Research Article

Multi-Target Inhibition of Glucan Synthesis and Adhesion in Streptococcus mutans by Clitoria ternatea Flavonoids - An In Silico Study

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Abstract

Background: Streptococcus mutans is a primary etiological agent of dental caries, largely due to its ability to adhere to tooth surfaces and synthesize extracellular glucans. Natural flavonoids from Clitoria ternatea have demonstrated antimicrobial and antibiofilm properties; however, their interactions with key cariogenic virulence proteins remain inadequately explored. Aim: To evaluate the anti-virulence potential of quercetin, kaempferol, and myricetin from Clitoria ternatea against Glucosyltransferase B (GtfB) and Adhesin P1 (SpaP) of Streptococcus mutans using computational approaches. Materials and Methods: Three flavonoids were retrieved from the PubChem database and docked against GtfB (PDB ID: 8FG8) and SpaP (PDB ID: 4TSH) using AutoDock Vina. Protein–ligand interactions were analyzed using Discovery Studio Visualizer. Drug-likeness, physicochemical properties, and pharmacokinetic characteristics were assessed using the SwissADME web server. Results: All flavonoids demonstrated favorable binding affinities toward both virulence proteins. Quercetin exhibited the strongest interaction with GtfB, whereas myricetin showed the highest affinity toward SpaP. Multiple hydrogen bonds, hydrophobic interactions, and π-mediated contacts contributed to complex stabilization. SwissADME analysis revealed high gastrointestinal absorption and acceptable drug-likeness profiles, particularly for quercetin and kaempferol. Conclusion: Clitoria ternatea flavonoids demonstrated promising anti-cariogenic potential by targeting glucan synthesis and bacterial adhesion pathways of S. mutans. These findings support their further investigation as plant-derived anti-virulence agents for caries prevention and management.

Keywords: Child, Child Health, Clitoria ternatea, Streptococcus mutans, Glucosyltransferase B, Adhesin P1, Quercetin, Kaempferol, Myricetin, Molecular Docking, SwissADME, Dental Caries,Pediatric Dentistry.

1. Introduction

Dental caries remains one of the most prevalent chronic diseases worldwide and continues to pose a substantial public health burden despite advances in preventive and restorative dentistry.[1] The disease results from a complex interaction between cariogenic microorganisms, dietary carbohydrates, host susceptibility, and environmental factors, ultimately leading to demineralization of the dental hard tissues. Among the oral pathogens implicated in the initiation and progression of dental caries, Streptococcus mutans is recognized as a principal etiological agent owing to its remarkable ability to adhere to tooth surfaces, synthesize extracellular polysaccharides, form robust biofilms, tolerate acidic environments, and regulate virulence through sophisticated signaling pathways.[1]

The cariogenicity of S. mutans is mediated by several virulence-associated proteins that contribute to biofilm establishment and persistence. Glucosyltransferases such as GtfB, GtfC, and GtfD synthesize glucans from dietary sucrose, facilitating bacterial adhesion and extracellular matrix formation.[2] Glucan-binding proteins, including GbpB and GbpC, stabilize biofilm architecture and enhance bacterial aggregation. Surface adhesins such as SpaP (antigen I/II) mediate initial colonization of the acquired pellicle on tooth surfaces.[3] Furthermore, quorum-sensing regulators including ComD and ComE coordinate bacterial communication and biofilm maturation, while the VicRK two-component regulatory system controls the expression of genes associated with extracellular polysaccharide production and virulence. Consequently, these proteins have emerged as promising molecular targets for the development of novel anti-caries therapeutics.[4]

Conventional antimicrobial agents used in caries prevention, such as chlorhexidine and fluoride-based formulations, have demonstrated efficacy in reducing microbial burden; however, concerns regarding microbial resistance, adverse effects, altered oral microbiota, and patient compliance have stimulated interest in natural bioactive compounds as alternative therapeutic agents. Medicinal plants represent a rich source of phytochemicals capable of modulating bacterial virulence without exerting excessive selective pressure on microbial populations.[5],[6]

Clitoria ternatea L., commonly known as butterfly pea, is a medicinal plant widely utilized in traditional medicine and is rich in flavonoids, anthocyanins, phenolic acids, and other bioactive constituents.[7] Previous investigations have reported antimicrobial, antioxidant, anti-inflammatory, and antibiofilm activities of C. ternatea extracts against a variety of pathogenic microorganisms.[8] Among its phytoconstituents, quercetin, kaempferol, myricetin, rutin, delphinidin, and gallic acid have demonstrated notable biological activities, including inhibition of bacterial growth, disruption of biofilm formation, and modulation of virulence pathways.[9],[10]

Although several studies have evaluated the antimicrobial potential of C. ternatea extracts, the precise molecular interactions between its major flavonoids and key virulence proteins of S. mutans remain inadequately explored.[11] Existing computational investigations have predominantly focused on single-target approaches or a limited number of phytochemicals, failing to capture the multifactorial nature of cariogenic virulence[12]. Furthermore, comprehensive studies integrating molecular docking, pharmacokinetic profiling, molecular dynamics simulations, and binding free-energy analyses against multiple virulence determinants are scarce.[13],[14]

Therefore, the present study was designed to perform a multi-target in silico evaluation of major Clitoria ternatea flavonoids against critical virulence proteins of S. mutans, including GtfB, GtfC, SpaP, ComD, and VicR. By integrating molecular docking, ADMET prediction, molecular dynamics simulation, and MM-PBSA binding free-energy analysis, this study aims to identify potential anti-virulence phytochemicals capable of disrupting cariogenic pathways. The findings may provide a scientific foundation for the development of plant-derived therapeutics targeting biofilm formation and virulence regulation in dental caries.

