



Evaluating the Effectiveness of Natural Antimicrobials and Chemical Disinfectants on the Inhibition of *E. coli* K12 Growth

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Abstract

E. coli is one of the most researched and common microorganisms found in the natural world, with many different strains that can be both harmless and harmful, and the growth of those more dangerous strains must be prevented inside and outside the household. Natural antimicrobials and chemical disinfectants are two common, but very different, forms of defense against the growth of *E. coli*, each containing different chemical components and mechanisms of action that can have both positive and negative impacts on food, as well as safe cleaning and hygienic practices. With the growth of the “clean eating” movement, many people are leaning more towards the usage of natural antimicrobials instead of chemical disinfectants. This influx of interest in natural antimicrobials brings about a necessity for further research on their mechanisms of action and ability to prevent the growth of bacteria. Therefore, this paper will evaluate the efficiency of both natural antimicrobials and chemical disinfectants to compare the inhibition of growth of *E. coli* K12. Within this study, chemical disinfectants were found to be more efficient than natural antimicrobials in the inhibition of *E. coli* K12 growth, however natural antimicrobials do still have a measurable effect. The difference in efficiency between the two cleaning agents demonstrated a significantly higher suppression of *E. coli* growth, which could help to inform individuals when making the choice between natural antimicrobial or chemical disinfectant products, especially in household cleaning and disinfecting.

Introduction

Escherichia Coli is one of the best researched microorganisms in the world, and can be separated into 11 different pathotypes, with small differences in each pathogen group that differentiate them (Geurtsen et al.), with different impacts on human health and wellness. The strains of *E. Coli* that most people are familiar with are the dangerous ones, for example, O157:H7, the strain that resulted in the infamous Jack in the Box outbreak of 1993 (FAQ). However, most strains of *E. Coli* are natural parts of the gut microbiome, aiding in digestion and protection against harmful microbes (FAQ). *E. Coli* strains are differentiated by certain markers in their DNA, like toxins (FAQ; Geurtsen et al.) with Shiga toxin 1 or 2 (*stx1* or *stx2*) found in Shiga toxin-producing *E. coli* (STEC), verocytotoxin-producing *E. coli* (VTEC), and enterohemorrhagic *E. coli* (EHEC), correlating with bloody diarrhea and kidney failure (Pakbin et al.; Geurtsen et al.).

The more dangerous strains of *E. coli* can inject different toxins, called effectors, into their host cells through sword-life apparatuses called secretion systems. Additionally, they can allow the *E. coli* to attach to the host's cell surface and initiate infection. This process is called “attaching and effacing,” and is known to create tissue damage in intestinal lining (Pakbin et al.; Johnson and Nolan). These more dangerous strands of *E. coli* are the culprits of thousands of cases of foodborne illness in the United States alone, with STEC O157:H7 causing an estimated 97,000 illnesses, and STEC non-O157:H7 causing an estimated 169,000 illnesses (CDC, “Technical Information”). STEC O157:H7 infections can cause traumatic infections like bloody diarrhea, hemorrhagic colitis, and can even result in kidney failure (Xu et al.; Pakbin et al.). As the demand for ready-to-eat foods increases, (Xu et al.) it is of the utmost importance to ensure

that food processing plants are safe from exposure to these dangerous *E. coli* strains.

Natural antimicrobials (or bio preservatives) are defined as naturally occurring compounds that are easily extracted, and can be organised into three main groups: from animals, microorganisms, or plants (Fan et al.; Karnwal and Malik). Antimicrobials from plants (phenolics, meaning that they contain one or more hydroxyl groups) are created through chemical synthesis of discarded parts of plants, making them more cost-effective and naturally occurring (Fan et al.). Many people are unaware that common household items, honey, rosemary, and cinnamon, are natural antimicrobials and all under this phenolic group. Honey works well as an antimicrobial due to its high sugar content, low pH, as well as the presence of hydrogen peroxide (H_2O_2) in its chemical makeup (Ogwu and Izah). These properties are known to create a hostile environment towards many pathogens, as a low pH is unfavorable to bacteria growth. A high sugar content works to draw the water out of bacteria cells, creating a hypertonic environment that is conducive to cell dehydration and death, while the inclusion of hydrogen peroxide helps to disrupt biofilm formation and protein synthesis in bacteria cells, while also working as a natural preservative (Ogwu and Izah).

