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ABSTRACT

S. marcescens is a gram-negative, facultatively anaerobic bacterium that is particularly aggressive in its growing capability. It is a concern in medical fields as well as home environments due to its resistance to most antibiotics, high mortality rate, and easy ability to colonize on soap and other fatty substances. Although testing of natural cleansers such as lemon juice, vinegar, and ammonia has been done on *S. marcescens* and essential oil efficacy has been tested on *Escherichia coli* and *Staphylococcus aureus*, no literature has been published on the effect of essential oils on *S. marcescens* growth. The purpose of this experiment therefore was to test the efficiency of essential oils in inhibiting the growth of this bacterium. Using Kirby-Bauer Assay and Minimum Inhibitory Concentration testing we compared the effectiveness of different harsh cleaners such as Lysol®, with peppermint and helichrysum essential oils. Although few significant values were obtained, noticeable zones of inhibition were seen, especially for peppermint oil. Based on this finding, further research should be done exploring this potency of peppermint and other essential oils on *S. marcescens*.

INTRODUCTION

When cleaning/disinfecting bacteria off bathroom counters found in the home, an individual may choose to use a store-bought, harsh chemical based cleaner or an "alternative" natural cleaner. Many alternative cleaners are marketed as better for the health of the inhabitants of the home as well as better for the environment. This may lead to individuals who prioritize using "green products" to reach for alternative cleaning products rather than a harsh disinfectant. These alternative products can range from cleaning agents branded as "natural" to more do-it-yourself cleaning agents made from items already found in the home (1). Vinegar and baking soda may be easily accessible in one's home as they can be used for many different purposes, which can be especially helpful for individuals struggling to leave their home or make it to the store.

During the COVID-19 pandemic, the efficacy of cleaning products were put under harsher scrutiny, with individuals paying more attention to what was being cleaned, how often, and what was being used (2). With the pandemic lockdown conditions making it harder to get to the store and to find cleaning supplies, resorting to items already purchased became a desirable source for those worried about the effects of harsh cleaners. Essential oils might be used as an alternative cleaner in these cases. Essential oils are derived from natural sources such as plant species, each retaining a unique chemical composition that can have various effects on a person as well as a strain of bacteria (3). This has led to the use of essential oils as natural disinfectants to decontaminate food contact surfaces (4) and water (5), which have been proven to be effective. Essential oils like peppermint oil are often and easily found as tools to make a space have a more pleasant smell, but can also serve a cleaning purpose; peppermint essential oil was able to effectively and efficiently disinfect a water source from E.coli and *S.aureus* strains (5). Chrysanthemum essential oil has been studied to determine the antibacterial properties as the chrysanthemum plant has been used to make tea in Chinese and other Eastern cultures for centuries (6). It has been determined to have a significant effect on certain bacterial strains and shows promise as a source of continued antibacterial study (6). *Serratia marcescens* is a gram-negative species of bacteria that is often found in bathrooms of homes. It can cause many kinds of illness in humans (7,8) and can often colonize liquid soap (9, 10). *S. marcescens* thrives in environments where fatty substances are abundant, such as bathtubs exposed to soap build-up. *S. marcescens* can be identified with a pigment called prodigiosin which is dark red to pink in color, and allows it to be easily seen in a bathtub or other light-colored surfaces (11). Other areas with soap build-up may include bathroom and kitchen sinks - both areas that encounter items that often go into an individual's mouth (toothbrushes, utensils, food, etc.) Making sure this bacterium is thoroughly cleaned from these surfaces

is incredibly important for the health of the frequenters of the area.

