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Curcumin targets *Enterococcus faecalis* virulence by inhibiting the enterococcal surface protein, offering a potential treatment for endodontic diseases

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Abstract

Background: The present study explores the inhibitory effect of curcumin on the Enterococcal Surface Protein (ESP) of Enterococcus faecalis (*E. faecalis*), a key pathogen in endodontic and post-endodontic diseases.

Methods: The 3D structure of ESP was modeled using SWISS-MODEL, and structural cavities were identified via CB-Dock2. Molecular docking was performed with DockingServer. ADMET properties were predicted using pkCSM, while curcumin's biological activity and potential macromolecular targets were assessed using PASS Online and SwissTargetPrediction, respectively. Cytotoxicity of curcumin on HEK293 cells was evaluated by MTT assay.

Results: Curcumin demonstrated good binding affinity (-11.06 kcal/mol) with ESP, supported by HBPlot analysis and SeamDock validation (-10.5 kcal/mol). Curcumin's deep binding within ESP's cavity suggests its potential to disrupt E. faecalis colonization and biofilm formation, offering a novel therapeutic strategy. ADMET predictions revealed favourable pharmacokinetic properties, including high intestinal absorption (82.19%) and no hepatotoxicity, positioning curcumin as a safe and effective candidate. PASS analysis highlighted curcumin's diverse biological activities, such as antioxidant, anti-inflammatory, and enzyme inhibitory effects, aligning with its therapeutic potential. SwissTargetPrediction further identified potential protein targets, including transcription factors and kinases, broadening its applicability. Concentration- and time-dependent assays confirmed curcumin's non-toxic nature toward normal HEK293 cells, highlighting its safety profile.

Conclusion: In conclusion, these findings collectively demonstrate curcumin's potential as a therapeutic agent for endodontic diseases, leveraging its ability to target *E. faecalis* virulence while addressing inflammation and oxidative stress.

Introduction

Enterococcus species, particularly *Enterococcus faecalis*, are Gram-positive, facultative anaerobic bacteria that are commonly associated with endodontic and post-endodontic infections [1,2,3]. These infections occur in the root canal system of teeth and can lead to persistent apical periodontitis, treatment failure, and systemic complications if left untreated [3]. *E. faecalis* is particularly notorious for its ability to survive in harsh environments, such as the nutrient-deprived and alkaline conditions of root canals, and its resistance to conventional endodontic treatments, including mechanical debridement and chemical irrigation [1,4].

One of the key virulence factors of *E. faecalis* is the Enterococcal Surface Protein (ESP), a cell wall-associated protein that plays a critical role in biofilm formation, adhesion to host tissues, and immune evasion [5,6,7,8]. ESP facilitates the colonization of *E. faecalis* in the root canal system and contributes to its persistence by enhancing bacterial adherence to dentin and extracellular matrix components [9,10]. Biofilm formation mediated by ESP makes *E. faecalis* highly resistant to antibiotics and disinfectants, posing a significant challenge in endodontic therapy [11,12,13].

Current treatment strategies for endodontic infections rely on mechanical cleaning, chemical irrigation (e.g., sodium hypochlorite), and intracanal medicaments (e.g., calcium hydroxide) [14,15,16]. However, these methods often fail to completely eradicate *E. faecalis* biofilms, leading to recurrent infections [13,16]. The rise of antibiotic-resistant strains further complicates the management of these infections, necessitating the development of novel therapeutic approaches [17,18,19].

Targeting the ESP with small molecules represents a promising strategy for combating *E. faecalis* infections in endodontic and post-endodontic diseases [11,20]. Small molecules offer several advantages, including high specificity, the ability to penetrate biofilms, and the potential for chemical optimization to enhance efficacy and reduce toxicity [20,13]. By inhibiting ESP, small molecules can disrupt critical virulence mechanisms, such as biofilm formation and adhesion, rendering *E. faecalis* more susceptible to host immune responses and conventional treatments [20,21].

