In Vitro Antibiofilm Activity of Ethyl Acetate Fraction of Coriander Seeds (*Coriandrum sativum*) Against *Enterococcus faecalis* ATCC 29212

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ABSTRACT

Background: *Enterococcus faecalis is frequently associated with persistent root canal infections due to its ability to form resistant biofilms. However, evidence regarding the antibiofilm activity of specific plant-derived fractions, particularly the ethyl acetate fraction of coriander seeds (Coriandrum sativum), remains limited. This study aimed to evaluate the effect of different concentrations of this fraction on E. faecalis ATCC 29212 biofilm formation.*
Method: *Dried coriander seeds were extracted using ethanol and subsequently fractionated with ethyl acetate to obtain the active fraction. Biofilm inhibition was assessed using a 96-well polystyrene microplate assay after exposure to various concentrations. Calcium hydroxide was used as a control. Biofilm biomass was quantified by measuring optical density (OD). Each group was tested in five replicates (n=5). Statistical analysis was performed using one-way ANOVA followed by a Bonferroni post-hoc test.*
Result: *All tested materials exhibited inhibitory activity against E. faecalis biofilms. Calcium hydroxide showed the highest mean inhibition (69.41%), while the 16% concentration of coriander seed ethyl acetate fraction demonstrated the strongest activity among the experimental groups (64.89%). Statistical analysis revealed significant differences between concentration groups ($p < 0.05$).*
Conclusion: *The ethyl acetate fraction of coriander seeds demonstrated concentration-dependent antibiofilm activity against E. faecalis ATCC 29212 in vitro. Higher concentrations showed greater inhibitory effects, however, further studies are required to confirm its clinical applicability.*

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INTRODUCTION

Exposed pulp provides a pathway for microbial invasion, leading to inflammation and ultimately pulp necrosis. In this condition, the dental pulp undergoes complete degeneration and may be replaced by serous or purulent exudate or by dry necrotic tissue. Necrotic pulp tissue creates a favorable environment for bacterial colonization and biofilm formation within the root canal system.[1] Biofilms consist of microbial communities embedded within a self-produced extracellular polymeric matrix that enables strong bacterial adhesion to biological surfaces. This structured environment enhances bacterial survival, metabolic activity, and virulence, contributing to persistent root canal infections and increasing the risk of endodontic treatment failure.[2,3]

Among the microorganisms associated with persistent endodontic infections, *Enterococcus faecalis* is one of the most frequently isolated species, detected in approximately 67–77% of cases. This bacterium demonstrates remarkable adaptability, including the ability to penetrate dentinal tubules, survive under nutrient-limited conditions, and tolerate harsh environmental changes within the root canal system.[4,5] These characteristics allow *E. faecalis* to persist even after conventional endodontic treatment and contribute to treatment failure.

Calcium hydroxide is widely used as an intracanal medicament due to its antibacterial properties. However, its effectiveness against *E. faecalis*, particularly when the bacteria are organized in biofilm structures, remains limited. In addition, prolonged use of certain intracanal medicaments may cause undesirable effects such as cytotoxicity, unpleasant taste, and potential weakening of the dentin structure.[6,7] Therefore, the development of alternative antimicrobial agents that are effective against biofilm-forming bacteria is necessary.

Natural products have attracted increasing interest in dentistry because of their diverse therapeutic properties, including antimicrobial activity.[8] Coriander (*Coriandrum sativum*) seeds contain several bioactive compounds, particularly essential oils rich in linalool, which have demonstrated antimicrobial activity against both Gram-positive and Gram-negative bacteria. Previous studies have reported that coriander seed extract exhibits antibacterial activity against *E. faecalis* in its planktonic form.[9,10] Extraction using ethanol followed by fractionation with ethyl acetate, a semi-polar solvent, may concentrate semi-polar bioactive compounds such as linalool, potentially enhancing antibacterial efficacy.[11,12]

However, previous studies have primarily evaluated the antibacterial activity of coriander seed extract against planktonic *E. faecalis*, whereas root canal infections are predominantly associated with biofilm forming bacteria. Biofilm associated bacteria exhibit significantly greater resistance than planktonic cells, requiring higher concentrations of antimicrobial agents for effective inhibition.[13,14] Moreover, there is still limited evidence specifically addressing the antibiofilm activity of the ethyl acetate fraction of coriander seeds, which may contain concentrated semi polar bioactive compounds such as linalool with potential enhanced efficacy. Therefore, this study aimed to evaluate the antibiofilm activity of the ethyl acetate fraction of coriander seeds against *Enterococcus faecalis* ATCC 29212.