Carnosic acid ($C_{20}H_{28}O_4$), caffeic acid ($C_9H_8O_4$), and rosmarinic acid ($C_{18}H_{16}O_8$) are found in rosemary, and these compounds are all known to have antimicrobial activities. Carnosic acid has been found to have stronger antimicrobial activities than rosmarinic acid, and is also known to have anti-inflammatory and antitumor capabilities. This highlights the idea that high concentrations of carsonic acid found in rosemary extracts may be helpful to fight diseases as well as foodborne illnesses (Moreno et al.). Also, the leaves and bark of the cinnamon plant, usually obtained from the inner of the tree, are known to have strong antimicrobial activity. The most prevalent metabolites in cinnamon extract include cinnamaldehyde (C_9H_8O), trans-cinnamaldehyde, cinnamic acid ($C_9H_8O_2$), and its conjugate base, cinnamate (Vasconcelos et al.). Trans-cinnamaldehyde, the trans isomer (two groups of molecules on either side of a double bond) of cinnamaldehyde, is mostly responsible for the antimicrobial activities of cinnamon extract, due to the acrolein group. (Vasconcelos et al.) The acrolein group is a group of atoms that are known to react strongly with sulfur and nitrogen atoms. These sulfur and nitrogen atoms are necessary for bacteria to survive, and the active compounds in cinnamon have the ability to disrupt cellular functions (Vasconcelos et al.).

Chemical disinfectants are commonly used in healthcare settings, food preparation, and even home environments. The term “disinfectant” refers to an antimicrobial agent that works best on non-living surfaces and living tissues (Maillard and Pascoe). Chemical disinfectants are used when intense heat cannot be used to kill off bacteria, viruses, or fungi (CDC, “About Chemical Disinfectants and Sterilants and Reproductive Health”). Disinfectants can reduce, but not entirely destroy, microorganisms, meaning that infection is highly unlikely (Curran et al.). Bleach and hydrogen peroxide (H_2O_2) are two of the most common household chemical disinfectants. Bleach can destroy bacteria by dissociating into two groups of molecules to form hypochlorous acid (HClO), which works to attack the amino acids that make up proteins within the bacteria (“How Does Bleach Bleach? | Nature”). Hydrogen peroxide, on the other hand, works by creating an oxidation burst, a large and rapid production of oxygen molecules, that disrupts and destroys cell functions (Juven and Pierson).

Chemical disinfectants greatly differ from natural antimicrobials because they are derived from different sources, with antimicrobials coming from natural sources like plants and animals, and disinfectants coming from chemicals. The differences in origin of these two compounds impact the implications and side effects of constant usage of them. With the growth of the “clean

eating” movement, more research is needed to compare the effectiveness of natural antimicrobials and chemical disinfectants. Therefore, this paper will investigate if natural antimicrobials (honey, cinnamon extract, and rosemary extract) work better than chemical disinfectants (hydrogen peroxide and bleach) to kill *E. coli*.

Methods

Strains and media.

E. coli strain K-12 sample was obtained from Carolina Biological Supply Company, grown in a Nutrient Agar plate. Prepoured Luria-Bertani Agar (LB) Petri dishes with a 10 cm diameter were obtained from Eevivia sciences through Amazon.com.

Natural and chemical disinfectants.

Cinnamon extract was obtained from Watkins 1868™ through Amazon.com, made up of alcohol, water, and cassia oil in a 2 fl oz plastic bottle. Rosemary extract was obtained from Botanic Choice® through Amazon.com, in a 5:1 ratio extract made up of rosemary extract, vegetable glycerin, and purified water in a 1 fl oz in a glass bottle. Appalachian Mountain Wildflower Honey was purchased through Wehrloom Honey, in a 16 fl oz glass container. Concentrated bleach solution was obtained from Harris Teeter™, with a 7.5% Sodium Hypochlorite solution, housed in a 43 fl oz plastic container. Hydrogen Peroxide in a 3% solution was also obtained from Harris Teeter™, in a 16 fl oz plastic container.

Zone of inhibition.

E. coli was streaked across a LB agar petri dish using a quadrant streak method to isolate an individual colony of bacteria. Petri dishes were then incubated at room temperature in a dark room for four days. Once bacteria colonies had been isolated, a single colony was then streaked across a new petri dish. 10 mm filter paper circles were soaked in each disinfectant until fully saturated, and then placed onto the new petri dishes. Three filter papers were then soaked in each individual disinfectant and placed in the dishes with the same bacteria colony. The entire process was then repeated two more times, for a total of three biological replicates. The plates were again incubated at room temperature in a dark room for four days before measurements were taken. As a control group, a filter paper disc was soaked in water and placed on a streaked bacteria plate, to insure inhibition of growth was not due to the filter paper itself. Zone of inhibition was measured in mm starting from the edge of the filter paper to the closest bacteria colony.

