Design of a Natural Preservative Manuscript

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By Teagan Warkentin, Dr. Liza Abraham and Dr. Chris Wang

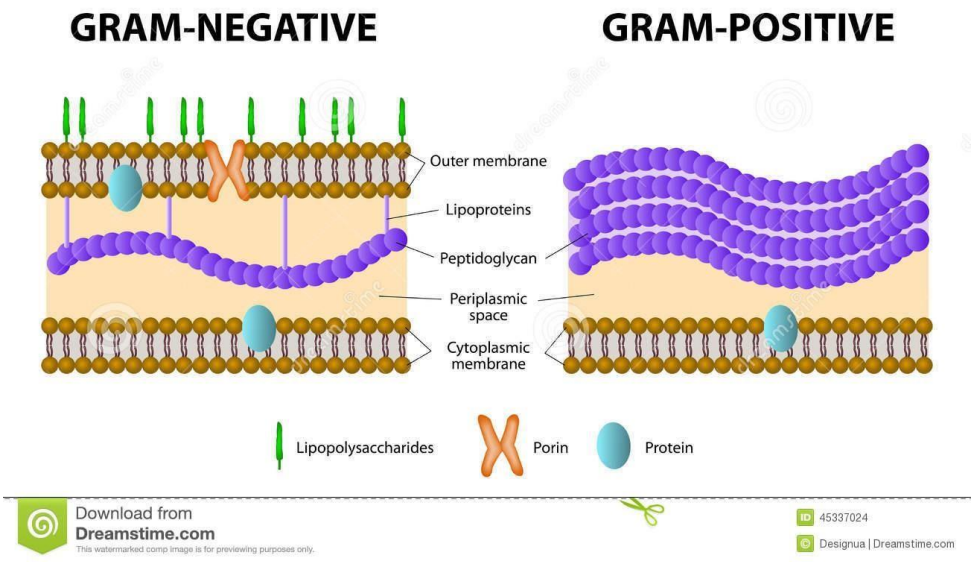
Introduction terms

Preservative: a substance that is used in food, cosmetics or other materials to prevent decay from microorganisms

Hans Christian Gram developed a stain that can divide the two groups of bacteria: one can retain the stain and show up purple the other cannot. Why is this? Due to the cell wall of the bacteria. (1)

Gram Negative Bacterium: Has a thin layer of peptidoglycan and the presence of a negatively charged lipopolysaccharides . E.coli is a gram negative (1)

Gram Positive Bacterium: Has no lipopolysaccharides and a thick layer of peptidoglycan. staphylococcus aureus is gram positive (2)



Picture showing Gram – and Gram + membrane structure (3)

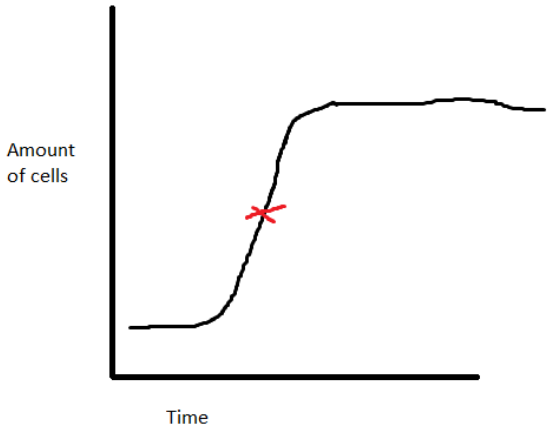
Zone of inhibition: The clear region around the drop of the compound on the agar surface. (4)

Optical Density: The amount of light which absorbed by bacteria cells and it is measured by a spectrophotometer. (5). This is used to determine the amount of cells in a culture. For E.coli it was 0.6 and for staphylococcus it is 2.0

<u>Research Statement</u>

Many preservatives are artificial but there are more healthy alternative compounds present in nature to kill the bacteria.

This study looked into essential oils, and lipids/fatty acids that are made by living organisms and determine its antimicrobial effects.



