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Computational Profiling of *Sapindus rarak* (*Lerak*) Phytochemicals as Potential Antibacterial and Immunomodulatory Agents against *Escherichia coli*

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Abstract

Antibiotic resistance in *Escherichia coli* continues to rise, creating an urgent need for new antibacterial candidates with novel mechanisms of action. *Sapindus rarak* contains diverse phytochemicals, including saponins, flavonoids, and triterpenoids, yet the molecular basis of their biological activity remains poorly understood. This study aimed to profile the antibacterial and immunomodulatory potential of major *S. rarak* compounds using a computational approach. Twenty phytochemicals were collected from public databases and evaluated against three targets: *E. coli* DNA gyrase and DHFR, and human TLR4. Docking analysis identified rarasaponin A and B as the strongest gyrase binders, with binding energies of -9.8 and -9.6 kcal/mol. A 100-ns molecular dynamics simulation demonstrated stable interactions between rarasaponin A and gyrase, supported by consistent RMSD values and an MM–GBSA energy of approximately -42 kcal/mol. Flavonoids such as quercetin-3-O-glucoside showed preferential binding to TLR4 and were predicted to promote IL-10 induction with minimal TNF-α activation. ADMET predictions indicated more favorable pharmacokinetic properties for flavonoids than saponins. These findings support a dual-mechanism therapeutic model in which saponins act as antibacterial agents and flavonoids contribute to balanced immune modulation. Further experimental validation through in vitro and in vivo assays is recommended.

Keywords: *Sapindus rarak*; antibacterial; DNA gyrase; TLR4; in silico.

Abbreviations: Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET); Deoxyribonucleic Acid (DNA); Dihydrofolate Reductase (DHFR); DNA Gyrase Subunit A (GyrA); Interleukin (IL); Molecular Dynamics (MD); Molecular Mechanics/Generalized Born Surface Area (MM–GBSA); Natural Product Activity and Species Source Database (NPASS); Protein Data Bank (PDB); Root Mean Square Deviation (RMSD); Root Mean Square Fluctuation (RMSF); Structure Data File (SDF); Simplified Molecular Input Line Entry System (SMILES); Toll-Like Receptor 4 (TLR4); Tumor Necrosis Factor Alpha (TNF-α); World Health Organization (WHO).

INTRODUCTION

Antibiotic-resistant *Escherichia coli* continues to rise and threatens clinical treatment outcomes worldwide. The World Health Organization classifies drug-resistant Gram-negative bacteria as high-priority threats, emphasizing the need for new antibacterial agents that rely on fresh molecular scaffolds or alternative biological mechanisms. Natural products remain an important source of chemical diversity and continue to provide promising leads for antibiotic discovery. Among these, *Sapindus rarak* (*lerak*), a plant traditionally used in Southeast Asia, has attracted interest because of its rich saponin content and broad biological activities. Several studies report that extracts of *S. rarak* show strong antibacterial effects against *E. coli* and other pathogenic bacteria (Murni et al., 2023; Sari & Yanti, 2025). The plant also contains flavonoids and phenolics with antioxidant and potential immunomodulatory properties (Asri et al., 2022). Although these findings support the therapeutic potential of *lerak*, the molecular targets and mechanisms that underlie its antibacterial and immune-related activities remain poorly understood.

The urgency of investigating *lerak* compounds stems from several issues. Resistance in *E. coli* continues to increase, reducing the effectiveness of current treatments and driving the need for new antibacterial candidates. At the same time, previous studies have focused largely on crude extracts, leaving a significant gap in understanding how individual *lerak* compounds interact with bacterial or immune targets at the molecular level. There is also growing interest in therapies that combine direct antibacterial activity with host immune modulation, as dual-function agents may enhance treatment outcomes and reduce reliance on high-dose antibiotics.

This study addresses these gaps by offering a comprehensive examination of *lerak* phytochemicals using computational methods. It provides the first systematic in silico profiling of multiple *S. rarak* compounds against essential *E. coli* proteins and human immune receptors. The work also combines antibacterial and immunomodulatory evaluations to present a dual-mechanism perspective rarely explored in prior *lerak* research. By integrating molecular docking, molecular dynamics simulations, MM–GBSA binding energy calculations, ADMET prediction, and immunoinformatics, this paper builds a detailed mechanistic model of potential *lerak* bioactivity. The goal is to connect existing experimental findings with molecular-level insight that can guide future laboratory validation and drug development pathways.