S. marcescens is known to cause meningitis and other blood infections. It is responsible for a significant portion of hospital-acquired infections, with pediatric intensive care units reporting up to 5% of cases and neonatal units up to 15% (10). The common source of *S. marcescens* in these medical settings is hand cleaners, medical equipment, soap dispensers, sinks, and other wet areas with high usage. The shift from manual pump soaps/sanitizers to automatic has been key to ending these endemics as well as increased usage of alcoholic hand disinfection (8). *S. marcescens* has also been found to be the cause of many secondary nosocomial infections (commonly respiratory, urinary tract, biliary tract, peritonitis, and wound infections) (12). A study in South Korea, done over the span of 6 years, resulted in a 28-day mortality of 22.4% (22/98 patients) (12). In the 1950s, the US military spread *S. marcescens* and *Bacillus globigii* for six days off the coast of California, near the Bay area, in an operation called Operation Sea Spray. The purpose of this was to see how a big city like San Francisco would respond to a bioweapon attack. A week later, 11 people went to the Stanford hospital with urinary tract infections (13). *S. marcescens* is concerning not only because of the aforementioned factors, but also because of its resistance to antibiotics (amoxicillin, ampicillin) (14, 15, 16). Minimum inhibitory concentration and plating treatment with ceftriaxone, kanamycin, gentamicin, and chloramphenicol have been performed to test their relative ability to interfere with bacterial growth (16). Research findings demonstrate that even at varying concentrations (0.08 and 0.08 mg/mL) all antibiotics except for chloramphenicol did not have a significant impact on *S. marcescens* growth. Sadly, it is also the more frequent killer in nosocomial infections as well, with some studies finding that its mortality rate in infected patients is close to 46.1% (17). Antibiotic resistance knowledge is key for effective health and safety guidelines to be enforced, and with the high frequency of *S. marcescens* associated death, this should be a pressing concern for medical personnel, epidemiologists, public healthcare specialists, and hospital management.

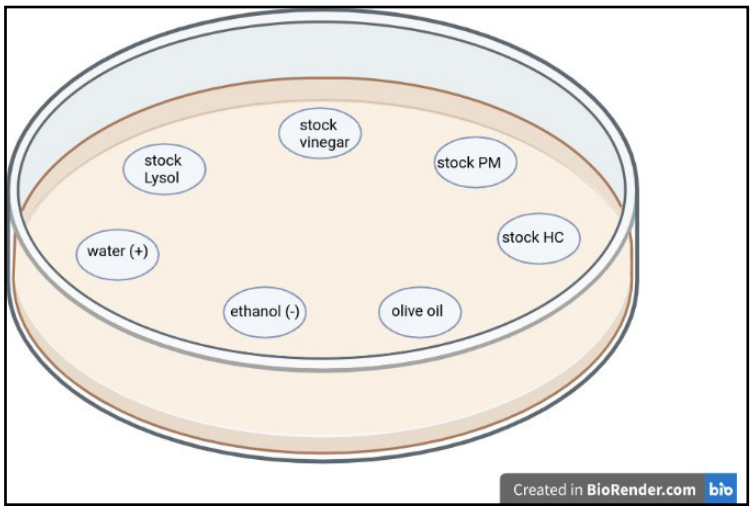
The efficacy of natural or alternative antiseptics on *S. marcescens* has been tested before (18); lemon juice, vinegar, baking soda, borax, and ammonia were all used as alternatives to store-bought chemical cleaning products. However, the performance of essential oils has not been studied on strains of *S. marcescens*. In particular, we are interested in studying the effects of peppermint and helichrysum essential oils on *S.marcescens* and how they compare to known efficient natural (vinegar) and harsh (Lysol® Disinfectant Spray) cleaners. Pure chrysanthemum oil is not easily commercially available, but helichrysum oil (from the *Helichrysum italicum* plant) is, with both genera found in the family Asteraceae. We also hope to explore the importance of helichrysum and if it has similar antimicrobial properties as seen in chrysanthemum. In this experiment we will use both helichrysum oil and peppermint oil as examples of essential oils that could serve as effective alternatives for harsh cleaning products using Kirby Bauer plates and Minimum Inhibitory Concentration tests. These tests will allow us to visualize the antibacterial properties of the essential oils and how they compare to other cleaning agents an individual might find in their home. Specifically, we would like to see if helichrysum oil and peppermint oil are effective in inhibiting *S. marcescens* growth at varying concentrations that the average person would use in day-to-day cleaning.

