DOI: 10.7860/JCDR/2025/82131.22040

Original Article

Dentistry Section

# In-silico Docking Analysis of Lupeol against the Enterococcal Surface Protein Receptor of *Enterococcus faecalis*: A Phytoendodontic Approach for Biofilm Disruption

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## ABSTRACT

**Introduction:** Persistent root canal infections often involve *Enterococcus faecalis*, a biofilm-forming pathogen resistant to conventional irrigants like sodium hypochlorite and Chlorhexidine (CHX). The Enterococcal Surface Protein (Esp) is a key virulence factor facilitating adhesion and biofilm development. Natural compounds like lupeol, derived from *Tinospora cordifolia*, have shown antimicrobial properties, but their specific interactions with bacterial virulence proteins remain underexplored.

**Aim:** To evaluate the binding affinity and molecular interaction of lupeol with the Esp receptor using in-silico docking, and compare it with CHX, a traditionally used endodontic irrigant.

**Materials and Methods:** The present in-silico docking study was performed using the Esp crystal structure (PDB ID: 6ORI) in the Department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospitals (SIMATS, Chennai, India). The protein and ligands were prepared and energy-minimised in AutoDockTools 1.5.6. Lupeol (PubChem CID: 259846) and CHX (PubChem CID: 9552079) were docked at the Esp active site with exhaustiveness set at 8. Binding affinities (kcal/mol) and key interactions were analysed.

**Results:** Lupeol exhibited a strong binding affinity of -9.14 kcal/mol, interacting specifically with the Histidine (HIS) residue of the Esp receptor. In comparison, CHX demonstrated a higher binding affinity (-11.83 kcal/mol) with multiple interactions involving Tyrosine (TYR), Phenylalanine (PHE), Methionine (MET), Leucine (LEU), Arginine (ARG), and Glutamic Acid (GLU), residues. Despite a narrower interaction profile, lupeol's targeted engagement and favourable binding energy suggest its potential as a natural Esp inhibitor.

**Conclusion:** Lupeol demonstrated promising molecular interaction with the *E. faecalis* Esp receptor, indicating its potential as a natural, target-specific anti-biofilm agent. These findings support further experimental validation and highlight the relevance of phytoendodontics and in-silico methods in the discovery of safe and effective alternatives to synthetic irrigants.


**Keywords:** Chlorhexidine, Endodontics, Molecular docking, Phytotherapy, Root canal therapy

## INTRODUCTION

The persistence of microbial biofilms within the root canal system remains a principal cause of endodontic treatment failure, even in cases where adequate mechanical preparation and conventional irrigation protocols have been followed [1]. *Enterococcus faecalis* (*E. faecalis*), a Gram-positive facultative anaerobe, is frequently isolated from cases of secondary or persistent apical periodontitis and has been reported to survive in failed endodontic treatments [2,3]. Its ability to invade dentinal tubules, withstand harsh environmental conditions, and form biofilms endows it with exceptional resistance to conventional antimicrobial strategies [4]. Biofilms are structured microbial communities encased in an extracellular polymeric matrix, which not only physically shields the bacteria from antimicrobial agents but also allows the bacteria to exhibit altered gene expression and metabolic activity [5]. This renders the bacteria within biofilms up to 1000 times more resistant than their planktonic counterparts [6]. Among the virulence factors of *E. faecalis*, the Esp has emerged as a critical adhesin implicated in initial attachment, biofilm maturation, and evasion of host immune responses [7]. Esp has thus become an attractive molecular target for strategies aimed at biofilm inhibition and disruption [1].

