

BSC2011L Plant Project Poster Examples:

Antibacterial Properties of Sage, Lime, and Shiitake Mushrooms

Veronica Beijo, Ngan Nguyen, Lissette Rodriguez, Carolina Arias, Dr. Jonelle Orridge
Department of Biology
Broward College

INTRODUCTION

Many plants in nature have been used for their antibacterial properties. They are used in traditional medicine to combat various illnesses and diseases. The plants that were selected for this project were chosen because they have been used in traditional medicine for centuries. These plants have been used for their antibiotic properties. The bacteria we chose through the literature review is *Escherichia coli*. This is a common bacterium found in the environment and can cause food poisoning. *E. coli* can cause intestinal infections, diarrhea, and resulting in the illness *colitis*.

The results of our project were that sage had the most inhibition zones, followed by lime and shiitake mushrooms. According to the *Anti-Cancer*, the antibiotic power of plants varies according to their parts. Sage has the highest antibiotic power, followed by lime and then shiitake mushrooms.

Using the Kirby-Bauer method, the antibiotic potential of sage, lime, and shiitake mushrooms was determined against *E. coli*. The *E. coli* was obtained from the environmental project of the selected plants. A colony of *E. coli* was isolated and grown on Mueller-Hinton agar plate. The diameter of the isolated *E. coli* was measured at 0.5 mm.

METHODOLOGY

This project has three parts: the extraction of the plant compounds, the testing of the bacteria, and finally determining the results for measuring zones of inhibition.

First, lime and shiitake mushroom were washed and cleaned to remove any dirt or debris. According to the *Anti-Cancer*, the antibiotic power of plants varies according to their parts. Sage has the highest antibiotic power, followed by lime and then shiitake mushrooms.

Second, the plants were washed and cleaned to remove any dirt or debris. According to the *Anti-Cancer*, the antibiotic power of plants varies according to their parts. Sage has the highest antibiotic power, followed by lime and then shiitake mushrooms.

Third, the plants were washed and cleaned to remove any dirt or debris. According to the *Anti-Cancer*, the antibiotic power of plants varies according to their parts. Sage has the highest antibiotic power, followed by lime and then shiitake mushrooms.

RESULTS

Figure 1 shows an agar plate with four wells containing different plant extracts. The zones of inhibition are visible around the wells.

Figure 2 shows an agar plate with a well containing a penicillin disc, showing a clear zone of inhibition.

DISCUSSION

In comparison to previous research performed on inhibition on the effectiveness of herbs against *E. coli*, the result of inhibition was similar. Inhibition of the *E. coli* was observed in all three plants. The *E. coli* was isolated from the environmental project of the selected plants. Another study, conducted by *Antibacterial*, also found that sage had the highest inhibition of *E. coli*. According to the *Anti-Cancer*, the antibiotic power of plants varies according to their parts. Sage has the highest antibiotic power, followed by lime and then shiitake mushrooms.

However, *Antibacterial* found that sage had the highest antibiotic power. The results of this project became reversed. The results of this project showed that sage had the lowest antibiotic power, followed by lime and then shiitake mushrooms. Another study, conducted by *Antibacterial*, also found that sage had the highest inhibition of *E. coli*. According to the *Anti-Cancer*, the antibiotic power of plants varies according to their parts. Sage has the highest antibiotic power, followed by lime and then shiitake mushrooms.

Furthermore, there are recommendations of local tea companies in the experiment. One of the areas that were recommended was to use the tea bags instead of the dried herbs. Another way to increase the effectiveness of the tea bags is to use the tea bags for a longer time. This will allow the tea bags to release more tannins into the water. Once the tea bags are removed, the tea bags should be rinsed with hot water to remove any remaining tannins. Another reason is due to spreading the tea bags with the high heat of the oven. This will allow the tea bags to release more tannins into the water. Another way to increase the effectiveness of the tea bags is to use the tea bags for a longer time. This will allow the tea bags to release more tannins into the water. Once the tea bags are removed, the tea bags should be rinsed with hot water to remove any remaining tannins. Another reason is due to spreading the tea bags with the high heat of the oven. This will allow the tea bags to release more tannins into the water. Another way to increase the effectiveness of the tea bags is to use the tea bags for a longer time. This will allow the tea bags to release more tannins into the water. Once the tea bags are removed, the tea bags should be rinsed with hot water to remove any remaining tannins. Another reason is due to spreading the tea bags with the high heat of the oven. This will allow the tea bags to release more tannins into the water. Once the tea bags are removed, the tea bags should be rinsed with hot water to remove any remaining tannins.

