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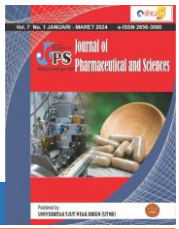

Antibacterial Activity of Sengir Mango (*Mangifera indica* L. Cultivar Sengir) Leaf Extract Against *Staphylococcus aureus*: *In vitro* and Molecular Docking Study

Aktivitas Antibakteri Ekstrak Daun Mangga Sengir (*Mangifera indica* L. Kultivar Sengir) terhadap *Staphylococcus aureus*: Studi *In vitro* dan *Molecular Docking*

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Abstract

The antibacterial potential of the underexplored *Mangifera indica* L. cultivar Sengir remains poorly understood. This study evaluated the antibacterial activity of Sengir mango leaf extract against *Staphylococcus aureus* using integrated *in vitro* and *in silico* approaches. Leaves were extracted by maceration with 70% ethanol. The extract contained 293.8 mg GAE/g extract of total phenolics and 34.8 mg QE/g extract of total flavonoids. Antibacterial activity was assessed using the disk diffusion method at extract concentrations of 40%, 60%, 80%, and 100%, corresponding to 488, 732, 976, and 1,220 mg/mL, respectively. Ampicillin and 10% DMSO served as the positive and negative control, respectively. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were also determined. For the *in silico* study, 60 *M. indica* phytochemicals from IMPPAT were screened using SwissADME and ProTox-III, and seven compounds were docked against penicillin-binding protein 2a (PBP2a; PDB ID: 4CJN) using PyRx. The extract produced concentration-dependent inhibition zones of 11.00–17.17 mm, whereas ampicillin produced a 34.50 mm inhibition zone and 10% DMSO showed no inhibition. MIC and MBC values were 48.87 and 73.20 mg/mL, respectively. Guaiol showed the lowest predicted binding energy (-7.0 kcal/mol), whereas PASS analysis identified Pogostol, Myrtenyl acetate, and Bicyclogermacrene as potential antibacterial compounds. Overall, Sengir mango leaf extract showed antibacterial activity against *S. aureus*, while computational analysis identified Guaiol and Pogostol as promising bioactive candidates for further validation.

***Keywords*: Sengir mango, *Mangifera indica*, *Staphylococcus aureus*, molecular docking, antibacterial activity.**

Abstrak

Potensi antibakteri kultivar Sengir dari *Mangifera indica* L. masih belum banyak diteliti. Penelitian ini bertujuan mengevaluasi aktivitas antibakteri ekstrak daun mangga Sengir terhadap *Staphylococcus aureus* menggunakan pendekatan *in vitro* dan *in silico*. Daun diekstraksi dengan metode maserasi menggunakan etanol 70%. Ekstrak mengandung fenolik total sebesar 293,8 mg GAE/g ekstrak dan flavonoid total sebesar 34,8 mg QE/g ekstrak. Aktivitas antibakteri diuji dengan metode difusi cakram pada konsentrasi ekstrak 40%, 60%, 80%, dan 100%, yang masing-masing setara dengan 488, 732, 976, dan 1.220 mg/mL. Ampisilin dan DMSO 10% digunakan sebagai kontrol positif dan kontrol negatif. Nilai *minimum inhibitory concentration* (MIC) dan *minimum bactericidal concentration* (MBC) juga ditentukan. Pada studi *in silico*, sebanyak 60 senyawa fitokimia *M. indica* dari basis data IMPPAT diseleksi menggunakan SwissADME dan ProTox-III, kemudian tujuh senyawa yang memenuhi kriteria dilakukan *molecular docking* terhadap *penicillin-binding protein 2a* (PBP2a; PDB ID: 4CJN) menggunakan PyRx. Ekstrak menghasilkan zona hambat yang meningkat seiring peningkatan konsentrasi, yaitu 11,00–17,17 mm, sedangkan ampisilin menghasilkan zona hambat sebesar 34,50 mm dan DMSO 10% tidak menunjukkan zona hambat. Nilai MIC dan MBC berturut-turut adalah 48,87 dan 73,20 mg/mL. Guaiol menunjukkan prediksi energi ikatan terendah (-7,0 kkal/mol), sedangkan analisis PASS mengidentifikasi Pogostol, Myrtenyl acetate, dan Bicyclogermacrene sebagai senyawa yang berpotensi memiliki aktivitas antibakteri. Secara keseluruhan, ekstrak daun mangga Sengir menunjukkan aktivitas antibakteri terhadap *S. aureus*, sementara analisis komputasional mengidentifikasi Guaiol dan Pogostol sebagai kandidat senyawa bioaktif yang menjanjikan untuk divalidasi lebih lanjut.

***Kata Kunci*: Mangga Sengir, *Mangifera indica*, *Staphylococcus aureus*, molecular docking, aktivitas antibakteri.**

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Introduction

Diarrheal disease remains a significant global public health problem and continues to contribute greatly to mortality, especially among children and the elderly [1,2]. According to the World Health Organization (WHO), diarrhea is one of the leading causes of death in children under 5 years of age, with approximately 525,000 deaths each year [3]. Although findings from the 2021 Global Burden of Disease (GBD) study show a fairly significant decline in diarrhea-related deaths and disability since 1990, the burden is still heavy in low- and middle-income countries, especially in South Asia and Sub-Saharan Africa [1,2]. In addition, recent epidemiological reports indicate that the global incidence of diarrheal disease has continued to increase since 2019 despite improvements in health systems [4]. This trend highlights the ongoing need for effective and sustainable therapeutic strategies.

