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# Comparison of the antibacterial activity of tea tree oil and tea seed oil with that of amoxicillin and ampicillin against *E. coli*

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## ABSTRACT

Essential oils have been growing in popularity in recent years as safer and more natural alternatives to modern medicine. Amoxicillin and ampicillin are two antibacterial drugs that are commonly prescribed to treat infections caused by both gram-negative and gram-positive bacteria. Recently these drugs have developed some problems associated with them such as increased cost and bacterial resistance and one possible solution to these issues is the use of natural oils that bacteria have not grown resistance to. Tea tree and tea seed oil were selected as possible alternatives. Since tea tree oil is a mix of active ingredients that differ in structure from the active ingredients of the chosen antibiotics it could offer an alternative if bacteria develop a resistance to pharmaceutical antibiotics. To compare the antibacterial properties of the oils and the antibacterial drugs the zone of inhibition was measured using the disc diffusion method. Results suggested the tea seed oil is totally ineffective against *E. coli* while tea seed oil is totally ineffective against it.

Keywords: *essential oils, tea tree oil, tea seed oil, antibiotics, ampicillin, amoxicillin, Escherichia coli*

## INTRODUCTION

Over the past century, the world has become pharmaceutical dependent. These drugs have saved countless lives. One type of pharmaceutical among the many that save lives is antibiotics. Antibiotics are used to either inhibit the growth of bacteria or kill them directly. These drugs are widely used to treat and prevent bacterial infections. Patients will insist on taking antibiotics for any illness they may contract, even illnesses such as the common cold which is caused by viral infection therefore, antibiotics cannot treat. However, along with antibiotics comes lists of side effects that, in some cases, can be even worse than the illness itself ranging from symptoms such as nausea to death. Another problem that arose with the continued use of antibiotics is the emergence of drug-resistant bacteria. One possible solution to these issues is the emergence of essential oils as an antibacterial agent.

Essential oils are naturally occurring aromatic oils obtained from plant materials through a variety of methods, however, the most common method used to obtain these oils is distillation (Prabuseenivasan, 2006). They contain volatile, hydrophobic molecules that are believed to be the active ingredients resulting in antibacterial properties, however the exact mechanism is not known. These oils are “essential” in the way that they contain the essence of the fragrance of the plant they were extracted from. Many essential oils have been known to show antibacterial properties when applied either topically or through aroma therapy as vapors (Sinthia, 2018). Essential oils are not meant to be taken orally and can be toxic when taken in high doses. These oils do have some side effects that are caused by allergies to the oil.

Tea tree oil (*Melaleuca alternifolia*) is an essential oil known for its broad-spectrum antibacterial properties (Singh, 2016). The oil is obtained through steam distillation of the leaves from the terminal branches of the *Melaleuca alternifolia* tree found in Australia (Carson, 2006). The oil was traditionally used by Australian Natives to treat wounds, aid in household cleaning, feminine care, and in the treatment of chronic illnesses (Shah, 2017). Tea tree oil has most recently been studied because it has shown to treat bacteria that have become drug resistant. As with most essential oils, the composition of tea tree oil is highly variable with variation that can exist even between individual plants of the same species (National Institutes of Health and Environmental Science). However, one of the molecules found in this oil are terpenes, which are organic molecules that are built up of $C_5H_8$ rings (Shah, 2017). Although the exact mechanism is unknown, one possible mechanism is that tea tree oil weakens the peptidoglycan membrane of the bacteria allowing for lysis to occur and has also shown to inhibit respiration (Carson, 2006). Tea tree oil has shown the ability to cause cells to get stuck in the $G_o$ phase of the cell cycle where the cell is neither dividing, nor preparing to divide (Antonietta). Since the cells are stuck in a phase where the cells cannot divide, tea tree oil is able to inhibit cell growth.

Tea seed oil (*Camellia oleifera*) is extracted from the defatted seeds of the evergreen plant *Camellia oleifera* which grows in central China and is used to make tea for drinking, unlike the plant that produces tea tree oil. Traditionally, along with making tea this oil has been used to stop itching and pain (Ye). This edible oil contains mostly unsaturated fatty acids making it edible for humans (9). Tea tree oil is also known for its antibacterial and anti-inflammatory properties (Yuefei, 2011). Much like tea seed oil the exact mechanism to kill bacteria is unknown, however, tea seed oil contains sapions which is believed to give the oil its antimicrobial properties. It is the sapions interactions that increase the permeability in gram-negative bacteria that cause lysis of the cell (Yuefei, 2011).

Ampicillin is a broad-spectrum antibiotic. In this case the drug is bactericidal. It is in the β-lactam class drug and more specifically, a penicillin type drug It works by binding to the penicillin binding proteins in bacterial cells and, while the cell is dividing, are able to stop the cell wall from completely forming, causing lysis (Yong, 2015). Over the year's bacteria have developed resistance to ampicillin (National Centers for Biotechnology Information, 2020). The exact mechanism of resistance is still being studied.

