Effects of Antibiotic Properties of Essential Oils on *E. coli* Growth

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Abstract

The aim of this experiment was to determine whether the different essential oils have antibacterial properties/activity. A total of six essential oils were used in the experiment and this included cinnamon oil, Eucalyptus, tea tree oil, almond, peppermint and penicillin oils. The methodology used included adding non-pathogenic *E.coli* in a petri dish with agar which was followed by adding paper discs each containing a different essential oil. This was then incubated and after two weeks after which the petri dish was checked to determine whether or not zones of inhibition were formed. According to the results, cinnamon formed a zone of inhibition of 28.5 mm, Eucalyptus 21 mm, tea tree 17.5 mm, almond oil 0 mm, peppermint 25 mm and lastly penicillin oil 29.5 mm. These results showed that apart from almond oil which failed the test, all other essential oils had significantly high antibacterial properties. Based on existing literature, it was shown that indeed the results of the various essential oils against *E. coli* were consistent with available empirical evidence. Consequently, the hypothesis that essential oils have an effect on the growth of *E. coli* was accepted. It is, therefore, possible that in the wake of antibiotic resistance, essential oils could be used as alternative antibiotics. A major challenge in the experiment was resources and getting the correct readings.

Introduction

Antibiotics can be described as substances that potentially inhibit the growth as well as growth of bacteria, fungi or protozoans. They are classified into two i.e. those obtained from natural sources, for example, penicillin and those that are manufactured synthetically. Their characteristic nature of selective life activity towards micro-organisms, and low toxicity make them suitable for use in the destruction and prevention of many microbes (Aminov, 2010). Following these characteristics, most antibiotics now play critical role especially in the fight against bacterial infections, both in among humans and animals, effectively making them indispensable in the well-being of both humans and animals (Korzybski, Kowszyk-Gindifer, Z., & Kurylowicz, 2013).

