Jurnal Jamu Indonesia (2025) 10(2): 102–109
https://jamu-journal.ipb.ac.id/index.php/JJI/index

ISSN 2407-7178
eISSN 2407-7763

Research Article
Doi: https://doi.org/10.29244/jji.v10i2.372

Virtual Screening of Flavonoid Compounds as Potential Antibiofilm Agents Targeting Glucosyltransferase

Penapisan Senyawa Flavonoid sebagai Antibiofilm melalui Penambatan Molekuler terhadap Glukosiltransterase

Popi Asri Kurniatin[1]*, Fadhilah Eka Putri[1], Ukhradiya Magharaniq Safira Purwanto[1]

[1]*Department of Biochemistry, Faculty of Mathematics and Natural Sciences, IPB University, Bogor, West Java, 16680, Indonesia*

ARTICLE INFO

Article history
Received on: 2025-02-11
Revised on: 2025-03-03
Accepted on: 2025-05-04

Keyword:

Biofilm
Streptococcus mutans
Glucosyltransferase
Flavonoids
Molecular docking

ABSTRACT

Dental caries are caused by plaque formation resulting from biofilm accumulation on tooth surfaces. The bacterium *Streptococcus mutans* plays a crucial role in biofilm formation, partly through the production of glucosyltransferase, which catalyzes biofilm synthesis. Flavonoids are secondary metabolites commonly found in plants, known for diverse biological properties, including antibiofilm effects. This study aimed to screen the potential of flavonoid compounds as antibiofilm agents through inhibition of glucosyltransferase using an in-silico approach. A total of 87 flavonoid compounds obtained from the ZINC database were evaluated via molecular docking methods. Screening results based on binding free energy (ΔG) values, analyzed using the PyRX-Virtual Screening Tool, indicated that 36 compounds had potential to inhibit glucosyltransferase. Further molecular docking using AutoDock Vina identified nine compounds with ΔG values more favorable than the natural ligand of glucosyltransferase (maltose). Molecular interaction analysis using LigPlot+ and PyMOL revealed that taxifolin, gallocatechin, and sakuranetin interacted with three catalytic residues of the enzyme, whereas the remaining six compounds interacted with two catalytic residues. Liquiritigenin exhibited the lowest ΔG (-7.0 kcal/mol) and an inhibition constant (Ki) of 7.39 μM, indicating high affinity for glucosyltransferase. This compound formed two hydrogen bonds and four hydrophobic interactions, engaging two catalytic residues of the enzyme, Asn481 and Trp517. These findings highlight the potential of flavonoids as antibiofilm agents via glucosyltransferase inhibition. Further experimental validation through in vitro studies is necessary to confirm these in-silico findings.

Kata kunci:

Biofilm
Streptococcus mutans
Glukosiltransferase
Flavonoid
Penambatan molekuler

ABSTRAK

Karies gigi disebabkan oleh pembentukan plak akibat akumulasi biofilm pada permukaan gigi. Bakteri *Streptococcus mutans* berperan penting dalam pembentukan biofilm, antara lain melalui produksi enzim glukosiltransferase yang mengkatalisis sintesis biofilm. Flavonoid merupakan metabolit sekunder yang umum ditemukan pada tumbuhan dan dikenal memiliki berbagai aktivitas biologis, termasuk efek antibiofilm. Penelitian ini bertujuan untuk menyaring potensi senyawa flavonoid sebagai agen antibiofilm melalui penghambatan enzim glukosiltransferase menggunakan pendekatan in-silico. Sebanyak 87 senyawa flavonoid yang diperoleh dari basis data ZINC dievaluasi menggunakan metode penambatan molekuler. Hasil penyaringan berdasarkan nilai energi bebas ikatan (ΔG) yang dianalisis menggunakan PyRX-Virtual Screening Tool menunjukkan bahwa 36 senyawa berpotensi menghambat glukosiltransferase. Penambatan molekuler lanjutan dengan AutoDock Vina mengidentifikasi sembilan senyawa dengan nilai ΔG yang lebih baik dibandingkan ligan alami glukosiltransferase (maltosa). Analisis interaksi molekuler menggunakan LigPlot+ dan PyMOL menunjukkan bahwa taxifolin, gallocatechin, dan sakuranetin berinteraksi dengan tiga residu katalitik enzim, sedangkan enam senyawa lainnya berinteraksi dengan dua residu katalitik. Liquiritigenin menunjukkan nilai ΔG terendah (-7,0 kcal/mol) dan konstanta inhibisi (Ki) sebesar 7,39 μM, yang mengindikasikan afinitas tinggi

terhadap glukosiltransferase. Senyawa ini membentuk dua ikatan hidrogen dan empat interaksi hidrofobik dengan dua residu katalitik enzim, yaitu Asn481 dan Trp517. Temuan ini menunjukkan potensi flavonoid sebagai antibiofilm melalui mekanisme penghambatan glukosiltransferase. Validasi lebih lanjut melalui studi in vitro diperlukan untuk mengonfirmasi hasil in-silico ini.