MATERIALS AND METHODS

Kirby-Bauer

Twenty-four-hour *S. Marcescens* culture broth was used to swab streak Luria Broth and Mueller-Hinton agar plates. Kirby-Bauer disks with stock, ½, ¼, and 1⁄8 dilutions of each experimental cleaning agent: peppermint oil, helichrysum oil, pure white distilled vinegar, and Lysol®, were placed onto dried bacteria streak plates to determine the diameter of arrested growth. Each plate contained a Kirby-Bauer disc with the negative control (70% ethanol), positive control (water), and experimental cleaning solutions at their varying dilutions. The oils were diluted with olive oil to ensure a consistent viscosity so another control for that was made as well. Lysol® and vinegar were diluted with DI water. Separate plates were made to test antibiotic resistance against ampicillin, chloramphenicol [25 ug/ml], and a mixture of both. Plates were left to incubate for 24 hrs at 30°C or room temperature. There were complications with the oils saturating the discs, sticking to the storage plates, and taking varying amounts of time to dry so they were in varying degrees of dryness prior to placing them on the plate. Due to this complication and the initial negative controls of chloramphenicol and bleach not having any effect, our first few sets of plates were invalid. We decided to pipette 5µL of each experimental and control solution directly onto the agar, affectionately naming this the "SHLP method". We did not counteract the spread with a boundary at which each fluid would leak out to. Both Luria Broth and Mueller-Hinton plates were used to determine how the effect of plate nutrient levels affect the growth and inhibition of experimental substances. Subsequently, the zones of inhibition of each disc were measured in mm and collated into a graph comparing the zone of inhibition to the associated dilution. This allows us to see

Figure 1.
Kirby-Bauer test plating layout. Each plate contained a dilution series of all the experimental substances along with positive and negative controls. PM- peppermint oil, HC- helichrysum oil. Created with BioRender.com.



how concentrated a solution you need for the largest zone of inhibition.

ANOVA testing was run to test statistical significance of results**.**

Minimum Inhibitory Concentration

To determine Minimum Inhibitory Concentration, we first performed dilution schemes with olive oil bases that were inoculated with bacteria and placed in the shaking incubator at 30°C for 24 hrs. This was done to ensure their homogeneity (which was successful), but they were not able to be checked until after 72 hrs, making the results invalid, despite observing visible turbidity across all the tubes. After this trial round (and realizing we had limited amounts of helichrysum oil left), we performed similar turbidity testing to compare the number of live cells from the Kirby-Bauer plates versus the live and dead amount from absorbance levels. Eppendorf tubes containing stock, ½, ¼, and ⅛ dilutions based on a 1mL volume of peppermint essential oil, helichrysum essential oil, pure white distilled vinegar, and Lysol® were inoculated with *S. Marscenens* and left to incubate for 24 hrs at 37°C. The base of each dilution consisted of 4% growth media containing Luria broth powder 5g, olive oil 8mL, and water 192mL. We had the following controls: water, bleach, ethanol, and growth media base. 25µL of each sample were placed into a 96 well plate and run in the spectramax to have their absorbance values read and were shaken to ensure homogeneity before each reading. This was done a total of five times with the same sample. All results were collected into graphs for comparison and ANOVA testing was run to test statistical significance of results.

Data Analysis. To determine if the essential oils and other natural cleaners had a significant effect on *S. marcescens* growth, we ran multiple two-way ANOVA tests without replication both for testing zones of inhibition, as well as the Minimum Inhibitory Concentration absorbance values. The measurements in mm for the zones of inhibition for the direct-pipetting "SHLP" method and absorbance values for MIC were run using a significant p-value of <0.05. The stock, ½, ¼, and ⅛ values were all included in this analysis.

For the plates-We found that the p-value of a two-way ANOVA test between all variables (helichrysum, peppermint, Lysol®, vinegar, ethanol, olive oil, and water) is 3.854E-05 (Table 1). The p-value of a two- way ANOVA test between peppermint vs controls (ethanol and water) is 0.385 (Table 2). The p-value of a two-way ANOVA test between vinegar vs controls (ethanol and water) is 0.438 (Table 3). The p-value of a two-way ANOVA test between Lysol® vs controls (ethanol and water) is 0.151 (Table 4).

For the MIC-The p-value of a two-way ANOVA test between Lysol® vs water vs growth media vs ethanol is 0.0153 (Table 5). The p-value of a two-way ANOVA test between peppermint vs water vs growth media vs ethanol is 0.00952 (Table 6). The p- value of a two-way ANOVA test between vinegar vs Water vs Growth Media vs ethanol is 0.00399 (Table 7). The p-value of a two-way ANOVA test between helichrysum vs water vs growth media vs ethanol is 0.413 (Table 8).

