A Natural Alternative: The Antibacterial Effects of Grapefruit Seed Extract and Grape Seed Extract on *Staphylococcus aureus*

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Abstract

This research paper describes a comparison of the antibacterial effectiveness of four antibacterial agents. Two are manufactured topical antibiotics, Neosporin® and neomycin, and two are herbal alternatives, grapefruit seed extract (GFS) and grape seed extract (GSE). The comparison involves observation of the effects on varying concentrations of the antibacterial agents to gram-positive bacterium, *Staphylococcus aureus*. The sensitivity and resistance of the bacteria to the bactericidal agents is measured by the zones of inhibition and analyzed to compare the effects of the two herbal versus the two chemical antibacterial agents. The Kirby-Bauer disk diffusion method and agar well diffusion test are used to determine the susceptibility of the *S. aureus* and the minimum inhibitory concentration (MIC) of the agents. The hypothesis predicts that the GFS and GSE will show similar antibacterial effects at similar concentrations to the Neosporin® and neomycin. The results obtained indicate that the sensitivity of *S. aureus* to the chemical agents is greater than the naturally extracted GFS and GSE. When commercially available extracts of GFS and GSE were tested, the results paralleled the effects of the chemical agents.

Introduction

Antibiotic-resistant bacteria, causally referred to as “superbugs,” are becoming more numerous and virulent primarily due to over usage of antibiotics. The prevalence and associated risks of antibiotic resistant bacteria have become an alarming medical risk. With multi-drug resistant infections on the rise, the development of new antibiotics is struggling to keep pace. For example *Staphylococcus aureus* is almost always resistant to benzyl penicillin, methicillin, and most over the counter drugs, such as Neosporin® and Polysporin® (Goodman, 2011, p.1). The resistant strain of *Staphylococcus aureus* known as Methicillin-resistant *Staphylococcus aureus* (MRSA) is contracted like any other contagious bacteria however the difference is that the inflections are difficult to cure and have resulted in deaths. The community-acquired MRSA has

become increasingly common but the severe morbidity and mortality attributable to MRSA lies in the infected hospitalized patients (Huang 176). Recently a deadly strain of MRSA has become resistant to the latest and standard last-line antibiotic, vancomycin (Thati, 2011, p.1). With the discovery of antibacterial properties in many natural herbal products, scientists have suggested this possibly sound alternative to the standard antibiotic.

Natural products have been known to have antibacterial effects against a variety of bacteria. A study in 2012 was conducted to show the antibacterial effects of plant-derived extracts on MRSA. The study showed that grape seed extract, one of the many agents tested, is rich in potent antioxidant polyphenolics, which have displayed antibacterial activity (Su 2012). Another natural herbal product known for containing the same active ingredient, polyphenols, is grapefruit seed. These two agents, grape seed and grapefruit seed, were tested on *Staphylococcus aureus* to analyze their antibacterial effects against the bacteria and lay a foundation for future studies that would test its antibiotic-resistant counterpart, MRSA.

Materials and Method

Experiment #1: Testing agents

Naturally extracted grapefruit seed, naturally extracted grape seed, Neosporin®, and neomycin

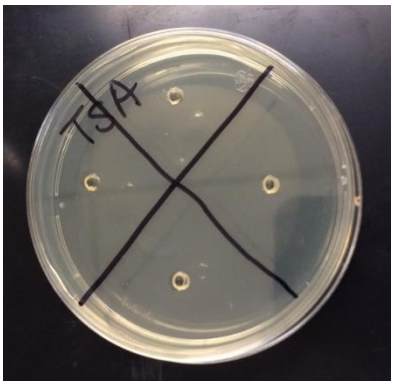
Step 1: Preparation of the agar plates and seed extracts

Six large and nine small petri dishes were poured with Mueller Hinton and Trypticase soy agar and allowed to set. Extraction of the grapefruit seed and the grape seed was accomplished using a mortar and pestle. The seeds and juices of the two fruits were grounded to a liquid substance to liberate the active antibacterial ingredient from each fruit, located inside the seed.