RESEARCH METHOD

This study employed a controlled laboratory-based in vitro experimental design to evaluate the antibiofilm activity of coriander (*Coriandrum sativum*) seed extract (ethyl acetate fraction) against *Enterococcus faecalis*. The study was conducted at the Integrated Research Laboratory, Faculty of Dentistry, Universitas Gadjah Mada, Indonesia, while extract preparation and fractionation were performed at the Pharmaceutical Extract

Standardization Laboratory, Faculty of Pharmacy, Universitas Muhammadiyah Surakarta. Coriander seeds used in this study were obtained from Boyolali Regency, Central Java, Indonesia. Ethical approval for this study was granted by the Ethics Committee of the Faculty of Dentistry, Universitas Gadjah Mada (No. 91/UN1/KEP/FKG-RSGM/EC/2023).

The selection of extract concentrations (4%, 8%, 12%, and 16%) was based on previous studies reporting the antimicrobial activity of coriander (*Coriandrum sativum*) extracts against *Enterococcus faecalis*, although the reported minimum inhibitory concentration (MIC) values vary depending on extraction methods and experimental conditions. A previous study reported MIC values in the range of 200–400 mg/mL for extracts against resistant microorganisms.[14] In general, antimicrobial activity of plant derived extracts against *E. faecalis* has been observed at relatively high concentrations, which may correspond to low percentage ranges depending on formulation. Furthermore, biofilm associated bacteria are known to exhibit significantly greater resistance than planktonic cells due to the presence of a protective extracellular matrix and altered metabolic activity, often requiring higher concentrations of antimicrobial agents for effective inhibition. Therefore, a range of concentrations above the reported antibacterial levels was selected to evaluate the antibiofilm activity in this study.

The preparation of coriander seed extract began with drying the seeds in a drying oven at 45°C, followed by grinding them into powder using a laboratory blender. A total of 1000 g of coriander seed powder was extracted using the maceration method with 70% ethanol as the solvent. The powder was mixed with 1000 mL of ethanol and homogenized for 30 minutes, then incubated for 24 hours. The mixture was filtered to remove solid residues, and the filtrate was concentrated using a vacuum evaporator to obtain the ethanolic extract. The extract was subsequently fractionated using ethyl acetate as a semi-polar solvent to isolate semi-polar bioactive compounds. The ethyl acetate fraction was collected and evaporated at room temperature for 48 hours to obtain the concentrated fraction. The resulting fraction served as the stock extract (100%). The extract was then diluted with sterile distilled water to obtain working concentrations of 4%, 8%, 12%, and 16% (v/v).

The bacterial strain used in this study was *Enterococcus faecalis* ATCC 29212 obtained from the bacterial culture collection of the Integrated Research Laboratory, Faculty of Dentistry, Universitas Gadjah Mada. The bacteria were cultured in 5 mL Brain Heart Infusion (BHI) broth and incubated at 37°C for 24 hours. The turbidity of the bacterial suspension was adjusted to the 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL.[15]

Biofilm inhibition was assessed using a 96-well flat-bottom polystyrene microtiter plate. Each well was inoculated with 20 µL of bacterial suspension and 148 µL of BHI broth. The treatment groups received 32 µL of coriander seed ethyl acetate fraction to obtain final concentrations of 4% (8 µL extract + 24 µL distilled water), 8% (16 µL extract + 16 µL distilled water), 12% (24 µL extract + 8 µL distilled water), and 16% (32 µL extract). The positive control group received 32 µL of a calcium hydroxide-glycerin mixture prepared in a 1:1 ratio (w/v) to simulate an intracanal medicament, while the negative control group received 32 µL of distilled water. The microplates were incubated at 37°C for 24 hours to allow biofilm formation.[16] Each experimental condition was performed in five independent replicates (n = 5).

