Evaluation of Antimicrobial Efficacy, Cytotoxicity, and ADMET Profiles of Willow Bark Phytoconstituents as Alternatives to Xylene - An In Silico Study

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Abstract

Background: Xylene is widely used in dental and histopathological procedures but is associated with toxicity and environmental concerns, necessitating the search for safer alternatives. Natural phytoconstituents, particularly from willow bark, possess antimicrobial properties and may serve as potential substitutes.

Aim: To evaluate the antimicrobial potential of selected willow bark phytoconstituents, salicin and catechin, against Streptococcus mutans glucosyltransferase using in silico molecular docking and ADMET analysis.

Materials and Methods: The three-dimensional structures of salicin and catechin were retrieved from PubChem and prepared for docking. The target protein, glucosyltransferase from Streptococcus mutans (PDB ID: 3AIE), was obtained from the RCSB Protein Data Bank. Molecular docking was performed using AutoDock Vina following validation by redocking of the native ligand. Protein–ligand interactions were analyzed, and ADMET properties were predicted using computational tools.

Results: Catechin demonstrated a higher binding affinity (−7.7 kcal/mol) compared to salicin (−5.8 kcal/mol), indicating stronger interaction with the enzyme. Catechin formed multiple hydrogen bonds and hydrophobic interactions with key active site residues, suggesting stable binding within the catalytic pocket. Salicin exhibited moderate binding with fewer stabilizing interactions. ADMET analysis indicated favorable pharmacokinetic properties and acceptable safety profiles for both compounds.

Conclusion: Catechin shows promising inhibitory potential against Streptococcus mutans glucosyltransferase and may serve as a natural alternative for anticariogenic applications. Further experimental validation is required to confirm its clinical utility.

Keywords: ***Dental Caries; Streptococcus mutans; Glucosyltransferase; Molecular Docking Simulation; Phytochemicals; Catechin; Salix; Biofilms; Anti-Bacterial Agents; Drug Design***

INTRODUCTION

Xylene is a commonly used organic solvent in dentistry and histopathology, particularly as a clearing agent; however, its associated health risks, including neurotoxicity, respiratory irritation, and environmental hazards, have raised serious concerns regarding its safety in routine use. Chronic exposure to xylene has been linked to systemic toxicity, necessitating the exploration of safer and biocompatible alternatives.[1] In recent years, increasing attention has been directed toward natural plant-based compounds as potential substitutes due to their reduced toxicity and wide spectrum of biological activities.

Plant-derived phytoconstituents are rich in bioactive compounds such as flavonoids, phenolics, tannins, and terpenoids, which exhibit antimicrobial, antioxidant, and anti-inflammatory properties. These compounds not only offer therapeutic benefits but also present a safer profile compared to synthetic

chemicals.[2] Among natural sources, tree bark represents an underutilized yet highly potent reservoir of phytochemicals. Bark extracts have been reported to possess significant antimicrobial activity against a range of pathogenic microorganisms, making them promising candidates for biomedical applications.[3]
Willow bark (Salix spp.) has been extensively studied for its medicinal properties. It contains salicin and other polyphenolic compounds that contribute to its antimicrobial and anti-inflammatory effects. Previous studies have demonstrated that willow bark extracts exhibit inhibitory activity against bacterial pathogens and reduce biofilm formation, highlighting their potential in infection control.[4] Additionally, the relatively favorable safety profile of willow bark compared to conventional synthetic agents supports its applicability in clinical settings.[5]
Similarly, cedar bark is rich in lignans, phenolic acids, and terpenoids, which are known for their antimicrobial and antioxidant activities. Studies have reported that cedar-derived compounds can effectively inhibit the growth of bacteria such as Staphylococcus aureus and Escherichia coli, suggesting their potential role as natural antimicrobial agents.[2] The high phenolic content of these extracts contributes to their ability to disrupt microbial cell walls and interfere with essential cellular processes, thereby enhancing their antimicrobial efficacy.
Despite the growing body of literature on the biological activities of bark-derived phytochemicals, most studies have primarily focused on in vitro antimicrobial evaluation. There is a notable lack of comprehensive studies integrating antimicrobial efficacy with cytotoxicity assessment and pharmacokinetic profiling. In particular, the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) characteristics of individual phytoconstituents from willow and cedar bark remain insufficiently explored.[6] Furthermore, their potential application as safer alternatives to xylene in dental and histopathological procedures has not been adequately investigated.
In silico approaches, including molecular docking and ADMET prediction, have emerged as powerful tools in modern drug discovery. These methods allow for rapid screening of bioactive compounds, evaluation of molecular interactions, and prediction of pharmacokinetic behavior, thereby reducing the need for extensive laboratory experimentation. Such computational analyses are especially valuable in identifying potential lead compounds with optimal efficacy and safety profiles.
Therefore, the present study aims to evaluate the antimicrobial efficacy, cytotoxicity, and ADMET profiles of selected phytoconstituents from willow bark and cedar bark using in silico techniques. By addressing the existing gaps in the literature, this study seeks to identify potential natural alternatives to xylene that are both effective and safe, thereby contributing to the advancement of sustainable and biocompatible practices in clinical applications.