Step 2: Serial Dilution

One milliliter of each seed extract liquid is pipetted into separate test tubes; this is the full strength concentration. The 1:2 concentration contains 0.5 mL of each full strength extract and 0.5 mL of sterile deionized water. The 1:4 concentration contains 0.25 mL of each full strength extract and 0.75 mL of sterile deionized water. Each concentration is tested in three trials to ensure accuracy and precision of the results. The Neosporin® came in the form of a cream ointment, so the full strength concentration will be the substance straight from the container. The process was repeated to obtain the 1:2 and 1:4 concentrations. The neomycin agent was in an antibacterial disk with a set concentration embedded in the disk.

Step 3: Culture the bacteria



With a cotton swap applicator, one day old prepared cultures were spread on the six large and five small agar plates used for disk diffusion. Before growing the lawn on the five plates for well diffusion, each agar plate was divided into four equal quadrants with a circular well punched in each center (Image 1).

Figure 1: Small agar plate for well diffusion

Step 4: Plate Organization

With the labeling system illustrated in Image 2 for disk diffusion and Image 3 for well diffusion, each concentration was tested in three trials to allow 36 spots for both tests. Each section of the plate was labeled by concentration, extract, and trial number.

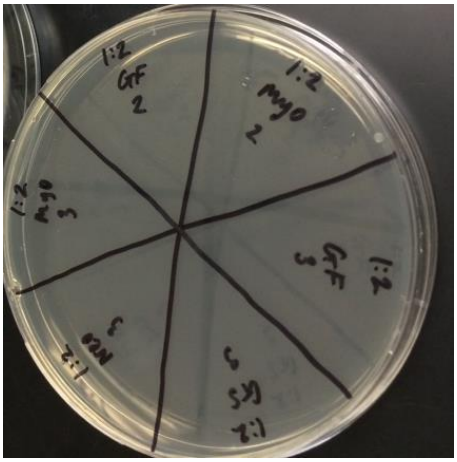


Figure 2: Large agar plate with labeling used for disk diffusion

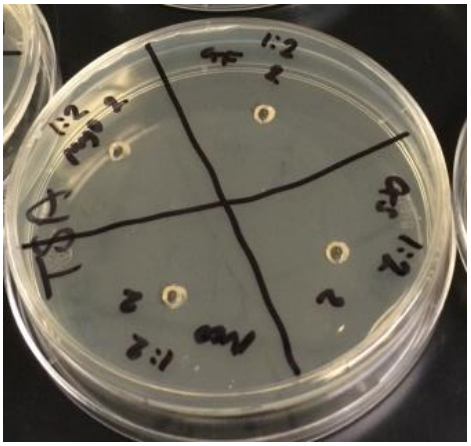


Figure 3: Additional large agar plate with labeling used for disk diffusion

Step 5: Disk Diffusion

Alcohol-flamed tweezers were used to place an empty, clean antibacterial disk into a spot plate. One drop of each concentration of each extract was pipetted onto a disk. Each disk was placed on the corresponding labeled agar spot (Image 2). Repeat this twice more for a total of three trials.

Step 6: Well diffusion

The different concentrations of the different agents were pipetted into the corresponding labeled wells (Image 3). The agent was pipetted to fill the well without overflow.

Step 7: Incubation

All petri plates were secured with masking tape and placed in an incubator for 48 hours to allow the bacteria to grow.

Step 8: Measuring the zones of inhibition

The antibacterial effect of the agents were visible as zones of inhibition, a clear circular shape around the disk to show the bacteria's sensitivity to the agents. After the 48 hours have passed for each experiment, each zone of inhibition (ZI) was measured in millimeters using a ruler.

Experiment #2: Testing agents

Commercially available grapefruit seed extract, commercially available grape seed extract, and neomycin

Step 1: Preparation of the agar plates and seed extracts

The procedure from Experiment #1 was repeated for preparing five small agar plates. Four grapefruit seed extract tablets were grounded into a fine powder using mortar and pestle. Six mL of sterile deionized water was added to the powder to turn the fine solid into a well infused liquid. The shell capsules of two grape seed extract tablet were removed to pour the powder inside the mortar. Three mL of sterile deionized water were added to liquefy the powder.

Step 2: Serial Dilution

The procedure from Experiment 1 was repeated.

Step 3: Culture the bacteria

The procedure to seed the lawn for disk diffusion from Experiment 1 was repeated. The well diffusion method was omitted.