Diarrhea is clinically characterized by the passage of loose or watery stools at least three times in a day [5]. Based on disease duration, diarrhea is generally classified as acute, resolving within 2 weeks, or chronic, persisting beyond this period [5]. In certain cases, the presence of blood in the stool indicates dysentery [6]. The condition may arise from multiple external factors, including contaminated food and water, poor sanitation, nutritional deficiencies, and infections caused by viruses, bacteria, or parasites [6,7]. These factors damage the intestinal wall and cause inflammation or impaired water absorption, which then lead to the physical symptoms of diarrhea [5].

Even though current treatments are widely used, managing diarrhea still presents many challenges regarding their effectiveness and long-term effects. Common solutions like Oral Rehydration Salts and zinc each have limits. Oral Rehydration Salts do not shorten the duration of symptoms, and zinc alone is often insufficient to prevent diarrhea from returning [8,9]. Other options, such as probiotics or adsorbents, also yield mixed results across different regions [10]. Antibiotics are sometimes used, but they are recommended only for certain invasive cases, as most acute diarrhea is caused by viruses [11]. Using these drugs too much is a big concern because it leads to antimicrobial resistance, which makes future treatments less effective [12].

Among bacterial pathogens associated with gastrointestinal infections, *Staphylococcus aureus* is a significant concern due to its ability to cause food poisoning and gastroenteritis. Contaminated dairy and meat products are important sources of transmission, resulting in hundreds of thousands of foodborne illness cases annually [13,14]. The pathogenicity of *S. aureus* is closely related to the production of heat-stable enterotoxins that act as superantigens and induce hyper-inflammatory responses [15]. Although many *S. aureus* isolates remain susceptible to conventional antibiotics, the emergence of methicillin-resistant *S. aureus* (MRSA) has become a major global public health concern. MRSA expresses Penicillin-Binding Protein 2a (PBP2a), a modified transpeptidase with low affinity for β-lactam antibiotics, enabling continued cell-wall synthesis despite antibiotic exposure [16]. Because of its central role in antimicrobial resistance, PBP2a has become one of the most extensively investigated molecular targets for the development of new antibacterial agents against resistant *S. aureus* strains [17,18].

Natural products continue to attract attention as potential sources of alternative antibacterial compounds capable of interacting with PBP2a through unique mechanisms compared to conventional β-lactam antibiotics. Mango (*Mangifera indica* L.) has been used for thousands of years in traditional medicine to treat gastrointestinal disorders such as diarrhea and dysentery [19]. Various parts of the plant, such as the leaves, bark, and seeds, contain bioactive compounds like flavonoids, phenols, and mangiferin [20]. Mango-derived phytochemicals might exhibit simultaneous multiple pharmacological effects, such as antimicrobial, anti-inflammatory, and antisecretory activities, in contrast to single-target synthetic drugs. While standard

medicines like loperamide mainly slow down gut movement, extracts from *M. indica* can reduce secretion, lower inflammation and fight microbes at the same time [8]. Mango extracts have been found to possess antibacterial activity against various pathogenic species that cause diarrhea, including resistant strains of *S. aureus* and *Escherichia coli* [21]. This plant has been used for a long time in traditional practice, and its safety profile makes it a candidate for natural therapeutic use [21].

Given this potential, this study aims to evaluate the antibacterial activity of Sengir mango leaf extract (*M. indica* L., Sengir cultivar) against *S. aureus* using complementary experimental and computational approaches. The antibacterial activity was evaluated through in vitro assays, whereas molecular docking was employed to investigate the interaction of selected phytochemicals with PBP2a, a clinically relevant molecular target associated with β-lactam resistance in *S. aureus*. The computational analysis was intended as an exploratory approach to assess whether phytochemicals from Sengir mango leaves exhibit potential interactions with this resistance-associated target, thereby providing preliminary mechanistic insights for future antibacterial drug discovery. The integration of both approaches is expected to support further investigations into the antibacterial potential of Sengir mango leaf phytochemicals.

Methods

Research Samples

In this study, fresh leaves of *M. indica* L. cultivar Sengir were collected from Sragen Regency, Central Java, Indonesia. The plant material was taxonomically identified at the Biology Laboratory, Universitas Muhammadiyah Surakarta, Indonesia, on 23 December 2025, with the identification certificate number is 051/A.E-I/LAB/BIO/XII/2025. *Staphylococcus aureus* was obtained from the Microbiology Laboratory, Faculty of Pharmacy, Universitas Muhammadiyah Surakarta. The isolate was routinely maintained as a laboratory stock culture and was used for antibacterial susceptibility testing. The materials employed in this research included 70% ethanol as the extraction solvent, 10% dimethyl sulfoxide (DMSO), sterile distilled water, and 0.9% sodium chloride solution. Nutrient Agar (NA) and Mueller-Hinton Agar (MHA) purchased from Merck were utilized as bacterial growth media. Ampicillin served as the positive control for comparison, and blank paper discs from Oxoid were used as negative controls.

Preparation of Simplicia and Extraction

The Sengir mango leaves collected first underwent wet sorting and were thoroughly washed under running water. After that, the leaves were drained, cut into smaller pieces, and dried in an oven at 40 °C for 3-5 days. The dry material that resulted is called simplicia. This simplicia was then extracted using maceration with 70% ethanol until the sample was fully covered. The liquid obtained was filtered and then evaporated in a water bath at 50-55 °C to get a thick extract. This extract was later diluted with 10% DMSO to prepare solutions at concentrations of 40%, 60%, 80%, and 100% for subsequent tests.

