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In Silico Screening of Phytochemical Efficacy Against Antimicrobial Resistance Proteins *via* Molecular Docking

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Abstract: Antimicrobial Resistance (AMR) is increasingly recognized as a major global health threat due to its persistent and widespread impact. Phytochemicals have emerged as potential candidates in the fight against antibiotic-resistant bacteria due to their reported antimicrobial properties. In this study, an *in silico* molecular docking method using AutoDock was performed to evaluate the binding affinities and interaction profiles of a selected set of phytochemicals against key bacterial target proteins associated with AMR. The compounds of quercetin, epigallocatechin gallate (EGCG), kaempferol, berberine, curcumin, and allicin were docked against the efflux pumps TetB and EmrD. Docking analysis was performed based on binding energy scores, with more negative binding energies (kcal/mol) indicating stronger predicted binding interactions for proteins associated with AMR. The docking results consistently identified quercetin as exhibiting the highest binding affinity towards TetB. These findings suggest that quercetin and other selected phytochemicals demonstrate potential interactions with AMR-associated proteins in silico, without experimental validation.

Keywords: antimicrobial; phytochemicals; computational; autodock; quercetin; kaempferol; berberine; curcumin; allicin; mechanisms.

1. Introduction

Antimicrobial Resistance (AMR) is identified as a major global public health challenge due to its increasing impact on the effectiveness of existing antimicrobial therapies [1]. Recent global studies suggest that, in projections for 2050, an estimated 1.91 million deaths were directly attributable to AMR and 8.22 million deaths were associated with AMR, compared to 1.14 attributable deaths and 4.71 million associated deaths reported in 2021 [2]. These projections indicate a substantial rise in AMR-related mortality, indicating a growing global clinical and economic burden associated with resistant infections. The emergence and dissemination of AMR microbes contribute to decreased efficacy of standard treatment regimens, leading to prolonged illness, increased healthcare costs, and higher rates of treatment failures [3]. Resistance mechanisms such as enzymatic drug inactivation, target modification, and efflux pump overexpression significantly reduce antimicrobial susceptibility [4]. Current strategies to mitigate AMR include infection prevention and control practices, vaccination

programs, surveillance of resistance patterns, rational and judicious use of antibiotics in both clinical and agricultural settings, and sustained research efforts focused on the discovery and development of novel antimicrobial agents and alternative therapeutic approaches [5].

Phytochemicals have been investigated in antimicrobial research due to their reported biological activities against a range of pathogens, including antibiotic-resistant strains. Several studies have suggested that phytochemicals may exhibit antimicrobial effects either alone or in combination with other antibiotics, making them of interest in AMR-related research. These compounds have been reported to interact with bacterial cells through multiple mechanisms such as membrane perturbation, interference with cellular signaling pathways, and modulation of resistance-associated processes. Additionally, certain phytochemicals have been suggested to enhance the activity of existing antibiotics or influence resistance mechanisms, highlighting the relevance for further investigations [6,7]. As shown in Table 1, the key details of the promising phytochemicals are summarized below. However, the extent of these effects and their clinical applicability require experimental validation.

Table 1. Promising phytochemicals for docking against AMR genes.

Phytochemical	Sources	Potential target	Activity notes
Curcumin	*Curcuma longa* (turmeric)	tetB, emrD	Inhibits efflux pumps, anti-inflammatory [8]
Berberine	*Berbers spp.*	EmrD	Efflux pump inhibitor, DNA binding [9]
Quercetin	Various fruits & vegetables	tetB	Binds DNA gyrase, modulates resistance genes [10]
Epigallocatechin gallate (EGCG)	Green tea	emrD	Blocks efflux pumps, synergizes with antibiotics [11]
Kaempferol	Tea, broccoli, apples	tetB	DNA/protein interaction, antioxidant [12]

The complex molecular structures of phytochemicals and reports suggesting comparatively lower toxicity have contributed to interest in their potential role in AMR research. Phenolics, alkaloids, and terpenoids are widely investigated for their interactions with the bacterial systems and are considered relevant for current AMR trends [13]. The major mechanisms of action against AMR include disruption of bacterial membranes [14], inhibition of essential enzymes [15], interference with cellular signaling pathways [16-18], and modulation of efflux pump activities [18]. The advantages and potential of phytochemicals as alternative antibiotics are driven by their ability to influence antibiotic resistance mechanisms [13], to act synergistically when combined with conventional antibiotics [19], to exert prebiotic effects [17], and by the diversity of naturally occurring phytochemicals [20].

