An Investigation of the Antimicrobial Resistance Patterns of *E. coli* to Essential Oils

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Abstract

Antibiotics are known for their lifesaving ability: attacking and killing the bacteria that cause dangerous diseases in humans. One complication with antibiotics is bacteria's ability to develop resistance and continue surviving in the body. This investigation focuses on essential oils (EOs) and tests whether the same resistance patterns are observed. Essential oils were selected for this investigation because of their disinfecting properties and possible uses throughout the home, in food preservation, and in medicinal applications. Our work focused on four oils: oregano, peppermint, cinnamon, and a disinfecting oil blend. Each oil was purchased from two different vendors, Young Living and Plant Therapy, at two drastically different price points. The research included verifying that the oils could kill *E. coli (*ATCC 25922) and testing this bacterium's ability to resist them. The minimum inhibitory concentration (MIC) values determined for the oils, with the exception of peppermint which was 2.5 µL/mL for Young Living and 1.2 µL/mL for Plant Therapy, did not differ between brands. The MIC of the cinnamon oils was 0.3 µL/mL and the MICs for the remaining oils were 1.2 µL/mL. Resistance propagations conducted over five days for the Young Living oils indicated that bacteria gained resistance and tolerated higher concentrations of peppermint oil before becoming stagnant, while the bacteria took multiple days to resist the oregano oil, and never grew past the highest tolerable concentration of cinnamon oil or Thieves (blend) oil.

Introduction

- Antibiotics work through various mechanisms, including interference with bacterial ribosomes, inhibition of bacterial DNA/RNA synthesis, and inhibition of cell wall synthesis. However, no antibiotic type or mode of action has been able to avoid the reality of bacterial resistance
- Essential oils (EOs) are oils extracted from various plants through steam distillation, and are used most notably in this context for their ability to limit the growth of microorganisms
- Although essential oils have been compared to and utilized alongside antibiotics in several studies, relatively little is known about whether bacteria develop resistance to EOs
- For this study, we investigated the antimicrobial and bacterial resistance patterns of eight essential oils that were marketed as either "disinfecting" or "cleaners". We purchased four oils from Young Living, a more expensive option, and the same four from Plant Therapy, which sells oils for a more competitive price.

Results

Essential Oil	*Young Living MIC (µL/mL)*	*Plant Therapy MIC (µL/mL)*	*Young Living Price per mL*	*Plant Therapy Price per mL*
Oregano	1.2	1.2	$1.90	$1.20
Peppermint	2.5	1.2	$1.47	$0.43
Cinnamon	0.3	0.3	$4.95	$1.39
Blend	1.2	1.2	$2.32	$0.49

Table 1. Minimum inhibitory concentration (MIC) values were determined by optical density and the observation of a visible pellet after centrifugation. All tests were done in triplicate and determined that, apart from peppermint, the oils performed similarly regardless of vendor and price.

