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Natural Inhibitors from *Ganoderma lucidum* Targeting BlaR1 Resistant *Staphylococcus aureus* in Athlete Skin Disease

Arija Qoshasi[1], D.Riani Pangestika[1], Rafly Prasetyo Priambodo[1], Arifa Rizqi Nafisa[1] and Anggiresti Kinasih[1,*]

[1] Department of Biology, Faculty of Science and Mathematics, Diponegoro University, Tembalang, Semarang, Central Java 50275, Indonesia.aqoshasi@gmail.com, dindariani10@gmail.com, prasetyorafly1402@gmail.com, Arifarizqi@live.undip.ac.id
* Correspondence: anggirestikinasih@live.undip.ac.id

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Abstrak:
Pendahuluan: Infeksi kulit yang disebabkan oleh beta lactam resistant Staphylococcus aureus menjadi perhatian di kalangan atlet karena penyebaran cepat melalui kontak kulit. Infeksi ini dapat mengganggu performa, menunda latihan, dan memengaruhi kesehatan tim secara keseluruhan, sehingga menjadi masalah penting dalam dunia olahraga. Penelitian ini bertujuan untuk mengeksplorasi potensi metabolit sekunder dari Ganoderma lucidum sebagai kandidat penghambat BlaR1, yang dapat menjadi pendekatan baru dalam mengatasi mekanisme resistensi pada Staphylococcus aureus. **Metode:** Sebanyak 324 metabolit sekunder dari G. lucidum diunduh dari KNAPSACK. Evaluasi farmakokinetik obat dilakukan dengan SwissADME berdasarkan Lipinski's Rule of Five, sedangkan prediksi toksisitas akut pada tikus dilakukan dengan platform GUSAR dan QSAR. Senyawa yang memenuhi kriteria farmakokinetik obat dan non-toksik, kemudian dianalisis lebih lanjut melalui simulasi molecular docking terhadap protein BlaR1 (PDB ID: 1XA1). **Hasil:** Hasil menunjukkan bahwa 26% (n=87/324) metabolit memenuhi kriteria Lipinski dan 4% (n=14/324) diprediksi tidak toksik. Berdasarkan hasil docking, Lucidenic acid F diidentifikasi sebagai kandidat paling potensial menghambat Staphylococcus aureus resisten antibiotik, dengan afinitas ikatan sebesar -7.4 kcal/mol pada situs aktif protein BlaR1. **Kesimpulan:** Lucidenic acid F potensial digunakan untuk mengobati penyakit kulit pada atlet yang disebabkan oleh S. aureus resisten antibiotik beta-laktam. Penelitian lebih lanjut perlu dilakukan secara in vitro dan in vivo untuk mengevaluasi keamanan Lucidenic acid F ketika diaplikasikan secara klinis.
Kata kunci: Antibiotik; In Silico; Laktamase; Olahraga; Senyawa

Abstract:
Introduction: Skin infections caused by Beta-lactam-resistant Staphylococcus aureus are a concern among athletes because they spread rapidly through skin contact. These infections can interfere with performance, delay training, and affect the team's overall health, making them a significant problem in the world of sports. This study explores the potential of secondary metabolites from Ganoderma lucidum as candidates for BlaR1 inhibitors, which can be a new approach to overcome the resistance mechanism in Staphylococcus aureus. **Methods:** 324 secondary metabolites from G. lucidum were downloaded from KNAPSACK. Drug pharmacokinetic evaluation was performed using SwissADME based on Lipinski's Rule of Five, while acute toxicity prediction in mice was performed using the GUSAR and QSAR platforms. Compounds that met the criteria for drug pharmacokinetics and non-toxicity were then further analyzed through molecular docking simulations against the BlaR1 protein (PDB ID: 1XA1). Results: The results showed that 26% (n=87/324) of metabolites met Lipinski's criteria and 4% (n=14/324) were predicted to be non-toxic. Based on the docking results, Lucidenic acid F was identified as the most potential candidate to inhibit antibiotic-resistant Staphylococcus aureus, with a binding affinity of -7.4 kcal/mol at the active site of BlaR1 protein. Conclusions: Lucidenic acid F has the potential to support future therapeutic development against skin disease in athletes caused by beta-lactam-resistant S. aureus. Further studies are needed in vitro and in vivo to evaluate the safety of Lucidenic acid F when applied clinically.
Keywords: *Antibiotics; Compounds; In Silico; Lactamase; Sports*

1. Introduction

Athletes often undergo high-intensity and repetitive physical training, temporarily suppressing the immune system and increasing susceptibility to infections. This immunosuppressive effect, combined with physical contact and skin trauma common in sports, creates a favorable environment for bacterial infections to develop. These infections impair performance and may lead to training interruptions and competition absences (1).

Skin infections are the most frequently reported among athletes, especially those engaged in contact or high-friction sports. One of the primary pathogens is *Staphylococcus aureus,* which affects athletes (2). According to Bilung et al. (3), swab tests conducted on gym equipment revealed that 73.81% of 42 tested surfaces were contaminated with *Staphylococcus aureus*, indicating a high risk of infection in the absence of proper hygiene practices. Transmission of infections caused by *Staphylococcus aureus* commonly occurs through cuts or abrasions on the skin. When the infection penetrates deeper layers, it can progress into cellulitis or erysipelas, which are characterized by redness, warmth, firmness, and pain (4).

Common infections include furunculosis (boils), impetigo, and folliculitis caused by *Staphylococcus aureus* (5,6). According to Jimenez-Truque et al. (2), the incidence of folliculitis caused by *Staphylococcus aureus* infection is significantly higher among athletes involved in contact sports, with a reported 61% increased risk of infection compared to non-contact sports. A study reported that 35% of 223 collegiate athletes experienced *Staphylococcus* infections, with a higher prevalence in wrestlers (76%) and baseball players (44%) (7).

Poor hygiene practices in shared facilities increase the risk of transmission among athletes (8). Shaban et al. (8) reported that the prevalence of *Staphylococcus aureus* skin infections can reach 10–20% in specific sports teams, particularly in football and wrestling. According to Anderson et al. (4), approximately 53.8% of reported skin infections among high school athletes in the United States were bacterial in origin, with a higher prevalence observed among wrestlers. While *Staphylococcus* infections can result in localized redness, swelling, and pain, more severe cases may lead to bacteremia or septicemia (9).

