Prize Winner

Scientific Inquiry

Year 9-10

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Oliphant Science Awards – Scientific Investigation

The synergistic effect of combining essential oils to create an antimicrobial blend for treating skin infections

Linh Bui, Maiar Elkhouly

Questioning and predicting:

<table>
<tr><td>Abstract</td><td>Staphylococcus Epidermidis is a typically non-pathogenic, innocuous and common bacterium found on human skin. However, its ubiquitous presence also allows for contamination of indwelling medical devices such as catheters, internal tubes, and prosthetics, prompting the bacteria to become opportunistic upon enter into the human body. " It is now the most frequent cause of nosocomial skin infections," (Otto, 2009). Although not viral in nature, S. epidermidis is difficult to treat since it forms biofilms, which enhances the bacteria's survivability against several antibiotics. Essential oils (EO/s) have antimicrobial properties, with evidence supporting their potentials against S. epidermis. The experiment builds upon this understanding to investigate the effectiveness of combining the three most statistically effective essential oils (thyme, tea tree and lemongrass) in observing its anti- bacterial scope against S. epidermis by comparison to the EOs individually.

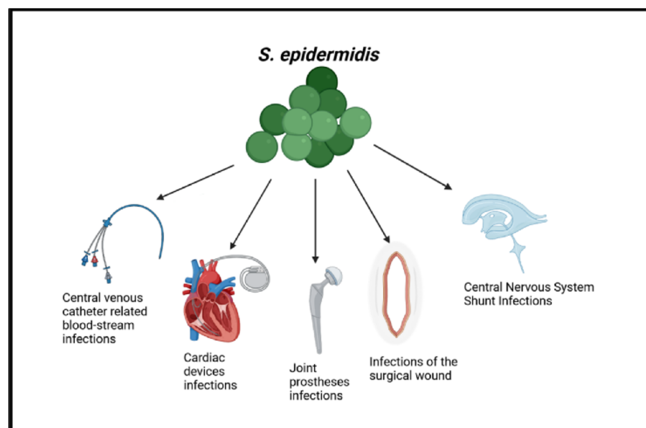

Figure 1: Locations for S.Epidermidis infection- https://www.mdpi.com/2075-1729/13/5/1126</td></tr>
<tr><td>Background</td><td>The mechanisms in which S. Epidermidis inhibits indwelling medical devices is a four-step process, consisting of adherence, accumulation, maturation, and dispersal to form biofilms, a matrix of extracellular polymeric substances (EPS) which is a major virulence attribute.

First, the bacteria's abundance on the skin adheres to an abiotic foreign body or biomaterial, (eg: plastic prosthetics) through surface proteins called adhesin, which anchors the bacteria's cell wall to the implant's extracellular matrix components such as fibronectin, collagen, and fibrinogen (Foster, 2020).

The Polysaccharide Intercellular Adhesin (PIA/PNAG) is then synthesized in the accumulation stage, facilitating the formation of microcolonies and the cell-cell adhesion "glue" that binds proteins and components within the biofilm together, protecting the bacteria within its impermeable matrix (Mack et al., 2015).

In maturation, the formation of Extracellular polymeric substances (EPS) like PIA/PNAG, eDNA, Protease and other proteins increases, enhancing the biofilm's structure and stability. Enzymes then release bacterial cells from the biofilm, allowing them to disperse and continue colonizing surfaces. The diagram below (Figure 2) illustrates these steps of bacteria formation.</td></tr>
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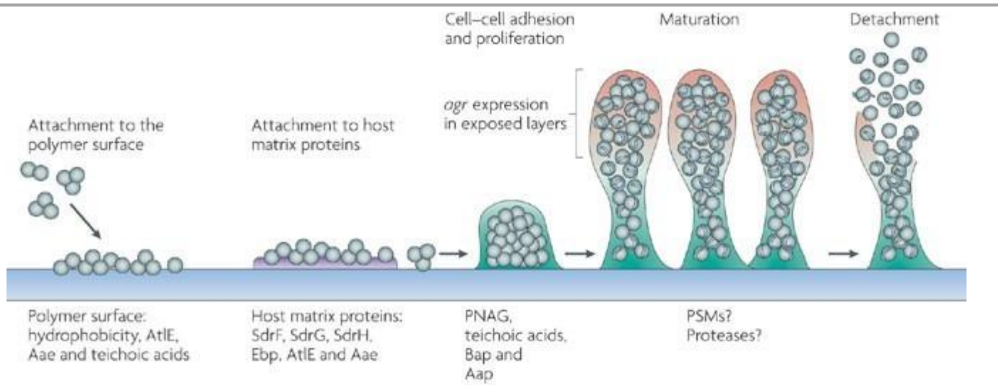


Figure 2 - A representation of the biofilm formation process-
https://www.nature.com/articles/nrmicro2182

S. Epidermidis causes virality issues when they enter the bloodstream or other sterile body systems, leading to various types of staph infection based on the interacted areas. The complex, impermeable matrix barrier also ensures that the bacteria has a slower metabolic rate, making antibiotics, phagocytosis and immune responses less susceptible. The bacteria's cementation onto the host's surface also allows for the infection to be sustainable, requiring persistent and prolonged treatments.

Thyme oil contains primarily thymol and carvacrol constituents, which are terpenes phenolic compounds known for their antimicrobial efficacy against S. Epidermidis, further inhibiting its biofilm formation. *(Walczac et al., 2021)*. The carvacrol molecule interferes with the gene coding of quorum sensing, the communication system facilitating biofilm formation, allowing for disruption in the bacteria's colony ability to form and maintain biofilms for virulence *(Walczak et al., 2021)*. Thymol and carvacrol works in cohesion to integrate into the biofilm's lipid layer, causing a chemical reaction that destabilizes the EPS, disrupting internal enzymes activities while increasing the structure's permeability. The carvacrol then inhibit enzymes that produces ATP, hindering bacterial metabolism.

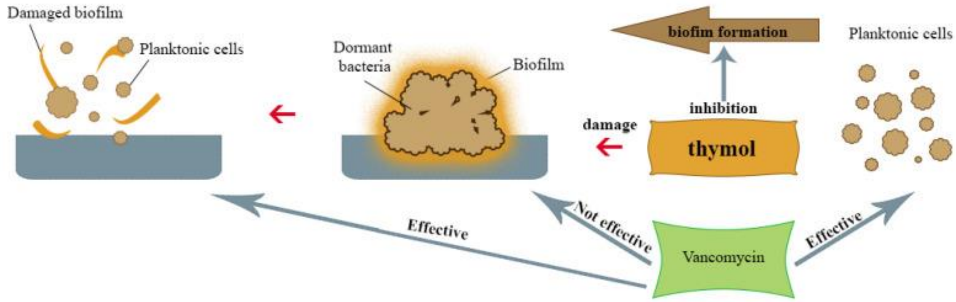


Figure 3 The mechanism in which thymol infiltrate the biofilm and bacteria cell
https://pdfs.semanticscholar.org/df8f/7ea4a494558dfc3a88088a1d81a2a59a316f.pdf

Tea Tree oil consists of primarily Terpinen-4-ol, which can interfere with the biofilm matrix's production, quorum sensing and structural integrity allowing it to subsequently inhibit the bacteria's respiration and metabolic events, causing cell death *(Cox et al., 2001)*. This action is identical to Thyme oil's, focusing on the inhibition of biofilm facilitating mechanisms and disruptions in the biofilm itself, although Terpinen-4-ol enhances the efficiency and lethality of the process.
Lemongrass oil's constituents are phenolic compounds, primarily citral, myrcene and

	geraniol *(Ariyanto et al., 2023)*. Tea Tree oil is especially found to be effective against planktonic cells *(Kavanaugh et al., 2012)*. Geraniol, the principal compound, inhibits S. epidermidis through disintegration of the EPS structure, with subsequent applications reducing the adaptive resistance capabilities of the formation *(Kannappan et al., 2017)*.
Predictions	**Question:** What are the synergistic effects of combining various essential oils to create a potent antimicrobial blend specifically tailored for skin infections? **Aim:** To assess the effectiveness of combining different essential oils for S.Epidermidis antibacterial efficacy in comparison to independent assays. **Hypothesis:** The combined application of Lemongrass, Thyme and Tea Tree oil will produce the most optimal outcome since the complementary mechanisms of their compounds can more effectively disrupt the biofilm and persister cells (carvacrol, thymol and terpinen-4-ol), while suppressing the cell's adaptive resistance (geraniol) for a faster process of synergistic inhibition.

Planning and conducting:

Method	Testing the antimicrobial effects of combinations of essential oil on S. Epidermidis at 1000 µL total application amounts
	Compare this to the antimicrobial effects of singular essential oil application on S. Epidermidis at 1000 µL each type

Independent Variables:	Combinations of EO and each included type's µL concerntration per application

Concerntration of each EO type (% of 1000µL)	**Combinations**
50%	Thyme EO + Tea Tree EO
50%	Lemongrass EO + Tea Tree EO
50%	Lemongrass EO + Thyme EO
33.3%	Thyme EO + Lemongrass EO + Tea Tree EO

Dependent Variables:	Remaining bacterial cell count after EO application (measured using the Jessen bacteria counting chamber technique)

Controlled (Fair Test)

Controlled Variables
Total volume of applied EO (1000µL)
Total bacteria suspension volume (supposed to be 2.5ml)
Type of EO used (Thyme, Lemongrass and Tea Tree)
Incubation temperature (37°C)
Incubation time (48h)
Brand of nutrient agar (Oxoid CM0003)

	Method of bacterial suspension application (grid formation swapping technique) Measurement system (Jessen grid calculation protocol) S.Epidermidis broth solution (B2 Southern Biological) (same vile used) Size and volume of Agar per plate (9cm diameter)
Control	see table below

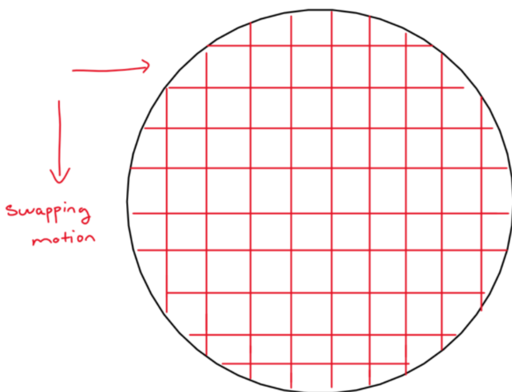
Concerntration of each EO type (% of 1000μL)	Type of oil
100%	Lemongrass
100%	Thyme
100%	Tea Tree

Apparatus:

- 8 x 90mm (diameter) Petri Dish
- Nutrient Agar Formula
- Fully grown bacteria colony
- 8 x Sterilized Pipette
- Metal Swap
- Bunsen Burner
- Heat Safety Mat
- Micropipette
- Incubator
- Ethanol Solution Spray
- Gloves
- Safety Goggles
- Face Mask
- Masking Tape
- Sharpie
- Jessen hemocytometer grid (1mm x 1mm chambers)
- 1 x Lemongrass Essential Oil Bottle
- 1 x Tea Tree Oil Bottle
- 1 x Thyme Essential Oil Bottle
- 7 x Micropipette tips
- 8 x Plastic Test Tube

Method:

1. Prepare the nutrient agar formula using the container's instructions.
2. Equip the gloves, safety goggles and mask.
3. Label control plates and experiment plates
4. Turn on the Bunsen Burner to a closed flame and hold the metal swap over the heat to sterilize. Remove once the metal tip is red.
5. Spray the ethanol solution on the workbench to sterilize and prevent experiment contamination.
6. Swipe the metal swap from the grown colony and draw the bacteria into the agar plate in a grid formation until all the broth is transffered.



7. Repeat steps 6 to 9 until all 8 plates are distributed with bacteria.
8. Incubate the plates at 37°C for 48h
9. After 48h, remove the plates from the incubator, the biofilm and colony should be fully formed. Then, use the Jesse hemocytometer grid to count the bacteria amount for each plate and record this in an excel sheet under the intended oil application.
10. For the singular oil controls, use the micropipette to transfer 1000 µL of each EO into their marked plate and swirl the liquid until it covers the entire plate's area.
11. For the binary combinations, use the micropipette to transfer 500 µL of each EO types into their marked plate, swirl the liquid to mix and until it covers the entire plate's area.
12. For the triple combinations, use the micropipette to transfer 333 µL of each EO types into their marked plate, swirl the liquid to mix and until it covers the entire plate's area.
13. Incubate the plates at 37°C for 48h.
14. After 48h, remove the plate from the incubator and use the Jessen hemocytometer grid to count the bacteria amount for each plate, repeat this process 3 times. Record this in an excel sheet under the oil's application and calculate the average count.

Method Explanation

1. Testing the different combinations at a systematic concerntration ensures that results variation and analysis can be wholly attributed in the change in oil types and their synergistic effectiveness instead of the physical total inputted amount changing. This allows for a valid experiment.
2. The incubation temperature at 37°C provides the most accurate enviroment that mimics the human body, allowing relevant growth conditions in context to human's pathogenic conditions.
3. The 48h time is the optimal period for full bacterial and biofilm formation, allowing for an accurate test to measure the oil's effectiveness against these factors for a comprehensive efficacy analysis.
4. The Jessen hemocytometer grid allows for an industry accurate counting technique for analyzing the results of EO's inputs on bacteria development. This allows for precise and quantifiable results.

	5. Spreading the EO solution across the entire colony as a comprehensive coverage enhances the sensitivity of the assay, where a maximized antimicrobial treatment can be more holistically analysed for its comprehensive effectiveness. 6. The singular oil serves as a controlled point of comparison to reliably asses the effectiveness of EO blends to their individual counterpart. 7. Counting the results 3 times and assesing an average reduces the risks of random sampling errors.

OSA RISK ASSESSMENT FORM

for all entries in (✓) ☐ Models & Inventions and ☑ Scientific Inquiry

This must be included with your report, log book or entry. One form per entry.

STUDENT(S) NAME: Linh Bui and Maiar Elkhouly ID: 0024-020

SCHOOL: Adelaide Botanic High School

Activity: Give a brief outline of what you are planning to do.

We are planning on growing the S.Epidermidis bacteria and using it to measure the effects of individual essential oils vs combination of essential oils, which are Thyme, Lemongrass and Tea Tree. The bacteria will be incubated at 37°C at periods of 48h for the biofilm to fully form.

Are there possible risks? Consider the following:

- Chemical risks: Are you using chemicals? If so, check with your teacher that any chemicals to be used are on the approved list for schools. Check the safety requirements for their use, such as eye protection and eyewash facilities, availability of running water, use of gloves, a well-ventilated area or fume cupboard.
- Thermal risks: Are you heating things? Could you be burnt?
- Biological risks: Are you working with micro-organisms such as mould and bacteria?
- Sharps risks: Are you cutting things, and is there a risk of injury from sharp objects?
- Electrical risks: Are you using mains (240 volt) electricity? How will you make sure that this is safe? Could you use a battery instead?
- Radiation risks: Does your entry use potentially harmful radiation such as UV or lasers?
- Other hazards.

Also, if you are using other people as subjects in an investigation you must get them to sign a note consenting to be part of your experiment.

Risks	How I will control/manage the risk
Listed Below	

(Attach another sheet if needed.)