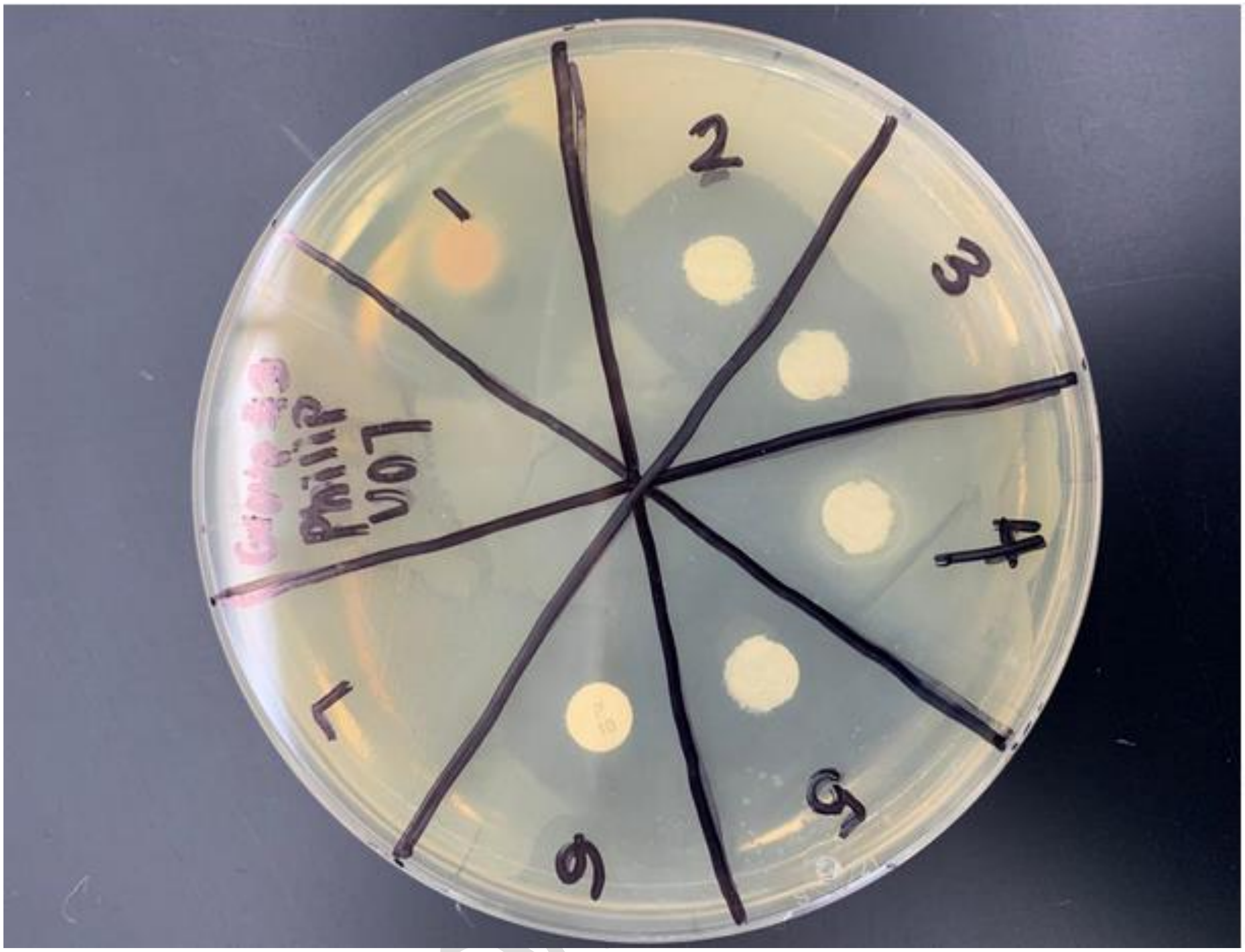
From a chemical point of view, antibiotics are composed of a variety of compounds. Generally, however, antibiotics tend to have a low relative molecular weight, exhibit different chemical structures as well as phyto-chemical properties. Following the unique nature of their properties, some antibiotics tend to be specialized and, therefore, effective against particular bacteria. Others, on the other hand, are broad-spectrum i.e. attack a wide range of bacteria (Bérdy, 2012). Based on the chemical composition, antibiotics target bacteria in different ways; firstly, by inhibiting bacterial protein synthesis usually by targeting bacterial ribosome that is composed of 50S and 70S subunit. Secondly, by killing the bacteria by preventing or inhibiting the process of building their cell walls which leads to loss of osmotic support and eventually cell lysis. Lastly, by inhibiting bacterial DNA synthesis (Lopez-Romero et al., 2015).

Essential oils are primarily produced by plants and have for a long time been used to treat various infections, for instance, respiratory tract diseases and currently used as ethical medicines for colds. Besides, essential oils play a critical function of aiding pollination as well as distribution of seeds and this is largely attributed to their strong smell which helps in attracting insects that carry out both processes. A majority of essential oils have unique properties and these include critical antimicrobial activity; antiseptic, antibacterial as well as antiviral properties (Inouye, Takizawa & Yamaguchi, 2001). It is these properties of essential oils that give them the ability to hamper growth of various pathogens. For instance, the antibacterial activity of essential oils allows them to slow down or completely destroy bacterial cells. Usually, the process of antibacterial action is aided by various biochemical reactions which occur on the bacteria's cell. It is, however, critical to note that the essential oils do not exhibit uniform antibacterial activity, rather, the activity differs based on a bacterial architecture. In general, the antibacterial properties of the oils depend, to a large extent on their chemical composition.