Diagram illustrating the penicillin disk diffusion method. A penicillin disk is placed on an agar plate. The antibiotic diffuses out from the disk, creating a zone of inhibition where bacterial growth is inhibited. The zone of inhibition is measured to determine antibiotic activity.

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Bacteria-Be-Gone!

Ashly Maximilien, Alyah Perer , Tahila Sadati, Laura Montano, Dr. Orridge •

Introduction

Staphylococcus aureus (S. aureus) or "staph" is *S. aureus* is recognized as one of the most dangerous of all *staphylococci* bacteria. It can cause disease in humans and animals and is often found in the environment reflecting its ability to survive in almost any type of environment. Some infections are not serious, however, *S. aureus* can induce a big problem like bloodstream infections, pneumonia, or bone and joint infections. In this view, we want to determine the susceptibility of *S. aureus* to three plants: Eggplant, Shiitake mushroom, and Spinach using the Bauer disk diffusion method. The plants that were chosen were spinach (Spinacia oleracea), eggplant (Solanum melongena) and the shiitake mushroom (Lentinula edodes). *S. aureus* is a Gram-positive, rod-shaped, non-spore-forming, facultatively anaerobic bacteria that grows best in temperatures between 25°C and 37°C. *S. aureus* can grow at temperatures as low as 4°C and as high as 45°C. The bacteria may be found on skin, hair, and in the nose and mouth. *S. aureus* can also be found in food, especially raw meat and dairy products. *S. aureus* can cause food poisoning, skin infections, and respiratory tract infections ("Foodborne Illnesses"). Eggplant has been found to treat skin diseases. Shiitake mushrooms are high in vitamin B and D, and they have been found to have antioxidant properties. Spinach is high in fiber, protein, and vitamins. Spinach has low caloric levels and has no fat and only 10 mg of saturated fat per cup. Spinach is similar help heart disease damage caused by arteries narrowing. This suggests spinach could be used as a potential natural cancer treatment." (Salvado, Chaitanya).

Methods

Extraction of the plant compounds: Three plant species belonging to three different families were selected for antibacterial properties. The plant parts were cleaned and weighed. Each one was extracted approximately 10g of each plant. The plants were extracted with 100ml of distilled water. The seeds were removed with a mortar and pestle by adding sterile distilled water. The extracts were added to a sterilized centrifuge tube with the plant name. After the liquid was removed as a residue, the supernatants were sterilized using a autoclave.

Bacterial strains: The rock agar plate was cleaned and sterilized with 70% isopropanol. These bacteria were isolated and placed a filter paper in each bottle with a small amount of the plant extracts. The bottom of the agar plate is labeled with name, date, and name of the bacteria.

The bacteria was spread with the glass spreader. The filter paper with the plant extract was placed over the bacteria. The filter paper was then sterilized with a sterilizer and placed over the plate and repeat the sterilizing process. Then, with sterilize the plant extract discs were applied on the rock plate. The filter paper on the agar plate labeled was the negative control and the penicilline disc in the middle of the plate was the positive control.

Results and Discussion

The basis of *Staphylococcus aureus* to their laboratory was tested for antibiotic susceptibility by the Kirby-Bauer disk diffusion method. A total of 9 plant extracts were tested for antibacterial activity that was illustrated in Figure 1. The results of the antibacterial activity of the results extracts of these plants (spinach, eggplant, and shiitake mushroom) were compared to the penicilline disc.

Figure 1 illustrates the antibacterial activity of *S. aureus* that produced a small (low activity), moderate (intermediate activity), and strong (high activity) inhibition zones. The strongest inhibition appeared to be from eggplant and the shiitake mushroom did not show any inhibition. The negative control and the penicilline disc did not show any inhibition. As shown in Figure 2, the negative control (penicilline disc) produced a large zone of inhibition. In contrast, the plants did not produce any zone of inhibition. The zone of inhibition was 24 mm. The inhibition was considered high activity. Thus, in this experiment, there was no evidence to prove that the plants have antibiotic activity of *S. aureus*. Sage, lime, and shiitake mushroom all showed the same level of inhibition against *S. aureus*.

