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Article

Molecular Docking of ftsZ Protein of *Staphylococcus aureus* to Indonesian Herbal Compound

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Abstract. Microbial resistance to antibiotics is a growing global problem, and new antibacterial agents are needed to overcome this. One of the bacteria with a high level of resistance is *Staphylococcus aureus*. Herbal compounds are an alternative as a source of new antibacterial agents. Molecular docking can be used in screening herbal compounds that can become new antibacterial agents against *Staphylococcus aureus*. Virtual screening was conducted using Ligandscout, and molecular docking was conducted via Autodock. LigPlot was used to analyze the interaction between hit compounds to the protein target, and finally, the pharmacokinetic characteristics were assessed in SWISSADME and ADMETsar programs. From 1377 compounds in the Indonesian Herbal Database, 12 hit compounds have an affinity to the target protein ftsZ of Staphylococcus aureus. Further analysis of the interaction with target protein and pharmacokinetics properties considers Alpha Santalol a compound with good potential for further development as an antibacterial agent against *Staphylococcus aureus*. However, in vitro and in vivo study is needed to validate this result.

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1. Introduction

Since their first discovery more than 70 years ago, antibiotics have saved millions of lives. Nonetheless, the incidence of antimicrobial resistance has continued to increase since the 21st century. In developing countries, main reasons of the resistance identified included: (1) poor quality of available antibiotics, (2) Lack of surveillance of resistance development, (3) ease of availability of antibiotics, and (4) clinical misuse [1].

This resistance causes a threatening global health problem and makes therapy challenging to implement [2-3]. If no action is taken, antimicrobial resistance could cause up to 10 million deaths by 2050 [4]. This high level of antibiotic resistance in bacteria makes the discovery of new antimicrobial agents an urgent matter [5], but ironically the development of new antibiotic is slowing down in the last 30 years [6].

One of the bacteria that has experienced widespread antibiotic resistance is *Staphylococcus aureus (S. aureus)* which has several resistant strains to almost all antibiotics and makes infection therapy difficult [7]. It is one of the six major bacteria causing more than one million resistance-associated deaths per year [8]. Herbal compounds have long been used as alternative sources of medicine, providing a safe and influential effect of therapy and possibly an excellent weapon to combat antibacterial resistance [9-10].

Indonesia, with its mega-biodiversity, is a country with abundant natural resources, including plants as a source of medicinal ingredients. Various original Indonesian herbal compounds databases have been developed and have become important libraries in developing medicinal ingredients [11-12]. The compound database can be screened effectively with the help of a computational program. It can identify which of the many herbal compounds have the potential to interact with the target protein.

Molecular docking is a useful method to simulate interactions between potential herbal compounds and target proteins so that compounds predicted to have the best interactions can be selected for further testing and development into new drug agents to combat antibiotic resistance [13-15]. This paper explains the screening, docking, interaction analysis, and visualization of the database of Indonesian herbal compounds owned by Universitas Indonesia to find a potential compound as a new antibacterial agent against the ftsZ target protein from *S. aureus* bacteria.

2. Methods

2.1 Identification of Protein Targets

The selection of the target protein is based on a combination of literature studies and looking at the availability of the 3-dimensional crystal structure of the protein in the Protein Data Bank database with good resolution [16]. One of the proteins known to play a significant role in S. aureus cell division is the ftsZ protein. This protein determines the formation of a contractile ring structure (often called the Z ring) in a position where cell division takes place.

Regulation of the Z ring assembly controls the timing and location of cell division. One of the functions of the ftsZ ring is to call other division proteins to the septum site to produce a new cell wall between the dividing cells [17]. By inhibiting the action of this protein, bacterial cell division does not occur. Thus, this is a potential protein to be used as a target for antibacterial agents. The ftsZ protein from *S. aureus* has a 3-dimensional structure available in the Protein Data Bank with code 4DXD and has a good resolution of 2.01Å to meet the requirements (below 2.5 Å) for use in molecular docking.

