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Tiny Earth, Tinier Microbes: An Experiential Learning Approach to Antibiotic Discovery

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TINY EARTH, TINIER MICROBES:
AN EXPERIENTIAL LEARNING APPROACH TO ANTIBIOTIC DISCOVERY

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Abstract

Antimicrobial resistance is one of the greatest global health challenges of the 21st century as antibiotic discovery has slowed even as scientific knowledge about AMR has progressed. At the same time, science education has turned to active learning approaches like CUREs, or course-based undergraduate research experiences, to achieve educational objectives while engaging students in real-life research. The Tiny Earth Project is a global research initiative that seeks to crowdsource antibiotic discovery by recruiting undergraduate students to screen soil samples for antibiotic producers. The goal of this study was to determine the viability of translating the Tiny Earth programming to a large-scale high school audience as assessed by the 3 aims of helping students develop 1) microbiology laboratory skills, 2) experimental design skills, and 3) positive scientific identity. Qualitative observations of 3 students in this pilot study suggest there is potential for expansion of the Tiny Earth project to a pre-undergraduate level, although data collection is still underway.

Key Words: Tiny Earth Project, antimicrobial resistance, CUREs, science education, EDAT assessment

Acknowledgments

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Introduction

Science educators face the complex challenge of teaching students core scientific principles while also encouraging them to seek out new knowledge and contribute to novel discovery. While traditional classrooms may succeed in the first objective, passive learning formats like lectures often fail to promote scientific curiosity. However, course-based undergraduate research experiences, or CUREs, may provide an alternate framework for active science education in a laboratory setting. CUREs are laboratory courses designed around research questions with unknown answers that are applicable and relevant to the larger science community (1). These experiences are intended to promote inquiry-based, experiential learning by asking students to utilize their classroom knowledge to solve real-world problems. By having students engage with research questions without established answers, CUREs aim to increase scientific activism and ownership even before students graduate and leave the educational setting.

One such research challenge currently facing the scientific community is the need for antibiotic drug discovery. The World Health Organization (WHO) has declared antimicrobial resistance (AMR) to be one of the biggest biomedical problems of the 21st century (2). By 2050, antimicrobial-resistant infections are expected to be responsible for 10 million deaths a year. Specifically, the WHO has issued a list of sixteen pathogen families, including a critical category of multi-drug resistant bacteria, that pose an urgent or serious threat to global health. The

problem with AMR is two-fold. The first part of the problem is that bacteria, viruses, and fungi continue to develop mechanisms for resistance against antibiotics already in use. Microbes are developing mechanisms that allow them to modify antibiotic targets; destroy, modify, and export antibiotics; and, most problematic for development purposes, limit antibiotic penetration into the cell (3). The second part of the problem is the slow progress of antibiotic discovery. Most antibiotics currently in use today were discovered with soil sampling techniques during the 1940s to 1960s, considered to be the "golden era of antibiotic discovery"(4). Since then, synthetic antimicrobials have had limited success, despite growing understanding of antibiotic targets, due to the difficulty of finding antibiotic products that penetrate the membrane. Gram-negative bacteria have made this particularly challenging.

The Tiny Earth Project is a CURE-like project which aims to address the problems of active science engagement and antimicrobial resistance by crowd-sourcing the search for new antibiotics. The project, in its original form, recruits STEM undergraduate students to perform microbiology techniques such as serial dilutions, bacterial culture, and PCR to screen soil samples for antibiotic producers. Since its inception, this programming has had profound gains at the undergraduate level, and more recently also been shown to be potentially adaptable to engage younger audiences, including high school students (5). However, little if any has been done to assess if the Tiny Earth platform can be implemented through formal educational programming at the secondary level. To close this gap and in collaboration with Nebraska EBSCoR, the University of Nebraska-Lincoln Microbiology Department, and the Lincoln Science Focus program (LSF), we are currently delivering an adapted version of the Tiny Earth program to pilot its potential functionality as a sustainable and formal curricular option within a high

school classroom setting. The *question* guiding this endeavor is "Can we use authentic research experiences to improve high school education relating to student attitudes and experimental design skills". To investigate this, we are leveraging various aspects of the Tiny Earth soil antibiotic discovery program to serve as a research platform for three high school students within the LSF to complete their senior thesis projects. Our *long-term goal for this project* is to establish a larger-scale, CURE-like platform that can be leveraged to serve surrounding high schools to promote science engagement and literacy at a pre-undergraduate level. The *overall objective for the current pilot study* is to develop, adapt, and fine-tune soil screening microbiology protocols and activities that are optimized for entire cohorts/classrooms of high school students in the future. With these protocols and weekly activities, we plan to achieve these specific aims:

1. **Develop basic student laboratory competencies in microbiology, while screening for novel (soil) antibiotic producers/resisters.**

   We will introduce and practice lab techniques **such as serial dilution, spread plate, and quadrant streak to culture bacteria. Students will practice these techniques** *in two stages—the first with a known monoculture sample, and the second with students' experimental soil samples—students will have the opportunity to practice techniques over multiple weeks and analyze successes and errors from different approaches. Importantly, culturing unknown microorganisms from soil samples provides an authentic research platform for students to investigate and identify potential new sources of antibiotic activity and or antimicrobial resistance.*

2. **Develop student experimental design skills.**

Students will be required to co-develop research protocols that include designating essential elements of experimental design, such as defining a research question or hypothesis, as well as utilizing positive and negative controls to address said question or hypothesis. In doing so, students will gain essential scientific thinking skills in both planning and performing authentic research.

3. **Promote a positive student science identity.**

   Students will be given opportunities to develop mastery over a variety of different experimental protocols. During this time, we will encourage students to focus on their developing skills and content knowledge, which includes improving their ability to troubleshoot issues that arise during experimentation. In this context, the goal is to promote student confidence and independence in the laboratory setting. Ultimately, we hope these gains result in a more robust science identity that enhances the number of future students that pursue STEM careers.

Materials and Methods

<u>Wet lab experimental procedures:</u>

Methods for soil sample preparation and antibiotic screening were adapted from the Tiny Earth Instructor and Student Guidebooks. The remaining protocols were adapted from the UNL Microbiology Department's protocols for BIOS 314 Microbiology Laboratory.

Serial dilutions and quantifying samples based on spectrophotometric signals:

A serial dilution activity was implemented to demonstrate Beer's law and its role in the creation of standard curves. The activity was carried out over two lab sessions, first using Nigrosin dye and then using *E. coli* liquid culture. Students performed serial dilutions according to the equation $M_1V_1=M_2V_2$ and then measured the optical density at 600 nm to demonstrate the use of spectrophotometric techniques in microbiology. The use of *E. coli* liquid culture also introduced the concept of colony-forming units (CFUs) and the necessity of dilutions when attempting to count and isolate colonies. As part of these lab sessions, students learned how to perform spread plate technique on liquid cultures.

Soil sample processing and culturing conditions

Students initially began plating their soil samples using a 1:10 dilution of 1 gram of soil per 9 mL distilled water to make a liquid culture for spreading. They performed serial dilutions on the soil slurry and plated dilutions 10^{-1} to 10^{-8} using spread plate technique to determine the optimal concentration for isolation of single colonies. Students then selected single colonies and performed quadrant streaks on LB agar plates with cycloheximide to produce monocultures.

Activity assays for identifying B. anthracis

B. anthracis is a gram-positive, rod-shaped bacteria naturally found in soil that causes the serious infections disease known as anthrax. While the likelihood of students culturing *B. anthracis* was incredibly low, additional safety measures were taken to promote student safety. This study followed Tiny Earth protocols and EHS recommendations to abstain from growing liquid culture from the soil samples to greatly reduce the possibility of culturing *B. anthracis* in the student laboratory. Students also used the catalase test, motility test, and MacConkey agar

activity assays, along with known bacteria controls, to screen their isolated cultures against *B. anthracis* to practice experimental design and learn other assay techniques.

Screening for antibiotic activity

The Tiny Earth curriculum asks students to screen for potential antibiotic activity against a specific subset of antibiotic-resistant bacteria known as the ESKAPE pathogens. The ESKAPE pathogens are *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species.* Non-pathogenic relatives of these bacteria deemed to be "safe relatives" were used to test soil sample cultures for antibiotic activity. For this study, the first round of screening was performed against *E. coli (*safe relative to *Klebsiella* species) and *S. epidermidis* (safe relative to the *S. aureus)*. A pick-and-patch method was used to generate antibiotic screening plates by examining the effects of students' monocultured bacteria on the growth of an *E. coli* lawn.

Samples with potential antibiotic activity will be submitted for colony PCR for identification.

Screening for antibiotic resistance

An antibiotic disk diffusion test was used to visualize monoculture antibiotic insensitivity and antibiotic resistance. K-12 *E. coli*, as well as an ampicillin-resistant *E. coli* strain and streptomycin-resistant *E. coli* strain, were cultured in the presence of ampicillin and streptomycin antibiotic disks to observe inhibitory effects of the antibiotics.

