The anti-bacterial quality of Tea Tree and Eucalyptus essential oils as determined by zones of inhibition over time

Gavin Osborn

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Dr. Matthew Neatrour

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Abstract

Increased usage of alternative anti-microbial agents and their potential use in combatting antibiotic resistance prompted an investigation into one such medium, essential oils. We examined the anti-bacterial potential of two essential oils, Tea Tree and Eucalyptus. To do so we measured the zones of inhibition on plates populated with *Escherichia coli* after placing the experimental solutions on sterile discs and placing them onto the plate, with water and bleach as negative and positive controls respectively. We found that the mean difference between Tea tree and the negative control was significant, but not for Eucalyptus. Our results suggest that some essential oils may have anti-bacterial qualities due to their chemical composition.

Introduction

Essential oils are organic concentrates of plant oils known for their pleasant aromas, and natural medicines such as essential oils have been used increasingly as anti-bacterial agents due to the problem of antibiotic resistance (Cermelli et al. 2008). Previous studies have reported the anti-microbial properties of essential oils generally and reported positive effects related to their chemical composition (Sartorelli et al. 2007), and other studies have been conducted specifically to test various Eucalyptus species' oil activity against viral and fungal infections of humans (Ameur et al. 2012).

Tea tree oil, typically of the species *Melaleuca alternifolia* native to Queensland Australia, has long been used for commercial purposes. It is utilized for the treatment of skin conditions, and more recently as an anti-bacterial (Pazyar et al. 2013). Eucalyptus oil comes from a wider variety of species under the family *Myrtaceae*, also native to Australia, and chemical composition between species may differ (Ameur et al. 2012). The oil of Eucalyptus leaves is used similarly to that of Tea Tree, typically as a fragrance, food flavoring, and even as an insect repellent (Batish et al. 2008). Because essential oils generally have anti-microbial properties and the two particular essential oils studied here are used as anti-bacterial agents, we believe they are good model species to use for our purposes.

The wide variety of known uses for essential oils and the need for alternative anti-bacterial treatments prompted us to inquire into the efficacy of essential oils as an anti-bacterial agent. Discovery of anti-bacterial qualities, or the lack thereof, in Tea Tree and Eucalyptus oil will provide a more substantial background for the use of essential oils more generally, dispelling some of the confusion surrounding them. In our study we investigated whether Tea Tree oil and Eucalyptus oil exhibit anti-bacterial qualities, possibly because of the presence of

anti-bacterial compounds such as aldehydes and phenolics. To test our hypothesis, we measured the zones of inhibition on plates of *E. Coli* over a period of one week, controlling with water and the known disinfectant bleach. Our initial prediction was that our findings would align with previous research on the topic and provide support for essential oils as an anti-bacterial treatment; that is, the zones of inhibition would be similar to that of known disinfectants such as bleach.

Materials and Methods

Experimental Design & Procedure

We used LB plates populated with the bacteria *Escherichia coli* as a substrate for which the anti-bacterial substances could work upon. We placed the anti-bacterial agents on sterile discs in 100 µl amounts and put one of each onto the plates, which were separated into two halves; a control and experimental group. Each anti-bacterial, tea tree and eucalyptus, had three plates comparing the opposed essential oil, water with hydrogen peroxide, and bleach zones of inhibitions to its own. We did not use water alone in order to control for the hydrogen peroxide added to the essential oil mixtures. There were nine plates total for replication, with three instances of each group appearing three times across these plates.

Essential Oil Mixture

Our essential oils had to be diluted in order to make them appropriate for household use. Our process to make each oil oil was 1 teaspoon of the given oil, 1/3 cup + 1 teaspoon of hydrogen peroxide, and 13 oz distilled water. The oil was shaken prior to use to ensure distributed dissolution of the oil into the liquid.

Statistical Analysis

Each zone of inhibition was measured after one week, which we used to calculate the mean and standard error for each group. A one-way ANOVA test was conducted on the data to test for overall significance and a Tukey HSD test determined the significance of the data relative to each group. We used a p-value of 0.05 to indicate significance.

