



## **Antimicrobial action of tea tree oil (*Melaleuca alternifolia*) on five common bacteria**

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**Abstract**

Microorganisms are ubiquitous in every habitat on earth. Some can be pathogenic and the need for controlling them is a topic of immense research. Tea tree oil (*Melaleuca alternifolia*) is known to have antibacterial effects and in this experiment we tested its abilities to control the growth of five bacteria *Bacillus subtilis*, *Escherichia coli*, *Micrococcus roseus*, *Sarcina luteus*, and *Serratia marcescens*. We also compared the antimicrobial effectiveness of fresh garlic (*Allium sativum*), an industrial cleaner and deodorizer Quad 10, and mouthwash Listerine a brand made by McNeil-PPC, Inc., contiguously with tea tree oil. Previous research lacks data as to how tea tree oil compares in its antimicrobial action to other commonly used antibacterial solutions, such as those tested in this experiment. We used a bacterial lawn technique on agar plates for each tested bacterium. Small disks of each of the antimicrobial were placed on plates of each strain with enough area surrounding each to calculate inhibitory effects. After a week of incubation, we determined their antimicrobial efficacy by measuring the zone of inhibition surrounding each sample. We report that tea tree oil had the best antibacterial action of the strains tested due to the clear zone of inhibition measured. The effectiveness of tea tree oil in controlling bacteria may have extensive application for common cleaning products to possibly inhibit bacteria growth on many surfaces. Our experiment reports tea tree oil's effectiveness inhibiting growth of several different bacteria, and because tea tree oil is a safe and sustainable material to use, its use as a common cleaning agent deserves further analysis.

## Introduction

Microorganisms exist ubiquitously around and inside us and we are bombarded with them from the moment we are born. They live in our bodies, our skin, our pets, food, furniture and some thrive in the air we breathe. They exist in every habitat on earth. Many are critical to human survival and some are pathogenic. Bacteria are unicellular prokaryotic microorganisms that reproduce by binary fission asexually, and may form colonies. Bacteria strains tested in this experiment include both gram positive and gram negative. We hoped to find a solution that would both inhibit bacteria growth and that is also safe and sustainable, suitable for common household use.

The bacterial strains tested in this experiment vary in their toxicity to humans and some have beneficial common uses. *Bacillus subtilis* for instance is not considered pathogenic and has many uses such as soil enhancement, food additives and alternative medicine. *Serratia marcescens* and *Escherichia coli*, both with a ubiquitous presence in our environments, can be pathogenic in some cases (Singleton 1999). These bacteria and many others that may be pathogenic require the use of antimicrobials to control their growth to inhibit infection.

Tea tree oil is extracted from the tree *Melaleuca alternifolia* that grows in Australia, and has been shown to have many beneficial medicinal uses as an antiseptic, antifungal and antibacterial agent (Carson and Riley 1995). Indigenous people of Australia have used it to treat coughs, colds, sore throats and skin ailments (Carson et al. 2006). Carson and Riley (1995) indicate that *Melaleuca alternifolia* is extracted from the leaves and twigs by steam distillation and the yield is about 1.8% and that the main chemical

component to have antimicrobial activity in tea tree oil is attributed to terpinen-4-ol. If our experiment shows tea tree oil's effectiveness as an antimicrobial agent, it would be suitable in many cleaning products and without adverse health or environmental effects.

Tea tree oil has been shown to inhibit cellular respiration in *E. coli*, and by disrupting the permeability barrier of microbial membranes the oil causes the cells to die (Cox et al. 1998). De Prijk et al. (2008) indicated death of *E. coli*, *Proteus mirabilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* after exposure to a mixture of tea tree oil and jojoba oil. We expected the same results with *E. coli* but few experiments have been performed for the other bacteria we tested, nor its comparison to the antibacterial/disinfectants used in this experiment.