Bacterial pathogens mostly rely on two major and well-structured mechanisms of antimicrobial resistance, namely efflux-mediated drug resistance and target-site modification. Efflux pumps such as TetB and EmrD actively reduce intracellular antibiotic concentrations, directly limiting drug efficacy, and contribute to multidrug resistance phenotypes. These transporters are widely distributed among clinically relevant bacteria and have been implicated in resistance to tetracyclines and other structurally diverse antimicrobial agents, making them relevant targets for studies of resistance modulation [21,22]. Together, these targets represent complementary resistance mechanisms, namely drug extrusion via efflux pumps, thereby allowing assessment of phytochemical interactions across distinct but clinically relevant AMR pathways without implying functional inhibition.

The phytochemicals selected in this study, namely quercetin, epigallocatechin gallate (EGCG), kaempferol, berberine, curcumin, and allicin, were chosen based on prior reports of resistance-modulating activity and their diverse chemical scaffolds [23,24]. These compounds possess structural features, such as polyphenolic rings, heterocyclic moieties, and multiple

hydrogen bond donors and acceptors, which may facilitate interactions with protein-binding pockets and membrane-associated targets [25]. Previous studies have suggested that several of these phytochemicals can influence efflux pump activity or interact with bacterial enzymes, supporting their selection for molecular docking against AMR-associated proteins [26]. Together, the selection of efflux pumps (TetB and EmrD) and structurally diverse phytochemicals provides a mechanistic framework for exploring potential ligand-protein interactions relevant to antimicrobial resistance using *in silico* approaches.

Although several *in silico* studies have investigated natural compounds against individual bacterial targets, comparatively fewer studies have focused on resistance-associated proteins that directly contribute to antimicrobial failure. The novelty of the present study lies in the integrated docking-based evaluation of structurally diverse phytochemicals against multiple AMR-relevant targets representing distinct resistance mechanisms, namely efflux-mediated drug extrusion (TetB and EmrD). This unified computational approach enables a comparative assessment of phytochemical interactions with key determinants of AMR, rather than focusing solely on essential growth-related proteins.

Despite these reported observations, further investigation is required to understand better the safety, efficacy, and clinical applicability of phytochemicals. *In silico* techniques have emerged as cost-effective preliminary tools for evaluating phytochemical interactions with bacterial targets. Computational techniques such as molecular docking enable the prediction of ligand-protein interactions and their relative binding affinities, thereby facilitating the identification of compounds with potential interaction capacity prior to experimental validation [27,28]. Such approaches serve as an initial screening step to support and guide subsequent laboratory-based studies.