For the MIC testing, we saw that the p- values were significant for Lysol® vs water vs growth media vs ethanol (Table 5), peppermint vs water vs growth media vs ethanol (Table 6), Vinegar vs Water vs Growth Media vs Ethanol (Table 7).

We found that the p-value of a two-way ANOVA test between all variables (helichrysum, peppermint, Lysol®, vinegar, ethanol, olive oil, and water) is 3.854E-05 (Fig.1). The p-value

Table 1.
Two-Factor ANOVA Without Replication for Zone of Inhibition with Helichrysum vs Peppermint vs Lysol® vs Vinegar vs Ethanol vs Olive Oil vs Water

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	607.886	27	22.514	2.779	$3.854\ E^{-05}$	1.555
Columns	3057.636	6	509.606	62.891	$8.235\ E^{-40}$	2.155
Error	1312.689	162	8.103	-	-	-
Total	4978.212	195	-	-	-	-

Table 2.
Two-Factor ANOVA Without Replication for Zone of Inhibition with Peppermint vs Controls (Ethanol and Water).

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	368.429	27	13.646	1.089	0.385	1.693
Columns	1509.652	2	754.826	60.225	$1.78\ E^{-14}$	3.168
Error	676.808	54	12.533	-	-	-
Total	2554.889	83	-	-	-	-

Table 3.
Two-Factor ANOVA Without Replication for Zone of Inhibition with Vinegar vs Controls (Ethanol and Water).

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	187.739	26	7.221	1.041	0.438	1.709
Columns	1458.268	2	729.134	105.089	$5.401\ E^{-19}$	3.175
Error	360.786	52	6.938	-	-	-
Total	2006.793	80	-	-	-	-

Table 4.
Two-Factor ANOVA Without Replication for Zone of Inhibition with Lysol® vs Controls (Ethanol and Water).

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	175.107	26	6.735	1.398	0.151	1.709
Columns	1615.309	2	807.655	167.601	$2.137\ E^{-23}$	3.175
Error	250.584	52	4.819	-	-	-
Total	2041	80	-	-	-	-

Table 5.
Two-Factor ANOVA Without Replication for MIC Test (600 nm) with Lysol® vs Water vs Growth Media vs Ethanol.

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	0.00251	3	0.000839	6.061	0.0153	3.863
Columns	0.00309	3	0.00103	7.447	0.00824	3.863
Error	0.00125	9	0.000138	-	-	-
Total	0.00685	15	-	-	-	-

Table 6.
Two-Factor ANOVA Without Replication for MIC Test (600 nm) with Peppermint vs Water vs Growth Media vs Ethanol.

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	0.00277	3	0.000923	7.106	0.00952	3.863
Columns	0.00784	3	0.00261	20.113	0.000250	3.863
Error	0.00117	9	0.000129	-	-	-
Total	0.0118	15	-	-	-	-

