

Treatment of *E. coli* biofilm using *Galla chinensis* and adjuvant chemicals

Abstract

Bacterial biofilms are present in about 60% of human infections, with those consisting of Gram-negative bacteria exhibiting particularly high resistance to antibiotics. To treat infections involving biofilms, it is important to investigate antibiotic alternatives with low toxicity. Chinese herbal medicines such as *Galla chinensis* have been shown to exhibit anti-biofilm activity, but are not strong enough to combat infections on their own. The purpose of this study was to determine the efficacy of using cinnamaldehyde (CAD), which interferes with substrate attachment and c-di-GMP-regulated biofilm formation pathways, and zinc, which interferes with ATP production and the aforementioned pathways, in conjunction with *G. chinensis* to inhibit *Escherichia coli* biofilm formation and disperse preformed biofilm. To conduct this study, biofilms were cultivated in the presence of 0.16 mg/mL *G. chinensis* water extract (GWE) and varying concentrations of either CAD or ZnCl₂ (0-10 mM), or were exposed to these treatments after cultivation. A crystal violet assay was used to quantify biofilm formation, and one-tailed t-tests were run to determine statistical significance of observed differences between treatments. The results demonstrated that GWE used in conjunction with CAD or ZnCl₂ at a concentration of 10 mM was significantly more effective at inhibiting biofilm formation and dispersing preformed biofilm than GWE alone. The most effective treatments for inhibiting biofilm formation and dispersing biofilm were GWE combined with 10 mM CAD and GWE combined with 10 mM ZnCl₂, respectively, with the former reducing formation to less than 10% of the control and the latter dispersing over 80% of the biofilm relative to the control. The synergistic anti-biofilm

effects of using *G. chinensis* in conjunction with other natural treatments is promising for its future application in combating chronic infections.

Background Research

The purpose of this experiment is to determine whether zinc or cinnamaldehyde used in conjunction with *Galla chinensis* water extract (GWE) is more effective at inhibiting *Escherichia coli* biofilm formation and which combination is more effective at dispersing preformed biofilm. Biofilms grown without exposure to GWE, zinc, or cinnamaldehyde will serve as the negative control. Biofilms grown with exposure to GWE only will serve as the positive control. The results of this experiment will help to determine whether the combination of GWE and the antibiotic alternatives, zinc and cinnamaldehyde, produce synergic effects on the inhibition of biofilm formation and the dispersal of preformed biofilm.

Antibiotics have been used to treat bacterial infections since Sir Alexander Fleming first discovered penicillin in 1928. They have since revolutionized the medical treatment of bacterial infections, saving millions of lives worldwide. However, antibiotic resistance, the immunity that bacteria develop against medication through natural selection, is a propagating global issue that is being fueled by excessive antibiotic usage. This means that antibiotics used to treat common culprits of bacterial infections, such as *Streptococcus pneumoniae* and *Mycobacterium tuberculosis*, are diminishing in effectiveness (Ventola, 2015).

This issue is further exacerbated by bacterial biofilms, sessile 3D aggregations of microorganisms that exhibit as high as 10,000 times the resistance to antibiotics as planktonic cells. They are involved in about 60% of human infections, including urinary infection, prosthetic joint infection, and cardiac valve infection. The first step in biofilm formation is the

attachment of planktonic cells to a surface. This stage of the process is often facilitated by flagella and type IV pili-mediated twitching motility, which enables cells to form colonies on a surface. Once attached, the cells form an extracellular polymeric substances (EPS) matrix consisting of various polysaccharides, proteins, and nucleic acids. The formation of the EPS matrix is known as the irreversible attachment phase. In the maturation stage, the bacterial cells accumulate on the surface and form a 3D structure organized according to metabolic activity of the cells (Rabin et al., 2015). The EPS matrix facilitates the exchange of nutrients, gases, and wastes within the complex microbial aggregation, allowing the bacterial cells to reduce metabolic activity. This reduction of metabolic activity makes it difficult for antibiotics to target key proteins, and the inability of the immune system to recognize the EPS matrix makes biofilm-related infections challenging to treat using traditional antibiotics (Wu, 2015).

