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In Silico Study of the Antibacterial Activity of *Acalypha indica* L. Compounds Against *Staphylococcus aureus* DNA Gyrase Protein

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Abstract

Flavonoids from *Acalypha indica* exhibit potential antibacterial activity against *Staphylococcus aureus*, particularly through inhibition of DNA gyrase B. This study evaluated molecular interactions of mauritanin, acalyphin, quercetin, and kaempferol using in silico approaches, including molecular docking, molecular dynamics simulation, and ADMET prediction. Docking results showed that mauritanin had the lowest binding energy (-8.5 kcal/mol) and formed stable interactions with key residues in the active site, corroborated by 50 ns molecular dynamics simulations. Pharmacokinetic and drug-likeness predictions indicated that mauritanin and acalyphin had favorable profiles, with high gastrointestinal absorption and low toxicity risk. The other flavonoids showed higher permeability across the central nervous system, potentially beneficial for CNS-targeted therapies. These findings support mauritanin as a promising lead compound for novel antibacterial agent development, warranting further in vitro and in vivo validation.

Keywords: *Acalypha indica*; flavonoid; *Staphylococcus aureus*; DNA gyrase B; molecular docking; ADMET.

Abbreviations: Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET); Adenosine Triphosphate (ATP); Blood–Brain Barrier (BBB); Chemistry at HARvard Macromolecular Mechanics (CHARMM); Central Nervous System (CNS); Deoxyribonucleic Acid (DNA); Density Functional Theory (DFT); Gastrointestinal (GI); Groningen Machine for Chemical Simulations (GROMACS); Molecular Dynamics (MD); Molecular Mechanics–Generalized Born Surface Area (MM-GBSA); Merck Molecular Force Field 94 (MMFF94); Methicillin-Resistant *Staphylococcus aureus* (MRSA); Constant Number of Particles, Pressure, and Temperature Ensemble (NPT); Constant Number of Particles, Volume, and Temperature Ensemble (NVT); Protein Data Bank (PDB); Pharmacokinetics using Graph-Based Signatures Model (pkCSM); Quantitative Structure–Activity Relationship (QSAR); Research Collaboratory for Structural Bioinformatics (RCSB); Root Mean Square Deviation (RMSD); Root Mean Square Fluctuation (RMSF); Structure Data File (SDF); Three-Dimensional (3D); Two-Dimensional (2D)

INTRODUCTION

Antimicrobial resistance remains one of the most pressing global health challenges of the 21st century. Among the resistant pathogens, *Staphylococcus aureus*—particularly methicillin-resistant *S. aureus* (MRSA)—continues to cause serious infections that are increasingly difficult to treat (Munir et al., 2022). DNA gyrase, a type II bacterial topoisomerase, is an essential enzyme responsible for introducing negative supercoils into DNA during replication and transcription. Because it has no human homolog, DNA gyrase serves as a selective and validated antibacterial drug target (Collin & Maxwell, 2011; Salman et al., 2023).

Fluoroquinolones, which target the A and B subunits of DNA gyrase, have been highly effective antibacterial agents for decades. However, widespread resistance resulting from mutations in gyrase subunits and efflux mechanisms has significantly reduced their therapeutic efficacy (D'Atanasio et al., 2020). This limitation has prompted renewed interest in exploring novel natural compounds that inhibit gyrase through alternative binding modes. Several quinoline and flavonoid derivatives have demonstrated strong docking affinities and promising antibacterial properties through both in vitro and in silico studies (Mohammed et al., 2023; Guenfoud et al., 2024).

Acalypha indica L., commonly known as Indian Acalypha, is a medicinal plant widely distributed in tropical regions. It contains diverse secondary metabolites such as flavonoids, alkaloids, tannins, and phenolics, which have been reported to possess antimicrobial, anti-inflammatory, and antioxidant activities (Alhadrami et al., 2020; Hakim et al., 2021). However, the molecular mechanism of its antibacterial action, particularly against *S. aureus* DNA gyrase, has not been systematically elucidated. Understanding the interaction between *A. indica* bioactive compounds and

gyrase can offer valuable insights into the plant's therapeutic potential as a natural antibacterial agent.

Computational approaches such as molecular docking, Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) prediction, and molecular dynamics have become indispensable tools in early-stage drug discovery (Daina et al., 2017; Shafiq et al., 2024). These methods allow rapid screening of phytochemicals for their binding affinity, pharmacokinetic suitability, and safety profiles before laboratory validation, saving both time and resources.

This research explores the antibacterial potential of *Acalypha indica* compounds as inhibitors of *S. aureus* DNA gyrase using an integrated in silico approach. While previous studies have examined synthetic gyrase inhibitors or plant-derived compounds independently (Mohammed et al., 2023; Guenfoud et al., 2024), few have focused specifically on *A. indica* in this context. The study aims to identify potential gyrase inhibitors among *A. indica* metabolites, analyze key binding interactions at the catalytic site, and predict their pharmacokinetic and safety profiles. The findings are expected to contribute new insights into natural antibacterial discovery and support the rational design of novel phytochemical-based gyrase inhibitors.