Determination of Total Phenolic and Flavonoid Content

The total phenolic content (TPC) of the Sengir mango leaf ethanol extract was determined using the Folin-Ciocalteu colorimetric method with gallic acid as the reference standard. The absorbance was measured spectrophotometrically and expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g). Total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method with quercetin as the reference standard. The absorbance was measured spectrophotometrically and expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g).

Preparation of Inoculum and Media

Mueller-Hinton Agar (MHA) was prepared by dissolving 19 g of powder medium in 500 mL of distilled water. The prepared medium was sterilized using an autoclave at 121 °C under 1-2 atm pressure for 60 minutes. For the bacteria, *S. aureus* was first rejuvenated on a slant of MHA and incubated at 37 °C for 24 hours [21]. A single bacterial colony was subsequently transferred into 10 mL of 0.9% NaCl. The solution was mixed until the cloudiness matched the McFarland standard.

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In vitro Antibacterial Diffusion Test

The antibacterial activity assay was conducted by a disk diffusion test. The sterilized MHA was poured onto Petri dishes and left to set. Then 200 µL of *S. aureus* culture suspension was streaked across the plate. Paper disks were prepared with 25 µL of the extract at concentrations of 40%, 60%, 80%, and 100%. Disks with ampicillin were used as the positive control, and disks with 10% DMSO were used as the negative control. All the paper disks were then placed on the agar, which was further incubated at 37 °C for 24 hours [21,26]. After incubation, the diameters of the inhibition zones were measured using a Vernier caliper. The data collected were analyzed using univariate analysis in SPSS.

Molecular Docking and ADMET Screening

Phytochemical data from the IMPPAT database (https://cb.imsc.res.in/imppat/), consisting of 60 compounds, were evaluated for drug-likeness using multiple filters (Lipinski, Ghose, Veber, Egan, and Muegge) via SwissADME (https://swissadme.ch/) and assessed for toxicity through ProTox-III (https://tox.charite.de/protox3/). Drug-likeness filters were applied as a prioritization strategy rather than absolute exclusion criteria. To identify the most promising candidates for future drug development, compounds meeting all drug-likeness rules and toxicity profiles were prioritized. This minimized the likelihood of selecting molecules with unfavorable pharmacokinetics. However, excluded compounds are not necessarily biologically inactive, as many natural products exert pharmacological effects despite violating conventional drug-likeness rules. After molecular docking, the selected compounds were further evaluated for predicted biological activity using PASS Online (https://way2drug.com/PassOnline/), focusing on antibacterial and related activities (Pa > 0.3). For the receptor protein, the three-dimensional structure of Penicillin-Binding Protein 2a (PBP2a) of *S. aureus* (PDB ID: 4CJN) was retrieved through the RCSB Protein Data Bank (PDB) (https://www.rcsb.org/).

Protein preparation included deletion of water molecules and separation of the native ligand using Biovia Discovery Studio 2021, followed by the addition of hydrogen atoms and Kollman partial charges using AutoDock Tools 1.5.7. Docking was carried out using PyRx with a grid box for PBP2a (PDB ID 4CJN). This structure is co-crystallized with a quinazolinone derivative (ligand ID: QNZ), which is a non-β-lactam allosteric inhibitor that binds to the allosteric site of PBP2a and induces conformational changes that impair the active site [19]. QNZ was therefore used as the native ligand for redocking validation.

The binding pocket of PBP2a (chain B) was defined using the binding site prediction tool in BIOVIA Discovery Studio Visualizer 2025 (Dassault Systèmes BIOVIA, San Diego, CA, USA). Key residues forming the pocket include Asn146, Lys148, Arg151, Tyr169, Tyr196, Gln200, Lys215, Thr216, Asn236, Glu237, Arg241, Asn242, Tyr243, Tyr255, Val256, Pro258, Asn260, Glu263, Tyr269, Tyr272, Lys273, Asp274, Asp275, Lys280, Lys281, Gln292, His293, Glu294, Asp295, Lys318, Lys322, Tyr369, and Met372. Therefore, the grid center was placed at the geometric center of the predicted binding site, which successfully encompassed the QNZ ligand (coordinates: x = -0.45, y = -5.13, z = -59.55), with a box dimension of 25 Å in each direction.

To verify that the setup was accurate, QNZ was redocked as the native ligand of PBP2a, and results were accepted only if the RMSD was less than 2 Å. Ligand-receptor interactions were subsequently visualized in both two-dimensional and three-dimensional formats using Biovia Discovery Studio 2021.

Data Analysis

The antibacterial activity was evaluated by measuring the diameter of the inhibition zone (mm) around each paper disc. For each extract concentration, three paper discs were placed on a single agar plate together with one ampicillin disc (positive control) and one 10% DMSO disc (negative control). The inhibition zone diameters obtained from the three paper discs were used as technical replicate measurements, and the results are presented as the mean ± standard error (SE). The data were analyzed descriptively, and no inferential statistical analyses were performed.

