

Effects of Arborvitae Essential Oil on Vancomycin-Resistant *Enterococcus faecalis* and Resistance Development

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Background

Antibiotic resistance is a major global health threat, contributing to over 1 million deaths annually¹.

Enterococci are bacteria that are naturally present in humans and the environment² that have acquired resistance to multiple antibiotics, including Vancomycin.

Vancomycin-resistant *Enterococcus* (VRE) is a leading cause of hospital-acquired infection in the United States³, and continues to develop resistance to additional antibiotics.

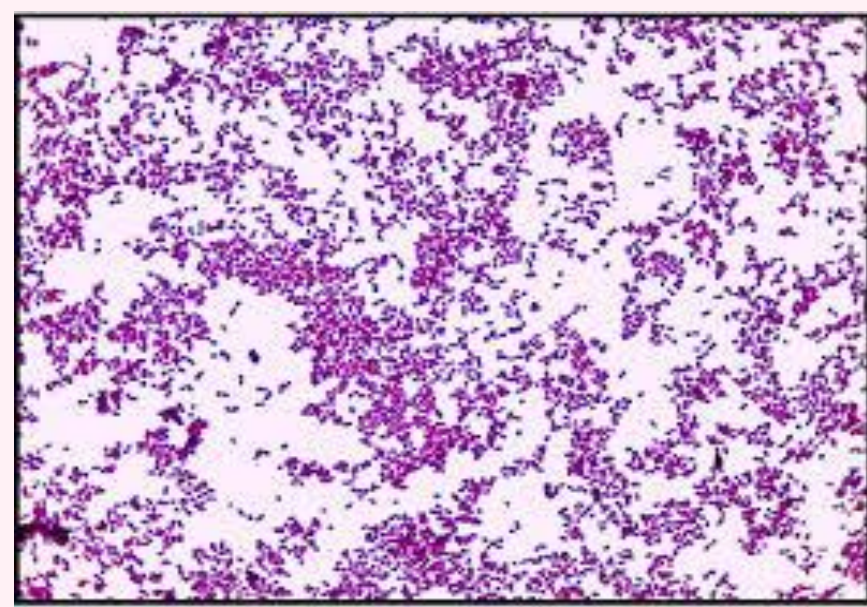


Figure 1: Gram-stained *Enterococci*; Gram-positive diplococci and short chains⁴

It is crucial to identify alternative treatment methods, as the rate of new antibiotic development cannot keep up with the growing resistance.

Essential oils have been used for thousands of years for anti-inflammatory, sedative, and digestive purposes⁵, as well as cosmetics and ointments dating back to Ancient Egyptians in 4500 BC⁶.

In prior independent research, arborvitae essential oil proved to be one of the most effective against *E. faecalis*. Arborvitae oil is derived from the Western Redcedar (*Thuja plicata*), which is native to western North America⁷.



Figure 2: Arborvitae essential oil from doTERRA⁸

Main components of arborvitae oil include methyl thujate, methyl myrtenate, and α -terpineol⁹, which may exhibit antibacterial activity.