The objective of the study was to test for antibiotic properties of the various essential oils by investigating their effect on the growth of *Escherichia coli.* The oils that were utilized in the experiment were as follows: cinnamon oil, tea tree oil (TTO), peppermint oil, eucalyptus as well as almond oil. It is important to note that information pertaining to the properties of the various essential oils was not given. It, therefore, follows that the experiment was designed with the aim of investigating or finding which of the provided essential oils did and did not exhibit antibacterial properties. Consequently, the hypothesis for the experiment was: H0: essential oils will have an effect on the growth of *E. coli.* Ha: essential oils will not have an effect on the on the growth of *E. coli.* An important feature of *E. coli* is that it is a gram-negative bacterium and usually tends to be resist attacks from the antibiotic compounds found in essential oils. It will be easier, therefore, to find out whether or not the essential oils have antibiotic properties. This is because they inhibit growth of the bacteria if they have antibiotic properties and not have any effect at all if they lack antibiotic characteristics.

Materials and Methods

In order to carry out the experiment successfully, the following materials were provided: one agar plate with lysogenic broth; one bacterial spreader (metal); one ethanol lamp; one micropipette set to 130 microliters; one forceps; filter paper discs; one beaker with ethanol; one ruler and permanent marker. In conducting the experiment, the following procedure was followed: firstly, a clean and sterilized Petri dish containing agar was held upside down and with the help of ruler and a permanent marker, divided into 8 different parts which were labeled 1-6. One space was left open for negative control and one free as shown below:



The spreader was then decontaminated by simply putting it in ethanol followed by passing it slowly but repeatedly over an ethanol flame until all the ethanol on it was burnt completely. In the next step, the spreader after being passed through the flame was allowed to cool after which it was dipped in the container holding the non-pathogenic liquid of *E.coli* culture. The picked *E.coli* was then spread evenly on the agar gel in the petri dish after which the lid was closed.

The next step that the paper discs were dipped in each of the various essential oils, aligned into the spaces marked at the bottom of the petri dish. To accomplish this task, the forceps were first put in ethanol and then over the ethanol flame for sterilization. After this was done, the forceps were cooled and then used to pick the paper discs. These were then dipped into the respective essential oils and carefully put on top of the agar by carefully aligning them with the numbers on the bottom of the petri dish. Each space marked on the agar was recorded against the essential oil that was placed on it to avoid confusion.

The experiment was conducted by six different groups of students and in a controlled environment and this was with a view of eliminating potential mistakes and increasing the accuracy of the experiments. Each class group was given one petri dish in which they applied *E.coli* on the agar and placed same essential oils on each of the petri dish in the labeled spaces. Important to note is that each petri dish had 5 different essential oils as well as two controls i.e. a negative and a positive control. In the experiment, the negative control was not treated in any way and, therefore, was expected to show bacterial growth. Conversely, the positive control contained penicillin and was, therefore, expected to show no bacterial growth.