Current treatments for skin infections caused by antibiotic-resistant *S. aureus* in athletes are increasingly limited due to rising resistance, leading to reduced efficacy of conventional antibiotics and prolonged recovery times. Mascaro et al. (5) reported that 42% of 101 tested athletes carried *Staphylococcus aureus*, with 24.8% of isolates resistant to clindamycin and 15.8% to erythromycin. Moreover, by the end of the 20th century, a study involving 364 members of the National Athletic Trainers' Association found that 53% had experienced infections caused by antibiotic-resistant *Staphylococcus aureus,* with 92% of those cases presenting as skin and soft tissue infections (SSTIs) (10).

There is a lack of effective treatment options for skin infections caused by antibiotic-resistant *Staphylococcus aureus*, especially in athletes who are prone to such infections due to frequent skin trauma and exposure. Traditional antibiotics, such as beta-lactams (e.g., penicillins and cephalosporins), are becoming less effective because resistant strains have evolved mechanisms to evade or neutralize these drugs. One key resistance mechanism involves the BlaR1 protein (11), which acts as a sensor for beta-lactam antibiotics and initiates bacterial defense responses that lead to antibiotic resistance (12). Currently, there are very few drugs designed to specifically inhibit BlaR1, and not clinically approved BlaR1-targeting agents exist. This limitation contributes to the therapeutic gap, as infections caused by resistant *S. aureus* strains persist despite standard treatments. For instance, beta-lactamase inhibitors like clavulanic acid target beta-lactamase enzymes but do not directly inhibit BlaR1, leaving this resistance pathway unaddressed.

This therapeutic gap highlights the urgent need for alternative agents that can effectively inhibit BlaR1. In this context, metabolites from *Ganoderma lucidum*, offer a potential novel strategy to overcome resistance and improve treatment outcomes. *Ganoderma lucidum*, a medicinal mushroom traditionally used in Asian medicine, has demonstrated promising antibacterial properties in various studies. Bioactive compounds from *Ganoderma lucidum*, such as triterpenoids and polysaccharides, are proven to be potential candidates as antibacterial agents. Recent studies suggest that these compounds may interfere with bacterial resistance mechanisms. However, to

date, no studies have investigated the specific mechanisms of *Ganoderma lucidum* metabolites against BlaR1, highlighting the novelty and importance of this research.This study aims to identify potential BlaR1 inhibitors from *Ganoderma lucidum* using an in silico molecular docking approach to support the development of novel therapeutics against antibiotic-resistant *S. aureus*.

2. Materials and Methods

2.1 Study design

This study employed an in silico molecular docking approach to evaluate the potential of secondary metabolites from *Ganoderma lucidum* as alternative antibacterial agents targeting the BlaR1 protein. The research followed a systematic sequence of steps: (1) retrieval of secondary metabolite data; (2) drug-likeness screening using Lipinski's Rule of Five; (3) toxicity prediction; (4) protein preparation; (5) molecular docking; and (6) docking validation and visualization. Each computational tool and software were selected based on established reliability and suitability for molecular docking studies.

2.2. Ethical statement

This study does not involve experiments with human participants or animals, so ethical approval was not required. All analyses were conducted using publicly available databases and computational tools.

2.3. Materials

Secondary metabolites from *Ganoderma lucidum* were retrieved from the KNApSAcK Family Metabolite Database (http://www.knapsackfamily.com/), a comprehensive metabolite information database widely used for phytochemical screening. The 3D structure with SDF format and Simplified Molecular Input Line Entry System (SMILE) of secondary metabolite was retrieved from PubChem to choose the best match (13). To evaluate the potential of these compounds as drug candidates, SwissADME (http://www.swissadme.ch/) was employed to analyze their drug-likeness based on Lipinski's Rule of Five such as bioavailability score, log P, violation, H-bond donor, H-bond acceptor, and molecular weight (14), while GUSAR (http://www.pharmaexpert.ru/GUSAR/ and http://www.way2drug.com/gusar/references.html) was utilized to predict acute oral toxicity in rats through in silico QSAR modeling through toxicity prediction with LD 50 (Lethal Dose, 50%) with Subcutaneous (SC), Intraperitoneal (IP), Intravenous (IV), and oral drug administration routes along with toxicity class classification (15). For protein preparation, the three-dimensional structure of the target protein BlaR1 was obtained from the Protein Data Bank (PDB) using the PDB ID 1XA1.

2.4. Procedures

The overall procedure began with collecting secondary metabolites as a ligand from *Ganoderma lucidum* via the KNApSAcK database (16). Conversely, several detected compounds, such as pesticides (e.g., DDT analogs, aldrin, heptachlor, malathion, etc.) and environmental contaminants (e.g., hexachlorobenzene, methamidophos) were flagged as likely exogenous contaminants and were excluded from further analysis. These compounds underwent screening for drug-likeness using SwissADME, and those violating more than one of Lipinski’s rules were excluded from further analysis (17). Inclusion criteria for selected compounds beyond KnapSack retrieval were due to endogenous secondary metabolites that met Lipinski’s rule criteria. Subsequently, GUSAR was used to predict the acute oral toxicity of each compound in rats, with only non-toxic compounds retained for docking. The BlaR1 protein structure (PDB ID: 1XA1) was then prepared by removing non-standard residues using UCSF Chimera. Initial cleaning and removal of non-standard amino acids were performed using UCSF ChimeraX (18), followed by the removal of water molecules, addition of polar hydrogens (protonation), and assignment of Gasteiger charges on the protein target using AutoDock Tools v1.5.7, with a total Gasteiger charge of 13.9754 (19). The molecular docking simulations between selected ligands and BlaR1 were carried out using PyRx v0.9.8, which integrates AutoDock Vina to calculate binding affinities (20). To validate the docking results, PyMOL Schrodinger LLC was used to superimpose ligand poses and assess binding orientations by selecting secondary metabolites with RMSD ≤ 2Å is a good solution of the docking solution because For a ligand-protein pair, when a pose is classified as a good solution, this means

the scoring function reproduces the crystallographic binding orientation. Detailed interaction analysis and visual representation in both 2D and 3D formats were generated using BIOVIA Discovery Studio Visualizer v21.1.0.20298 (21).

2.5. Statistical Analysis

This study was a silico molecular docking simulation, therefore, the statistical analysis involved comparing binding affinity scores (in kcal/mol) obtained from AutoDock Vina for each ligand. The binding energy values were used to rank the ligands based on their predicted binding strength to BlaR1. Ligands with the most negative binding energy values were considered to have the highest potential as BlaR1 inhibitors. Descriptive statistics, including minimum, maximum, and mean binding affinities, were calculated using IBM SPSS Statistics.

3. Results

3.1. Identification of G. lucidum Metabolites with Potential Antibacterial Activity

Table 1 summarize the total number of metabolite groups in G lucidum.