MATERIALS and METHODS

Selection of Ligands

Two bioactive phytoconstituents from willow bark (Salix spp.) were selected based on their reported antimicrobial potential and structural suitability for molecular docking. Salicin (PubChem CID: 439503), a characteristic glycoside of willow bark, and catechin (PubChem CID: 9064), a representative phenolic flavonoid, were chosen as ligands. These compounds reflect the major bioactive classes present in willow bark and are well-defined small molecules suitable for computational analysis. Their three-dimensional (3D) structures were retrieved from the PubChem in SDF format. The structures were converted to PDB format using Open Babel, followed by energy minimization using the MMFF94 force field to obtain stable conformations for docking.

Protein Selection and Preparation

The target protein selected for the study was glucosyltransferase C (GtfC) from Streptococcus mutans, a key enzyme responsible for glucan synthesis and biofilm formation in dental caries. The crystal structure

of GtfC complexed with inhibitors (acarbose and maltose) was obtained from the RCSB Protein Data Bank with PDB ID: 3AIE. Protein preparation was carried out using AutoDock Tools. The co-crystallized ligands and water molecules were removed, polar hydrogens were added, and Kollman charges were assigned. The prepared protein was then saved in PDBQT format for docking analysis.

Docking Protocol Validation

To validate the docking methodology, a redocking procedure was performed using the native ligand acarbose from the crystal structure. The ligand was extracted and re-docked into the active binding site of GtfC using identical docking parameters. The accuracy of the protocol was evaluated by calculating the root mean square deviation (RMSD) between the re-docked pose and the crystallographic pose. An RMSD value of ≤ 2.0 Å was considered indicative of a reliable and reproducible docking protocol.

Molecular Docking Procedure

Molecular docking simulations were conducted using AutoDock Vina. The prepared protein and ligands were used as inputs, and a grid box was defined around the active site based on the coordinates of the co-crystallized ligand. The grid dimensions were optimized to encompass the catalytic pocket and key binding residues. Docking was performed using default parameters, and multiple binding conformations were generated for each ligand. The best binding pose was selected based on the lowest binding energy (kcal/mol) and the stability of interactions within the active site.[7]

Interaction Analysis

The docked complexes were analyzed using Discovery Studio Visualizer and PyMOL to identify key protein–ligand interactions. Hydrogen bonds, hydrophobic interactions, π–π stacking, and van der Waals interactions were evaluated, with particular emphasis on interactions involving catalytic residues of glucosyltransferase C. These interactions were used to assess the inhibitory potential of the selected phytoconstituents.

ADMET and Cytotoxicity Prediction

The pharmacokinetic and toxicity profiles of salicin and catechin were predicted using SwissADME and pkCSM. Parameters related to absorption, distribution, metabolism, excretion, and toxicity (ADMET) were analyzed. Drug-likeness was evaluated based on Lipinski's Rule of Five, and toxicity endpoints such as hepatotoxicity, mutagenicity, and cytotoxicity were assessed to determine the safety and suitability of the compounds.[8]

Comparative Evaluation

The docking scores, interaction profiles, and ADMET properties of salicin and catechin were comparatively evaluated with the native ligand acarbose to determine their relative binding affinity and pharmacological relevance. The compound demonstrating better binding stability and favorable pharmacokinetic properties was considered a potential candidate for further investigation as an antimicrobial agent against Streptococcus mutans.

