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Comparative analysis of cinnamaldehyde with conventional endodontic irrigants against *Enterococcus faecalis* surface protein receptor for targeted biofilm inhibition: an in-silico docking study

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Aim: Enterococcus faecalis biofilm-mediated root canal infections are a significant challenge in endodontics due to their resistance to conventional disinfection, often leading to treatment failure. This study aims to evaluate the inhibitory potential of cinnamaldehyde, a phtyochemical from cinnamon, on the *E. faecalis* surface protein (Esp) receptor, using computational docking simulations and compare it with standard disinfectants, sodium hypochlorite (NaOCl) and chlorhexidine (CHX)
Materials and methods: In-silico docking simulations were performed to assess the binding affinities, amino acid interactions, and binding energies of cinnamaldehyde, NaOCl, and CHX with the Esp receptor.
Results: CHX showed the highest binding affinity (-11.83 kcal/mol) with diverse amino acid interactions. Cinnamaldehyde exhibited moderate binding energy (-5.5 kcal/mol) with targeted interactions involving HIS, VAL, TYR, and ALA. NaOCl had the lowest binding affinity (-0.93 kcal/mol), likely due to fewer interactions. However, NaOCl's known clinical effectiveness is supported by other mechanisms than receptor binding, such as hypochlorous acid formation and a high pH.
Conclusion: The findings underscore CHX's potential as an Esp receptor inhibitor. Cinnamaldehyde, as a natural alternative, shows promise with targeted interactions. Although NaOCl's in-silico affinity is lower, its established clinical efficacy and alternative mechanisms affirm its value in root canal disinfection. These findings highlight the potential of integrating natural alternatives like cinnamaldehyde to complement conventional disinfection strategies against biofilms in endodontic protocols.

Keywords: Enterococcus faecalis, Esp receptor, Cinnamaldehyde, endodontic irrigants, root canal disinfection

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Introduction

Root canal infections represent a significant and persistent challenge in the field of endodontics, often causing severe discomfort for the patient and posing a threat to overall oral health. Among the pathogens responsible for these infections, Enterococcus faecalis is particularly notorious. E. faecalis, a facultative anaerobic gram positive coccus is one of the most commonly found species in persistent root canal infections, being present in 30-89% of cases of post-endodontic treatment failures, often as monoculture. [1] It shows remarkable resilience to harsh conditions and capacity to form robust biofilms within the intricate root canal system. [2,3] Biofilms are complex structured communities of microorganisms embedded within a self-produced extracellular matrix. In the context of root canal infections, E. faecalis biofilms present a multifaceted challenge[4]. The ability of E. faecalis to establish biofilms is a primary factor contributing to the persistence of root canal infections as these biofilms offer protection to the enclosed microorganisms, making them highly resistant to the host immune responses and conventional antimicrobial agents used in endodontic treatment.[5,6]

The initial step in the formation of E. faecalis biofilms is the adherence of the bacterium to the root canal walls followed by colonization. This adherence is mediated by specific surface proteins, with the Enterococcus faecalis surface protein (Esp) receptor playing a central role.[7–9] The Esp receptor functions as an adhesin, facilitating the initial attachment of E. faecalis to host tissues and the subsequent colonization of the root canal system with the formation of biofilms.[10,11] Thus, targeting the Esp receptor offers a promising strategy for the prevention and treatment of root canal infections. In recent years, research efforts have focused on identifying compounds capable of inhibiting the Esp receptor, thereby interfering with the initial stages of biofilm formation.

While conventional disinfectants such as sodium hypochlorite (NaOCl) and chlorhexidine (CHX) have played pivotal roles as endodontic irrigants, the pursuit of more targeted and effective inhibitors continues. Phytochemicals or natural compounds derived from plants, have emerged as promising candidates for this purpose. [12,13] Recent research has highlighted the antimicrobial[12,13] properties of various phytochemicals and their capacity to interact with specific bacterial receptors.[14,15] Among these phytochemicals, cinnamaldehyde, a major constituent of cinnamon that is responsible for its taste and aroma, has garnered attention for its potential antimicrobial activity. It possesses a well-documented history of use as antimicrobial agent against a variety of pathogens,[16–19] suggesting its suitability to offer a sustainable and effective alternative for applications in root canal disinfection. However, the specific interactions between cinnamaldehyde and the Esp receptor, as well as its comparative efficacy against established disinfectants like NaOCl and CHX, remain subjects of investigation.

