OPEN ACCESS Journal of Interdisciplinary Research in Allied Health and Pharmacy
ISSN: (Print) (Online)
Journal homepage: https://jirap.ubas.edu.pk/index.php/jirap/index

RESEARCH ARTICLE

In-Silico-Driven Identification of Epigallocatechin Gallate (EGCG) and Berberine as Novel Candidates to Overcome Salmonella Antibiotic Resistance via acrB and gyrB Inhibition

Naima Mukhtar[1], Maaz Ahmad[1], Umme Hani Mustafa[1], Abeera Iftikhar[1]

[1]Informatix BioLabs, Lahore, Pakistan

Correspondence
Naima Ali
E-mail: naimaali469@gmail.com

Conflict of Interest
All the authors have no conflict of interest

Reference
Mukhtar. N.,Ahmad.M.,Mustafa.H., Iftikhar.A.

Published: 30th December 2024

ABSTRACT
Salmonellosis is a common bacterial infection caused by *Salmonella* species, primarily affecting the gastrointestinal tract. It is a major foodborne illness typically transmitted through contaminated water or food. Most *Salmonella* infections are self-limiting, and in severe cases, antibiotics become necessary. However, the problem is that *Salmonella* has developed resistance to multiple antibiotics due to several factors (misuse and overuse of antibiotics, horizontal gene transfer (HGT), efflux pumps, and mutation in target sites). Therefore, antibiotic resistance in *Salmonella is* a growing health concern, making infection harder to treat and increasing mobility and mortality rates. These drastic outcomes have led to the urgent discovery of alternative and highly targeted therapeutic drugs. Key targets, such as Efflux pump and DNA gyrase, help in overcoming Multidrug Resistance (MDR) in *Salmonella*, as they play a crucial role in antibiotic expulsion and bacterial DNA replication. A library of natural inhibitors was selected and assessed for their drug-likeness. Structural Validation and Refinement of both targeted proteins were conducted using ERRAT and PROCHECK, ensuring high-quality models for further interaction. The binding affinities of complex molecules and the dynamic behaviour of ligands with the active sites of acrB and gyrB over time were predicted using molecular docking and molecular dynamics simulations. Protox-3 evaluated the toxicity level of selected drugs. Strong binding affinities of EGCG and berberine with receptor proteins and their pharmacophore properties, highlighting them as novel therapeutic agents against the antibiotic-resistant strains of *Salmonella*.

Keywords: Multidrug resistance (MDR), efflux pump, DNA gyrase, molecular docking, molecular dynamics simulations, natural inhibitors, acrB, gyrB, EGCG, berberine.

1. INTRODUCTION

Salmonella is a rod-shaped, facultative anaerobic, Gram-negative bacterium that causes salmonellosis. It belongs to the Enterobacteriaceae family. The two species that make up the Salmonella genus are *Salmonella enterica* and *Salmonella bongori* (Punchihewage-Don, Ranaweera, & Parveen, 2024). Salmonella can be broadly classified as either typhoidal or non-typhoidal. *Salmonella typhi* and *Salmonella paratyphi* A are typhoidal serovars that infiltrate human cells and produce systemic or potentially fatal illnesses like typhoid and paratyphoid fever. (Johnson, Mylona, & Frankel, 2018). On the other hand, it has been determined that the two serotypes of Salmonella that cause non-typhoidal invasive illness in humans are Typhimurium and Enteritidis (Marchello et al., 2022). According to the World Health Organization (WHO), it is estimated that 550 million people fall ill every year as a result of Salmonella infections caused by unsafe food, including 220 million children younger than five. Antibiotics are commonly prescribed to treat salmonellosis, caused by Salmonella spp (M. A. S. Khan & Rahman, 2022). Unfortunately, Salmonella has developed antimicrobial resistance (AMR) due to the overuse and misuse of antibiotics in human and animal health as well as in agriculture. This has increased the risk of morbidity and mortality by making AMR strains

more severe and challenging to treat (Naushad, Ogunremi, Huang, & Treatment, 2023).

Increased virulence and multidrug resistance in Salmonella have been linked to resistance development driven by genetic alteration and genomic evolution (Talukder et al., 2023). Many Salmonella strains developed resistance to various antibiotic classes. For example, the recently discovered multidrug-resistant Salmonella enterica serovar Typhimurium DT104, which is resistant to ampicillin, chloramphenicol, florfenicol, streptomycin, sulfonamides, and tetracycline, is a serious public health concern because it can cause infections that are fatal and cannot be treated with conventional antibiotics (Alenazy, 2022). Antibiotic resistance from multidrug (MDR) and extensive resistance (XDR) may lead to a post-antibiotic era when bacterial infections are no longer controlled by antibiotics (M. Khan & Shamim, 2022). Pathogenic bacteria employ diverse mechanisms to counteract antibiotics, their most critical environmental threat. Classic examples of resistance mechanisms that bacteria develop are a) Development of Efflux pumps, b) Antibiotic-degrading enzymes, c) Target site modification, and d) L-form switching (Thakur, Uniyal, & Tiwari, 2021). Targeting efflux pumps and other resistance mechanisms is one of the novel therapeutic approaches that must be developed to address the problem of multidrug resistance in Salmonella.

Multidrug-resistant bacteria possess highly developed efflux pump systems that enable the expulsion of various classes of antibiotics. In addition to drug transportation, it supports heavy metal resistance, biofilm formation, colonisation, cell invasion, adhesion, and virulence regulated by quorum sensing (Ebbensgaard, Løbner-Olesen, & Frimodt-Møller, 2020). Among the efflux systems are the resistance-nodulation-division (RND) superfamily, the adenosine triphosphate (ATP)-binding cassette (ABC) superfamily, the major facilitator superfamily (MFS), the small multidrug resistance (SMR) family, and the multidrug and toxic compound extrusion (MATE) family (Dawan, Li, Lu, He, & Ahn, 2022). The RND efflux pumps are directly responsible of multidrug resistance in Salmonella strains since they are polyspecific transporters with the tripartite system (AcrAB-ToIC). Also the acrAB-TolC efflux pump consists of 3 subunits of proteins namely TolC (a porin protein that is a part of the outer membrane of the cell), AcrA (periplasmic adaptor protein) and acrB (occurs on the inner membrane of the cell as a transporter protein) (Colclough et al., 2020). According to (Cheema, Maurya, Kumar, Pandey, & Singh, 2024), As polyspecific transporters with the tripartite system (AcrAB-ToIC), the RND efflux pumps are directly in charge of Salmonella strains' multidrug resistance. Additionally, the three protein subunits that make up the acrAB-TolC efflux pump are TolC (the outer membrane porin protein), AcrA (periplasmCompounds such as lysergol, chanaoclavine, niacin, 4-hydroxy-α-tetralone, ursolic acid, phytol, and others are examples of botanically derived efflux pump inhibitors (EPIs) that have demonstrated great promise as useful therapeutic strategies in treating efflux-induced antimicrobial resistance.ic adaptor protein), and acrB (inner membrane transporter protein). As the evidence provided by (Wang-Kan et al., 2017), the loss of efflux function causes loss of virulence in *S. Typhimurium*. Therefore, AcrB can be a potential target to combat the antibacterial resistance strain of Salmonella. Plant-based polyphenols can be tested to reverse the antibacterial resistance activity in salmonella.