Biofilm biomass was quantified using the crystal violet staining method. After incubation, non-adherent cells were removed by gently washing the wells twice with phosphate-buffered saline (PBS). The remaining biofilm was stained with 0.1% crystal violet solution and incubated at room temperature for 15 minutes. Excess

stain was removed by washing the wells twice with PBS. Subsequently, 200 µL of ethanol was added to each well to solubilize the bound crystal violet dye. The optical density (OD) was then measured at a wavelength of 540 nm using a microplate reader.[17,18] The percentage of biofilm inhibition was calculated using the following formula:

$$\%\text{biofil inhibition} = \frac{(\text{OD control } (-) - \text{OD Blank}) - (\text{OD sample} - \text{OD Blank})}{\text{OD control } (-) - \text{OD Blank}}\ x100\%$$

Statistical analysis was performed using IBM SPSS Statistics version 25. Data normality was assessed using the Shapiro–Wilk test, while homogeneity of variance was evaluated using Levene's test. Since the data were normally distributed and homogeneous, differences among groups were analyzed using one-way analysis of variance (ANOVA) with a significance level of $p < 0.05$. When significant differences were detected, pairwise comparisons were conducted using the Bonferroni post hoc test.

RESULTS

This study evaluated the inhibitory effect of different concentrations of coriander seed (*Coriandrum sativum*) ethyl acetate fraction on *Enterococcus faecalis* ATCC 29212 biofilm formation. Biofilm inhibition was quantified using the crystal violet microtiter plate assay, and optical density (OD) values were measured at a wavelength of 540 nm using a microplate reader. Each experimental group was tested in five replicates (n = 5). The obtained OD values were converted into percentages of biofilm inhibition relative to the negative control.

The mean percentage of biofilm inhibition for each experimental group is presented in Table 1. The positive control group (calcium hydroxide) demonstrated the highest inhibitory activity, with a mean inhibition value of 69.41 ± 5.40%. Among the treatment groups, the inhibitory effect increased progressively with increasing extract concentration. The 4% concentration showed minimal inhibition (6.33 ± 3.98%), while the 8% concentration demonstrated moderate inhibition (21.10 ± 7.63%). A substantial increase in antibiofilm activity was observed at the 12% concentration (51.63 ± 8.44%), and the 16% concentration exhibited strong inhibitory activity (64.89 ± 6.84%), approaching the effect observed in the calcium hydroxide group.

These findings indicate a concentration-dependent inhibitory effect of the ethyl acetate fraction of coriander seed extract against *E. faecalis* biofilm formation. A marked increase in inhibition was observed between the 8% and 12% concentrations, suggesting that higher extract concentrations substantially enhance antibiofilm activity. The comparative inhibition levels among the experimental groups are illustrated in Figure 1.

Table 1. Mean Percentage of Biofilm Inhibition of *E. faecalis* ATCC 29212

No	Sample	Mean % ± SD
1	CaOH	69.413 ± 5.395
2	CE 4%	6.328 ± 3.984
3	CE 8%	21.099 ± 7.631
4	CE 12%	51.631 ± 8.438
5	CE 16%	64.891 ± 6.843