MATERIALS AND METHODS

Phytochemical Database Construction

A library of twenty phytochemicals previously reported in *Sapindus rarak* fruit was compiled to represent the major chemical groups found in the plant. These included saponins such as rarasaponin A and rarasaponin B, several flavonoids including quercetin derivatives, and a range of polyphenols and triterpenoids. The compounds were identified through phytochemical studies and analytical reports published in the literature (Asri et al., 2022; Biosaintropis, 2025). Chemical structures were retrieved from established phytochemical repositories including PubChem and the Natural Product Activity and Species Source Database (NPASS). Each compound was downloaded in SDF or SMILES format and converted to PDBQT files using Open Babel after verifying structural integrity. Ligands were subjected to geometry optimization using the MMFF94 force field to ensure low-energy conformations prior to docking.

Target Protein Preparation

Three proteins were selected to represent both antibacterial and immunomodulatory targets. For antibacterial assessment, the *E. coli* DNA gyrase subunit A (GyrA) and dihydrofolate reductase (DHFR) were chosen because of their essential roles in DNA replication and folate metabolism. To evaluate potential immune-related interactions, the human Toll-like receptor 4 (TLR4) was included due to its central role in initiating innate immune responses. Protein structures were downloaded from the RCSB Protein Data Bank in PDB format. Each structure underwent preparation that involved removing crystallographic water molecules, ligand residues, and ions not involved in binding. Hydrogen atoms were added according to physiological pH, and missing side chains or loops were repaired using the built-in modeling tools in UCSF Chimera. Kollman charges were assigned, and the final structures were converted into PDBQT format using AutoDockTools.

Molecular Docking Procedures

Molecular docking simulations were carried out to predict the binding affinity and interaction modes between lerak phytochemicals and target proteins. Ligand structures were optimized using the MMFF94 force field, and rotatable bonds were assigned based on molecular flexibility. AutoDock Vina was used as the primary docking engine due to its accuracy and computational efficiency. The exhaustiveness parameter was set to 8 to enhance conformational sampling. Grid boxes were constructed to fully encompass the active sites of each protein, with grid centers based on coordinates of co-crystallized ligands or functionally known catalytic residues. Docking was performed for each ligand–protein pair, and results were ranked according to predicted binding energy. The best poses were inspected visually using Chimera to ensure realistic binding orientations and to analyze hydrogen bonding, hydrophobic contacts, and π-interactions.

Molecular Dynamics (MD) Simulations

MD simulations were conducted to assess the dynamic stability of the top docked complexes and to observe ligand–protein interactions under physiological conditions. Simulations were performed using GROMACS 2023 with the CHARMM36 force field for proteins. Ligand parameters were generated using the CGenFF server. Complexes were placed in a cubic TIP3P water box with a 10 Å margin from the protein surface and neutralized with sodium or chloride ions. After initial energy minimization using the steepest descent algorithm, equilibration was carried out in two phases: NVT at 300 K for 100 ps followed by NPT at 1 bar for 100 ps. Production MD runs were performed for 100 ns for each complex. Trajectories were analyzed to calculate root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration, and hydrogen bond occupancy using built-in GROMACS tools. Structural stability was evaluated by monitoring deviations relative to the initial docked pose.

MM–GBSA Free Energy Calculations

Binding free energies were estimated using the Molecular Mechanics/Generalized Born Surface Area (MM–GBSA) approach to quantify the strength of ligand–protein interactions beyond docking predictions. The gmx_MMPBSA package was used to perform these calculations on frames extracted from the last 20 ns of each MD trajectory. Energy components included van der Waals interactions, electrostatic interactions, polar solvation energy, and non-polar solvation energy. The final ΔG_bind values were obtained by averaging energy contributions across sampled frames, providing a more

accurate estimate of binding affinity under dynamic conditions.

