In Silico Evaluation of Quercetin, Epigallocatechin Gallate, and Curcumin as Potential Therapeutic Agents Against Early Childhood Caries Through Targeting MMP-8,MMP-9 and TLR- 2

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ABSTRACT

Background: Early childhood caries (ECC) is a multifactorial disease characterized by microbial dysbiosis, inflammation, and degradation of the dentinal extracellular matrix. Matrix metalloproteinases (MMP-8 and MMP-9) and Toll-like receptor-2 (TLR-2) play critical roles in ECC progression and represent potential therapeutic targets.

Aim: To evaluate the molecular interactions, binding affinity, and drug-likeness properties of quercetin, epigallocatechin gallate (EGCG), and curcumin against MMP-8, MMP-9, and TLR-2 using an in silico approach.

Materials and Methods: Crystal structures of MMP-8 (1BZS), MMP-9 (1GKC), and TLR-2 (2Z7X) were retrieved from the Protein Data Bank. Quercetin, EGCG, and curcumin structures were obtained from PubChem. Molecular docking was performed using AutoDock Vina, and interaction analysis was carried out using Discovery Studio Visualizer. Drug-likeness and pharmacokinetic properties were assessed using SwissADME.

Results: EGCG exhibited the strongest binding affinity toward MMP-8 (−8.4 kcal/mol) and TLR-2 (−7.0 kcal/mol), while curcumin demonstrated the highest affinity toward MMP-9 (−8.8 kcal/mol). Quercetin showed favorable multitarget interactions across all proteins. SwissADME analysis revealed favorable drug-likeness profiles for quercetin and curcumin.

Conclusion: Quercetin, EGCG, and curcumin demonstrated promising inhibitory potential against ECC-associated targets. These phytochemicals may serve as potential adjunctive therapeutic agents for ECC prevention and management, warranting further experimental validation.

Keywords: *Early childhood caries, matrix metalloproteinase-8, matrix metalloproteinase-9, Toll-like receptor-2, molecular docking, quercetin, epigallocatechin gallate, curcumin, phytochemicals, in silico analysis, Good Health and Well-Being, oral health, pediatric dentistry, pediatric oral healthcare, sustainable healthcare innovation.*

INTRODUCTION

Early childhood caries (ECC) remains one of the most prevalent chronic diseases affecting children worldwide and continues to represent a significant public health concern.[1] The condition is characterized by the presence of one or more decayed, missing, or filled tooth surfaces in any primary tooth of children younger than six years of age. Despite advances in preventive dentistry, ECC affects millions of children globally and can lead to pain, infection, impaired nutrition, reduced quality of life, and increased healthcare costs.[2] The multifactorial etiology of ECC involves microbial dysbiosis, host susceptibility, dietary factors, and immune-inflammatory responses that collectively contribute to the initiation and progression of dental caries.[3]

Recent evidence suggests that the pathogenesis of ECC extends beyond bacterial acid production and includes complex host-mediated inflammatory mechanisms. Matrix metalloproteinases (MMPs), particularly MMP-8, have been implicated in the degradation of dentinal collagen during caries progression. MMP-8, also known as neutrophil collagenase, is capable of degrading type I collagen, the principal organic component of dentin, thereby accelerating structural destruction of the tooth matrix.[4] Elevated salivary and dentinal levels of MMP-8 have been reported in children with active carious lesions, indicating its potential role as a therapeutic target in caries management.[5]

Another important mediator involved in ECC-associated inflammation is Toll-like receptor 2 (TLR-2). TLR-2 is a pattern-recognition receptor expressed on immune and odontoblastic cells that recognizes bacterial cell wall components and initiates innate immune responses. Activation of TLR-2 triggers downstream inflammatory signaling pathways, resulting in the production of cytokines, chemokines, and matrix-degrading enzymes that contribute to pulpal inflammation and tissue destruction.[6] Increased expression of TLR-2 has been observed in carious dentin and inflamed pulpal tissues, highlighting its importance in the host response to cariogenic microorganisms.[7]

Naturally derived phytochemicals have attracted considerable attention as potential adjunctive agents for caries prevention because of their antimicrobial, antioxidant, and anti-inflammatory properties.[8] Quercetin, a flavonoid widely distributed in fruits and vegetables, has demonstrated antibacterial activity against cariogenic microorganisms and inhibitory effects on inflammatory mediators.[9],[10] Epigallocatechin gallate (EGCG), the principal catechin found in green tea, has been reported to suppress matrix metalloproteinase activity, reduce oxidative stress, and inhibit biofilm formation by oral pathogens.[11] Curcumin, the bioactive component of turmeric, exhibits potent anti-inflammatory and immunomodulatory activities through the regulation of multiple signaling pathways involved in tissue destruction and inflammation.[12]

Several studies have independently investigated the antimicrobial and anti-inflammatory effects of these phytochemicals in oral diseases; however, limited evidence exists regarding their direct molecular interactions with ECC-associated therapeutic targets such as MMP-8 and TLR-2. Furthermore, comparative investigations evaluating the binding affinity and interaction patterns of quercetin, EGCG, and curcumin against both targets within a single computational framework are scarce. This represents an important gap in the literature, as identifying phytochemicals capable of simultaneously modulating collagen degradation and inflammatory signaling may facilitate the development of novel preventive or therapeutic strategies for ECC.

Therefore, the present study aimed to evaluate the molecular interactions of quercetin, epigallocatechin gallate, and curcumin with MMP-8, MMP-9 and TLR-2 using molecular docking techniques. By identifying compounds with favorable binding affinities and interaction profiles, this study seeks to provide preliminary evidence supporting the development of plant-derived therapeutics for the management of ECC and establish a foundation for future in vitro and in vivo investigations.

MATERIALS AND METHODS

Study Design

The present investigation was designed as an in silico molecular docking study to evaluate the potential therapeutic efficacy of selected phytochemicals against key molecular targets implicated in the pathogenesis of early childhood caries (ECC). The study focused on assessing the binding affinity and interaction profiles of quercetin, epigallocatechin gallate (EGCG), and curcumin against matrix metalloproteinase-8 (MMP-8),matrix metalloproteinase-9 (MMP-9) and Toll-like receptor-2 (TLR-2). Molecular docking was selected as a computational approach because it enables prediction of protein–ligand interactions, estimation of binding energies, and identification of crucial amino acid residues involved in molecular recognition before conducting laboratory-based investigations.[13] The study workflow included target protein selection, ligand retrieval and optimization, protein preparation, active-site identification, molecular docking, visualization of molecular interactions, and pharmacokinetic evaluation.

Selection of Target Proteins

MMP-8, MMP-9 and TLR-2 were selected as molecular targets based on their established roles in ECC progression and host inflammatory responses. MMP-8, commonly known as neutrophil collagenase, is a zinc-dependent endopeptidase involved in the degradation of dentinal collagen and extracellular matrix proteins during carious lesion progression. Previous studies have demonstrated increased expression of MMP-8 in active dentinal caries and inflamed pulpal tissues, suggesting its contribution to tooth structure destruction and disease advancement.[14],[15] TLR-2 is an innate immune receptor responsible for recognizing pathogen-associated molecular patterns present on cariogenic microorganisms. Activation of TLR-2 initiates intracellular signaling cascades leading to the production of pro-inflammatory cytokines, chemokines, and tissue-degrading enzymes that contribute to pulpal inflammation and immune dysregulation. The three-dimensional crystal structures of human MMP-8 (PDB ID: 3DPE) MMP-9 (PDB ID: 1GKC) and TLR-2 (PDB ID: 2Z7X) were retrieved from the Protein Data Bank (PDB), a publicly accessible repository containing experimentally validated protein structures.[16]

Ligand Selection and Preparation

Three phytochemicals, namely quercetin, epigallocatechin gallate (EGCG), and curcumin, were selected based on previous reports describing their antimicrobial, antioxidant, anti-inflammatory, and matrix metalloproteinase inhibitory properties relevant to oral diseases [8-10]. The three-dimensional structures of the selected compounds were obtained from the PubChem database in Structure Data File (SDF) format. Quercetin (CID: 5280343), EGCG (CID: 65064), and curcumin (CID: 969516) were downloaded and subsequently converted into Protein Data Bank (PDB) format using Open Babel software version 3.1.1. To obtain energetically stable molecular conformations, geometry optimization and energy minimization were performed using the MMFF94 force field. Hydrogen atoms were added, bond orders were corrected, and molecular structures were optimized to eliminate steric hindrance and unfavorable conformations prior to docking analysis.[17]

