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Evaluation of Essential Oils as an Alternative to Conventional Antibiotics

Megan Leinenbach

The purpose of this study was to determine if synergistic interactions between essential oils can increase their antibacterial efficacy by testing essential oils. The following combinations were assessed for effective inhibition of E.coli and S. aureus growth: tea tree oil as the control solution, tea tree/oregano solution, tea tree/thyme solution, and tea tree/wintergreen solution. A Kirby-Bauer disc diffusion was employed to assess antibiotic efficacy. When comparing the different combinations to the control solution, the results of this study signify the presence of synergistic interactions between select essential oils; the combinations that were proven to increase antibacterial efficacy when compared to the control were the tea tree/oregano and tea/tree thyme solution. The combination with the greatest antibacterial efficacy for inhibiting E. coli and S. aureus growth was tea tree and oregano oil, which had an average inhibition percentage of 59.53% (E. coli) and 55.91% (S. aureus). This result was found to be statistically significant ($p < 0.05$) using an ANOVA single factor test.

Keywords: Essential oils, natural medicine, bacterial growth, synergism

Introduction

An increasing number of antibiotic resistant strains of bacteria are posing a health risk to Americans. Since the introduction of penicillin in 1940, antibiotics have been the dominant method used to treat infections; however, antibiotic resistance has been increasing among bacteria populations due to selective pressures (Ott & Morris, 2008). In fact, antibiotic resistance has been detected in bacteria for all of the more than 100 antibiotics in use as of 2008 (Ott & Morris, 2008). The Center for Disease Control (2017) details the impact of antibiotic resistance by stating that at least 2 million people in the United States are infected with antibiotic resistant bacteria each year, and 23,000 people die each year as a direct result of these infections. With antibiotic resistance being the cause of such a large number of deaths, it is vital that the public is aware of this biological phenomenon and that this be addressed to meet the needs of patients with antibiotic-treatable illnesses.

While multiple bacteria have developed a resistance to antibiotics, this study will focus exclusively on *Escherichia coli* and *Staphylococcus aureus*. *E. coli* is a gram-negative bacterium[1] that is a common inhabitant of the intestinal tract of warm-blooded animals; there are four strains of *E. coli* that can cause diarrheal illness and disease (Jenson, 2003). Further demonstrating this assertion, Christina Gorman (2011) of University of California: Santa Barbara writes that disease-causing strains of *E. coli* are resistant to fourteen different antibiotics and are consistently the subject of research evaluating alternative treatment options. Roa et al. (2015) classify *Staphylococcus aureus* as a gram-positive[2] bacterium that can be found on the skin and

1 Gram-negative bacteria have cell walls composed of a thin layer of peptidoglycan, which may affect the way essential oils enter through the cell wall and membrane of the bacteria.

in the nares of humans. Additionally, *Staphylococcus aureus* has become an acute threat to infection control with the increasing prevalence of methicillin-resistant *S. aureus* (MRSA) and its ability to resist multiple drugs (Rao et al., 2015). Although the work of the aforementioned authors focused on two different bacteria, their findings collectively demonstrate the consequences of antibiotic resistance and its implications regarding public health.

Due to the prevalence of antibiotic resistance, various research studies have been conducted to gain a deeper understanding of how this phenomenon occurs. Laxminarayan and Brown (2000), had an environmental perspective when analyzing the mechanisms of antibiotic resistance, asserting that natural selection fosters antibiotic resistance. The authors go further in depth, attributing the development of antibiotic resistant strains to plasmid transfer[3], mutation, and the overuse of broad-spectrum antibiotics[4] in hospitals (Laxminarayan & Brown, 2000). Building on Laxminarayan and Brown's argument, Lauren Richardson, who has a PhD in pharmacology from University of Washington, offers similar information in her PLOS biology article; Richardson identifies the overuse of antibiotics as a primary source of growing antibiotic resistance, indicating that "overuse threatens [antibiotics] efficacy", again, taking an environmental approach to explaining antibiotic resistance. Richardson also ascertains mutation as a mechanism of antibiotic resistance, attributing the mutation of one bacterium to the development of further mutation, leading resistance to become a property of the microbial community (Richardson, 2017). Both Richardson and Laxminarayan's ideas attribute the overuse of antibiotics to the evolution of bacterial communities in response to environmental conditions, meaning that an alternative to conventional antibiotics must be identified in order to limit antibiotic use.