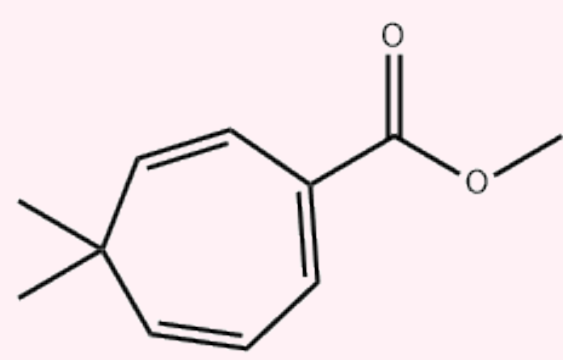


Figure 3: Methyl thujate structure¹⁰

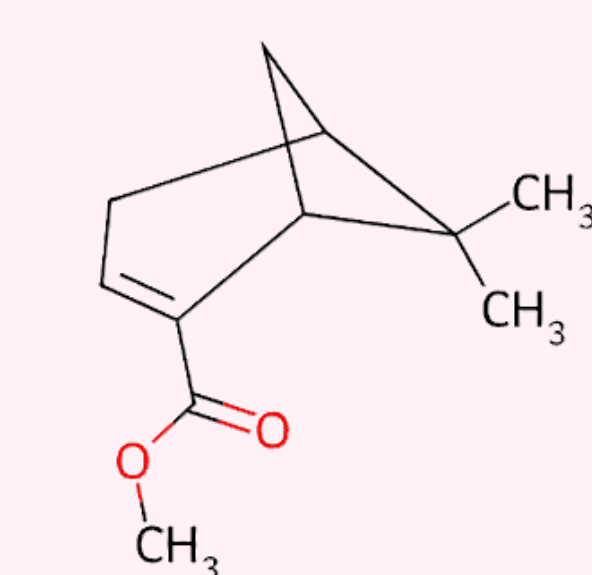


Figure 4: Methyl myrtenate structure¹¹

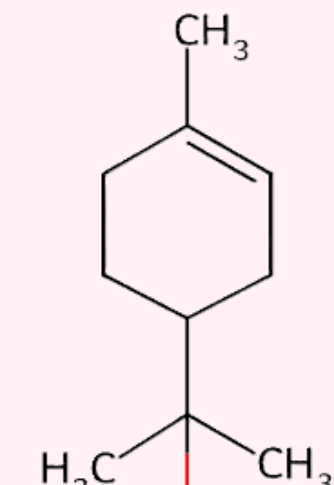


Figure 5: α -terpineol structure¹²

Research

Research Question

Can arborvitae essential oil inhibit VRE growth, and does repeated exposure lead to resistance development?

Hypothesis

Arborvitae essential oil will inhibit the growth of *E. faecalis* in a concentration-dependent manner, and prolonged exposure will result in minimal resistance development.

Objectives

- Evaluate antibacterial activity using disk diffusion
- Determine the minimum inhibitory concentration (MIC)
- Assess resistance development under sub-inhibitory exposure

Methods

Disk Diffusion (Kirby-Bauer)

- 0.5 McFarland *Enterococcus faecalis* (ATCC 700802) suspension
- Mueller-Hinton II agar plates inoculated
- 6 mm paper discs with 5 μ L arborvitae oil
 - Concentrations tested: 100%, 75%, 50%, 25%, 10%, 5%
 - Dilutions using jojoba oil
- Incubated at 37°C for 24 hours
- Zones of inhibition (ZOI) measured (mm)

Resistance Testing

- Sub-inhibitory concentration: 12.5% arborvitae oil
 - Two parallel lines were maintained:
 - Selection line: VRE + arborvitae oil (12.5%)
 - Passage control: VRE only
 - Daily passaging
 - Susceptibility measured via disk diffusion

Controls

- Vancomycin (30 μ g disc)
- Linezolid (30 μ g disc)
- Carrier oil (jojoba)
- Positive control (bacteria only)
- Negative control (media only)

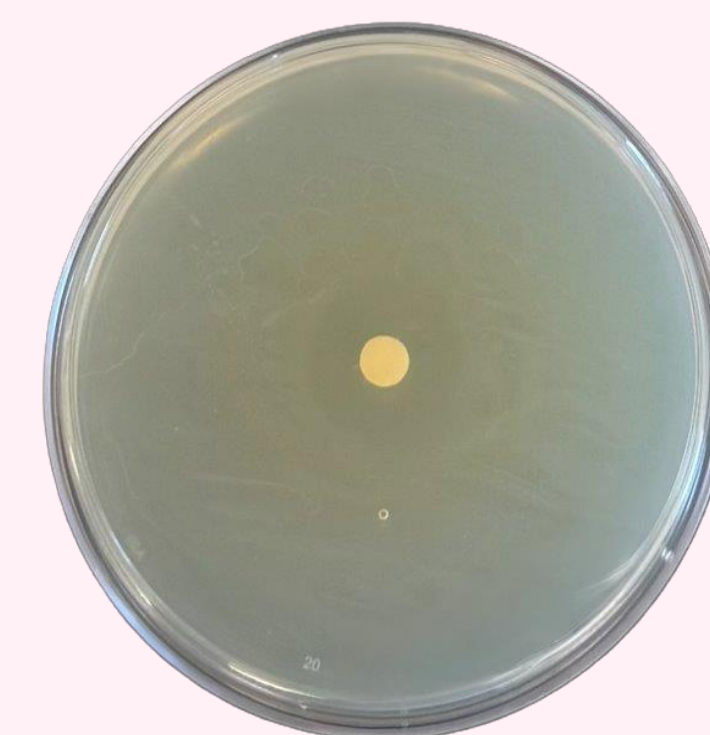


Figure 7: Zone of inhibition produced by 100% arborvitae essential oil against *E. faecalis* using the Kirby-Bauer disk diffusion method

Results

Table 1: Mean Zone of Inhibition (ZOI) produced by arborvitae essential oil concentrations against *Enterococcus faecalis* using the Kirby-Bauer disk diffusion assay.

