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Nature's Defense: Studying the Antibiotics Properties of the Goldenrod Plant

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ABSTRACT

Plants have long been known to possess antimicrobial properties and treat various conditions, including cancer (Gonelimali et al., 2018). Recent research has accelerated the use of plant-derived drugs and supplements, pivotal in reducing the strain on fungal-based antibiotics (Veeresham, 2012). However, there have been very few studies to evaluate the individual parts of the plants and their contribution to the antimicrobial properties. This study will be crucial in creating the most potent treatment and using plants more conservatively, potentially leading to the production of different types of antibiotics. This paper focuses on evaluating the antimicrobial properties of the various parts of the Goldenrod plant and the inhibitory mechanisms they use. Tests conducted on the Goldenrod plant against gram-positive and gram-negative bacteria indicate that the roots had the highest antimicrobial effect for the gram-positive bacteria, while the leaves had the highest antimicrobial effect for the gram-negative bacteria. The findings show that specific parts of the plant are specialized in specific types of antimicrobial compounds. When the antibiotics found in different parts of the plant were compared with commercial antibiotics, it was found that the antibiotics present in leaves worked similarly to Streptomycin, while the roots worked differently than any antibiotic that was available to us at the time of the study. While mass spectrometry of the plant compounds is underway, the findings of this study will be extended to other medicinal plants and will help prevent antibiotic winter and the discovery of new antibiotics.

Figure 1. The Goldenrod plant (Solidago Canadensis)

Introduction

Since the discovery of commercial antibiotics in the late 1920s, they have been overused in clinical practices (Llor & Bjerrum, 2014) and in other forms such as agriculture, mainly in Concentrated Animal Feeding Operations (CAFOs). The overuse of the limited number of antibiotics is quite detrimental to not only the human population but the ones in the future (Clardy, Fischbach, & Currie, 2009). Through this repeated overuse, bacteria are able to develop resistance to many types of antibiotics with relative ease. However, for the past few decades, there has been an increased focus

on developing antibiotics from other kingdoms, mainly Plantae, that can relieve the current strain on fungal-based antibiotic compounds (Veeresham, 2012). Plants have long been known to have antimicrobial properties and have been used in traditional medicines across various cultures (Cowan, 1999). The current state of literature involving the study of the Goldenrod plant as an antibiotic is quite minimal. While there have been some research articles that do validate claims that Goldenrod does have antibiotic properties (Elshafie et al., 2019), no study has compared the properties of specific parts of the Goldenrod plant nor tried to understand the mechanisms that contribute to the antibiotic effect.

The study involves comparing plant antibiotics to commercial antibiotics to help understand the inhibitory mechanisms. Commercial antibiotics use the following methods to achieve an antibiotic effect:

a) Inhibition of cell wall synthesis: This mechanism involves disrupting the production of bacterial cell walls, which can lead to cell death. An example of an antibiotic that inhibits cell wall synthesis is Penicillin, which works by binding and inhibiting the enzyme responsible for creating the cross-links between peptidoglycan chains in the cell wall (Sullivan, Delgado, Maharjan, & Cain, 2020). Penicillin was used in this study as a positive control against the gram-positive bacteria, Lactobacillus.

b) Inhibition of protein synthesis: This mechanism involves targeting the ribosomes in bacterial cells responsible for synthesizing proteins, which can inhibit bacterial growth. Examples of antibiotics that inhibit protein synthesis include Tetracyclines, Macrolides, and Streptomyces (Sullivan, Delgado, Maharjan, & Cain, 2020).

c) Inhibition of nucleic acid synthesis: This mechanism involves disrupting the replication or transcription of bacterial DNA or RNA, which can prevent bacterial growth. One example of an antibiotic that inhibits nucleic acid synthesis is Rifampin, which works by binding to and inhibiting the enzyme responsible for bacterial RNA synthesis (Sullivan, Delgado, Maharjan, & Cain, 2020).

d) Disruption of cell membrane function: This mechanism involves interfering with the function of bacterial cell membranes, which can cause leakage of cellular contents and cell death. Examples of antibiotics that disrupt cell membrane function include Polymyxins and Daptomycin (Sullivan, Delgado, Maharjan, & Cain, 2020).

e) Inhibition of metabolic pathways: This mechanism involves disrupting key metabolic pathways in bacterial cells, which can lead to a lack of energy and other essential cellular processes, ultimately leading to cell death. One example of an antibiotic that inhibits metabolic pathways is Sulfonamides, which work by inhibiting the synthesis of folic acid, an important component of bacterial metabolism (Sullivan, Delgado, Maharjan, & Cain, 2020).

