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An in Vitro Study of Chinese Goldthread as a Natural Cleaning Agent When Compared to a QAC Containing Agent on *E. coli*.

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ABSTRACT

Traditional Chinese Medicine (TCMs) have been used for centuries for their wide array of properties. Studies conducted on these properties have shown especially strong antibacterial properties, specifically for a TCM named Chinese Goldthread rhizome. Conversely, Quaternary ammonium compounds (QACs) are toxic ingredients in cleaning products that are used to kill microorganisms that lead to adverse health effects in humans. With this, studies involving the ability of Chinese Goldthread rhizome to be used in a cleaning product because of its antibacterial properties as a replacement for QACs are absent. Therefore, the purpose of this study is to test the effectiveness over time of Chinese Goldthread on a strand of a bacteria that is present on hard surfaces against a QAC containing agent. By using an experimental method, both qualitative and quantitative data has been yielded. Positive results have added to the current body of knowledge in the scientific field, and added the possibility of a more natural approach to toxic cleaning ingredients.

Introduction

With the increased usage of more developed cleaning products and disinfectants, in conjunction with the COVID-19 outbreak, there has been an increase in the amount of reports of people who have been exposed to the harmful ingredients from such products. Between January and March of 2020, poison centers had received over forty-five thousand calls related to toxic exposures from cleaning and disinfecting agents, which is a 20.4% increase for cleaners and 16.4% increase for disinfectants from the previous year.[1]

This is a concern as people are becoming sick trying to stay safe from an outbreak by keeping their living spaces clean from harmful microorganisms. But what they do not know is that the ingredients in the agents being used are doing the same, if not more, damage to people's health than what they are using it to fight against. The ingredients that are designed to fight off germs, known as quaternary ammonium compounds (QACs) are what make the agents antimicrobial. Many disinfectants, sprays, wipes, and soaps among other products include QACs on the ingredient list. Long term exposure from QACs have been shown to reduce fecundity and fertility in mice in laboratory experiments.[2] QACs have also been shown to induce work-related asthma in a study done on healthcare workers.[3]

With that being said, one of the most common hard surface bacteria that these QACs are combating is *E. coli,* which was determined to be one of the most prevalent bacteria on toilets, and door knobs at hospitals and schools,[4] which is a gut bacteria that can lead to UTIs, pneumonia, food poisoning, and in severe cases, death.[5] As alternatives to toxic ingredients like the QACs, research for natural sources to fight off such bacteria has been conducted.

For centuries, China has used herbal medicines and plant extracts, commonly called Traditional Chinese Medicine (TCM), as a means to holistically treat common ailments ranging from the common cold to insomnia and heart disease. Many of these TCMs have been shown to exhibit antibacterial properties as well.[6] One such TCM, Chinese Goldthread, especially the rhizome (root) of the plant has portrayed promising antibacterial properties that

could potentially be used as a more natural cleaning product. However, not much research has been conducted on the effectiveness of the plant's ability to be so in this regard. This has led to a gap in modern research. Therefore, an experiment will be conducted to evaluate the effectiveness of Chinese Goldthread rhizome's antibacterial properties as a potential natural cleaning agent against *E. coli*, as it is one of the most prevalent bacteria on hard surfaces.

Literature Review

Traditional Chinese Medicine (TCM) ranges from practices such as acupuncture and Tai Chi to herbal concoctions and remedies. For thousands of years these practices have been used and evolved. However, it wasn't until the 1970s until Americans began to recognize TCMs after it was brought over during the nineteenth century when the Chinese came to the US in search of gold during the Gold Rush. Since then, numerous studies have been conducted on their benefits.

The extract specific to this experiment, Chinese Goldthread rhizome, traditionally has been used as a heat purgative, anti-inflammatory, and a detoxicant,[7] and recently has been studied extensively for its properties. It was found to contain an alkaloid called Berberine, which can be used to treat a range of diseases from hepatic disease to cancer.[8] In a study done to determine if it has neuroprotective properties it was found that the extract from the rhizome of the plant does indeed have neuroprotective properties, which could be put forth into therapeutic agents for diseases that are associated with oxidative stress such as Alzheimers or Parkinsons due to its determined antioxidant and anti-inflammatory properties.[9]