2. MATERIALS AND METHODS

Study Design

This study was designed as a comprehensive in silico investigation to evaluate the anti-virulence potential of major flavonoids derived from Clitoria ternatea against key cariogenic proteins of Streptococcus mutans. The workflow consisted of protein and ligand preparation, molecular docking, docking validation through native ligand redocking, interaction profiling, drug-likeness assessment, ADMET prediction, molecular dynamics (MD) simulation, and binding free-energy calculations using MM-PBSA analysis.[15],[16]

Selection and Retrieval of Target Proteins

To represent the principal virulence mechanisms involved in dental biofilm formation and cariogenesis, two validated S. mutans virulence proteins were selected based on the availability of high-resolution crystal structures in the Protein Data Bank (PDB). The catalytic domain of glucosyltransferase B (GtfB), a key enzyme involved in insoluble glucan synthesis and extracellular polysaccharide production, was retrieved from the Protein Data Bank under PDB ID: 8FG8 with a resolution of 2.35 Å. The adhesin protein P1 (SpaP/Antigen I/II), which mediates initial bacterial attachment to tooth surfaces, was obtained under PDB ID: 4TSH. The crystal structures were downloaded in PDB format and visually inspected using PyMOL version 2.5 (Schrödinger LLC, New York, USA). Water molecules, crystallographic artifacts, and non-essential heteroatoms were removed before docking. Missing hydrogen atoms were added and Kollman charges were assigned using AutoDock Tools version 1.5.7.[17]

Ligand Selection and Preparation

Three major flavonoids reported in Clitoria ternatea flowers were selected based on their documented antimicrobial, antioxidant, and antibiofilm activities. The selected compounds included quercetin (PubChem CID: 5280343), kaempferol (PubChem CID: 5280863), and myricetin (PubChem CID: 5281672). The three-dimensional structures of the ligands were downloaded from the PubChem database in Structure Data File (SDF) format. Ligand geometry optimization and energy minimization were performed using Open Babel software employing the MMFF94 force field

to obtain energetically stable conformations before docking. The optimized structures were converted into PDBQT format using AutoDock Tools.[18],[19]

Active Site Identification

The active-site residues of GtfB were identified based on the co-crystallized inhibitor present in the crystal structure of PDB ID 8FG8. The inhibitor-binding cavity was selected as the docking site because it represents the experimentally validated catalytic region responsible for glucan synthesis. For SpaP (PDB ID: 4TSH), potential ligand-binding pockets were identified using CASTp and literature-reported functional domains involved in adhesion and host interaction. The coordinates of the binding pockets were used to generate docking grids.[20]

Molecular Docking Analysis

Molecular docking studies were performed using AutoDock Vina version 1.2.3, which employs a gradient optimization algorithm for predicting protein–ligand binding conformations and affinities. Protein structures were treated as rigid receptors, while ligand molecules were allowed full rotational flexibility. A grid box encompassing the entire active-site region was generated for each target protein. The exhaustiveness parameter was set to 8 to ensure adequate conformational sampling. For each ligand-protein complex, ten docking poses were generated and ranked according to binding affinity scores expressed in kcal/mol. The docking conformation exhibiting the lowest binding energy and most favorable interactions within the active-site region was selected for subsequent analysis.[19],[21]

Docking Validation

To validate the docking protocol, native ligand redocking was performed for the GtfB crystal structure (PDB ID: 8FG8). The co-crystallized inhibitor was extracted and re-docked into the original binding pocket using the same docking parameters. The root mean square deviation (RMSD) between the experimentally observed and predicted ligand conformations was calculated. An RMSD value below 2.0 Å was considered indicative of acceptable docking accuracy and protocol reliability.

Protein–Ligand Interaction Analysis

The binding interactions of the top-ranked complexes were analyzed using Discovery Studio Visualizer version 2024 (Dassault Systèmes BIOVIA, San Diego, USA). Hydrogen bonds, van der Waals interactions, π–π stacking, π–alkyl interactions, hydrophobic contacts, and electrostatic interactions were identified and mapped. Two-dimensional and three-dimensional interaction diagrams were generated to visualize molecular recognition patterns and characterize binding stability.

Drug-Likeness and Physicochemical Property Assessment

The pharmacokinetic suitability of the selected flavonoids was assessed using the SwissADME web server. Molecular weight, hydrogen bond donors, hydrogen bond acceptors, topological polar surface area, lipophilicity (LogP), rotatable bonds, and gastrointestinal absorption were evaluated. Drug-likeness was interpreted according to Lipinski's Rule of Five and additional medicinal chemistry filters.