Results

In order to discover the efficacy of essential oils as an anti-microbial, we examined the zones of inhibition for each essential oil relative to bleach and water, measured on plates of bacteria *E. coli*. Our two experimental solutions were Tea Tree and Eucalyptus oil, and our positive control was bleach while the negative control was water with hydrogen peroxide. Proportional to bleach, Eucalyptus had a mean ZOI of 0.8x, Tea Tree 0.6x, and water 0.7x, over two weeks of incubation. The One-Way ANOVA indicated significance, but the Tukey HSD found significance only within Tea Tree/Water and Water/Bleach ($p = 0.018$, std.dev = 0.5, std.err = 0.16, Table 1). Overall, Eucalyptus and Bleach exhibited the largest mean ZOI (Figure 1). These data suggest Eucalyptus oils show some signs of anti-bacterial qualities, but tea tree was not significantly different from the negative control.

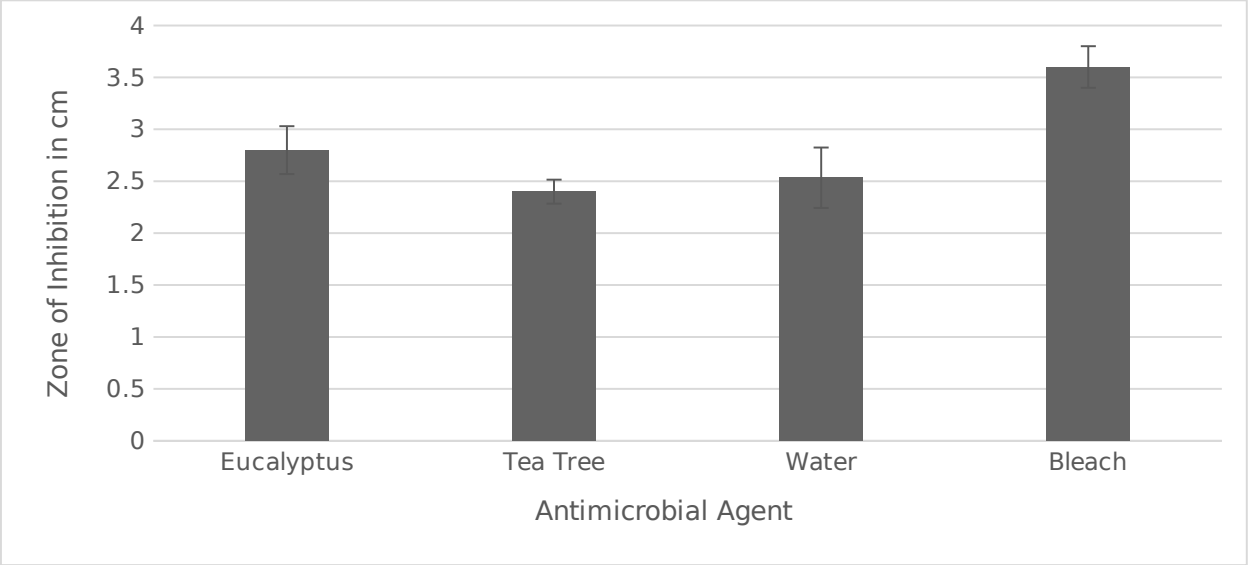

Figure 1. Eucalyptus and Tea Tree Oil zones of inhibition compared to water and bleach. Measured after one week of bacterial growth. Values are means and error bars represent standard error. Significance was found in water against bleach and tea tree against bleach.

P-Value	0.018724
Std. Dev	0.5836
Std. Err.	0.1685
Eucalyptus vs Tea Tree	Insignificant
Eucalyptus vs Water	Insignificant
Eucalyptus vs Bleach	Insignificant
Tea Tree vs Water	Insignificant
Tea Tree vs Bleach	Significant
Water vs Bleach	Significant

Table 1. One-Way ANOVA and Tukey HSD results. The first three rows are from the ANOVA, the rest are tests of significance from the Tukey HSD. Only Tea Tree against bleach and water against bleach were measured to have significance.

Discussion

We measured the zones of inhibition across several bacterial plates for four different groups, Tea Tree Oil, Eucalyptus Oil, water with hydrogen peroxide, and bleach. This was to determine whether they had significant anti-bacterial qualities for which we could pose the question of whether this was due to the chemical composition of these oils. Our results partially supported our hypothesis. Eucalyptus oil exhibited a ZOI that was insignificant from bleach, indicating anti-bacterial qualities, but was also insignificant from water and Tea Tree oil. Tea Tree oil exhibited results similar to water. These results suggest Eucalyptus oil to have moderate anti-bacterial qualities, possibly due to its chemical composition.