To address this challenge, this study aims to employ molecular docking simulations to assess the binding affinities and specific amino acid interactions of cinnamaldehyde, NaOCl and CHX, with the Esp receptor. This methodology provides a cost effective and efficient platform to assess potential of cinnamaldehyde as an Esp receptor inhibitor and its suitability for root canal disinfection.

Materials and methods

Protein preparation

Selection of protein structures**:** The crystallographic structure of the Enterococcus faecalis surface protein (Esp) receptor was retrieved from the Protein Data Bank (PDB) database [PDB ID: 6ORI]. To ensure data integrity, the structure with the highest resolution and overall structural quality was selected for subsequent downstream analysis.

Cleaning and optimisation**:** The selected crystal structure of the Esp receptor was cleaned by eliminating water molecules and any co-crystallized ligands to isolate the protein of interest. The structure was then optimized using the molecular modeling software AutoDockTools 1.5.6 to minimize steric clashes and enhance overall structural integrity.

Adding hydrogen atoms and assigning partial charges**:** Hydrogen atoms were added to the protein structure using Chimera, and their positions were optimized through energy minimisation to ensure realistic hydrogen bonding geometries. Partial charges were assigned to the protein atoms to accurately represent the electrostatic properties. Force field parameters consistent with the chosen docking software were applied.

Ligand preparation

Cinnamaldehyde**:** Cinnamaldehyde (C_9H_8O), the phytochemical compound from cinnamon was chosen as the potential inhibitor for the present study. The three dimensional structure of cinnamaldehyde was retrieved from the PubChem database (PubChem CID: 637511) in PDB format. This structure was further optimized using AutoDockTools 1.5.6 software. Energy minimization was performed to achieve a stable and accurate three dimensional conformation for docking.

Sodium hypochlorite (NaOCl) and Chlorhexidine (CHX)**:**

Sodium hypochlorite (NaOCl) and chlorhexidine (CHX), conventional root canal irrigants, were included in the study for comparative analysis. The three-dimensional structures of NaOCl and CHX were retrieved from the PubChem database (PubChem CID: 23665760 and 9552079) and optimized using the same protocol applied to cinnamaldehyde.

Grid generation and molecular docking simulations**:** A three-dimensional grid was generated around the active site of the Esp receptor using the AutoGrid module in AutoDockTools 1.5.6 program. The grid dimensions were configured to encompass relevant amino acid residues involved in ligand binding, ensuring a comprehensive sampling of potential binding sites. In-silico molecular docking simulations were performed employing and the following docking parameters were used:

- Multiple docking runs to explore various binding conformations.
- Ligand flexibility was considered to account for potential conformational changes (flexible ligand docking).
- Exhaustiveness parameter set to an appropriate level to ensure a thorough exploration of binding modes

Analysis of docking results: Docking results were analyzed using AutoDock Tools 1.5.6 program. The following analyses were undertaken:

- Visualization of docking poses of cinnamaldehyde, NaOCl, and CHX within the Esp receptor's active site to assess binding modes and orientations.
- Calculation of docking scores/binding affinity values (kcal/mol) for each ligand to quantify their interaction strength with the Esp receptor
- Identification of key amino acid residues within the active site involved in ligand binding and their interaction patterns.

Results

The results obtained by molecular docking analysis are reported in **Figure 1, 2** and **Table 1.** The present in-silico docking study aimed to investigate the binding affinities of cinnamaldehyde towards the Enterococcus faecalis surface protein (Esp) receptor of E.faecalis. In particular, the study also investigated the binding affinity of two conventional endodontic irrigants, sodium hypochlorite (NaOCl) and chlorhexidine (CHX) toward Esp receptor for comparison. The binding energy for the stable docking poses for cinnamaldehyde, CHX, NaOCl was calculated as -5.5 kcal/mol, -11.83 kcal/mol and -0.93 kcal/mol respectively. These binding energies indicate the strength of interaction between each compound and the Esp receptor representing the thermodynamic stability of the ligand-

receptor complexes. A more negative value suggests a stronger binding affinity.