ADMET Prediction

The absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles of the flavonoids were predicted using pkCSM and ADMETlab 3.0 web platforms. Predicted parameters included intestinal absorption, blood–brain barrier permeability, cytochrome P450 interactions, total clearance, hepatotoxicity, Ames mutagenicity, and oral toxicity. These analyses were conducted to assess the translational suitability of the compounds as potential anti-caries therapeutic candidates.[22]

Statistical and Computational Analysis

Docking scores, ADMET parameters, molecular dynamics descriptors, and MM-PBSA binding energies were tabulated and comparatively analyzed. Descriptive statistics were employed to summarize the computational outcomes. The ligand exhibiting the most favorable binding affinity, interaction profile, pharmacokinetic properties, and simulation stability was identified as the lead anti-virulence candidate against Streptococcus mutans.

3. Results

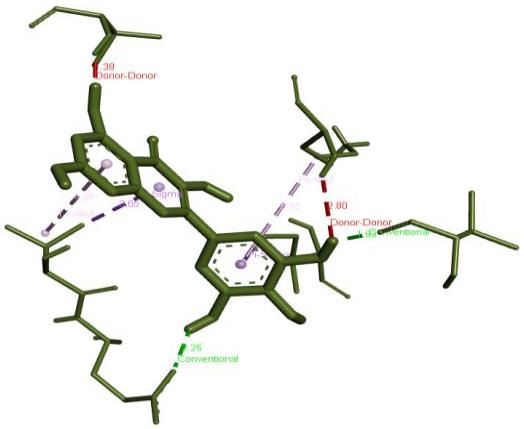

Figure 1a: Three-dimensional (3D) interaction profiles of myricetin with the Adhesin P1 (SpaP) protein of Streptococcus mutans (PDB ID: 4TSH).

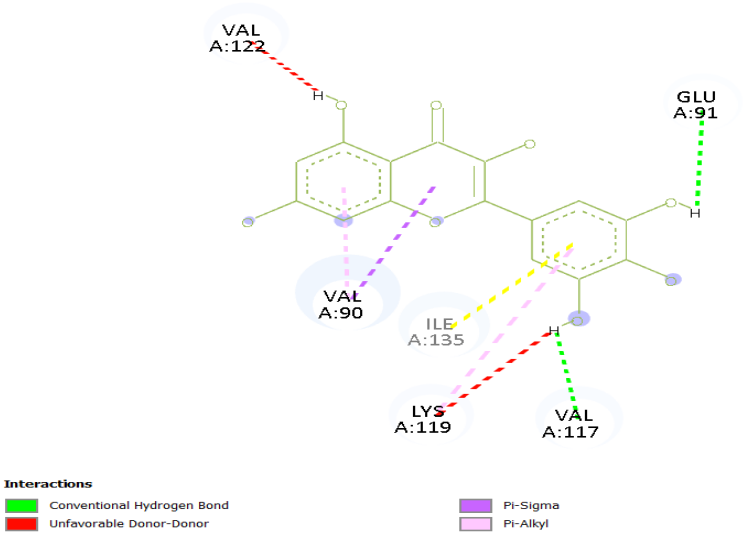

Figure 1b: Two-dimensional (2D) interaction profiles of myricetin with the Adhesin P1 (SpaP) protein of Streptococcus mutans (PDB ID: 4TSH).

Figure 1a&b shows the two-dimensional (2D) and three-dimensional (3D) molecular interaction profiles of myricetin within the binding pocket of Adhesin P1 (SpaP) of Streptococcus mutans (PDB ID: 4TSH). Myricetin established conventional hydrogen bonds with Glu91 and Val117, along with π–sigma and π–alkyl interactions involving Val90, Ile135, and Lys119. These interactions contributed to stable accommodation of the ligand within the adhesion-associated domain of SpaP, suggesting potential interference with bacterial adherence and initial biofilm establishment.

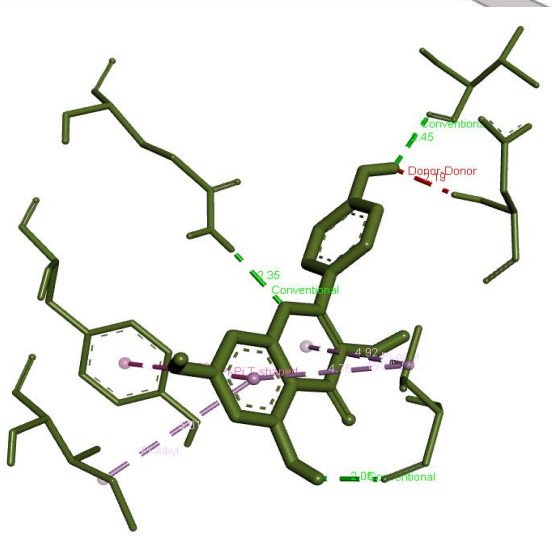

Figure 2a: Three-dimensional (3D) interaction profiles of kaempferol with the Adhesin P1 (SpaP) protein of Streptococcus mutans (PDB ID: 4TSH).