Inhibition of topoisomerases is another approach that has been used as a therapeutic target for developing antibiotics (Salman, Sharma, Kumar, Ethayathulla, & Kaur, 2023). Several antibacterial and anticancer agents target topoisomerases due to their crucial role in enhancing DNA replication, transcription, and chromosomal segregation processes (Gupta, Sachdeva, Salman, & Kaur, 2025). Depending on their mechanism of action, bacterial topoisomerases can be divided into two categories: type I and type II. An enzyme called DNA gyrase, a type IIA topoisomerase found in bacteria, is capable of breaking both strands of one segment of DNA (G-DNA) resulting in the separation of strands of DNA. Gyrase A (gyrA) forms covalent bonds to the single-stranded DNA overhangs via tyrosine amino acids in their active sites, and Gyrase B (gyrB) hydrolyzes ATPs; these are the two subunits of DNA gyrase (Pozdeev, Mogre, & Dorman, 2021). Currently, aminocoumarin medications and fluoroquinolones target DNA gyrase. Both the ATPase site and the quinolone-binding site are targeted by FDA-approved medications. To assess the type and mode of the interaction, a number of experimentally determined crystal structures of inhibitory substances have been reported with DNA gyrase. Furthermore, ongoing efforts are focused on designing novel inhibitors targeting this enzyme. However, drug resistance often compromises the effectiveness of existing inhibitors due to the accumulation of mutations at the binding sites.

This study evaluated plant-based natural inhibitors against acrB and gyrB proteins using molecular docking and molecular dynamics simulations. Additionally, the toxicity and drug-like properties of

these natural inhibitors were assessed using the respective software.

2. MATERIALS & METHODS

2.1 Retrieval of Sequences & Structure Prediction

The amino acid sequences of acrB (CCW73233.1) and gyrB (CCW76519.1) from Salmonellaenterica subsp. enterica serovar Typhimurium DT104 (GeneBank HF937208.1) were obtained from the NCBI GenBank database (https://www.ncbi.nlm.nih.gov/genbank/). AlphaFold 3.0 (https://alphafoldserver.com/) was utilized to create three-dimensional protein structures from the amino acid sequences retrieved from NCBI GenBank (Abramson et al., 2024). The quality of both proteins was assessed by examining their ERRAT score and Ramachandran plot using the web server UCLA-DOE LAB — SAVES v6 (https://saves.mbi.ucla.edu/) (Colovos & Yeates, 1993). The 3D structure of the natural plant polyphenols epigallocatechin-3-gallate (EGCG) (CID 65064), Berberine (CID 2353), Gallic acid (CID 370), and Resveratrol (CID 445154) was downloaded in SDF format from the compound database, PubChem (https://pubchem.ncbi.nlm.nih.gov).

2.2 Molecular Docking Analysis

The docking analysis of all ligands with the target proteins was performed using an online CB-Dock2 server (https://cadd.labshare.cn/cb-dock2/php/blinddock.php)(Liu et al., 2022). The PDB file of acrB and gyrB proteins and the SDF file of all ligands were uploaded to CB-Dock2. Each time one ligand and protein were selected for docking in this way, eight different docking analyses were performed.

2.3 Molecular Dynamics Simulations

2.3.1 iMODS Normal Mode Analysis (NMA)

Normal mode analysis was conducted using the iMODS server (https://imods.iqf.csic.es/)(López-Blanco, Aliaga, Quintana-Ortí, & Chacón, 2014) to investigate the intrinsic dynamics of acrB and gyrB. This approach evaluates large-scale protein motions by analyzing deformability, eigenvalues, and B-factor profiles. The results provide insights into structural stability and functional motions, helping to understand the impact of EGCG binding on protein dynamics.

2.3.2 CABS-Flex 2.0 Molecular Dynamics Simulation

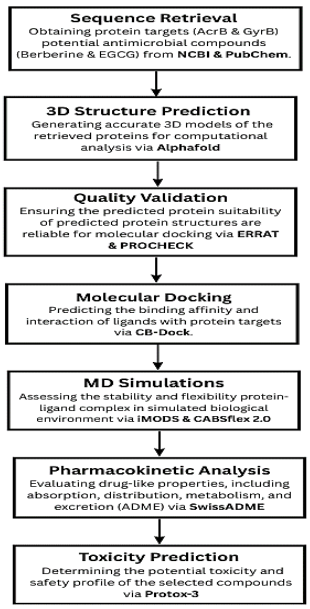
To assess the flexibility of acrB and gyrB proteins upon EGCG binding, molecular dynamics simulations were performed using CABS-flex 2.0 (https://biocomp.chem.uw.edu.pl/CABSflex2/index)(Kurcinski et al., 2019). The input protein structures were subjected to coarse-grained modeling, generating an ensemble of conformations to capture dynamic fluctuations. Residue-level flexibility was quantified through Root Mean Square Fluctuation (RMSF) analysis, identifying regions with significant movement.

2.4 Drug-Likeness Analysis

SWISS-ADME was used for drug-likeness analysis. The files of ligands were downloaded in the SDF format. The files were uploaded on the SwissADME online tool (http://www.swissadme.ch/)(Daina, Michielin, & Zoete, 2017). Ciprofloxacin was used as a standard for Salmonella.

2.5 Toxicity Prediction Using ProTox 3.0

The toxicity characteristics of the two plant-sourced natural inhibitors, epigallocatechin gallate (EGCG) and berberine, were assessed using the ProTox 3.0 online platform (https://tox-new.charite.de/protox_3/)(Banerjee, Kemmler, Dunkel, & Preissner, 2024), a computational resource for predicting rodent acute toxicity, organ toxicity, and toxicity endpoints. The chemical structures of



EGCG (PubChem CID: 65064) and berberine (PubChem CID: 2353) were retrieved in SMILES (Simplified Molecular Input Line Entry System) format from the PubChem database.

These structures were uploaded to ProTox 3.0 to estimate their toxicological properties

Salmonella, illustrating key steps from sequence retrieval to toxicity prediction.

Figure-1: Computational screening workflow for antimicrobial compounds against antibiotic-resistant

3. RESULTS

3.1 Assessing acrB & gyrB Protein Quality

The ERRAT Overall Quality Factor of acrB and gyrB are 97.8304 and 97.3046, respectively, confirming the protein structure is best for further analysis.

The Ramachandran plot analysis of acrB and gyrB reveals that most residues fall within the most favored regions, indicating a high-quality protein model. Specifically, 95.8% of the residues (872 out of 910 non-glycine and non-proline residues) and 94.8% of non-glycine and non-proline residues (676 of 713) are located in the most favored regions [A, B, L] of acrB and gyrB, respectively. This high percentage suggests that the protein's backbone dihedral angles are within the expected ranges for stable secondary structures such as alpha-helices and beta-sheets.

Additionally, 4.2% of the amino acids (38aa) of acrB and a minor proportion of amino acids (5.0%, 36aa) of gyrB populate in the additional allowed regions [a, b, l, p], which are also considered acceptable for protein structures. Notably, there are no residues of both proteins in the generously allowed regions [~a, ~b, ~l, ~p] in the disallowed regions, indicating both models are best for molecular docking.