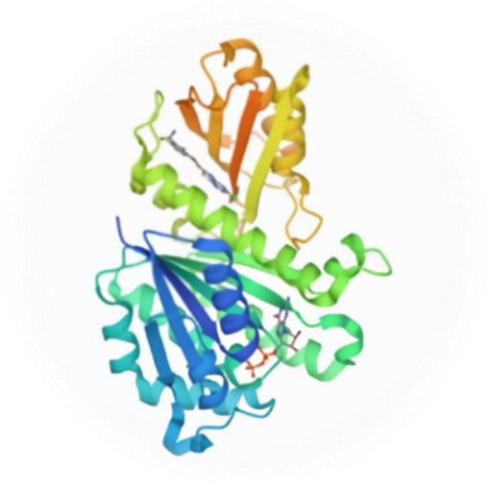

Figure 1. 3-dimensional structure of the ftsZ protein from S. aureus from the PDB database with the code 4DXD

2.2 Indonesian Herbal Compound Database

The database used in this study is the herbal compound database owned by Universitas Indonesia, with 1377 compounds [18].

2.3 Screening, Molecular Docking, Protein-Ligand Interaction Analysis, and Visualisation

Screening, molecular docking, analysis of protein-ligand interactions, and visualisation of docking results are carried out according to the following workflow

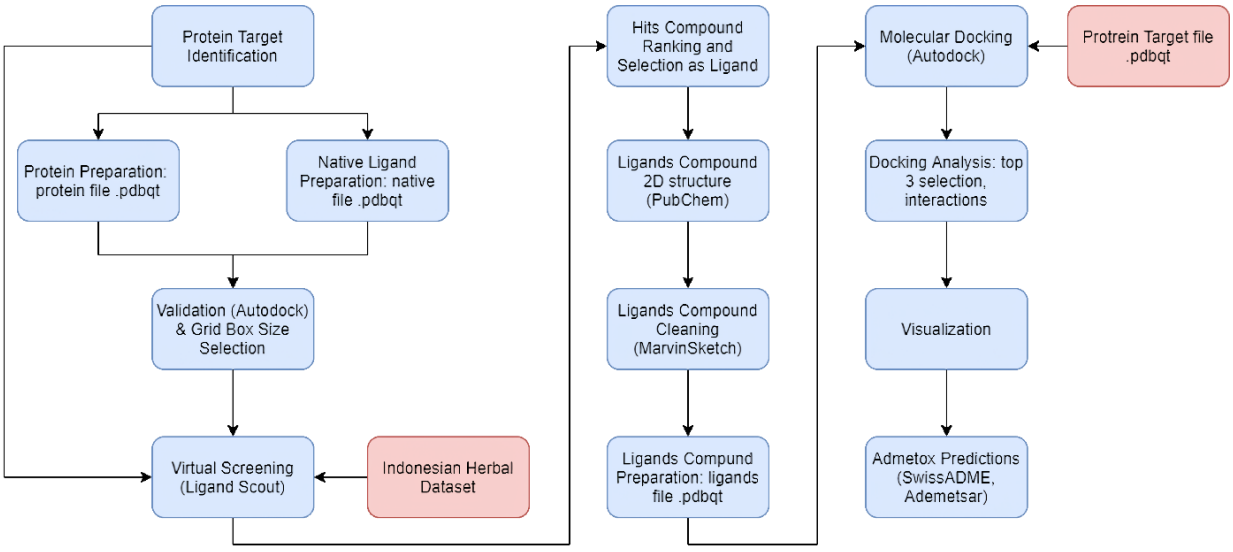

Figure. 2. Molecular docking workflow of Indonesian herbal compounds to 4DXD protein from *Staphylococcus aureus*

After obtaining the structure of the target protein from PDB, the protein is separated from its native ligand and stored as a separate file. In protein molecules, water molecules are removed, and all molecules apart from amino acid residues are separated from the protein structure and stored in pdbqt format. The pdbqt file loading was also carried out for existing native ligand molecules.

