Phytocompounds present in essential oils used as alternative antimicrobial agents against *Escherichia coli* O157:H7.

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Background

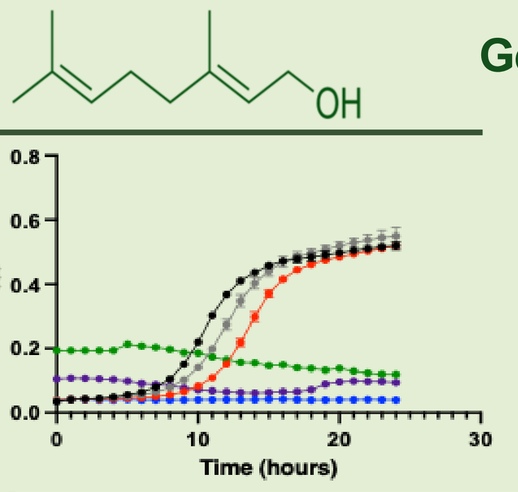
Enterohemorrhagic *E. coli* O157:H7 (EHEC) is a foodborne enteropathogen that causes hemorrhagic colitis and hemolytic uremic syndrome (HUS), with approximately 30% of infections in children and the elderly progressing to HUS and potential mortality. Currently, treatment is limited to supportive care, highlighting the need for alternative therapeutic strategies. Essential oils have long been used by various ethnic communities to combat bacterial infections. Recent studies have demonstrated the antibacterial activity of certain essential oils against O157:H7. In this study, we evaluated three major essential oil constituents: geraniol from *Cymbopogon martini*, cinnamaldehyde from *Cinnamomum verum* bark oil, and citral from *Cymbopogon flexuosus* for their ability to exert antibacterial activity against **EHEC O157:H7**.

Minimal Inhibitory Concentration (MIC) Minimal Bactericidal Concentration (MBC)

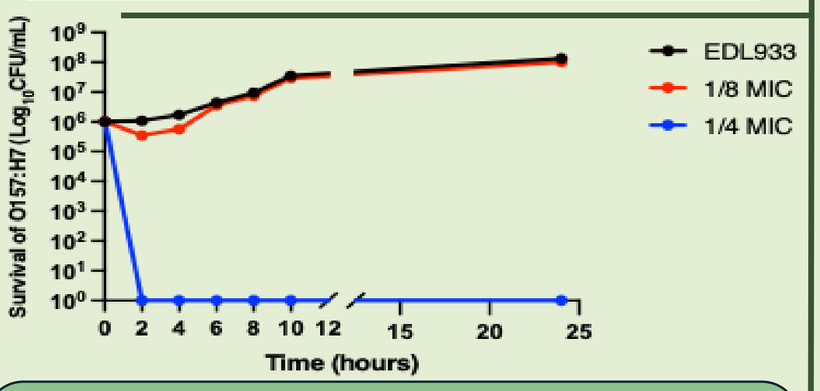
phytocompound	MIC		MBC	
	K12	EDL 933	K12	EDL 933
Geraniol	6.4%	0.8%	6.4%	0.8%
Cinnamaldehyde	0.0125%	0.00625%	0.1%	0.025%
Citral	6.4%	0.2%	6.4%	0.4%

MIC and MBC assays showed that the phytocompounds possess antibacterial activity against both pathogenic and non-pathogenic *E. coli* strains. However, a significantly higher concentration was required to inhibit the **K12 strain** compared to **O157:H7**, indicating selective targeting of pathogenic strains. Unlike conventional antibiotics, these compounds may suppress pathogens while leaving the gut microbiota largely unaffected.

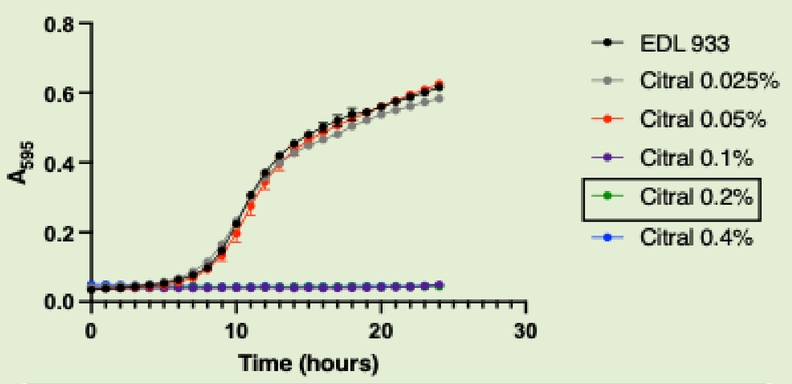
Geraniol



Geraniol exerted a bactericidal effect at the MIC concentration alike 0.4% and 0.2%. At 0.1%, it initially slowed cell growth, but the cells later recovered to levels comparable to the untreated control. Initial higher OD_{595} of MIC and ½ MIC due to density of Geraniol.