CE = Coriander Extract

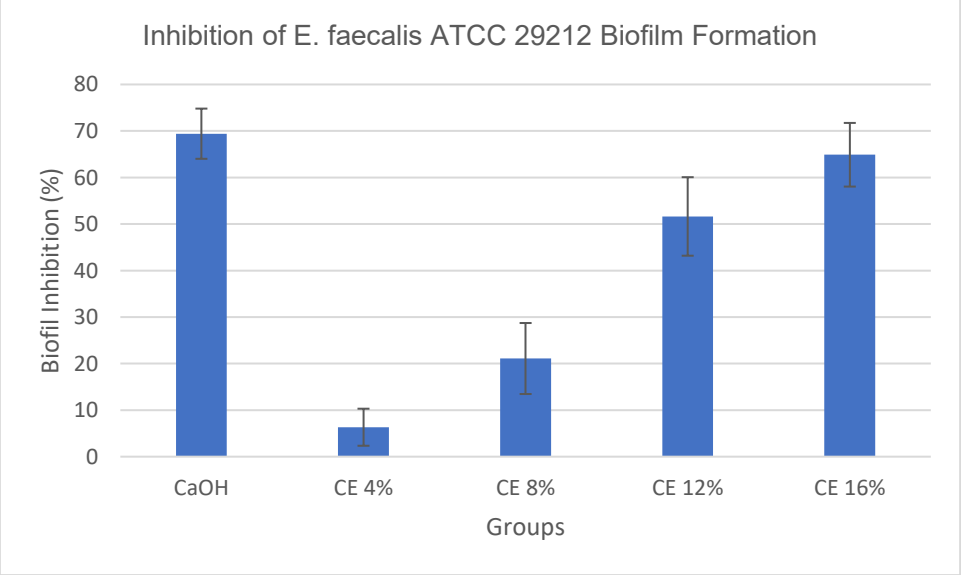

Figure 1. Inhibitory effect of coriander seed (*Coriandrum sativum*) ethyl acetate fraction on *Enterococcus faecalis* ATCC 29212 biofilm formation.

Prior to inferential statistical analysis, the assumptions of normality and homogeneity were assessed. The Shapiro-Wilk test confirmed that the data were normally distributed across all groups ($p > 0.05$), while Levene's test indicated homogeneity of variance among groups ($p > 0.05$). Based on these results, parametric analysis using one-way analysis of variance (ANOVA) was performed.

The one-way ANOVA revealed a statistically significant difference among the experimental groups ($p < 0.001$), indicating that extract concentration significantly influenced biofilm inhibition. To further identify differences between specific groups, Bonferroni post hoc analysis was conducted.

The post hoc results demonstrated no statistically significant difference between the 16% extract concentration and the calcium hydroxide group ($p = 0.817$), suggesting comparable inhibitory efficacy. However, significant differences were observed between the 16% concentration and the lower concentrations, including 12% ($p = 0.036$), 8% ($p < 0.001$), and 4% ($p < 0.001$). Additionally, the 12% concentration exhibited significantly greater inhibition than the 8% ($p < 0.001$) and 4% ($p < 0.001$) groups.

Table 2. Bonferroni Post Hoc Comparison of Biofilm Inhibition

Group	CE 4%	CE 8%	CE 12%	CE 16%	CaOH
CE 4%	–	0.017	<0.001	<0.001	<0.001
CE 8%		–	<0.001	<0.001	<0.001
CE 12%			–	0.036	0.003
CE 16%				–	0.817
CaOH					–

Significance level: $p < 0.05$

Overall, the results demonstrate that the ethyl acetate fraction of coriander seed extract exhibits significant antibiofilm activity against *E. faecalis* ATCC 29212, with inhibitory efficacy increasing proportionally with extract concentration. At the highest tested concentration (16%), the antibiofilm activity was statistically comparable to calcium hydroxide, indicating a strong inhibitory potential.

DISCUSSION

The present study demonstrated that the ethyl acetate fraction of coriander seed extract exhibited concentration-dependent inhibitory activity against *Enterococcus faecalis* ATCC 29212 biofilm formation. All tested concentrations (4%, 8%, 12%, and 16%) were able to reduce biofilm biomass, with the highest inhibition observed at the 16% concentration. This finding indicates that higher extract concentrations contain greater amounts of active phytochemical compounds capable of interfering with bacterial adhesion and early biofilm development. The progressive increase in inhibition across concentrations supports previous reports showing that increasing concentrations of coriander extract enhance antibacterial and antibiofilm activity against oral microorganisms.