2. Materials and Methods

2.1. Structure protein acquisition.

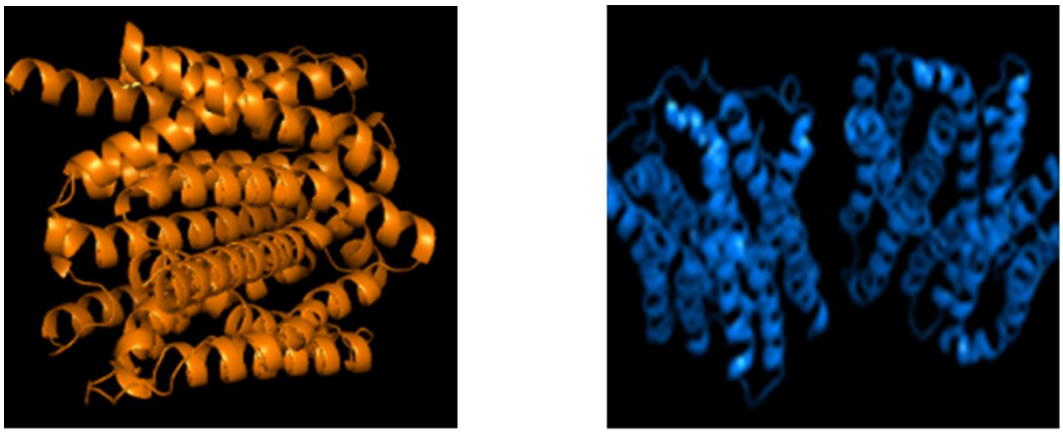
The 3D structure of the emrD efflux pump was obtained from the RCSB Protein Data Bank (PDB ID:2GFP) [29]. The tetB efflux protein structure was obtained from the AlphaFold Protein Structure Database (AlphaFold ID: AF-P23054-F1, UniProt ID: P23054) since the structure data was not available in RCSB PDB (Figure 1) [30]. The AlphaFold model showed high reliability, with an average predicted Local Distance Difference Test (pLDDT) score of 87.94, and most residues exhibited high to very high confidence. Structural validation was performed using visual inspection in UCSF Chimera to confirm stereochemical quality and the absence of major steric clashes.

2.2. Retrieval of bioactive ligand structure.

A set of bioactive compounds, namely allicin, curcumin, berberine, quercetin, epigallocatechin gallate (EGCG), and kaempferol, was selected for this study based on their prior reports of their efflux pump inhibition, antimicrobial activity, and resistance-modulating potential. Ligand selection was guided through an extensive literature review focusing on natural compounds known to interact with bacterial resistance mechanisms. The 2D and 3D structures of the selected phytochemicals were downloaded in SDF format from the PubChem database for further molecular docking analysis [31].

2.3. Protein preparation.

The 3D crystal structure of emrD (PDB ID:2GFP) and tetB were prepared for docking. Since the TetB structure was not available in the RCSB PDB, the AlphaFold predicted model was used. The protein preparation process consisted of the following steps: removal of water molecules, addition of polar hydrogen atoms, assignment of Kollman charges, setting the AD4 atom type, and saving the structure in PDBQT format for docking.



(a) **(b)**

Figure 1. Structure of proteins **(a)** tetB*;* **(b)** emrD.

2.4. Ligand preparation.

The 3D molecular structures of curcumin, allicin, berberine, quercetin, epigallocatechin gallate (EGCG), and kaempferol were downloaded in SDF format from the PubChem database. The ligands were prepared for docking by opening and reading the ligands and by adding polar hydrogens and deleting unnecessary water molecules (Figure 2). Then, Gasteiger charges were assigned, and the structure was converted into PDBQT format for compatibility with AutoDock.

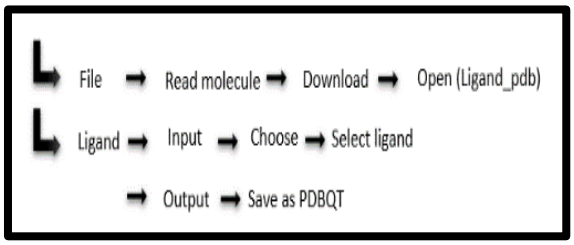


Figure 2. Ligand preparation.

2.5. Binding site identification.

The active site of the target proteins was identified using UCSF Chimera. The amino acid residue located within active sites was selected for grid generation and subsequent molecular docking analysis.

2.6. Grid generation.

Grid boxes were generated to define the binding site region for each target protein (tetB and emrD). The grid box dimensions were optimized to encompass the active site of the proteins completely. For both targets, the grid size was maintained at 40 x 40 x 40 x Å. For tetB, the grid center was set at X = -0.705 Å, Y = 1.373 Å, and Z = 2. 372 Å, whereas for emrD, the grid center was at X = 16.887 Å, Y = 8.601 Å, and Z = 24. 108 Å, corresponding to the

predicted binding pockets. Grid parameter files (txt) were generated using AutoDock Tools for subsequent molecular docking analysis (Figure 3).

