

Biology Extended Essay- May 2024 Session

An Experimental Examination of Antibacterial Properties of Essential Oils

Research Question: “To what extent do the essential oils of peppermint, Ginger and Rosemary show antibacterial properties, indicated by their inhibition on the growth of *Escherichia Coli* cultures?”

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Contents

1.Introduction.....	3
1.1 Personal Involvement and Significance.....	3
1.2 Background Information.....	4
1.2.1 Testing for antibacterial properties.....	4
1.2.2 Use of spices.....	5
1.2.3 Prior research conducted into topic.....	5
2.Experiment.....	7
2.1 Aim.....	7
2.2 Hypothesis.....	8
2.3 Variables.....	9
2.4 Method.....	10
2.4.1 Materials.....	10
2.4.2 Sterilising with a pressure cooker.....	11
2.4.3 Sterilising the workspace.....	11
2.4.4 Preparing the Stock Culture of E.Coli.....	12
2.4.5 Disc Diffusion.....	13
2.4.6 Turbidity.....	14
2.5 Risk assessment.....	15
3.Results.....	16
3.1 Qualitative observations.....	16
3.2 Tables and Graphs.....	18
3.2.1 Tables and graphs from measurements.....	18
3.2.2 Calculating Reduction in Absorbance.....	22
3.2 Analysis and Discussion.....	24
4.Conclusion.....	26
4.1 Comment on results/hypothesis.....	26
4.2 Evaluation of method.....	27
4.3 Extension.....	28

1.Introduction

1.1 Personal Involvement and Significance

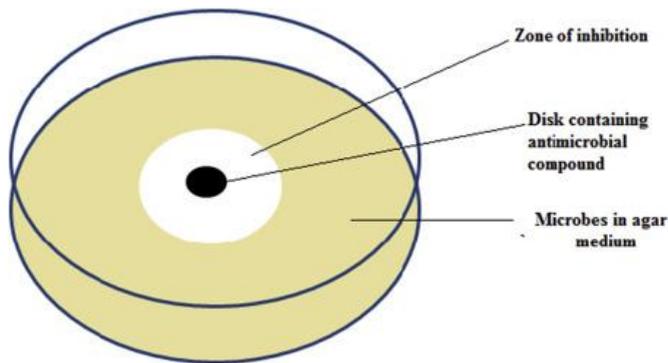
The research question that will be investigated is “To what extent do the essential oils of peppermint, Ginger and Rosemary show antibacterial properties, indicated by their inhibition on the growth of *E. coli* cultures?”. This question initially sought to test the antibacterial qualities of these herbs as well as crushed garlic, because I had heard from my family that herbs were a great home remedy, and that garlic was an antibiotic. I wanted to test out that story by taking the extracts of commonly used herbs and seeing how they inhibited bacterial growth. However, it proved difficult to obtain herb extracts, and prior research which tested extracts and essential oils demonstrated that essential oils show more antibacterial character. Hence, I decided to test the essential oils of herbs. Essential oils can be used as flavouring in small amounts, and can be added to hand creams and other products, which makes it important to understand if there is any benefit to using them outside of improving taste and smell.

It is significant to research this topic because herbs have been traditionally used in many cultures to treat symptoms of illness such as nausea or headaches. By observing the activity that the oils of these herbs have on bacteria, the health benefits of herbs can be better understood, as well as the interaction of plant-produced chemicals with different entities. These interactions can further the remedies that we have for different ailments.

1.2 Background Information

1.2.1 Testing for antibacterial properties

There are different methods to measure the antibacterial properties of samples that revolve around the concept of measuring the growth of bacteria in environments close to



or immersed in the tested material. One procedure testing antibacterial activity is disc diffusion, which can be performed in a variety of ways. Disc diffusion measures the zone of inhibition on an agar plate where a **Fig**

1- a visual representation of disc diffusion (Prusty) sample of an organism is growing (Eliopoulos and Moellering). The organism can be a variety of microorganisms which makes the method easy to regulate. This method is not expensive and can be replicated in a school environment with the use of filter paper to take the place of a disc to contain the tested material, and the inhibition zone data can be measured with a ruler. The use of this method will make the data of this experiment reliable since it is an adaptation of an effective and contemporary method.

Another method used to measure antibacterial activity would be to measure the bacterial growth of samples and compare the growth of samples with added essential oils in contrast to a control sample which had only water added to it. Turbidity can be used to measure this kind of bacterial growth. This method measures the light absorbance of a liquid culture using a photometer (“Factors influencing”), but can also be done with a colorimeter which takes an absorbance value. This method will also be tested to see the antibacterial

effect of the essential oils on a non solid sample of bacteria which can be encountered in situations like dirty water.

All incubation will take place under 30°C to ensure safety in a school environment.