Coriander (*Coriandrum sativum*) seeds are known to contain several bioactive phytochemicals, particularly monoterpenes such as linalool, geraniol, and camphor. Previous studies have reported that linalool is the dominant compound in coriander essential oil, typically representing approximately 60-80% of the total composition.[19,20] These phytochemical compounds have been widely associated with antimicrobial and antibiofilm activities.[21] The antibiofilm activity observed in this study may be primarily attributed to linalool, a monoterpene alcohol that has been reported to disrupt bacterial cell membrane integrity and alter membrane permeability, thereby impairing nutrient transport and metabolic activity.[22,23] In addition, linalool has been shown to interfere with bacterial motility, reduce cell surface hydrophobicity, and affect quorum sensing pathways involved in bacterial communication, which are critical in the early stages of biofilm formation.[24] Recent studies have further demonstrated that linalool exerts antibiofilm effects through multiple mechanisms, including modulation of gene expression related to metabolic pathways and stress responses.[23] A previous study reported that linalool was able to inhibit biofilm formation by approximately 77.15%–83.21%, primarily through reduction of extracellular polymeric substance (EPS) production, decreased cell surface hydrophobicity, and inhibition of bacterial motility.[24] These findings are consistent with the present study, in which a concentration-dependent reduction in biofilm biomass was observed, with the highest inhibition at 16% (64.89%). The slightly lower inhibition observed in this study may be attributed to differences in bacterial species, extract composition, and experimental conditions. Overall, these results support the role of linalool as a key contributor to the antibiofilm activity of the ethyl acetate fraction of coriander seed extract against *Enterococcus faecalis*.

Other constituents such as geraniol and camphor may also contribute to the overall antibacterial activity of coriander extract.[25] Geraniol has been reported to disrupt bacterial membranes, reduce extracellular polysaccharide (EPS) production, and decrease cell surface hydrophobicity, which are important factors in biofilm maturation and structural stability. Although camphor also exhibits antimicrobial activity, its antibacterial effect is generally considered weaker compared with other monoterpenes.[26] The combined activity of these phytochemical compounds may produce a synergistic effect that enhances the overall antibiofilm potential of coriander seed extract.[27,28]

The antibiofilm activity observed in this study is consistent with previous research demonstrating the antimicrobial potential of herbal essential oils containing linalool against endodontic pathogens. Several investigations have reported that plant-derived essential oils exhibit inhibitory effects against *E. faecalis*, a bacterium frequently associated with persistent root canal infections. However, when compared with conventional endodontic antimicrobial agents such as chlorhexidine and sodium hypochlorite, herbal extracts often demonstrate variable levels of antibacterial efficacy depending on the experimental conditions.[29] For

example, some essential oils rich in linalool have shown antibacterial activity comparable to sodium hypochlorite in certain microbial models but remain less effective than chlorhexidine in others.[30] Despite these differences, plant-derived compounds may offer potential advantages, including lower toxicity, reduced adverse effects, and natural bioactive properties that may support their development as adjunctive intracanal medicaments.[31,32]

In the present study, calcium hydroxide was used as a positive control and showed the highest percentage of biofilm inhibition. The antibacterial activity of calcium hydroxide is primarily attributed to its strong alkaline pH (approximately 12.5-12.8), which results in the release of hydroxyl ions capable of damaging bacterial cell membranes, denaturing proteins, and disrupting bacterial DNA.[33] Statistical analysis indicated that the 16% concentration of coriander extract showed no significant difference compared with calcium hydroxide, suggesting that higher concentrations of the extract may achieve antibiofilm effects approaching those of commonly used intracanal medicaments. However, despite its strong antibacterial properties, *E. faecalis* has been reported to exhibit resistance to calcium hydroxide. This resistance is attributed to its ability to survive in highly alkaline environments by maintaining intracellular pH homeostasis through proton pump activity and adaptive stress response mechanisms.[34] Additionally, the presence of biofilm structures further enhances bacterial tolerance by limiting the penetration of hydroxyl ions. These factors may explain why alternative or adjunctive antimicrobial agents are required to effectively eliminate persistent endodontic infections.

Biofilm biomass in this study was quantified using the crystal violet assay, a widely used method for measuring total biofilm mass. Crystal violet is a positively charged dye that binds to negatively charged components within the biofilm matrix, including bacterial cell walls and extracellular polymeric substances. The optical density values obtained are therefore proportional to the total biofilm biomass attached to the surface. However, it is important to note that the crystal violet assay measures total biofilm biomass rather than distinguishing between viable and non-viable bacterial cells. Consequently, reductions in optical density reflect decreases in biofilm mass but do not directly indicate bacterial killing. Additional techniques, such as viable cell counting or metabolic activity assays, may provide more comprehensive information regarding bacterial viability within the biofilm.[35]

Several limitations should be considered when interpreting the results of this study. First, the experiment was conducted in vitro using a single-species biofilm model, whereas root canal infections are typically polymicrobial and involve complex microbial interactions. Second, the study focused on biofilm biomass inhibition and did not evaluate bacterial viability, gene expression, or cytotoxic effects on host tissues. Third, the crystal violet assay does not differentiate between live and dead cells within the biofilm matrix. Therefore, further investigations are necessary to evaluate the antimicrobial mechanisms, cytotoxicity, and biocompatibility of coriander seed extract before its potential application as an intracanal medicament can be considered.