Protein Preparation

Protein preparation was carried out using Discovery Studio Visualizer and AutoDock Tools to ensure structural suitability for docking simulations. The downloaded protein structures were inspected for structural integrity, and all crystallographic water molecules, heteroatoms, and co-crystallized ligands not directly involved in active-site stabilization were removed. Polar hydrogen atoms were added to the protein structures, and Kollman charges were assigned to facilitate accurate electrostatic interaction calculations during docking. The prepared proteins were then converted into PDBQT format, which is required for molecular docking using AutoDock Vina. Care was taken to preserve the native conformation of the proteins while ensuring that the active binding pockets remained accessible to the ligands.[18]

Active Site Identification

Identification of biologically relevant active sites is essential for reliable docking predictions. The active-site residues of MMP-8 & 9 were identified based on previously reported catalytic domains and zinc-binding regions involved in collagen degradation . Similarly, the ligand-binding pocket of TLR-2 was identified using information from crystallographic studies and published literature describing receptor activation mechanisms. Grid boxes were generated around the active binding regions to ensure complete coverage of the interaction sites while allowing sufficient space for ligand flexibility and conformational sampling. The coordinates and dimensions of the grid box were optimized to encompass all key amino acid residues implicated in protein function.[19]

Molecular Docking

Molecular docking simulations were performed using AutoDock Vina version 1.2.0, a validated and widely used docking platform for predicting protein–ligand interactions. The prepared ligand and protein structures were imported into the docking environment, and docking calculations were conducted using predefined grid coordinates corresponding to the active sites of MMP-8 ,MMP-9 and TLR-2. Multiple conformational poses were generated for each ligand, and the pose exhibiting the lowest binding free energy and most favorable interaction profile was selected for further analysis. Six docking combinations were evaluated: quercetin–MMP-8, EGCG–MMP-8, curcumin–MMP-8, quercetin–TLR-2, EGCG–TLR-2, and curcumin–TLR-2. Binding affinity scores were recorded in kcal/mol, with more negative values indicating stronger predicted interactions and greater inhibitory potential.[19]

Interaction Analysis

The docked complexes were visualized and analyzed using Discovery Studio Visualizer version 2021. Detailed examination of the protein–ligand complexes was performed to identify hydrogen bonds, hydrophobic contacts, van der Waals interactions, electrostatic interactions, π–π stacking, and π–alkyl interactions. The amino acid residues participating in ligand stabilization within the binding pocket were recorded, and interaction maps were generated to illustrate the molecular basis of binding. Particular attention was given to interactions involving catalytic and functionally important residues because these interactions are often associated with enhanced inhibitory activity. Both two-dimensional and three-dimensional interaction diagrams were generated to provide a comprehensive understanding of ligand-binding behavior.[20]

Drug-Likeness Assessment

To evaluate the pharmaceutical suitability of the selected phytochemicals, drug-likeness analysis was performed using the SwissADME web server. Physicochemical parameters including molecular weight, hydrogen bond donor count, hydrogen bond acceptor count, lipophilicity (LogP), topological polar surface area, and number of rotatable bonds were determined. Compliance with Lipinski's Rule of Five was assessed to estimate oral bioavailability and drug-like characteristics. Compounds exhibiting minimal violations of Lipinski's criteria were considered more favorable candidates for further therapeutic development.[21]

ADMET Prediction

Pharmacokinetic and toxicity profiles of quercetin, EGCG, and curcumin were predicted using the pkCSM and SwissADME online platforms. Parameters analyzed included gastrointestinal absorption, aqueous solubility, blood-brain barrier permeability, cytochrome P450 enzyme interactions, skin permeability, total clearance, hepatotoxicity, and mutagenic potential. ADMET prediction provides an early assessment of the pharmacological feasibility and safety of candidate molecules, thereby reducing the likelihood of failure during subsequent experimental stages. The generated pharmacokinetic profiles were compared among the three phytochemicals to identify compounds possessing favorable absorption, distribution, metabolism, excretion, and toxicity characteristics.[19]

Statistical and Comparative Analysis

Binding affinity values obtained from AutoDock Vina were tabulated and comparatively analyzed across all protein–ligand combinations. The phytochemical demonstrating the most negative docking score together with the highest number of favorable interactions with active-site residues was considered the most promising candidate. Drug-likeness and ADMET data were integrated with docking results to provide a comprehensive evaluation of the therapeutic potential of quercetin, EGCG, and curcumin against ECC-associated targets. The overall findings were interpreted to identify phytochemicals capable of simultaneously modulating collagen degradation mediated by MMP-8 and inflammatory signaling mediated by TLR-2, thereby supporting their potential application as adjunctive agents for the prevention and management of early childhood caries.

RESULTS

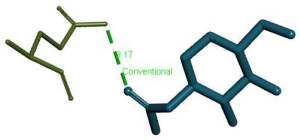

Figure 1a: Three-dimensional molecular interaction of Quercetin with TLR-2 (PDB ID: 2Z7X).

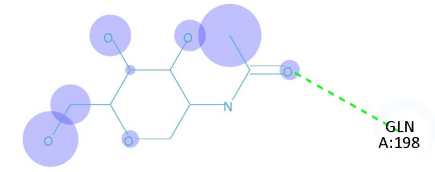

Figure 1b: Two-dimensional interaction map of Quercetin with TLR-2 (PDB ID: 2Z7X).

The 3D visualization in figure 1a revealed the orientation of quercetin within the active binding cavity of TLR-2. A conventional hydrogen bond was observed between quercetin and GLN198, indicating favorable receptor-ligand interaction. The binding pose supports the moderate docking affinity obtained for the quercetin–TLR-2 complex. The 2D interaction analysis in figure 1b demonstrated that quercetin formed a conventional hydrogen bond with GLN198 of TLR-2. Hydrogen bonding contributes to stabilization of the ligand within the receptor binding pocket and may facilitate modulation of TLR-2-mediated inflammatory signaling. Additional van der Waals contacts surrounding the ligand further supported complex stabilization.

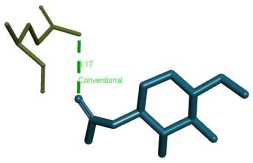

Figure 2a: Three-dimensional molecular interaction of EGCG with TLR-2 (PDB ID: 2Z7X).

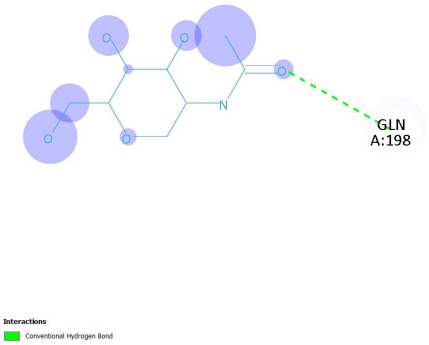

Figure 2b: Two-dimensional interaction map of EGCG with TLR-2 (PDB ID: 2Z7X).

The 3D docking model illustrated in figure 1a the binding orientation of EGCG inside the receptor pocket. EGCG formed a hydrogen bond with GLN198, which is likely to contribute to stabilization of the protein-ligand complex and may influence TLR-2-associated inflammatory pathways. The interaction profile demonstrated in figure 2b that EGCG established a conventional hydrogen bond with GLN198 within the TLR-2 binding region. The presence of hydrogen-bond interactions suggests stable ligand accommodation and may contribute to the strong binding affinity observed during molecular docking analysis.

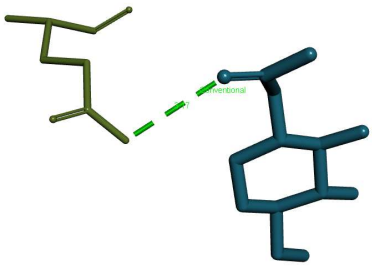

Figure 3b: Three-dimensional molecular interaction of Curcumin with TLR-2 (PDB ID: 2Z7X).