1.2.2 Use of spices

Spices are dried parts of plants, such as stems and leaves, which are used in many cuisines across the world. There has been evidence showing that a reason why we use spices is not just because they enhance a dish's flavour, but also because the chemicals in plants have important attributes. Plants have many phytochemicals which they produce as byproducts of their metabolism (Sherman and Billing) , and each have their own properties. These chemicals play a role in protecting the plant against pathogens and insects (Sherman and Billig), and humans over time have picked up on that fact and use spices to keep their food fresh. This idea is also supported by statistics which show that a higher number of spices are used in countries with warmer climates (Sherman and Billing). As for other uses of spices, oils and extracts derived from plants have been long used for treating various ailments (Grieve).

1.2.3 Prior research conducted into topic

Peppermint

The major ingredients making up peppermint essential oil are menthol, menthone, menthofuran and menthyl acetate (Singh et al.). Other components include limonene, inactive pinene and isovalerate.(Grieve)

Other research indicates that alcohols like menthol show antibacterial activity (Sienkiewicz et al.). Previous tests into peppermint's antibacterial properties on *E.coli* observed both distilled essential oil and extracts of the leaves utilising different solutes

(Singh et al.). Other trials used store bought essential oils (Thompson et al.). Peppermint oil proved effective in deterring bacterial activity measured by disc diffusion (Singh et al.) and turbidity (Thompson et al.). Peppermint essential oil resulted in zones of inhibition whose sizes were 7.7 ± 3.5 mm in diameter (Thompson et al.). In other trials, the inhibition zone range varied based on the concentration of essential oil used, and extracts showing varying levels of antibacterial activity (Singh et al.).

In herbal medicine, peppermint is described as the most extensively used essential oil, used to treat cholera, nausea and colds (Grieve).

Rosemary

Rosemary essential oil's major components include 1,8 cineole, camphor, α -pinene, β -pinene and camphene (Sienkiewicz et al.).

Previous studies have observed antibacterial activity in rosemary essential oil. When tested against a specific strain (DH5 alpha), zones of inhibition were produced with diameter 13.3 ± 0.3 mm (Thompson et al.). Rosemary essential oil was also effective against multiple strains of *E.coli* obtained from patients in hospitals which were resistant to antibiotics , although this efficacy wasn't deemed a very significant one (Sienkiewics et al.).

Traditionally, rosemary has been utilised in oil form for herbal medicine to treat headaches and problems with digestion (Grieve). It has also been described as a great oil to use in hair washes (Grieve).

Ginger

Ginger's main component chemicals are sesquiterpenoids which include zingiberene, β - sesquiphellandrene bisabolene and farnesene (Malu et al.).

Ginger extracts have been demonstrated to display antibacterial activity in disc diffusion against non *E.coli* bacteria(Malu et al.). The extracts which showed antibacterial activity were non water-based ones. Previous research has shown ginger essential oil can

inhibit the growth of *E. coli* , with inhibition zone size being measured at 19.0 ± 2.0 mm

(Silva et al.)

Ginger roots have been used in herbal medicine both ingested and used externally. Externally, ginger can be used to improve blood flow to the skin, and it can be used to aid with issues in the gastric system and make the flavour of other medicines less bitter (Grieve).

2. Experiment

2.1 Aim

The objective is to see if the essential oils of Peppermint, Ginger, and Rosemary will inhibit the growth of *E. coli* by measuring the oils' impact on the growth in solid and liquid mediums.

2.2 Hypothesis

My hypothesis is that all oils tested will inhibit the growth of *E. coli*, and ginger will inhibit growth the most, displaying the largest inhibition zone sizes. This hypothesis is rooted in the experimental data, as ginger essential oil created the large inhibition zones and also had an effect on harmful *E. coli* strains. I hypothesise that rosemary will also show a strong antibacterial effect due to its efficacy on antibiotic-resistant strains. However, it should also be noted that when looking at the use of the herbs in traditional remedies, peppermint has been used as a treatment for cholera and mild colds where rosemary tends to be used more for headaches only and ginger solely for gastric issues and taste improvement, which may point to peppermint having more effect on bacteria.