After obtaining each protein and native ligand file, the protein-ligand binding was validated by redocking with the default docking parameters using the Autodock program [19]. Redocking was performed on three grid box sizes (40x40x40, 50x50x50, and 60x60x60). Before redocking, the protein and native ligands were prepared first. Protein preparation was carried out by adding hydrogen to the polar portion and adding charge, while ligand preparation was carried out by adjusting the torque.

The parameter file grid and the parameter file docking follow the default settings of the Autodock program. In setting the grid parameter file, it is important to notice the grid centre's position, which will be used in the next docking process. Furthermore, redocking was carried out between the protein and the native ligand at each grid box size; then, the results were compared and analysed for binding free energy and Root Mean Square Deviation (RMSD). The grid box with the best results is selected for the next work step.

Virtual screening of the database of herbal compounds on target proteins was carried out using the LigandScout application [16], and the compounds resulting from the screening which were predicted to have interactions with the target protein were test compounds which would then be carried out by molecular docking one by one to the target protein using the Autodock program. The structures of the screening compounds were obtained from the PubChem database, and the 2D and 3D structures were cleaned using the Marvin Sketch program to ensure that the torques, distances, angles, and atomic positions were correct and that each test compound was stored in a .pdb file. For each test compound, when molecular docking is carried out using Autodock, the torque setting is done beforehand. The file is changed to .pdbqt format so that docking can be carried out with the target protein that has previously been owned.

The docking results of each test compound were then analyzed for its pharmacophore fit score, binding free energy, and inhibition constant. Score ranking was carried out, and three compounds with the best docking results were selected. The three chosen compounds were then subjected to interaction analysis and visualization using the LigPlot program [20] and compared with the native ligand. In addition, metabolism and toxicity prediction was also carried out using the SWISSADME [21] and ADMETSar [22] programs.

3. Results and Discussion

3.1 Protein-Native Ligand Docking Validation

The grid center was obtained at positions x=-14.15, y=41.334, and z=18.46. From the redocking process with three grid box size options (Table 1.), it is found that the grid box size of 40x40x40 is the optimal size. It produces a smaller binding free energy and inhibition constant with the RMSD value range in ten docking attempts, all within a reasonable RMSD limit, between 0.63 – 1.06. RMSD is a standard parameter to evaluate the difference between the obtained docking orientation and the corresponding co-crystallized pose of the same ligand molecule.

The smaller number of RMSD represents a more similar docking pose of a ligand concerning the biological configuration of the same ligand in the crystal structure of a complex protein, which means the docking is validated [23-24]. As for the other grid box sizes, although the binding free energy is almost the same and the best RMSD value is also good, at ten attempts of the redocking experiment, an RMSD value is out of standard. Considering the RMSD value, a 40x40x40 grid box size is chosen for the docking process.

Table 1. Protein-native ligand docking validation in three grid box size

Grid Box Size	40x40x40	50x50x50	60x60x60
Binding Energy	-10.32 kkcal/mol	-10.32 kkcal/mol	-10.30 kkcal/mol
Grid Center		X: -14.15 Y: 41.334 Z: 18.46	
Best RMSD	0.63	0.60	0.59
Inhibition Constant	27.47 nM	27.38 nM	28.14 nM
RMSD Range	0.63 – 1.06	0.60 – 5.76	0.59 – 5.67

3.2 Virtual Screening

Virtual screening is an in silico technique used in the drug discovery process where it possible to automatically evaluated large databases of known 3D structures are using computational methods [25]. Virtual screening assists in identifying the most promising hits that bind to the target protein. It works like a funnel by selecting more promising molecules for the following process in the drug discoveries and development process. Only the most promising molecules are synthesized. Virtual screening can also identify compounds that may be toxic or have unfavorable pharmacodynamic as well as pharmacokinetic properties. Thus, virtual screening techniques play a prominent role among strategies for the identification of new bioactive substances [26].

The results of the virtual screening database of Indonesian herbal compounds against 4DXD target proteins show that of the 1377 compounds in the database, 12 compound hits (Figure 1) are predicted to interact with the target protein. These 12 compounds are Angiolensin, Z,Z,Z,)-3,6,9-Dodecatrien-1-ol, Dehydrosafynol, Tetrahydroxystilbene, (E)-alpha-Santalol, Phytol, (R)-beta-Citronellol, Beta-Santalol, (Z)-2-Methyl-6-methylene-2,7-octadiene-1-ol, Geraniol, Trans,trans-Farnesol, and Safynol. This result confirmed that virtual screening increases the speed of the drug discovery process by automatically evaluating large compound libraries through computational simulations.