*Corresponding author:
Popi Asri Kurniatin (popi_asri@apps.ipb.ac.id)

1. INTRODUCTION

Dental caries is a chronic infectious disease affecting the hard tissues of teeth, characterized by the demineralization and subsequent breakdown of organic material (Nelwan, 2016). One of the main causes of dental caries is plaque formation. Dental plaque is an accumulation of microorganisms on the tooth surface in the form of a biofilm. If left uncontrolled, the biofilm can thicken significantly, creating a conducive surface for bacterial colonization and proliferation (Reokistiningsih et al., 2013; Gao et al., 2024). Numerous oral microorganisms contribute to the formation of cariogenic biofilm. Among them, *S. mutans* is particularly linked to the development of dental caries due to its ability to tolerate acidic environments, produce acid, and synthesize glucan, which aids in biofilm formation (Takahashi and Nyvad, 2011).

Streptococcus mutans metabolizes carbohydrates into acids, reducing the pH of the oral cavity and initiating tooth surface demineralization—one of the earliest steps in dental caries development (Reokistiningsih et al., 2013; Nigel and Pitts, 2017). Moreover, *S. mutans* produces the enzyme glucosyltransferase, which converts sucrose into extracellular polysaccharide matrices essential for bacterial adhesion and microbial consortium growth within biofilms (Egi et al., 2018; Kriswandini et al., 2019). Therefore, glucosyltransferase is a critical target for biofilm inhibition (Atta et al., 2024; Gao et al., 2024).

Flavonoids are a group of polyphenolic secondary metabolites abundantly found in plants and recognized for their broad spectrum of biological activities (Towaha, 2014; Panche et al., 2016; Saxena et al., 2013). Several in vitro and in vivo studies have demonstrated the ability of flavonoids to inhibit glucosyltransferase and reduce the progression of dental caries (Amanda et al., 2017; Egi et al., 2018). Notable flavonoids include catechins from tea leaves (*Camellia sinensis*) and apigenin from bee propolis (*Apis mellifera*) (Ren et al., 2016).

Given the abundance of flavonoids in nature, systematic screening for their potential to inhibit glucosyltransferase is crucial for translational applications. Screening flavonoids for glucosyltransferase (Gtf) inhibition has many practical uses. In dentistry, it can help develop toothpaste, mouthwash, and treatments that prevent plaque and cavities. In drug development, it may lead to new anti-cavity medications. The food industry could also use flavonoids in functional foods and drinks to support oral health. Additionally, research on flavonoids may reveal antibacterial properties useful for treating infections. These applications make flavonoid screening valuable for improving oral care and overall health.

An in-silico approach using molecular docking offers a rapid and efficient method for predicting the inhibitory potential of numerous flavonoid compounds. This study aims to screen flavonoid compounds through molecular docking against glucosyltransferase. Flavonoids from various plants available in the ZINC database were used as ligands (Irwin et al., 2012). Virtual screening was conducted using the PyRX-Virtual Screening Tool with binding free energy parameters, followed by molecular docking using AutoDock Vina. Ligand-glucosyltransferase molecular interaction analysis was performed using LigPlot + and PyMOL.

The PyRX-Virtual Screening Tool was chosen for virtual screening due to its user-friendly interface, automation capabilities, and integration with multiple docking algorithms, making it efficient for high-throughput screening. AutoDock Vina was selected for molecular docking because of its improved accuracy in predicting binding affinities, enhanced speed, and flexible docking algorithms compared to other docking software. Both tools have been validated in previous studies for their effectiveness in identifying bioactive compounds with favourable binding profiles, supporting their selection for this study.

2. METHODS

2.1. Tools and Materials

The tools used in this study included a laptop with an Intel® Core™ i3-6100U processor, 8192 MB RAM, and a Windows 10 Pro 64-bit operating system. Software used included AutoDock Vina Tools (The Scripps Research Institute, USA) version 1.5.6 (Trott & Olson, 2010), Discovery Studio Visualizer 2017 Client (BIOVIA, 2016), PyRx (Dallakyan & Olson, 2015), LigPlot + version 2.2 (Laskowski & Swindells, 2011), and PyMOL (DeLano, 2009).

The materials included the three-dimensional structure of glucosyltransferase (PDB code: 3AIB) as the receptor, along with its natural ligand (maltose) and Ca^{2+} ions, obtained from the RCSB Protein Data Bank (Ito et al., 2011). The three-dimensional structures of the reference ligand apigenin (Ren et al., 2016) and 87 flavonoid compounds from the ZINC database (zinc.docking.org) were used as test ligands (Irwin et.al, 2012).

2.2. Receptor Preparation

The three-dimensional structure of the glucosyltransferase enzyme (PDB ID: 3AIB) was obtained from the RCSB Protein Data Bank (http://www.rcsb.org) in *.pdb format. The downloaded receptor structure was prepared using Discovery Studio 2016 Client and

AutoDock Vina Tools version 1.5.6. Receptor preparation in Discovery Studio involved removing non-relevant chains (A, B, C, D, E, F, H), retaining only chain G, which contains the native ligand (maltose) along with the Ca^{2+} metal ion. The Ca^{2+} ion was retained as it is coordinated with the enzyme. Subsequently, water molecules, non-essential residues, and the native ligand were removed from chain G. The receptor structure in *.pdbqt format was further refined by adding polar hydrogen atoms and Gasteiger charges using AutoDock Vina version 1.5.6 (Modified from Nosrati et al., 2018).