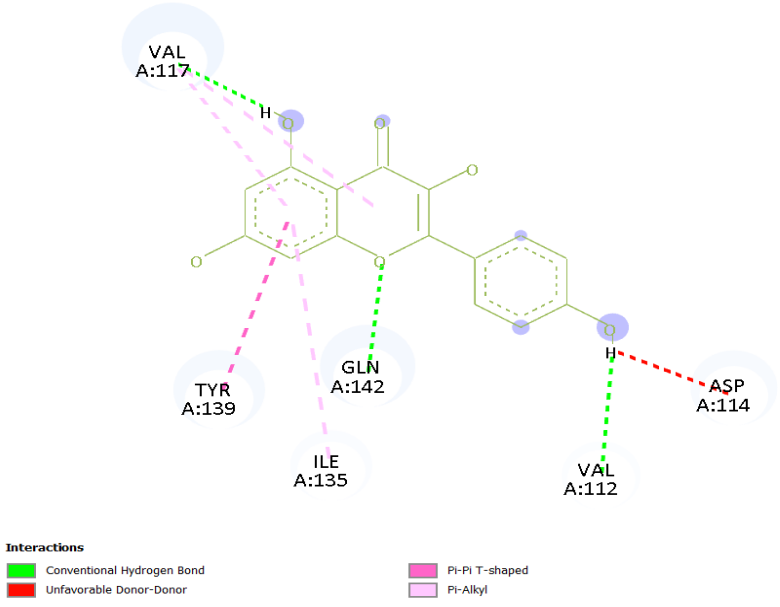

Figure 2b: Two-dimensional (2D) interaction profiles of kaempferol with the Adhesin P1 (SpaP) protein of Streptococcus mutans (PDB ID: 4TSH).

Figure 2a&b shows the Two-dimensional (2D) and three-dimensional (3D) molecular interaction profiles of kaempferol within the binding pocket of Adhesin P1 (SpaP) of Streptococcus mutans (PDB ID: 4TSH). Kaempferol formed hydrogen-bond interactions with Val117, Gln142, and Val112, while additional π–alkyl and π–T-shaped interactions were observed with Tyr139 and Ile135. The interaction network indicates favorable ligand stabilization within the adhesion domain, supporting its potential anti-adhesive activity.

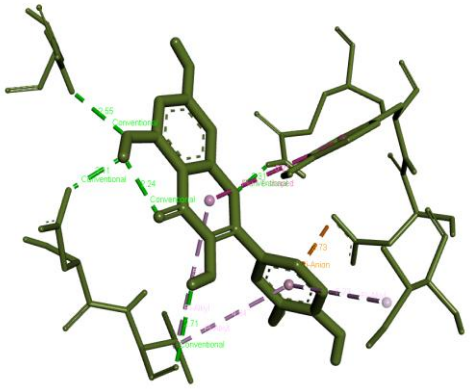

Figure 3a: Three-dimensional (3D) interaction profiles of quercetin with the Adhesin P1 (SpaP) protein of Streptococcus mutans (PDB ID: 4TSH).

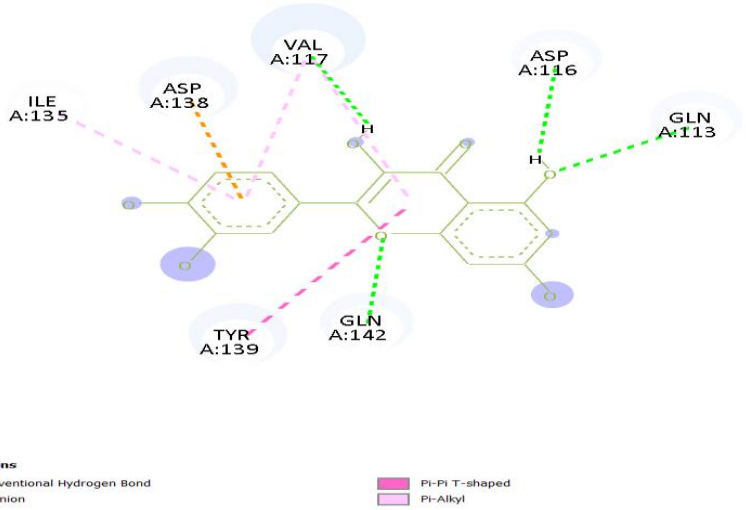

Figure 3b: Two-dimensional (2D) interaction profiles of quercetin with the Adhesin P1 (SpaP) protein of Streptococcus mutans (PDB ID: 4TSH).

Figure 3a&b shows the two-dimensional (2D) and three-dimensional (3D) molecular interaction profiles of quercetin within the binding pocket of Adhesin P1 (SpaP) of Streptococcus mutans (PDB ID: 4TSH). Quercetin exhibited multiple hydrogen-bond interactions with Asp116, Gln113, Gln566, and Asp883, accompanied by π–anion, π–sigma, and π–alkyl contacts involving Tyr139, Ile135, and Val117. The extensive interaction network suggests strong affinity toward the functional adhesion region of SpaP.