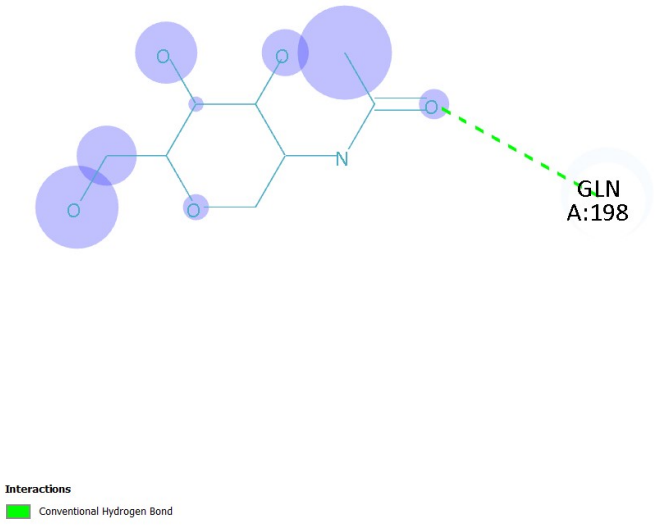

Figure 3a: Two-dimensional interaction map of Curcumin with TLR-2 (PDB ID: 2Z7X).
The three-dimensional docking visualization demonstrated the spatial orientation of curcumin within the active binding pocket of TLR-2. Curcumin formed a conventional hydrogen bond with GLN198, contributing to the stability of the docked complex. The observed binding pose supports the favorable interaction of curcumin with the receptor and its potential anti-inflammatory activity. The 2D interaction analysis showed that curcumin interacted with TLR-2 primarily through a conventional hydrogen bond involving GLN198. Such interactions indicate favorable accommodation of curcumin within the receptor cavity and support its predicted anti-inflammatory potential. The 3D docking visualization demonstrated the spatial orientation of curcumin within the TLR-2 binding pocket. A conventional hydrogen bond with GLN198 was observed, contributing to complex stability and supporting the docking-derived binding affinity of the curcumin–TLR-2 complex.
Interestingly, interaction analysis revealed that quercetin, EGCG, and curcumin all formed conventional hydrogen bonds with the same amino acid residue, GLN198, within the TLR-2 binding pocket. This finding suggests that GLN198 may represent a critical binding hotspot involved in ligand recognition and stabilization. Despite differences in their chemical structures, all three phytochemicals adopted binding conformations that enabled interaction with this residue, highlighting its potential importance in receptor-ligand complex formation. The conservation of this interaction among all tested compounds may indicate that GLN198 plays a key role in mediating the inhibitory effects of phytochemicals against TLR-2-associated inflammatory signaling pathways. However, variations in binding affinity among the compounds suggest that additional non-bonded interactions, including van der Waals forces and hydrophobic contacts, may also contribute to the overall stability of the docked complexes.

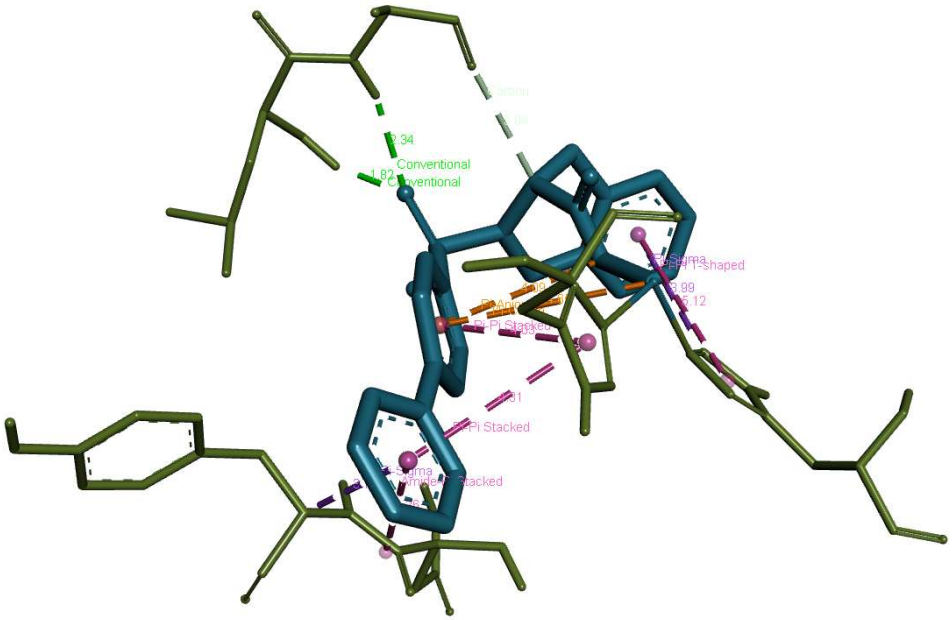

Figure 4a: Three-dimensional interaction model of Quercetin with MMP-8.
The 3D docking visualization revealed that quercetin occupied the catalytic cavity of MMP-8 and interacted closely with key active-site residues and the catalytic zinc ion. The presence of hydrogen bonding, π-cation interactions, and aromatic stacking interactions suggests a stable binding conformation that may contribute to inhibition of collagenolytic activity.

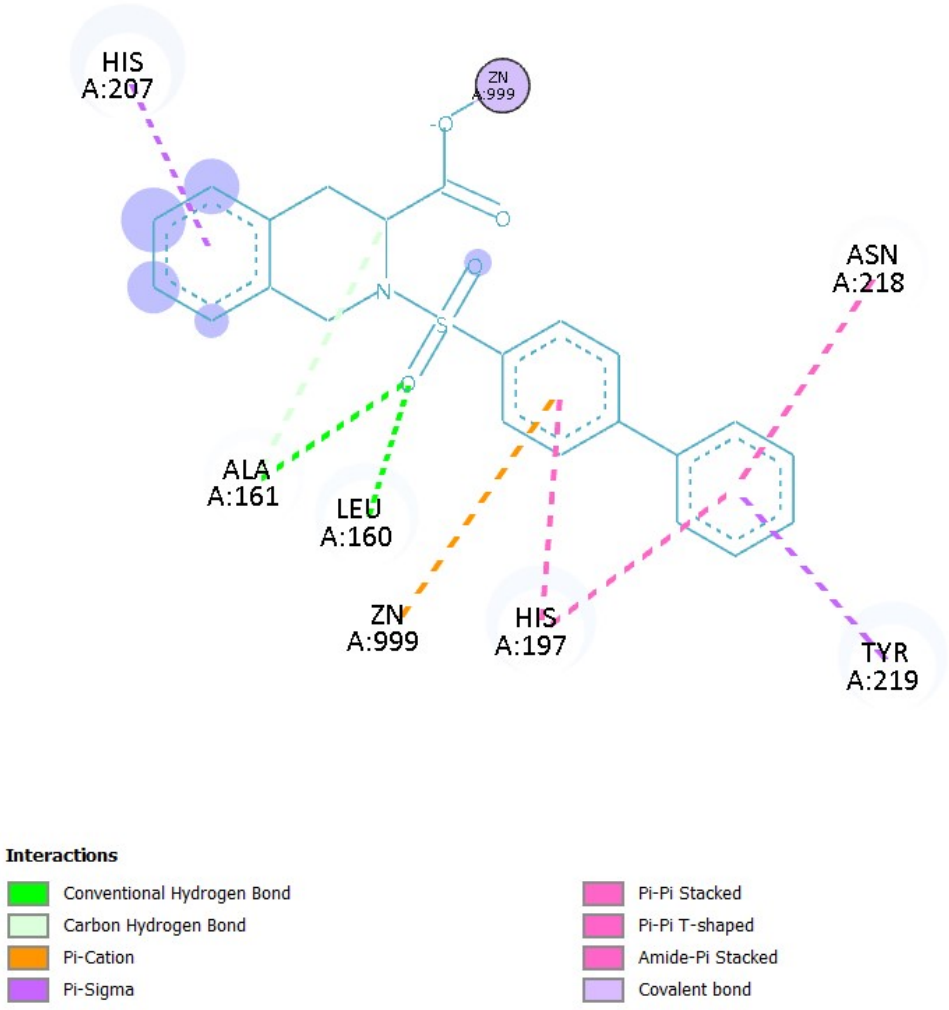

Figure 4a: Two-dimensional interaction map of Quercetin with MMP-8. The 2D interaction analysis demonstrated that quercetin established multiple favorable interactions within the active site of MMP-8. Conventional hydrogen bonds were observed with ALA161 and LEU160, contributing to ligand stabilization within the catalytic pocket. Additional π-cation interactions with the catalytic zinc ion (ZN999), π–π stacking interactions with HIS197 and ASN218, and π-sigma interactions involving HIS207 and TYR219 further strengthened the protein–ligand complex. These interactions indicate effective accommodation of quercetin within the active site of MMP-8.

