Research Article

iMedPub Journals
www.imedpub.com

Journal of Biomedical Sciences
ISSN 2254-609X

2019
Vol.8 No.3:17

The Effect of Essential Oils on the Inhibition Zones of Antibiotic-Resistant Bacteria

Charitha Vennapusa*

Oakton High School (Vienna, VA), United States

***Corresponding author:** Charitha Vennapusa, Oakton High School (Vienna, VA), United States, Tel: +5714855279; E-mail: charithausa@gmail.com

Received date: September 03, 2019; **Accepted date:** September 16, 2019; **Published date:** September 22, 2020

Citation: Vennapusa C (2019) The Effect of Essential Oils on the Inhibition Zones of Antibiotic-Resistant Bacteria. J Biomedical Sci Vol.8 No.3:17.

Abstract

This experiment depicts the antibacterial properties of different types of essential oils in comparison to ampicillin in antibiotic-resistant bacteria. The purpose of this experiment is to determine whether essential oils are more effective at killing antibiotic-resistant *E. coli* bacteria than ampicillin. Antibacterial strength was determined by doing a Kirby Bauer assay and measuring the inhibition zone in millimeters that each substance created. The data showed that the cinnamon essential oil had the strongest antibacterial properties in the resistant bacteria. All the essential oils had inhibition zones on the experimental group that was equal to or greater than the inhibition zones created in the control group meaning that essential oils are more effective in resistant bacteria compared to non-resistant bacteria. Thus, it has been shown that some natural ingredients may work better than antibiotics in resistant and non-resistant bacteria.

Keywords: Antibiotics; Resistant bacteria; Essential oils, *E. coli*

Introduction

80 years ago, penicillin was first introduced into the market as a lifesaving antibiotic. Until that point, 30% of all deaths in America were due to bacterial infections. At first, the rise in antibiotics was truly beneficial saving many lives but over time, the misuse and overuse of antibiotics led to antibiotic-resistant bacteria. The decrease in the development of new antibiotics in recent years also means that there are fewer antibiotics to replace the ineffective ones. In fact, a study conducted in 2016 by the Review on Antimicrobial Resistance stated that by 2050, more than 10 million people could die globally from just resistance to antibiotics alone, a number greater than deaths caused by cancer each year. Antibiotic-resistant bacteria have been on the rise with no reliable, consistent solution. The goal of conducting this experiment was to see whether natural substances are more effective at killing antibiotic-resistant *E. coli* bacteria than antibiotics [1]. There are two main questions that this experiment is trying to answer: Are essential oils more effective than antibiotics at fighting antibiotic-resistant bacteria? If so, which essential oil exhibits the greatest antibacterial properties? This experiment will be useful and applicable to many other scientists who are trying to find a natural, safe cure for antibiotic-resistant bacteria. Natural substances, such as herbs and spices, have been used as a way to treat illnesses since ancient times as medicine. Today, there is an increasing amount of focus and research being conducted on plants exhibiting strong antibacterial properties as a successful and inexpensive alternative to pharmaceutical drugs [2]. This experiment will show if natural substances could potentially replace antibiotics or have their natural chemical compounds incorporated into current antibiotics.

Methods

This experiment was done using a Kirby Bauer assay. *E. coli* bacteria were transformed with pUC19-ampicillin so that the *E. coli* bacteria could become resistant to the ampicillin. Six Petri dishes contained the ampicillin-resistant *E. coli* bacteria and one of the Petri dishes had regular *E. coli* bacteria which were used as a comparison. 200 microliters were deposited in all of the Petri dishes [3]. All seven of the Petri dishes were divided in the following manner **(Figure 1)**.

Then, using forceps, each filter paper disc was dipped into its respective essential oil/ water and placed in the petri dish except for vancomycin and ampicillin where ready-made filter paper discs were used. Between different substances, make sure to sterilize the forceps by dipping it into ethanol [4]. After you have completed this process, place the Petri dishes in an incubator at 37°C for 48 hours.

After 48 hours, record the results by using a ruler and measuring the zone of clearance from the outside of the filter disc to the beginning of the bacteria. If there are no bacteria from the edge of the filter disc to the edge of the box designated to this, then state the length from the edge of filter paper to the side of the box [5]. All measurements should be done in millimeters. Take pictures and write down any observations. Lastly, dispose of all materials properly **(Figure 2)**.

