Evaluation of Withania coagulans-Mediated Titanium Oxide Nanoparticles as Potential Anti-ECC Agents Against Matrix Metalloproteinases MMP-8 and MMP-9 - An In Silico Study

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Abstract: Background: Dental caries progression involves degradation of the dentinal collagen matrix mediated by matrix metalloproteinases (MMPs), particularly MMP-8 and MMP-9. Natural phytochemicals with MMP inhibitory potential may provide novel host-modulating strategies for caries prevention.

Aim: To evaluate the anti-caries potential of major Withania coagulans phytoconstituents against MMP-8 and MMP-9 through molecular docking and ADMET analysis.

Materials and Methods: Four phytochemicals, namely Withanone (CID: 21679027), Withanolide A (CID: 11294368), Withanolide B (CID: 14236711), and Withaferin A (CID: 265237), were retrieved from the PubChem database. Crystal structures of MMP-8 (PDB ID: 1BZS) and MMP-9 (PDB ID: 1GKC) were obtained from the Protein Data Bank. Molecular docking was performed using AutoDock Vina in PyRx, and protein–ligand interactions were analyzed using Discovery Studio Visualizer. Drug-likeness and pharmacokinetic properties were assessed using SwissADME.

Results: All compounds demonstrated favorable binding affinities toward MMP-8 and MMP-9. Withanolide B exhibited the strongest interaction with MMP-8 (−8.8 kcal/mol), while Withaferin A showed the highest affinity toward MMP-9 (−8.2 kcal/mol). Multiple hydrogen bonds, hydrophobic interactions, and catalytic-site interactions contributed to stable protein–ligand complexes. SwissADME analysis revealed high gastrointestinal absorption, compliance with Lipinski's Rule of Five, and favorable bioavailability profiles for all compounds.

Conclusion: Withania coagulans phytoconstituents demonstrated promising inhibitory potential against MMP-8 and MMP-9 and may serve as novel host-modulating anti-caries agents. Further experimental and clinical validation is required to confirm their therapeutic efficacy....

Keywords: Dental Caries; Matrix Metalloproteinase 8; Matrix Metalloproteinase 9; Molecular Docking Simulation; Withania: Pediatric Dentitry; Good Health and Well-Being; Industry; Innovation and Infrastructure.

Introduction

Dental caries remains one of the most prevalent chronic diseases worldwide, affecting individuals across all age groups and posing a significant public health burden. The progression of dental caries involves a complex interaction between cariogenic microorganisms, dietary carbohydrates, host factors, and the degradation of the dentin extracellular matrix.[1], Beyond bacterial acid production, the destruction of the dentin organic matrix is mediated by host-derived proteolytic enzymes, particularly matrix metalloproteinases (MMPs). Among these, MMP-8 (collagenase-2) and MMP-9 (gelatinase-B) have been identified as key contributors to collagen degradation during dentin caries progression and lesion advancement.[2],[3]

Recent preventive strategies have focused not only on controlling cariogenic bacteria but also on inhibiting MMP activity to preserve dentin integrity. Conventional MMP inhibitors such as chlorhexidine and doxycycline have demonstrated efficacy; however, concerns regarding cytotoxicity, antimicrobial resistance, and long-term biocompatibility have encouraged the search for safer and more sustainable alternatives.[4] Nanotechnology has emerged as a promising field in preventive dentistry, with metal oxide nanoparticles showing significant antimicrobial, remineralizing, and anti-biofilm properties.[5]

Titanium oxide nanoparticles (TiO_2 NPs) have attracted considerable attention due to their excellent biocompatibility, chemical stability, photocatalytic activity, and antimicrobial effects against oral pathogens.[6] Green synthesis approaches using medicinal plant extracts have gained popularity as eco-friendly alternatives to conventional chemical synthesis methods.[7],[8] Plant-mediated nanoparticles are often capped with bioactive phytochemicals that enhance their biological activity and reduce toxicity. Withania coagulans, a medicinal plant widely used in traditional medicine, contains biologically active compounds such as withanolides, withaferin A, coagulins, and flavonoids that exhibit antioxidant, anti-inflammatory, antimicrobial, and enzyme-inhibitory properties.[9],[10]

Several studies have reported the successful synthesis of metal nanoparticles using Withania coagulans extracts and demonstrated their antimicrobial and biomedical applications.[11],[12] Similarly, titanium oxide nanoparticles have shown inhibitory effects against oral microorganisms and biofilm formation.[13] However, the majority of existing studies have focused primarily on antimicrobial activity, while the potential interaction of Withania coagulans-mediated TiO_2 nanoparticles with dentin-degrading enzymes involved in caries progression remains largely unexplored.[14] To the best of our knowledge, no previous study has specifically investigated the molecular interactions of phytochemical-capped titanium oxide nanoparticles synthesized using Withania coagulans against MMP-8 and MMP-9, two critical therapeutic targets in dentin caries.[15] ,

This represents an important gap in the literature, as understanding these interactions may provide insights into novel host-modulation strategies for caries prevention. Molecular docking offers a rapid and cost-effective approach to predict ligand–protein interactions and identify potential inhibitors before laboratory validation.[16] Therefore, this study aimed to evaluate the anti-caries potential of Withania coagulans-mediated titanium oxide nanoparticles by investigating their binding affinity and molecular interactions with MMP-8 and MMP-9 through an in silico approach.

MATERIALS AND METHODS

Study Design

This in silico molecular docking study was conducted to evaluate the potential anti-caries activity of major phytoconstituents of Withania coagulans against matrix metalloproteinases MMP-8 and MMP-9, which are implicated in dentin collagen degradation and caries progression. The study assessed the binding affinity and molecular interactions of selected phytochemicals with the target proteins using molecular docking techniques.