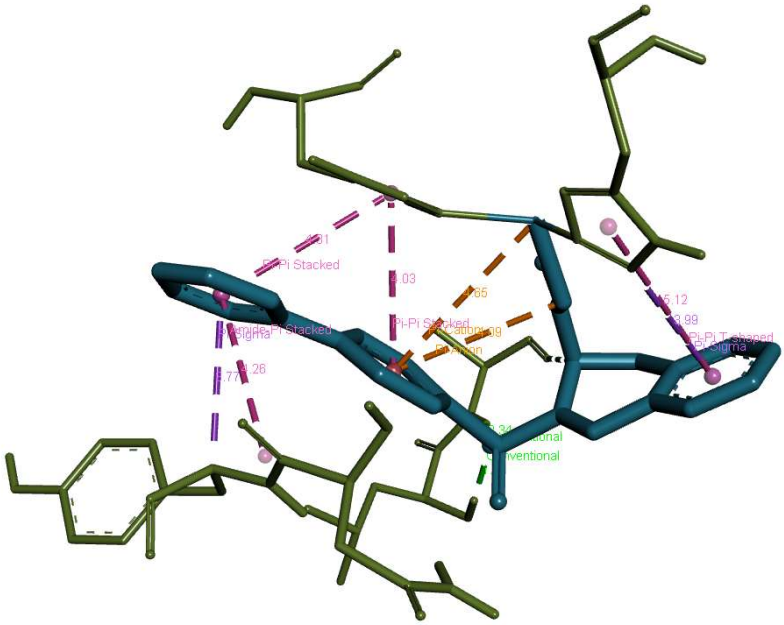

Figure 5a: Three-dimensional interaction model of EGCG with MMP-8. The 3D docking model illustrated deep insertion of EGCG into the MMP-8 catalytic pocket. Multiple hydrogen bonds and aromatic interactions with surrounding amino acid residues created a highly stable protein–ligand complex. The interaction network observed around the zinc-binding region may explain the strong docking affinity exhibited by EGCG.

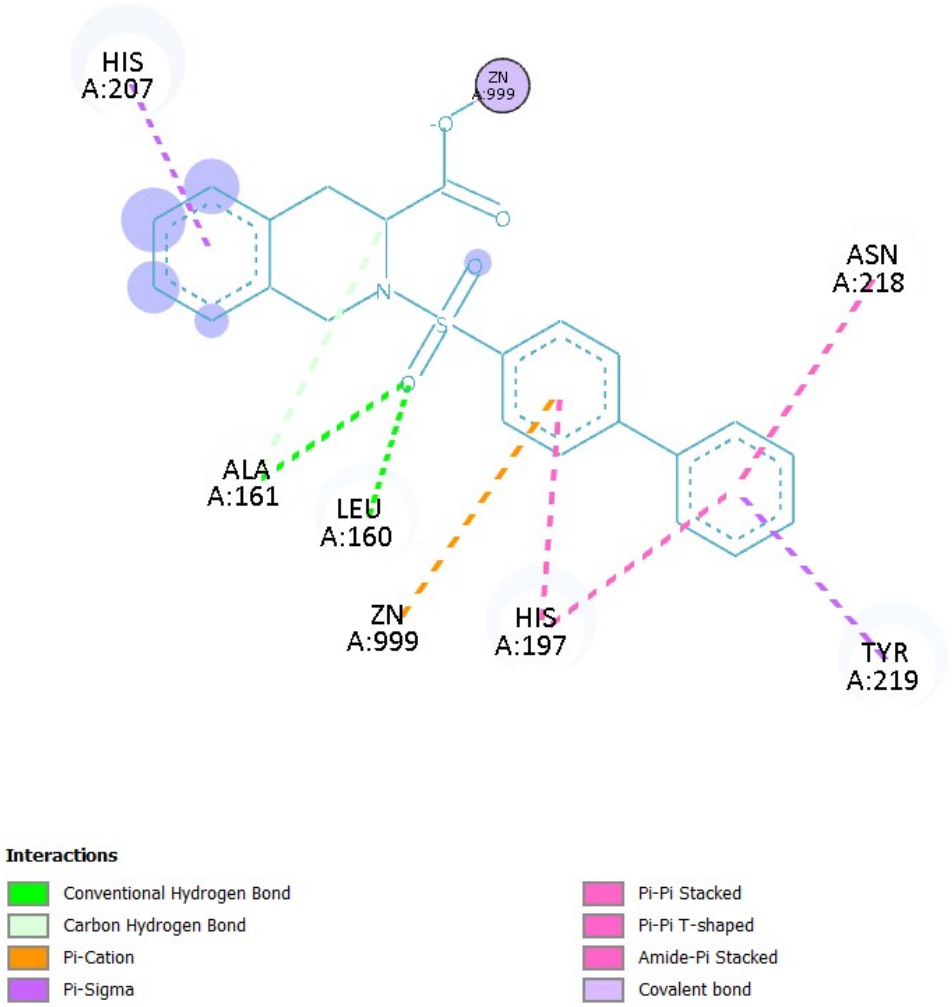

Figure 5b: Two-dimensional interaction map of EGCG with MMP-8. The interaction profile demonstrated that EGCG formed multiple non-covalent interactions within the active site of MMP-8. Conventional hydrogen bonds were observed with residues located near the catalytic region, while aromatic interactions including π–π stacking, π–π T-shaped interactions, and amide–π stacking contributed to stabilization of the ligand. Interaction with the catalytic zinc ion through π-cation contacts further enhanced binding affinity and supported strong ligand accommodation within the active-site cavity.

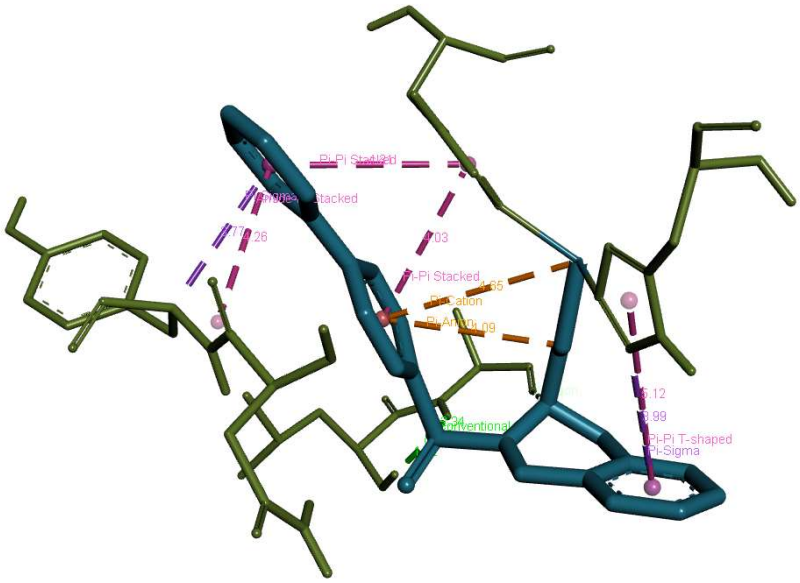

Figure 6a: Three-dimensional interaction model of Curcumin with MMP-8. The 3D visualization demonstrated that curcumin occupied the active-site cavity of MMP-8 and formed a stable interaction network involving hydrogen bonds, aromatic interactions, and zinc-associated contacts. The spatial arrangement of curcumin within the catalytic pocket indicates its potential to interfere with enzyme activity and dentinal collagen degradation.