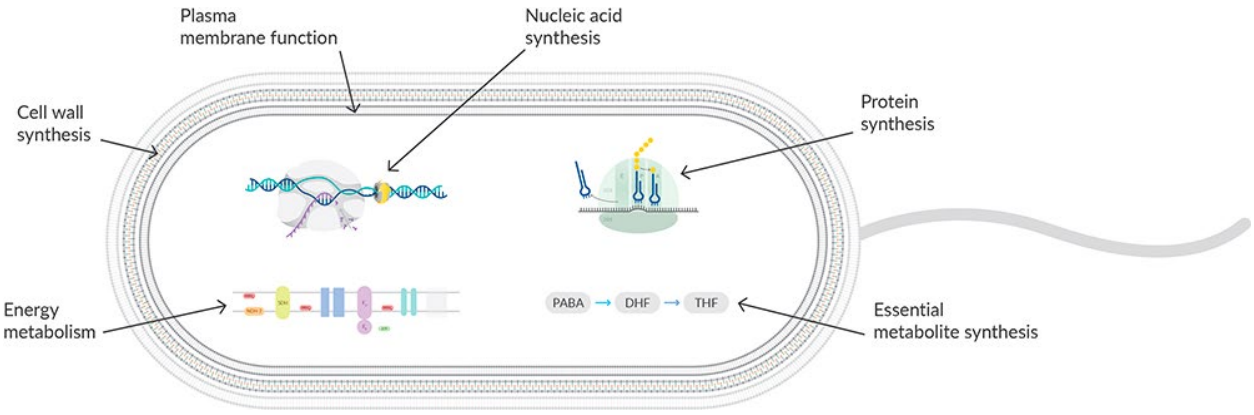

Figure 2. Showing the five main targets of antibiotics ("How Do Antibiotics Work? - Nordic Biosite," 2021)

Methods and Materials

a) Collection of plant material: The Goldenrod plant was chosen due to its prevalence in Native American literature, where it has been cited to have antibiotic properties (*GOLDEN ROD*, n.d.). The Goldenrod plant was also readily available in the vicinity of the lab and collected from the local area outside of Acton-Boxborough Regional High School. The samples were collected in late November 2022. There were no flowers left on the Goldenrod plant at this time. Hence flowers could not be included in our test case. Care was taken to collect only healthy and mature plants.

b) Preparation of plant extracts: Once collected, the plant samples were stored in an incubator for 7 days at 37℃. This helped to take out any moisture that might have contained additional chemicals from the outside environment. Once dried, the samples were rinsed in distilled water and dried for 24 hours at 37℃. The leaves stems, and roots were then separated and crushed individually using a coffee grinder. Each powder was then stored in 5 ml Eppendorf tubes, with 1 gram of plant extract and 3 ml of methanol. The powders were then run through a series of cycles - vortexed for 1 minute, allowed to steep for 15 minutes, vortexed for 15 seconds, and allowed to steep for 1 minute. After the three Eppendorf tubes had undergone this process, they were all kept in a centrifuge that was spun for 1 minute at 13,400 rpm. The final step was to extract the supernatant which served as the final extract from my plant as shown in Figure 3. The yield rate for my experiment was quite satisfactory, and there was enough to conduct the experiment.

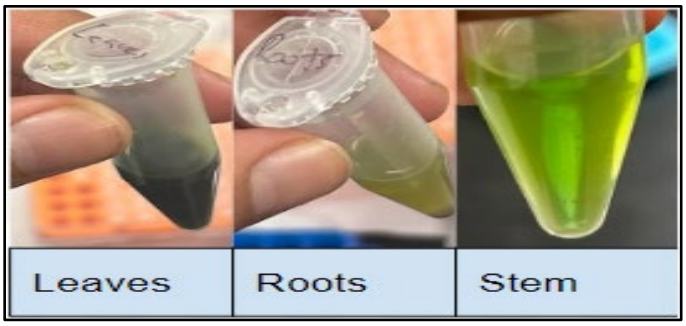

Figure 3. Final extracts from the plant

c) Preparation of cell plates: Two different types of bacteria - Lactobacillus, and E.Coli were used to study the antimicrobial patterns. Two different types of cell plates, therefore, had to be prepared as shown in Figure 4. For Lactobacillus, readily available MRS plates were used as they are designed to selectively grow only Lactobacillus which reduced the probability of contamination. However, for E.Coli, Trypsin Soy Agar (TSA) plates had to be made. To make the TSA plates, 12g of TSA powder was mixed with 100 ml of distilled water, creating enough agar to pour 15 plates ("Tryptic Soy Agar TSA | Principle | Preparation | Interpretation," n.d.).