Risk Assessment indicates that this activity can be safely carried out

RISK ASSESSMENT COMPLETED BY (student name(s)): Linh Bui and Maiar Elkhouly

SIGNATURE(S): L.B M.E

☑ By ticking this box, I/we state that my/our project adheres to the listed criteria for this Category.

TEACHER'S NAME: Karen Bang

SIGNATURE: [signature] DATE: 26/06/2024

<table>
<tr><td>Risk Assesment</td><td>
<table>
<tr><th>Risk</th><th>Why?</th><th>Controlling Risk</th></tr>
<tr><td>Bunsen Burner
(Thermal Risk)</td><td>This is an ignition source, with temperature ranging above 1000°C, meaning that fatal burns and injury can occur with improper handling. Risks to other equipment and the facility also presents as the heat can ignite flammable materials and cause subsequent damage.</td><td>Ensure that in use, the Bunsen Burner is only activated on a stable and heat safe mat/ surface.
Keep all materials far away from the open flame, especially combustible substances and volatile chemicals.
Use flame resistant tools to handle items near the flame and keep a safe distant. Never leave the equipment unattended.
Equip the required eye ware and PPE, ensure that long hair is tied up.
Have a fire extinguisher readily available in case of an emergency.</td></tr>
<tr><td>Incubator
(Electrical Risk)
(Biological Risks)</td><td>This is an electrical device, meaning that malfunctioning components and faulty wires could cause electrical shocks and overheating, leading to potential fire-hazards. Aditionally, bio-hazard also presents as the incubator's temperature</td><td>Ensure that it is recently inspected and maintained for preventing technical damage. Keep in a dry enviroment.
Always properly seal petri dishes with bacteria</td></tr>
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</td></tr>
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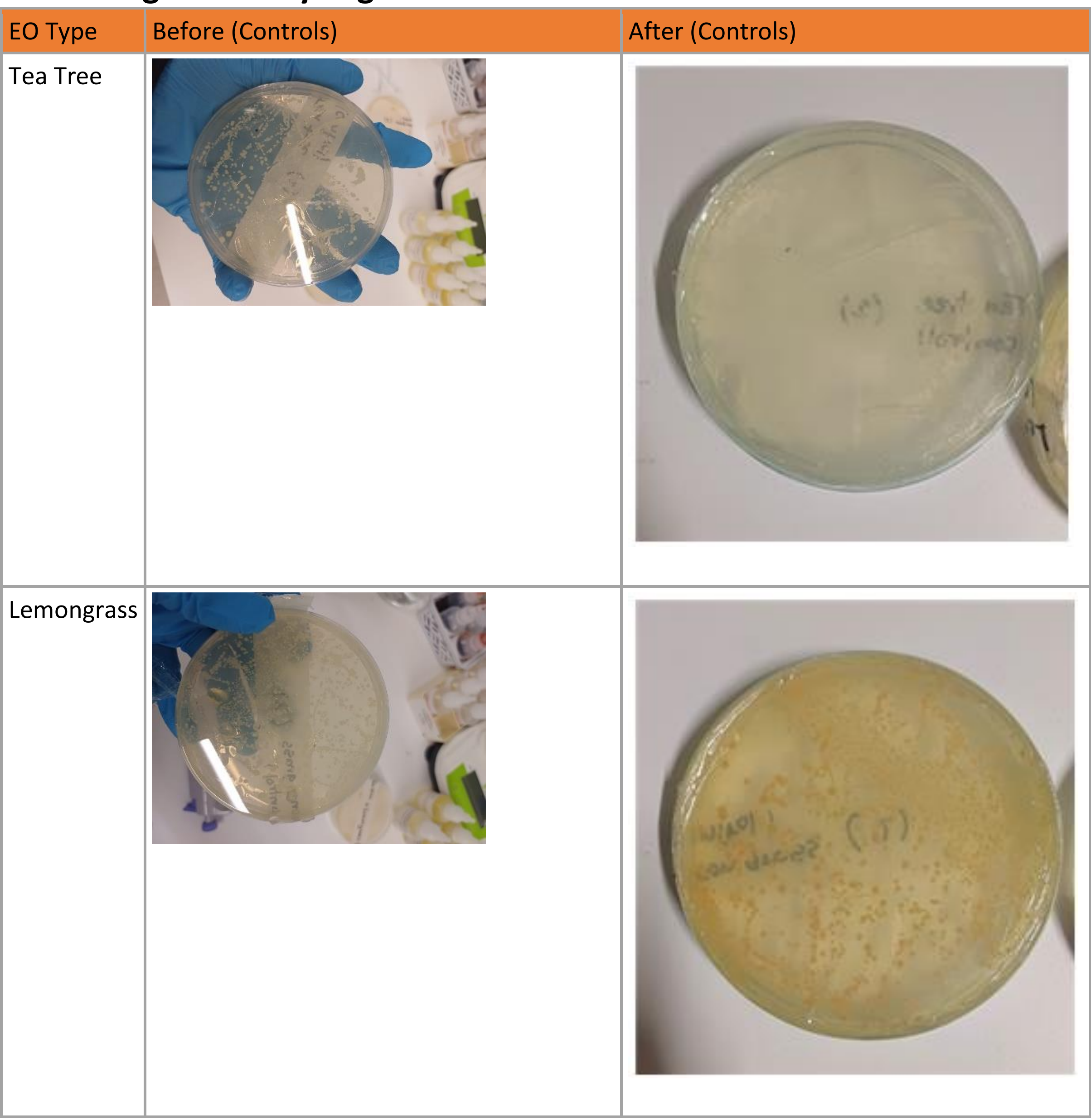
	can develop bacteria culture, meaning that potential contamination could spread with improper handing and monitoring.	culture to prevent spread into the external enviroment • Regularly disinfect the inside of the incubator.
S.Epidermidis	The bacteria, once entered inside the human body can become opportunistic, leading to potential severe and viral infections.	• Ensure to equip all the neccesary PPE (gloves, safety goggles..etc) in contact with the bacteria. Also check and bandage any • open wounds. • Regularly disinfect surfaces housing the bacteria (eg: lab counter, incubator) to kill any bacterial residuals. • Dispose bacteria contaminated equipment (eg: pipette) in biohazard specified container to prevent spread. • Only handle and extract bacteria broth with a certified supervisor present. • Only use commercially obtained pure nonpathogenic strains.
Agar Plate	Agar is harmless, but bacteria or fungi grown on agar may be	• Knowledge of microbiology and aseptic

		pathogenic, causing bio-safety hazards.	techniques is required to minimise risks to people • Dispose agar in bio-hazard specified container to regulate contamination. • Regularly monitor agar infiltrated bacteria temperature and enviroment. • Always equip the required PPE when making agar or using it grow bacteria broth. Always disinfect the cultivation area.
	Essential Oil (Chemical Risk)	Eos are volatile organic compounds (VOCs), meaning that they have high vapors at room temperature and can cause irritation and health issues for allergic individuals through inhalation, eye contact or skin contact. Even in small quantities, ingestion of active chemicals inside EO can be fatal	• Always equip the required PPE when in contact with the chemical (eg: safety goggles, gloves, face mask..etc) • Locate a nearby eye water fountain in case of irritation. • Always disinfect the area after a spillage, use absorbable materials and store the EOs in the labelled adequate conditions.

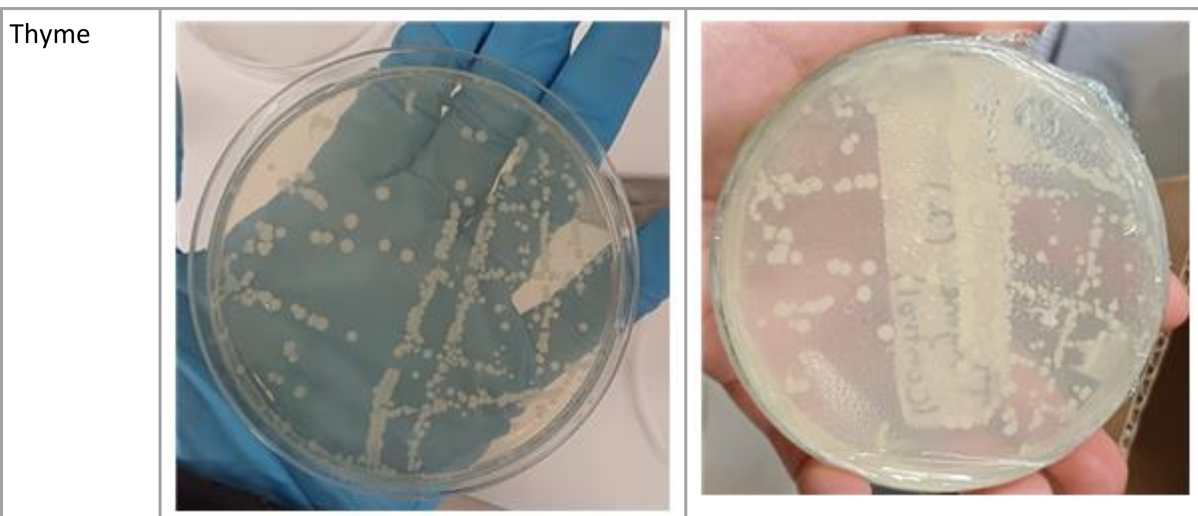
Fair Test?

Although aspects of the investigation limited external influences on the experiment's attributions, the bacteria suspension volume consistency was compromised so we took grown bacteria from another colony and populate the agar plates via the same grid technique. This meant that the total bacteria volume was inconsistent in each dish. However, we've decided to analyse the percentage changed in bacteria grid before and after EO application, so the initial variations would not significantly compromise the data's validity. Aditionally, the EO brand should've been the same to ensure a consistent oil extraction process. Otherwise, the repeated 3 counting times ensures that potentials for human calculation errors is decreased and that results are more reliable.

Processing and analysing:

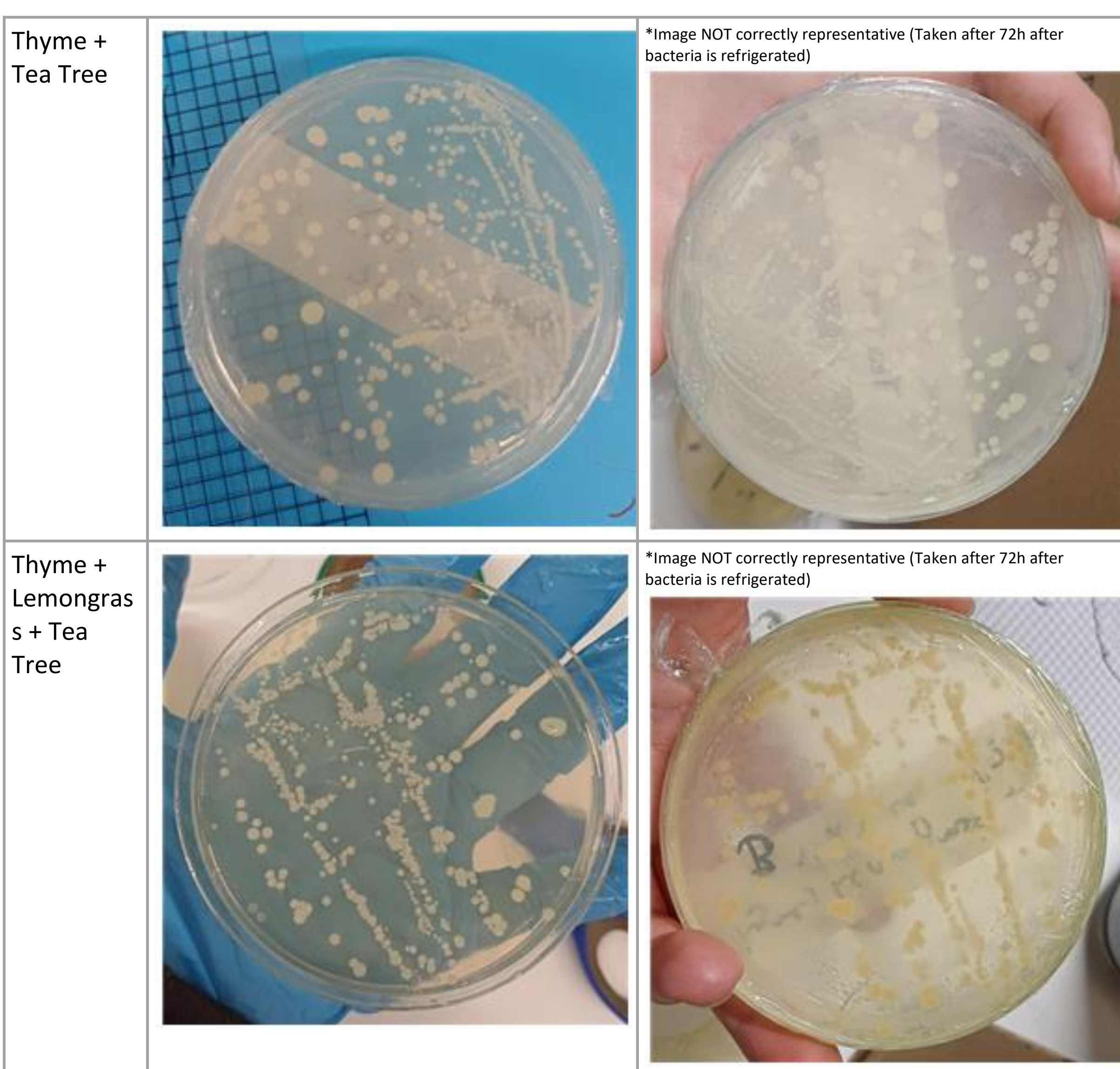


EO Type	Before (Controls)	After (Controls)
Tea Tree		
Lemongrass		

Thyme		

Observations: The bacterium for lemongrass is stained yellow due to the EO's extract being a yellow, orange hue. Tea tree is the most effective in removing whole strands of bacteria, while also decreasing the dollop sizes.

EO Type	Before (Experiment)	After (Experiment)
Tea Tree + Lemongrass		*Image NOT correctly representative (Taken after 72h after bacteria is refrigerated)
Lemongrass + Thyme		*Image NOT correctly representative (Taken after 72h after bacteria is refrigerated)



Observations: Similar to the controls, any combination with lemongrass demostrated a colour change. Additionally, both the Thyme + Lemongrass combination and the Tea Tree + Lemongrass combinations had the least noticeable volume change. Unlike the results with Lemongrass, combinations with the inclusion of Tea Tree proved effective. Tea tree individually showcased drastic density changes. However, it is worth noting that the changes included with lemongrass application in this trial is more effective than the last, and that EOs decreases the size of the bacteria strands instead of wiping out clusters entirely.
(*Observations were noted at the 48h period*)

Table 1: A table showing the grid count of S. Epidermidis colony after applied EO treatment. The average is conducted over three different counting times to ensure maximal results accuracy.

EO Type	**Grid Count (Before)**	**Grid Count 1**	**Grid Count 2**	**Grid Count 3**	**Average Count**
Thyme (Control)	123	115	118	115	116
Tea Tree (Control)	155	138	140	135	137.7

Lemongrass (Control)	176	170	173	171	171.3
Lemongrass + Thyme + Tea Tree	158	136	137	140	137.7
Thyme + Tea Tree	139	126	122	120	122.7
Lemongrass + Thyme	160	150	147	152	149.7
Lemongrass + Tea Tree	185	176	172	172	173.3

Table 2: A table showing the average difference in S.Epidermidis colony before and after applying the EO treatments, averaged over the three counting times.