Figure 1 shows an agar plate with four wells containing plant extracts. The zones of inhibition are visible around the wells. The wells are labeled with the plant names: Shiitake Mushroom, Eggplant, and Spinach, and a negative control.

Figure 2 shows an agar plate with a well containing a penicillin disc, showing a large zone of inhibition.

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Antibacterial Properties of Plants

Stephanie Batista • Clifford Jackson • Luciana Lavilaire • Miriam Munoz • Dr. Jonelle Ormida
Broward College Juvilene A. Semuels South Campus

INTRODUCTION

This study seeks to find natural alternatives in preventing and treating the evolutionary resistance of bacteria to prevent day antibiotic. Staphylococcus aureus is a gram positive bacterium which is a major cause of pathogenic infections. Penicillin, a first choice in medical treatment for bacterial infections, will be compared against the efficacy of the different plant extracts described below. Sage is rich in various phytochemicals which has been reported to have antibacterial properties for its antimicrobial properties.

Oregano possesses various antioxidants and polyphenols that have been recorded to inhibit S. aureus in in vitro studies. Eggplant contains various polyphenols which in ethanolic extract the plant have shown positive antibacterial inhibitions. These plants have been prized for their health benefits throughout the world for centuries. If other studies have shown positive antibacterial results for the plant extracts it is predicted the study will find positive inhibition on S. aureus.

METHODOLOGY

To extract the plants, 25g of the plant material was weighed out and cut into small pieces. The pieces were put into a mortar and grinded with a pestle and extracted with distilled water. It was then filtered through a cheesecloth into a 250ml beaker. The filtrate was then poured into a labeled 100ml centrifuge tube. Next, the centrifuge was turned on another tube containing an equal amount of water which was then centrifuged for 20 minutes at 1500 rpm. Lastly, the supernatant was collected into a clean labeled centrifuge tube.

After the work area was disinfected, filter discs and a small amount of plant extract were placed into labeled beakers with their plant name on it. An agar plate was then divided into four sectors using a permanent marker to label three sectors with the plant extract and the fourth with the negative control. The S. aureus cultures were then added to each sector of the culture using the swabbing technique. A nutrient agar plate was incubated by streaking the entire surface with a sterile swab that was dipped into the sterilized water. After leaving with a sterile swab that was dipped into the sterilized water, the plate was then rotated 90° of a turn once again where the swabbing process was done for a first time. After letting it sit for five minutes with sterile forceps the discs were removed into the filter tubes. The filter tube with the code for each plant or the control was placed where the negative control would be recorded. Once the plating steps were followed in order to plate the penicillin disc. Lastly, the plates were sealed with parafilm and were then put to incubate at 37 degrees Celsius for 24 hours.

Once the 24 hours were up the plates were retrieved and data was recorded. Zones of inhibition were measured in millimeters and measuring the distance from one edge to the other on the bottom of the petri dish. Once measurements were taken, photos of the plants were taken and the plates were then disposed of in the bio-hazard bag.

RESULTS

Depicted in Figure 1 shown below are the plates that were used with *Staphylococcus aureus* bacterium. In the first photo on the left, the bacterium coated plate was divided into four test areas that correlated with the plant extracts used for this experiment: 1) Sage 2) Oregano 3) Control 4) Penicillin. The second photo shows the negative control of one large test area for the test subject. Penicillin that served as our positive control. After measuring the zone of inhibition surrounding each disc, the zone of inhibition was measured in millimeters. Figure 2 shows the zone of inhibition for the plant extracts as well as the negative control. Plate 3 (right) is the test area for Penicillin.

DISCUSSION

OREGANO EXTRACT:

The findings are inconsistent with Maselli, Taddeo, & Pappalardo (2012), which state that S. aureus is resistant to some oregano plant extracts with the exception of Oregano water shielded by Gómez et al (2004). Oregano water shielded by Gómez et al (2004) showed Moderate activity this compound contained additional active ingredients via phytonics. Lastly, findings with Oregano water shielded by Gómez et al (2004) and Oregano tea made from Oregano had a disk diameter of 15 mm. The disk diameter of both the Control and Eggplant test compounds measured out to be 0 mm. Penicillin's disk diameter measured out to be 30 mm. The results are further amplified in the Data Table below.

