Analysis of Common Food Remedies as Quorum Sensing Inhibitors

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Since traditional antibiotics have been known to surface extremely potent forms of pathogenic bacteria, allowing them to survive and reproduce in the human body, a new pathway to treating bacterial infections needs to be found to combat this cycle, commonly referred to as antibiotic resistance. In the recent past, research has been done about quorum sensing, or the communication network found in bacteria, and how stopping it or slowing it down can cause individual bacterium to become isolated and die. This study tested eight different extracts, commonly found in households and used as home remedies for common bacterial infections, on four species of bacteria, three of them being human pathogens, for their ability to inhibit quorum sensing in these bacteria. The only extract that proved to be effective in this manner was cinnamon when used with the pathogen *Staphylococcus aureus.*

Bacteria are some of the Earth's oldest inhabitants; the oldest species have been present on our planet for about 3.5 billion years. These beautiful organisms can vary in shape, size, and color, and can serve many different purposes. Bacteria such as those present in dairy products like yogurt and sour cream can play a large role in their appealing texture and taste as well as aid in digestion. Furthermore, other kinds of bacteria are responsible for the decomposition of dead organic matter. If it weren't for these kinds of bacteria, the earth would be overrun with the waste of deceased plants and animals.

Unfortunately, in our society, the word *bacteria* tends to have a negative connotation associated with "germs" and disease. It is true that bacteria can have extremely harmful effects, and bacterial infections in the human body can make us very sick and can even be fatal. For example, bacteria from the *salmonella* genus are responsible for food borne illness and typhoid fever, two ailments that kill hundreds of thousands of people worldwide. Also, the deadly *Mycobacterium tuberculosis*, responsible for tuberculosis, has infected millions throughout the course of history, and was responsible for one third of all deaths in the early nineteenth century.

Many courses of action have been taken by scientists and medical professionals to prevent the spread of bacterial infections and to help provide treatment and a cure to those suffering from them. These forms of prevention and treatment include standard antibiotics that simply kill the bacteria inside your body, and vaccines that introduce your body to weakened or dead forms of the bacteria, and trick your immune system into thinking that your body has already had the infection. However, the overuse of antibiotics including antibacterial soaps, hand wipes, hand sanitizers, cleaning products, eye drops, shampoo, mouth and face wash, and many other products have led to antibiotic resistance that only weakens the effect of such products. Antibiotic resistance occurs when a product infused with antibiotics kills off the majority of

bacteria present, but leaves behind a small amount of bacteria- usually the most potent forms that are then able to reproduce and thrive, unable to be affected by antibiotics. This deadly cycle hassled to “superbugs” that are left behind, that have the potential to wreak havoc on the human body. Fortunately, antibiotic resistance can be avoided. First, antibacterial products, contrary to popular belief, should be avoided unless one is suffering from a bacterial infection. Second, it is important that antibiotics are only used when prescribed by a medical professional and that only the suggested dose is taken.

A third approach can also be taken to avoid the perils of antibiotic resistance. Quorum sensing (QS), or the communication between individual bacterium, is a process studied in the recent past by scientists that has shown promising potential for alternate treatment of bacterial infections by which antibacterial resistance can be altogether avoided. When QS stops in a colony of bacteria, individual cells are no longer able to communicate with each other, and therefore become isolated and die. Many natural substances, like the antioxidants found in different fruits, vegetables, spices and herbs can inhibit QS, and if these substances are introduced to a colony of bacteria, they are effective in killing the cells and drastically reducing the growth of the colony. Thus, with further research these substances can eventually be used on humans to treat infections and kill bacteria without the fear of developing antibiotic resistance.