EO Type	Grid Count 1 Difference	Grid Count 2 Difference	Grid Count 3 Difference	Average Difference
Thyme (Control)	8	5	8	7
Tea Tree (Control)	17	15	20	17.3
Lemongrass (Control)	6	3	5	4.6
Lemongrass + Thyme + Tea Tree	22	21	18	20.3
Thyme + Tea Tree	13	17	19	16.3
Lemongrass + Thyme	10	13	8	10.3
Lemongrass + Tea Tree	9	13	13	11.6

Graph 1: A graph showcasing the average numerical difference in bacteria colony grid count after the application the individual EOs and EO combinations over three different counts.

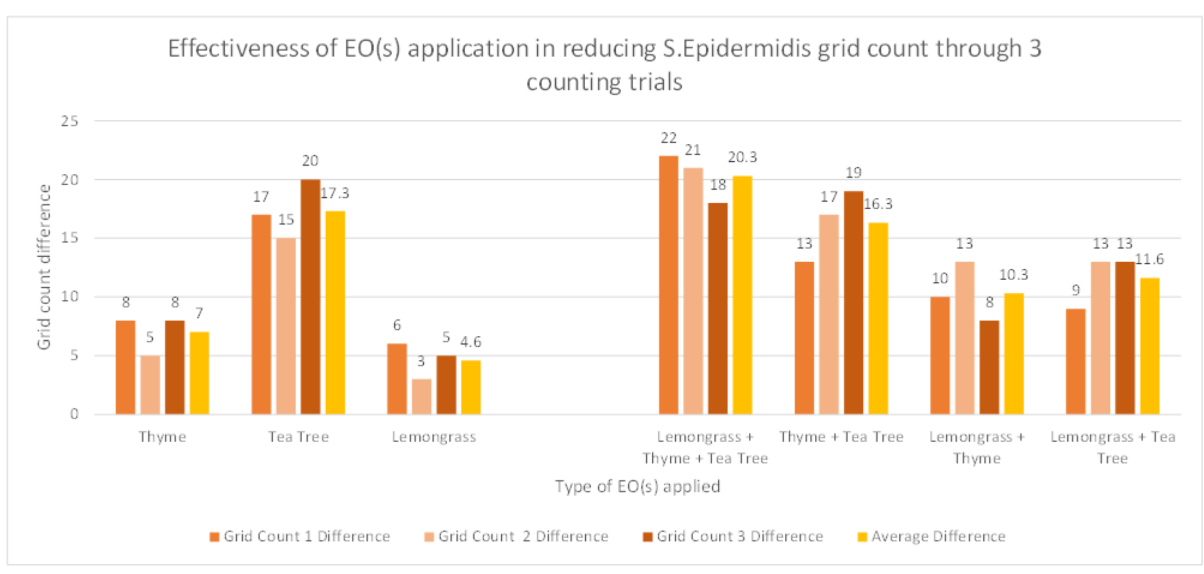
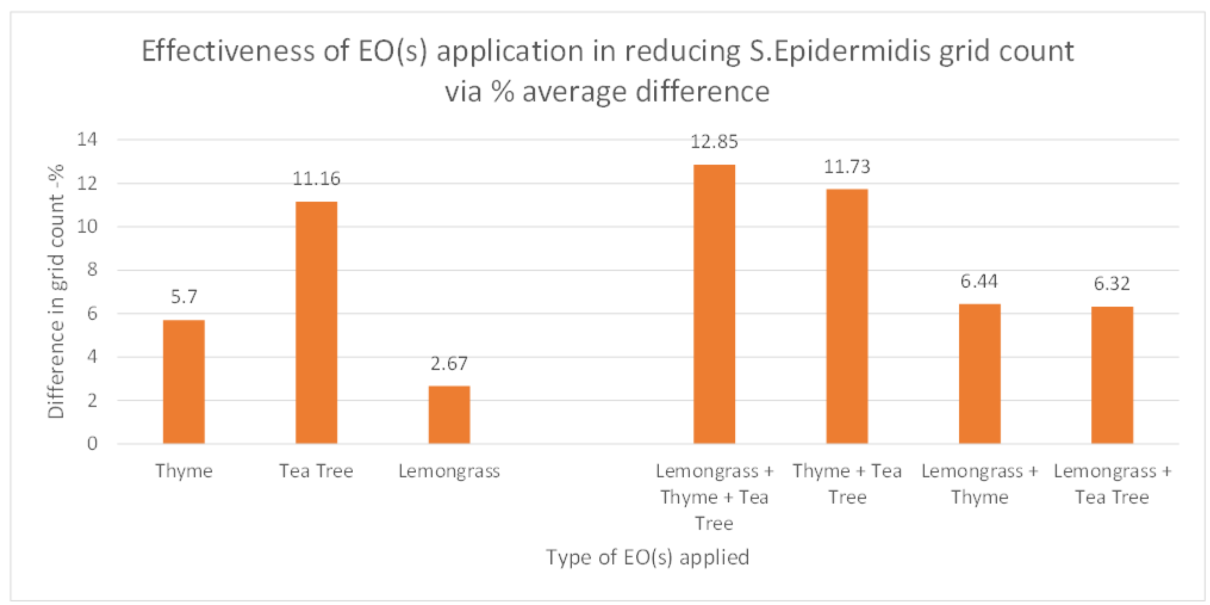


Table 3: Average - A table showing the percentage difference in S.Epidermidis colony before and after applying the EO treatment, calculated through the average difference.

EO Type	Average Count	Average Difference	Average % Difference
Thyme (Control)	116	7	5.7
Tea Tree (Control)	137.7	17.3	11.16
Lemongrass (Control)	171.3	4.7	2.67
Lemongrass + Thyme + Tea Tree	137.7	20.3	12.85
Thyme + Tea Tree	122.7	16.3	11.73
Lemongrass + Thyme	149.7	10.3	6.44
Lemongrass + Tea Tree	173.3	11.7	6.32

Graph 2: A graph showcasing the average % difference in bacteria colony grid count after the application of different individual EOs and EO combinations:



Analysis and Patterns:

The investigation compared the binary and triple combinations of Thyme, Lemongrass and Tea Tree EO in their synergestic effects with individual application. Results shows that all three Lemongrass, Tea Tree and Thyme was the most statistically effective oil combination because it had the highest bacteria percentage decreasement and also the highest average numerical decreasement. The solution's major chemical compounds include, terpinen-4-ol, thymol, carvacrol, citral and geraniol, which, as described in the hypothesis, provides the most effective antimicrobial effect due to their ability to infiltrate biofilms and compromise the enzyme production to halt subsequent biofilm developments. This also explains that while all the mixture's compound concerntration is lessened, their synergestic abilities together allows for a more effective and comprehensive bacteria wipeout, leading to the substance blend having a greater antimicrobial effect than its singular counterpart. Graph 2 further demostrates that an individual EO has less antimicrobial efficacy than the same EO mixed with another compound since their infliction on the bacteria and biofilm is less comprehensive. For example, even at a 1000 µL concerntration, Thyme's lack of ability to kill

persister will reduce its potency, compared to Thyme and Tea Tree at 500µL each complementing one another to infiltrate the biofilm, while further utilising Tea Tree's ability to disintegrate persister cells. Aditionally, although the triple combination was most effective, it only exceeded Tea Tree (11.16%) and Thyme + Tea Tree (11.73%), meaning that the terpinen-4-ol and thymol/ carvacrol compounds in Thyme and Tea Tree are also highly synergestic to one another. Tea Tree especially has the best average synergestic and individual effects, being in both the 1st, 2nd and 3rd most effective composition/s, as demostrated in the data. Throughout, lemongrass has significantly lower individual and complementary effects compared to the other EOs, this could be attributed to their singular capability to reduce the biofilm without having the same mechanism of disrupting enzyme functions like Thyme and Tea Tree.

Evaluation:

Source of Error	Improvements
Carrier oil and Dilution *(Concerntration: 50µL, 100µL..etc in comparison)*	Test a broader range of EO concerntration through dilution methods to determine the MIC (Minimum Inhibitory Concerntration), providing a baseline comparison model for each EO's effectiveness, enhancing results reliability. This information would allow for us to conduct further analysis to attribute a certain percentage of the bacteria's decreasement to a specific oil type. Aditionally, dilution further enhances the relevancy of the study as when applying EO to bacterial infections in real life, individuals usually dilute the solution to reduce the chemical irritation of a high concerntration.
Data collection method	Although this collection procedure was effective, it doesn't account for size differences in the bacteria strands, meaning that although the bacteria dollop may reduce in size, if it occupies a grid, it must be counted as a one. This leads to over-estimations of bacteria colonies and data inaccuracies since detailed changes is not fully noted. We would instead be using an inhibition diameter to assess the EO's effectiveness since it accounts for minor conditions changes.
Incubation Periods	The bacteria were incubated and checked after 48h, however, to assess the oil's influence more holistically over time, the incubation time should be more periodic at 1h, 10h, 24h, 48h and 72h. This reduces the risk of systematic error and allows for the results to be more comprehensive.
Negative Control	A negative control would allow us to observe and analyse the impacts of EO and EO compositions on a bacteria colony more holistically.
Microscope	Assesment of the bacterial changes under a microscope would further enhance the contextual understanding of the study, providing specific information on the how and why some compounds were more effective than other.
Trial Accuracy	The practical was only conducted once, meaning that its findings and results are less reliable due to potential errors, in future studies, we would be conducting this same method over more occasions to reduce the risk of errors severely infiltrating the results.

Aditional Research:

- Antiseptics treatment vs EO and EO combinations?

- Expanding the research to diverse types and concerntrations of EO vs EO combinations and analyse their effectiveness?
- Specific study into analysing the biofilm disruption of each EO and EO combination?
- What is the most effective application method for reducing S. Epidermidis growth *(eg: dressing model)*?

Usefulness: The result offers aditional ideation for researchers to develop more effective, and cost-effective cures for persistent skin infections, with sustainably sourced ingredients that can disrupt the microfilm structure and help reduce the frequency of hospital-induced infections for better global healthcare outcomes. It is recommended that the three combinations of EOs is the most effective and should be applied to the affected area however, aditional safety research must be conducted to determine the safety of the solution before application, considering the chemically active compounds that could cause irritation.

Conclusion:

Overall, the combination of Lemongrass, Tea Tree and Thyme holds the most effective antimicrobial effect for S. Epidermidis, decreasing the bacteria count be an average of 20.3 and 12.85% due to their complementary antimicrobial capabilities in a blend. This result supports the hypothesis' understanding. Tea Tree was the most potent compound both individually and synergistically, being a constituent of the 2nd most effective blend Thyme + Tea Tree at 11.73% bacterial decreasement, and also the most significantly effective control compound at 11.16%. Evidently, lemongrass was the least effective compound in singular application and also in a mixture, however, on average, an EO in combination has more antimicrobial efficacy than the individual application.

Assistance:	We would like to extend a special acknowledgement to our lab supervisor Karen Bang for her guidance and support in the investigation. Also thank you to ABHS for providing us with the neccesary equipment and materials.

2526 Words – not including appendix, references, headings, titles, figure, captions, tables and log book/journal

Appendix:

Count 1:

EO Type	Grid Count (Before)	Grid Count (After)	Difference
Thyme	123	115	8
Tea Tree	155	138	17
Lemongrass	176	170	6
All 3	158	136	22
Thyme + Tea Tree	139	126	13
Lemongrass + Thyme	160	150	10
Lemongrass + Tea Tree	185	176	9

Count 2:

EO Type	Grid Count (Before)	Grid Count (After)	Difference
Thyme	123	118	5
Tea Tree	155	140	15

Lemongrass	176	173	3
All 3	158	137	21
Thyme + Tea Tree	139	122	17
Lemongrass + Thyme	160	147	13
Lemongrass + Tea Tree	185	172	13

Count 3:

EO Type	Grid Count (Before)	Grid Count (After)	Difference
Thyme	123	115	8
Tea Tree	155	135	20
Lemongrass	176	171	5
All 3	158	140	18
Thyme + Tea Tree	139	120	19
Lemongrass + Thyme	160	152	8
Lemongrass + Tea Tree	185	172	13

Bibliography:

Alu'datt, M.H., Rababah, T., Alhamad, M.N., Gammoh, S., Al-Mahasneh, M.A., Tranchant, C.C. and Rawshdeh, M. (2018). Chapter 15 - Pharmaceutical, Nutraceutical and Therapeutic Properties of Selected Wild Medicinal Plants: Thyme, Spearmint, and Rosemary. [online] ScienceDirect. Available at: https://www.sciencedirect.com/science/article/abs/pii/B9780128146255000145.

Ariyanto, E. (2023). Peningkatan Pengetahuan Masyarakat Melalui Pelatihan Pembuatan Minyak Atsiri di Kelurahan Plaju Darat Kota Palembang. [online] Available at: https://www.researchgate.net/figure/The-structures-of-the-flavonoids-and-phenolic-compounds-in-C-citratus_fig1_335484905#:~:text=Lemongrass%20contains%20essential%20oils%20such,insect%2C%20and%20anti%2Dcancer. [Accessed 25 Jun. 2024].

BÃ¼ttner, H., Mack, D. and Rohde, H. (2015). Structural basis of Staphylococcus epidermidis biofilm formation: mechanisms and molecular interactions. Frontiers in Cellular and Infection Microbiology, [online] 5. doi: https://doi.org/10.3389/fcimb.2015.00014.

Cleveland Clinic (2022). Staph Infection (Staphylococcus Infection). [online] Cleveland Clinic. Available at: https://my.clevelandclinic.org/health/diseases/21165-staph-infection-staphylococcus-infection [Accessed 25 Jun. 2024].

El Abed, S., Koraichi Saad, I., Latrache, H. and Ghizlane, Z. (2011). Carvacrol and thymol components inhibiting Pseudomonas aeruginosa adherence and biofilm formation. [online] ResearchGate. Available at: https://www.researchgate.net/publication/272291292_Carvacrol_and_thymol_components_inhibiting_Pseudomonas_aeruginosa_adherence_and_biofilm_formation [Accessed 25 Jun. 2024].

Fey, P.D. and Olson, M.E. (2010). Current Concepts in Biofilm Formation of Staphylococcus Epidermidis. Future Microbiology, [online] 5(6), pp.917–933. doi: https://doi.org/10.2217/fmb.10.56.

Foster, T.J. (2020). Surface Proteins of Staphylococcus epidermidis. Frontiers in Microbiology, 11. doi: https://doi.org/10.3389/fmicb.2020.01829.

Gao, S., Liu, G., Li, J., Chen, J., Li, L., Li, Z., Zhang, X., Zhang, S., Thorne, R.F. and Zhang, S. (2020). Antimicrobial Activity of Lemongrass Essential Oil (Cymbopogon flexuosus) and Its Active Component Citral Against Dual-Species Biofilms of Staphylococcus aureus and Candida Species. Frontiers in Cellular and Infection Microbiology, [online] 10, p.603858. doi: https://doi.org/10.3389/fcimb.2020.603858.