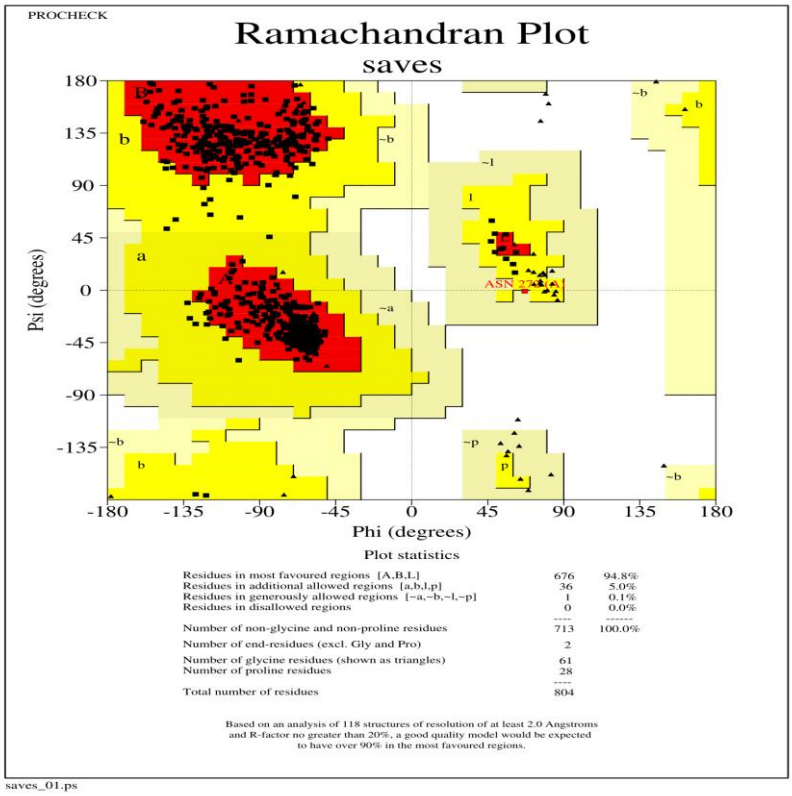


Figure-2: shows the Ramachandran plot of the gyrB protein

3.2. Potential Affinity of all Ligands toward acrB and gyrB Proteins

The results of the comparative analysis revealed that EGCG had the highest binding affinity for acrB and gyrB, represented by the lowest binding energies of -9.8 and -10.3 Kcal/mol, respectively. Among all ligands interacting with the acrB and gyrB protein, EGCG demonstrates the highest degree of polar interaction, evidenced by its binding to seven distinct amino acid residues ARG 815, PHE 617, ASN 81, GLN 89, THR 676, GLY 861, TYR 77 of acrB and three amino acid residues PHE 458, ARG 480, GLN 263 of gyrB protein as shown in table 1.

Similarly, berberine and resveratrol showed binding energies of (-8.3, -7.4) Kcal/mol with acrB and (-9.3, -7.7) Kcal/mol upon interacting with gyrB protein respectively. Both ligands exhibited weak binding affinities for the acrB and gyrB proteins, as berberine forms polar interactions with ARG815 and TYR77 in the acrB protein and ARG 389 in the gyrB protein. In contrast, Resveratrol interacts with hydrophobic residues (TYR 35, ALA 299, ALA 297, THR 295, and ALA 42) and ASP 481, ARG 480, and GLN 517 in both acrB and gyrB proteins respectively as depicted in figure 1.

Finally, the gallic acid ligand exhibited the lowest binding energies with acrB and ayrB. It demonstrated a binding affinity of -6.3 kcal/mol with acrB by interacting with the amino acid residues ILE-38, ALA-39, and ALA-294. In the case of GyrB, it showed a binding affinity of -6.5 kcal/mol, forming polar interactions with GLU-454, LYS-233, ASN-259, GLN-263, and ASN-265 as shown in Table 1.

Table-1: Docking interaction of all ligands with acrB and gyrB

Receptor protein	Ligands	Vina Score (kcal/mol)	Polar Interaction with Ligands
acrB	EGCG	-9.8	ARG 815, PHE 617, ASN 81, GLN 89, THR 676, GLY 861, TYR 77
	Berberine	-8.3	ARG 815, TYR 77
	Gallic acid	-6.3	ILE 38, ALA 39, ALA294
	Resveratrol	-7.4	TYR 35, ALA 299, ALA 297, THR 295, ALA 42
gyrB	EGCG	-10.3	PHE 458, ARG 480, GLN 263
	Berberine	-9.3	ARG 389
	Gallic acid	-6.5	GLU-454, LYS-233, ASN-259, GLN-263, ASN-265
	Resveratrol	-7.7	ASP-481, ARG-480, GLN-517

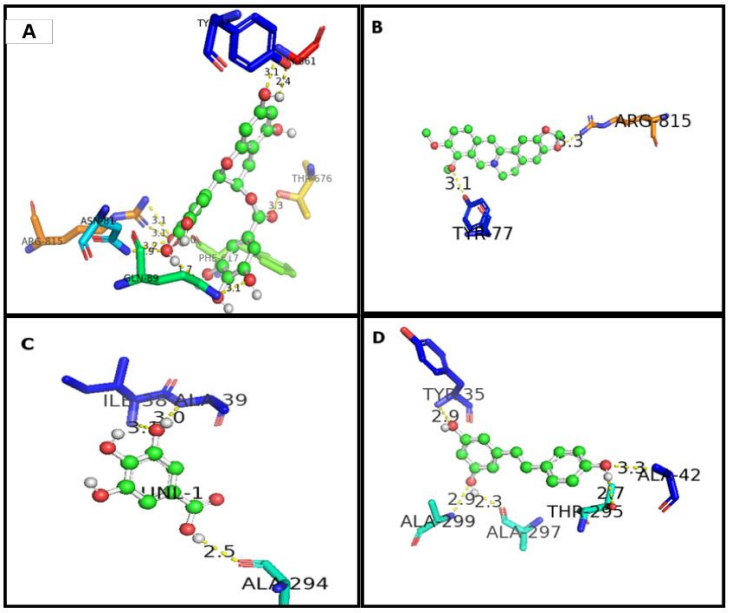


Figure-4: Docked Complexes selected ligands with acrB protein: (A) ECGC (B) berberine (C) gallic acid (D) resveratrol

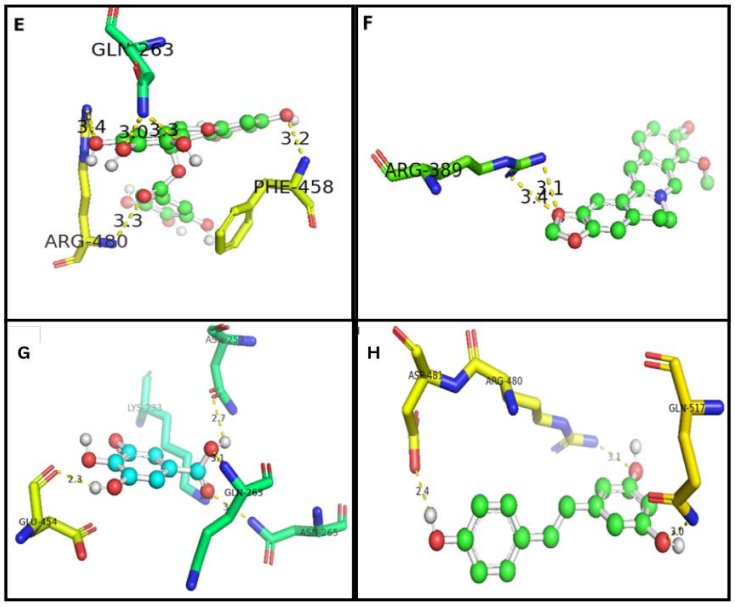


Figure-5: Docked complexes of selected ligands gyrB protein: (E) EGCG (F) berberine (G) gallic acid (H) Resveratrol

3.3 Molecular Dynamics Simulations

3.3.1 Via iMODS

Normal Mode Analysis (NMA) of acrB and gyrB (with EGCG) reveals distinct differences in flexibility, residue interactions, and overall dynamics. AcrB remains notably flexible at specific residues, showing strongly correlated and anti-correlated motions, while gyrB undergoes structural shifts influenced by EGCG binding. The covariance matrices highlight the collective atomic motion of acrB, whereas gyrB displays changes suggesting stabilization or disruption due to ligand interaction. Additionally, acrB's lower rigidity contrasts with gyrB's increased structural constraints in the presence of EGCG, indicating a shift in dynamic behavior.

