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Research Article

In-Silico Studies of *Musa balbisiana* Colla with Proteins Promoting Antibiotic Resistance in Bacterial and Fungal Pathogens

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Abstract

Fruit of klutuk banana (*Musa balbisiana* Colla) has been used by Indonesian people to stop the diarrhea caused by the effect of dysentery and fungal infections of the skin. In this study, we used molecular docking methods to investigate the mechanism of inhibition of their main bioactive compounds cholesta-5,7-dien-3beta-ol, cycloartenol, cyclomusalenone and were compared with the native ligand (the control drugs: amphotericin B, erythromycin, and fluconazole) against the proteins responsible for antibiotic resistance in bacterial and fungal pathogens. The target receptor that plays a role were cytochrome P450 14-alpha-sterol demethylase (CYP51), N-myristoyl transferase (NMT), and penicillin-binding protein 3 (PBP3). Swiss ADME servers were used for the determination of Lipinski rule of 5 and drug-likeness prediction respectively. admetSAR and Protox-II tools for toxicity prediction. Cyclomusalenone performed the best binding affinity value -6.0, -8.5, and -6,1 (kcal.mol^{-1}) compared than the control drugs. All the bioactive compounds are non-carcinogenic, non-cytotoxic, and conform to the Lipinski rule. We recommend predicting the targets of bioactive compound for testing antifungal activity *in vitro* and *in vivo*.

Keywords: amphotericin B, binding affinity, erythromycin, fluconazole, molecular docking, *Musa balbisiana* Colla.

Studi *In-Silico Musa balbisiana* Colla dengan Protein yang Meningkatkan Resistensi Antibiotik pada Patogen Bakteri dan Jamur

Abstrak

Buah pisang klutuk (*Musa balbisiana* Colla) telah lama dimanfaatkan oleh masyarakat Indonesia untuk menghentikan diare akibat efek disentri dan infeksi jamur pada kulit. Penelitian ini menggunakan metode penambatan molekul untuk menyelidiki mekanisme penghambatan tiga senyawa bioaktif utama dari buah pisang klutuk yaitu *cholesta-5,7-dien-3beta-ol*, *cycloartenol, cyclomusalenone* dan dibandingkan dengan ligan asli (obat kontrol) amfoterisin B, eritromisin, dan flukonazol dalam penghambatan protein yang bertanggung jawab pada mekanisme resistensi antibiotik pada patogen bakteri dan jamur. Target reseptor yang berperan adalah sitokrom P450 *14-alpha-sterol demethylase* (CYP51), *N-myristoyl transferase* (NMT), dan *penisilin-binding protein 3* (PBP3). *Server* ADME Swiss digunakan untuk penentuan aturan Lipinski 5 dan memprediksi kemiripan obat. admetSAR dan Protox-II untuk memprediksi toksisitas. Siklomusalenon memiliki nilai afinitas pengikatan terbaik -6,0, -8,5, dan -6,1 (kkal.mol^{-1}) dibandingkan obat kontrol. Semua senyawa bioaktif bersifat non-karsinogenik, non-sitotoksik, dan mematuhi aturan Lipinski. Kami merekomendasikan prediksi target senyawa bioaktif untuk pengujian aktivitas antijamur secara *in vitro* dan *in vivo*.

Kata Kunci: amphotericin B, aktifitas pengikatan, *erythromycin, fluconazole, Musa balbisiana* Colla, penambatan molekul.

1. Introduction

The development of new bioactive chemicals for use in pharmaceutical research and development can be found in abundance in medicinal plants, which have been essential in the provision of essential medicine.[1,2,3] Globally, infectious diseases are a major cause of death and a challenge for public health. Drug resistance cases are increasing, existing antibiotics have adverse reactions, and previously known illnesses are resurfacing, necessitating the development of novel, safe, and efficient antimicrobial medicines. Especially when it comes to multidrug-resistant (MDR) Gram-negative and Gram-positive bacteria. Antimicrobial resistance to bacterial and fungal strains is currently a significant global issue.[4,5,6]