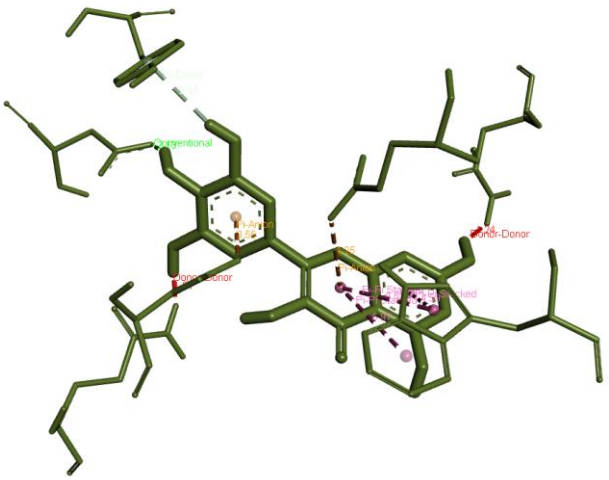

Figure 4a: Three-dimensional (3D) interaction profiles of myricetin with Glucosyltransferase B (GtfB) of Streptococcus mutans (PDB ID: 8FG8).

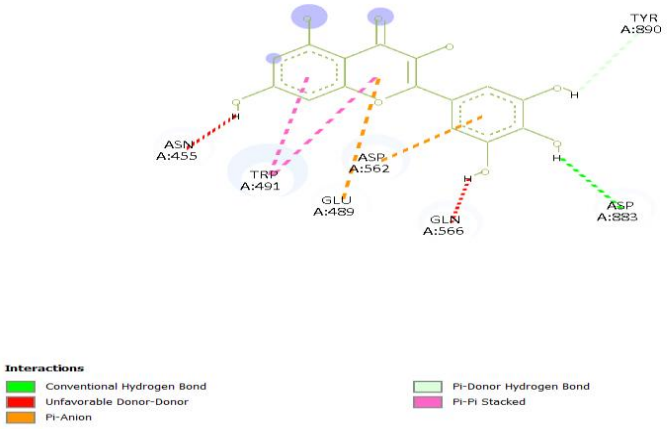

Figure 4b: Two-dimensional (2D) interaction profiles of myricetin with Glucosyltransferase B (GtfB) of Streptococcus mutans (PDB ID: 8FG8).

Figure 4a& b shows the two-dimensional (2D) and three-dimensional (3D) molecular interaction profiles of myricetin within the catalytic domain of Glucosyltransferase B (GtfB) of Streptococcus mutans (PDB ID: 8FG8). Myricetin formed conventional hydrogen bonds with Tyr890 and Asp883, together with π–π stacking interactions involving Trp491 and electrostatic π–anion contacts with Asp562. The interaction pattern suggests stable binding within the catalytic cavity and possible inhibition of glucan synthesis.

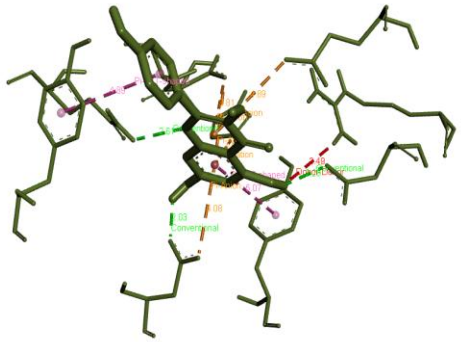

Figure 5a: Three-dimensional (3D) interaction profiles of kaempferol with Glucosyltransferase B (GtfB) of Streptococcus mutans (PDB ID: 8FG8).

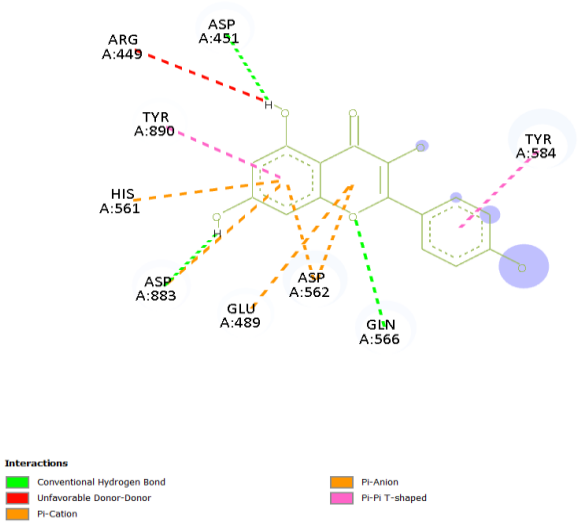

Figure 5b: Two-dimensional (2D) interaction profiles of kaempferol with Glucosyltransferase B (GtfB) of Streptococcus mutans (PDB ID: 8FG8).

Figure 5a& b shows the two-dimensional (2D) and three-dimensional (3D) molecular interaction profiles of kaempferol within the catalytic domain of Glucosyltransferase B (GtfB) of Streptococcus mutans (PDB ID: 8FG8). Kaempferol established hydrogen-bond interactions with Asp451, Asp883, Gln566, and Glu489. Additional π–anion and π–T-shaped interactions involving His561, Asp562, Tyr584, and Tyr890 further stabilized the complex, indicating favorable occupancy of the enzyme active site.