EGGPLANT EXTRACT:

Finding are partially consistent with Maselli, Taddeo, & Pappalardo (2012) which used an eggplant extract as opposed to water distilled. Results are consistent with Generali et al (2012) where tested different phenophanes of the sage leaf extract measured moderate activity via phytonics. Lastly, findings with Eggplant water shielded by Gómez et al (2004) and Eggplant tea made from Eggplant extract which was partial in reducing bacterial growth.

PENICILLIN:

Other studies state antibiotic properties are found on the leaves and not on the fruit. Sitap et al (2015). Not much comprehensive available regarding distilled water extracts, mostly water extracts are used. Eggplant extract has not been used but have produced positive results. Sitap et al (2015). It should be considered that Balsamic vinegar is influenced by environmental factors, Van et al (2012). Further studies with variety of eggplant species and parts are needed under controlled growing conditions to rule out negative antibacterial inhibition.

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Figure 1 Antibacterial Disk showing four extracts and their zones of inhibition - area of inhibition measured in millimeters and compared to the plant extract as well as the Negative Control. Plate 3 right is the test area for Penicillin.

Figure 2 The Data Table gathered above depicts the listed extracts in this study and their corresponding measured zone diameter in millimeters. The subjects' Antibiotic Activity Level was determined as either having No activity (N, 0-mm), Little activity (L, 1-10mm), Moderate activity (M, 11-16mm), or High activity (H, >16mm).

BROWARD COLLEGE

Laura Indigo Antebiols, Dr. Edwardo Tadevoshe Zardon, Valentina Merys, Dr. Jonelle Ormida

ANTIBACTERIAL PROPERTIES OF MEDICINAL PLANTS AGAINST S. AUREUS

Department of Sciences, Broward College South Campus, 7280 Pines Blvd, Pembroke Pines, FL 33064

INTRODUCTION

Plants have been used for thousands of years due to their medicinal properties. Nature has been our long-time friend against disease and the unknown. The plants used in this study, tested for their antibacterial properties against *Staphylococcus aureus*, are: garlic (*Allium sativum*), which is known to control the growth of pathogenic strains, and for having a zone of inhibition of 16mm (Nair & Aspa, 2012); ginger (*Zingiber officinale*), known to relieve nausea and motion sickness; and eucalyptus (*Eucalyptus globulus*), used to treat respiratory congestions such as bronchitis and asthma. *E. globulus* leaves might be especially as a natural antibiotic for the treatment of several infectious diseases (Sachin & Benali, 2012).

Unfortunately, the misuse of antibiotics has led to drug-resistant mutants where the bacteria adapt or resist to the antibiotic and is still able to replicate or even cause more harm to the human body. The purpose of this study is to know whether the plants are able to kill or stop the S. aureus from growing. Based on its antibacterial property, out of the three plants used, garlic will have the greatest zone of inhibition.

HISTORY OF S. AUREUS AND PENICILLIN

In the 1880's, S. aureus was discovered by a surgeon from Scotland named Sir Alexander Ogston. This bacterium is well known for causing skin and soft tissue infections. Although S. aureus is not known for being a deadly bacteria, it can cause serious infections throughout the bloodstream, and lungs (Mandal, A., 2012). It wasn't until the 1880's, that Alexander Fleming discovered an antibiotic to fight this bacteria. Penicillin is a well-known antibiotic that is used to treat most bacteria. As time passed, scientists began to realize that S. aureus was becoming resistant to penicillin. Thus pushed them to create a new drug to counteract the resistance, which then became a new form of penicillin. After creating a new antibiotic (Merchitskin), S. aureus became more resistant to it. Because of the stronger resistance, MRSA (Merchitskin-resistant S. aureus) came into existence. MRSA is resistant to numerous beta-lactam antibiotics such as penicillin, amoxicillin, cefaclor, and methicillin.

METHODOLOGY

(1)Plant Extraction (Bacteriostatic performed this step)

20g of the plant material was weighed out. The plant material was cut into small pieces. It was then transferred to a mortar and ground down with the pestle, and 5ml of sterilized distilled water was added. After the 20g of plant material was fully ground out, it was filtered through a noted cheesecloth. The filtrate was then placed into a labeled 100ml centrifuge tube. Next, the filtrate inside the tube was carefully labeled with the plant name. The tube was put into the centrifuge for 20 minutes at 1500 rpm. The supernatant was collected and put in new, cleaned, labeled, 10ml centrifuge tube.