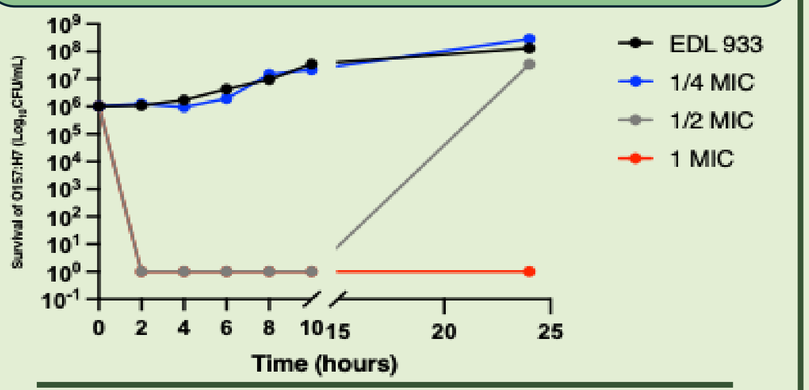


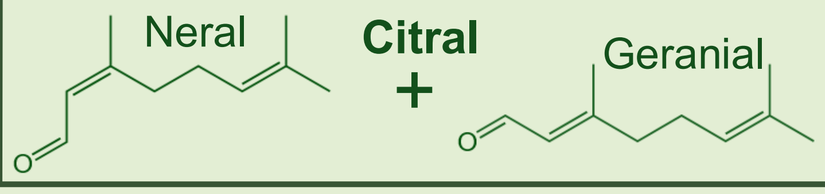
One-quarter MIC induced complete cell death within 2 hours post-treatment. In contrast, 1/8 MIC had minimal effect between 2–6 hours compared to the untreated control, with recovery observed after 6 hours.



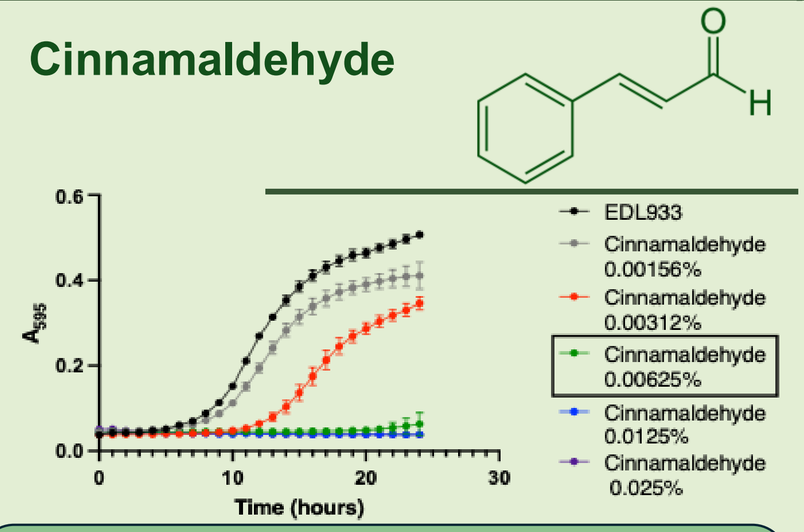
Citral reduced the bacterial population. At lower concentrations, cells eventually recovered to levels comparable to the untreated control, whereas MIC and 2×MIC reduced the population below detectable levels.

Citral exhibited a bactericidal effect at the MIC concentration. At half the MIC, cells began to recover after 10 hours, while lower concentrations had no significant effect.

