ISSN: 3065-758X
Research Article

Journal of Infectious Diseases and Viruses Research

The Potential of Selected Essential Oils in Combating Enterococcus Faecalis

Sebastian Such[1]* & Gabriela Chowaniec[2]

[1]*Institute of Economy, Department of Herbal Studies, State University of Sciences in Krosno, ul. Dmochowskiego 12, 38-400 Krosno* https://orcid.org/0009-0001-4750-6012

[2]*Institute of English Studies, Faculty of Philology, University of Rzeszów, al. mjr. W. Kopisto 2B, 35-315 Rzeszów* https://orcid.org/0009-0008-4034-9166

***Corresponding author:** Sebastian Such, Institute of Economy, Department of Herbal Studies, State University of Sciences in Krosno, ul. Dmochowskiego 12, 38-400 Krosno.

Submitted: 26 July 2025 **Accepted:** 06 August 2025 **Published:** 13 August 2025

https://doi.org/10.63620/MKJIDVR.2025.

Citation: *Sebastian Such & Gabriela Chowaniec. (2025). The Potential of Selected Essential Oils in Combating Enterococcus Faecalis. J of Infec Dise and Vir Res, 4(4), 01-10.*

Abstract

Background: *Enterococcus faecalis represents a significant clinical challenge as an etiological agent of endodontic infections, characterized by high resistance to conventional antibacterial agents and the ability to form biofilms [1-4]. Growing interest in natural alternatives to synthetic antibiotics has led to investigations on essential oils as potential therapeutic agents [5-9].*

Objective: *The aim of this study was to determine the antibacterial potential of selected essential oils against E. faecalis, with particular emphasis on marjoram, eucalyptus, cananga, Ylang-Ylang, caraway, clementine, and Petitgrain oils.*

Materials and Methods: *The study was conducted using the disk diffusion method according to Janusz Borysiewicz on an E. faecalis strain [10]. Essential oil samples at a concentration of 20% were prepared by diluting them in a Tween 80–water mixture. Next, the disks were applied onto Petri dishes containing a prepared medium with a pH of 7 ± 0.2, which consisted of enzymatic casein hydrolysate, yeast extract, dextrose, and agar, and then inoculated with E. faecalis. The plates were incubated for 48 hours at 37 °C, and measurements were taken after 24 and 48 hours.*

Results: *The study demonstrated variable antibacterial activities among the tested essential oils. The 20% Petitgrain oil solution showed the highest efficacy, achieving an inhibition zone diameter of 7.5 mm after 48 hours. This was followed by Cananga oil (4.5 mm), Ylang-Ylang oil (2 mm), and eucalyptus oil (1.5 mm). Marjoram and clementine oils exhibited no antibacterial activity. After 24 hours of incubation, the greatest activity was observed for marjoram oil (22.5 mm) and Petitgrain oil (20.5 mm), suggesting strong effects of phenolic and terpenoid alcohol compounds such as thymol and linalool [11].*

Conclusions: *Some of the selected essential oils demonstrate promising potential as alternative antibacterial agents against E. faecalis. Further research to optimize their composition and elucidate their mechanisms of action may contribute to development of new therapeutic strategies for infections caused by this pathogen [12]. Additionally, these oils could serve as alternatives to antibiotic therapy.*

Keywords: Essential Oils, Enterococcus Faecalis, Antibacterial Activity, Natural Antiseptics, Antibiotic Resistance.

Introduction

Enterococcus faecalis is a Gram-positive bacterium belonging to the family Enterococcaceae, representing one of the most important etiological agents of nosocomial infections worldwide

[14]. This organism is characterized by an exceptional ability to survive under adverse environmental conditions, high levels of antibiotic resistance, and the capacity to form persistent biofilms on both biotic and abiotic surfaces [15, 16]. These properties make E. faecalis a particularly problematic pathogen in the context of endodontic infections, where it can proliferate within root canals despite intensive treatment with conventional disinfectants [17].

The pathogenicity of E. faecalis results from a complex interaction of multiple virulence factors, including lipoteichoic acid (LTA), aggregation substance, gelatinase, hemol, enterococcal surface protein (ESP), and cytolysin. These factors enable the bacterium to adhere to host tissues, form biofilms, modulate immune responses, and induce inflammatory processes. Particularly important is its biofilm-forming ability, which provides protection against antibacterial agents and contributes to the chronic nature of infections [18,19].