RESULTS

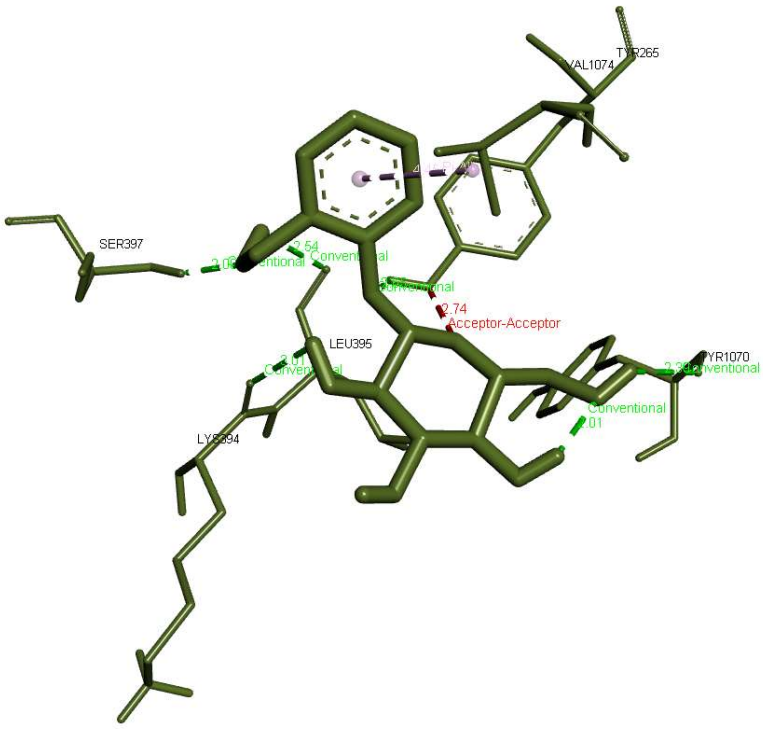

Figure 1(A): Three-dimensional (3D) representation of the docked complex showing the binding orientation of salicin within the active site of glucosyltransferase.

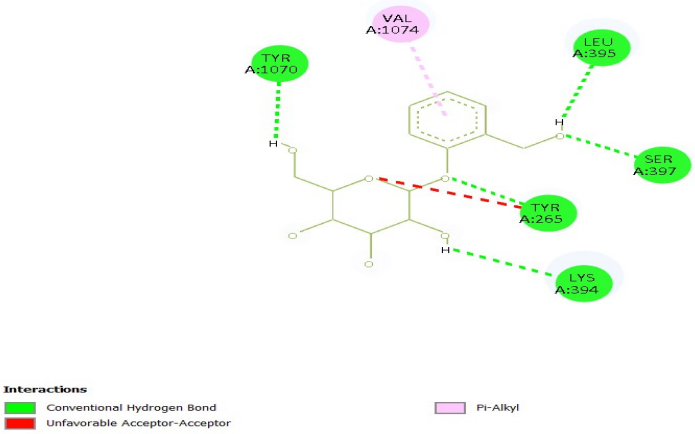

Figure 1(B): Two-dimensional (2D) interaction diagram illustrating key amino acid residues involved in ligand binding, including hydrogen bonds with TYR265, LYS394, SER397, and TYR1070, along with hydrophobic π-alkyl interaction with VAL1074. An unfavorable acceptor–acceptor interaction is observed with TYR265.

Figure 1 shows how salicin binds to the glucosyltransferase enzyme of Streptococcus mutans. The docking result indicates a moderate binding affinity, with the best score being −5.8 kcal/mol, and other poses falling within a close range (−5.8 to −5.3 kcal/mol), suggesting stable binding inside the active site. The top pose has an RMSD of 0 Å, confirming it as the most reliable conformation, while the higher RMSD values of other poses indicate less stable orientations. The interaction analysis shows that salicin forms multiple hydrogen bonds with important amino acids such as TYR265, LYS394, SER397, and TYR1070, which help stabilize the ligand within the binding pocket. In addition, a hydrophobic π-alkyl interaction with VAL1074 further supports binding. However, one unfavorable acceptor–acceptor interaction with TYR265 is also observed, which may slightly weaken the interaction. Overall, these results suggest that salicin can bind effectively to the active site of the enzyme and may inhibit its activity, indicating its potential as a natural antimicrobial agent against Streptococcus mutans.