Results

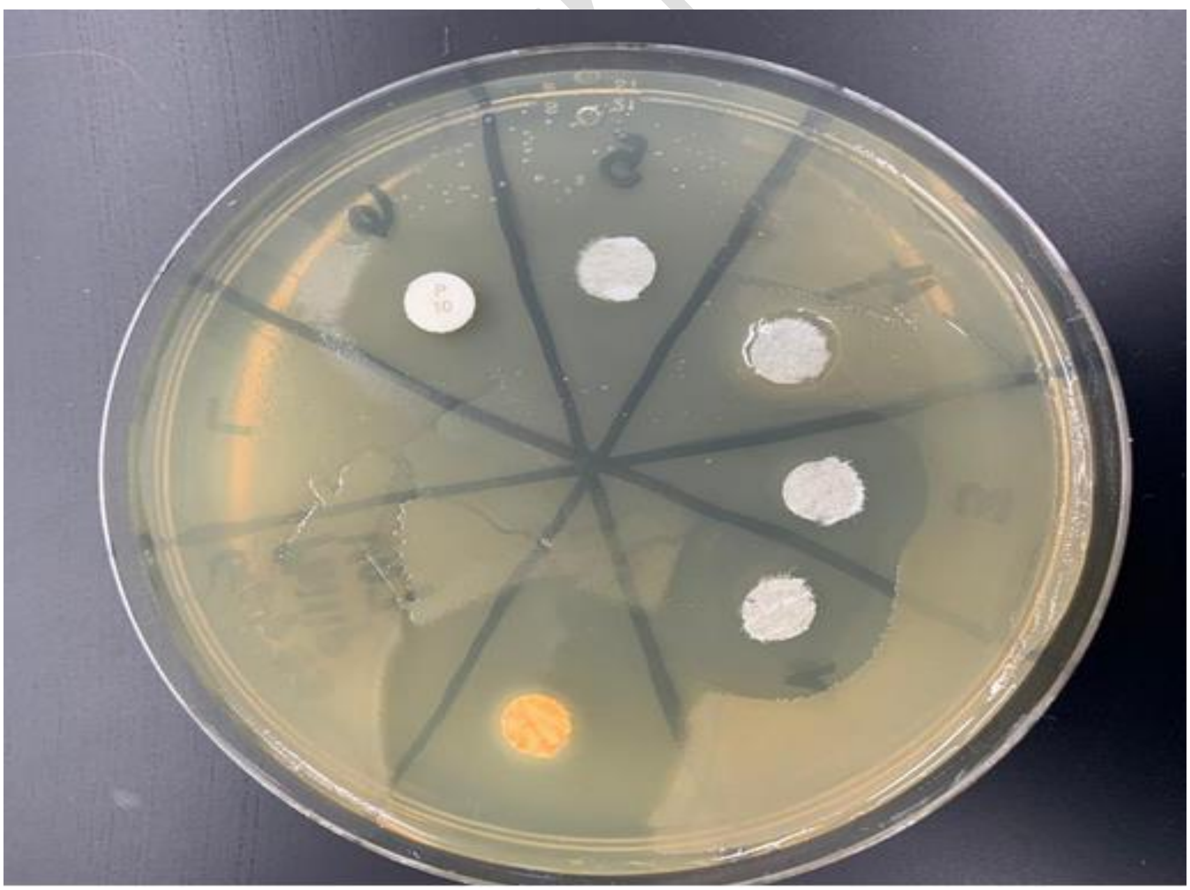
The results attained from the experiment to a large extent portrayed what was expected. Before the experiment, the following were the predictions put forth by the author.

Table 1

Paper Disc No	**Compound**	**Expected Result**
1	Cinnamon Oil	No inhibition expected

2	Eucalyptus Oil	Inhibition expected
3	Tea Tree Oil	No inhibition expected
4	Almond Oil	No inhibition expected
5	Peppermint Oil	Inhibition expected
6	Penicillin Oil	Inhibition expected

Upon keeping the petri dishes for two weeks, the following results were obtained. Group 3 where the author was a member obtained the following results.