The increasing issue of antibiotic resistance and the toxicity of conventional disinfectants such as sodium hypochlorite (NaOCl) drive the search for alternative treatment methods [20]. Essential oils-natural plant metabolic products-have gained significant attention as potential broad-spectrum antibacterial agents. Their biological activity arises from a diverse array of bioactive compounds, including monoterpenes, sesquiterpenes, phenolic compounds, and aromatic aldehydes.

Previous studies have shown that essential oils can act at various levels of the bacterial cell, including disruptions of the cytoplasmic membrane, cell wall, and metabolic processes [21]. These mechanisms include membrane permeabilization, disruption of membrane potential, inhibition of ATP synthesis, and induction of oxidative stress. Additionally, some essential oils exhibit antibiofilm activity, which is particularly relevant for combating endodontic infections.

The aim of this study was to determine the antibacterial potential of selected essential oils against E. faecalis, with particular emphasis on marjoram, eucalyptus, cananga, ylang-ylang, caraway, clementine, and petitgrain oils. The study also sought to evaluate the clinical potential of these natural compounds as alternatives to conventional antibacterial agents.

Materials and Methods

Test Organism

The study used an Enterococcus faecalis strain, a standard test organism for antibacterial activity research. The strain was propagated in 2% distilled water solution and incubated for 24 hours at 37 °C. After incubation, cultures were stored in the laboratory refrigerator. Prior to experiments, bacteria were equilibrated to room temperature for 4 hours.

Essential Oils

The study used marjoram, eucalyptus, cananga, ylang-ylang, caraway, clementine, and petitgrain essential oils (Fig. 2). Caraway oil was obtained via 3-hour steam distillation of Carum carvi fruits „Rafex" brand using a Deryng apparatus, while the remaining oils were sourced from "Mystic Moments" (Fig. 1). All oils were stored at room temperature in a dark room prior to use.

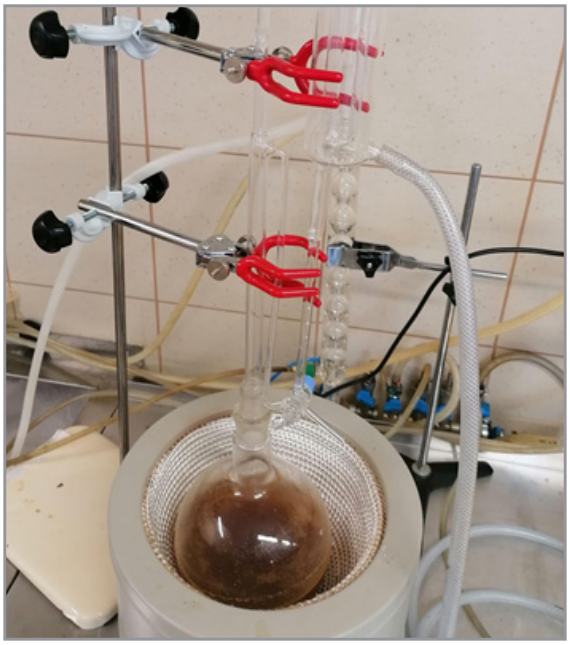

Figure 1: Steam distillation of caraway oil using a Deryng apparatus

Figure 2: Caraway oil after distillation

Antibacterial Activity Assay – Disk Diffusion Method

The antibacterial activity of the essential oils was evaluated using the disk diffusion method described. Petri dishes were filled with sterile agar medium, and after the agar had solidified, each plate was uniformly inoculated with a bacterial suspension. Sterile paper disks were impregnated with a 20% solution of the test essential oil and placed on the agar surface. The plates were then incubated at 37 °C for 48 hours, and inhibition zone diameters were measured after 24 and 48 hours of incubation [22].

Statistical Analysis

All inoculations were performed in triplicate. Results are expressed as the mean of the three measured inhibition zone diameters.

Results

The study revealed significant differences in the antibacterial activities of the individual essential oils against E. faecalis. In the disk diffusion assay, after 24 hours, marjoram oil produced zones measuring 22.5 mm (Fig. 3). Petitgrain oil showed similar efficacy, with an inhibition zone of 20.5 mm (Fig. 4).