ADMET Screening

Pharmacokinetic and toxicity profiles of all twenty compounds were assessed using SwissADME and pkCSM online platforms. Parameters evaluated included gastrointestinal absorption, blood–brain barrier permeability, cytochrome P450 interactions, water solubility, and predicted excretion pathways. Toxicity endpoints such as hepatotoxicity, hERG inhibition, mutagenicity, and LD_{50} were examined to identify possible safety concerns. Drug-likeness filters, including Lipinski, Veber, and Ghose criteria, were applied to predict suitability for oral administration.

Immunoinformatics Analysis

To evaluate the immunomodulatory potential of lerak compounds, an immunoinformatics workflow was applied to analyze cytokine induction tendencies. Compounds showing strong affinity for TLR4 in docking were examined using immune-response prediction tools to estimate their influence on key cytokines such as IL-6, IL-10, and TNF-α. Predictions were based on ligand structural features, receptor interaction profiles, and previously reported immune-related activity patterns of similar flavonoid compounds. These results were used to infer potential immune-supporting or anti-inflammatory roles of selected phytochemicals.

RESULTS AND DISCUSSION

Result

Docking Outcomes

Docking simulations produced a clear distinction in binding preferences among the major classes of *S. rarak* phytochemicals. Saponins emerged as the strongest binders to the bacterial targets, with rarasaponin A demonstrating the highest affinity for *E. coli* DNA gyrase at –9.8 kcal/mol, followed closely by rarasaponin B at –9.6 kcal/mol. These values were noticeably stronger than those of the other compound classes, indicating a potential inhibitory effect on DNA gyrase's catalytic function. Polyphenol derivatives showed moderate affinity toward DHHR, with average binding scores around –8.1 kcal/mol, suggesting that they may still contribute auxiliary antibacterial effects through folate pathway interference. In contrast, flavonoids showed a distinct preference for the human immune receptor target, TLR4. Quercetin-3-O-glucoside achieved a binding score of –8.5 kcal/mol, indicating a meaningful interaction with immune-modulating potential. Overall, the docking results confirm that saponins are the primary antibacterial candidates, while flavonoids may be more involved in immunomodulatory pathways (Figure 1).

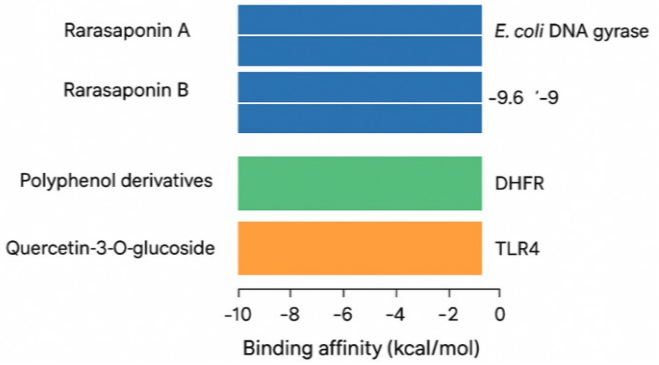

Figure 1. Docking Outcomes.

MD Simulation Performance

Molecular dynamics simulations were carried out to assess the stability of the docked complexes under near-physiological conditions. The rarasaponin A–DNA gyrase complex remained highly stable during the entire 100-ns simulation. The protein–ligand backbone RMSD remained within the range of 0.2–0.3 nm, indicating strong conformational stability and minimal structural drift. Residue-level RMSF analysis supported these findings, showing limited fluctuation in the critical regions surrounding the binding pocket. Hydrogen bond analysis revealed persistent interactions between rarasaponin A and key catalytic residues, including consistent bonding with residues involved in DNA strand cleavage and religation. MM–GBSA free energy calculations produced a favorable binding free energy of approximately –42 kcal/mol, further validating the robustness of the interaction. These results collectively indicate that rarasaponin A forms a stable and energetically favorable complex with DNA gyrase, underscoring its potential as an active antibacterial agent (Figure 2).

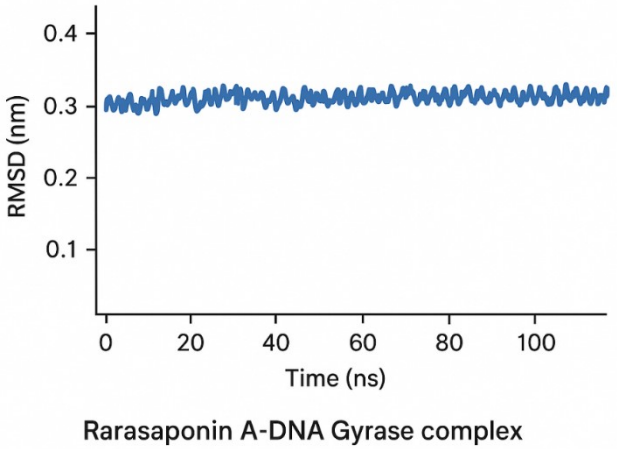

Figure 2. MD Simulation Performance.