Quad 10 is an industrial cleaner, disinfectant and deodorizer, which we expect to have equal or greater antibacterial effects than tea tree oil due to its popular use as an industrial cleaning solution. The active ingredient is N-alkyl dimethyl benzyl ammonium chloride, but safety precautions are recommended when using this product (MSDS sheet). Unfortunately Quad 10 and many commonly antimicrobial products used today, such as bleach, can be harmful to use, inhale or dangerous to the environment. Listerine manufactured by McNeil-PPC, Inc., is a popular mouthwash brand that claims to "kill germs that cause bad breath", however the ethanol content of Listerine (21% to 26%) is controversial and argued as being unsafe (Lachenmeier 2008). Garlic (*Allium sativum*) has been found to possess substantial and broad-spectrum antibacterial activity (Ross et al. 2001). We anticipated that garlic will have some effect on bacteria inhibition and that it is certainly a safe material to use.

The purpose of this experiment was to determine if tea tree oil is effective in controlling many bacteria rather than merely a few, and how its antimicrobial action

compares to other products that are commonly used. We hypothesized that tea tree oil would not be as effective as other commonly used antibacterial/disinfectants, but that it would have some inhibitory action on at least a few of the bacteria strains tested. We expected to find minimal zone of inhibition with tea tree oil and that Quad 10 would measure the clearest zone of inhibition based on the experiments by Carson (1995) and the common industrial use of Quad 10 as an disinfectant.

Our testing method was a bacterial lawn technique on an agar plate with samples of each of the antimicrobial placed with a few clear centimeters surrounding each sample. After an incubation period of 48 hrs we measured the zone of inhibition. Any product tested that did not create a zone of inhibition we considered null. If we determined that tea tree oil was at least effective in controlling all these bacteria in our test strains and that it was at least equal to the effectiveness of Quad 10 and Listerine, then it would certainly make a safe and sustainable antimicrobial for common use.

### **Materials and Methods**

Procedures to prepare bacterial lawns on agar plates, placement of antiseptic/disinfectant samples, incubation temperature and duration, and methods for measurement of the zone of inhibition by Morgan and Carter (2008) were followed for this experiment. Lawn cultures were created by applying swabs dipped in broth cultures of the bacteria over the entire surface of agar plates in a cross hatch pattern of five neutralized bacteria: *Bacillus subtilis*, *Escherichia coli*, *Micrococcus roseus*, *Sarcina luteus*, and *Serratia marcescens*. Small discs approximately 3.0 mm dia. soaked in antibacterial/disinfectants solutions and sterilized water (the control), were placed on

each plate with at least 2.0 cm clearance between discs to avoid confusion in zone of inhibitions. A small piece of fresh garlic purchased from a local grocer was used rather than a disc to test its inhibitory efficacy. Sanitary procedures such as sterile gloves and flame sterilized forceps were used throughout to avoid any contamination with materials or other environmental bacteria as much as possible.

The antibacterial/disinfectants used were Desert Essence Inc. 100% Pure Australian Tea Tree Oil (*Melaleuca alternifolia*), Majestic Foods Inc. fresh garlic clove (*Allium sativum*), W.W. Grainger Quad 10 an industrial cleaner and deodorizer, and McNeil-PPC, Inc. Listerine a common brand of mouthwash. The prepared plates of *Micrococcus r.*, *Sarcina l.* and *Serratia m.* were incubated at 28 C for 48 hours. The *E. coli* and *Bacillus s.* were incubated at 36 C for 48 hours. The zone of inhibition was measured for each of the antibacterial/disinfectant and recorded.

## Results

**Table 1** | Measured zone of inhibition for five bacteria and four known antimicrobials. Solid circle represents the zone of inhibition measured more than one centimeter from sample of antiseptic, disinfectant or control. An open circle indicates that the zone of inhibition was less than one centimeter.

