Biointerface Research in Applied Chemistry
Open-Access Journal (ISSN: 2069-5837)

Article
Volume 16, Issue 1, 2026, 32
https://doi.org/10.33263/BRIAC161.032

Molecular Docking, QSAR, and Bioactivity Prediction of *Uncaria gambir* Flavonoids as Antibacterial Agents Targeting MurA Enzyme

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Received: 10.07.2025; Accepted: 15.11.2025; Published: 15.02.2026

Abstract: Antimicrobial resistance is a critical global health threat due to the declining effectiveness of existing treatments. The overuse and misuse of antibiotics have accelerated this crisis, rendering many antimicrobial drugs ineffective and leaving previously treatable infections potentially life-threatening. This study employs Quantitative Structure-Activity Relationship (QSAR) modelling as a computational approach to predict the biological activity of novel compounds. QSAR has gained significant recognition in medicinal chemistry and drug discovery due to its ability to establish mathematical correlations between molecular properties and pharmacological effects. The optimal QSAR model was selected via multilinear regression, ensuring statistical robustness. The final model incorporates three key molecular descriptors: electronic, hydrophobic, and steric parameters, which provide a framework for evaluating and designing new antimicrobial agents with improved efficacy. The best QSAR model obtained is $LogIC_{50}$ = (2.731) - (0.084 x AM1_dipole) + (0.005 x ASA_H) - (0.651 x LogP) - (1.096 x LogS) + (2.863 x mr) - (0.093 x vol). QSAR model predictions, molecular docking, and bioactivity predictions suggest that the novel compound's design can be recommended as an antibacterial, as reflected in lower IC50 values compared to fosfomycin or the original compounds. The integrated computational approach successfully established a predictive QSAR model and identified new inhibitors targeting the MurA enzyme with enhanced efficacy.

Keywords: catechin; antibacterial resistance; QSAR; *Uncharia gambir*.

1. Introduction

The ongoing rise in antibiotic resistance among bacterial pathogens necessitates the search for innovative and effective antibacterial agents. Natural products, particularly plant-derived compounds, have attracted significant attention for their diverse biological activities. Epigallocatechin (EGC), Epicatechin Gallate (ECG), and Epicatechin (EC), found abundantly in green tea and other plants, have demonstrated notable antibacterial properties. Molecular docking has emerged as a key computational tool for predicting interactions between biomolecules and ligands. It simulates the binding of small molecules to a target enzyme's active site and evaluates binding affinity using energy calculations and interaction patterns. Numerous studies have utilized molecular docking to assess the binding capabilities of various flavonoids and phenolic compounds to specific target proteins. For instance, the molecular

docking studies of catechins to viral proteases indicate strong binding affinities, positioning them as potential therapeutic agents against SARS-CoV-2 [1, 2]. Similar approaches could be adapted to assess the binding of EGC, ECG, and EC against the MurA enzyme. These phenolic compounds exhibit a wide spectrum of biological properties, including antioxidant, anti-inflammatory, and antibacterial activities, which render them crucial in the fight against various pathogens [3–5].

Although existing docking reports on Uncaria gambir catechins are useful for identifying putative activity, they mostly depict interactions of naturally occurring compounds. Our study attempted to focus on the nuanced rational design of new derivative compounds. Modifying chemical structures to design novel compounds derived from epigallocatechin, epicatechin gallate, and epicatechin from *Uncaria gambir* leaves is a promising strategy for developing effective antibacterial agents targeting the MurA enzyme. MurA is an essential enzyme involved in the biosynthesis of peptidoglycan, a critical component of bacterial cell walls. It represents a prime antimicrobial target due to its unique structure and role in bacterial physiology [6–8].

Recent studies have illuminated the mechanisms by which catechins exert their antibacterial effects. Research indicates that catechins can inactivate key bacterial enzymes, disrupt cell membranes, and form complexes with cell wall components, leading to bacterial lysis and inhibition of cell wall synthesis [9–11]. For instance, ECG and EC have shown efficacy against numerous Gram-positive and Gram-negative bacteria, which highlights their potential as therapeutic agents for treating infections caused by resistant microbial strains [5, 12]. Moreover, their ability to function synergistically with conventional antibiotics further enhances their appeal as potential adjunct therapies to combat multidrug-resistant infections, such as those caused by *Staphylococcus* aureus and *Escherichia coli* [10, 13].

The MurA enzyme, a crucial component in the bacterial cell wall biosynthesis pathway, has garnered attention as a target for antibacterial drug design. This enzyme's role in peptidoglycan synthesis makes it a strategic target for inhibiting bacterial growth and survival, particularly in pathogenic strains that are resistant to conventional antibiotics [3, 14]. The potential of catechins to inhibit MurA presents an innovative avenue for developing novel antibacterial agents. Recent molecular docking studies have shown that EGC, ECG, and EC can bind effectively to the active site of MurA, suggesting that these compounds hold promise as inhibitors [15, 16].

In silico technologies, particularly molecular quantum and quantitative structure–activity relationship (QSAR) modeling, play vital roles in the discovery and development of new antibacterial agents. Molecular docking allows researchers to predict ligand-enzyme interactions and assess binding affinities, providing insights into the most promising candidates for further exploration [16, 17]. Based on these preliminary docking findings, our study used them as a basic guideline to direct systematic chemical optimization of the catechin scaffold. Likewise, QSAR modeling can help elucidate the relationship between catechin chemical structure and biological activity, thereby aiding the optimization of lead compounds for enhanced efficacy [18]. Unlike previous QSAR studies on catechins, which often correlate structure with general antibacterial activity, our QSAR model will be specifically designed based on MurA inhibition data, enabling us to identify the most crucial in-target structural features. This integrated *in silico* strategy anticipates a predictive and iterative design process that goes beyond the prescriptive nature of previous reports. Together, these computational

approaches streamline the drug development process by enabling the identification of innovative therapeutic candidates derived from natural sources.

The systematic structural modifications of epigallocatechin, epicatechin gallate, and epicatechin are poised to unlock new avenues for antimicrobial agents targeting the MurA enzyme. Through the strategic use of in silico modeling, rigorous screening of antibacterial activity, and consideration of pharmacokinetic properties, the refinement of these natural products into viable therapeutic agents against resistant bacterial strains is not only ambitious but crucial in the field of pharmacology. However, several previous studies have reported the antibacterial activity of catechin derivatives from Uncaria gambir, and this study reinforces and extends these findings by developing a new, more accurate QSAR model and performing RMSD-validated docking analysis. The main novelty lies in integrating an optimized quantitative QSAR model with validated molecular docking simulations, and in predicting bioactivity and toxicity using an integrated in silico approach. This approach is expected to provide a deeper understanding of the structure–activity relationship and improve the accuracy of predicting antibacterial activity against the MurA enzyme.

2. Materials and Methods

This research has been conducted through QSAR analysis and molecular docking simulations as a form of computational drug discovery, using a computer with the following specifications: AMD Ryzen 5, 24 GB RAM, NVIDIA GeForce GTX 1050 graphics card, and Radeon Vega 8 Graphics, with Windows 10 Professional 64-bit as the primary operating system. The software used is Molecular Operating Environment (MOE) 2019.0102, UCSF Chimera 1.17.3, Discovery Studio 3.5 Client, Auto Dock Vina Tools 4.2 (The Scripps Research Institute, USA), Avogadro, Marvin View, and SPSS version 25.

The 3D structures used in this study are files with the extension PDB and PDBQT from PubChem (https://pubchem.ncbi.nlm.nih.gov/), National Center for Biotechnology Information (NCBI). The 3D structure of MurA enzyme with PBD code 1UAE was acquired from The (https://www.rcsb.org/) Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB), the chemical structure of ligand such as epigallocatechin PubChem code 72277, epicatechin gallate PubChem Code 107905, epicatechin PubChem code 72276, epigallocatechin gallate PubChem 65064, catechin PubChem 9064, gallocatechin gallate PubChem code 5276890, and the chemical structure of fosfomycin. The database of the ligand was obtained from the PubChem Substance and Compound Database.

2.1. Ligand optimization and preparation.

Firstly, all the 3D structures used in this study are files with the extension PDB from PubChem (https://pubchem.ncbi.nlm.nih.gov/), National Center for Biotechnology Information (NCBI). Thr optimization of this structure has been completed by Avogadro using molecular mechanics/geometry optimization with 500 steps of the steepest descent algorithm, 10e-7 convergence of the MMFF94 force field. The structure has been saved in the PDB file for further computational studies, such as quantitative structure-activity relationships, molecular docking, toxicity properties, and bioactivity prediction.