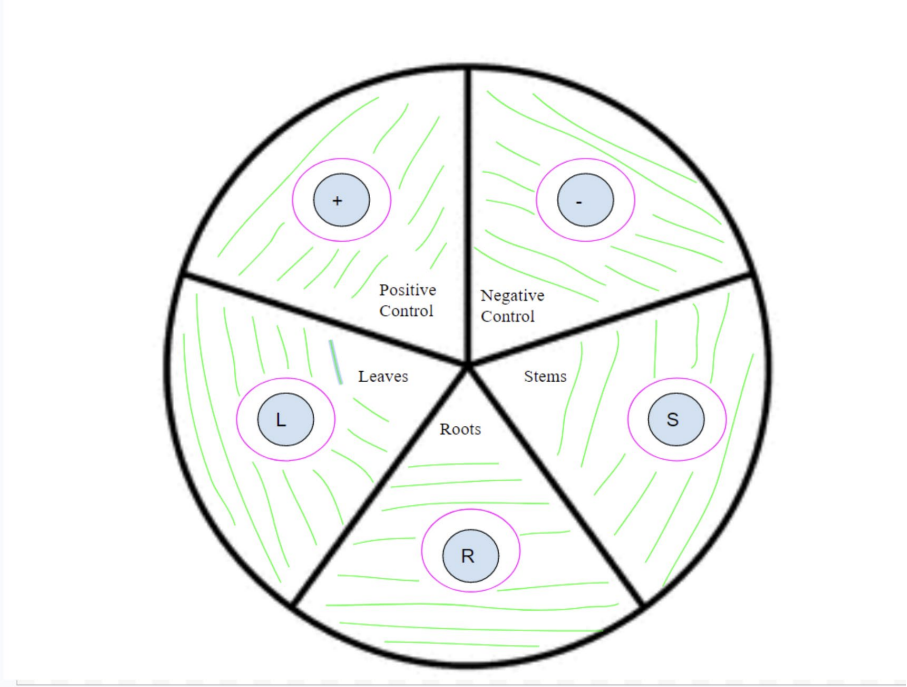

Figure 4. Design of a cell plate. Purple denotes the inhibition zone. Green denotes bacterial growth. Inhibition zones are not to scale.

d) Preparation of discs: First, the bacteria were spread onto the Petri dishes using cell spreaders. Blank discs were then soaked in each plant part extract and then placed on the Petri dishes as shown in Figure 5. The positive controls for this experiment were Penicillin for Lactobacillus and Tetracycline for E.Coli. These came in pre-prepared disks, so they just had to be put on the surface of the cell plate. Methanol was used as the negative control, as it was the base of all the plant extracts. The experiment involved comparing the inhibition zones in order to evaluate the antibiotic effects of each part of the plant.

e) Measurement of inhibition zones: After 24 hours of incubation, the plates were examined for inhibition zones around the discs. The radius of the inhibition zones was not uniform, so the distance to the nearest growing colony was calculated as the 'zone of inhibition'. A total of 12 trials were performed for the experiment, 6 using Lactobacillus, and 6 using E. coli. Measuring the zones of inhibition provided us with data on the antibiotic strength exhibited by each part of the Goldenrod plant.

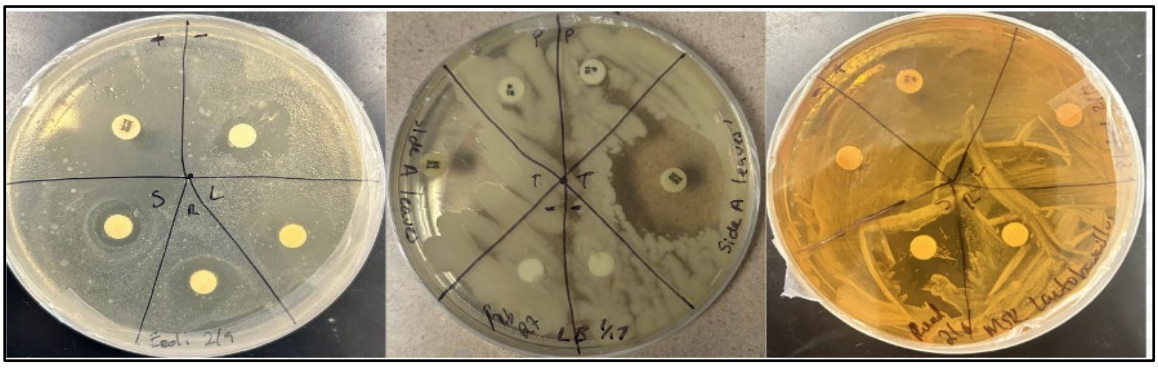

Figure 5. Assortment of cell Petri dishes, showing the experimental process

f) Comparison with Commercial Antibiotics: After 72 hours of incubation for E.Coli and Lactobacillus, some bacteria had started to creep closer and closer to the disc, effectively breaking the zone of inhibition. This indicated that the bacteria had mutated in some way rendering the antibiotic property from the specific plant part ineffective. To understand the mutation, these mutant bacteria were tested against four commercially available antibiotics. The results were then compared with the parent strain of these bacteria against the same commercial antibiotics. The bacteria that had grown the closest to the discs inhibited the plant antibiotic the most. This bacteria was then collected and grown on a different cell plate. Due to a shortage of materials, only the bacteria that could inhibit the roots or leaves of the Goldenrod plant were tested.