2.3. Ligand Preparation

The three-dimensional structures of 87 test ligands and the reference ligand (apigenin) were retrieved from the ZINC database (http://www.zinc.docking.org) in *.sdf format. These structures were converted to *.pdb format using Discovery Studio Client 2016. Ligand preparation involved adding polar hydrogens and defining torsional flexibility using AutoDock Vina (Modified from Nosrati et al., 2018).

2.4. Molecular Docking Validation

Molecular docking validation was performed using AutoDock 4 with the Lamarckian genetic algorithm. Ligand conformations were clustered based on a Root Mean Square Deviation (RMSD) threshold of ≤ 2 Å. Validation was conducted by redocking the native ligand (maltose) to the 3AIB receptor (chain G) with the Ca^{2+} ion. Docking validation was carried out using four different grid box sizes, with specified spacing and center coordinates, and the results were saved in *.txt format. The grid box parameters were set to 10 Å × 5 Å × 6 Å with a center point of x = 178.845, y = –19.166, z = 159.222, and a spacing of 0.375 Å. Twenty redocking runs were performed using these parameters, and results were evaluated based on the RMSD threshold (Modified from Nosrati et al., 2018, and Ravindranath et al., 2015).

2.5. Virtual Screening

Virtual screening was performed using the prepared receptor, the native ligand, and the reference ligand (apigenin). The ligands were saved in *.pdb format. A total of 87 test ligands were screened based on their binding free energy using the PyRx-Virtual Screening Tool. The program was executed using a grid box centered at x = 178.6896, y = –19.0833, z = 159.0276, with dimensions of 19 Å × 9 Å × 11 Å and spacing of 1.0 Å (Modified from Dallakyan & Olson, 2015).

2.6. Molecular Docking

Molecular docking of the test ligands and the reference ligand with glucosyltransferase was performed using AutoDock Vina version 1.5.6. The docking technique employed was “targeted docking,” focusing on the maltose binding site, using the validated grid box center and dimensions. Docking accuracy (exhaustiveness) was set to 8, and the number of docking modes (num_modes) was set to 20. The Vina folder was placed in C:/Vina, containing a configuration file (config.txt) with the specified parameters. Docking was executed via the command line using: vina –config config.txt –log log.txt. The docking produced output files in *.pdbqt format, which were visualized in Discovery Studio, and a log.txt file containing binding free energy values. These files were stored for subsequent energy evaluation and interaction analysis (Modified from Nosrati et al., 2018, and Ravindranath et al., 2015).

2.7. Molecular Interaction Analysis

Ligand-receptor binding interactions were visualized in two dimensions using LigPlot+ version 2.2 and in three dimensions using PyMOL. The docking result files in *.pdbqt format were imported into these tools for interaction analysis (Modified from Nosrati et al., 2018).

3. RESULTS AND DISCUSSION

3.1. Molecular Docking Validation

Ligand-macromolecule interaction prediction can be performed using molecular docking, as it enables high-accuracy predictions of ligand conformations that fit precisely within the target binding site (Nur et al., 2023). Molecular docking methods require validation by redocking the native ligand into the receptor’s active site. In this study, the receptor used was glucosyltransferase (PDB ID: 3AIB), consisting of 844 amino acids with a resolution of 3.09 Å. The structure forms a complex with the native ligand maltose, and it includes two unique ligands: MES (2-(N-Morpholino)-ethanesulfonic acid) and a calcium ion (Ca^{2+}). The calcium ion binding site is located near the +1 subsite at the interface of domains A and B (Ito et al., 2011).

The validation was conducted using a grid box with dimensions 10 Å × 5 Å × 6 Å, and a center at x = 178.845, y = -19.166, z = 159.222. A grid spacing of 0.375 Å was applied, and 20 docking repetitions were performed, yielding stable RMSD (Root Mean Square Deviation) values across all iterations. The chosen grid box dimensions were selected to accurately encompass the binding site while allowing sufficient space for ligand flexibility and movement, ensuring accurate docking predictions without unnecessary computational burden. The grid center coordinates were set based on the known active site location, optimizing interactions between the ligand and target.

The 20 repetitions confirmed pose stability, as indicated by consistent RMSD values. The 18th iteration produced the lowest RMSD value (1.4781 Å) (**Table 1**), reflecting the highest consistency with the reference structure. This ensures that the most reliable pose is used for further analysis. While averaging multiple iterations could broaden insight, selecting the iteration with the lowest RMSD prioritizes accuracy.

RMSD measures the positional deviation between two ligand poses by comparing atomic coordinates between the experimental and docked conformations. “Molecular docking validation by re-docking co-crystallized ligands is considered successful if the RMSD value is ≤ 2.0 Å,” indicating a valid docking method (Agistia et al., 2013). As shown in Figure 1, the two poses from redocking closely resemble the native structure. The low RMSD

indicates a high-fidelity prediction, approximating the native ligand (Susanti et al., 2018).