While antibiotic resistance is a concern for the medical community, society itself tends to underestimate the significance of this biological phenomenon; although a multitude of medical discoveries have been made recently, the discovery of new antibiotics is a rare event (Richardson, 2017); thus, the drugs that can be used to combat infections caused by resistant bacteria are limited. In a study done by Adabara (2012), a member of the Department of Microbiology at Federal University of Technology, it was found the 60% of bacterial samples in a Nigerian hospital contained antibiotic resistant bacteria, encompassing many different species. With such a large percentage of collected bacterial isolates being resistant, it is apparent that the resistance of bacterial communities has become a serious issue. The fortuitous results of this study substantiate the critical nature of antibiotic resistance and its global reach. While antibiotic resistance allows all bacterial species to evolve in response to their surroundings, MRSA is the most frequent cause of antibiotic resistant infection in humans Richardson, 2017. Rao et al. elaborate on Richardson's claims about *S. aureus* by stating that it often infects healthy individuals who do not possess risk factors, such as surgery or residence in a long-term facility (2015). The presence of antibiotic-resistant bacteria in both the healthy community and hospitals makes containing the growth of resistance ever more urgent, leading medical researchers to pursue an innovative technique with which to treat antibiotic resistant infections; one of these proposed methods is the application of essential oils.

According to Stephen Bent, a professor at University of California: San Francisco's School of Medicine, the United States has seen a surge in the popularity of herbal products in the last decade, and, as of 2007, were used by twenty percent of the American population for aromatherapy, depression, and nausea among other uses (2008). Various oils and their antibiotic efficacies have been studied extensively; however, in this study, the focus will remain on four essential oils that have been shown to have antibiotic properties: tea tree, thyme, oregano, and wintergreen oil. Tea tree oil, obtained from *Melaleuca alternifolia,* has been found to inhibit the growth of multiple bacteria, including *Escherichia coli* and *Candida* species (Ott & Morris, 2008). Of the multiple essential oils that have been researched, tea tree oil has shown the greatest potential

2 Gram positive bacteria have a cell wall that is composed of a thicker layer of peptidoglycan than gram-negative bacteria.

3 Plasmid transfer: the ability to directly transfer genetic material between bacteria

4 Broad-Spectrum Antibiotics: antibiotics that act against a wide range of disease-causing bacteria, including both gram-negative and gram-positive bacteria.

as an inhibitory agent for bacterial growth, and oregano oil has been shown to have consistent antibacterial effects against common bacteria (Ott & Morris, 2008). Additionally, both wintergreen and thyme oil are recognized as having antibiotic and antimicrobial properties (European Medicines Agency, 2014). While essential oils are currently popular for their commercial use, they were first used by classical civilizations, most notably in India, for their medicinal properties (Bodeker, Buford, Chamerlain, and Bhat, 2001). Medical professionals have turned to these past treatments in the hopes that they will illuminate the solution to current medical issues, such as antibiotic resistance.

Bodeker et al. (2001) studied the medicinal potential of two popular agroforestry[5] species; this research revealed that both species were suitable for the treatment of priority diseases in Sub-saharan Africa, such as AIDS and Malaria, also including treatment of urinary diseases, boils, skin diseases, ulcers, malaria, fever, colic, and inflammation. Research studies evaluating the effectiveness of essential oils to address antibiotic resistance, such as those by Ott, Morris, Bodeker, Bufort, Bhat, and Chamberlain, have made it apparent that it is possible to utilize essential oils as a treatment option opposing antibiotics; the use of essential oils could be especially effective in countries where new medical technology has yet to become readily available. While the work of Bodeker et al. does demonstrate the pharmaceutical potential of essential oils, it is relatively generalized, making its application to antibiotic resistance particularly onerous. However, the potential of essential oils as bacterial growth inhibitors was further analyzed by S. Alizadeh-Salteh, K. Arzani, R. Omidgeigi, and N. Safaie in their work discussing how essential oils can be used to inhibit the growth of *Rhizopus stolonifer,* a chief source of destructive postharvest disease of fruit (2010). In addition to the work of Bodeker et al., which generally classified essential oils as having medicinal potential, this new research highlighted the use of essential oils specifically to inhibit bacterial growth rather than treat symptoms of disease. These results further demonstrated the potential of essential oils as bacterial growth inhibitors, demonstrating how essential oils could be advantageous for the inhibition of *R. stolonifer* growth as well as other bacterial species.