Some commercial antibiotics were less effective against the mutant bacteria. This was evident because the inhibition zone of these antibiotics was less. We compared the inhibition zones of the commercial antibiotics (against mutant bacteria) with the inhibition zones of parts of the plants (against parent strain). If they were found to be similar quantitatively, we hypothesized that the underlying antibiotic mechanism in both the commercial antibiotic and the part of the plant might be the same. These results are explained in Figure 10.

Results

In this study, the antimicrobial properties of different parts of the Goldenrod plant were tested against gram-positive and gram-negative bacteria, and their inhibitory mechanisms were found. The roots, leaves, and stems of the plants were tested using a disc diffusion assay, and methanol was used as a negative control with penicillin as the positive control for gram-positive bacteria, and tetracycline as the control for gram-negative bacteria.

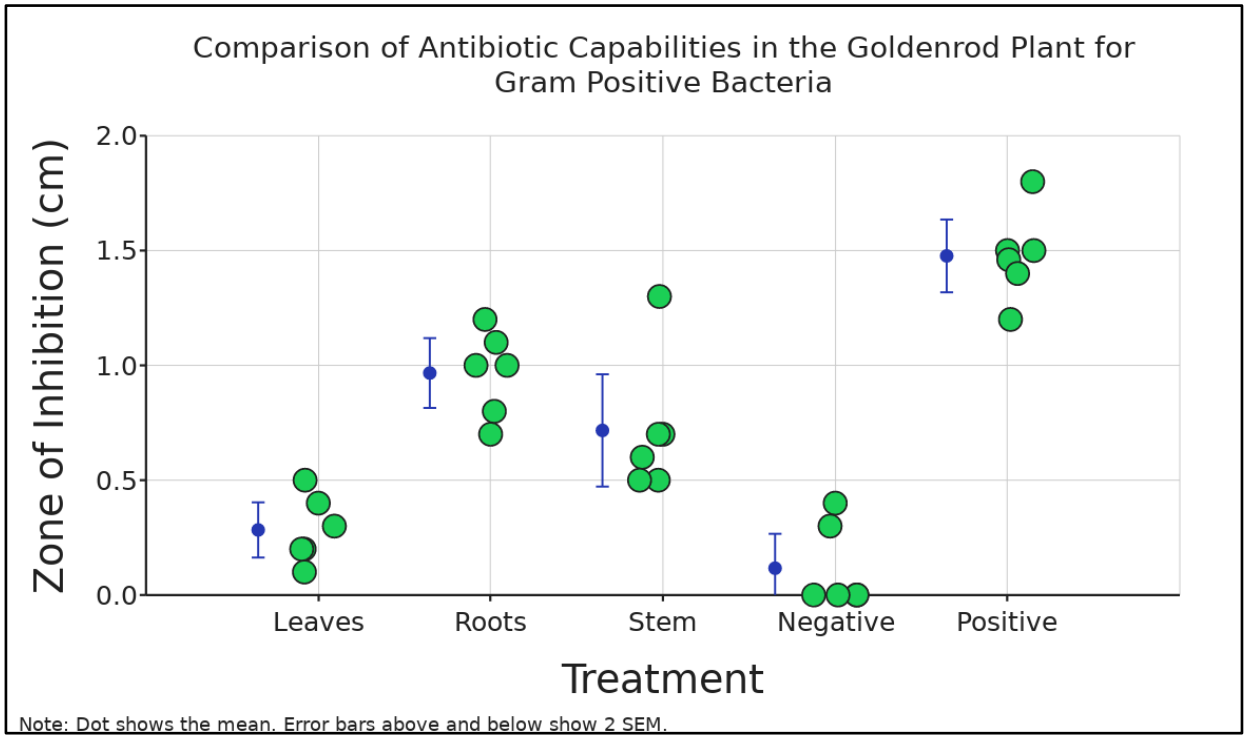

Figure 6. Antibiotic effects of the various parts of the Goldenrod plant against gram-positive bacteria, Lactobacillus

As shown in Figure 6, the plant showed antibiotic sectionalization - certain parts had a much larger effect than others. The roots of the Goldenrod plant had the highest antimicrobial effect against gram-positive bacteria, with an average zone of inhibition radius of 0.97 cm, compared to the leaves and stem with average radius of 0.283 cm and 0.716 cm respectively.

