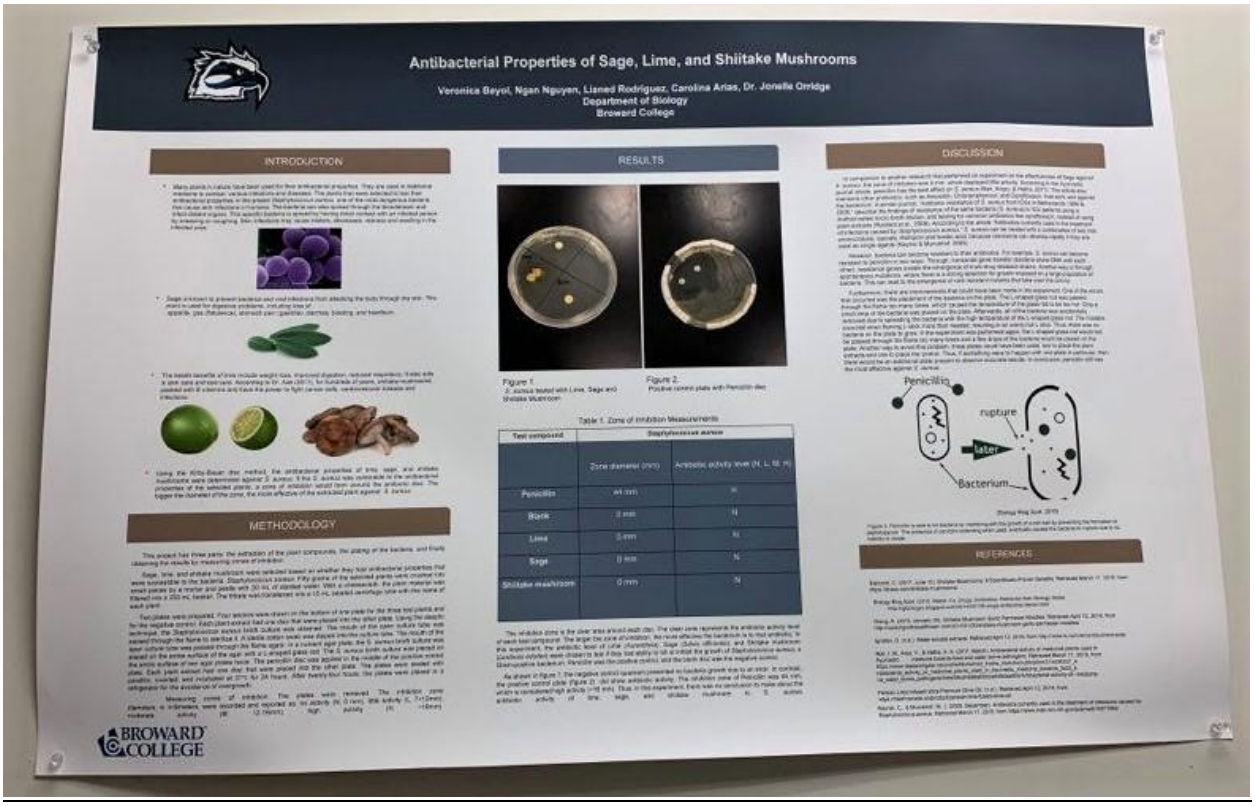
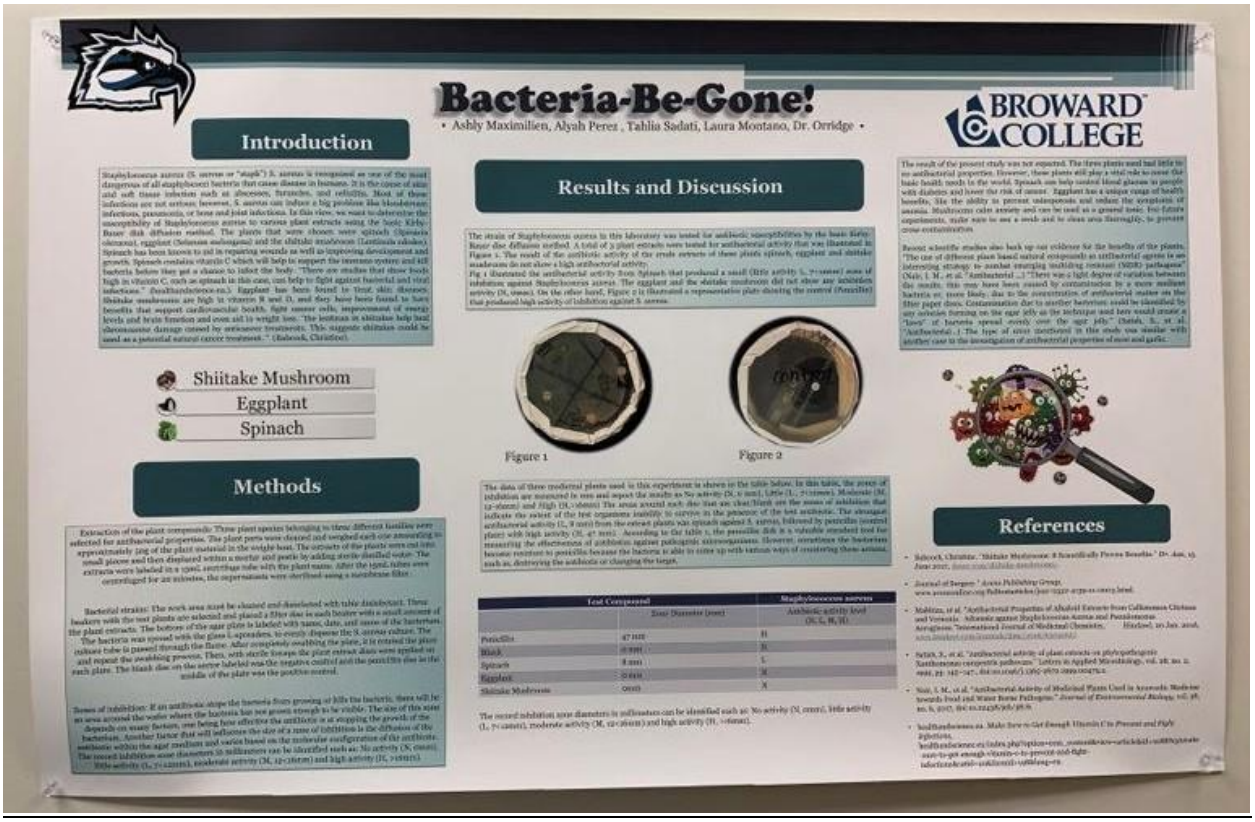
BSC2011L Plant Project Poster Examples:





Antibacterial Properties of Plants

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INTRODUCTION

This study seeks to find natural alternatives in preventing and treating the evolutionary resistance of bacterium to present day antibiotics. *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:

1. Sage is rich in various phytochemical components which has been extensively studied for its antimicrobial properties.
2. Oregano possesses various antioxidants and polyphenols that have been recorded to inhibit *S. aureus* in scientific trials.
3. Eggplant possesses a compound Solanesol in which ethanolic extracts of the plant have shown positive antibacterial inhibition.

These plants have been prized for their health benefits through out 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 plant, 25g of the plant material was weighed out and cut into small pieces. The pieces were then put into a mortar and grinded with 5ml of autoclaved distilled water. It was then filtered through a cheesecloth into a 250ml beaker. The filtrate was then poured into a labeled 15ml centrifuge tube. Next, the centrifuge was balanced with 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 in order to label three sections with the plants names and the fourth with the negative control. The *S. aureus* contents were then spread throughout the culture using the aseptic technique. A nutrient agar plate was inoculated by streaking the entire surface of the agar with a sterile swab that was dipped into the sterile culture tube, leaving no un-swabbed areas. Once it was swabbed, the plate was rotated 1/3 of a turn where the swabbing process was repeated. It was then rotated 1/3 of a turn once again where the swabbing process was done for a final time. After letting it sit for five minutes, using sterile forceps the discs were placed into the different sectors of the plate with the code facing up and a blank one was placed where the negative control would be recorded. Then the same plating steps were followed in order to plate the penicillin disc. Lastly, the plates were sealed with paraffim and were then put to incubate at 37 degrees Celsius for 24 hours.

