Antibacterial activity of cinnamon oil and its synergy with antibiotics against *Pseudomonas aeruginosa*

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Research on the antimicrobial efficacy of essential oils has revealed promising effects of cinnamon oil (Lo 2012, Poaty 2015). Studies revealed that essential oils may have synergistic properties when combined with other agents (Patrone 2009, Khan 2011, Naveed 2013). It was hypothesized that synergistic effects on bacterial inhibition would occur if cinnamon oil was combined with antibiotics. The bacteria chosen for investigation was *Pseudomonas aeruginosa*, commonly associated with hospital-acquired infections. The antibiotics used were cefuroxime 30µg, clindamycin 2µg, trimethoprim 5µg, and ofloxacin 5µg. The experiment required the inoculation of 36 plates with four plates per treatment. The treatments were prepared on filter paper chads then placed in the plates. Measurements of the zones of inhibition on each plate were taken after 48 hours of incubation. Ofloxacin was the only antibiotic to successfully inhibit the growth of *P. aeruginosa*. An analysis of variance (ANOVA) suggested a significant difference between ofloxacin treatments and the cinnamon oil control ($p = 0.000393$, ANOVA). A Tukey HSD post-hoc analysis revealed no significant difference between the ofloxacin and the ofloxacin-cinnamon combination ($p = 0.121$, Tukey HSD). Between the other groups, there likely existed significant differences due to the cinnamon's ability to inhibit bacterial growth ($p = 0.001$, cinnamon v. antibiotic; $p = 0.0016$, antibiotic v. combination, Tukey HSD).

Introduction

Antimicrobial resistance in bacteria is a pressing and threatening issue, with the Center of Disease Control (CDC) recently publishing several web pages on the matter. It is estimated that two million people each year become infected with a resistant strain of bacteria, and 23,000 of them end up dying (CDC 2017). Essential oils contain several different applications such as aromatherapy, with some revealed to contain antimicrobial properties. Recent research has investigated the individual antimicrobial properties of such oils by testing their abilities to inhibit bacterial colonization and growth (Lo et al., 2012, Naveed et al., 2013, Poaty et al., 2015). Being able to cross such avenues of bacterial inhibition could uncover new pathways of treatment against bacterial resistance. The idea of combining such treatments stems from earlier research conducted on the effectiveness of essential oils as antifungals or food preservatives when combined with specified treatments (Patrone et al., 2009, Park et al. 2010, Khan et. al. 2013). As research efforts sway toward discovering the true antimicrobial potential of essential oils, some research groups have compared the antimicrobial effectiveness of several different essential oils to discover *Cinnamomum verum* oil is the most active in bacterial inhibition due to it mainly being comprised of cinnamaldehyde (Naveed et al., 2013). Cinnamaldehyde directly affects FtsZ in bacteria, which is a cell division protein that functions similarly to tubulin, and plays a significant role in the cell separation of bacteria. Specifically, it prevents the polymerization and assembly of the FtsZ compound in bacteria (Domadia et al., 2007). To further explore this mechanism, a different bacterium was chosen to investigate the versatility of cinnamaldehyde.

Pseudomonas aeruginosa is most commonly a hospital acquired infection (HAI), which highlights it as an ideal candidate for investigation. Of the *P. aeruginosa* infections found in hospitals, 13% of them are multi-drug resistant (CDC 2014). *P. aeruginosa* is a gram-negative rod bacteria that is associated with many diseases. It usually manifests itself as a localized infection following surgery or burns, as well as urinary tract infections, in cystic fibrosis patients, necrotizing pneumonia, and corneal infections. The overall mortality in association with *P. aeruginosa* is 50%, and accounts for 15% of all gram-negative bacteremia. It has been reported that from 51,000 infections of *P. aeruginosa*, 6,700 are multidrug resistant, with 440

deaths associated with *P. aeruginosa* (CDC 2017). Due to multiple antibiotic resistant strains of *P. aeruginosa,* it is mandatory to perform antibiotic susceptibility testing of clinical isolates. It has been found that gentamicin and carbenicillin are quite effective against acute infections of *P. aeruginosa* (Iglewski 1996).

