Assessment of Anti-Caries Potential of Maqui Berry Cystatin for Early Childhood Caries Prevention - An In Silico Study

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Abstract: Background: Matrix metalloproteinases (MMPs), particularly MMP-8 and MMP-9, play a crucial role in dentin collagen degradation and progression of Early Childhood Caries (ECC). Natural anthocyanins derived from Maqui berry have demonstrated antioxidant and anti-inflammatory properties, but their interaction with caries-associated MMPs remains poorly understood.
Aim: To evaluate the binding affinity and interaction profile of Delphinidin-3-O-glucoside, a major Maqui berry anthocyanin, against MMP-8 and MMP-9 using molecular docking analysis.
Materials and Methods: An in silico molecular docking study was performed using AutoDock Vina integrated within PyRx. Delphinidin-3-O-glucoside (PubChem CID: 443650) was docked against MMP-9 (PDB ID: 1GKC) and MMP-8 (PDB ID: 1BZS). Binding affinity, interaction residues, hydrogen bonding, hydrophobic contacts, and electrostatic interactions were analyzed using Discovery Studio Visualizer.
Results: Delphinidin-3-O-glucoside demonstrated favorable binding toward both MMP-9 and MMP-8, with docking scores of −7.5 kcal/mol and −6.9 kcal/mol, respectively. Multiple hydrogen bonds, π-alkyl interactions, and electrostatic contacts contributed to complex stability. The ligand exhibited stronger affinity toward MMP-9, suggesting enhanced inhibitory potential against dentin matrix degradation.
Conclusion: Delphinidin-3-O-glucoside showed promising interactions with MMP-8 and MMP-9, supporting its potential as a natural inhibitor of matrix metalloproteinase-mediated dentin degradation. These findings provide a molecular basis for further experimental studies exploring Maqui berry anthocyanins as preventive agents for Early Childhood Caries...
Keywords: Early Childhood Caries; Matrix Metalloproteinase-8; Matrix Metalloproteinase-9; Molecular Docking Simulation; Anthocyanins; Good Health and Well-Being and Pediatric Dentistry

Introduction

Early Childhood Caries (ECC) is one of the most prevalent chronic childhood diseases worldwide and remains a significant public health concern despite advances in preventive dentistry. The disease is characterized by the presence of one or more decayed, missing, or filled tooth surfaces in any primary tooth of a child younger than six years of age. ECC results from a complex interaction among cariogenic microorganisms, dietary sugars, susceptible tooth surfaces, and host-related factors such as saliva and acquired enamel pellicle composition. The progression of ECC can lead to pain, infection, impaired nutrition, reduced quality of life, and increased treatment costs, emphasizing the need for effective preventive strategies. [1],[2]

Fluoride remains the cornerstone of modern caries prevention due to its ability to enhance enamel remineralization, inhibit demineralization, and reduce acid solubility of hydroxyapatite crystals.[3],[4],[5] Numerous clinical trials and systematic reviews have demonstrated the effectiveness of fluoride-containing products in reducing caries incidence in both primary and permanent dentitions.[6],[7] However, despite widespread fluoride use, ECC continues to affect millions of children globally, suggesting that fluoride alone may not be sufficient to completely control the disease process, particularly in high-risk populations.[8]

In recent years, increasing attention has been directed toward biomimetic and biological approaches that enhance the protective functions of the acquired enamel pellicle. Salivary cystatins are naturally occurring proteins involved in regulating mineral homeostasis and protecting enamel surfaces. Cystatin molecules have been identified as important components of the acquired enamel pellicle and contribute to the maintenance of tooth integrity through protein–mineral interactions.[7] Plant-derived cystatins have emerged as promising candidates for dental applications. Experimental studies involving sugarcane cystatin (CaneCPI-5) demonstrated reduced enamel mineral loss, decreased biofilm activity, and enhanced protection against erosive challenges.[9] More recently, recombinant Maquiberry cystatin (MaquiCPI-3) has been investigated for its potential role in acquired pellicle engineering and enamel protection.[10]

Stannous compounds have also gained considerable interest in preventive dentistry. Stannous fluoride and stannous ions exhibit antibacterial, antiplaque, anti-erosive, and remineralizing properties that extend beyond the conventional actions of fluoride alone. The incorporation of stannous ions into enamel surfaces promotes the formation of acid-resistant protective layers and reduces biofilm viability, thereby providing additional protection against demineralization. These findings suggest that combining fluoride with stannous compounds may offer enhanced preventive benefits compared with fluoride monotherapy.[11],[12]

A recent in vitro investigation demonstrated that formulations containing Maquiberry cystatin, sodium fluoride (NaF), and stannous chloride ($SnCl_2$) provided superior protection against initial enamel erosion compared with individual agents alone, indicating a potential synergistic interaction among these components.[13],[14] No comprehensive in silico studies have evaluated the interactions of Maquiberry cystatin with enamel-associated proteins, salivary pellicle components, or cariogenic targets relevant to ECC development.

The existing literature primarily focuses on the individual effects of fluoride, stannous compounds, or cystatin-based proteins. There is a paucity of computational evidence exploring their combined molecular behavior, binding affinity, and potential cooperative mechanisms involved in enamel protection and caries prevention. Understanding these interactions at the molecular level may provide valuable insights into the development of novel preventive formulations specifically targeted toward ECC.

Therefore, the present in silico study was designed to evaluate the synergistic anti-caries potential of Maquiberry cystatin through molecular docking and computational interaction analyses. By elucidating their molecular interactions with enamel- and caries-related targets, this study aims to provide a mechanistic rationale for the development of next-generation biomimetic preventive strategies for Early Childhood Caries.