The curative potential of essential oils in combatting infection by antibiotic-resistant bacteria was addressed by Ott and Morris (2008), in their article *Homeopathic Alternatives to Conventional Antibiotics*. Ott and Morris addressed how a myriad of essential oils and herbs are capable of hindering the growth of microorganisms, including *E. coli* and *S. aureus* (Ott & Morris, 2008). The work of Ott and Morris not only deemed essential oils as effective bacterial growth inhibitors, but it also distinguished between the efficacy of different essential oils. While data obtained prior to this point was limited to a single bacteria or disease, the work of Ott and Morris applied essential oils to multiple bacteria, greatly increasing knowledge regarding natural medicine. The data collected suggests that tea tree oil is an extremely effective inhibitor of *E. coli* growth and identified oregano, thyme, and wintergreen oil as supplementary growth inhibitors (Ott & Morris, 2008).

The present body of research has led to an augmented interest in the homeopathic use of essential oils. In fact, many articles have been published delineating the procedure used to test the efficacy of essential oils as antibiotic inhibitors (Morris, 2010; Scheppler, Sethakorn, Styer, 2003). However, the current body of research on this topic has yet to address several gaps in the research of natural medicine, such as the effects of combining essential oils or how the concentration of bacteria affects the efficacy of essential oils. The lack of knowledge about essential oils and natural medicine has led physicians to develop a penchant for conventional antibiotics and become skeptical of the reliability of essential oils as a common treatment (Bent, 2008). While plant pathologists and microbiologists have already conducted considerable research on the efficacy of individual essential oils and their inhibitory effects on multiple strains of bacteria, research concerning the effects of these oils in combination is lacking. By researching the potential synergistic interactions of essential oils, this study can contribute to the growing body of work concerning alternative medicine and address the knowledge gap regarding the effects of combining essential oils. Additionally, the use of gram-negative (*E. coli*) and gram-positive (*S. aureus*) bacteria in the research will allow for a deeper understanding of how the classifi-

5 Agriculture incorporating the cultivation and conservation of trees

cation of bacteria may affect the efficacy of essential oils in combination. This fostered the question: *How do the synergistic interactions of select essential oils – tea tree, thyme, oregano, and wintergreen oil – affect their antibacterial efficacy as an* Escherichia coli *and* Staphylococcus aure*us growth inhibitor?* Analyzing the results of prior studies and the efficacy of individual essential oils led to the hypothesis that a combination of tea tree and oregano oil would have the greatest antibiotic efficacy.

Methodology

The methodology chosen for this study was a modified Kirby-Bauer disc diffusion, which was chosen due to its success in producing results for prior studies conducted by the aforementioned researchers. This method was chosen because a Kirby-Bauer disc diffusion poses minimal risk to healthy individuals and is a standard procedure used in clinical laboratories to test the susceptibility of patient's bacterial isolates to antibiotics (Scheppler et al., 2003); Since this methodology is often used to test antibiotic susceptibility and the goal of this study was to test susceptibility to essential oils, a Kirby-Bauer disc diffusion was an appropriate choice for this study. Additionally, this method utilizes filter paper discs, which can absorb any solution, making the creation of precise essential oil combinations and their exposure to bacteria a relatively simple process. Finally, the quantitative nature of a Kirby-Bauer disc diffusion allows for easy comparison between control and test groups. Overall, this was an optimal methodology for this study because it provided a safe way to obtain accurate data comparing the inhibitory potential of essential oils in combination.