2.3 Variables

	Name of Variable	Explanation/measurement
Independent Variable	Type of Essential Oil	The essential Oils of Peppermint, Ginger, and Rosemary bought from a local store were used. The Peppermint oil is from <i>Mentha Piperita</i> , the Ginger from <i>Zingiber Officinalis</i> (Sri Lanka), and Rosemary from <i>Rosmarinus pyramidalis</i> (France).
Dependent Variable	Inhibition of growth in <i>E.coli</i> samples	The antibacterial properties of essential oils will inhibit the growth rate of <i>E.coli</i> , and the extent to which will indicate the potency. This will be measured through two methods; turbidity measurements and disc diffusion. With turbidity the inhibition of bacteria growth in a liquid sample will be measured through light absorbance at 470 nm . With Disc Diffusion, the zone of inhibition (area with no bacterial growth around the disc containing essential oil) on the solid agar medium will be measured in cm with a ruler.
Control Variables	Incubator Temperature	The incubator was kept at 29°C during its use to ensure that bacteria growth wouldn't be impacted by changes in temperature. This temperature was chosen to ensure that the bacteria could grow effectively but not at near human body temperatures.
	Quantity of essential oil, <i>E.coli</i> and agar used	The quantity needs to be controlled in order to ensure data accuracy and minimise anomalies. The proportions used are as follows: <ul style="list-style-type: none"> • Disc Diffusion: 200µl <i>E.coli</i> per dish, ~300µl for 15 filter papers (~20µl per zone of inhibition tested) [1:10 proportion] • Turbidity <ul style="list-style-type: none"> ○ Petri Dish: 12 ml agar, 1ml <i>E.coli</i> taken from a preculture 200µl oil ○ Test Tube: 7ml medium, 500µl <i>E. coli</i>, 100µl oil
	Trial Duration	All samples will be inoculated for around 28 hours to ensure that no additional growth occurs in specific samples due to additional time, impacting the measurements taken.
	Reference Value for colorimeter	The colorimeter was calibrated with distilled water at 470 nm in order to ensure data consistency .
	Radius of Filter Paper	The diameter of the filter paper will be 0.5 cm to ensure that all inhibition zones tested will have the same initial radius and similar amounts of essential oil.

2.4 Method

2.4.1 Materials

	Section of Experiment				
	Pressure cooker sterilisation	Workspace sterilisation	Bacteria Culture	Disc Diffusion	Turbidity
Materials	<ul style="list-style-type: none"> -Aluminium foil -Hot plate -1L beaker -Pressure cooker 	<ul style="list-style-type: none"> -70% ethanol or other disinfectant -Paper towel 	<ul style="list-style-type: none"> Bunsen burner -Conical flask -Liquid agar solution -<i>E.coli</i> sample -Wooden stick/inoculation loop -Incubator -Refrigerator 	<ul style="list-style-type: none"> -Flow hood -Bunsen burner -Tweezers -Petri dishes -Filter paper circles -Hole puncher -Essential oils -Pipette -Glass spreader -Labelling paper -Pen -Incubator -Ruler -Pipette heads -Distilled water 	<ul style="list-style-type: none"> -Nutrient agar -Granulated cylinder -Petri dishes -Liquid <i>E.coli</i> culture (made in Bacteria culture) -Pipette -Pipette heads -Essential oils -Distilled water -Aluminium foil -Incubator -Colorimeter -Data collection software (like LoggerPro) -Cuvette -Paper towel

2.4.2 Sterilising with a pressure cooker

1. Use aluminium foil to cover the mouths of beakers and test tubes making the foil easy to take off and put on
2. Wrap the wooden sticks, filter paper circles in aluminium foil
3. Turn on the hot Plate to highest heat
4. Add 2L of tap water into the pressure cooker, measuring with 1L beaker (enough to submerge the strainer)
5. Place the materials into the pressure cooker and close the lid, allowing for air to escape
6. After 45 minutes, turn off the hot plate
7. open the pressure cooker after 10 minutes
8. Carefully take out the materials and store in a box with lid until use
9. Open the sink with hot water, then empty the contents of the pressure cooker

2.4.3 Sterilising the workspace

1. Turn on the flow hood's ventilation
2. Spray the workspace with 70% ethanol and wipe down with a paper towel
3. Repeat after experiment is done, and repeat whenever bacteria is being used

2.4.4 Preparing the Stock Culture of *E. Coli*

1. In a sterile environment, connect Bunsen burner to gas, take off lid of sterile conical flask, and open the Bunsen burner's flame with a match
2. Using the flame, sterilize the mouth of the flask
3. Open beaker containing liquid agar solution, and sterilize mouth of beaker with flame
4. Pour agar solution into flask by making the mouths of the containers touch
5. Place the lid on the beaker and put it aside
6. Open the lid of the test tube containing *E. coli*.
7. Sterilize the mouth of the conical flask with Bunsen burner flame
8. Sterilize the wooden stick by quickly putting it in the flame
9. Stick the stick into the mouth of the test tube and gently wave it against the very top to clean whatever was burned by the flame
10. Lightly rub the stick against the *E. coli* to obtain a sample to culture
11. Place the stick into the flask and scrape the bacteria off into the nutrient agar
12. Sterilize the mouth of the flask one more time before sealing it with aluminium and placing into the incubator set to 29°C
13. After around 28 hours, take the sample out of the incubator and store in a refrigerator until use