MATERIALS AND METHODS

Materials and Methods

Study Design

This study was designed as an in silico molecular docking analysis to evaluate the binding affinity and interaction pattern of selected Maqui berry-derived anthocyanin compounds against matrix metalloproteinase-8 and matrix metalloproteinase-9. The selected ligands were docked against MMP-9 and MMP-8 to assess their possible inhibitory potential against dentin matrix degradation-related enzymes relevant to Early Childhood Caries.

Selection of Ligands

Four phytochemical ligands were selected from PubChem based on their reported presence as anthocyanin-related compounds in berry sources. The selected compounds were Delphinidin 3-O-glucoside cation, Petunidin 3-galactoside, Kuromanin/Cyanidin 3-O-glucoside cation, and Delphinidin cation. Their respective PubChem compound identifiers were CID 443650, CID 14311149, CID 441667, and CID 128853. The three-dimensional conformer structures were downloaded from PubChem in SDF format. The ligands were subsequently converted into PDB format using Open Babel software. Energy minimization was performed to obtain stable ligand conformations before docking. The prepared ligands were then imported into PyRx and converted into PDBQT format using the built-in Open Babel tool.

Selection and Retrieval of Target Proteins

Two target proteins were selected for the docking analysis: MMP-9 and MMP-8. The crystal structure of MMP-9 was retrieved from the RCSB Protein Data Bank using PDB ID 1GKC. The crystal structure of MMP-8 was retrieved using PDB ID 1BZS. These targets were selected because MMP-8 and MMP-9 are collagenolytic matrix metalloproteinases associated with extracellular matrix breakdown, dentin collagen degradation, and caries progression.

Docking Plan

Each ligand was docked separately against both target proteins. Therefore, a total of eight docking runs were performed.

Ligand	PubChem CID	Target 1	PDB ID	Target 2	PDB ID
Delphinidin 3-O-glucoside cation	443650	MMP-9	1GKC	MMP-8	1BZS
Petunidin 3-galactoside	14311149				
Kuromanin / Cyanidin 3-O-glucoside cation	441667				
Delphinidin cation	128853				

Protein Preparation

The downloaded PDB structures of MMP-9 and MMP-8 were prepared before docking. The structures were opened in Discovery Studio Visualizer, and co-crystallized ligands, water molecules, and unwanted heteroatoms were removed. The native zinc ion present in the catalytic domain was retained because MMPs are zinc-dependent enzymes and removal of zinc may affect the biological relevance of docking. The cleaned receptor structures were saved in PDB format. The receptor files were then imported into AutoDock Tools for addition of polar hydrogen atoms and Kollman charges. The prepared protein structures were saved in PDBQT format for docking.

Ligand Preparation

The four ligand structures downloaded from PubChem were inspected for structural errors. The ligands were energy-minimized using Open Babel with the MMFF94 force field. The minimized structures were imported into PyRx. Gasteiger charges were assigned, rotatable bonds were detected, and the ligands were converted into PDBQT format. Each ligand was prepared individually before docking with MMP-9 and MMP-8.[15]

Molecular Docking Procedure

Molecular docking was performed using PyRx software with AutoDock Vina as the docking engine. The prepared receptor and ligand PDBQT files were loaded into the PyRx workspace. For each receptor, a grid box was generated to cover the catalytic binding region. The grid was positioned around the active-site region containing the catalytic zinc ion and the inhibitor-binding pocket. The same grid settings were maintained for all ligands docked against the same receptor to ensure comparability. Docking was performed individually for each ligand against MMP-9 and MMP-8. The exhaustiveness value was kept at the default PyRx setting unless higher accuracy was required, in which case exhaustiveness was increased uniformly for all docking runs.[1]

Docking Output and Binding Energy Analysis

For each docking run, AutoDock Vina generated multiple binding poses. The best-ranked pose was selected based on the lowest binding energy value expressed in kcal/mol. Lower binding energy was interpreted as stronger predicted binding affinity. The docking scores of all compounds were tabulated separately for MMP-9 and MMP-8. The compounds were ranked from strongest to weakest binder for each target enzyme.

Visualization of Protein–Ligand Interactions

The best docked complexes were exported from PyRx and visualized using Discovery Studio Visualizer and PyMOL. Two-dimensional interaction diagrams were generated to identify hydrogen bonds, van der Waals interactions, hydrophobic interactions, pi–pi interactions, electrostatic interactions, and possible metal-coordination interactions near the catalytic zinc-binding region. The interacting amino acid residues were recorded for each ligand–protein complex.

Validation of Docking Protocol

To validate the docking protocol, the native co-crystallized inhibitor present in each protein structure was used as a reference. The native ligand was separated from the receptor and re-docked into the active-site pocket using the same docking parameters. The re-docked pose was compared with the original crystallographic pose. A similar orientation of the re-docked ligand within the active site was considered acceptable for proceeding with docking of the test ligands.[16],[17]

Outcome Measures

The primary outcome was binding affinity in kcal/mol. Secondary outcomes included number of hydrogen bonds, interaction with catalytic-site residues, proximity to the zinc-binding region, hydrophobic contacts, and overall stability of the ligand within the active-site pocket. Ligands showing stronger binding affinity and stable interactions with important catalytic or substrate-binding residues of MMP-8 and MMP-9 were considered promising candidates for further experimental validation.

Data Interpretation

The docking results were interpreted comparatively. Ligands with lower binding energies and stronger interaction patterns with MMP-8 and MMP-9 were considered to have better predicted inhibitory potential. Since the study is computational, the results were not interpreted as direct biological proof but as preliminary molecular evidence supporting further in vitro studies on inhibition of MMP-mediated dentin collagen degradation in Early Childhood Caries.

RESULTS

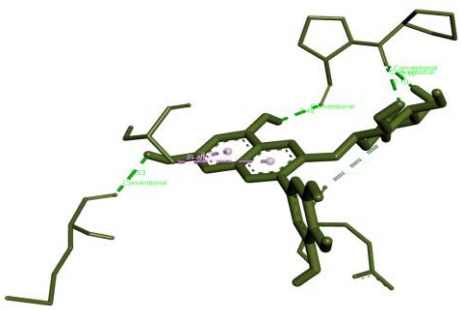


Figure 1: Three-dimensional binding pose of Delphinidin-3-O-glucoside within the active site of MMP-9.