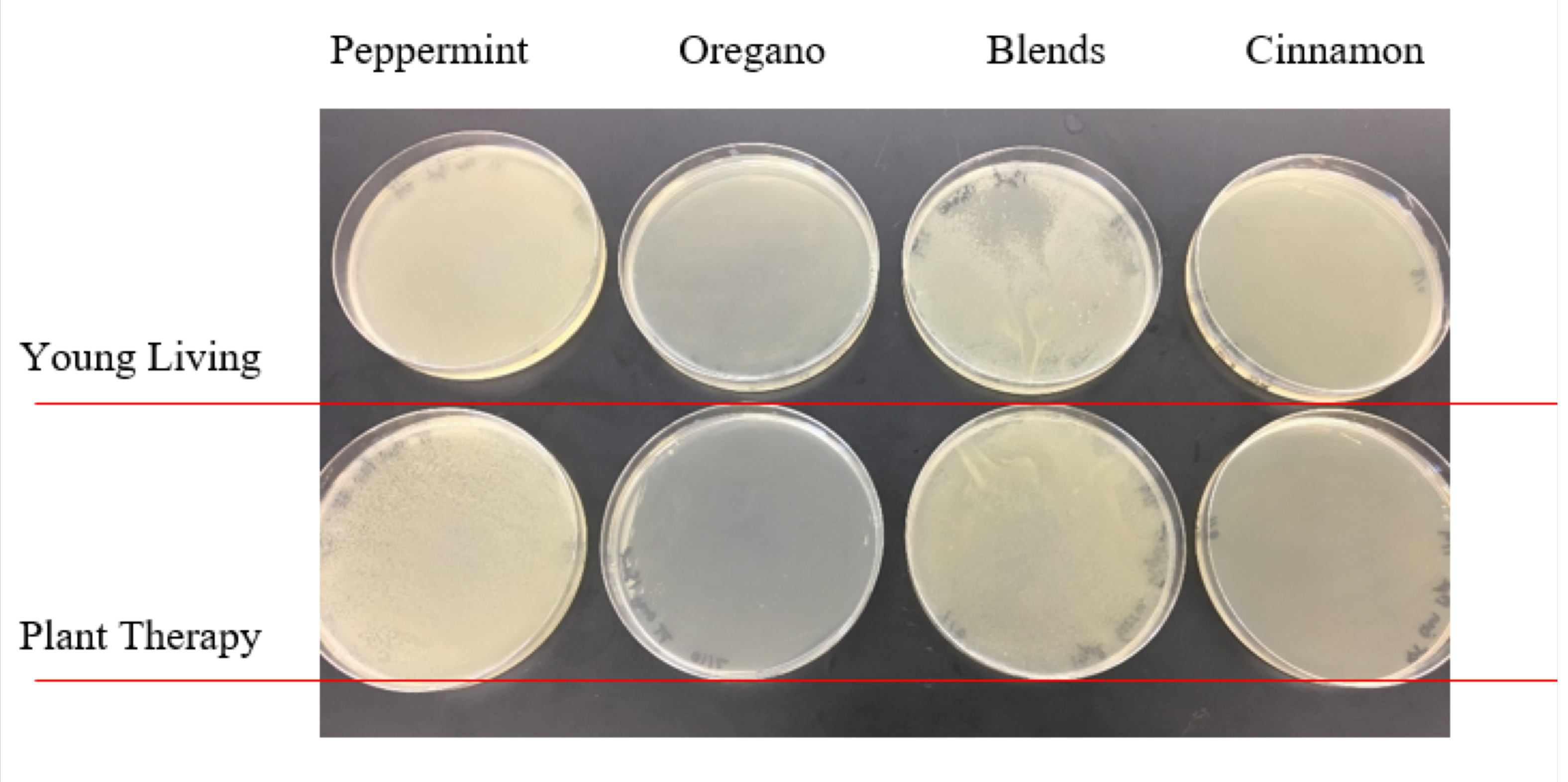

Figure 1. Essential oils (15 µL) were plated first, followed by E. coli, and the bacteria was allowed to grow overnight at 37 °C. Plates with peppermint and multi-oil blends had lawns of growth, while those with oregano or cinnamon oils had no visible growth.