Overall, the findings of this study demonstrate that the ethyl acetate fraction of coriander seed extract exhibits significant concentration dependent antibiofilm activity against *E. faecalis* ATCC 29212. At higher concentrations, its inhibitory effect approaches that of calcium hydroxide, suggesting its potential as a natural adjunctive agent in endodontic therapy. However, considering the limitations of this in vitro study, further investigations involving multispecies biofilm models, cytotoxicity evaluation, and in vivo studies are necessary to validate its clinical applicability and safety.

CONCLUSION

The results of this investigation indicate that the ethyl acetate fraction of coriander seed extract effectively suppresses the development of Enterococcus faecalis ATCC 29212 biofilms at all evaluated concentrations (4%, 8%, 12%, and 16%). Furthermore, the inhibitory response followed a concentration-dependent trend, where higher concentrations yielded more substantial reductions in biofilm biomass.

Among the tested groups, the 16% concentration demonstrated the highest antibiofilm activity and showed no statistically significant difference compared with calcium hydroxide. These findings suggest that coriander seed extract may represent a promising natural source of antibiofilm compounds against *E. faecalis*.

Further research is recommended to evaluate its effectiveness in multispecies biofilm models, assess cytotoxicity, and investigate its clinical applicability.

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REFERENCES

1. Anand P, Kasarla RR. Role of *Enterococcus faecalis* in endodontic treatment failure. Med J East Nepal. 2024;3(2):27-29. https://doi.org/10.3126/mjen.v3i2.73052
2. Liu RZ, Bai LA, Luo Y, Liu P, Hua F, Fan W, Huang D, Chen Y, Zhang C. The presence of *Enterococcus* in root canal infections based on next-generation sequencing: a systematic review and meta-analysis. Int Endod J. 2025;58(9):1331-1353. https://doi.org/10.1111/iej.14266
3. Yang S, Meng X, Zhen Y, Baima Q, Wang Y, Jiang X, Liu Y, Zhang H. Strategies and mechanisms targeting *Enterococcus faecalis* biofilms associated with endodontic infections: a comprehensive review. Front Cell Infect Microbiol. 2024;14:1433313. https://doi.org/10.3389/fcimb.2024.1433313
4. Pratiwi RD, Kuswandari S, Utomo RB. Antibacterial effect of ethanol extract of *Moringa oleifera* seeds against *Enterococcus faecalis* ATCC 29212. Odonto Dent J. 2023;10(2):180-187. https://doi.org/10.30659/odj.10.2.180-187
5. Sharma J, Jhamb S, Mehta M, Bhushan J, Bhardwaj SB, Kaur A. Characterization of *Enterococcus faecalis* associated with root canal failures: virulence and resistance profile. J Conserv Dent Endod. 2025;28(7):602-606. https://doi.org/10.4103/JCDE.JCDE_190_25
6. Diouchi J, Marinković J, Nemoda M, El Rhaffari L, Toure B, Ghoul S. 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 Protoc. 2024;7(4):50. https://doi.org/10.3390/mps7040050
7. Wu B, Zhou Z, Hong X, Xu Z, Xu Y, He Y, Li J, Chen L. Novel approaches on root canal disinfection methods against *Enterococcus faecalis*. Pathogens. 2025;17(1):2475947. https://doi.org/10.1080/20002297.2025.2475947
8. Duggal S, Chandrika P, Nasyam F, Singh D, Indira A, Anand C. Herbal dentistry: nurturing oral health with natural remedies. J Pharm Bioallied Sci. 2024;16:S1932-S1934. https://doi.org/10.4103/jpbs.jpbs_18_24
9. Ahmed FBM, Alla AEJ. Effectiveness of *Coriandrum sativum* extract as antimicrobial by applying on six bacterial strains. J Dis Med Plants. 2019;5(6):103-106. https://doi.org/10.11648/j.jdmp.20190506.13
10. Al-Khayri JM, Banadka A, Nandhini M, Nagella P, Al-Mssallem MQ, Alessa FM. Essential oil from *Coriandrum sativum*: a review on its phytochemistry and biological activity. Molecules. 2023;28(2):696. https://doi.org/10.3390/molecules28020696
11. Dewi K, Prasetyo A, Nugroho W. Effect of different solvents in Soxhlet extraction on the characteristics of basil oil (*Ocimum americanum* L.). J Bahan Alam Terbarukan. 2023;12(1):63-69. https://doi.org/10.15294/jbat.v12i1.41903