Health, P.A. (2018). Essential oils: Mechanism of action on pathogenic bacteria | PlusVet Animal Health. [online] Plusvet. Available at: https://plusvet.eu/2018/01/18/essential-oils-mechanism-of-action-on-pathogenic-bacteria/#:~:text=Adenosine%20triphosphate%20(ATP)%20is%20the.

Joubert, I.A., Otto, M., Strunk, T. and Currie, A.J. (2022). Look Who's Talking: Host and Pathogen Drivers of Staphylococcus epidermidis Virulence in Neonatal Sepsis. International Journal of Molecular Sciences, 23(2), p.860. doi: https://doi.org/10.3390/ijms23020860.

Kannappan, A., Sivaranjani, M., Srinivasan, R., Rathna, J., Pandian, S.K. and Ravi, A.V. (2017). Inhibitory efficacy of geraniol on biofilm formation and development of adaptive resistance in Staphylococcus epidermidis RP62A. Journal of Medical Microbiology, 66(10), pp.1506–1515. doi: https://doi.org/10.1099/jmm.0.000570.

Kavanaugh, N.L. and Ribbeck, K. (2012). Selected Antimicrobial Essential Oils Eradicate Pseudomonas spp. and Staphylococcus aureus Biofilms. Applied and Environmental Microbiology, 78(11), pp.4057–4061. doi: https://doi.org/10.1128/aem.07499-11.

Kowalczyk, A., Przychodna, M., Sopata, S., Bodalska, A. and Fecka, I. (2020). Thymol and thyme essential oil—new insights into selected therapeutic applications. Molecules, [online] 25(18), p.4125. doi: https://doi.org/10.3390/molecules25184125.

Martínez-Santos, V.I., Torres-Añorve, D.A., Echániz-Aviles, G., Parra-Rojas, I., Ramírez-Peralta, A. and Castro-Alarcón, N. (2022). Characterization of Staphylococcus epidermidis clinical isolates from hospitalized patients with bloodstream infection obtained in two time periods. PeerJ, 10, p.e14030. doi: https://doi.org/10.7717/peerj.14030.

Mollea, C., Bosco, F. and Fissore, D. (2022). Agar Plate Methods for Assessing the Antibacterial Activity of Thyme and Oregano Essential Oils against S. epidermidis and E. coli. Antibiotics, 11(12), p.1809. doi: https://doi.org/10.3390/antibiotics11121809.

Nguyen, L., DeVico, B., Mannan, M., Chang, M., Rada Santacruz, C., Siragusa, C., Everhart, S. and Fazen, C.H. (2023). Tea Tree Essential Oil Kills Escherichia coli and Staphylococcus epidermidis Persisters. Biomolecules, [online] 13(9), p.1404. doi: https://doi.org/10.3390/biom13091404.

Oliveira, S.F., SaraivaL.M. and Carvalho, S.M. (2023). Staphylococcus epidermidis biofilms undergo metabolic and matrix remodeling under nitrosative stress. Frontiers in Cellular and Infection Microbiology, [online] 13. doi: https://doi.org/10.3389/fcimb.2023.1200923.

Orchard, A. and van Vuuren, S. (2017). Commercial Essential Oils as Potential Antimicrobials to Treat Skin Diseases. Evidence-Based Complementary and Alternative Medicine, [online] 2017, pp.1–92. doi: https://doi.org/10.1155/2017/4517971.

Otto, M. (2009). Staphylococcus epidermidis — the 'accidental' pathogen. Nature Reviews Microbiology, [online] 7(8), pp.555–567. doi: https://doi.org/10.1038/nrmicro2182.

SA Health (n.d.). Infection and injury management. [online] www.sahealth.sa.gov.au. Available at: https://www.sahealth.sa.gov.au/wps/wcm/connect/public+content/sa+health+internet/clinical+resources/clinical+programs+and+practice+guidelines/infection+and+injury+management/ [Accessed 25 Jun. 2024].

Science Direct (2010). Tea Tree Oil - an overview | ScienceDirect Topics. [online] Sciencedirect.com. Available at: https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/tea-tree-oil.

Sharma, P., James, P., Mack and Rojtman, D. (2012). TEN HIGHLY EFFECTIVE ESSENTIAL OILS INHIBIT GROWTH OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) AND METHICILLIN SENSITIVE STAPHYLOCOCCUS AUREUS (MSSA). [online] Available at: https://naturalingredient.org/wp/wp-content/uploads/4863.pdf.

Siciliano, V., Rosa Anna Passerotto, Chiuchiarelli, M., Gabriele Maria Leanza and Ojetti, V. (2023). Difficult-to-Treat Pathogens: A Review on the Management of Multidrug-Resistant Staphylococcus epidermidis. Life, 13(5). doi: https://doi.org/10.3390/life13051126.

Soković, M., Glamočlija, J., Marin, P.D., Brkić, D. and Griensven, L.J.L.D. van (2010). Antibacterial Effects of the Essential Oils of Commonly Consumed Medicinal Herbs Using an In Vitro Model. Molecules, 15(11), pp.7532–7546. doi: https://doi.org/10.3390/molecules15117532.

Swamy, M.K., Akhtar, M.S. and Sinniah, U.R. (2016). Antimicrobial Properties of Plant Essential Oils against Human Pathogens and Their Mode of Action: an Updated Review. Evidence-Based Complementary and Alternative Medicine, [online] 2016(PMC5206475), pp.1–21. doi: https://doi.org/10.1155/2016/3012462.

Walczak, M., Michalska-Sionkowska, M., Olkiewicz, D., Patrycja Tarnawska and Oliwia Warżyńska (2021). Potential of Carvacrol and Thymol in Reducing Biofilm Formation on Technical Surfaces. Molecules, [online] 26(9), pp.2723–2723. doi: https://doi.org/10.3390/molecules26092723.

Wińska, K., Mączka, W., Łyczko, J., Grabarczyk, M., Czubaszek, A. and Szumny, A. (2019). Essential Oils as Antimicrobial Agents—Myth or Real Alternative? Molecules, 24(11), p.2130. doi: https://doi.org/10.3390/molecules24112130.

Oliphant Science Awards- STEM Inquiry - What are the synergistic effects of combining various essential oils to create a potent antimicrobial blend specifically tailored for skin infections? *- Linh Bui and Maiar Elkhouly*

Friday, 28 June 2024 12:57 PM

<table>
<tr><th>Dates</th><th>Photos and Comments</th></tr>
<tr><td>15/5/24</td><td>Introduction

Name: Linh Bui and Maiar Elkhouly
Category: Scientific Inquiry (A topic related to biology)
School: Adelaide Botanic High School</td></tr>
<tr><td>17/5/24</td><td>• From the beginning, we knew that the inquiry should be regarding a topic in biochemistry since we're both highly interested and relatively knowledgeable in the topic. The brainstorming included a variety of topics:

1. Seaweed extracts as UV-blocking agents for sunscreen formulations?
○ Find the UV-blocking compounds from different species of seaweed.
○ Evaluate the UV absorption spectra of the extracts.
○ Formulate sunscreen products incorporating the seaweed extracts.
○ Test the effectiveness of these formulations in blocking UV radiation.
○ Compare the performance with commercial sunscreen products.

2. Effects of combining various essential oils to create a potent antimicrobial blend specifically tailored for oral hygiene?
○ Select a common oral pathogen to cultivate
○ Staphylococcus aureus
○ Streptococcus pyogenes
○ Pseudomonas aeruginosa
○ Test the effectiveness of some combinations of EO on reducing bacteria colony and compare this to individual application

3. Food waste extract as a natural UV absorber (eg: citrus peel)
○ Extract the food waste from selected ingredients
○ Evaluate the UV absorption spectra of the extracts.
○ Formulate sunscreen products incorporating the seaweed extracts.
○ Test the effectiveness of these formulations in blocking UV radiation and compare to a typical brand of sunscreen

4. Synergistic effects of combining various essential oils to create a potent antimicrobial blend for skin infections?
○ Built on the premise of utilising antiseptics in bandages
○ Select a common skin infection to cultivator to grow
○ Staphylococcus aureus
○ Staphylococcus epidermidis
○ Test the effectiveness of some combinations of EO on reducing bacteria colony and compare this to individual application</td></tr>
</table>

- From these ideas, which is centered around the theme of sunscreen and essential oil, we decided to investigate the synergestic effects of combining EOs, to create an antimicrobial blend and assessing the effectiveness of the mixture to the individual application for tackling a common skin infection bacteria.

Title: What are the synergistic effects of combining various essential oils to create a potent antimicrobial blend specifically tailored for skin infections?

Aim: To asses the effectiveness of combining different essential oils for a bacteria (TBD) and assesing its antibacterial efficacy in comparison to a singular EO application.

Bacteria: Staphylococcus aureus or Staphylococcus epidermidis were the two options since both are prominent causes noscomical skin infections. However, when discussing this with our lab supervisor, we've been notified that only non-pathogenic bacterias can be used, meaning that our remaining option is **S.Epidermidis**. This bacteria is a typical component of the skin flora, meaning that it is commonly harmless, but once entered into the human body, the optimal temperature and sterile enviroment allows the bacteria to cultivate into an opportunistic and viral pathogen. Under safety guidelines, monitorisation and with the adequate PPE, this can be closely replicated using an incubator set at the human body temperature condition.

To: Bui, Linh (School SA) <Sunny.BuiHuynh363@schools.sa.edu.au>
Subject: Re: Oliphant Science Award Experiment

Hi Linh
I have received your mail and the list of bacteria you would like to grow are pathogens. We are not allowed to grow any live pathogens at school unfortunately. Commercially obtained pure non-pathogenic stains can only be used at school.

20/5/24

Research Background (bacteria) (how it is contracted..etc)

- S.Epidermidis is especially hard to treat since it forms biofilms that embeds the bacteria inside a matrix of impermeable "glue". There are 4 key steps in a biofilm's formation, these are adhesion, accumulation, maturation and dispersion.

1. **Adhesion:** In this stage, the bacteria adhere/ stick to an abiotic host, specifically those with extracellular matrix components of fibronectin, collagen, and fibrinogen through the release of a series of surface proteins called adhesins. First, An autolysin degrades the bacterial cell wall and expose adhesins that bind to surfaces. , SdrG specifically binding to fibrinogen, aiding in the initial adherance process. Through the biofilm, S.Epidermidis is protected from external impacts, while being glued to a surface, aiding its ability tp spread.

2. **Accumalation:** Next, the accumalation stage is when the cell-cell adhesion is further developed, enhancing the density, structure and stability of the biofilm. The IcaADBC operon enzyme synthesizes PIA/PNAG, a polysaccharide responsible for the action and also for the facilitation of microcolonies. In this stage, the Embp further binds to ECM components and further sticks the biofilm on the host's surface. This also includes Aap, facilitating cell-cell adhesion and biofilm accumulation, and Bhp which further contributes to this intercellular adhesion, and also the development of cells in the biofilm.

3. **Maturation:** In this stage, PIA/PNAG and Embp continues working to maintian the structure and stability of the biofilm. eDNA is released at the stage through a cell lysis, and it binds with PIA/PNAG to enhance the robustness of the biofilm through connecting with other proteins and components in this matrix.

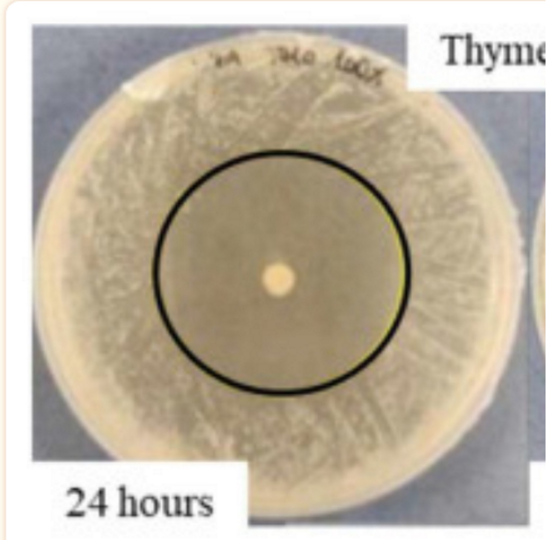
4. **Dispersal:** Enzymes and other factors that degrade the biofilm matrix, such as proteases and DNases, facilitate the release of bacterial cells from the biofilm, allowing them to spread and colonize new surfaces.

21/5/23

Research how essential oil helps
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5435909/

Patchoulol CH_3 CH_3 CH_3 OH
p-Cymene
Limonene
Pogostone OH O O O
Germacrene D
Caryophyllene
Caryophyllene oxide O
Geranyl acetate CH_2OCOCH_3
Geraniol CH_3 OH
Eugenol O HO
Carvacrol OH
Thymol OH

- The chemical compounds inside each essential oil is the key ingredient to their antimicrobial capabilities.

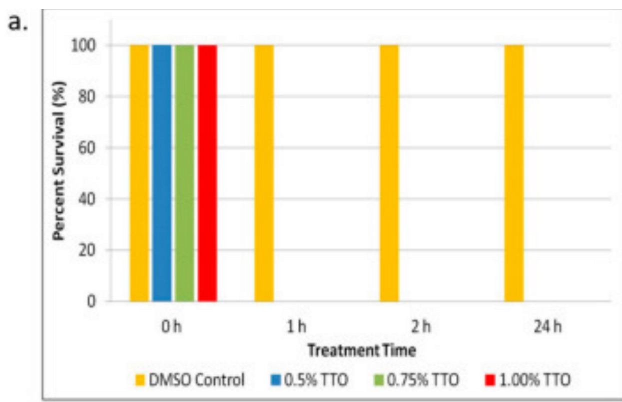


https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9774300/

Thyme oil's trial effectiveness against S.Epidermidis showcasing its halo of inhibition.

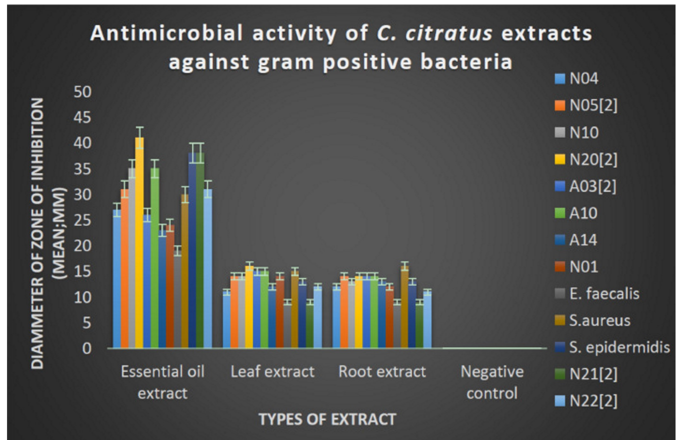
Thyme oil contains primarily thymol and carvacrol constituents, which are terpenes phenolic compounds known for their antimicrobial efficacy against S.Epidermidis, further inhibiting its biofilm formation.. The carvacrol molecule interferes with the gene coding of quorum sensing, the communication system facilitating biofilm formation, allowing for disruption in the bacteria's colony ability to form and maintain biofilms for virulence . Thymol and carvacrol works in cohesion to intergrate into the biofilm's lipid layer,

causing a chemical reaction that destabilizes the EPS, disrupting internal enzymes activities while increasing the structure's permeability. The carvacrol then inhibit enzymes that produces ATP, hindering bacterial metabolism.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8125478/



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10526169/
This graph details Tea Tree Oil's effectiveness against persister S.Epidermidis cells, which are "a small subpopulation of non-growing bacteria within a population that can survive long exposures to antibiotic treatment. Following antibiotic removal, persister cells can regrow and populate, playing a key role in the chronic reoccurrence of bacterial infections" (Nguyen et al., 2023).