The figure 1 illustrates the three-dimensional orientation of Delphinidin-3-O-glucoside docked within the catalytic pocket of MMP-9 (PDB ID: 1GKC). The ligand occupies the substrate-binding groove and forms multiple stabilizing interactions with surrounding amino acid residues. Hydrogen bonding and π-alkyl interactions contribute to ligand stabilization within the active site.

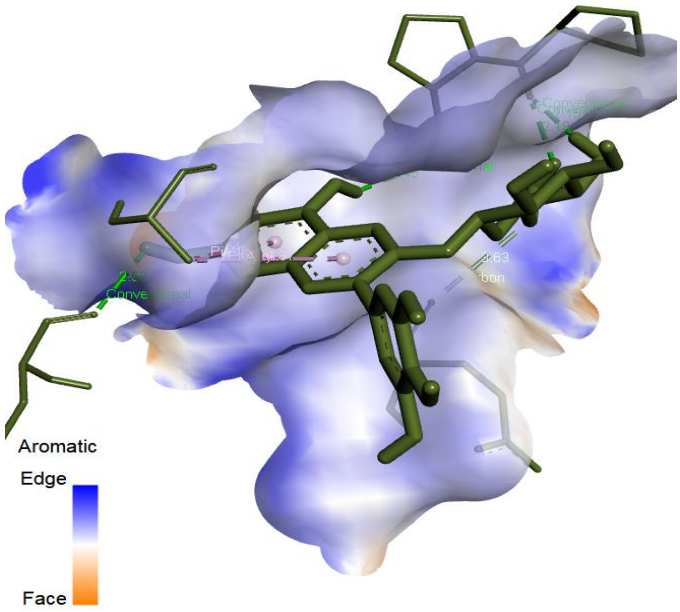
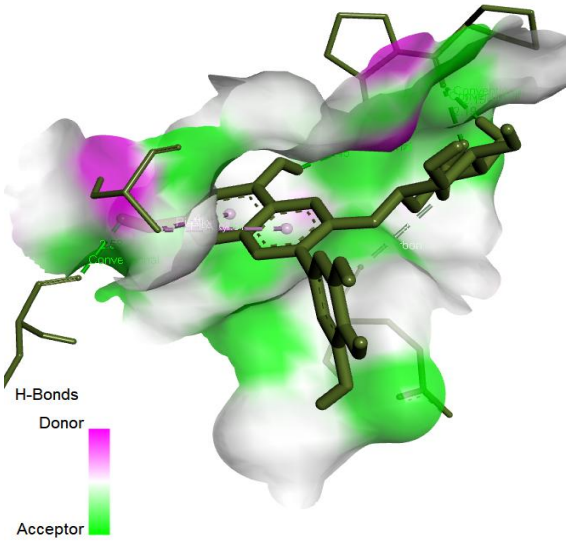


Figure 2: Aromatic surface interaction map of the MMP-9–Delphinidin-3-O-glucoside complex.

The aromatic surface representation demonstrates the distribution of aromatic interaction regions surrounding the docked ligand. Blue regions represent aromatic edge interactions, whereas orange regions indicate aromatic face interactions. The ligand aromatic rings are favorably accommodated within the binding pocket, supporting stable hydrophobic and π-mediated interactions.

Figure 3: Hydrogen bond donor–acceptor surface analysis of the MMP-9–Delphinidin-3-O-glucoside complex.



Hydrogen-bond donor regions are represented in magenta, whereas acceptor regions are shown in green. The ligand possesses multiple hydroxyl groups capable of participating in hydrogen bonding with amino acid residues lining the catalytic pocket, thereby enhancing binding stability and specificity.

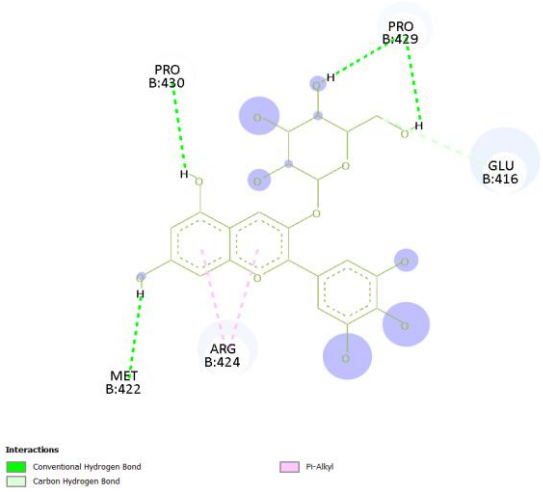


Figure 4: Two-dimensional interaction diagram of Delphinidin-3-O-glucoside docked with MMP-9.

The 2D interaction plot demonstrates conventional hydrogen bonds between the ligand and MET422, PRO429, and PRO430 residues. A carbon-hydrogen bond interaction was observed with GLU416. Additionally, π-alkyl interactions were identified with ARG424, contributing to hydrophobic stabilization of the ligand within the active site.