2.4.5 Disc Diffusion

1. Sterilize the laminar flow hood
2. Connect Bunsen burner to gas
3. Place materials into sterile environment
4. Using tweezers, place 15 filter paper circles on an empty Petri dish, spacing them out
5. Sterilize the lid of one essential oil using bunsen burner
6. Taking 100 μ l at a time with the pipette, place 1 drop of oil onto each piece of filter paper
7. Sterilize tweezers and mouth of conical flask containing *E. Coli* sample
8. Using pipette, put 200 μ l *E.coli* onto each agar plate
9. Sterilize pipette heads after use with Bunsen burner before disposing
10. Sterilize glass spreader with burner before using it to spread *E.coli* inside agar plate in a circular motion
11. Sterilize spreader between different agar plates
12. Using tweezers, place the filter papers onto the agar plates, 5 per plate and spread out as evenly as possible
13. Close the lid of complete agar plates and label with labelling paper
14. Repeat with all 3 essential oils and one with control (distilled water)
15. Place the plates into incubator at 29 $^{\circ}$ C
16. Take out the petri dishes from the incubator after around 28 hours into sterile environment
17. Using a ruler, hold the dishes against the light and measure the zone of inhibition's diameter by putting the ruler against the bottom of the plate
18. If no zone of inhibition is visible, note down 0.5cm, the diameter of the filter paper

19. After measurements, dispose of Petri dishes responsibly

Note: During the experiment, the petri dishes were incubated for an extra 25 hours and measured again using method described in step 17 then disposed of.

2.4.6 Turbidity

1. Using Bunsen burner, sterilize mouth of liquid nutrient agar container and graduated cylinder
2. Measure out 12 ml for each Petri dish, and 7ml for each test tube and pour into respective container
3. Sterilize mouth of liquid *E.coli* sample flask
4. Using pipette, pipette 1 ml into each Petri dish and 500 μ l into each test tube, making sure to not let the pipette head touch the nutrient agar or test tube walls. If it does, sterilize head in Bunsen burner and get a new head
5. Sterilize the head of one essential oil bottle
6. Using a pipette, pipette 200 μ l into each Petri dish and 100 μ l into each test tube, making sure to not touch the liquid of test tube walls
7. Repeat for each essential oil and once for control (Distilled water), replacing the pipette head with each oil
8. Label each Petri dish and test tube
9. Seal the containers with lids/aluminum foil
10. Incubate in incubator at 29 Celsius for around 28 hours
11. Take samples out of incubator into sterile environment
12. Plug colorimeter into computer and open logging program
13. Calibrate colorimeter using a cuvette filled with distilled water

14. Fill cuvette with one liquid sample using pipette, sterilising and then disposing tips between samples
15. Place full cuvette into colorimeter and take 5 measurements
16. Label measurements and repeat for each sample, cleaning and drying cuvette with water and paper towel between each measurement
17. After taking measurements, dispose of samples responsibly

2.5 Risk assessment

Below are some equipment which should be treated with caution

- Hot plate should be kept out for a while before storing after use to make it softer to touch
- Bunsen burner should be closed when not in use, those with long hair should tie their hair before dealing with the open flame
- When sterilizing equipment, the pressure cooker should be able to let out air, and before opening it, it must be ensured that there is no pressure left in the cooker to avoid burns and accidents.

In addition, additional precautions need to be taken in order to avoid bacterial contamination;

- Wear disposable gloves when working with and transporting material with bacteria (Petri dishes, Test tubes)
- Wear a lab coat to avoid getting material on clothes worn outside of the lab
- Sterilize using 70% ethanol workspace before and after starting work with bacteria cultures
- Before disposing of any equipment which has come in contact with bacteria, sterilise them.