Table 1. Molecular docking validation

Docking repetitions	RMSD (Å)	Docking repetitions	RMSD (Å)
1	1.5411	11	1.5137
2	1.5707	12	1.4862
3	1.5205	13	1.5462
4	1.5705	14	1.5203
5	1.5544	15	1.5239
6	1.5410	16	1.5260
7	1.5947	17	1.5755
8	1.5460	18	1.4781
9	1.5401	19	1.5117
10	1.5434	20	1.5300

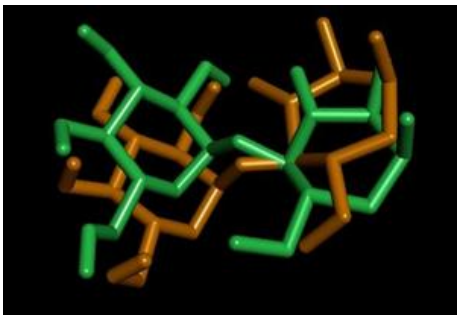

Figure 1. Visualization of native ligand-receptor redocking. Native conformation (orange), redocking conformation (green).

3.2. Virtual Screening

Virtual screening using the PyRx-Virtual Screening Tool on 87 flavonoid compounds identified 36 compounds that exhibited binding affinity to glucosyltransferase with lower ΔG (binding free energy) than the native ligand maltose (**Table 2**). Subsequent molecular docking of these 36 compounds using AutoDock Vina revealed that 9 compounds had lower ΔG than maltose (**Table 3**).

Further docking confirmed these 9 compounds exhibited lower ΔG values, with inhibition constants (Ki) ranging from 5.2 to 28.53 μM (**Table 3**). ΔG reflects the Gibbs free energy needed for ligand-receptor interaction (Kesuma et al., 2018). A negative ΔG indicates spontaneous binding, while a positive value reflects a non-spontaneous interaction (Amelia, 2014).

More negative ΔG values indicate stronger ligand-protein interactions, suggesting better inhibitory potency. The magnitude of ΔG correlates with the stability of the complex (Du et al., 2016). Stronger binding (more negative ΔG) implies greater inhibition (Hasan et.al, 2024). According to Zheng and Polli (2010), compounds with Ki < 100 μM are considered strong inhibitors. **Table 3** shows that all tested compounds, including apigenin and the 9 lead candidates, meet this criterion. Since Ki is directly proportional to ΔG, lower ΔG corresponds to stronger inhibition (Pratama, 2016).

Table 2. Virtual screening flavonoids by PyRX-*Virtual Screening*

Compounds	ΔG (kcal/mol)	Compounds	ΔG (kcal/mol)
Maltose (native ligand)	-5.5	Taxifolin	-7
Phloretin	-6.3	Hispidol	-7
Isosalipurposide	-6.5	Tectirogenin	-7
Daidzein	-6.5	Meletin	-7.1
Fisetinidin	-6.5	Aureusidin	-7.2
Fustin	-6.5	Hirsutrin	-7.2
Gallocatechin	-6.6	Naringenin	-7.2
Formononetin	-6.6	Liquiritigenin	-7.3
Sakuranetin	-6.6	Cyanidin	-7.3
Pelargonidin	-6.7	Cyanidin 3-Galactoside	-7.4
Akasetin	-6.7	Chrysin	-7.4
Leptosin	-6.7	Daidzin	-7.5
Biochanin A	-6.8	Scutellarein	-7.6
Butein	-6,8	Sulfuretin	-7.6
Herbacetin	-6,8	Apigenin (reference ligand)	-7.7
Guaiaverin	-6.9	Apigetrin	-7.9
Baicalein	-7	Glycitin	-7.9
Eriodictyol	-7	Fragarin	-7.9
Galangin	-7	Neodiosmin	-8.3

Table 3. Gibbs free energy and inhibition constant obtained from molecular docking

Compounds	ΔG (kcal/mol)	Ki (μM)
Apigenin (reference ligand)	-7.2	5.2
Liquiritigenin	-7	7.39
Pelargonidin	-6.9	8.75
Cyanidin	-6.8	10.36
Hirsutrin	-6.7	12.27
Taxifolin	-6.6	14.52
Galangin	-6.5	17.19
Fisetinidin	-6.5	17.19
Gallokatekin	-6.2	28.53
Sakuranetin	-6.2	28.53

3.3. Ligand-Receptor Molecular Interaction

Ligand-receptor interaction analysis was conducted by visualizing the molecular docking results in two dimensions (2D) using LigPlot+ and three dimensions (3D) using PyMOL. The two types of visualizations provide valuable and complementary insights into ligand-receptor interactions. 2D visualization using LigPlot+ simplifies interactions by displaying hydrogen bonds and hydrophobic contacts in a schematic format, making it easier to identify key interacting residues. 3D visualization using PyMOL offers a spatial representation of the ligand within the binding pocket, illustrating the molecular orientation and interaction depth.

The Binding Site Similarity (BSS) between the test ligands and the native ligand maltose was either 80% or 60% (**Table 4**). Seven ligands (fisetinidin, gallocatechin, liquiritigenin, cyanidin, taxifolin, and sakuranetin) exhibited 80% BSS, while two ligands

(galangin and pelargonidin) exhibited 60% BSS. A BSS percentage above 50% indicates that all test ligands can bind at the enzyme's active site. BSS is a parameter used to assess the similarity of amino acid residues between the native ligand, reference ligand, and test ligands (Fakhruri et al., 2021). A binding site is the region where a protein interacts with molecules and ligands, involving key amino acid residues essential for ligand binding. These interactions result in a stable ligand-receptor conformation, which in turn influences the structure and function of the receptor (Arwansyah & Hasrianti, 2014).