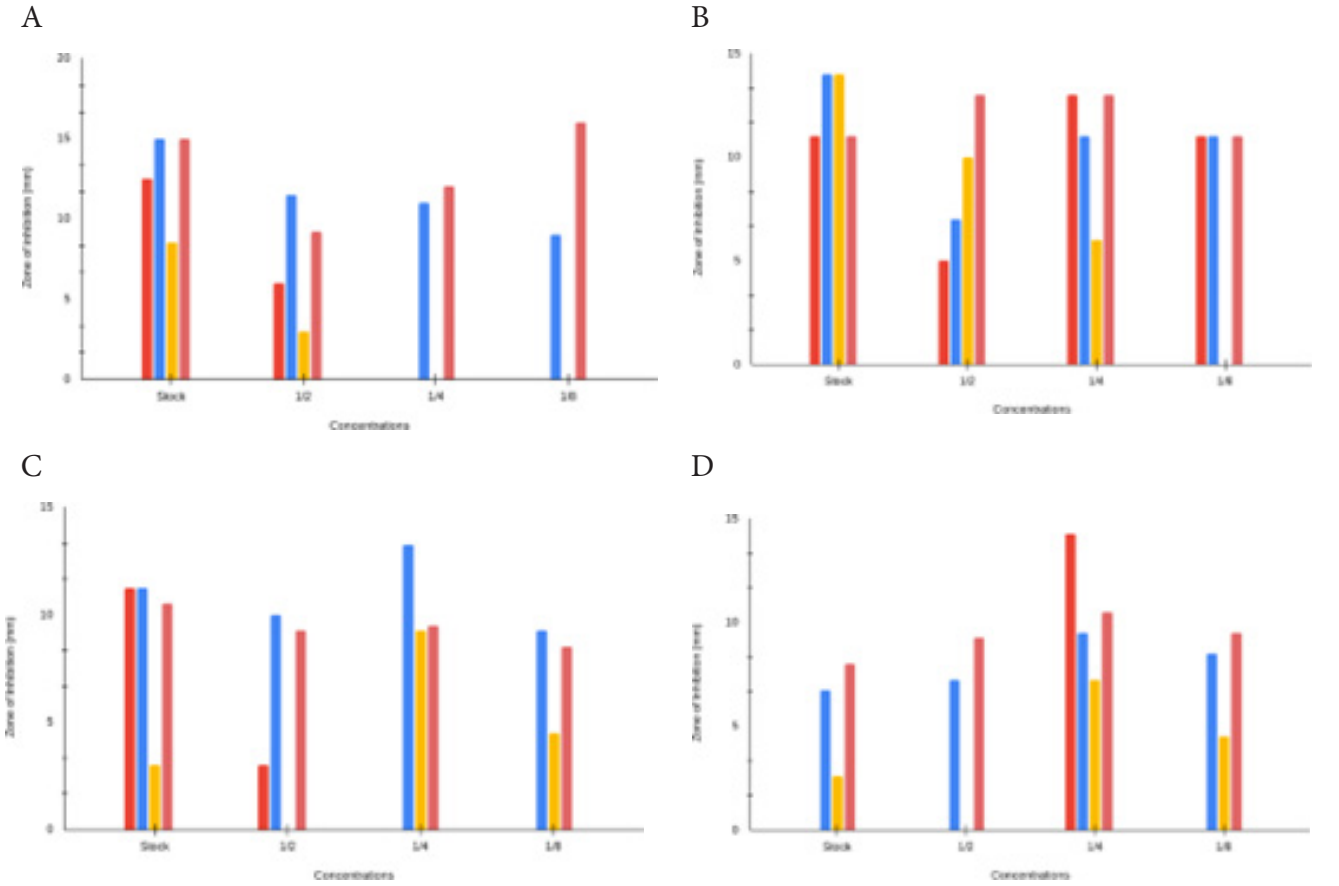
Table 7.
Two-Factor ANOVA Without Replication for MIC Test (600 nm) with Vinegar vs Water vs Growth Media vs Ethanol.

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	0.00289	3	0.000964	9.332	0.00399	3.863
Columns	0.00349	3	0.00117	11.285	0.00209	3.863
Error	0.000930	9	0.000103	-	-	-
Total	0.00732	15	-	-	-	-

Table 8.
Two-Factor ANOVA Without Replication for MIC Test (600 nm) with Helichrysum vs Water vs Growth Media vs Ethanol.

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	0.00188	3	0.000626	1.0612	0.413	3.863
Columns	0.00107	3	0.000356	0.603	0.629	3.863
Error	0.00531	9	0.000590	-	-	-
Total	0.00826	15	-	-	-	-

Figure 2.
Zones of inhibition measured in mm. All plates were streaked with undiluted bacteria and incubated for 24 hrs. Stock, ½, ¼, ⅛ dilutions of experimentals were on their own plate. For plate testing we used water (orange), ethanol (mauve), olive oil (teal) as controls and Lysol® (dark blue), vinegar (yellow) peppermint oil (red), helichrysum oil (green) as experimental substances. (A) Mueller-Hinton plates at room temperature. (B) Mueller-Hinton plates at 30°C. (C) Luria broth plates at room temperature. (D) Luria broth plates at 30°C.



of a two-way ANOVA test between Lysol® vs controls (ethanol and water) is 0.151 (Table 4).