ESP is essential for the initial attachment of *E. faecalis* to surfaces and the development of biofilms [10,12,22]. Small molecules that inhibit ESP can prevent biofilm formation, thereby reducing bacterial resistance to antibiotics and disinfectants. This approach can enhance the effectiveness of existing endodontic treatments. ESP mediates the adhesion of *E. faecalis* to dentin and extracellular matrix proteins [22,23,24]. Inhibiting ESP can reduce bacterial colonization in the root canal system, preventing the establishment of persistent infections. Overall, inhibiting the ESP of Enterococcus bacteria using small molecules represents a novel and promising therapeutic strategy for the management of endodontic and post-endodontic diseases. By targeting a key virulence factor, this approach has the potential to enhance the efficacy of existing treatments, overcome antibiotic resistance, and improve clinical outcomes for patients with persistent root canal infections.

Material and Methods

Modelling and preparation of structure of ESP

Homology modelling is a widely used technique for predicting the three-dimensional structure of a protein by leveraging the known structures of similar proteins. This process is often carried out using SWISS-MODEL, a well-known web-based platform (accessible at https://swissmodel.expasy.org/). In this study, SWISS-MODEL was utilized to generate the 3D structure of the ESP. The FASTA sequence of the protein was obtained from UniProt (UniProt id: B1PCJ4), and a suitable template structure was chosen based on factors such as sequence similarity, structural quality, and biological relevance. The high-resolution 3D model of the ESP was downloaded from the SWISS-MODEL server and visualized using PyMOL and Chimera for detailed structural analysis. To prepare the protein for molecular docking, standard protocols were followed to refine the structure, including checking for steric clashes or missing atoms. Partial charges were then assigned to ensure compatibility with molecular docking software, and the final structure was saved in PDBQT format for molecular docking with ligand molecules.

Ligands preparation for molecular docking

Based on the therapeutic value of turmeric, six small ligand molecules derived from turmeric, namely Alpha-Turmerone, Beta-Turmerone, ar-Turmerone, Curcumin, Demethoxycurcumin, and Bisdemethoxycurcumin, were selected. The ligand structures were obtained from PubChem in SDF, MOL, or SMILES format. Open Babel was used to convert the 2D structures to 3D, ensuring that the conversion preserved stereochemistry. Chimera was employed to perform energy minimization and optimize the 3D structure of the ligand molecules. Hydrogen atoms were added to the ligand structures to account for protonation states at physiological pH 7.4. Partial atomic charges were assigned to the ligand structure using AutoDock Tools. The ligand files were generated in a docking-compatible format. The prepared ligands were then saved in a PDBQT format compatible with the docking software, AutoDock. Finally, the prepared ligands were further visualized using PyMOL to ensure no errors and check for any steric clashes or unusual geometries.

Cavity detection for the molecular docking

The identification of cavities within the Enterococcal Surface Protein structure was conducted using CB-Dock2, a specialized tool for protein-ligand docking, accessible at

http://cadd.labshare.cn/cb-dock2/. The protein structure, prepared as a PDB file, was uploaded to the CB-Dock2 platform. Specific parameters, including probe radius and cavity size, were configured according to the target protein's properties. The tool automatically detected binding sites and cavities within the protein, computed their dimensions and positions, and optimized the docking box size to ensure comprehensive coverage during the docking process. Additionally, it provided visual representations of the identified cavities, facilitating detailed analysis of potential binding sites.

Molecular docking

Molecular docking was conducted using the DockingServer platform (https://www.dockingserver.com). The prepared protein structure and ligand structure were uploaded to the server. After setting the docking parameters, the binding site on the target protein was automatically predicted by the server. The docking parameters, such as grid size, grid center, and exhaustiveness, were adjusted to ensure optimal coverage of the binding site. The appropriate docking algorithm, AutoDock Vina, provided by the platform was selected to run the docking simulation. Once the docking was complete, the results were downloaded, which typically include the binding affinity scores, predicted binding poses, and interaction details. Additionally, the protein-ligand complex was redocked using CB-Dock2 to validate the binding affinity of protein-ligand complex.

Assessment of ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of curcumin

The pharmacokinetic and toxicity profile of a compound is critical in drug discovery and development. The pkCSM online tool (https://biosig.lab.uq.edu.au/pkcsm/) is a widely used platform for predicting Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) properties of small molecules. It employs graph-based signatures and machine learning algorithms to provide accurate and reliable predictions, making it a valuable resource for early-stage drug screening. The SMILES string of curcumin was directly entered into the pkCSM platform to determine the ADMET properties associated with curcumin. The pkCSM provides numerical outputs and qualitative assessments (e.g., high/low permeability, toxic/non-toxic). The use of the ADMET predictions is to prioritize compounds with favourable pharmacokinetic and safety profiles for further experimental validation. Identify potential liabilities early in the drug development process to reduce the risk of failure in later stages.