Results

Zone of inhibition was the largest for bleach

Table 1 shows the average zones of inhibition across the five different disinfectants tested. Bleach, with a zone of inhibition of 27 mm (Table 1) across all three biological replicates, was found to have the largest zone of inhibition when compared to both the chemical disinfectants and natural antimicrobials. Hydrogen peroxide (H₂O₂) was a close second, with an average of 20.33 mm (Table 1) across all three biological replicates. Of the natural antimicrobials, cinnamon extract had the largest zone of inhibition across the three biological replicates, about 3.22 mm (Table 1). Honey and rosemary followed up, with average zones of inhibition of 1.44 mm and 0.39 mm respectively (Table 1).

Table 1. *Zones of inhibition produced by different disinfectants across biological replicates.* The table shows the average zone of inhibition (mm) measured for rosemary extract, cinnamon extract, honey, bleach, and hydrogen peroxide (H_2O_2), which were diluted as stated in the methods. The average across three technical replicates is shown for three biological replicates. The final column represents the overall zone of inhibition across the three biological replicates.

Disinfectant	Replicate 1 average zone of inhibition (mm)	Replicate 2 average zone of inhibition (mm)	Replicate 3 average zone of inhibition (mm)	Average of biological replicates zone of inhibition (mm)
Control (H_2O)	0.0	N/A	N/A	0.0
Rosemary extract	1.0	0.0	0.16	0.39
Cinnamon extract	3.0	3.0	3.7	3.22
Honey	1.3	1.0	2.0	1.44
Bleach	23.0	26.3	31.7	27.0
Hydrogen Peroxide (H_2O_2)	14.3	21.3	25.3	20.3

Chemical disinfectants produced larger zones of inhibition than natural disinfectants

Figure 1 shows a visual representation of the average zones of inhibition across the five different disinfectants. Overall, the chemical disinfectants were shown to create larger zones of inhibition than the natural antimicrobials. Bleach had the largest zone of inhibition (Fig. 1, Fig. 2), followed closely by hydrogen peroxide (H_2O_2), the two had a difference of 6.67 mm (Fig. 1, Fig. 2). Cinnamon had the largest zone of inhibition of the antimicrobials, with a difference of 1.78 mm (Fig. 1, Fig. 2) between that of honey, and a difference of 2.83 mm (Fig. 1, Fig. 2) between that of rosemary. Honey and rosemary had a difference of 1.05 mm (Fig. 1, Fig. 2) in their zones of inhibition.

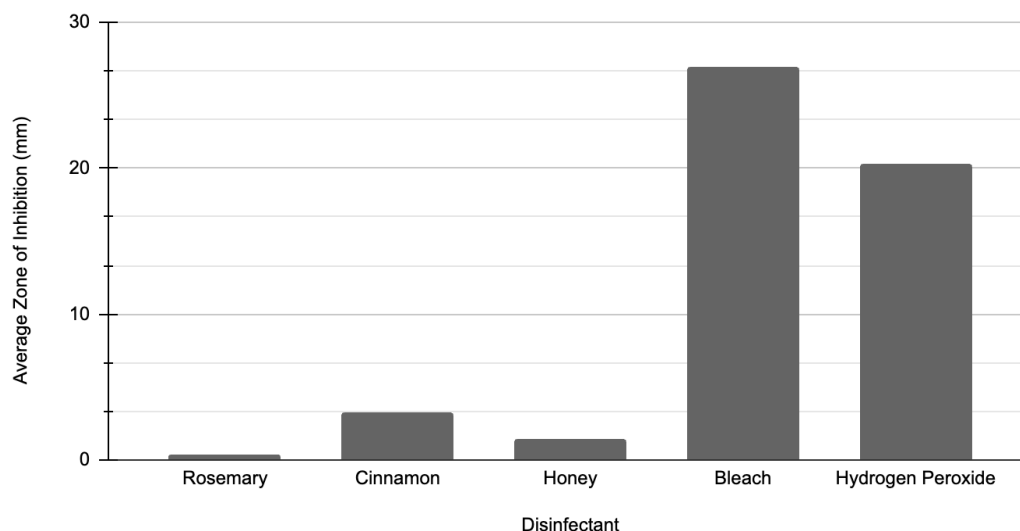


Figure 1. Average zone of inhibition produced by different disinfectants. The graph shows the mean diameter (mm) of *E. coli* growth inhibition zones for rosemary extract, honey, cinnamon extract, 10% bleach, and hydrogen peroxide.