The search for antibiotic alternatives in order to improve treatment of bacterial infections, especially those involving biofilm formation, has spawned many new areas of research. One of these budding fields of research is the use of Chinese herbal medicines, or CHMs, to combat bacterial infections through disruption of QS. *Galla chinensis* is a CHM with anti-inflammatory properties, and it contains a high concentration of gallotannins, strong antioxidants that reduce oxidative stress in cells by decreasing the amount of damaging free radicals. The primary components of GWE are gallic acid ($71.3 \pm 0.2\%$ w/w), which is a type of gallotannin, and its isomer. Multiple studies on the inhibition of biofilm formation using both gallic acid alone and GWE have been conducted. In one study, it was found that a concentration of 4 mg/ml of GWE had an inhibitory effect on an oral multispecies biofilm. In other studies, it has been shown that gallic acid at a concentration of 4 mg/ml inhibits biofilm formation of *Staphylococcus aureus* and that gallic acid at a concentration of 1 mg/ml inhibits biofilm formation of both

Pseudomonas aeruginosa and *E. coli*. The molecular mechanisms of biofilm inhibition by *G. chinensis* are still unknown (Wu, 2015).

The study of how essential oils (EOs) from medicinal plants can interfere with microbial growth has also produced promising results. EOs are liquid extracts that protect plants against insects, herbivores, competing plants, bacteria, fungi, and viruses. Cinnamaldehyde (CAD) is an EO that is a primary component of Chinese cinnamon essential oil (*Cinnamomum cassia*) and cinnamon bark oil (*Cinnamomum zeylanicum*). In one study, it was found that CAD inhibited QS-dependent phenotypes, such as biofilm formation and motility, in *Pseudomonas fluorescens*. In another study, CAD was shown to inhibit *P. aeruginosa* biofilm formation and motility, as well as disperse preformed biofilm through cytoplasmic membrane disruption and reduction of intracellular levels of c-di-GMP, a second messenger involved in signaling processes that stimulate biofilm formation (Topa et al., 2018). Overall, studies conducted using cinnamaldehyde as an antimicrobial agent indicate that it could potentially be used to treat infections involving biofilms.

Zinc is another substance that has been shown to exhibit antimicrobial properties. It is a trace mineral in the human body that is necessary for a number of biological processes, including cell division, cell growth, and immune system function. It is also environmentally friendly and has low toxicity. However, zinc is a bacteriostatic, rather than a bactericidal agent, meaning that while it is capable of reducing bacterial growth, it cannot eradicate entire bacterial communities on its own. It has been shown to inhibit the growth of oral bacteria by interfering with glycolysis and increasing proton permeability of the cell membrane, thus reducing adenosine triphosphate (ATP) production, amongst other molecular mechanisms of inhibition (Almoudi et al., 2018). One study showed that zinc was capable of inhibiting biofilm formation of multiple strains of

bacteria, including *E. coli*, but it was ineffective in dispersing preformed biofilm. The molecular mechanisms by which zinc inhibit biofilm formation still remain to be elucidated, although some studies indicate that its antibiofilm properties may stem from the disruption of gene regulation and signaling pathways, including the c-di-GMP pathway necessary for biofilm formation. Although zinc is not a bactericidal agent on its own, it has been shown to act synergistically with other antibacterial agents, such as biocides, indicating that when combined with other antimicrobial substances, treatments involving zinc may be very effective (Wu et al., 2013).

The growing problem of antibiotic resistance has made it necessary to search for effective alternative treatments. This has led to research involving the use of natural compounds and substances, such as *G. chinensis*, CAD, and zinc, to treat bacterial infections. As many of these natural compounds have a generally weaker antimicrobial effect than antibiotics, it is important to better understand their molecular mechanisms of inhibition and to test their effectiveness when used in conjunction with one another, which is what this study will investigate. The expected results of this experiment are that if tested for their effect on *E. coli* biofilm, then the combination of *G. chinensis* and CAD will inhibit *E. coli* biofilm formation and disperse preformed *E. coli* biofilm more effectively than a combination of *G. chinensis* and zinc. CAD has been shown to interfere with motility, which is necessary for attachment to a substrate, the first step of biofilm formation. However, zinc has not been demonstrated to have such an effect on bacterial cells, indicating that CAD's prevention of initial attachment may make it more effective at inhibiting biofilm formation. Zinc has also been shown to have minimal effects on preformed biofilms of various bacterial strains, while CAD has been shown to disperse preformed *P. aeruginosa* biofilm. The ability of CAD to disperse preformed biofilm may extend to other bacterial strains, such as *E. coli*.