2.2. Calculation of typical compound descriptors from Gambir leaves.

The QSAR calculation and modeling process includes the following steps: structure optimization and preparation using the MMFF94 force field; evaluation of chemical structure descriptors; descriptor selection; structural diversity analysis of the database based on the selected descriptor set; and evaluation of the model's significance level and each of the specified descriptors. Molecular descriptors are quantitative parameters derived from a simple mathematical equation known as a QSAR model [19]. The proposed model should be explicitly correlated with the physicochemical, biological, and toxicological properties of molecules under investigation [20]. Stepwise regression is used to identify the best subset of molecular descriptors. The integrated backward-forward selection approach efficiently minimises the number of molecular descriptors while maintaining robust predictive performance in QSAR modelling. Careful selection of optimal descriptor subsets is essential, as their effectiveness in meeting predefined objective criteria can vary significantly based on the composition of the training dataset. Furthermore, superior model training performance does not always correlate with enhanced predictive accuracy in external validation sets, highlighting the need for rigorous model validation.

The descriptors used in this study represent the three main parameters of QSAR: hydrophobic parameters, including the n-octanol/water partition coefficient (LogP, lipophilicity), globularity (inverse condition number, Glob), area of van der Waals surface (vdw_area), and solubility level (LogS). Then the electronic parameters include the highest energy occupied molecular orbital (AM1_HOMO), the lowest energy occupied molecular orbital (AM1_LUMO), the total energy (AM1_E), the heat of formation (AM1_HF), the electronic energy (AM1_Eele), and the dipole moment (AM1_dipole). Furthermore, the steric parameters in the form of Van der Waals volume (vol), total hydrophobic surface area (ASA_H), molecular weight (weight), and molar reactivity (mr) [21].

2.3. Statistical analysis and QSAR equations validation.

SPSS 25 was used to perform multilinear regression analysis with Log IC_{50} as the dependent variable and the value of the independent variable as the descriptor. The regression results yielded several equations that explained the relationships between the structures and activities of catechin and its derivative compounds found in Gambir leaves. The equation model chosen for further testing in this study has a value of $r > 0.9$. Validation aims to produce a reliable and tested QSAR equation. Validation using the cross-validation Leave One Out (LOO) method, with each compound's predicted activity data being removed from the regression analysis. The LOO (q^2) cross-validation square measures the model's performance and stability. One QSAR equation will be obtained from LOO validation. The criteria for a good QSAR equation have a value of $r^2 \geq 0.8$ and $q^2 \geq 0.5$ [21].

2.4. Modification of chemical structure.

Designing novel epigallocatechin, epicatechin gallate, epicatechin, epigallocatechin gallate, catechin, and gallocatechin gallate compounds by adding, reducing, or changing substituents is predicted to increase their antibacterial activity. The novel compound design considered the QSAR formula; modifications to catechin compounds are based on functional groups that play a key role in biological activity, particularly as antibacterials. This modification also refers to the study of structure-activity relationships (SAR) in the literature,

enabling effective modifications while accounting for potential side effects and without compromising the original biological activity. Then, we performed geometry optimization and descriptor calculations to obtain the predicted IC_{50} value [21, 22].

2.5. Drug-like parameter analysis and prediction of activity spectra for substances.

Prediction of Activity Spectra for Substances (PASS) is a software tool designed to evaluate the general biological potential of an organic drug-like molecule. PASS provides simultaneous predictions of many types of biological activity based on the structure of organic compounds. PASS algorithm enables *in silico* estimation of potential biological activities for designed compounds prior to their synthesis and experimental evaluation. Computational predictions were conducted using the publicly accessible PASS online web server (available at: http://way2drug.com/PassOnline/predict.php). This platform demonstrates the capability to predict diverse pharmacological properties, including, but not limited to, antimicrobial (antibacterial, antifungal), antiviral (notably anti-HIV), psychotropic (antidepressant), anticancer (via tumor necrosis factor modulation), and contraceptive activities. Such *a priori* activity spectrum prediction represents a crucial component in contemporary drug discovery pipelines, significantly facilitating the identification of promising candidate molecules for subsequent development [23].

2.6. Bioactivity prediction and toxicity evaluation.

Bioactivity prediction of the test ligands was conducted using online software on the website https://molinspiration.com/cgi-bin/properties. All ligands were downloaded from the PubChem website in SMILES format. The prediction was performed by uploading the SMILES structure of the ligand on the website and selecting the Predict [24]. ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiling is a critical tool in early-stage drug discovery and development. It enables researchers to evaluate key pharmacokinetic and drug-like properties, helping to identify the most promising drug candidates. By predicting parameters such as oral bioavailability, blood-brain barrier permeability, acute toxicity, and carcinogenic potential, ADMET analysis streamlines the selection of compounds with optimal safety and efficacy profiles before advancing to clinical trials [25]. Additionally, drug metabolism refers to the enzymatic transformation of pharmaceutical compounds in the body, which influences their clearance rates and toxicity thresholds. This process determines how a drug is broken down in the bloodstream, its overall elimination from the system, and the maximum safe dosage before adverse effects occur [26].

2.7. Receptor preparation and molecular docking.

Receptor preparation is done by trimming the structure from water, unwanted ligands, and co-crystallized ligand. Then, the receptor was further optimized by adding polar hydrogen, and Kollman and Gasteiger charges are calculated using the AMBER force field based on the molecule's molecular orbital electron density in molecular simulation and quantum mechanics. Kollman charges are more physically accurate as they take into account the molecule's chemical environment [27]. Molecular docking studies were performed using the free software AutoDock Tools 4.2 on the MurA protein/enzyme, whose structure was downloaded from the RCSB PDB under the access code 1UAE [28]. The active site was created using a grid of 16 x 16 x 18 Å on the XYZ side with a grid point spacing of 1.0 Å, following the binding site of the

co-crystallized and positive control ligand (fosfomycin). To validate the docking protocol, the native ligand (fosfomycin) was redocked into the MurA receptor's binding pocket. The method's accuracy was assessed by calculating root-mean-square deviation (RMSD) values; results below 2 Å were considered acceptable for reliable docking predictions. Molecular docking was repeated 20 times. Other parameters are used following the default. Visualization of molecular docking results was performed using the Discovery Studio Visualizer 2016 and UCSF Chimera [29].

The overall methodological workflow is illustrated in Figure 1 (research flowchart), which outlines the stages from compound data collection and structure optimization to QSAR modeling, chemical modification design, and biological activity prediction (PASS and Molinspiration), to molecular docking simulation and result analysis. E

ach stage was validated using relevant statistical parameters (r, r^2, q^2, and F-test), and the docking procedure was verified by RMSD calculations (< 2 Å). The inclusion of a methodological flow diagram facilitates readers' understanding of the interrelationships among the computational processes.

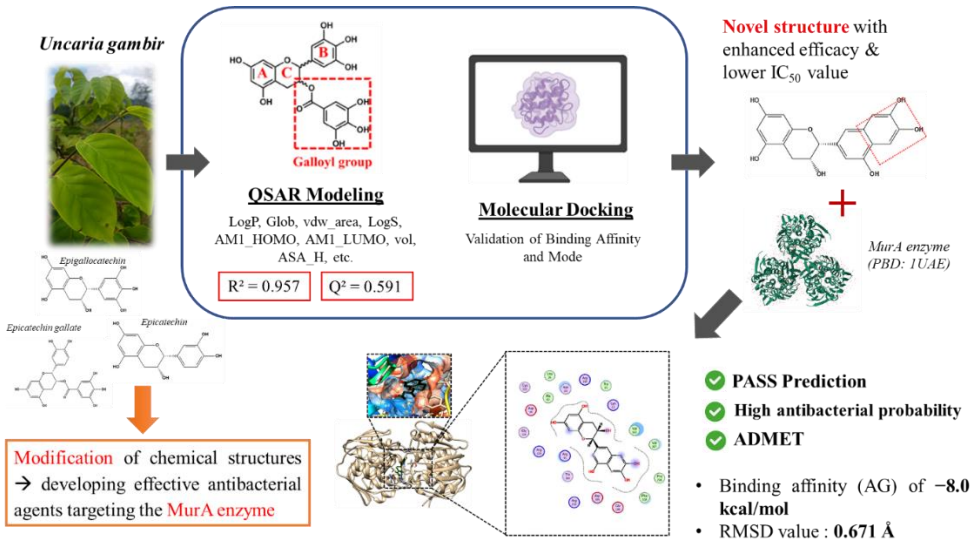


Figure 1. The flowchart of research methodology.