It is known that MDR infections are increasing due to β-lactamase production (e.g. metallo- β-lactamases, carbapenemases, and extended spectrum β-lactamases [ESBLs] enzymes), resulting in third-generation carbapenem and cephalosporin resistance. Drug resistance mechanisms can be divided into three categories: modifications in enzymes, mutation in antibiotics and a rise in efflux pump activity.[7,8,9,10] The outcomes of the emergence of medication resistance in order to combat drug resistance, new antimicrobial treatments must be developed quickly for bacterial and fungal strains. The appealing, efficient, and all-encompassing therapeutic action against infections provided by plant-based phytochemicals is safe from many side effects.[11,12,13,14]

One of the most consumed fruits around the globe is the banana. Banana fruit is produced in enormous quantities in Indonesia. Traditionally, unripe banana fruits have been used to treat dysentery and diarrhea, especially the klutuk banana (*Musa balbisiana* Colla). Bananas and fruit peels have been found to be rich in tannins, triterpenes, and flavonoids.[15,16,17] Complexes of flavonoids can prevent the formation of bacterial cell walls and extracellular proteins. Tannins have antibacterial properties by destroying enzymes, genetic information, cell walls, and other protein building blocks. It could possess an effect on bacterial cell death and bacterial replication inhibition. The chemical compound of this plant is reported to consist of several compounds with the main composition being Cholesta-5,7-dien-3beta-ol, Cycloartenol, Cyclomusalenone.[18,19,20,21]

Molecular docking serves as essential to the rational development of drugs. Docking is a technique used in molecular modeling to predict the preferred orientation of one molecule in comparison to another when they interact together to create a stable complex. The "best-fit" orientation of a ligand that binds to a particular protein of interest is what is referred to as a "best-fit" orientation in this setting of molecular docking, which is an optimization challenge.[22,23,24] The application of medicinal plants in the treatment of many diseases is essential. There are many potential solutions for overcoming drug resistance offered by traditional medicine (TM). It is complicated and currently unclear how phytocompounds can work synergistically. The potential processes include switching the host targets that cause drug resistance, altering antibiotics so they are no longer sensitive to drug resistance effectors, preventing antibiotic efflux, etc.[25,26,27]

The purpose of this work is to investigate the possible synergistic mechanism of phytocompounds from Musa balbisiana Colla interacting with bacterial and fungal proteins that cause drug resistance and antibacterial and fungal inactivation. The target receptors used in this study were CYP51, NMT, and PBP3 by comparing the interactions with native ligands as controls (Amphotericin B, Erythromycin, and Fluconazole).[28,29]

2. Method

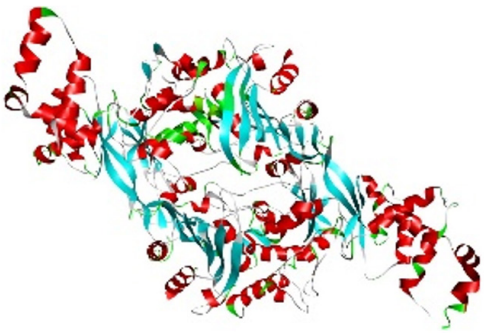
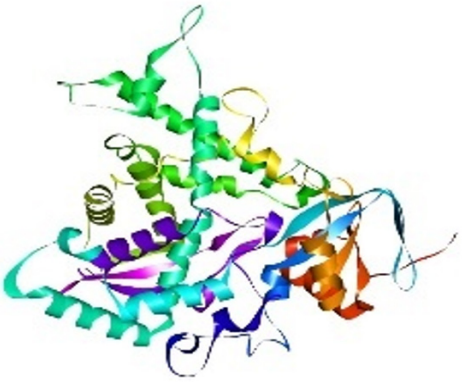
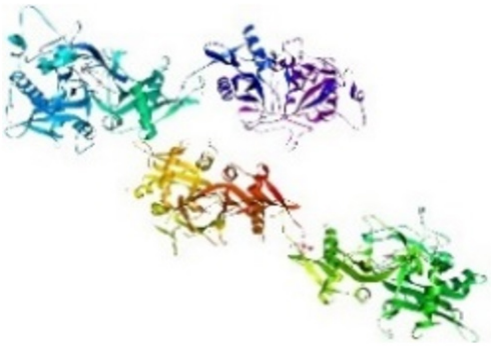
2.1. Instruments

A personal computer with AMD Ryzen 5 3550H with Radeon Vega Mobile Gfx 2.10 GHz and 16.0 GB RAM was employed to run the computational simulation.