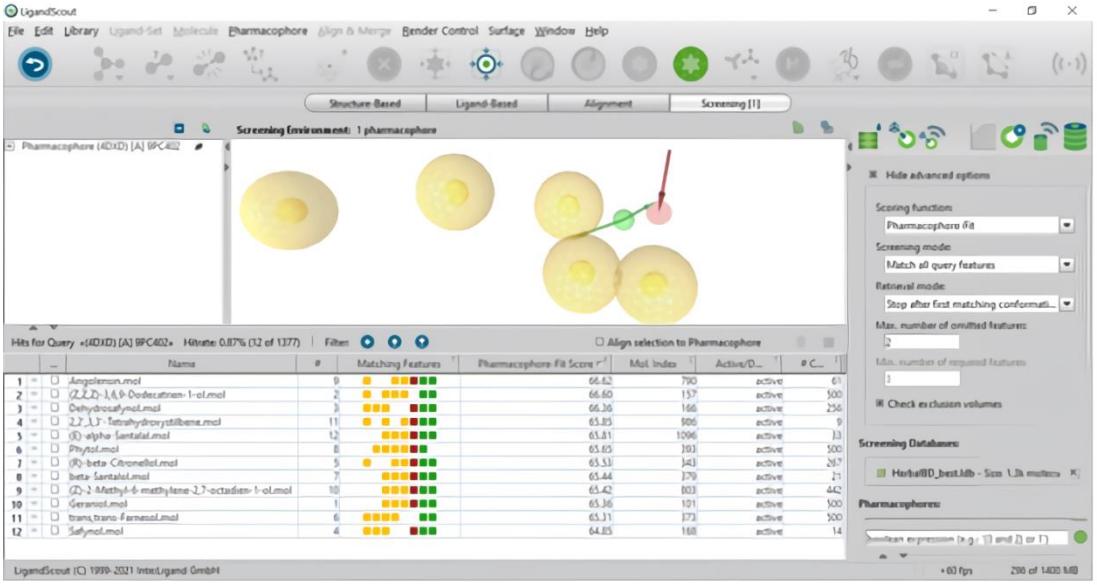

Figure 3. Virtual screening result on LigandScout

3.3 Molecular Docking

The 12 hit compounds are then docked to the protein target to evaluate which makes the stable complex. Three parameters are used to evaluate the docking; pharmacophore fit score, binding free energy, and inhibition constant [27-28]. The result of these three parameters of each compound can be seen in Table 2.

Table 2. Pharmacophore fit score, binding free energy, and inhibition constant resulted from the molecular docking of twelve compounds from the virtual screening process.

Compounds	Pharmacopore Fit Score	Binding free energy (kkcal/mol)	Inhibition Constant
Native	-	-10.32	27.47 nM
Angolensin	66.62	-8.37	3.42 nM
(Z,Z,Z,)-3,6,9-Dodecatrien-1-ol	66.60	-5.63	75.2 uM
Dehydrosafynol	66.36	-7.48	3.3 uM
Tetrahydroxystilbene	65.85	-7.16	5.68 uM
(E)-alpha-Santalol	65.81	-7.78	1.97 uM
Phytol	65.65	-6.15	31.24 uM
(R)-beta-Citronellol	65.53	-5.43	104.8 uM
Beta-Santalol	65.44	-6.77	10.98 uM
(Z)-2-Methyl-6-methylene-2,7-octadiene-1-ol	65.42	-5.44	103.09 uM
Geraniol	65.36	-5.56	83.42 uM
Trans,trans-Farnesol	65.31	-7.00	7.39 uM
Safynol	64.85	-7.32	4.33 uM

Pharmacophore fit score measures the fitness of the geometric aspect of a molecule to the 3D-structure-based pharmacophore model. The higher score indicates a better fit to the model, and therefore molecules that fit the pharmacophore model should also show the activity of binding to the target protein [16], [29]. The pharmacophore fit score ranges from 64.85 to 66.62, and the top three scores are Angiolensin (Z,Z,Z,)-3,6,9-Dodecatrien-1-ol, and Dehydrosafynol.