Materials

- *Escherichia coli* suspension- BSL1 organism (“Biological Safety Levels,” n.d.)
- Lysogeny broth (LB)
- 10 nutrient agar plates
- Inoculating loops
- Cell spreaders
- *Galla chinensis* water extract
- 0.1 M ZnCl_2
- Cinnamaldehyde
- Test tubes
- Micropipettes
- Vortex
- Flat-bottom 96-well plates
- Crystal violet (CV)
- Small trays
- Distilled water
- 30% acetic acid
- Microplate reader

Procedure

General Safety Precautions

1. Wear gloves and goggles at all times during the experiment. Do not directly smell any chemicals.
2. If CAD comes into contact with skin during handling, wash thoroughly with soap and water. Dispose of CAD by washing it down the drain with soap (HiMedia, 2013).
3. When handling ZnCl_2 , inspect gloves regularly for chemical break-through and wash immediately with plenty of water if it comes into contact with skin. Dispose of ZnCl_2 by sealing it into a glass bottle and putting it in the biological hazard bin (Carolina Biological, 2018). Follow the same procedures for acetic acid (Fisher Scientific, 2008).

4. Dispose of *E. coli* and all disposable materials that come into contact with it in the biological hazard bin. Sterilize glassware in an autoclave.

Inhibition of E. coli Biofilm Formation by G. chinensis & CAD and by G. chinensis & Zinc

1. Add 100 μ l of 10^6 CFU/ml bacterial suspension to 48 wells in a flat-bottom 96-well plate.
2. Add 50 μ l of 0.640 mg/ml GWE to all of the wells, with the exception of eight wells, which will serve as the negative control.
3. Add 50 μ l of 10.00 mM CAD solution, 5.000 mM CAD solution, 2.500 mM CAD solution, 1.250 mM CAD solution, and 0.6250 mM CAD solution each to 8 of the wells.
4. Add 100 μ l of pure LB to the 8 wells containing neither GWE nor CAD solution. There should now be a volume of 200 μ l per well.
5. Incubate the microtiter plate at 37°C for 24 hours.
6. Repeat steps 1 through 5 using a new microtiter plate and varying concentrations of ZnCl_2 .
7. After incubation, use the crystal violet assay to quantify biofilm formation for both plates.

Dispersion of Preformed Biofilms by G. chinensis & CAD and by G. chinensis & Zinc

1. Add 100 μ l of 10^6 CFU/ml bacterial suspension to 48 wells in a flat-bottom 96-well plate.
2. Incubate the plate at 37°C for 24 hours.
3. After incubation, remove the liquid medium from the wells, and wash away planktonic cells by submerging the plate in distilled water.

4. Add 50 μ l of 0.640 mg/ml GWE to all of the wells, with the exception of eight wells, which will serve as the negative control. Then, add 50 μ l of 10.00 mM CAD solution, 5.000 mM CAD solution, 2.500 mM CAD solution, 1.250 mM CAD solution, and 0.6250 mM CAD solution each to 8 of the wells. Add 100 μ l of LB to the 8 control wells.
5. Incubate the plate at 37°C for another 24 hours.
8. Repeat steps 1 through 5 using a new microtiter plate and varying concentrations of ZnCl₂.
6. After incubation, use the crystal violet assay to quantify biofilm formation for both plates.