In 2006 at Florida International University, a study in which 50 different plant species found in various locations in southern Florida were tested for their QS-inhibiting ability. This study was called “Anti-quorum sensing activity of medicinal plants in southern Florida” (Adonozio, et. al, 2006), and it proved the effectiveness of natural substances in QS inhibition. This article also rejected the theory that microbes growing on the surface of plants like lichens and epiphylls were the cause of QS inhibition instead of the plants themselves, because these

were removed and screened separately before the start of the experiment, and they showed no effect on the growth of cells. “Quorum sensing inhibitors as anti-pathogenic drugs” and “Quorum sensing in *Escherichia coli* and *Salmonella*”, both published in 2006, provide an overview as to how quorum sensing works in pathogenic bacteria and the general process of using quorum sensing inhibitors to treat infections.

In the 2007 article entitled “Dietary phytochemicals as quorum sensing inhibitors” (Vattem, et.al, 2007), a study was conducted with thirteen different fruit and spice extracts on *Chromobacterium violaceum*, which was used as a wildtype because of the dark pigmentation produced, and *Pseudomonas aeruginosa*, a pathogen that can cause infections in the lungs, kidneys, and urinary tract. The study showed that all thirteen extracts, including common fruits, spices, and herbs such as strawberry, blueberry, raspberry, basil, and oregano showed inhibition of QS and inhibition of the production of Acyl-homoserine lactone(AHL), a chemical that acts as an autoinducer and that transfers communicatory signals between bacteria. The extracts most effective in both categories were blueberry, strawberry, and ginger. From this study, one can conclude that the consumption of the food items used can help strengthen one’s immune system and prevent and treat bacterial infections without risk.

A similar study conducted in 2011 at Nanyang Technological University in Singapore entitled “Screening of traditional Chinese medicinal plants for quorum-sensing inhibitors activity” (Koh & Tham, 2011) featured a similar concept and tested plants used in traditional Chinese culture for medicinal purposes. In this study, 10 different species of traditional Chinese medicinal (TCM) plants were tested to see if they inhibited QS in the way that the extracts did in Vattem’s study. The results showed that seven out of the ten TCM plants tested showed anti-QS activity, and that *Panax notoginseng*, also known as ginseng, demonstrated the highest level of

QS inhibition. This study is significant because it shines light on the use of traditional medicinal plants from ancient cultures as effective ways to treat bacterial infections in the modern day and age.

For my own experiment, I decided to do an experiment similar to the one inVattem's study, except with different natural substances and with pathogenic bacteria that more commonly cause infections in the human population. Instead of testing fruits and vegetables, I decided to take the approach of testing substances that one usually consumes when sick, such as ginger ale, chicken broth, and rice water. If my study proves to be significant, it will shed light on different foods that one can consume to help protect their bodies from the wrath of pathogenic bacteria, as well as provide a healthy alternate approach to antibiotic resistance, and lessen the ghastly effects that bacteria have on the modern human population.

Purpose

The purpose of this study was to challenge the scientific validity of "old wives' tales" claiming that certain household items can provide relief to people suffering from bacterial infections. I hypothesized that most if not all of the extracts would prove to be scientifically significant by creating zones of inhibition when introduced into a colony of bacteria.

Methodology

The strains of bacteria used for this study were the human pathogens *Staphylococcus aureus* and *Escherichia coli* and the non-pathogen *Chromobacterium violaceum. S. aureus* and *E. coli* were chosen because of the harmful effects they have on the human body: *S. aureus* is known to cause respiratory illnesses such as sinusitis, skin infections such as boils, while both *E. coli* and *S. aureus* are known to cause serious cases of food poisoning. *C. violaceum* was used as

a wildtype for this study because of the dark violet-colored pigment produced while the species undergoes quorum sensing. To prepare the bacteria, they were grown in a nutrient broth and refrigerated.

The extracts used were garlic, honey, orange juice, cinnamon, cumin, mustard seed, anise seed, and cayenne pepper. Extracts were first chosen due to their widespread consumption during times of illness or anecdotal evidence of antiseptic properties. To prepare each extract for inoculation, they were ground with a mortar and pestle and then soaked in a 50% water and 50% acetone mixture and then filtered with filter paper. The extracts were then set aside in room temperature for about an hour for the acetone to evaporate. Blank sterile paper discs were placed into the extract solutions and left overnight to soak in the liquid.