3. Results

3.1 Qualitative observations

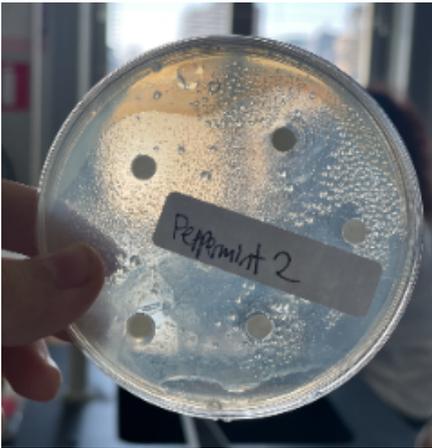


Fig 2a- Peppermint 2 Sample

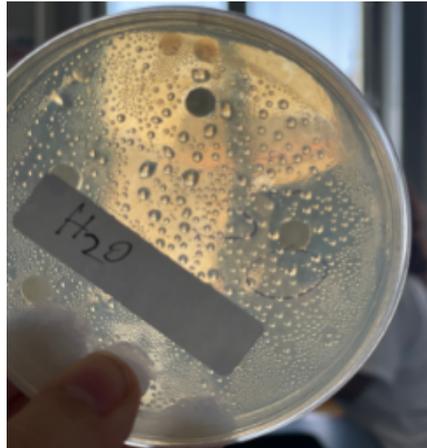


Fig 2b- Control Sample



Fig 2c- Rosemary 2 Sample

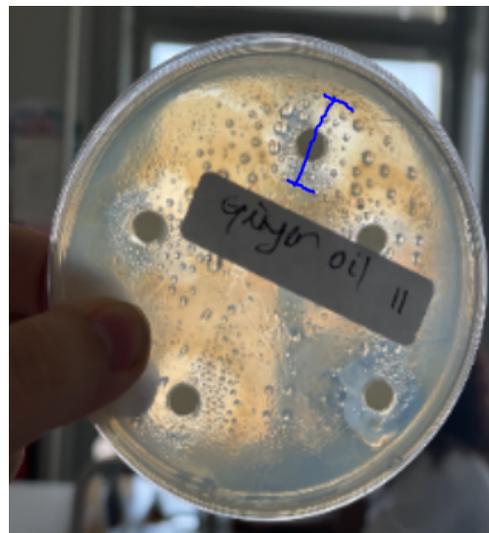


Fig 2d- Ginger Oil 2 Sample with the diameter of one inhibition zone clearly marked

The above images were taken after the additional 25 hours of incubation to clearly display the inhibition zones. As seen in the images, zones of inhibition were distinctly visible on the petri dishes, with a clearer colour in the zones. The control petri dishes still didn't have any zones of inhibition, and the zones' sizes increased in the samples. Most notably the zone of inhibition on the peppermint samples had grown so large that it was impossible to measure accurately (see fig. 2a).

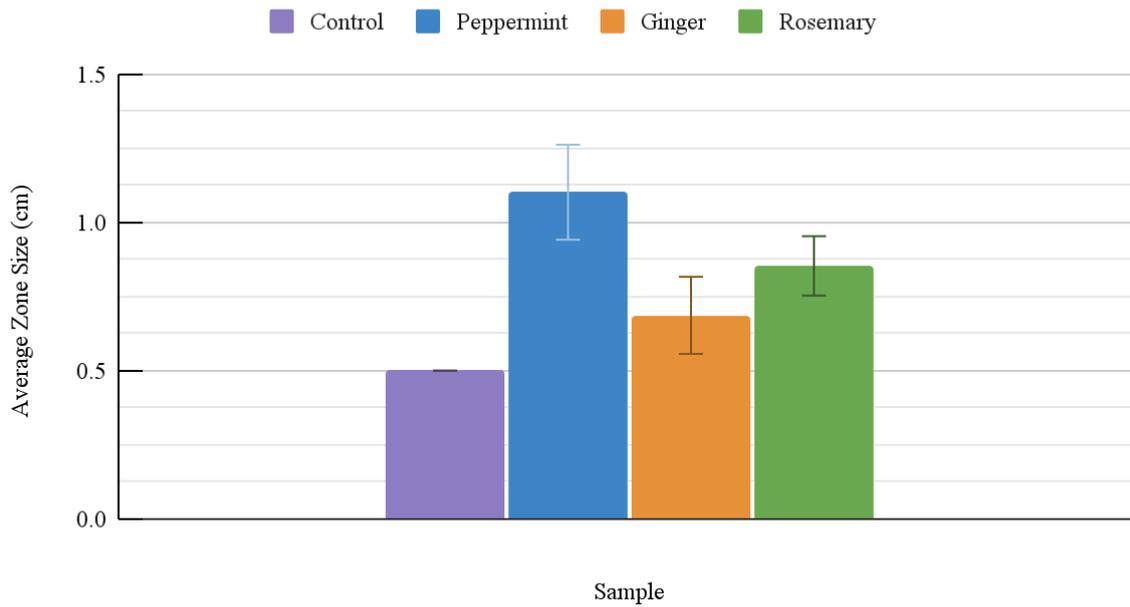
3.2 Tables and Graphs

3.2.1 Tables and graphs from measurements

		Table 1: Disc Diffusion Inhibition Zone (cm) $\pm 0.05\text{cm}$					
Sample		Zone 1	Zone 2	Zone 3	Zone 4	Zone 5	Mean Zone Size
Control	Petri Dish 1	0.5	0.5	0.5	0.5	0.5	0.50
Peppermint	Petri Dish 1	0.9	0.9	1	0.8	1	0.92
	Petri Dish 2	1.2	1.2	0.9	1.2	1.3	1.16
	Petri Dish 3	1.3	1.2	1.3	1.1	-	1.23
Ginger	Petri Dish 1	0.8	0.9	0.8	0.7	0.9	0.82
	Petri Dish 2	0.6	0.6	0.7	0.9	0.6	0.68
	Petri Dish 3	0.7	0.6	0.5	0.5	0.5	0.56
Rosemary	Petri Dish 1	0.7	0.9	0.8	0.7	0.7	0.76
	Petri Dish 2	0.9	1	0.8	0.7	-	0.85
	Petri Dish 3	1	1	0.8	1	-	0.95