Table 1. Potential Activity and Total Number of Metabolite Groups in *G. lucidum*

Groups	Total	Potential Activity	Citation
Terpenoid	206	Antimicrobial activities are antibiotic-susceptible and antibiotic-resistant bacteria, via their ability to promote cell rupture and inhibit protein and DNA synthesis.	(22)
		Lucidenic acid can inhibit bacterial proliferation by disrupting critical cellular processes, leading to reduced bacterial viability and growth suppression.	(23, 24)
		Ganoderic acid disrupts bacterial membranes, causing leakage of essential components and cell death.	(25, 26, 27)
		(+)-Cedrol has been shown to inhibit biofilm formation, reducing bacterial protection and resistance, which enhances susceptibility to antibiotics.	(28, 29)
		Alpha-Terpineol showed strong antibacterial activity against MDR *Escherichia coli* strains and was able to reduce the bacterial colonies in a short time.	(30, 31)
Steroids and sterol derivatives	15	Ergosterol exhibits activity against *Staphylococcus aureus* by disrupting membrane integrity and inducing oxidative stress.	(32)
		Cerevisterol disruption of bacterial membrane integrity and inhibition of bacterial enzymes.	(33)
		Steroidal derivatives are attributed to their ability to disrupt bacterial membrane integrity and inhibit essential bacterial enzymes.	(34)
Fatty acid derivatives	10	Caproic acid has the potential to be an antibiotic.	(35)
		Lauric acid and oleic acid show antibacterial activity against pathogenic bacteria *Staphylococcus aureus* and *Escherichia coli*.	(36)
		Methyl ester antibacterial activity against pathogenic bacteria.	(37)
		Myristate enhances the bactericidal activity of aminoglycoside antibiotics against *Staphylococcus aureus*.	(38)
		Linoleic acid exhibits antibacterial activity by inhibiting the FabI enzyme.	(39)
Aldehydes and ketones	19	Bis-chalcone exhibits antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and S*almonella enteritidis*.	(40)
		Totarol has antibacterial activity against *Staphylococcus aureus*.	(41)
Other	74	Benzyl phenyl ketone derivatives have the potential to be selective and competitive inhibitors of the enzyme 5-lipoxygenase (5-hLOX) and show antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*.	(42,43)
Total		324	

A comprehensive metabolite profiling of *Ganoderma lucidum* was conducted using data from the KNAPSACK family database. Over 334 secondary metabolites were identified, including 206 compounds as terpenoids, 1 alkaloid, 15 compounds as steroids and sterol derivatives, and 10 compounds as fatty acids derivatives. Among these, several metabolites were recognized as unique or characteristic markers of *G. lucidum*, notably the ganoderic acids, lucidenic acids, and ganoderiols. Table 1 indicate of 324 secondary metabolites from G. lucidum were assessed for their drug-likeness properties using Lipinski's Rule of Five, which evaluates five key physicochemical parameters: Log P, molecular weight (Mr), number of hydrogen bond donors (HBD), number of hydrogen bond acceptors (HBA), and the total count of rule breaches.

When considered collectively across all six parameters (Figure 1), only 87 out of the 324 metabolites fully satisfied every aspect of Lipinski's Rule of Five and thus can be classified as Lipinski-compliant. The remaining 237 compounds showed non-compliance in one or two parameters. Among the remaining 237 compounds, various deviations from the ideal ranges were observed. Specifically, 49 compounds exceeded the recommended threshold for Log P, suggesting a potential reduction in aqueous solubility. Regarding hydrogen bonding characteristics, 94 compounds showed an excess in the number of hydrogen bond donors, while 17 exceeded the recommended number of hydrogen bond acceptors. Additionally, 92 compounds had molecular weights above the 500 Da threshold, which may affect membrane transport and metabolic stability. These excesses reflect the chemical complexity typically found in natural products such as those derived from G. lucidum.

3.2. Drug-Likeness Profiling of G. lucidum Compounds Based on Lipinski's Rule

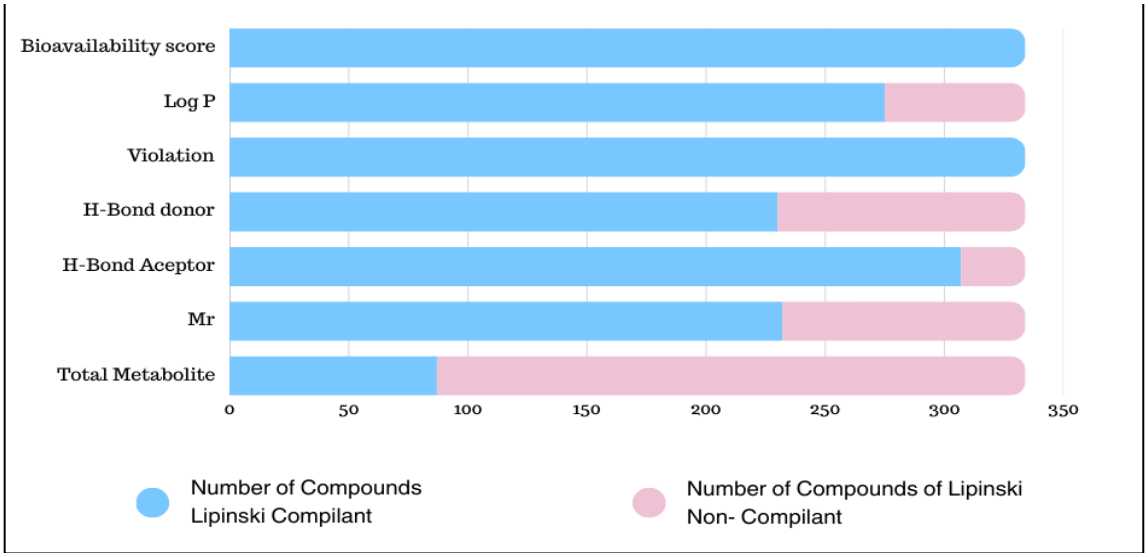

Figure 1. Druglikeness Criteria Based on Lipinski's Rule on G. *lucidum*. Mr= Molecular weight, H-Bond donor= Hydrogen bond donors, H-bond acceptor= Hydrogen bond acceptor.

Notably, none of the compounds exhibited multiple simultaneous breaches of Lipinski's parameters, meaning each compound only exceeded one criterion rather than showing broader non-compliance. This partial deviation pattern indicates that most compounds contain a significant degree of drug-likeness despite falling outside a single recommended range.

