

# Inhibitory Effect of Garlic Oil, Clove Oil, and Thyme Oil on *Micrococcus Luteus* and *Staphylococcus Epidermidis*

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## ABSTRACT

This study is aimed to investigate the antimicrobial activity of Garlic, Thyme and Clove essential oils against *Micrococcus luteus* and *Staphylococcus epidermidis*. Using natural oils to fight the bacteria will limit the usage of antibiotics, reducing the probability of antibiotic resistance which is a global increasing problem. Also, it will eliminate antibiotic side-effects such as vomiting, diarrhoea and abdominal pain, Which occurs around 1 in 10 people (NHS, 2019). Antibiotics also have an effect the biofilm layer, causing a decrease in immunity. *Micrococcus luteus* has shown no growth in the trail run when the extracts where undiluted, nor in the main investigation when dilutions took place. This reveals the susceptibility of the bacteria to the following essential oils. *Staphylococcus epidermidis* has shown to be more resistant than *micrococcus luteus*. The oils however have produced a diameter of inhibition zone (DIZ), which means the oils are effective. Clove essential oil has produced the smallest inhibition zones in all concentrations carried out, suggesting that it's the least effective extract. Thyme oil and clove oil have produced similar results; however, Thyme has shown a stronger antimicrobial effect at the 30 and 40% concentrations, whereas garlic has shown a stronger effect using the 20% concentration which has the highest coefficient of variation at 32.00% suggesting that it's the least precise result. These results indicate that these essential oils have strong antimicrobial properties suggesting a potential clinical relevance in tackling bacteria.

## 1. Introduction

The success of herbal medicine in curing infectious diseases shows that many plants have beneficial effects in various bacterial, fungal, viral or parasitic infections. For a long time ancient healers used various plants for healing diseases, often they combined the medical plants to produce a recipe(Ionescu, 2017). Since 1928, the discovery of penicillin by Sir Alexander Fleming. Antibiotics have been the prefect medical treatments to fight bacteria and heal diseases. Millions of lives were saved form extremely fatal illnesses and thus our understanding of herbal components have not evolved as rapidly as in comparison with antibiotics. The emergency of antibiotic-resistance has evolved mainly due to overuse of antibiotics and we have started to become more desperate than ever to win the fight against bacterial resistance. Our shift have turned back to the natural remedies and how they can aid us in this fight. Discovering natural remedies is important not only to fight bacterial resistance but to also eliminate side effects of antibiotics. The human bodies respond well to natural remedies, as Since ancient times people have faced diseases from urinary tract to world pandemics and during that time they relied on what's nearby which is herbs and other natural substances. There are many studies that are being carried out to prove the importance of natural products; for instance, a Recent study has proved that Clove Oil has a Antimicrobial effect on the antibiotic-resistance gram positive bacteria "Clove oil can damage the cell membrane of *L. monocytogenes*."(Cui, Zhang, Li, Lin, 2018).

### 1.1. *Micrococcus luteus*

*Micrococcus luteus* (*M. luteus*) is a gram-positive pathogenic bacteria which belongs to the Micrococcaceae family. It was firstly discovered by Alexander Fleming in 1928 and was first named *Micrococcus lysodeikticus*. *M. Luteus* is found in soil, water, dust and mammalian skin and also have been found in goat's cheese and milk (Wickham Laboratories, no date). Researchers in 2013 have found the *M. Luteus* can absorb wavelengths of ultraviolet light from 350 to 475 nano-meters. It was observed that such wavelengths of UV light have shown strong correlation with increased cases of skin cancer (Science daily, 2013). *M. Luteus* is a bacteria which is considered to be generally harmless and rarely causing diseases thus it's generally over looked; however, it's recently been stated to be a very opportunistic pathogen which can cause severe problems with patients that have compromised immune system (Kocur, M., Klosss, W. E., Schliefer K, 2006). These individuals have a very low immune system defence which affects how their body preforms to fights infections and diseases (Penn Medicine, 2020). Patients that have gone through organ transplant surgeries will be given immunosuppressant drugs to prevent organ rejection however they lower the immune system leaving the patient vulnerable. Also HIV positive (human immunodeficiency viruses) patients are at risk. Patients have been known to get chronic cutaneous infections, septic shock, septic arthritis, endocarditis, meningitis, intracranial suppuration and cavitating pneumonia from *M. luteus* (Kocur, M., Klosss, W. E., Schliefer K, 2006). It can be also responsible for causing nosocomial infections (Wickham Laboratories, no date), which is the most common type of a health care facility acquired illness and they account for 7% in developed and 10% in developing countries as stated in a scientific article published in 2017 (Khan, Baig, Mehboob, 2016), which furthermore states that according to the WHO (world health organisation) "approximately 15% of all hospitalised patients suffer from these infections". These infections are surgical wound site infections, urinary tract infections, respiratory infections, gastrointestinal infections and genitourinary infections. They cause prolonged stay in a hospital environment, disability and economic burden which all the following will cause a huge mental strain on the patients. During Hospital stay the patients are exposed to the pathogen via non-sterile environment, healthcare professionals and other infected patients and the most vulnerable are ICU (intensive care Unit) patients with decreased immunity (Khan, Baig, Mehboob, 2016). A recent study shows that Dead *Micrococcus* cells Helped enhance *Staphylococcus aureus* (a common serious bacteria that causes infections and potentially death) this caused researchers to probe the components of *M. luteus* such as the peptidoglycan (bacteria's cell wall made of polymers), to find their ability and contribution to "facilitate *S. aureus*" (Nguyen, 2018).

### 1.2. *Staphylococcus epidermidis*

*Staphylococcus epidermidis* also known as (staph. Albus) is a gram-positive bacteria. It's normally found on human skin and non-pathogenic in most circumstances. It prefers to live in sweaty places, such as armpits, but are also found on your back and in your nostrils. It works together with other micro-organisms to produce substances from sweat to cause body odour associated with perspiration (Artis micropia, no date). Analysis of the bacteria's genome showed that the species is well adapted to the harsh environment such as to cope with high salt concentration and osmotic pressure. *S. epidermidis* has eight sodium ion/proton exchangers and six transport systems for osmoprotectants to aid survival (Otto, 2010). Previously this bacteria was only regarded as an innocuous (non-harmless) commensal, it's now seen as a opportunistic pathogen and now it's the most frequent cause of nosocomial infections, at a rate about as high as that due to it's more dangerous cousin *staphylococcus aureus*. Moreover, it's infections rarely develop into life-life-threatening diseases, their frequency and the fact that they are apparently extremely difficult to tackle represent a serious stress on the public health system. Also the financial cost related to vascular catheter-related bloodstream infections caused by *S. epidermidis* is estimated \$2 billion annually in the United States. Furthermore, *S. epidermidis* represents the most frequent causative agent involved with via medical equipments such as peripheral or central intravenous catheters; it now accounts for 22% of bloodstream infections in Intensive care units (ICU) in the US. lastly,

second only to *Staphylococcus aureus*, *S. epidermidis* causes 13% of prosthetic valve endocarditis (PVE) infections, with high rate of intracardiac abscesses 38% and 24% mortality (Otto, 2010). This information is supported by another study (Palraj, Wilson, 2016) which illustrates that although PVE is rare, it's caused by coagulase-negative staphylococci which includes *S. epidermidis* in 15% to 40% cases (resulting from inoculation at the time of surgery) and manifests within 12 months of valve placement. The bacteria are also a significant cause of many joint replacement implant infections. In the United Kingdom staphylococcus epidermidis was isolated in 36% of total hip arthroplasty and 49% of total knee arthroplasty surgeries. Another study has showed that infected hips and knees arthroplasties are about 77% staphylococcus epidermidis related (Buttner, Mack, Rohde, 2015).