Molecular docking results yield the Gibbs free energy (ΔG) value in negative numbers, indicating that binding running on constant temperature and pressure and is a conformational stability parameter between the ligand and the receptor. The value of the ΔG predictor of the spontaneity of a reaction, when ΔG is negative, then a reaction occurs spontaneously and when ΔG is positive then the reaction is not spontaneous.

ΔG values are lowest used for selection the best ligand results of molecular docking. In addition to ΔG analysis, inhibition constant analysis (Ki) is also done to determine the power inhibition of a compound against its receptor, where the smaller the value of Ki, then the more potent the resistance of the compound. Constant inhibition is a parameter showing the ability to inhibit the interaction of the target protein with the hit compounds as alternative ligands. The smaller Ki value obtained will allow the interaction of the active site on the ligand with the receptor to be maximized, and the bond formed will be stable. Both of these values can be into two benchmarks in determining best compound [30-34].

Our computational docking study revealed that all the docked compounds had a smaller binding efficiency, represented by binding free energy value ranging from -5.63 to -8.37 kcal/mol, compared to the native ligand's -10.32 kcal/mol. Considering the binding free energy and inhibition constant as well as pharmacophore fit score, we specify the three best compounds were obtained are Angolensin,

E-alpha-Santaral, and Dehydrosafynol which had the smallest binding free energy and inhibition constant which was still close to the inhibition constant value of the native ligand and also reasonable pharmacophore fit score.

3.4 Interaction Analysis

The three best compounds were then analyzed for their interactions with the target protein and compared with the native ligand by looking at which amino acid residues the interactions and hydrogen bonds occurred (Table 3).

Table 3. Interaction of Angolensin, Alpha Santalol, Dehydrosafenol and native ligand against 4DXD Protein Target

Amino Acid	Gln192	Gly193	Gly196	Ile197	Asp199	Leu200	Val203	Gly205	Val207	Asn208
Native	Y	Y	Y	N	Y	Y	Y	Y (H) 3.07 Å	Y (H) 2.78 Å	Y
Angolensin	N	N	Y	N	Y	Y	Y	Y (H) 3.33 Å	N	Y (H) 2.67 Å
Alpha Santalol	Y	Y	Y	Y	Y	Y	N	N	N	N
Dehydrosafynol	Y	Y	Y	N	N	Y	Y	Y	Y (H) 3.99 Å	Y

Amino Acid	Leu209	Met226	Gly227	Leu261	Asn263	Thr265	Val297	Thr309	Val310	Ile311
Native	Y (H) 2.94 Å	Y	Y	Y	Y	N	Y	Y	Y	Y
Angolensin	Y (H) 2.88 Å	N	N	Y	Y	Y (H) 3.08 Å	Y	Y	Y	Y
Alpha Santalol	N	Y	Y	Y	Y	N	N	Y (H) 2.08 Å	Y	Y
Dehydrosafynol	Y (H) 3.11 Å	Y	Y	N	Y	N	Y	Y	N	N

Y = Yes, having interaction; N = No, have no interaction; (H) = Hydrogen bond as main interaction; number in Å = highest binding free energy among others amino acid

Main interactions are shown in all compounds, native ligands and the top three compounds, on the amino acid residues Gly196, Leu200, Asn263, and Thr309. The interactions are not hydrogen bonds at the amino acids Gly196, Leu200, and Asn263 in all ligands. At the amino acid Thr309 the Alpha Santalol compound shows a different hydrogen bond than the other three compounds.