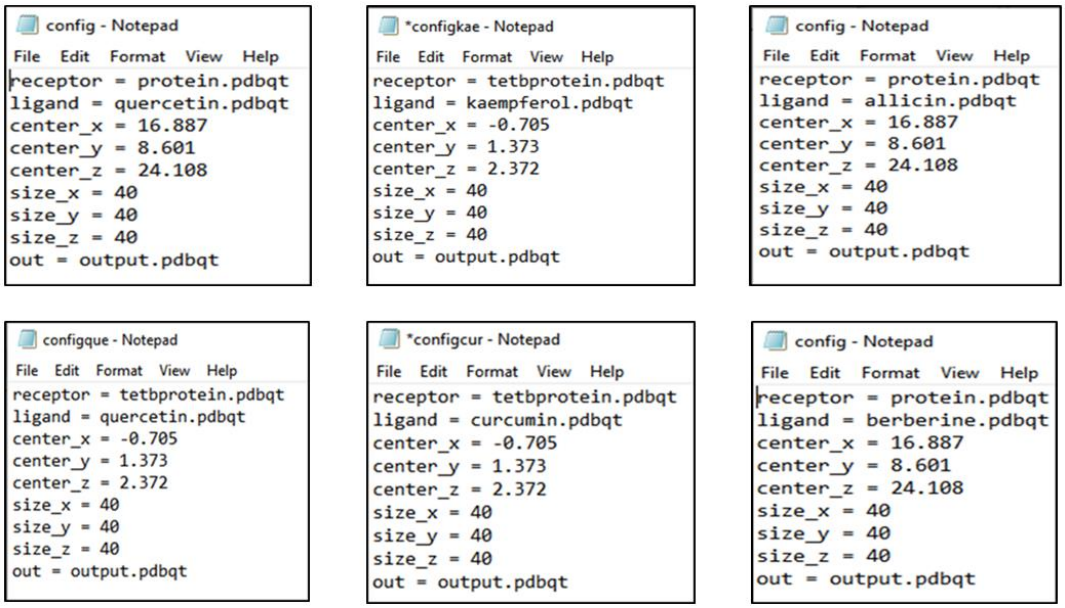


Figure 3. Configuration file.

2.7. Molecular docking.

AutoDock was used for molecular docking to analyze the possible binding orientation and affinity of the ligand with the amino acid residues in the target protein's active site.

2.8. Identification of optimal docked conformation.

Following successful molecular docking, the resulting protein-ligand complexes were evaluated based on their docking scores and binding affinities. A more negative binding energy indicates a stronger interaction between the ligand and the target receptor. Therefore, conformations exhibiting lower binding energy than the reference (known drug) were selected for subsequent analyses.

2.9. Protein ligand interaction.

Finally, the top-scoring docked complexes were visualized to examine the non-bonding interactions between the protein and ligand, providing insights into the docking behavior. The 2D interaction diagrams generated highlighted the key amino acid residues and the types of bonds involved in binding. Additionally, all ligands were visualized for their hydrophobicity profiles using the UCSF Chimera program.

3. Results and Discussion

The molecular docking results provide a comparative assessment of the binding affinity of the selected phytochemicals towards the two proteins involved (tetB and emrD efflux pumps) [Table 2]. Rather than serving as a definitive measure of inhibitor activity, the docking scores were interpreted as an indicator of relative binding preferences and interaction feasibility. Binding affinities were expressed as binding energy values (kcal/mol), and the more negative the values indicated, the relatively stronger the interactions. Emphasis was placed on

comparative trends, recurring interaction patterns, and the consistency of ligand behavior across both targets rather than on minor numerical differences in binding energies.

Table 2. Optimal binding affinity.