2.2. Materials

Selected target proteins with antibacterial and antifungal properties 3VSL, 1EA1, 1IYL (table 1) had their 3D crystal

Table 1. Target proteins in fungi and bacteria that promote resistance to antibacterial

PDB ID	Name of protein	3D Structure
3VSL	Penicillin Binding Protein 3: Penicillin-binding proteins are targets for antibacterial therapy and alteration of the antibiotic target, responsible for bacteria infections' resistance to antibiotics.	
1EA1	Antifungal medications (azoles) work by suppressing the cytochrome P450 14 alpha-sterol demethylase, which is a key enzyme in fungal, responsible for fungal infections' resistance to antibiotics.	
1IYL	Drug development therapeutic targets for bacterial and fungal infections include N-myristoyl transferase, responsible for fungal infections' resistance to antibiotics.	

structures downloaded from the RCSB PDB (http://www.rscb.org/pdb).

2.3. Softwares and Tools

AutoDockVina 1.1.2, ChemBio3D, admetSAR, PROTOX-II, Avogadro, Open Babel, Protein Data Bank (PDB), PubChem.

In their binding sites, all proteins had co-crystallized ligands (X-ray ligands). These complexes, which included heteroatoms and non-essential water molecules, were removed from the target receptor molecule. Finally, the target receptor molecule obtained hydrogen atoms.

2.4. Procedures

2.4.1. Ligand preparation

Cholesta-5,7-dien-3beta-ol, Cycloartenol, Cyclomusalenone, the three primary phytocompounds of the Musa balbisiana Colla plant, were further studied to determine the molecular mechanism of their interactions with bacterial and fungal pathogens. The standard of control included the use of antibiotics such as erythromycin, fluconazole, and amphotericin B. All of the phytocompounds and antibiotics' 2-dimensional structures were downloaded in.sdf format from Pubchem (www.pubchem.com). Additionally, using Open Babel, the phytocompounds.sdf file was converted to PDB format.

2.4.2. In-silico Drug-Likeness and Toxicity Predictions

Based on an established theory, Lipinski

et al. predicted that isolated molecules would have drug-like properties. Bioactive chemicals' structures were transformed into their standard simplified molecular-input line-entry system (SMILE). They provided data to the SwissADME and PreADMET tools to calculate in silico pharmacokinetics, including the quantity of hydrogen donors and acceptors, rotatable bonds, and total polar surface area of a chemical.

2.4.3. PreADMET

PreADMET was used to predict the organ toxicities and toxicological endpoints of the isolated chemicals. A factor termed drug score was used to select compounds as drug candidates. The chance of a chemical being regarded as a drug candidate increases with increasing drug score value. The molecular PDB structures have been uploaded to the website's server (http://www.scfbio-iitd.res.in/utility/LipinskiFilters.jsp).

2.4.4. Docking Studies Using AutoDock Vina

Using AutoDock Vina 1.1.2, the energy-minimized structures of phytocompounds and ligands (positive control) were docked with target proteins. The atomic charges were included in the PDBQT file format, a modified version of pdb, which was used to represent the receptor and ligand files, definitions of ligand atom types and topological details (rotatable bonds). For docking, a grid box with a grid spacing of 1 Å was used to surround the entire receptor, keeping the ligand a flexible molecule and the receptor a rigid molecule. The side-chain and backbone of the ligand were adaptable, allowing it to interact with the receptor in all potential configurations.

The preparation of the receptor-ligand and the binding site are defined, the command prompt was used to start docking runs. The whole binding site's interaction energy between the ligand and receptor was computed and reported as affinity (kcal/mol). The total binding site interaction energy was computed and reported as affinity (kcal/mol) for the ligand and receptor.