3.3. In Silico Toxicity Evaluation of Selected G. lucidum Metabolites

This study investigated the acute toxicity profiles of 87 secondary metabolites derived from *G. lucidum* that comply with Lipinski's Rule of Five, indicating favorable drug-likeness properties. The compounds were assessed for their predicted rat acute toxicity (LD_{50}) across four different routes of administration: Subcutaneous (SC), Intraperitoneal (IP), Intravenous (IV), and Oral. The results are visualized in the heatmap presented in **Figure 2**.

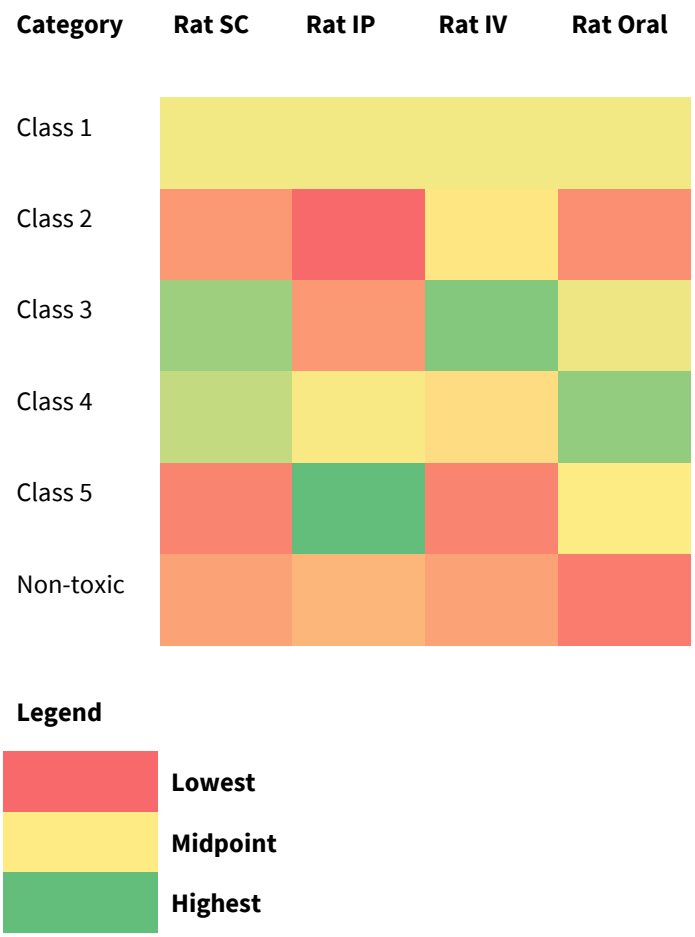

Figure 2. Heatmap of Compounds Lipinski Rule of Five Compliant Based on LD50 Classification by Route of Administration. Abbreviation: SC= subcutaneous, IP= intraperitoneal, IV=intravena

The heatmap reveals that the majority of *G. lucidum* metabolites fall into Class 3 and Class 4 toxicity levels, suggesting moderate toxicity with LD_{50} values typically ranging between 50 and 300 mg/kg. This trend is most prominent in the IV and Oral administration routes, with 37 compounds in Class 3 (IV) and 34 in Class 4 (Oral). These findings suggest that when administered via these routes, many *G. lucidum* metabolites exhibit moderate acute toxicity, which is acceptable for early-stage pharmacological evaluation.

Table 2 shows the predicted LD_{50} values of five secondary metabolites from *Ganoderma lucidum* across different administration routes in rats. Among the compounds, (+)-Cedrol exhibited the highest oral LD_{50} (5252 mg/kg), indicating the lowest predicted acute toxicity, followed by alpha-Terpineol (2209 mg/kg) and Lucidenic acid F (1285 mg/kg). In contrast, Trinorlanostane pentaoxo acid demonstrated the lowest oral LD_{50} (834.2 mg/kg), suggesting relatively higher toxicity. Notably, intravenous LD_{50} values were consistently lower than oral LD_{50} across all compounds, highlighting the influence of the administration route on systemic toxicity. These in silico results suggest that Lucidenic acid F and (+)-Cedrol may be relatively safe candidates for oral administration based on their higher LD_{50} values, which reflect lower toxicity per unit body weight.

Table 2. Toxicity Evaluation of Selected Secondary Metabolite Compounds in *G. lucidum*

Secondary Metabolite	Rat SC LD50 (mg/kg)	Rat IP LD50 (mg/kg)	Rat IV LD50 (mg/kg)	Rat Oral LD50 (mg/kg)
Lucidenic acid F	326.200	796.400	104.800	1285.000
Trinorlanostane pentaoxo acid	178.200	575.000	73.810	834.200
(+)-Cedrol	259.700	964.500	61.590	5252.000
Caproic acid	935.400	339.500	910.800	1024.000
alpha-Terpineol	266.500	371.000	82.080	2209.000
4-Terpineol	366.200	308.600	54.120	2320.000
(6E)-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	1207.000	380.400	257.200	4499.000
(-)-linalool	674.500	109.600	81.030	3271.000
(-)-4-Terpineol	366.200	308.600	54.120	2320.000
(+)-S-Linalool	674.500	109.600	81.030	3271.000
Lauric acid	3003.000	1802.000	1218.000	2799.000
Nonanoic acid	1496.000	609.500	876.100	2680.000
Ricinoleic acid	3519.000	1600.000	466.200	6327.000
Nonanol	5269.000	2468.000	537.700	4338.000

3.4. Molecular Docking of G. lucidum Metabolites Against BlaR1 Protein

All the metabolites tested in this study are constituents of *G. lucidum*, a medicinal mushroom known for its diverse bioactive compounds. The results highlight the presence of secondary metabolites in *G. lucidum* with promising binding affinities, especially Lucidenic Acid F, demonstrating a strong interaction with the target protein BlaR1. These findings are significant in drug discovery as they suggest that *G. lucidum* holds considerable potential as a source of bioactive compounds.

Molecular docking analysis revealed that among the evaluated secondary metabolites from *Ganoderma lucidum*, four compounds exhibited the strongest binding affinity toward the BlaR1 protein such as Lucidenic Acid F (-7.4 kcal/mol= strong), Trinorlanostane Pentaoxo Acid (-7.2 kcal/mol= strong), (+)-Cedrol (-6.4 kcal/mol= strong), and Caproic Acid (-6.2 kcal/mol= strong) and met the criteria of lipinski's rule of five (RO5). Based on general thresholds in molecular docking studies, binding energies lower than -6.0 kcal/mol are typically considered indicative of strong and potentially biologically relevant interactions, whereas values between -4.0 and -6.0 kcal/mol suggest moderate affinity. Compounds such as Lucidenic Acid F and Trinorlanostane Pentaoxo Acid, with binding energies below -7.0 kcal/mol, are therefore considered promising candidates due to their high binding strength, which may reflect enhanced inhibitory potential against the BlaR1 sensor domain. Further experimental validation is warranted to explore the therapeutic potential of these metabolites, particularly Lucidenic Acid F, in drug development.