Figure 2. Representative images of the average zone of inhibition by different disinfectants. These representative images of the data collected to find the average zones of inhibition of *E.*



coli growth for rosemary extract, honey, cinnamon extract, 10% bleach, and hydrogen peroxide. The H quadrant is that of honey, C is the cinnamon extract, R is the rosemary extract.

Discussion

Investigating the differences between chemical disinfectants and natural antimicrobials was done with the goal of discovering which type of disinfectant is more effective at preventing the growth of *E. coli* bacteria. Within the “clean eating” movement, there is a large push for an increase in natural antimicrobials instead of chemical disinfectants. However, a comparative analysis between chemical disinfectants and common active agents in natural disinfectants is needed to allow for the consumer to have a thorough knowledge on the antimicrobial activities of both chemical and natural disinfectants. When making the decision to choose between a natural antimicrobial or a chemical disinfectant, it is important to understand the depth at which either of these are able to properly kill bacteria and reduce the overall amount of pathogens within one’s living space. Through a thorough analysis of the data collected, it was found that the chemical disinfectants were much more successful at inhibiting the growth of *E. coli* K-12.

Both of the chemical disinfectants showed large zones of inhibition: Bleach had an average of 27.0 mm of inhibition (Table 1, Fig. 1, Fig. 2) and hydrogen peroxide followed closely with an average 20.3 mm zone of inhibition (Table 1, Fig. 1, Fig. 2). All the trials were compared to a control group (H_2O), which had a zone of inhibition of 0 mm (Table 1). The lack of zone of inhibition for the control group supports the conclusion that the zone of inhibition that was observed was due to the disinfectants themselves, not the filter paper or any other variables. Compared to the chemical disinfectants, the natural antimicrobials showed a much smaller zone of inhibition, but they did still have measurable effects. The highest performing natural antimicrobial observed was cinnamon, with an average zone of inhibition of 3.22 mm (Table 1, Fig. 1, Fig. 2). Honey and rosemary followed behind, with average zones of inhibition of 1.44 mm (Table 1, Fig. 1, Fig. 2) and 0.39 mm (Table 1, Fig. 1, Fig. 2) respectively. Comparison of the zones of inhibition for all the cleaning agents tested showed clearly that both of the chemical disinfectants had stronger results compared to the natural antimicrobials (Fig. 1, Fig. 2). These results are also reinforced given that the control group had a zone of inhibition of 0 mm. Overall, these results strongly indicate a strong growth inhibition with chemical disinfectants compared to natural antimicrobials.

There are many aspects of the chemical makeup of a disinfectant that can contribute to its overall high success in preventing the growth of *E. coli* bacteria when in comparison to a natural antimicrobial. Chemical disinfectants can work in many different ways, each one specific to that particular disinfectant. The mechanism of action of bleach is well established: bleach attacks the different amino acids that make up the proteins that the bacteria need to survive by disassociating into two groups of molecules and forming hypochlorous acid ($HClO$), a weak acid with strong oxidation capabilities ("How Does Bleach Bleach? | Nature"). Similarly, hydrogen peroxide inhibits the growth of bacteria by increasing the amount of hydroxyl radicals within a bacterial cell. The production of these hydroxyl radicals can oxidize and damage DNA, cell membranes, and proteins. This process is known as an oxidation burst, and can ultimately disrupt and destroy cell functions within the bacteria itself (Juven and Pierson). While the exact mechanisms of these two chemical disinfectants are not the same, both target essential cell components, leading to cell death.

Natural antimicrobials had a much smaller impact on bacterial growth inhibition. This result could be due to low concentrations of the active compounds with the antimicrobial capabilities contained within the natural antimicrobials. In fact, research has shown that natural antimicrobials have been found to be more effective in higher concentrations (Gonelimali et al.). This leads to the possibility that there was a low concentration of antimicrobial compounds present within the extracts used in this study, as they were not pure substances. Another possibility for the low levels of bacterial growth inhibition observed with natural antimicrobials could be that the active agents contained within the antimicrobial agents themselves could be highly chemically unstable, meaning that they degrade quickly over time. Thereby losing their antimicrobial capabilities (Pinto et al.; Yarmolinsky et al.). In fact, antimicrobials are highly susceptible to changes in pH, temperature, and concentration (Pinto et al.), all of which can be impacted during processing and storage, and which could have contributed to the low levels of bacterial growth inhibition observed. Additionally, within this study, the products used were not standardized by concentration of active ingredient, making a direct comparison difficult. Overall, this presents the possibility that the natural antimicrobials may have the capacity to increase bacterial growth inhibition, but the commercially available versions may not be standardized, stable, or have high enough concentrations to demonstrate the full effect.