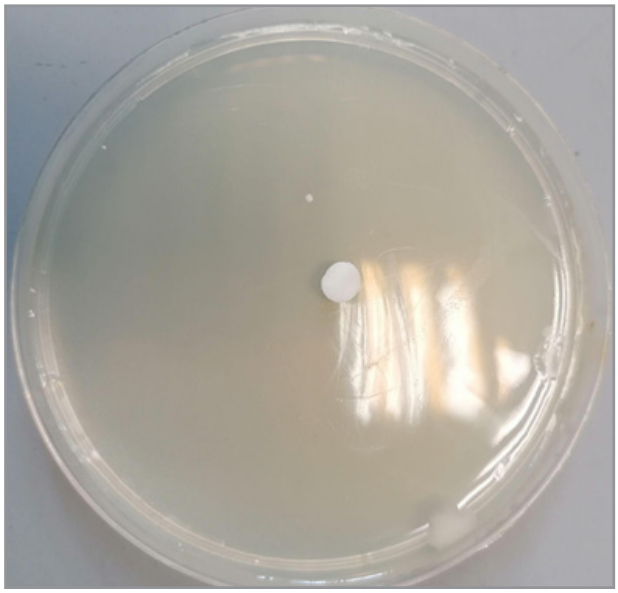

Figure 3: The inhibition zone of the 20% marjoram oil sample after 24 hours.

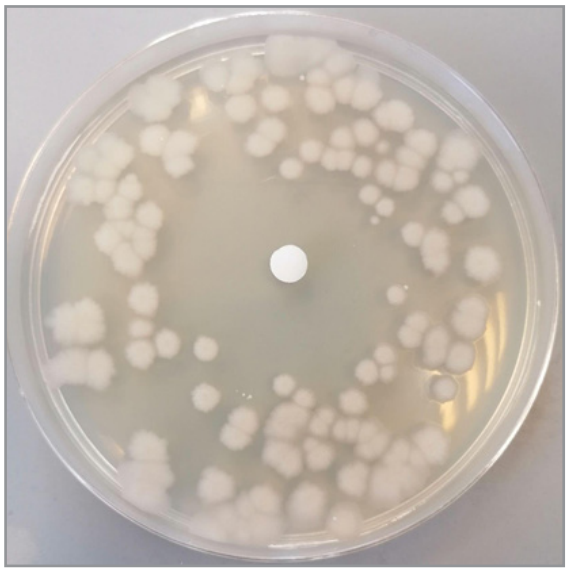

Figure 4: The inhibition zone of the 20% Petitgrain oil sample after 24 hours.

Cananga oil also demonstrated similar antibacterial activity with an inhibition zone of 18 mm, while ylang-ylang oil showed a zone of 4.8 mm (Fig. 5). Caraway oil exhibited activity with a zone of 2.3 mm (Fig. 6). Eucalyptus oil likewise showed low activity with an 8 mm inhibition zone (Fig. 7). Clementine oil had the weakest activity, with a 1.5 mm inhibition zone (Fig. 8).

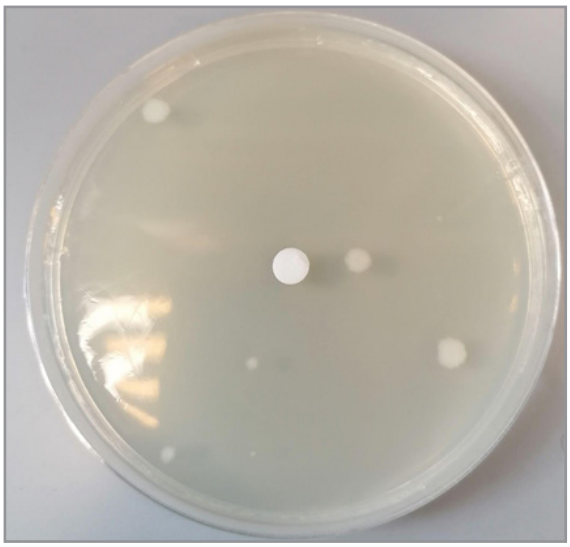

Figure 5: The inhibition zone of the 20% Ylang-Ylang oil sample after 24 hours.

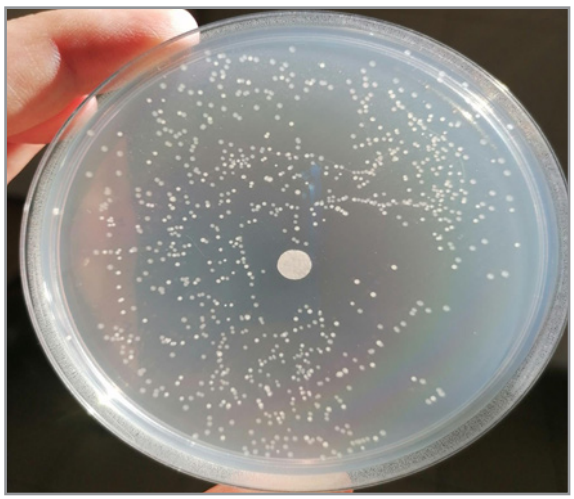

Figure 6: The inhibition zone of the 20% caraway oil sample after 24 hours.