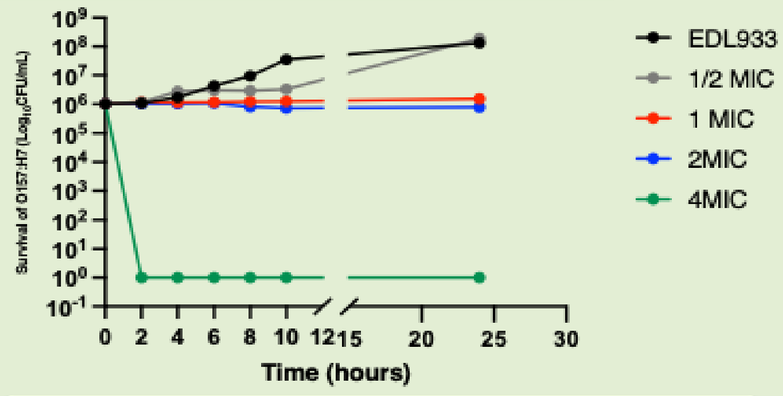




Cinnamaldehyde

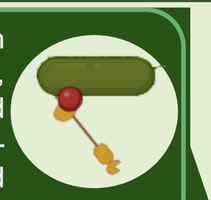


Cinnamaldehyde exhibited concentration-dependent antibacterial activity. At 24 hours post-treatment, a significant reduction in the total bacterial population was observed, with complete cell death at 4×MIC.

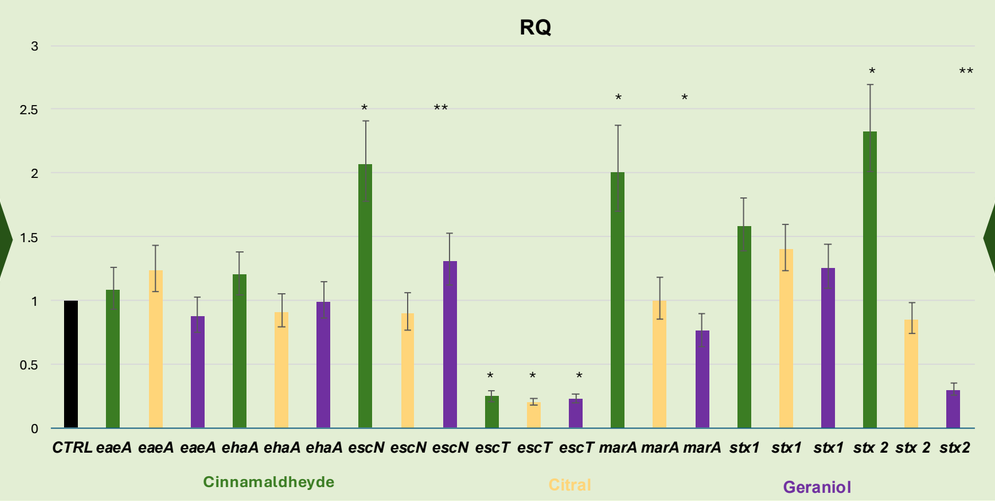


In accordance with the growth curve experiment, 4×MIC completely killed the bacterial cells. Substantial reductions in cell count were observed at MIC and 2×MIC, while treatment with half the MIC had minimal effect after 24 hours.

The intimin-encoding *eaeA* gene, which confers attaching and effacing lesions, was unaffected by phytocompound treatment. The adhesin-encoding *ehaA* gene also remained unchanged.



Among pathogenic genes associated with the type III secretion system (TTSS), *escN* was upregulated only upon cinnamaldehyde treatment, while *escT* was significantly downregulated by all three phytocompounds, potentially disrupting TTSS.



The *marA* gene, a key regulator of multiple antibiotic resistance that enhances bacterial survival, was upregulated only upon Cinnamaldehyde treatment and remained unchanged under other treatments.

The expression of the less virulent Shiga toxin subtype *stx1* remained unchanged following treatment with the phytocompounds. In contrast, Cinnamaldehyde significantly increased expression of the more potent *stx2* gene, while Geraniol down regulates *stx2* levels.

References

1. Scotti, R., Stringaro, A., Nicolini, L., Zanellato, M., Boccia, P., Maggi, F., & Gabbianelli, R. (2021). Effects of essential oils from cymbopogon spp. And cinnamomum verum on biofilm and virulence properties of escherichia coli o157:H7. *Antibiotics*, *10*(2), 1–15. https://doi.org/10.3390/antibiotics10020113.
2. Scotti, R., Casciaro, B., Stringaro, A., Maggi, F., Colone, M., & Gabbianelli, R. (2024). Fighting Microbial Infections from Escherichia coli O157:H7: The Combined Use of Three Essential Oils of the Cymbopogon Genus and a Derivative of Esculentin-1a Peptide. *Antibiotics*, *13*(1). https://doi.org/10.3390/antibiotics13010086.
3. Critzer, F. J., D'souza, D. H., & Golden, D. A. (2008). Transcription Analysis of stx 1 , marA, and eaeA Genes in Escherichia coli O157:H7 Treated with Sodium Benzoate. In Journal of Food Protection (Vol. 71, Number 7).

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Acknowledgement

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