(2)Plates

The work area was disinfected. Three small beakers were labeled: methicillin, garlic, and ginger. A small amount of water was added to the plant extracts. Three sectors were labeled with the plant names, as well as the control. The L-shaped glass rod was dipped in ethanol with the plant extract and the control. The glass rod was then placed in the center of the plate for sterility before withdrawing a small amount. The glass rod was passed through the flame to be sterilized, then it was cooled. The contents of S. aureus were swirled gently until spread evenly throughout the plate.

(3)Petri dish

The work area was disinfected. Three small beakers were labeled: methicillin, garlic, and ginger. A small amount of water was added to the plant extracts. Three sectors were labeled with the plant names, as well as the control. The L-shaped glass rod was dipped in ethanol with the plant extract and the control. The glass rod was then placed in the center of the plate for sterility before withdrawing a small amount. The glass rod was passed through the flame to be sterilized, then it was cooled. The contents of S. aureus were swirled gently until spread evenly throughout the plate.

RESEARCH APPLICATIONS

Figure 1 Penicillin Agar Plate depicting its zone of inhibition

Figure 2 Agar plate of the zones of inhibition of control, garlic, eucalyptus, and ginger.

Table 1 shows the four test compounds used, and map of the zones of inhibition along with the antibiotic activity level of each. Activity level was determined as follows: No activity (N, 0-1mm); Little activity (L, 1-10mm); Moderate activity (M, 11-16mm); High activity (H, >16mm).

CONCLUSIONS / RESULTS

In this study, extracts from plants have been tested for antibacterial activity against S. aureus. The diameter of the zone of inhibition from each plant has been measured to determine which plant has the greatest zone of inhibition. Ginger demonstrated a zone of inhibition of 16mm, garlic activity 7-12mm, eucalyptus demonstrated a zone of inhibition of 11mm, and methicillin demonstrated a zone of inhibition of 15mm (high activity). As predicted, garlic obtained the greatest zone of inhibition. The diameter of penicillin was of 33mm and the control was of 0mm. For future experiments, we will use a higher concentration of the plant extracts. The control did not show any success. As can be seen in picture 1, the control can't be measured. While placing the control disc, it got smeared which is also why we had to change the place of where it was placed.

Table 1 shows the plants used, the diameter of the zones of inhibition, and the activity level of each. Penicillin and the control have been included in the table. Figure 1 shows the control of inhibition of penicillin. As seen in Figure 2, there was a wavy shape for the zone of inhibition of the control. Garlic had a much-deeper zone of inhibition. Eucalyptus and ginger had a much smaller zone of inhibition.

DISCUSSION

The zone of inhibition is the clear area around each disc, which shows whether the bacteria is reproducing or not—the greater the zone of inhibition, the better the antibiotic. Research has shown that the size of the zone of inhibition may not be the best measure of the results obtained. Since it's already known that penicillin is an effective antibiotic it provides an insight on whether the procedures were done successfully or not, and on reliability of the results. In this experiment, the control was not successful because the bacteria was allowed to grow. Instead of looking at area of inhibition, the bacteria was allowed to grow. In the experiment, the plants were compared to penicillin. Penicillin had a much greater area of inhibition than the plants. The control had no inhibition, which means the bacteria was able to grow. The difference in the size of the zone of inhibition between penicillin and garlic, eucalyptus, and ginger is significant. Penicillin had a zone of inhibition of 33mm, while the plants had a zone of inhibition of 15mm (high activity). As the antibiotic activity rises, ginger had a zone of inhibition of 16mm in this experiment; however, the diameter obtained for ginger in this experiment was 5mm.

The bacterium can genetically evolve to become resistant, allowing for bacteria to keep growing despite the use of antibiotics. The outer membrane of the bacterium was destroyed by penicillin, which is why it is effective. According to the American Journal of Medical Microbiology, penicillin had a zone of inhibition of 23mm—same diameter as the garlic extract in this experiment. Based on the British Microbiology Research Journal's study, garlic had a zone of inhibition of 16mm, while the plants had a zone of inhibition of 15mm (high activity). As the antibiotic activity rises, ginger had a zone of inhibition of 16mm in this experiment; however, the diameter obtained for ginger in this experiment was 5mm.

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