Table 4. Molecular interaction of ligand-receptors

Compounds	Hydrogen interaction		Hydrophobic interaction	% BSS
	Residues on receptor	Molecules in the ligand and bond length	Residues on receptor	
Maltose (native ligand)	**Trp517**	3.07	Asp480, **Asn481, Glu515**, Arg540	
Apigenin (Ligan pembanding)	**Asn481**	O_3-OD_1 (2.84)	Gly429, Tyr430, Asp480, Ser509, **Trp517**	80%
	Arg540	O_6-NB_1 (3.30)		
Liquiritigenin	**Asn481**	O_4-OD_1 (2.82)	Gly429, Tyr430, Asp480, **Trp517**	80%
	Arg540	O_3-NB_1 (3.23)		
Pelargonidin	Asp480	O_3-NB_2 (3.29)	Tyr430, Ala478, **Asn481**, **Trp517**, Ser589	60%
	Asn481	O_{11}-N (3.17) O_{15}-OD_1 (2.52)		
Cyanidin	Arg540	O_{17}-OD_1 (3.35) O_{17}-ND_2 (3.21) O_5-NH_2 (3.21)	Tyr430, Leu433, Asp480, **Trp517**,Ser589, Asp593	80%
Hirsutrin	**Asn481**	O_{23}-ND_2 (2.81)	Gly429, Tyr430, Asp480, **Trp517**, Arg540, Ser589	80%
	Tyr430	O_1-OH (3.21)		
Taxifolin	**Asn481**	O_5-O_{D1} (3.09)	Ala478, **Glu515**, **Trp517**, Ser589	80%
	Arg540	O_{19}-NH_2 (3.34)		
Galangin	**Asn481**	O_{19}-ND_2 (2.80)	Tyr430, Arg540, **Trp517**, Ser589, *Asp593*	60%
Gallocatechin	Tyr430	O_{18}-O_8 (2.92)	Ala478, **Asn481**, **Glu515**, **Trp517**, Arg540, Ser589	80%
Fisetinidin	Asn481	O_{16}-ND_2 (3.03) O_{16}-OD_2 (2.85) O_{14}-OD_2 (3.25)	Gly429, Tyr430, Leu433, Asp480, Ser509, **Trp517**	80%
	Arg540	O_1-NH_1 (3.21) O_1-NH_2 (3.30)		
Sakuranetin	**Asn481**	O_2-ND_2 (2.96)	Ala478, Asp480, **Glu515**, **Trp517**, *Asp593*	80%

Note: Catalytic residues (bold), catalytic domain (italics)

All ligands formed between one and three hydrogen bonds with the receptor (**Table 4**), with bond lengths greater than 2.0 Å, classifying them as weak hydrogen bonds. Literature suggests that hydrogen interactions between ligands and receptors can impact compound activity, as the distance between specific ligand atoms influences binding energy strength (Ruslin et al., 2020). Weak hydrogen bonds such as C–H...O typically have H...O distances greater than 1.85 Å and may be replaced by other interaction types depending on conditions at the binding interface. A shorter hydrogen bond distance between the ligand and key amino acid

residues in the receptor enhances binding strength (Patil et al., 2010).

Hydrophobic interaction analysis revealed 3–4 hydrophobic interactions per ligand (**Table 4**). The receptor residues involved were Asp480, Asn481, Glu515, Trp517, and Arg540, which also interacted with maltose. This indicates that the test ligands may occupy the same active site as maltose. Interacting residues may undergo conformational shifts in hydrophobic interaction patterns depending on the ligand. Though weak, hydrophobic interactions stabilize the ligand conformation within the protein. They can enhance ligand-receptor binding affinity and biological activity (Fitriana et al., 2018).

ΔG (binding free energy) is influenced by interactions between receptor and ligand. Liquiritigenin (–7.0 kcal/mol) formed two hydrogen bonds and four hydrophobic interactions, while pelargonidin (–6.9 kcal/mol) formed one hydrogen bond and five hydrophobic interactions. According to Kartasasmita et al. (2009), the number of hydrogen bonds does not solely determine ΔG, which is also influenced by van der Waals forces, bond rotation, and other structural features.

Hydrogen bonds occur between receptor and ligand atoms with high electronegativity, such as fluorine (F), nitrogen (N), or oxygen (O), whereas hydrophobic interactions occur between nonpolar residues and ligand atoms (Arwansyah & Hasrianti, 2014).

All ligands (**Table 4**) formed fewer than three hydrogen bonds, consistent with Malau & Azzahra (2020), who reported that hydrogen bonding minimally contributes to overall molecular stability due to its lower energy output. However, a greater number of hydrogen bonds can enhance molecular stability by providing additional binding energy.

3.4. Interaction of Test Ligands with Catalytic Residues

Glucosyltransferase (the receptor in this study) has three key catalytic residues: Asn481, Glu515, and Trp517 (Ito et al., 2011). According to **Table 4**, the native ligand maltose interacts with all three catalytic residues, along with Asp480 and Arg540. Among the test ligands, taxifolin, gallocatechin, and sakuranetin interacted with all three catalytic residues, while the remaining test ligands interacted with only two (Asn481 and Trp517). Simultaneous engagement of all three catalytic residues may enhance binding stability and inhibitory potential, as multiple interactions typically contribute to stronger and more specific ligand-receptor affinity. However, the actual inhibitory strength also depends on factors such as binding free energy (ΔG), hydrogen bond strength, and the extent of hydrophobic interactions (Chen et al., 2016; Patil et al., 2010).