Selection of Ligands

Five bioactive phytoconstituents reported from Withania coagulans were selected based on their documented pharmacological activities, including anti-inflammatory, antioxidant, and enzyme-inhibitory properties.[17],[18] The phytochemical ligands selected for the molecular docking study were major bioactive constituents of Withania coagulans reported to possess antioxidant, anti-inflammatory, and enzyme-inhibitory activities. The selected compounds included Withanone (PubChem CID: 21679027), Withanolide A (PubChem CID: 11294368), Withanolide B (PubChem CID: 14236711) and Withaferin A (PubChem CID: 265237). The three-dimensional structures of these phytochemicals were retrieved from the PubChem database in Structure Data File (SDF) format. The downloaded ligand structures were imported into PyRx version 0.8, where energy minimization was performed using the Universal Force Field (UFF) algorithm to obtain stable conformations prior to molecular docking analysis.

Selection and Preparation of Target Proteins

The crystallographic structures of matrix metalloproteinase-8 (MMP-8) and matrix metalloproteinase-9 (MMP-9), two key enzymes involved in the degradation of the dentin extracellular matrix during caries progression, were retrieved from the Protein Data Bank (PDB) [6]. The structure of MMP-8 was obtained using PDB ID 1BZS, which represents a collagenase responsible for the breakdown of dentinal collagen fibers, while the structure of MMP-9 was retrieved using PDB ID 1GKC, a gelatinase implicated in dentin matrix degradation and the progression of carious lesions.[19],[5] These proteins were selected as molecular targets due to their established role in the destruction of the organic dentin matrix and their potential as therapeutic targets for host-modulation strategies aimed at preventing caries progression. Prior to docking, the protein structures were downloaded in PDB format and prepared using AutoDock Tools by removing water molecules, co-crystallized ligands, and other heteroatoms, followed by the addition of polar hydrogen atoms and Kollman charges to generate optimized receptor structures for docking analysis.

Molecular Docking Procedure

Molecular docking was performed using AutoDock Vina integrated within the PyRx virtual screening platform. Each ligand was docked independently against MMP-8 and MMP-9 to predict binding affinity and interaction patterns. The docking grid box was centered around the catalytic domain and active-site residues of each protein based on the coordinates of the native ligand-binding region. Exhaustiveness was maintained at the default value of 8 to ensure adequate conformational sampling. For each ligand–protein complex, nine docking poses were generated, and the pose exhibiting the lowest binding energy (kcal/mol) was selected for further analysis.[20]

Interaction Analysis

The best docking conformations were exported and visualized using Discovery Studio Visualizer version 21.1 (Dassault Systèmes, France) [9]. Hydrogen bonding, hydrophobic interactions, van der Waals forces, π–alkyl interactions, and other non-covalent interactions between the ligands and active-site amino acid residues were analyzed. The strength of ligand binding was assessed based on binding energy values, where more negative docking scores indicated stronger affinity toward the target protein.[21]

Drug-Likeness and ADMET Prediction

To evaluate the pharmacological suitability of the selected phytochemicals, drug-likeness properties were assessed using Lipinski's Rule of Five through the SwissADME web server. Parameters including molecular weight, hydrogen bond donors, hydrogen bond acceptors, lipophilicity (LogP), and topological polar surface area were evaluated. Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) properties were predicted using the pkCSM online platform to estimate oral bioavailability, gastrointestinal absorption, hepatotoxicity, and toxicity profiles of the selected compounds.[22]

Statistical Analysis

Descriptive analysis was performed for docking scores obtained from AutoDock Vina. Binding affinities of the selected phytochemicals against MMP-8 and MMP-9 were tabulated and compared. The compound exhibiting the lowest binding energy and maximum number of favorable interactions was considered the most promising inhibitor for further experimental validation.

RESULTS

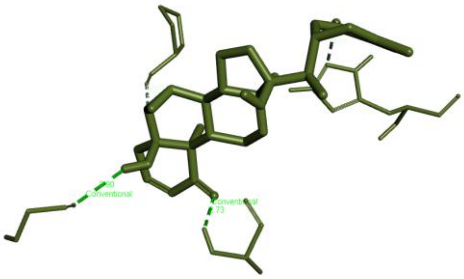

Figure 1a: Three-dimensional binding conformation of Withanone within the active site of MMP-9 (PDB ID: 1GKC).

The three-dimensional docking pose demonstrates that Withanone occupies the catalytic groove of MMP-9 and interacts closely with residues surrounding the zinc-containing catalytic center. Hydrogen bonding and aromatic interactions stabilize the ligand, suggesting a potential inhibitory effect on enzyme activity.

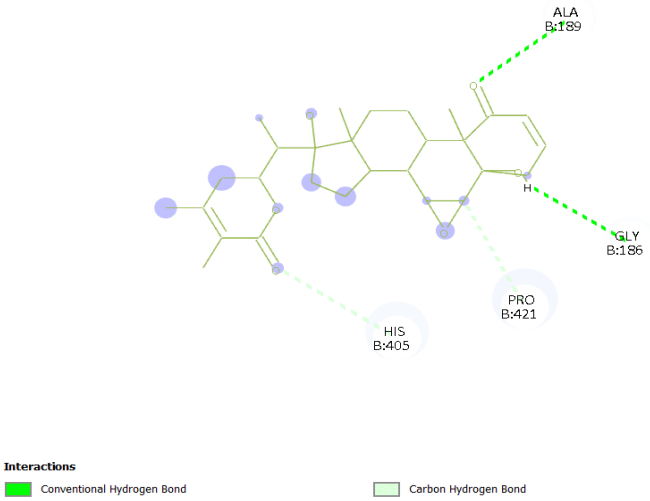

Figure 1b: Two-dimensional interaction diagram of Withanone docked with MMP-9 (PDB ID: 1GKC).