Step 4: Plate Organization, Step 5: Disk Diffusion, Step 7: Incubation, and Step 8: Measuring the zones of inhibition

The procedure from Experiment 1 was repeated.

Results

Raw data tables are in Appendix A.

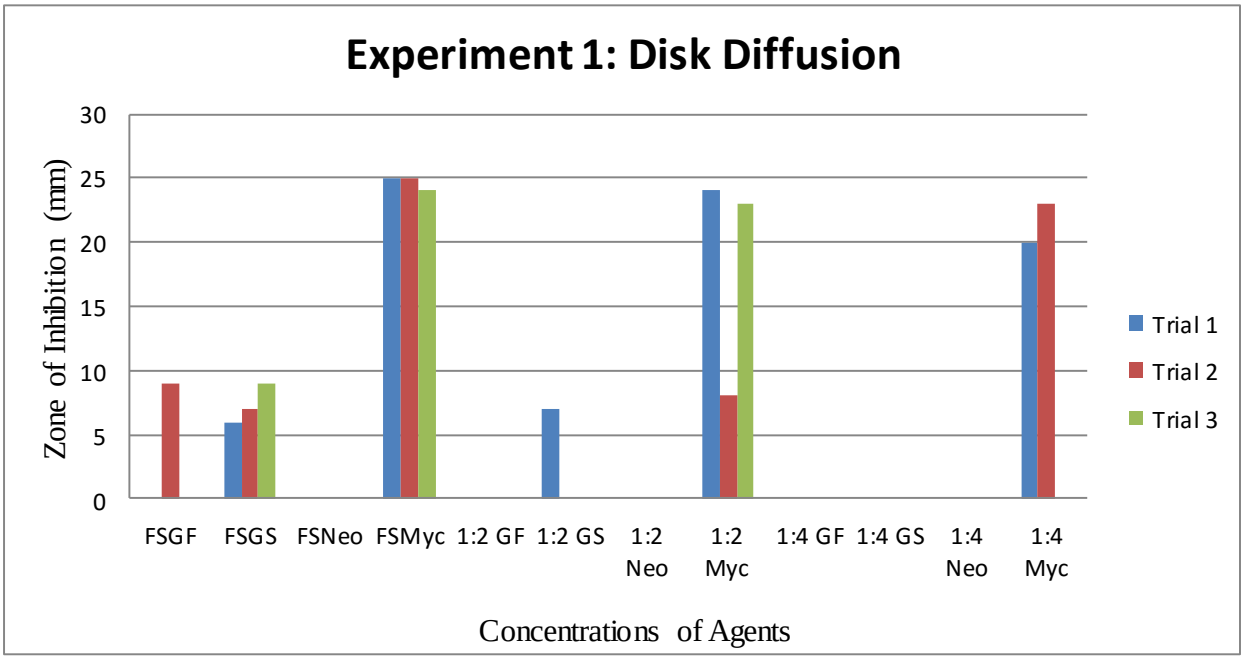


Table 1: Antibacterial results of the tested variables of Experiment 1: Disk Diffusion