The amino acid interactions were analyzed using a two-dimensional representation (Figure 1, 2). The specific amino acid residues involved in the interactions between each compound and the Esp receptor were identified and reported in Table 1 Chlorhexidine displayed diverse interactions with tyrosine (TYR), phenylalanine (PHE), histidine (HIS), methionine (MET), leucine (LEU), arginine (ARG) and glutamine (GLU) residues. Cinnamaldehyde also exhibits specific interactions involving HIS, valine (VAL), TYR, and alanine (ALA), suggesting targeted inhibition. NaOCl primarily interacted with the HIS residue, indicating weaker binding patterns.

Table 1. Data obtained by molecular docking protocols with the AutoDock server.

Ligands	Docking scores (Binding affinity values in kcal/mol)	Amino acid interaction between the ligand & Esp receptor
Cinnamaldehyde	-5.5 kcal/mol	HIS, VAL, TYR, ALA
Chlorhexidine	-11.83 kcal/mol	TYR, PHE, HIS, TYR (second occurrence), MET, LEU, ARG, LEU, and GLU
Sodium hypochlorite	-0.93 kcal/mol	HIS

HIS (Histidine), VAL (Valine), TYR (Tyrosine), ALA (Alanine), PHE (Phenylalanine), MET (Methionine), LEU (Leucine), ARG (Arginine), LEU (Leucine), GLU (Glutamic Acid).

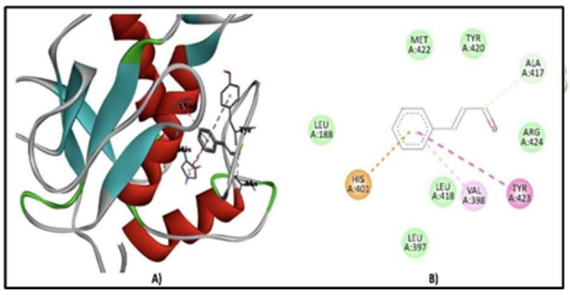

Figure 1. Molecular docking interaction between cinnamaldehyde and E. faecalis surface protein (Esp) receptor. A) 3D docked pose of cinnamaldehyde with Esp receptor obtained with the AutoDock server. B) 2D docked pose representing interaction types of cinnamaldehyde with Esp receptor.

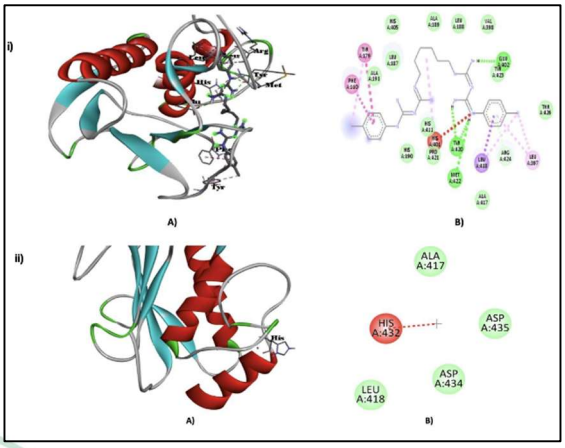

Figure 2. i) Molecular docking interaction between chlorhexidine (CHX) and E. faecalis surface protein (Esp) receptor. A) 3D simulation of the docked pose of CHX with Esp receptor obtained with the AutoDock server. B) 2D representation of ligand interaction types of CHX with Esp receptor. ii) Molecular docking interaction between sodium hypochlorite (NaOCl) and E. faecalis surface protein (Esp) receptor. A) 3D simulation of the docked pose of NaOCl with Esp receptor obtained with the AutoDock server. B) 2D representation of ligand interaction types of NaOCl with Esp receptor.