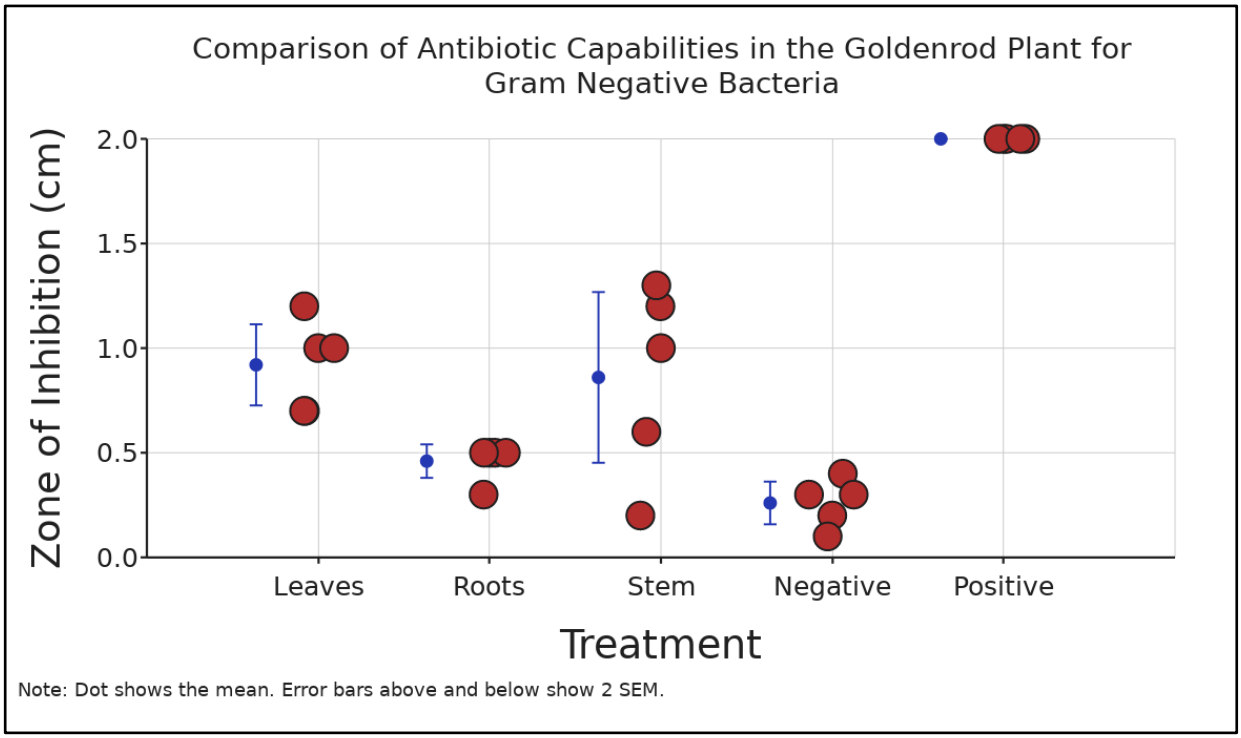

Figure 7. Antibiotic effects of the various parts of the Goldenrod plant against gram-negative bacteria, E. coli k-12

Conversely, Figure 7 indicates that the leaves of the plant had the highest antimicrobial effect against gram-negative bacteria, with an average radius of 0.92 cm, compared to the roots and stem with average radius of 0.46 cm and 0.86 cm, respectively. These first two tests showed that the Goldenrod plant was sectionalized in terms of antibiotic compound production. The roots produced an antibiotic that was able to inhibit gram-positive bacteria very well, while it struggled in inhibiting gram-negative bacteria. Contrariwise, the leaves' antibiotic compound was able to inhibit gram-negative bacteria but struggled with gram-positive. The antibiotic from the stem performed average for both. After 72 hours bacteria that broke these zones of inhibition were harvested and then compared to commercial antibiotics, following the logic sequence shown in Figure 10.