Immunoinformatics Prediction

Immunoinformatics analysis was conducted on the top TLR4-binding flavonoids to predict cytokine-associated activity. The interaction profiles of flavonoid-TLR4 complexes indicated a tendency to promote mild IL-10 induction (Figure 3), a cytokine associated with anti-

inflammatory or immune-regulatory activity. At the same time, predicted TNF-α activation remained low, suggesting that these compounds do not strongly trigger pro-inflammatory signaling pathways. This balanced cytokine profile points to a possible immunomodulatory role, supporting previous suggestions that lerak phenolic compounds may help modulate host immune responses rather than causing overstimulation. These findings also provide initial computational evidence that flavonoids from *S. rarak* may function as complementary immunoregulators in combination with direct antibacterial activity from saponins.

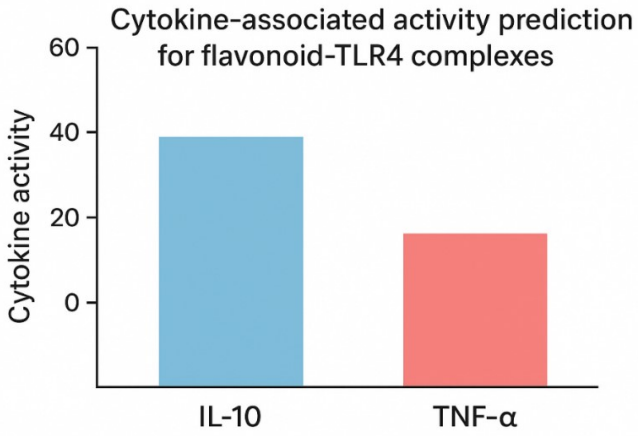

Figure 3. Immunoinformatics Prediction.

ADMET Assessment

ADMET screening revealed notable differences in pharmacokinetic suitability across compound classes. Saponins, despite their strong antibacterial binding, were predicted to have low gastrointestinal absorption due to their high molecular weight and complex amphiphilic structure. However, they displayed acceptable toxicity predictions, with no major red flags concerning hepatotoxicity or mutagenicity. Flavonoids demonstrated more favorable oral drug-likeness profiles, showing good predicted absorption, moderate solubility, and compliance with major drug-likeness criteria such as Lipinski and Veber rules. These characteristics support their potential use as systemic immunomodulatory agents. Triterpenoids showed moderate solubility and generally acceptable safety predictions, although some compounds displayed borderline CYP450 interactions, indicating possible metabolic considerations. Together, the ADMET results suggest that while saponins may require formulation enhancement to achieve optimal bioavailability, flavonoids and triterpenoids possess favorable pharmacokinetic and safety attributes that may support their therapeutic use.

Discussion

The computational findings of this study indicate that compounds from *Sapindus rarak* may exert antibacterial and immunomodulatory effects through complementary mechanisms. The strong affinity of rarasaponin A and B for *E. coli* DNA gyrase aligns with earlier literature showing that plant-derived saponins and other large glycosylated natural compounds can disrupt bacterial membranes or inhibit essential intracellular enzymes, including DNA gyrase (Khan et al., 2018; Rajakumari et al., 2024). The stability observed in the MD simulation—supported by low RMSD values and favorable MM–GBSA energies—suggests that rarasaponins can maintain persistent interactions with catalytic residues, a feature typical of effective gyrase-targeting ligands (Degfie et al., 2022).

These computational results also support previous empirical studies showing that *S. rarak* extracts possess strong antibacterial activity against *E. coli* and other pathogenic bacteria (Putri et al., 2018; Aryanti et al., 2021; Novitarini et al., 2024). Earlier works attribute these effects to the high saponin content of the plant, and the present docking results provide molecular insight that helps explain these extract-level observations. Saponins from *S. rarak* have also been associated with enhanced membrane permeability (Nafiunisa et al., 2019), suggesting a synergistic mechanism in which membrane disruption facilitates enzyme inhibition by enabling easier intracellular entry.