Plot showing exponential growth of bacteria cells

Introduction

Many Studies show that thyme and oregano worked against E. Coli

(Burt, 2003 Essential Oils their antimicrobial properties) (Bozin et al., 2006, Characterization of the Volatile Composition of Essential Oils of Some Lamiaceae Spices and the Antimicrobial and Antioxidant Activities of the Entire Oils) (Gutierrez, Barry-Ryan, Bourke, 2008). The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients)

Cinnamon and Clove against E. coli and staphylococcus

Goni, 2009, Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils

Eucalyptus against E. Coli and staphylococcus

Bachir and Banili, 2012, Antibacterial activity of the essential oils from the leaves of Eucalyptus globulus against Escherichia coli and Staphylococcus aureus

Lauric Acid can kill staphylococcus

Nakatsuji, 2009 Antimicrobial Property of Lauric Acid Against Propionibacterium acnes: Its Therapeutic Potential for Inflammatory Acne Vulgaris

Octanoic Acid

Nair et al., 2005 Antibacterial Effect of Caprylic Acid and Monocaprylin on Major Bacterial Mastitis Pathogens

Methods

E.coli was grown in an LB liquid broth culture. The brain heart fusion broth was used for the plates. Optical Density at 600nm needed was 0.6. 1.2 was used instead for the experiments

	Control	Sample	Time
Trial 1	0.09	2.5+ (Brain-Heart Broth)	18 hours
Trial 2	0.05	1.2	24 hours
Trial 3	0.07	1.2 (1.31-1.17)	15 hours

Table 1. Showing OD recordings for *E. Coli*

For *staphylococcus* brain heart fusion broth was used to make the plates and grow the culture. The optical density needed was 2.0 ,2.5. was used/

	Control	Sample	Time
Trial 1	0.05	1.08	17 hours 40 min
Trial 2	-0.02	2.5	19 hours and 30 min

Table 2. Showing OD recordings for *staphylococcus*

For the plates 15ml of broth was used but that was too little for most of the *E.coli* plates anyways we used 20ml of broth for plates.

Some of the plates used were not flat they were bumpy which could explain some of the results later on

The broth was sterilized using an autoclave 1-2 hours of sterilizing the liquid and agar broth. A flame was used to sterilize constantly and pop bubbles in the agar

On to the actual experiment now.

Dimethylsufloxide DMSO was used to dilute the compounds that was used.

After doing extensive research these natural compounds were chosen:

6 essential oils: Thyme, Oregano, Lavender, Clove, Cinnamon, and Eucalyptus

2 lipids (fatty acids): Octanoic acid, and lauric acid

2 preservatives: Phenoxyethanol, and Potassium sorbate

1 Vitamin: E

For making the plates 500µL of liquid broth containing the microbes was used to plate them

A pipette was used mix each compound with DMSO to make dilutions of the compound to see what would better. So used special containers mix in and pipettes to mix it.

Each solution was put directly onto the plate. From each solution 5µL of solution was used with a pipette

The plates incubated for about 20-24+ hours

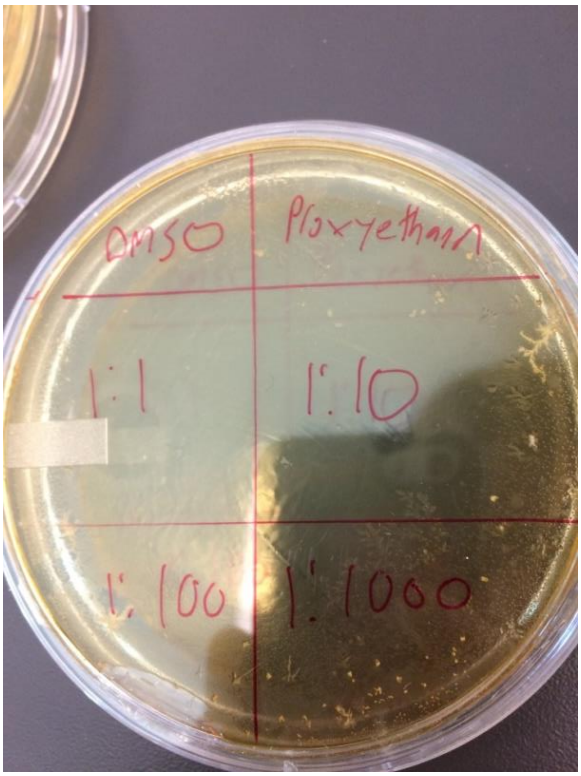
Concentrations	DMSO (µL)	Compound (µL)
Pure Compound	0	5
DMSO control	5	0
1:1	5	5