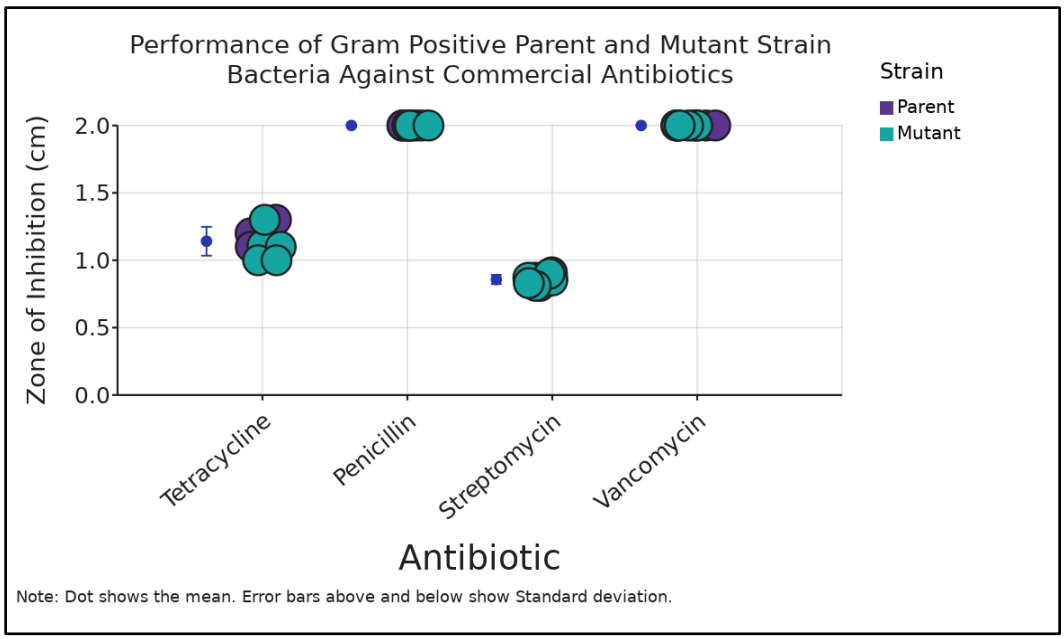

Figure 8. Inhibition zones of parent and mutant gram-positive bacteria against commercial antibiotics

Figure 8 shows that the results were quite unusual. None of the four antibiotics showed any significant difference in their inhibition of the parent strain of Lactobacillus or the mutant. The inhibition values of Tetracycline, Penicillin, Streptomycin, and Vancomycin were 1.12, 2, 0.87, and 2 cm respectively.

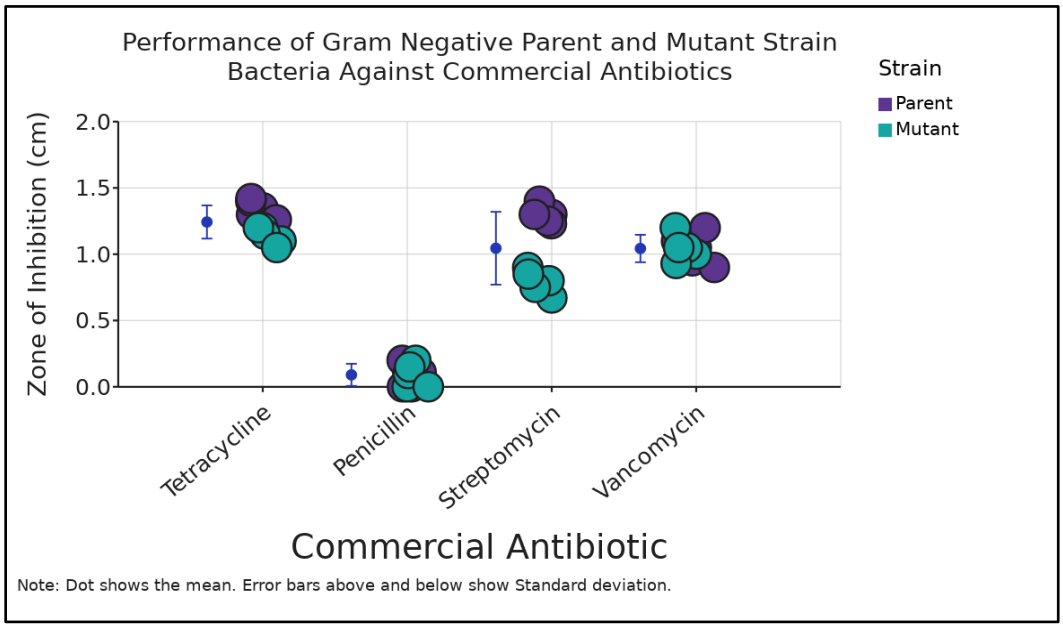

Figure 9. Inhibition zones of parent and mutant gram-negative bacteria against commercial antibiotics

As can be seen in Figure 9, both Penicillin and Vancomycin had a minimal difference in inhibiting the parent strain of E. coli as compared to the mutant strain of E.Coli. Penicillin had inhibition values of 0.1,0.1 cm and Vancomycin had inhibition values of 1.05, 1 cm for parent E.Coli and mutant E.Coli respectively. On the other hand, Tetracycline had a larger difference. It had an inhibition value for the parent E.Coli of 1.35 cm, while for the mutant E.Coli, it was only 1.2 cm. Ultimately, Streptomycin showed the largest difference by far with an inhibition zone of 1.3 cm for the parent strain of E. coli, while only having an inhibition zone of 0.8 cm for the mutant strain.

Statistical Significance

Statistical tests were done on all the trials to prove that the difference between the antibiotic capabilities of each part of the plant was not noise, instead there were some intrinsic differences contributing to the same. Later the performance of the antibiotics against the gram-positive/gram-negative mutants were analyzed using tools from DataClassroom® and the ANOVA test.