Crystal Violet Biofilm Quantification Assay

1. Set up two small trays, one to collect waste and the other to wash the microtiter plate. Fill the latter with 1 to 2 inches of distilled water.
2. Shake the microtiter plate over the waste tray in order to remove planktonic bacteria.
3. Submerge the plate into the water tray, and shake it over the waste tray to remove water afterwards. Repeat this a second time.
4. Add 200 μ l of 0.1% CV solution to each well, and allow the stain to sit for 10 minutes at room temperature.
5. To remove the CV solution, shake the plate over the waste tray, and wash it in the water tray, making sure to shake out water after submersions. Replace the water if it becomes clouded with color.
6. Place the dish on a paper towel in an inverted position, and allow it to air-dry for a few hours or overnight.

7. Add 200 μ l of 30% acetic acid to each stained well to solubilize the CV.
8. Let the microtiter plate sit at room temperature for 10 to 15 minutes.
9. Transfer 200 μ l of the solubilized CV of each well to a well in a new microtiter plate.
10. Quantify the absorbance of the samples using a microplate reader set at 550 nm. Use 30% acetic acid in water as the blank.

Statistical Analysis

1. Calculate the standard error for control and experimental groups and graph as error bars.
If error bars overlap, the difference seen between two experimental groups is not statistically significant.
2. Run a two-tailed t-test assuming variance to determine whether there is a statistical difference between the effectiveness of *G. chinensis* and 10.00 mM CAD treatment vs. *G. chinensis* and 10.00 mM ZnCl₂ treatment on biofilm formation. If the absolute value of the t-statistic is greater than the t-critical value, and the p-value is less than 0.05 for the test, then the difference can be determined to be statistically significant.
3. Run another two-tailed t-test assuming variance to determine whether there is a statistical difference between the effectiveness of *G. chinensis* and 10.00 mM CAD treatment vs. *G. chinensis* and 10.00 mM ZnCl₂ treatment on the dispersal of preformed biofilm.

Data

Table 1. Relative absorbance of negative and positive control groups for biofilms grown in the presence of treatment.

Concentration	0 mg/mL	0.16 mg/mL GWE
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Sample 1	0.965	0.433
Sample 2	0.986	0.436
Sample 3	0.952	0.454
Sample 4	1.075	
Sample 5	0.981	
Sample 6	1.051	
Sample 7	1.025	
Sample 8	0.968	

Table 2. Relative absorbance of solubilized crystal violet from stained *E. coli* biofilms grown in the presence of 0.16 mg/ml GWE and varying concentrations of CAD.

Concentration	0.16 mg/mL GWE and 0.6250 mM CAD	0.16 mg/mL GWE and 1.250 mM CAD	0.16 mg/mL GWE and 2.50 mM CAD	0.16 mg/mL GWE and 5.00 mM CAD	0.16 mg/mL GWE and 10.00 mM CAD
Sample 1	0.524	0.580	0.588	0.463	0.135
Sample 2	0.551	0.680	0.588	0.496	0.075
Sample 3	0.628	1.148	0.524	0.479	0.104
Sample 4	0.684	0.605	0.599	0.380	0.080
Sample 5	0.650	0.638	0.705	1.080	0.078
Sample 6	0.531	0.611	0.624	0.668	0.073
Sample 7	0.642	0.748	0.577	0.549	0.080
Sample 8	0.529	0.528	0.589	0.592	0.068

Table 3. Relative absorbance of solubilized crystal violet from stained *E. coli* biofilms grown in the presence of 0.16 mg/ml GWE and varying concentrations of ZnCl₂.

Concentration	0.16 mg/mL GWE and 0.6250 mM ZnCl₂	0.16 mg/mL GWE and 1.250 mM ZnCl₂	0.16 mg/mL GWE and 2.50 mM ZnCl₂	0.16 mg/mL GWE and 5.00 mM ZnCl₂	0.16 mg/mL GWE and 10.00 mM ZnCl₂
Sample 1	0.477	0.988	1.059	1.186	0.076
Sample 2	0.468	1.108	1.095	1.154	0.129
Sample 3	0.535	1.104	1.193	1.180	0.122
Sample 4	0.444	1.134	0.982	1.214	0.164
Sample 5	0.452	1.147	1.178	1.165	0.222
Sample 6	0.475	1.177	1.198	1.203	0.193
Sample 7	0.511	0.454	1.183	1.223	0.283
Sample 8	0.351	0.572	1.163	1.239	0.231

Table 4. Relative absorbance of solubilized crystal violet from stained *E. coli* biofilms incubated for 24 hours before being exposed to 0.16 mg/ml GWE and varying concentrations of CAD.