Aside from its neuroprotective properties, Chinese Goldthread rhizome exhibits strong antibacterial properties as well. In a 2011 experiment done by Francesca Leach, she had tested this TCM along with Chinese Skullcap, another plant in Chinese medicine, on varying bacteria and yeast such as *E. coli*, *Staphylococcus*, and *Saccharomyces cerevisiae*.[7] The purpose was to determine both of their potential to be natural antibiotics in medicine. This was conducted through a disc-diffusion method, which is a method where sterile discs are impregnated with the antibiotic to be tested and placed onto an inoculated agar petri dish in order to determine how the antibiotic will impact the growth of the inoculated bacteria. Leach concluded that both of the TCMs had exhibited powerful antimicrobial effects on all of the bacteria tested after reading the zone of inhibition — which is the ring that forms around each impregnated disc that determines how much growth of the bacteria the antibiotic inhibited. The bigger the ring, the more sensitive the bacteria was to that antibiotic, the smaller the ring, the more resistant the bacteria was to the antibiotic. The mean zone of inhibition determines the MIC (Minimum Inhibitory Concentration) of the antibiotic to that bacteria, which is the smallest amount needed for the tested antibiotic to inhibit the bacteria growth. Chinese Goldthread rhizome's zone of inhibition value on *E. coli* was a large 10.3 mm when it was at only 12.5% of the original infusion, which led to the MIC value being a smaller concentration that is needed to inhibit the growth of the bacteria. As shown in Figure 1, the Chinese Goldthread rhizome (labeled *C. chinensis* for its scientific classification) along with the other TCM tested, *S. baicalensis* (Chinese Skullcap), and the mixture of the two (50% mixture of both herbs) were tested against *E. coli.* The zone of inhibition (labeled mean kill zone on graph) for the Chinese Goldthread was significantly higher at around 14 mm than it was for Chinese Skullcap (~4 mm) and the mixture of the two (~5 mm).[7] This pattern is consistent with the other tested bacteria and yeast (*Staphylococcus* and *Saccharomyces cerevisiae*) in the paper, where the Chinese Goldthread rhizome had the highest mean kill zone when compared to the other TCMs.

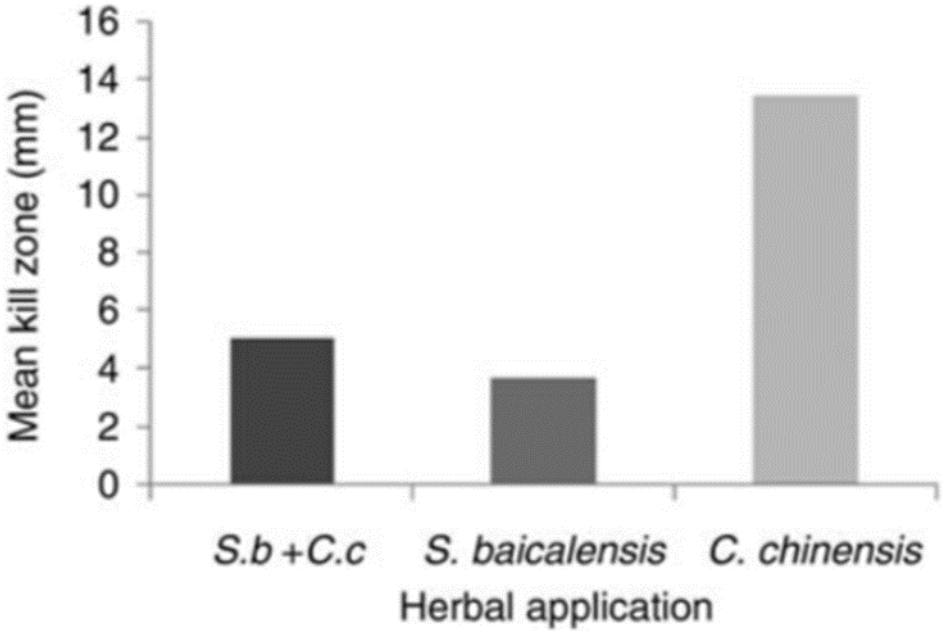

Figure 1. Comparison of the mean kill zone width produced in E. coli B by the two herbs acting alone or in combination.[7]

It was concluded in this experiment that Chinese Goldthread rhizome could be comparable to an existing antibiotic on the market - vancomycin, due to its strong antibacterial properties. This was valuable due to it being a baseline for a future experiment using a similar method.

In a similar experiment done in 2020 by Gowoon Kim, over 200 TCMs were tested for their potential as antibiotics to drug-resistant *S. aureus* using the agar diffusion method (another name for the disc diffusion method). Out of the 239 tested, the Chinese Goldthread rhizome was a selected TCM that showed promising antibacterial activity and a low cytotoxicity (how toxic it is to cells).[10] With that being said, both papers had reached the conclusion that TCMs, especially Chinese Goldthread rhizome could potentially be used as a natural antibiotic, and potentially an antibacterial agent in pharmaceutical, food, and animal feed industries.[10]

In a 2012 study done by Michael Popp, an experiment was conducted to determine if a plant extract could be used to be used as a natural alternative to chemicals in detergents, disinfectants, or cleaning agents that contains at least one plant hydrolyzate (which in the experiment was defined as an aqueous phase retrieved from the extract of the plant that could be obtained through an ethanol extraction of the plant). Multiple plant extracts were tested, many being TCMs, none of which, however, was Chinese Goldthread rhizome. The agar dilution test (another method to determine an MIC) was applied against varying bacteria strains. By the end of the experiment the majority of the plant extract's hydrolyzates had shown high levels of antibacterial activity.[11] It was then concluded from this experiment that multiple of the extracts tested could be put forth into one of the previously listed agents, building on top of the previous possibilities from the aforementioned studies.