Prediction of biological activity profile of curcumin

PASS Online (https://www.way2drug.com/passonline/) was used to predict the biological activity profile of curcumin. This online server predicts over 4000 types of biological activities associated with ligand molecules. The SMILES string of curcumin was directly entered into the PASS Online platform (https://www.way2drug.com/passonline/) to generate the predicted the biological activity profile.

Prediction of most probable macromolecular targets of a curcumin

Swiss Target Prediction tool (http://www.swisstargetprediction.ch/about.php) was used to predict the most probable macromolecular targets of a curcumin. This tool allows to estimate the most probable macromolecular targets of a small molecule, assumed as bioactive. The prediction is founded on a combination of 2D and 3D similarity with a library of 37 0'000 known actives on more than 3000 proteins from three different species.

Cytotoxicity assay of curcumin

MTT assay was used to determine the cytotoxicity effect of curcumin using HEK293 cell line [25]. The MTT assay is a widely used colorimetric method to assess cell viability, proliferation, and cytotoxicity. It is based on the reduction of the yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to purple formazan crystals by metabolically active cells. HEK293 cells were Seeded into a 96-well plate at a density of 5,000-10,000 cells per well in 100 µL of culture medium. Serial dilutions (0-1000 µM) of the test compound was prepared in culture medium and 100 µL of the treatment solutions was added to the respective wells. Plate was incubated for the desired treatment period (e.g., 24 hours) in a CO_2 incubator at 37°C. After the treatment period, culture medium was carefully removed from the wells and 100 µL of fresh medium containing 10 µL of MTT solution (final concentration: 0.5 mg/mL) was added to each well. Plate was incubated for 2-4 hours in a CO_2 incubator at 37°C. During this time, metabolically active cells will reduce MTT to formazan crystals. MTT-containing medium was removed from the wells and 100 µL of DMSO (solubilization solution) was added to each well to dissolve the formazan crystals. Plate was gently shaken on an orbital shaker for 10-15 minutes to ensure complete dissolution. Plate was transferred to a microplate reader and absorbance was measured at 570 nm (reference wavelength: 630 nm or 690 nm), ensuring that the blank wells (medium only) are used to zero the instrument. Readings were utilized to calculate the cell viability and IC_{50} value.

Results

Homology modelling and preparation of ESP

Figure 1a displays the high-resolution structure of the ESP model generated using Swiss-MODEL. The model demonstrates 100% sequence identity and complete coverage. The model showed good quality with GMQE score of 0.91 and a QMEANDisCo Global score of 0.83 ± 0.05. These scores, which range from 0 to 1, reflect the overall quality of the model, with higher values indicating better reliability. The GMQE score is influenced by sequence coverage, meaning a model covering only half of the target sequence is unlikely to score above 0.5. In contrast, the QMEANDisCo score evaluates model quality independently of coverage. Figure 1b provides a graphical representation of the QMEANDisCo local quality estimate, highlighting the local reliability of the model. Figure 1c shows the Ramachandran Plot of the modelled structure, illustrating the distribution of backbone dihedral angles for amino acid residues. This plot identifies energetically favoured regions and distinguishes between General, Glycine-only, Proline-only, and Pre-Proline-specific regions, offering further validation of the model's structural integrity. Figure 1D shows the sequence representation of C1, C2, C3, C4, & C5 and Figure 1E-I shows structural visualization of all identified cavities: (E) C1; (F) C2; (G) C3; (H) C4; and (I) C5 within ESP.

Cavity detection on ESP

The key findings related to cavity detection in the Enterococcal Surface Protein (ESP) are summarized in Table 1A, which outlines the number and properties of cavities identified using CB-Dock2. A total of five significant cavities (C1-C5) were detected, with detailed information on their dimensions, volume, and other relevant features provided in the table. Among these, Cavity 1 (C1) stood out with a maximum cavity size of X, Y, and Z (18, 12, 11) and a cavity volume of 1117 Å^3, making it a primary focus for molecular docking studies with ligand molecules. Figure 1D illustrates the sequence representation of cavities C1 through C5, while Figure 1E provides a structural visualization of these cavities within ESP, including a cartoon representation of the Curcumin-ESP complex.