1:10	9	1
1:100	99	1
1:1000	1000	1

Table 3. Showing the amounts of each compound in each ratio/concentration

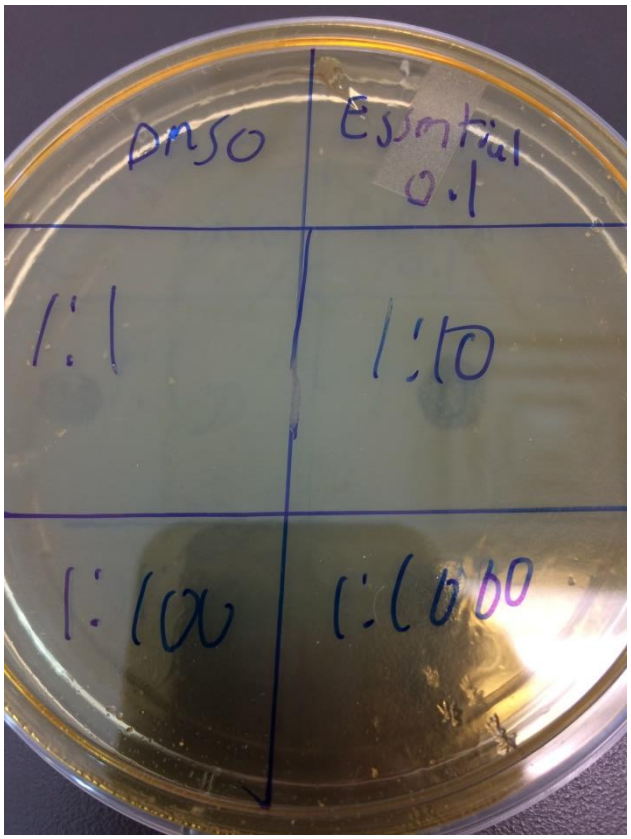
Results

E.coli results: wild Orange and phenoxyethanol

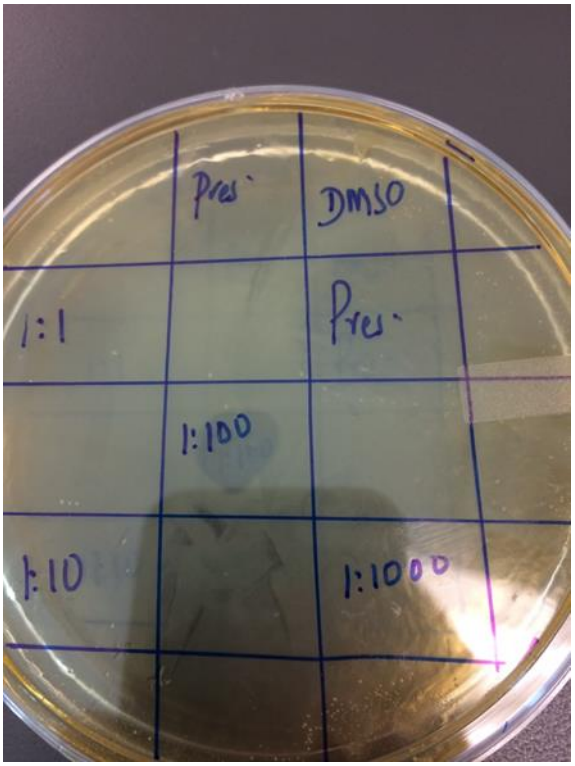


Concentrations	Phenoxyethanol
Phenoxyethanol	10 mm
1:1	7 mm
1:10	5 mm

1:100	3 mm
1:1000	none



Concentration	Wild Orange Essential Oil
1:1	5mm
1:10	5 mm

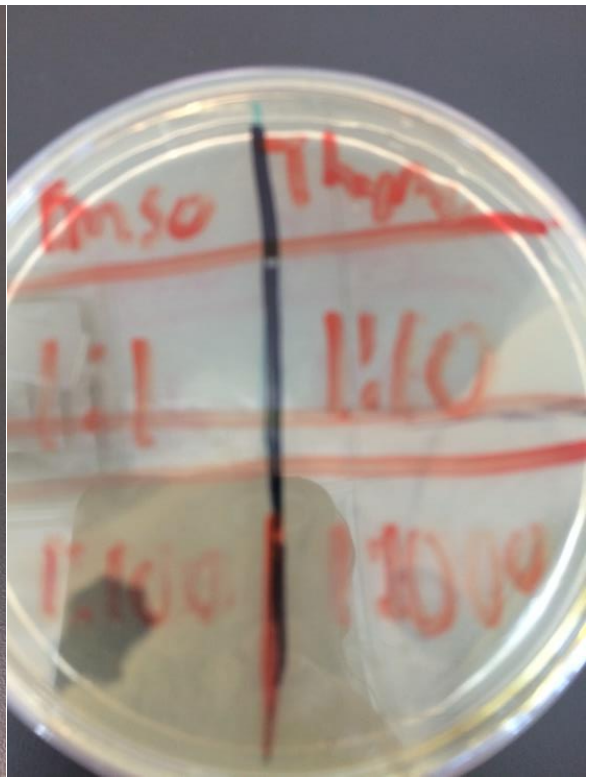