Assessing student science identities, skills, and content gains

The previously validated experimental design tool (EDAT) was used as a pre-project assessment worksheet to serve as a baseline measurement for scientific experimental design skills (6). Additionally, a formative assessment derived from a 2012 rat empathy study was completed mid-curriculum to formally teach and reinforce key experimental design elements, including generating hypotheses, defining experimental controls, as well as labeling independent and dependent variables of an experiment (7). The worksheet was also used to practice scientific brainstorming and cartooning in a collaborative setting. At the conclusion of the project, students will also complete a variety of assessments to determine any post-gains that potentially correlate with completing the adapted Tiny Earth curriculum. This includes completing (i) a post-EDAT assessment worksheet to probe gains in experimental design skills and (ii) two Likert-based self-assessment surveys including a modified learning gains assessment based on the student assessed learning gains tool (i.e., SALG), and a modified version of the Student Self-Rated Abilities, Attitudes, and Beliefs Survey, (i.e., SAAB); Both surveys assess various aspects of science identity and experimental design skills, and ongoing efforts are being made to adapt these tools to address specific aspects of the Tiny Earth curriculum (8). In addition, we are currently developing an open-ended response sub-section, which will enable students to further elaborate on their experiences.

Results

Currently, two of the three students have submitted their pre-EDAT assessment (The remaining pre-EDAT assessment has been completed, but the student has not yet submitted it. See

supplemental data); Based on the submitted results, both students were familiar with and comfortable with the concepts of experimental design. One student pointed out the need for a (negative) control and indicated some variables that would need to be held constant in the experiment. The other student made reference to the need for a metric for measuring the dependent variable and the complicated factors that could influence that criterion. The shared sense with both responses was that the students had learned or been taught the general components of experimentation but had not been asked to creatively design an experiment themselves before.

Currently, educational post-project assessment results and complete antibiotic activity results from student soil samples are unavailable. Select sample result photos from the UNL Microbiology Department are included as proof of concept. Two student photos from the current stage of testing are also included, although complete identification data from these samples is not yet available. Students will complete the post-project assessment and submit potential antibiotic producers for colony PCR in early April.

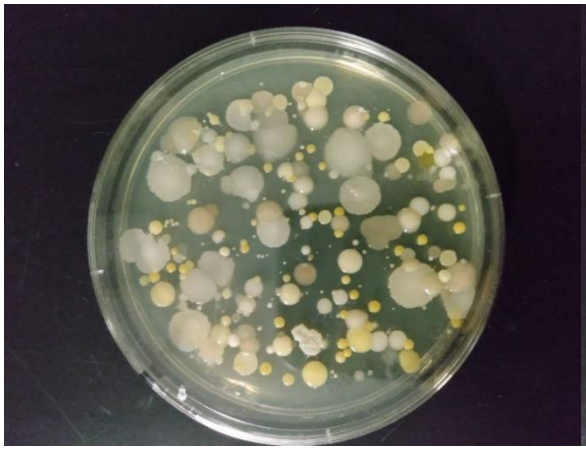

Figure 1: A soil sample cultured on TSA medium. Photo courtesy of UNL Microbiology Department

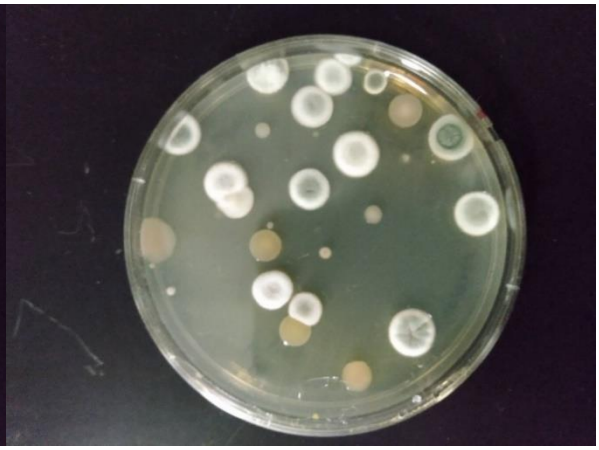

Figure 2: A soil sample cultured on TSA medium + ampicillin. Photo courtesy of UNL Microbiology Department