ID (CurPocket)	Volume of cavity (Å^3)	Centre (x, y, z)	Size of cavity (x, y, z)
C1	1117	9, 5, 12	18, 12, 11
C2	234	-12, 5, 41	11, 11, 5
C3	134	-34, 2, 33	10, 5, 4
C4	134	-31, -8, 41	8, 8, 9
C5	133	-12, -4, 22	9, 7, 7

Table 1A: Detailed information on the size, volume, or other relevant characteristics of the detected cavities (C1-C5) on ESP.

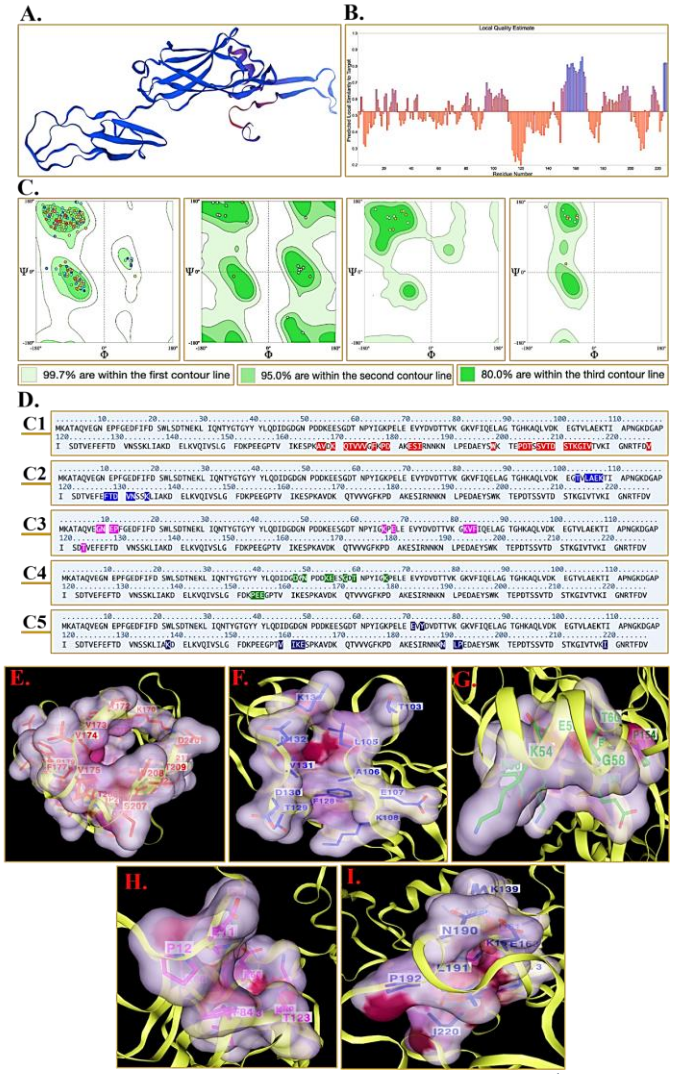


Figure 1 A: Cartoon representation of target protein 'ESP"; (B) QMEANDisCo local quality estimate graphically denotes the model's local worth; (C) Plot (Ramachandran) imagines dynamically ideal areas for mainstay dihedral viewpoints, by means of contours delineating these regions in the modelled structure; (D) Sequence representation of C1, C2, C3, C4, & C5; (E) Structural visualization of all identified cavities: (E) C1; (F) C2; (G) C3; (H) C4; and (I) C5 within ESP.

Molecular docking studies of curcumin with ESP

After screening six small ligand molecules derived from turmeric, curcumin was identified as the most promising candidate, exhibiting an exceptional fitness score of 99.31% and the highest predicted binding affinity among the tested compounds. The binding affinities of all six molecules are summarized in Table 1B. Based on these results, curcumin was chosen as the top candidate and further analysed through molecular docking with the target protein ESP.

The molecular docking results for the curcumin-ESP complex are visually represented in Figure 2A-E. The analysis revealed that curcumin had a strong binding

affinity score of -11.06 kcal/mol with ESP. Figure 2A shows the cartoon representation of the curcumin-ESP complex, while Figure 2B provides a surface view of the interaction. Figure 2C displays a 2D plot highlighting the specific amino acid residues of ESP involved in various interactions with curcumin. The docking results confirmed that curcumin successfully bound to the deep cavity of ESP, forming multiple polar, hydrophobic, and other interactions with the protein.

