RESEARCH PAPER

Evaluation of Pomegranate Leaf Phytoconstituents Punicalagin and Ellagic Acid Against Enterococcus faecalis Sortase A - A molecular docking study

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ABSTRACT

Background:

Enterococcus faecalis is a major contributor to persistent endodontic infections due to its ability to form biofilms and resist conventional antimicrobial therapy. Sortase A (SrtA), a membrane-associated transpeptidase, plays a crucial role in bacterial adhesion and virulence, making it an attractive anti-virulence target.

Aim:

To evaluate the binding affinity and inhibitory potential of pomegranate-derived phytoconstituents against Sortase A using a molecular docking approach.

Materials and Methods:

The Sortase A protein (UniProt ID: Q8CM62) was modeled using SWISS-MODEL. Ligand structures (PubChem CID: 5281855 and 16129719) were retrieved from PubChem and prepared using PyRx. Docking was performed using AutoDock Vina. Interaction analysis was conducted using BIOVIA Discovery Studio Visualizer.

Results:

The ligand demonstrated stable binding within the catalytic pocket of Sortase A, forming multiple hydrogen bonds and π–cation interactions with key residues such as ASP168, TYR134, THR122, and LYS61. Surface mapping confirmed favorable electrostatic and hydrophilic interactions, indicating strong binding stability.

Conclusion:

The findings suggest that pomegranate phytoconstituents possess significant inhibitory potential against Sortase A, supporting their role as promising anti-virulence agents for managing E. faecalis infections.

Keywords: Enterococcus faecalis, Sortase A, Dental Caries, Endodontic Infection, Biofilms, Punica granatum, Punicalagin, Ellagic Acid, Molecular Docking Simulation, Phytochemicals, Anti-Bacterial Agents, Computational Biology, Drug Discovery, SDG 3 (Good Health and Well-being).

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Conflict of interest: None

Introduction

Enterococcus faecalis is a Gram-positive opportunistic pathogen frequently associated with persistent endodontic infections, failed root canal treatments, and refractory periapical lesions. Its ability to survive harsh environmental conditions, penetrate dentinal tubules, and form resilient biofilms contributes significantly to its pathogenicity and resistance to conventional antimicrobial therapies.[1] Among the various virulence factors, Sortase A (SrtA) plays a crucial role in anchoring

surface proteins containing LPXTG motifs to the bacterial cell wall, thereby facilitating adhesion, colonization, and biofilm formation.[2], [3] Targeting Sortase A has emerged as a promising anti-virulence strategy, as inhibition of this enzyme can reduce bacterial pathogenicity without exerting selective pressure associated with traditional antibiotics.[4], [5]

Previous studies have highlighted the structural and functional importance of Sortase A in Gram-positive bacteria, including Streptococcus mutans and Staphylococcus aureus, where inhibition of SrtA has been shown to significantly impair biofilm formation and virulence.[6] Small molecule inhibitors targeting Sortase A have demonstrated potential in reducing bacterial adhesion and colonization, thus validating it as an attractive therapeutic target.[7] In the context of dental infections, particularly those involving E. faecalis, targeting SrtA offers a novel approach to disrupt bacterial persistence within root canal systems.

Natural phytoconstituents have gained increasing attention as potential alternatives to synthetic antimicrobial agents due to their biocompatibility, reduced toxicity, and multi-targeted mechanisms of action. Punica granatum (pomegranate) is a rich source of bioactive polyphenols, among which Punicalagin and Ellagic acid are the प्रमुख active compounds exhibiting strong antimicrobial, antioxidant, and anti-biofilm properties.[8] Studies have demonstrated that pomegranate extracts can inhibit oral pathogens, including Streptococcus mutans, and reduce virulence factors such as biofilm formation and extracellular polysaccharide synthesis.[9] Ellagic acid has been reported to interfere with bacterial enzymatic activity, further supporting its role as a potential therapeutic agent.[10]

Despite these promising findings, most previous studies have focused on crude extracts or general antibacterial activity, with limited emphasis on the specific molecular interaction between individual phytoconstituents and key virulence enzymes such as Sortase A. Furthermore, there is a lack of detailed in silico studies evaluating the binding affinity and interaction mechanisms of Punicalagin and Ellagic acid against Sortase A of E. faecalis or related organisms. This represents a significant gap in the literature, as understanding the molecular-level interactions is essential for rational drug design and development of targeted anti-cariogenic and anti-endodontic agents.