Previous studies have measured the anti-bacterial effects of Eucalyptus and Tea Tree oil. Ameur et al. (2021) measured eight different species for their antibacterial efficacy and found significance to differ between species used (2012). In a study by Widiyastuti et al. (2022), the anti-bacterial qualities of Tea Tree Oil differed by extraction method for the acquisition of the oil. Notably, their results for the antibacterial quality differed from our own, with their data indicating antibacterial qualities. This suggests a possible flaw on our part.

Our results may have been from our solution process. An inappropriate amount of ethanol may have been added, or too much water diluting the solution. Importantly, this could've made our water solution far more effective than it should be relative to the essential oils themselves. Another limitation of our study may have been that we do not know the species of either oil that we use, with both Tea Tree and Eucalyptus anti-microbial efficacy varying between species (Noumi, et al., 2011). In the future, we suggest that more research be done that combines different essential oils and more adeptly dilutes the essential oil solutions. Previous

studies, such as that of de Almeida et al., (2023) have done something similar to this in which they found synergistic effects when combining two or more essential oils.

We found that, generally, Eucalyptus oil can function as an anti-bacterial. Tea Tree Oil may also serve this purpose but has a chemical composition that necessitates a different solution recipe than what we used, or varies by species. Our findings are valuable to researchers that want to find alternatives to current antibacterial treatments given the rise of antibiotic resistance among bacteria species, or for the proper health guidance of the commercial anti-bacterial industry.

References

Ameur, E., Sarra, M., Yosra, D., Mariem, K., Nabil, A., Lynen, F., & Larbi, K. M. (2021). Chemical composition of essential oils of eight Tunisian Eucalyptus species and their antibacterial activity against strains responsible for otitis. *BMC Complementary Medicine & Therapies*, *21*(1), 1. https://doi-org.xavier.idm.oclc.org/10.1186/s12906-021-03412-0

Batish, D. R., Singh, H. P., Kohli, R. K., & Kaur, S. (2008). Eucalyptus essential oil as a natural pesticide. *Forest Ecology & Management*, *256*(12), 2166–2174. https://doi-org.xavier.idm.oclc.org/10.1016/j.foreco.2008.08.008

Cermelli, C., Fabio, A., Fabio, G., & Quaglio, P. (2008). Effect of eucalyptus essential oil on respiratory bacteria and viruses. *Current Microbiology*, *56*(1), 89–92. https://doi-org.xavier.idm.oclc.org/10.1007/s00284-007-9045-0

De Almeida, J. M., Lourenço Crippa, B., Vilaça Martins Alencar de Souza, V., Pereira Perez Alonso, V., da Motta Santos Júnior, E., Siqueira Franco Picone, C., Silvia Prata, A., Cristina Cirone Silva, N. (2023). Antimicrobial action of Oregano, Thyme, Clove, Cinnamon and Black pepper essential oils free and encapsulated against foodborne pathogens. Science Direct. https://www.sciencedirect.com/science/article/pii/S0956713522005497

Noumi, E., Snoussi, M., Hajlaoui, H., Trabelsi, N., Ksouri, R., Valentin, E., & Bakhrouf, A. (2011). Chemical composition, antioxidant and antifungal potential of Melaleuca alternifolia (tea tree) and Eucalyptus globulus essential oils against oral Candida species. *Journal of Medicinal Plants Research*, *5*(17), 4147–4156.

https://search.ebscohost.com/login.aspx?direct=true&db=edswsc&AN=000297465300022&site=eds-live&scope=site

Pazyar, N., Yaghoobi, R., Bagherani, N., & Kazerouni, A. (2013). A review of applications of tea tree oil in dermatology. *International Journal of Dermatology*, *52*(7), 784–790. https://doi-org.xavier.idm.oclc.org/10.1111/j.1365-4632.2012.05654.x.

Sartorelli P, Marquioreto AD, Amaral-Baroli A, Lima MEL, & Moreno PRH. (2007). Chemical composition and antimicrobial activity of the essential oils from two species of Eucalyptus. *Phytotherapy Research*, *21*(3), 231–233. https://doi-org.xavier.idm.oclc.org/10.1002/ptr.2051

Widiyastuti, Y., Matoha, H. K., & Fitriana, F. (2022). Antibacterial Activity of Melaleuca alternifolia Extract from Different Extraction Method. *Biosaintifika: Journal of Biology & Biology Education*, *14*(2), 285–292. https://doi-org.xavier.idm.oclc.org/10.15294/biosaintifika.v14i2.36223