Picture 19. Showing plate for preservative, possibly phenoxyethanol otherwise unknown

Picture 20. Showing plate for Vitamin E. There was no inhibition

Picture 21 Showing plate for potassium sorbate an preservative used in foods. No inhibition is shown

Results Thyme

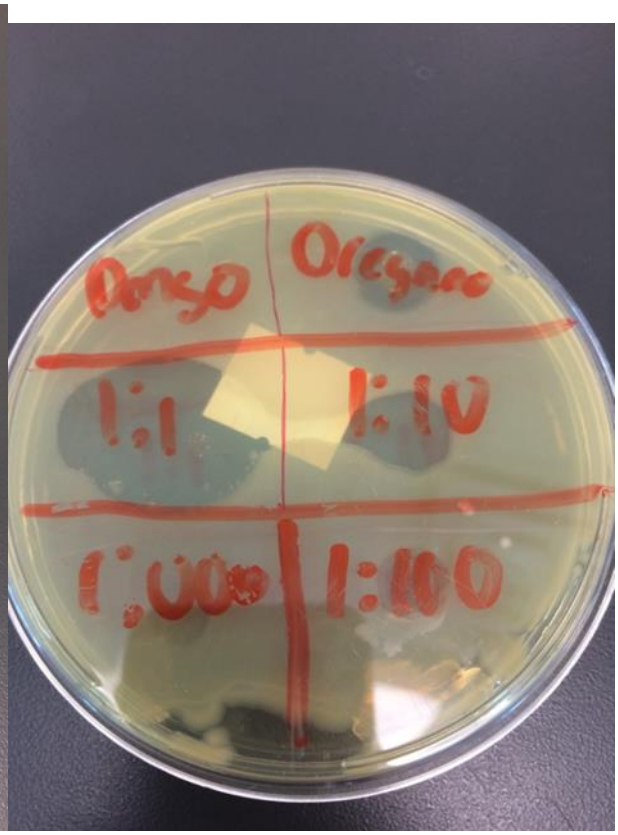


Staphylococcus

E. Coli

Concentration	*staphylococcus*	*E.coli*
Essential Oil	30 mm	40 mm
1:1	20 mm	Can't tell
1:10	10 mm	Can't tell
1:100	5 mm	7mm
1:1000	5 mm	5 mm