These experiments had led to a gap in modern research. The potential of Chinese Goldthread rhizome to be used as a natural cleaning agent. While there have been studies conducted on the TCM against *E. coli* as either an antibiotic (medicinal purposes) or means of food preservation, there have yet to be studies done on the effectiveness of Chinese Goldthread rhizome on *E. coli* as a surface bacteria to be used as a cleaner. This leads to the research

question: Does Chinese Goldthread rhizome extract inhibit the growth of non-pathogenic *E. coli* in order to be used as a potential natural cleaning agent when compared to a QAC containing agent? This question will attempt to be answered through the Kirby-Bauer Disc Diffusion method by comparing the TCM to an existing agent containing QACs over the course of a week. It is hypothesized that the cleaning agent will have a higher zone of inhibition over the TCM in the beginning, but throughout the course of the week the effectivity of the cleaner will decline and the TCM's effectivity will remain the same throughout. This is important to modern research as it could help protect people sensitive to harsh chemicals and instead replace them with natural alternatives.

Method

To be able to determine if Chinese Goldthread rhizome extract would inhibit the growth of non-pathogenic *E. coli* and potentially be used as a natural cleaning agent through means of comparing it to a QAC containing product, an experiment was conducted to determine qualitative and quantitative data using the scientific method through means of the Kirby-Bauer method. This method is a specific iteration of the disc-diffusion method. This was chosen over other methods due to it being the most commonly used for antibiotic susceptibility testing of pure bacterial cultures (*E. coli* for this experiment). An experimental design is best suited for tests of this type because a survey would not yield objective results, rather, more subjective opinions than hard evidence. An experimental design would ensure data that would support the research question by supplying numerical data from numerous trials. For the purpose of this experiment, the Chinese Goldthread rhizome extract and HDQ Neutral, which is a traditional cleaning agent that is widely used at public schools during the 2020-2021 school year for cleaning hard surfaces that contains QACs, will be referred to as antibiotics, not for the meaning of medicinal uses, but for their antibacterial usages.

Materials

Material:	Justification:
• *E. coli* K12 (non-pathogenic strain)	Bacteria must be non-pathogenic for a safe experimenting environment at a school-based BSL-1 Lab. Purchased from Carolina Biological.
• Mueller-Hinton Agar Plates (15)	Mueller-Hinton in order to meet the specifications for the Kirby-Bauer method. Fifteen are needed for three time recording periods with five trials for each.
• Chinese Goldthread rhizome extract (alcohol-free tincture to test the pure extract) • HDQ Neutral solution	Alcohol-free (closest to natural form) for the Chinese Goldthread rhizome in order to avoid potential variables that could negatively affect results. This was purchased from an online provider specializing in alternative treatments. HDQ Neutral was used to compare the extract to, this agent was chosen as it is what schools are currently using to disinfect hard surfaces during the COVID-19 pandemic.
• 6mm Sterile Antibiotic discs (45)	In order to meet the method standards, 6mm discs are needed to be placed on the agar plates.
• Sterile Swabs (15) • Alcohol Burner • Forceps • Rubbing Alcohol • Micropipette	Rubbing alcohol and alcohol burner to sterilize forceps for disc placement. Sterile swabs to inoculate agar plates with the bacteria. Micropipette to impregnate discs with specified concentration of both items tested.
• Incubator	To incubate the agar plates inoculated with bacteria for quick results.
• Gloves • Safety goggles	PPE equipment to ensure safety while experimenting.

Procedure

First, the bottom of each agar plate was divided into three sections in a Y shape using a sharpie and labeled on the bottom with the time period tested, as well as which trial number the plate was. One section for a disc of the extract, another for the disc with HDQ Neutral, and the third to be used as the control.

The Chinese Goldthread extract was then diluted to 25 mg/ml using tap water. This was decided over distilled water in order to match how the HDQ Neutral (and other cleaning agents) are diluted in real-world applications, which can lead to a limitation in results. This amount of dilution was chosen from a similar study conducted by Cepta F.

Duffy and Ronan F. Power in which they determined the MIC (Minimum Inhibitory Concentration) value from the same extract.[6] After the HDQ Neutral was freshly diluted at 1 ounce per gallon, as per the product guidelines, (due to the HDQ Neutral only being good to use for seven days after initial dilution), it was impregnated onto the discs with 10 µL (determined from the same previous study mentioned) using a micropipette. The purpose of diluting both to these amounts was to compare their effectiveness over time with their recommended serving size values. Figure 1 below visualizes this process.