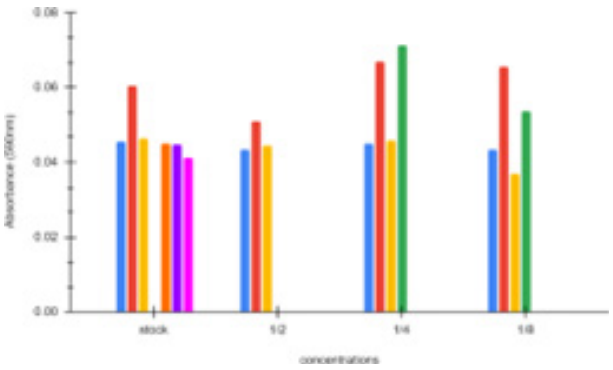
RESULTS

We expected to see smaller zones of inhibition as each experimental substance became more diluted. This was not seen in this data set, but it may have been if we had a larger number of replicates to give variation. Lysol® demonstrated this trend in Fig. 2A along with vinegar in Fig. 2C. Peppermint oil and vinegar also had some unexpected results in Fig. 2D, by showing no zones of inhibitions at stock or ½ dilution but then a large zone of inhibition at ¼ dilution. Helichrysum oil showed no zones of inhibition no matter the level of dilution. The p-values that indicated variance that was significant were produced by measuring the difference between the means for all the experimentals and negative and positive controls on the directly pipetted plates. This p-value came in at 3.854E-05, indicating a high likelihood that the cleaners have a varying degree of effectiveness in regards to their ability to inhibit *S.marcescens* growth.

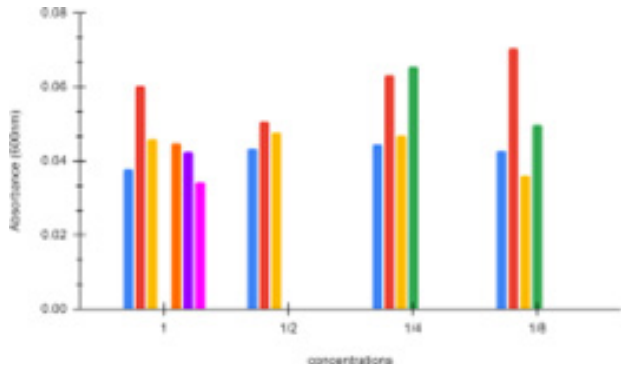
For Spec Max data we expected to see an overall increase of absorbance values the more diluted each substance was since more bacteria, live and dead would be present in solution when less experimental cleaners were used. This would cause a larger absorbance of light in these tubes. There was visible growth and turbidity in each inoculated tube. Red growth was at the top due to prodigiosin production since the olive oil separated itself to the top of the tube which encouraged bacterial growth. There was some yellow/cloudy growth within the growth media, but it was difficult to visualize due to the color similarity of the growth media. This round of testing did give significant results when comparing Lysol® (P-value 0.0153), peppermint oil (P-value 0.00952), and vinegar (P-value 0.00399) against the controls, water, growth media, and ethanol at 600nm. Overall, these results demonstrate that at varying concentrations the same number of bacteria grows but does not specify the amount of death as seen in

Figure 3.
Spec Max absorbance values read through 25uL samples of controls water (orange), ethanol (pink), growth media (purple) and inoculated experimental substances, Lysol ® (dark blue), vinegar (yellow) peppermint oil (red), helichrysum oil (green). There was not enough sample to produce stock or ½ dilutions of helichrysum oil. One sample was read five times with the values averaged. The following absorbance levels were used to account for the baseline yellow hue of the growth media: (A) Absorbance read at 590 nm. (B) Absorbance read at 600nm.

A



B



the plates. We performed this test to compare the amount of overall growth of bacteria compared to inhibition zones found through plating.

DISCUSSION

We could not make any definitive conclusions after our testing. However, we could determine definite points of interest for continued research, both for methods and the experimentals we used.

Based on our ANOVA results, we were unable to conclude that essential oils have an effect on *S. marcescens* that differs significantly from other harsher cleansers (or our controls). We did find that comparing all of the experimentals with the controls (Table 1) produced a p-value that was significant for the plates, indicating that the cleaners have a varying degree of effectiveness in regards to their ability to inhibit *S.marcescens* growth overall. This significant value indicates that we can reject any notion that all cleaners are made the same. For the MIC testing, we saw that the p-values were significant for nearly all the experimental solutions other than helichrysum (Table 7). This supports the idea of varying "kill strength" of the cleaners. Interestingly, peppermint showed a fairly strong degree of variance when compared to the controls, indicating that it was able to inhibit *S. marcescens* growth to a degree (Table 6). These findings should be interpreted with the consideration that the MIC and Spec absorption is measuring growth of the live and dead cells of our bacterium, unlike the plates which just measures the live count.