12. Prismawan D, Michael, Gunawan GY, Kambira PFA. Antibacterial property evaluation of ethanolic extract of *Plantago major* L. and its fractions. J Riset Sains Teknol. 2025. https://doi.org/10.30595/jrst.v9i1.26388
13. Mokhtari F, Modaresi J, Salmasi AH, Khorshidi H, Yazdani J. In vitro comparison of *Enterococcus faecalis* survival in dentinal tubules following root canal therapy with different sealers. BMC Oral Health. 2024;24:1488. https://doi.org/10.1186/s12903-024-05192-8
14. Afrin S, Goswami B, Rahman S, Bhuiyan MNI, Shamima KAA, Nandi NC. Antimicrobial potential of *Coriandrum sativum*, *Lactuca sativa* and *Mentha spicata* against antibiotic resistant microorganisms. J Herbs Spices Med Plants. 2022;28(2):193-205. https://doi.org/10.1080/10496475.2022.2040684
15. Agarwal A, Gupta N, Gupta A, Saxena P, Sharma P. Evaluation of antimicrobial efficacy of intracanal medicaments against *Enterococcus faecalis*. J Clin Diagn Res. 2016;10(6):ZC71-ZC77. https://doi.org/10.7860/JCDR/2016/17911.8021
16. Dewi M, Darmawi, Nurliana, Karmil TF, Helmi TZ, Fakhrurrazi. Antibiotic activities to *Staphylococcus aureus* biofilms of Aceh cattle preputium isolate. J Sain Veteriner. 2020;38(1):1-6. https://doi.org/10.22146/jsv.35219
17. Stephens S, Mahadevan R, Allen DG. Establishing a quantitative link between crystal violet absorbance and biomass in biofilms. MethodsX. 2025;15:103630. https://doi.org/10.1016/j.mex.2025.103630
18. Adusei EBA, Adosraku RK, Kyekyeku JO, Amengor CDK, Jibira Y. Resistance modulation action, time-kill kinetics assay, and inhibition of biofilm formation effects of plumbagin from *Plumbago zeylanica* Linn. J Trop Med. 2019;2019:1250645. https://doi.org/10.1155/2019/1250645
19. Duarte A, Luís Â, Oleastro M, Domingues FC. Antioxidant properties of coriander essential oil and linalool and their potential to control *Campylobacter* spp. Food Control. 2016;61:115-122. https://doi.org/10.1016/j.foodcont.2015.09.024
20. Wibowo DP, Nuari DA, Apionita S, Mariani R. Antimicrobial activity of coriander (*Coriandrum sativum* Linn.): a review. Trop J Nat Prod Res. 2023;7(5):2844-2858. https://doi.org/10.26538/tjnpr/v7i5.1
21. Guo F, Chen Q, Liang Q, Zhang M, Chen W, Chen H, Yan Z. Antimicrobial activity and proposed action mechanism of linalool against *Pseudomonas fluorescens*. Front Microbiol. 2021;12:562094. https://doi.org/10.3389/fmicb.2021.562094
22. Nouhaila Z, Naima E, Soumya E, Saad IKA. A comprehensive review on medicinal plant extracts as antibacterial agents: factors, mechanism insights and future prospects. Sci Afr. 2024;26:e02395. https://doi.org/10.1016/j.sciaf.2024.e02395
23. Li Y, Ren F, Chen D, Chen H, Chen W. Antibacterial mechanism of linalool against *Pseudomonas fragi*: a transcriptomic study. Foods. 2022;11(14):2058. https://doi.org/10.3390/foods11142058
24. Shen G, Yang L, Lv X, Zhang Y, Hou X, Li M, Li Y. Antibiofilm activity and mechanism of linalool against *Bacillus amyloliquefaciens*. Int J Mol Sci. 2023;24(13):10980. https://doi.org/10.3390/ijms241310980