CHX has long been recognised as a gold standard adjunctive irrigant in endodontics due to its broad-spectrum antimicrobial activity and substantivity [8]. However, despite its bactericidal efficacy, CHX exhibits several limitations. It lacks tissue dissolving capacity, cannot penetrate deep biofilm layers effectively [9], and may exert cytotoxic effects on periapical tissues [10]. Furthermore, it may interact unfavourably with sodium hypochlorite, producing a precipitate that occludes dentinal tubules and potentially forms toxic byproducts such as Para-Chloroaniline (PCA) [11]. As a result, there has been a growing focus on developing safer, biocompatible natural alternatives capable of targeting biofilms more selectively and with fewer adverse effects [12,13]. Lupeol, isolated from the medicinal plant *Tinospora cordifolia* (Giloy), is a naturally occurring pentacyclic triterpenoid with promising bioactivity [14]. It demonstrated a wide range of pharmacological properties, including anti-inflammatory, antioxidant, anticancer, and antimicrobial effects [15,16]. Importantly, lupeol's plant based origin confers it with a favourable biocompatibility profile and a reduced likelihood of inducing microbial resistance, making it a compelling molecule for endodontic applications [16,17].

Computational drug discovery has become an essential tool in modern biomedical research, enabling rapid identification of potential therapeutic phytochemicals against specific bacterial targets in-vitro or in-vivo testing [18,19]. In-silico molecular docking is a widely used technique within this framework, allowing prediction of binding affinity, interaction profiles and orientation of candidate molecules with proteins of interest. These approaches are advantageous because they are cost-effective, time-efficient, and capable of screening large compound libraries to prioritise candidate ligands [20,21]. In addition, docking provides detailed insights into the molecular basis of binding, which can guide subsequent laboratory validation and precision drug design [18,20].

Prior research demonstrated *E. faecalis* biofilms in post-treatment disease [1,22] and reported antimicrobial effects of lupeol, yet most studies have evaluated non specific bactericidal effects or whole-extract activity [14,15]. In particular, there is limited evidence on phytochemicals that directly engage the Esp, a key adhesin for attachment and biofilm maturation and no docking comparisons of lupeol versus a standard agent such as CHX at the Esp binding domain exist in an endodontic context [1]. This gap justifies a target-based, computational approach to rapidly screen and visualise ligand-receptor interactions. Therefore, the present study evaluates the binding affinity and interaction profile of lupeol with Esp and compares it to CHX, providing residue-level insights that support the development of precision, receptor-directed anti-biofilm strategies for endodontic disinfection.

## MATERIALS AND METHODS

The present study was designed as an in-silico molecular docking analysis conducted in the Department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospitals (SIMATS, Chennai, Tamil Nadu, India) from January-April 2024. The study protocol was reviewed and approved by the Scientific Review Board (SRB/SDC/ENDO-2202/23/233). As this was a computational analysis not involving patients or biological samples, inclusion and exclusion criteria were not applicable. Only publicly available molecular structures of the target protein and ligands were used. Similarly, no formal sample size estimation was required. Instead, reproducibility was ensured by performing 10 independent docking runs for each ligand.

### Study Procedure

For target protein preparation, the three-dimensional crystallographic structure of the *Enterococcus faecalis* Esp was retrieved from the Protein Data Bank (PDB ID: 6ORI). The entry with the highest resolution and structural completeness was chosen. The protein was pre processed using AutoDockTools 1.5.6 by removing heteroatoms, co-crystallised ligands, and water molecules, followed by energy minimisation to relieve steric hindrances and ensure accurate geometry for docking. For ligand preparation, lupeol (test ligand), a pentacyclic triterpenoid derived from *Tinospora cordifolia*, was selected for its documented antimicrobial and anti-biofilm properties. Its three-dimensional structure was obtained from the PubChem database (PubChem CID: 259846) in SDF and converted to PDB format. Geometry optimisation was performed using AutoDockTools 1.5.6, followed by energy minimisation to ensure a stable low-energy conformation suitable for docking. CHX, a conventional endodontic irrigant, was used as the comparative control. Its three-dimensional structure was similarly retrieved from PubChem (PubChem CID: 9552079), converted to PDB format, and minimised using the same parameters to maintain methodological consistency.

