UNC PEMBROKE | MAYNOR HONORS COLLEGE

Antimicrobial Properties of *Diospyros virginiana* Against Human Dwelling Bacteria

Senior Project

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Abstract

Approximately three-fourths of medically important antibiotics are being wasted on healthy livestock in an effort to make up for unsanitary and overcrowded growing conditions. This misuse of antibiotics has led to the prevalence of antibiotic resistant bacteria. Before the creation of these modern medicines, Indigenous People relied heavily on medicinal plants. Many medicinal plants possess antimicrobial properties that allow them to inhibit the growth of bacteria, however most have not been charact. The plant used in this project was *Diospyros virginiana*. Previous studies have documented the antimicrobial properties of this plant against a variety of bacterial strains. Here, it was specifically used against six human dwelling bacteria: *S. epidermidis, K. pneumonia, N. sicca, M. luteus, B. subtilis, C. xerosis, and E. faecalis.* Teas created from the leaves of this plant as well as the plant material were tested against these bacteria in disk assays. The strain most inhibited during this process was *S. epidermidis*. With the exception of *S. epidermidis*, there seems to be a pattern of inhibition based on Phylum and gram stain.

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Introduction

Until recently, scientists believed the ratio of bacterial to human cells in the body to be 10:1. According to the American Microbiome Institute, "A recent study out of the Weizmann Institute in Israel states that the number of bacteria may actually be very similar to the number of human cells in the body."[1] The bacteria native to our bodies are typically harmless; they do, however, have the potential to cause harm, such as when bacteria colonize parts of the body, leading to bacterial infections. With the help of modern medicine, and more specifically antibiotics, these infections can often be treated. Antibiotics are antimicrobial drugs that can be obtained from bacteria, mold, and other organisms.[7,9] These drugs fight bacterial infection by inhibiting the growth of bacteria and hindering their ability to form cell walls.[9] While antibiotics are currently still serving their purpose, we are now seeing a rise in antibiotic resistant bacteria. These drugs are no longer as effective as they once were and this is likely due to their misuse.[7] Use of antibiotics is a sort of double-edged sword. Misuse of antibiotics has led to the creation of "superbugs", and even when taken correctly, antibiotics destory good bacteria that is present.[7,11] While both doctor and patient-related misuse and overuse contribute to the creation of antibiotic resistant bacteria, the industrial livestock system is also contributing to this dilemma.[7,8,9] Farmers feed chickens antibiotics in order to facilitate faster growth and fight off disease. These antibiotics are given as a preventative mesuare; the chickens are not actually in need of the antibiotics they are given. A whopping 74% of medically important antibiotics are utilized in animal agriculture practices.[8] To make matters worse, approximately 44% of retail chicken contain bacteria resistant to a variety of antibiotics.[8] While modern medicine has been pivotal in fighting bacteria, the prolonged misuse is leading us down an unstoppable bath booming with antibiotic resistant bacteria.

Before modern medicine, communities relied on traditional/herbal medicines to heal wounds, ward off diseases, and fight bacteria. These remedies were in the form of teas and ointments that originated from harvested raw plant material. The majority of our knowledge on this type of traditional medicine comes directly from transcribed traditional plants used in herbal medicine as well as the ailments they treated can be found in variety of literature sources. Two sources are discussed here. "Herbal remedies of the Lumbee Indian" describes medicinal plant use by the Lumbee Nation in southeastern North Carolina.[2] "Native American Plants: An Ethnobotanical" details the use of over 3000 medicinal plants by 218 different Native American tribes.[6] *Diospyros virginiana* (Figure 1) contains leaves, flowers, fruits, twigs, and bark.[12] Its physical characteristics have allowed it to be used as an antidiarrheal syrup, a dermatological aid for sore throat and mouth, gastrointestinal aid for heartburn, hemorrhoid remedy, liver aid, oral, throat aid, toothache remedy and venereal aid.[6] While there is not a large amount of literature detailing the use of medicinal plants in place of modern medicine, there are studies detailing the antimicrobial properties of *Diospyros virginiana.*

Figure 1: A depiction of the plant Diospyros virginiana, commonly known as Persimmon.