*S. epidermidis* is an anaerobic bacteria but also grows quite well under aerobic conditions. An article presents more evidence which states that “*S. epidermidis* is responsible for 35% to 60% of infections of synthetic urinary sphincters and penile prostheses in which overall infections rate of 2% to 4% is observed” (Science direct, 2015). It has been introduced that *S. epidermidis* may have a probiotic effect. As this bacteria is generally harmless *Staphylococcus aureus* is anything but that, fortunately *S. epidermidis* helps our body to defend against *Staphylococcus aureus*. To do this it uses serine protease Esp. This is an enzyme that has an effect of slowing the growth of *S. Aureus*, however not all produce the enzyme (Artis micropia, no date). Our biofilm are in constant war as there is dense competition. Thus bacteria have mechanisms to kill other bacteria; they compete for nutrients leaving little food for invaders to survive. Taking antibiotics against a bacteria such as *S. Aureus* will remove all defences present by a healthy microbiome leaving potential breeding ground for invaders especially those antibiotic-resistant.

### 1.3. Essential oils

I will be investigating how three different natural herbal extracts will have an impact on the growth of *M. Luteus* compared with *S. Albus* at different concentrations of the essential oil extracts when incubated. In this study we aim to characterise Clove oil, Thyme oil and garlic oil as a potential effective way to attack and minimise the growth of the bacteria leading to a clinical relevance and developing our understanding of natural remedies as we take more interest of it in this modern era.

Clove is a tree, which especially in Asia has been traditionally used a spice seasoning for centuries and in tea. The extract of clove oil has been essential in the manufacturing of Chinese and Indian medicine as it contains many medical properties. Studies have shown the it has an effect at maintaining oral health as it has an effect on plaque, gingivitis and mouth bacteria thus it's used in dentistry. Importantly it shown medical properties like antioxidant and anti-inflammatory. The clove Buds contain 14 to 20 percent of essential oil and it contains components which are phenylpropanoides such as carvacrol, thymol, eugenol and cinnamaldehyde. It is used as a local anaesthetics in dentistry (Encyclopaedia Britannica, no date), due to the component eugenol which can act on the ions channels preventing a threshold for a action potential (Khalilzadeh, Hazrati, Gholamreza, 2016). Despite clove oil being antimicrobial as proven in many studies. In one case however that was carried out to investigate the effect of clove oil on *E. Coli* it showed a negative effect and therefor proved to be not very effective in inhibiting the growth of clinical strains of *E. Coli* (Packyanathan, Prakasam, 2017).

Thyme, is a greenish-gray aromatic leaves. It originates from southern Europe and countries on the mediterranean borders. Its used as a flavouring spice in many foods around the world. Traditional remedies included tackling pulmonary diseases, Roman soldiers have been known to bathe in thyme to become courageous, in the 19th century, thyme oils were used by dentists to treat oral obsesses, inflammation and as a antiseptic for endodontics. The main components of thyme are thymol and carvacrol which have important attributes to thyme's numerous biological actions. According to this article it's stated that thyme oil in some cases proved to be the most potent antioxidant among many other natural oils (Singletary, 2016). A study carried out in 2006 showed that both thyme and clove oil have antimicrobial activities and the origin of the herbs (geographical locations) have no effect on their antimicrobial activity (Nzeako, Al-Kharousi, Al-Mahrooqui, 2016).

Garlic oil (Nech- shinkurt in Amharic/Local name/) (*Allium sativum* L.) is under family Liliacea. Is potentially the oldest and most commonly used for its antioxidant properties. It has a ageing tradition as a medical plant from approximately about 3000 BC. Investigations on garlic have began in 1939 and there are many studies that have assessed the value of garlic's clinical relevance(Ejigu, 2016). Garlic has shown to be an effective antibiotic and further more similar effect as penicillin, even vapour of sliced garlic as shown to kill bacteria at a distance of 20 cm! Many diseases such as tuberculosis was treated with garlic by breathing in the vapour. Allicin is found to be the component in garlic that has a antibacterial effect, which is proven to be capable of illuminating different infections(Abiy, Berhe, 2016). Methicillin-resistant staphylococcus aureus (MRSA) has been uncovered to be susceptible to allicin. Different from conventional antibiotics, allicin can kill Bactria via the gas phase and thus many lung bacteria are susceptible. Allicin is toxic to human cells and therefore a correct approach has to be taken in order to control the damage(Leontiev, Hohaus, claus, Gruhlke, Slusarenko, 2018).

## 2. Materials and Methods

### 2.1. Reagents

The essential oil Garlic, Thyme and Clove where purchased from Holland & Barrett. This is an international, world leading health and wellness retailer and the largest in Europe. The brand that supplies the extracts is known as Mi- aroma(Holland and Barrett, no date).

#### *Bacteria strains and Growth conditions*

The study was carried out within the college lab, which meant that the two bacteria strains were supplied to us via the school's science lab department. The bacteria were swapped onto the agar petri dish by the lab technicians prior to us inoculating the extracts. Both bacteria's were incubated within the college lab at 35-37°C for 48 hours.

### 2.2. Apparatus

Bunsen burner, sterile forceps, 60 Sterile filter paper discs, hazard tape, marker pen, ruler, 18 Cotton wool swap, McCartney bottle with the bacteria, incubator, 18 Petri dish for Agar culture (one sterile dish for placing the soaked discs to dry before a repeat) , 2 Pipettes (one measures 0.1-1ml and one measures 1-10ml), different essential oils used, disinfectant solution and cloth for sterilising, 6 sterile distilled water of 20ml and a rack with containers that will be used to contain oils with all diluted concentrations (1 rack 9 containers).

