Essential oils from thyme and rosemary in combination as an antibiotic-sparing agent to treat *Escherichia coli*-caused urinary tract infections

Mallika Subramanian

Abstract

Antimicrobial resistance (AMR) is one of the largest healthcare emergencies today, with urinary tract infections (UTIs) caused by antibiotic-resistant uropathogenic *Escherichia coli* (*E.coli*) (UPEC) as a critical offender. The development of biofilms, matrices of UPEC, further complicate treatment. Antibiotics struggle to pierce the biofilm, leading to longer infections and antibiotic use, increasing AMR. Little has been done to identify efficient UTI treatments which inhibit both bacterial and biofilm growth. Previous research demonstrates that essential oils (EOs), especially *T.zygis* (thyme) and *R.officinalis* (rosemary) EOs, can combat bacterial growth as effectively as antibiotics, as well as inhibit biofilm growth. EO combinations have shown enhanced antibacterial and antibiofilm activity over individual EOs, but ratios of EOs in combination have not been optimized. *T.zygis* and *R.officinalis* EOs were tested on *E.coli* in different ratios to establish an optimal EO combination to inhibit bacterial and biofilm activity. Agar disk diffusion evaluated antibacterial activity(n=2) and a colony forming unit/mL assay measured antibiofilm activity(n=30). 100% *T.zygis*, 0% *R.officinalis* and 90% *T.zygis*, 10% *R.officinalis* had the highest antibacterial activities, with similar activity to ciprofloxacin control. 90% *T.zygis*, 10% *R.officinalis* and 60% *T.zygis*, 40% *R.officinalis* had the highest antibiofilm activities, with inhibition levels of 80.89% and 80.09%, respectively. 90% *T.zygis*, 10% *R.officinalis* was more effective than *T.zygis* (70.87% inhibition;p=0.03034) or *R.officinalis* (37.95% inhibition;p=0.0011) alone, and the most effective treatment overall. These results could indicate EO combinations for utilization in alternative antibiotic-sparing treatments for UPEC-caused UTIs.

Introduction

Antimicrobial Resistance

- Antimicrobial resistance (AMR) is one of the biggest healthcare emergencies today[10]

UPEC Resistance

- Urinary tract infections (UTIs) affect ~ 274 million people annually worldwide[4]
- Uropathogenic *E. coli* (UPEC) causes 75% of uncomplicated UTIs and 65% of complicated UTIs[4]
- UPEC resistance has increased against common antibiotics ranging from 37-72% of all UPEC isolates (Table 1)
- Biofilms, bacterial cells in polysaccharide matrices, are a large contributor to resistance[4] because antibiotics do not effectively kill biofilms[8]
- New treatments to combat both biofilm and bacterial growth are needed

Previous Research

- *T.zygis*(T) and *R. officinalis*(R) essential oils (EOs) have been proven to inhibit bacterial and biofilm growth of UPEC[6]
- Optimal ratio of combinations of these EOs is unknown
- This study evaluated the effects of varied ratios of *T. zygis* and *R. officinalis* EOs on inhibiting *E. coli* growth and biofilm formation

Antibiotic	*E. coli* Resistance (%)
Amoxicillin	72%
Ciprofloxacin	8%-65%
Co-trimoxazole	63%
Ampicillin/sulbactam	> 50%
Levofloxacin	38.3%
Doxycycline	37.5%

Table 1. Extremely high E. coli resistance rates to common antibiotics.[2,3,5,7,9]

	Ciprofloxacin (+ control)	Penicillin (- control)	*T. zygis*	*R. officinalis*
Group 1	✓			
Group 2		✓		
Group 3			0%	100%
Groups 4-12			Increase in 10% increments	Decrease in 10% increments
Group 13			100%	0%

Table 2. Treatment groups and controls-percentage by volume of essential oil.

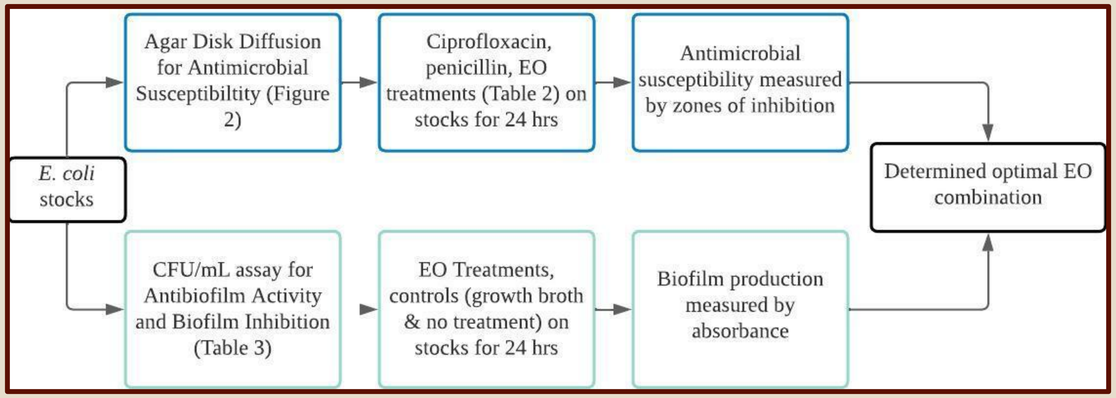

Figure 1. Study design - using 2 different experiments to find optimal combination.