The interactions between curcumin and ESP's amino acid residues were further validated using HBPlot analysis, as illustrated in Figure 2D. To ensure the reliability of the docking results, the binding affinity of the curcumin-ESP complex was re-evaluated using SeamDock (https://bioserv.rpbs.univ-paris-diderot.fr/services/SeamDock/), which yielded a binding affinity of -10.5 kcal/mol (Figure 2E). These findings consistently support curcumin's potential as a strong inhibitor of ESP, highlighting its relevance in addressing endodontic or post-endodontic diseases.

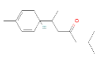
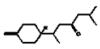
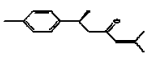
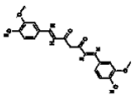
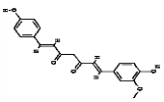
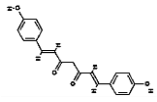
Name of the ligand	Target protein	Binding Energy (kcal/mol)
Alpha-Turmerone 	Enterococcal Surface Protein (ESP)	-6.10
Beta-Turmerone 	Enterococcal Surface Protein (ESP)	-6.22
ar-Turmerone 	Enterococcal Surface Protein (ESP)	-8.55
Curcumin 	Enterococcal Surface Protein (ESP)	-11.06
Demethoxycurcumin 	Enterococcal Surface Protein (ESP)	-7.01
Bisdemethoxycurcumin 	Enterococcal Surface Protein (ESP)	-7.21

Table 3: List of the molecular method used to find SNPs in codons 167, 198, and 200 as well as the presence of SNPs and the codon that it was found on.

ADMET predictions for curcumin

The summarized outcomes of ADMET predictions for curcumin, presented in Table 2A, indicate that it meets key pharmacokinetic criteria. Curcumin demonstrated favourable ADMET properties, including 82.19% intestinal absorption, a volume of distribution of -0.215 Numeric (log L/kg), blood-brain barrier permeability of -0.562 Log BB, central nervous system permeability of -2.99 Log PS, and total clearance of -0.002 log ml/min/kg. Curcumin showed no AMES or hepatotoxicity. These findings position curcumin as a promising candidate for therapeutic development targeting ESP inhibition in endodontic or post-endodontic diseases.

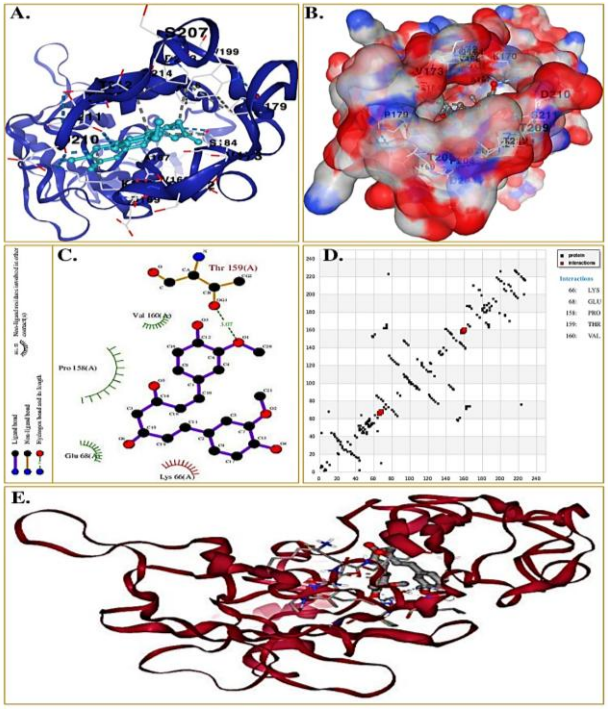


Figure 2: Illustration of the ESP-Curcumin Docking complex: (A) Cartoon representation, (B) Surface view, (C) 2D plot, (D) HBPlot analysis, (E) Redocking validation of binding affinity.