Table 2: Average Zone Size and Standard Deviation		
Sample	Average Zone Size (cm)	Standard Deviation
Control	0.5	0
Peppermint	1.10	0.16
Ginger	0.69	0.13
Rosemary	0.85	0.10

Average Zone Size (cm) vs. Sample



Graph 1: Mean Zone of Inhibition size by Petri Dish (Based off Table 2)

		Table 3: Light Absorbance of Samples Over Time					
Sample	Time (seconds)	0	3	6	9	12	Mean
Control	Petri Dish 1	0.56	0.561	0.561	0.561	0.561	0.561
	Petri Dish 2	0.573	0.573	0.573	0.573	0.573	0.573
Peppermint	Petri Dish 1	0.081	0.084	0.084	0.084	0.084	0.083
	Petri Dish 2	0.075	0.078	0.078	0.079	0.079	0.078
Ginger	Petri Dish 1	0.265	0.269	0.27	0.27	0.271	0.269
	Petri Dish 2	0.252	0.252	0.253	0.254	0.254	0.253
Rosemary	Petri Dish 1	0.113	0.114	0.114	0.114	0.114	0.114

	Petri Dish 2	0.108	0.108	0.108	0.108	0.108	0.108
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Table 3: Light Absorbance of Samples Over Time in Test Tubes

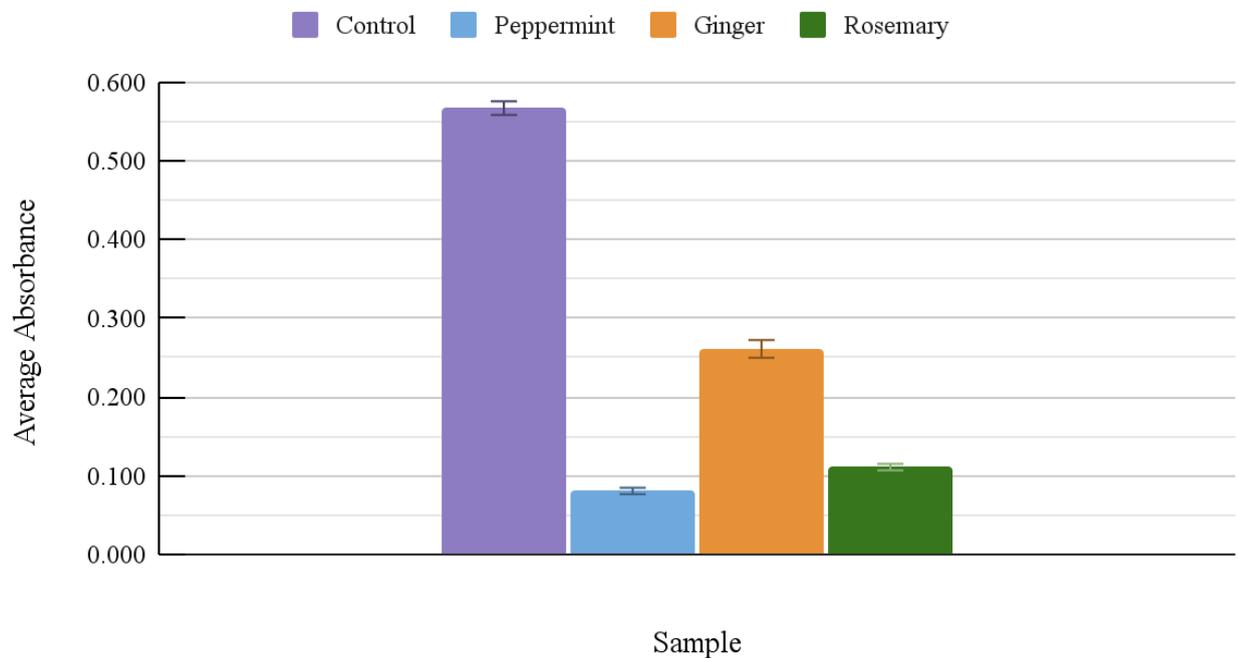
Time (seconds)	0	3	6	9	12	Mean
Control	0.182	0.184	0.184	0.184	0.184	0.184
Peppermint	0.165	0.164	0.164	0.164	0.164	0.164
Ginger	0.409	0.421	0.421	0.415	0.411	0.415
Rosemary	0.285	0.284	0.825	0.822	0.28	0.499