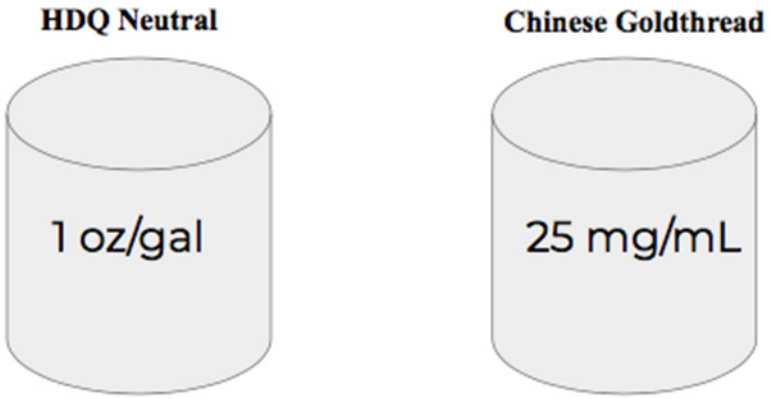

Figure 1. Both the HDQ Neutral and Chinese Goldthread are diluted in separate containers. The HDQ Neutral to 1 oz/gal and the Chinese Goldthread to 25mg/mL based on recommended usage values and similar experiments.

Secondly, the sterile swab was used to inoculate the bacteria onto the plates by firstly sterilizing the top of the tube of the *E. coli* by running it through the flame, dipping the swab into the nutrient broth (where *E. coli* is kept), and streaking it across the plate and rotating the plate, and repeating to ensure full coverage (as per Kirby-Bauer standards) and allowing it to dry for five minutes. Between each repetition the top of the tube is sterilized as per method guidelines.

Next the sterile forceps were used to place the discs of each antibiotic into two of the three sections (one for control with no product on it), and in between each placement the forceps were sterilized by dipping them in rubbing alcohol and running it through the alcohol burner flame and letting all of the rubbing alcohol burn off. This was repeated for five plates (one plate is one trial) to have comparable results.

The plates were then topped with a lid and flipped upside down. They were then placed into an incubator for 18 hours at 37 degrees Celsius (as per method guidelines) in order to quicken the growing process. After 18 hours, the zone of inhibition was recorded (mm), that is, the ring that forms around the antibiotic disc that demonstrates the susceptibility of the *E. coli* to the antibiotic. In Figure 2 below it visually demonstrates what the zone of inhibition looks like and what is being recorded.

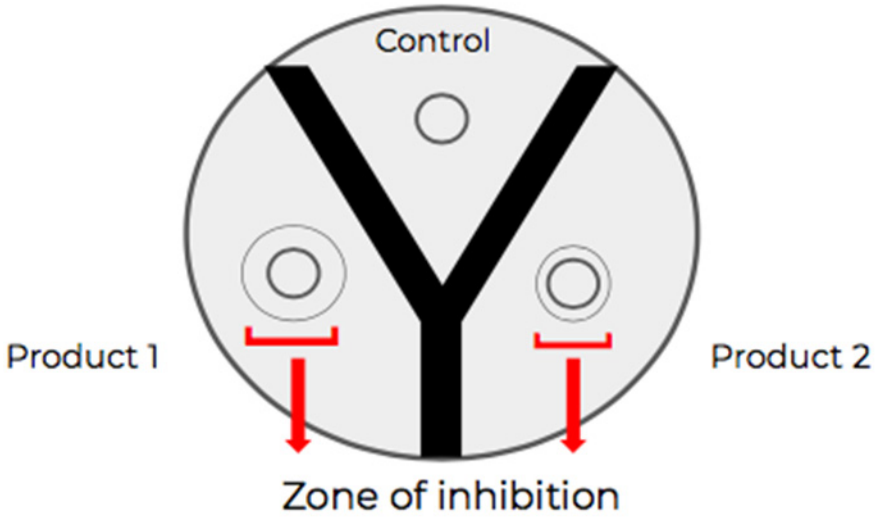

Figure 2. An agar plate with a zone of inhibition visual that demonstrates what is being recorded. The red brackets show the zone of inhibition from the rings that formed around the discs.

It is important to note that the plates from 0 hours had not properly incubated, therefore the process was repeated for the HDQ Neutral and results were gathered for that product at this period (0 hours). This had led to a limitation in results since there was no Chinese Goldthread data available for this time period, but was still able to draw conclusions from what was provided among other trials.

Once both antibiotics had sat out for 96 hours (approximately 4 days after initial dilution, due to time limitations and not being able to conduct the experiment at 3.5 days after, the process had to be done after 4 days), this process was repeated for five more trials to create a second period of data gathering at halfway through the week instead of the right after initial mixture. Once again, after 18 hours data was recorded.