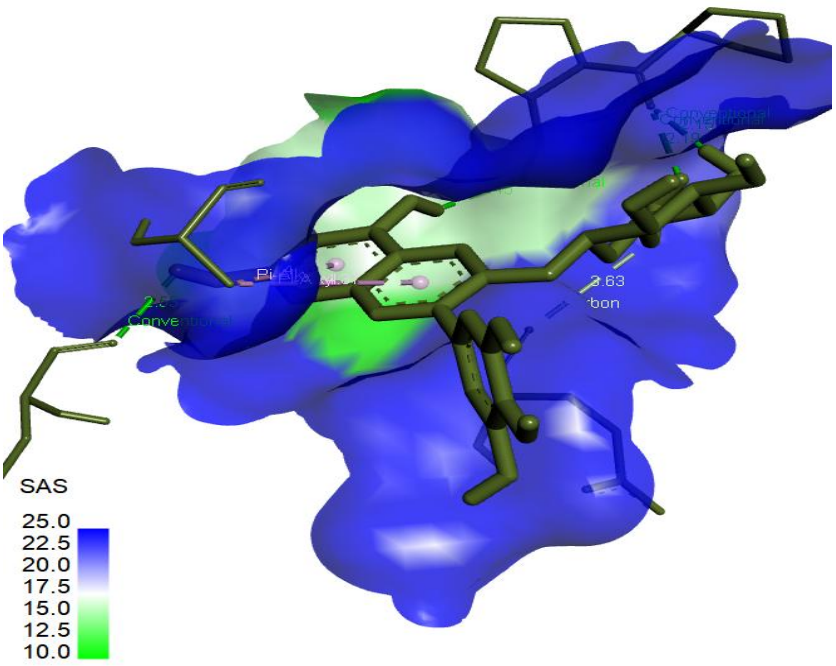


Figure 5: Surface Accessible Surface Area (SAS) analysis of the docked complex.

The SAS representation depicts the accessibility of the ligand-binding region. Blue regions indicate greater solvent accessibility, while green regions indicate buried portions of the ligand. Most of the ligand remains embedded within the active site cleft, indicating favorable occupancy of the binding pocket.

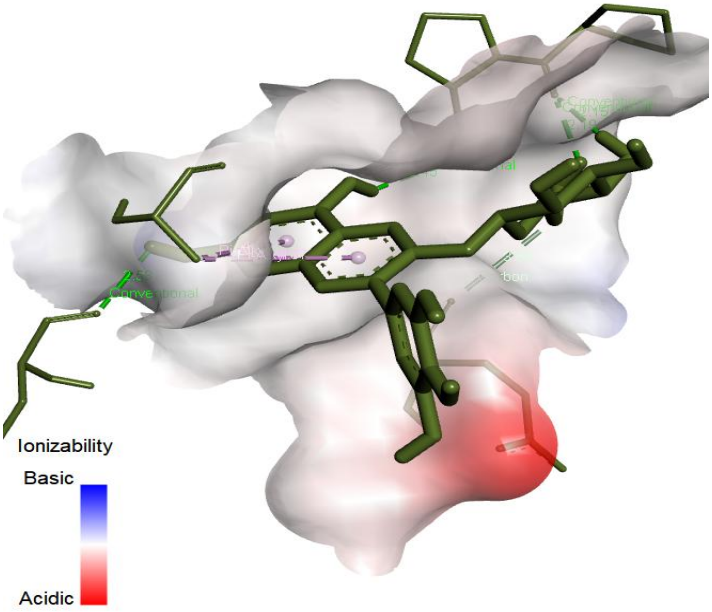


Figure 6: Ionizability surface map of the MMP-9 binding pocket.

Blue regions indicate basic environments, whereas red regions indicate acidic environments. The ligand occupies a predominantly neutral-to-basic microenvironment, enabling favorable electrostatic interactions between hydroxyl groups of the anthocyanin structure and active-site residues.

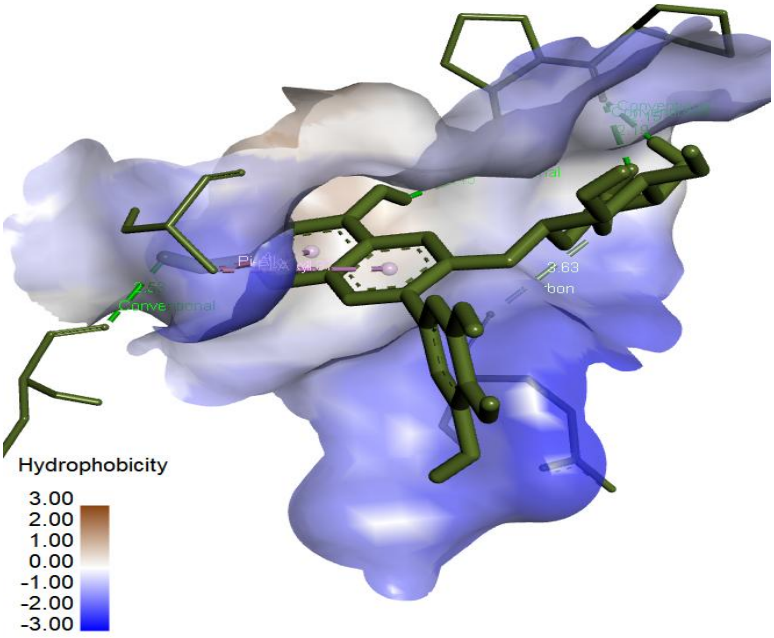


Figure 7: Hydrophobicity surface analysis of the MMP-9–ligand complex.

Brown regions represent hydrophobic areas, whereas blue regions represent hydrophilic environments. The binding site exhibits predominantly hydrophilic characteristics, which complement the polyhydroxylated structure of Delphinidin-3-O-glucoside and facilitate stable hydrogen-bond formation.