The flowchart illustrates a systematic research methodology that begins with collecting compound data from databases such as PubChem and PDB, followed by structure optimization using computational chemistry software. After that, QSAR modeling is performed to determine the relationship between molecular structure and biological activity, which serves as the basis for chemical modification of compounds. Next, the modification results are tested in silico using biological activity predictions through PASS and Molinspiration, as well as ADMET analysis to assess pharmacokinetic feasibility. The next stage is a molecular docking simulation to assess ligand interactions with the MurA enzyme, followed by analysis of the results to conclude the antibacterial potential of the designed compounds.

3. Results and Discussion

3.1. Quantitative structure activity relationships.

Quantitative structure-activity relationship (QSAR) models have become a crucial computational approach in modern drug discovery, accelerating the process of optimizing

initial compounds. These models establish mathematical correlations between molecular descriptors and biological activities, enabling the prediction of the pharmacological or toxicological properties of new compounds prior to experimental validation. The main advantage of QSAR is its ability to accelerate screening and improve the efficiency of identifying potential compounds. Developments in QSAR methodology continue to refine the accuracy, reliability, and applicability of these in silico techniques [30–32].

In pharmaceutical research, QSAR modeling is essential for correlating structural features with biological functions. Structural properties include physicochemical parameters, pharmacokinetic (ADMET), and toxicology [33, 34]. The Hansch method is one of the methods used for the current rendition. The Hansch method is based on the concept of the chemical structure of the compound and the biological activity (log X) of the compound, which can be quantified by the hydrophobic (π), electronic (σ), and steric (Es) parameters of the substituents [35].

All compounds derived from *Uncaria gambir* leaves (epigallocatechin, epicatechin gallate, epicatechin, epigallocatechin gallate, catechin, and gallocatechin gallate) were used as a training set to develop the QSAR model. The descriptors used in this study represent the three main QSAR parameters: hydrophobic parameters, including the n-octanol/water partition coefficient (LogP, lipophilicity); globularity or inverse condition number (Glob); area of van der Waals surface (vdw_area); and solubility level (LogS). Then the electronic parameters include the highest energy occupied molecular orbital (AM1_HOMO), the lowest energy occupied molecular orbital (AM1_LUMO), the total energy (AM1_E), the heat of formation (AM1_HF), the electronic energy (AM1_Eele), and the dipole moment (AM1_dipole). Furthermore, the steric parameters in the form of Van der Waals volume (vol), total hydrophobic surface area (ASA_H), molecular weight (weight), and molar reactivity (mr) are listed in Table 1.

Table 1. Descriptors used in QSAR analysis.

Symbol	Type	Unit	Definition
AM1_HOMO	Quantum/ Electronic	eV	The highest occupied molecular orbital energy
AM1_LUMO		eV	The lowest occupied molecular orbital energy
AM1_dipole		Debye	Dipole moment or dipolar moment
AM1_E		Kcal/mol	The total energy
AM1_HF		Kcal/mol	The heat of formation
AM1_Eele		Kcal/mol	The electronic energy
ASA_H	Steric	$Å^2$	Total hydrophobic surface area
mr		$Å^3$	Molar refractivity
vol		$Å^3$	Van der Waals Volume
weight		Da	Molecular weight
glob	Hydrophobic/ Topological	-	Globularity, or inverse condition number
LogP		-	N-octanol/water partition coefficient
LogS		mol/L	Solubility level
vdw_area		$Å^2$	Area of van der Waals Surface
lip_acc	Pharmaco	-	The number of O and N atoms
lip_don		-	The number of OH and NH atoms
lip_druglike		-	One if and only if lip_violation < 2, otherwise zero
lip_violation		-	The number of violations of Lipinski's Rule of Five

The descriptor value for each compound was calculated using MOE 2019.0102 software and then used to build a QSAR equation, with bioavailability of the research endpoint (IC50) as the independent variable. The multilinear regression statistic by average is shown in

Table 2. This analysis resulted in five models being chosen based on the best validation of the equations.

The equation model was created using regression analysis and the backward method, resulting in a set of equations and statistical parameters, as shown in Table 2. At the 95% confidence level, not all QSAR equation models obtained were significant. Table 2 shows that the price of the F_{count}/Ftable ratio is less than 1, indicating that the Fcount/Ftable equation model is not acceptable statistically [17]. The equation 3 model, with an r-value of 0.978, is said to meet the requirements if R> 0.9, the closer the value to 1; the value of r^2 0.957 meets the requirements of r^2 >0.6; and F_{count}/F_{table} is 1.251, which is greater than 1.0, and a low value of SE [21, 36].

Table 2. QSAR equation model based on multilinear regression analysis.

Model	Descriptor	r	r^2	SE	F_{cou}/F_{tab}
1	vol, AM1_HOMO, AM1_dipole, logS, ASA_H, logP, AM1_LUMO, mr	0.984	0.968	0.309	0.016
2	vol, AM1_HOMO, AM1_dipole, logS, ASA_H, logP, mr	0.983	0.966	0.226	0.420
3	vol, AM1_dipole, logS, ASA_H, logP, mr	0.978	0.957	0.207	1.251
4	vol, logS, ASA_H, logP, mr	0.964	0.930	0.229	1.692
5	vol, logS, ASA_H, logP	0.947	0.897	0.249	2.089

r = correlation coefficient; r^2 = coefficient of determination; SE= Standard Error; F= Fisher's Test.

The model 3 equation was chosen because fewer descriptors had a value that satisfied the statistical parameter, namely,≥ 0.9. The descriptors in the equation represent the four main QSAR parameters in the form of quantum/electronic, hydrophobic/topological, pharmacokinetic, and steric parameters. This parameter will serve as a quantitative metric for evaluating novel molecular structures in QSAR modeling. $LogIC_{50}$ = (2.731) - (0.084 x AM1_dipole) + (0.005 x ASA_H) - (0.651 x LogP) - (1.096 x LogS) + (2.863 x mr) - (0.093 x vol) is the QSAR model 3 formula, which will be validated. The developed QSAR model equation demonstrates a linear relationship between biological activity values and six molecular descriptors. To assess model performance, several statistical parameters were evaluated, including R, R^2, SE, and F_{count}/F_{table}. A higher value of R and R^2 and a low SE value indicate that the proposed model is reliable and predictive in Figure 2.

Validation aims to produce a good QSAR equation with proven repeatability. Cross-validation techniques are used for validation. The test method employs cross-validation with Leave One Out (LOO). The LOO approach omitted each predictable compound from experimental activity data in linear regression analysis. The LOO cross-validation square (q^2) measures the model's performance and stability. The value of q^2 must be greater than 0.5, indicating the method's repeatability [21]. The LOO (Leave-One-Out) validation yields a q2 value of 0.591 for the model 3 equation, indicating that it is valid and can be used for modification and QSAR calculations. To strengthen the validation of the QSAR model, a Y-randomization test was also conducted with 100 random permutations. The results showed that the permutation r^2 was much lower (< 0.3), indicating that the model did not overfit and produced stable predictions. In addition, the external test set calculation using 20% of the data yielded an external r^2 of 0.85, indicating that the model has strong predictive capabilities outside the training data.

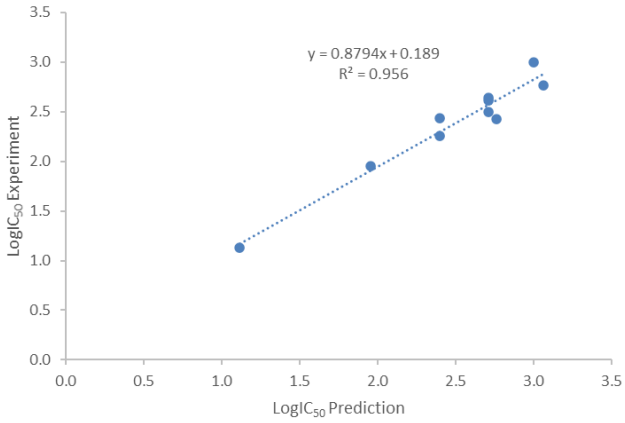


Figure 2. The correlation between the experiment or observed and the predicted activities is established.

3.2. Structure modification of epigallocatechin, epicatechin gallate, and epicatechin.

Molecular modification is a key approach driven by QSAR to improve the biological activity, bioavailability, and selectivity of lead compounds. By adding or replacing functional groups, molecular modification can increase enzyme inhibition potential while reducing toxicity [37, 38]. Investigations into compounds derived from *Uncaria gambir* have illustrated their potential as natural inhibitors of the MurA enzyme. Notably, research has identified catechins as having a significant binding affinity for MurA, as demonstrated by *in vitro* and *in silico* analyses [7, 8]. These studies have revealed that catechins exert their antimicrobial effects by mimicking substrate interactions at the active site of MurA, effectively displacing key substrates necessary for enzymatic activity [8, 39, 40].