Binding energy	Binding strength/interpretation
< -10 kcal/mol	Very strong binding, high affinity, and stable complex
-9 to -7 kcal/mol	Strong binding, suitable for drug-like interactions
-7 to -5 kcal/mol	Moderate binding, possibly effective, but needs further evaluation
-5 to -4 kcal/mol	weak binding, unlikely to be biologically significant
>-4 kcal/mol	Very weak or no binding

The docking results obtained using AutoDock revealed multiple binding conformations of the ligand.

Variations in binding energy were observed among the tested compounds, and since the differences were comparatively modest, they should not be interpreted as decisive indicators of superior inhibitory efficacy. Such variations are primarily reflected as the differences in ligand size, flexibility, and interaction geometry within the binding pocket.

3.1. The docking results obtained using AutoDock revealed multiple binding conformation of the ligand (curcumin, kaempferol, quercetin) with the tetB efflux protein.

The docking results obtained with AutoDock revealed multiple binding conformations for all ligands, with varying binding affinities. Among the tested compounds, quercetin showed the highest binding affinity with a minimum binding energy of -7.743 kcal/mol, followed closely by kaempferol -7.696 kcal/mol [Figure 4].

Curcumin		Quercetin		Kaempferol	
Mode	Affinity (kcal/mol)	Mode	Affinity (kcal/mol)	Mode	Affinity (kcal/mol)
1	-7.743	1	-7.696	1	-6.943
2	-7.318	2	-7.403	2	-6.848
3	-7.268	3	-7.163	3	-6.821
4	-7.226	4	-7.152	4	-6.818
5	-7.121	5	-6.951	5	-6.557
6	-7.058	6	-6.864	6	-6.472
7	-7.033	7	-6.854	7	-6.429
8	-7.006	8	-6.754	8	-6.346
9	-6.993	9	-6.671	9	-6.345

Figure 4. Binding affinity of the ligands (quercetin, kaempferol, curcumin) with tetB.

These results indicate that quercetin and kaempferol have stronger and more stable interactions with the target protein than the other ligands, suggesting greater potential for biological activity.

3.2. Docking visualization using PyMol.

PyMol is a molecular visualization tool used to generate high-quality three-dimensional representations of proteins, ligands, and other biomolecules. It facilitates structural analysis and visualization of molecular interactions [31]. In PyMol, docking visualization is performed by loading the protein-ligand complex obtained from docking results. The 3D visualization tool PyMol enables detailed examination of the binding poses and orientations within the active site.

Molecular docking of curcumin, kaempferol, and quercetin with the tetB efflux protein showed stable binding within the active site. Curcumin interacted with residue THR-399 through hydrogen bonding.

Kaempferol formed hydrogen bonds with LYS-366 and GLY-310, while quercetin interacted with LYS-366, SER-363, and GLY-310, showing the strongest binding affinity among the three (Figure 5). Overall, all three ligands exhibited stable interactions, with quercetin showing the highest potential to inhibit tetB efflux activity by occupying the binding pocket. Hydrogen bonding interactions contributed to the stabilization of several docked complexes, but the hydrogen bonds alone do not directly imply inhibitory activity. Effective inhibition of efflux pumps is likely governed by a combination of factors, including hydrophobic interactions, steric complementarity, and the ability of a ligand to occupy a functionally relevant region of the transporter. Therefore, hydrogen bonds were considered supportive interactions rather than sole determinants of binding effectiveness.