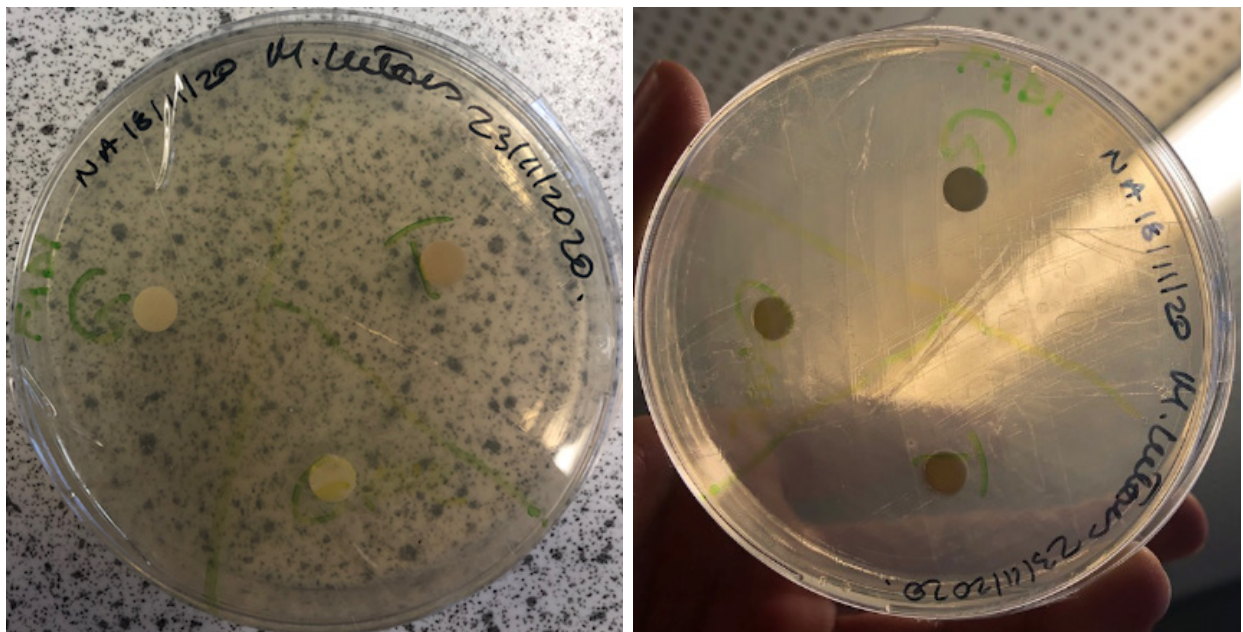
### 2.3. Method

1. Clean and sterile the workspace thoroughly with the disinfectant solution.
2. Turn on Bunsen burner to draw air currents away from the sterile workspace to prevent any external bacteria from the atmosphere drawn in and changing the variability of the investigation.
3. Work closely to the Bunsen burner. Use a sterile clean swap and swap the bacteria on the surface of one agar petri dish ensuring that the swap overlaps each time to cover the entire surface area evenly and close the dish.
4. Make the concentrations of the chosen extract and place them ready on the rack. Flame the forceps to prevent contamination and pick up the sterile filter paper discs and place on the second testing petri dish ensuring which disc is which. Dip the disc into the pure mineral concentration which is being tested and allow to dry for approximately five minutes on a desperate open sterile dish before repeating for a two more times.
5. For our negative control test add distilled water to the filter paper disk using a sterile pipette before Repeating it similarly to step 4 with the other three substances.

6. Use the agar plate that with the prepared bacteria on it. Divide the plate into four sections evenly using a marker pen on the bottom of the dish and label each section with your name and date.
7. Open the dish and flame the forceps in order to pick up the discs with the substances on them and place each one on the centre of the section.
8. Tape the lid closely and incubate.

#### 2.4. Trial run

Prior to performing the investigation procedure, it was appropriate to carry out a trial run to investigate whether these extracts actually have antimicrobial effect on the bacteria. This will give an indication whether results will be achieved and if it's appropriate to still go ahead with the study. The trial was done using a small amount of undiluted extracts (0.1ml) on the bacteria *Micrococcus luteus*. This will give a clear indication if the extracts will produce any inhibitions before carrying out dilutions.



**Figure 1:** Results of *Micrococcus luteus*

The results of the trial show no bacterial growth on the agar petri dish which can either be due to an error made while inoculating the bacteria on the dish or simply the extracts have completely inhibited the growth of the *Micrococcus luteus*. To investigate this further, another trial was made using *Micrococcus luteus* alongside *Staphylococcus epidermidis*, using the same undiluted concentration (0.1ml).





Figure 2. Micrococcus luteus



Figure 3. Staphylococcus epidermidis

In figure 2, Micrococcus luteus has again shown similarly to (figure 1) no bacterial growth which eliminates the option of human error and shows that the bacteria are completely susceptible to the three extracts. There is a small amount of bacterial growth at the edges of the clove oil region which may indicate that clove was the least strong out of the three extracts. In Figure 3 however, we can see clear inhibition zones which means that the bacteria is susceptible to the extracts; however, in comparison with figure 2 staphylococcus epidermidis can resist the extract better than Micrococcus luteus.

#### Results for Staphylococcus epidermidis - trial 2

Essential Oils	Diameter of inhibition zone (cm)	Area = $\pi r^2$
Thyme	4.5	15.9cm <sup>2</sup>
Garlic	2.6	5.3cm <sup>2</sup>
Clove	2.2	3.8cm <sup>2</sup>

From the findings of the two trials, the concentrations and dilutions used in the study were determined. Due to Micrococcus luteus being much weaker a lower concentration was used.

#### Concentrations used for Micrococcus luteus in the study

	20% concentration	30% concentration	40% concentration
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Concentrations used for *Micrococcus luteus* in the study

Thyme essential oil	0.2ml oil/0.8ml distilled water	0.3ml oil/0.7ml distilled water	0.4ml oil/0.6ml distilled water
Clove essential oil	0.2ml oil/0.8ml distilled water	0.3ml oil/0.7ml distilled water	0.4ml oil/0.6ml distilled water
Garlic essential oil	0.2ml oil/0.8ml distilled water	0.3ml oil/0.7ml distilled water	0.4ml oil/0.6ml distilled water

Concentrations used for *Staphylococcus epidermidis* in the study

	20% concentration	30% concentration	40% concentration
Thyme essential oil	2ml oil/8ml distilled water	3ml oil/7ml distilled water	4ml oil/6ml distilled water
Clove essential oil	2ml oil/8ml distilled water	3ml oil/7ml distilled water	4ml oil/6ml distilled water
Garlic essential oil	2ml oil/8ml distilled water	3ml oil/7ml distilled water	4ml oil/6ml distilled water