PASS Analysis for curcumin

PASS analysis uses a compound's structure to reveal information about its biological activity. The output includes predicted pharmacological effects, mechanisms of action, & potential toxicity, expressed as Pa and Pi values, helping researchers efficiently assess a compound's drug-like properties. PASS analysis results (Table 2B) highlight the favourable biological properties of curcumin and its involvement in multiple key biological processes. The compound shows potential as an inhibitor of several enzymes, including feruloyl esterase, beta-carotene 15,15'-monooxygenase, aspulvinone dimethylallyltransferase, linoleate diol synthase, gluconate 2-dehydrogenase, chlordecone reductase, ubiquinol-cytochrome-c reductase, vanillyl-alcohol oxidase, 4-coumarate-CoA ligase, HIV-1 integrase, and others. It also exhibits activities such as membrane integrity agonism, reductant and carminative properties, mucomembranous protection, free radical scavenging, and antihypercholesterolemic and fibrinolytic effects. Additionally, curcumin modulates several molecular pathways by enhancing hmox1 expression, inhibiting mmp9 and tnf expression, and stimulating

caspase-3 and MAP kinase activity. Furthermore, it interacts with multiple metabolic substrates, including GST M/A and UDP-glucuronosyltransferase, and serves as an inhibitor of membrane permeability.

Swiss Target Prediction analysis for ESP

Swiss Target Prediction analysis provides insights into potential protein targets for a given small molecule. The output includes a ranked list of predicted targets based on similarity to known ligands, along with probability scores. It also categorizes targets by species, protein class, and interaction type, aiding in drug discovery and

Table 2B: PASS analysis of curcumin likelihood "to be active" was set at Pa>0,7. molecular docking studies. Results of Swiss Target Prediction analysis are represented in figure 3A. Results showed that the curcumin influence the activity of various biological macromolecules such as transcription factors, isomerases, membrane receptors, toll-like and II-1 receptors, unclassified proteins, writer, proteases, kinases, oxidoreductases, and other enzymes. This Swiss Target Prediction analysis not only confirms but also elaborates on the diverse biological properties linked to curcumin, as elucidated in Figure 3A.

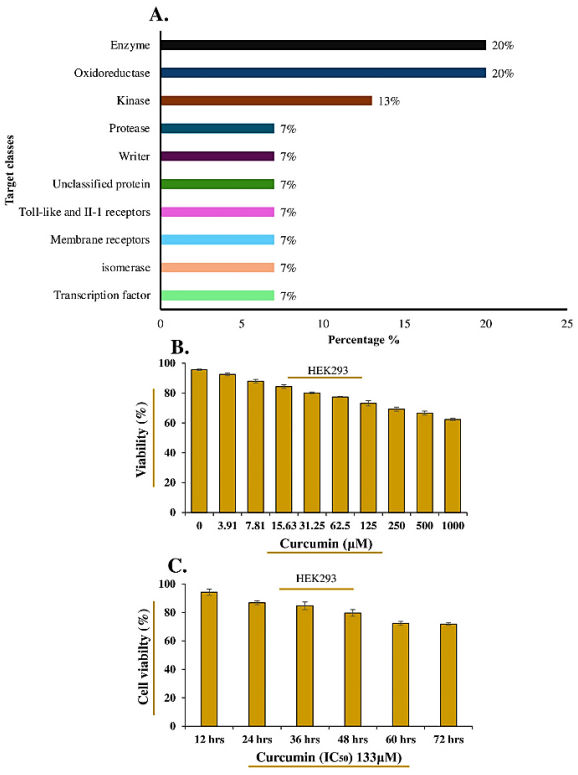


Figure 3: (A) Swiss Target Prediction analysis showing a variety of potential protein targets for curcumin (B) Concentration dependent cell viability of curcumin treated HEK293 cells (C) Time dependent cell viability of curcumin treated HEK293 cells.