Results and Discussion

Antibacterial Activity of *M. indica* 'Sengir' Leaf Extract Against *S. aureus*

The solvent selected for this study was 70% ethanol due to its polarity, which enables efficient extraction of diverse secondary metabolites, ranging from polar to semi-polar compounds [22,23]. Through the maceration method, the Sengir mango leaves were processed and evaporated to produce a highly

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concentrated crude extract. For the purposes of this study, the maximum concentration of the test sample was set at 1,220 mg/mL, the highest concentration at which the extract could be fully dissolved in the solvent.

Further antibacterial activity tests showed that Sengir mango leaf extract effectively suppressed the tested pathogens in a concentration-dependent manner. At the lowest tested concentration of 40%, the extract produced an average inhibition zone of 11.00 mm, which gradually increased to 15.00 mm and 16.33 mm at concentrations of 60% and 80%, respectively Figure 1 and Table 1. The highest antibacterial activity was observed at 100% concentration, yielding an average inhibition zone of 17.17 mm. All three replications for the 40% and 60% concentrations yielded identical inhibition zone diameters (11.0 mm and 15.0 mm, respectively), resulting in a standard deviation of 0.00. This consistency reflects the high reproducibility of the disc diffusion test under experimental conditions. The MIC and MBC values were determined as 48.87 mg/mL and 73.20 mg/mL, respectively. The lower MIC relative to the MBC indicates that bacterial growth inhibition occurred at concentrations lower than those required for complete bacterial killing. This concentration-dependent response is consistent with the increasing inhibition zone diameters observed in the disc diffusion assay.

Table 1. Phytochemical characterization, antibacterial activity, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) of *M. indica* L. cultivar Sengir leaf ethanol extract against *S. aureus*.

A. Phytochemical characterization			
Parameter	Amount	Unit	Reference standard
Total Phenolic Content (TPC)	293.8	mg GAE/g extract	Gallic acid
Total Flavonoid Content (TFC)	34.8	mg QE/g extract	Quercetin

B. Antibacterial activity (disk diffusion)	
Concentration	Mean ± SD*
DMSO (Negative control)	0.00±0.00
40%	11.00±0.00
60%	15.00±0.00
80%	16.33±0.57
100%	17.17±0.28
Ampicillin (Positive control)	34.50±0.00

**Values represent measurements obtained from three technical replicate discs placed on a single agar plate for each treatment.*

C. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)		
Parameter	Value (mg/mL)	Equivalent concentration
MIC	48.87	4%
MBC	73.2	6%

Total Phenolic and Flavonoid Contents

To further characterize the phytochemical composition of the Sengir mango leaf extract, the TPC and TFC were determined prior to antibacterial evaluation. As shown in Table 1, the extract exhibited a relatively high phenolic content of 293.8 mg GAE/g extract and a total flavonoid content of 34.8 mg QE/g extract (Table 1). These findings indicate that the ethanol extract contains abundant phenolic constituents together with a considerable amount of flavonoids, both of which are widely recognized as important contributors to the biological activities of medicinal plants [26,27].

The TPC value was over 8-fold higher than the TFC value, indicating that non-flavonoid phenolics constitute the major phenolic fraction of the Sengir mango leaf extract. This observation suggests that the extract is likely rich in phenolic acids, tannins, benzophenones, and xanthones, including mangiferin, which are commonly reported in *M. indica* leaves and are recognized for their antioxidant and antibacterial activities. Compared with other *M. indica* cultivars reported in previous studies, the phytochemical profile of the Sengir cultivar falls within the reported range, although variations in TPC and TFC are expected because of differences in genetic background, geographical origin, climatic conditions, leaf maturity, and extraction procedures. These compositional differences may partly explain the variability in antibacterial activity reported among mango cultivars and suggest that the antibacterial efficacy of the Sengir extract depends not only on the total amount of phenolics but also on the specific composition and synergistic interactions among its bioactive constituents.

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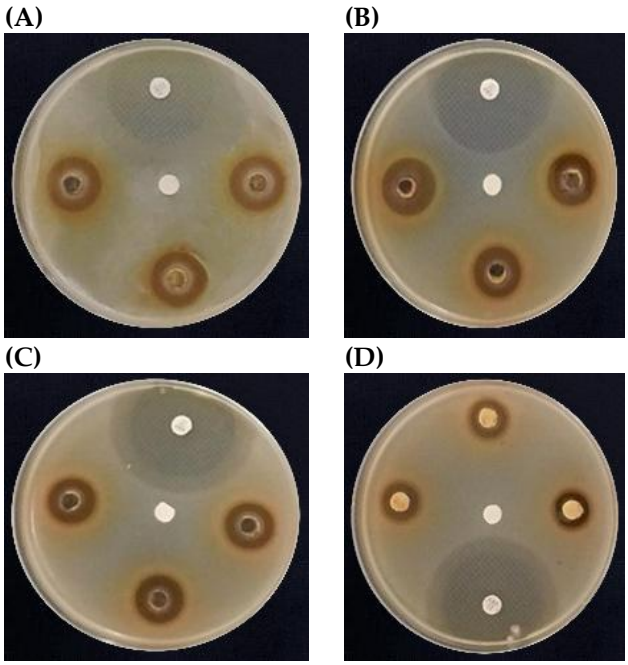

Figure 1. Representative inhibition zones produced by the ethanol extract of *M. indica* cultivar Sengir leaves against *S. aureus* using the disk diffusion method at concentrations of (**A**) 100%, (**B**) 80%, (**C**) 60%, and (**D**) 40%.

