Surface Soldiers: The War Against Bacteria-Infested Fomites

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Abstract

Many disease-causing bacteria can be transmitted indirectly via fomites (Castaño, Cordts, Kurosu Jalil, Zhang, Koppaka, Bick, Paul, & Tang, 2021). While there exists antibacterial and chemical cleaners to kill off bacteria, bacteria are evolving to resist antibacterials, and chemical cleaners often have undisclosed toxins that can lead to asthma, anemia, developmental and fertility issues, and pollution (Frack & Sutton, 2010)(Lobanovska & Pilla, 2017). It is important to have new, natural alternatives to kill bacteria found on surfaces. This project looks at the antibacterial properties of 8 essential oils: calendula, oregano, rosemary, lemon, cinnamon bark, jojoba, peppermint, and ginger in fighting bacteria found on high-contact objects. 7 bacteria samples were tested (2 lab grown samples and 5 environmental samples) by all 8 essential oils by disk diffusion. Cinnamon bark and oregano essential oil showed the greatest inhibition of growth and therefore the strongest antibacterial properties, while jojoba and calendula essential oils had no visible effect on any of the bacteria tested.

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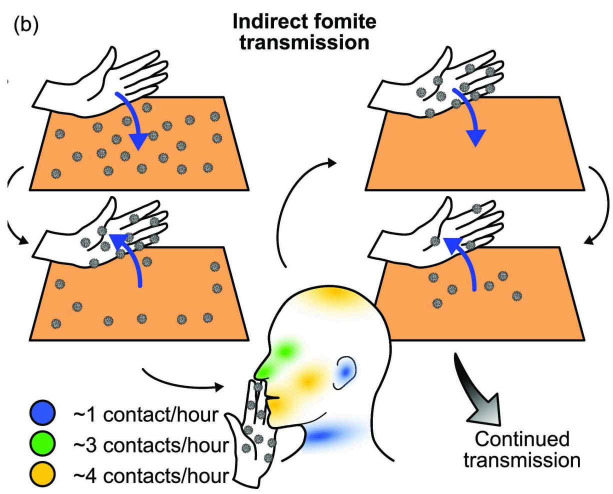
Introduction

Everywhere around us exists invisible life forms that often go unnoticed. Microbes are microscopic life forms that include bacteria, viruses, protists, and even some fungi. Though they are not visible to the naked eye, microbes play a large role in our day-to-day life, and without them, the world would be a very different place. Bacteria belong to the kingdom of Archaebacteria which is estimated to be comprised of upwards of millions of different species (Dykhuizen, 2005). While bacteria can cause disease and infection (parasitic) in organisms such as humans, they can also work in tandem with organisms in a behavior referred to as symbiosis. Symbiosis is a relationship characterized by mutual gain. An example of this is the bacteria that resides in your gut that aids in digestion.

On the other hand, there are many diseases caused by harmful bacteria and microbes that are called "pathogens." Before bacteria were discovered, scientists believed that disease was transmitted via an "invisible badness" called miasma (Green, 2018). As the first microbiologists made headway in understanding microbes, we gained a greater understanding of how to combat these diseases that used to kill thousands of people.

After the discovery of the first antibiotic, penicillin, by Scottish microbiologist Alexander Fleming in the 1920's, death rates from diseases sensitive to penicillin dropped by an estimated 58% (McKnight, 2019). Since the discovery of penicillin, more than 150 different kinds of antibiotics have been found (Lobanoska & Pilla, 2017). Unfortunately, over time, bacteria have become resistant to many common antibiotics. Eventually there will come a day where our antibiotics are no longer as effective in the fight against pathogens. Before that happens, we should be prepared with new ways to guard against them (Lobanoska & Pilla, 2017).