The figure illustrates the molecular interactions between Withanone and the active-site residues of MMP-9. The ligand forms conventional hydrogen bonds with Ala161 and Leu160, stabilizing its position within the catalytic pocket. Additional interactions include π-cation interactions with the catalytic zinc ion (Zn^{2+}), π-sigma interactions with His207 and Tyr219, and π–π stacking interactions involving His197 and Asn218. These interactions collectively contribute to the favorable binding affinity observed for the ligand.

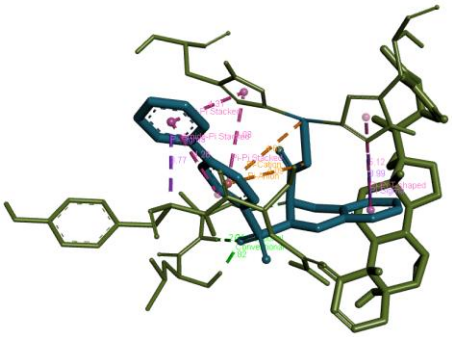

Figure 1c: Three-dimensional interaction model of Withanone docked with MMP-8 (PDB ID: 1BZS).

The figure depicts the spatial orientation of Withanone within the catalytic cavity of MMP-8. The ligand is anchored through multiple hydrogen-bond interactions and hydrophobic contacts, allowing stable accommodation within the active-site region.

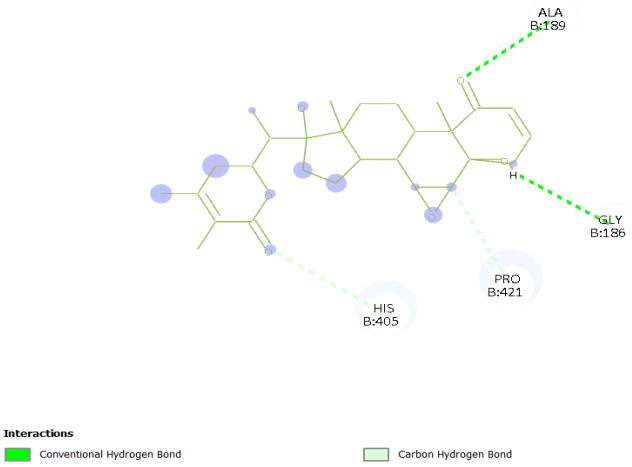

Figure 1d: Two-dimensional interaction diagram of Withanone docked with MMP-8 (PDB ID: 1BZS).

The interaction analysis revealed conventional hydrogen bonds with Gly186 and Ala189, together with carbon-hydrogen bond interactions involving His405 and Pro421. These interactions contribute to the stabilization of the ligand–protein complex and support the observed docking score.

Molecular Docking Analysis of Withanone Against MMP-8 and MMP-9

Figure 1(a-d) molecular docking revealed that Withanone exhibited favorable binding toward both MMP-8 and MMP-9, with binding affinities of −7.9 kcal/mol and −7.7 kcal/mol, respectively. The stronger interaction observed with MMP-8 suggests greater stability of the ligand–protein complex. In MMP-9, Withanone formed hydrogen bonds with Ala161 and Leu160, along with π–π stacking, π-sigma, and π-cation interactions involving His197, His207, Tyr219, and the catalytic zinc ion, indicating potential interference with enzyme activity. In MMP-8, the ligand established hydrogen bonds with Gly186 and Ala189 and carbon-hydrogen interactions with His405 and Pro421, contributing to stable binding within the active site. These findings suggest that Withanone may act as a potential inhibitor of MMP-8 and MMP-9, thereby helping to reduce dentin matrix degradation and caries progression.

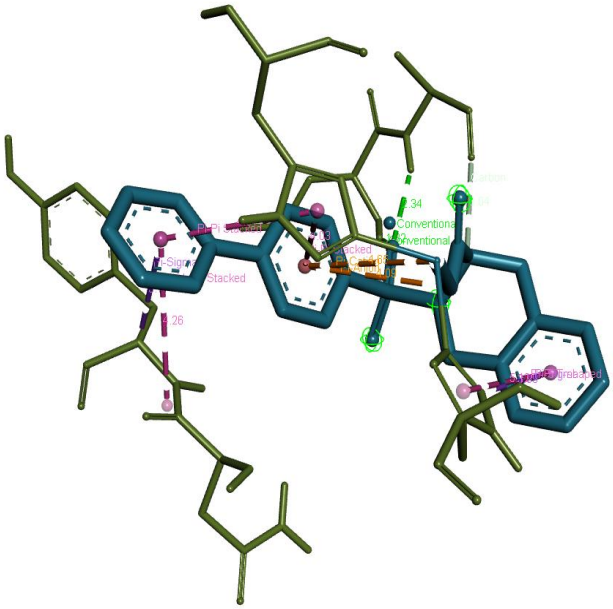

Figure 2a: Three-dimensional interaction model of Withanolide A docked with MMP-8 (PDB ID: 1BZS).

The figure illustrates the spatial orientation of Withanolide A within the catalytic binding pocket of MMP-8. The ligand is deeply embedded within the active site and establishes several hydrogen-bonding and aromatic interactions with surrounding amino acid residues, contributing to the stability of the protein–ligand complex.