## 2.5. Statistical Analysis

After collecting the findings of the diameters of each inhibition zones, the area of the zones will be calculated using equation 1. From this we can make observations to determine which extract has the greatest effect on the bacteria. The variability of the data will be measured using standard deviation to determine the average distance of each data set from the mean. This is important to find out the average distance of the points from the centre of distribution, whether the points are scattered apart widely or closely packed near the centre. The formula that will be used For standard deviation used is shown in equation 2. Furthermore, in order to compare the data from different essential oils the coefficient of variation will be calculated from the standard deviation measured. Also the coefficient variation shows the rate of dispersion around the mean value, this is shown in equation 3.

$$\text{Area} = \pi r^2 \quad (1)$$

$\pi = 3.14$   $r =$  radius of zone

$$SD = \sqrt{\frac{\sum (X - \bar{X})^2}{N - 1}} \quad (2)$$

SD=Standard deviation

N=the size of population

$\bar{X}$ = each value from a population

$\bar{X}$ = mean

$$Cv = \frac{SD}{\bar{X}} \cdot 100 \quad (3)$$

Cv= coefficient of variation

SD=standard deviation

$\bar{X}$ = mean

### 3. Results

#### 3.1. Micrococcus luteus

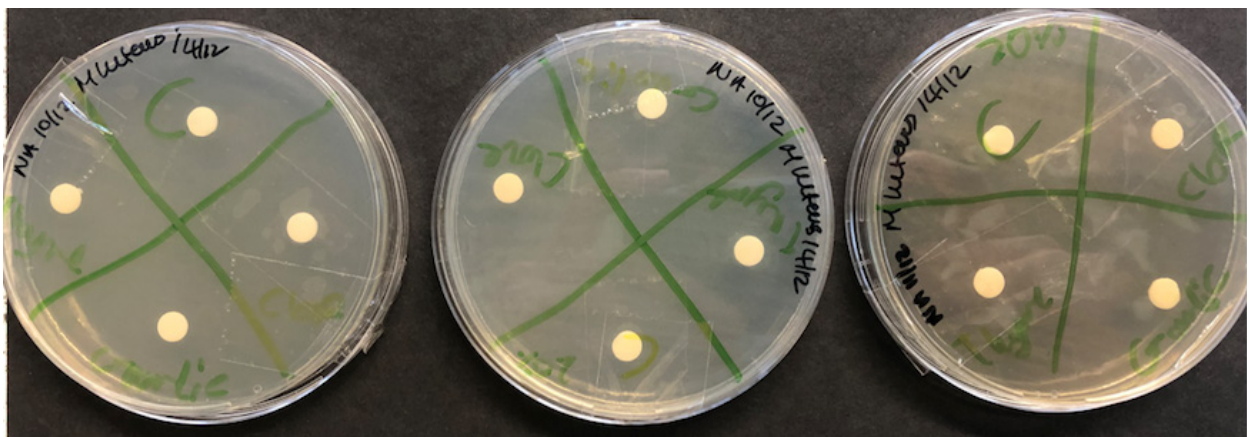


Figure4. Micrococcus luteus - 20% concentration with control

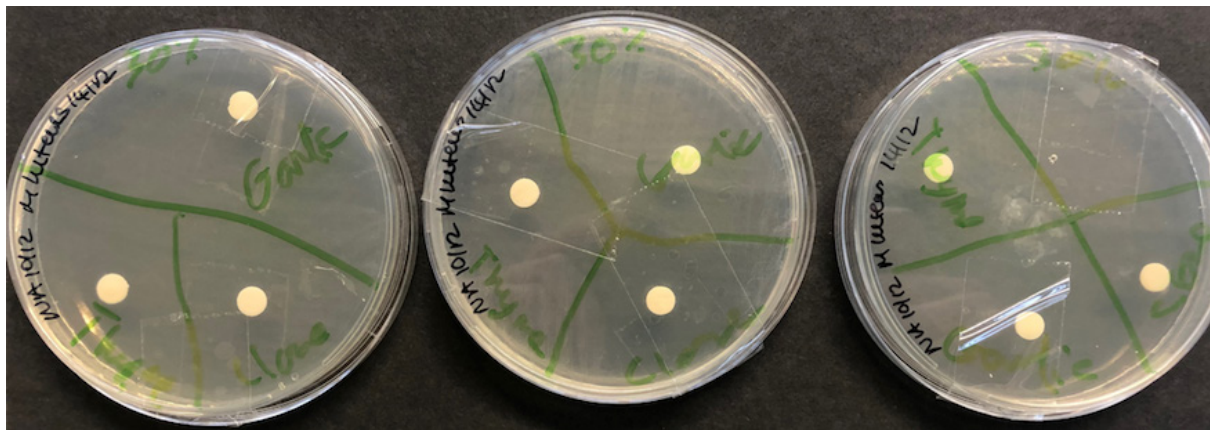
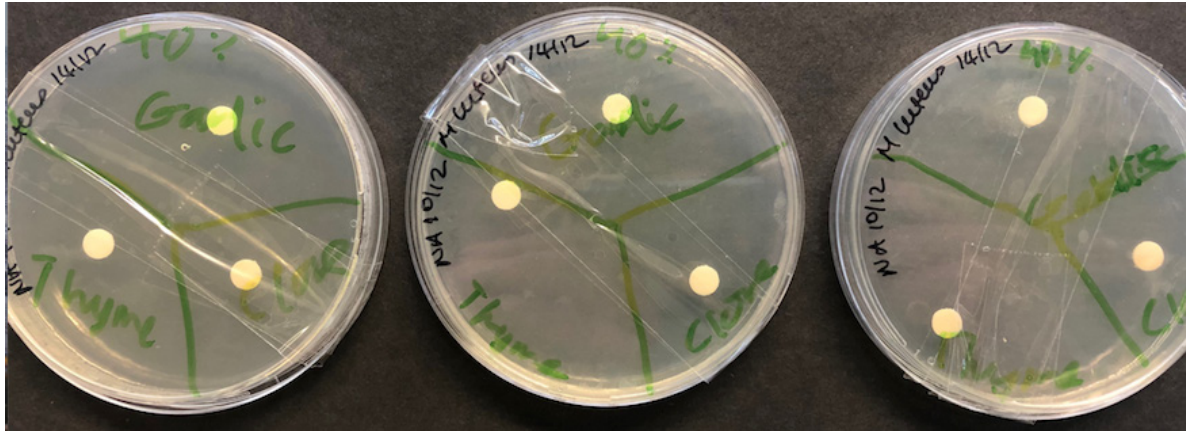