At 168 hours (7 days) after the initial dilution, the process was repeated for five more trials to create the third period of data gathering at the end of the week. The zone of inhibition diameter was measured in millimeters using a ruler for each trial done at each time period and recorded into a table. In Figure 3 below it depicts the table that the results were recorded in, with the top left corner changing for each time period after mixture (0 hours, 96 hours, 168 hours).

It is predicted for the zone of inhibition size for the HDQ Neutral to be large initially as it is freshly diluted, but throughout the week as the effectivity decreases for the HDQ Neutral, it is predicted that the zone of inhibition size will decrease with it. On the contrary, it is predicted that the Chinese Goldthread rhizome will be effective, but not as much initially as the HDQ Neutral (due to the QAC component), and that throughout the week the effectiveness of the extract will remain the same. This method will determine if the extract will last longer and still remain as effective over time while being safe to health (if put forth into a cleaning agent), when compared to HDQ Neutral (a representative of a cleaning agent with QACs) that does not last as long and is more harmful to health. Possible complications could be cross contaminations with other bacteria or improperly sterilizing. This could be combated by going slowly through the steps with caution and having backup equipment in the case that complications ensue.

0/96/168 hours after dilution	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	
Antibiotic	Zone diameter (mm)	Zone diameter (mm)	Zone diameter (mm)	Zone diameter (mm)	Zone diameter (mm)	Average Zone Diameter (mm)
Chinese Goldthread rhizome						
HDQ Neutral						

Figure 3. Data table used to record the zone of inhibition size for each trial during each period of experimentation throughout the week.

Results

So, did this experiment answer the research question as to whether or not Chinese Goldthread could be used as a natural cleaning agent when compared to a QAC containing agent? Results show that the initial hypothesis has been supported in that the HDQ Neutral had declined in effectiveness over time while the Chinese Goldthread remained constant.

From the initial dilution after 18 hours of incubation, the *E. coli* within the agar plates had not grown. An error involved with the initial incubation of the *E. coli* (the medium the bacteria was stored in needed to be liquified, but was not for the first set of data) had led to no results for both products since the bacteria medium was not in the proper form for it to incubate properly. However, the HDQ Neutral was salvageable and data was able to be construed for this particular antibiotic through properly liquifying the medium and re-mixing leftover HDQ Neutral, unfortunately the Chinese Goldthread was not able to be construed due to no extract being left over and not enough time to acquire more. This had led to no data available for 0 hours for the Chinese Goldthread. Another petri dish was used just for the HDQ Neutral divided into five sections (trials). This can be seen below in Figure 1.

Figure 1. Salvageable HDQ Neutral sample showing trials 1-5.

So, for the zone of inhibition for the HDQ Neutral that was usable, the discs had shown an average of 10 mm. As for the control group for this time period, it had remained unchanged and no inhibition had occurred — which was the anticipated outcome. The data for each trial at 0 hours can be seen in Figure 2 below. The zone of inhibition among the discs can be seen in Appendix A.

0 hours after dilution	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	
Antibiotic	Zone diameter (mm)	Zone diameter (mm)	Zone diameter (mm)	Zone diameter (mm)	Zone diameter (mm)	Average Zone Diameter (mm)
Chinese Goldthread rhizome	N/A	N/A	N/A	N/A	N/A	N/A
HDQ Neutral	18 mm	15 mm	17 mm	12 mm	16 mm	15.6 mm
Control	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm

Figure 2. Chinese Goldthread was unusable for this set of data, however, the HDQ Neutral was salvageable and the zone of inhibition (mm) was measured above, leading to its average of 10 mm.

The data table above was broken down into two rows depicting the antibiotic's name, which then ran alongside the column that represented the trial number (six columns for five trials and one for the average from all of them). From there the results that were gathered were broken down into each category and averaged out at the end to determine what the zone of inhibition approximately was for that time period (i.e. 0 hrs, 96 hrs, 168 hrs).

It can be seen in the table that the zone of inhibition for the HDQ Neutral is large, however, it cannot be compared to the size of the Chinese Goldthread as no data was available for the extract. However, this data from the HDQ Neutral could be used for the next period of data gathering (96 hours) for comparison.

After 96 hours from the initial dilution and after having it incubated for 18 hours, the zone of inhibition size between trials 1-5 had shown that the HDQ Neutral did have a larger zone of inhibition average than that of Chinese Goldthread, with the HDQ Neutral averaging in 8.4 mm and the Chinese Goldthread averaging in 4.4 mm. Once again, the control remained constant with the first set of data at 0 hours with no zone of inhibition surrounding it. This data can be seen in Figure 3 below.

96 hours after dilution	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	
Antibiotic	Zone diameter (mm)	Zone diameter (mm)	Zone diameter (mm)	Zone diameter (mm)	Zone diameter (mm)	Average Zone Diameter (mm)
Chinese Goldthread rhizome	0 mm	6 mm	0 mm	8 mm	8 mm	4.4 mm
HDQ Neutral	7 mm	7 mm	8 mm	7 mm	13 mm	8.4 mm
Control	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm

Figure 3. HDQ Neutral averaged a larger zone of inhibition (8.4 mm) overall than Chinese Goldthread (4.4 mm), indicating that so far the E. coli is more susceptible to the HDQ Neutral, as predicted.