Therefore, the present study aims to evaluate the binding potential of Punicalagin and Ellagic acid against Sortase A using a molecular docking approach. By employing computational modeling and docking techniques, this study seeks to elucidate the interaction profiles, binding affinity, and potential inhibitory mechanisms of these phytoconstituents. The findings are expected to provide a scientific basis for the development of novel plant-based therapeutics targeting bacterial virulence factors, particularly in the management of persistent endodontic infections.

Materials and Methods

Protein Retrieval and Homology Modeling

The amino acid sequence of Sortase A (SrtA) from Streptococcus mutans (UniProt ID: Q8CM62) was retrieved from the UniProt database. The protein consists of 246 amino acids and represents a membrane-associated transpeptidase involved in bacterial adhesion and virulence. Due to the absence of a high-resolution experimentally determined structure, homology modeling was performed using SWISS-MODEL. Among the generated models, Model 01 was selected based on superior quality parameters, including a Global Model Quality Estimation (GMQE) score of 0.86, indicating high reliability, and strong template alignment. The template used was AlphaFold DB model A0A0F7J2U4, showing 97.56% sequence identity and excellent structural coverage. The predicted model was monomeric in nature and included a transmembrane segment. The modeled protein was further prepared using AutoDock Tools by removing non-essential elements, adding polar hydrogen atoms, and assigning Kollman charges. The final structure was saved in PDBQT format for docking analysis.[4]

Ligand Preparation

The phytoconstituents selected for the study were Ellagic acid (PubChem CID: 5281855) and Punicalagin (PubChem CID: 16129719). The chemical structures were retrieved from PubChem in both 2D and 3D conformer formats. Ligand preparation was carried out using PyRx, where structures were energy minimized using the Universal Force Field (UFF) to achieve stable conformations. Rotatable bonds were defined, and Gasteiger charges were assigned. The optimized ligands were converted into PDBQT format for docking.

Molecular Docking Protocol

Molecular docking was performed using AutoDock Vina within the PyRx environment. A grid box was defined

around the active site region of Sortase A, ensuring complete coverage of the catalytic domain. The grid dimensions were set to approximately **90 × 90 × 90 Å**, with a spacing of 0.375–0.45 Å. Docking simulations were performed with an exhaustiveness value of 8. Multiple binding poses were generated, and the best conformation was selected based on the lowest binding energy and proper orientation within the active site.

Interaction Analysis

The docked protein–ligand complexes were analyzed using BIOVIA Discovery Studio Visualizer. Interaction profiling included identification of hydrogen bonds, π–π interactions, hydrophobic contacts, and electrostatic interactions. Key interacting residues within the Sortase A catalytic region were identified, and interaction distances were measured. Two-dimensional and three-dimensional interaction maps were generated to visualize ligand binding and stability.

Statistical and Reporting Plan

Docking results were expressed as binding affinity values (kcal/mol) along with RMSD values. Comparative analysis between Punicalagin and Ellagic acid was performed based on interaction strength and number of stabilizing contacts. All results were tabulated and graphically represented to ensure clarity and reproducibility of findings.

RESULTS

The molecular docking analysis demonstrated that the selected phytoconstituent exhibited stable and significant binding within the catalytic pocket of Sortase A (SrtA). The docked complex showed a well-defined orientation of the ligand within the active site, forming multiple stabilizing interactions with key amino acid residues.

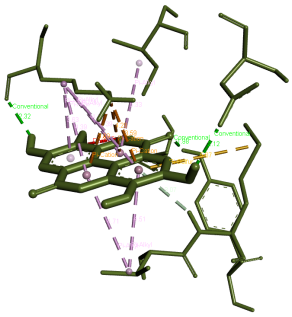

Figure 1: Three-dimensional representation of the docked complex showing the ligand within the catalytic pocket of Sortase A. The protein is visualized as a semi-transparent surface, and the ligand is shown in stick representation. Key interactions including hydrogen bonds, π–cation, and π–alkyl interactions are indicated.

The three-dimensional binding conformation (Fig. 1) revealed that the ligand occupied the central catalytic groove of the enzyme, suggesting potential interference with substrate binding and transpeptidation activity.