Figure5. Micrococcus luteus - 30% concentration

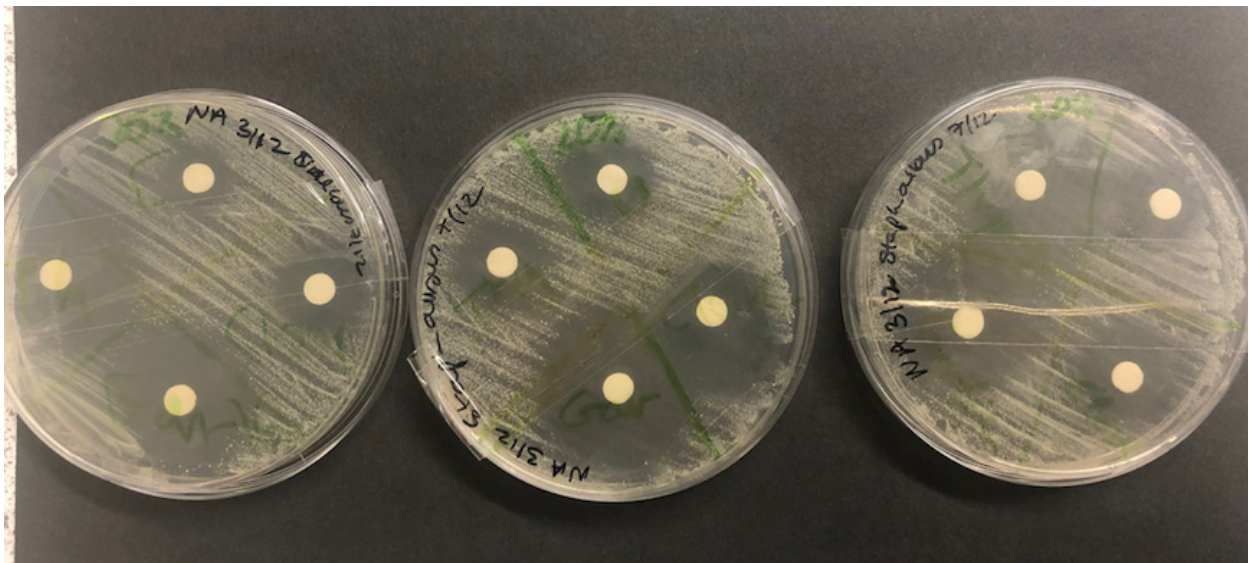




**Figure6.** *Micrococcus luteus* - 40% concentration

No data will be shown for the results of *Micrococcus luteus* as it hasn't shown any growth despite the dilutions being carried out. These results are promising despite there being no data collected, the observations show the susceptibility of the bacteria towards the essential oils. The extract with the strongest antimicrobial properties will not be determined however, as there are no inhibition zones present thus, further investigations can be undertaken to determine this.

### 3.2. *Staphylococcus epidermidis*



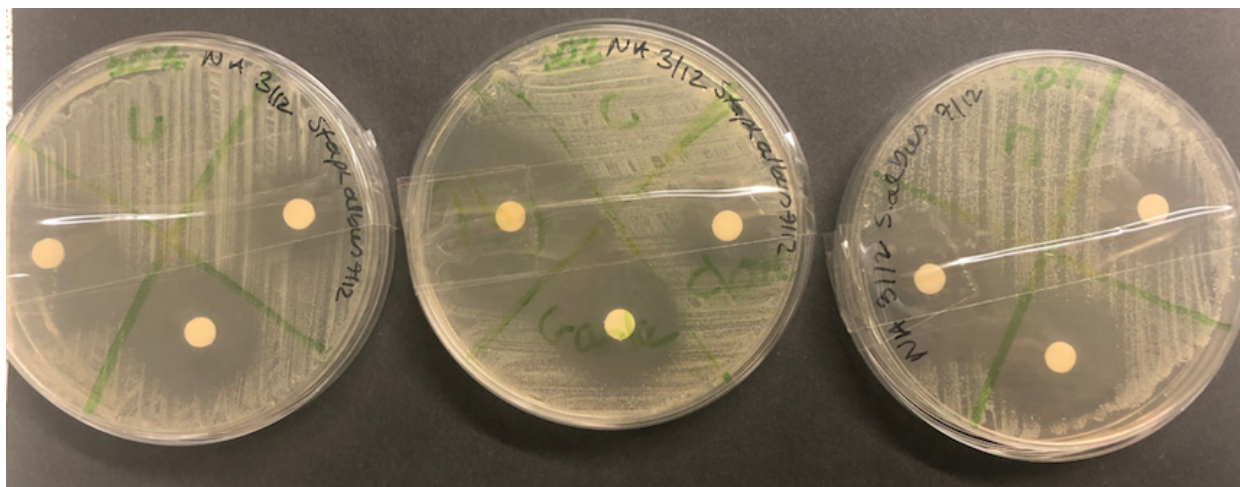
**Figure7.** *Staphylococcus epidermidis* - 20% concentration with control discs

*Staphylococcus epidermidis* - 20% concentration diameter of inhibition zones

	Thyme - Diameter (cm)	Clove - Diameter (cm)	Garlic - Diameter (cm)	Control disc - Diameter (cm)
Repeat 1	3	2.3	3.3	1.3

Staphylococcus epidermidis - 20% concentration diameter of inhibition zones

Repeat 2	2.8	2.5	4.6	1.5
Repeat 3	3.3	2.3	3.4	1.5



**Figure 8.** Staphylococcus epidermidis - 30% concentration

Staphylococcus epidermidis - 30% concentration diameter of inhibition zones

	Thyme - Diameter (cm)	Clove - Diameter (cm)	Garlic - Diameter (cm)
Repeat 1	3.5	2.2	3
Repeat 2	3.6	2	2.8
Repeat 3	4	2	3.1