Results Oregano

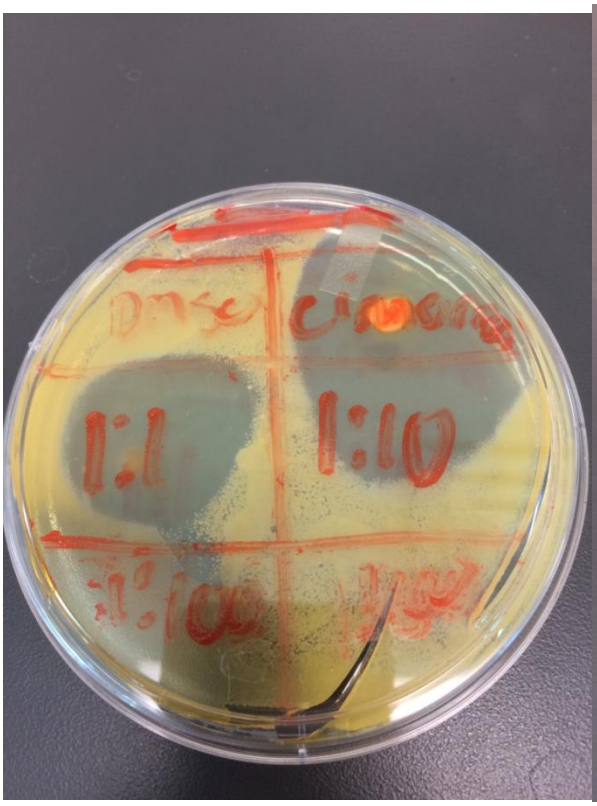


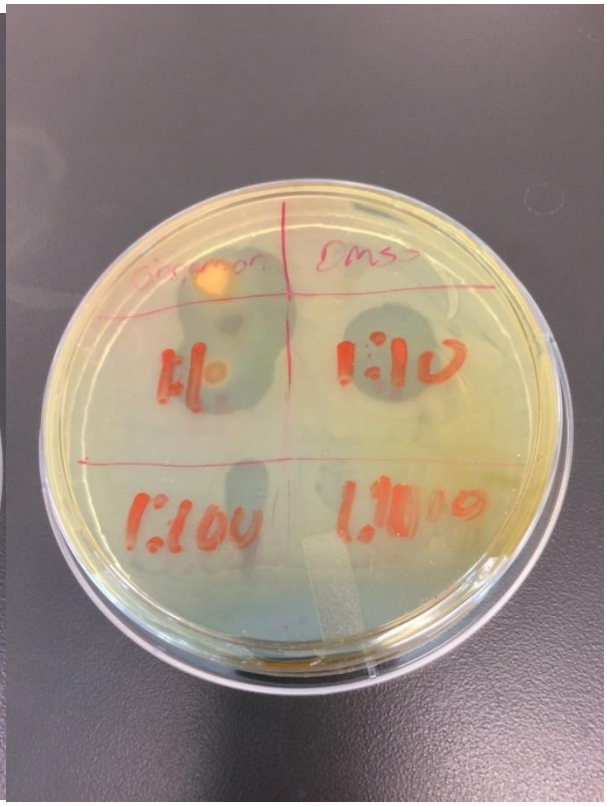
Staphylococcus

E. Coli

Concentration	*staphylococcus*	*E.coli*
Essential Oil	none	10 mm
1:1	8 mm	30 mm
1:10	10 mm	14 mm
1:100	none	5mm
1:1000	none	7 mm

Results Cinnamon





Staphylococcus *E. Coli*

Concentration	*staphylococcus*	*E.coli*

Essential Oil	40 mm	40 mm
1:1	25 mm	Can't tell
1:10	Can't tell	Can't tell
1:100	20 mm	7mm
1:1000	none	5 mm