In the effort to limit the effects of disease caused by exposure to harmful microbes, it is important to understand how to stop the microbes from spreading. In recent years, studies on what are referred to as “fomites” contribute largely to the spread of some diseases, such as diseases commonly referred to as Covid-19 and the flu (Stephens, Azimi, Thoemmes, Heidarinejad, Allen, & Gilbert, 2019). A fomite is an inanimate (non-living) object that can carry and pass along harmful microbes and promote the spread of disease. People are exposed to bacteria or viruses from fomites when they pick up bacteria and germs from a surface and proceed to touch areas around the face, near areas where germs can enter the body, such as the eyes and mouth (see figure 1). In the case of pathogenic bacteria, once within the body the bacteria will begin multiplying and/or releasing toxins that can make you sick. Fever, a common symptom of many diseases, is a result of a person’s body trying to kill off the unwelcome bacteria by raising its body temperature. In many places, people lack the resources necessary to fight an infection or disease. Understanding how diseases spread via fomites allows scientists to better limit the spread of infectious microbes.



(Figure 1.) [1]

[1] Castaño, N., Cordts, S. C., Kurosu Jalil, M., Zhang, K. S., Koppaka, S., Bick, A. D., Paul, R., & Tang, S. K. Y. (2021, March 5). Fomite transmission, physicochemical origin of virus-surface interactions, and disinfection strategies for enveloped viruses with applications to SARS-COV-2. ACS omega. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7944398/

Synthetic cleaners often contain harmful or toxic chemicals, such as 2-butoxyethanol, alkylphenol ethoxylates, and artificial fragrances, often a mix of many, potentially toxic, chemicals to replicate a desired smell. These substances are a risk to both the individual using it as well as the environment, as they can lead to anemia, impaired fertility and development, asthma, and air pollution (Lisa & Sutton, 2010). My experiment focuses on testing the antibacterial properties of organic substances via essential oils that can lead to healthier, more sustainable alternatives to kill off bacteria found on fomites. The research question being asked is the following:

> Which essential oils display antibacterial properties? If successful in killing the bacteria, to what extent and is this result consistent over all bacteria samples?

Methods

Using proper sterile technique, environmental samples were collected from 8 classroom objects/fomites. First a sterile swab was dipped in phosphate buffered saline (PBS) and rubbed against the fomite. Then, the swab was immediately streaked onto a LB agar plate. Positive and negative controls were also taken. The negative control was streaked by the sterile swab dipped in PBS that had not made contact with any other surface. The positive control was streaked with E. coli from a pregrown plate using an inoculation hoop. These plates were then incubated upside down at 37℃ for 22 hours.

After observing the plates, bacteria colonies were selected to be treated by essential oils. These colonies were then purified by streaking each onto their own plate with a sterile inoculation hoop to be incubated for 48 hours. To confirm that the selected bacteria had been purified, bacteria from each plate was heat-fixed into a slide and gram stained, following standard protocol prescribed by Cornell University's College of Veterinary Medicine.

The bacteria that was successfully purified into a single shape and gram result were then resuspended in luria broth. A sterile pipette tip picked up a colony of the desired bacteria and was transferred into a sterile 15mL conical tube, containing 2mL of luria broth. Each tube is then vortexed for a few seconds and placed into a shaker to be incubated at 37℃ overnight. After incubation, the samples were re-vortexed.

Each bacteria sample (5 environmental samples and 2 lab samples, E.coli and S.epidermidis) was treated with 8 different 100% pure essential oils (EOs) and sterile PBS by method of disk diffusion. The EOs tested were cinnamon bark, ginger, lemon, oregano, jojoba, calendula, peppermint, and rosemary. Each plate was inoculated with 100µLs of bacteria grown in luria broth and spread using proper sterile technique and allowed to dry. Each essential oil was placed in its own sterile tray. The paper disks were dipped into the desired essential oil and placed onto the inoculated plate, so that each plate had 3 disks of the same essential oil. These plates were then incubated at 37℃ for 24 hours. (see figure 2.)