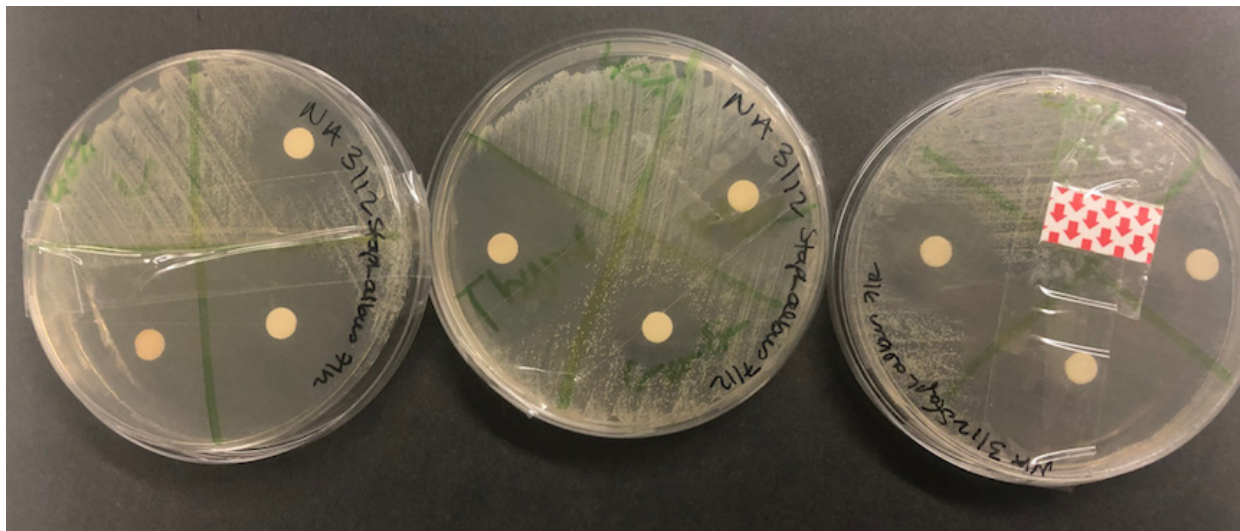


Figure 9. Staphylococcus epidermidis - 40% concentration

Staphylococcus epidermidis - 40% concentration diameter of inhibition zones

	Thyme - Diameter (cm)	Clove - Diameter (cm)	Garlic - Diameter (cm)
Repeat 1	4.1	2.4	3.5
Repeat 2	3.9	2.5	2.8
Repeat 3	3	2.6	3

It is clear that a contamination has occurred in the 20% concentration of the investigation as inhibition zones are present by the control discs. This can be due to a human error done while the investigation being carried such as using the wrong pipette head or a leakage of excess oil which was absorbed by the control disc. Therefore, an extra test was carried out to test the 20% concentration. In figure 10, we can see that the results shown on the petri dish has showed no inhibition zone by the control disc.

Inhibition zones shown by figure 10

	Thyme	Clove	Garlic	Control disc
Diameter (cm)	1.9	2.1	4.2	0

The results of figure 10, prove that the control disc should no show inhibition. Although the result for thyme oil is lower than expected, the results of clove and garlic are within the expected range according to the three main three repeats of the 20% concentration. In this case it is appropriate that when calculating the results the main three repeats shown in figure 7 will be used except the control disc which has been proved here to show no inhibition.





Figure 10. Staphylococcus epidermidis - 20% concentration repeat

### 3.3. Table results

#### Staphylococcus epidermidis - Thyme essential oil results

Concentration	20%	30%	40%
Repeat 1 (area cm <sup>2</sup> )	7.02	9.60	13.20
Repeat 2 (area cm <sup>2</sup> )	6.20	10.20	11.90
Repeat 3 (area cm <sup>2</sup> )	8.60	12.60	7.10
Mean area	7.29	10.80	10.73
Standard deviation	0.99	1.30	2.62
Coefficient variation (%)	13.61	12.00	24.44

#### Staphylococcus epidermidis - Clove essential oil results

Concentration	20%	30%	40%
Repeat 1 (area cm <sup>2</sup> )	4.20	3.80	4.50
Repeat 2 (area cm <sup>2</sup> )	4.90	3.10	4.90



Staphylococcus epidermidis - Clove essential oil results

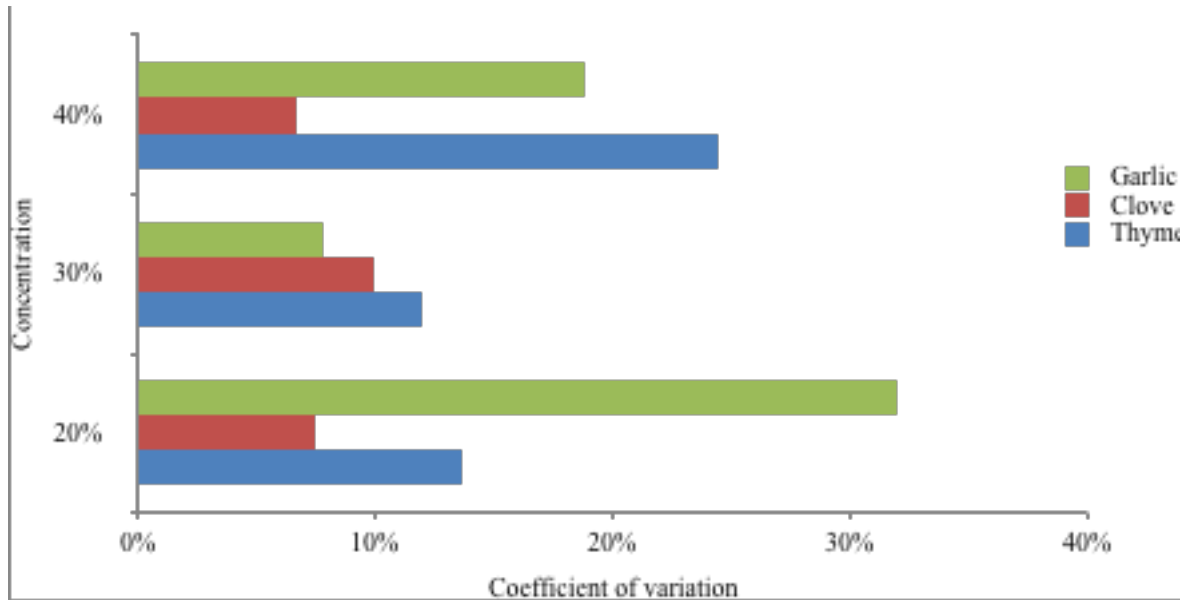
Repeat 3 (area cm <sup>2</sup> )	4.20	3.10	5.30
Mean area	4.43	3.33	4.90
Standard deviation	0.33	0.33	0.33
Coefficient variation (%)	7.44	9.90	6.67

Staphylococcus epidermidis - Garlic essential oil results

Concentration	20%	30%	40%
Repeat 1 (area cm <sup>2</sup> )	8.60	7.10	9.60
Repeat 2 (area cm <sup>2</sup> )	16.60	6.20	6.20
Repeat 3 (area cm <sup>2</sup> )	9.10	7.50	7.10
Mean area	11.43	6.93	7.63
Standard deviation	3.66	0.54	1.44
Coefficient variation (%)	32.00	7.84	18.84