Bacteria	Sterilized Water (control)	Tea Tree Oil ( <i>Melaleuca alternifolia</i> )	Fresh Garlic ( <i>Allium sativum</i> )	Quad 10	Listerine
<i>Bacillus Sutilis</i>	○ > 1 cm	● > 1 cm	○ > 1 cm	○ > 1 cm	○ > 1 cm
<i>Escherichia Coli</i>	○ > 1 cm	● > 1 cm	○ > 1 cm	○ > 1 cm	○ > 1 cm
<i>Micrococcus Roseus</i>	○ > 1 cm	● > 1 cm	○ > 1 cm	● > 1 cm	○ > 1 cm
<i>Sarcina Luteus</i>	○ > 1 cm	○ > 1 cm	○ > 1 cm	○ > 1 cm	○ > 1 cm
<i>Serratia Marcescens</i>	○ > 1 cm	● > 1 cm	○ > 1 cm	○ > 1 cm	○ > 1 cm

○ > 1 cm = zone of inhibition null and less than 1 cm

● > 1 cm = zone of inhibition greater than 1 cm, showed success of controlling the bacteria

Tea tree oil showed the most consistent inhibitory action with all bacteria, except *Sarcina luteus*, controlled to at least one centimeter radius when measured from the edge of the disk solution (Table 1). Fresh garlic and Listerine had no effect on controlling the bacteria as they showed no or little zone of inhibition (Table 1). Garlic showed slight control of *B. subtilis* and *M. roseus* but was less than 1 cm. Listerine showed no zone of inhibition for any bacteria tested. Quad 10 controlled only the growth of *M. roseus* to more than one centimeter (Table 1). Sterilized water, the control, showed no activity on controlling any of the bacteria as no zone of inhibition was observed (Table 1).

### **Discussion**

Several experiments show success of the antimicrobial action of tea tree oil, (Carson et al. 2006 Carson and Riley 1995, Cox et al. 1998, De Prijck et al. 2008) but few have tested on all the strains of bacteria used in this experiment or have compared their effectiveness to common household products. Tests have shown how tea tree oil is capable of controlling growth of *E. coli*. In an experiment by Cox et al. (1998) the antimicrobial action of tea tree oil to control the growth of *E. coli* can be attributed to its ability to inhibit cellular respiration and cause leakage of cellular potassium. Tea tree oil has shown inhibitory effects on bacteria with *E. coli* (Carson et al. 2006, Carson and Riley 1995, Cox et al. 1998), and our experiment tested its antimicrobial action on other bacteria but our experiment also determined how tea tree oil compared to other common products thought to control bacteria.

Our hypothesis that tea tree oil would not be as effective as other commonly used antibacterial/disinfectants, was based on the limited product options that use tea tree oil as an active cleaning ingredient. We expected to find minimal zone of inhibition with tea

tree oil and that Quad 10 would have the clearest zone of inhibition but we were incorrect in this prediction. Tea tree oil was not predicted to control all the bacteria tested, nor be superior to Quad 10 or Listerine. The results indicated that the other products showed no or little zone of inhibition on the agar plates tested. Tea tree oil was shown to be more effective in its antibacterial actions even to Quad 10, which is an industrial cleaner and unlike tea tree oil has some safety precautions for its use (MSDS sheet). Compared to the other products tested, tea tree oil far exceeded our expectations.

Listerine, that claims to “kill germs that cause bad breath”, was expected to show some success in controlling the bacteria examined but showed no success and all tests were null and equal in effect to sterilized water and garlic. Garlic was expected to show some inhibiting behavior as Ross et al. (2001) showed that both gram positive and gram negative bacteria were susceptible to garlic oil and powder. Why our fresh garlic sample showed no antimicrobial action was unclear. Further tests would include other forms of garlic as it would be a safe and sustainable ingredient should it show success as an antimicrobial agent. Another possible future experiment would test tea tree oil in comparison to Listerine on any bacteria that cause bad breath, although many other hygiene regimens, such as lack of regular brushing and flossing, likely cause bad breath rather than the existence of “germs”.