As seen above in the table, the Chinese Goldthread ranged between 0-8 mm for each trial, indicating that the *E. coli* is more resistant to the extract, or the bacteria is more likely to grow when this is present. While on the other hand the HDQ Neutral ranged between 7-13 mm for each trial, showing that the bacteria was more susceptible to the product, or that the bacteria is less likely to grow when this product is present. A picture of trial 3 from 96 hours is displayed below in Figure 4 as an example of what is being measured.

Figure 4. Trial 3 from 96 hours. Visible growth is demonstrated except for around the bottom white disc (HDQ Neutral), which is the zone of inhibition. No inhibition occurred around the other two discs (the brown disc is Chinese Goldthread and the other white disc is the control) which suggests that the bacteria is resistant to these discs.

This data is consistent with the original hypothesis in that the HDQ Neutral was predicted to have a higher zone of inhibition than the extract throughout the week and would gradually decline, which it has— starting at 15.6 mm at 0 hours and then declining to 8.4 mm at 96 hours. Since the Chinese Goldthread could not be used for data gathering due to the lack of data from 0 hours, the hypothesis for the Chinese Goldthread could not be supported at that period in time. However, with more data to come from 168 hours the hypothesis for the extract could still be supported or not depending on the incoming data.

The possible variability in the sizes among each trial could be attributed to an uneven distribution of the *E. coli* among the plates — meaning not each plate got the exact same amount of coverage in certain spots. Which was attempted to be avoided by fully streaking the bacteria across the plates and turning the swab and plate periodically to reach more coverage. However, with possible uneven distribution still in mind, this then could have possibly led to either a slightly larger or smaller zone of inhibition, hence the differing sizes of the rings among trials. Nevertheless, the data from these numerous trials was enough to draw an average out and make conclusive statements about the antibiotics and their susceptibility to *E. coli*, which so far is showing that the bacteria is more susceptible to the HDQ Neutral, and resistant to Chinese Goldthread.

Once again after 168 hours from the initial dilution the zone of inhibition size for the Chinese Goldthread for trials 1-5 was on average 4.84 mm, which is similar to the data from 96 hours which was 4.4 mm, showing consistency. As for the HDQ Neutral, it had no zone of inhibition form, resulting in an average of 0 mm, which means that the bacteria had been more resistant (more likely to grow when the product is present) to the HDQ Neutral at this point in the week. This can be seen in Figure 5 below.

168 hours after dilution	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	
Antibiotic	Zone diameter (mm)	Zone diameter (mm)	Zone diameter (mm)	Zone diameter (mm)	Zone diameter (mm)	Average Zone Diameter (mm)
Chinese Goldthread rhizome	0 mm	6.1 mm	6.1 mm	8 mm	0 mm	4.84 mm
HDQ Neutral	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm
Control	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm

Figure 5. 168 hours after the initial solution had shown no zone of inhibition for the HDQ Neutral, however, an average of 4.84 mm for the Chinese Goldthread.

The results for 168 hours had shown promising data that supported the hypothesis. With the HDQ Neutral showing no inhibition of the bacteria, this has demonstrated a trend of decreasing effectivity on the bacteria over time. With the Chinese Goldthread averaging at 4.84 mm, and it being consistent in size with the data for 96 hours, it is able to be concluded that at 0 hours the zone of inhibition average would have been around the same average (~4 mm) for the extract, since there was no data for that period. This is significant due to it showing that Chinese Goldthread had remained consistent in how effective it was against the bacteria over the course of the week, and the HDQ Neutral rapidly declined in how effective it was at stopping the growth of the bacteria, showing how the hypothesis was supported. This trend can be seen below in Figure 6.