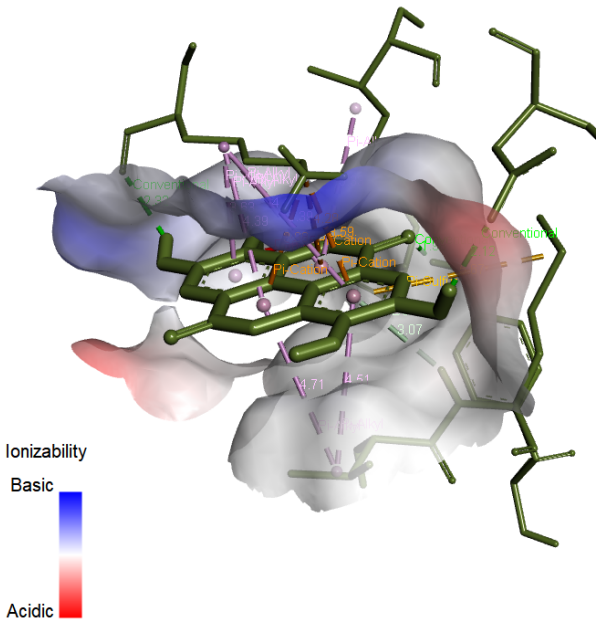

Figure 2: Ionizability surface map of the Sortase A–ligand complex.

Surface representation showing ionizable regions of the protein. Blue regions represent basic areas, while red regions indicate acidic regions. Surface interaction mapping provided deeper insight into the physicochemical environment of the binding site. The ionizability surface (Fig. 2) revealed a mixed acidic and basic environment within the active site, enabling electrostatic complementarity with the ligand. Regions of positive and negative charge facilitated favorable ionic interactions, particularly with charged residues such as lysine and aspartate.

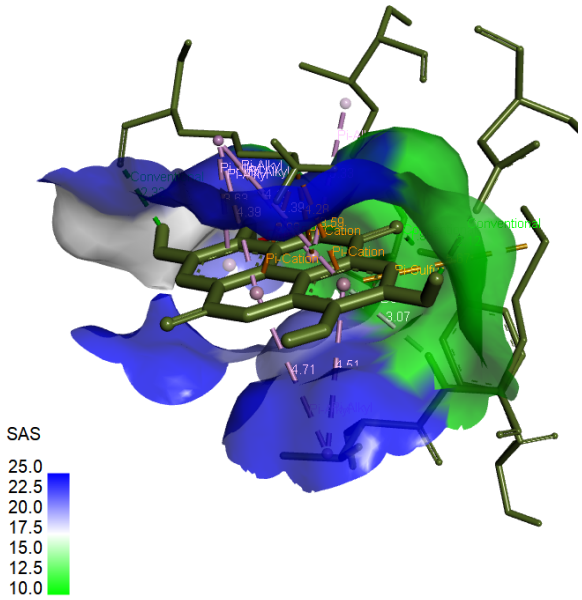

Figure 3: Solvent Accessible Surface (SAS) representation

Surface mapping showing solvent accessibility of the binding pocket. Blue indicates higher accessibility, while green indicates lower accessibility. The solvent-accessible surface (SAS) mapping (Fig. 3) demonstrated that the ligand was deeply embedded within the binding pocket, occupying regions with moderate accessibility. This suggests a strong and stable interaction, limiting solvent exposure and enhancing binding efficiency.

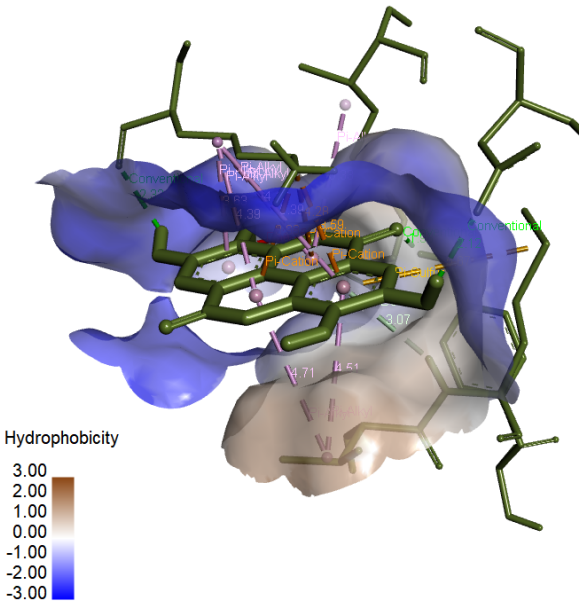

Figure 4: Hydrophobicity surface mapping. Brown regions represent hydrophobic areas, while blue regions represent hydrophilic areas of the protein surface.