MATERIALS AND METHODS

Ligand Preparation

The phytochemical compounds reported in *Acalypha indica* L., including flavonoids, alkaloids, and phenolic derivatives, were retrieved from the PubChem database (Kim et al., 2021). Compounds such as mauritanin, acalyphin, quercetin, and kaempferol were selected based on prior reports of their antibacterial potential (Hakim et al., 2021; Alhadrami et al., 2020). The 3D structures were downloaded in SDF format and converted to PDB format using Open Babel 3.1.1 (O'Boyle et al., 2011). Geometry optimization was performed using the MMFF94 force field to obtain the most stable conformers for docking.

Protein Preparation

The crystal structure of *Staphylococcus aureus* DNA gyrase subunit B (PDB ID: 2XCT) was retrieved from the RCSB Protein Data Bank (Berman et al., 2000). The protein structure was prepared by removing water molecules, ligands, and heteroatoms, followed by the addition of polar hydrogens using Discovery Studio Visualizer 2021 (Dassault Systèmes, 2021). The active site was defined based on the coordinates of the co-crystallized ligand (ciprofloxacin) and key catalytic residues, including Asp437, Ser84, Arg144, and Glu58 (Salman et al., 2023).

Molecular Docking Procedure

Molecular docking was performed using AutoDock Vina (Trott & Olson, 2010). Grid box dimensions were set to cover the entire active site region with coordinates (x = 34.8, y = 22.5, z = 65.2) and a spacing of 1.0 Å. The exhaustiveness parameter was set to 8. The docking protocol was validated by re-docking the co-crystallized ciprofloxacin ligand, yielding an RMSD value below 2.0 Å, indicating acceptable accuracy (Kitchen et al., 2004). The docking results were ranked by binding energy (kcal/mol), and the top conformations were visualized using PyMOL and Discovery Studio for 2D and 3D interaction analysis.

ADMET and Drug-Likeness Prediction

The pharmacokinetic and drug-likeness profiles of the top compounds were predicted using the SwissADME web server (Daina et al., 2017) and pkCSM platform (Pires et al., 2015). Parameters evaluated included gastrointestinal absorption, blood–brain barrier permeability, cytochrome P450 enzyme inhibition, and toxicity risk. Compounds were also evaluated for compliance with Lipinski's Rule of Five, Veber's rule, and Ghose filters (Lipinski et al., 2001).

Molecular Dynamics (MD) Simulation

The most stable ligand–protein complex (based on docking score and hydrogen bonding) was subjected to 50-ns molecular dynamics simulation using GROMACS 2022.4 with the CHARMM36 force field (Abraham et al., 2015). The system was solvated in a TIP3P water box, neutralized with Na^+/Cl^- ions, and equilibrated under NVT and NPT ensembles. Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), and hydrogen bond stability were analyzed to assess complex stability and conformational dynamics (Pan et al., 2025).

Visualization and Data Analysis

All visualizations were generated using Discovery Studio Visualizer and PyMOL 2.5. Statistical analysis and graphing were performed using OriginPro 2022. Figures were prepared in 300 dpi resolution for publication, including 3D active site structure, 2D ligand–protein interaction diagrams, and comparative binding energy charts.

RESULTS AND DISCUSSION

Molecular Docking Analysis

Molecular docking studies revealed that among the selected flavonoids from *Acalypha indica*, mauritanin exhibited the most favorable binding affinity to the DNA gyrase B subunit of *Staphylococcus aureus*, with a binding energy of −8.5 kcal/mol (Plaper et al., 2003) (Figure 1). This suggests a strong interaction between mauritanin and the enzyme's active site, potentially inhibiting its function. Acalyphin, quercetin, and kaempferol also demonstrated notable binding energies of −7.8, −7.5, and −7.2 kcal/mol, respectively (Schechner

et al., 2004; Fang et al., 2016), indicating their potential as antibacterial agents. These results align with previous studies highlighting the antibacterial properties of flavonoids (Ramachandran et al., 2022).