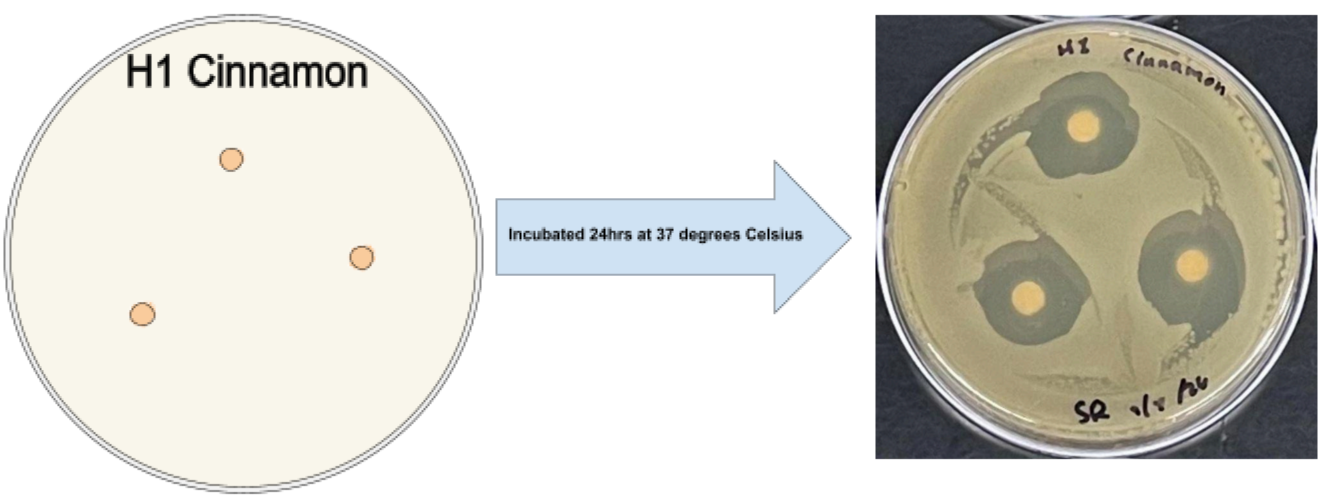


Figure 2. Shows what an ideal sample of bacteria+treatment should look like.

A polymerase chain reaction (PCR) test was performed on the 5 environmental samples to easily compare it with other outside experiments. The PCR was set up with a 1X concentration of the "GoTaq G2 Green Master Mix" from the company Promega and 27F and 1492R primers. This amplified the 16S rRNA gene region, resulting in an approximately 1,500 base pair long PCR product. The products were sequenced by Genewiz using the 27F primer. The nucleotide base sequences were analyzed by the program DNA Subway, where the sequences can be BLASTed to identify the genus/species.

<u>Data Collection</u>

Each plate held 3 paper disks with the same essential oil to provide a complete set of data. Each disk was measured in mm 3 times around, from the end of the disk to the edge of the bacteria. These three measurements were then averaged to provide a single distance for each disk. These distances were then recorded into a spreadsheet. The values seen on the graphs in figure 3 were found by finding the mean value of the average distances of each disk on a plate.

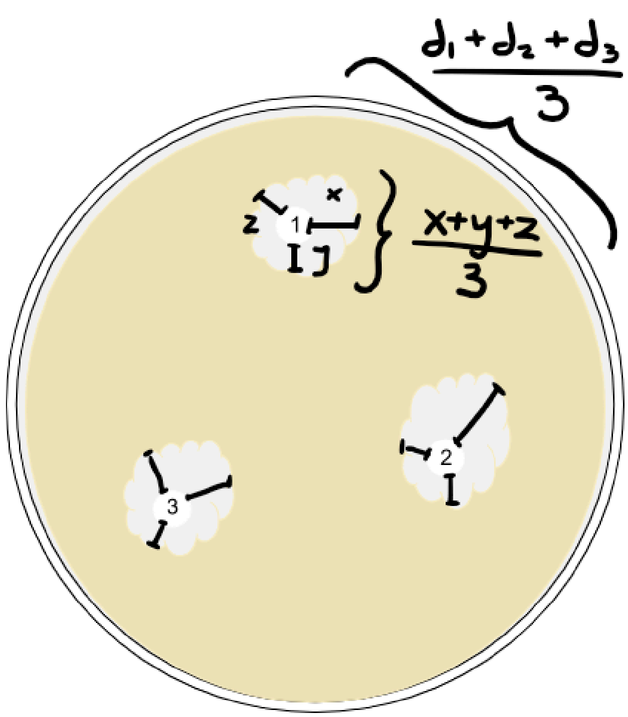


Figure 3. Visual representation of how data was collected and calculated. (d1=disk 1)