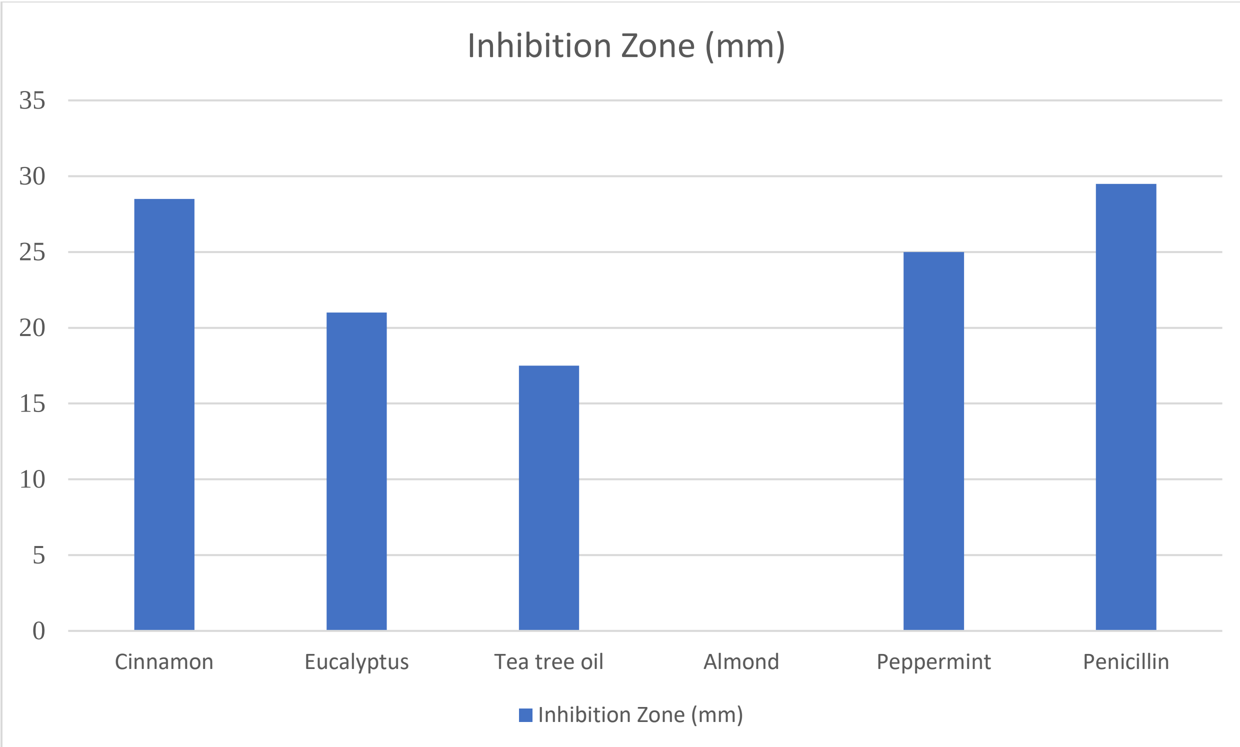


To determine the inhibition zone, the author used a ruler to measure from the disc center to the nearest bacterial colony that appeared. Accordingly, the author converted the distance from cm to mm and the data was recorded as follows:

Table 2

Compound	**Plate 1**	**Plate 2**	**Average**
1	25mm	32mm	28.5 mm
2	20mm	22mm	21mm
3	15mm	20mm	17.5mm
4	0mm	0mm	0mm
5	20mm	30mm	25mm
6	15mm	44mm	29.5mm

Graphical Representation



A quick scan of the graph shows that the various essential oils have antibacterial properties and this can be confirmed by looking the inhibition zone against *E.coli.*

Discussion

The aim of the experiment was determine the potency of the antibacterial properties of each of the essential oils towards *E.coli* growth (determined by the extent of growth inhibition) when placed in a *E.coli* growth medium. Based on the predicted results and doing a comparison with the collected information particularly from the next table, one can easily make a conclusion on the various essential oils used in the experiment. To understand inhibition, it is usually said that an inhibition zone of less than 6mm is not antibacterial. On the other hand, an inhibition zone that falls between 6 and 16mm is usually considered slightly antibacterial and any area that exceeds 16mm is usually deemed to be significantly antibacterial. Consequently, the following deductions can be made.

Essential Oil	**Plate 1**	**Plate 2**	**Average**	Activity level
Cinnamon	25mm	32mm	28.5 mm	Highly antibacterial
Eucalyptus	20mm	22mm	21mm	Highly antibacterial
Tea tree	15mm	20mm	17.5mm	Highly antibacterial
Almond	0mm	0mm	0mm	Not antibacterial
Peppermint	20mm	30mm	25mm	Highly antibacterial
Penicillin	15mm	44mm	29.5mm	Highly antibacterial

The results above confirm that indeed a majority of essential oils have antibacterial properties. As can be deduced, Cinnamon is highly antibacterial. These findings are consistent with other studies conducted on Cinnamon to determine its antibacterial properties Nabavi et al., (2015) lists a number of studies whose findings revealed that the essential oil has well established antibacterial activity against both Gram-positive and Gram-negative bacteria that often cause diseases not only to humans but also responsible for the degradation of food or cosmetics. The researchers attribute the antibacterial properties of Cinnamon to the fact that it contains bioactive phytochemicals, for example, cinnamaldehyde eugenol.