Phenolic compounds are well known for their antibacterial properties through multiple mechanisms, including disruption of bacterial cell membranes, protein denaturation, inhibition of essential enzymes, metal ion chelation, and induction of oxidative stress. Likewise, flavonoids exhibit antibacterial activity by increasing membrane permeability, inhibiting nucleic acid synthesis, suppressing energy metabolism, and interfering with biofilm formation. Therefore, the relatively high TPC and measurable TFC observed in the Sengir mango leaf extract may contribute to the antibacterial activity demonstrated against *S. aureus* in the present study. The presence of these bioactive compounds provides a plausible explanation for the concentration-dependent antibacterial activity observed in the disc diffusion assay. These findings support the hypothesis that the antibacterial activity of the Sengir mango leaf extract results from the synergistic action of multiple phytochemicals rather than a single constituent [30].

Drug-Likeness Screening and PASS Prediction

To elucidate the molecular basis of the antibacterial activity, computer-based molecular docking simulations were performed. Before docking, a selection process was done by screening compounds from *M. indica* for drug-likeness and toxicity using SwissADME and ProTox III. This analysis evaluated key ADME (Absorption, Distribution, Metabolism, and Excretion) properties together with established drug-likeness criteria, including the Lipinski, Ghose, Veber, Egan, and Muegge rules.

From the initial set of 60 compounds, 12 fulfilled all drug-likeness criteria without any violations. The 12 compounds that passed the drug-likeness screening were then subjected to toxicity testing to ensure their safety profiles across various parameters. This screening assessed potential organ toxicity, including hepatotoxicity, neurotoxicity, and cardiotoxicity, as well as critical endpoints such as mutagenicity and cytotoxicity. Of the 12 candidates analyzed, 7 compounds demonstrated acceptable safety profiles and were deemed suitable for proceeding to the molecular docking stage (Figure 2).

PASS online prediction (Pa > 0.3) was used to evaluate the antibacterial potential of the seven docked compounds (Table 2). Among them, Pogostol showed the highest predicted antibacterial activity (Pa = 0.417), followed by Myrtenyl acetate (Pa = 0.384) and Bicyclogermacrene (Pa = 0.332). In contrast, Guaiol, despite having the strongest binding affinity to PBP2a (-7.0 kcal/mol), lacked any predicted antibacterial activity (Pa < 0.3). Instead, Guaiol was predicted to be carminative (Pa = 0.722). Notably, 2-Tridecanone also lacked antibacterial prediction but showed moderate anti-inflammatory activity (Pa = 0.602) and peptidoglycan glycosyltransferase inhibition (Pa = 0.575). These results suggest that the antibacterial effect of the crude extract may not be attributed to a single compound but rather to synergistic interactions among multiple phytochemicals.

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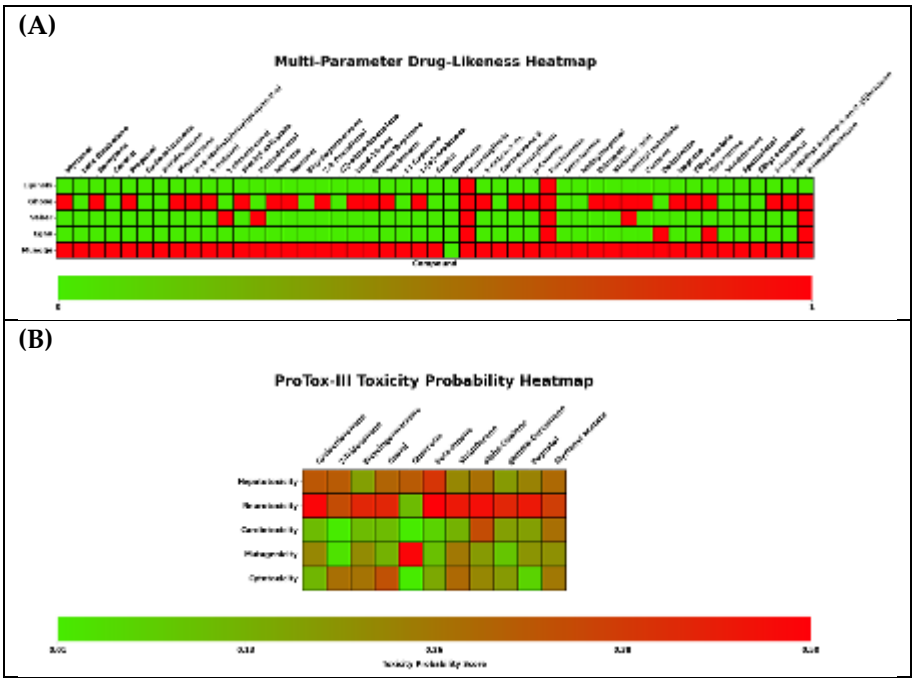

Figure 2. Drug-likeness and toxicity screening of phytochemicals identified from *M. indica* cultivar Sengir leaves. (**A**) Multi-parameter drug-likeness evaluation using Lipinski, Ghose, Veber, Egan, and Muegge filters. (**B**) Toxicity prediction using ProTox-III.