Drug resistance is becoming an increasing problem in the healthcare system. For as long as drug treatment has been around bacteria have been developing resistance to pharmaceuticals. This can lead to treatment failure which can result in death for patients and allows infections to spread more and be harder to control. Against penicillin type drugs bacteria have developed multiple mechanisms of resistance. One mechanism involves enzymes that destroy the antibacterial agent before it can work. Another is the ability to expel the drug from the bacterial cell before it can do its job. The final way bacterial cells have resisted antibiotics is by altering their cell walls to not contain the binding site for the active ingredient of the drug (Sakoulas, 2012). So far there has been no evidence that bacteria have developed resistance to tea tree oil and their resistance to antibiotics has no effect on the antibacterial properties of tea tree oil (Singh, 2016).

Along with their intended side effects, pharmaceuticals have side effects which are any effect that the drug has other than its intended use. According to the Mayo Clinic, there are around 60 common side effects linked to the use of ampicillin, come of which include vomiting, headaches, and fever. When compared to other topical ointments tea tree oil has shown to have much fewer side effects than pharmaceuticals (Arikekpar). Most side effects from essential oils are rash or skin irritation unless the patient has an allergy.

Although antibiotics have helped keep society healthy for decades there are downfalls to their use. With the emergence of drug resistant bacteria and the side effects associated with drugs many people are searching for alternate treatment methods. This study will compare the antibacterial properties of the essential oils tea tree and tea seed oil and compare those to the effects of the broad-spectrum antibiotic, ampicillin.

## MATERIALS AND METHODS

### Preparation of bacterial plates

Nutrient agar plates using 11.5 g of nutrient agar mixed with 500 mL of water. This was heated and mixed with a magnetic stir bar until translucent. Once heated this was then put in the autoclave and left at the maximum pressure for 15 minutes. Five tongs, a bacterial roller, the antibiotic discs, micropipette tips, and the beakers for the oils were sterilized in the autoclave as well. After cooling, about 20 mL of agar was poured into each petri dish and allowed to solidify. Plates were then placed in the refrigerator for storage until use.

### Preparation of working culture of *E. Coli*

A working culture of *E. coli* to be tested against the antibacterial agents was made by heating the opening of the stock *E. coli* and the nutrient broth tube then using a sterile, disposable transfer loop to scrape some of the bacteria off the stock colony. The loop was transferred with the bacteria into the nutrient broth and swirled to remove all bacteria from the transfer loop. The transfer loop was disposed of in the hazardous waste container and stored the working culture in an incubator at 37 ºC. 24 hours before the start of the antibacterial study another transfer loop was used to move *E. coli* to a new nutrient broth tube using the same method to grow the colony for study.

### Initial antibacterial study

This study compared the activity of two known antibiotics, ampicillin purchased from Fisher BioReagents, and amoxicillin purchased from Acros Organics and two essential oils, therapeutic grade tea tree oil purchased from Maple Holistics and cooking grade tea seed oil purchased from Arette, against *E. coli*. Cooking grade tea seed oil was purchased because therapeutic grade oil could not be found.

Twelve samples were tested for each antibacterial agent. There were three plates for each agent with four, six mm disks on each plate.

To spread the bacterial plates, 100 uL of *E. coli* were pipetted onto each plate using a micropipette with a sterile tip. The bacterial were spread using a roller to get an even lawn of growth. On the control discs 15 uL of DMSO was pipetted onto each of the twelve discs. On the discs containing tea tree and tea seed oil 15 uL of pure oil was pipetted onto each disc. The pure oil was used to determine if the oil was effective against the E. coli. For the ampicillin solution 0.0396 g of ampicillin was dissolved in 100 mL of DMSO. It is standard for 6 mm discs to hold 10 ug of ampicillin when being tested. For the amoxicillin solution 0.133 g of amoxicillin was dissolved in 100 mL of DMSO. 15 uL of each antibiotic was pipetted onto each disc. Using tongs, the discs were each placed into a quadrant of the corresponding plate and lightly pressed down to make sure they were stuck to the agar. After all discs are placed on the plate the bacteria were incubated for 24 hours at 37 ºC and then the zone of inhibition was measured.

**Tea tree oil activity study**

Based on the results of the results from the initial antibacterial study it was decided to further study the antibacterial activity of Tea tree oil at different concentrations for comparison to the pharmaceutical antibiotics. Since the tea seed oil did not showed to be ineffective against *E. coli* was no longer studied. To study the antibacterial activity of Tea tree Oil a series of dilutions to concentrations of 10%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, and 90% Tea tree oil in DMSO were made. The plates were prepared in the same way as the initial study. The plates were incubated for 24 hours and the zone of inhibition was measured.

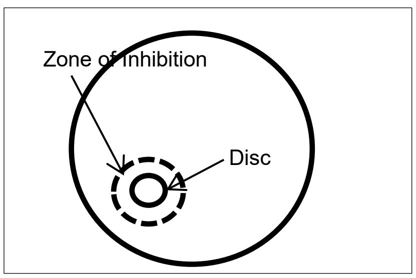


**Figure 1.** Zone of Inhibition- The dashed line represents the zone of inhibition which is the distance from the disk in which that antibacterial agent killed bacteria.