Table 5: Average Petri Dish Absorption and Standard Deviation

Sample	Average Absorbance	Standard Deviation
Control	0.567	0.0086
Peppermint	0.081	0.0040
Ginger	0.261	0.0113
Rosemary	0.111	0.0041

Graph 2: Mean Absorbance of Petri Dish samples (Based off Table 2)

Average Absorbance vs. Sample



3.2.2 Calculating Reduction in Absorbance

To better understand the data from the turbidity experiment, I processed the data in the following manner. This calculation will demonstrate how much each oil reduces the growth in bacteria in direct comparison to the control. The average petri dish absorption values were used for these calculations.

- Determine the percentage change of sample's absorption in comparison to control sample's absorption.

$$\begin{aligned} \text{Percentage change} &= \left(\frac{(\text{Absorbance value of sample}) - (\text{control's absorbance value})}{\text{Control's absorbance value}} \right) \times 100 \\ &= \left(\frac{(\text{Absorbance value of sample}) - (0.567)}{0.567} \right) \times 100 \end{aligned}$$

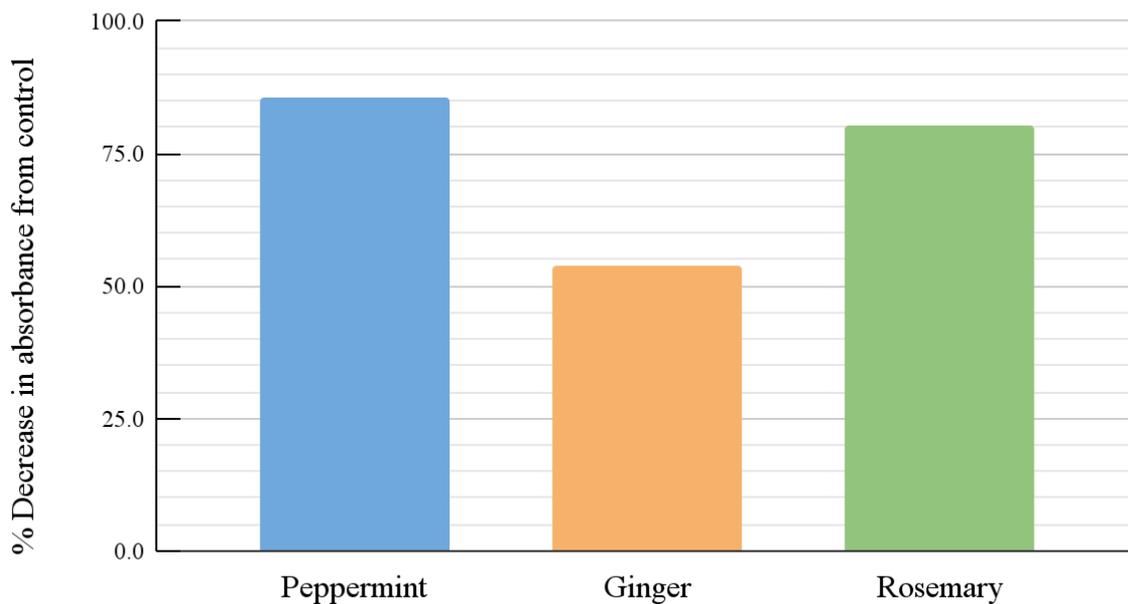
- Since all percentage changes were negative, the absolute value was taken and introduced as Percentage decrease from control

$$\text{Percentage decrease} = \left| \frac{(\text{Absorbance value of sample}) - (0.567)}{0.567} \right| \times 100$$

The calculation's results are below:

Table 6: Reduction in Absorbance	
% Decrease in absorbance from control	
Peppermint	85.8
Ginger	54.0
Rosemary	80.4

Reduction in Absorbance in Comparison to Control



3.2 Analysis and Discussion

In both turbidity and disc diffusion , all three essential oils displayed antibacterial properties, indicated by the decrease of bacterial growth in comparison to controls. Overall, peppermint essential oil performed best, followed by rosemary then ginger.

In the control disc diffusion, no zone of inhibition beyond the 0.5cm radius of the filter paper was observed, confirming that any zone of inhibition in the other petri dishes was due to the oils. The largest inhibition zones produced by ginger were 0.9cm, which are comparable to the inhibition zones created by rosemary oil. However, since the smallest inhibition zones produced by ginger are much smaller with some not inhibiting growth at all, the overall efficacy of ginger is lower. It must also be noted that since some inhibition zones were not measurable on some petri dishes , it should be kept in mind that there are less samples for some oils. This however can be ignored as the individual zone size data supports the overall trend. The qualitative observations demonstrate that peppermint has the highest antibacterial activity, and that the ginger samples had larger inhibition zones than rosemary (fig 2c and 2d) , suggesting that ginger may be more effective in long-term inhibition.