Tea tree oil was shown to inhibit growth of all five common bacteria tested, except *Sarcina luteus*, and showed superior results to Quad 10, garlic and Listerine for all others. The zone of inhibition for tea tree oil on *S. luteus* showed a radius slightly less than 1 cm extension from the tea tree oil disc sample. Beyond tea tree oil’s current application in specialty household cleaning agents, based on the results of this experiment, its use for a broader spectrum of readily available antimicrobial agents such as easily attainable

products in common grocery stores and even for industrial uses should be considered. With more tests in comparison to other commonly used products tea tree oil may be a viable and sustainable ingredient for this purpose.

Future tests would examine its behavior of cellular damage on healthy bacteria along side *E. coli* and other possible pathogens in a mutual ecosystem. Bacteria exist in complex communities interacting with other organisms and rarely exist on their own (Zinder and Salyers 2001). Although helpful in our understanding of bacteria growth inhibition, the controlled environment of a monoculture as produced in this experiment does not test the effectiveness of tea tree oil in controlling these bacteria in real world habitats and deserves further testing. Tea tree oil should be tested continuously on the bacteria it sets to control as all living things evolve and bacteria is not immune. The high price of industrial cleaners warrant more research as to their effectiveness as a quality disinfectant in comparison to other safe and sustainable products. Overuse of antibacterial and disinfectants should be of serious consideration due to the inherent evolution of all living organisms and tea tree oil's actions on controlling bacteria deserves further testing in this inherent evolution.

## References

- Carson, C.F., Hammer, A., Riley, T.V. (2006). *Melaleuca alternifolia* (Tea Tree) Oil: a Review of Antimicrobial and Other Medicinal Properties. *Clinical Microbiology Reviews*, Jan. 2006, p. 50–62.
- Carson, C.F. and Riley, T.V. (1995). Antimicrobial activity of the major components of the essential oil of *Melaleuca alternifolia*.. *Journal of applied bacteriology*. 78 (3), 264-269.
- Cox, S.D., Gustafson, J.E., Mann, C.M., Markham, J.L., Liew, Y.C., Hartland, R.P., Bell, H.C., Warminton, J.R. and Wyllie, S.G. (1998) Tea tree oil causes K<sup>+</sup> leakage and inhibits respiration in *Escherichia coli*. *Letters in Applied Microbiology* 78, 264-269.
- De Prijk, K., Peeters, E., & Nelis, H. (2008). Comparison of solid-phase cytometry and the plate count method for the evaluation of the survival of bacteria in pharmaceutical oils. *Letters in Applied Microbiology*, 47(6), 571-573. doi:10.1111/j.1472-765X.2008.02464.x.
- Lachenmeier, D.W. (2008). Safety evaluation of topical applications of ethanol on the skin and inside the oral cavity. *Journal of Occupational Medicine and Toxicology* 2008, 3:26doi:10.1186/1745-6673-3-26.
- Morgan, J. and Carter, MEB. (2008). *Investigating Biology Laboratory Manual*, 6<sup>th</sup> ed. San Francisco, CA: Pearson Benjamin Cummings Publishers.
- Ross, Z.M., O'Gara, E.A., Hill, D.J., Sleightholme, H.V., & Maslin, D.J. (2001). Antimicrobial properties of garlic oil against human enteric bacteria: evaluation of methodologies and comparisons with garlic oil sulfides and garlic powder. *Applied and Environmental Microbiology*. 67 (1), 475-80.
- Singleton, P. (1999). *Bacteria in Biology, Biotechnology and Medicine*, 5<sup>th</sup> ed. England. John Wiley & Sons Ltd.
- Zinder, S.H and Salyers, A.A. (2001). Microbial Ecology-New Directions, New Importance. In Boone, D.R., Castenholz, R.W. (Eds.), *Bergey's Manual of Systematic Biology* (Vol. 1 101-109). New York, NY: Springer-Verlag.