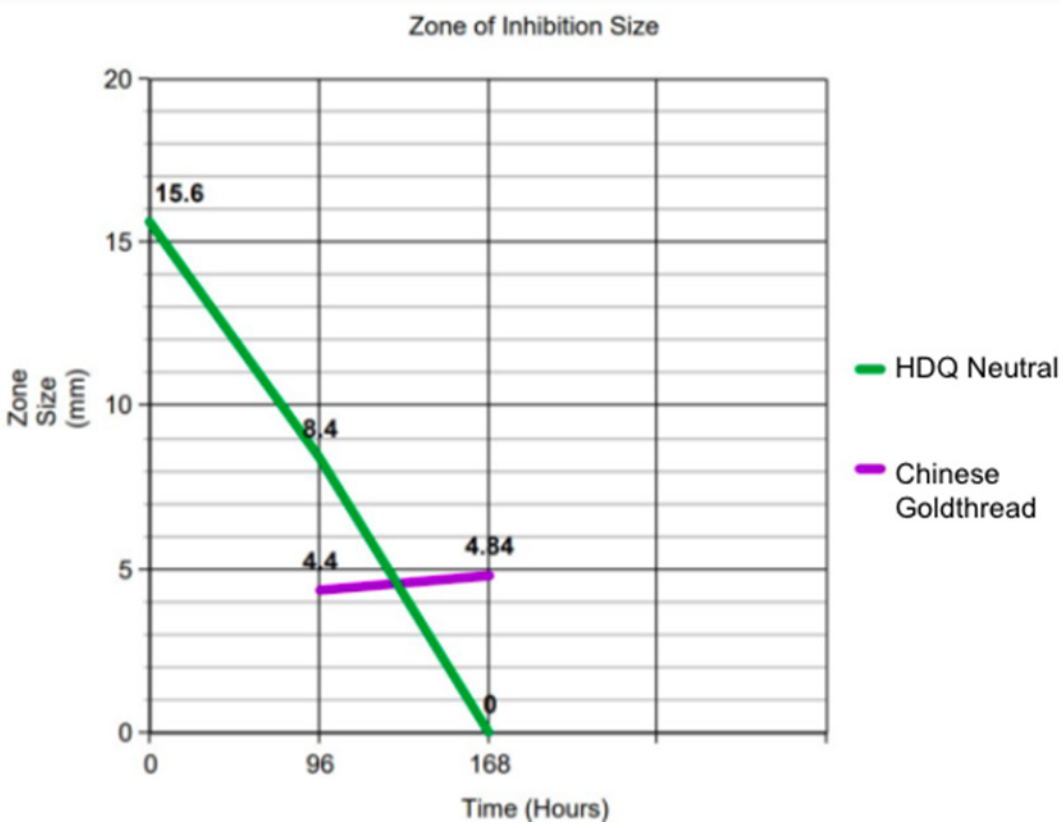

Figure 6. Trend of zone of inhibition averages for both HDQ Neutral and Chinese Goldthread (not showing data for 0 hours due to insufficient data). HDQ Neutral decreased rapidly throughout the week while Chinese Goldthread remains at around 4 mm.

While there are occasions that may occur that could have possibly skewed data, such as possible cross contamination, an uneven distribution of the *E. coli* among the plates (which can be seen below in Figure 7) having to do the experiment at 96 hours (four days) instead of 84 (exactly halfway through the week) due to time constraints, the use of tap water to match how it would be mixed in real world applications (as the HDQ Neutral is), and the unusability of the Chinese Goldthread discs at 0 hours, the 25 usable samples from the three data gathering periods support the original hypothesis — a decrease in the effectivity of the HDQ Neutral over time and consistency with how effective the Chinese Goldthread was over time.

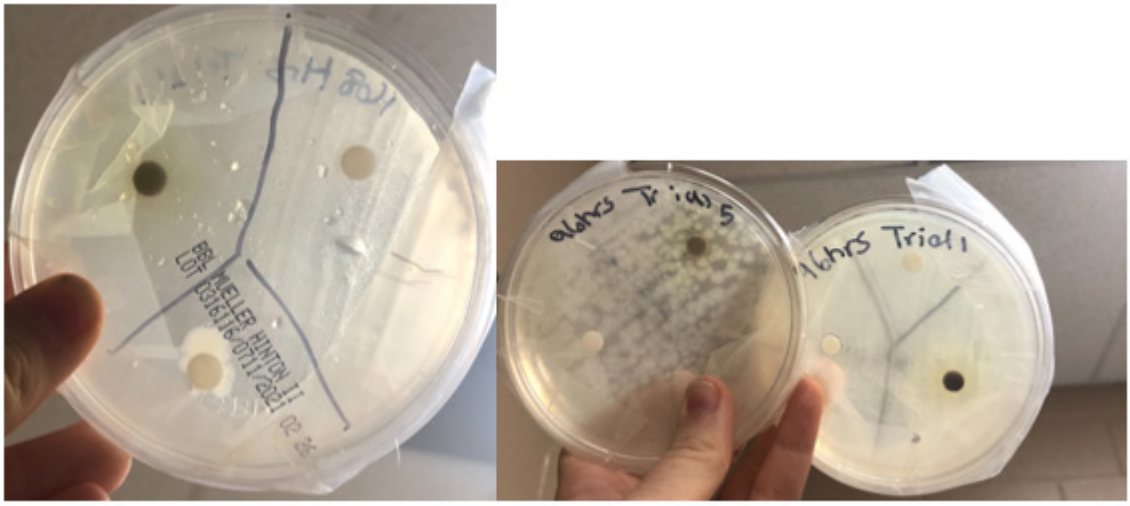

Figure 7. The control disc (bottom section) shows a cross-contamination that occurred, fungus had grown which was picked up from the air when inoculating the plates. The second picture on the right shows an uneven distribution of the E. coli among the plates.