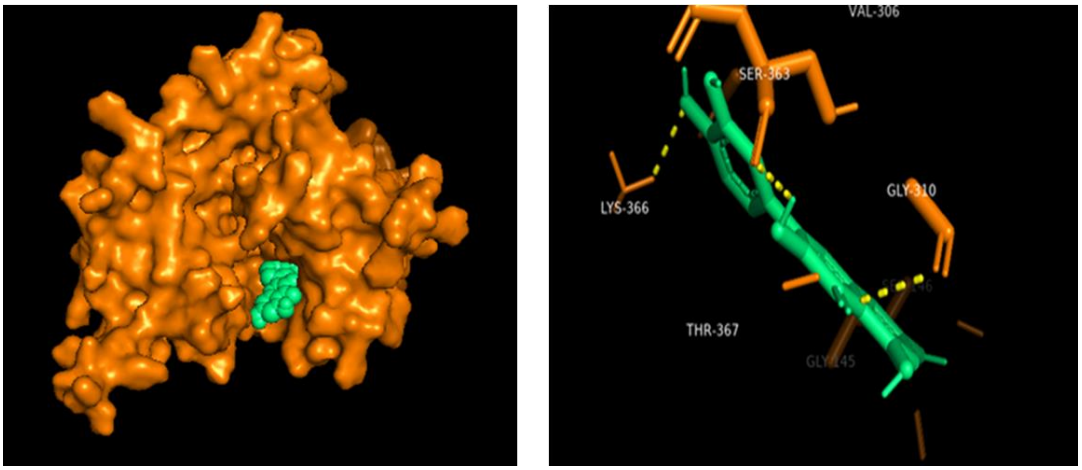


Figure 5. Docking visualization of quercetin against tetB.

3.3. The docking results obtained using AutoDock revealed multiple binding conformations of the ligand (EGCG, curcumin, and berberine) with the emrD protein.

Molecular docking of EGCG, curcumin, and berberine against the emrD efflux pump protein was performed using AutoDock. Among them, EGCG showed the strongest binding affinity (−7.494 kcal/mol), followed by curcumin (−6.819 kcal/mol) and berberine (−6.819 kcal/mol). The results indicate that EGCG has the highest potential to inhibit emrD activity.

EGCG		Curcumin		Berberine	
Mode	Affinity (kcal/mol)	Mode	Affinity (kcal/mol)	Mode	Affinity (kcal/mol)
1	-7.494	1	-6.819	1	-6.819
2	-7.405	2	-6.533	2	-6.533
3	-7.367	3	-6.037	3	-6.037
4	-7.333	4	-6.03	4	-6.03
5	-7.222	5	-5.911	5	-5.911
6	-7.216	6	-5.809	6	-5.809
7	-7.085	7	-5.799	7	-5.799
8	-6.988	8	-5.789	8	-5.798
9	-6.968	9	-5.697	9	-5.697

Figure 6. Binding affinity of ligands (EGCG, curcumin, and berberine).

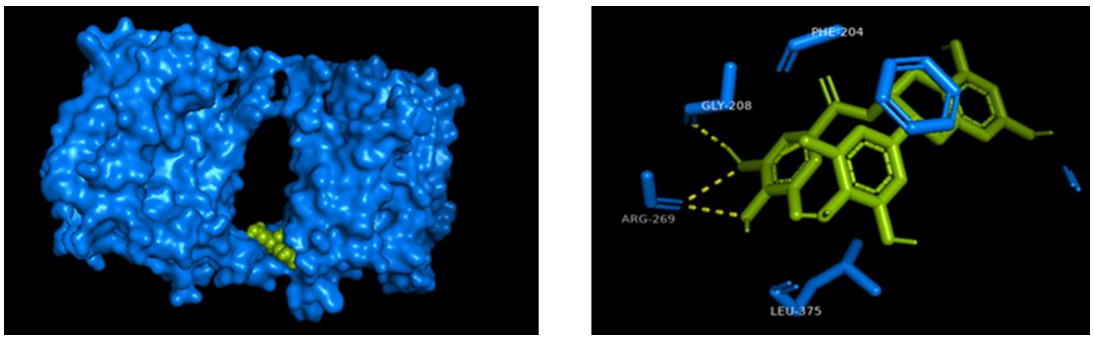


Figure 4. Docking visualization of EGCG against emrD.

Docking analysis showed that EGCG binds stably within the active pocket, forming hydrogen bonds with key residues GLY-208 and ARG-269, suggesting strong inhibitory potential by occupying the binding cavity (Figure 8).