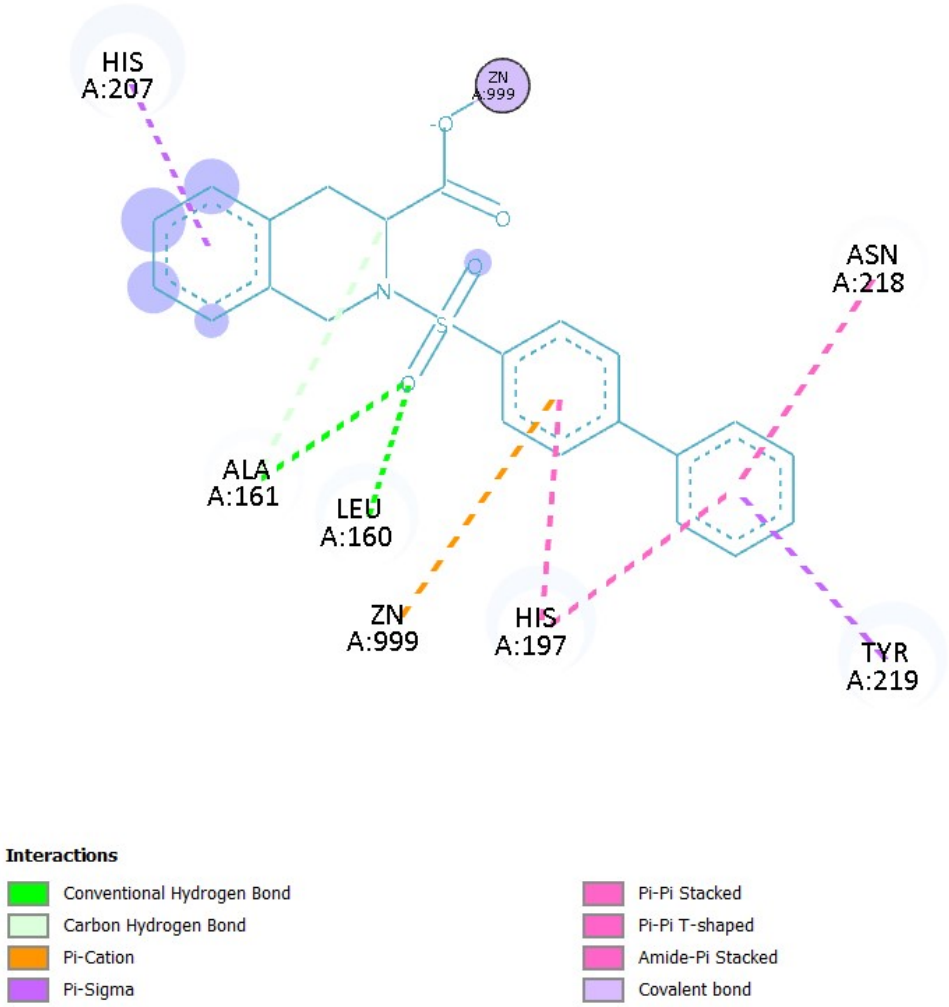

Figure 6b: Two-dimensional interaction map of Curcumin with MMP-8. The 2D interaction analysis showed that curcumin established favorable interactions with MMP-8 through conventional hydrogen bonds, carbon hydrogen bonds, π-cation interactions with the catalytic zinc ion, and multiple aromatic contacts including π–π stacking and π–π T-shaped interactions. Residues such as HIS197, ASN218, HIS207, and TYR219 participated in stabilizing the docked complex, suggesting effective interaction with the catalytic region.

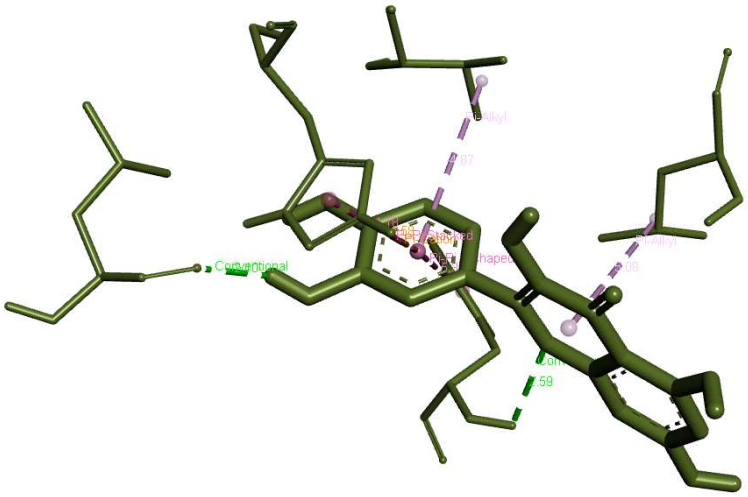

Figure 7a: Three-dimensional interaction model of Quercetin with MMP-9. The 3D docking visualization revealed that quercetin occupied the catalytic pocket of MMP-9 and established several hydrophobic and aromatic interactions with surrounding amino acid residues. The interaction network formed by π-cation, π–π stacked, and π-alkyl contacts contributed to stabilization of the protein–ligand complex and supported the strong binding affinity observed for quercetin.

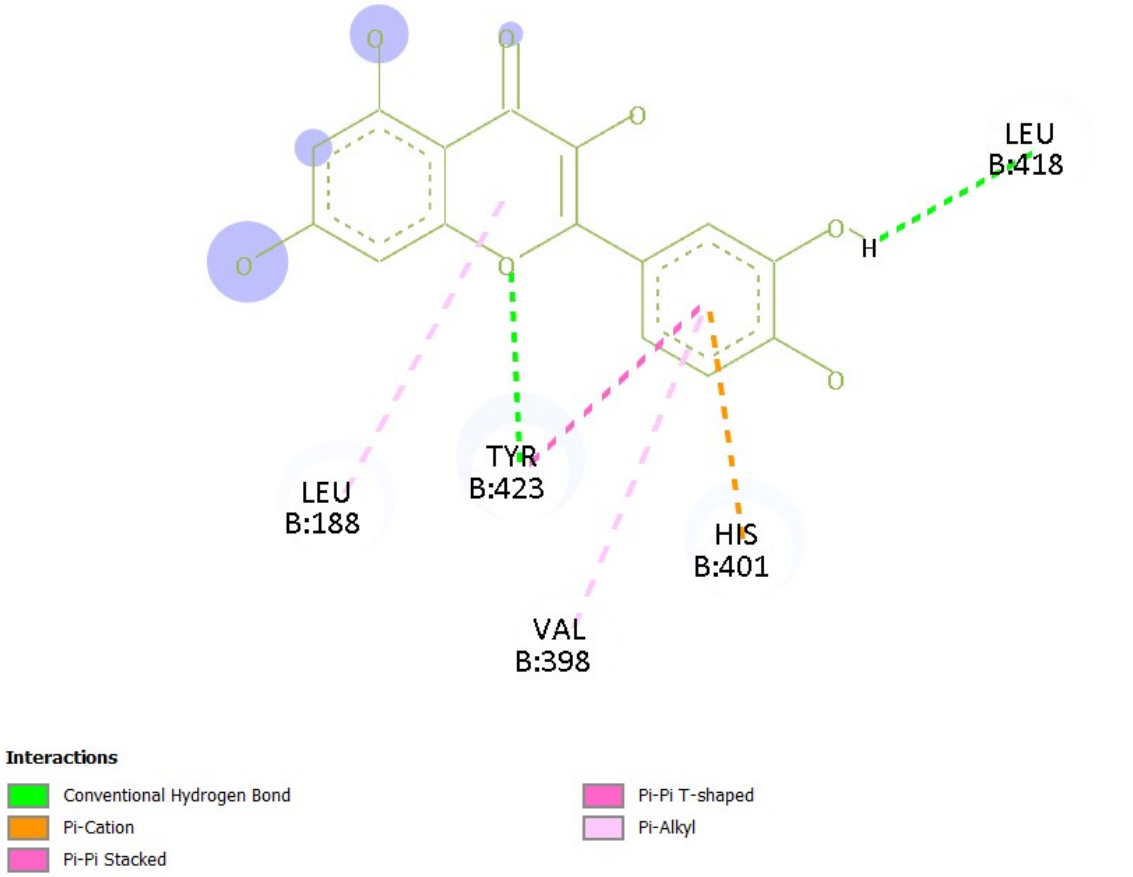

Figure 7b: Two-dimensional interaction map of Quercetin with MMP-9. The 2D interaction analysis demonstrated that quercetin interacted with MMP-9 through multiple non-covalent interactions involving THR426, LEU188, VAL398, HIS401, and TYR423. The complex was stabilized by π-sigma interactions with THR426, π-cation interactions with HIS401, π–π stacked interactions with VAL398, and π-alkyl interactions with neighboring hydrophobic residues. These interactions indicate favorable accommodation of quercetin within the active site of MMP-9.

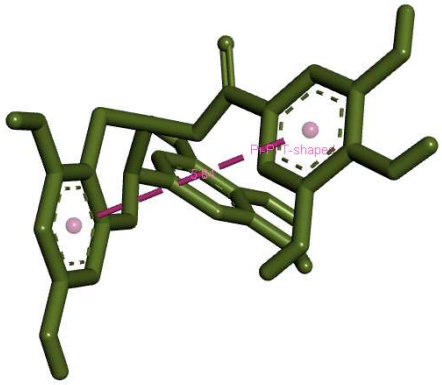

Figure 8a: Three-dimensional interaction model of EGCG with MMP-9. The 3D docking model demonstrated the binding orientation of EGCG within the MMP-9 active pocket. The ligand adopted a stable conformation and interacted with surrounding residues through hydrophobic and aromatic interactions, supporting the favorable docking score obtained during molecular docking analysis.