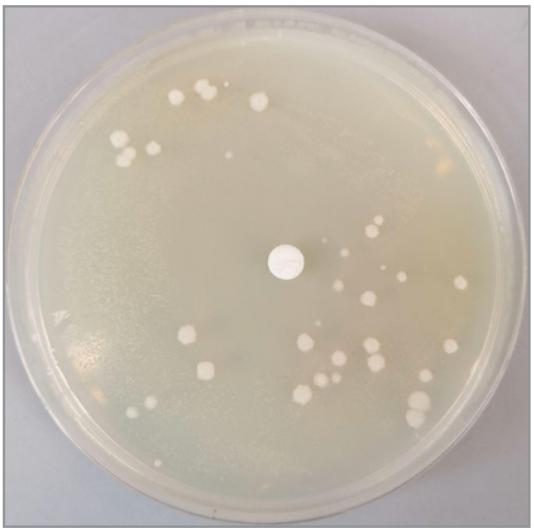

Figure 7: The inhibition zone of the 20% eucalyptus oil sample after 24 hours.

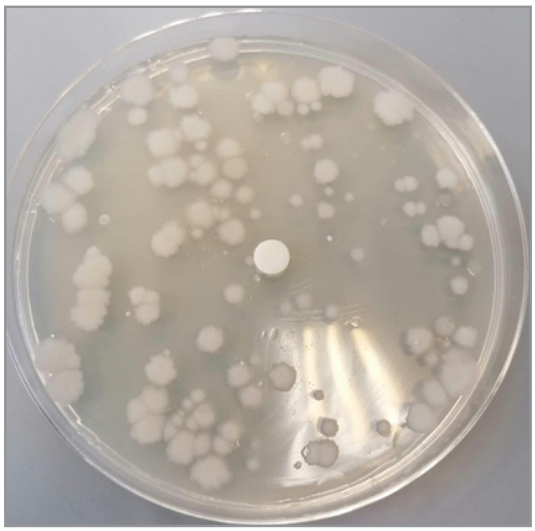

Figure 8: The inhibition zone of the 20% clementine oil sample after 24 hours.

After 48 hours of incubation, the most pronounced antibacterial effect was observed for Petitgrain oil, whose inhibition zone reached 7.5 mm (Fig. 9). Next in size was Cananga oil with a zone 4.5 mm. Ylang Ylang oil produced an inhibition zone measuring 2 mm (Fig. 10), while eucalyptus (Fig. 11) and cumin oils showed comparable effects (Fig. 12), forming zones with diameters of 1.5 mm and 1 mm, respectively. The weakest results were seen for marjoram (Fig. 13) and clementine oils (Fig. 14), which exhibited no antibacterial activity against E. faecalis, as no inhibition zones were formed.

Figure 9: The inhibition zone of the 20% Petitgrain oil sample after 48 hours.

Figure 10: The inhibition zone of the 20% Ylang-Ylang oil sample after 48 hours.

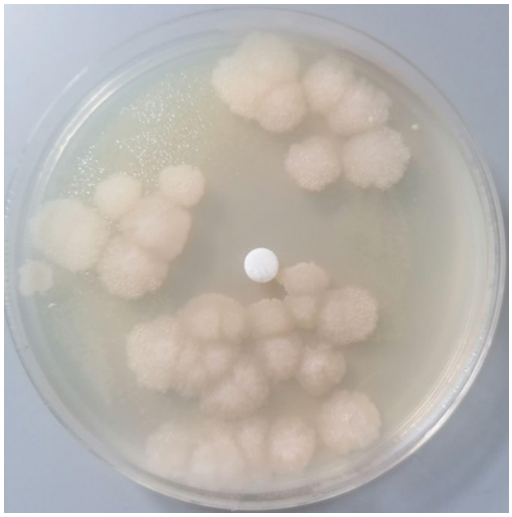

Figure 11: The inhibition zone of the 20% eucalyptus oil sample after 48 hours.