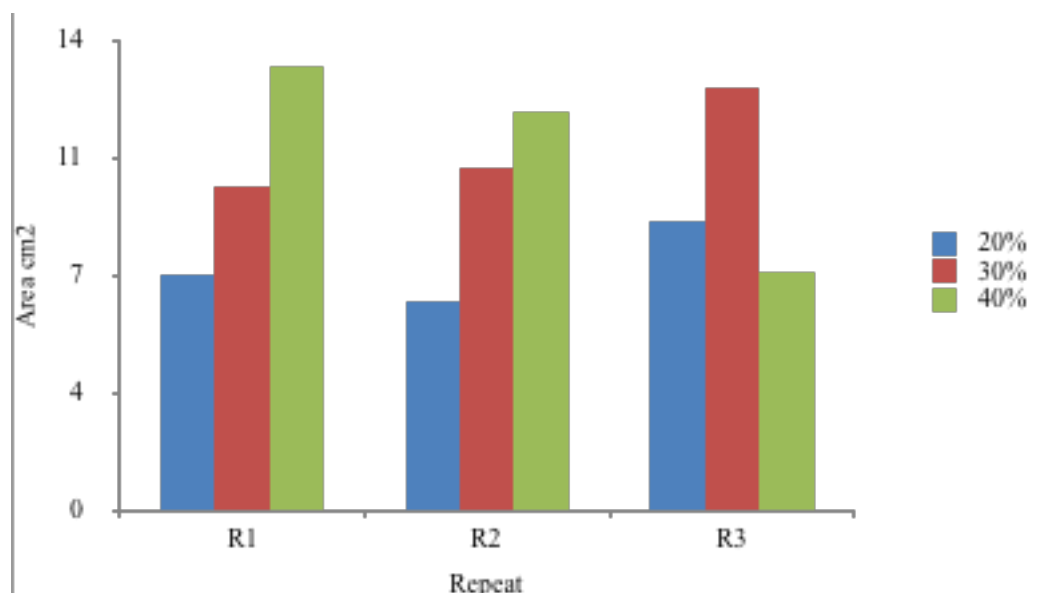
#### 4. Analysis of results

Thyme essential oil results show that the 30% concentration is the most reliable data, as the coefficient variation value is the smallest percentage at 12.00%. Suggesting that it's the most precise estimate. Whereas, the 40% concentration of thyme oil has the greatest dispersion from the mean value as it's coefficient of variation value is 24.44%. Clove essential oil shows the value of coefficient variation 6.67% is the lowest at 40% concentration suggesting it's the least dispersed from the mean. In comparison 30% concentration of clove oil has the biggest dispersion from the mean, with the value of coefficient variation at 9.90%. Garlic's 30% concentration has the lowest coefficient variation at 7.84% showing the least amount of variation around the mean, meaning the standard deviation is 7.84% of the mean, whereas garlic greatest dispersion is at 20% concentration with a coefficient variation value of 32.00%. We can see this below in figure 11.



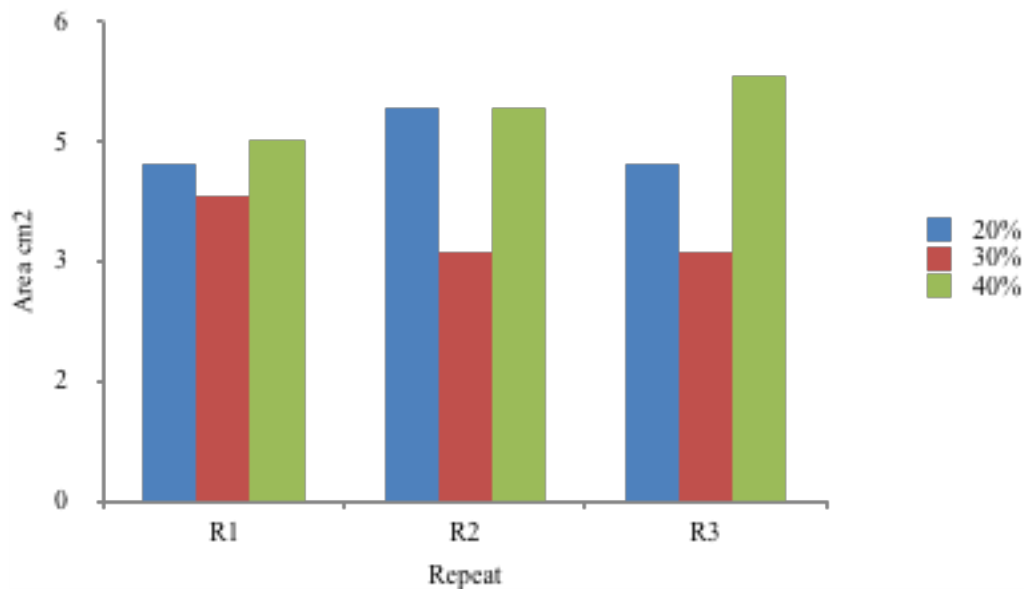
**Figure 11.** The Coefficient of variation obtained by the three essential oils.

Thyme oil's 20% concentration has shown the smallest overall inhibition zone area out of the three concentrations. However, it has the second highest coefficient of variation due to slight increased difference between the first and third repeat. Thyme's 30% concentration has shown to be more effective than the 20% on all repeats and it has shown a bigger zone against one of the 40% repeat. The 40% concentration of thyme oil has shown very dominant results for the first two repeats but a smaller third repeat by a huge amount thus it has the highest coefficient of variation. Overall the 30% and 40% concentration difference in the mean area is very small as the 30% mean is 10.80 and the 40% mean is 10.73. This is shown in figure 12.



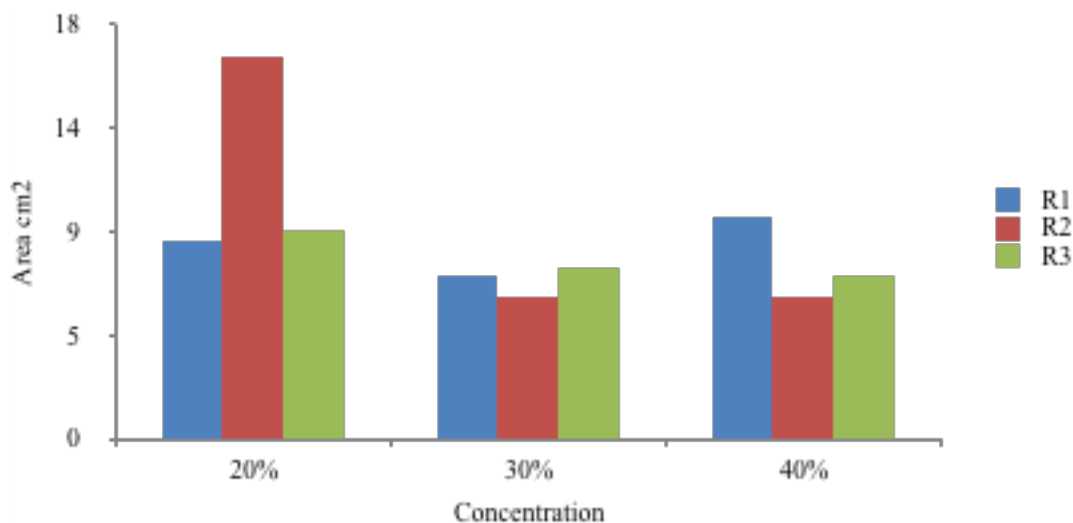
**Figure 12.** Thyme essential oil

Clove oil's 30% concentration has proved to be the least effective due to the three repeats showing the smallest areas on inhabitation despite the results being varied due to the high coefficient of variation. The 20% concentration has bigger inhibition areas than the 30% however two of those areas are smaller than the 40% and it ties with one repeat which proves to be an outlier as the mean is much smaller than the 40% concentration's mean. The 40% concentration of clove oil has the highest inhibition zone areas on two repeats and the smallest coefficient of variation. This proves that 40% is the most effective concentration for clove oil. This is shown in figure 13.