Discussion

The present study investigates the inhibitory effect of curcumin on the Enterococcal Surface Protein (ESP) of *Enterococcus faecalis* (*E. faecalis*), a pathogen implicated in endodontic and post-endodontic diseases. The findings provide compelling evidence for curcumin's potential as a therapeutic agent, supported by robust structural, molecular, and pharmacological analyses. Molecular docking plays a pivotal role in identifying and developing new therapeutic molecules by predicting the binding interactions between ligands and target proteins [26,27]. It enables researchers to screen potential drug candidates, assess their binding affinity, and understand the molecular basis of their interactions [28]. By identifying key binding sites and residues, docking studies provide insights into the mechanism of action and optimize lead compounds for enhanced efficacy and specificity [29]. This computational approach accelerates drug discovery, reduces experimental costs, and facilitates the design of novel therapeutics for various diseases. In the current study, among the six small ligand molecules derived from turmeric, curcumin exhibited the highest fitness score (99.31%) and binding affinity for ESP. Molecular docking studies revealed a strong binding affinity score of -11.06 kcal/mol, indicating a stable and energetically favourable interaction between curcumin and ESP. The 2D interaction plot highlighted specific amino acid residues involved in polar, hydrophobic, and other interactions, further validating curcumin's binding efficacy. HBPlot analysis and re-evaluation using SeamDock confirmed these findings, with a consistent binding affinity score of -10.5 kcal/mol. The deep binding of curcumin within ESP's cavity suggests that it can effectively inhibit the protein's function, potentially disrupting *E. faecalis* colonization and biofilm formation [13]. This is particularly relevant in the context of endodontic and post-endodontic diseases, where *E. faecalis* is a major pathogen [1]. By targeting ESP, curcumin could mitigate the virulence of *E. faecalis*, offering a novel therapeutic strategy. The determination of ADMET properties is crucial for identifying and developing new therapeutic molecules. It provides essential insights into a drug candidate's pharmacokinetics and safety profile, ensuring it can reach the target site, exert its effect, and be eliminated without causing adverse effects [30]. By evaluating parameters such as bioavailability, blood-brain barrier permeability, and hepatotoxicity, ADMET studies help prioritize compounds with optimal drug-like properties [31]. This process reduces the risk of failure in later stages of drug development, accelerates the discovery of safe and effective therapeutics, and enhances the success rate of clinical trials. In the present study, the ADMET predictions for curcumin revealed favourable pharmacokinetic properties, including high intestinal absorption, moderate blood-brain barrier permeability,

Properties													
-	Absorption	Distribution			Metabolism							Excretion	Toxicity
Models	Intestinal absorption (human)	VDss (human)	BBB permeability	CNS permeability	CYP							Total Clearance	AMES toxicity
					Substrate		Inhibitor						
					2D6	3A4	1A2	2C19	2C9	2D6	3A4		
Unity	Numeric (% absorbed)	Numeric (log L/kg)	Numeric (log BB)	Numeric (log PS)	Categorical (yes/ no)							Numeric (log ml/min/ kg	Categorical (yes/no)
Projected values													
CM	82.19	-0.215	-0.562	-2.99	No	No	No	No	Yes	No	Yes	-0.002	No/No

Table 2A: ADME & Toxicity properties for Curcumin (CM).

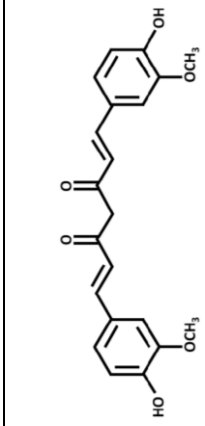
Composite	Structure	Pa	Pi	Action
Curcumin		0.936	0.003	Feruloyl esterase inhibitor
		0.898	0.001	Beta-carotene 15.15'-monooxygenase inhibitor
		0.900	0.008	Aspulvinone dimethylallyltransferase inhibitor
		0.887	0.014	Membrane integrity agonist
		0.872	0.003	Monophenol monooxygenase inhibitor
		0.864	0.003	Reductant
		0.833	0.003	Carminative
		0.832	0.005	Linoleate diol synthase inhibitor
		0.833	0.010	Gluconate 2-dehydrogenase (acceptor) inhibitor
		0.826	0.003	HMOX1 expression enhancer
		0.833	0.014	Chlordecone reductase inhibitor
		0.836	0.021	Ubiquinol-cytochrome-c reductase inhibitor
		0.812	0.002	Vanillyl-alcohol oxidase inhibitor
		0.814	0.004	Antimutagenic
		0.816	0.014	Mucomembranous protector
		0.805	0.003	MMP9 expression inhibitor
		0.803	0.008	Apoptosis agonist
		0.783	0.003	Steroid N-acetylglucosaminyltransferase inhibitor
		0.777	0.003	GST M substrate
		0.778	0.012	GST A substrate
		0.766	0.003	Free radical scavenger
		0.764	0.004	TNF expression inhibitor
		0.747	0.009	Chlordecone reductase inhibitor
		0.747	0.011	Ubiquinol-cytochrome-c reductase inhibitor
		0.737	0.004	Caspase 3 stimulant
		0.736	0.007	UDP-glucuronosyltransferase substrate
		0.731	0.013	MAP kinase stimulant
		0.708	0.003	Antihypercholesterolemic
		0.706	0.002	Fibrinolytic
		0.724	0.029	4-Coumarate-CoA ligase inhibitor