In a study led by Gowoon Kim, who is part of the Department of Food Science & Technology, School of Agriculture and Biology, a similar study done on antibiotic resistant *S. aureus* that was used to determine Chinese Goldthread's cytotoxic activities (toxicity to cells) had determined the zone of inhibition to be approximately 23 mm.[10] Of course, the type of bacteria will define the size of the zone of inhibition and what is being measured (cytotoxic activities in this scenario). Naturally, the size will be different when compared to an experiment done on *E. coli*, especially non-pathogenic. However, it does make a good reference point for Chinese Goldthread and which bacteria is more resistant or susceptible to it, which is important to determine since for the purpose of being in a cleaning product, the extract would have to combat multiple bacteria at once since there are varying bacteria present on hard surfaces.

Similarly, Francesca Leach, a researcher who published her findings in Bioscience Horizons, did a related experiment on *E. coli* with Chinese Goldthread, however, not in comparison to cleaning agents.[7] She had determined that the zone of inhibition on *E. coli* was 10.3 mm for 12.5% of her original concentration (which she defined as a 1 in 10 dilution of water to 24hr old bacteria). This relates to this experiment as it is also done on *E. coli* and sets a point of reference for *E. coli* and the zone of inhibition for Chinese Goldthread. While her concentration is 12.5 percent of the original, whereas this one contained a concentration of 25mg/ml, and hers consisted of using distilled water, this could account for the slight variability in the average zone of inhibition sizes, with this experiment consisting of an average of ~4 mm for the extract and hers consisting of 10.3 mm.

This experiment has contributed back to the body of knowledge through seeing the extract's effectiveness on inhibiting *E. coli* specifically over the course of a week and comparing it to a cleaning product, which has not been researched before, has added new knowledge to the field of science. Through this it can be compared to more QAC containing agents or even isolated QACs themselves, as well as other bacteria over a specific course of time to determine its effectiveness.

This could imply that Chinese Goldthread, and moreover, TCMs, could possibly be applied to combat harmful ingredients, such as QACs, in cleaning products to prevent some future health risks and hazards, such as asthma or irritants to the respiratory system, that these QACs attribute to. This experiment has shown how the Chinese Goldthread is better for human health, as it is used as a medicine already, and remains as effective over time on inhibiting the growth of *E. coli* for cleaning purposes. Whereas the HDQ Neutral is worse for human health and does

not remain as effective over time on bacteria. This is a significant finding to go off of for future experiments as it shows the ability of some natural medicines to possibly replace QACs in effectivity and health safety.

Conclusion

As people continuously use cleaning products to clean their home and work spaces, these numbers associated with exposures to harmful ingredients will continue to rise — especially during the COVID-19 pandemic as more people are cleaning and disinfecting on a daily basis to protect themselves. A need for a natural alternative is prominent to combat these ingredients, such as QACs — which attribute the most to exposures. Traditional Chinese Medicines (TCMs), such as specific plant extracts are a good alternative to investigate as they have been used for hundreds of years to treat common ailments, and exhibit strong antibacterial properties.

This research concluded that Chinese Goldthread rhizome (a TCM) is an effective and consistent TCM to use against hard surface bacteria — specifically *E. coli* — as shown by the consistent zone of inhibition size throughout the week when compared to HDQ Neutral, which had declined in contrast. With this outcome, it becomes possible to envision Chinese Goldthread as a combatant against hard surface bacteria in place of QACs, which adds a new understanding to research as this ability to be a cleaning agent was not known before. Through this, it could result in a possible decrease in exposures and risk to humans' health from these ingredients, and a safer environment.

Adding to a study by Micheal Popp,[11] which addressed the possibility of plant extract hydrolyzates to be put forth into a detergent or cleaning agent, this research expands upon the body of knowledge Popp had previously researched through contributing another plant extract into the data pool that could be used for further research. It allows further conclusions to be drawn from these extracts in their ability to be put forth into something better for health.

However, the overarching limitations of this research has been cross-contamination of outside fungus improper initial incubation of the *E. coli*, which could be avoided in future research by placing the lids back onto their respective plates after each plate has been inoculated to ensure no outside air particles land on the agar, and to through properly preparing the bacteria at hand prior to experimentation. While these may have prevented secure data in areas, it provides a gateway to future research that could be improved upon.

Further research would have to conducted with careful attention to fully conclude the Chinese Goldthread could be used as a complete substitute for QACs in cleaning agents through testing it against more QAC containing cleaning products, combining it with other TCMs, or even testing against different bacteria, viruses, or fungus due to their presence on hard surfaces as well.

Going forward, this experiment could be replicated and perfected further through changing slight variables such as the concentrations, type of water used, or ensuring full plate coverage in order to work towards a solid conclusion that Chinese Goldthread can be used against other agents and microorganisms in a cleaning agent as opposed to QACs.

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