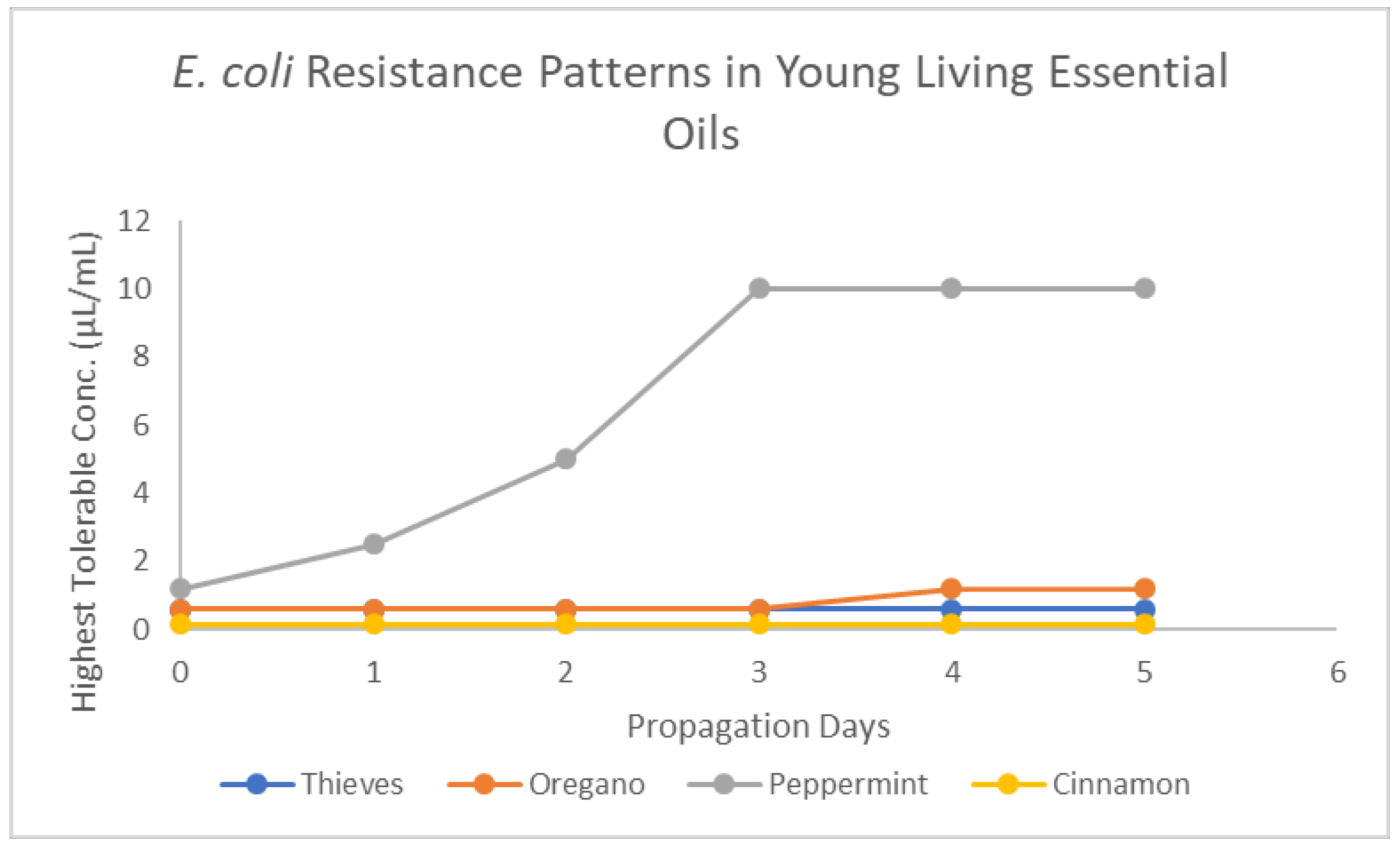

Figure 2. E. coli resistance patterns were tested for all four Young Living essential oils, and found that, while E. coli did develop some resistance to peppermint oil, the E. coli struggled to resist the other oils. E. coli developed no resistance to Thieves or cinnamon oils over the five days of testing and took 4 days of propagations to develop slight resistance to the oregano oil.

Methods

- Growth medium was prepared by pipetting 1.8 mL of Difco™ LB Broth into each tube and adding 200 µL of 10% Tween® 80, resulting in a 1% Tween solution to aid in solubility
- The desired amount of essential oil was added directly into each tube
- A wild-type *E. coli* sample was grown from a frozen stock and diluted with LB to an optical density of 0.1 using the ThermoSpectronic Genesys 20 spectrophotometer, 600 nm
- From this diluted sample, 20 µL (10^6 CFU) were pipetted into each tube and grown overnight (~18 hours, shaking water bath at 37 °C)
- When the essential oil/ Tween mixture caused the solutions to be cloudy, 100 µL of the culture was pipetted into an Eppendorf tube along with 400 µL ethanol to solubilize the oil. It was then centrifuged, and growth determination was based on the presence or absence of a round, solid pellet

Conclusion

- Essential oils were approximately three orders of magnitude less potent than traditional antibiotics
- Plating experiments showed that some oils are more effective than others at killing *E. coli* growth on a surface (Figure 1)
- Acquiring resistance to EOs was uncommon and usually took multiple rounds of exposure, if it did occur. (Figure 2)
  - Bacteria initially resisted the peppermint oil, but were unable to grow beyond 10 µl/mL
  - Bacteria eventually gained slight resistance to the oregano oil, but required four passes to do so
  - The other two Young Living oils did not allow the *E. coli* to jump beyond the initial highest tolerable concentration (HTC) over five consecutive passes

Acknowledgements

I would like to acknowledge the Virginia Ellis Franta Endowment Fund as well as the entire Wittenberg Chemistry Department for making my research possible.

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Celebration of Learning, Wittenberg University, April 2020