Table 2B: PASS analysis of Curcumin. Likelihood "to be active" was set at Pa>0.7.

and no hepatotoxicity. Further, prediction of activity spectra for substances is important in drug discovery. By providing Pa (probability of activity) and Pi (probability of inactivity) values, PASS analysis helps researchers identify promising therapeutic candidates with desired pharmacological effects [32]. This approach accelerates the screening process, highlights multi-target potential, and reduces the risk of adverse effect [33]. In the current study, PASS analysis further highlighted curcumin's diverse biological activities, including its roles as an antioxidant, anti-inflammatory agent, and enzyme inhibitor. These properties align with its potential therapeutic applications, particularly in managing infections and inflammation associated with endodontic diseases [34]. By guiding the selection of ligands with optimal drug-like properties, PASS analysis contributes significantly to the development of novel and effective therapeutics for various diseases. Moreover, SwissTargetPrediction analysis significantly contributes to drug discovery by predicting potential protein targets of ligand molecules based on their structural similarity to known ligands [35]. This approach provides a ranked list of probable targets, categorizing them by species, protein class, and interaction type, which aids in understanding the ligand's mechanism of action [36]. In this study, SwissTargetPrediction analysis expanded on these findings by identifying potential protein targets, such as transcription factors, kinases, and oxidoreductases, which could mediate curcumin's broader biological effects. By identifying novel targets and pathways, SwissTargetPrediction helps researchers explore new therapeutic applications and repurpose existing molecules. This approach accelerates the identification of drug candidates, enhances target specificity, and supports the development of innovative treatments for various diseases. Additionally, the non-toxic nature of curcumin towards normal HEK293 cells, as demonstrated by concentration- and time-dependent viability assays, underscores its safety profile [37]. All these properties together position curcumin as a viable candidate for oral administration, with minimal risk of adverse effects.

The findings of this study have significant implications for the treatment of endodontic and post-endodontic diseases. *E. faecalis* is notoriously resistant to conventional endodontic treatments, including root canal therapy, due to its ability to form biofilms and survive in harsh environments [1]. By targeting ESP, curcumin could disrupt these virulence mechanisms, enhancing the efficacy of existing treatments. Moreover, curcumin's anti-inflammatory and antioxidant properties could address the chronic inflammation and oxidative stress often associated with endodontic infections [38,39,40]. This dual action, targeting both the pathogen and the host response, positions curcumin as a multifaceted therapeutic agent. Its natural origin and established safety profile further enhance its appeal as a complementary or alternative treatment option. While the results are promising, there are some limitations associated with this study. First, the study relies on computational models and in vitro assays, which may not fully replicate the complex environment of endodontic infections. Future research should include in vivo studies to validate curcumin's efficacy and safety in a clinical context. Second, the precise mechanisms by which curcumin inhibits ESP and other virulence factors of *E. faecalis* remain to be elucidated. Detailed mechanistic studies, including transcriptomic and proteomic analyses, could provide deeper insights.

This study demonstrates that curcumin exhibits strong inhibitory effects on the Enterococcal Surface Protein (ESP) of *E. faecalis*, supported by robust structural, molecular, and pharmacological evidence. Its favourable pharmacokinetic properties, non-toxic nature, and diverse biological activities make it a promising candidate for the treatment of endodontic and post-endodontic diseases. While further research is needed to validate these findings in clinical settings, the results underscore the potential of curcumin as a novel therapeutic agent in endodontics. By targeting *E. faecalis* virulence and modulating host responses, curcumin could revolutionize the management of persistent endodontic infections, offering a safe, effective, and natural alternative to conventional therapies.

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Generative AI Statement:

The author(s) declare that Generative AI tools, including Grammarly and QuillBot, were used to enhance the language and clarity of this work. We take full responsibility for the accuracy and integrity of the content.

Conflict of Interest

The authors declare no conflict of interest.

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