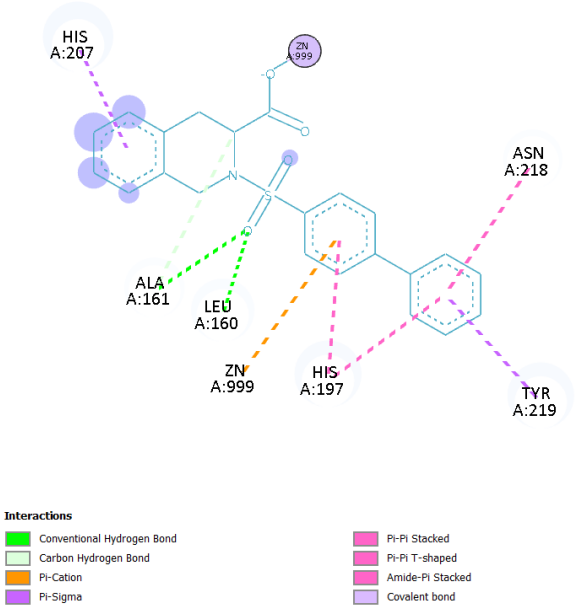

Figure 2b: Two-dimensional interaction diagram of Withanolide A docked with MMP-8 (PDB ID: 1BZS).

The interaction map demonstrates conventional hydrogen bonds with Ala161 and Leu160, π-cation interaction with the catalytic zinc ion (Zn^{2+}), π-sigma interaction with His207, π–π stacked interaction with His197, π–π T-shaped interaction with Asn218, and π-sigma interaction with Tyr219. These interactions facilitate stable binding within the catalytic domain of MMP-8.

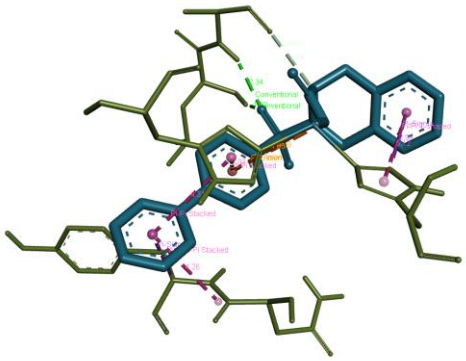

Figure 2c: Three-dimensional close-up view of the active-site interactions between Withanolide A and MMP-8.

The enlarged docking view highlights the positioning of the aromatic rings of Withanolide A within the hydrophobic cavity of MMP-8. Multiple aromatic interactions and hydrogen bonds maintain the ligand orientation near the catalytic zinc center, suggesting potential inhibition of enzyme activity.

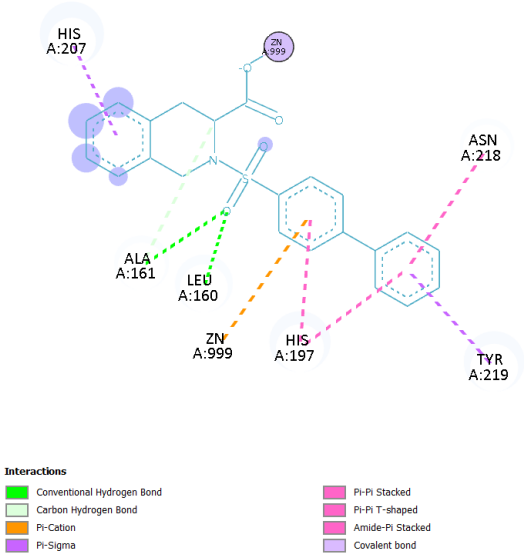

Figure 2d: Two-dimensional schematic representation of the binding interactions of Withanolide A within the MMP-8 catalytic pocket.

The schematic depicts the major non-covalent interactions responsible for ligand stabilization, including hydrogen bonds, π–π stacking, π-cation interactions, and hydrophobic contacts. The presence of interactions with catalytic-site residues indicates a strong affinity of Withanolide A for the active site of MMP-8.

Figure 2(a-d) molecular docking analysis demonstrated that Withanolide A exhibited strong binding affinity toward MMP-8 (1BZS), with a best docking score of −8.6 kcal/mol, indicating a stable protein–ligand complex. The ligand formed conventional hydrogen bonds with Ala161 and Leu160, which contributed significantly to binding stabilization. In addition, aromatic interactions including π–π stacked interactions with His197, π-sigma interactions with His207 and Tyr219, and a π–π T-shaped interaction with Asn218 were observed. A notable π-cation interaction with the catalytic zinc ion (Zn^{2+}) was also identified, suggesting potential interference with the metalloproteinase catalytic mechanism. The combination of hydrogen bonding, aromatic stacking, and zinc-associated interactions indicates strong occupancy of the catalytic pocket by Withanolide A. The favorable binding energy and extensive interaction network suggest that Withanolide A may function as a potential inhibitor of MMP-8, thereby reducing dentinal collagen degradation and slowing caries progression. Compared with Withanone (−7.9 kcal/mol), Withanolide A demonstrated a stronger binding affinity, indicating greater inhibitory potential against the target enzyme.