Tea Tree oil consists of primarily Terpinen-4-ol, which can interfere with the biofilm matrix's production, quorum sensing and structural integrity allowing it to subsequently inhibit the bacteria's respiration and metabolic events, causing cell death *(Cox et al., 2001)*. This action is parallel to Thyme oil's, focusing on the inhibition of biofilm facilitating mechanisms and disruptions in the biofilm itself, although Terpinen-4-ol enhances the efficiency and lethality of the process. Lemongrass oil's constituents are phenolic compounds, primarily citral, myrcene and geraniol *(Ariyanto et al., 2023)*. Tea Tree oil is especially found to be effective against planktonic cells *(Kavanaugh et al., 2012)*.



https://www.sciencedirect.com/science/article/pii/S1026918520300238

Research showcasing Lemongrass Oil's effectiveness against S.epidermidis amongst other bacterias. The pure essential oil was more effective against S. epidermidis in comparison to S. aureus, which showed 1-fold wider zone of inhibition in S. epidermidis. Geraniol, the principal compound, inhibits S. epidermidis through disintegration of the EPS structure, with subsequent applications reducing the adaptive resistance capabilities of the formation. This means that especially the geraniol component in lemongrass is most responsible for its effectiveness against S.Epidermidis and its biofilm.

	https://pubmed.ncbi.nlm.nih.gov/28893370/
22/5/24	Understanding the mechanism of action in which S.Epidermidis forms biofilm and its survival capabilities as a result, along with the potential chemical compounds that separately, have been proven to be effective against the bacteria, we are now going to develop a hypothesis and a method to test the EO's effectiveness in synergy.

Hypothesis: The combined application of Lemongrass, Thyme and Tea Tree oil will produce the most optimal outcome since the complementary mechanisms of their individual compounds can more effectively disrupt the biofilm and the planktonic cells in complementary, then, suppressing the cell's adaptive resistance (geraniol) for a faster process of synergistic production inhibition (carvacrol, thymol and terpinen-4-ol).

Method Draft
Preparation of Essential Oil Solutions Preparation of Bacterial Culture Cultivate Staphylococcus epidermidis - 2.5ml Control Groups: 1. Individual Essential Oils: - Apply Individually Thyme Oil - Apply Individually Tea Tree Oil - Apply Individually Lemongrass Oil Antimicrobial Testing: Essential Oil Combinations: 1. Thyme Oil + Tea Tree Oil 2. Lemongrass Oil + Tea Tree Oil 3. Lemongrass Oil + Thyme Oil 4. Thyme Oil + Lemongrass Oil + Tea Tree Oil Procedure 1. Label and prepare nutrient agar plates. 2. Spread the adjusted bacterial suspension (2.5ml) evenly across the agar surface using a sterile cotton swab. 3. Apply 1000 µL of each test solution onto the inoculated agar plates. 4. For combination treatments, mix equal volumes of the oils to achieve desired final concentrations before application. 5. Incubate the plates at 37°C for 48 hours. 6.Count the bacteria using a Jesse hemocytometer grid. 7. Record the count for all control and test groups.

Upon evaluation, the draft lacked many details to ensure that replication of the procedure would minimize assumptions made for the steps. So we built upon the draft to enhance the specificity of the study for optimal results similarity with subsequent investigations:

Independent Variables:	Combinations of EO and each included type's µL concerntration per application

Concerntration of each EO type (% of 1000µL)	**Combinations**
50%	Thyme EO + Tea Tree EO

<table>
<tr><td></td><td>
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<tr><td>50%</td><td>Lemongrass EO + Tea Tree EO</td></tr>
<tr><td>50%</td><td>Lemongrass EO + Thyme EO</td></tr>
<tr><td>33.3%</td><td>Thyme EO + Lemongrass EO + Tea Tree EO</td></tr>
</table>
</td></tr>
<tr><td>Dependent Variables:</td><td>Remaining bacterial cell count after EO application (measured using the Jessen bacteria counting chamber technique)</td></tr>
<tr><td>Controlled (Fair Test)</td><td>
<table>
<tr><th>Controlled Variables</th></tr>
<tr><td>Total volume of applied EO (1000μL)</td></tr>
<tr><td>Total bacteria suspension volume (2.5ml)</td></tr>
<tr><td>Type of EO used (Thyme, Lemongrass and Tea Tree)</td></tr>
<tr><td>Incubation temperature (30°C)</td></tr>
<tr><td>Incubation time (48h)</td></tr>
<tr><td>Brand of nutrient agar</td></tr>
<tr><td>Method of bacterial suspension application (grid formation swapping technique)</td></tr>
<tr><td>Measurement system (Jessen grid calculation protocol)</td></tr>
<tr><td>S.Epidermidis broth solution (same vile)</td></tr>
<tr><td>Size of Agar per plate (9cm diameter)</td></tr>
</table>
</td></tr>
<tr><td>Control</td><td>
<table>
<tr><th>Concerntration of each EO type (% of 1000μL)</th><th>Type of oil</th></tr>
<tr><td>100%</td><td>Lemongrass</td></tr>
<tr><td>100%</td><td>Thyme</td></tr>
<tr><td>100%</td><td>Tea Tree</td></tr>
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Apparatus:	• 8 x 90mm (diameter) Petri Dish • Nutrient Agar Formula • Fully grown bacteria colony • 8 x Sterilized Pipette • Micropipette • Incubator • Gloves • Safety Goggles • Face Mask • Masking Tape • Sharpie • Jesse hemocytometer grid • 1 x Lemongrass Essential Oil Bottle • 1 x Tea Tree Oil Bottle • 1 x Thyme Essential Oil Bottle

	• 7 x Micropipette tips • 8 x Plastic Test Tube

Method:	1. Prepare the nutrient agar formula using the container's instructions 2. Equip the gloves, safety goggles and mask 3. Label control plates and experiment plates 4. Use a sterilized pipette to input 2.5ml of S.Epidermidis broth and swirl the bacteria around until the plate is covered. 5. Repeat steps 6 to 9 until all 8 plates are distributed with bacteria 6. Incubate the plates at 30°C for 48h 7. After 48h, remove the plates from the incubator, the biofilm and colony should be fully formed. Then, use the Jesse hemocytometer grid to count the bacteria amount for each plate and record this in an excel sheet under the intended oil application. 8. For the singular oil controls, use the micropipette to transfer 1000 µL of each EO into their marked plate and swirl the liquid until it covers the entire plate's area. 9. For the binary combinations, use the micropipette to transfer 500 µL of each EO types into their marked plate, swirl the liquid to mix and until it covers the entire plate's area. 10. For the triple combinations, use the micropipette to transfer 333 µL of each EO types into their marked plate, swirl the liquid to mix and until it covers the entire plate's area. 11. Incubate the plates at 30°C for 48h 12. After 48h, remove the plate from the incubator and use the Jesse hemocytometer grid to count the bacteria amount for each plate, repeat this process 3 times. Record this in an excel sheet under the oil's application and calculate the average count.

23/5/24 **Risk Assessment**

We conducted 2 risks assessments, the school one and OSA's.

OSA RISK ASSESSMENT FORM

for all entries in (✓) ☐ Models & Inventions and ☑ Scientific Inquiry

This must be included with your report, log book or entry. One form per entry.

STUDENT(S) NAME: Linh Bui and Maiar Elkhouly ID: 0024-020

SCHOOL: Adelaide Botanic High School

Activity: Give a brief outline of what you are planning to do.

We are planning on growing the S.Epidermidis bacteria and using it to measure the effects of individual essential oils vs combination of essential oils, which are Thyme, Lemongrass and Tea Tree. The bacteria will be incubated at 37°C at periods of 48h for the biofilm to fully form.

Are there possible risks? Consider the following:

- Chemical risks: Are you using chemicals? If so, check with your teacher that any chemicals to be used are on the approved list for schools. Check the safety requirements for their use, such as eye protection and eyewash facilities, availability of running water, use of gloves, a well-ventilated area or fume cupboard.
- Thermal risks: Are you heating things? Could you be burnt?
- Biological risks: Are you working with micro-organisms such as mould and bacteria?
- Sharps risks: Are you cutting things, and is there a risk of injury from sharp objects?
- Electrical risks: Are you using mains (240 volt) electricity? How will you make sure that this is safe? Could you use a battery instead?
- Radiation risks: Does your entry use potentially harmful radiation such as UV or lasers?
- Other hazards.

Also, if you are using other people as subjects in an investigation you must get them to sign a note consenting to be part of your experiment.

Risks	How I will control/manage the risk
Listed Below	

(Attach another sheet if needed.)

Risk Assessment indicates that this activity can be safely carried out

RISK ASSESSMENT COMPLETED BY (student name(s)): Linh Bui and Maiar Elkhouly

SIGNATURE(S): L.B M.E

☑ By ticking this box, I/we state that my/our project adheres to the listed criteria for this Category.

TEACHER'S NAME: Karen Bang

SIGNATURE: [signature] DATE: 26/06/2024

Risk	Why?	Controlling Risk
Bunsen Burner (Thermal Risk)	This is an ignition source, with temperature ranging above 1000°C, meaning that fatal burns and injury can occur with improper handling. Risks to other equipment and the facility also presents as the heat can ignite flammable materials and cause	• Ensure that in use, the Bunsen Burner is only activated on a stable and heat safe mat/ surface. • Keep all materials far away from the open flame,

Risk	Why?	Controlling Risk
Bunsen Burner (Thermal Risk)	This is an ignition source, with temperature ranging above 1000°C, meaning that fatal burns and injury can occur with improper handling. Risks to other equipment and the facility also presents as the heat can ignite flammable materials and cause subsequent damage.	• Ensure that in use, the Bunsen Burner is only activated on a stable and heat safe mat/ surface. • Keep all materials far away from the open flame, especially combustible substances and volatile chemicals. • Use flame resistant tools to handle items near the flame and keep a safe distant. Never leave the equipment unattended. • Equip the required eye ware and PPE, ensure that long hair is tied up. • Have a fire extinguisher readily available in case of an emergency.
Incubator (Electrical Risk) (Biological Risks)	This is an electrical device, meaning that malfunctioning components and faulty wires could cause electrical shocks and overheating, leading to potential fire-hazards. Aditionally, bio-hazard also presents as the incubator's temperature can develop bacteria culture, meaning that potential contamination could spread with improper handing and monitoring.	• Ensure that it is recently inspected and maintained for preventing technical damage. Keep in a dry enviroment. • Always properly seal petri dishes with bacteria culture to prevent spread into the external enviroment • Regularly disinfect the inside of the incubator.

S.Epidermidis	The bacteria, once entered inside the human body can become opportunistic, leading to potential severe and viral infections.	• Ensure to equip all the neccesary PPE (gloves, safety goggles..etc) in contact with the bacteria. Also check and bandage any open wounds. • Regularly disinfect surfaces housing the bacteria (eg: lab counter, incubator) to kill any bacterial residuals. • Dispose bacteria contaminated equipment (eg: pipette) in biohazard specified container to prevent spread. • Only handle and extract bacteria broth with a certified supervisor present. • Only use commercially obtained pure nonpathogenic strains.
Agar Plate	Agar is harmless, but bacteria or fungi grown on agar may be pathogenic, causing bio-safety hazards.	• Knowledge of microbiology and aseptic techniques is required to minimise risks to people • Dispose agar in bio-hazard specified container to regulate contamination. • Regularly monitor agar infiltrated bacteria temperature and enviroment. • Always equip the required PPE when making agar or

		using it grow bacteria broth. Always disinfect the cultivation area.
Essential Oil (Chemical Risk)	Eos are volatile organic compounds (VOCs), meaning that they have high vapors at room temperature and can cause irritation and health issues for allergic individuals through inhalation, eye contact or skin contact. Even in small quantities, ingestion of active chemicals inside EO can be fatal	• Always equip the required PPE when in contact with the chemical (eg: safety goggles, gloves, face mask..etc) • Locate a nearby eye water fountain in case of irritation. • Always disinfect the area after a spillage, use absorbable materials and store the EOs in the labelled adequate conditions.

I/we consider the inherent level of risk (risk level without control measures) to be:

Low risk **Medium risk** High risk Extreme risk

Control measures:

Students will be under teacher supervision and use careful handling and safety protection when using the bacteria.
Additional measures: safety glasses, gloves, apron

With the specified control measures in place, I/we have found that all the risks are "low risk". Risks will therefore be managed by routine procedures in the classroom, in combination with the specified control measures.

Certification by Teacher

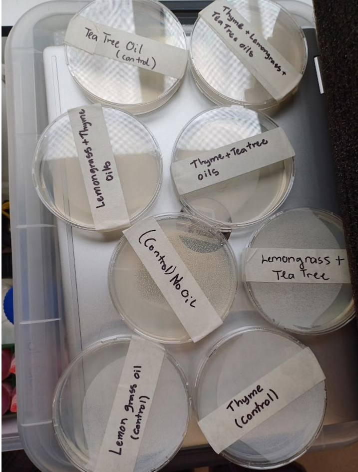
I have assessed the risks associated with performing this experiment in the classroom on the basis of likelihood and consequences using the School's risk matrix, according to International Organization for Standardization Standard ISO 31000:2018. I confirm that the risk level and control measures entered by student(s) above are correct and appropriate.

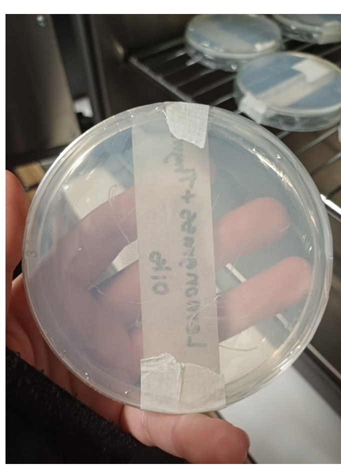
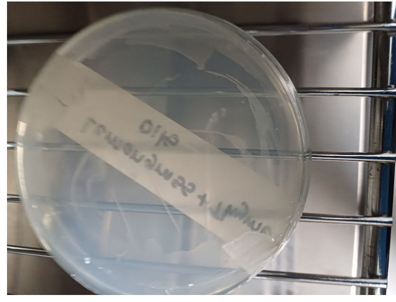
Electronic Signature: Lauren Scarfe **Date:** 23 May 2024

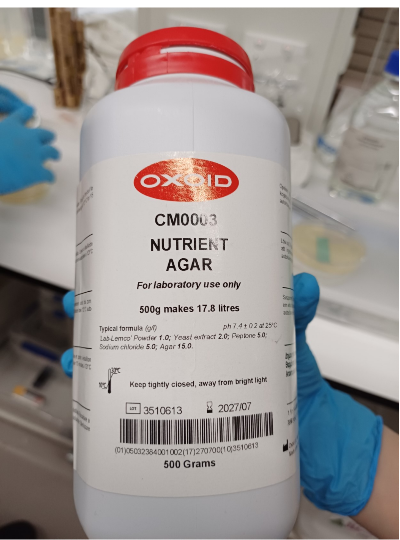
You have provided an electronic signature which is the equivalent of signing your name with a pen and as such will constitute a legally binding agreement between the relevant parties. We can give no warranty in respect to fraud or security breach resulting from the use of an electronic signature.