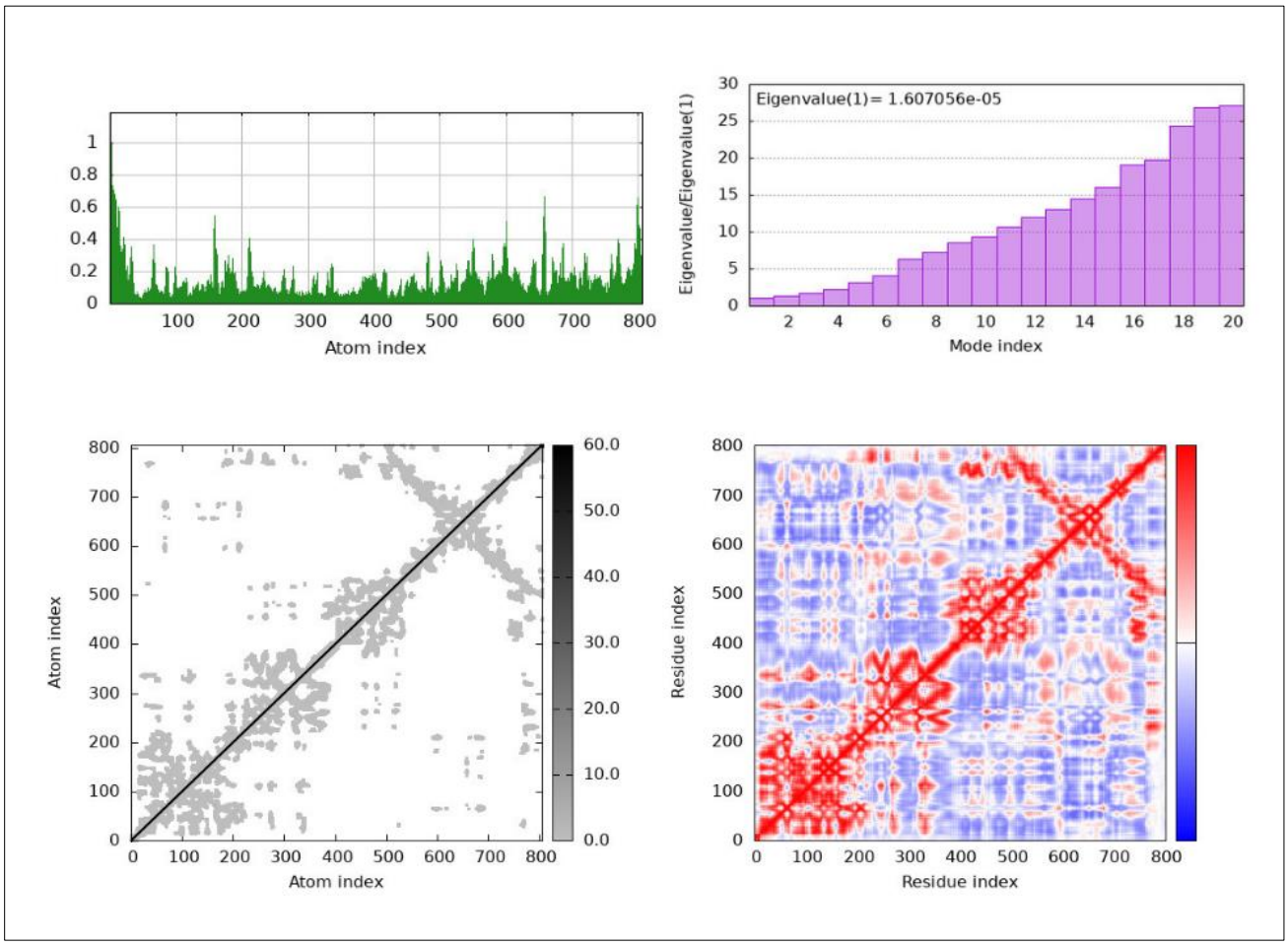


Figure 6: Normal mode analysis (NMA) results for the gyrB protein. (A) Atomic fluctuations illustrate the flexibility of each atom. (B) Eigenvalue distribution highlights the significance of each mode. (C) The covariance matrix represents correlations in atomic motion. (D) The cross-correlation map depicts residue-residue dynamic interactions, with red regions indicating correlated movements and blue regions reflecting anti-correlated movements.