The interaction comparison, it was found that the native ligand shows four hydrogen bonds. The Angolensin and Dehydrosafinol compounds show the same type of hydrogen bond at two amino acid positions. In contrast, the Alpha Santalol compound does not have the same hydrogen bond pattern as the native ligand. However, the binding free energy values sequentially from the largest to the smallest are Native Ligand, Angolensin, Alpha Santalol, and dehydrosafinol. This result shows that the hydrogen bonding pattern does not directly determine the binding free energy in its interaction with the target protein and the number of amino acids interacting.

The number of interacting amino acids of each ligand is 18 positions for the native ligand, 14 for Angolensin, 13 for Alpha Santalol, and 14 for Dehydrosafinol (Table 4). From this interaction

analysis, it is known that the test compound which has the most robust interaction with the target protein to the weakest is Angolensin, followed by Alpha Santalol, and the last is Dehydrosafinol.

Table 4. List of amino acid interaction positions between each ligand and the target protein

Coumpounds	Amino Acid Interactions and Position
Native	Gln192, Gly193, Gly196, Asp199, Leu200, Val203, Gly205, Val207, Asn208, Leu209, Met226, Gly227, Leu261, Asn263, Val297, Thr309, Vall310, Ile311 (18)
Angolensin	Gly196, Asp199, Leu200, Val203, Gly205, Asn208, Leu209, Leu261, Asn263, Thr265, Val297, Thr309, Val310, Ile311 (14)
Alpha-Santalol	Gln192, Gly193, Gly196, Ile197, Asp199, Leu200, Met226, Gly227, Leu261, Asn263, Thr309, Val310, Ile311 (13)
Dehydroxysafynol	Gln192, Gly193, Gly196, Leu200, Val203, Gly205, Val207, Asn208, Leu209, Met226, Gly227, Asn263, Val297, Thr309 (14)

The results of the interaction of each of these ligands were visualized using Ligplot (Figure 4) and Autodock (Figure 5) as follows