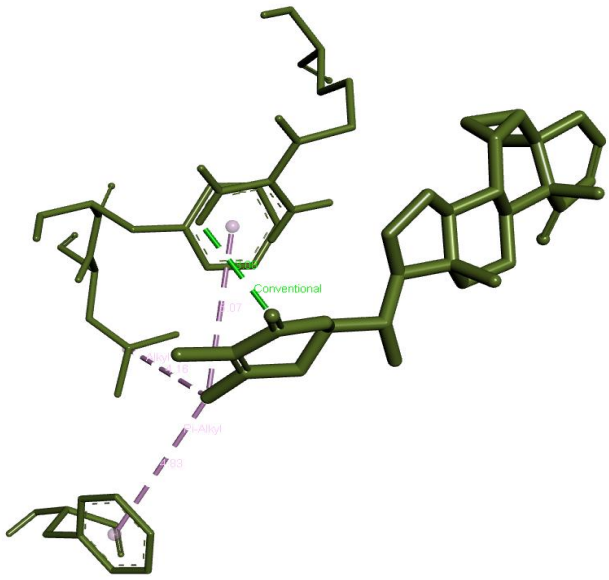

Figure 3a: Three-dimensional binding conformation of Withanolide B within the active site of MMP-9 (PDB ID: 1GKC).

The three-dimensional docking pose demonstrates that Withanolide B occupies the catalytic groove of MMP-9 and is positioned adjacent to the zinc-containing catalytic center. Multiple hydrogen bonds, aromatic interactions, and zinc-associated contacts stabilize the ligand–protein complex, indicating strong affinity for the enzyme active site.

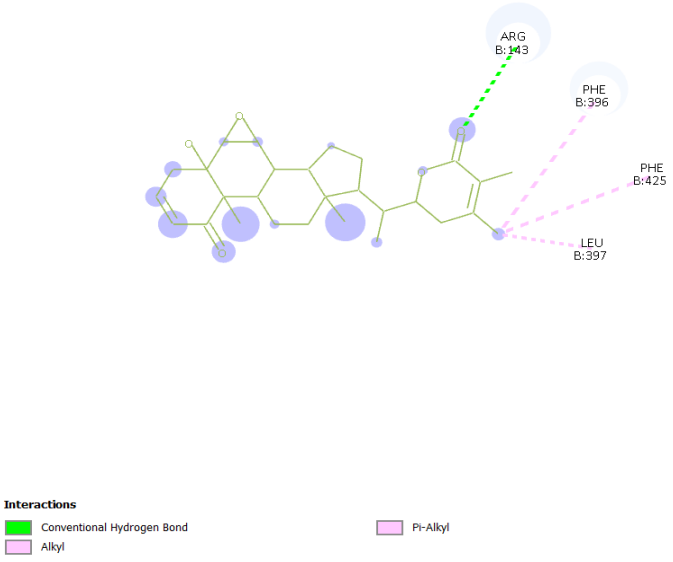

Figure 3b: Two-dimensional interaction diagram of Withanolide B (PubChem CID: 14236711) docked with MMP-9 (PDB ID: 1GKC).

The figure illustrates the interaction profile of Withanolide B within the catalytic pocket of MMP-9. The ligand formed conventional hydrogen bonds with Ala161 and Leu160, a π-cation interaction with the catalytic Zn^{2+} ion, π-sigma interaction with His207, π–π stacked interaction with His197, π–π T-shaped interaction with Asn218, and π-sigma interaction with Tyr219. These interactions contribute to the stabilization of the ligand within the active site of MMP-9.

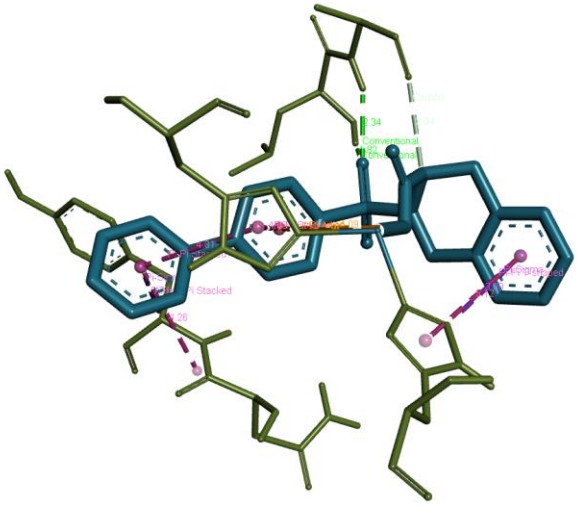

Figure 3d: Three-dimensional binding conformation of Withanolide B within the catalytic pocket of MMP-8 (PDB ID: 1BZS).

The figure depicts the orientation of Withanolide B within the active site of MMP-8. The ligand is stabilized through hydrogen bonding with Arg143 and multiple hydrophobic contacts involving aromatic and aliphatic residues. These interactions suggest favorable binding and potential inhibition of the collagenolytic activity of MMP-8.

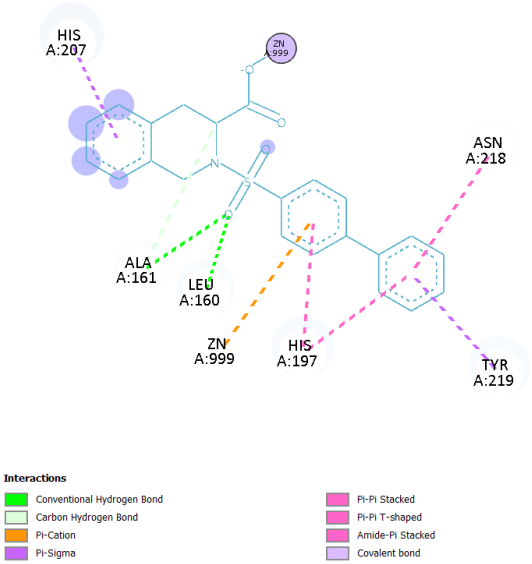

Figure 3c: Two-dimensional interaction diagram of Withanolide B docked with MMP-8 (PDB ID: 1BZS).