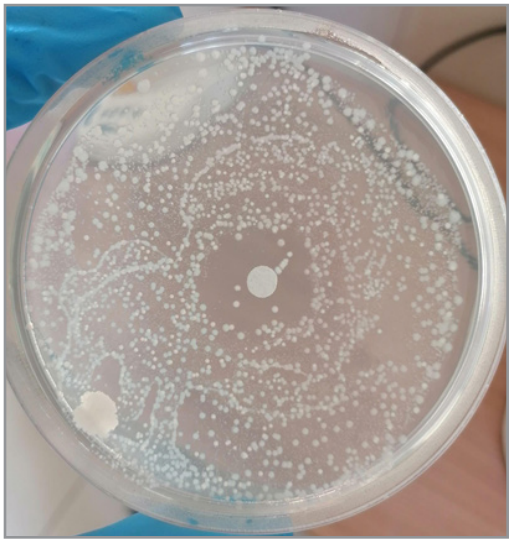

Figure 12: The inhibition zone of the 20% caraway oil sample after 48 hours.

Figure 13: The inhibition zone of the 20% marjoram oil sample after 48 hours.

Figure 14: The inhibition zone of the 20% clementine oil sample after 48 hours.

Discussion

The conducted studies clearly indicate that the selected essential oils exhibit markedly diverse antibacterial activity against E. faecalis in an in vitro model, which is confirmed by the rigorous methodology and classification in the literature regarding measurement of bacterial growth inhibition zones using the disk diffusion method. A standardized approach to zone measurements and categorization of results according to established susceptibility categories (resistant, poorly susceptible, intermediately susceptible, susceptible) allows for reliable interpretation of the obtained data and enables comparison with findings from other authors (Table 1). The bacterial susceptibility levels to the specified essential oils were determined based on the data from the table by Janusz Borysiewicz. The observed differences in activity among individual oils can be attributed to their varied chemical compositions, particularly the content of bioactive compounds with documented antibacterial properties.

The highest antibacterial activity after 24 hours of incubation was exhibited by marjoram oil (22.5 mm inhibition zone) and Petitgrain oil (20.5 mm), suggesting that these are highly effective during the initial phase of bacterial exposure. These results are consistent with the literature highlighting the role of phenols and terpenoid alcohols (e.g., thymol, linalool) as key constituents responsible for strong interactions with bacterial cell walls and cytoplasmic membranes [23-25]. The large inhibition zones also indicate the potential use of these oils as disinfectants in endodontic practice, particularly during the preliminary elimination of pathogens.

Cananga and Ylang-Ylang oils exhibited moderate (18 mm) and low (4.8 mm) efficacy, respectively. Their activity may be related to lower levels of compounds such as eugenol or linalool, which at higher concentrations are known for their antibacterial effects [26]. Eucalyptus, caraway, and clementine oils demonstrated weak or very weak activity (Fig. 15). This outcome corroborates reports that eucalyptol—the main constituent of eucalyptus oil-shows limited efficacy against Gram-positive pathogens, and that citrus oils high in limonene are generally less active against E. Faecalis.

Table 1: Degrees of Sensitivity and the Sizes of Microbial Growth Inhibition Zones

Degrees of Sensitivity and the Sizes of Microbial Growth Inhibition Zones

(disk diffusion method) (according to WWSS-4 standards)

Susceptibility category	Mean inhibition zone diameter (mm)
Resistant	0-19
Poorly susceptible	20-24
Intermediately susceptible	25-29
Susceptible	≥30