These three catalytic residues play a crucial role in glucosyltransferase (GtfC) inhibition and are key targets for competitive inhibitors. This is supported by findings from Ito et al. (2011), which indicate that Trp517 provides a platform for the glycosyl acceptor, Glu515 functions as a general acid/base catalyst, and Asn481 participates in calcium ion (Ca^{2+}) coordination, contributing to hydrogen bond formation. In the GtfC–maltose complex, maltose competes at the active site, acting as a competitive inhibitor of the enzyme.

Additionally, cyanidin, galangin, and sakuranetin interacted not only with catalytic residues but also with Asp593, located in the catalytic domain. These ligands bind to residues that are critical for glucosyltransferase inhibition. This observation is consistent with Ito et al. (2011), who reported that glucosyltransferase enzymes (GtfC, GtfB, and GtfD) share more than 40% sequence similarity, with Asp593 and Glu431 identified as key residues involved in determining glucan production specificity. Asp593 in GtfC is essential for orienting the acceptor sugar, thereby influencing the type of glucan produced by GtfB, GtfC, and GtfD. Glu431, located opposite the active center, plays a role in metal ion coordination and influences the enzyme's transglycosylation specificity, determining whether the enzyme produces insoluble glucans (α-1,3 linkages) or soluble glucans (α-1,6 linkages).

Asp593 is positioned within an additional helix in domain A. According to Shimamura et al. (1994), replacing the threonine residue at the corresponding position in GtfD with either Asp or Glu promotes the synthesis of soluble glucans over insoluble glucan formation.

3.5. Liquiritigenin: The Promising Inhibitor

Among the test ligands, liquiritigenin exhibited the lowest binding free energy (ΔG) of –7.0 kcal/mol. Although its ΔG was lower than that of maltose, it was slightly higher than that of the reference ligand, apigenin. Apigenin is a flavonoid compound found in *Apis mellifera* propolis (beehive resin), with in vivo studies demonstrating its ability to inhibit *S. mutans* growth, suppress glucosyltransferase activity, reduce biofilm viability, and prevent dental caries development in rats (Ren et al., 2016).

The inhibitory strength of a ligand is influenced not only by its binding free energy (ΔG), but also by the nature and number of interactions it forms, such as hydrogen bonds and hydrophobic contacts (Meng et al., 2011). In this study, liquiritigenin formed two hydrogen bonds with glucosyltransferase at Asn481 and Arg540, similar to apigenin. Liquiritigenin formed four hydrogen bonds overall, whereas apigenin formed five. The slightly lower number of hydrogen bonds in liquiritigenin may account for its comparatively higher ΔG.

However, liquiritigenin interacts with critical catalytic residues—Asn481 and Trp517—which are essential for Ca^{2+} binding and stabilization at the +1 subsite. These interactions suggest that liquiritigenin, despite forming fewer hydrogen bonds, maintains strong inhibitory potential through targeted engagement with the active site.

CONCLUSION

Virtual screening of 87 flavonoid compounds from the database through molecular docking against glucosyltransferase identified nine flavonoids with ΔG values lower than that of the native

ligand, maltose, ranging from –6.2 to –7.0 kcal/mol. These nine flavonoids interacted with key residues of the receptor involved in enzymatic activity. The most promising compound was liquiritigenin, which exhibited the lowest ΔG (–7.0 kcal/mol), an inhibition constant (Ki) of 7.39 μM, formed two hydrogen bonds and four hydrophobic interactions, and engaged two catalytic residues.

This study demonstrates the potential of flavonoid compounds as glucosyltransferase inhibitors, which could be further developed as antibiofilm agents. Further studies involving a broader library of compounds and molecular dynamics simulations may uncover additional flavonoids with enhanced inhibitory potential. Additionally, in vitro and in vivo experiments are essential to validate their inhibitory activity and assess their suitability for clinical or preventive applications.

REFERENCES

Agistia, D. D., Tegar, M., & Nugroho, A. E. (2013). Interaction between active compounds from *Aegle marmelos* Correa as anti-inflammatory agent with COX-1 and COX-2 receptor. *Traditional Medicine Journal, 18*(2), 80–87.

Amanda, Kunarti, S., & Subiwahyudi, A. (2017). Daya hambat aktivitas enzim glukosiltransferase (*Gtf*) *Streptococcus mutans* oleh ekstrak temulawak (*Curcuma xanthorrhiza* Roxb.). *Conservative Dentistry Journal, 7*(1), 32–36.

Amelia, F. (2014). Studi interaksi ligan peptinoid dan peptida dengan enzim protease NS3/NS2B virus dengue. *Jurnal Saintek, 1*(1), 24–29.

Arwansyah, & Hasrianti. (2014). Simulasi molecular docking senyawa kurkumin dan analognya sebagai selective androgen receptor modulators (SARMs) pada kanker prostat. *Jurnal Dinamika, 5*(2), 60–75.