Discussion

Root canal infections are often associated with biofilm formation,[1,6] which confers resistance to conventional disinfection methods, contributing to treatment failures.[5] The physiological and functional characteristics of bacteria in biofilms distinguish them from planktonic bacteria. Biofilm bacteria exhibit reduced metabolic activity and altered physiology, with the biofilm structure creating a physical barrier that limits the penetration of antimicrobial agents into its deeper layers. Consequently, bacteria within biofilms develop heightened tolerance to antibiotics and increased resistance to host immune responses. With the escalating concern over antibiotic resistance, there is an urgent need to develop novel strategies capable of inhibiting biofilm formation.[20] The Enterococcus faecalis surface protein (Esp) receptor has emerged as a key player in biofilm formation and persistence,[7,8] making it an attractive target for therapeutic intervention.

Throughout history, natural plant products, particularly phytochemicals and their derivatives, have served as significant sources of effective therapeutic agents and are increasingly considered as alternatives to conventional antibacterial agents. Their benefits include cost-effectiveness, widespread availability from sources like fruits, seeds, and vegetables, low cytotoxicity, diverse chemical composition, and specificity, along with a reduced tendency to develop antibiotic resistance.[20,21] Cinnamaldehyde, an aromatic aldehyde derived from cinnamon essential oil, is commonly utilized as a flavoring and aromatic agent in various industries. It has demonstrated efficacy against both Gram-positive and Gram-negative bacterial biofilms, including those formed by Pseudomonas aeruginosa, Staphylococcus aureus and enterococcus faecalis.[17,19,22,23]

In this study, in-silico docking simulations were performed to investigate the potential of a phytochemical compound - cinnamaldehyde, as an Esp receptor inhibitor. The choice of an in-silico approach allowed for a rapid and cost-effective assessment. The valuable structural insights provided can be a powerful tool for preliminary screening [24] and comparative analysis with traditional endodontic irrigants such as CHX and NaOCl. This may aid in the development of targeted compounds and further testing using in vitro or in vivo models for root canal disinfection especially against persistent biofilm forming bacteria. [25]

Binding affinities, Amino acid interactions and relative efficacy:

The binding energies obtained in the study revealed crucial insights into the comparative efficacy of cinnamaldehyde, NaOCl, and CHX as Esp receptor inhibitors. Chlorhexidine (CHX) displayed the highest binding affinity with a binding energy of -11.83 kcal/mol. This exceptionally negative value indicates a robust and energetically favorable interaction between CHX and the Esp receptor. These results of CHX's binding affinity align with the established efficacy of CHX as an antimicrobial agent in endodontics, suggesting its potential for inhibiting E. faecalis biofilm formation in line with various in vitro studies.[26,27] The docking simulations provided detailed insights into the specific amino acid interactions between the compounds and the Esp receptor. Chlorhexidine (CHX) engaged a diverse array of amino acid residues, including tyrosine (TYR), phenylalanine (PHE), histidine (HIS), methionine (MET), leucine (LEU), arginine (ARG) and glutamic acid (GLU). These interactions encompassed aromatic, polar, and aliphatic residues, highlighting the versatility and robust binding profile of CHX. TYR and PHE participated in pi-pi interactions, while HIS and GLU likely engaged in hydrogen bonding, illustrating CHX's multifaceted inhibition potential

Cinnamaldehyde, a natural phytochemical, displayed a binding energy of -5.5 kcal/mol. While this value is less negative than that of CHX, it signifies a favorable interaction with the Esp receptor. Notably, the specificity of Cinnamaldehyde's interactions with specific amino acids, such as HIS, valine (VAL), TYR, and alanine (ALA), suggests a targeted mode of action. These suggest potential hydrogen bonding and pi-pi stacking interactions, indicative of a targeted mode of action. The inclusion of HIS and TYR in the interaction network underscores the significance of aromatic and polar interactions in cinnamaldehyde's binding profile, meriting further investigation of its potential as a focused inhibitor.