Table 1: Methods, including extracts used an method of preparation. This table also includes faulty trials in which extracts were present in the form of oils rather than using the acetone method.

Trial #	Extracts	Procedure
1	cinnamon	Stirred into a 1:1 mixture of cinnamon and olive oil, then boiled and left to cool
	honey	Stirred into a 1:1 mixture of honey and tap water, then boiled and left to cool
	garlic	Stirred into a 1:4 mixture of garlic and tap water until dissolved
	orange juice	Stirred into a 1:1 mixture of orange juice and tap water
2	cinnamon	Same as trial 1
	honey	
	garlic	
	orange juice	
3	cinnamon	Solvents were stirred into a 1:1

	honey	mixture of acetone and water so that the solute to solvent volume ratio was 1:6, then vacuum filtrated with a coffee filter and left to evaporate
	garlic	
	orange juice	
4	cinnamon	Same as trial 3
	honey	
	garlic	
	orange juice	
5	anise seed	Same as trial 3
	mustard seed	
	cumin	
	cayenne pepper	
6	anise seed	Same as trial 3
	mustard seed	
	cumin	
	cayenne pepper	

To grow the bacteria, sterile cotton-tipped applicators were placed into each broth containing the three species of bacteria, and a standard agar solution was poured into growth plates and left to cool so that the bacteria had a medium to grown on. Once the cotton tip was soaked in the broth, it was dragged along the plate so that the broth fully coated the agar. Then, metal tweezers were used to transfer the blank sterile disks from the extract solution onto the plate, sterilizing with a Bunsen burner flame in between each transfer. After inoculation, the plates were placed in an incubator and left to sit for 24 hours.

Results

Contrary to my hypothesis, the majority of the extracts did not show zones of inhibition in any of the bacterial strains. The only extract that proved to be significant was cinnamon, and the only strain in which zones of inhibition could be found was S. aureus. A clear halo 1.5 centimeters in diameter was found for that particular section of the plate, while for all other extracts and bacterial species, no interruption of growth was found.

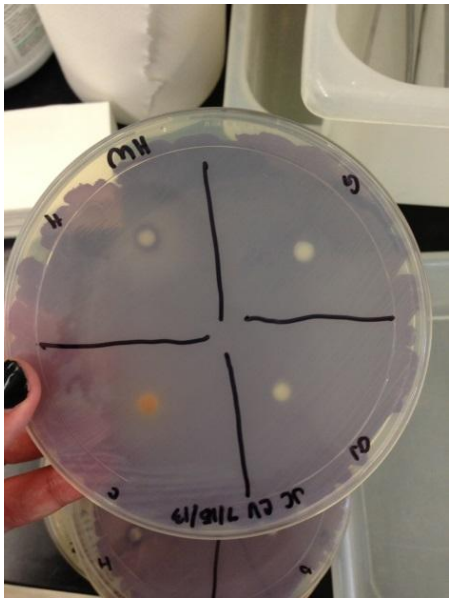


Figure 1.1: The results of Trial 4 on *C. violaceum*; no extracts show any signs of inhibition. Notice the dark pigment in comparison with the other two species of bacteria.