The study focuses on observing any cooperative effects of bacterial inhibition between several antibiotics when they are combined with cinnamon leaf essential oil. Ofloxacin is a treatment meant to combat optical bacterial infections known to inhibit DNA gyrase in bacteria (RXList 2017b). Clindamycin is known to inhibit protein synthesis in bacteria by binding to specific subunits of bacterial ribosomes, specifically the 23S RNA of the 50S ribosomal subunit (RXList 2017a). Trimethoprim is an antibiotic known to inhibit the synthesis of tetrahydrofolic acid, which is essential for the synthesis of proteins and nucleic acids in bacteria. This is done by interfering with the folic acid pathway by binding to dihydrofolate reductase (AMRLS 2011). Finally, the mechanism of action for cefuroxime includes its ability to inhibit the synthesis of the bacterial cell wall (Brogden 1979). It is hypothesized that synergistic effects will be observed when cinnamon oil and these antibiotics are combined against *P. aeruginosa.*

Materials and Methods

Agar plates were made using standard nutrient agar powder dissolved and heated with deionized water. Approximately one hundred agar plates were made using a standard recipe of 23.0 grams of nutrient powder for every 40 plates, dissolved in 1000mL of water. After mixing and heating the solution, the flask was autoclaved and the agar mixture was poured evenly into sterile petri plates. After the agar cooled and solidified, the plates were inoculated using sterile techniques and nutrient broths provided by Senior Microbiology Technician Anita Bandekar of Saddleback College Biological Sciences Department. Sterile cotton swabs were used to create a lawn of bacteria on the agar plates, using sterile techniques including flaming the mouths of the nutrient broth tube and disposing of cotton swabs in disinfectant solution.

After inoculation of the agar plates, the antibiotic treatments were prepared. Cinnamon oil controls were prepared using 5 µL of cinnamon oil infused on a sterile filter paper chad. Antibiotic treatments were pre-prepared at fixed dosing and provided by Saddleback College's Biological Sciences Department. The antibiotic strengths were as follows: cefuroxime 30µg, clindamycin 2µg, trimethoprim 5µg, and ofloxacin 5µg. Each antibiotic chad was infused with 5 µL of cinnamon oil, with one chad for each antibiotic acting as a control. Once ready, the chads were placed in the middle of the agar plate (Fig. 1). The plates were then inoculated for 48 hours at 37°C to insure maximum growth of bacteria. Upon removal of the plates, results were analyzed using the zones of inhibition, measured in diameter using a 6-inch ruler.

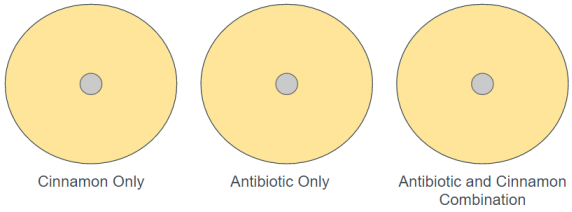

Fig. 1 *Diagram of plate arrangement and organization. Four plates allocated for each treatment per antibiotic.*

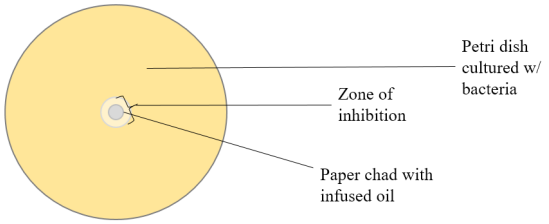

Fig. 2 *Diagram illustrating zone of inhibition after removal from incubation.*

Results

Data collected was organized by treatments and separated by antibiotic. The data revealed no zones of inhibition for the cefuroxime 30µg, clindamycin 2µg, trimethoprim 5µg antibiotics thus revealing identical p-values during statistical analysis. An Analysis of Variance (ANOVA) was run on each antibiotic treatment data set including the cinnamon oil control ($p = 0.000393$, ANOVA, ofloxacin; $p = 0.000496$, ANOVA, other antibiotics). A Tukey HSD post-hoc analysis revealed significant differences between the cinnamon control and the ofloxacin-cinnamon combination ($p = 0.0010053$, Tukey HSD). There was also significance between the cinnamon control and ofloxacin control ($p = 0.0059001$, Tukey HSD). However, there was no statistically significant difference between the ofloxacin control and the ofloxacin-cinnamon combination, thus failing to reject the null hypothesis.

Between the other antibiotics, there was significance between the cinnamon control and the antibiotic control ($p = 0.0010053$, Tukey HSD). Furthermore, there was significance between the antibiotic control and the antibiotic-cinnamon combination ($p = 0.0016246$, Tukey HSD). Lastly, there was no significant difference between the cinnamon control and the antibiotic-cinnamon control, which is attributed to the equivalent values measured for each data set.