Concentration	Control (no treatment)	0.16 mg/mL GWE and 0.6250 mM CAD	0.16 mg/mL GWE and 1.250 mM CAD	0.16 mg/mL GWE and 2.50 mM CAD	0.16 mg/mL GWE and 5.00 mM CAD	0.16 mg/mL GWE and 10.00 mM CAD
Sample 1	0.449	1.686	1.800	1.795	1.618	0.523
Sample 2	1.100	1.734	2.291	1.712	2.397	0.589
Sample 3	0.763	2.239	2.246	2.464	1.630	0.483
Sample 4	0.766	2.888	1.667	2.030	2.282	0.399
Sample 5	0.658	2.215	2.882	2.407	2.274	0.524
Sample 6	2.163	2.706	2.161	2.642	2.392	0.964
Sample 7	1.219	2.377	2.634	2.545	2.109	0.629
Sample 8	0.882	1.476	1.867	1.712	2.361	0.460

Table 5. Relative absorbance of solubilized crystal violet from stained *E. coli* biofilms incubated for 24 hours before being exposed to 0.16 mg/ml GWE and varying concentrations of ZnCl₂.

Concentration	Control (no treatment)	0.16 mg/mL GWE and 0.6250 mM ZnCl ₂	0.16 mg/mL GWE and 1.250 mM ZnCl ₂	0.16 mg/mL GWE and 2.50 mM ZnCl ₂	0.16 mg/mL GWE and 5.00 mM ZnCl ₂	0.16 mg/mL GWE and 10.00 mM ZnCl ₂
Sample 1	0.449	0.614	0.665	0.243	0.152	0.351
Sample 2	1.100	0.558	0.326	0.342	0.140	0.393
Sample 3	0.763	0.534	0.742	0.321	0.177	0.212
Sample 4	0.766	0.421	0.451	0.379	0.168	0.171
Sample 5	0.658	1.450	0.715	0.445	0.181	0.200
Sample 6	2.163	1.322	0.799	0.383	0.185	0.444
Sample 7	1.219	1.352	0.680	0.244	0.151	0.213
Sample 8	0.882	1.748	0.535	0.352	0.375	0.240