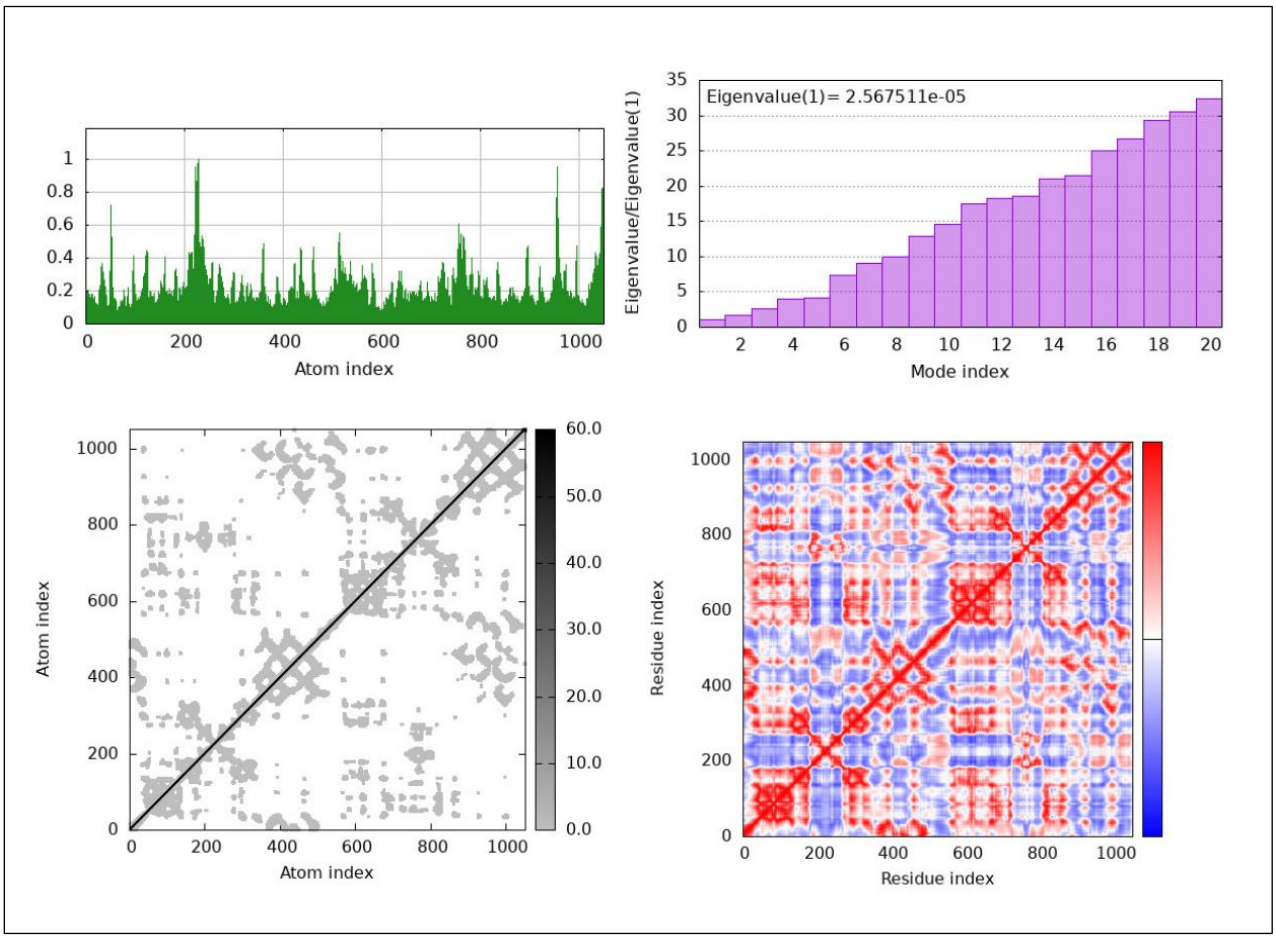


Figure 7: Normal mode analysis (NMA) results for the acrB protein after docking with EGCG (A) Atomic fluctuations represent the mobility of each atom. (B) Eigenvalue distribution depicting the contribution of different modes to the overall motion. (C) Covariance matrix illustrating the correlation between atomic motions. (D) A cross-correlation map shows the dynamic coupling between residues, where red and blue regions indicate correlated and anti-correlated movements, respectively.

Upon docking with Berberine, acrB exhibits lower eigenvalues in the analyzed modes, indicating softer, more flexible movements, whereas gyrB has slightly higher eigenvalues, suggesting relatively stiffer dynamics. The residue fluctuation profiles further emphasize this contrast—acrB shows broader fluctuations across multiple residues, implying a more flexible structural framework, while gyrB displays more localized fluctuations, indicative of a rigid core with specific flexible regions. Additionally, the mode index plots suggest that acrB's collective motions involve larger-scale conformational changes, whereas gyrB's dynamics are more restricted to localized vibrations, highlighting the functional adaptations of each protein, with AcrB emphasizing dynamic motion for transport and gyrB maintaining a stable conformation for enzymatic efficiency.

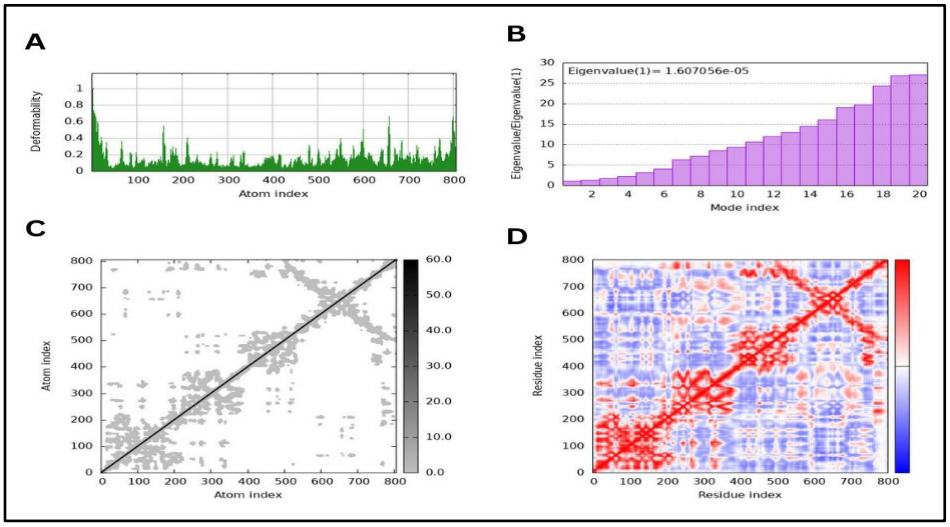


Figure-8: Normal Mode Analysis (NMA) of gyrB after docking with Berberine: (A) Deformability plot showing residue-specific flexibility, with peaks indicating highly flexible regions. (B) Eigenvalue distribution, where lower eigenvalues suggest increased flexibility and large-scale motions. (C) Covariance matrix representing correlated atomic movements, with darker regions indicating stronger correlations. (D) Correlation matrix illustrating inter-residue communication, with red indicating correlated motions and blue indicating anti-correlated motions.

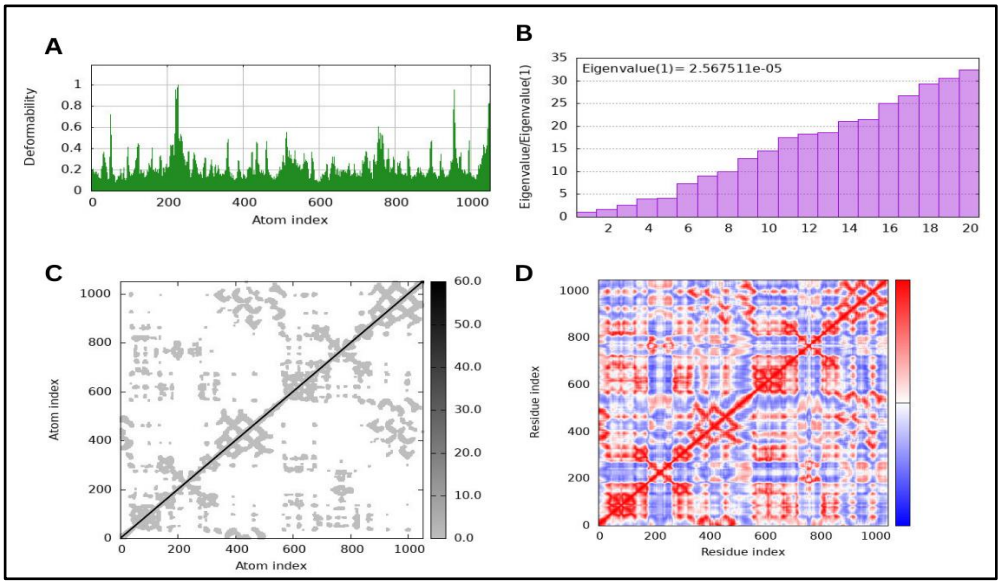


Figure-9: Normal Mode Analysis (NMA) of acrB after docking with Berberine: (A) Deformability plot highlighting residue-specific flexibility, with peaks indicating highly flexible regions. (B) Eigenvalue distribution, where lower eigenvalues suggest increased structural flexibility and larger conformational changes.(C) Covariance matrix illustrating correlated atomic motions, with darker regions representing stronger correlations.(D) Correlation matrix depicting inter-residue interactions, where red indicates correlated movements and blue represents anti-correlated motions.

Via CABS-flex 2.0

The molecular dynamics (MD) simulation using the CABS-flex approach provided valuable insights into the structural flexibility of the acrB and gyrB proteins when complexed with EGCG (Epigallocatechin gallate) and berberine. The Root Mean Square Fluctuation (RMSF) plots illustrate the residue-specific fluctuations, revealing key regions of structural variability as shown in Figure-10.

For acrB docked with EGCG, the RMSF data highlights a significant peak around residue A202, indicating a highly flexible region. This suggests the presence of an unstructured loop or a domain prone to conformational changes. Aside from this peak, the fluctuations remain relatively moderate, suggesting that most of the protein maintains structural stability. The 3D ensemble visualization confirms this observation, displaying a well-formed secondary structure with some flexible loops (shown in Figure 10, B). In contrast, gyrB exhibits lower overall fluctuations than acrB, with the most prominent peak occurring near the N-terminal region. The structural ensemble of gyrB demonstrates a more compact and stable conformation, with localized flexibility in certain regions (shown in Figure-10, A)

While docking with berberine the data showed significant peaks in acrB observed around residue 202 and residue 502, indicating enhanced dynamic behavior. Similarly, in gyrB, fluctuations were more widespread, with multiple peaks suggesting structural adaptability. Structural representations of ensemble models further illustrated these flexible regions (Top panels of figure-11), emphasizing potential sites for conformational changes.