3. Result

3.1. In silico Pharmacokinetics (Drug-Likeness) and Toxicity Analysis.

The SwissADME tool was used to predict in silico pharmacokinetic characteristics (drug-likeness qualities) based on Lipinski's rule of five. Bioactive chemicals' structures were translated to their canonical simplified molecular-input line entry system (SMILE). The five-parameter rule should be followed by the medications and/or candidates, according to Lipinski's rule of five, which specifies that hydrogen-bond acceptors (HBAs) should be fewer than 10, and hydrogen-bond donors (HBDs) should be less than 5, molar refractivity (MR) between 70 and 239, log P should not be less than 5, and molecular mass should be less than 500 Da.

Lipinski's rule of five was verified on the docking-ready ligands. The target proteins were also evaluated using the described or commercially available peptide inhibitors/inducers (positive controls) (Table 2). A prediction known as "drug-likeness" determines whether a specific organic molecule exhibits characteristics that are typical of an orally active drug. The evaluated compounds in this study adhered to Lipinski's rule of five and are therefore expected to be orally active, according to SwissADME

Table 2. Physiochemical parameters of ligand molecules screened for Lipinski's rule

Compounds	MW (g/mol)	NHD	NHA	Log P	MR	Lipinski's Rule
1	384.6	1	1	4.79	123.1	Yes
2	426.7	1	1	5.17	135.1	Yes
3	424.7	0	1	4.78	134.4	Yes
4	924.1	12	18	3.76	239.1	No
5	733.9	5	14	4.52	189.3	No
6	306.3	1	7	0.41	70.7	Yes

Abbreviations: NHD, number of hydrogen donor; NHA, number of hydrogen acceptor; MR, molar refractivity

Table 3. ADME Predictions of Bioactive Compounds

No	Skin Permeation Value (log Kp) cm/s	GI Absorption	BBB Permeability	Inhibitor Interactin (SwissADME/PreADMET)					
				P-gp Substrate	CYPIA2	CYP2CI9	CYP2C9	CYP2D6	CYP3A4
1	-2.99	Low	No	No	No	No	Yes	No	No
2	-1.96	Low	No	No	No	No	No	No	No
3	-1.98	Low	No	No	No	No	No	No	No
4	-11.94	Low	No	Yes	No	No	No	No	No
5	-8.6	Low	No	Yes	No	No	No	No	No
6	-7.92	High	No	Yes	No	Yes	No	No	No

Abbreviations: GI, gastro-intestinal; BBB, blood brain barrier; P-gp, P-glycoprotein; CYP, cytochrome-P

prediction.

3.2. ADMET Properties

TSwiss ADMET was used to predict the results of research on the absorption, distribution, metabolism, excretion, and toxicity (ADMET) of bioactive compounds. The skin's ability to absorb molecules is indicated by its skin permeability value (Kp), which is measured in cm/s. All compounds' skin permeability, Kp, values varied from -1.96 to -11.94 cm/s in silico, indicating low skin permeability.

Furthermore, the penetration of the blood-brain barrier (BBB) and the gastrointestinal (GI) system provide evidence of drug molecules' absorption and distribution. The findings of the in-silico predictions for absorption, distribution, metabolism, and excretion (ADME) of the investigated substances are shown in (Table 3).

Table 4 displays the results of the PreADMET and ProTox property explorer prediction assessments. Therefore, bioactive compounds may be appropriate candidates for this inquiry based on ADMET prediction analysis.

3.3. Docking Studies Using AutoDock Vina

The optimal docked conformation between the ligand and protein was evaluated using the docking algorithm offered by Auto Dock Vina. AutoDock Vina was used to complete the docking operation. Table 5 shows the binding affinities of ligands in kcal/mol. The molecular docking evaluation showed that the binding affinities of all the compounds range from (- 3.2 and -18.5 kcal/mol).