Materials

Four essential oils that are commonly used for their medicinal properties and commercial purposes [tea tree *(leaf) (Melaleuca alternifolia)*, oregano *(flower) (Origanum vulgare)*, thyme linalool *(flowering tops) (Thymus vulgaris)*, and wintergreen *(leaf) (Gaultheria procumbens)*] were purchased from Plant Therapy at planttherapy.com; one 10 mL container of each essential oil was ordered. The brand "Plant Therapy" was chosen because of their company imposed regulations to ensure the consistency and quality of their oils; Plant Therapy tests their oils multiple times, sending them to a third-party laboratory to undergo Gas Chromatography and Mass Spectrometry tests. In addition, two microorganisms were used to determine the inhibitory properties of these essential oils; the bacterial strains that were used included *Escherichia coli* (ATCC 11775) and *Staphylococcus aureus* (ATCC 29213).

Disc Diffusion

Plate Preparation

The susceptibility of bacteria to essential oil combinations was determined using a modified Kirby-Bauer disc diffusion assay. Twenty-four Mueller-Hinton agar plates were used to cultivate bacterial cultures; twelve plates were used for each bacteria. Each Mueller-Hinton agar plate was divided into four separate quadrants, labelling the respective quadrant on the bottom of the plate. Two categories of plates were tested: control plates and test plates. Control plates were used to determine the inhibitory potential of each essential oil individually; therefore, no combinations were tested on control plates. Test plates were used to obtain data for combinations of essential oils. For test plates, these quadrants included "Control", "Oregano and Control", "Wintergreen and Control", and "Thyme and Control". For the purpose of this project, the "Control" solution is tea tree oil. For control plates, these quadrants included "Tea Tree", "Oregano", "Wintergreen", and "Thyme". Plates were swabbed evenly with bacteria, *E. coli* and *S. aureus* respectively. To do this, a sterile swab was dipped into the bacterial suspension broth and streaked over the surface of the plate.

Disk preparation

Control Plates: Following the swabbing of all agar plates, filter paper discs were prepared. Each quadrant of an agar plate contained one filter paper disc that had absorbed a solution for testing; for discs on control plates, individual oils were absorbed. Each plate was comprised of one disc per essential oil: tea tree, oregano, wintergreen, and thyme oil (four total oils were tested, meaning one oil was tested per quadrant); each disc contained 6 μL of solution. Once these discs were prepared, each was placed in its respective quadrant using sterilized forceps. Plates were incubated at 37 °C for 24 hours.

Test Plates: Filter paper discs on the test plates tested combinations of essential oils; these plates were also composed of one quadrant dedicated to a control disc for tea tree oil. For the control quadrant of the test plates, 6 μL of tea tree oil was absorbed by the filter paper discs by directly applying the solution to the disc using a micropipette. Since each disc must contain a total 6 μL of solution total and the combinations were mixed at a 1:1 ratio, combination discs contained 3 μL of tea tree oil (control solution) and 3 μL of a supplementary oil. Once these discs were prepared, each disc was placed in its respective quadrant using sterilized forceps. Plates were incubated at 37 °C for 24 hours.

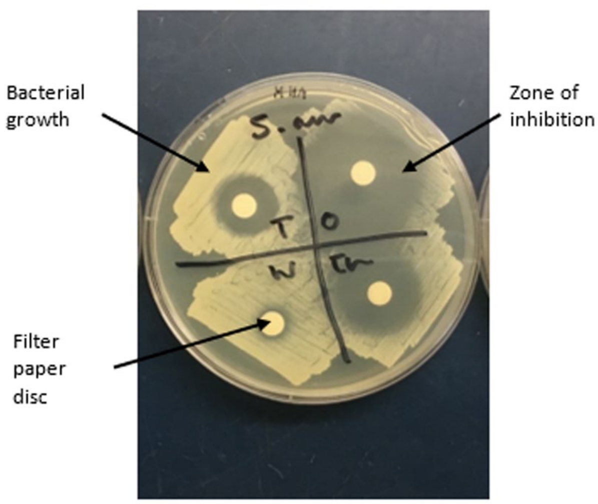

Figure 1: Agar Plate Diagram
This picture provides an example of what a correctly prepared plate will look like after incubation. This plate has four correctly labelled quadrants, each with one filter paper disc containing it respective solution, as described previously.