In contrast, sodium hypochlorite (NaOCl), a widely used root canal irrigant, displayed the weakest binding affinity with a considerably lower binding energy of -0.93 kcal/mol. This result indicates a less effective interaction between NaOCl and the Esp receptor. An unfavorable donor-donor bond was observed between NaOCl and the amino acid histidine (HIS). Thus, the binding affinity of NaOCl to the receptor appears to be weaker compared to

Cinnamaldehyde and CHX. This weaker binding may arise from various factors, including computational limitations inherent in docking simulations, possible steric hindrance in the binding site, and potential electrostatic repulsion due to charge distribution.

Implications for root canal disinfection

The implications of these findings for root canal disinfection strategies are significant, especially in the context of combating persistent infections due to E. faecalis biofilms. Chlorhexidine (CHX) stands out as a highly promising Esp receptor inhibitor. Its strong binding affinity and versatile interaction network suggest effectiveness in preventing biofilm formation and potentially eradicating established biofilms. CHX's well-documented antimicrobial properties align with the findings of the present study, solidifying its status as a compelling choice for antibacterial agent for root canal disinfection. It also has substantive action due to its unique ability to adhere to root dentine. However, in spite of being used routinely as a final irrigant, CHX has cytotoxic effects on periapical tissues, can cause tooth discolouration and forms flocculate on interaction with NaOCl.[28]

Sodium Hypochlorite (NaOCl), commonly employed in endodontics, exhibited a lower binding affinity in our study. While these findings raise questions about NaOCl's binding affinity in-silico, it's crucial to interpret them cautiously, considering that NaOCl is a well-established and clinically effective root canal irrigant. Further experimental validation is essential to confirm the true nature and strength of NaOCl's interactions with the Esp receptor in a real biological system. Also, NaOCl may have alternative mechanisms that can affect E.faecalis other than receptor inhibition. These may include formation of hypochlorous acid, high pH, neutralization of amino acids, saponification reaction, solvent action and cell lysis.[29] NaOCl acts primarily through the generation of hypochlorous acid which is a potent oxidizing agent that disrupts bacterial cell membranes by oxidizing essential components such as lipids, proteins, and nucleotides, leading to cell lysis and death. This oxidative stress damages bacterial cell walls and significantly disrupts biofilm structures. The high pH denatures bacterial proteins, including key enzymes and structural components, rendering them non-functional. This protein denaturation extends to the extracellular polymeric substance (EPS) matrix of biofilms, which is vital for the structural integrity and protective nature of biofilms, thereby disrupting biofilms at multiple levels. Moreover, NaOCl dissolves organic tissue by breaking down proteins into smaller peptides through saponification and amino acid degradation, a property that is especially valuable in the cleaning of necrotic tissue and pulp remnants from the root canal system. This broad-spectrum mechanism makes NaOCl effective not only against E. faecalis biofilms but also against a wide range of bacteria and fungi commonly found in infected root canals. Clinically, NaOCl's ability to penetrate dentinal tubules and reach areas inaccessible to mechanical instrumentation is another crucial aspect of its effectiveness especially in cases where necrotic tissue is deeply embedded within the canal system.[5,30,31] Although its receptor binding affinity may appear limited in computational models, its overall disinfection ability in vivo is significantly enhanced by these chemical properties and actions. This explains why NaOCl remains the most clinically effective irrigant due to its multi-pronged mechanism of antibacterial action, which is not solely dependent on receptor inhibition. Though its effectiveness against well established biofilms may be inferior to that against planktonic cells, several methods such as increasing the contact time or the concentration of NaOCl and activation[32] may give more positive outcomes as shown by previous research. Gomes et al. concluded that among various concentrations of sodium hypochlorite,

5.25% solution exhibited the highest efficacy as an irrigant.[31] In a separate investigation by Van der Waal et al., a hyperosmotic sodium hypochlorite solution demonstrated substantial antibiofilm activity after a 10-minute exposure.[33] However, the use of sodium hypochlorite is accompanied by notable drawbacks. These include irritation to periapical tissues, high toxicity, inability to remove smear layer and a decrease in flexural strength and elastic modulus of dentin.[30]

Thus, conventional synthetic irrigants have limitations, including their inability to effectively disrupt biofilms, aggressiveness on root dentine or their potential cytotoxicity to host tissues. Therefore, there is a need for alternative irrigants that can efficiently eliminate biofilms while maintaining biocompatibility without deleterious effects on tooth structure [34]. In the present in-silico study, although precise physical concentrations are not applicable, the molecular structure of cinnamaldehyde was modeled based on concentrations proven effective in biological systems. Previous in vitro studies have demonstrated that cinnamaldehyde exhibits significant antimicrobial activity against biofilm-forming bacteria like E. faecalis at concentrations ranging from 0.01% to 2%.[35,36] These effective concentrations guide the theoretical interactions examined in the docking simulations.