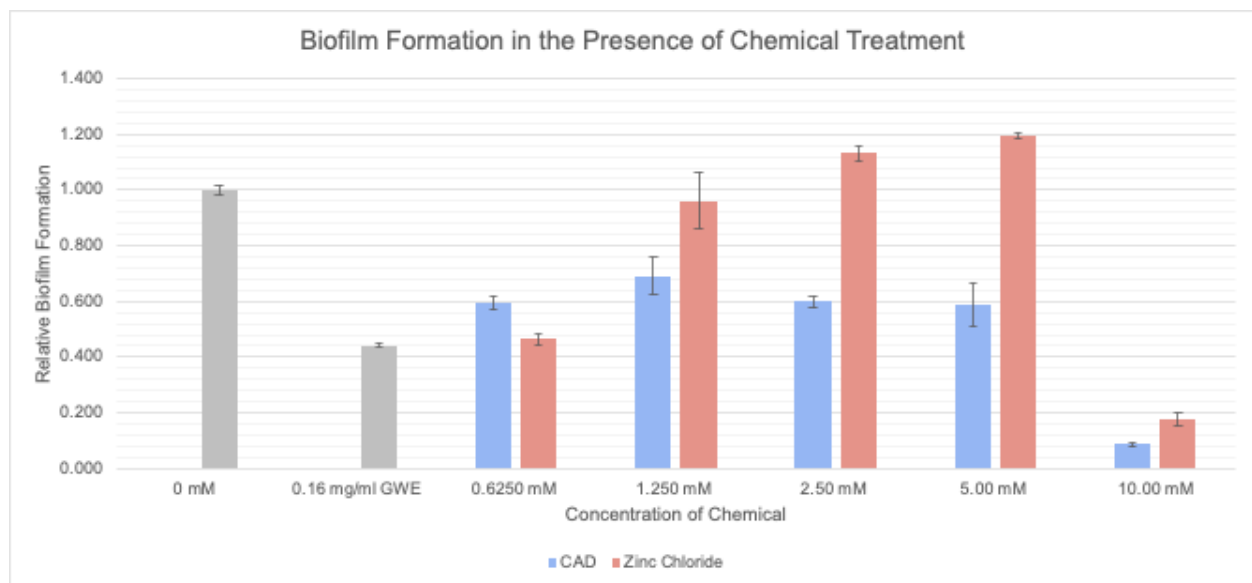


Figure 1. Fold change ($\pm$ SEM) of *E. coli* biofilm formation relative to negative and positive controls after exposure to 0.16 mg/ml GWE and varying concentrations of CAD and ZnCl₂.

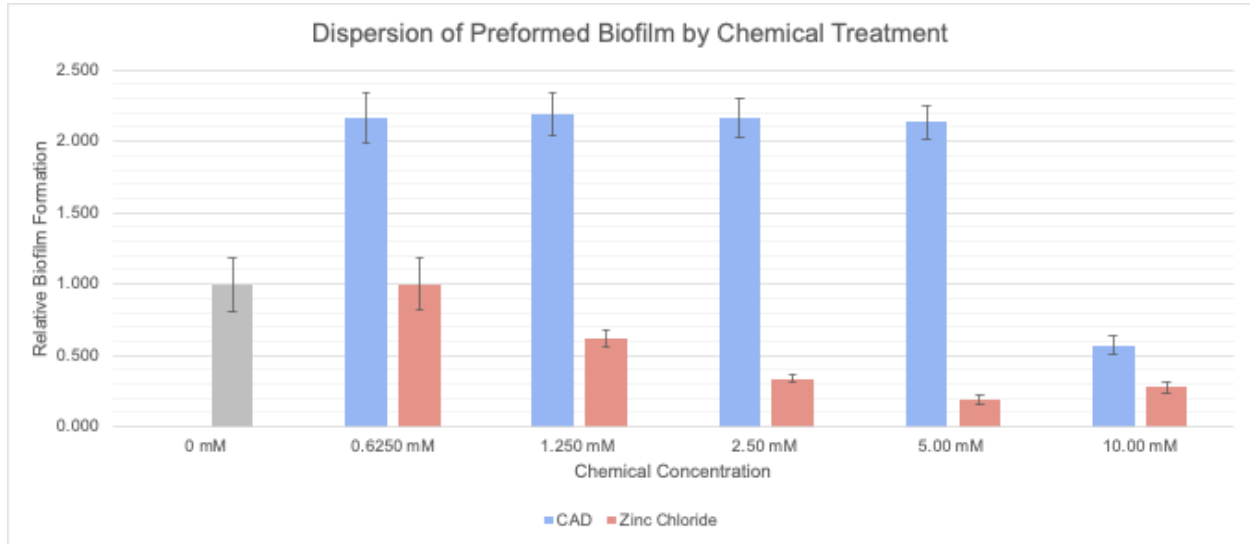


Figure 2. Fold change ($\pm$ SEM) of dispersion of preformed *E. coli* relative to negative control after exposure to 0.16 mg/ml GWE and varying concentrations of CAD and ZnCl₂.

Table 6. Standard error of results for sample groups used to test the effect of treatment on biofilm formation.