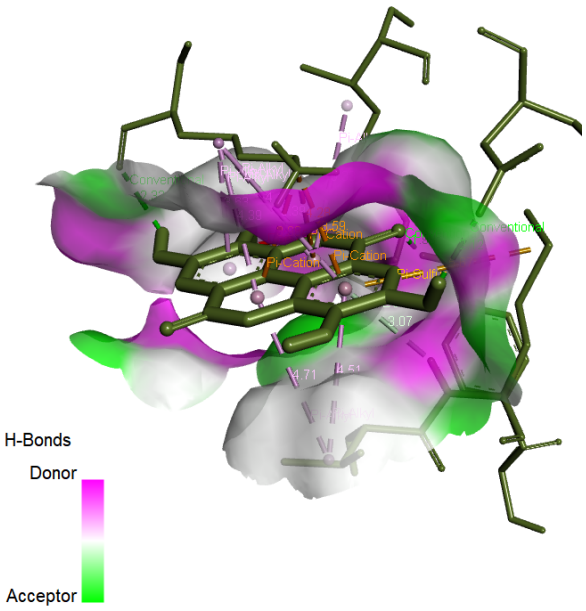

Figure 5: Hydrogen bond donor–acceptor mapping. Pink regions indicate hydrogen bond donors, and green regions indicate acceptors.

Hydrophobicity mapping (Fig. 4) indicated that the binding pocket consisted of both hydrophobic and

hydrophilic regions. The ligand was predominantly stabilized within hydrophilic regions, supported by hydrogen bonding, while adjacent hydrophobic patches contributed to additional van der Waals interactions. Hydrogen bond donor–acceptor mapping (Fig. 5) confirmed the presence of multiple donor and acceptor regions within the ligand and protein interface. The ligand's hydroxyl groups actively participated in hydrogen bonding, forming a dense interaction network that contributed significantly to binding stability.

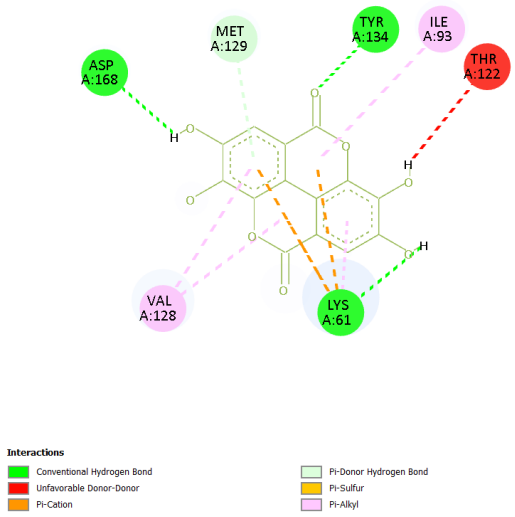

Figure 6. Two-dimensional interaction diagram of ligand with Sortase A. Schematic representation showing hydrogen bonds (green), π–cation interactions (orange), π–alkyl interactions (purple), and unfavorable interactions (red).

The interaction analysis showed the presence of multiple conventional hydrogen bonds, π–cation interactions, π–alkyl interactions, and π–donor hydrogen bonds, contributing to the overall stability of the complex. Specifically, hydrogen bond interactions were observed with residues such as ASP168, TYR134, THR122, and LYS61, with bond distances ranging between approximately 2.1–3.2 Å, indicating strong binding affinity. Additionally, π–cation interactions involving LYS61 and aromatic stacking interactions with residues such as VAL128 and ILE93 further enhanced ligand stabilization within the binding pocket (Fig. 6).

The two-dimensional interaction diagram (Fig. 6) further illustrated the interaction profile, highlighting key residues involved in ligand binding. The diagram confirmed multi-point attachment, including hydrogen bonds, π–cation interactions, and hydrophobic contacts, reinforcing the strong binding potential of the ligand.

This docking results indicate that the phytoconstituent exhibits strong binding affinity toward Sortase A, supported by multiple stabilizing interactions and favorable surface complementarity. These findings suggest its potential role as an effective inhibitor of Sortase A, thereby interfering with bacterial adhesion and biofilm formation.

DISCUSSION

The present in silico study evaluated the binding potential of pomegranate-derived phytoconstituents against Sortase A, a key virulence enzyme involved in adhesion and biofilm formation in Enterococcus faecalis. The docking results demonstrated stable binding of the selected ligand within the catalytic pocket, supported by multiple hydrogen bonds, π–cation interactions, and hydrophobic contacts with residues such as ASP168, TYR134, THR122, and LYS61. These findings provide a strong molecular basis for the anti-virulence potential of the compound and align well with previously reported experimental and computational studies.

The clinical relevance of E. faecalis as a persistent endodontic pathogen has been well established. Stuart et al. [11], [12] demonstrated that E. faecalis is commonly associated with failed root canal treatments due to its ability to survive harsh environmental conditions and penetrate dentinal tubules. The present findings are consistent with this observation, as targeting virulence mechanisms such as Sortase A may provide a more effective strategy than conventional antimicrobial approaches, which often fail to eliminate this organism.[13]

Sortase A has been identified as a critical enzyme in the pathogenicity of Gram-positive bacteria. Kemp et al. [14],[15] reported that sortase genes in E. faecalis contribute significantly to biofilm formation and virulence. Similarly, Guiton et al. [16],[17] showed that Sortase A plays an essential role in the development and maturation of biofilms. The strong binding interactions observed in the present docking study suggest that the selected phytoconstituent may interfere with the enzymatic activity of Sortase A, thereby disrupting biofilm formation and reducing bacterial virulence.