The interaction between curcumin and the emrD protein demonstrated stable binding within the active pocket, as shown in the surface and binding site models. Key amino acid residues, including ARG-269, MET-372, and ARG-269, form hydrogen bonds, indicating that curcumin has strong potential to inhibit emrD efflux activity by occupying the binding site.

The interaction between berberine and the emrD protein shows binding within the active pocket, as visualized in the surface and binding-site representations; however, no significant hydrogen-bond interactions were observed. This may suggest a weaker or non-specific binding.

The computational analysis successfully investigated the potential of six phytochemicals, which are quercetin, EGCG, kaempferol, berberine, curcumin, and allicin inhibitors, against several critical antimicrobial resistance (AMR) proteins, namely the efflux pumps tetB and emrD. The study used molecular docking, employing the criterion that a more negative binding energy (kcal/mol) signifies stronger interaction and greater therapeutic potential.

Among the tested compounds, quercetin exhibited consistent binding interactions with both tetB and emrD. While these observations suggested potential for broad interaction capability, such findings should be interpreted cautiously. The docking results are insufficient to confirm simultaneous inhibition of multiple resistance targets, and experimental validation would be required to substantiate any multitarget inhibitory effects. Against the tetB efflux pump, quercetin demonstrated the highest binding affinity (minimum binding energy of -7.743 kcal/mol), forming strong hydrogen bonds with key amino acid residues such as LYS-366, SER-363, and GLY-310. EGCG displayed the strongest binding potential against the emrD efflux pump, achieving a minimum binding affinity of -7.494 kcal/mol through stable hydrogen bonds with GLY-208 and ARG-269. Meanwhile, berberine and curcumin demonstrated moderate-to-strong binding across targets, with affinities ranging from -7.306 kcal/mol and -6.819 kcal/mol, respectively. In contrast, allicin showed the weakest affinity, -4.149 kcal/mol in the experiments, suggesting a non-specific binding mode and limited inhibitory potential. The discussion therefore, focuses on comparative interaction trends and biological relevance rather than on reiterating fundamental principles of molecular docking.

4. Conclusions

This study utilized molecular docking as a preliminary computational screening tool to assess the binding potential of selected phytochemicals against antimicrobial resistance–related

targets, including the tetB and emrD efflux pumps. Quercetin consistently ranked among the highest-scoring ligands across multiple targets, while EGCG, kaempferol, berberine, and curcumin showed moderate to strong binding affinities depending on the protein. These results indicate that certain phytochemicals exhibit favorable in silico interaction profiles with proteins involved in bacterial resistance mechanisms.

However, these findings represent predictive computational evidence rather than functional or translational validation. Molecular docking is limited by its reliance on static protein structures and simplified scoring algorithms and does not capture protein flexibility, membrane dynamics, solvent effects, or cellular pharmacokinetics. As a result, high docking scores alone cannot be equated with biological activity or antimicrobial efficacy.

Further validation should include molecular dynamics simulations to examine interaction stability, followed by in vitro enzymatic assays, efflux pump inhibition studies, and antimicrobial susceptibility testing. Overall, this work provides a hypothesis-generating framework for prioritizing phytochemicals for subsequent experimental evaluation in antimicrobial resistance research.

Author Contributions

Conceptualization, J.P. and S.N.; methodology, M.D. and J.P.; formal analysis, Y.S. and C.B.; writing—original draft preparation, Y.S., C.B., S.N., M.D., R.M., and J.P. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

Not applicable.

Data Availability Statement

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

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Declared none.

Conflicts of Interest

There is no conflict to declare.

Abbreviations

The following abbreviations are used in this manuscript:

Abbreviation	Definition
AMR	Antimicrobial Resistance
EGCG	Epigallocatechin Gallate
DNA	Deoxyribonucleic acid
PDB	Protein Data Bank
SDF	Structured Data File
UCSF	University of California, San Francisco

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