4. Discussions

Due to their capacity to discover and create novel, promising compounds, computational methodologies have become extremely valuable in pharmaceutical research, particularly when using the molecular docking methodology. These strategies have been used by researchers from many research groups to find prospective novel compounds that could treat a range of diseases. Cholesta-5,7-dien-

Table 4. Toxicity Prediction of Compounds Computed by PreADMET and ProTox

No	LD50 (mg/kg)	Toxicity Class	Organ Toxicity				
			Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
1	42	2	Inactive	Inactive	active	Inactive	Inactive
2	3450	5	Inactive	Inactive	active	Inactive	Inactive
3	5000	5	Inactive	Inactive	active	Inactive	Inactive
4	100	3	Inactive	Inactive	active	Inactive	Inactive
5	2000	4	active	Inactive	active	Inactive	Inactive
6	1271	4	active	Inactive	Inactive	Inactive	Inactive

Table 5. Comparative binding affinity of different ligands with receptors

No	Receptor	Ligand	Binding Affinity (kcal/mol)
1	Penicillin Binding protein 3 (3VSL)	Erythromycin	-7.1
		Cholesta-5,7-dien-3beta-ol	-5.8
		Cycloartenol	-6.2
		Cyclomusalenone	-8.5
2	Cytochrome P450 14 alpha-sterol demethylase (1EA1)	Amphotericin B	-5.3
		Cholesta-5,7-dien-3beta-ol	-6.3
		Cycloartenol	-7.5
		Cyclomusalenone	-6.1
3	N-myristoyl transferase (1IYL)	Fluconazole	-5.8
		Cholesta-5,7-dien-3beta-ol	-6.5
		Cycloartenol	-6.7
		Cyclomusalenone	-6.0

3beta-ol, cycloartenol, and cyclomusalenone are the main phytocompounds of the fruit of the klutuk banana (*Musa balbisiana* Colla), and molecular docking experiments were utilized to investigate the interactions with antibacterial and antifungal receptor proteins. As a control, standard antibacterial (erythromycin) and antifungal (fluconazole and amphotericin B) drugs were used.

The optimal docked conformation between the ligand and protein was evaluated using the docking algorithm offered by Auto Dock Vina. The higher the docking score, the better it is for this type of docking. Discovery studio visualizer identified the conformations with the most advantageous (least) free binding energy to study the interactions between the target receptor and ligands (table 6).

The Swiss ADME prediction parameters indicated that only compound (6) showed high gastrointestinal (GI) absorption, whereas (1-5) displayed low absorption. In the same way, Swiss ADME prediction showed that all compounds were not show blood–brain barrier (BBB) permeation.

Moreover, a range of cytochromes (CYP's) regulates the drug metabolism, in which CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 are vital for the biotransformation of drug molecules. Thus, in silico Swiss ADME prediction, only compound (1-3) has permeability glycoprotein (P-gp). In addition, all compound could not inhibited CYPIA2, CYP2D6 and CYP3A4, but only compound (6) could inhibit CYP2CI9 and compound (1) could inhibit CYP2C9.

None of the identified compounds have acute toxicity, according to results of acute toxicity prediction such as toxicity class categorization and LD_{50} values. Results from endpoints like hepatotoxicity, carcinogenicity, mutagenicity, and cytotoxicity are provided by the toxicological prediction. The evaluated compounds were expected to be neither irritating nor carcinogenic. All of the bioactive compounds tested negative for hepatotoxicity, carcinogenicity, mutagenicity, and cytotoxicity.

Our studies showed the efficacy of cholesta-5,7-dien-3beta-ol, cycloartenol, and cyclomusalenone in terms of their pharmacokinetic or binding affinity. The three main compounds (cholesta-5,7-dien-3beta-ol, cycloartenol, cyclomusalenone) showed a binding affinity value that was more dominant towards the N-myristoyl transferase receptor target, compared to fluconazole as the native ligand (positive control), with affinity scores of -6.0, -6.7, and -6.5 (kcal. mol-1) respectively against the control - 5.8 (kcal.mol-1). These results show that the three active compounds of *Musa balbisiana* Colla have an inhibitory mechanism as antifungal compounds on the N-myristoyl transferase receptor. Furthermore, the three