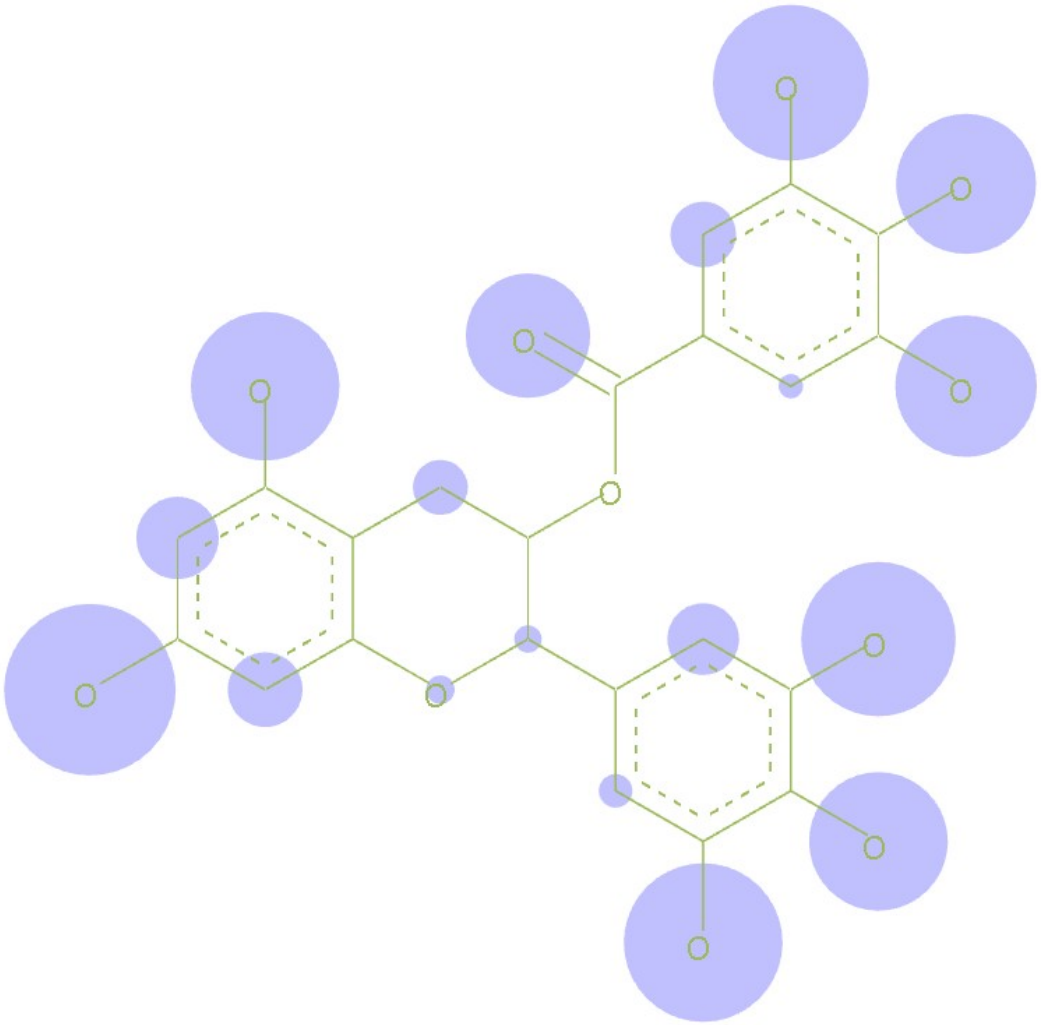

Figure 8b: Two-dimensional interaction map of EGCG with MMP-9. The interaction profile showed that EGCG established extensive contacts with residues surrounding the active site of MMP-9. Multiple hydroxyl groups of EGCG contributed to van der Waals interactions, while aromatic regions participated in hydrophobic contacts that stabilized the docked complex. The interaction pattern suggests effective ligand accommodation within the enzyme binding cavity.

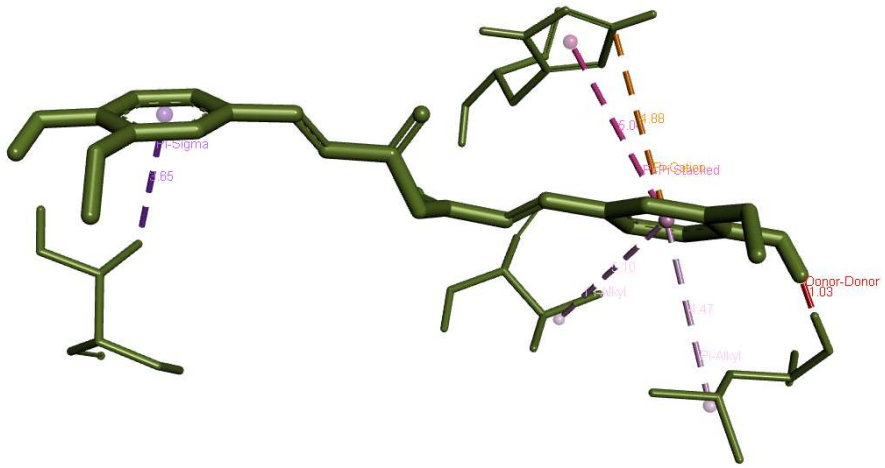

Figure 9a:. Three-dimensional interaction model of Curcumin with MMP-9. The 3D docking visualization demonstrated that curcumin occupied the active-site cavity of MMP-9 and established a dense interaction network involving hydrogen bonds, π-cation contacts, and aromatic interactions. These interactions contributed to stabilization of the protein–ligand complex and may explain the strong binding affinity exhibited by curcumin toward MMP-9.

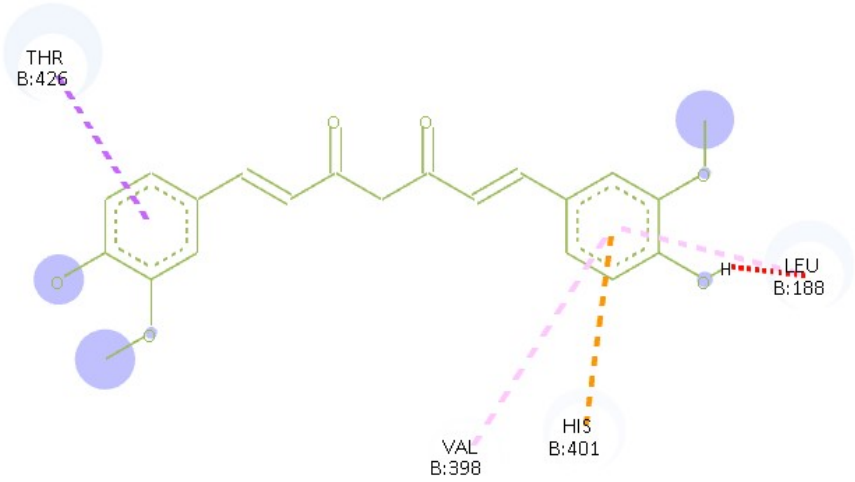

Figure 9b: Two-dimensional interaction map of Curcumin with MMP-9. The 2D interaction analysis revealed that curcumin formed conventional hydrogen bonds with LEU418 and TYR423, along with π-cation interactions involving HIS401. Additional π–π stacked, π–π T-shaped, and π-alkyl interactions with VAL398 and neighboring residues further enhanced complex stability. The presence of both hydrogen bonding and aromatic interactions suggests strong binding within the catalytic region of MMP-9.

The molecular docking analysis demonstrated favorable interactions of quercetin, EGCG, and curcumin with MMP-9 (PDB ID: 1GKC). Curcumin exhibited the highest binding affinity (−8.8 kcal/mol), followed by quercetin (−8.7 kcal/mol) and EGCG (−7.8 kcal/mol). The docked complexes were stabilized by hydrogen bonds, π-cation interactions, π–π stacked interactions, and hydrophobic contacts involving key residues such as HIS401, VAL398, TYR423, LEU418, and THR426. These findings suggest that curcumin and quercetin may possess strong inhibitory potential against MMP-9 and could contribute to reducing matrix degradation associated with early childhood caries.