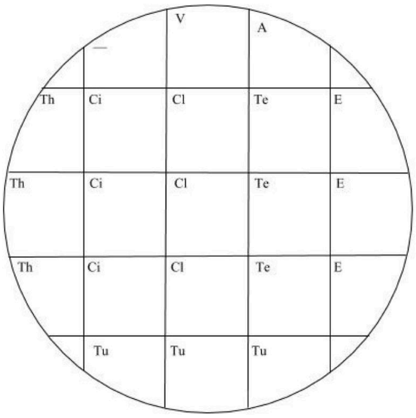

Figure 1: Water (constant) V: Vancomycin (an antibiotic used as a comparison); A: Ampicillin; Th: Thyme; Ci: Cinnamon; Te: Tea Tree.

Results

A Student's t-test was used to determine the statistical difference between the means of the antibiotic or essential oil to water **(Figure 3)**.

Type of Essential Oil on Inhibition Zone

Inhibition Zone (mm)

		Water	Vancomycin	Ampicillin	Thyme	Cinnamon	Clove	Tea Tree	Eucalyptus	Turmeric
		Essential Oil								
Petri Dish 1 (non resistant)	1	0	0	6	3	8	11	5	1	0
	2				2	7	6	5	0	0
	3				2	5	8	5	1	0
	Average	0	0	6	2.333	6.667	8.333	5	0.667	0
	Range	0	0	0	2-3	5-8	6-11	0	0-1	0
	SD	0	0	0	0.577	1.528	2.517	0	0.577	0
Petri Dish 2	4	2	0.5	2	6	13	12	6	2	0
	5				3	13	10	9	2	0
	6				5	10	13	12	3	0
Petri Dish 3	7	0	0	3	10	12	13	11	2	0
	8				10	13	10	7	1	0
	9				7	12	9	5	1	0
Petri Dish 4	10	0	0	3	6	11	11	9	2	0
	11				10	9	10	10	1	0
	12				10	11	5	5	1	0
Petri Dish 5	13	0	0.5	4	9	12	10	11	1	0
	14				10	10	10	8	1	0
	15				10	12	9	9	1	0
Petri Dish 6	16	1	0.25	0	10	14	11	9	2	0
	17				9	10	10	6	1	0
	18				8	10	11	5	0	0
Petri Dish 7	19	0	0	3	10	11	11	8	2	0
	20				10	10	10	10	1	0
	21				6	13	11	12	2	0
	Average	0.5	0.208333333	2.5	8.277777778	11.44444444	10.33333333	8.444444444	1.444444444	0
	Range	0-2	0-0.5	0-4	3-10	9-14	5-13	5-12	0-3	0
	SD	0.836660027	0.24579802	1.378404875	2.217724206	1.423426777	1.748949264	2.357022604	0.704792186	0

Figure 2: Type of essential oil on inhibition zone.

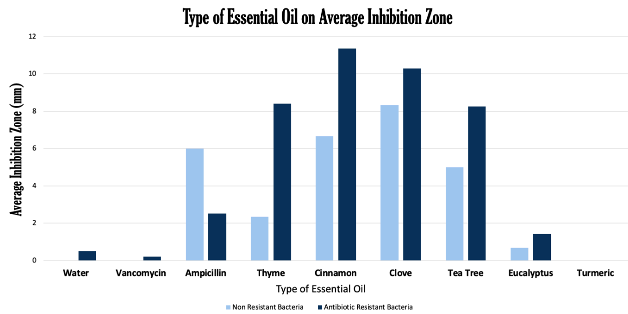

Figure 3: Type of essential oil average inhibition zone.

The t-test was used regarding only the resistant bacteria trials as the data in those samples were independent of each other with a normal distribution and were two-tailed [6]. The significance level was set to 0.05. Comparisons with a degree of freedom of 10 had a critical value of 2.228 and comparisons with a degree of freedom of 22 had a critical value of 2.074 **(Table 1)**.

Vancomycin had one of the lowest inhibition zones in nonresistant bacteria suggesting that if necessary to use an antibiotic for a bacterial infection, ampicillin may be more effective than vancomycin. Vancomycin had the second-lowest average inhibition zone after turmeric which was surprising considering that it is a commonly used antibiotic [7].

Vancomycin also had the smallest range, other than turmeric, between the non-resistant and the resistant bacteria with a difference of only 0.2083 mm. Vancomycin had a t-value less than the critical value of 2.228 and a p-value greater than 0.05 meaning that the null hypothesis cannot be rejected and there is no statistical significance between vancomycin and water **(Table 2)**.

Ampicillin was the only independent variable that worked better in the non-resistant bacteria than the resistant bacteria. This was an expected result as ampicillin has a strong effect against regular *E. coli* but ampicillin starts to lose that strength once it has been already introduced in the bacteria which are what was seen in the resistant bacteria.

Table 1: Vancomycin v/s Water.

Vancomycin v/s Water		
t- Value	**df**	**p-value**
0.81928803	10	0.43171457

Table 2: Ampicillin v/s Water.

