Alli et al. (2011) investigated effects of garlic extract on two bacteria: *Pseudomonas aeruginosa* and *Staphylococcus aureus*, which are known to have developed recalcitrant antibiotic resistance. They quantified the amount of extract they used by measuring a dry weight of garlic, homogenizing it, adding water and then sterile filtering it through a 2 µm MilliporeTM filter. They found that a range of from 67 to 201mg/mL concentrations of garlic was necessary to kill the bacteria. Jazani et al. (2007) found that garlic extracts caused zones of inhibition in some mulit-drug resistant strains of *Acinetobacter spp.*. Du et al. (2009) found that the essential oils of allspice, cinnamon, and clove bud inhibited bacterial growth when employed in edible films placed on plates inoculated with *Escherichia coli O157:H7*, *Salmonella enterica*, and *Listeria monocytogenes*,. This is an example of an applied use of antibacterials in food preservation.

Al-Naama, R. (2009) studied the effects of honey from a

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certain region in Iraq on *Escherichia coli,Pseudomonas spp.* and *Staphylococcus aureus* bacteria. Their experiment was very thorough in that they used a set of controls in which they performed serial dilutions to quantify the bacteria and another set of serial dilutions of the bacteria in which various concentrations of honey were added. They were searching for the minimal inhibitory concentration (MIC). When this was determined, they plated out the best dilution that would just barely produce a complete lawn, then conducted the disc diffusion method in the usual way with a range of honey concentrations. They found that the largest halos (zones of inhibition) were produced with 50-100% honey concentrations in *E. coli* and 75-100% honey concentrations in *S. aureus*. Kwakman and Zaat (2012) discussed what some of these antibacterial components of honey are and describe how they work. Jenkins and Cooper (2012) elucidated how Manuka honey can act synergistically with antibiotics in acting against certain bacteria. In an experiment conducted in Taiwan, Liu et al. (2007) found that 26 out of 56 Chinese herbal extracts were effective against *Pseudomonas aeruginosa*, which is the most common opportunistic infection in those with cystic fibrosis. Thomsen et al. (2011) found that tea tree oil in various formulations produced zones of inhibition against Escherichia coli, *Staphylococcus aureus*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, and *Candida albicans*.

Student Outline

1. Obtain a fresh clove of garlic and crush with a mortar and pestle. Filter through a funnel fitted with a Whatman #1™ filter paper. (If available, substitute a sterile filter.)
2. Punch out disks of Whatman #1™ filter paper with a hole puncher (these should match the size of the antibiotic disks.)
3. Using sterile forceps, (sterilize by dipping or rubbing forceps briefly in 70% alcohol and flame off alcohol carefully with a gas or alcohol burner) dip four separate disks respectively in: a. garlic juice b. apple cider vinegar, c. cinnamon oil and d. tea tree oil
4. Place each disks in the center of an agar plate that has been inoculated (spread evenly across with an applicator stick so that a lawn would normally grow) with *Staphylococcus. aureus* or *Escherichia coli*.
5. Directly pipet 100 µL of Manuka honey on the center of a separate plate of bacteria.
6. Incubate at 37°C overnight and measure the zone of diffusion (halo) in mm the next day, as in Figure 1.
7. Make some control plates using commercially available antibiotic discs such as erythromycin and tetracycline
8. Make a table in which you compare the zones of inhibition among the various natural substances used against the two bacteria, and list the measurement, in mm, of the zone of inhibition.

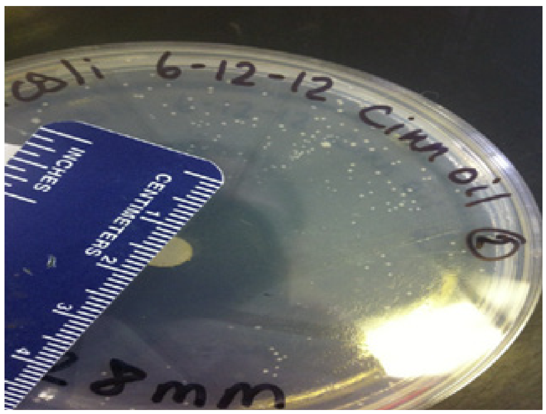

Figure 1. Measurement of a zone of diffusion or halo. (Photo by Hannah Singer)