Implications, Limitations and Future Directions

Though the chemical disinfectants showed an obvious larger zone of inhibition and ability to stop the growth of bacteria, the natural antimicrobials did show an effect. The disinfectants were much superior in potency when compared to the antimicrobials, but both did have a quantifiable effect on the *E. coli* growth. Since the chemical disinfectants were able to kill much more bacteria than the natural antimicrobials, it is safe to assume that the natural antimicrobials may not be the best choice for eliminating pathogens, especially in household cleaning and food production and storage. However, this does not mean that they are useless; natural antimicrobials may best serve as preventative or complementary measures for cleaning. Using natural antimicrobials before, or alongside, chemical disinfectants could allow for a decrease in chemicals in food products, and households, whilst also decreasing the number of pathogens present.

Optimization of the natural antimicrobials is crucial to ensure that they are being used to their full potential. The extraction and concentrations of these antimicrobials from their respective plants, microorganisms, and animals can influence their ability to show antimicrobial capabilities. Specifically, the solvent used to extract the active ingredient in natural antimicrobials may play a substantial effect in the overall quality and stability of these substances in inhibiting the growth of bacteria. Ethanol is found to be the most effective solvent throughout all extraction techniques, and is shown to extract the highest matter of bioactive compounds (Haido et al.). This is because ethanol can act as a solvent for both polar and nonpolar molecules due to its molecular structure. In addition, the concentration of active antimicrobials extracted from natural products strongly impacts the antimicrobial activities of the compounds (Bernier and Surette). The higher the concentration achieved, the more likely that the antimicrobial will be successful (Bernier and Surette). When the concentration is too low, the bacteria have the possibility to sense the presence of an antimicrobial, triggering different cellular functions to best employ defensive measures (Bernier and Surette).

One of the main limitations of this experiment is that only one strain of *E. coli* (K-12) was tested. *E. coli* O157 is most correlated with foodborne illness. Given this, O157 is a pathogenic strain of *E. coli*, whilst K-12 is a nonpathogenic, laboratory strain. Due to this difference in type of strain, it is possible that O157 would behave differently than K-12 when exposed to different natural antimicrobials or chemical disinfectants. So, the direct impact of these cleaning agents on the growth of *E. coli* K-12 cannot be directly compared to the growth inhibition of another strain, without directly testing that strain. Another limitation is that the antimicrobials and disinfectants were not laboratory standardized. Standardization would ensure that all the compounds are at the optimal concentration for the experiment, and would also standardize the purity of the extracts used. Finally, disc diffusion was the method used to evaluate *E. coli* growth inhibition. While this is a common and reliable laboratory technique, it may not be the most representative of *E. coli* growth changes in food or *in-vivo* (taking place inside an organism). For disc diffusion to be truly successful, it is crucial that high-quality reagents are used in conjunction with quality control and exact compliance with the methodology of the experiment (*Disk Diffusion - an Overview* | ScienceDirect Topics). Additionally, these findings should be paired with other techniques to validate the findings.

Future directions for a related experiment should be tested with multiple strains of *E. coli*, both pathogenic and nonpathogenic. Additionally, standardized plant extracts and purified compounds should be used. Future experiments could help to validate these results, and could



help to answer the question of the efficacy of natural antimicrobials, and what context they may be useful in. Finally, future experiments could investigate this topic using different methodologies, to clarify the efficacy of antimicrobials in a context more applicable to food production and storage processes. While chemical disinfectants were found to be superior to natural antimicrobials in eliminating *E. coli*, there is always a food safety concern. Applying practical considerations to this experiment, it could be dangerous to support an increased use of chemical disinfectants, as they can leave residues and affect food quality. Natural antimicrobials may be preferred by some, due to some of the negative impacts of chemical disinfectants, as well as the alignment with the “clean eating” movement. As this movement grows in popularity, it is important to discover the level of actual antimicrobial activities that these extracts show, and to decide if they are strong enough to be used in food safety to prevent the growth and spread of bacteria. It is possible that it might offer safer, consumer-friendly alternatives, but there must be more research on their stability and reliability. In conclusion, the choice between a natural antimicrobial and a chemical disinfectant is heavily reliant on the context of the situation, however it is important to remember the efficacy that each can offer.

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