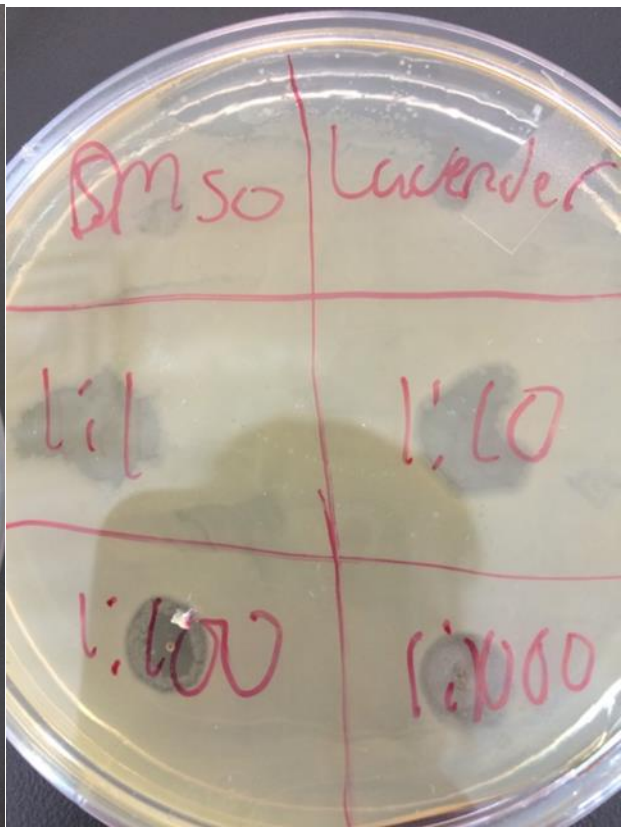
Results Clove

Staphylococcus *E.Coli*

Concentration	*staphylococcus*	*E.coli*
Essential Oil	15 mm	15 mm
1:1	5 mm	15 mm

1:10	10 mm	13 mm
1:100	7 mm	7mm
1:1000	none	5 mm

Results Lavender

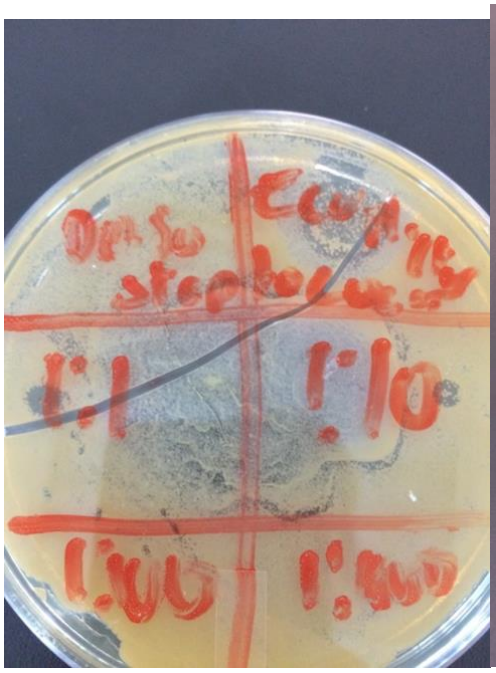


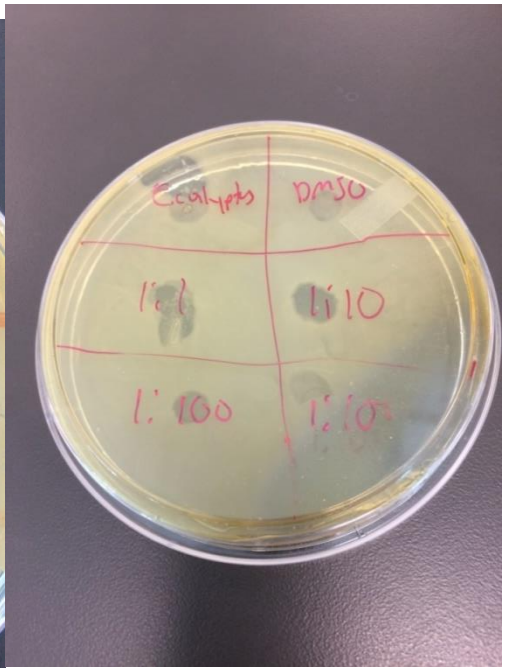
Staphylococcus *E. Coli*

Concentration	*staphylococcus*	*E.coli*
Essential Oil	25 mm	15 mm
1:1	5 mm	10 mm
1:10	10 mm	7 mm
1:100	none	5mm