**Figure 13.** Clove essential oil

Garlic oil has shown the biggest overall inhibition zones at 20% concentration, as the three repeats are the biggest data collected. The precision of the data is questionable as repeat number two has a huge amount of difference causing the biggest coefficient of variation of any data collected. This is an outlier suggesting an error while performing the procedure, as the data of the other two repeats are close together. Garlic's 30% and 40% concentration have similar results as they tie together with one data and have both have bigger data then the other on the other two repeats. However, the 40% concentration has a bigger mean result. Figure 14 shows these results.



**Figure 14.** Garlic essential oil

Overall, the three extracts have shown to be effective against staphylococcus epidermidis. Thyme essential oil's strongest concentration will not be determined due to the close difference in mean of the 30% and the 40% concentration. Garlic oil has shown to be just as effective as thyme oil however it is arguable that thyme is the more effective oil as it has stronger mean at 30% and 40% concentration whereas, Garlic has a bigger mean at 20% concentration which is the least precise result. Clove oil has proved to be the least effective out of the three essential oils in tackling staphylococcus epidermidis. It has lower inhibition zones in every concentration in comparison with the other two oils. The 40% concentration for clove oil is the strongest concentration at producing the overall biggest inhibition zones.

## 5. Discussion

As the two gram positive bacteria micrococcus luteus and staphylococcus epidermidis are classed as opportunistic bacteria's they are generally overlooked. These bacteria can cause serious problems if they are in the right spot and they are treated with antibiotics and their ability to develop resistance as increased our interest to tackle the crisis.

In this current study, We have used the disk diffusion method to investigate the effect of three essential oils on the bacteria. The three essential oils have shown to be effective against both bacteria. Micrococcus luteus has shown to be much more susceptible to the three extract than staphylococcus epidermidis. In both the trial where undiluted extracts where tested and the main study where dilution took place, Micrococcus luteus has shown sufficient growth to produce inhibition zones despite testing lower concentration in comparison with staphylococcus epidermidis. On the other hand, Staphylococcus epidermidis is also susceptible but has shown clear inhibition zones. There was clear results suggesting that clove oil is the least effective extract whereas, Garlic and Thyme have shown to be similarly effective.

The most alarming finding of this investigation is the extreme susceptibility of Micrococcus luteus. The bacteria wasn't capable to show any growth on the petri dish suggesting that it's completely vulnerable to these natural oils. The results from this study (Chan, Kong, Yee, Chua, Loo, 2012) shows that Micrococcus luteus diameter of inhibitory zone (DIZ) was determined from various oils and one of them is thyme. This suggests that thyme wasn't the extract which caused the biggest inhibition when we carried out the test. The results we obtained show that the bacteria is effected and so we can use this as a foundation to investigate further the most effective oil, the MIC and how likely it is to be used clinically for improving patient care. The results of staphylococcus epidermidis showing



the vulnerability of these bacteria are important for a number of things. Such as, the bacteria has shown to cause extreme damage to patients with prosthetic valve endocarditis (PVE) and according to this study (Science direct, 2019) it's one of the most common. A report (Verhoef, Fleer, 1983) has stated that antibiotics have proven to not be satisfactory alone and carries a mortality rate of 70-80%. And so this finding allow for a possible helper compound to lead for a stronger treatment and also limit the potential antibiotic resistance.

Overall, the plan to conduct this experiment is well suited to investigate the hypothesis. Literature review was conducted to understand how likely it is that the extracts will show antimicrobial effect of the following bacteria *Micrococcus luteus* at first. We've reviewed the different methods used to investigate antimicrobial activity and chosen the best suited to work with. And the factors that influenced that were: personal experience, school laboratory capabilities and the complexity of my experiment. It is clear that the equipment used was very limited within a school college lab this is a possible risk for contamination. When the extracts were added on the sterile filter paper discs they were placed within a sterile petri dish like container whiteout the agar, this was a risk as there was nothing separating the discs from each other except they were carefully placed at distance. This meant that if excess extract was accidentally poured on the disc, it could flood into the other disc causing contamination and possibly what happened with the control disc test used on *staphylococcus epidermidis*. The equipment is the right size for both concentrations planned for the two bacteria as the pipette measures 1-10ml, which is useful for testing *Staphylococcus epidermidis*. The accuracy of this pipette is the following: the pipette measures 10ml, so one deviation is 0.1ml (for 10ml pipette) therefore uncertainty is  $0.1\text{ml}/2 = \pm 0.05$ . The other pipette measures 0.1-1ml, which is well suited for *Micrococcus luteus* concentrations planned. So the accuracy is calculated as follows: the pipette measures 1ml, so one deviation is 0.01ml thus the uncertainty of this pipette is  $0.01\text{ml}/2 = \pm 0.005$ . As the standard deviation and coefficient of variation are taken. They are easily influenced by outliers. So due to the factor that we did only three repeats for each concentration, this meant that one outlier has caused a big variation.

These results build a foundation to further studies which need to be conducted. To determine the most effective essential oil on *Micrococcus luteus*, an investigation has to be conducted at lower concentrations and possibly testing each essential oil in a separate petri dish so that overlaps can be avoided. Further investigations on *Staphylococcus epidermidis* has to be further conducted with larger amount of repeats to determine the strongest essential oil. The MIC can then be investigated for each of the extracts on the different bacteria. When these are determined, the essential oils can be further tested on human cells to investigate their effect and ensure that they wouldn't inflict harm if tested clinically.

## 6. Conclusion

We presented a study to investigate the antimicrobial properties of three essential oils on two gram positive bacteria which can cause serious complications to medical treatments if given the opportunity to do so. Through the trial experiment, I was interestingly found the *micrococcus luteus* was unable to show any growth due to the strength of undiluted extracts at 0.1ml; whereas, *staphylococcus epidermidis* shows a level of growth in comparison but the extracts are still presenting inhibition zones. Despite dilutions carried out *micrococcus luteus* was unable to produce any growth. Results for *staphylococcus epidermidis* was collected, suggesting that clove essential oil was the least effect. Garlic and Thyme showed equal results and so the more effective oil was undetermined.

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