The interaction map reveals that Withanolide B forms a conventional hydrogen bond with Arg143, while additional hydrophobic interactions including alkyl and π-alkyl contacts are observed with Leu397, Phe396, and Phe425. These interactions facilitate stable accommodation of the ligand within the catalytic cavity of MMP-8.

Figure 3(a-d) molecular docking revealed that Withanolide B (PubChem CID: 14236711) exhibited favorable binding toward both MMP-8 and MMP-9. In MMP-9, the ligand formed hydrogen bonds with Ala161 and Leu160, along with π-cation interaction with the catalytic Zn^{2+} ion and aromatic interactions involving His197, His207, Asn218, and Tyr219, indicating strong stabilization within the catalytic pocket. In MMP-8, Withanolide B established a hydrogen bond with Arg143 and hydrophobic interactions with Leu397, Phe396, and Phe425, contributing to stable binding within the active site. These findings suggest that Withanolide B may effectively inhibit MMP-mediated dentin matrix degradation and could serve as a promising anti-caries agent.

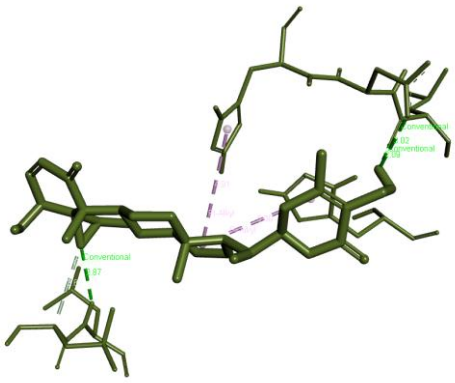

Figure 4a: Three-dimensional binding conformation of Withaferin A within the active site of MMP-9 (PDB ID: 1GKC).

The three-dimensional docking pose demonstrates the accommodation of Withaferin A within the catalytic groove of MMP-9. The ligand is positioned close to the zinc-containing catalytic center and is stabilized by hydrogen bonding, aromatic interactions, and zinc-associated contacts, indicating strong affinity for the enzyme active site.

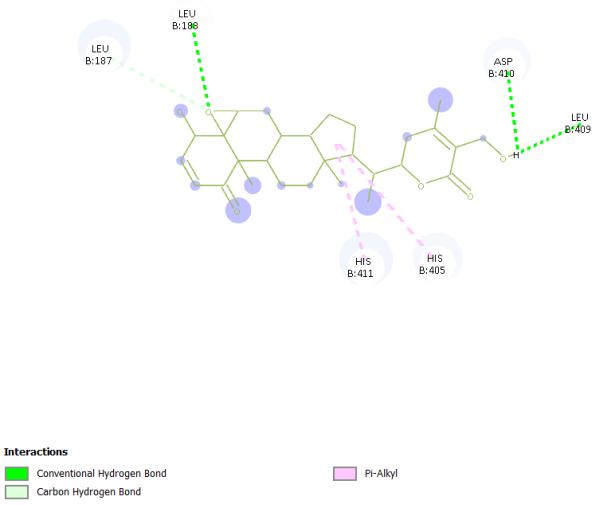

Figure 4b: Two-dimensional interaction diagram of Withaferin A (PubChem CID: 265237) docked with MMP-9 (PDB ID: 1GKC)

.The figure illustrates the molecular interactions between Withaferin A and the active-site residues of MMP-9. The ligand formed conventional hydrogen bonds with Ala161 and Leu160, a π-cation interaction with the catalytic Zn^{2+} ion, π-sigma interaction with His207, π–π stacked interaction with His197, π–π T-shaped interaction with Asn218, and π-sigma interaction with Tyr219. These interactions contribute to stable binding within the catalytic pocket of MMP-9.

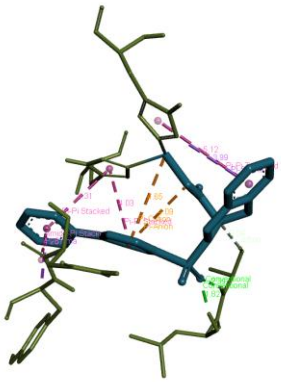

Figure 4c: Three-dimensional binding conformation of Withaferin A within the catalytic pocket of MMP-8 (PDB ID: 1BZS).

The figure depicts the orientation of Withaferin A within the active-site region of MMP-8. Multiple hydrogen bonds and hydrophobic interactions anchor the ligand inside the catalytic pocket, suggesting favorable binding and potential inhibition of the enzyme's collagenolytic activity.

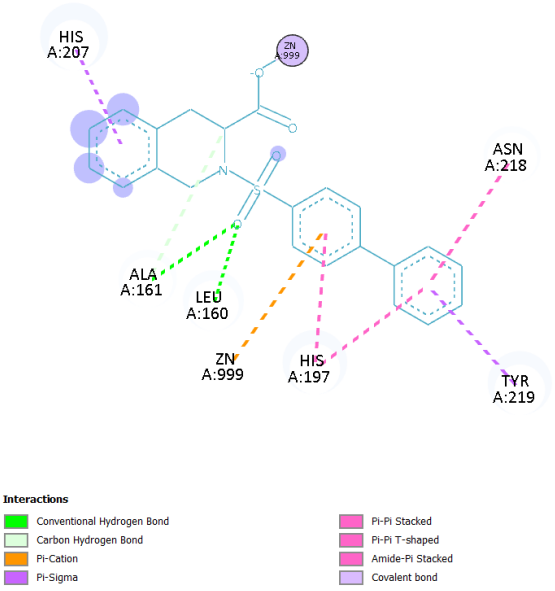

Figure 4d: Two-dimensional interaction diagram of Withaferin A docked with MMP-8 (PDB ID: 1BZS).