Based on the lack of observable growth and measurable zones of inhibition for our plates, we believe that our testing allows for a clear avenue for continued study. Previous literature indicated the success of vinegar as a cleaning agent, but our results did not definitively support this based on the ANOVA testing (Table 3 and 8). However, it is worth mentioning that throughout the vinegar dilution series we were able to measure a clear zone of inhibition on the plates. The results for our harsh cleaner of choice, Lysol® Disinfectant Spray were also not statistically significant when comparing against the controls (Table 4), but consistently produced large zones of inhibition near 10mm across all plates and dilutions. A 10- 15 mm zone of inhibition measurement was seen for the ethanol, Lysol®, and peppermint at stock solutions, suggesting a similar strength fighting against *S. marcescens* for these cleaners (Figure 10A and 10B).

The most interesting results were from the essential oils and antibiotics we had chosen to be a part of our experiment. Helichrysum oil did not have any visual zones of inhibition and therefore had no data visible in our results. Peppermint oil on the other hand did have a visible zone of inhibition at its stock concentration, leading us to believe that further research should be pursued for this essential oil.

Literature supported the use of chloramphenicol as an effective antibiotic against *S.marcescens* (16). However, our use of chloramphenicol was unsuccessful, with growth continuing even in the presence of chloramphenicol even when high concentrations [25 ug/ml] were pipetted directly onto the culture.

We were able to determine some aspects of our study that might have caused limitations affecting our results. Our biggest limitation was the lack of a working antibiotic for a negative control. Without a working negative control present on our

Kirby-Bauer plates, we were unable to consider those plates and that round of dilutions as valid; we had nothing to compare the inhibition to and therefore could not use that data to make any conclusions regarding antimicrobial properties. However, we believe that the chloramphenicol available to us was made using a powder that might have been too old. By using fresh chloramphenicol, we would hope to get cleaner, more accurate results.

Our next limitation came to us in our choice of experimentals and how they interacted with the method we chose to determine their properties. The viscosity of the oils we were using for our study did not penetrate the filter discs we were using in accordance with the Kirby-Bauer method. Without an automatic disc dispenser, the saturation and drying of each oil disc was done manually, but led to uneven saturation, solidification, and lack of absorption by the discs. In a future study, we should find a way to lower the viscosity of the oils, decrease the drying time of the discs, and ensure the same saturation on each to allow the Kirby-Bauer method to work as expected.

Lastly, we believe that the carrier oil we decided to use to dilute our essential oils might have skewed our results. We did include a disc saturated with just olive oil as a control disc; we wanted to verify that the olive oil itself did not have any kind of inhibitory properties. This proved to be correct; olive oil itself had no visible antimicrobial properties. However, it did seem to encourage the growth of *S.marcescens*, as there was a visible dense ring of growth around each disc. We believe that this could have affected the results of the diluted essential oils. For a future study, we would find a less preferable oil for the bacteria to dilute with or get rid of the dilutions and focus on the stock concentrations themselves.

LITERATURE CITED

Farias CBB, Almeida F, Silva I, Souza T, Meira H, Soares da Silva R, Luna J, Santos V, Converti A, Banat I, Sarubbo L. 2021. Production of green surfactants: Market prospects. Electron J Biotechnol51: 28-39. https://doi.org/10.1016/j.ejbt.2021.02002

Vayisoglu SK, Oncu E. 2021. The use of cleaning products and its relationship with the increasing health risks during the COVID-19 pandemic. NIH 75(10). https://doi.org/10.1111%2Fijcp.1453 4

U.S. Department of Health and Human Services. 2022. Essential oils. National Institute of Environmental Health Sciences. https://www.niehs.nih.gov/health/topics/agents/essential-oils/index.cfm

Falco I, Verdeguer M, Aznar R, Sanchez G, Randazzo W. 2019. Sanitizing food contact surfaces by the use of essential oils. Innovative Food Science & Emerging Technologies 51: 220-228. https://doi.org/10.1016/j.ifset.2018.0 2.013