The structural modifications of these catechins could enhance their enzyme-inhibitory capabilities. For instance, incorporating different functional groups or structural motifs may improve binding affinity and selectivity for MurA over non-target enzymes, thereby reducing potential toxicity to host cells [7, 8, 39]. Similar strategies have been successful with other classes of MurA inhibitors, whereby small molecular changes have led to significant enhancements in antibacterial potency and selectivity [40-42]. *In silico* simulations have emerged as a vital tool for guiding the design of such modification applications, such as molecular docking studies, which allow researchers to predict how alterations in the chemical structure of catechins affect their binding to MurA [40, 43, 44]. These computational insights are instrumental for elucidating the optimal chemical modifications required to enhance efficacy against target bacterial strains, particularly in the context of multidrug-resistant phenomena observed in clinical isolates such as *Escherichia coli* and *Staphylococcus aureus* [6, 44].

Furthermore, the evaluation of structure-activity relationships (SAR) is critical in this endeavor. By systematically investigating how changes in the catechin skeleton correlate with their inhibitory effects on MurA, it becomes possible to identify potent leads for further development [7, 41]. The literature indicates that higher levels of hydroxylation, for instance, can amplify antibacterial properties, suggesting that modifications aimed at increasing the number of hydroxyl groups could be strategically beneficial [41].

Moreover, emulsifying agents and permeability enhancers are sometimes used alongside these modified compounds to improve their bioavailability and facilitate greater tissue penetration, which is crucial for achieving therapeutic concentrations at potential infection sites. Strategies such as encapsulation in lipid formulations or combination with adjuvants may also be considered to mitigate the effects of efflux pumps, which are frequently

employed by resistant bacterial strains to expel antibiotics [8]. Multiple studies demonstrate that a dual approach combining chemical modification of catechins from Uncaria gambir with advanced drug delivery systems may yield significant advances in antimicrobial therapy [39, 45, 46]. This comprehensive strategy not only seeks to address the immediate issue of bacterial resistance but also lays the foundation for the development of new classes of antibiotics capable of circumventing the pitfalls that have beset existing drugs.

The galloyl group of the typical Gambir leaves compound epigallocatechin, epicatechin gallate, epicatechin, epigallocatechin gallate, catechin, and gallocatechin gallate was modified, as was the amount of galloyl group or catechol in the structure, to increase the compound's biological activity as an antibacterial (Figure 3 and Table 3) [22, 47, 48]. Modification of the structure of epigallocatechin, epicatechin gallate, epicatechin, epigallocatechin gallate, catechin, and gallocatechin gallate compounds typical of Gambir leaves is predicted to increase its biological activity as an antibacterial through inhibition or enzyme inhibitors, especially in inhibiting the activity of the MurA enzyme in the formation of cell walls so that the cell wall in bacteria is not formed which causes the bacteria to undergo morphological changes, agglutination and lysis in bacteria [49]. Modifications were carried out to increase the bioactivity value and reduce the inhibition concentration (IC50) of compounds derived from Gambir leaves. Table 4 shows the design of the novel compounds.

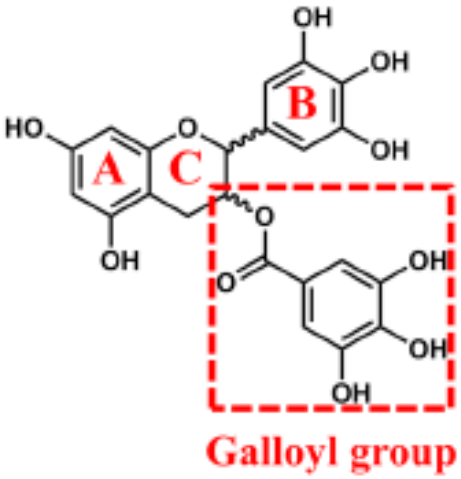


Figure 3. Galloyl group of catechin.

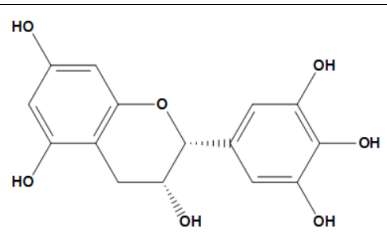
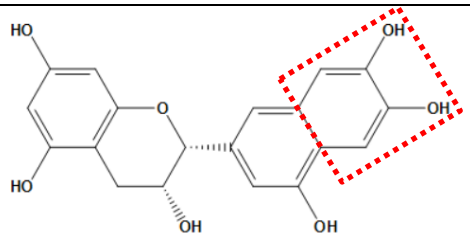
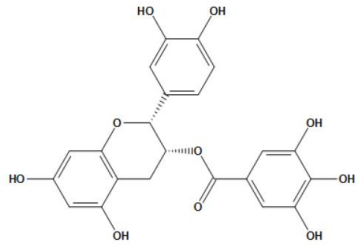
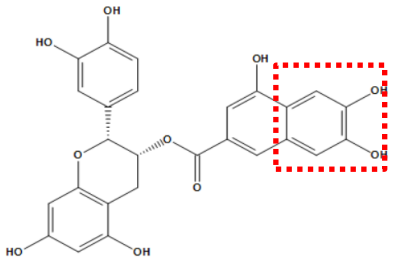
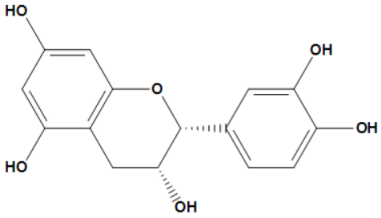
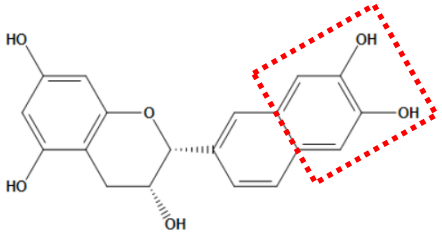
Table 3. Structure-activity relationship to increase antibacterial activity.

Activity / Group	Antiviral/Anti-bacterial
Catechol	↑
Number of -OH	↓
O-Me	↓
C_2=C_3	↑
3-OH	↑
4-Carbonyl	↑
Glycosylation	↑

The MMFF94 force field for small organic molecules in medicinal chemistry must be used to optimize the structure of the novel compounds (medicinal chemistry). The validated equation is used to calculate the descriptor for the novel compound that has been optimized. The results of the descriptor calculations for the three novel compounds are in Table 4. Table 5 shows that the novel compound can reduce the IC_{50} values of epigallocatechin, epicatechin gallate, and epicatechin. The novel compound had an IC_{50} smaller than fosfomycin or the

compound without modification; the predicted IC_{50} for novel epigallocatechin was 2.46 µg/mL, the predicted IC_{50} for novel epicatechin gallate was 2.27 µg/mL, and the predicted IC_{50} for novel epicatechin was 18.77 µg/mL—table 5. IC_{50} values of epigallocatechin, epicatechin gallate, and epicatechin had IC_{50} experimental values of 512, 512, and 1145 µg/mL, respectively [50–52]. These results suggest enhanced inhibition potential relative to fosfomycin (IC_{50} = 121.1 µM or 28.1 mg/L).

Table 4. Modification of compounds typical of Gambir leaves.

Compound Name	Original Structure	Novel structure	Ref
epigallocatechin modified			[48, 53]
epicatechin gallate modified			[48, 53]
epicatechin modified			[48, 53]

Molecules inside the red dashed box are modified substituents.

Table 5. Results of the descriptor value of novel compounds typical of Gambir leaves

Compounds	AM1_dipole	ASA_H	LogP	LogS	mr	vol	IC_{50} exp*/IC_{50} Predict
epigallocatechin	2.374	488.015	1.706	-1.375	7.357	256.125	512*
epicatechin gallate	4.222	652.503	3.376	-3.029	10.675	374.5	512*
epicatechin	4.236	476.323	1.979	-1.737	7.240	250.875	1145*
epigallocatechin modified	3.035	644.939	3.002	-3.253	8.976	350.750	2.46
epicatechin gallate modified	4.337	870.281	4.672	-4.907	12.293	472.000	2.27
epicatechin modified	2.883	667.781	3.275	-3.615	8.855	341.250	18.77

3.3. Drug-like parameter analysis and prediction of activity spectra for substances.

PASS (Prediction of Compound Activity Spectrum) is used to evaluate the potential biological activity of designed compounds. This tool predicts various biological activities directly from molecular structures, especially for antibacterial, antibiotic, and antineoplastic properties. The novel compounds were analyzed using the PASS webserver, and we have estimated their biological activities for antibacterial, antibiotic, and antineoplastic properties.