Table 1. Statistical significance for all four experiments conducted in the study

Experiment	Variable	F-Statistic	P Value	Interpretation	Coincides with findings?
Plant V. Gram-Positive	Plant Extract	41	<0.01	A P-value of <0.01 means that the groups are different.	Yes
Plant V. Gram-Negative	Plant Extract	41	<0.01	A P-value of <0.01 means that the groups are different.	Yes
Antibiotics V. Parent/Mutant strains of Gram-Positive bacteria	Interaction (X*Z) Commercial Antibiotic (X) Strain of Bacteria (Z)	1.2	0.34	A lower F-Statistic indicates that the four commercial antibiotics behaved similarly on both strains of bacteria.	Yes
Antibiotics V. Parent/Mutant strain of Gram-Negative bacteria	Interaction (X*Z) Commercial Antibiotic (X) Strain of Bacteria (Z)	19	<0.01	A higher F-Statistic indicates that the four commercial antibiotics show a significant difference in effectiveness, depending on the strain of the bacteria	Yes

Discussion

The question this study sought to answer was, 'Do different parts of the Goldenrod plant have different antibiotic capabilities, and if so, how do they work'? The data collected in the experiment shows that there is sectionalization in the Goldenrod plant when it comes to antibiotic production. The roots outperformed the leaves and stems, as the zone of inhibition was on average 0.4 cm more when it came to gram-positive bacteria. On the other hand, the leaves outperformed the roots by a similar magnitude with gram-negative bacteria. While the stems were average for both. According to recent studies, gram-positive bacteria are very prevalent in soil (Liu et al., 2019). It, therefore, makes sense that the roots were the best at inhibiting gram-positive bacteria as they are found underground. Being surrounded primarily by gram-positive bacteria throughout the evolution of the Goldenrod plant would act as a selective pressure, causing the roots to have the most potent antibiotics against gram-positive bacteria. In other literature, it has been concluded that gram-negative bacteria, specifically Proteobacteria, are found in the air (Ruiz-Gil et al., 2020). It is therefore reasonable that the leaves have higher antibiotic potency against gram-negative bacteria as compared to gram-positive bacteria. Finally, the results showed that the antibiotic effect from the stems was average. This could be a result of the diffusion of antibiotics from the leaves and roots into the stem thereby averaging it out.

In the second part of the study, attempts were made to understand the mechanisms behind the antibiotic effect found in the roots and leaves. The logic diagram in Figure 10 explains how the antibiotic mechanism for leaves and roots was determined. For the leaves, Streptomycin and Tetracycline had the largest difference in zones of inhibition between parent and mutant strains as the mutation would have affected the commercial antibiotics' inhibitory mechanism. Additionally, because the mutant bacteria also successfully inhibited the leaf's antibiotic it can be surmised that they work in a similar fashion. It can be inferred that the leaves inhibit translation, specifically by binding to the 30s subunit of a bacterial ribosome (Humayun & Ayyappan, 2013). A similar process was used to understand the mechanism behind the root's antibiotic effect, however, results showed that there was no major difference in the inhibition zones for any of the four commercial antibiotics on the gram-positive bacteria. This indicates that mutation in the gram-positive bacteria did not affect any mechanism of the commercial antibiotics. Additionally, because the same mutation also impaired the inhibition from the roots, one can conclude that the antibiotic mechanism in the roots works differently from the four antibiotics available.

In this study, the general antibiotic mechanism was examined. However, pinpointing the exact inhibitory mechanism will take additional time and be highlighted in future work.

Conclusion

In my research, I have found compelling evidence supporting the potential of the Goldenrod plant in treating a wide range of bacteria. What makes this discovery even more promising is the likelihood that the mechanisms behind the plants' compounds differ from those of existing antibiotics. This opens the possibility of uncovering novel antibiotics that are not derived from fungi, which is crucial amidst the looming threat of antibiotic resistance and the alarming concept of an 'antibiotic winter'. An antibiotic winter is a grim future where none of our current antibiotics are effective against bacterial infections, leading to a rapid increase in disease rates. As we rely heavily on fungal-based antibiotics, the repeated overuse of these drugs has enabled bacteria to develop resistance at an alarming pace. Therefore, it is imperative that we explore alternative sources to discover new antibiotics that can combat this growing problem effectively. Through my experimentation, I have observed the potential for isolating unique antibiotics from the Goldenrod plant, which are distinct from fungal-based antibiotics. Not only do these newly discovered compounds show promising efficacy against bacteria, but they also possess a higher potential to thwart resistance development. Furthermore, my research has focused on utilizing the Goldenrod plant in multiple ways, targeting various types of bacteria and broadening the scope of potential treatments. It is crucial for the scientific community to conduct similar

experiments, exploring different avenues and sources for antibiotic discovery. By collectively working towards finding new solutions and deepening our understanding of antibiotic mechanisms, we can actively prevent the alarming antibiotic winter from becoming a reality. The Goldenrod plant holds significant promise as a source of novel antibiotics. Its unique compounds provide hope for combating bacterial infections in a more effective manner, while simultaneously reducing the risk of resistance development. The pursuit of alternative sources for antibiotics, as demonstrated in my research, is essential in our ongoing battle against antibiotic resistance and the potential defeat of an antibiotic winter.