Molecular Docking and Protein-Ligand Interaction Analysis

The molecular docking analysis and the in vitro antibacterial assay addressed distinct biological objectives. The in vitro assay evaluated the antibacterial activity of Sengir mango leaf extract against *S. aureus* using the disk diffusion method, whereas molecular docking investigated the interaction of selected phytochemicals with PBP2a, a validated resistance-associated protein. Consequently, the docking results should not be interpreted as the direct molecular mechanism underlying the inhibition zones observed in vitro. Instead, they provide preliminary evidence that Sengir mango leaf phytochemicals possess structural compatibility with PBP2a, supporting their potential for future investigation against resistant *S. aureus* isolates.The computational study began by selecting PBP2a from *S. aureus* as the primary molecular target. This protein was selected because it plays a key role in antibiotic resistance. To validate the docking setup, the co-crystallized ligand (QNZ) was extracted and re-docked into the same binding pocket. The root mean square deviation (RMSD) between the original (crystal) and re-docked ligand conformations was calculated, yielding a value of 0.051 Å (Figure 3).

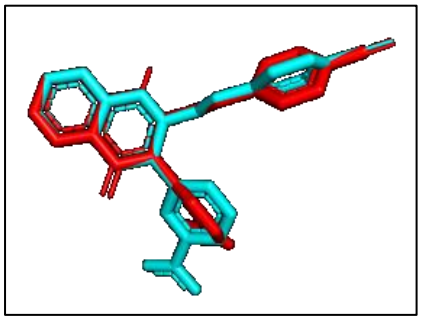

Figure 3. Superimposition of native ligand QNZ (blue, from crystal structure) and re-docked QNZ (red) in the allosteric binding pocket of *S. aureus* PBP2a (PDB ID: 4CJN). The root mean square deviation (RMSD) between the two conformations is 0.051 Å, confirming docking parameter reliability.

Since this value is well below the 2 Å threshold, it confirms that the binding site coordinates are highly reliable for further simulation. The molecular docking simulations showed that several compounds from *M. indica* leaf have promising inhibitory potential against the PBP2a protein. As summarized in Table 2, the binding affinities ranged from −5.5 to −7.0 kcal/mol. Among the candidates, Guaiol had the strongest binding

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affinity at -7.0 kcal/mol. This value was comparable to those of the control antibiotics Vancomycin and Ampicillin, which were -7.6 and -7.4 kcal/mol, respectively.

The interaction analysis shows that Vancomycin has the highest binding affinity because it forms the most extensive network of hydrogen bonds with the receptor. These hydrogen bonds help stabilize the complex by forming strong electrostatic attractions, thereby providing an enthalpy advantage [25]. Among the *M. indica* compounds, Guaiol is the only ligand that forms a hydrogen bond. This bond contributes directly to its stronger binding affinity compared to the other samples. Hydrogen bonds play a critical role in molecular recognition because they are stronger than van der Waals forces and provide the precise directional specificity required to fit into the binding site [26].

Forming these bonds also means the ligand must overcome a hydrogen bonding penalty by displacing the water molecules that originally surround the protein [27]. The high affinity of Vancomycin and Guaiol suggests that their new hydrogen bonds are strong enough to offset this energy cost and stabilize the interaction. If hydrogen bonds are poorly oriented or too polar, they can actually reduce binding affinity due to desolvation penalties and a loss of entropy [28]. Guaiol's potential in this study likely comes from its ability to form a well-positioned bond that works with the surrounding environment to overcome the presence of water [29].

Although Guaiol formed a hydrogen bond with His293 and showed the best docking score, PASS online did not predict antibacterial activity for this compound. This suggests that its contribution to the extract's antibacterial effect, if any, is unlikely to be direct. In contrast, Pogostol, which had a slightly lower binding affinity (-6.6 kcal/mol), was predicted to have moderate antibacterial activity (Pa = 0.417), making it a more plausible contributor to the extract's efficacy. Therefore, Pogostol may be a more suitable lead compound for future antibacterial studies because it combines acceptable target affinity with a positive prediction of biological activity, whereas Guaiol may require further experimental validation to clarify its actual contribution to the antibacterial activity of the crude extract.

Guaiol formed a hydrogen bond with His293, which is one of the key residues in PBP2a, as shown in Figures 3 and 4. Several studies report that His293 is located in the allosteric pocket where ligands such as β-lactams, peptidoglycan, quinazolinones, and oxadiazoles bind via hydrogen-bonding interactions [30]. Residues like His293 that appear repeatedly in allosteric ligand binding are part of a communication network linking the allosteric site to the active site [31]. Ligand binding at the allosteric site involving His293 may induce conformational changes that disrupt the salt-bridge cascade and keep the active site in a closed or inactive state [32]. This suggests that environmental perturbations around His293 can alter the active state of PBP2a.

While hydrogen bonding drives stability for the controls and Guaiol, other compounds such as Pogostol and Bicyclogermacrene rely only on hydrophobic interactions to stay in the PBP2a binding pocket. Hydrophobic contacts, including van der Waals forces, pi-alkyl interactions, and pi-stacking, can significantly stabilize ligand-protein binding even in the absence of hydrogen bonds [33]. Studies have shown that strong van der Waals forces can lead to higher binding affinity because hydrophobic amino acids are often concentrated in protein binding areas [34]. As shown in Figure 4b, Pogostol has the highest number of hydrophobic contacts among the tested ligands, which means its binding is supported mainly by these nonpolar forces. This profile indicates that phytochemicals can achieve strong binding scores by fitting well in the binding pocket and forming a lipophilic network with the target protein. This mechanism explains why Bicyclogermacrene and Pogostol remain competitive in binding affinity with Vancomycin and Ampicillin, even without hydrogen bonding.