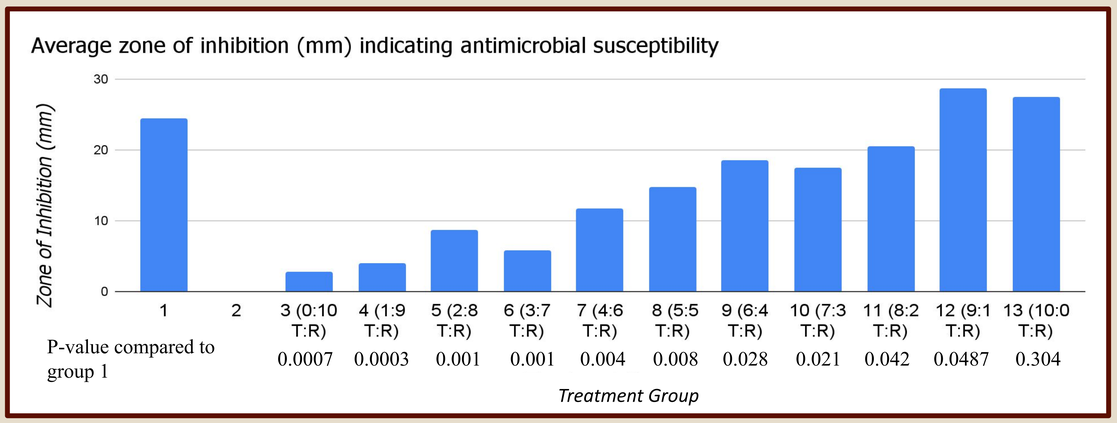

Figure 2. Effectiveness of inhibiting bacterial growth.

	Treatment										
	3 (0:10 T:R)	4 (1:9 T:R)	5 (2:8 T:R)	6 (3:7 T:R)	7 (4:6 T:R)	8 (5:5 T:R)	9 (6:4 T:R)	10 (7:3 T:R)	11 (8:2 T:R)	12 (9:1 T:R)	13 (10:0 T:R)
% Inhibition (CFU/mL)	37.95	49.61	40.63	75.79	50.99	44.68	80.09	67.78	63.08	80.89	70.87
Standard Deviation	65.88	24.93	79.74	49.98	70.99	88.21	9.24	23.80	47.67	8.47	23.29
P-value compared to group 13	0.013	0.001	0.050	0.622	0.147	0.119	0.047	0.607	0.418	0.030	N/A

Table 3. Effectiveness of inhibiting biofilm formation.

Methods

- Varied combinations of EOs (Table 2) were tested on non-pathogenic *E. coli* with 2 different experiments (Figure 1)
- Bacterial growth inhibition was measured through a disk diffusion assay (n=2) [1] → quantified by inhibition zones
- Biofilm inhibition was measured through a crystal violet colony forming unit/mL assay (n=30)[11] → quantified by optical density
- Formula used to calculate % biofilm inhibition:
  % Inhibition = 100 - [(absorbance of sample/ absorbance of control) * 100]
- No ethical issues because *E. coli* in this study is non-pathogenic

Optimization

- Analyzed for combination with optimal efficacy
- Determined type of interaction (synergistic, additive, antagonistic, or indifferent effect)

Statistical Analysis

- 2-sample t-tests used to confirm significant differences in antibacterial activities between ciprofloxacin control and other treatments
- 2-sample t-tests used to confirm significant differences in antibiofilm activities between *100% R. officinalis, 0% T. zygis* and other treatments

Results

- 100% *T. zygis*, 10% *R. officinalis* and 90% *T. zygis*, 10% *R. officinalis* had the highest antibacterial activities, similar to ciprofloxacin control (Figure 2)
- 90% *T. zygis*, 10% *R. officinalis* and 60% *T. zygis*, 40% *R. officinalis* had the highest antibiofilm activities (Table 3)

Conclusion

- Optimal combination was **90% *T.zygis*, 10% *R. officinalis*** because it was the intersection between the combinations with highest antibacterial and antibiofilm activities
- *T. zygis* and *R.officinalis* enhance each others effects, but do not reach an additive or synergistic interaction

Implications

- Hinted to effects of interaction between *T. zygis* and *R. officinalis* EOs - further research is needed
- EO combinations have potential as part of alternative antibiotic-sparing therapies that inhibit both bacterial and biofilm growth

References

[1] Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016, 04). *Journal of Pharmaceutical Analysis*, *6*. NCBI. 10.1016/j.jpha.2015.11.005
[2] Bryce, A., Costelloe, C., & Hawcroft, C. (2016, 07 25). 10.1186/s12879-016-1697-6
[3] Dimitrova, L., Kaleva, M., & Zaharieva, M. M. (2021, 08 4)
[4]Flores-Mireles, A., Walker, J., & Caparon, M. (2015, 04 08). *Nat Rev Microbiol*, *13*, 269-284. Nature. https://doi.org/10.1038/nrmicro3432
[5]*High levels of antibiotic resistance found worldwide, new data shows*. (2018, January 29). WHO
[6]Lagha, R., Abdallah, F. B., AL-Sarhan, B. O., & Al-Sodany, Y. (2019, 03 23). *NCBI*. 10.3390/molecules24061161
[7]Nji, E., Kazibwe, J., & Hambridge, T. (2021).https://www.nature.com/articles/s41598-021-82693-4
[8]Sanchez, C. J., Mende, K., & Beckius, M. L. (2013, 01 29). *BMS Infect Dis.* PubMed. 10.1186/1471-2334-13-47
[9]Terahara, F., & Nishiura, H. (2019, 04 23).
[10]WHO. (2021). WHO. https://www.who.int/publications/i/item/9789240027336
[11]Wilson, C. (2017, 10 24). NCBI. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6133255/#R21