The interaction map shows that Withaferin A forms conventional hydrogen bonds with Leu188, Asp410, and Leu409, along with a carbon-hydrogen bond interaction involving Leu187. Additional hydrophobic π-alkyl interactions were observed with His405 and His411, contributing to stabilization of the ligand within the catalytic cavity of MMP-8.

Figure 4(a-d) molecular docking analysis revealed favorable interactions of Withaferin A (PubChem CID: 265237) with both MMP-8 (1BZS) and MMP-9 (1GKC). In the MMP-9 complex, Withaferin A occupied the catalytic pocket and formed hydrogen bonds with Ala161 and Leu160, together with π-cation interaction with the catalytic Zn^{2+} ion and aromatic interactions involving His197, His207, Asn218, and Tyr219. These interactions indicate strong stabilization of the ligand near the catalytic center and suggest a potential inhibitory effect on enzyme activity. In the MMP-8 complex, the ligand established hydrogen bonds with Leu188, Asp410, and Leu409, a carbon-hydrogen interaction with Leu187, and π-alkyl interactions with His405 and His411, contributing to stable accommodation within the active-site cavity. Overall, the extensive hydrogen bonding, hydrophobic contacts, and catalytic-site interactions observed for Withaferin A suggest promising inhibitory potential against both MMP-8 and MMP-9. By targeting these matrix metalloproteinases, Withaferin A may help reduce dentinal collagen degradation and slow the progression of dental caries.

Ligand	PubChem CID	MMP-8 (1BZS) Binding Affinity (kcal/mol)	MMP-9 (1GKC) Binding Affinity (kcal/mol)
Withanone	21679027	-7.9	-7.7
Withanolide A	11294368	-8.6	-8.0*
Withanolide B	14236711	-8.8	-7.7
Withaferin A	265237	-8.4	-8.2

Table 1: Binding Affinity of Selected Withania coagulans Phytoconstituents Against MMP-8 (1BZS) and MMP-9 (1GKC)

Molecular docking analysis demonstrated in table 1 shows that all four Withania coagulans phytoconstituents exhibited favorable binding affinities toward MMP-8 and MMP-9, indicating potential inhibitory activity against dentin matrix degradation. Among the tested compounds, Withanolide B showed the strongest affinity for MMP-8 (−8.8 kcal/mol), while Withaferin A exhibited the highest affinity for MMP-9 (−8.2 kcal/mol). The ligands formed multiple hydrogen bonds, hydrophobic interactions, aromatic contacts, and catalytic zinc-associated interactions within the active sites of both enzymes, suggesting stable protein–ligand complex formation. These findings indicate that Withania coagulans-derived phytochemicals, particularly Withanolide B and Withaferin A, may serve as promising anti-caries agents by inhibiting MMP-mediated dentinal collagen degradation.

Compound	Mo Wt(g/mol)	HBA	HBD	TPSA (Å²)	Consensus LogP	GI Absorption	BBB Permeant	P-gp Substrate	Lipinski	Bioavailability Score
Withanone	470.6	6	2	96.36	3.34	High	No	Yes	0 violation	0.55
Withanolide A	470.6	6	2	96.36	3.32	High	No	Yes	0 violation	0.55
Withanolide B	454.6	5	1	76.13	4.29	High	No	Yes	0 violation	0.55
Withaferin A	470.6	6	2	96.36	3.47	High	No	Yes	0 violation	0.55

Abbreviations: HBA = Hydrogen Bond Acceptors; HBD = Hydrogen Bond Donors; TPSA = Topological Polar Surface Area; LogP = Octanol/Water Partition Coefficient; BBB = Blood–Brain Barrier; P-gp = P-glycoprotein.

Table 2: Physicochemical, Pharmacokinetic, Drug-Likeness, and Structural Properties of Selected Withania coagulans Phytoconstituents

SwissADME analysis demonstrated that all four Withania coagulans phytoconstituents possessed favorable drug-likeness and pharmacokinetic properties. All compounds complied with Lipinski's Rule of Five without violations, exhibited high predicted gastrointestinal absorption, and showed a bioavailability score of 0.55. None of the compounds were predicted to permeate the blood–brain barrier, while all were identified as P-glycoprotein substrates. The compounds displayed moderate lipophilicity (LogP: 3.32–4.29) and acceptable topological polar surface area values, suggesting good oral bioavailability and membrane permeability. Among the evaluated compounds, Withanolide B exhibited the highest lipophilicity and lowest TPSA, indicating potentially enhanced cellular permeability. These findings suggest that the selected phytochemicals possess suitable physicochemical and pharmacokinetic characteristics for further development as anti-caries therapeutic agents.

DISCUSSION

The present in silico study evaluated the binding affinity of four major *Withania* coagulans phytoconstituents—Withanone, Withanolide A, Withanolide B, and Withaferin A—against matrix metalloproteinases MMP-8 and MMP-9, which are key enzymes involved in dentinal collagen degradation during caries progression. Among the tested compounds, Withanolide B exhibited the highest affinity toward MMP-8 (−8.8 kcal/mol), whereas Withaferin A demonstrated the strongest interaction with MMP-9 (−8.2 kcal/mol). These findings support the potential use of Withania coagulans-derived compounds as host-modulating anti-caries agents.