Data Measurement

After 24 hours of incubation, the zone of inhibition[6] was measured in millimetres for each quadrant; this data was used to arbitrate the inhibition percentage. The inhibition percentage of the bacterial growth was calculated by utilizing the data obtained from test plates. The three quadrants of the test plate were compared to the control quadrant of that respective plate. The following equation was used:

Inhibition Percentage = 100 (T-C) / C, where C is the diameter of the zone of inhibition for the control quadrant (tea tree oil individually) and T is the diameter of the zone of inhibition for the test quadrant.

Considering the goal of this research project was to evaluate the antibacterial properties of essential oils, this methodology was an appropriate choice. The modified Kirby-Bauer disk diffusion allowed for data to be obtained for all combinations and individual essential oils; The dependent variable of the Kirby-Bauer disc diffusion (the diameter of the zone of inhibition) corresponded to the goal of this study: measuring the inhibitory potential of essential oil combinations.

Results

Herbs, including essential oils, are defined as any form of a plant product, including leaves, stems, flowers, roots, and seeds (Noller, Kumar, Lajis, & Ali, 2008). When evaluating the possible presence of synergistic interactions between select essential oils as bacterial inhibitors, the data that was obtained confirmed that combining certain essential oils can increase their efficacy in certain cases.

6 The area where bacterial growth was inhibited

TABLE 1: ANOVA SINGLE FACTOR (ESCHERCHIA COLI)

SUMMARY						
Groups	Count	Sum	Average	Variance		
Column 1	9	142	15.77778	0.694444		
Column 2	9	226	25.11111	3.111111		
Column 3	9	155	17.22222	5.944444		
Column 4	9	187	20.77778	1.444444		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	467.6667	3	155.8889	55.70223	8.43E-13	2.90112
Within Groups	89.55556	32	2.798611			
Total	557.2222	35				

The ANOVA single factor test for E. coli test plates shows a P-value of 8.43×10^{-13}, signifying that the results of this study concerning the inhibition of E. coli growth are statistically significant.

TABLE 2: T TEST: TWO-WAY SAMPLE ASSUMING UNEQUAL VARIANCES (ESHERICHIA COLI)

Key	A (Control)	B (Control + Oregano)	C (Control + Wintergreen)	D (Control + Thyme		
Combinations	*A-B	A-C	*A-D	*B-C	*B-D	*C-D
T--test P-value	1.8109E-08	0.123522	6.82E-08	1.06E-06	2.79E-05	0.00202
Adjusted P-value	0.008	0.008	0.008	0.008	0.008	0.008

To determine which combinations had a statistical difference, a t test: two-way sample assuming unequal variances was conducted. The P-value was adjusted from .05 to .008 to account for the six tested combinations. The resulting P-values from the t-test demonstrated statistically significant results for all combinations, excluding the tea-tree and wintergreen oil combination (A-C).

FIGURE 1: ESCHERICHIA COLI: TEST PLATES

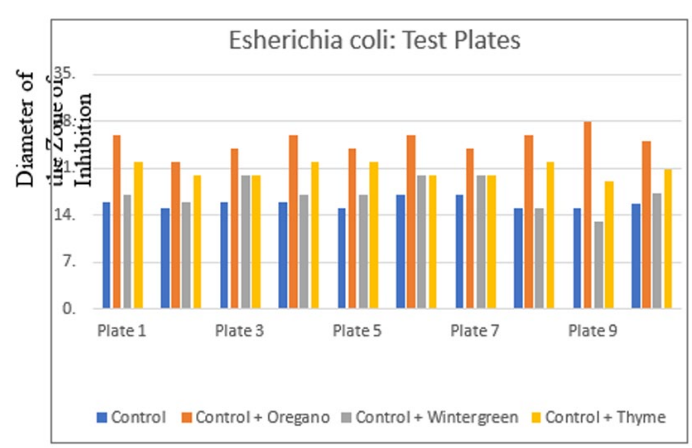

TABLE 3: SINGLE FACTOR ANOVA (STAPHYLOCOCCUS AUREUS)

SUMMARY						
Groups	Count	Sum	Average	Variance		
Column 1	9	142	15.77778	0.694444		
Column 2	9	226	25.11111	3.111111		
Column 3	9	155	17.22222	5.944444		
Column 4	9	187	20.77778	1.444444		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	467.6667	3	55.70223	55.70223	8.43E-13	467.6667
Within Groups	89.55556	32	2.798611			
Total	557.2222	35				

A single factor ANOVA test was conducted to determine if the results concerning the microorganism *Staphylococcus aureus* were statistically significant. With a P-value of 8.43 x 10^{-13}, the results can be deemed statistically significant.