Arborvitae Concentration	Mean ZOI (mm)
100%	18.8
75%	16.4
50%	15.5
25%	11.2
10%	0
5%	0

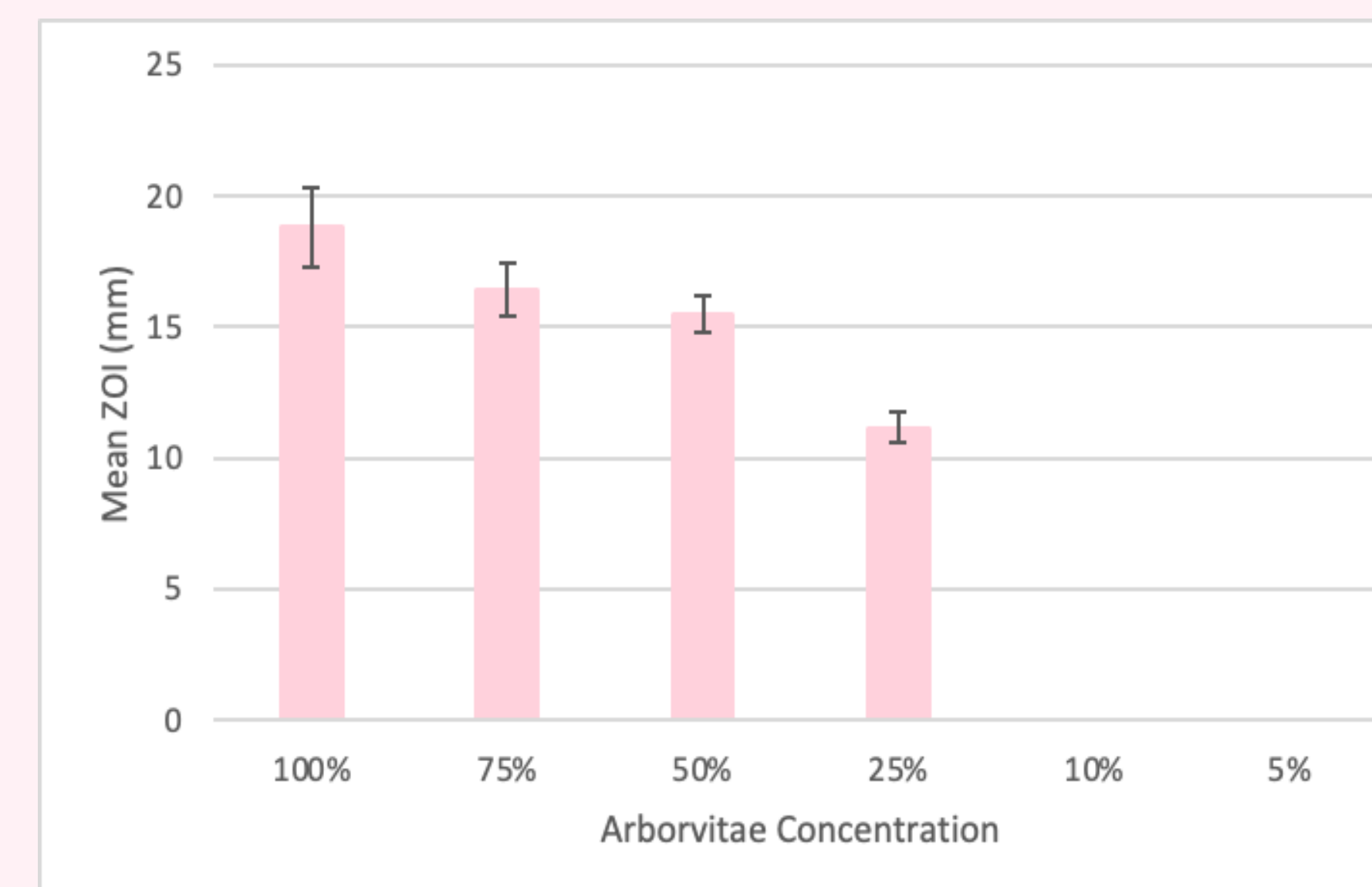


Figure 6: Dose-Dependent Antibacterial Activity of Arborvitae Oil
Antibacterial activity increased with concentration, with inhibition observed at \geq 25%

Key Observations

- Dose-dependent response observed
- No inhibition at 10% or 5%
- Jojoba oil shows no antibacterial activity
- Antibiotic controls produced expected ZOI values

Resistance Analysis

- Control group maintained stable susceptibility (22-25 mm ZOI)
- Arborvitae-exposed cultures showed limited early growth
- No clear resistance trends observed under current conditions

Conclusion

Arborvitae essential oil demonstrated measurable antibacterial activity against *Enterococcus faecalis*, with effectiveness increasing in a dose-dependent manner. Inhibition was observed at concentrations \geq 25%, while lower concentrations showed no measurable effect.

No clear resistance development was observed. Continued inhibition at sub-inhibitory concentrations suggests potential antibacterial activity beyond levels indicated by diffusion-based assays.

Significance

These findings support the potential of plant-based antimicrobials as alternative strategies to combat antibiotic-resistant bacteria

Future Research

- Test lower sub-inhibitory concentrations
- Extend resistance study across additional passages
- Investigate active compounds in arborvitae oil
- Evaluate effectiveness against other pathogens

Key Takeaway

Arborvitae essential oil exhibits dose-dependent antibacterial activity against VRE, demonstrating potential as a plant-based alternative to conventional antibiotics

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