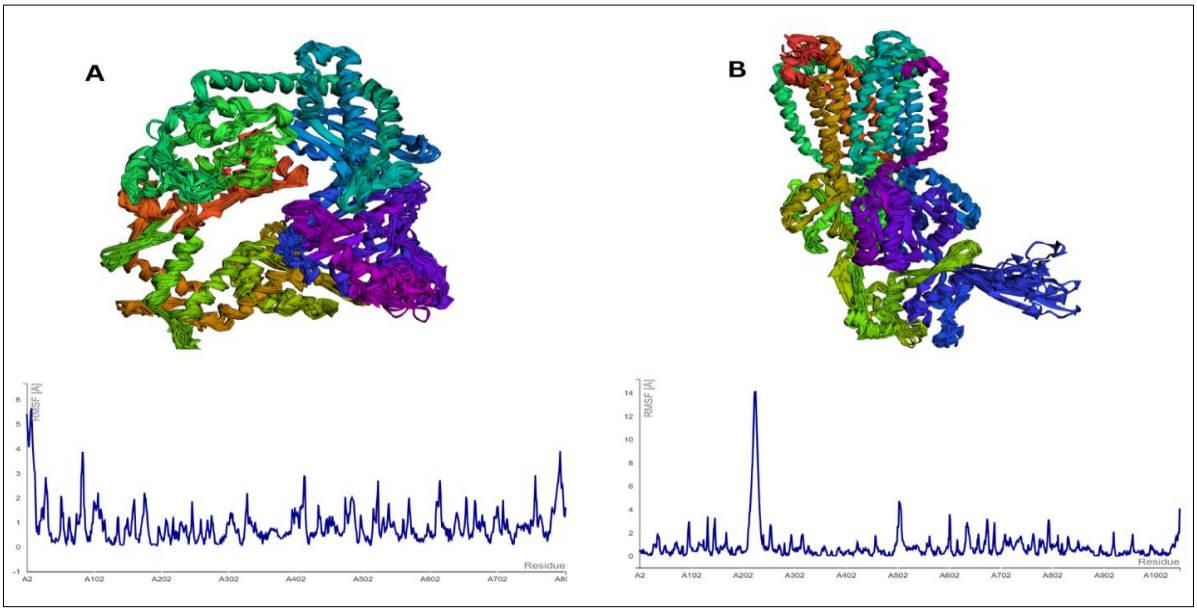


Figure-10: CABS-flex MD simulation results for gyrB (A) and acrB (B) docked with EGCG. The top panels depict structural ensembles, highlighting flexibility in different regions. The bottom panels show RMSF plots, where a significant peak in *acrB* around residue A202 indicates high flexibility, while *gyrB* exhibits a more compact structure with localized fluctuations.

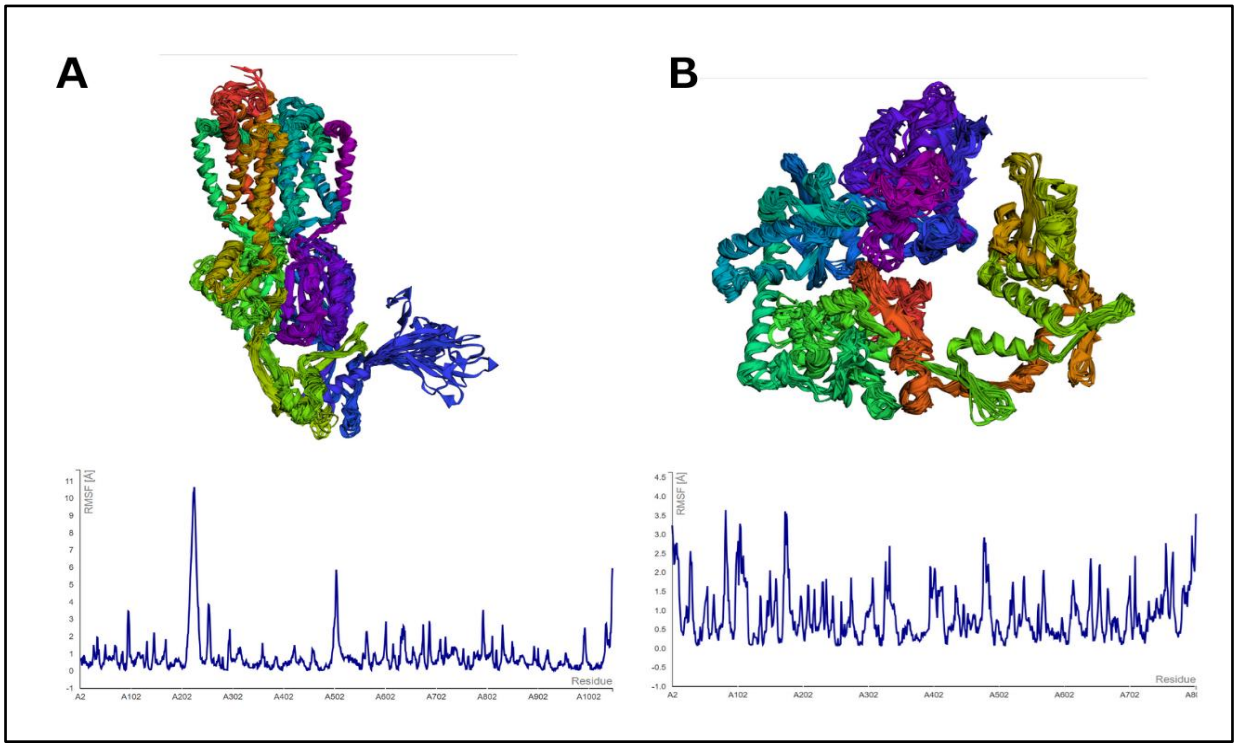


Figure-11: CABS-flex MD simulation results for (A) acrB & (B) gyrB docked with Berberine. The top panels depict structural ensembles, highlighting flexibility in different regions. The bottom panels show RMSF plots showing similar localized fluctuations as that of docking complexes with EGCG.

3.4 Pharmacokinetic Analysis

SwissADME was used to perform pharmacokinetic analysis. According to the evaluations, Berberine shows that it has a molecular weight of 336.36 Da, falling within the drug-like range. It has a moderate lipophilicity (LogP = 3.1), indicating good permeability and high gastrointestinal (GI) absorption, making it suitable for oral administration. Additionally, Berberine can cross the blood-brain barrier (BBB), suggesting potential effects on the central nervous system (CNS). Its bioavailability score of 0.55 suggests moderate oral bioavailability, and it follows Lipinski's Rule of Five without any violations, indicating good drug-likeness. The SwissADME analysis of EGCG (Epigallocatechin gallate) shows that it has a molecular weight of 371.52 Da, which is within the drug-like range but violates Lipinski's Rule of Five twice due to its high number of hydrogen bond donors and acceptors. It has low gastrointestinal (GI) absorption, indicating poor oral bioavailability (0.17), and high lipophilicity (LogP = 6.0), suggesting poor permeability. Additionally, EGCG does not cross the blood-brain barrier (BBB), limiting its potential for CNS-related effects.