Interpretation of Computational Findings

Among the seven selected compounds, Pogostol appears to be the most promising antibacterial candidate because it demonstrated both favorable binding affinity toward PBP2a (-6.6 kcal/mol) and the highest predicted antibacterial activity according to PASS Online (Pa = 0.417). In contrast, although Guaiol exhibited the strongest binding affinity (-7.0 kcal/mol), it was not predicted to possess antibacterial activity. This finding suggests that candidate prioritization should not rely solely on docking scores but should also consider biological activity prediction, as the integration of multiple computational approaches provides a more comprehensive assessment of antibacterial potential.

It is important to note that molecular docking and PASS prediction evaluate different aspects of compound activity. Molecular docking estimates the binding affinity of a compound toward a specific protein

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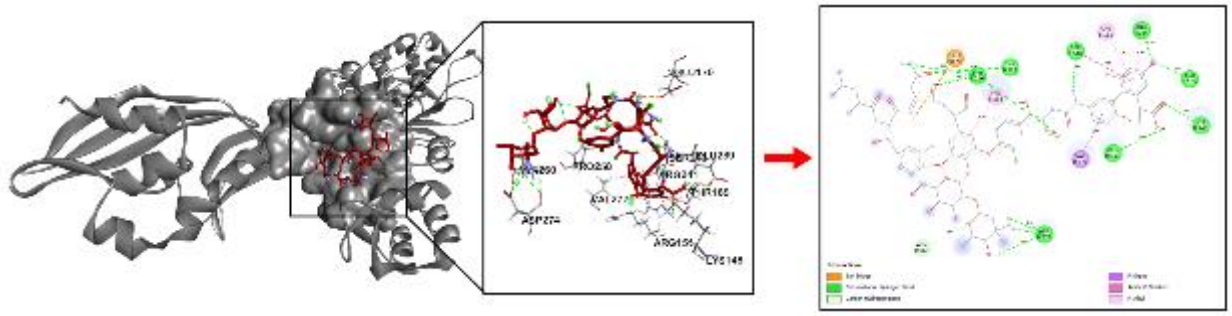
target based on molecular interactions, whereas PASS predicts the probability of biological activity using structure-activity relationships derived from large datasets of experimentally characterized compounds. Consequently, a compound with excellent binding affinity does not necessarily exhibit a high probability of antibacterial activity, and vice versa. This explains why Guaiol demonstrated the strongest binding affinity toward PBP2a but was not predicted to be antibacterial by PASS Online, whereas Pogostol exhibited moderate binding affinity and the highest predicted antibacterial activity.

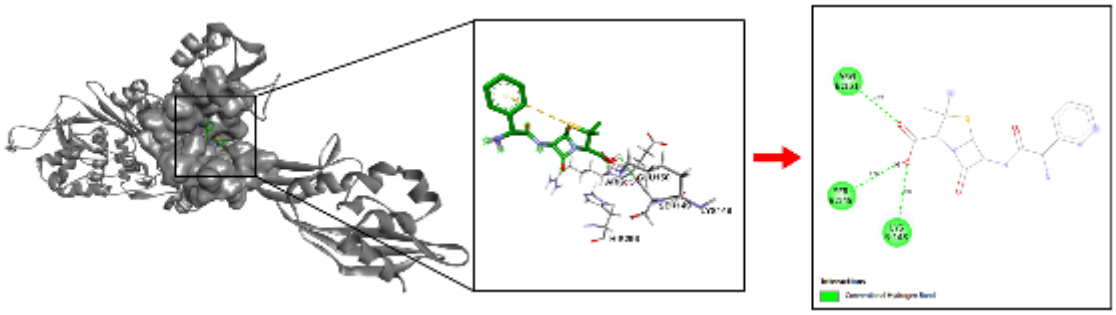
Table 2. Molecular docking results and PASS Online biological activity prediction of selected phytochemicals from *M. indica* cultivar Sengir leaves against PBP2a.

Compound	Binding Affinity (kcal/mol)	Antibacterial (Pa)	Main Predicted Activity
Vancomycin (control)	-7.6	-	Control
Ampicillin (control)	-7.4	-	Control
Guaiol	-7	-	Carminative (0.722)
Bicyclogermacrene	-6.7	0.332	Spasmolytic (0.609)
Pogostol	-6.6	0.417	Carminative (0.862)
Viridiflorene	-6.6	-	Spasmolytic (0.556)
γ-Curcumene	-6.3	-	Carminative (0.765)
Myrtenyl acetate	-6.2	0.384	Anti-inflammatory (0.753)
2-Tridecanone	-5.5	-	Prostaglandin E_2 inhibitor (0.613)

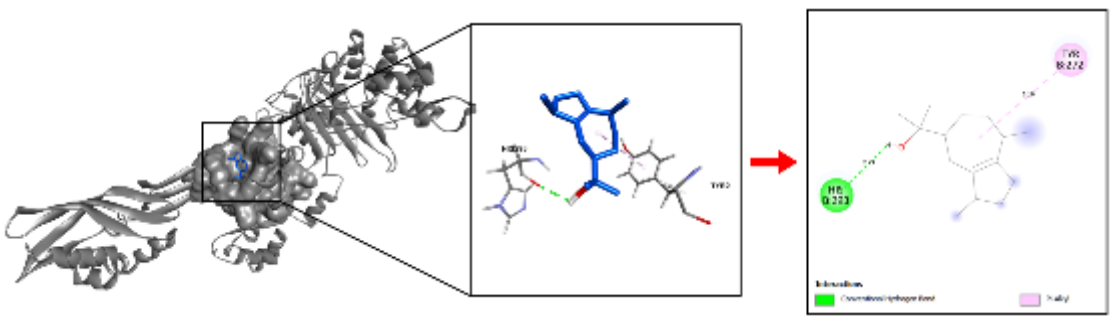
(A)