For absorbance measurements, a smaller absorbency value indicated that there was less bacterial growth since bacteria would prevent light from passing through. The data collected from the liquid petri dish samples supported the trend from the disc diffusion that peppermint is more effective than rosemary and ginger, however the data from the test tube liquid samples in table 3 were anomalous due to all oils having more growth than the control. In the petri dishes, the samples with essential oil had a lower absorbance value, indicating that there was less *E.coli* growth than the control petri dishes. Through the calculations which gave the data used for table 4 and graph 3, it can also be determined how much of a reduction in growth was observed, and the different essential oils can be compared to each other. A higher % difference in these results indicated a higher antibacterial property. Overall, the

difference in efficacy between peppermint and rosemary oil was around 7%, the difference between rosemary and ginger 27% and peppermint and ginger 34%. Through this data it can be determined that there is a large difference between ginger and the other essential oils regarding the strength of their antibacterial properties' strength.

In both experiments, the standard deviation values were noticeably small, indicating that there was little variation in the data range. A small variation demonstrates that essential oils are consistent in the degree of antibacterial activity they have, and that the same oil will have the same overall effect. This is important as it demonstrates that essential oils have an consistent and significant effect on reducing bacterial growth.

Comparing these results to those obtained in previous studies, there is some overlap. The observed range in inhibition zone size for peppermint and rosemary were different from those in studies, with rosemary having smaller zones (8.5 ± 5 mm observed compared to 13.3 ± 0.3 mm in literature (Thompson et al.)) and peppermint having larger ones. The inhibition zones of ginger were very different from the values seen in previous studies, a reason for that potentially being regarding the specific essential oil used.

4. Conclusion

4.1 Comment on results/hypothesis

All three oils demonstrated antibacterial properties, and these properties were shown to be consistent. The trends in the data are different to my hypothesis. Previous studies indicated that ginger would have the largest zones of inhibition, but this experiment demonstrated that they had the smallest inhibition zones and highest absorbance, indicating it had the weakest antibacterial properties of all the oils. These results also show that peppermint and rosemary oil are comparable to each other, with rosemary being more

effective than ginger in the first 28 hours, and ginger eventually becoming more effective in solid samples.

A possible reason for this result can be reflected in the traditional use of these herbs, which already indicated that peppermint may be more effective in dealing with bacteria. However, determining which chemical in particular this effect is owed to would be more difficult since both of these essential oils have many different components. With further research, it might be possible to determine which chemical groups may be the ones contributing to antibacterial properties.

In conclusion, essential oils can be considered as a method to reduce bacterial growth on solid and liquid mediums.

4.2 Evaluation of method

These results are reliable as at least 4 test zones per petri dish and 5 measurements per sample for turbidity absorbance testing were taken. In addition, since all samples were prepared from the same bottle of essential oil at the same time and incubated at the same place, the conditions subjected were exactly identical, eliminating any uncertainty which could be caused by the experiment conditions. The small standard deviation values indicate that the individual results were consistent, indicating its reliability in producing trends. As for the anomalous data in the test tubes, since the anomaly was caused by the control absorbance value being lower than the ginger and rosemary values but not the peppermint values, it can be attributed to a mistake when adding the *E.Coli* sample, nutrient agar or essential oil into the test tube, or a small mistake while handling the cuvettes.

The strength of the method used came from the large number of data points, as each petri dish gave 4 zones to measure, and having multiple zones from one dish also ensured that the zones would develop in near identical conditions. All of the petri dishes and liquid

samples used the same oil and liquid culture. This made the data reliable as it also ensured that all samples would be in similar conditions. The use of absorbance was also a strength because it reinforced the trends seen in the inhibition zone trials. As for its weaknesses, the incubation time wasn't very well controlled, which led to the samples for peppermint being unmeasurable. The method can be improved by planning multiple rounds of incubation and measurement to better observe the longer-term impact of essential oils.

4.3 Extension

There are multiple ways to extend upon this investigation. Firstly, previous research has demonstrated that various oils such as Basil essential oil have shown a stronger antibacterial character (Sienkiewicz et al.), as well as a variety of other oils demonstrating such ability such as Thyme oil (Thompson et al.). Second, the scope can be further expanded by testing extracts and bits of ground up plants such as garlic. This extension would allow for better application of the findings to daily life and implementation in cuisine, which was my initial intention with this trial. Furthermore, the duration of the experiment can be extended to study long-term efficacy.

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