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

RESULTS

Depicted in Figure 1 shown below are the plates that were swabbed with *Staphylococcus aureus* bacterium. In the first plate 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) Eggplant (clockwise direction). The second plate consisted of one large test area for the test subject, Penicillin that served as our positive control. After measuring the zone of inhibitions surrounding each test subject, we found that Sage had a disk diameter of 8 mm. 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 50 mm. The results are further simplified in the Data Table below.

Figure 1 Antimicrobial disks displaying test subjects and their zones of inhibition – area of no growth. Plate 1 (left) is divided into four sections and separated by the plant extract tested as well as the Negative Control. Plate 2 (right) is the test area for Penicillin.

DATA TABLE

Test Compound	Staphylococcus Aureus	
	Zone Diameter (mm)	Antibiotic Activity Level (N, L, M, H)
Penicillin	50	H
Sage	18	M
Oregano	5	L
Control	0	N
Eggplant	0	N

Figure 2 The Data Table pictured above depicts the tested subjects in this study and their corresponding measured zone diameters. Based on the zone diameter, the subject's Antibiotic Activity Level was classified as either having: No activity (N, 0 mm), Little activity (L, 7-12mm), Moderate activity (M, 13-16 mm), or High activity (H, >16mm).

DISCUSSION

OREGANO EXTRACT

The findings are inconsistent with Dahiya & Purkayastha (2012), which state that *S. aureus* is resistant to water distilled plant extracts of oregano. Although, Oregano water studied by Gulmez et al (2006) showed Moderate activity this compound contained additional active ingredients viz. thymus. Lastly, findings were somewhat consistent with Gulmez et al (2006) whom also tested Oregano tea made from pure plant extract which was partial in reducing bacterial growth.

SAGE EXTRACT:

Findings are partially consistent with Moulefo, Yahyaoui, & Douifi (2012) whom used an ethanolic extract as opposed to water distilled. Results are consistent with Genaidie et al (2012) which tested different phenophases of the sage leaves and measured quantification of individual phenols soluble in H2O. The overall results were positive for antimicrobial activity. It must be considered there are seasonal variations in phenolic compounds that influence the antibacterial activity of Sage.

EGGPLANT EXTRACT:

Other studies state antibacterial properties are found on the leaves and not on the fruit, Sitap et al (2015). Not much comparison available regarding distilled water extracts most studies are based on Methanolic and Ethanolic extracts that have produced positive results. Sitap et al (2015), it should be considered that Solanesol availability is influenced by environmental factors, Yan et al (2015). Further studies with variety of eggplant species and parts are needed under controlled growing conditions to rule out negative antibacterial inhibition.

PENICILLIN

The findings are consistent with current medical uses. Although, this is sometimes not the best choice due to development of resistance. Resistance is developed by bacterium via recombination or mutation.

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ANTIBACTERIAL PROPERTIES OF MEDICINAL PLANTS AGAINST S. AUREUS

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1

INTRODUCTION

Plants have been used for thousands of years due to their medicinal properties. Nature has been our longtime 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 28mm (Nair & Anju, 2017); 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 exploited as a natural antibiotic for the treatment of several infectious diseases (Bachir & Benali,2012).

Unfortunately, the misuse of antibiotics has led to drug resistant mutants where the bacteria adapts or resists 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 selected 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.

2

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 1940'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. This pushed them to create a new drug to counteract this resistance, which then became a new form of penicillin. After creating a new antibiotic (Methicillin), *S. aureus* became more resistant to it. Because of the stronger resistance, MRSA (Methicillin-resistant *S. aureus*) came into existence. MRSA is resistant to numerous beta-lactam antibiotics such as penicillin, amoxicillin, oxacillin, and methicillin.

3

METHODOLOGY

(1)Plant Extraction (Rotary clave performed this step)

25g of the plant material was weighed out. The plant material was cut into small pieces. It was then transferred to a mortar and grinded down with the pestle, and 5ml of autoclaved distilled water was added. After the 25g of plant material was fully grounded up, it was filtered through a folded cheesecloth. Then, it was filtered into a 250ml beaker. The 15ml centrifuged tube with 15ml of the filtrate inside the tube was correctly labeled with the plant name. The tubes were put into the centrifuge for 20 minutes at 1500 rpm. The supernatant was collected and put in new, cleaned, labeled, 15ml centrifuge tube.