TABLE 4: T TEST: TWO-WAY SAMPLE ASSUMING UNEQUAL VARIANCES (STAPHYLOCOCCUS AUREUS)

Key A (Control)	B (Control + Oregano)	C (Control + Wintergreen)	D (Control + Thyme)			
Combination	*AB	*AC	AD	*BC	*BD	*CD
T-test P-value	4.32E-08	1.88E-05	0.218088	6.94E-12	8.93E-08	2.31E-08
Adjusted P-value	0.008	0.008	0.008	0.008	0.008	0.008

A t test: two way sample assuming unequal variances was conducted to determine which combinations had statistically significant results concerning their potential synergistic interactions and the inhibition of *Staphylococcus aureus* growth. The P-value was adjusted from .05 to .008 to account for the six combinations. The results show that all combinations proved a statistical difference except for control and thyme oil (tea tree and thyme oil).

FIGURE 2: STAPHYLOCOCCUS AUREUS: TEST PLATES

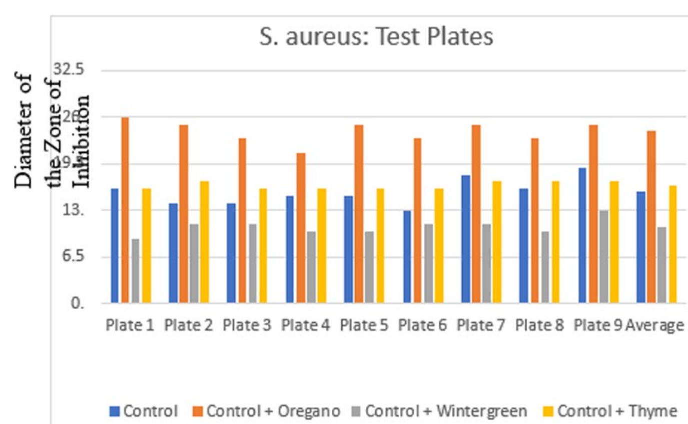

TABLE 5: INHIBITON PERCENTAGE (ESCHERICHIA COLI) TABLE 6: INHIBITON PERCENTAGE (STAPHYLOCOCCUS AUREUS)

Plate number	Control + Oregano	Control + Wintergreen	Control + Thyme
Plate 1	62.50%	6.25%	37.50%
Plate 2	46.67%	6.67%	33.33%
Plate 3	50%	25%	25%
Plate 4	62.50%	6.25%	37.50%
Plate 5	60%	13.33%	46.67%
Plate 6	52.94%	17.65%	17.65%
Plate 7	41.18%	17.65%	17.65%
Plate 8	73.33%	0%	46.67%
Plate 9	86.67%	-13.33%	26.67%
Average	59.53%	8.83%	32.07%

TABLE 6: INHIBITON PERCENTAGE (STAPHYLOCOCCUS AUREUS)

Plate number	Control + Oregano	Control + Wintergreen	Control + Thyme
Plate 1	62.50%	-43.75%	0%
Plate 2	78.57%	-21.43%	21.43%
Plate 3	64.29%	-21.43%	14.29%
Plate 4	40%	-33.33%	6.67%
Plate 5	66.67%	-33.33%	6.67%
Plate 6	76.92%	-15.34%	23.08%
Plate 7	38.89%	-63.64%	-5.56%
Plate 8	43.75%	-37.50%	6.25%
Plate 9	31.58%	-31.58%	-10.53%
Average	55.91%	-33.48%	7%

Discussion

The results of this study signify the presence of synergistic interactions between tea tree and oregano oil, making it the combination that is most effective at inhibiting bacterial growth for both *E. coli* and *S. aureus*. These findings supported the initial hypothesis that the combination with the greatest antibacterial efficacy would be tea tree and oregano oil.