Certification by Laboratory Technician

I have assessed the risks associated with preparing the equipment, chemicals and and biological items, including living organisms, for this experiment and subsequently cleaning up after the experiment and disposing of wastes, on the basis of likelihood and consequences using the School's risk matrix, according to International Organization for Standardization Standard ISO 31000:2018.

	I/we consider the inherent level of risk (risk level without control measures) to be: Low risk **Medium risk** High risk Extreme risk *Control measures:* Students will be under teacher supervision and use careful handling and safety protection when using the bacteria. Additional measures: safety glasses, gloves, apron With the specified control measures in place, I/we have found that all the risks are "low risk". Risks will therefore be managed by routine procedures in the classroom, in combination with the specified control measures. **Certification by Teacher** I have assessed the risks associated with performing this experiment in the classroom on the basis of likelihood and consequences using the School's risk matrix, according to International Organization for Standardization Standard ISO 31000:2018. I confirm that the risk level and control measures entered by student(s) above are correct and appropriate. **Electronic Signature:** Lauren Scarfe **Date:** 23 May 2024 *You have provided an electronic signature which is the equivalent of signing your name with a pen and as such will constitute a legally binding agreement between the relevant parties. We can give no warranty in respect to fraud or security breach resulting from the use of an electronic signature.* **Certification by Laboratory Technician** I have assessed the risks associated with preparing the equipment, chemicals and and biological items, including living organisms, for this experiment and subsequently cleaning up after the experiment and disposing of wastes, on the basis of likelihood and consequences using the School's risk matrix, according to International Organization for Standardization Standard ISO 31000:2018. I consider the inherent level of risk (risk level without control measures) to be: **Low risk** Medium risk High risk Extreme risk Risks will therefore be managed by routine procedures in the laboratory. **Electronic Signature:** Alistair Smith **Date:** 30 May 2024 *You have provided an electronic signature which is the equivalent of signing your name with a pen and as such will constitute a legally binding agreement between the relevant parties. We can give no warranty in respect to fraud or security breach resulting from the use of an electronic signature.* **Monitoring and review** This risk assessment will be monitored using electronic review notes or hand-written notes on a printout. It will be reviewed within 15 months as part of the regular review process.
30/5/24	**Preliminary Trial 1**  In this trial, we're using a different formula composition to grow the bacteria, causing the agar to have a white/ clear gelatin instead of the typical yellow tone. First, we labelled each plate with the intended EO application name, Al, our stand in supervisor when Karen was away told us that this is extremely important to avoid confusion later on. (*Editor's note: Initially, we had plans for a negative control to serve as an aditional point of comparison, however, due to issues with the broth later on. This control was removed)*

	    Next, we applied 2.5ml of S.Epidermidis broth into the nutrient agar using a sterilized pippette, and swirled the liquid around until it covers the entire plate. This was replicated for every plate. We then incubated the bacterias at 30°C for 48h, the closest human temperature condition for allowed for ensuring a low risk school experiment . 48h is also the period required for S.Epidermidis to form their biofilms.
31/6/24	 The next day, we went to check on the bacteria and noticed that only a very small strand was able to develop. It could be mistaken for scratches on the agar surface if you weren't paying attention So, we subsequently increased temperature to 37 degrees for optimising the conditions for growth, however, we have to be much more careful when handling this as the bacteria could develop to become opportunistic.
3/6/24	 On Monday, we came back to check on the bacteria and noticed that a few more white strands has developed. However, this is still nowhere near the population we were expecting and needed to accurately undertake the experiment.



So, Karen suggested that there my have been issues with the new formula causing it to limit bacteria growth. There may have also been technical issues with the S.Epidermidis broth, so we decided that to ensure a populus and optimal bacteria colony, we would develop Agar using the trusted original formula (Oxoid CM003), and use bacteria from another grown colony to populate our dishes. This meant that the total bacteria volume was now inconsistent in each dish. However, we've decided to analyse the percentage and numerical changed in bacteria grid before and after EO application, so the initial variations would not significant compromise the data's validity.

7/6/24

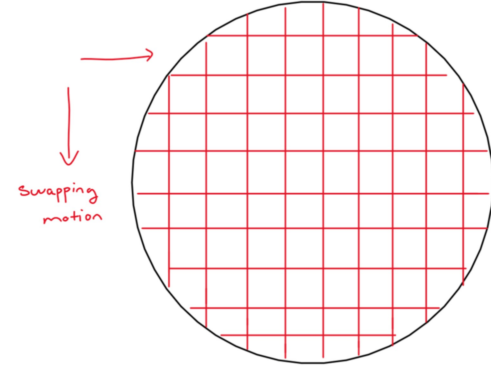
Main 2 Beginning

We made altercations to the controlled variables table and the method, as described in the changes above. We've also noticed that for the previous experiment, there was small contaminations on the agar, meaning that greater measures should be taken to sanitise and sterilise the work area. So, we sterilized the metal swapper using flame from a Bunsen burner until the tip is red. Cleaned the surface using ethanol and then use the metal swapper to transfer bacteria from one grown plate to our dishes. This transfer was conducted in a grid formation, as suggested by our lab supervisor Karen. The bacteria dishes were incubated at 37°C for 48h.

Controlled Variables
Total volume of applied EO (1000µL)
Total bacteria suspension volume (supposed to be 2.5ml)

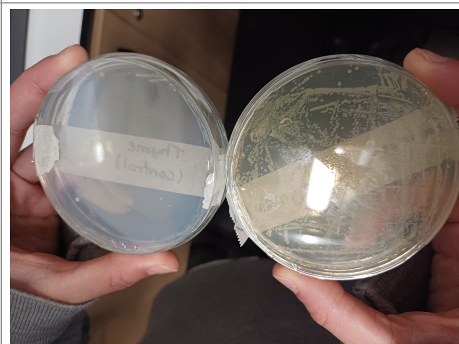
Type of EO used (Thyme, Lemongrass and Tea Tree)
Incubation temperature (37°C)
Incubation time (48h)
Brand of nutrient agar (Oxoid CM0003)
Method of bacterial suspension application (grid formation swapping technique)
Measurement system (Jessen grid calculation protocol)
S.Epidermidis broth solution (B2 Southern Biological) (same vile used)
Size and volume of Agar per plate (9cm diameter)

Apparatus:	• 8 x 90mm (diameter) Petri Dish • Nutrient Agar Formula • Fully grown bacteria colony • 8 x Sterilized Pipette • Metal Swap • Bunsen Burner • Heat Safety Mat • Micropipette • Incubator • Ethanol Solution Spray • Gloves • Safety Goggles • Face Mask • Masking Tape • Sharpie • Jesse hemocytometer grid • 1 x Lemongrass Essential Oil Bottle • 1 x Tea Tree Oil Bottle • 1 x Thyme Essential Oil Bottle • 7 x Micropipette tips • 8 x Plastic Test Tube

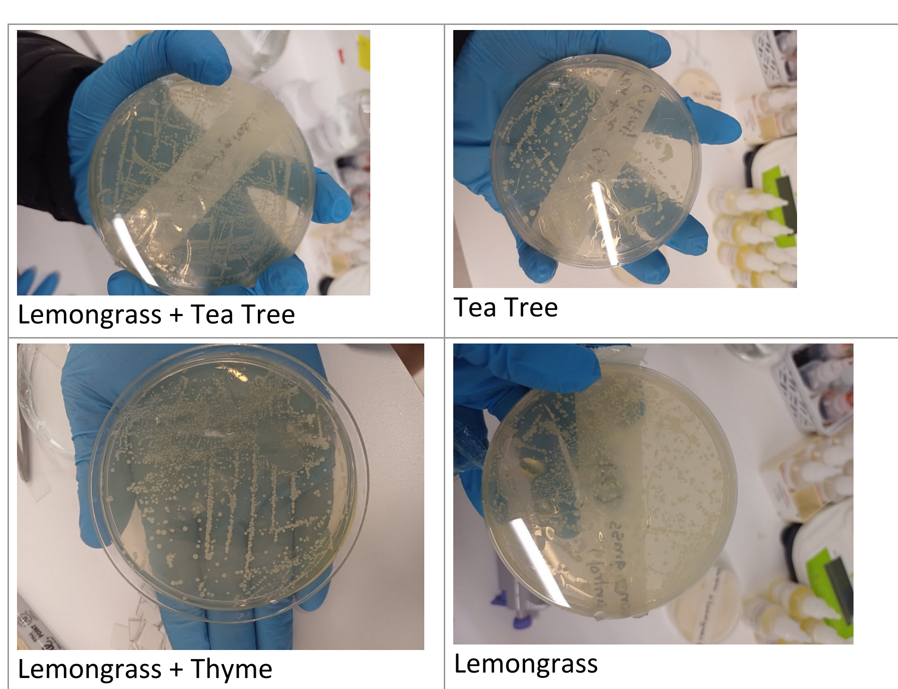
Method:	1. Prepare the nutrient agar formula using the container's instructions 2. Equip the gloves, safety goggles and mask 3. Label control plates and experiment plates 4. Turn on the Bunsen Burner to a closed flame and hold the metal swap over the heat to sterilize. Remove once the metal tip is red. 5. Spray the ethanol solution on the workbench to sterilize and prevent experiment contamination 6. Swipe the metal swap from the grown colony and draw the bacteria into the agar plate in a grid formation until all the broth is transfffered.  7. Repeat steps 6 to 9 until all 8 plates are distributed with bacteria

8. Incubate the plates at 37°C for 48h
9. After 48h, remove the plates from the incubator, the biofilm and colony should be fully formed. Then, use the Jesse hemocytometer grid to count the bacteria amount for each plate and record this in an excel sheet under the intended oil application.
10. For the singular oil controls, use the micropipette to transfer 1000 µL of each EO into their marked plate and swirl the liquid until it covers the entire plate's area.
11. For the binary combinations, use the micropipette to transfer 500 µL of each EO types into their marked plate, swirl the liquid to mix and until it covers the entire plate's area.
12. For the triple combinations, use the micropipette to transfer 333 µL of each EO types into their marked plate, swirl the liquid to mix and until it covers the entire plate's area.
13. Incubate the plates at 37°C for 48h
14. After 48h, remove the plate from the incubator and use the Jesse hemocytometer grid to count the bacteria amount for each plate, repeat this process 3 times. Record this in an excel sheet under the oil's application and calculate the average count.

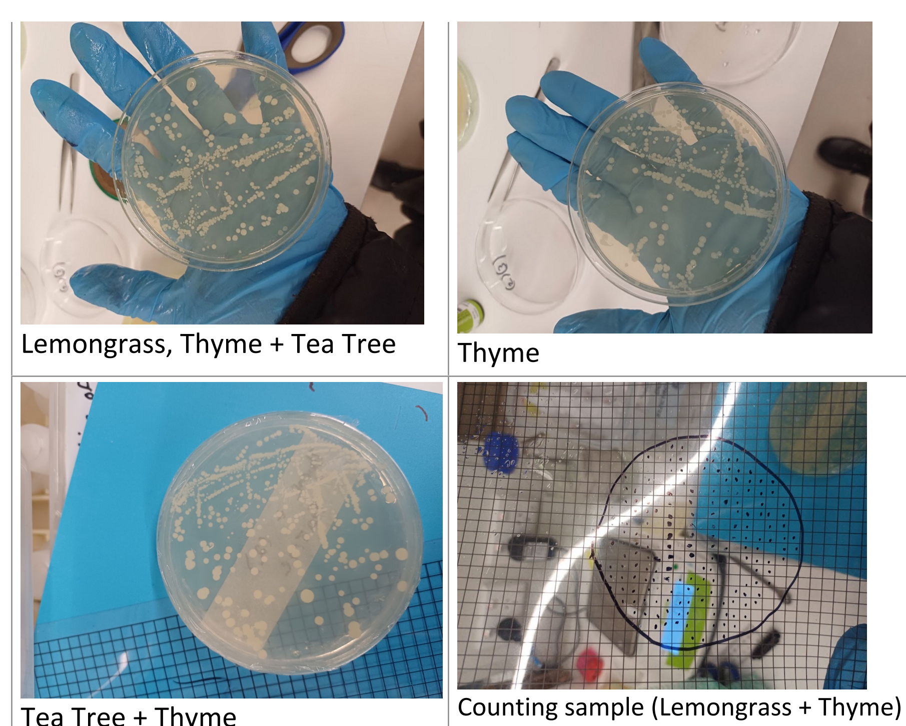
11/6/24



Compared to the previous trial, this bacteria colony growth was much more sucessful, as evidenced by the clearly defined clumps and strands in the right dish.



Lemongrass + Tea Tree	Tea Tree
Lemongrass + Thyme	Lemongrass



Lemongrass, Thyme + Tea Tree

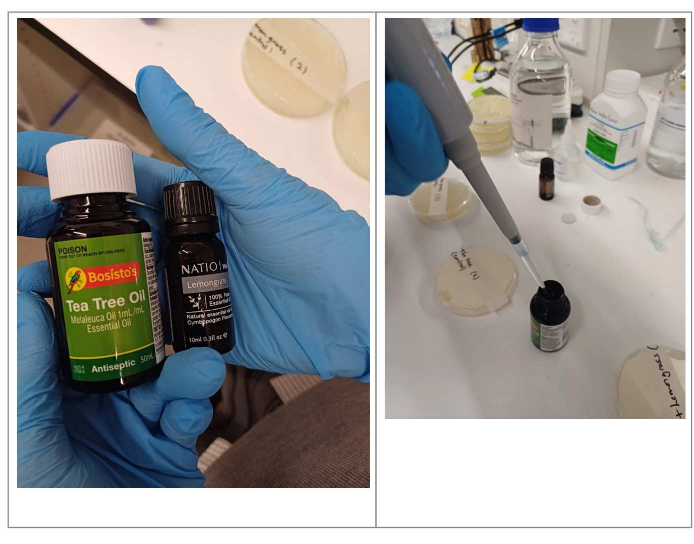
Thyme

Tea Tree + Thyme

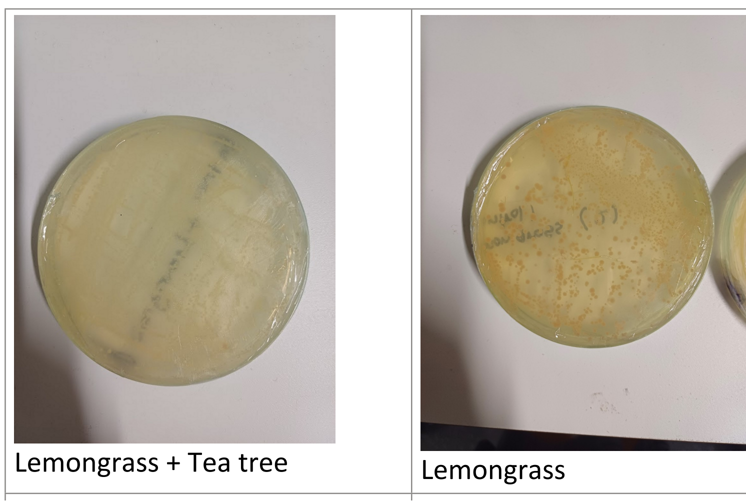
Counting sample (Lemongrass + Thyme)

EO Type	Grid Count (Before)
Thyme (Control)	123
Tea Tree (Control)	155
Lemongrass (Control)	176
Lemongrass + Thyme + Tea Tree	158
Thyme + Tea Tree	139
Lemongrass + Thyme	160
Lemongrass + Tea Tree	185

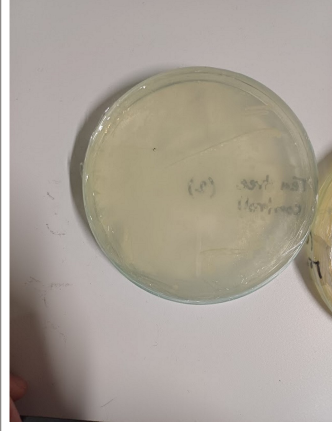
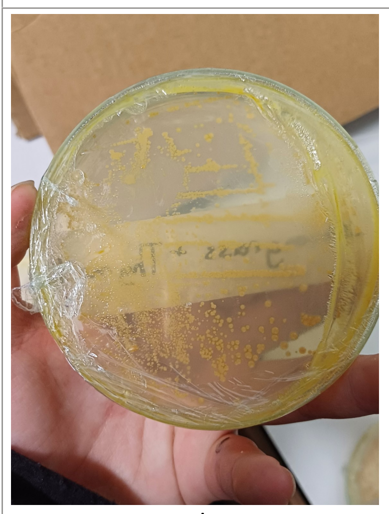
We then counted the bacteria amount in each plate using the grid as a metric, and then inputted those details into the table as seen above.