(B)



(C)



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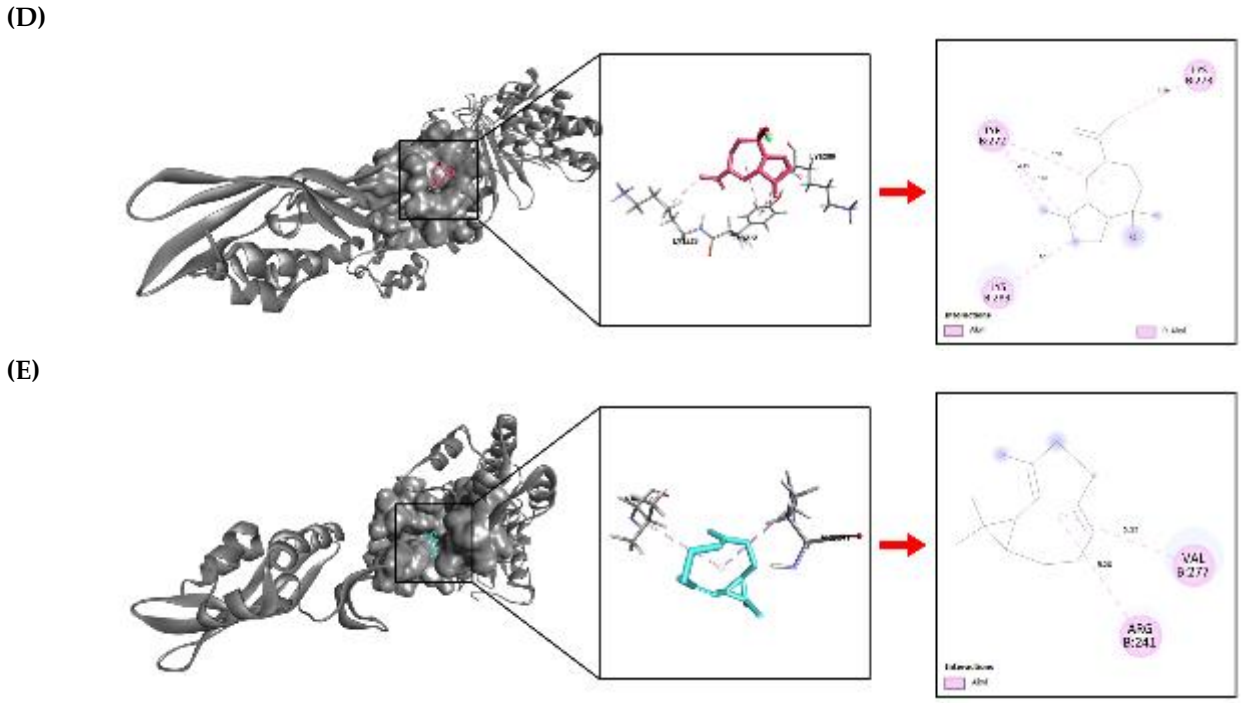

Figure 4. Two-dimensional (2D) and three-dimensional (3D) interaction profiles of selected ligands docked into the PBP2a binding pocket of *S. aureus*. (**A**) Vancomycin, (**B**) Ampicillin, (**C**) Guaiol, (**D**) Bicyclogermacrene, and (**E**) Pogostol.

One limitation of this study is that the molecular docking analysis and the in vitro antibacterial assay addressed different biological objectives. The docking results should therefore be interpreted as an exploratory assessment of phytochemical interactions with PBP2a rather than as direct mechanistic evidence explaining the antibacterial activity observed in vitro. Another limitation is the discordance between docking scores and PASS predictions. Although Guaiol exhibited the strongest binding affinity, it showed no predicted antibacterial activity, whereas Pogostol displayed moderate binding affinity but the highest predicted antibacterial activity. These findings indicate that binding affinity alone is insufficient to predict biological activity and that the antibacterial effect of the crude extract may result from the synergistic action of multiple phytochemicals. Future studies should isolate the major bioactive compounds and validate their antibacterial activity experimentally.

Conclusions

The *in vitro* and molecular docking studies demonstrated that the ethanol extract of Sengir mango leaves (*M. indica* L. cultivar Sengir) exhibits antibacterial activity against *S. aureus*. The extract produced concentration-dependent inhibition, with the largest inhibition zone (17.17 mm) observed at 100% concentration, and MIC and MBC values of 48.87 and 73.20 mg/mL, respectively. Molecular docking identified Guaiol as the strongest binder to PBP2a (-7.0 kcal/mol), whereas PASS Online predicted Pogostol (Pa = 0.417) as the most promising antibacterial candidate. These findings indicate that docking affinity alone is insufficient to predict antibacterial activity. As the computational analysis and the in vitro antibacterial assay addressed different biological objectives, the docking results should be interpreted as preliminary mechanistic insights rather than as a direct explanation of the observed antibacterial activity. Overall, the combined experimental and computational findings provide a basis for further investigation of the active constituents of Sengir mango leaf extract and their antibacterial mechanisms of action.

Conflict of Interest

The authors declare no conflict of interest.

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