Sample Group	Standard Error (for samples grown in the presence of treatment)	Standard Error (for samples exposed to treatment after 24 hours of incubation)
0 mg/mL	± 0.016	± 0.187
0.16 mg/mL GWE	± 0.007	N/A
0.16 mg/mL GWE and 0.6250 CAD	± 0.023	± 0.177
0.16 mg/mL GWE and 1.250 CAD	± 0.069	± 0.148

0.16 mg/mL GWE and 2.500 CAD	± 0.018	± 0.139
0.16 mg/mL GWE and 5.000 CAD	± 0.077	± 0.116
0.16 mg/mL GWE and 10.000 CAD	± 0.008	± 0.062
0.16 mg/mL GWE and 0.6250 ZnCl ₂	± 0.019	± 0.184
0.16 mg/mL GWE and 1.250 ZnCl ₂	± 0.100	± 0.057
0.16 mg/mL GWE and 2.500 ZnCl ₂	± 0.028	± 0.024
0.16 mg/mL GWE and 5.000 ZnCl ₂	± 0.010	± 0.027
0.16 mg/mL GWE and 10.000 ZnCl ₂	± 0.024	± 0.036

Table 7. Two-tailed t-test assuming unequal sample variances. Used to determine statistical significance of the difference between treatment effectiveness on inhibiting biofilm formation.

	0.16 mg/ml GWE and 10.00 mM CAD	0.16 mg/ml GWE and 10.00 mM ZnCl₂
Mean	0.087	0.178
Variance	0.000496	0.00459
df	8	
t-Statistic	-3.604	
t-Critical two-tail	2.306	
P-value two-tail	0.00694	

Table 8. Two-tailed t-test assuming unequal sample variances. Used to determine statistical significance of the difference between treatment effectiveness on dispersing preformed biofilm.

	0.16 mg/ml GWE and 10.00 mM CAD	0.16 mg/ml GWE and 10.00 mM ZnCl₂
Mean	0.571	0.278
Variance	0.0303	0.0105
df	11	
t-Statistic	4.105	
t-Critical two-tail	2.201	
P-value two-tail	0.00174	

Conclusion

The findings of the experiment supported the first half of the hypothesis that CAD used in conjunction with GWE would be more effective at inhibiting *E. coli* biofilm formation than ZnCl₂ used in conjunction with GWE. At concentrations of 0.6250 mM, 1.250 mM, 2.50 mM, and 5.00 mM, CAD used in conjunction with 0.16 mg/mL GWE actually inhibited *E. coli* biofilm formation less than when 0.16 mg/mL GWE was used alone (Fig. 1). However, at a concentration of 10.00 mM, CAD used in conjunction with 0.16 mg/mL GWE inhibited biofilm formation to $8.7\% \pm 0.8$ of the control (Table 2), while exposure to 0.16 mg/mL GWE alone inhibited biofilm formation to $44.1\% \pm 0.7$ of the control (Table 1). 0.6250 mM ZnCl₂ used in conjunction with 0.16 mg/mL GWE had the same inhibitory effect on biofilm formation as 0.16 mg/mL GWE alone, while using ZnCl₂ at concentrations of 1.250 mM, 2.500 mM, and 5.000 mM either had no inhibitory effect on biofilm formation or promoted it (Fig. 2). As with CAD, ZnCl₂ used in conjunction with 0.16 mg/mL GWE did not appear to have a greater inhibitory effect on biofilm formation than 0.16 mg/mL GWE alone until increased to a concentration of

10.00 mM, at which biofilm formation was reduced to $17.8\% \pm 2.4$ of the control (Table 3). The null hypothesis that the two treatments had the same inhibitory effect on biofilm formation at a chemical concentration of 10.00 mM was rejected (two-sample t-test, $t=3.604$, 8 df, $p<0.01$; Table 7). This showed that while both treatments had a greater inhibitory effect on biofilm formation than 0.16 mg/mL GWE alone, the treatment involving CAD was more effective.

The findings of the experiment rejected the second half of the hypothesis that CAD used in conjunction with GWE would be more effective at dispersing preformed biofilm than ZnCl_2 used in conjunction with GWE. At concentrations of 0.6250 mM, 1.250 mM, 2.500 mM, and 5.000 mM, CAD used in conjunction with 0.16 mg/mL GWE promoted the growth of preformed biofilm to over 200% of the control. However, at a concentration of 10.00 mM, CAD used in conjunction with 0.16 mg/mL GWE dispersed preformed biofilm to $57.1\% \pm 6.2$ of the control. As the concentration of ZnCl_2 used in conjunction with 0.16 mg/mL GWE increased in two-fold increments from 0.6250 mM to 10.00 mM, a general trend of increasing dispersal activity was seen. The null hypothesis that the dispersal activity of the two treatments was the same at a chemical concentration of 10.00 mM was rejected (two-sample t-test, $t=4.105$, 11 df, $p<0.01$; Table 8). This showed that while both treatments dispersed preformed *E. coli* biofilm at a chemical concentration of 10.00 mM, the treatment involving ZnCl_2 was more effective, thus rejecting the original hypothesis that the treatment involving CAD would be more effective.