Previous scientific studies characterize some of the antimicrobial properties of *Diospyros virginiana*. Methanolic (80%) extracts from the fruits of D. *virginiana*, as well as phenolic compounds isolated from the extract, have inhibited the growth of *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimorium*, *Listeria monocytogenes*, and

Staphylococcus aureus. The bacterium P. *aeruginosa* was the most resistant to inhibition. The phenolic compounds were even shown to hinder growth better than some antibiotics.[10] Another study examined the plant's ability to hinder the growth of *Mycobacterium tuberculosis*. Leaf extracts were made using methanol 70%. The results concluded that the extract's highest percentage of inhibition was 33.3% .[4] Very little is published, however, about aqueous based extracts, which is the manner in which plants were used traditionally (e.g. as teas). The purpose of this experiment is to expand our knowledge on traditional medicine specifically using *Diospyros virginiana*. The project will characterize how well a 60% concentrated tea made from the leaves of this plant can inhibit the growth of six strains of human dwelling bacteria.

Materials and Methods

Six laboratory strains of human dwelling bacteria - *S. epidermidis, K. pneumonia, N. sicca, M. luteus, B. subtilis*, *C. xerosis*, and E. *faecalis* - were grown in Luria Broth (LB) and on Nutrient Agar (NA) medium. Other materials required to run the disk assay include: 6mm filter disks, a heat lamp, forceps, 70% isopropanol, ddH_2O, a heat source to boil teas, nutrient agar plates and centrifuge tubes. Scientific instruments used in this experiment include: an incubator shaker, a conventional incubator, and a -4° Celsius cold room. Most importantly, *Diospryros virginiana* was needed for the experiment. After the plant was harvested from Sampson's Landing (Figure 2), it was placed in a -80° Celsius freezer for preservation.

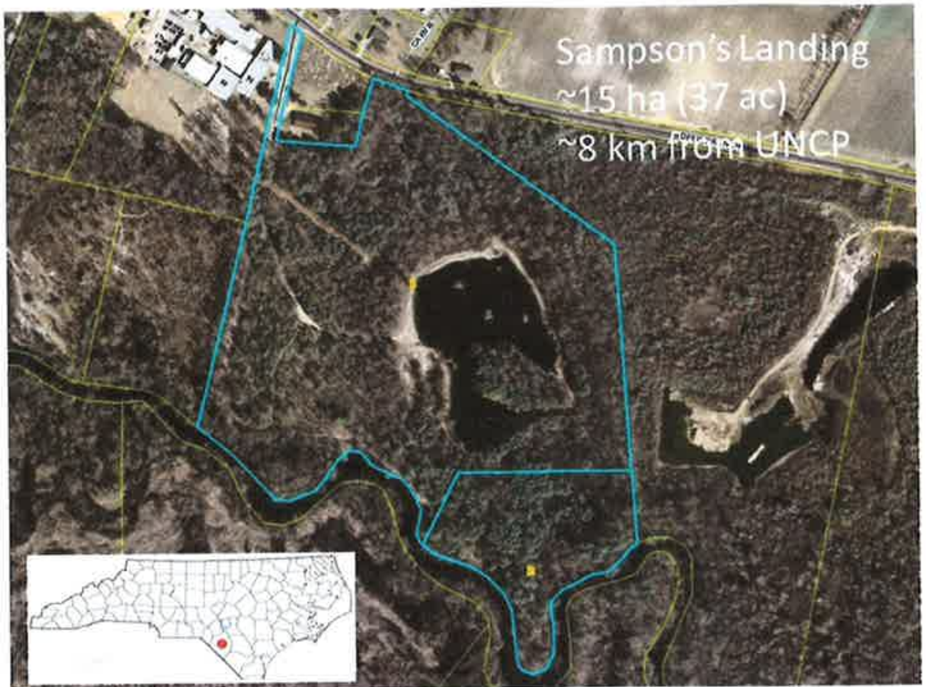

Figure 2: The image shows an aerial view of the area from which the plant was harvested. This area is called Sampson's Landing.

In order to begin the experiment, LB tubes and NA plates were made. Bacteria were inoculated into liquid media and left overnight in a 37° Celsius incubator shaker to grow. The following morning, teas were made by adding raw plant material to ddH_20 in a centrifuge tube. The centrifuge tube was then placed in a heat block to heat the tea to100° Celsius for at least one hour. The concentration of the teas were calculated by taking the amount of plant material and dividing it by the amount of water used and multiplying by one hundred (Eq 1).