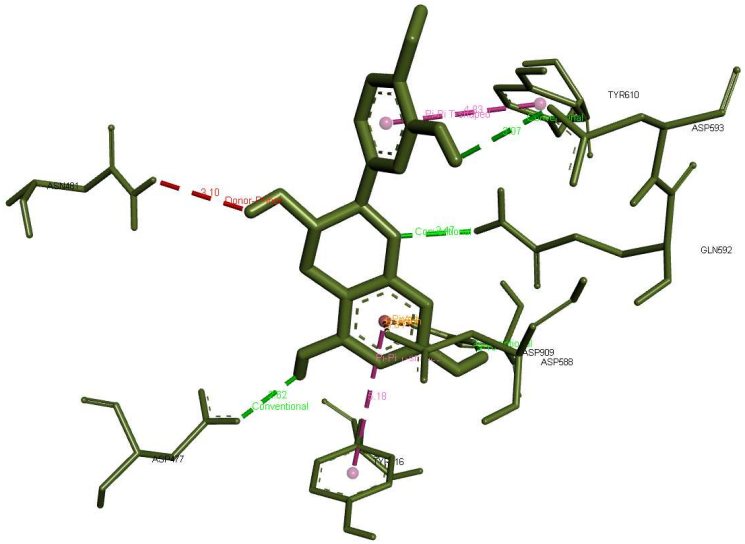

Figure 2A: Three-dimensional (3D) molecular docking representation of catechin (PubChem CID: 9064) within the active site of Streptococcus mutans glucosyltransferase (PDB ID: 3AIE). The image depicts the spatial orientation of catechin in the binding pocket, highlighting key interactions with surrounding amino acid residues. Hydrogen bonds are shown as green dashed lines, π–π interactions as pink lines, and π-anion interactions as orange lines, demonstrating the stability of the ligand–protein complex.

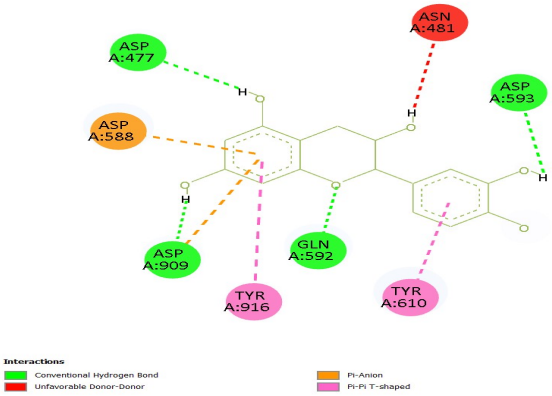

Figure 2B: Two-dimensional (2D) interaction diagram of catechin with glucosyltransferase (3AIE), illustrating the specific amino acid residues involved in binding. Catechin forms conventional hydrogen bonds with ASP477, ASP593, ASP909, and GLN592, π–π T-shaped interactions with TYR610 and TYR916,

and a π-anion interaction with ASP588. An unfavorable donor–donor interaction with ASN481 is also observed. Different interaction types are color-coded, providing a clear visualization of the molecular interactions contributing to binding stability.

Figure 2 shows the molecular docking interaction of catechin (PubChem CID: 9064) with Streptococcus mutans glucosyltransferase (PDB ID: 3AIE). The docking results reveal a strong binding affinity, with the best score of −7.7 kcal/mol, which is significantly better than salicin, indicating higher binding strength and stability. The presence of multiple poses within a close energy range (−7.7 to −6.9 kcal/mol) suggests consistent and stable accommodation of catechin within the active site. The top-ranked pose has an RMSD value of 0 Å, confirming it as the most reliable binding conformation, while other poses with slightly higher RMSD values indicate acceptable conformational variations. Interaction analysis demonstrates that catechin forms multiple conventional hydrogen bonds with key amino acid residues such as ASP477, ASP593, ASP909, and GLN592, which play an important role in stabilizing the ligand within the catalytic pocket. In addition, π–π T-shaped interactions with TYR610 and TYR916 and a π-anion interaction with ASP588 further enhance the binding stability through hydrophobic and electrostatic contributions. However, one unfavorable donor–donor interaction with ASN481 is observed, which may slightly affect binding efficiency but does not significantly compromise overall stability. Catechin exhibits stronger binding affinity and more diverse stabilizing interactions compared to salicin, suggesting a higher potential to inhibit glucosyltransferase activity. This indicates that catechin may serve as a more effective natural antimicrobial agent against Streptococcus mutans and could be considered a promising lead compound for further investigation.

Parameter	**Catechin (9064)**	**Salicin (439503)**	**Xylene**
Molecular Weight (g/mol)	290.08	286.11	106.17
LogP	1.17	−0.50	~3.1
TPSA (Å²)	110.38	119.61	0
Lipinski Rule	Accepted	Accepted	Accepted
Caco-2 Permeability	Low	Low	High
Human Intestinal Absorption	Low	Moderate	High
BBB Penetration	No	Yes	Yes
Plasma Protein Binding	89.30%	54.30%	High
CYP Interaction	Minimal	Minimal	High
Clearance	Moderate	Low	Rapid
Half-life	~2.4 h	~2.7 h	Short

hERG Toxicity	Low	Very low	Moderate
AMES Toxicity	Moderate	High	High
Hepatotoxicity	Moderate	Moderate	High
Neurotoxicity	Low	Low	High