Ampicillin v/s Water		
t- Value	**df**	**p-value**

3.0382181	10	0.01250054

Ampicillin also had a t-value higher than the critical value of 2.228 meaning the null hypothesis is rejected and the alternative hypothesis is accepted stating that there is a statistical difference between ampicillin and water **(Table 3)**.

Table 3: Thyme v/s Water.

Thyme v/s Water		
t- Value	**df**	**p-value**
15.160514	22	2.87993E-11

The greatest difference in the average size of inhibition zones between the non-resistant and the resistant bacteria was seen in thyme which had a difference of 5.94 mm. All of the other oils showed similar antibacterial properties between resistant and non-resistant bacteria except for thyme. Seeing that the range and standard deviation was also one of the largest compared to other essential oils, this data may not be as reliable [8]. However, thyme had a t-value greater than the critical value of 2.074 and a p-value less than 0.05 meaning that the null hypothesis is rejected and the alternative hypothesis is true meaning there is a significant statistical difference between thyme and water **(Table 4)**.

Table 4: Cinnamon v/s Water.

Cinnamon v/s Water		
t- Value	**df**	**p-value**
22.859029	22	3.67906E-13

Cinnamon bark essential oil had the highest inhibition zone of 11.44 mm against the resistant bacteria suggesting that cinnamon may have strong antibacterial properties, especially, against resistant bacteria. Cinnamon also had a fairly average range and standard deviation compared to other oils suggesting that there were not many outliers and that the data is accurate and reliable. Cinnamon had the largest t-value and the smallest p-value compared to all other independent variables showing that the null hypothesis can be rejected as cinnamon had the largest statistical difference against water. Overall, cinnamon was shown to be the most effective compared to the antibiotics and essential oils against ampicillin-resistant bacteria **(Table 5)**.

Table 5: Clove v/s Water.

Clove v/s Water		
t- Value	**df**	**p-value**
18.367992	22	2.2856E-13

Regarding non-resistant bacteria, clove had the greatest antibacterial properties as it had the largest average inhibition zone. Clove indicated to be more effective than both of the antibiotics. However, clove had the highest range with a difference of 8 mm suggesting that clove may be effective but not necessarily reliable. Yet, clove did have the second-highest t-value and second-lowest p-value meaning the null hypothesis can be rejected and that there is a strong significant difference between clove and water **(Table 6)**.

Table 6: Tee tree v/s Water.

Tee Tree v/s Water		
t- Value	**df**	**p-value**
12.181799	22	3.51771E-11

Tea Tree had similar antibacterial properties as thyme in the resistant bacteria proposing that it has the ability to fight antibiotic resistance. However, it had the highest standard deviation and one of the highest range values meaning that the data is not very precise. Results from the tea tree data showed that the null hypothesis could be rejected but there was not as great of a statistical difference between tea tree and water versus the other essential oils that also rejected the null hypothesis and water **(Table 7)**.

Table 7: Eucalyptus v/s Water.

Eucalyptus v/s Water		
t- Value	**df**	**p-value**
2.4865609	22	0.03953101

Eucalyptus exhibited low antibacterial properties compared to the other essential oils. The low range and standard deviation further prove that the data is accurate and precise meaning that although eucalyptus oil had some antibacterial properties, it is not as effective as other essential oils. Since the p-value of 0.0395 for Eucalyptus is close to but still greater than the significance level of 0.05, the null hypothesis cannot be rejected. Eucalyptus did not have a significant statistical difference against water **(Table 8)**.

Table 8: Turmeric v/s Water.

Turmeric v/s Water		
t- Value	**df**	**p-value**
1.4638501	22	0.20311066

Turmeric showed no antibacterial properties at all in both the non-resistant and the resistant bacteria. None of the trials had an inhibition zone greater than 0 mm suggesting that turmeric is not reliable or effective at fighting bacteria. In addition, turmeric had a low t-value and a large p-value meaning the null hypothesis failed to be rejected and that there is negligible statistical difference between turmeric and water.