Target Protein	PDB ID	Ligand	PubChem CID	Binding Energy (kcal/mol)	Binding Strength
MMP-8	1BZS	Quercetin	5280343	-7.6	Strong
MMP-8	1BZS	EGCG	65064	-8.4	Very Strong
MMP-8	1BZS	Curcumin	969516	-6.6	Moderate

MMP-9	1BZS	Quercetin	5280343	-8.7	Strong
MMP-9	1BZS	EGCG	65064	-7.8	Moderate
MMP-9	1BZS	Curcumin	969516	-8.8	Very Strong
TLR-2	2Z7X	Quercetin	5280343	-6.7	Moderate to Strong
TLR-2	2Z7X	EGCG	65064	-7	Strong
TLR-2	2Z7X	Curcumin	969516	-6.3	Moderate

Table 1: Summary of Molecular Docking Results of Quercetin, EGCG, and Curcumin Against MMP-8 (PDB ID: 1BZS), MMP-9 (PDB ID: 1GKC) and TLR-2 (PDB ID: 2Z7X)

The molecular docking results presented in Table 1 demonstrated favorable interactions of all three phytochemicals with the selected ECC-associated targets. Among the compounds evaluated, EGCG exhibited the strongest binding affinity toward MMP-8 (−8.4 kcal/mol) and TLR-2 (−7.0 kcal/mol), indicating its potential to inhibit collagen degradation and inflammatory signaling pathways. In contrast, curcumin showed the highest affinity for MMP-9 (−8.8 kcal/mol), followed closely by quercetin (−8.7 kcal/mol), suggesting strong inhibitory potential against matrix degradation. Quercetin demonstrated consistently favorable binding across all three targets, indicating a multitarget therapeutic profile. These findings suggest that EGCG and curcumin possess superior target-specific interactions, while quercetin exhibits balanced activity against MMP-8, MMP-9, and TLR-2, supporting their potential application as phytotherapeutic agents for the prevention and management of early childhood caries.

Parameter	**Quercetin**	**EGCG**	**Curcumin**
Molecular formula	C15H10O7	C22H18O11	C21H20O6
Molecular weight	302.24 g/mol	458.37 g/mol	368.38 g/mol
H-bond acceptors	7	11	6
H-bond donors	5	8	2
TPSA	131.36 Å²	197.37 Å²	93.06 Å²
GI absorption	High	Low	High
BBB permeant	No	No	No
P-gp substrate	No	No	No
Lipinski rule	Yes; 0 violation	No; 2 violations	Yes; 0 violation
Bioavailability score	0.55	0.17	0.55
PAINS alert	1 alert	1 alert	0 alert
Lead-likeness	Yes	No	No
Synthetic accessibility	3.23	4.2	2.97
Overall ADME suitability	Good	Limited	Good

Table 2: SwissADME Summary Table for Quercetin, EGCG and Curcumin

SwissADME analysis showed that quercetin and curcumin had better drug-likeness profiles, with no Lipinski rule violations and high predicted gastrointestinal absorption. EGCG showed low GI absorption, high TPSA, increased hydrogen bond donors/acceptors, and two Lipinski violations, suggesting poor oral bioavailability. None of the compounds were predicted to cross the blood–brain barrier, which may indicate a lower risk of central nervous system-related effects. Although these phytochemicals showed promising docking affinity, especially EGCG and curcumin, their direct clinical use may be limited by poor bioavailability, solubility issues, and medicinal chemistry

alerts. Therefore, they may be better suited for topical dental formulations, gels, varnishes, mouth rinses, or nanoparticle-based delivery systems rather than direct systemic use. Quercetin and curcumin showed more favorable ADME profiles, while EGCG may require formulation improvement to enhance absorption and therapeutic effectiveness.

DISCUSSION

The present in silico investigation evaluated the binding interactions of quercetin, epigallocatechin gallate (EGCG), and curcumin against three key molecular targets implicated in the pathogenesis of early childhood caries (ECC), namely MMP-8, MMP-9, and TLR-2. The docking results demonstrated favorable interactions for all three phytochemicals, with EGCG exhibiting the strongest affinity toward MMP-8 (−8.4 kcal/mol) and TLR-2 (−7.0 kcal/mol), whereas curcumin showed the highest affinity toward MMP-9 (−8.8 kcal/mol). These findings support the growing evidence that plant-derived bioactive compounds may serve as multitarget therapeutic agents capable of modulating both matrix degradation and inflammatory pathways associated with ECC.

Quercetin demonstrated strong binding affinities against MMP-8 (−7.6 kcal/mol), MMP-9 (−8.7 kcal/mol), and TLR-2 (−6.7 kcal/mol), indicating broad-spectrum activity across all evaluated targets. These findings are consistent with the study by Zeng et al., who reported that quercetin significantly inhibited *Streptococcus mutans* biofilm formation and reduced cariogenic activity, suggesting its potential role in caries prevention.[22] Yang et al. demonstrated that quercetin incorporated into dental adhesives inhibited *S. mutans* biofilms while also exhibiting matrix metalloproteinase inhibitory properties, thereby preserving dentin bond stability.[23] The favorable interaction of quercetin with both MMP-8 and MMP-9 observed in the present study may explain its previously reported protective effects on dentinal collagen and extracellular matrix integrity. Furthermore, nano-quercetin-mediated antimicrobial photodynamic therapy has been shown to disrupt bacterial virulence and biofilm metabolism, supporting the multifunctional role of quercetin in oral disease management.[19]

Among the tested compounds, EGCG exhibited the highest affinity toward MMP-8 and TLR-2, suggesting superior anti-inflammatory and anti-collagenolytic potential. Although direct docking studies against MMP-8 and TLR-2 are limited, previous literature has consistently demonstrated the ability of EGCG to suppress matrix metalloproteinase activity and inflammatory mediator production.[11] The strong interaction of EGCG with TLR-2 observed in the present study may indicate its potential to regulate innate immune signaling pathways activated by cariogenic microorganisms.[24] However, SwissADME analysis revealed low gastrointestinal absorption, high topological polar surface area, and multiple drug-likeness violations, suggesting that systemic administration may be challenging. Nevertheless, topical delivery systems such as varnishes, gels, and nanoparticle formulations could overcome these limitations and maximize local therapeutic effects within the oral cavity.

Curcumin demonstrated the strongest binding affinity toward MMP-9 (−8.8 kcal/mol), indicating a high potential for inhibiting extracellular matrix degradation and inflammatory tissue destruction. This finding is in agreement with previous studies reporting significant anti-cariogenic and anti-biofilm effects of curcumin. Lan et al. demonstrated that curcumin reduced *S. mutans* biofilm formation, bacterial metabolism, and virulence factor expression.[25],[26] Another investigation involving clinical isolates from severe ECC showed enhanced antibiofilm activity of curcumin against highly cariogenic strains.[27] Senthil et al. further reported that curcumin inhibited sortase A activity, thereby reducing bacterial adhesion and colonization on tooth surfaces.[28] In addition, curcumin has been shown to suppress adherence of *S. mutans* to extracellular matrix proteins and dental surfaces, an important mechanism in the prevention of early biofilm formation.[29] The strong interaction of curcumin with MMP-9 identified in the present study may therefore complement its previously established antimicrobial properties.

The observed affinity of the phytochemicals toward MMP-8 and MMP-9 is particularly relevant because these enzymes play a critical role in dentinal collagen degradation during caries progression. Elevated salivary concentrations of MMP-8 have been associated with severe ECC and increased tissue destruction.[12] MMP-9 contributes to extracellular matrix remodeling and inflammatory tissue breakdown in oral diseases. Therefore, inhibition of these enzymes may slow caries progression and preserve dentinal integrity. The strong interactions observed with MMP targets in the present study suggest a potential mechanism through which these phytochemicals may exert protective effects beyond their antimicrobial activity.[6]

The docking results against TLR-2 are equally significant because TLR-2 is a key mediator of innate immune responses in carious lesions. Previous studies have reported significantly elevated salivary TLR-2 levels in children with active dental caries, indicating its involvement in disease progression and host inflammatory responses. The strong binding affinity of EGCG and quercetin toward TLR-2 suggests that these compounds may interfere with receptor activation and downstream cytokine signaling pathways, thereby reducing inflammation associated with ECC.

A key strength of this study is the simultaneous evaluation of quercetin, EGCG, and curcumin against multiple

ECC-associated targets (MMP-8, MMP-9, and TLR-2), along with assessment of their pharmacokinetic properties using SwissADME. However, the study is limited by its purely computational design, and the predicted interactions require experimental validation. Future research should focus on molecular dynamics simulations, in vitro and in vivo validation, and the development of advanced delivery systems such as nanoparticles, hydrogels, or varnishes to enhance the clinical applicability of these phytochemicals in early childhood caries prevention and management.

CONCLUSION

Within the limitations of this in silico study, quercetin, EGCG, and curcumin demonstrated favorable interactions with ECC-associated targets involved in dentinal matrix degradation and inflammatory signaling. EGCG emerged as the most promising candidate against MMP-8 and TLR-2, whereas curcumin showed the strongest affinity toward MMP-9. The consistent binding profile of quercetin across all three targets further highlights its potential as a multitarget phytochemical. These findings suggest that naturally derived compounds may offer a novel adjunctive approach for ECC prevention by targeting both microbial-host interactions and tissue destruction pathways. However, further in vitro, in vivo, and clinical investigations are required to confirm their biological efficacy and therapeutic applicability.