Cinnamaldehyde, despite displaying a moderate binding energy, presents an intriguing pathway for use of indigenous phytochemicals for root canal disinfection. Cinnamaldehyde's moderate binding affinity (-5.5 kcal/mol) compared to CHX (-11.83 kcal/mol) suggests that while it may not be as potent as CHX, its targeted interactions with key amino acids (HIS, VAL, TYR, ALA) still hold promise for disrupting biofilm formation. Its natural origin and the specific amino acid interactions observed in the study suggest a targeted mode of action against the Esp receptor. These findings align with previous in vitro studies that have reported positive results for cinnamaldehyde's antibiofilm activity against E. faecalis.[18,19,21] Notably, previous research also indicates its lower toxicity profile with its biocompatibility with fibroblasts and osteoblasts and antioxidant activity which are essential for tissue repair and healing in the periapical region compared to other medicaments.[37–39] This makes cinnamaldehyde a potentially safer alternative or adjuvant to NaOCl and CHX, which are known for their toxic effects on soft tissues and dentin. The moderate binding energy suggests that cinnamaldehyde may be more effective when used as an adjunct to conventional irrigants rather than a standalone agent. Synergistic effects between natural antimicrobials and synthetic disinfectants allows for reduced concentrations of the more cytotoxic agents without compromising antimicrobial efficacy of the combination. This could be a promising approach to reduce tissue toxicity while maintaining or even enhancing disinfection efficiency in root canal therapy. However, further in vivo studies are necessary to substantiate its safety and efficacy within the complex root canal environment.

Limitations and Future Research Directions

In-silico docking simulations, while informative, are based on computational models with inherent simplification and limitations. Experimental validation through in vitro biofilm models that mimic the physiological conditions of the root canal system is essential to verify the efficacy of cinnamaldehyde, NaOCl, and CHX against E. faecalis biofilms. These in vitro studies should focus not only on biofilm disruption but also on the agents' ability to penetrate the biofilm matrix and sustain their antimicrobial activity over time. Combination studies to evaluate potential synergistic effects when used alongside conventional irrigants such as NaOCl or CHX can also be pursued. Further, in vivo studies on animal models

could provide data on the biocompatibility, tissue toxicity, and overall therapeutic effectiveness of cinnamaldehyde in real-world endodontic applications. Clinical trials involving human subjects could further elucidate cinnamaldehyde's therapeutic potential in endodontic procedures, focusing on factors such as patient outcomes, post-treatment sensitivity, and success rates in root canal treatments. Moreover, structural and molecular studies could provide deeper insights into the exact mechanisms of interaction between these compounds and the Esp receptor. If validated, cinnamaldehyde could offer a natural alternative or adjunct to conventional root canal irrigants, addressing the challenges associated with E. faecalis biofilms and improving treatment outcomes in endodontic therapy.

Conclusion

The findings underscore CHX's potential as an Esp receptor inhibitor with the highest binding affinity and versatile amino acid interactions. The weaker interaction of NaOCl prompts further scrutiny on its effectiveness against biofilm-mediated infections, though other mechanisms of actions may be involved instead of specific receptor inhibition. Cinnamaldehyde, as a natural alternative, shows promise with targeted interactions despite displaying moderate binding energy. HIS and TYR, both aromatic and polar residues, played pivotal roles in cinnamaldehyde's binding profile. These insights emphasize the potential of natural alternatives like cinnamaldehyde to synthetic disinfectants as viable options to augment conventional disinfection strategies in endodontics especially against biofilm mediated persistent root canal infections.

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