Results

PCR Results

The five environmental bacteria were sequenced through a polymerase chain reaction (PCR) test. A perfect match was not seen for *Bacillus* species 1 (sp1) and *Bacillus* species 2 (sp2), either with multiple 100% matches or none at all.

Bacteria "name"	Species
H1	*Bacillus sp1*
3A	*S. epidermidis*
7A	*S. hominis*
8A	*S. epidermidis*
5G	*Bacillus sp2*

Essential Oil Disk Diffusion Results

The graphs below show the essential oils that resulted in the inhibition of growth by a bacteria. If the essential oil is not shown on graph, there was no visible inhibition of growth of that given bacteria or an average distance of 0mm. In the special case of the plates growing *Bacillus sp1* and *sp2* that were treated with peppermint oil, there was insufficient lawn growth to collect data. Another thing to note, on the graph showing "Essential oil's effects on *S. epidermidis*" 8A's negative control, treated with sterile PBS, showed significant inhibition of the growth of *S. epidermidis*.

Cinnamon bark and oregano EOs showed consistent inhibition of growth with average distances of 5.0835mm and 15.222mm, excluding 8A that was treated with oregano and showed no signs of inhibition. The only other EO that exceeded 5.0835mm was peppermint EO on all *S.*

epidermidis trials. Calendula and jojoba EOs, not shown on any of the graphs, exhibited no visible inhibition of growth over any of the bacteria tested.