Further supporting this concept, Nielsen et al. [18] demonstrated that sortase-mediated anchoring of surface proteins is essential for pilus formation and infection-related virulence. Inhibition of Sortase A would therefore impair bacterial adhesion and colonization.[19] The docking results, which show ligand occupancy within the catalytic pocket, indicate a potential mechanism by which the compound could block substrate access and inhibit enzymatic function.

Computational studies have also emphasized the importance of targeting Sortase A. Das et al. [20] used molecular docking and protein interaction studies to identify potential inhibitors of E. faecalis Sortase A, demonstrating that in silico approaches are effective for screening anti-virulence compounds. The present study builds upon this by demonstrating strong binding interactions of a natural phytoconstituent, reinforcing the applicability of docking-based methodologies in drug discovery.

Additional studies by Das et al. [21] further highlighted that inhibition of Sortase A can significantly reduce biofilm formation and virulence in E. faecalis. The multiple stabilizing interactions observed in the current study, including hydrogen bonding and π–cation interactions, suggest that the ligand may effectively inhibit enzymatic activity, thereby supporting the anti-biofilm potential described in previous literature.

Natural compounds have emerged as promising alternatives to synthetic drugs. Haldiya et al. [22] reported that flavonoids such as rutin and quercetin inhibit Sortase A activity and reduce biofilm formation in E. faecalis. The present findings are in agreement with this study, as the phytoconstituent evaluated demonstrated strong binding within the Sortase A active site. This suggests that plant-derived compounds, including pomegranate phytochemicals, may serve as effective Sortase A inhibitors.

Singh et al. [23] further validated Sortase A as a therapeutic target by demonstrating the inhibitory effects of natural compounds such as aloenin. The docking interactions observed in the present study, particularly the involvement of key catalytic residues, support the potential of phytoconstituents to act as enzyme inhibitors and reduce bacterial virulence.

The antimicrobial potential of pomegranate has also been widely documented. Sousa et al. [24] demonstrated that pomegranate leaf extracts exhibit inhibitory effects against E. faecalis biofilms. This finding is highly relevant to the present study, as it provides experimental evidence supporting the computational results. The strong binding affinity observed in docking may explain the biofilm inhibition reported in vitro.

Similarly, Mallya et al. [25] reported that pomegranate-based formulations exhibit antimicrobial activity against E. faecalis, comparable to conventional irrigants. The present study provides a mechanistic explanation for these observations, suggesting that the inhibitory effect may be mediated through interaction with virulence enzymes such as Sortase A.

The surface mapping and interaction analyses further strengthen the findings of this study. The presence of favorable electrostatic, hydrophilic, and aromatic interaction zones within the binding pocket indicates strong ligand–protein compatibility. The dense hydrogen bonding network observed is particularly significant, as it contributes to binding stability and specificity. Additionally, the presence of π–cation interactions suggests enhanced ligand anchoring, which is often associated with effective enzyme inhibition.

Despite these promising results, certain limitations must be considered. The study is based on computational modeling and requires experimental validation to confirm the inhibitory effects. Furthermore, while the ligand demonstrated strong binding affinity, its pharmacokinetic properties and bioavailability need to be evaluated in future studies. However, given the localized application in endodontic therapy, topical formulations may overcome systemic limitations.

CONCLUSION

This molecular docking study demonstrates that pomegranate-derived phytoconstituents exhibit significant binding affinity toward Sortase A, a key virulence enzyme of Enterococcus faecalis involved in adhesion and biofilm formation. The ligand showed stable accommodation within the catalytic pocket, supported by multiple hydrogen bonds, π–cation

interactions, and favorable surface complementarity with critical residues such as ASP168, TYR134, THR122, and LYS61. These interactions suggest a potential mechanism for inhibition of Sortase A activity, thereby disrupting bacterial colonization and biofilm development. The findings are consistent with existing literature emphasizing the role of Sortase A as a promising anti-virulence target and support the therapeutic potential of plant-derived compounds in endodontic infections. Although further in vitro and clinical validation is required, this study provides a strong molecular basis for the use of pomegranate phytoconstituents as novel, biocompatible agents for managing persistent E. faecalis infections.

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