Molecular docking simulations were performed using AutoDockTools 1.5.6. The docking grid was centered on the active site of the Esp receptor, with grid box dimensions adjusted to sufficiently encompass key amino acid residues involved in biofilm adhesion. Flexible ligand docking was enabled to allow conformational variability of the ligands during interaction. The exhaustiveness parameter was set to 8 to ensure comprehensive conformational sampling. Each ligand underwent multiple docking runs to ensure reproducibility and to identify the most energetically favourable binding pose based on the lowest binding affinity (kcal/mol). Docking results were analysed using AutoDock Tools 1.5.6 program. The docked complexes were visualised to analyse the spatial orientation of the ligands within the Esp receptor binding site. For each ligand, interaction profiles were mapped, focusing on hydrogen bonding, hydrophobic interactions, van der Waals forces, and π-π stacking. Amino acid residues involved in stabilising the ligand-receptor complex were documented and two-dimensional interaction diagrams were generated to facilitate comparison between lupeol and CHX.

## STATISTICAL ANALYSIS

The data was analysed using descriptive statistics.

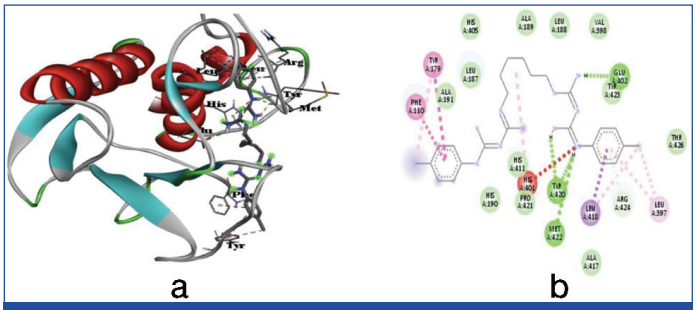
## RESULTS

The molecular docking simulations conducted using AutoDock Tools 1.5.6 provided comparative insights into the binding affinity and interaction profiles of lupeol and CHX with the Esp receptor of *Enterococcus faecalis*. CHX [Table/Fig-1] exhibited the strongest binding affinity, with a docking score of -11.83 kcal/mol, forming interactions with multiple amino acid residues.

| Ligands | Docking scores (Binding affinity values in kcal/mol) | Amino acid interaction between the ligand and Esp receptor* |
|---|---|---|
| Lupeol | -9.14 kcal/mol | HIS |
| Chlorhexidine (CHX) | -11.83 kcal/mol | TYR, PHE, HIS, TYR (second occurence), MET, LEU, ARG, LEU, and GLU |

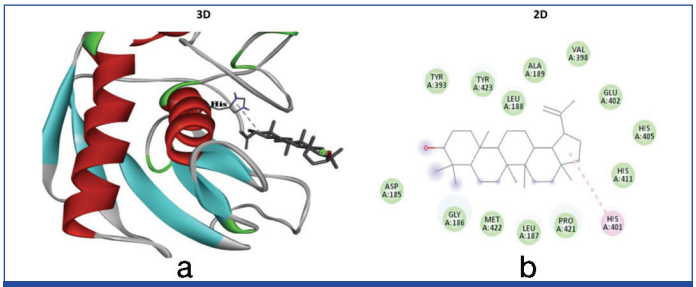
**[Table/Fig-1]:** Summary of docking scores and amino acid interactions between ligands and the *E. faecalis* Esp receptor.
*HIS (Histidine), VAL (Valine), TYR (Tyrosine), ALA (Alanine), PHE (Phenylalanine), MET (Methionine), LEU (Leucine), ARG (Arginine), LEU (Leucine), GLU (Glutamic Acid)

The interaction profile of CHX was characterised by multiple non-covalent interactions involving key amino acid residues including Tyrosine (TYR), Phenylalanine (PHE), Histidine (HIS), Methionine (MET), Leucine (LEU), Arginine (ARG), and Glutamic Acid (GLU), indicating a broad and stable binding network within the receptor's active site [Table/Fig-2].