1:1000	none	5 mm

Results Eucalyptus

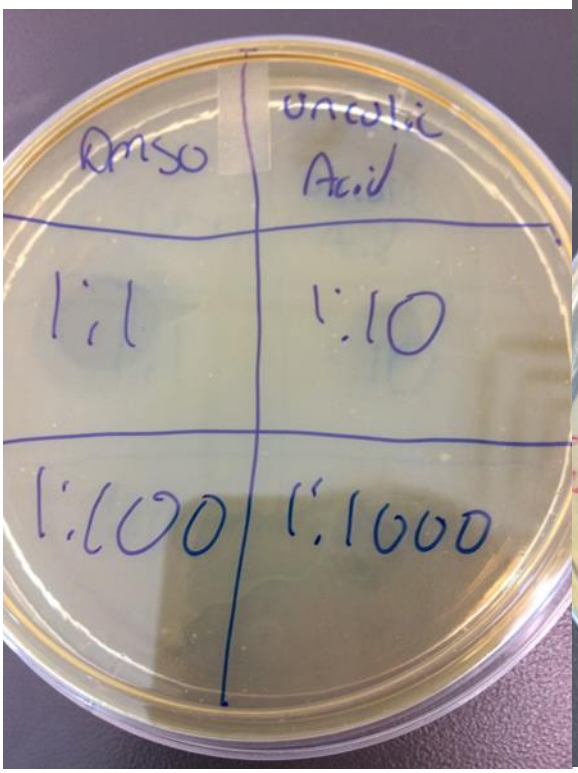


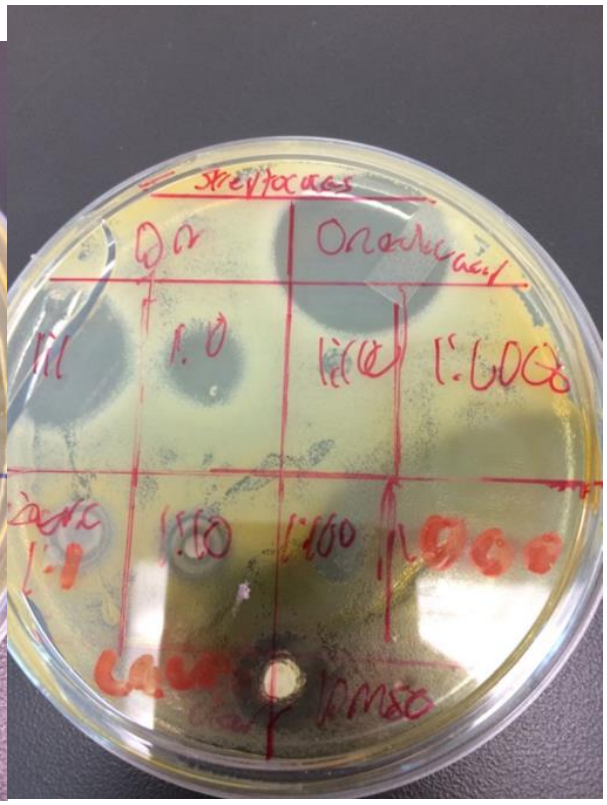


Staphylococcus *E.Coli*

Concentration	*staphylococcus*	*E.coli*
Essential Oil	1 mm	15 mm
1:1	4 mm	14 mm
1:10	3 mm	8 mm
1:100	none	4mm
1:1000	none	5 mm

Results Octanoic acid



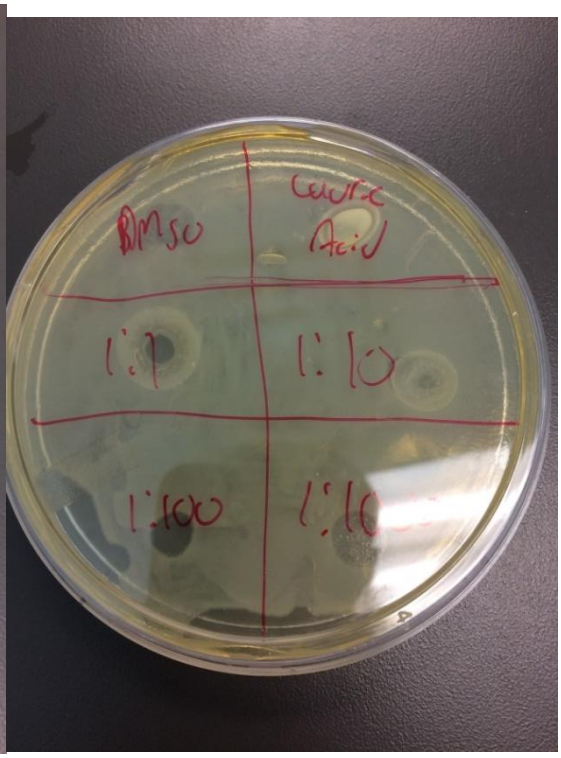


E. Coli

Staphylococcus

Concentration	*staphylococcus*	*E.coli*
Octanoic acid	25 mm	20 mm
1:1	20 mm	10 mm
1:10	10 mm	5 mm
1:100	5 mm	none
1:1000	none	none