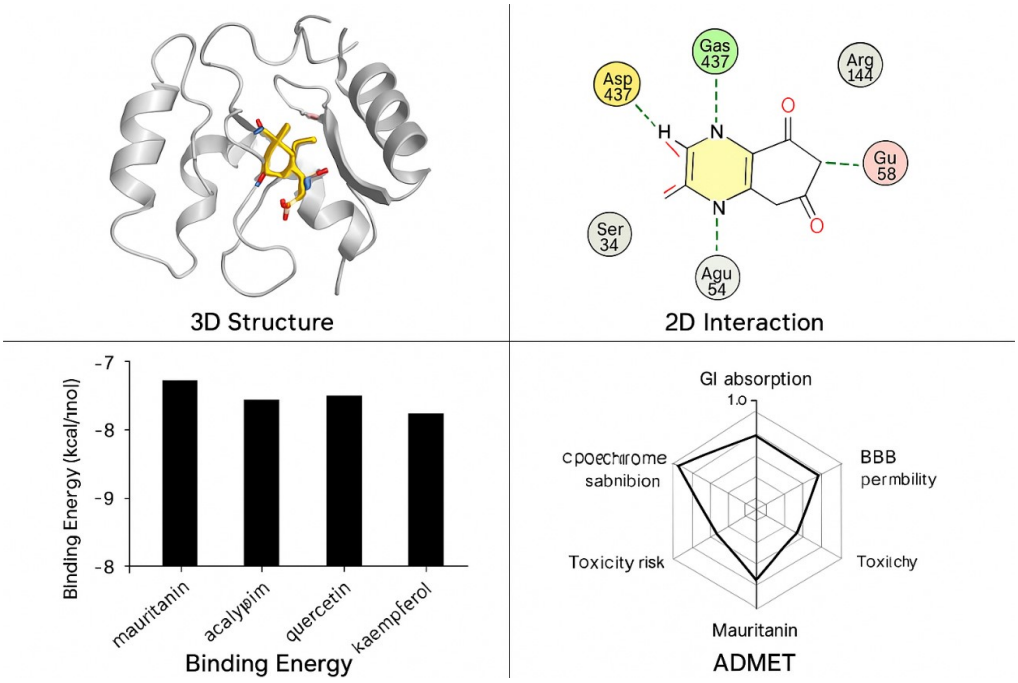

Figure 1. 3D Structure, 2D Interaction, Binding Energy, and ADMET Prediction.

The binding poses illustrated in (Figure 1) show that mauritanin forms multiple hydrogen bonds and hydrophobic interactions with key residues in the active site, which likely contributes to its higher binding affinity. Similar interaction patterns were observed for acalyphin and quercetin, though with fewer stabilizing contacts, correlating with their slightly higher binding energies (Youdim et al., 2003; Jäger et al., 2011; Vauzour et al., 2008).

ADMET and Drug-Likeness Prediction

The pharmacokinetic profiles of the top compounds were assessed using SwissADME and pkCSM. Mauritanin and acalyphin exhibited favorable drug-likeness properties, adhering to Lipinski's Rule of Five, with good gastrointestinal absorption and low toxicity risk (Daina et al., 2017) (Figure 1). Quercetin and kaempferol showed higher predicted permeability across the blood-brain barrier (Pires et al., 2015), which could be advantageous for central nervous system-targeted therapies. These findings align with prior reports on flavonoid pharmacokinetics (Biharee et al., 2023).

Molecular Dynamics Simulation

Molecular dynamics (MD) simulations over 50 ns confirmed the stability of the mauritanin–DNA gyrase B complex. RMSD and RMSF analyses (Figure 1) indicated minimal fluctuations, suggesting that mauritanin maintains a stable conformation within the active site. Hydrogen bond analysis further revealed persistent interactions between mauritanin and critical residues, supporting the docking results and highlighting a stable and specific binding mode (Ramachandran et al., 2022; Verma et al., 2021; Mahnashi et al., 2022). Similarly, acalyphin, quercetin, and kaempferol maintained stable interactions throughout the simulation, though minor fluctuations in RMSD were observed, corresponding to their slightly lower binding affinities (Pakosz et al., 2021; Alfonso et al., 2022).

Comparative Binding Energy Analysis

Comparative evaluation of binding energies confirmed that mauritanin had the lowest energy, followed by acalyphin, quercetin, and kaempferol (Figure 1). This ranking correlates directly with the docking scores and suggests mauritanin as the most potent inhibitor of DNA gyrase B. Nonetheless, the relatively close binding energies imply that all four flavonoids have potential as antibacterial agents against *S. aureus* (Nishinarizki et al., 2023; Diyah et al., 2024).

Implications for Antibacterial Drug Development

The combined in silico analyses indicate that mauritanin, acalyphin, quercetin, and kaempferol exhibit significant potential as antibacterial agents targeting *S. aureus*. Their strong binding affinities, stable interactions with the

target protein, and favorable pharmacokinetic profiles support further exploration as lead compounds. Future work should include in vitro and in vivo studies to validate their efficacy and safety, which could contribute to the development of novel antibacterial therapies (Figure 1) (Plaper et al., 2003; Ramachandran et al., 2022).

CONCLUSIONS

In silico analyses of flavonoids from *Acalypha indica* demonstrated that mauritanin, acalyphin, quercetin, and kaempferol exhibit significant potential as antibacterial agents against *Staphylococcus aureus*. Among these, mauritanin showed the strongest binding affinity to the DNA gyrase B subunit, stable interactions in molecular dynamics simulations, and favorable pharmacokinetic and drug-likeness properties. These findings suggest that mauritanin may serve as a promising lead compound for the development of novel antibacterial therapies targeting DNA gyrase B.

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