Based on the results, it is also apparent that Eucalyptus oil is also highly antibacterial with an activity level of 21mm. These results are consistent with various studies, notably that of Bachir and Benali (2012) which confirmed that Eucalyptus oil formed a large inhibition zone with *E.coli* indicating as significant antibacterial properties. However, the authors noted that inhibition depends on the level of concentration of Eucalyptus oil with higher concentration leading to higher inhibition and vice-versa. Additionally, the authors noted that Eucalyptus oil was more effective on Gram-negative bacteria as opposed to Gram-positive bacteria and this is as a result of the structural differences in their cell walls particularly the presence of lipoproteins and lipopolysaccharides in gram-negative bacteria which is responsible for creating a barrier to hydrophobic compounds.

The results also show that tea tree oil exhibited significant levels of inhibition thereby leading to a conclusion that it is an effective antibacterial essential oil. In their work on tea tree, Cox et al., (2001) found out that the essential oil consists of cyclic monoterpenes. Consequently, this compound contributes to the tea tree exhibiting antimicrobial activity. The sentiments of being a strong antibiotic are further carried by a study conducted by Mumu and Hossain (2018) who upon incubation for 24 hours, reported that tea tree oil (TTO) showed minimum 96.94% against *E.coli.* In fact, in their conclusion, the researchers opine that there is a possibility that over time, TTO will gradually take the place of conventional antibiotics to treat bacterial infection.

A look at the results indicate that Almond oil did not have antibacterial properties and this is clear in the sense that there was no zone of inhibition. This is true because Almond oil contains emollient and sclerosant properties that are used primarily for skin care. As per the results, peppermint showed significant antibacterial properties meaning it is was able to form a significant zone of inhibition with

E.coli. These findings are consistent with those of a study conducted by Saeed et al., (2006) who found that essential oil of peppermint showed an antibacterial activity with 11.78 mm inhibition zone. However, as per the zoning rules, this could be classified as having a slight antibacterial activity. According to the results, penicillin has the highest zone of inhibition. Penicillin is a natural antibiotic whose mode of action is inhibiting cell wall synthesis. However, natural penicilins have been found to have a narrow spectrum of activity while the newer penicillins have been found to have a wider spectrum activity. A significant number of studies have been carried out and shown that penicillin has significant antibacterial activity.

With the above information, it is clear that essential oils have an impact on the growth of *E.coli.* Consequently, the hypothesis that essential oils have an effect on the growth of *E. coli* is accepted. This is because a majority of the essential oils formed a zone of inhibition against *E.coli* meaning they have antibacterial properties. With the rising cases of antibacterial resistance, Essential oils, therefore, provide an alternative that can be used to create alternative antibiotics for infectious bacterial diseases.

While carrying out this experiment, the author experienced some challenges which she believes could have contributed to some errors. For instance, the area of inhibition for all essential oils used remained consistently high. However, findings from similar studies seem to suggest different zones of inhibition for different essential oils. Errors could have arisen from the fact that the experiments were done in a class set up with limited resources to monitor the experiment properly. It is also possible that there could have been contamination which may have interfered with the results. Another challenge could have been as a result of working as a group. It is possible that some members did not perform their duties well and this could have interfered with the final results. To eliminate such challenges, there is need for more experiments on the same or related topics as this will help improve the competence of the author as well as that of the classmates.

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