25. Salinas C, Florentín G, Rodríguez F, Alvarenga N, Guillén R. Terpene combinations inhibit biofilm formation in *Staphylococcus aureus* by interfering with initial adhesion. Microorganisms. 2022;10(8):1527. https://doi.org/10.3390/microorganisms10081527
26. He R, Zhang Z, Xu L, Chen W, Zhang M, Zhong Q. Antibacterial mechanism of linalool emulsion against *Pseudomonas aeruginosa*. World J Microbiol Biotechnol. 2022;38:56. https://doi.org/10.1007/s11274-022-03233-4
27. Kannappan A, Sivaranjani M, Srinivasan R, Rathna J, Pandian SK, Ravi AV. Inhibitory efficacy of geraniol on biofilm formation and development of adaptive resistance in *Staphylococcus epidermidis* RP62A. J Med Microbiol. 2017;66(10):1506-1516. https://doi.org/10.1099/jmm.0.000597
28. Rai M, Mohanalakshmi V, Rajashree R, Renuka P, Meenakshi. Exploring the leafy coriander (*Coriandrum sativum* L.): a comprehensive review on metabolomics properties. S Afr J Bot. 2025;181:258-271. https://doi.org/10.1016/j.sajb.2025.04.017
29. Nagy-Bota MC, Man A, Santacroce L, Brinzaniuc K, Pap Z, Pacurar M, Moldovan M. Essential oils as alternatives for root-canal treatment and infection control against *Enterococcus faecalis*: a preliminary study. Appl Sci. 2021;11(4):1422. https://doi.org/10.3390/app11041422
30. Teja KV, Janani K, Kaligotla VA, Harini K. Comparative antimicrobial efficacy of oregano oil, chlorhexidine, and sodium hypochlorite against *Enterococcus faecalis*: an in vitro study. Endodontology. 2021;33(2):97-101. https://doi.org/10.4103/endo.endo_124_20
31. Lahiri D, Nag M, Dutta B, Dey S, Mukherjee S, Joshi SJ, Mukherjee A. Antibiofilm and anti-quorum sensing activities of eugenol and linalool from *Ocimum tenuiflorum* against *Pseudomonas aeruginosa* biofilm. J Appl Microbiol. 2021;131(6):2821-2837. https://doi.org/10.1111/jam.15171
32. Islam AA, Cheung BPK, Matinlinna JP, Lévesque CM, Neelakantan P. Trans-cinnamaldehyde potently kills *Enterococcus faecalis* biofilm cells and prevents biofilm recovery. Microb Pathog. 2020;149:104482. https://doi.org/10.1016/j.micpath.2020.104482

33. Besan EJ, Rahmawati I, Saptarini O. Antibiofilm activity of butterfly pea flower (*Clitoria ternatea* L.) extract and fractions against *Staphylococcus aureus*. Pharmacy. 2023;20(1):1-11. https://doi.org/10.15416/ijcp.2023.20.1.1
34. Reyhani MF, Ghasemi N, Milani AS, Abbasi Asl M. Antimicrobial Effect of Nano-Calcium Hydroxide on the Four- and Six-Week-Old Intra-Canal *Enterococcus Faecalis* Biofilm. J Dent (Shiraz). 2023 Jun;24(2):194-199. doi: 10.30476/dentjods.2022.94792.1812. PMID: 37388199; PMCID: PMC10300140.
35. Azeredo J, Azevedo NF, Briandet R, Cerca N, Coenye T, Costa AR, Desvaux M, Di Bonaventura G, Hébraud M, Jaglic Z, Kačániová M, Knøchel S, Lourenço A, Mergulhão F, Meyer RL, Nychas G, Simões M, Tresse O, Sternberg C. Critical review on biofilm methods. Crit Rev Microbiol. 2017;43(3):313-351. https://doi.org/10.1080/1040841X.2016.1208146