	Next, we used a micropipette to apply extremely precise amounts of EOs, into their designated bacteria colony. For the binary combinations, it was 500 µL of each EO, for the singular oil controls it was 1000 µL of each EO types, for the triple combinations it was 333 µL of each EO types. We then swirled to combine the essential oils and ensured that it covered the entire bacteria colony's surface area.
13/6/24	Today we observed the outcome from the second trial. Below are our observations for each E/o and its effectiveness. **Observations**: Similar to the controls, any combination with lemongrass demostrated a colour change. Additionally, both the Thyme + Lemongrass combination and the Tea Tree + Lemongrass combinations had the least noticeable volume change. Unlike the results with Lemongrass, combinations with the inclusion of Tea Tree proved effective. Tea tree individually showcased drastic density changes. However, it is worth noting that the changes included with lemongrass application in this trial is more effective than the last, and that EOs decreases the size of the bacteria strands instead of wiping out clusters entirely. **Observations:** By visual analysis, Lemongrass had the most distinct change in the bacteria's colour composition, which is attributed to the EO's orange hue compared to the other oil's clear ones. Additionally, although the image is blurry, Tea Tree oil demostrated the most drastic visual change, with the majority of its bacteria clearing, whereas visually, thyme and lemongrass only demostrated very minor change. To compare the Eos's effectiveness, we used the Hemocytometer grid sheets to count each agar plate three times to ensure an accurate probable average, taking into account human errors. After counting the bacteria growth using the grid sheets, we thought about other ways that will help us collect accurate results and realised that using a microscope is a great way to count bacteria but also take a closer look at the bacteria's behaviour. *Please note that the pictures attached below are taken 72 hours after the allocated 48hr incubation period. However the subsequent bacteria count is still accurate as it was conducted on the day the agar plates taken out.*



Lemongrass + Tea tree | Lemongrass

 Thyme + Tea Tree	 Tea Tree
 Thyme + Lemongrass + Tea Tree	 Thyme
 Lemongrass + Thyme	

14/6/24

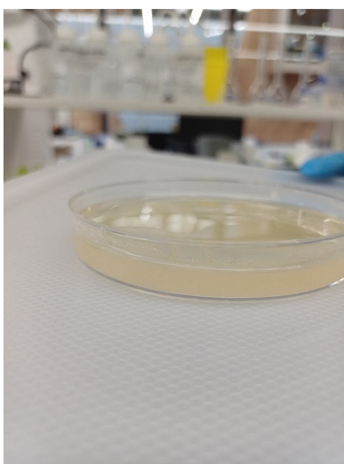
Objective: To investigate the effectiveness of essential oils in reducing bacterial colonies on agar plates, and to evaluate potential improvements for EO application.

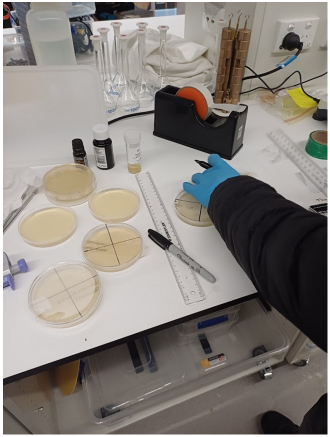
Method	1. Prepare workspace by spraying ethanol spray on workbench to sterilise. 2. Ensure the appropriate PPE is used. 3. Using a ruler and sharpie draw four quadrants on the agar plates' lids to use a guide for oil placement. 4. Use a micropipette and set the volume indicator to the following: - For the singular oil controls, use the micropipette to transfer 1000 µL of each EO into their marked plate and swirl the liquid until it covers the entire plate's area. - For the binary combinations, use the micropipette to transfer 500 µL of each EO types into their marked plate, swirl the liquid

- to mix and until it covers the entire plate's area.
- For the triple combinations, use the micropipette to transfer 333 µL of each EO types into their marked plate, swirl the liquid to mix and until it covers the entire plate's area.

5. Eject the Eos into the designated quadrant, and give it time to fully absorb into the agar plate.
6. Gently apply the bacterial broth using plastic pipette vertically and then horizontally to ensure an even spread.
7. Close the lid and wrap it using thin glad wrap to secure the lid and protect the bacteria from any external contamination.
8. Place the agar plates into the incubator for 48 hours and set the temperature to 37°C to stimulate human body temperature
9. Take the agar out and count the bacteria colony using the Jessen hemocytometer grid. Log this into a table and compare data to Trial 2's bacterial reduction.

Today we worked on our third trial. We thought about different ways that can enhance the EO/s effectiveness by ejecting the EO/s into the agar plate before the bacteria. When the EO/s was initially inputted, the plan, as discussed was to allow the liquid to fully soak into the agar composition. In the scenario, the agar represents the human skin condition that is lathered with EO/s before the bacteria could become viral. This is to asses its effectiveness before excess bacterial development vs the previous trial, which studies the EO/s effectiveness after virulence.


Essential oil initially applied


Bacteria in 2.5ml applied to each square

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<td>17/6/24</td>
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As discussed, the idea was to compare bacteria development with intial EO/s application vs EO/s application after the bacteria has developed virulence. However, the bacteria never cultivated in this practical since we did not leave enough time for EO/s to fulling soak into the agar before putting in S.Epidermidis broth, meaning that it mixed with the oil and never contacted the agar surface. If a second trial was to be conducted, we would first allow 12h for the EO/s to fully absorb into the agar surface before inputitng the bacterial broth. Unfortunately, due to these issues, this study cannot be included in our report.
</td>
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<tr>
<td>18/6/24</td>
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Objectives:

Method:

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<td>Method:</td>
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1. Prepare the nutrient agar formula using the container's instructions
2. Equip the gloves, safety goggles and mask
3. Label 1 plate as a control plate and 1 plate as the experiment plate
4. Divide the control plate into 4 equal quadrants. Mark each as (Thyme (3), Lemongrass (2), Tea Tree (3))
5. Divide the experiment plate into 5 equal quadrants. Mark each as combinations of the represented EO number
6. Using a pipette, collect 2.5ml from the S.Epidermidis broth and insert this into a plastic test tube
7. Turn on the Bunsen Burner to a closed flame and hold the metal swap over the heat to sterilize. Remove once the metal tip is red.
8. Spray the ethanol solution on the workbench to sterilize and prevent experiment contamination
9. Transfer the broth onto the plate in the same method as described.
10. Repeat steps 6 to 9 until all 8 plates are distributed with bacteria
11. Incubate the plates at 37°C for 48h
12. After 48h, remove the plates from the incubator, the biofilm and colony should be fully formed. Select a bacteria dollop from each quadrant and measure using caliper.
13. For the singular oil controls, use the micropipette to transfer 200 µL of each EO into 6 pieces of filtration paper. Place 1 piece of filtration paper on the selected dollop in its individual quadrant.
14. For the combinations, use the micropipette to transfer 33.3 µL of each type into the plastic test tubes and shake to combine.
</td>
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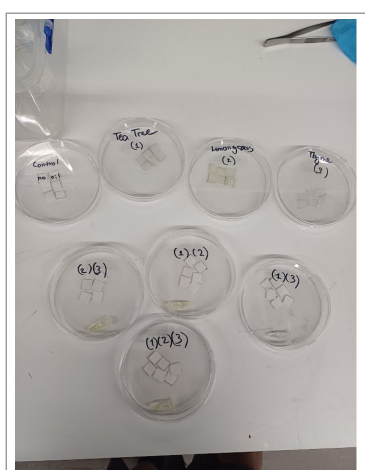
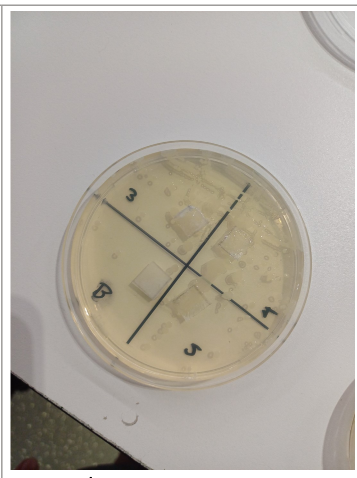
Distribute this over 6 pieces of filtration paper per application. Place 1 piece of filtration paper on the selected dollop in each combination's quadrant.

15. Incubate the plates at 37°C for 48h
16. After 48h, remove the plate from the incubator and use the caliper to measure the bacteria size underneath. Record this in an excel sheet under the oil's application.

By using filter papers, we aimed to represent a guaze and assess the EO/s' effectiveness if it was absorbed into a bandage first rather than just applying it directly onto the skin. 1x1 cm grids were drawn onto filter papers to standardise the application area and ensure consistent dosing for more accurate comparison of antibacterial efficacy. After the application EO/s we let placed the agar plates into the incubator which is set to 37° C , to leave for the next 48 hours.

 Each EO used is represented by a number	Experimental Group	 Control Group

20/4/24

Today, we put the data we counted from Trial 2 into a grid for further synthesis and calculation. As seen, The combos of Thyme + Tea Tree + Lemongrass was the most effective, having the highest average difference of 18,21 and 22 reduction counts. Aditionally, the lemongrass control, as described in the above observation was less statistically effective, at a reduction difference of only 3, 6 and 5 grid counts. Our visual observation also proved this statistics. The next step would be to synthesize these data into graphs that showcases the trends clearly.

Count 1:

EO Type	**Grid Count (Before)**	**Grid Count (After)**	**Difference**
Thyme	123	115	8
Tea Tree	155	138	17
Lemongrass	176	170	6
All 3	158	136	22
Thyme + Tea Tree	139	126	13
Lemongrass + Thyme	160	150	10
Lemongrass + Tea Tree	185	176	9

Count 2:

EO Type	Grid Count (Before)	Grid Count (After)	Difference
Thyme	123	118	5
Tea Tree	155	140	15
Lemongrass	176	173	3
All 3	158	137	21
Thyme + Tea Tree	139	122	17
Lemongrass + Thyme	160	147	13
Lemongrass + Tea Tree	185	172	13

Count 3:

EO Type	Grid Count (Before)	Grid Count (After)	Difference
Thyme	123	115	8
Tea Tree	155	135	20
Lemongrass	176	171	5
All 3	158	140	18
Thyme + Tea Tree	139	120	19
Lemongrass + Thyme	160	152	8
Lemongrass + Tea Tree	185	172	13

21/4/24

From the initial observation, Tea Tree infused with Thyme was the most effective in reducing bacteria growth, in comparison to the other statistic from Trial 2, which resulted in Thyme + Tea Tree + Lemongrass being the most potent bacterial blend in reducing S.Epidermidis development, this showcases that Thyme and Tea Tree is highly effective at a minimum concerntration model. Similarly, Lemongrass infusions was also the least potent in this minimum concerntration enviroment. These observations, however, will not be logged and utilized in the report since the nature of the bacteria strand structure (*view bellow*), in that grid formation mean that it was not possible to measure the diameter of intial bacteria habitation. Due to the tight time frame until the deadline, we were not able to replicate the experiments with these criterias considered.

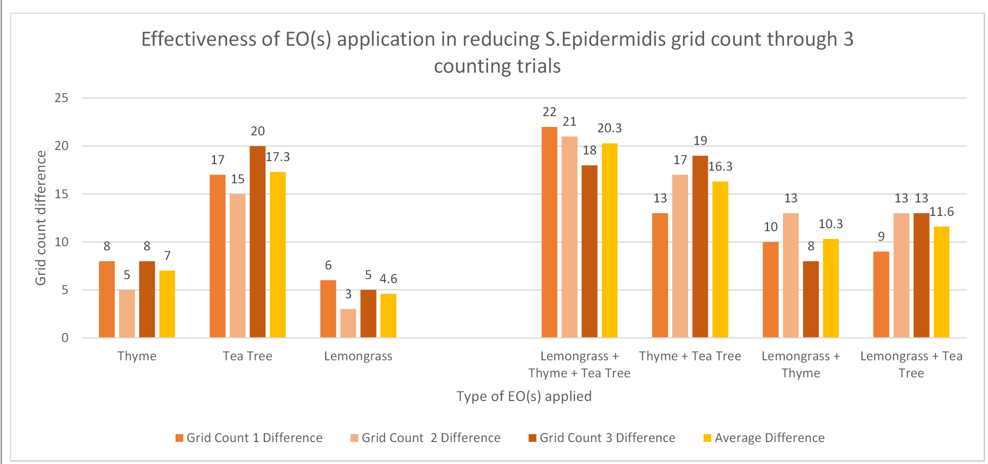
22/4/24

Today, we placed the collated data into more relevant tables and graphs for better visualization, allowing us to comprehensively analyse the antimicrobial effects of the different EO properties and generate potential conclusion.

Table 2: A table showing the average difference in S.Epidermidis colony before and after applying the EO treatments, averaged over the three counting times.

EO Type	Grid Count 1 Difference	Grid Count 2 Difference	Grid Count 3 Difference	Average Difference
Thyme (Control)	8	5	8	7
Tea Tree (Control)	17	15	20	17.3
Lemongrass (Control)	6	3	5	4.6
Lemongrass + Thyme + Tea Tree	22	21	18	20.3
Thyme + Tea Tree	13	17	19	16.3
Lemongrass + Thyme	10	13	8	10.3
Lemongrass + Tea Tree	9	13	13	11.6

Graph 1: A graph showcasing the average numerical difference in bacteria colony grid count after the application the individual EOs and EO combinations over three different counts.