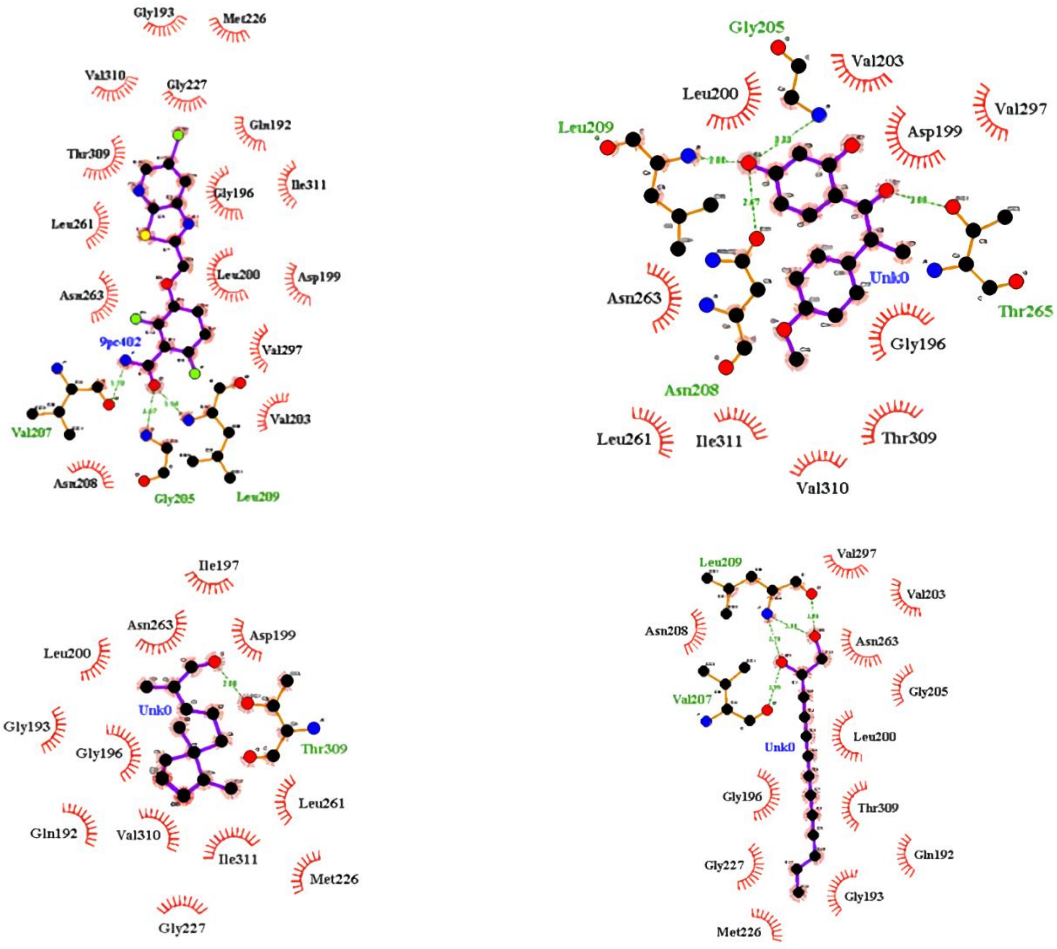

Figure 4. Visualization of the interaction of each ligand with the target protein using Ligplot. Top left: native ligand complex, top right: Angolensin complex, bottom left: Alpha Santalol complex, bottom right: Dehydrosafinol complex.