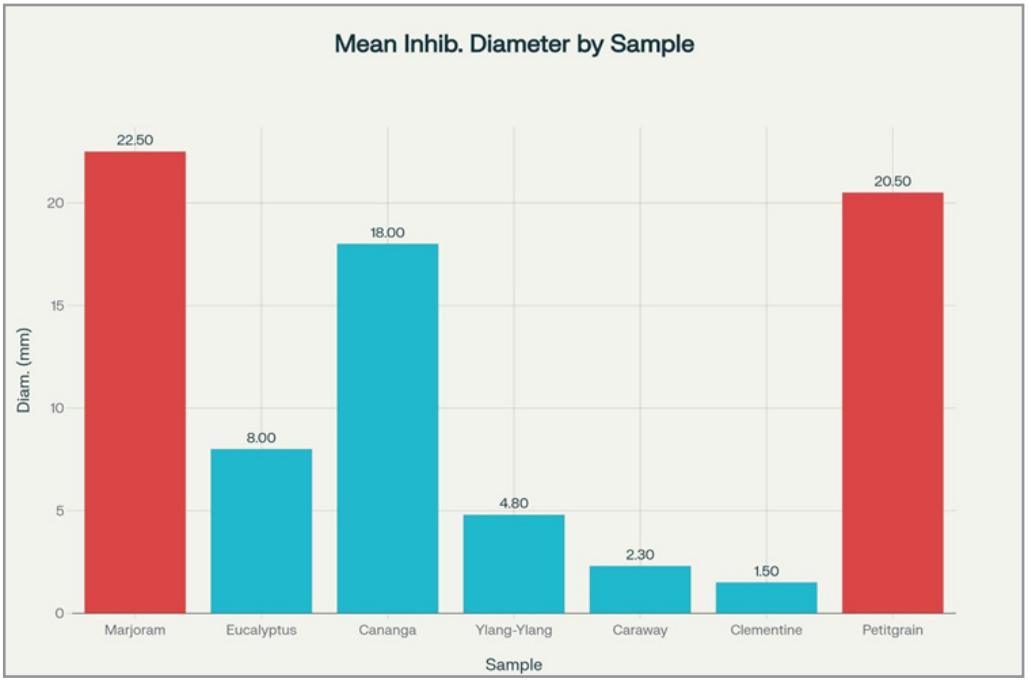

Figure 15: Mean Inhibition Zone Diameter by Sample after 24 hours

After 48 hours, most of the tested essential oils showed a general decline in antibacterial activity. Petitgrain oil demonstrated the greatest resistance to loss of efficacy, although its inhibition zone still decreased significantly to 7.5 mm. This may indicate that its constituents are more stable under prolonged incubation or that it interacts more strongly and durably with E. faecalis compared to the other extracts. Cananga oil maintained limited activity (4.5 mm), while the remaining oils—marjoram included—exhibited no activity after extended exposure (Fig. 16). The absence of marjoram oil's effect at 48 hours is particularly noteworthy, given its initially highest potency; this could suggest instability of its active components or a possible reactive regrowth of the pathogen once the oil's action wanes. Such observations highlight the importance of evaluating not only the initial antibacterial effect but also the sustained efficacy of essential oils in clinical applications.

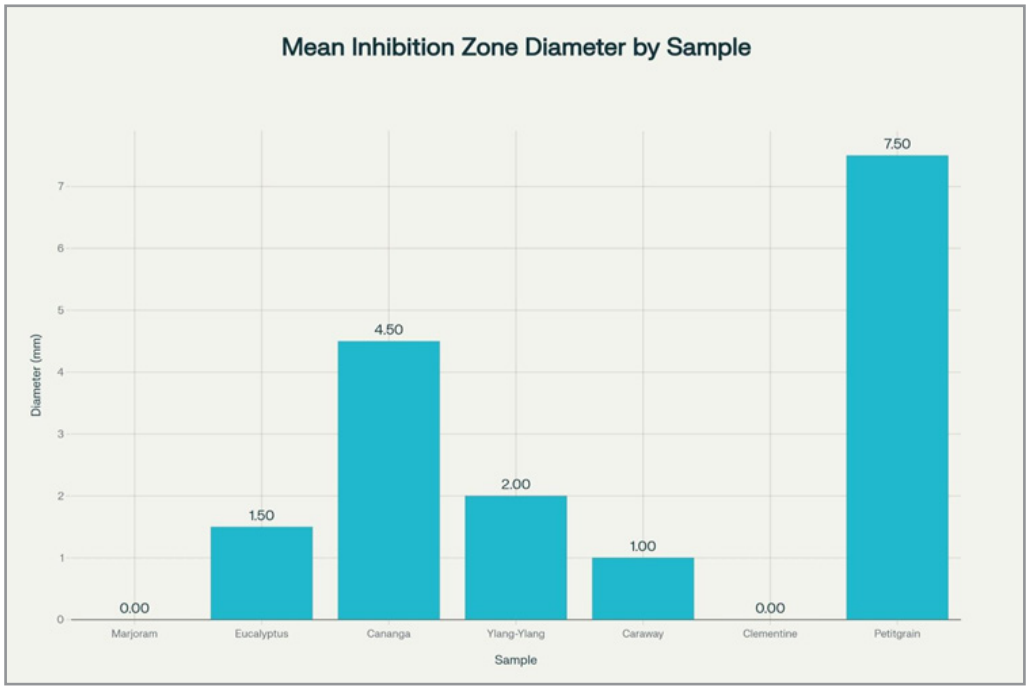

Figure 16: Mean Inhibition Zone Diameter by Sample after 48 hours

The results indicate that selected essential oils may be effective as short-term antiseptic agents; however, their long-term stability and practical applicability require further investigation, including in models simulating the biological conditions the root canal. The data obtained confirm literature reports of the high efficacy of certain essential oils against E. faecalis over a short exposure time, but also underscore the challenges related to their stability and the maintenance of activity over extended periods.