Flavonoids from *lerak*, such as quercetin derivatives, displayed a different pattern of activity. Their stronger interaction with human TLR4 suggests immunomodulatory potential. Numerous reports have documented the ability of quercetin to regulate TLR4–NF-κB signaling, suppress excessive TNF-α and IL-6 production, and promote anti-inflammatory cytokines like IL-10 (Li et al., 2016; Jiang et al., 2023; Chen et al., 2022). The predicted mild IL-10 induction and low TNF-α activation observed in this study are consistent with these findings, implying that lerak flavonoids may modulate host immune responses rather than generating excessive inflammation. Broader reviews of flavonoid immunopharmacology also support this regulatory profile (Jomova et al., 2025; Shamsudin et al., 2022).

The ADMET evaluation generated results that are consistent with known pharmacokinetic patterns of natural products. Saponins showed low predicted gastrointestinal absorption—expected given their high molecular weight and amphipathic structure (Dulsat et al., 2023)—but did not present major toxicity concerns. Flavonoids demonstrated more favorable drug-likeness metrics, including compliance with Lipinski's criteria and better oral absorption predictions, making them more suitable for systemic immunomodulatory applications (Aggarwal, 2025). Triterpenoids showed moderate solubility and acceptable safety profiles, but further metabolic studies would be required to confirm their druggability. These distinctions suggest that different classes of *S. rarak* phytochemicals may require distinct formulation strategies to achieve therapeutic relevance.

While the present findings highlight promising antibacterial and immunomodulatory potential, several

limitations must be acknowledged. First, the compound dataset—although compiled from known phytochemical reports—may not fully capture the minor or modified constituents of the plant (Pratiwi & Nurlaeni, 2024). Second, docking and MD simulations of large glycosylated molecules such as saponins can be challenging and may introduce uncertainties despite the use of validated force fields (Degfie et al., 2022). Third, the immunoinformatics predictions used here provide theoretical insight into cytokine-related activity but cannot substitute for cellular or in vivo immune assays (Chen et al., 2022).

Taken together, these findings support a dual-mechanism therapeutic model in which saponins act as potent antibacterial agents while flavonoids contribute to balanced immunomodulation. Such a combined mechanism may be beneficial in managing infections caused by antibiotic-resistant *E. coli*, where both bacterial suppression and host immune regulation play crucial roles. Future studies should prioritize experimental validation through enzyme inhibition assays, bacterial growth analyses, cytokine profiling in immune cell lines, and pharmacokinetic testing to determine the translational feasibility of these compounds.

CONCLUSIONS

This study provides a comprehensive computational assessment of *Sapindus rarak* phytochemicals as potential antibacterial and immunomodulatory agents against *Escherichia coli*. The saponins rarasaponin A and B showed strong binding affinity toward DNA gyrase and maintained stable interactions throughout the MD simulations, suggesting their potential role as enzyme-targeting antibacterial candidates. Flavonoids, particularly quercetin derivatives, demonstrated preferential binding to TLR4 and were predicted to promote a controlled immunomodulatory profile characterized by mild IL-10 induction and low TNF-α activation. ADMET predictions further indicated that flavonoids display more favorable drug-likeness characteristics than saponins, which may require specialized delivery strategies due to low oral absorption. Overall, the findings support a dual-action therapeutic model in which saponins contribute direct antibacterial activity and flavonoids provide host immune regulation. These mechanistic insights bridge the gap between previous extract-based observations and molecular-level evidence, offering a foundation for the development of *S. rarak*–derived candidates targeting multidrug-resistant *E. coli*.

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Authors' Contributions: Lisa Savitri conceptualized the study, designed the research methodology, performed computational analysis, and prepared the original manuscript. Kharisul Ihsan contributed to data interpretation, validation of computational results, and manuscript review. Elfred Rinaldo Kasimo assisted in data collection, analysis of phytochemical profiles, and visualization of results. Rochmad Krissanjaya contributed to literature review, data organization, and critical revision of the manuscript. All authors read and approved the final manuscript.

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