Atta, L., Mushtaq, M., Siddiqui, A. R., Khalid, A., & Ul-Haq, Z. (2024). Targeting glucosyltransferases to combat dental caries: Current perspectives and future prospects. *International Journal of Biological Macromolecules, 278*(Pt 2), 134645. https://doi.org/10.1016/j.ijbiomac.2024.134645

Chen, D., Oezguen, N., Urvil, P., Ferguson, C., Dann, S. M., & Savidge, T. C. (2016). Regulation of protein–ligand binding affinity by hydrogen bond pairing. *Science Advances, 2*(3), e1501240. https://doi.org/10.1126/sciadv.1501240

Dallakyan, S., & Olson, A. J. (2015). Small-molecule library screening by docking with PyRx. In H. B. & J. W. (Eds.), *Methods in molecular biology* (Vol. 1263, pp. 243–250). Humana Press. https://doi.org/10.1007/978-1-4939-2269-7_19

DeLano, W. L. (2009). PyMOL: An open-source molecular graphics tool. DeLano Scientific.

Du, X., Li, Y., Xia, Y. L., Ai, S. M., Liang, J., Sang, P., Ji, X. L., & Liu, S. Q. (2016). Insights into protein–ligand interactions: Mechanisms, models, and methods. *International Journal of Molecular Sciences, 17*(2), 144. https://doi.org/10.3390/ijms17020144

Egi, M., Soegiharto, G. S., & Evacuasiany, E. (2018). Efek berkumur sari buah tomat (*Solanum lycopersicum* L.) terhadap indeks plak gigi. *SONDE, 3*(2), 70–85. https://doi.org/10.28932/sod.v3i2.1784

Fakhruri, M., Rahmayanti, Y., & Isfanda. (2021). Potensi fitokimia *Citrus aurantium* (hesperetin, naringenin) dalam menghambat xantin oksidase pada hiperurisemia secara in-silico. *Jurnal Health Sains, 2*(1), 79–89.

Fitriana, N., Lestari, S. R., & Lukiati, B. (2018). Senyawa alami bawang putih tunggal sebagai inhibitor LpxC bakteri *Pseudomonas aeruginosa* melalui virtual screening. *Jurnal Kedokteran dan Kesehatan, 18*(1), 25–33.

Gao, Z., Chen, X., Wang, C., Song, J., Xu, J., Liu, X., Qian, Y., & Suo, H. (2024). New strategies and mechanisms for targeting *Streptococcus mutans* biofilm formation to prevent dental caries: A review. *Microbiological Research, 278*, 127526. https://doi.org/10.1016/j.micres.2023.127526

Hassan, A. M., Gattan, H. S., Faizo, A. A., Alruhaili, M. H., Alharbi, A. S., Bajrai, L. H., Al-Zahrani, I. A., Dwivedi, V. D., & Azhar, E. I. (2024). Evaluating the binding potential and stability of drug-like compounds with the monkeypox virus VP39 protein using molecular dynamics simulations and free energy analysis. *Pharmaceuticals, 17*(12), 1617. https://doi.org/10.3390/ph17121617

Irwin, J. J., Sterling, T., Mysinger, M. M., Bolstad, E. S., & Coleman, R. G. (2012). ZINC: A free tool to discover chemistry for biology. *Journal of Chemical Information and Modeling, 52*(7), 1757–1768. https://doi.org/10.1021/ci3001277

Ito, K., Ito, S., Shimamura, T., Weyand, S., Kawarasaki, Y., Misaka, T., Abe, K., Kobayashi, T., Cameron, A. D., & Iwata, S. (2011). Crystal structure of glucansucrase from the dental caries pathogen *Streptococcus mutans*. *Journal of Molecular Biology, 408*, 177–186.

Kartasasmita, R. E., Herowati, R., Harmastuti, N., & Gusdinar, T. (2009). Quercetin derivatives docking based on study of flavonoids interaction to cyclooxygenase-2. *Indonesian Journal of Chemistry, 9*(2), 297–302.

Kesuma, D., Siswadon, Purwanto, B. T., & Hardjon, S. (2018). Uji in-silico aktivitas sitotoksik dan toksisitas senyawa turunan N-(benzoil)-N'-feniltiourea sebagai calon antikanker. *Journal of Pharmaceutical Science and Clinical Research, 3*(1), 1–11.

Kriswandini, I. L., Diyatri, I., & Putri, I. A. (2019). Density of *Streptococcus mutans* biofilm protein induced by glucose, lactose, soy protein and iron. *Dental Journal, 52*(2), 86–89.

Laskowski, R. A., & Swindells, M. B. (2011). LigPlot+: Multiple ligand–protein interaction diagrams for drug discovery. *Journal of Chemical Information and Modeling, 51*(10), 2778–2786. https://doi.org/10.1021/ci200227u

Malau, N. D., & Azzahra, S. F. (2020). Molecular docking studies of potential quercetin 3,4'-dimethyl ether 7-alpha-L-arabinofuranosyl-(1-6)-glucoside as inhibitor antimalarial. *Journal of Physics: Conference Series, 1428*, 012057. https://doi.org/10.1088/1742-6596/1428/1/012057

Meng, X. Y., Zhang, H. X., Mezei, M., & Cui, M. (2011). Molecular docking: A powerful approach for structure-based drug discovery. Current Computer-Aided Drug Design, 7(2), 146–157. https://doi.org/10.2174/157340911795677602

Nelwan, J. J. (2016). Faktor-faktor yang mempengaruhi tingginya kasus karies gigi pada anak panti asuhan Yataama Al-Firdaus di wilayah kerja Puskesmas Ngesrep tahun 2011. *Jurnal Kesehatan Gigi, 3*(2), 106–113.