Overall, based on the results obtained, petitgrain oil appears to possess the greatest therapeutic potential, having demonstrated relatively sustained activity. The findings for marjoram oil suggest that its use may require repeated dosing or combination with other agents to prolong its effect. The weak activity of clementine and caraway oils, on the other hand, highlights the need to assess each oil individually and to explore the synergistic potential of their mixtures.

A limitation of this study is its in vitro model, which does not fully replicate clinical conditions. Although the disk diffusion method is widely accepted, the efficacy of the oils may be influenced by the presence of matter, pH variations, or interactions with biological substrates. In subsequent phases, it would be desirable to extend the research to tissue-based models and to assess cytotoxicity in human cell lines.

In summary, according to current findings and the scientific literature, marjoram and Petitgrain oils exhibit the greatest potential for short-term elimination of Enterococcus faecalis, whereas the durability of and efficacy of the other oils require further detailed evaluation.

References

1. Benbelaïd, F., Khadir, A., Abdoune, M. A., Bendahou, M., Muselli, A., & Costa, J. (2014). Antimicrobial activity of some essential oils against oral multidrug–resistant Enterococcus faecalis in both planktonic and biofilm state. Asian Pacific journal of tropical biomedicine, 4(6), 463-472.
2. Bansal, D., Chandola, I., & Mahajan, M. (2020). Antimicrobial activity of Five Different Essential oils against Enterococcus Faecalis: An In vitro study. Journal of Dental Materials & Techniques, 9(3).
3. Nagy-Bota, M. C., Man, A., Santacroce, L., Brinzaniuc, K., Pap, Z., Pacurar, M., ... & Kovacs, M. (2021). Essential oils as alternatives for root-canal treatment and infection control against enterococcus faecalis-a preliminary study. Applied Sciences, 11(4), 1422.
4. Sena, G., De Rose, E., Crudo, M., Filippelli, G., Passarino, G., Bellizzi, D., & D'Aquila, P. (2024). Essential Oils from Southern Italian Aromatic Plants Synergize with Antibiotics against Escherichia coli, Pseudomonas aeruginosa and Enterococcus faecalis Cell Growth and Biofilm Formation. Antibiotics, 13(7), 605.
5. Kędzia, A., Kusiak, A., Kochańska, B., Kędzia, A. W., Półjanowska, M., Gębska, A., & Ziółkowska-Klinkosz, M. (2011). The susceptibility of aerobic bacteria to clove oil (Oleum caryophylli). Postępy Fitoterapii.
6. Bogojević, J., Nikolić, M., Marković, T., Ćirić, A., & Marković, D. (2016). Analysis of chemical composition of the most efficient essential oils towards Enterococcus faecalis referent strain ATCC 29212 and clinical isolates. Lekovite sirovine, (36), 3-25.
7. Javed, S., Javaid, A., Nawaz, S., Saeed, M. K., Mahmood, Z., Siddiqui, S. Z., & Ahmad, R. (2014). Phytochemistry, GC-MS analysis, antioxidant and antimicrobial potential of essential oil from five citrus species. Journal of Agricultural Science, 6(3), 201.
8. Zhan, X., Tan, Y., Lv, Y., Fang, J., Zhou, Y., Gao, X., ... & Shi, C. (2022). The antimicrobial and antibiofilm activity of oregano essential oil against Enterococcus faecalis and its application in chicken breast. Foods, 11(15), 2296.
9. Diouchi, J., Marinković, J., Nemoda, M., El Rhaffari, L.,