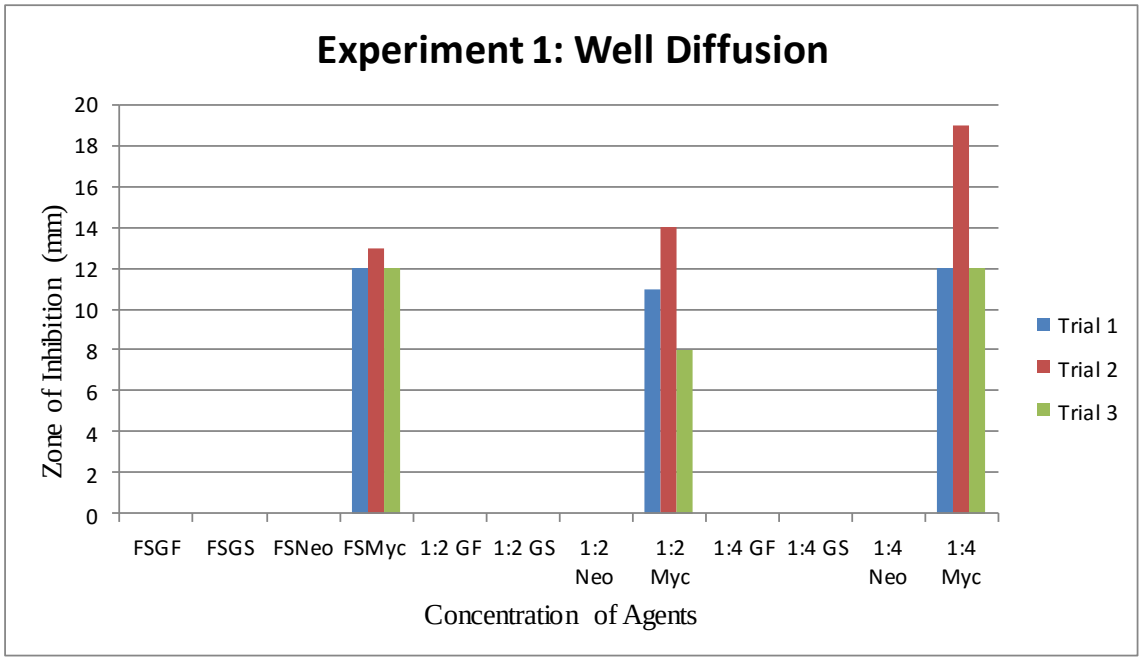


Table 2: Antibacterial results of the tested variables of Experiment 1: Well Diffusion