Dixit D, Bhandari V, Mane M, Balapure K. 2022. Studies in instant water disinfection using natural oils. Biochemical Engineering Journal 187. https://doi.org/10.1016/j.bej.2022.10 8631

Cui H, Bai M, Sun Y, Abdel-Samie M, Lin L. 2018. Antibacterial activity and mechanism of Chuzhou chrysanthemum essential oil. Journal of Functional Foods 48: 159-166. https://doi.org/10.1016/j.jff.2018.07. 021

Maki D, Hennekens C, Phillips C, Shaw W, Bennet, J. 1973. Nosocomial Urinary Tract Infection with *Serratia marcescens*: An Epidemiologic Study. ISDA 128(5): 579-587. https://doi.org/10.1093/infdis/128.5. 579

Khanna A, Khanna M, **Aggarwal,** 2012. Serratia Marcescens- A Rare Opportunistic Nosocomial Pathogen and Measures to Limit its Spread in Hospitalized Patients. Journal of Clinical and Diagnostic Research 7(2): 243-246. https://doi.org/10.7860%2FJCDR%2 F2013%2F5010.2737.

Sartor C, Jacomo V, Duvivier C, Tissot-Dupont H, Sambuc R, Drancourt M. Nosocomial *Serrartia marcescens* Infections Associated With Extrinsic Contamination of a Liquid Non Medicated Soap. 2000. Infection Control & Hospital Epidemiology 21(3): 196-199. https://doi.org/10.1086/501743

Buffet-Bataillon S, Rabier V, Bétrémieux P, Beuchée A, Bauer M, Pladys P, Le Gall E, Cormier M, Joliet-Gougeon A. 2009. Outbreak of *Serratia marcescens* in a neonatal intensive care unit: contaminated unmedicated liquid soap and risk factors. Journal of Hospital Infection 72(1):17-22. https://doi.org/10.1016/j.jhin.2009.0 1.010

Khanna A, Khanna M, & Aggarwal 2013. Serratia marcescens- a rare opportunistic nosocomial pathogen and measures to limit its spread in hospitalized patients. Journal of clinical and diagnostic research *: JCDR*, *7*(2), 243–246. https://doi.org/10.7860/JCDR/2013/5010.2737

Kim SB, Jeon YD, Kim JH, Kim JK, Ann HW, Choi H, Kim MH, Song JE, Ahn JY, Jeong SJ, Ku NS, Han SH, Choi JY, Song YG, & Kim JM. 2015. Risk factors for mortality in patients with Serratia marcescens bacteremia. Yonsei medical journal, 56(2), 348–354 https://doi.org/10.3349/ymj.2015.56. 2.348

Mahlen SD. 2011. Serratia infections: from military experiments to current practice. Clinical microbiology reviews, 24(4), 755–791. https://doi.org/10.1128/CMR.00017- 11

Stock I, Grueger T, & Wiedemann B. 2003. Natural antibiotic susceptibility of strains of Serratia marcescens and the S. liquefaciens complex: S. liquefaciens sensu stricto, S. proteamaculans and S. grimesii. International journal of antimicrobial agents, 22(1), 35–47. https://doi.org/10.1016/s0924-8579(02)00163-2

Haddix PL, Paulsen ET, & Werner TF. 2000. Measurement of Mutation to Antibiotic Resistance: Ampicillin Resistance in Serratia marcescens https://citeseerx.ist.psu.edu/documen t?repid=rep1&type=pdf&doi=a0d730bf0ef310fdd58c9e724ceef177a7274 3b7

Christopher R, Shenoy A, Orihuela CJ, & González-Juarbe N. 2017. Killing of Serratia marcescens biofilms with chloramphenicol. Ann Clin Microbiol Antimicrob, 16(19). https://doi.org/10.1186/s12941-017- 0192-2

Soltani J, Poorabbas B, Miri N, & Mardaneh J. 2016. Health care associated infections, antibiotic resistance and clinical outcome: A surveillance study from Sanandaj, Iran. World journal of clinical cases, *4*(3), 63–70. https://doi.org/10.12998/wjcc.v4.i3.6 3

Olson W, Vesley D, Bode M, Dubbel P, Bauer T. 1994. Hard Surface Cleaning Performance of Six Alternative Household Cleaners Under Laboratory Conditions. Journal of Environmental Health 56(6):27-31. https://www.jstor.org/stable/4453557 6