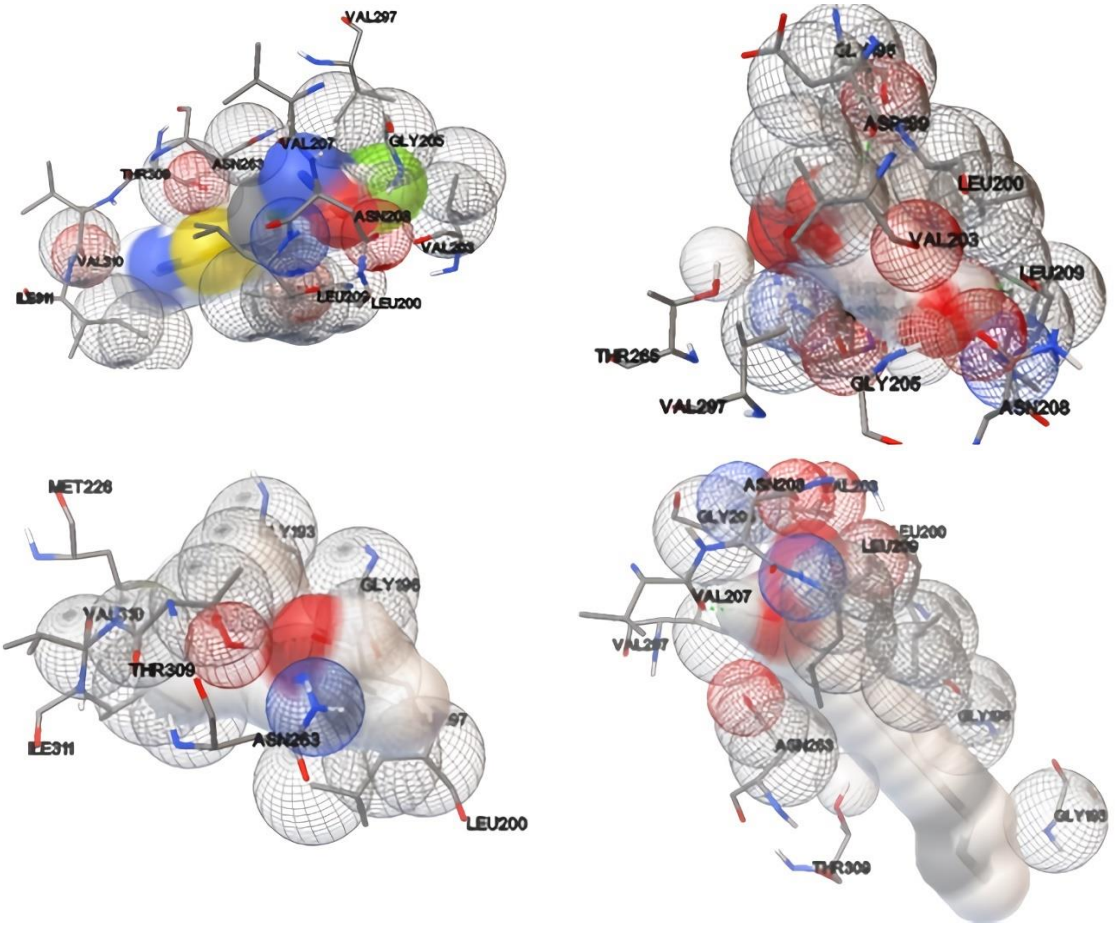

Figure 5. Visualization of the interaction of each ligand with the target protein using Autodock. Top left: native ligand complex, top right: Angolensin complex, bottom left: Alpha Santalol complex, bottom right: Dehydrosafinol complex

3.5 Pharmakokinetic Profile and Toxicity Assays

After knowing the interactions in the complex formed between each ligand and the target protein, pharmacokinetic profile prediction was analyzed for each test compound using SwissADME and Ademetsar, including absorption, distribution, metabolism, excretion, and toxicity (admet). Admet prediction results can be seen in Table 5. Efficacy and safety are the two major causes leading to drug failure, which means chemicals' absorption, distribution, metabolism, excretion, and toxicity properties play vital roles in every drug discovery and development stage. Therefore, it is necessary to find efficacious molecules with better admet properties [35].

Admet prediction results show that all compounds show good characteristics [36] in terms of molecular weight, toxicity, and absorption in the digestive tract. All compounds have a molecular weight of less than 500g/mol and do not indicate a possible cause of carcinogenesis; AOT all fall into category III, which is classified as safe for consumption, and show high absorption through the digestive tract indicating that the test compounds are easily absorbed . Nevertheless, the Angolensin compound shows a pattern of mutations in the CYP1A2, CYP2C19, and CYP3A4 genes. Moreover, when viewed from the LogP value, the most optimal is around 3, so there are two compounds whose logP values are pretty optimal [37], namely Angolensin and Alpha Santalol. Combining all parameters

in molecular docking, interaction analysis, and admet assays Alpha Santalol is considered as the best hit compound in this study.

Table 5. Predictions of Pharmakokinetic Profile and Toxicity for Angiolensin, Alpha Santalol, Dehydroxysafinol

Ligands	Druglikeness					Pharmacokinetics					Toxicities		
	MW (g/mol)	HBA	HBD	LogP	GI Absorbtion	Inhibitor CYP					AMES	Carcinogenesis	AOT
						CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4			
Angolensin	272.30	4	2	2.7	High	Yes	Yes	No	No	Yes	-	-	III
Alpha Santalol	218.33	1	0	3.57	High	No	No	No	No	No	-	-	III
Dehydroxysafynol	198.22	2	2	1.73	High	No	No	No	No	No	-	-	III

Alpha Santalol Alpha-santalol is a phytochemical derived from sandalwood oil (*Santalum album Linn*) that has been extensively studied for its anti-inflammatory, antifungal properties, cancer preventive,etc. It is the most abundant sesquiterpenoids found in sandalwood, contributing to its pleasant fragrance and wide-spectrum bioactivity. It is a very hydrophobic alcohol that emanates a cedar-like sweet smell and is typically purified from sandalwood oil by fractional distillation, column chromatography, solvent extraction [38-39]. The anti-bacterial properties of saldalwood oil have also been studied. It is reported by several study investigating the antimicrobial activities of a mixture of alpha, beta-santalol, and different sandalwood oil from various origins against yeast *Candida albicans*, the Gram-positive bacterium and the Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and concluded that santalol concentrations in sandalwood oils' affects their antimicrobial properties [40-41].

4. Conclusion

This study confirms that molecular docking is an effective and efficient method for simultaneously screening large quantities of herbal compounds. Alpha sotalol was found to be a compound that has an affinity for the ftsZ protein in *S. aureus*, showing good pharmacokinetic characteristics and potential for further development as a new antibacterial agent. However, it is necessary to carry out in vitro and in vivo studies to validate the results of this study in finding new antibacterial agents against *S. aureus*.

5. Acknowledgement

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