Table 6 shows the PASS result for the novel compound and the outcome results for Probability to be active (Pa) and Probability to be inactive (Pi), as shown in the data for the novel compound. The largest Pa value has been found to be 0.431 for antibacterial activity in novel epicatechin gallate, 0.219 for novel epigallocatechin, and 0.208 for antineoplastic antibiotic activity in novel epicatechin. The data showed that novel epigallocatechin, epicatechin gallate, and epicatechin were more effective against bacteria and antibiotics and have been taken for further computational studies, such as bioactivity and toxicity prediction and molecular docking. Although the predicted IC_{50} values indicate a significant increase in antibacterial activity, these results are in silico and do not account for in vitro biological variability. Therefore, quantitative interpretation needs to be followed by experimental testing to ensure that the very low IC_{50} values (2.27–2.46 μg/mL) can be reproduced biologically. These results still provide strong indications that the structural modifications made have successfully increased affinity for the MurA enzyme.

Table 6. Result of prediction activity spectra for novel compounds.

Compounds	Antibacterial		Antibiotic		Antineoplastic antibiotic	
	Pa	Pi	Pa	Pi	Pa	Pi
fosfomycin	0,373	0,037	0,222	0,024	N/A	N/A
epigallocatechin	0,387	0,033	0,173	0,037	0,201	0,018
epicatechin gallate	0,379	0,035	0,154	0,046	0,147	0,033
epicatechin	0,320	0,053	0,149	0,049	0,190	0,021
epigallocatechin modified	0.425	0.055	0.219	0.024	0,205	0,017
epicatechin gallate modified	0.431	0.024	0.194	0.030	0,150	0,032
epicatechin modified	0,331	0,049	0.160	0.043	0.208	0.066

3.4. Bioactivity prediction and toxicity evaluation.

The beneficial or harmful effects of a drug on cell tissue or living matter as a targeted drug in the body are described as biological activity or pharmacological activity. Drug targets or biological targets can have specific effects, such as desired therapeutic effects or undesirable side effects [54]. Bioactive compounds as drug candidates must meet certain bioactivity criteria using Molinspiration: active with a value > 0.0, moderately active with a value of -5.0 to 0.0, and inactive with a value < -5.0 against enzyme inhibitors [55]. According to the findings, the bioactivity values of the novel epigallocatechin and novel epicatechin gallate as GPCR ligands, nuclear receptors, and enzyme inhibitors increased. Novel epigallocatechin compounds increased from 0.40 to 0.43 for GPCR ligand, 0.57 to 0.65 for nuclear receptor, and 0.25 to 0.49 for enzyme inhibitor. Novel epicatechin gallate compounds increased from 0.17 to 0.20 for GPCR ligand, 0.34 to 0.41 for nuclear receptor, and 0.25 to 0.27 for enzyme inhibitor, and novel epicatechin compounds increased from 0.40 to 0.45 for GPCR ligand, 0.57 to 0.60 for nuclear receptor, and the same value is 0.49 for enzyme inhibitor. Table 4 shows that all of the compounds found in novel Gambir leaves are predicted to have active bioactivity as a MurA enzyme inhibitor in antibacterial formation.

All of the original or novel compounds have been predicted to be non-carcinogens, non-toxic to the blood-brain barrier (BBB), and non-toxic in acute oral toxicity tests. Toxicity is an important property for choosing the best drugs. The designed compounds are not toxic. From Table 7, we notice that novel original and novel compounds meet all the properties of in-silico ADMET property evaluation. Therefore, all compounds can be used as drugs to treat bacterial infections by inhibiting the enzymatic activity of MurA. The PASS results indicate that all three modified compounds have an antibacterial activity probability (Pa) > 0.4, indicating a

high tendency toward the expected biological potential. However, the Pa–Pi values still need to be compared with those of standard compounds, such as fosfomycin, to provide relative context for effectiveness. Thus, the PASS results table should be accompanied by a brief interpretation explaining that Pa values close to 1.0 indicate a high probability of actual activity in biological tests.

Table 7. IC_{50}, bioactivity, and toxicity value of novel compounds of catechin.

Compounds	Bioactivity Value			Toxicity Value		
	GPCR ligand	Nuclear receptor	Enzyme inhibitor	Carcinogenicity	BBB	Acute oral toxicity
epigallocatechin	0.40	0.57	0.25	NC	-	Class IV
epicatechin gallate	0.17	0.34	0.25	NC	-	Class IV
epicatechin Non carcinogen	0.49	0.57	0.49	NC	-	Class IV Class IV
epigallocatechin modified	0.43	0.65	0.49	NC	-	Class IV
epicatechin gallate modified	0.20	0.41	0.27	NC	-	Class IV
epicatechin modified	0.45	0.60	0.49	NC	-	Class IV

* Value in IC_{50} meaning is IC_{50} based on the experiment from references; Bioactivity value: active= >0; moderate= -5.0 - 0; inactive= <-5.0; Toxicity value: NC=non carcinogen; - = negatif.

3.5. Molecular docking against pathogenic bacteria on the MurA enzyme.

Molecular docking simulations were performed on the MurA enzyme (PDB ID: 1UAE) to predict ligand-receptor interactions. Redocking validation produced RMSD = 0.379 Å, confirming docking accuracy. If the RMSD value is less than 2 Å, the ligand position is good; if it is more than 2 Å, a molecular shift in the tethering process has occurred, and the tethered ligand position is not relative to the X-ray conformation. RMSD values greater than 2 Å can be caused by various factors, including scoring, which occurs when the energy required to bind to molecules with low RMSD is insufficient [50].

The MurA-Fosfomycin as co-crystallized ligand was also redocked into the binding pocket or active site of the MurA Receptor and complex formed hydrogen bonds interaction with amino acid residues Cys^{115}, Arg^{120}, Arg^{397}, Gly^{114}, and formed hydrophobic bonds with amino acid residues Asn^{23}, Asp^{305}, Arg^{371}, Arg^{331}, Ile^{117}, and Arg^{91}, formed salt bridge with Asp^{49} and Lys^{22}. To validate the simulation results, the root-mean-square deviation (RMSD) was used to assess how different the obtained orientations are from the corresponding co-crystallized poses of the same ligand molecules [51]. The lower value of RMSD obtained is 0.379 Å, and the MurA-Fosfomycin interaction has a binding affinity of -4.5 kcal/mol and inhibition constant of 498.67 μM in Figure 4. An amino acid residue of Cys^{115} with approximately Arg^{397}, Arg^{120}, and Lys^{22} defines the active site of the MurA enzyme [52]. Other residues, such as Asp^{305}, which acts as a base in the initiation of UDP-N-acetylglucosamine deprotonation, Cys^{115}, which acts as an acid in the C-3 protonation process of phosphoenolpyruvate during the reaction, and Lys^{22}, which is involved in PEP binding and affects the conformational change of the enzyme, is involved in PEP binding and affect the conformational change of the enzyme [51].

The ligand interactions of epigallocatechin on the binding site of MurA enzyme, builded MurA-epigallocatechin complex and formed hydrogen bonds with the amino acids Cys^{115} and hydrophobically or alkil reaction with the amino acids Gly^{114}, Val^{327}, Val^{163}, Arg^{120}, and Arg^{91} and formed salt bridge between MurA-epigallocatechin on the amino acid Asp^{305} and Lys^{22}, the MurA-epigallocatechin complex exhibited a binding affinity of -8.1 kcal/mol

and an inhibition constant of 1.138 μM, with a docking RMSD value of 0.665 Å, indicating a highly stable and well-fitted ligand conformation within the active site in Figure 5.