**[Table/Fig-2]:** Docking interaction of Chlorhexidine (CHX) with *E. faecalis* Esp receptor. a) Three-dimensional visualisation of the CHX-Esp complex illustrating the docked pose; b) Two-dimensional schematic interaction map showing key binding residues.

Lupeol demonstrated a notably strong binding affinity of -9.14 kcal/mol with the Esp receptor, reflecting a thermodynamically favourable and stable interaction [Table/Fig-1]. This value falls well within the threshold of high-affinity ligand binding (typically ≤6 kcal/mol) [23,24]. The docking results revealed that lupeol formed a specific and stable interaction with the HIS residue of the Esp receptor, as visualised in both three-dimensional and two-dimensional interaction maps [Table/Fig-3]. While the interaction was primarily localised, its precise engagement with a functionally relevant amino acid suggests that lupeol may effectively interfere with Esp-mediated processes such as adhesion and biofilm stability.




**[Table/Fig-3]:** Docking interaction of lupeol with *E. faecalis* Esp receptor. a) Three-dimensional visualisation of the lupeol-Esp complex illustrating the docked pose; b) Two-dimensional schematic interaction map showing key binding residues.

## DISCUSSION

The persistence of *Enterococcus faecalis* in previously treated root canals poses a major challenge in contemporary endodontics, often contributing to treatment failure, reinfection, and persistent apical periodontitis [1,22]. Its survival is primarily attributed to its capacity to form resilient biofilms, invade dentinal tubules, and withstand the effects of traditional intracanal irrigants and medicaments [6,25]. Among the virulence factors of *E. faecalis*, the Esp plays a pivotal role in adhesion, biofilm formation, and colonisation within the root canal system [7]. The Esp is a well-characterised adhesion molecule and its role in facilitating initial attachment and biofilm maturation makes it a compelling molecular target for anti-biofilm strategies [1].

In recent years, the integration of plant based therapeutics into dental care has led to the emergence of phytoendodontics which refers to the application of plant based agents in root canal therapy for microbial control, tissue healing, and biocompatibility [13,26]. Several herbal agents such as *Azadirachta indica* (neem), *Morinda citrifolia*, *Curcuma longa*, *Ocimum sanctum*, and *Tinospora cordifolia* have demonstrated promising antimicrobial efficacy against endodontic pathogens, including *E. faecalis* [17]. These plant derived solutions are often favoured for their low cytotoxicity, natural origin, and minimal environmental impact compared to synthetic irrigants [13,17]. Despite promising in-vitro outcomes, the broader clinical application of herbal irrigants in endodontics remains limited due to incomplete mechanistic insights, inconsistent extract standardisation, and a lack of molecular evidence for target-specific antimicrobial activity [26].

The present study sought to explore the molecular interaction of lupeol, a pentacyclic triterpenoid phytochemical, with the Esp receptor of *E. faecalis* using in-silico docking analysis, and to compare its binding profile with CHX, a routinely used endodontic irrigant. The results demonstrated that lupeol possesses a favourable binding affinity of -9.14 kcal/mol, forming a specific interaction with the HIS residue of the Esp receptor. Although CHX exhibited a stronger binding affinity (-11.83 kcal/mol) and a broader interaction spectrum involving multiple residues (TYR, PHE, MET, LEU, ARG, and GLU), the specificity and stability of lupeol's interaction suggest a promising inhibitory potential.

Earlier computational research has established general benchmarks for interpreting docking scores [18]. It has been reported that binding energies more negative than -5 kcal/mol usually reflect strong ligand-receptor interactions, with many approved drugs displaying affinities in the -5 to -10 kcal/mol range [24]. Similarly, other studies note that binding energies lower than -6.0 kcal/mol are indicative of active drug candidates, and interactions stronger than -8.0 kcal/mol often signify compounds with particularly high potential in structure-based drug design [23,27-29]. In this context, lupeol's binding affinity of -9.14 kcal/mol falls well within the range, supporting its potential as a strong Esp receptor binder when compared to conventional agents. The specific interaction of lupeol with a key active site residue (HIS) in the Esp receptor suggests it could potentially disrupt protein functionality, thereby interfering with the bacterium's ability to adhere to dentinal walls and form mature biofilms. This could impair bacterial adhesion and destabilise biofilm integrity, two critical aspects in the pathogenicity of *E. faecalis* in root canal infections. While CHX exhibited a stronger and broader binding profile, engaging multiple residues within the Esp binding domain, lupeol's targeted interaction with the HIS residue suggests molecular specificity rather than generalised binding. The concept of precision disinfection, where agents are designed to interfere with specific microbial targets, represents a novel direction in endodontic pharmacotherapy, particularly relevant in cases involving resistant or recurrent infections [30].