Nigel, B., & Pitts, D. T. Z. P. (2017). Dental caries. *Nature Reviews Disease Primers, 3*, 17031. https://doi.org/10.1038/nrdp.2017.31

Nur, S., Setiawan, H., Hanafi, M., & Elya, B. (2023). Phytochemical composition, antioxidant, in vitro and in-silico studies of active compounds of *Curculigo latifolia* extracts as promising elastase inhibitor. Saudi Journal of Biological Sciences, 30(8), 103716. https://doi.org/10.1016/j.sjbs.2023.103716

Nosrati, M., Behbahani, M., Mohabatkar, H., & Shakeran, Z. (2018). Antibacterial and antibiofilm activities of *Prangos acaulis* Bornm. extract against *Streptococcus mutans*: An in-silico and in vitro study. *Journal of Herbmed Pharmacology, 7*(3), 176–184.

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: An overview. *Journal of Nutritional Science, 5*, e47. https://doi.org/10.1017/jns.2016.41

Patil, R., Das, S., Stanley, A., Yadav, L., Sudhakar, A., & Varma, A. K. (2010). Optimizing hydrophobic interactions and hydrogen bonding at the target–ligand interface leads the pathways of drug-designing. *PLOS ONE, 5*(8), e12029. https://doi.org/10.1371/journal.pone.0012029

Pratama, M. R. F. (2016). Studi docking molekular senyawa turunan kuinolin terhadap reseptor estrogen-α. *Jurnal Surya Medika, 2*(1), 1–7.

Ravindranath, P. A., Forli, S., Goodsell, D. S., Olson, A. J., & Sanner, M. F. (2015). AutoDockFR: Advances in protein–ligand docking with explicitly specified binding site flexibility. *PLOS Computational Biology, 11*(12), e1004586. https://doi.org/10.1371/journal.pcbi.1004586

Ren, Z., Chen, L., Li, J., & Li, Y. (2016). Inhibition of *Streptococcus mutans* polysaccharide synthesis by molecules targeting glycosyltransferase activity. *Journal of Oral Microbiology, 8*, 31095. https://doi.org/10.3402/jom.v8.31095

Reokistiningsih, H., Hapsari, D. N., & Almira, H. (2013). Efek ekstrak daun mahkota dewa (*Phaleria macrocarpa*) sebagai penghambat pembentukan biofilm pada *Streptococcus mutans* secara in vitro. *Prodenta Journal of Dentistry, 1*(1), 1–11.

Rollando, R. (2017). Isolasi, identifikasi, karakterisasi, dan uji antibiofilm derivat asam galat dari batang *Sterculia quadrifida* R. Br. *Jurnal Kefarmasian Indonesia, 7*(2), 105–111. https://doi.org/10.22435/jki.v7i2.6433105-111

Ruslin, Y., Yana, N. R. A., & Leorita, M. (2020). Desain turunan senyawa leonurine sebagai kandidat obat antiinflamasi. *Jurnal Farmasi Galenika, 6*(1), 181–191. https://doi.org/10.22487/j24428744.2020.v6.i1.15025

Saxena, M., Saxena, J., Nema, R., Singh, D., & Gupta, A. (2013). Phytochemistry of medicinal plants. *Journal of Pharmacognosy and Phytochemistry, 1*(6), 168–182.

Shimamura, A., Nakano, Y. J., Mukasa, H., & Kuramitsu, H. K. (1994). Identification of amino acid residues in *Streptococcus mutans* glucosyltransferases influencing the structure of the glucan product. *Journal of Bacteriology, 176*(16), 4845–4850.

Susanti, N. M. P., Saputa, D. P. D., Hendrayati, P. L., Parahyangan, L. P. D. N., & Swandari, L. A. D. G. (2018). Molecular docking sianidin dan peonidin sebagai antiinflamasi pada aterosklerosis secara in-silico. *Jurnal Farmasi Udayana, 7*(1), 28–33.

Takahashi, N., & Nyvad, B. (2011). The role of bacteria in the caries process. *Journal of Dental Research, 90*(3), 294–303. https://doi.org/10.1177/0022034510379602

Towaha, J. (2014). Kandungan senyawa polifenol pada biji kakao dan kontribusinya terhadap kesehatan. *Sirinov, 2*(1), 1–16.

Trott, O., & Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry, 31*(2), 455–461.

Zheng, X., & Polli, J. E. (2010). Identification of inhibitor concentrations to efficiently screen and measure inhibition Ki values against solute carrier transporters. *European Journal of Pharmaceutical Sciences, 41*(1), 43–52.

Citation format:

Kurniatin, P. A., Putri, F. E., & Purwanto, U. M. S. (2025). Virtual Screening of Flavonoid Compounds as Potential Antibiofilm Agents Targeting Glucosyltransferase, *10*(2), 102–109. https://doi.org/10.29244/jji.v10i2.372