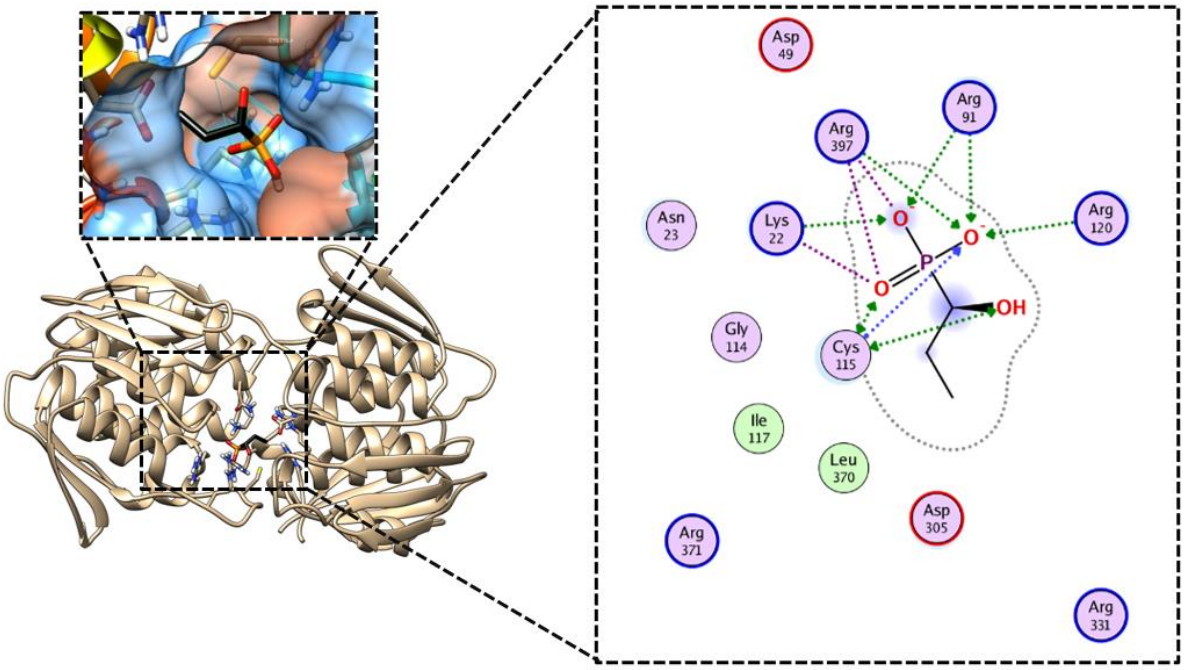


Figure 4. Co-crystallized ligand interactions (fosfomycin) on the binding site of MurA enzyme.

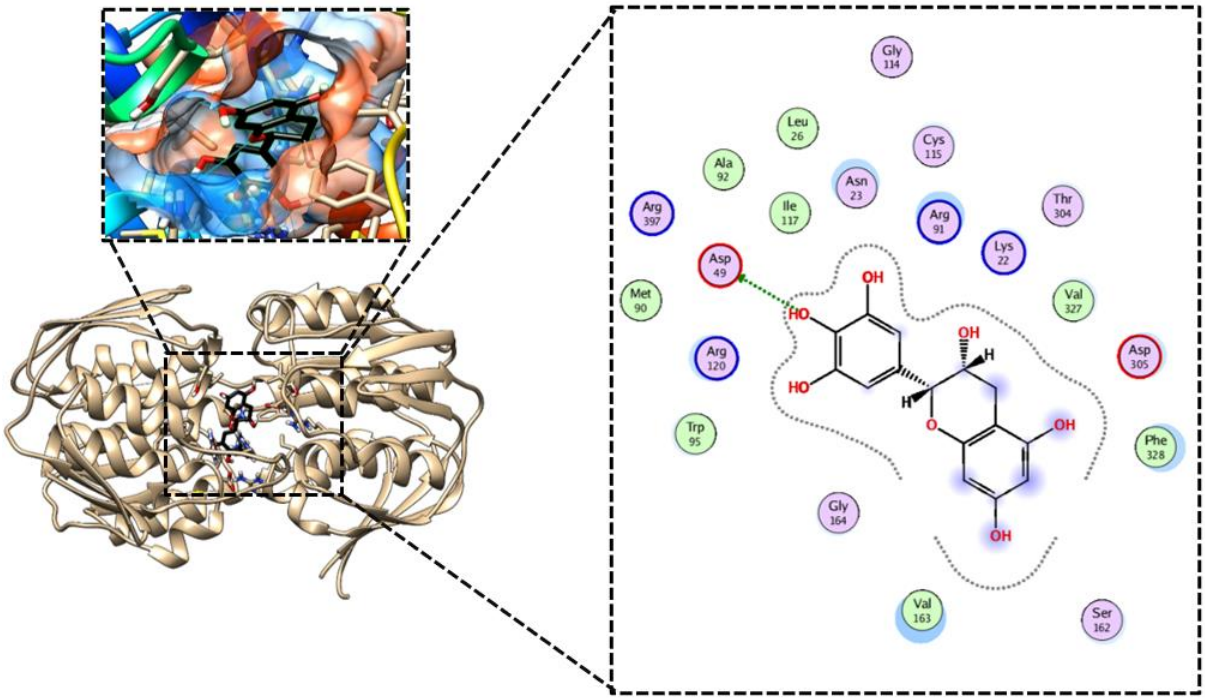


Figure 5. Ligand interactions of epigallocatechin on the binding site of MurA enzyme.

The ligand interactions of novel epigallocatechin form eight hydrogen bonds with the amino acids Cys[115] as the active site, Arg[120], Arg[397], Asp[49], Ala[92], Val[163], Gly[164], and Arg[91]. A favorable positive-positive interaction for Lys[22] with the hydroxyl group. A pi-alkyl interaction is observed with Phe[328] with the ring of benzene. The MurA-novel epigallocatechin complex exhibited a binding affinity of –8.7 kcal/mol and an inhibition constant of 0.413 μM, with a docking RMSD value of 0.578 Å, indicating a reliable and stable ligand conformation within the MurA active site (Figure 6).

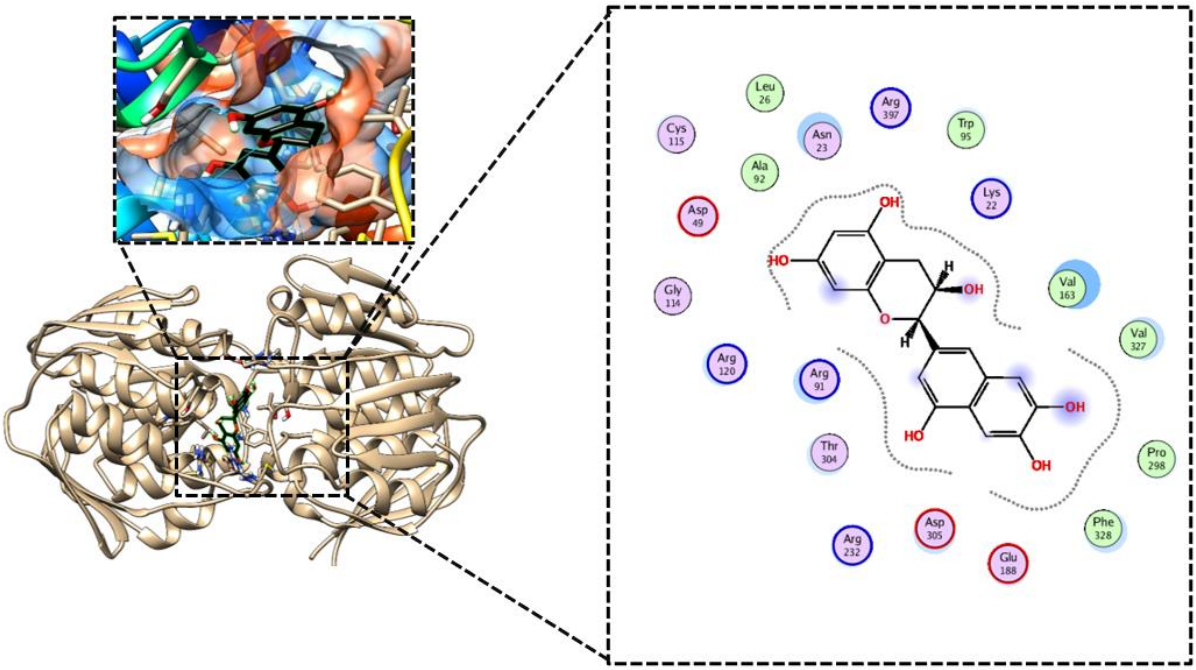


Figure 6. Ligand interactions of novel epigallocatechin on the binding site of MurA enzyme.

The ligand interactions of epicatechin gallate on the binding site of MurA enzyme to build MurA-epicatechin gallate by forming hydrogen bonds with the amino acids Asp[49], Arg[371], Arg[91], Asn[23], and Asp[305]. Four favorable positive-positive interactions for Lys[22], Leu [370], Ala[165], and Arg [331] with the hydroxyl group. Alkyl bonds are observed with Val[163] and Cys[115]. Hydrophobically with the amino acids Arg[120], the MurA-epicatechin gallate complex exhibited a binding affinity of –7.3 kcal/mol and an inhibition constant of 1.138 μM, with a docking RMSD value of 0.995 Å, indicating a consistent and well-aligned ligand orientation within the MurA active site (Figure 7).