Table 3. Affinity and Biophysical Profile Based on Lipinski's Rule of Selected Secondary Metabolite Compounds in *G. lucidum*

Secondary Metabolite	Binding Affinity (kcal/mol)	RMSD Value	Bioavailability Score	LogP	Violation	H-bond aceptor	H-bond donor	Mr (gr/mol)
Lucidenic acid F	-7.4	2.046	0.56	1.88	0	6	1	456.57
Trinorlanostane pentaoxo acid	-7.2	1.902	0.56	1.81	1	7	1	470.55
(+)-Cedrol	-6.4	0	0.55	2.99	0	1	1	222.37
Caproic acid	-6.2	2.173	0.85	1.57	0	2	1	116.16
alpha-Terpineol	-5.2	1.519	0.55	2.51	0	1	1	154.25
4-Terpineol	-5.1	2.016	0.55	2.51	0	1	1	154.25
(6E)-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	-4.7	3.793	0.55	3.64	0	1	1	222.37
(-)-linalool	-4.5	2.36	0.55	2.71	0	1	1	154.25
(-)-4-Terpineol	-4.4	1.355	0.55	2.52	0	1	1	154.25
(+)-S-Linalool	-4.4	1.685	0.55	2.70	0	1	1	154.25
Lauric acid	-4.1	3.035	0.85	2.70	0	2	1	200.32
Nonanoic acid	-4.1	1.851	0.85	2.30	0	2	1	158.24
Ricinoleic acid	-4	2.225	0.85	3.86	0	3	2	298.46
Nonanol	-3.8	0.825	0.55	2.72	0	1	1	144.25
Penicillin G	-7.3	1.589	0.56	1.96	0	4	2	334.39

3.5. Interaction Profile of Top-Ranked G. lucidum Compounds with BlaR1 Active Site

Lucidenic Acid F (-7.4 kcal/mol= strong) demonstrated a strong binding affinity to BlaR1, interacting with several key amino acid residues through hydrogen bonds and van der Waals interactions. Hydrogen bonds were formed with GLN105, MET104, LYS196, THR197, THR199, and van der Waals interactions were observed with ILE185, SER59, SER107, ILE201, ASN109, TRP94, PHE91, ASN106, and GLU239. These interactions are consistent with its bioactive potential, where hydrogen bonding with polar residues like SER59, SER107, LYS196, and THR197 may stabilize the ligand in the binding pocket. In contrast, van der Waals interactions with hydrophobic residues such as ILE185, ILE201, and PHE91 contribute to the overall binding affinity. The involvement of LYS196 and THR197, crucial for the β-lactamase activity of BlaR1, suggests that Lucidenic Acid F could potentially inhibit BlaR1 by disrupting its catalytic mechanism.

Trinorlanostane Pentaoxo Acid (-7.2 kcal/mol= strong) exhibited a similar binding pattern, with hydrogen bond interactions with MET104, THR197, LYS196, and THR199, and van der Waals interactions with LYS236, GLY235, SER107, SER59, ILE201, ASN109, TRP94, PHE91, ASN106, GLU239, and GLN105. The presence of van der Waals interactions with LYS236 and GLY235 suggests that the compound may occupy a slightly different orientation in the binding pocket than Lucidenic Acid F but stabilizes the binding through interactions with key residues. Notably, the hydrogen bonds with LYS196 and THR197, which are critical for the enzyme's function, further highlight the potential of Trinorlanostane Pentaoxo Acid to inhibit BlaR1 activity.

Table 4. Interaction Profile of Top-Ranked *G. lucidum* Compounds with BlaR1 Active Site

Secondary Metabolite	Molecular formula	Group	Binding Affinity (kcal/mol)	Binding Pocket
Lucidenic acid F	C27H36O6	Terpenoid	-7.4	Hydrogen bond: GLN105, MET104, **LYS196**, **THR197**, **THR199**, Van der waals: ILE185, **SER59**, **SER107**, ILE201, **ASN109**, TRP94, **PHE91**, ASN106, GLU239
Trinorlanostane pentaoxo acid	C27H34O7	Terpenoid	-7.2	Hydrogen bond: MET104, **THR197**, **LYS196**, **THR199**; Van der waals: **LYS236**, **GLY235**, SER107, **SER59**, ILE201, **ASN109**, **TRP94**, **PHE91**, ASN106, GLU239, GLN105
(+)-Cedrol	C15H26O	Terpenoid	-6.4	Hydrogen bond: **THR197**, Van der waals: GLN105, PHE211, GLY195, GLU239, MET104, **SER107**; Alkyl: ILE185, **LYS196**
Caproic acid	C6H12O2	Fatty acid	-6.2	Hydrogen bond: **TRP94**, **THR199**, **SER59**; Van der waals: ALA93, PHE91, SER107, MET146, ASN58, GLY198, THR197, ASN 106
Penicillin G	C16H18N2O4S	Control (+)	-7,3	Hydrogen bond: **SER59**, **SER107**, **THR197**, **LYS196**, **GLY235**, **LYS236**; Van der waals: MET149, LYS62, **ASN109**, GLY198, **THR199**, SER234; Pi-Alkyl: **TRP94**, **PHE91**

Penicillin G, a known β-lactamase inhibitor, serves as the positive control. The docking results for *Penicillin* G (-7.3 kcal/mol= strong) showed hydrogen bond interactions with SER59, SER107, THR197, LYS196, GLY235, and LYS236, which are critical residues involved in the hydrolysis of the β-lactam ring. Van der Waals interactions were also observed with MET149, LYS62, ASN109, GLY198, THR199, and SER234, while Pi-Alkyl interactions were observed with TRP94 and PHE91. These interactions align with the known mechanism of *Penicillin* G, which involves the inhibition of BlaR1 by binding to its active site and preventing the hydrolytic activity of the enzyme.

Lucidenic Acid F and Trinorlanostane Pentaoxo Acid exhibited binding affinities similar to Penicillin G's, suggesting their potential as β-lactamase inhibitors. The shared interaction sites, particularly with residues like SER59, SER107, LYS196, and THR197, indicate that these compounds may inhibit BlaR1 through a mechanism analogous to that of *Penicillin* G. Notably, the presence of hydrogen bonds and van der Waals interactions with these critical residues suggests that Lucidenic Acid F and Trinorlanostane Pentaoxo Acid could effectively disrupt BlaR1's β-lactamase activity, similar to how *Penicillin* G functions by binding to and blocking the active site. The significant interactions with hydrophobic residues such as TRP94, PHE91, and ILE185 further suggest that these compounds may achieve favorable binding through polar and non-polar interactions. Including both hydrogen bonding and van der Waals interactions enhances the stability of the binding complex, potentially contributing to the observed high binding affinity.