REFERENCES

1. Janani, Ramesh R, Ramakrishnan M. Association of salivary catalase activity and oxidative stress in early childhood caries: A biomarker for caries risk. Journal of South Asian Association of Pediatric Dentistry [Internet]. 2026 Mar 5;9(1):51–6. Available from: http://dx.doi.org/10.5005/jp-journals-10077-3403
2. Douglas G. Dental caries: the disease and its clinical management (4th edition). Br Dent J [Internet]. 2025 May;238(10):763. Available from: http://dx.doi.org/10.1038/s41415-025-8727-y
3. Pitts NB, Zero DT, Marsh PD, Ekstrand K, Weintraub JA, Ramos-Gomez F, et al. Dental caries. Nat Rev Dis Primers [Internet]. 2017 May 25;3:17030. Available from: http://dx.doi.org/10.1038/nrdp.2017.30
4. Nishitha D, Ramesh D, Mahesh D. Evaluation of glyphosate interaction with Nrf2 and heme oxygenase-1 and its implications for oxidative stress in early childhood caries: An in silico study. Int J Drug Deliv Technol [Internet]. 2026 Apr 21;16(15s). Available from: http://dx.doi.org/10.25258/ijddt.16.15s.69
5. Senthil R, Angamuthu D, Geetha Sravanthy P, Pradeep Kumar R. Integrative nanoformulation of paclitaxel, ruthenium (II), and curcumin for enhanced oral cancer cell suppression. J Oral Biol Craniofac Res [Internet]. 2025 Nov;15(6):1824–30. Available from: http://dx.doi.org/10.1016/j.jobcr.2025.10.024
6. Tjäderhane L, Buzalaf MAR, Carrilho M, Chaussain C. Matrix metalloproteinases and other matrix proteinases in relation to cariology: the era of "dentin degradomics." Caries Res [Internet]. 2015 Feb 6;49(3):193–208. Available from: http://dx.doi.org/10.1159/000363582
7. Kaczor-Urbanowicz KE, Carreras-Presas M, Aro C, Tu K, Garcia-Godoy M, Wong F. Toll-like receptor 2. Takeda K, Akira S. Toll-like receptors in innate immunity. Exp Biol Med. 2005;242(5):1–14.
8. Kishorwara R, Pillai DS, Madhumitha M, Mohanprasanth A, Snega R. Research article Structural and optical characteristics of green-synthesized ZnO/SrO nanocomposites using pomegranate peel extract and their biological activity.
9. Veerayutthwilai O, Byers MR, Pham TTT, Darveau RP, Dale BA. Differential regulation of immune responses by odontoblasts. Oral Microbiol Immunol [Internet]. 2007 Feb;22(1):5–13. Available from: http://dx.doi.org/10.1111/j.1399-302X.2007.00310.x
10. Anand David AV, Arulmoli R, Parasuraman S. Overviews of biological importance of quercetin: A bioactive flavonoid. Pharmacogn Rev [Internet]. 2016 Jul;10(20):84–9. Available from: http://dx.doi.org/10.4103/0973-7847.194044
11. Reygaert WC. Green tea catechins: Their use in treating and preventing infectious diseases. Biomed Res Int [Internet]. 2018 Jul 17;2018:9105261. Available from: http://dx.doi.org/10.1155/2018/9105261
12. Hewlings SJ, Kalman DS. Curcumin: A review of its effects on human health. Foods [Internet]. 2017 Oct 22;6(10):E92. Available from: http://dx.doi.org/10.3390/foods6100092
13. Mohamed A, Ramesh D. Antimicrobial evaluation of Cassia auriculata extracts against streptococcus mutans and Enterococcus faecalis: An in vitro study. Int J Drug Deliv Technol [Internet]. 2026 Apr 13;16(8s). Available from: http://dx.doi.org/10.25258/ijddt.16.8s.114
14. Maheswari UK, Ramesh R, Alex P, Babu AA, George R, Mathew A. Assessment of Anti-Caries Potential of Maqui Berry Cystatin for Early Childhood Caries Prevention -An In Silico Study.
15. Harshini S, Ramesh R, Mahesh R, Alex P, Babu AA, George R. Assessment of Acetic Acid Interaction with MMP-8 and MMP-9: Implications for Dentin Matrix Degradation in Early Childhood Caries -An in silico study.
16. Dallakyan S, Olson AJ. Small-molecule library screening by docking with PyRx. Methods Mol Biol [Internet]. 2015;1263:243–50. Available from: http://dx.doi.org/10.1007/978-1-4939-2269-7_19
17. Kim S, Chen J, Cheng T. PubChem in 2023: new data content and improved web interfaces. Nucleic Acids Res. 2023;51(D1):D1373–80.
18. Pagadala NS, Syed K, Tuszynski J. Software for molecular docking: a review. Biophys Rev [Internet]. 2017 Apr;9(2):91–102. Available from: http://dx.doi.org/10.1007/s12551-016-0247-1

19. Joshy R, Ramesh D, Mahesh D. Structural insights into curcumin interaction with inflammatory cytokines and proliferative protein targets in early childhood caries - an in silico docking study. Int J Drug Deliv Technol [Internet]. 2026 Apr 9;16(6s). Available from: http://dx.doi.org/10.25258/ijddt.16.6s.91
20. Prasad R, Roopa S, Mudhol MB, Khan A, Jayaraman S, Jayaraman T. In silico evaluation of ligand interaction with Nrf2, V1a receptor, SOD, and Angiotensin II: A molecular docking study. Int J Drug Deliv Technol [Internet]. 2026 Apr 4;16(5s). Available from: http://dx.doi.org/10.25258/ijddt.16.5s.53
21. Noordam ER, Haryanto K, Iin Hardiyati IH, Wilapangga A, Yudianto D, Komarudin D, et al. Microbiological evaluation of garlic extract and SwissADME profiling of allicin as an antimicrobial candidate. Indonesian Journal of Pharmaceutical Education [Internet]. 2026 May 10;6(2):190–200. Available from: http://dx.doi.org/10.37311/ijpe.v6i2.37287
22. Zeng Y, Nikitkova A, Abdelsalam H, Li J, Xiao J. Activity of quercetin and kaemferol against Streptococcus mutans biofilm. Arch Oral Biol [Internet]. 2019 Feb;98:9–16. Available from: http://dx.doi.org/10.1016/j.archoralbio.2018.11.005
23. Chen W, Jiang Q, Yan G, Yang D. The oral microbiome and salivary proteins influence caries in children aged 6 to 8 years. BMC Oral Health [Internet]. 2020 Oct 28;20(1):295. Available from: http://dx.doi.org/10.1186/s12903-020-01262-9
24. Mahalingam M, Pillai DS, Muthupandian S. Assessment of anti-inflammatory and antioxidant properties of trypsin, bromelain, rutoside and glucosamine combination gel: An in-vitro study. J Clin Diagn Res [Internet]. 2026 Jan 1; Available from: http://dx.doi.org/10.7860/jcdr/2025/79703.22219
25. Lan C, Yang Y, Li J, Zhang X, Wang X. Curcumin attenuates lipopolysaccharide-induced inflammation in human dental pulp stem cells via inhibition of the NLRP3 inflammasome and NF-κB signaling pathway. J Endod [Internet]. 2023;49(3):372–80. Available from: http://dx.doi.org/10.1016/j.joen.2022.11.012
26. Madhumitha M, Pillai DS, Kumar RS. Assessment of Antimicrobial Efficacy of Saffron - Withania Somnifera Herbal gel Against oral ulcers-An Invitro Study.
27. Sruthi MA, Mani G, Ramakrishnan M, Selvaraj J. Dental caries as a source of Helicobacter pylori infection in children: An RT-PCR study. Int J Paediatr Dent [Internet]. 2023 Jan;33(1):82–8. Available from: http://dx.doi.org/10.1111/ipd.13017
28. Senthil R, R PK, Sravanthy PG. Dual-drug-loaded carbopol gel for enhanced oral wound healing: a curcumin-ciprofloxacin-based therapeutic approach. Oral Maxillofac Surg [Internet]. 2025 Nov 28;30(1):3. Available from: http://dx.doi.org/10.1007/s10006-025-01489-2
29. Harshini S, Ramesh R, Mahesh R. Evaluation of Amoxycillin with Host Immune Mediators and Streptococcus mutans Virulence Proteins Associated with Early Childhood Caries - An In-Silico Study. Int J Drug Deliv Technol [Internet]. 2026;16(12s):779–90. Available from: http://dx.doi.org/10.25258/ijddt.16.12s.92