## RESULTS

The zone of inhibition was analyzed using the disc diffusion method as shown in Figure 1. The width of the zone of inhibition was considered as a measure of effectiveness of a test compound against *E. coli*.

The initial study showed tree oil to be completely effective against *E. coli* and it killed all the bacteria on the plate. It also showed that tea seed oil was completely ineffective and killed no bacteria on the plate. The average zone of inhibition of amoxicillin and ampicillin were measured from the initial study and considered as the basis to compare the antibacterial activity of tea tree oil. The average of the zone of inhibition of tea tree oil at different concentrations are shown in figure 2. The same graph shows the concentration of tea tree oil that gives the same antibacterial activity comparable to amoxicillin and ampicillin.

**Table 1.** Average Zone of Inhibition measured for each antibacterial agent and concentrations of tea tree oil.

| Antibacterial Agent | | Average Zone of Inhibition |
|---|---|---|
| **amoxicillin** | | 9.49 mm |
| **ampicillin** | | 7.87 mm |
| **tea tree oil** | | |
| | **100%** | Totally Effective |
| | **90%** | 9.49 mm |
| | **80%** | 8.20 mm |
| | **70%** | 6.63 mm |
| | **60%** | 4.93 mm |
| | **50%** | 4.00 mm |
| | **40%** | 3.34 mm |
| | **25%** | 1.43 mm |
| **tea seed oil** | | Totally Ineffective |

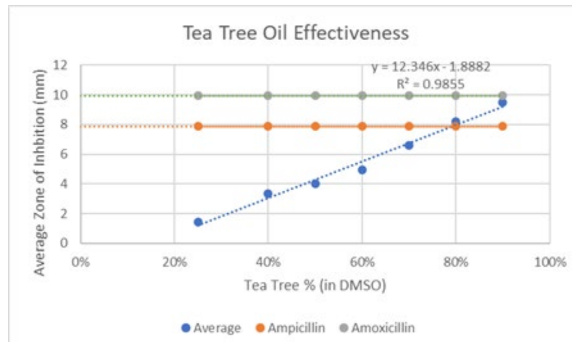


**Figure 2**. Average zone of inhibition measurements of tea tree oil compared to antibiotics

## DISCUSSION

The test for the control DMSO showed the solvent was totally ineffective against *E. coli*. The test for tea seed oil at 100% showed the oil to be totally ineffective against *E. coli*. The test for tea tree oil at 100% showed the oil to be highly effective and the plate was cleared of all bacteria. The tea tree oil was then tested at different concentrations to set a standard to compare the antibiotics with. The tea seed oil was determined to not be effective against *E. coli* at all.

The tea tree oil showed a linear correlation between greater concentrations and greater zone of inhibitions. Using this linear graph, the antibiotics were compared to the oil by substituting the average zone of inhibition for each into the equation of the trendline. This gave the percent tea tree oil that is equal to that zone of inhibition measurement. The ampicillin would be as effective as 76% tea tree oil. The amoxicillin would be as effective as 92% tea tree oil. Therefore, the ampicillin is comparable to tea tree oil, however, the amoxicillin is more effective than the tea tree oil at killing bacteria.

The exact mechanisms of the antibacterial properties of tea tree oil are still not known for certain. However, the mechanisms of both amoxicillin and ampicillin are known. Both drugs are penicillin type drugs. Drugs in this family contain 3- carbon 1 nitrogen containing rings with a carbonyl located on the ring.

These active sites in the drugs bind penicillin-binding protein which is an enzyme in bacteria which cross links peptides during peptidoglycan synthesis (Arikekpa, 2016). The enzyme activity is inhibited, and the cells undergo lysis without the peptidoglycan to provide structural support.

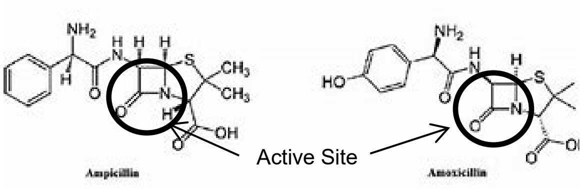


**Figure 3**. Structures of amoxicillin and ampicillin.

When the composition of tea tree oil was examined it was found to have about 20 components. Published literature states the major components are terpinen-4-ol at about 40%, Terpinene at about 23%, and Terpinene at about 10%. The individual structures of these components were also examined and there were no apparent similarities to the antibiotic's structures. So, it could be that instead of one active bactericidal component it could be multiple components working together to kill bacteria. Since tea tree oil is a mix of different active ingredients bacteria could find it harder to develop a resistance to tea tree oil as an antibiotic.

These findings suggest that in place of both amoxicillin and ampicillin, tea tree oil could offer a cheaper and over the counter option to some infections caused by *E. coli*. Further tests would be needed to determine how tea tree oil could be used in a medical setting. Also, the recommended use of tea tree oil is either aromatherapy or for topical use at a concentration at 1%. At this concentration this study suggests that tea tree oil would not be effective as an antibiotic in that way and further tests would need to be done to determine how to use tea tree oil effectively as a realistic alternative.

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