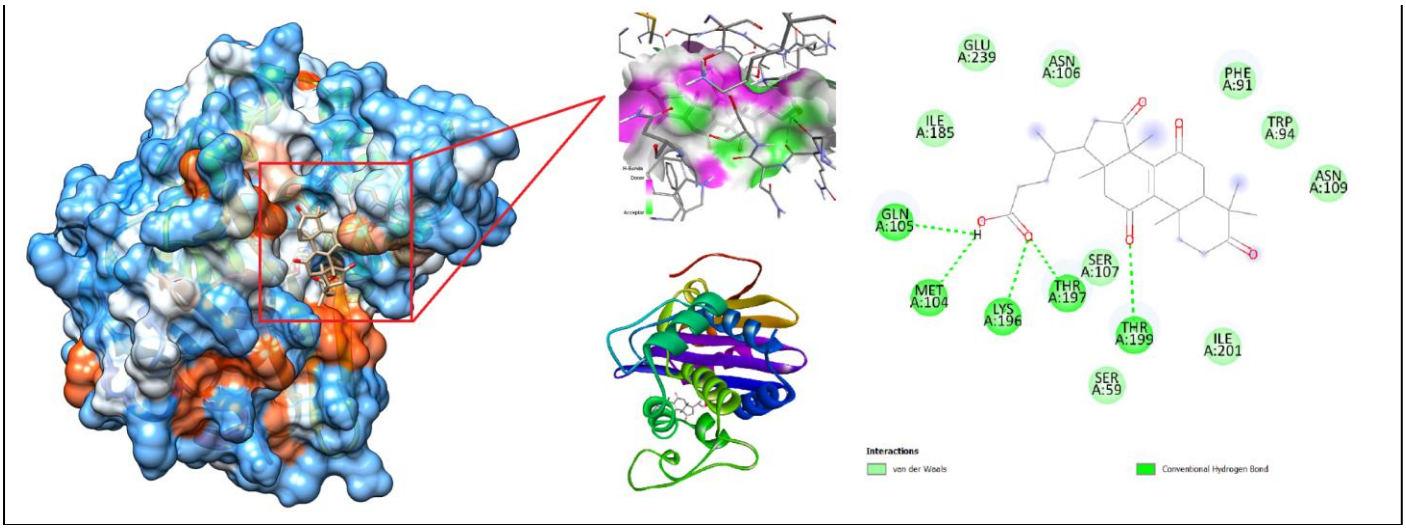

Figure 3. Binding Pocket Interactions of the Secondary Metabolite Lucidenic acid F from *G. lucidum* with the BlaR1 Protein Target

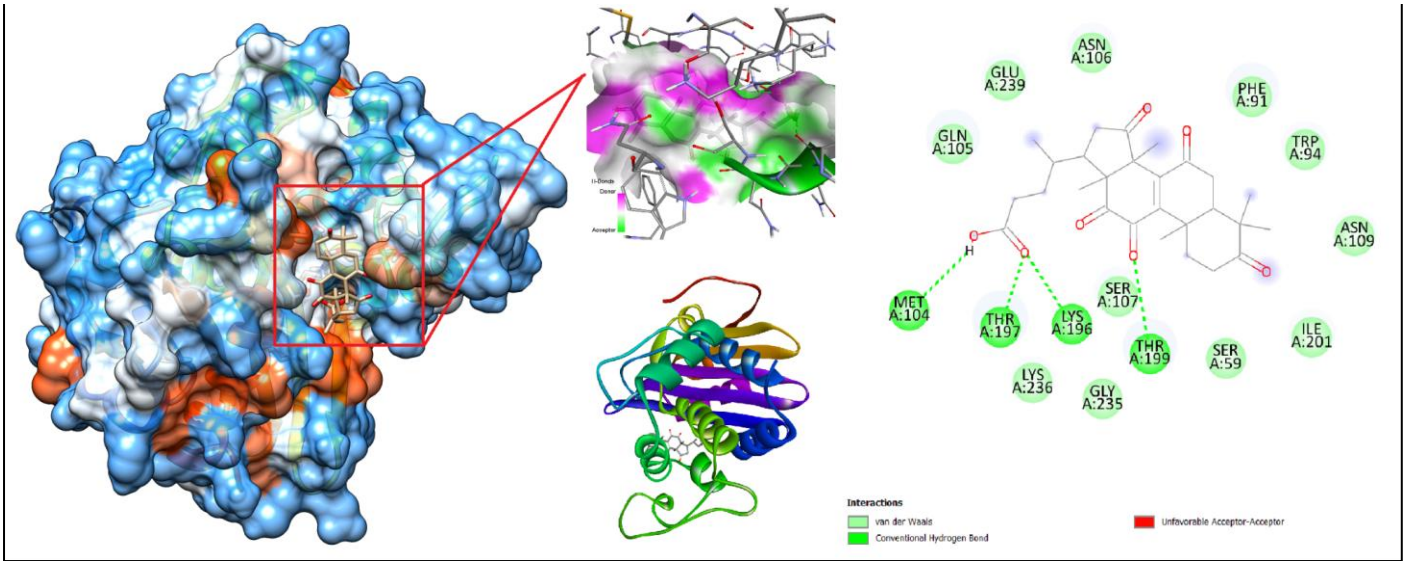

Figure 4. Binding Pocket Interactions of the Secondary Metabolite Trinorlanostane Pentaoxo Acid from *G. lucidum* with the BlaR1 Protein Target