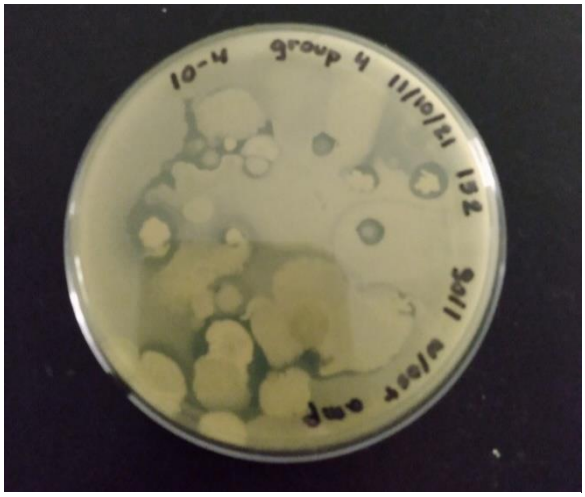

Figure 3: Antibiotic screening test with bacterial lawn. Note zones of inhibition around select colonies. Photo courtesy of UNL Microbiology Department

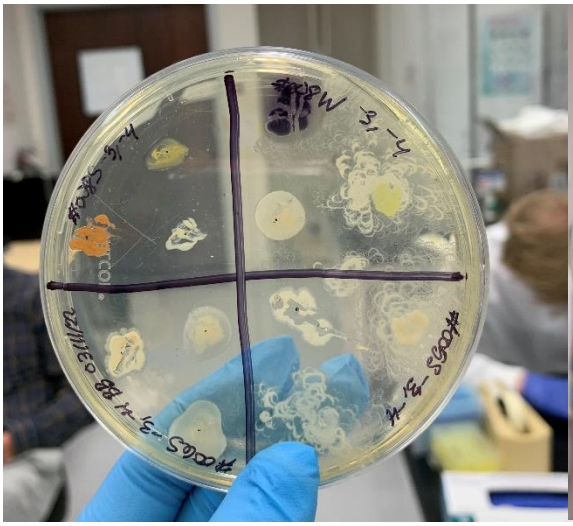

Figure 4: Colony master plate from student unknown soil samples

Figure 5: Antibiotic screening test performed with student unknown soil sample colonies compared to a streptomycin disk. Note slight reduction in growth of E. coli lawn around black colonies in top right section of the plate.

Discussion and Conclusion

Current analysis of the results is limited by time constraints of this report versus the timeline of the overall project. However, qualitative observations of the students during laboratory sessions suggest that progress is being achieved in all three aims.

Gains towards achieving Aim I: Develop basic student laboratory competencies in microbiology, while screening for novel (soil) antibiotic producers/resisters

During the first spread plate exercise, the plates developed contamination likely due to errors made during the plating protocol. After a discussion about potential sources of contamination and error, students independently adjusted their techniques and began more consistently wiping down the lab bench area and exercising greater caution while plates and samples were exposed to the air. These changes were associated with improved plating technique and diminished contamination issues. Another example of student improvement in laboratory competency was observed when students were culturing their soil samples: Initial attempts to isolate single colonies to make monocultures had limited success but following repeated trials of plating the soil produced better growth. While there are several unknowns related to the soil samples themselves, such as the overall effects of different storage methods and different properties of the soil taken from different locations, successive attempts on every protocol produced more viable results. This suggests that the students developed familiarity with the procedures such that they made conscious adjustments to reduce error and optimize techniques with repeated trials. Additionally, students are currently beginning to screen for antibiotic activity/identifying AMR.

Gains towards achieving Aim II: Develop student experimental design skills

At the beginning of the project, students were familiar with the need for controls in experimental design but did not have a fully developed understanding of positive versus negative controls. They initially struggled in recognizing whether a control was positive or

negative and had difficulty predicting what results would be expected from each control in successful or unsuccessful experiments. After a group discussion, they had the opportunity to practice recognizing controls and predict results in the form of the motility and catalase tests and the MacConkey agar activity. After the second lab session working with the assays, students were better able to articulate the role of positive and negative controls in laboratory discussions. They could identify both types of control in successful activity assays, where the identities of the controls (but not necessarily their role in the experiment) were provided. Additionally, they were able to use their understanding of the implications of positive and negative controls to troubleshoot an unsuccessful experimental antibiotic screening. In the initial run of antibiotic screening, the plates did not demonstrate bacterial growth of the *E. coli* lawn. In discussion, students recognized that this was a failure of the positive control and that it suggested error with the experimental system which we ultimately attributed to a dead *E. coli* liquid culture.