When analyzing the statistically significant results and looking at the potentially synergistic combinations, it is apparent that the combination with the highest mean inhibition zone diameter (IZD) is Control-Oregano (tea tree and oregano oil) for both bacteria. This illustrates that the combination with the greatest antibacterial potential is control-oregano (Figure 1 & Figure 2). The inhibition percentages for *E. coli* further support the claim that the control-oregano combination has the greatest antibacterial efficacy; the average inhibition percentage for the control-oregano combination was 59.53 percent, meaning that this combination's IZD was 59.53 percent larger than the control groups IZD. Furthermore, this inhibition percentage is considerably larger than that of the other two combinations, further supporting the data demonstrated by the ANOVA, TTEST, and averages IZDs (Table 5). The inhibition percentages for *S. aureus* also demonstrate the antibacterial efficacy of the control-oregano combination; the average inhibition percentage for the control-oregano combination was 55.91 percent. This inhibition percentage is considerably larger than that of the other two combinations. In addition to the synergistic interaction that is demonstrated by this data, one can see how the combination of wintergreen and tea tree oil is antagonistic with an inhibition percentage of -33.48%.

These results gave way to a variety of fascinating findings. For both *E. coli* and *S. aureus,* the tea tree and oregano oil combination had the greatest degree of antibacterial efficacy with an inhibition percentage of 59.53% (*E. coli* – Table 5) and 55.91% (*S. aureus* – Table 6); however, the t-test proved that the same combinations did not elicit the same results for both bacteria. The combinations that proved a statistical difference for *E. coli* were tea tree/oregano oil and tea *tree/thyme oil* (Table 3) when the values were compared to the control (tea tree oil alone). The combinations that proved a statistical difference for *S. aureus* were tea tree/oregano oil and *tea tree/wintergreen* oil (Table 4). These results show that combining essential oils does cause a change in efficacy, although this change is not consistent between bacterial species. Further analyzing these results, it is apparent that utilizing essential oils as a medical treatment would require a deeper understanding of how the inhibitory potential of an oil will change in the presence of various bacteria. This assertion is further supported by the work of Ott and Morris (2008), whose study demonstrates that the inhibitory potential of essential oils individually will vary based on the bacterial species being tested.

Furthermore, the results demonstrated that combining essential oils does not always lead to an increase in antibiotic efficacy. For *E. coli*, the combination of tea tree and wintergreen oil was not proven to have a statistical difference from the control, meaning that the addition of wintergreen oil to tea tree oil did not have any effect on the solutions antibiotic efficacy; however, this combination did prove a statistical difference for *S. aureus*. Unlike the tea tree/oregano oil combination, the tea tree/wintergreen oil combination had antagonistic effects on the growth of *S. aureus*. While the average IZD for tea tree oil alone was 15.6 mm, the average IZD for tea tree and wintergreen oil was 10.7 mm, decreasing the zone of inhibition by 33.5% (Figure 2). This is a significant finding because the tea tree/wintergreen combination was the only tested solution that led to a smaller IZD for either bacteria. From this case, one can conclude that although combining essential oils consistently led to an increase in efficacy, it is possible for a combination to have adverse effects. Should the tea tree/wintergreen solution be used in a medical setting to treat a *S. aureus* infection, the results would have been detrimental to the patient because of the antagonistic relationship between these oils; with this in mind, it is important that one understands that combining essential oils can increase and decrease antibacterial efficacy in certain cases.

Prior studies and literature stress the urgent need to address the increasing growth of antibiotic resistant bacteria, as the increasing use of antibiotics has led to greater antibiotic resistance and there is a need to identify an alternative treatment method. When analyzing the data presented in the study, it is apparent that essential oils do have antibacterial properties and their efficacy can be increased by combining them with supplementary oils; in particular, the combination of tea tree and oregano oil had the greatest increase in antibacterial efficacy; however, the next step, after identifying the particularly effective essential oil solutions, is to implement natural and holistic medicine into treatment regimens. While holistic medicine is unconventional in the United States, it is the main form of medicine in 80% of the currently developing world and was used by ancient civilizations for over 5,000 years; furthermore, plants and plant-based solutions make up 20% of pharmaceuticals in the United States (Noller, Kumar, Lajis, & Ali, 2008). Considering their widespread use, essential oils could be used as