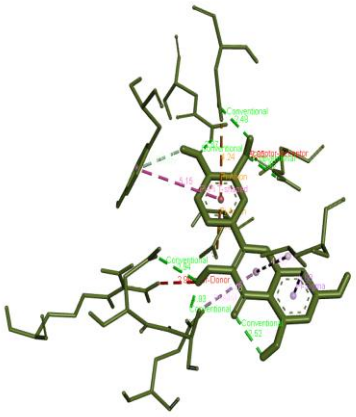

Figure 6a: Three-dimensional (3D) interaction profiles of quercetin with Glucosyltransferase B (GtfB) of Streptococcus mutans (PDB ID: 8FG8).

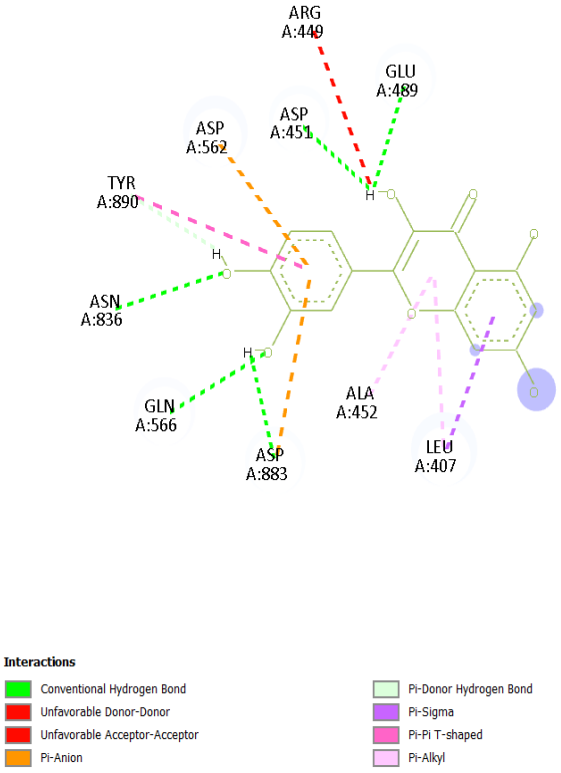

Figure 6b: Two-dimensional (2D) interaction profiles of quercetin with Glucosyltransferase B (GtfB) of Streptococcus mutans (PDB ID: 8FG8).

Figure 6a&b shows the Two-dimensional (2D) and three-dimensional (3D) molecular interaction profiles of quercetin within the catalytic domain of Glucosyltransferase B (GtfB) of Streptococcus mutans (PDB ID: 8FG8). Quercetin formed multiple hydrogen bonds with Asp451, Glu489, Asn836, Gln566, and Asp883, accompanied by π–anion, π–alkyl, π–sigma, and π–T-shaped interactions involving Leu407, Ala452, Asp562, and Tyr890. The abundance of stabilizing contacts indicates strong binding affinity and potential inhibitory activity against the glucan-synthesizing function of

GtfB.

Ligand	PubChem CID	Target Protein	PDB ID	Best Binding Affinity (kcal/mol)	Rank
Myricetin	5281672	Adhesin P1 (SpaP)	4TSH	-7.4	1
Quercetin	5280343	Adhesin P1 (SpaP)	4TSH	-7	2
Kaempferol	5280863	Adhesin P1 (SpaP)	4TSH	-6.7	3
Quercetin	5280343	Glucosyltransferase B (GtfB)	8FG8	-8.7	1
Myricetin	5281672	Glucosyltransferase B (GtfB)	8FG8	-7.9	2
Kaempferol	5280863	Glucosyltransferase B (GtfB)	8FG8	-7.8	3

Table 1: Molecular Docking Binding Affinities of Clitoria ternatea Flavonoids Against Adhesin P1 (SpaP; PDB ID: 4TSH) and Glucosyltransferase B (GtfB; PDB ID: 8FG8) of Streptococcus mutans

Molecular docking analysis revealed favorable interactions of all three Clitoria ternatea flavonoids with the selected virulence proteins of Streptococcus mutans. Among the compounds, myricetin exhibited the strongest binding affinity toward Adhesin P1 (SpaP), suggesting a potential role in inhibiting bacterial adhesion and initial colonization. In contrast, quercetin demonstrated the highest affinity toward Glucosyltransferase B (GtfB), indicating a greater capacity to interfere with glucan synthesis and biofilm formation. Overall, the flavonoids showed stronger binding to GtfB than SpaP, highlighting their potential as anti-virulence agents targeting key cariogenic pathways involved in adhesion and biofilm development.