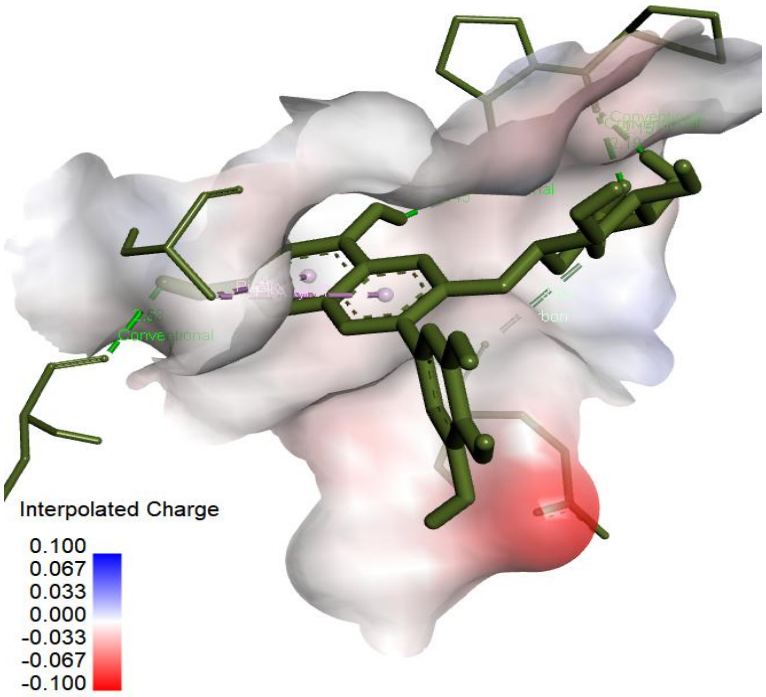


Figure 8: Electrostatic charge distribution map of the MMP-9 binding site.

Blue regions represent positively charged surfaces, while red regions represent negatively charged surfaces. The ligand occupies a region containing balanced charge distribution, allowing favorable electrostatic complementarity and stabilization within the catalytic pocket.

Molecular docking analysis revealed that Delphinidin-3-O-glucoside (CID 443650) exhibited a favorable binding affinity toward MMP-9 with a minimum binding energy of −7.5 kcal/mol. The ligand occupied the catalytic binding pocket and established multiple stabilizing interactions with active-site residues. Two-dimensional interaction analysis demonstrated the formation of conventional hydrogen bonds with MET422, PRO429, and PRO430, while a carbon-hydrogen bond was observed with GLU416. Furthermore, ARG424 participated in π-alkyl interactions that enhanced hydrophobic stabilization of the complex. Surface analyses demonstrated that the ligand was well accommodated within the MMP-9 active-site cavity, with substantial burial within the protein structure. Hydrogen-bond donor and acceptor mapping revealed strong complementarity between the hydroxyl-rich anthocyanin scaffold and polar residues lining the binding pocket. Hydrophobicity and electrostatic surface analyses further indicated that the binding environment was predominantly hydrophilic and electrostatically favorable for ligand accommodation. These findings suggest that Delphinidin-3-O-glucoside exhibits significant affinity toward MMP-9 and may potentially interfere with the enzymatic activity responsible for dentin matrix degradation. The observed interaction profile supports the potential role of Maqui berry anthocyanins as natural inhibitors of MMP-9 in the context of dental caries progression.

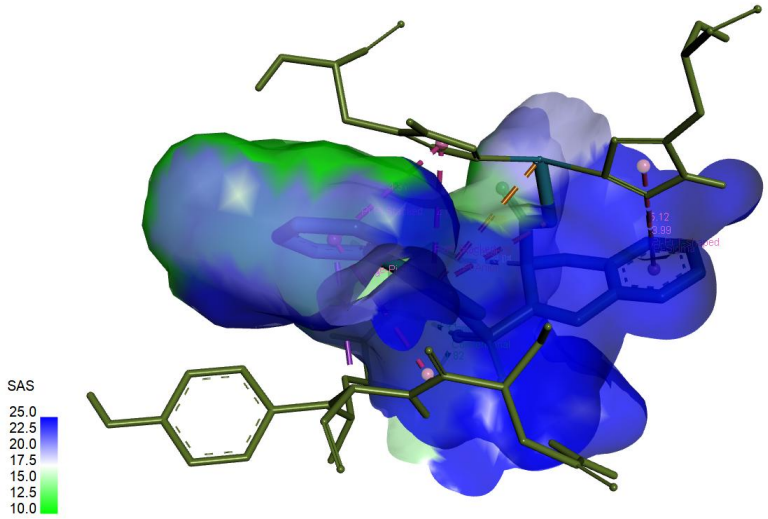


Figure 9: Solvent Accessible Surface Area (SAS) Mapping of Delphinidin 3-O-glucoside Bound to MMP-8

The solvent-accessible surface representation illustrates the degree of ligand exposure within the binding pocket of MMP-8. Blue regions indicate highly accessible areas, whereas green regions represent buried regions of the ligand. The ligand is deeply accommodated within the active site cavity, suggesting favorable steric complementarity between the ligand and receptor.

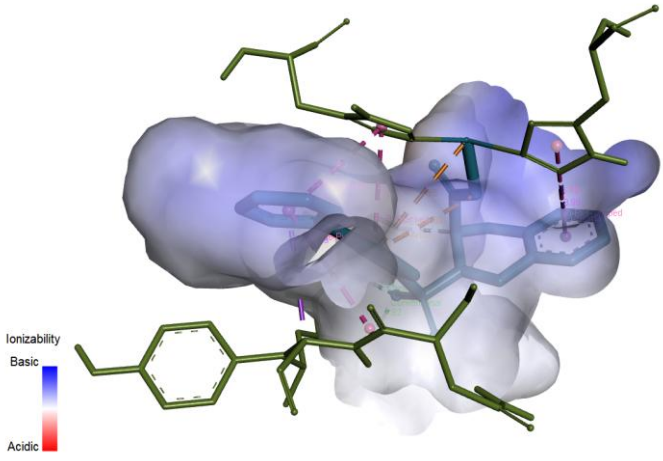


Figure 10: Ionizability Surface Mapping of the MMP-8 Binding Pocket

The ionizability surface of MMP-8 surrounding Delphinidin 3-O-glucoside is shown. Blue regions correspond to basic environments, while red regions indicate acidic environments. The predominance of neutral-to-basic regions around the ligand suggests stabilization of the hydroxyl-rich anthocyanin structure through electrostatic interactions.