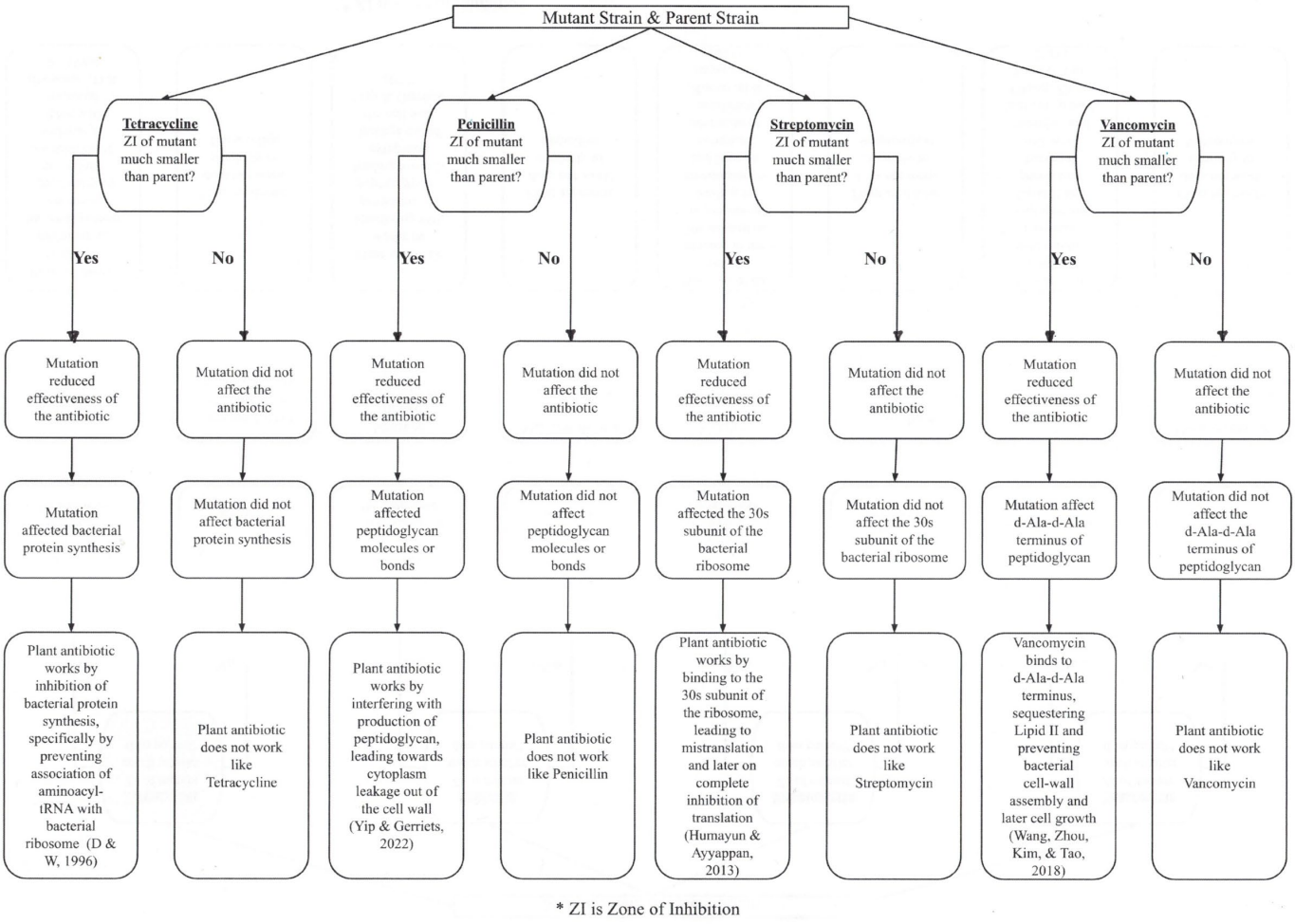

Figure 10. Flow chart detailing the logic used in testing mutant strain on commercial antibiotics.

Limitations

As stated before, the understandings of the antibiotic mechanisms present in the Goldenrod plant are only preliminary, they have only been tested against four commercially available drugs. To improve the accuracy of this study, they could be tested alongside many other drugs, or computer models can be created to get an accurate outcome. To account for the variation across Goldenrod plants, comprehensive experimental testing will be needed.

Future Work

I am working with the Barnett Lab at Northeastern University to locate specific antibiotic compounds found in my extracts. The next possible steps could be developing methods to manufacture this compound, refine it to become a viable FDA-approved drug, or study the metabolic systems present in the plant that produces it. Additionally, the mutant and parent strain for the gram-positive and gram-negative bacteria exposed to roots and leaves respectively is being sequenced to identify the exact changes. This is being done at the Chai Lab at Northeastern University. This will allow us to pinpoint a specific organelle, protein, or other part of the cell that changed, leading us to the exact inhibitory mechanism that the antibiotic compound uses. Pairing this with the results from the Barnett Lab, both the compound and the mechanisms can be soundly understood.

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