(1) $\frac{Plant\ Material\ (grams)}{ddH_2O\ (milliliters)}\ x\ 100$

Once the teas were heated, they were removed from the heat source (Figure 4) and the liquid cultures were removed from the incubator shaker. Nutrient agar plates were labelled and then it was time to set up disk assay. First, the liquid bacterial cultures were swabbed onto the nutrient agar petri dishes. Once all the petri dishes were swabbed, filter disks were dipped into the positive control (70% isopropanol), negative control (ddH_2O), and tea (60% w/v) and placed in their corresponding quadrant on the dish using forceps making sure to disinfect in between each (Figure 5).

Figure 4: Representative image of the teas made in this experiment.

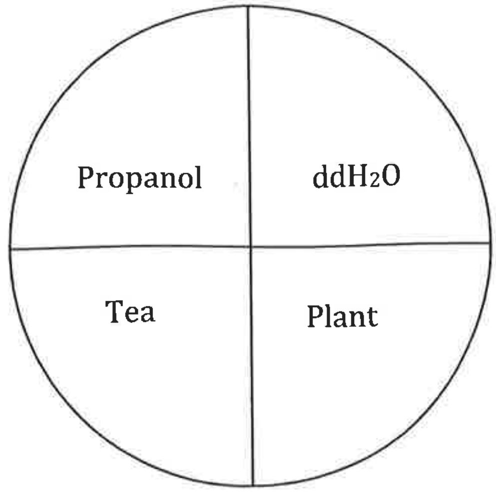

Figure 5: Representative image of disk assay setup.

Once all the disk assays were completed, they were placed upside down in a 36°Celsius incubator and left overnight to grow on the assay. The next day the plates were analyzed.

Figure 6: Representative image of disk assay after it was left in incubator overnight.

Results

The growth of all six strains of bacteria used in this experiment were inhibited by *Diospyros virginiana*, with varying degrees of inhibition. A total of seven replicate disk assays were completed in the duration of this project. However, the data for one assay was not included here because the plates were contaminated. The area where no bacteria growth is present is known as the zone of inhibition. The average inhibition for each plant from the tea and plant material are located in Table 1 and a graph depicting this information is present in Figure 7. The shaded area in Figure 7 indicates a zone of inhibition that is larger than the negative control. The greatest inhibition by the tea and plant material were both against *S. epidermidis*.

Table 1: Average inhibition done by plant and tea material.

Bacteria	Average Inhibition by Plant (mm)	Average Inhibition by Tea (mm)
S. epidermidis	14.67	10.45
K. pneumoniae	8.92	8.82
N. sicca	8.22	7.87
M. luteus	7.80	8.48
B. subtillis	6.83	6.20
C. xerosis	6.82	6.77
E. faecalis	6.68	7.33