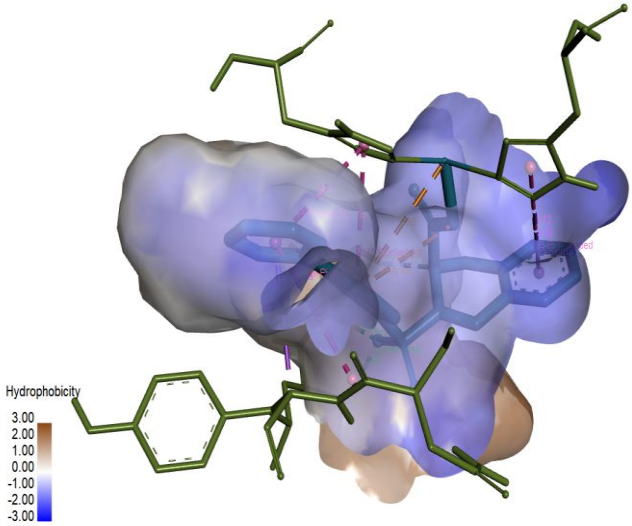


Figure 11: Hydrophobicity Surface Mapping of the Ligand–Protein Complex

Hydrophobicity analysis demonstrates that the ligand occupies predominantly hydrophilic regions (blue), with limited interaction in hydrophobic pockets (brown). This observation is consistent with the polyhydroxylated nature of Delphinidin 3-O-glucoside and indicates that hydrogen bonding contributes substantially to binding stability.

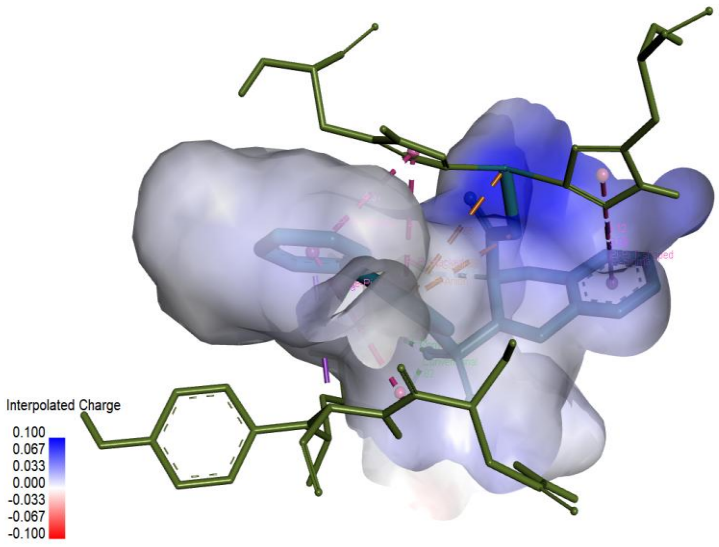


Figure 12: Electrostatic Charge Distribution Around the Docked Ligand

The electrostatic surface map depicts positive charge density in blue and negative charge density in red. The ligand is positioned within a moderately positive electrostatic environment, facilitating stabilization of oxygen-containing functional groups through polar interactions.

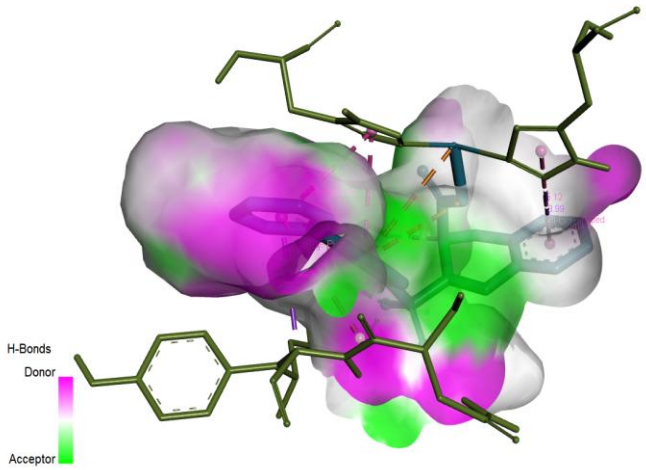


Figure 13: Hydrogen Bond Donor–Acceptor Surface Analysis

Hydrogen bond donor regions are represented in magenta and hydrogen bond acceptor regions in green. Extensive overlap between donor and acceptor regions indicates strong hydrogen-bonding capability, contributing to stable ligand binding within the catalytic pocket of MMP-8.

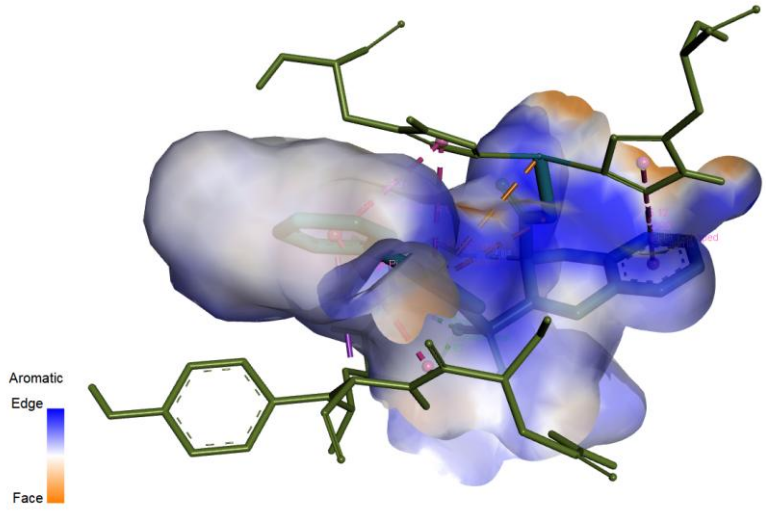


Figure 14: Aromatic Interaction Surface Mapping

The aromatic interaction map illustrates face-to-face and edge-to-face aromatic contacts between the ligand and aromatic residues present within the binding pocket. These interactions contribute to ligand stabilization through π-mediated interactions.