Tjäderhane et al. reported that host-derived MMPs are activated within demineralized dentin and significantly contribute to collagen matrix degradation during caries progression.[23] The present findings support this concept by demonstrating that all selected phytochemicals effectively interacted with MMP-8 and MMP-9 active sites. By occupying catalytic regions and forming stable protein–ligand complexes, these compounds may potentially inhibit MMP-mediated degradation of dentinal collagen.

Sulkala et al. demonstrated increased expression of MMP-8 in human dentinal caries lesions and identified it as a major collagenolytic enzyme involved in tissue destruction. In this study, Withanolide B exhibited the strongest affinity toward MMP-8, suggesting its potential to inhibit collagenase activity and preserve dentinal matrix integrity. This observation aligns with the therapeutic rationale proposed by Sulkala et al.[24]

Chaussain et al. reported that MMP-mediated destruction of the dentin organic matrix continues even after bacterial acids initiate demineralization.[2] The strong binding affinities observed in the present study suggest that Withania coagulans phytochemicals may interrupt this secondary degradation process. Therefore, these compounds may complement traditional antimicrobial approaches by targeting host-mediated tissue destruction.

Shimada et al. localized MMP-2, MMP-8, MMP-9, and MMP-20 within carious dentin and highlighted their involvement in lesion progression.[25] This docking analysis specifically targeting MMP-8 and MMP-9 extends these findings by identifying natural phytochemicals capable of interacting directly with these enzymes. Such interactions may reduce enzymatic activity and slow lesion advancement.[26]

Chibinski et al. observed strong expression of MMP-8 and collagen within dentinal lesions, indicating active collagen degradation during caries progression.[27] The ability of Withanolide B and Withaferin A to bind effectively to MMP-8 supports the possibility of preserving collagen architecture by reducing enzymatic breakdown.

Mazzoni et al. emphasized the importance of MMP inhibition in maintaining dentin stability and preventing degradation of collagen-rich substrates.[28] Similar observations were obtained in the present study, where multiple hydrogen bonds, hydrophobic interactions, and zinc-associated contacts stabilized the ligand–protein complexes. Such interactions are characteristic of effective enzyme inhibitors and support the potential therapeutic role of these phytochemicals.

Jain and Bahuguna described MMPs as critical mediators of dental caries and pulpal inflammation.[29] These findings suggest that Withania coagulans phytochemicals may offer dual benefits owing to their reported anti-inflammatory properties and their ability to interact with MMP targets. This combination may provide a broader host-modulating effect compared with conventional antimicrobial agents alone.

Guirado et al. identified dentinal MMPs as promising therapeutic targets and advocated the development of novel inhibitors for caries prevention.[30] This study results directly support this recommendation by identifying naturally derived compounds capable of interacting strongly with MMP-8 and MMP-9. Particularly, Withanolide B and Withaferin A emerged as promising candidates for future therapeutic development.

Besinis et al. demonstrated that titanium dioxide nanoparticles possess antibacterial activity against Streptococcus mutans and may be useful in preventive dentistry.[31] Combining these antimicrobial properties with the MMP-inhibitory potential demonstrated in the present study could provide a dual-action anti-caries strategy. Titanium dioxide nanoparticles synthesized using Withania coagulans may therefore offer both antibacterial and host-modulatory effects.

Ahrari et al. reported antimicrobial activity of metal oxide nanoparticles against oral streptococci.[20] This study results expands upon this concept by suggesting that phytochemical-capped nanoparticles may additionally inhibit host-derived collagenolytic enzymes, thereby addressing both microbial and host factors involved in caries progression.

Khan et al. reviewed the phytochemistry of Withania coagulans and reported the presence of biologically active withanolides possessing antioxidant, anti-inflammatory, and antimicrobial properties.[32] The favorable docking interactions observed in the current study further support the therapeutic potential of these compounds and provide a mechanistic basis for their application in dental caries management.

Maher et al. demonstrated the successful green synthesis of nanoparticles using Withania coagulans

extracts and confirmed their biological activity.[33] These findings complement their work by suggesting that phytoconstituents present in Withania coagulans may contribute directly to therapeutic activity through inhibition of MMP-mediated dentin degradation.

This study highlight the potential of Withania coagulans phytochemicals as inhibitors of MMP-8 and MMP-9, two key enzymes involved in dentinal collagen degradation during caries progression. A major strength of this study is the simultaneous evaluation of multiple bioactive compounds along with ADMET and drug-likeness assessments, which revealed favorable pharmacokinetic properties and compliance with Lipinski's rule. However, the study is limited by its purely computational nature, and the docking results require experimental validation. Future research should focus on the green synthesis and characterization of Withania coagulans-mediated titanium dioxide nanoparticles, followed by in vitro enzyme inhibition, antimicrobial, cytotoxicity, and molecular dynamics studies. Further animal and clinical investigations are necessary to confirm their efficacy and safety as novel anti-caries therapeutic agents.

CONCLUSION

Within the limitations of this in silico investigation, the phytoconstituents of Withania coagulans demonstrated promising inhibitory potential against MMP-8 and MMP-9, which are critically involved in dentinal matrix degradation during caries progression. The favorable binding affinities observed for Withanolide B, Withaferin A, Withanolide A, and Withanone, together with their satisfactory drug-likeness and pharmacokinetic profiles, suggest that these compounds may serve as effective host-modulating agents for the prevention of dental caries. By targeting matrix metalloproteinases, these phytochemicals may help preserve dentinal collagen integrity and reduce lesion progression. The present findings provide a scientific basis for the future development of Withania coagulans-mediated titanium dioxide nanoparticle formulations as innovative anti-caries therapeutics. Further in vitro, in vivo, and clinical studies are warranted to confirm their efficacy, safety, and applicability in preventive and minimally invasive dentistry..

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