7 Nephropathy- medical term used to denote disease or damage of the kidney, which can eventually result in kidney failure

a first line of defence for fighting bacterial infections in the United States and ultimately across the world; thus, patients could initially address their infections with natural medicine and utilize conventional antibiotics if the essential oils or herbs were not producing the desired effects. This solution would allow physicians to prescribe fewer antibiotics, limiting the survival of antibiotic resistant bacteria and the toxic side effects of antibiotic usage.

While the results of this study demonstrate an optimistic future for essential oils as an alternative to conventional antibiotics, there are limitations concerning this research project and the use of essential oils as a treatment option. Regarding the execution of this methodology, there was potential for contamination when swabbing the agar plates and transitioning them from the biosafety cabinet to the incubator.

One limitation regarding the use of natural medicine as a treatment is a lack of regulation. According to Stephen Bent (2008), herbs are currently defined by law as dietary supplements, meaning that manufacturers can produce, sell, and market herbs without demonstrating their efficacy or safety. Additionally, in order for a "dietary supplement" (i.e. natural medicine and essential oils) to be removed from the market, it must be deemed unsafe by the FDA, directly contrasting to the regulation of drugs, which must be proven as safe and effective by the FDA before they can be sold (Bent, 2008). This regulatory structure, or lack thereof, limits the reliability of essential oils and natural medicine and their use as a medical treatment. In conjunction with a lack of regulation is the potential for contamination when producing essential oils (Hosihuzzaman & Iqbal Chaudhary, 2008). This contamination, in extreme cases, can lead to the toxicity of essential oils; in the past, contamination has led to reports of nephropathy[7] caused by commonly used Chinese herbs; of the nephropathy cases, 43 patients developed end-stage renal failure and 39 had prophylactic renal removal (Bent, 2008). These cases have demonstrated how contamination and lack of regulation of essential oils can have implications regarding their medical usage. With potential contamination putting the health of the patient at risk, it is difficult to implement essential oils into treatment regimens. Should more stringent regulations concerning the marketing and production of dietary supplements (essential oils) be put in place, patients and medical professionals would likely become more confident in the efficacy of essential oils.

The results of this research examined the presence of synergistic interactions between select essential oils, with the combination of tea tree and oregano oil having the greatest antibacterial efficacy. However, this study only addressed four essential oils: tea tree, oregano, wintergreen, and thyme oil; further research could be conducted to look into potential synergistic, or antagonistic interactions, between other essential oils. Prior research shows that other oils that were not tested in this study, such as citrus and curry oil, also exhibited relatively high antibacterial efficacies (Ott & Morris, 2008). Conducting research on these oils could reveal a combination that has a higher degree of efficacy than those in this study. Furthermore, this study only addressed oils in combination with tea tree oil; in the future, combining other oils, such as pure oregano oil with other oils, may provide additional information surrounding medical implications.

Not only could one look into other combinations in the future, but using the tested combinations on other bacteria would allow researchers to gain a deeper understanding of how changing the bacteria affects the efficacy of the essential oils. As discussed earlier, the results for *E. coli* and *S. aureus* were different, showing that the results for one bacteria are not applicable to all. In addition, this study did not compare essential oils directly to antibiotics, but rather compared combinations to individual oils. In the future, a study comparing the combinations with the greatest efficacy to antibiotics directly would not only allow for a greater understanding of how essential oils inhibit bacterial growth but also demonstrate their efficacy in comparison to other treatment options.

Overall, the results of this study demonstrated that synergistic interactions are present between select essential oils and of all the combinations that were tested, tea tree and oregano oil had the greatest inhibitory potential. Using this data, one can identify new, innovative ways to combat bacterial infections, particularly of the bacteria that were studied: *Escherichia coli* and *Staphylococcus aureus*. While antibiotics are commonly used to treat patients with bacterial infections, the results of this study show that essential oils can be an effective treatment option, particularly if combinations with high efficacy are utilized.

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