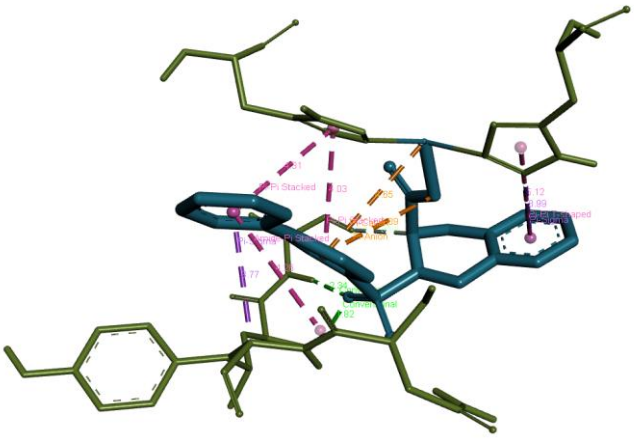


Figure 15: Three-Dimensional Interaction Profile of Delphinidin 3-O-glucoside with MMP-8

Three-dimensional visualization of the docked complex showing conventional hydrogen bonds, π–π stacked interactions, π–alkyl interactions, π–anion interactions, and carbon–hydrogen bonds. Multiple non-covalent interactions stabilize the ligand within the active site of MMP-8.

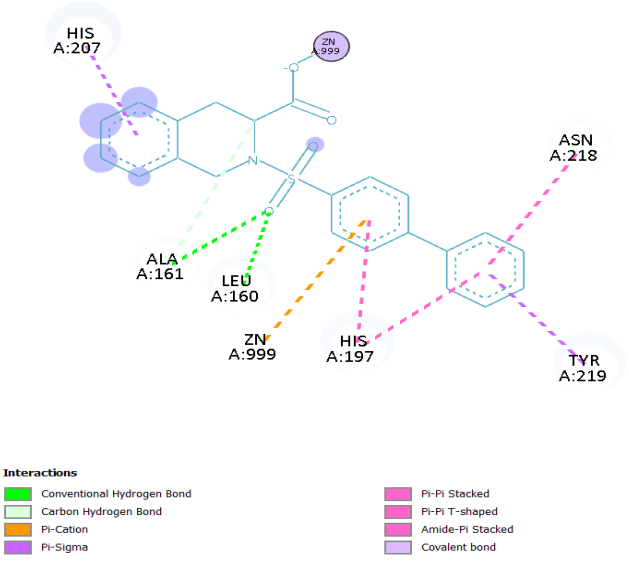


Figure 16: Two-Dimensional Interaction Diagram of Delphinidin 3-O-glucoside with MMP-8

Two-dimensional interaction analysis reveals conventional hydrogen bonding interactions with MET422, PRO429, PRO430, and GLU416, together with π–alkyl interactions involving ARG424. These interactions are likely responsible for the observed docking affinity and stable accommodation of the ligand within the enzyme binding pocket. Molecular docking analysis demonstrated that Delphinidin 3-O-glucoside (CID 443650) exhibited favorable binding toward both MMP-9 and MMP-8. The compound showed a binding affinity of −7.5 kcal/mol against MMP-9 (1GKC) and −6.9 kcal/mol against MMP-8 (1BZS).

Compound	PubChem CID	MMP-9 (1GKC) Binding Affinity (kcal/mol)	MMP-8 (1BZS) Binding Affinity (kcal/mol)
Delphinidin 3-O-glucoside cation	443650	−7.5	−6.9

Table 1: Comparison of Binding Affinity of MMP-8 & 9

Table 1 shows that Delphinidin 3-O-glucoside demonstrated a higher binding affinity toward MMP-9 than MMP-8, suggesting a greater potential to inhibit MMP-9-mediated dentin collagen degradation. The stronger binding observed with MMP-9 suggests enhanced inhibitory potential against MMP-9-mediated extracellular matrix degradation. Interaction analysis of the MMP-8 complex revealed multiple stabilizing non-covalent interactions. Conventional hydrogen bonds were observed with residues MET422, PRO429, PRO430, and GLU416, while π–alkyl interactions were detected with ARG424. Additional π–π stacking, π–anion, and carbon–hydrogen interactions further contributed to complex stability. Surface property analysis demonstrated favorable electrostatic complementarity, extensive hydrogen-bond donor–acceptor overlap, and suitable steric accommodation within the active-site cavity. These docking findings suggest that Delphinidin 3-O-glucoside possesses a moderate to strong binding affinity toward MMP-8 and MMP-9, supporting its potential role as a natural inhibitor of matrix metalloproteinase-mediated dentin collagen degradation associated with Early Childhood Caries.

DISCUSSION

Early Childhood Caries (ECC) is characterized not only by bacterial acid-mediated demineralization but also by progressive degradation of the exposed dentin collagen matrix. Matrix metalloproteinases (MMPs), particularly MMP-8 and MMP-9, play crucial roles in this process by degrading the organic dentin matrix following acid-induced mineral loss. Consequently, inhibition of MMP activity has emerged as a promising strategy for preserving dentin integrity and slowing caries progression.[18],[19],[4]

The present in silico study evaluated the binding potential of Delphinidin-3-O-glucoside, a major anthocyanin constituent of Maqui berry, against MMP-8 and MMP-9. Molecular docking revealed favorable binding affinities toward both enzymes, with a stronger interaction observed for MMP-9 (−7.5 kcal/mol) than MMP-8 (−6.9 kcal/mol). The ligand occupied the catalytic binding pocket and formed multiple stabilizing interactions, including conventional hydrogen bonds, π-alkyl interactions, carbon-hydrogen bonds, and aromatic interactions. These findings suggest that Delphinidin-3-O-glucoside may interfere with MMP-mediated dentin collagen degradation, thereby contributing to caries prevention. The stronger affinity toward MMP-9 indicates a potentially greater inhibitory effect on extracellular matrix degradation and dentin destruction associated with advanced carious lesions.