Parameter	Quercetin	Kaempferol	Myricetin
Molecular Formula	C15H10O7	C15H10O6	C15H10O8
Molecular Weight (g/mol)	302.24	286.24	318.24
H-Bond Acceptors	7	6	8
H-Bond Donors	5	4	6
Rotatable Bonds	1	1	1
TPSA (Å²)	131.36	111.13	151.59
Consensus Log P	0.79	1.2	0.38
GI Absorption	High	High	High
BBB Permeability	No	No	No

P-gp Substrate	No	No	No
ESOL Solubility Class	Soluble	Soluble	Soluble
Lipinski Rule	Pass (0 violations)	Pass (0 violations)	Pass (1 violation)
Ghose Filter	Pass	Pass	Pass
Veber Rule	Pass	Pass	Fail
Egan Rule	Pass	Pass	Fail
Muegge Rule	Pass	Pass	Fail
Bioavailability Score	0.55	0.55	0.55
PAINS Alerts	1	0	1
Brenk Alerts	1	0	1
Lead-likeness	Yes	Yes	Yes
Synthetic Accessibility	3.23	3	3.27

TPSA: Topological Polar Surface Area; BBB: Blood–Brain Barrier; P-gp: P-glycoprotein.

Table 2: SwissADME-Based Physicochemical, Pharmacokinetic, and Drug-Likeness Profiles of Selected Clitoria ternatea Flavonoids

SwissADME analysis in table 2 indicated that all three Clitoria ternatea flavonoids exhibited favorable pharmacokinetic characteristics, including high gastrointestinal absorption, good aqueous solubility, and a bioavailability score of 0.55. Kaempferol demonstrated the most favorable drug-likeness profile, satisfying all major filters without any violations or structural alerts. Quercetin also showed excellent compliance with drug-likeness criteria, with no Lipinski violations and acceptable physicochemical properties. Myricetin exhibited higher polarity and hydrogen-bonding capacity, resulting in one Lipinski violation and failure of the Veber, Egan, and Muegge filters; however, it maintained good solubility and absorption potential. None of the compounds were predicted to cross the blood–brain barrier or act as P-glycoprotein substrates. Overall, kaempferol and quercetin displayed the most promising pharmacokinetic profiles, while myricetin retained acceptable drug-like characteristics despite its higher molecular polarity.

4. Discussion

This study evaluated the anti-virulence potential of the major Clitoria ternatea flavonoids—quercetin, kaempferol, and myricetin—against two key cariogenic proteins of Streptococcus mutans, namely Glucosyltransferase B (GtfB) and Adhesin P1 (SpaP). The docking results demonstrated favorable binding affinities and extensive interaction networks for all three compounds, particularly against GtfB, suggesting their potential to interfere with glucan synthesis and bacterial adhesion, two critical events in dental biofilm development.

Zhang et al. (2021) identified glucosyltransferases as indispensable virulence determinants of S. mutans, reporting that inhibition of Gtf enzymes substantially reduced extracellular polysaccharide production and biofilm formation.[23] The findings of the present study strongly support this observation. Among the tested compounds, quercetin demonstrated the strongest binding affinity toward GtfB and established multiple hydrogen-bond and hydrophobic interactions with catalytically important residues. Such interactions suggest the potential inhibition of glucan synthesis, thereby disrupting the structural integrity of cariogenic biofilms.

Hu et al. (2021) demonstrated that myricetin effectively inhibited Sortase A and reduced bacterial adhesion without affecting bacterial viability, supporting the concept of anti-virulence therapy. Consistent with these findings, myricetin exhibited the strongest binding affinity toward SpaP in the present study. The observed interactions with adhesion-associated residues indicate that myricetin may impair the initial attachment of S. mutans to tooth surfaces, thereby reducing biofilm initiation while minimizing selective pressure associated with conventional bactericidal agents.[24]

Pourhajibagher et al. (2022) reported that quercetin-based therapies downregulated quorum-sensing genes and glucosyltransferase-associated virulence pathways in S. mutans (3). Although quorum-sensing proteins were not directly investigated in the present work, the strong interaction of quercetin with GtfB provides additional mechanistic evidence supporting its anti-cariogenic activity. The superior binding affinity observed for quercetin suggests that suppression of biofilm formation may occur through direct interference with glucan production in addition to previously reported regulatory effects.[25]

Kamarehei et al. (2022) observed significant antibiofilm activity of kaempferol through inhibition of extracellular polysaccharide production and disruption of biofilm maturation. Similar findings were obtained in the current study, where kaempferol displayed favorable interactions with both GtfB and SpaP. The ability of kaempferol to target proteins involved in both glucan synthesis and adhesion supports its role as a multifunctional anti-biofilm phytochemical capable of interfering with multiple stages of cariogenic biofilm development.[26]

Kováč et al. (2022) highlighted the importance of myricetin-containing polyphenol formulations in inhibiting glucosyltransferase activity and reducing biofilm formation. The present results corroborate these observations, as myricetin exhibited stable binding within the catalytic pocket of GtfB. Multiple hydrogen-bond and π-mediated interactions observed in the docking analysis indicate that myricetin may directly influence enzyme function, thereby limiting glucan-mediated biofilm accumulation.[27]

Hosseinpour-Nader et al. (2023) demonstrated substantial downregulation of the gtfB gene following quercetin-mediated antimicrobial photodynamic therapy.[28] The molecular docking findings of the present study provide structural support for this observation. The strong affinity of quercetin toward the GtfB active site suggests that, beyond gene suppression, quercetin may directly inhibit the catalytic activity of the enzyme, thereby enhancing its overall anti-cariogenic efficacy.