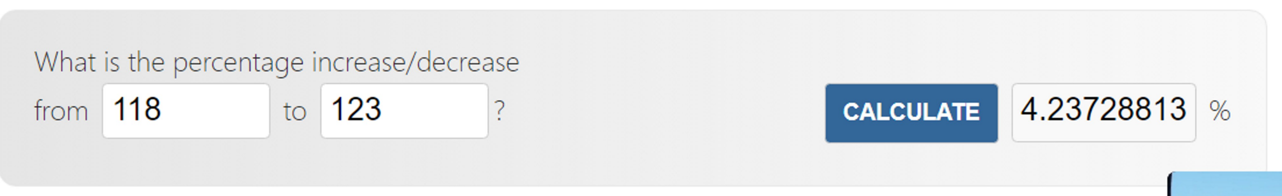
Key Findings:

- The combination of Lemongrass, Tea Tree, and Thyme was the most statistically effective oil combination.
- This combination had the highest percentage decrease in bacteria and the highest average numerical decrease.
- Lemongrass has significantly lower individual and complementary effects compared to Thyme and Tea Tree. This may be due to its singular capability to reduce biofilm without disrupting enzyme functions like Thyme and Tea Tree.

Table 3: Average - A table showing the percentage difference in S.Epidermidis colony before and after

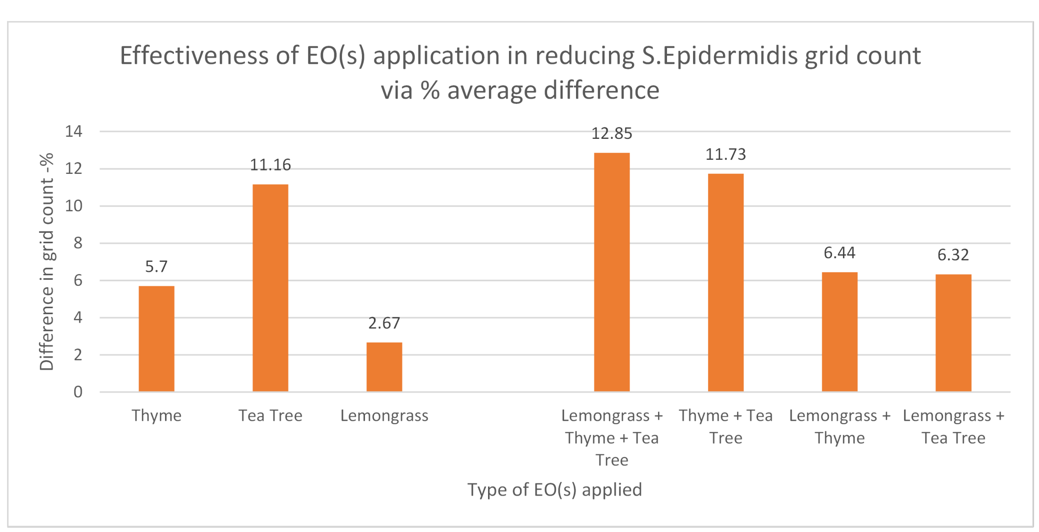
applying the EO treatment, calculated through the average difference.

- We used a percentage calculator to gage the percentage decrease or increase, enhancing points of analysis.



EO Type	Average Count	Average Difference	Average % Difference
Thyme (Control)	116	7	5.7
Tea Tree (Control)	137.7	17.3	11.16
Lemongrass (Control)	171.3	4.7	2.67
Lemongrass + Thyme + Tea Tree	137.7	20.3	12.85
Thyme + Tea Tree	122.7	16.3	11.73
Lemongrass + Thyme	149.7	10.3	6.44
Lemongrass + Tea Tree	173.3	11.7	6.32

Graph 2: A graph showcasing the average % difference in bacteria colony grid count after the application of different individual EOs and EO combinations:



- Lemongrass, thyme and tea tree EOs together were the most effective, reducing the bacterial volume through the grid count metric by 12.85%.
- Thyme + Tea were the second most effective combination, showcasing that thymol, carvacrol and terpinen-4-ol's similar mechanism of infiltrating the biofilm and production enzyme was especially effective synergistically.
- Tea Tree alone was the 3rd most effective, at a reduction rate of 11.16%, this is due to their comprehensive capabilities to infiltrate the biofilm, disrupt enzyme functions and also diminish

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<td>subsequent bacteria development from persisting cells.
• With singular application, Thyme and Lemongrass was less effective at reducing bacteria colony at only 5.7% (Thyme) and 2,67% (Lemongrass), than them in synergestic combination, with a higher decreasement of 6.44%.
• Lemongrass has significantly lower individual and complementary effects compared to Thyme and Tea Tree, with an average decreasement of only 2.67% for its individual application. In combination, this was the least effective oil with a statistic of 6.44% reduction in Thyme + Lemongrass and a 6.32% reduction in Tea Tree, which was still more effective than lemongrass in itself. In comparison, Tea Tree + Thyme was more effective, leading to the potential link between Lemongrass and a decrease in synergestic effectiveness. As a total, however, EOs in combination was more effective in inhibiting S.Epidermidis colony.</td>
</tr>
<tr>
<td>23/4/24</td>
<td>Today, we started compiling all the relevant data into our final report. We managed to get the Introduction and methods completed. However, continuous work over the next few days would be critical for getting this done on time as we are very tight to the deadline.</td>
</tr>
<tr>
<td>25/4/24</td>
<td>We managed to complete the report and are now editing to enhance the communications criteria while also working on finishing up the bibliography and citation aspects.

skin infections?

Questioning and predicting:
<table>
<tr>
<td>Abstract

200 words max</td>
<td>Staphylococcus Epidermidis is a typically non-pathogenic, innocuous and common bacterium found on human skin. However, its ubiquitous presence also allows for contamination of indwelling medical devices such as catheters, internal tubes, and prosthetics, prompting the bacteria to become opportunistic upon enter into the human body. " It is now the most frequent cause of nosocomial skin infections," (Otto, 2009). Although not viral in nature, S. epidermidis is difficult to treat since it forms biofilms, which enhances the bacteria's survivability against several antibiotics. Essential oils (EO/s) are (have) antimicrobial properties, with evidence proving their potency (potentials might be a better word but idk) against S. epidermis. The experiment builds upon this understanding to investigate the effectiveness of combining the three most statistically cohesive essential oils (thyme, tea tree and lemongrass) in observing its anti- bacterial scope (hmm synonyms?) against S. epidermis by comparison to the EOs individually.</td>
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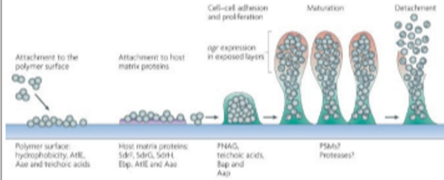
<tr>
<td>Background</td>
<td>The mechanisms in which S. Epidermidis inhibits indwelling medical devices is a four steps process, consisting of adherence, accumulation, maturation and dispersal to form biofilms, a matrix of extracellular polymeric substances (EPS) which is a major virulence (too complex?) attribute.

First, the bacteria's abundance on the skin adheres to an abiotic foreign body or biomaterials, (eg: plastic prosthetics) through surface proteins called adhesin, which anchors the bacteria's cell wall to the implant's extracellular matrix components such as fibronectin, collagen, and fibrinogen (Foster, 2020).

The Polysaccharide Intercellular Adhesin (PIA/PNAG) is then synthesized in the accumulation stage, facilitating the formation of microcolonies and the cell-cell adhesion "glue" that binds proteins and components within the biofilm together, protecting the bacteria within its impermeable matrix (Mack et al., 2015).

In maturation, the formation of Extracellular polymeric substances (EPS) like PIA/PNAG, eDNA, Protease and other proteins increases, enhancing the biofilm's structural intergrity (integrity?) and stability. Enzymes then releases bacterial cells from the biofilm, allowing them to disperse and continue colonizing surfaces.

The diagram below (Figure 1) illustrates these steps of bacteria formation



(Figure 1 - label, and then state it's source to avoid plagiarism)
S.Epidermidis causes virality issues when they enter the bloodstream or other sterile body systems, leading to various types staph infection based on the interacted areas. The complex, impermeable matrix barrier also ensures that the bacteria has a slower metabolic rate, making antibiotics, phagocytosis and immune responses less susceptible. The bacteria's cementation onto the host's surface also allows for the infection to be sustainable, requiring persistent and prolonged treatments.

Thyme oil contains primarily thymol and carvacrol constituents, which are terpenes phenolic compounds known for their antimicrobial efficacy against S.Epidermidis, further inhibiting its biofilm formation. (Marsili et al., 2021). The carvacrol molecule interferes with the gene coding of quorum sensing, the communication system facilitating biofilm formation, allowing for disruption in the bacteria's colony ability to form and maintain biofilms for virulence (Marsili et al., 2021). Thymol and carvacrol works in cohesion to intergrate into the biofilm's lipid layer, causing a chemical reaction that destabilizes the EPS, disrupting internal enzymes activities while increasing the</td>
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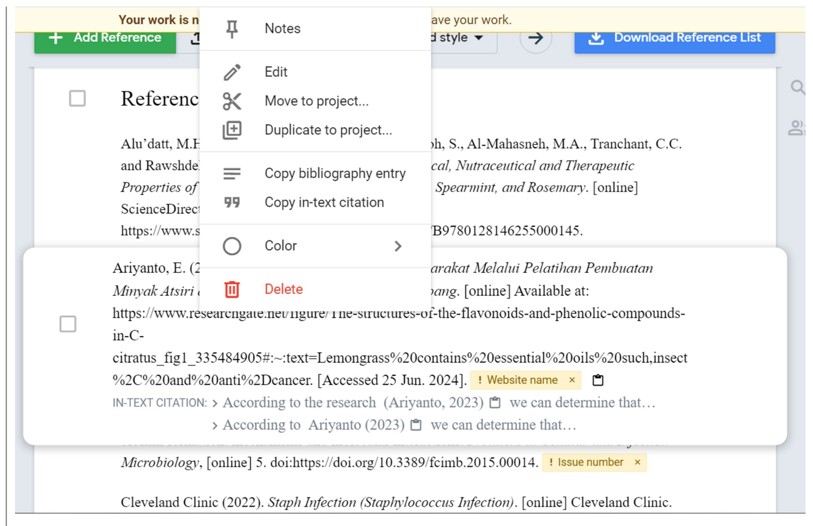
| | Your work is n
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Microbiology, [online] 5. doi:https://doi.org/10.3389/fcimb.2015.00014.
! Issue number
Cleveland Clinic (2022). Staph Infection (Staphylococcus Infection). [online] Cleveland Clinic. |

OSA RISK ASSESSMENT FORM

for all entries in (✓) ☐ Models & Inventions and ☑ Scientific Inquiry

This must be included with your report, log book or entry. One form per entry.

STUDENT(S) NAME: Linh Bui and Maiar Elkhouly ID: 0024-020

SCHOOL: Adelaide Botanic High School

Activity: Give a brief outline of what you are planning to do.

We are planning on growing the S.Epidermidis bacteria and using it to measure the effects of individual essential oils vs combination of essential oils, which are Thyme, Lemongrass and Tea Tree. The bacteria will be incubated at 37°C at periods of 48h for the biofilm to fully form.

Are there possible risks? Consider the following:

- Chemical risks: Are you using chemicals? If so, check with your teacher that any chemicals to be used are on the approved list for schools. Check the safety requirements for their use, such as eye protection and eyewash facilities, availability of running water, use of gloves, a well-ventilated area or fume cupboard.
- Thermal risks: Are you heating things? Could you be burnt?
- Biological risks: Are you working with micro-organisms such as mould and bacteria?
- Sharps risks: Are you cutting things, and is there a risk of injury from sharp objects?
- Electrical risks: Are you using mains (240 volt) electricity? How will you make sure that this is safe? Could you use a battery instead?
- Radiation risks: Does your entry use potentially harmful radiation such as UV or lasers?
- Other hazards.

Also, if you are using other people as subjects in an investigation you must get them to sign a note consenting to be part of your experiment.

Risks	How I will control/manage the risk
Listed Below	

(Attach another sheet if needed.)

Risk Assessment indicates that this activity can be safely carried out

RISK ASSESSMENT COMPLETED BY (student name(s)): Linh Bui and Maiar Elkhouly

SIGNATURE(S): L.B M.E

☑ By ticking this box, I/we state that my/our project adheres to the listed criteria for this Category.

TEACHER'S NAME: Karen Bang

SIGNATURE: [signature] DATE: 26/06/2024

Risk	Why?	Controlling Risk
Bunsen Burner (Thermal Risk)	This is an ignition source, with temperature ranging above 1000°C, meaning that fatal burns and injury can occur with improper handling. Risks to other equipment and the facility also presents as the heat can ignite flammable materials and cause subsequent damage.	• Ensure that in use, the Bunsen Burner is only activated on a stable and heat safe mat/ surface. • Keep all materials far away from the open flame, especially combustible substances and volatile chemicals. • Use flame resistant tools to handle items near the flame and keep a safe distant. Never leave the equipment unattended. • Equip the required eye ware and PPE, ensure that long hair is tied up. • Have a fire extinguisher readily available in case of an emergency.
Incubator (Electrical Risk) (Biological Risks)	This is an electrical device, meaning that malfunctioning components and faulty wires could cause electrical shocks and overheating, leading to potential fire-hazards. Aditionally, bio-hazard also presents as the incubator's temperature can develop bacteria culture, meaning that potential contamination could spread with improper handing and monitoring.	• Ensure that it is recently inspected and maintained for preventing technical damage. Keep in a dry enviroment. • Always properly seal petri dishes with bacteria culture to prevent spread into the external enviroment • Regularly disinfect the inside of the incubator.

S.Epidermidis	The bacteria, once entered inside the human body can become opportunistic, leading to potential severe and viral infections.	• Ensure to equip all the neccesary PPE (gloves, safety goggles..etc) in contact with the bacteria. Also check and bandage any open wounds. • Regularly disinfect surfaces housing the bacteria (eg: lab counter, incubator) to kill any bacterial residuals. • Dispose bacteria contaminated equipment (eg: pipette) in biohazard specified container to prevent spread. • Only handle and extract bacteria broth with a certified supervisor present. • Only use commercially obtained pure nonpathogenic strains.
Agar Plate	Agar is harmless, but bacteria or fungi grown on agar may be pathogenic, causing bio-safety hazards.	• Knowledge of microbiology and aseptic techniques is required to minimise risks to people • Dispose agar in bio-hazard specified container to regulate contamination. • Regularly monitor agar infiltrated bacteria temperature and enviroment. • Always equip the required PPE when making agar or

		using it grow bacteria broth. Always disinfect the cultivation area.
Essential Oil (Chemical Risk)	Eos are volatile organic compounds (VOCs), meaning that they have high vapors at room temperature and can cause irritation and health issues for allergic individuals through inhalation, eye contact or skin contact. Even in small quantities, ingestion of active chemicals inside EO can be fatal	• Always equip the required PPE when in contact with the chemical (eg: safety goggles, gloves, face mask..etc) • Locate a nearby eye water fountain in case of irritation. • Always disinfect the area after a spillage, use absorbable materials and store the EOs in the labelled adequate conditions.