Results Lauric Acid



Staphylococcus *E. Coli*

Concentration	*staphylococcus*	*E.coli*
Lauric Acid	30 mm	10 mm
1:1	5 mm	15 mm
1:10	7 mm	10 mm
1:100	5 mm	5mm
1:1000	Can't really tell	7 mm

Analysis of results

So the essential oil to have the largest inhibition zone for *staphylococcus* was: cinnamon (40mm) followed by thyme (30mm) and lauric acid (30mm). The least was oregano with none, and Eucalyptus

The one that worked the best at the lowest concentration for *staphylococcus* were: Thyme

For E.coli the essential oil to have the largest inhibition zone was: cinnamon and thyme (40mm)

The one that worked the best at the lowest concentration for *E.coli* was: Oregano and lauric acid at 7mm with 1:1000.

More compounds in total however where able to inhibit *E.coli* at 1:1000 (a total of 6 compounds inhibited the *E.coli* at the lowest concentration) whereas there weren't that many for staphylococcus only one which was thyme

For concentrations 1:1 the winners for staphylococcus , cinnamon at 25mm and E.coli are: oregano at 30mm

1:10 *staphylococcus* are thyme oregano clove and lauric acid all tied for 10 mm and *E.coli* are: oregano at 14

1:100 the winners for *staphylococcus* are: cinnamon at 2 mm and E.coli it was , thyme cinnamon and clove at 7mm

Discussion

Synergism is the wide belief of how these compounds can kill the bacteria. They may inhibit a common biochemical pathway to these bacteria, inhibit protective enzymes and cell wall components. (9) Carvacrol the main ingredient in cinnamon is a close structure to hydrocarbons and monoterpenes (9). These can interact with the cell wall and therefore carvcrol and thymol can penetrate the cell wall (9).

Thymol and Carvacrol can disintegrate the cell wall of the *E. Coli* thus why they worked so well (9). Therefore it is important to note that when combined with other essential oils because they break the cell wall the other components can enter the cell. Also because gram positive bacteria and gram negative bacteria have different structures of the cell wall each of these essential oils will have a different effect. Because gram negative are negatively charged lipoproteins, hydrophobic compounds (9).

Components within Eucalyptus oil are: 1,8 cineole, citronellal, citronellal, linalool, and others (6) page 742). No one really knows exact mechanism but it seems to inhibit the growth of the bacteria.

In terms of clove , lavender and other essential oils it is usually cell membrane damage. (7) Essential oils are hydrophobic and can acclimate in the lipid membrane structures which can cause structural and functional damage (7). This may not be the only factor involved because

toxicity may be linked to hydrophobicity, so the hydrophobic compounds can accumulate to the lethal levels. ((7) Page 998)

Lauric acid works against gram positive bacteria but against a range of gram negative bacteria (8). It seems to cause a separation in the inner and outer membranes and cytoplasmic disorganization (8).

Octanoic acid

The shorter the fatty acid the more antimicrobial it is, because octanoic acid is an 8 carbon fatty acid chain it was more soluble and more effective at killing bacteria. (10)

Hypothesises as to why fatty acids like lauric acid and Octanoic acid work may be that they act as non-ionic surfactants that penetrant the cell plasma membrane and alter the permeability. (10)

Another one might be that short and medium fatty acids can actually go into the bacterial/fungal cells and can become dissociated. When they are dissociated it becomes acidic therefore increasing cytoplasmic pH which in turn inactivates enzymes, and can alter protein structure. (10)

Conclusions

There is a lot more work to do

Ran out of time to study fungi and combinations of different essential oils to determine which combination would work the best at killing both prokaryote and eukaryote cells

However this study did find out whether or not these things can inhibit these gram negative and gram positive bacteria.

The essential oils seemed to work at a lower concentration for gram negative bacteria than for gram positive bacteria.

Cinnamon and thyme worked the best for E.coli and thyme worked the best for staphylococcus

Most of the essential oils work by damaging the cell membrane

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