Table 1 *Data table representing raw data collected from zone of inhibition measurements.*

Cinnamon	Cefuroxime	Cefuroxime Combo
9	6	8
9	6	8
8	6	8
7	6	8
Cinnamon	Ofloxacin	Ofloxacin Combo
9	14	15
9	11	12
8	12	16
7	13	16
Cinnamon	Trimethoprim	Trimethoprim Combo
9	6	8
9	6	8
8	6	8
7	6	8
Cinnamon	Clindamycin	Clindamycin Combo
9	6	8
9	6	8
8	6	8
7	6	8

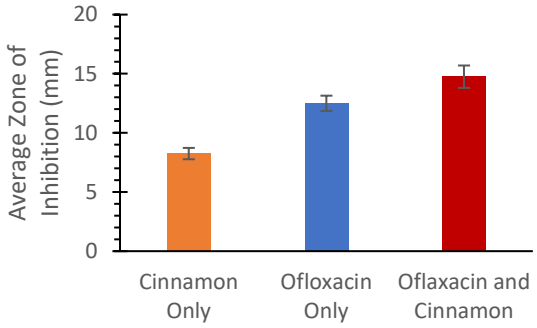

Fig. 3 *Average zone of inhibition for ofloxacin treatments versus the cinnamon control. Error bars represent mean ± SEM.*

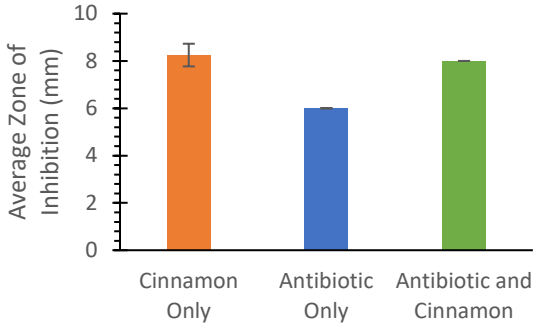

Fig. 4 *Average zone of inhibition for each antibiotic treatment versus cinnamon control. Error bars represent mean ± SEM.*

Discussion

Three out of the four antibiotics used in the study failed to inhibit the growth of *P. aeruginosa.* Ofloxacin was the only antibiotic to successfully inhibit bacterial growth. Although a greater average zone of inhibition was observed, the cinnamon-ofloxacin combination had no significant difference compared to the ofloxacin control. The results of the experiment failed to reject the null hypothesis. There was no significant increase in any of the treatments' zones of inhibition when compared to the cinnamon leaf oil control. Despite these results, it is important to note the significant difference between the cinnamon oil control and the antibiotic controls. In the three failed antibiotics, the cinnamon oil was still able to inhibit the growth of *P. aeruginosa*, with statistical significance being observed across all the treatments. This can be attributed to the mechanism of action of cinnamon oil's active ingredient, cinnamaldehyde, on the bacteria gene FtsZ, which controls mechanisms of binary fission (Domadia 2007).

Furthermore, the results can be associated with the antibiotics mostly targeting gram-positive bacteria. *P. aeruginosa* is a gram-negative rod bacteria that is ubiquitous and free-living. Trimethoprim is an antibiotic that has been found to inhibit the growth of a large range of gram-positive bacteria, with a few gram-negative bacteria (AMRLS 2011). However, it is unable to inhibit the growth of *P. aeruginosa*, and our findings suggest that cinnamon oil does not have a synergistic effect with trimethoprim. Clindamycin is an antibiotic that is mostly used against gram-positive anaerobic bacteria, which may explain its failure to inhibit the growth of *P. aeruginosa* (RXList 2017a). Lastly, although cefuroxime is indicated for use in both gram-positive and gram-negative bacteria, it has been confirmed that it has no effect of *P. aeruginosa* (Brogden 1979).

Given the antibiotics' mechanisms of action, the effect cinnamon oil has on *P. aeruginosa* did not allow means of synergy to occur between the oil and the antibiotic. Moreover, the resilience and adaptations of *P. aeruginosa* made it a difficult bacterium to inhibit, which can also explain the failure of synergy to occur. The aim of future studies hope to explore the effect cinnamon leaf oil has on antibiotic resistant bacteria, after breeding resistance with a select antibiotic. More narrow-spectrum and specific antibiotics can be used and cinnamon oil efficacy after antibiotic-resistance development can be explored.

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