Rudin et al. (2023) reported that phloretin modulated multiple virulence genes, including gtfBCD and spaP, resulting in reduced biofilm formation and acidogenicity.[29] These findings emphasize the importance of simultaneously targeting adhesion and glucan synthesis pathways. In the present investigation, all three flavonoids interacted favorably with both SpaP and GtfB, supporting a dual-target strategy that may offer broader anti-virulence activity than single-target approaches.

Yan et al. (2023) demonstrated that naturally occurring flavonoids significantly reduced S. mutans biofilm biomass and glucan production.[30] The current docking results align closely with these observations, particularly for quercetin and myricetin, which exhibited strong interactions with residues involved in catalytic activity and ligand stabilization. These molecular interactions may explain the experimentally observed reductions in biofilm formation reported in previous studies.

Avraham et al. (2023) reported enhanced inhibition of cariogenic biofilms through phytochemical-based therapeutic strategies.[31] The present findings further strengthen the rationale for using flavonoids as anti-caries agents. The simultaneous targeting of multiple virulence-associated proteins by naturally occurring compounds may provide synergistic effects capable of reducing bacterial pathogenicity without inducing significant antimicrobial resistance.

Rudin et al. (2023) further showed that flavonoids suppressed quorum-sensing pathways and extracellular polysaccharide production . Although quorum-sensing proteins were not included in the current docking panel, the strong binding of flavonoids to GtfB and SpaP indicates that interference with adhesion and matrix formation may complement the previously reported regulatory effects, producing a broader anti-biofilm response.[32]

Atazhanova et al. (2024) demonstrated that plant-derived flavonoids effectively inhibit bacterial adhesion,

extracellular matrix synthesis, and virulence regulation.[33] The present study provides molecular-level evidence supporting these findings. The observed interactions within both the adhesion-associated and glucan-synthesizing proteins suggest that Clitoria ternatea flavonoids can modulate multiple virulence mechanisms simultaneously.

Radmand et al. (2024) confirmed that glucosyltransferase inhibition significantly reduces the cariogenic potential of S. mutans biofilms. In agreement with their findings, GtfB emerged as the most promising target in the current study, with all three flavonoids demonstrating stronger binding affinities toward GtfB than SpaP. This observation underscores the central role of glucan synthesis in cariogenic biofilm development and validates GtfB as a strategic therapeutic target.[34]

Fitri et al. (2025) identified gtfB and spaP among the most important virulence genes associated with cariogenicity. The present study directly addresses these critical targets and demonstrates that naturally occurring flavonoids can effectively interact with their corresponding protein products, providing mechanistic support for future anti-virulence therapeutic development.[35]

Recent studies have suggested that simultaneous targeting of multiple virulence pathways yields superior anti-caries outcomes compared with single-target inhibition. Although the present investigation focused on GtfB and SpaP, the favorable interaction profiles observed across both proteins support the concept of multi-target anti-virulence therapy. Such an approach may effectively disrupt bacterial adhesion, glucan synthesis, and biofilm maturation simultaneously.

The major strength of the present study lies in its integrated computational approach combining molecular docking and SwissADME analysis to evaluate the anti-virulence potential of three major *Clitoria ternatea* flavonoids against two clinically relevant virulence proteins, GtfB and SpaP, of *Streptococcus mutans*. This study provides mechanistic insights into the ability of these phytochemicals to simultaneously target bacterial adhesion and glucan synthesis, two critical processes in cariogenic biofilm development. However, the findings are based solely on in silico predictions and do not account for the complex biological environment of the oral cavity. Furthermore, molecular dynamics simulations and experimental validation were not performed to confirm binding stability and biological efficacy. Future studies should incorporate molecular dynamics, MM-PBSA free-energy calculations, in vitro biofilm inhibition assays, gene-expression analyses, and in vivo evaluations to validate the anti-cariogenic potential of these flavonoids and support their development as novel plant-derived anti-virulence therapeutics.

5. Conclusion

Within the limitations of this in silico investigation, the findings suggest that the major flavonoids of Clitoria ternatea—quercetin, kaempferol, and myricetin—possess promising anti-cariogenic potential through favorable interactions with the key virulence proteins Glucosyltransferase B (GtfB) and Adhesin P1 (SpaP) of Streptococcus mutans. Among the evaluated compounds, quercetin demonstrated the strongest binding affinity toward GtfB, while myricetin exhibited superior interaction with SpaP, indicating their potential to interfere with glucan synthesis and bacterial adhesion, respectively. SwissADME analysis further revealed acceptable pharmacokinetic and drug-likeness characteristics, particularly for quercetin and kaempferol. Collectively, these findings support the potential application of Clitoria ternatea flavonoids as natural anti-virulence agents targeting multiple cariogenic pathways. Further molecular dynamics simulations and experimental validation are warranted to confirm their therapeutic efficacy and facilitate the development of novel plant-derived strategies for the prevention and management of dental caries.

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