Gains towards achieving AIM III: Promote a positive student science identity

Based on qualitative observation, students have become more comfortable in the laboratory setting and, consequently, have developed a greater sense of inquiry and investment in their projects. When students had questions in the first couple of weeks, they were primarily questions about ***what*** to do, but over the course of the project, their questions have become more about ***why*** they are supposed to perform protocols in certain ways. Moreover, they have started making suggestions and giving input as to how to approach certain protocols. When prompted, the students have played more active roles in group brainstorming during error analysis, responding to open-ended questions with less hesitancy and more creativity. During

an informal discussion, the students explicitly reported feeling more confident in the laboratory and personal areas of growth over the course of the project. While these observations are limited by researcher perspective, the students' future responses to the post-project student attitude surveys may provide more detailed information about the students' perception of any scientific and educational growth.

As this program was a pilot study, there were limitations to the study that must be addressed. One of the most challenging elements in obtaining results was the organization and coordination of meeting times. Initially, the students were only able to attend laboratory sessions once or twice a month. This made it difficult to reinforce techniques and discussion topics because the material was not consistently relevant to the students. Another difficulty with the timing was the long delay between beginning the project and seeing experimental results. The project was always directed toward screening soil samples, but it was multiple lab sessions and four months before students could begin working with their collected samples. While this was in part due to the time it took for students to become comfortable with basic lab techniques such as pipetting and plating, it seems this delay could have been reduced with greater consistency and frequency in initial lab sessions. Another element of this as a pilot study is that it is limited to a small sample size of three students. It is unclear how these educational gains would translate to a larger representative setting, and it is difficult to measure educational gains in a quantifiable way even with the three current students. Additionally, these specific students already came from strong science backgrounds as of a science-centered high school program. Thus some of the gains observed may have accelerated or assisted by pre-existing interest in the field. Still, the observable gains in competency and

confidence in the lab setting are not insignificant. While further results and an expanded study will be needed, the current findings suggest this authentic research experience *has* improved student attitudes and experimental design skills. This study has also determined that the Tiny Earth Project specifically can be translated to a high school audience. The project has both the approachability and flexibility to promote student engagement while also giving students the opportunity to ask questions and make educated decisions about their experiments based on those inquiries. Expanded studies will be needed in the future to determine how successes in this project will translate to a larger setting, but current findings suggest that these ideas are worth further investigation.