Discussion

Through this experiment, it has been suggested that essential oils work the same or are more effective in antibiotic-resistant bacteria than non-resistant bacteria. In non-resistant bacteria, some of the essential oils were stronger than the ampicillin. Thus, some natural ingredients could work better than antibiotics at killing bacteria such as *E. coli*, whether the bacteria is resistant or not. The hypothesis that the cinnamon bark essential oil would have the largest inhibition zone was supported in the resistant bacteria but clove had the largest inhibition zone in the non-resistant bacteria. This was expected as other studies that looked at essential oils found that cinnamon and clove showed the strongest antibacterial properties. Studies have shown that cinnamaldehyde, an organic compound found in cinnamon that gives cinnamon its odor and flavor, has the ability to break up biofilms which are the strong, complex layers formed by bacteria that antibiotics cannot reach leading to persistent and continuous infections. It was also shown to prevent the formation of new biofilms or bacteria spreading. Additionally, eugenol, a chemical compound found in cinnamon and especially cloves, is one of the strongest and most powerful antioxidants in the world. It also has the ability to inhibit the growth of various types of bacteria which is why clove had the largest zone of clearance in the non-resistant bacteria. One problem in this experiment was that the turmeric essential oil was not from the same company as the other oils. This may be why the turmeric oil showed absolutely no antibacterial properties. Another problem was that some essential oils diffused quicker than others which meant that the oil spread to other places killing bacteria that were maybe outside the inhibition zone of other bacteria. If this happened then the data would show another essential oil having high antibacterial properties when it was truly the other diffused oil that killed those bacteria. The vancomycin also showed very low strength against both the resistant and non-resistant bacteria but the ampicillin showed strength against the resistant and non-resistant bacteria. Since both of them are common antibiotics, it is likely that another experimental error was that the vancomycin filter paper discs were expired or dried out. In addition, because the filter paper discs were dipped in the essential oil and dabbed onto a paper towel to get rid of excess oil, there may have been varying amounts of oil on each filter disc which could have affected the results. Since there is a possibility that the oils diffused and may have mixed, using Petri dishes with only one type of essential oil or switching the position of the essential oils could also change the results. Antibiotic-resistant bacteria are a new concept that is become more and more of an urgent threat that scientists are struggling to find a cure for. Experiments such as this one can help to find natural ingredients that would be more productive than using more antibiotics and worsening the problem. People today could incorporate foods with the ability to kill bacteria into their meals to prevent infections or use essential oils in their homes. Even if it is not possible to fully replace antibiotics, the chemical compounds in the natural substances exhibiting strong antibacterial properties could be used such as the cinnamaldehyde in the cinnamon or the eugenol in the clove and be incorporated into current and

2019
Vol.8 No.3:17

new antibiotics to at least take a step in a more natural approach to fighting bacterial infections.

Conclusion

Experiments in the past have proven the antibacterial properties of some essential oils such as the ones in this experiment. This experiment specifically has suggested that some of these natural substances can work just as well or even better than antibiotics. Of course, this conclusion has to be tested out by many other scientists as well to determine its validity but it is a step and guideline for other experiments similar to this. More research needs to be done on whether essential oils or natural substances, in general, are more effective than multiple different types of antibiotics since this experiment only used two, ampicillin and vancomycin. Furthermore, since the null hypothesis failed to be rejected in the comparisons between vancomycin, eucalyptus oil, and turmeric oil against water, more data should be collected to provide enough evidence supporting the alternative hypothesis. Additionally, since the placement and proximity of the filter paper discs could have affected the results, experiments using a different filter paper set up within the petri dish would have to be conducted. This would also have to be tested long term to see whether antibiotics or essential oils would work better over multiple bacterial reproductions. There are also many other essential oils that have been proven to be antibacterial such as oregano oil, ginger oil, and more which could be tested against the essential oils used in this experiment to determine which ones are the most antibacterial. This experiment is useful for real-world applications such as creating new medicines that are more natural or taking steps at home to decrease the possibility of a bacterial infection.

Acknowledgment

This experiment would not have been possible without the help and support of Dr. Charles Madden at George Mason University who gave up his time and resources to help the experimenter understand how to use specific machinery and equipment.

References

1. Outpatient Antibiotic Prescriptions-United States (2018).
2. Burt SA, Reinders RD (2003) Antibacterial activity of selected plant essential oils against Escherichia Coli O157:H7. Letters in Appl Microbio 36: 162-167.
3. Creation of recombinant bacmids for gene expression in insect cells (2011).
4. Mukhtar S, Ghori I (2012) Antibacterial activity of aqueous and ethanolic extracts of garlic, cinnamon and turmeric against Escherichia coli ATCC 25922 and Bacillus subtilis DSM 3256. Int J App Bio and Pharma Technol 3: 131-136.
5. Nascimento GGF, Locatelli J, Freitas PC, Silva GL (2000) Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Brazilian J of Microbio 31: 247-256.
6. Noormandi A, Dabaghzadeh F (2015) Effects of green tea on Escherichia coli as a uropathogen. J Traditional and Complementary Med 5: 15-20.
7. https://resistancemap.cddep.org/AntibioticUse.php
8. Venugopal A, Shalini R, Dasani S (2009) Antibacterial effect of herbs and spices extract on Escherichia coli. Electronic J Biol 5: 40-44.