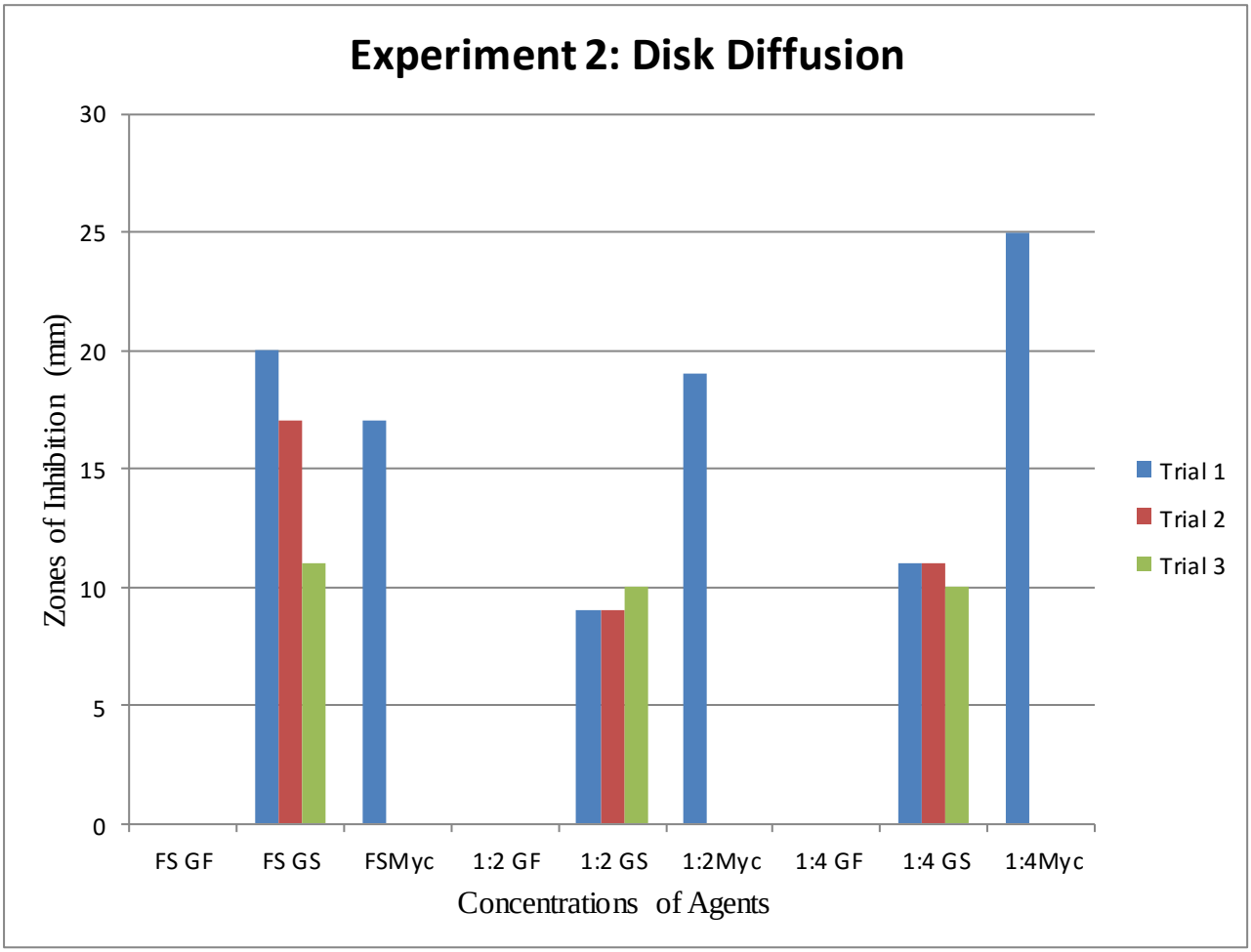


Table 3: Antibacterial results of the tested variables of Experiment 2: Disk Diffusion

Discussion of Results and Experiment

From the results shown above, on average the bacteria showed more sensitivity to the commercially available agents in Experiment #2. This is consistent since the commercially available extracts had a known composition of antioxidant while the naturally extracted grapefruit seed extract (GFS) and grape seed extract (GSE) qualitatively yielded a lower amount of the active ingredient. The commercially available grapefruit seed extract, from NutriBiotic®, contains 125 mg of the GFS in one tablet which has 95% of the active ingredient from the seed and pulp and 5% of the binder, filler, and lubricant. The commercially available grape seed extract, from the brand Nature's Way®, contains 100 mg of the GSE with 95% of the active ingredient and 5% of cellulose, gelatin, and magnesium stearate. The manual extraction process was limited due to being the qualitative nature of the extraction and the concentration of the active ingredients were variable since the amount of polyphenols couldn't be measured in the agents with the available equipment. A quantitative measure of the active ingredients in Experiment #1 using an organic extraction process would allow better controls.

The GSE performed better than the GFS in the disk diffusion of Experiment #1 and Experiment #2, obtaining results that were 50% or higher than GFS. On average, the full strength concentration showed the highest values of inhibition. In addition, the disk diffusion method was more effective than the well diffusion because the zones of inhibitions were larger in all trials with that test. The test allowed the agents to seep through the surface of the agar where most of the bacteria grew. Clearer, more consistent results were obtained using the disk diffusion method, so Experiment #2 used only this method.

In the first experiment the Neosporin® proved to be ineffective, showing little to no zones of inhibition. In two cases (Figure 1 in Appendix A) it appeared that the bacteria grew around the antibacterial disk and in the area of inhibition, showing signs of its resistance against the lowest concentration of the agent. The low dosage of the antibacterial agent supports the claim that the bacterium is resisting the chemical antibacterial agent. This occurrence is not seen in any of the natural agents (Figure 2 and 3 in Appendix A).

The performance of Neosporin® was affected by application in its creamy state which could result in limited diffusion. One gram of Neosporin® contained 400 units of Bacitracin, 5,000 units of Polymyxin B, 3.5 mg of neomycin, along with multiple inactive ingredients. The small percentage of the active ingredients and the additional inactive additives could have hindered the antibacterial effects of the antioxidants.

Although in Experiment #2 GSE showed comparatively high results, the values were not consistent in each trial. Neomycin proved to be the most reliable and effective antibacterial agent since it had the largest zones of inhibition. However, it could only be measured for one concentration because of limited resources. The neomycin is a control for the Neosporin® since it is an active ingredient of the ointment.

Improvements that would minimize errors and maximize results include: better methods of extraction for a more potent full strength concentration, selecting a liquid or aqueous form of Neosporin® or similar over-the-counter drug, and testing extracts with the only ingredient being the active antioxidant.