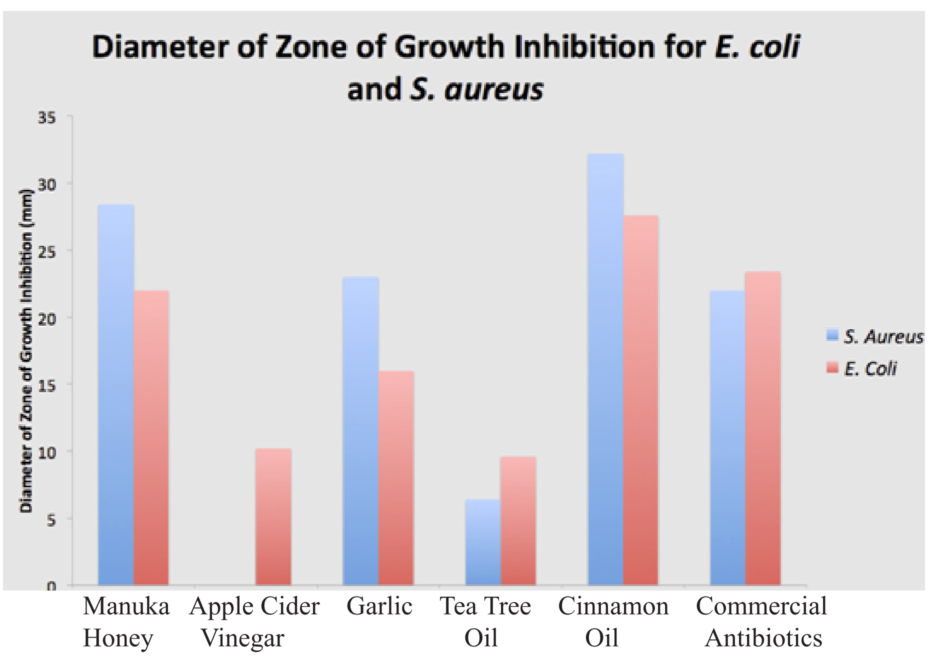

Figure 2. A comparison of zones of inhibition across treatments.

The results (Fig. 2 and poster) signified that there is measurable evidence that natural remedies are just as, or sometimes more, effective in killing the bacteria *S. aureus* and *E. coli*. (Apple cider vinegar did not kill *S. aureus*.) The zone of inhibitions differed slightly between each remedy (garlic oil, cinnamon oil, Manuka honey, tea tree oil, and apple cider vinegar, and the commercial antibiotics (erythromycin on *S. aureus*, and tetracycline on *E. coli*) implying that there are differences in the effectiveness of each remedy for different bacterial species.

Notes for the Instructor

This experiment could be conducted as part of an inquiry-based lab in which students pick the substances to test. We require the students to search the literature for what has been tested, and also to read about how various known antibiotics work. This project was the result of a group independent research assignment that is part of the St. Francis College Bio 1201-Bio 1202-laboratory curriculum. It could be incorporated into a microbiology or botany course as well. It really requires very little prep; additional components that might fine-tune it could include autoclaving the paper discs and performing serial dilutions to get a better idea of the bacteria count.

Finally, we would like to address concerns expressed by several participants at the ABLE Conference about using a species in decline for the classroom laboratory. Even though the adults are in decline, the eggs are quite plentiful. We feel that several hundred eggs used for a lab exercise to awaken a student interest in this topic is worth it. These students will now be aware of the complexities surrounding the ecosystem of the horseshoe crab, and will be in a better position to participate in policy-making that would enhance the organism's survival.

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Literature Cited

Alli, J. A., B. E. Boboye, I.O. Okonko, A.F. Kolade, and J. C. Nwanze. 2011. In-vitro assessments of the effects of garlic (*Allium sativum*) extract on clinical isolates of *Pseudomonas aeruginosa* and *Staphylococcus aureus. Advances in Applied Science Research*, 2(4):25-36.

Al-Naama, R. 2009. Evaluation of inhibitory effect of honey on some bacterial isolates. *Iraqi Journal of Medical Sciences*, 7(4):67-72.

Ching-Shen, L., C. Thau-Ming, Y. Cheng-Hong, C. Hsueh-Wei, C. Chia-Hong, and C. Li-Yeh. 2007. Antibacterial properties of Chinese herbal medicines against nosocomial antibiotic resistant strains of *Pseudomonas aeruginosa* in Taiwan. *American Journal of Chinese Medicine*, 35(6):1047-1060.

Du, W. X., C.W. Olsen, R.J. Avena-Bustillos, T.H. McHugh, C.E. Levin, and M. Friedman. 2009. Effects of allspice, cinnamon, and clove bud essential oils in edible apple films on physical properties and antimicrobial activities. *Journal of Food Science*, 74(7):M372-M378.

Jazani, N., S. Shahabi, A. Ali, S. Zarrin, and N. Daie. 2007. In vitro antibacterial activity of garlic against isolates of *Acinetobacter sp. Journal of Biological Sciences,* 7(5), 819-822.

Jenkins, R., R. Cooper, and J. Vadivelu. 2012. Improving antibiotic activity against wound pathogens with Manuka honey in vitro. *Plos ONE*, 7(9):1-9.

Kwakman, P.S., and S.J. Zaat. 2012. Antibacterial components of honey. *IUBMB Life*, 64(1), 48-55. doi:10.1002/iub.578

Thomsen, P. S., T.M. Jensen, K.A. Hammer, C.F. Carson, P. Mølgaard, and T. V. Riley. 2011. Survey of the antimicrobial activity of commercially available Australian tea tree (*Melaleuca alternifolia*) essential oil products in vitro. *Journal of Alternative and Complementary Medicine*, 17(9):835-841.

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