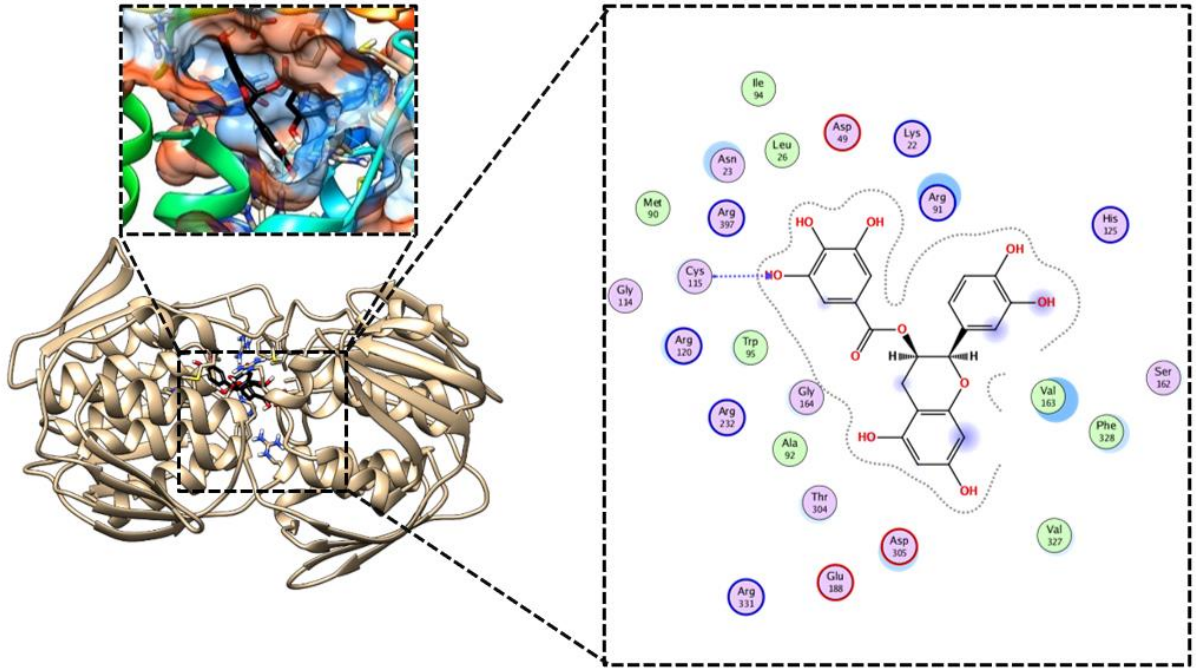


Figure 7. Ligand interactions of epicatechin gallate on the binding site of MurA enzyme.

The ligand interactions of novel epicatechin gallate on the binding site of MurA enzyme to build MurA-novel epicatechin gallate by forming four hydrogen bonds with the amino acids Cys[115], Arg[91], Asp[305], Gly[114], and Asp [49]. Salt bridge are forming with the amino acids Arg[120]

and Glu[188], and interacting two favorable positive-positive interaction for Lys[22], Arg[397] with the ring benzene, the MurA-novel epicatechin gallate complex exhibited a binding affinity of –8.0 kcal/mol and an inhibition constant of 1.347 μM, with a docking RMSD value of 0.671 Å, indicating a stable and well-fitted ligand orientation within the MurA active site (Figure 8).

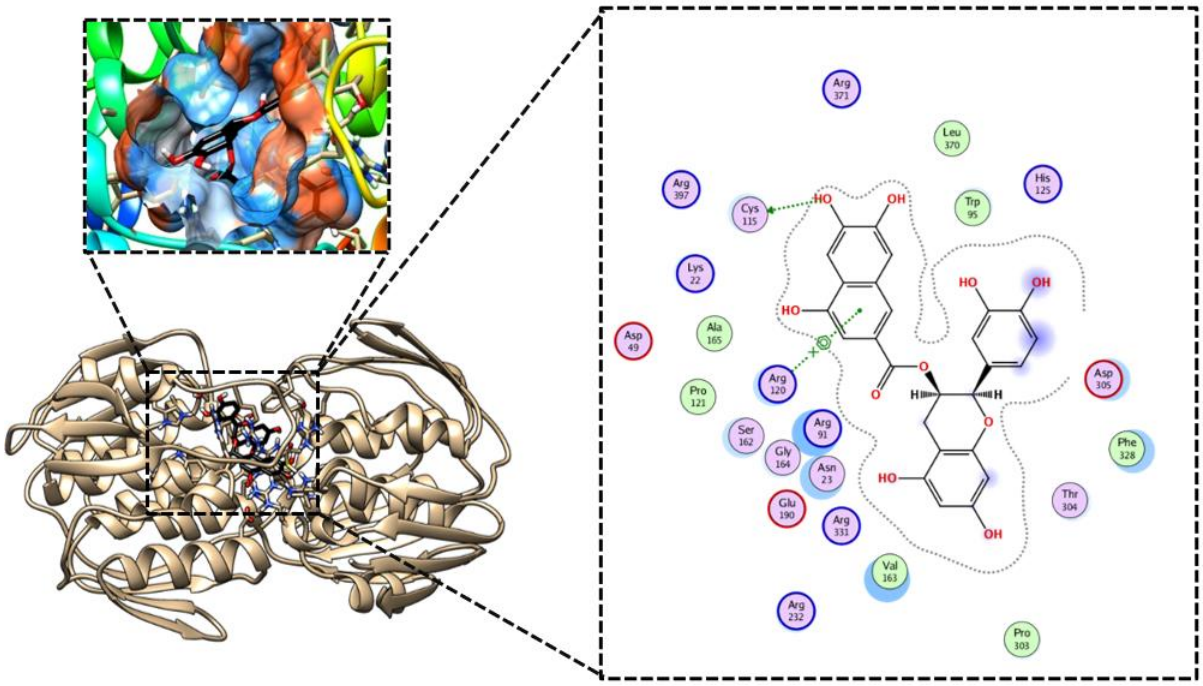


Figure 8. Ligand interactions of novel epicatechin gallate on the binding site of MurA enzyme

The ligand interactions of epicatechin on the binding site of MurA enzyme to build MurA-epicatechin by forming a hydrogen bond with the amino acid Cys[115]. Two favorable positive-positive interactions for Arg[397] and Arg[91] with the hydroxyl group.

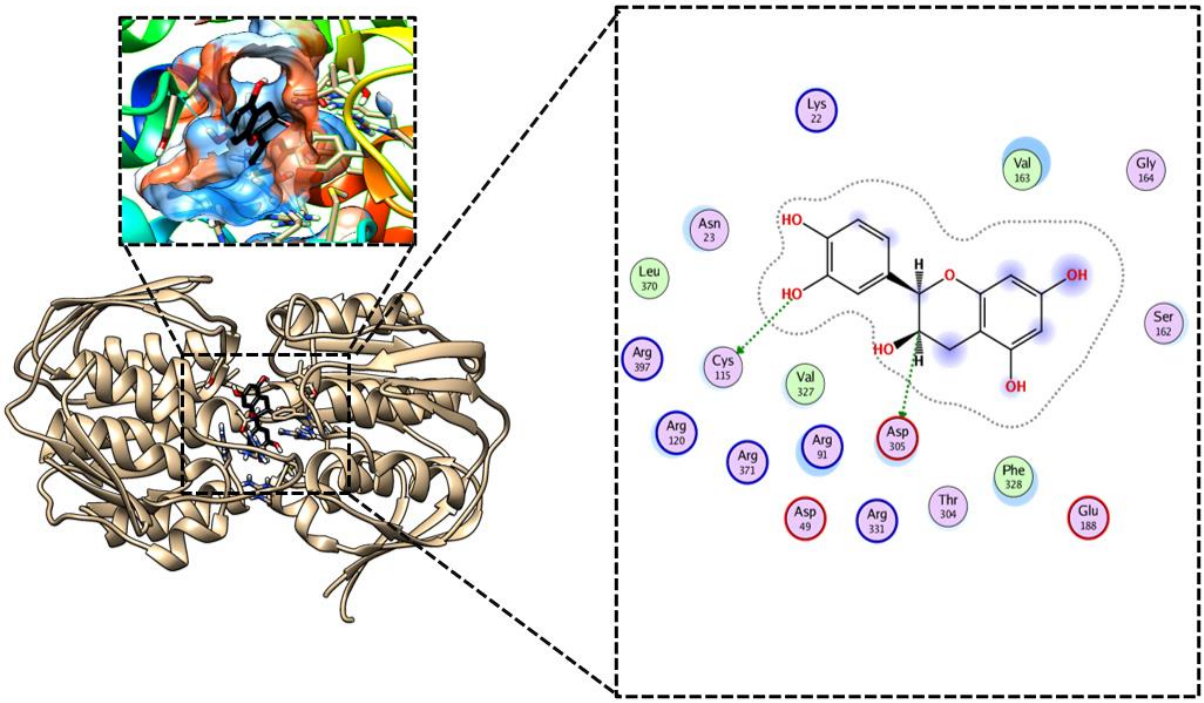


Figure 9. Ligand interactions of epicatechin on the binding site of MurA enzyme.