Conclusion of Research

An aim of the experiment was to determine the minimum inhibitory concentration (MIC) of each agent, excluding the neomycin. The MIC refers to the lowest concentration of an antimicrobial that will inhibit the visible growth of a bacterium. The concentrations for the naturally extracted grapefruit seed extract, commercially available grapefruit seed extract, naturally extracted grape

seed extract, and the Neosporin® showed zero to very small zones of inhibition for all three concentrations under the conditions used, so the MIC is inconclusive.

In the case of the commercially available grape seed extract, the concentrations yielded scattered results, ranging from 10-20 mm per zone of inhibition (Graph 3). There was no gradual increase of zone size to show which concentrations inhibited the most or least. Therefore, more trials and concentrations of the commercially available grape seed extract would be necessary to determine the MIC and test the level of susceptibility of the bacteria.

There is a possibility of considering commercially available grape seed extract as an alternative to treating *Staphylococcus aureus* related infections, such as community acquired MRSA. The experiment results (Graph 3) show the similarity of bacteria inhibition between commercially available GSE and Neomycin. A study in 2010, which shows the bactericidal effect of grape seed extract on methicillin-resistant Staphylococcus aureus (MRSA), found grape skin and seed extracts to completely inhibit 43 strains of MRSA at a concentration of 3 mg/mL (Al-Habib). The study, in addition to the experiment results, indicates that the considerable antibacterial activity of commonly available grape seed extract could signify a major advancement in the treatment of MRSA diseases.

Studies have provided evidence showing bacteria with the ability to build resistance against antibiotics. Although the science of medicine is rapidly developing, the concern of non-treatable antibiotic resistant bacteria is present. Natural antibacterial products could become a plausible alternative in medicine provided that they obtain the same results as chemical antibiotics and show no signs of resistance. Therefore further studies are necessary to verify that a natural alternative is effective enough to replace known medicinal and chemical treatments, such as antibiotics.

Past research suggests that increased use of commercially available GSE on community acquired MRSA could decrease the growing prevalence of antibiotic resistant bacteria. Whether this conclusion can be extended to all cases of MRSA, is unlikely since the deadlier MRSA strains aren’t preventable by medication let alone a natural treatment. Nevertheless, it would be beneficial to look into more natural forms of treatment as an alternative medication provided these natural agents obtain the antibacterial results with no signs of developing antibiotic resistance. In this study, all of the concentrations of commercially available GSE were inhibitory to *Staphylococcus aureus* and in past studies GSE is shown to inhibit MRSA. This research suggests the agent’s potential as a natural alternative treatment and pushes for more studies to conclude that GSE is a successful alternative to neomycin.

Literature Cited

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Appendix A

Experiment #1 Results Data Table for Disk Diffusion

Figure 1

Disk Diffusion	Trial 1: ZI (mm)	Trial 2: ZI (mm)	Trial 3: ZI (mm)
FS GF	0	9	0
FS GS	6	7	9
FS Neo	0	0	0
Mycin	25	25	24
1:2 GF	0	0	0
1:2 GS	7	0	0
1:2 Neo	0	0	0
Mycin	24	8	23
1:4 GF	0	0	0
1:4 GS	0	0	0
1:4 Neo	0 *R	0 *R	0
Mycin	20	23	----

*R- bacteria showed resistance to agent

Experiment #1 Results Data Table for Well Diffusion

Figure 4

Well Diffusion	Trial 1: ZI (mm)	Trial 2: ZI (mm)	Trial 3: ZI (mm)
FS GF	0	0	0
FS GS	0	0	0
FS Neo	0	0	0
Mycin	12	13	12
1:2 GF	0	0	0
1:2 GS	0	0	0
1:2 Neo	0	0	0
Mycin	11	14	8
1:4 GF	0	0	0
1:4 GS	0	0	0
1:4 Neo	0	0	0
Mycin	12	19	12

Experiment #2 Results Data Table for Disk Diffusion

Figure 3

Disk Diffusion	Trial 1: ZI (mm)	Trial 2: ZI (mm)	Trial 3: ZI (mm)
FS GF	0	0	0
FS GS	20	17	11
Mycin	17	----	----
1:2 GF	0	0	0
1:2 GS	9	9	10
Mycin	19	----	----
1:4 GF	0	0	0
1:4 GS	11	11	10
Mycin	25	----	----