Table 6. Molecular interactions of ligands with amino acids of bacterial and fungal targets

No	Receptor	Ligand	Affinity (kcal/mol)	Interacting amino acids	
				H-Bond	Hydrophobic interaction
1	3VSL	Erythromycin	-7.1	3	Asn501, Asp498, His259, Gln548, Glu 255, Leu576, Leu256, Val493, Gly496,Glu 502, Lys494, Asn501
		Cholesta-5,7-dien-3beta-ol	-5.8	-	Ile555, Val283, Asp282, Gln279, Gln279, Ile671, Asp498, Asn572, Glu258, Thr574, Gln548, Lys569
		Cycloartenol	-6.2	-	Asp498, Ile555, Val283, Asp282, Lys569, Ile571, Gln548, Asn572, Glu258, Thr574, Gln279, Tyr280
		Cyclomusalenone	-8.5	-	Lys494, Gly496, His259, Gln548, His259, Leu576, Tyr278, Pro500, Asn501, Lys273, Tyr275, Arg504, Ser274, Glu255
2	1EA1	Amphotericin B	-5.3	2	Leu152, Ala104, Met253, Ala103, Ile153, Met225, Asp224, Gly154, Lys155, Ala150, His113, Gly112, Gln109
		Cholesta-5,7-dien-3beta-ol	-6.3	-	Asp159, Lys155, Asp222, Lys156, Arg223, Asp224, Ala104 Gly154, Ile153, Met225, Leu152, Cys151, His113, Gln109
		Cycloartenol	-7.5		Ala104, Arg223, Asp222, Lys155, Asp154, Asp224,Ile153, Met225, Leu152, Lys151 His113, Gln109
		Cyclomusalenone	-6.1	-	Arg223, Asp224, Lys156, Met225, Lys155, Asp222, Ile153, Gly154, His113, Cys151, Gln109, Leu152, Ala104, Ala103
3	1IYL	Fluconazole	-5.8	3	His307, Asp91, Lys135, Arg134, Gly132, Thr296, Glu298
		Cholesta-5,7-dien-3beta-ol	-6.5	-	Ile205, Trp137, Pro157, Gln203, Gly132, Gln203, Arg134, Gly132, Glu299, Pro131, Thr296, Glu298, Thr159, Asp170
		Cycloartenol	-6.7	-	Trp137, Ile205, Pro157, Gln203, Arg134, Gly132, Glu299, Pro131, Thr296, Glu298, Thr159, Asp170, Ser171, Val172
		Cyclomusalenone	-6.0	-	Trp133, Arg134, Lys135, Gly132, Pro131, Asp91, Glu298, His307, Lys129

active compounds of *Musa balbisiana* Colla also showed inhibitory activity as antifungal compounds on cytochrome P450 14 alpha-sterol demethylase receptor targets, compared to the control drug (amphotericin B) with a binding affinity score value of -6.3, -7.5, -6.1 respectively to the drug amphotericin B.

Meanwhile, only the cyclomusalenone compound showed activity as an antibacterial through an inhibition mechanism on the Penicillin Binding protein 3 receptor target with a dominant binding affinity score of -8.5 (kcal.mol-1) compared to the standard drug (Erythromycin) -7.1 (kcal.mol-1). These antibacterial activity results are in line with our previous research on testing against S. dysenteriae ATCC 13313.[18] Cycloartenol, cholesta-5,7-dien-3-ol, and cyclomusalenone also meets every requirement of both ADMET and Lipinski's rule of five.

5. Conclusion

The present study provides confirmation that *Musa balbisiana* Colla bioactive compounds bind to bacterial and fungal proteins responsible for altering

and protecting antibiotics, increasing their effectiveness, and helping us to understand the mechanisms underlying the synergistic potential of these compounds.

Additionally, in silico studies offer other qualities including drug-likeness, ADMET prediction, and toxicity analysis, which may be useful in creating safe and and efficient formulations of phytocompounds and antibiotics. efficient formulations of phytocompounds and antibiotics which may be useful in creating safe and efficient formulations of phytocompounds and antibiotics.

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