Toure, B., & Ghoul, S. (2024). In Vitro Methods for Assessing the Antibacterial and Antibiofilm Properties of Essential Oils as Potential Root Canal Irrigants—A Simplified Description of the Technical Steps. Methods and Protocols, 7(4), 50.
10. Borysiewicz, J. (1976). Podstawy mikrobiologii lekarskiej. Wydawnictwo Lekarskie PZWL.
11. Martos, J., Luque, C. M. F., González-Rodríguez, M. P., Arias-Moliz, M. T., & Baca, P. (2013). Antimicrobial activity of essential oils and chloroform alone and combinated with cetrimide against Enterococcus faecalis biofilm. European journal of Microbiology and Immunology, 3(1), 44-48.
12. John, S., Lee, J. W., Lamichhane, P., Dinh, T., Nolan, T., & Yoon, T. (2023). Potential Synergistic Inhibition of Enterococcus faecalis by Essential Oils and Antibiotics. Applied Sciences, 13(19), 11089.
13. Madsen, K. T., Skov, M. N., Gill, S., & Kemp, M. (2017). Virulence factors associated with Enterococcus faecalis infective endocarditis: a mini review. The open microbiology journal, 11, 1.
14. Najafi, K., Ganbarov, K., Gholizadeh, P., Tanomand, A., Rezaee, M. A., Mahmood, S. S., ... & Kafil, H. S. (2020). Oral cavity infection by Enterococcus faecalis: virulence factors and pathogenesis. Reviews and Research in Medical Microbiology, 31(2), 51-60.
15. Momotaz, T., Afroz, F., Chowdhury, S., islam, N., Sarwar, M. T., Khan, R. R., & Sattar, A. N. I. (2023). Phenotypic and genotypic detection of the virulence factors and their association with antibiotic resistance in Enterococcus species. bioRxiv, 2023-04.
16. Turan, D., & Gürler, B. (2009). Detection of virulence factors of Enterococcus faecalis isolated from the urinary system and evaluation of antibiotic resistance. Journal of Health Sciences and Medicine, 7(5), 543-548.
17. Bakhti, M., Akhondnezhad, M., Gholami, M., Nasrolahei, M., & Goli, H. R. (2021). Antibiotic resistance and virulence genes in Enterococcus faecalis isolated from human dental plaque. Infectious Diseases in Clinical Practice, 29(6), e366-e370.
18. Anderson, A. C., Jonas, D., Huber, I., Karygianni, L., Wölber, J., Hellwig, E., ... & Al-Ahmad, A. (2016). Enterococcus faecalis from food, clinical specimens, and oral sites: prevalence of virulence factors in association with biofilm formation. Frontiers in microbiology, 6, 1534.
19. Gorski, D. B., Vlainić, J., Škrlec, I., Novak, S., Novosel, Ž., Biloglav, Z., ... & Kosalec, I. (2024). Virulence Factors and Susceptibility to Ciprofloxacin, Vancomycin, Triclosan, and Chlorhexidine among Enterococci from Clinical Specimens, Food, and Wastewater. Microorganisms, 12(9), 1808.
20. Kumar, P., Kararia, N., & Raju, M. S. (2025). Comparative Evaluation of Herbal Extract and Chlorhexidine as Root Canal Irrigants Against Enterococcus Faecalis-An In vitro Study. Journal of Pharmacy and Bioallied Sciences, 17(Suppl 2), S1643-S1645.
21. Zhou, Q., Hu, Z., Du, L., Liu, F., & Yuan, K. (2020). Inhibition of Enterococcus faecalis growth and cell membrane integrity by Perilla frutescens essential oil. Foodborne pathogens and disease, 17(9), 547-554.
22. Narayanan, N., Sabour, J., Chiswell, B., & Weiland, M. (2024). Evaluation of Plant Essential Oils as Natural Alternatives for Alcohol-based Mouthwashes: Spotlight—Lemongrass and Citronella Java. European Journal of General Dentistry, 13(01), 060-068.
23. Alves, M. D. S., Medeiros, M. A. A. D., Santos, B., Simões, M. M., Farias, J. H. A. D., Pessôa, H. D. L. F., ... & Oliveira Filho, A. A. D. (2024). Evaluation of the antibacterial effect of (R)-(+)-Limonene against Enterococcus faecalis and Enterobacter cloacae strains isolated from food. Semina ciênc. agrar, 1201-1214.
24. Gniewosz, M., Krasniewska, K., Kosakowska, O., Pobiega, K., & Wolska, I. (2017). Chemical compounds and antimicrobial activity of petitgrain (Citrus aurantium L. var. amara) essential oil. Herba Polonica, 63(4).
25. Deans, S. G., & Svoboda, K. P. (1990). The antimicrobial properties of marjoram (Origanum majorana L.) volatile oil. Flavour and fragrance journal, 5(3), 187-190.
26. Abd El-Baky, R. M., & Hashem, Z. S. (2016). Eugenol and linalool: Comparison of their antibacterial and antifungal activities. African Journal of Microbiology Research, 10(44), 1860-1872.

Copyright: *©2025 Sebastian Such, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.*