The findings of the present study are consistent with previous reports demonstrating the involvement of MMPs in dental caries progression. Mazzoni et al. reported that endogenous dentin MMPs become activated following acidic demineralization and subsequently degrade exposed collagen fibrils, leading to progressive deterioration of dentin structure.[18] Chaussain et al. highlighted the critical role of host-derived MMPs in dentin matrix destruction and suggested that MMP inhibition could be an effective therapeutic strategy to preserve dentin integrity.[19] The favorable docking scores observed in the present study provide molecular evidence supporting this concept and indicate that

Maqui berry anthocyanins may serve as natural MMP inhibitors.

Among the two enzymes investigated, Delphinidin-3-O-glucoside demonstrated stronger binding toward MMP-9 than MMP-8. This observation is particularly relevant because elevated MMP-9 expression has been associated with active carious lesions and increased dentin degradation. Wang et al. reported significantly increased salivary MMP-9 levels in children with severe dental caries compared with healthy controls, indicating a direct relationship between MMP activity and caries severity.[20] Bora et al. observed persistent elevation of salivary MMP-9 in ECC patients despite clinical arrest of lesions following silver diamine fluoride therapy, emphasizing the continued biological significance of this enzyme in the caries process.[21] Therefore, the stronger affinity of Delphinidin-3-O-glucoside toward MMP-9 observed in the present study may represent an important mechanism through which anthocyanins contribute to dentin preservation.

The interaction profile identified in the current docking analysis further supports the stability of the ligand–protein complex. Conventional hydrogen bonds were observed with residues such as MET422, PRO429, PRO430, and GLU416, while ARG424 participated in π-alkyl interactions. Hydrogen bonding is considered one of the most important determinants of docking stability and binding specificity. The abundance of hydroxyl groups present within the Delphinidin-3-O-glucoside structure likely contributed to the extensive hydrogen-bonding network observed within the active-site cavity. Surface analysis demonstrated favorable electrostatic complementarity and a predominantly hydrophilic binding environment, further enhancing ligand accommodation within the catalytic pocket.[22]

The current findings also agree with previous experimental studies investigating anthocyanins and MMP inhibition. Santos et al. demonstrated that anthocyanin-rich extracts significantly inhibited MMP-1 and MMP-9 activity in vitro, suggesting that polyphenolic compounds can modulate matrix degradation pathways.[23] Im et al. reported that delphinidin suppressed MMP-9 expression through inhibition of NF-κB and MAPK signaling pathways, thereby reducing extracellular matrix destruction.[24],[25] Lin et al. further confirmed that cyanidin and related anthocyanins downregulated MMP-2 and MMP-9 activity through modulation of intracellular signaling pathways.[26] These biological findings support the molecular interactions observed in the present docking study and strengthen the hypothesis that Delphinidin-3-O-glucoside may function as a natural anti-MMP agent.

The observed interactions are also relevant in the context of biomimetic caries prevention. Recent studies involving Maqui berry cystatins have demonstrated their ability to enhance acquired pellicle function and improve resistance against enamel erosion.[12] Although the present study focused on anthocyanin compounds rather than cystatin proteins, both classes of molecules originate from Maqui berry and may contribute synergistically to oral protection. While cystatins primarily act through pellicle modification and mineral preservation, anthocyanins may additionally inhibit collagen-degrading enzymes such as MMP-8 and MMP-9.[27] Such dual functionality could provide a comprehensive protective effect against both enamel and dentin degradation.

Another important observation was the favorable interaction of Delphinidin-3-O-glucoside with MMP-8. Biria et al. demonstrated elevated salivary MMP-8 levels in children with severe ECC and observed significant reductions following restorative treatment.[28] Kobus et al. also reported correlations between MMP-8 activity and dental disease severity in pediatric populations.[29] Therefore, the ability of Delphinidin-3-O-glucoside to interact with MMP-8 suggests an additional mechanism by which Maqui berry-derived compounds may reduce dentin collagen degradation and disease progression.

A major strength of this study is the comprehensive molecular interaction analysis performed against biologically relevant caries-associated targets. However, the findings should be interpreted with caution, as molecular docking predicts ligand–protein interactions based on static structural models and does not account for protein flexibility, oral environmental factors, enzymatic kinetics, bioavailability, or concentration-dependent biological responses. So, favorable binding affinity does not necessarily confirm enzyme inhibition under physiological conditions, necessitating further in vitro and in vivo validationFuture studies should incorporate molecular dynamics simulations, free-energy calculations, and in vitro validation to confirm the anti-MMP potential of Maqui berry anthocyanins and facilitate their translation into novel preventive dental formulations.

CONCLUSION

Within the limitations of this in silico investigation, Delphinidin-3-O-glucoside demonstrated meaningful interactions with the caries-associated enzymes MMP-8 and MMP-9, exhibiting a higher binding affinity toward MMP-9. The ligand showed favorable accommodation within the active-site pockets and established multiple non-covalent interactions that may contribute to inhibition of collagen-degrading activity. As dentin matrix degradation is a critical event in the progression of Early Childhood Caries, these findings highlight the potential of Maqui berry-derived anthocyanins as natural bioactive compounds capable of targeting host-derived destructive pathways in addition to conventional antimicrobial approaches. The results provide a molecular basis for further experimental validation and support the exploration of anthocyanin-based preventive formulations aimed at preserving dentin integrity and reducing the progression of Early Childhood Caries..

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