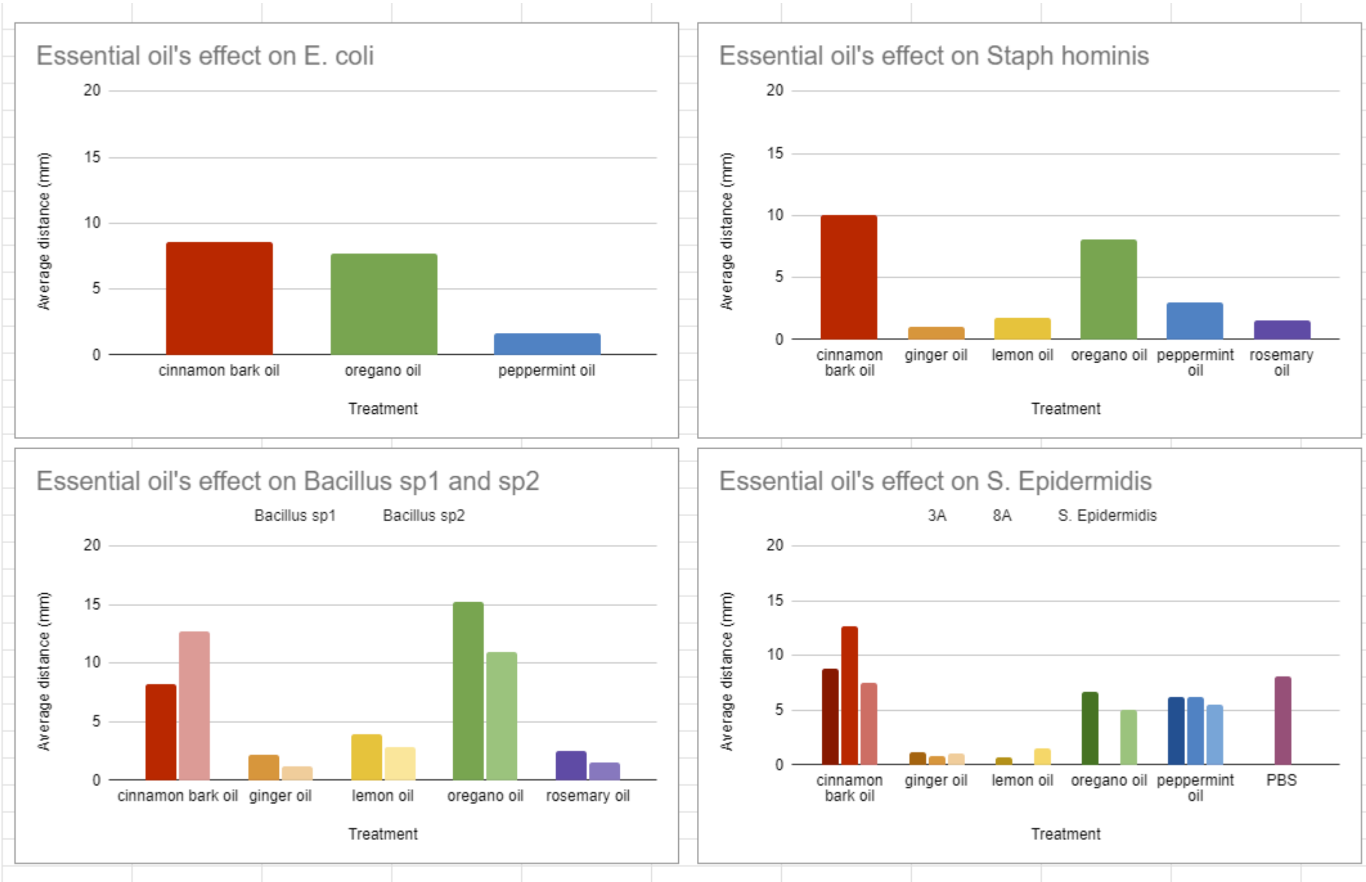


Figure 4.

If the graph shows multiple bacteria, the sets of data are in the order of the bacteria listed just below the title (i.e. On the graph showing Bacillus sp1 and sp2, the left column represents Bacillus sp1's data and the right column shows Bacillus sp2's data.)

Discussion

The average distance between the disk and the bacteria growth is being used to quantify the strength of the antibacterial properties of each essential oil. Following this line of logic, cinnamon bark EO and oregano EO have the greatest antibacterial properties, as they resulted in the greatest average distance across all the bacteria tested. While other EOs, ginger, lemon, and rosemary, showed some antibacterial properties, the results were not consistent over all tested bacteria and resulted in average distances less than that of cinnamon and oregano. The exception to this trend was peppermint EO's effect on *S. epidermidis* which was on par, if not greater to the effect of oregano EO on S. epidermidis.

Contrary to the hypothesis, lemon EO did not prove to be the strongest antibacterial of the treatments tested. While lemon EOs did show some antibacterial properties, most likely due to its high acidity, cinnamon bark and oregano exhibited the strongest antibacterial properties of the EOs tested. These EOs antibacterial properties can largely be accredited to their chemical composition of phenols and polyphenols. Oregano EO is largely made up of two phenols, carvacrol and thymol (Fournomiti, Kimbaris, Mantzourani, Plessas, Theodoridou, Papaemmanouil, Kapsiotis, Panopoulou, Stavropoulou, Bezirtzoglou, & Alexopoulos, 2015). As for cinnamon, the bulk of its polyphenol composition is vanillic, caffeic, gallic, protocatechuic, *p*-coumaric, and ferulic acids (Nabavi, Lorenzo, Izadi, Sobarzo-Sanchez, Daglia, & Nabavi, 2015). Jojoba EO, on the other hand, has a much less offensive composition that is primarily wax and oil based (Gad, Roberts, Hamzi, Gad, Touiss, Altyar, Kensara, & Ashour., 2021).

Conclusion

Cinnamon bark and oregano EOs serve as the most effective, natural antibacterials to kill bacteria out of the EOs tested, due to their high composition of phenols and potency over all the bacteria samples tested.

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