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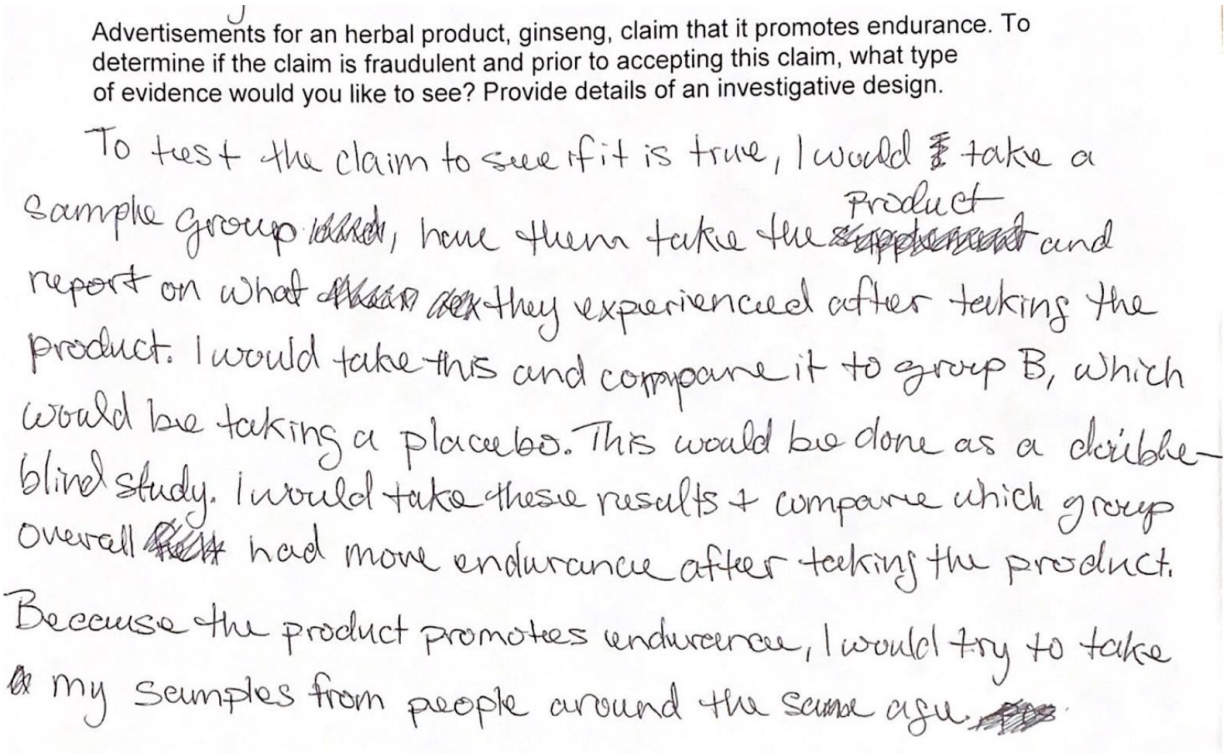
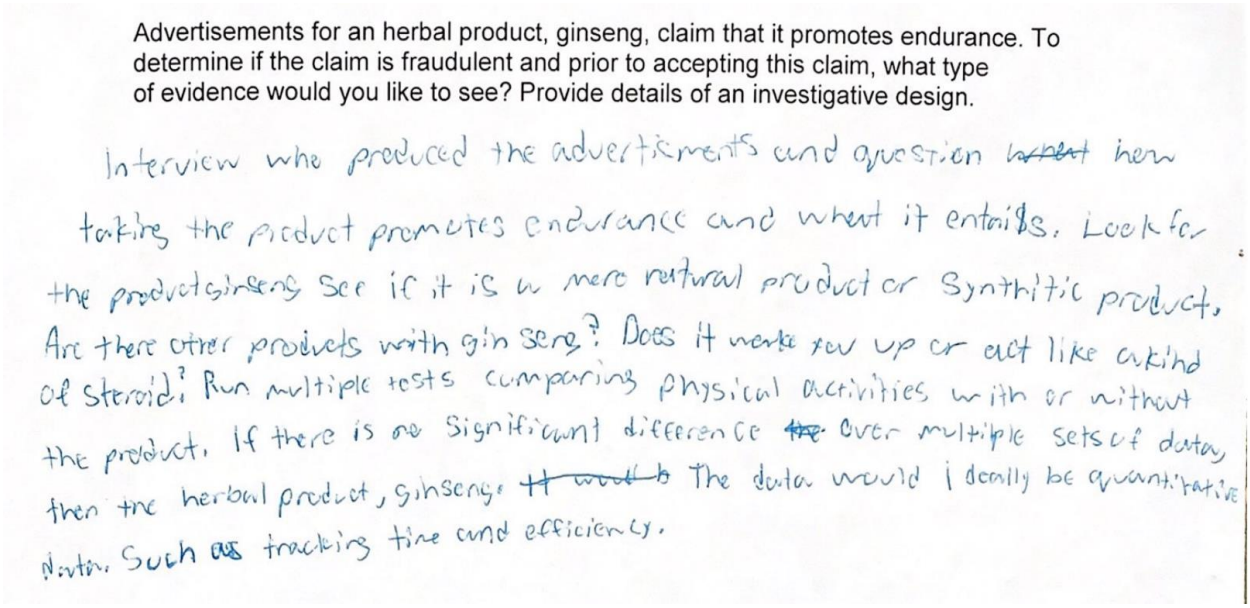
Supplemental Data



Advertisements for an herbal product, ginseng, claim that it promotes endurance. To determine if the claim is fraudulent and prior to accepting this claim, what type of evidence would you like to see? Provide details of an investigative design.

To test the claim to see if it is true, I would take a sample group, have them take the product and report on what they experienced after taking the product. I would take this and compare it to group B, which would be taking a placebo. This would be done as a double-blind study. I would take these results + compare which group overall had more endurance after taking the product. Because the product promotes endurance, I would try to take my samples from people around the same age.



Advertisements for an herbal product, ginseng, claim that it promotes endurance. To determine if the claim is fraudulent and prior to accepting this claim, what type of evidence would you like to see? Provide details of an investigative design.

Interview who produced the advertisements and question how taking the product promotes endurance and what it entails. Look for the product ginseng See if it is a more natural product or Synthitic product. Are there other products with gin seng? Does it work rev up or act like a kind of steroid? Run multiple tests comparing physical activities with or without the product. If there is no significant difference over multiple sets of data then the herbal product, ginseng, The data would ideally be quantitative data. Such as tracking time and efficiency.

Figure 6: Pre-EDAT assessment results from two of the three students. Names have been cropped out for student privacy