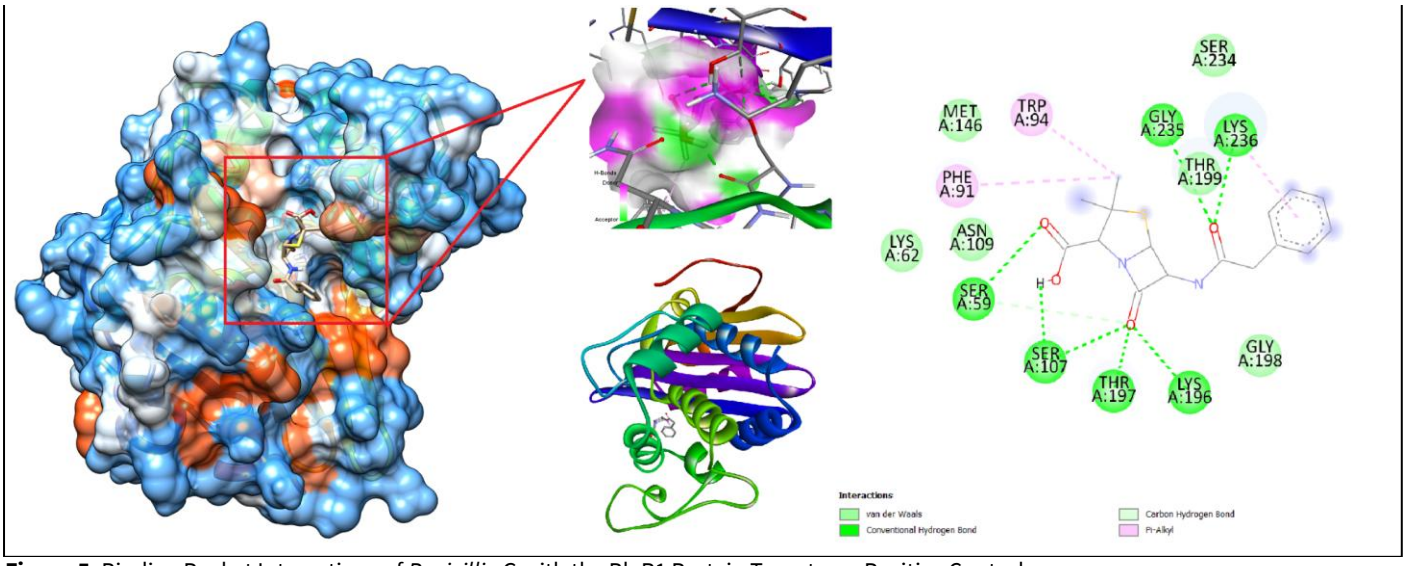

Figure 5. Binding Pocket Interactions of *Penicillin* G with the BlaR1 Protein Target as a Positive Control

4. Discussion

This study presents computational evidence suggesting that Lucidenic Acid F, a triterpenoid compound derived from *Ganoderma lucidum*, may act as a potential inhibitor of BlaR1, a key regulatory protein involved in β-

lactam antibiotic resistance in *Staphylococcus aureus*. Given that BlaR1 plays a pivotal role in sensing the presence of β-lactam antibiotics and initiating the expression of resistance genes such as blaZ, its inhibition could represent a novel strategy to combat resistant *S. aureus* strains, particularly those implicated in skin infections commonly observed in athletes due to frequent skin contact and humid environments.

Supporting the bioactivity of *G. lucidum* extracts, previous studies by Surahmaida et al. (44) and Nuraeni & Sembiring (45) reported an IC_{50} value of 94.83 ppm for the 70% ethanol extract, indicating moderate antimicrobial potential of its phytochemical constituents. While Lucidenic Acid F was not individually tested in these studies, the reported IC_{50} value provides indirect evidence of bioactive compounds within *G. lucidum*, supporting its continued exploration as a source of potential therapeutic agents.

Based on Fatturahman et al. (46), the ethanol extract of *Ganoderma lucidum* has the most effective concentration for inhibiting *Staphylococcus aureus* at the 40% concentration with inhibition zone diameters of 12 mm. Handrianto (47) stated that the highest antibacterial activity of the ethanol extract of *G.lucidum* .

According to Parameswari et al. (48), *Ganoderma lucidum* extract exhibits potential as an antimicrobial agent, including inhibitory activity against *Staphylococcus aureus*, a common pathogenic bacterium known to produce beta-lactamase (BORSA). According to Nguyen et al. (49), at a concentration of 12.5 mg/mL *G.lucidum* extract, 99.9% of *Staphylococcus aureus* cells were killed.

Preliminary in silico analysis shows that Lucidenic Acid F complies with Lipinski's Rule of Five, a widely accepted guideline for predicting oral drug-likeness. The compound has a molecular weight below 500 Da, fewer than 5 hydrogen bond donors, fewer than 10 hydrogen bond acceptors, and a logP value under 5 (50). These properties suggest that Lucidenic Acid F may possess favorable pharmacokinetic characteristics such as good bioavailability in absorption and permeability, although experimental ADME (Absorption, Distribution, Metabolism, and Excretion) studies are still needed to confirm this.

The importance of bioavailability in determining therapeutic efficacy and absorption speed has been highlighted by Daina et al. (18). Since most drugs are administered orally, a minimum oral bioavailability score of >10% is necessary to ensure sufficient systemic circulation, as noted by Kim et al. (51). Lucidenic Acid F fulfills these preliminary pharmacokinetic requirements because has fewer than two drug-likeness violations in RO5 (52). Lipinski's Rule of Five (RO5) sets criteria for drug absorption and stability, including logP <5 to ensure appropriate lipophilicity (53), and limits on hydrogen bond donors (<5) and acceptors (<10) to maintain membrane permeability (50, 54). Molecular weight also plays a key role in drug transport and should be <500 g/mol to support better absorption and distribution (50).

Based on the results (**Figure 1**), out of 334 secondary metabolites from *Ganoderma lucidum*, 87 compounds complied with Lipinski's Rule of Five. This finding is supported by a study by Chen et al. (55), which reported that among 180 acaricide-derived compounds, a total of 80 compounds met Lipinski's criteria.

In this study, Lucidenic Acid F was computationally predicted to have low toxicity and may be suitable for oral administration at a dose of 1285 mg/kg rat body weight, based on QSAR (Quantitative Structure-Activity Relationship) modeling. However, this estimation is based solely on in silico data and has not yet been validated through experimental or in vivo testing. QSAR and QSPR methods are widely utilized in early-phase drug development due to their ability to assess toxicity and side effects, and to support the design of low-toxicity compounds from known chemical structures (15). Zakharov et al. (16) further emphasized that QSAR models can reliably predict compound potency, even when using unbalanced datasets, by correlating molecular descriptors with biological activity. While this approach offers valuable initial insight, it does not replace the need for acute toxicity tests, which remain a critical step before any clinical application (56).

LD_{50} remains a standardized parameter for evaluating acute toxicity (57), commonly expressed in mg/kg body weight and convertible to log mmol/kg body weight for computational modeling (58, 59). Although oral administration appears theoretically feasible for Lucidenic Acid F, its actual bioavailability, metabolic stability, and pharmacokinetic profile in vivo remain untested. Jin et al. (60) also highlighted that most injectable drugs are

limited to one specified administration route, suggesting that further investigation is required to confirm the practicality and efficacy of Lucidenic Acid F when delivered orally.