Limitations

Although this experiment demonstrated the increased effectiveness of GWE treatment when combined with chemical adjuvants, the limited scope of research on the effectiveness of CHM treatments combined with other chemicals means that the ability of certain chemicals to

reinforce CHM treatment cannot necessarily be generalized. In addition, due to time constraints and limited access to resources, the molecular mechanisms by which the treatments affected biofilm formation and dispersed preformed biofilm could not be further analyzed. To investigate some of these mechanisms, a motility test could be used to determine whether the treatments affected flagella motility, which is essential for initial cell attachment, and confocal laser scanning microscopy could be used to assess variations in biofilm structure by staining the biofilms with fluorescent dye.

Error Analysis

Errors during the experimental procedure could have impacted the accuracy of the recorded results. Although the initial and diluted suspension of bacteria were stored at 4°C to prevent growth, a small amount of microbial growth during this time could have altered the concentrations of the solutions. As a result, the concentration of the bacterial suspension pipetted into the microtiter plates may have been slightly off from 10^6 CFU/mL. The concentration of GWE may also have been slightly different from the 0.16 mg/mL GWE solution used in last year's experiment. This is due to fluctuations in the displayed measurement on the electronic scale when measuring out a specific mass of GWE.

Applications

Due to the increasing prevalence of antibiotic resistance, the need to find alternative treatments that can similarly combat bacterial infections is becoming more and more urgent. In recent years, the study of using CHMs to treat bacterial infections, including those involving bacterial biofilms, has emerged. However, as many CHMs do not have as significant of an antibacterial effect as antibiotics, it is important to determine whether using them alongside

chemical adjuvants can produce a synergistic effect. This experiment demonstrated that the combined treatment of the CHM *G. chinensis* and CAD, as well as the combined treatment of *G. chinensis* and ZnCl_2 were both more effective at inhibiting *E. coli* biofilm formation than *G. chinensis* treatment on its own. This shows that if the inhibitory effect of a CHM treatment on its own is relatively weak, it can be supplemented with chemical adjuvants, such as CAD and ZnCl_2 , making it more probable that antibiotics can be substituted by these natural alternatives in the future. Both treatments were also effective at dispersing preformed biofilm at higher concentrations, and as most bacterial infections involve biofilms that have already formed (such as in prosthetic joint infections and dental plaque), CHMs used in conjunction with chemical adjuvants can be considered as a potential substitute for antibiotics in the treatment of preformed biofilm. The results also showed that the ability of a treatment to inhibit biofilm formation is not necessarily correlated with its ability to disperse preformed biofilm. This is a factor that should be considered when treating biofilms with natural alternatives, such as those used in this experiment.

Future Research

The use of CHMs, such as *G. chinensis*, to treat bacterial infections is still a relatively new field of research that requires further investigation before it can be successfully applied as an antibiotic alternative. The use of chemical adjuvants in conjunction with a wider range of CHMs should be tested to determine whether the synergistic effect of chemical and GWE treatment observed in this experiment can be generalized to other CHMs as well. If it can be consistently demonstrated that the effectiveness of CHM treatment can be reinforced by chemical adjuvants, then these natural treatments could replace antibiotics in treating bacterial

infections. Additionally, the effectiveness of CHM treatment on biofilms composed of other types of bacterial species should be investigated, as this experiment was limited to the study of *E. coli* biofilm only. This will help determine trends between certain bacteria characteristics and their susceptibility and/or resistance to certain types of treatment. Conducting studies on how treatments involving CHMs can best be administered to human patients, such as through oral or injection routes, will also assist in eventually making these treatments applicable to actual infections.

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