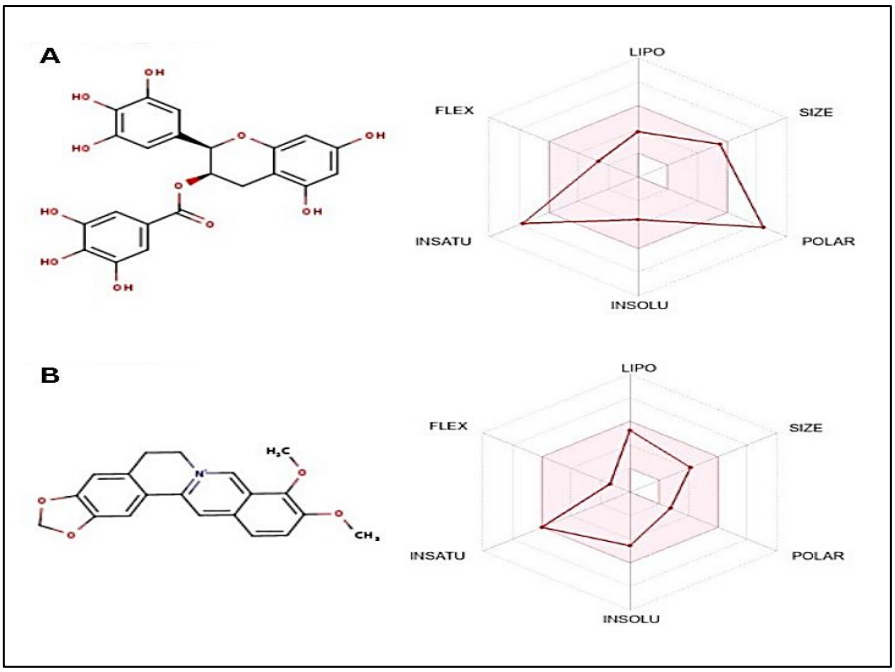


Figure-12: **SwissADME Analysis of EGCG & Berberine:** Two bioactive compounds are selected, including (A) Epigallocatechin Gallate (EGCG) and (B) Berberine, and the main attention is paid to the SwissADME analysis. On the left of each picture, there is a molecular formula of EGCG and Berberine. Radar plots of their physicochemical properties include the lipophilicity (LIPO), the size, the polarity (POLAR), the insolubility (INSOLU), the unsaturation (INSATU), and the flexibility (FLEX) on the right hand side. The compounds depend on these properties to determine their drug-likeness and drug pharmacokinetics. This SwissADME analysis identifies two bioactive compounds such as Epigallocatechin Gallate (EGCG) and Berberine. At the left to both images, the molecular description of EGCG and Berberine appear. Their physicochemical properties are represented by radar plots on the right side; they are lipophilicity (LIPO), size, and polarity (POLAR), insolubility (INSOLU), and unsaturation (INSATU) and flexibility (FLEX). Such properties help in the prediction of drug-likeness and pharmacokinetic of the compounds.

3.5 Toxicity Prediction

The ProTox-3 toxicity prediction classifies Berberine as moderately toxic (LD50: 200 mg/kg, Toxicity Class 3). It exhibits active neurotoxicity (0.55), suggesting potentially harmful effects on the nervous system, and active respiratory toxicity (0.76), indicating possible lung toxicity. However, hepatotoxicity is inactive (0.82), meaning Berberine poses a low risk of liver toxicity. Additionally, it shows active carcinogenicity (0.56) and mutagenicity (0.62), implying a potential risk of cancer and genetic mutations. Berberine also has high immunotoxicity (0.99**)**, suggesting significant effects on the immune system. Furthermore, it inhibits multiple CYP enzymes (CYP1A2, CYP2C19, CYP2D6, and CYP3A4), indicating the potential for drug interactions.

The ProTox-3 toxicity prediction classifies EGCG as less toxic (LD50: 1190 mg/kg, Toxicity Class 4) compared to Berberine. However, it shows active hepatotoxicity (0.69), indicating a risk of liver toxicity, and active neurotoxicity (0.87**),** suggesting potential harm to the nervous system. Unlike Berberine, EGCG is not carcinogenic or mutagenic, making it a safer option in terms of genetic and cancer-related risks. However, it exhibits high immunotoxicity (0.96) and active respiratory toxicity (0.98), indicating possible immune system and lung-related toxicity. In terms of metabolism, EGCG inhibits CYP3A4 (0.71) but does not significantly

affect other CYP enzymes, reducing the likelihood of drug interactions.

Table 2: Toxicity Assessment of EGCG and Berberine as Potential Therapeutic Agent

Toxicity Parameters	EGCG	Berberine
Toxicity Class	4 (Less toxic)	3 (Moderately toxic)
LD50 (Lethal Dose, mg/kg)	1190 mg/kg (Less toxic)	200 mg/kg (More toxic)
Hepatotoxicity (Liver Toxicity)	Active (Risky)	Inactive (Safe)
Neurotoxicity	High Risk	Moderate Risk
Carcinogenicity (Cancer Risk)	Inactive (No Risk)	Active (Possible Risk)
Mutagenicity (Genetic Mutation Risk)	Inactive (No Risk)	Active (Possible Risk)
Immunotoxicity (Immune System Risk)	High Risk	Higher Risk
Blood-brain barrier (BBB) Penetration	Cannot Cross	Can Cross

4. DISCUSSION

Molecular docking was carried out using berberine, EGCG, resveratrol, and gallic acid to evaluate their interactions with acrB and gyrB. Among these, berberine and EGCG demonstrated the strongest binding affinities, making them the most promising candidates for further analysis. This selection was based on their lower binding energy profiles, indicating more stable interactions with the target proteins.

The computational analysis of acrB and gyrB proteins with EGCG and berberine ligands provided valuable insights into their potential as drug targets in overcoming *Salmonella's* multiple antibiotic resistance. The docking results indicated strong binding affinities between the ligands and their targeted protein, suggesting a promising inhibitory effect.

The molecular docking analysis revealed that EGCG has a high binding affinity with acrB, a key efflux pump protein responsible for antibiotic resistance in *Salmonella*. The identified binding site showed strong interactions with critical residues, including hydrophobic links and hydrogen bonds that are essential for stabilizing ligands within the active site. This suggests that EGCG may effectively block the efflux activity of acrB, thereby enhancing the intracellular retention of antibiotics. Moreover, molecular dynamics simulations supported the stability of the acrB- EGCG complex, further reinforcing its potential as an effective efflux pump inhibitor.

Docking study analysis between berberine and gyrB, a sub unit of DNA gyrase, which plays a pivotal role in replication of bacteria DNA, established high binding affinity. The gyrB-berberine complex formed very strong hydrogen bonding and π- π stacking with some key active site residues which indicate efficient binding of gyrB inhibition. As gyrB is a well characterized target of fluoroquinolone antibiotics, the high binding affinity demonstrates that berberine may be used as a natural antimicrobial reagent to stop bacterial DNA synthesis and alleviate resistance phenomenon.

AcrB inhibition could in itself limit the expulsion capacity of Salmonella to antibiotics to a low level and turn the resistant bacteria vulnerable to drugs and this is likely to affect the drug efficacy positively. In the meantime, gyrB inhibition by berberine might directly harm the bacterial replication thereby exhibiting a synergistic effect when combined with other available antibiotics. These results indicate that the dual-target method may be a chance to reduce multi-drug resistance (MDR) of Salmonella infection. However, the hurdle is that EGCG has shown good docking results because EGCG has an effective binding affinity with both acrB and gyrB protein, but berberine has minimal toxicity risk and high bioavailability. Overall, this study highlights the potential of EGCG and berberine as novel inhibitors of efflux and DNA gyrase proteins in *Salmonella*, paving the way for developing alternative therapeutic strategies to combat antibiotic resistance.