4.1. Comparative Docking Analysis Between G. lucidum Metabolites and Known BlaR1 Native Ligand

Based on **Table 4**, the results clearly show that Lucidenic acid F exhibits stronger binding energy (-7.3 kcal/mol= strong) compared to penicillin G (-7.2 kcal/mol= strong) on BlaR1. While penicillin G is well known as a BlaR1 substrate or native ligand, Lucidenic Acid F as a structurally distinct triterpenoid derived from *Ganoderma* species, demonstrates promising potential as a viable substitute. Lucidenic acid F may offer a novel mechanism of action, by reverse resistance of BlaR1 mechanism, potentially reducing the risk of cross-resistance in *Staphylococcus aureus* (61). The BlaR1 protein is an integral membrane that plays a key role in sensing the presence of β-lactam antibiotics, such as penicillin. The presence of BlaR1 protein, makes *Staphylococcus aureus* survive longer because BlaR1 can detect the presence of penicillin and subsequently activate a signal transduction pathway that leads to antibiotic resistance. Penicillin G can interact with *Staphylococcus aureus* BlaR1 by binding to the active site of the protein, which contains a critical serine residue involved in its enzymatic function (62). So, in this study, penicillin G was used as a positive control.

According to Ewunken et al. (63) the percentage of *Staphylococcus* isolates harboring the BlaR1 gene ranges from 30% to 70%, depending on the population and methodologies used. The study indicates that BlaR1-positive isolates frequently display greater resistance to antibiotic treatments, which may contribute to therapeutic failure.

Research by Hashizume et al. (64) stated that the secondary metabolite can reverse resistance to the β-lactams possessed by *Staphylococcus aureus* BlaR1 strains. By reducing the expression of the blaZ and mecA genes. Based on research by Dawod et al. (65), the BlaR1 gene functions as a sensor receptor that regulates the expression of the blaZ gene, which encodes the production of the beta-lactamase enzyme. The molecular docking analysis reveals that Lucidenic Acid F (-7.4 kcal/mol= strong) may similarly affect BlaR1 and potentially inhibit its function in a manner analogous to Penicillin G.

Lucidenic acid F has a van der Waals interaction with SER59 and SER107 in the binding pocket of BlaR1 and hydrogen bond with LYS196, THR197, THR199. These serine residues are involved in the hydrolysis of β-lactam rings in antibiotics, a key feature of β-lactamase activity. In the case of Penicillin G, these serines may act as nucleophiles, attacking the β-lactam ring and inhibiting the resistance antibiotic's mechanism of action. Meanwhile, threonine residues are predicted to contribute to the stabilization of the inhibitor–protein complex, potentially enhancing its binding affinity compared to other compounds such as Trinorlanostane pentaoxo acid, (+)-Cedrol, and Caproic acid. The lysine residue is positively charged and involved in forming salt bridges also electrostatic interactions with negatively charged or polar groups on the ligands. In BlaR1, these lysine residues contribute to the binding of β-lactam antibiotics by stabilizing interactions between the antibiotic and the enzyme.

By targeting these key residues within the binding pocket of BlaR1, Lucidenic Acid F compounds will bind effectively to the active site of BlaR1, preventing the interaction between BlaR1 with β-lactam antibiotics, and potentially inhibiting the β-lactamase activity. This interaction may block the ability of BlaR1 to inactivate the antibiotics, rendering the bacterial resistance mechanism ineffective. Nevertheless, several limitations must be acknowledged. The current findings are derived exclusively from in silico approaches, including molecular docking and dynamic simulations. Although these results suggest a stable interaction between Lucidenic Acid F and the BlaR1 binding domain, no in vitro or in vivo experimental data are yet available to confirm the biological activity or functional inhibition of BlaR1. Furthermore, the actual binding affinity and selectivity in a complex biological system remain unknown. Further in vitro and in vivo testing of identified inhibitors is needed in future studies before clinical trials are conducted.

Potential pharmacological limitations must also be considered. The bioavailability of Lucidenic Acid F, including its solubility, membrane permeability, and metabolic stability, may hinder its translation into an effective

therapeutic agent. Moreover, the predicted activity does not account for the possibility of alternative or compensatory resistance pathways in *S. aureus* that could reduce the impact of targeting BlaR1 alone.

5. Conclusions

Through rigorous in silico molecular docking and toxicity prediction analyses, Lucidenic Acid F demonstrated favorable drug-likeness properties, including a bioavailability score of 56%, a logP value of 1.88, no violations of Lipinski's Rule of Five, six hydrogen bond acceptors, one hydrogen bond donor, and a molecular weight of 456.57 g/mol. The Lucidenic Acid F compound also exhibited an acceptable safety profile, falling under Class 5 (non-toxic category), when administered via the intraperitoneal (IP) route, with an LD50 of 796,400 mg/kg. Additionally, Lucidenic Acid F showed strong binding affinity (-7.4 kcal/mol= strong), comparable to the conventional antibiotic Penicillin G (-7.3 kcal/mol= strong). Importantly, Lucidenic Acid F formed stable and critical interactions with key BlaR1 residues, namely LYS196, THR197, THR199, SER59, SER107, ASN109, and PHE9 which are essential for β-lactamase activity. These findings provide compelling evidence supporting the continued exploration of *Ganoderma lucidum* as a valuable source of bioactive secondary metabolites with potential applications against antibiotic-resistant bacterial strains. This study underscores the potential of integrating traditional medicinal resources with modern computational drug discovery approaches in the development of novel antimicrobial agents. However, in silico studies require in vitro and in vivo validation before clinical application because computational models are often only abstract representations of complex biological systems. In addition, in silico methods may fail to identify unexpected side effects or drug interactions that may arise in real biological systems.

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List of Abbreviations

BORSA	Borderline oxacillin-resistant *Staphylococcus aureus*
SMILE	Simplified Molecular Input Line Entry System
SDF	Standard Data Format
PDB	Protein Data Bank
MR	Molecular Weight
HBD	Hydrogen Bond Donors
SC	Subcutaneous
IP	Intraperitoneal
IV	Intravenous
Pop	Pyrophosphatase
GLN	Glutamine
MET	Metionine
LYS	Lysine
THR	Threonin
SER	Serine
ILE	Isoleucine
ASN	Asparagine

TRP	Tryptophan
PHE	Phenylalanine
GLU	Glutamic acid
ALA	Alanine
PSA	Surface Area
FDA	Food and Drug Administration
RO5	Lipinski's Rule of Five
GLY	Glysine
GUSAR	General Unrestricted Structure-Activity Relationships
QSAR	Quantitative Structure-Activity Relationships
QSPR	Quantitative Structure-Property Relationships
LD	Lethal Dose

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