Alkyl bonds are observed with Val[163] and Val[327] with the ring benzene and salt bridge on Lys[22] amino acid. Hydrophobically with the amino acids Glu[168], Thr[304], Val [161], Ser[162], Phe[328],

Asn[23], Gly[164], Trp[95], Leu[26], Ala[92], Asp[49], Met[90], Gly[114], Arg[120], and Asp[305]. The MurA-epicatechin complex exhibited a binding affinity of –7.7 kcal/mol and an inhibition constant of 2.237 μM, with a docking RMSD value of 0.822 Å, suggesting a stable ligand orientation and reliable docking conformation within the MurA active site (Figure 9).

The ligand interactions of novel epicatechin on the binding site of MurA enzyme to build MurA-novel epicatechin by forming six hydrogen bonds with the amino acids Cys[115], Arg[120], Asp[305], Lys[22], Asn[23], and Arg[397]. Pi-Akyl are forming with the amino acids Arg[91]and Val[163], and interacting Pi-Pi T-Shaped for Phe[328] with the ring benzene, the MurA-novel epicatechin complex exhibited a binding affinity of –8.6 kcal/mol and an inhibition constant of 0.489 μM, with a docking RMSD value of 0.694 Å, indicating a stable and well-aligned ligand orientation within the MurA active site (Figure 10)

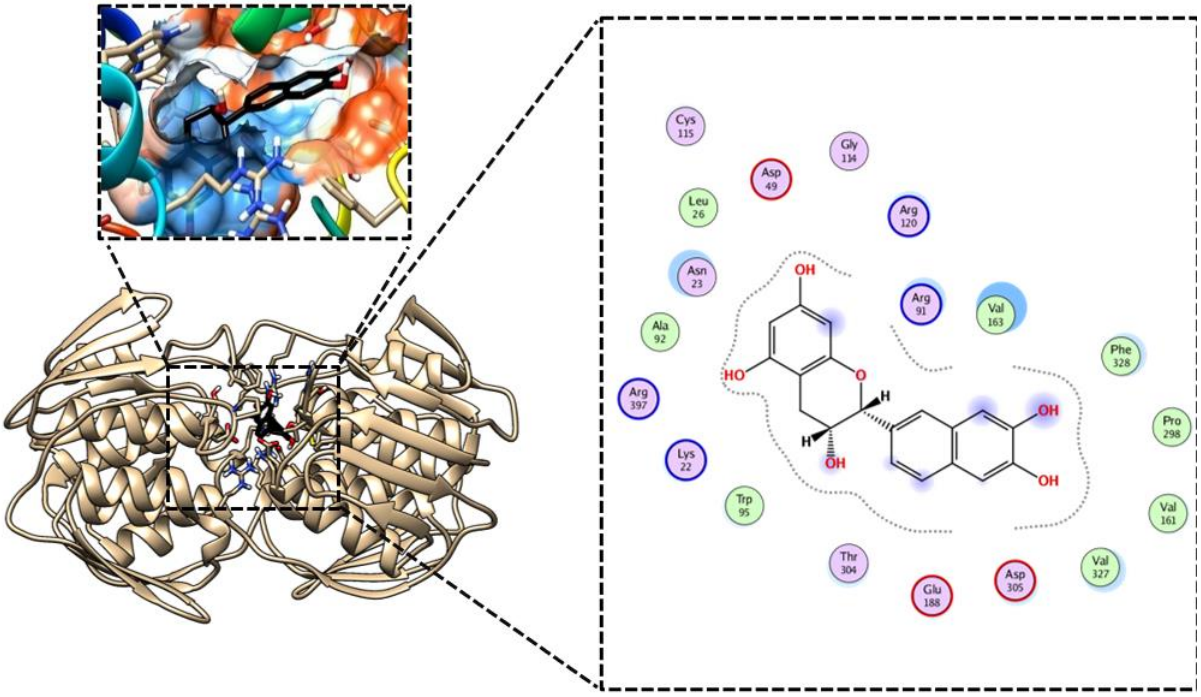


Figure 10. Ligand interactions of novel epicatechin on the binding site of MurA enzyme.

The interaction of the ligand-binding site on the target protein's active site was demonstrated by novel epigallocatechin, novel epicatechin gallate, and novel epicatechin research, and the predicted energy affinity of each ligand showed that it had a much higher potential or lower energy than the natural ligand or co-crystal ligand. The Gibbs free energy or binding affinity (G), which is used to determine the value of the Inhibition Constant (Ki), is a partial derivative of affinity energy. The concentration required to inhibit an enzyme is measured in Ki. As the Ki value decreases, the concentration required to produce an inhibitory effect decreases [56].

Molecular modification involves chemically altering existing compounds to enhance their biological activity, selectivity, and pharmacokinetic properties. Catechins from Uncaria gambir are structurally simple flavonoids that can be modified to improve their binding affinity and inhibitory efficacy against the MurA enzyme. Research suggests that modifications to the catechin scaffold can enhance binding interactions by strategically placing functional groups [7, 8, 45]. Structure-activity relationship (SAR) studies are pivotal, as they elucidate how molecular changes can influence the inhibitory activity of potential drugs [40, 57]. One study showed that systematic modifications of various catechins resulted in significantly increased inhibitory potencies towards the MurA enzyme, indicating that specific structural

configurations are crucial for effective enzyme binding [7]. Advances in synthetic methods enable the rational design of such modifications, guided by both computational modeling and empirical testing [43]. The identification of lead compounds through molecular modification strategies is critical, given the diversity of chemical interactions that may enhance bioactivity [7]. According to the findings, the novel ligand had a much lower inhibition constant than the positive control ligand in the form of fosfomycin.

Docking analysis shows that all modified compounds interact strongly with MurA active residues, particularly Cys115, Arg120, and Asp305, which play an important role in the enzymatic process of bacterial cell wall formation. The binding affinity values ranged from –8.0 to –8.7 kcal/mol, which is lower than that of the control ligand fosfomycin (–4.5 kcal/mol), indicating a more stable interaction. However, the difference in interaction energy between compounds is relatively small (<1 kcal/mol). Hence, the interpretation of effectiveness needs to be confirmed by molecular dynamics simulations to assess the stability of the complex over time.

4. Conclusions

This study successfully developed a valid QSAR model ($r = 0.978$; $r^2 = 0.957$; $q^2 = 0.591$) and identified three novel catechin derivatives from *Uncaria gambir* with enhanced antibacterial potential against the MurA enzyme. The predicted IC_{50} values of novel epigallocatechin (2.46 μg/mL), novel epicatechin gallate (2.27 μg/mL), and novel epicatechin (18.77 μg/mL) were considerably lower than those of the parent compounds and the reference antibiotic fosfomycin. Molecular docking confirmed strong binding affinities at the MurA active site, particularly involving Cys115, Arg120, and Asp305 residues. These interactions suggest that the modified compounds may inhibit bacterial cell wall biosynthesis, leading to cell damage and autolysis. Although promising, these findings are predictive; thus, *in vitro* and *in vivo* studies, including synthesis, toxicity evaluation, and experimental IC_{50} validation, are essential to confirm their antibacterial efficacy and stability before progressing toward clinical investigation.

Author Contributions

Conceptualization, I.K. and A.U.; methodology, I.K.; software, I.K.; validation, I.K. and A.U.; formal analysis, I.K.; investigation, I.K.; resources, A.U.; data curation, I.K.; writing—original draft preparation, I.K.; writing—review and editing, A.U.; visualization, I.K. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

Funding

This research received no external funding.

Acknowledgments

The author would like to thank all parties who have contributed to the completion of this research.

Conflicts of Interest

The authors declare no conflict of interest

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