This study lays the groundwork for developing more effective treatment strategies against multidrug-

resistant (MDR) *Salmonella*. Integrating predictive models and advanced simulations into preclinical research will enhance drug screening and clinical decision-making, paving the way for more personalized and effective treatments. These advancements could significantly reduce the burden of antibiotic resistance, contributing to better patient outcomes and global public health.

5. CONCLUSION

This study provides valuable insights into the importance of targeting efflux pumps and DNA gyrase to combat multidrug-resistant **(***MDR***)** *Salmonella*. Molecular docking and simulation studies revealed that EGCG and berberine exhibit strong interactions with acrB and gyrB**,** suggesting their potential as alternative therapeutic agents. However, toxicity assessments indicate that EGCG's hepatotoxicity and berberine's mutagenic risks require further exploration. Moving forward**,** in vivo studies and structural modifications will be essential to refine these compounds for safer and more effective clinical applications.

6. LIMITATION

Computational tools like molecular docking, virtual screening, and in silico drug design are valuable in identifying potential treatments for *Salmonella*. However, their predictions require validation through in vitro and in vivo studies to confirm safety and efficacy. These models simplify biological systems and cannot fully account for bacterial resistance, metabolism, or host interactions. Limitations include the inability to predict drug degradation, efflux pump activity, or precise toxicity and bioavailability. While essential for early drug discovery, computational approaches must be combined with laboratory testing to develop effective treatments for antibiotic-resistant *Salmonella*.

7. REFERENCE

1. Abramson, J., Adler, J., Dunger, J., Evans, R., Green, T., Pritzel, A., . . . Jumper, J. M. (2024). Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature, 630*(8016), 493-500. doi:10.1038/s41586-024-07487-w
2. Alenazy, R. J. J. o. K. S. U.-S. (2022). Antibiotic resistance in *Salmonella*: Targeting multidrug resistance by understanding efflux pumps, regulators and the inhibitors. *34*(7), 102275.
3. Banerjee, P., Kemmler, E., Dunkel, M., & Preissner, R. (2024). ProTox 3.0: a webserver for the prediction of toxicity of chemicals. *Nucleic Acids Res, 52*(W1), W513-w520. doi:10.1093/nar/gkae303
4. Cheema, H. S., Maurya, A., Kumar, S., Pandey, V. K., & Singh, R. M. J. M. C. (2024). Antibiotic potentiation through phytochemical-based efflux pump inhibitors to combat multidrug resistance bacteria. *20*(6), 557-575.
5. Colclough, A. L., Alav, I., Whittle, E. E., Pugh, H. L., Darby, E. M., Legood, S. W., . . . Blair, J. M. J. F. m. (2020). RND efflux pumps in Gram-negative bacteria; regulation, structure and role in antibiotic resistance. *15*(2), 143-157.
6. Colovos, C., & Yeates, T. O. (1993). Verification of protein structures: patterns of nonbonded atomic interactions. *Protein Sci, 2*(9), 1511-1519. doi:10.1002/pro.5560020916
7. Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports, 7*(1), 42717. doi:10.1038/srep42717
8. Dawan, J., Li, Y., Lu, F., He, X., & Ahn, J. J. P. (2022). Role of efflux pump-mediated antibiotic resistance in quorum sensing-regulated biofilm formation by *Salmonella* Typhimurium. *11*(2), 147.
9. Ebbensgaard, A. E., Løbner-Olesen, A., & Frimodt-Møller, J. J. A. (2020). The role of efflux pumps in the transition from low-level to clinical antibiotic resistance. *9*(12), 855.
10. Gupta, D., Sachdeva, E., Salman, M., & Kaur, P. (2025). Topoisomerases as targets for halting bacterial DNA replication. In *Bacterial Enzymes as Targets for Drug Discovery* (pp. 187-214): Elsevier.
11. Johnson, R., Mylona, E., & Frankel, G. J. C. m. (2018). Typhoidal *Salmonella*: Distinctive virulence factors and pathogenesis. *20*(9), e12939.
12. Khan, M., & Shamim, S. J. M. (2022). Understanding the mechanism of antimicrobial resistance and pathogenesis of *salmonella* enterica serovar typhi. *10*(10), 2006.
13. Khan, M. A. S., & Rahman, S. R. J. V. S. (2022). Use of phages to treat antimicrobial-resistant *Salmonella* infections in poultry. *9*(8), 438.
14. Kurcinski, M., Oleniecki, T., Ciemny, M. P., Kuriata, A., Kolinski, A., & Kmiecik, S. (2019). CABS-flex standalone: a simulation environment for fast modeling of protein flexibility. *Bioinformatics, 35*(4), 694-695. doi:10.1093/bioinformatics/bty685
15. Liu, Y., Yang, X., Gan, J., Chen, S., Xiao, Z.-X., & Cao, Y. (2022). CB-Dock2: improved protein–

ligand blind docking by integrating cavity detection, docking and homologous template fitting. *Nucleic Acids Research, 50*(W1), W159-W164. doi:10.1093/nar/gkac394 %J Nucleic Acids Research
16. López-Blanco, J. R., Aliaga, J. I., Quintana-Ortí, E. S., & Chacón, P. (2014). iMODS: internal coordinates normal mode analysis server. *Nucleic Acids Res, 42*(Web Server issue), W271-276. doi:10.1093/nar/gku339
17. Marchello, C. S., Birkhold, M., Crump, J. A., Martin, L. B., Ansah, M. O., Breghi, G., . . . Kim, J.-H. J. T. L. I. D. (2022). Complications and mortality of non-typhoidal *salmonella* invasive disease: a global systematic review and meta-analysis. *22*(5), 692-705.
18. Naushad, S., Ogunremi, D., Huang, H. J. S.-P. f. L.-C. P., Control, & Treatment. (2023). *Salmonella*: a brief review.
19. Pozdeev, G., Mogre, A., & Dorman, C. J. (2021). Consequences of producing DNA gyrase from a synthetic gyrBA operon in *Salmonella* enterica serovar Typhimurium. *115*(6), 1410-1429. doi:https://doi.org/10.1111/mmi.14689
20. Punchihewage-Don, A. J., Ranaweera, P. N., & Parveen, S. J. F. i. A. (2024). Defense mechanisms of *Salmonella* against antibiotics: a review. *3*, 1448796.
21. Salman, M., Sharma, P., Kumar, M., Ethayathulla, A., & Kaur, P. J. B. i. F. G. (2023). Targeting novel sites in DNA gyrase for development of anti-microbials. *22*(2), 180-194.
22. Talukder, H., Roky, S. A., Debnath, K., Sharma, B., Ahmed, J., Roy, S. J. J. o. e., & health, g. (2023). Prevalence and antimicrobial resistance profile of *Salmonella* isolated from human, animal and environment samples in South Asia: a 10-year meta-analysis. *13*(4), 637-652.
23. Thakur, V., Uniyal, A., & Tiwari, V. J. E. J. o. P. (2021). A comprehensive review on pharmacology of efflux pumps and their inhibitors in antibiotic resistance. *903*, 174151.
24. Wang-Kan, X., Blair, J. M. A., Chirullo, B., Betts, J., Ragione, R. M. L., Ivens, A., . . . Piddock, L. J. V. (2017). Lack of AcrB Efflux Function Confers Loss of Virulence on *Salmonella* enterica Serovar Typhimurium. *8*(4), 10.1128/mbio.00968-00917. doi:doi:10.1128/mbio.00968-17