(2) Plating

The work area was disinfected. Three small beakers were labeled: eucalyptus, 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 entire short end fully submerged. The culture tube containing the bacterial broth was flamed 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.

4

METHODOLOGY

The mouth of the culture was passed through the flame. The pipette was used to withdraw a small amount of the broth from the culture tube. The mouth was passed through the flame again and cap was replaced. The lid of the agar plate was partially removed, and with the pipette, two drops of the broth culture was added to the surface of the agar. The agar plate was placed on the turn table. The lid was partially removed and the bacteria was spread on the entire surface of the agar with the sterilized glass rod. The rod was moved back and forth to make sure the bacteria was spread to the entire plate, then it was returned to the beaker with ethanol. Allowed the surface area to dry out for about 5 minutes. The sterile forceps were used to place the discs, with the coded side faced upward, on the agar plate in their separate sectors. Each disc was gently pressed with a sterile forceps so that full contact was permitted with the agar surface. The steps were repeated for the other plant extracts, with the forceps being sterilized in between uses. Parafilm was used to seal the plates. The plates were incubated at 37 C for 24 hours.

(3) Measuring Zones of Inhibition

The plates were retrieved and examined for the presence of antibiotic activity. With a metric ruler placed at the bottom of the petri dish, the zones of inhibitions were measured. The diameter of the zone of inhibition was recorded in millimeters. Pictures were taken of all of the plates. The plates were disposed in a biohazard bag.

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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.

Test compound	Staphylococcus aureus	
	Zone diameter (mm)	Antibiotic activity level (N, L, M, H)
Penicillin	32 mm	H
Blank	0 mm	N
Garlic	28 mm	H
Ginger	8 mm	L
Eucalyptus	7 mm	L

Table 1 shows the test compounds used, and their zone of inhibition along with the antibiotic activity level of each. Activity level: No activity (N, 0 mm); Little activity (L, 7-12 mm); Moderate activity (M, 12-16mm); High activity (H, >16 mm).

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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 had been measured to determine which plant has the greatest zone of inhibition. Ginger demonstrated a zone of inhibition of 8mm (little activity, 7 < 12mm), eucalyptus demonstrated a diameter of 7mm (little activity, 7 < 12mm), and garlic showed a diameter of 28mm (high activity, >16mm). As predicted, garlic obtained the greatest zone of inhibition. The diameter of penicillin was of 50mm and the control was of 0mm. For future experiments, there are things that could be done differently in order to assure complete 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 initially located.

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 weird shape for the zone of inhibition of the control. Garlic had a well-depicted zone of inhibition. Eucalyptus and ginger had a much smaller zone of inhibition.

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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 antibacterial property of the plant or antibiotic. The penicillin disc was used to test the efficiency 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 results. Like penicillin, the blank disc was used to test the efficiency of the results, but, instead of looking at area of inhibition, the bacteria was allowed to grow. In the experiment, the plant extracts were compared to penicillin. Penicillin had a much greater area of inhibition with a diameter of 50mm. Of the three plants, garlic had the greatest zone of inhibition with a diameter of 28mm (ginger had a diameter of 8mm and eucalyptus of 7mm). There was a 20mm difference in the zone of inhibition between penicillin and garlic. Garlic has been used to help with hypo- or hypertension, cholesterol, cardiovascular diseases, leveling blood sugar, and for its antibacterial properties (Ayaz, E., & Alpsoy, 2016). Eucalyptus is known for treating respiratory problems, such as asthma, pneumonia, and bronchitis (Aas, J., 2017). Ginger has been used to help with nausea, pain, loss of appetite, colds, and digestion (Eid, N., 2017). The antibiotic properties of eucalyptus and ginger are being further studied. There are numerous ways a bacterium may become resistant to penicillin.

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 can also prevent the antibiotic from reaching inside the bacterium. According to the Ayurvedic journal article, garlic had a zone of inhibition of 28mm– same diameter as the garlic extract in this experiment. Based on the British Microbiology Research Journal's study, garlic showed a zone of inhibition of 25mm. As the Ayurvedic article states, ginger had a zone of inhibition on 8mm in the experiment; however, the diameter obtained for ginger in this experiment was 8mm.

8

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