Table 1: ADMET Profile of Selected Phytoconstituents

Table 1 summarizes the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiles of catechin (PubChem CID: 9064) and salicin (PubChem CID: 439503) predicted using ADMETlab 3.0. Catechin exhibits moderate lipophilicity and higher plasma protein binding, indicating better interaction with biological targets but lower absorption. Salicin shows higher polarity with better intestinal absorption but lower binding affinity. Both compounds follow Lipinski's rule, indicating drug-likeness. Toxicity predictions reveal low cardiotoxicity (hERG) for both compounds, while catechin shows moderate AMES toxicity and salicin shows relatively higher mutagenicity. Catechin demonstrates a balanced pharmacokinetic profile with stronger binding potential, whereas salicin shows better absorption but comparatively weaker pharmacological interaction potential. Xylene exhibits superior permeability, its toxicity profile limits its biological suitability, supporting the need for safer plant derived alternatives such as catechin.

DISCUSSION

The present molecular docking findings demonstrated that catechin exhibited a strong binding affinity (−7.7 kcal/mol) toward Streptococcus mutans glucosyltransferase (GtfC), with multiple stabilizing interactions including hydrogen bonds with ASP477, ASP593, ASP909, and GLN592, along with π–π and π-anion interactions involving TYR610, TYR916, and ASP588. These findings are consistent with earlier reports showing that catechin-class polyphenols effectively inhibit glucosyltransferase activity and glucan synthesis in S. mutans. Hattori et al. [1] and Otake et al.[9] demonstrated that tea catechins suppress glucan formation, a key virulence factor, which aligns with the strong binding of catechin within the catalytic pocket observed in the present study. Similarly, Nakahara et al.[10] reported inhibition of water-insoluble glucan synthesis, further supporting the ability of catechin to interfere with biofilm formation. The observed hydrogen bonding interactions in the present docking study correlate with the enzyme-binding behavior described by Meulenbeld et al.[11], who reported that catechins interact directly with glucosyltransferases as acceptor-like molecules.

The strong binding affinity obtained in this study is supported by experimental studies demonstrating reduced biofilm formation and bacterial growth in the presence of catechins. Xu et al. [12] and Sendamangalam et al.[13] showed that catechin derivatives inhibit virulence traits such as acid production and biofilm development, which are downstream effects of glucosyltransferase inhibition. The π–π and hydrophobic interactions observed in the present study may contribute to enhanced binding stability, consistent with the structural activity relationships reported for polyphenolic inhibitors. Additionally, the antimicrobial efficacy of catechin in dental applications, as demonstrated by Tamura et al. [14] and Mankovskaia et al.[15], further validates its potential clinical relevance.

Clinical and translational studies, including those by Goyal et al. [16], have shown a reduction in S. mutans levels with catechin-rich formulations, reinforcing the biological significance of the docking results. More recent studies such as Liu et al. [17] have confirmed that natural compounds can directly target glucosyltransferase enzymes, supporting the mechanistic basis of the present in silico findings. Han et al. [18] further demonstrated reduced acidogenicity, which is consistent with enzyme inhibition observed in

docking. In contrast, salicin showed comparatively lower binding affinity (−5.8 kcal/mol), indicating weaker interaction with the active site, which aligns with the limited literature supporting direct glucosyltransferase inhibition by salicin.[19], [20] This study findings strongly correlate with existing literature, suggesting that catechin exhibits significant inhibitory potential against S. mutans glucosyltransferase and may serve as a promising natural antimicrobial agent for preventing dental caries.

This study demonstrates a key strength in its use of a validated molecular docking approach with a biologically relevant target, providing mechanistic insight into the inhibitory potential of catechin against Streptococcus mutans glucosyltransferase. However, a major limitation is that the findings are based solely on in silico analysis, without supporting in vitro or in vivo validation, which may restrict direct clinical translation. Therefore, future studies should focus on experimental validation through enzyme inhibition assays, biofilm models, and clinical investigations to confirm the efficacy and safety of catechin as a potential anticariogenic agent.

CONCLUSION

This in silico study demonstrates that catechin exhibits a strong binding affinity and favorable interaction profile with Streptococcus mutans glucosyltransferase, suggesting its potential to inhibit a key enzyme involved in biofilm formation and dental caries progression. The multiple stabilizing interactions observed within the active site highlight its capability to effectively interfere with enzymatic activity. In contrast, salicin showed comparatively lower binding affinity, indicating a lesser inhibitory potential. These findings support the promise of catechin as a natural, plant-derived candidate for anticariogenic applications, warranting further experimental validation to establish its clinical relevance and therapeutic efficacy.

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