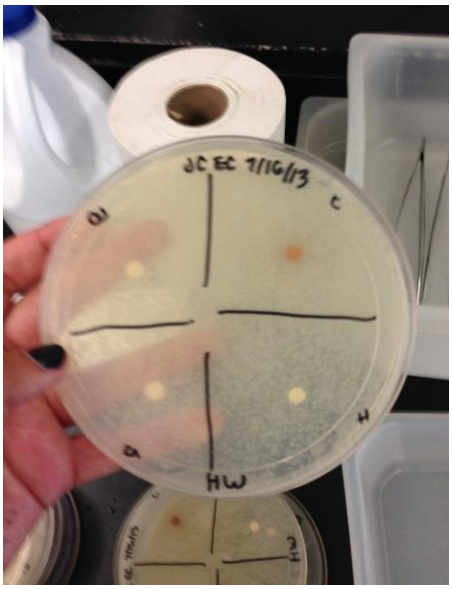
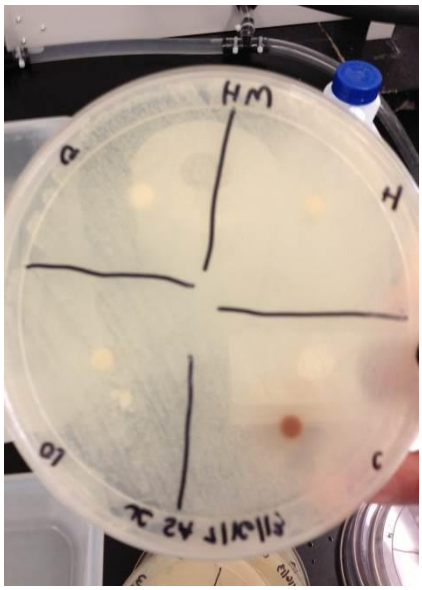


Figure 1.2: The results of Trial 4 on *E. coli.* Similar to in *C. violaceum*, no signs of inhibition were found.



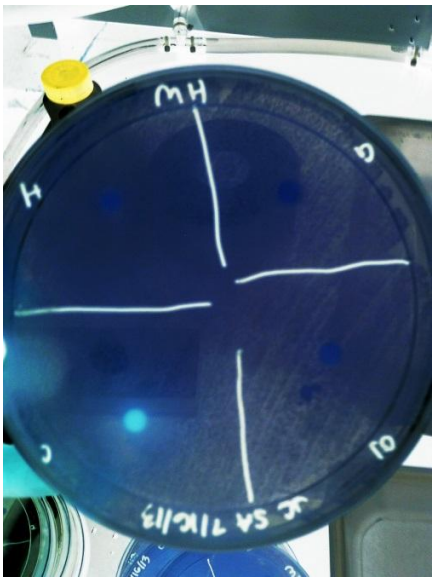


Figure 1.3(left): The results of Trial 4 on *S. aureus*. Zones of inhibition can be found for cinnamon only in the bottom right.

Figure 1.4(right): The results of Trial 3 on *S. aureus*. Again, zones can be found for cinnamon, this time on the bottom left. Colors were inverted to make viewing easier for the reader.

Discussion

Generally speaking, the results of this study disproved my hypothesis that the majority of the extracts would inhibit bacterial growth in some way. This provides some kind of insight into the "mind-over-matter" debate when it comes to treating illnesses with household remedies: maybe it isn't the food content that helps us feel better, but the placebo effect. Often times, if we tell our brains that a certain food, drink, or spice is going to help relieve our symptoms, it does.

On the other hand, cinnamon could possess characteristics that inhibit quorum sensing while other extracts do not. Cinnamon is known to contain essential oils such as cinnamyl acetate and cinnamyl alcohol, as well as significant amounts of manganese, calcium, iron and fiber, while other spices and herbs do not.

Furthermore, for extracts like anise seed, an oil needs to be made to expose its ethno-botanical significance, so this was one flaw in this study. In order for this study's results to become more valid, it should be repeated several more times in a sterile laboratory setting to confirm that the results were not that of human error.

Conclusion

It was concluded that substances that can be found in a common household do have some effect on the growth of pathogenic bacteria. However, the hypothesis that most, if not all, of the extracts would exhibit inhibition in some form, was refuted. The fact that only one of the substances was effective in stopping growth in only one bacterial strain could be attributed to human error; the experiment was mostly done in a fume hood where spillage and cross-contamination could have easily occurred.

In order to further elaborate on the results of this study and to develop more knowledge in this field, one could extend the species and bacteria used to include more spices with structural similarities to cinnamon and to include more human pathogens, in order to find more effective extract-bacteria combinations. Also, it would also prove interesting to conduct a follow-up study with human subjects to test whether or not household remedies for illnesses have a distinct scientific effect on us, or whether it's all in the heads of the patients being treated.

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