These results are particularly promising given the natural origin and known bioactivity of lupeol, including its antimicrobial, anti-inflammatory, and antioxidant properties [14]. A notable strength of this study is its target-specific approach. Prior investigations have demonstrated lupeol's efficacy against a range of bacterial species, and its specificity in this context adds a new dimension to its pharmacological potential in endodontics [15,31,32]. While CHX remains an effective synthetic irrigant with proven antimicrobial efficacy and substantivity [8], it has several limitations. It lacks tissue-dissolving properties, can be cytotoxic to periapical tissues, and when used in combination with sodium hypochlorite, may result in the formation of a toxic precipitate (PCA) [10,11]. In contrast, natural compounds like lupeol offer the advantages of biocompatibility, lower toxicity, lesser effect of dentin microhardness and reduced risk of resistance development, making them attractive alternatives or adjuncts in endodontic disinfection protocols [17,33]. Specific parts of the lupeol molecule that influence its binding can be explored to develop improved versions of the compound from herbal scaffolds. This approach is known as Structure Activity Relationship (SAR) analysis [34], such as hydroxylation at specific carbon positions or conjugation with functional groups may produce semi-synthetic derivatives with improved binding affinity or broader interaction profiles. This strategy could lead to the development of more potent agents that retain the safety and biocompatibility of natural compounds, while offering improved performance against biofilm-forming pathogens like *E. faecalis*.

### Limitation(s)

The present study was conducted entirely using in-silico molecular docking, which, while efficient for preliminary screening, has inherent constraints. The docking approach assumes a static protein structure and may not fully capture receptor flexibility or dynamic conformational changes. Moreover, in-silico results do not account for biological factors such as bioavailability, metabolic degradation, or host-tissue interactions. Experimental validation through in-vitro assays, molecular dynamics simulations, and in-vivo studies is therefore essential to confirm the biological relevance of the predicted interactions. Future research should evaluate lupeol's performance against other conventional and novel irrigants as well as in relation to additional resistance mechanisms within endodontic biofilms, for a more comprehensive understanding of its potential.

## CONCLUSION(S)

The present study highlights the potential of lupeol, a plant derived compound, as a target-specific inhibitor of the Esp receptor in *Enterococcus faecalis*. Its strong binding affinity, natural origin, and specific interaction with a key virulence factor support its candidacy as an adjunctive or alternative endodontic irrigant, particularly in retreatment and persistent cases where conventional endodontic disinfection protocols may be insufficient. By impairing Esp-mediated adhesion and biofilm stability, lupeol-based formulations could offer a biocompatible and effective strategy for managing persistent endodontic infections. The present study findings provide a strong rationale for further in-vitro and in-vivo investigations into lupeol's antimicrobial efficacy, cytocompatibility, and clinical applicability in both regenerative and conventional endodontic therapy.

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**PLAGIARISM CHECKING METHODS:** [Jain H et al.]
- Plagiarism X-checker: Jul 27, 2025
- Manual Googling: Sep 03, 2025
- iThenticate Software: Sep 11, 2025 (6%)

**ETYMOLOGY:** Author Origin

**EMENDATIONS:** 6

**AUTHOR DECLARATION:**
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? NA
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jul 18, 2025**
Date of Peer Review: **Aug 08, 2025**
Date of Acceptance: **Sep 13, 2025**
Date of Publishing: **Nov 01, 2025**