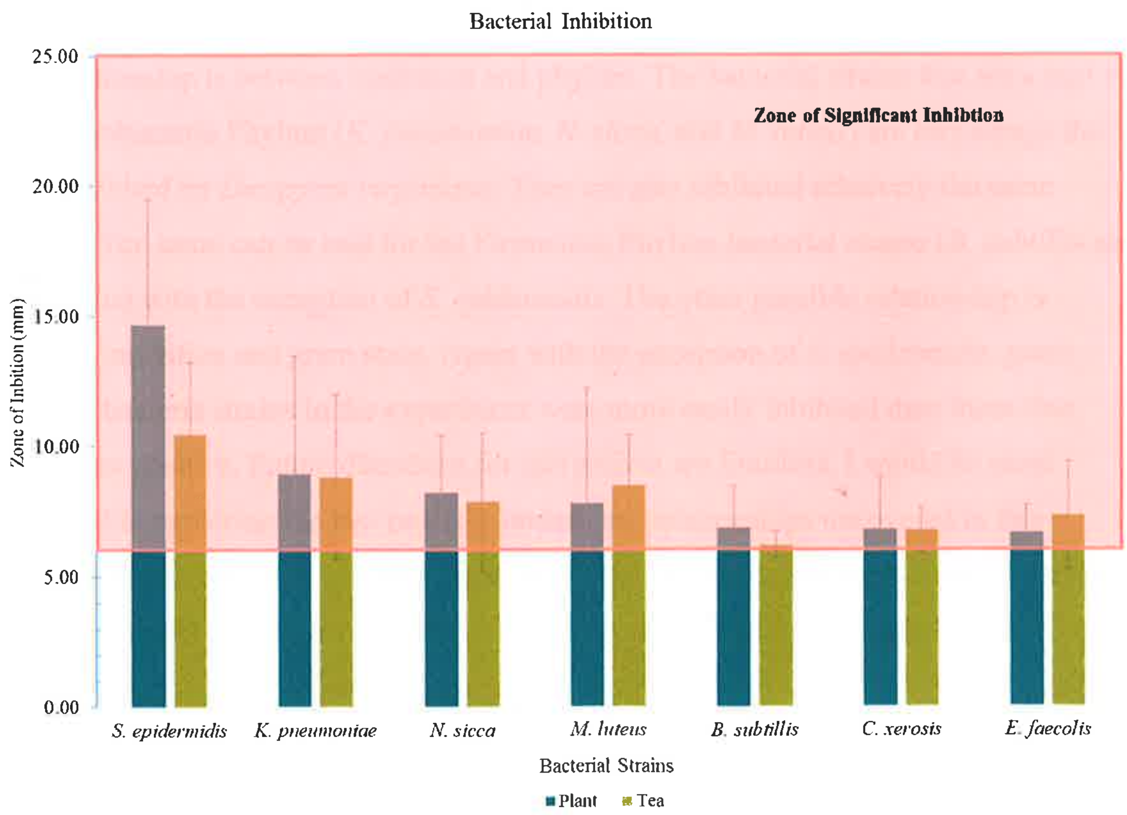

Figure 7: Bar graph depicting the data found in Table 1.

Discussion

The results show that *Diospyros virginiana* does in fact possess antimicrobial properties. Teas made from the leaves of this plant as well as plant material were successful in inhibiting the growth of human dwelling bacteria. In order to establish a direct link between inhibition and bacteria strain, characteristics of each bacterial strain were examined (Figure 8).

Bacteria	Phylum	Family	Genus	Gram	Avg Inbt for Plant (mm)	Avg Inbt for Tea (mm)
S. epidermidis	Firmicutes	Staphylococcaceae	Staphylococcus	+	14.67	10.45
K. pneumoniae	Proteobacteria	Enterobacteriaceae	Klebsiella	-	8.92	8.82
N. sicca	Proteobacteria	Neisseriaceae	Neisseria	-	8.22	7.87
M. luteus	Proteobacteria	Micrcoccaceae	Micrococcus	+	7.80	8.48
B. subtillis	Firmicutes	Bacillaceae	Bacillus	+	6.83	6.20
C. xerosis	Actinobacteria	Corynebacteriaceae	Corynebacterium	+	6.82	6.77
E. faecalis	Firmicutes	Enterococcaceae	Enterococcus	+	6.68	7.33

Figure 8: Phylum, family, genus, and gram stain for each bacterial strain.

With the exception of *S. epidermidis*, there seems to be two underlying relationships. The first relationship is between inhibition and phylum. The bacterial strains that are a part of the Proteobacteria Phylum (*K. pneumoniae, N. sicca,* and *M. luteus*) are on average the most inhibited by *Diospyros virginiana*. They are also inhibited relatively the same amount. The same can be said for the Firmicutes Phylum bacterial strains (*B. subtillis* and *E. faecalis*) with the exception of *S. epidermidis*. The other possible relationship is between inhibition and gram stain. Again with the exception of *S. epidermidis*, gram negative bacteria strains in the experiment were more easily inhibited than those that were gram positive. Future directions for this project are limitless. I would be most interested in exploring the two possibly underlying relationships uncovered in this project. I would also like to explore how the use of this medicinal plant in the industrial livestock system could slow impact of the emergence of antibiotic resistant bacteria.

Conclusion

This project was successful in demonstrating antimicrobial properties of *Diospyros virginiana* against human dwelling bacteria. The project also led to the discovery of two possible factors that contribute to or hinder inhibition; however, there is still a lack of scientific research on this plant used in traditional medicine. With the emergence of antibiotic resistant bacteria caused by the overuse of antibiotics, we need to act now and act fast. Traditional medicine could be just what the doctor ordered.

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