In Silico Antibacterial Activity of Selected Active Compounds of Cherry Leaf (*Muntingia calabura*) to *Staphylococcus aureus*

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Abstract. In addition to physical problems, acne caused by *Staphylococcus aureus* frequently leads to psychological problems as well. As a result, Treatment therapy using antibiotics in the long term and at inappropriate doses increases the rate of antibiotic resistance. Alternative treatments can be performed by using natural ingredients, such as cherry leaves (*Muntingia calabura*), which exhibit antibacterial properties. This study aimed to determine the active compound of *M. calabura* extract which has the potential as an antibacterial agent for *S. aureus*. The research was conducted in September 2022 at Universitas Negeri Surabaya. Using PyRx software, we carried out a molecular docking (blind docking) analysis. The drug-likeness test indicated stigmasterol, tetradecanoic acid, and dodecanoic acid, ethyl ester compounds met the criteria as medicinal compounds. The results of the molecular docking analysis showed that stigmasterol has a binding affinity of -7.8 kcal/mol better than other compounds and RMSD of 1.482 Å indicating its high potential. The three selected active compounds have the potential as antibacterial agents for *S. aureus*. The application of this product as a treatment for acne, however, requires further research.

1 Introduction

Skin disease may be caused by microorganisms (fungi, bacteria, and viruses) that attack tissues [1]. Acne is a skin disease frequently experienced by most people, both in their teens and adults. Acne breaks on several parts of the body such as the face, chest, and back [2]. Acne also affects psychosocial health [3].

The bacteria that trigger acne is *Staphylococcus aureus* [4]. *S. aureus* is the microflora of the skin but may turn pathogenic in susceptible hosts. This bacterium is capable of causing various suppurative infections of varying severity in tissues [5]. In accordance, Dreno *et al* [6] state that changes in the psychological condition of the skin can affect the balance of microflora and trigger a shift of *S. aureus* to be invasive and pathogenic.

Antibiotics are still an option in the treatment of acne. However, excessive and prolonged consumption of antibiotics may trigger antibiotic resistance [6]. The East Kalimantan Provincial Health Laboratory recorded that in 2013, a total of 79.5% of *S. aureus* isolates are resistant to penicillin, 34.6% resistant to gentamicin, and 33.3% resistant to ciprofloxacin [7]. Resistant bacteria are more difficult to handle and require even higher doses of antibiotics, leading to toxic and increasing costs [8]. Therefore, alternatives are needed in acne treatment such as the application of natural ingredients.

Cherry leaf (*Muntingia calabura*) is one of the plants that are effective as a traditional medicine. Based on phytochemical tests conducted by Zebua *et al* [9], the ethanol extract of cherry leaves (*M. calabura*) contains flavonoids, phenolics, terpenoids, tannins, and saponins. Empirically, this plant can be used as an antibacterial, antioxidant, antidiabetic, anticancer, and anti-inflammatory [10]. Pramiastuti *et al* [11], revealed that cherry leaf extract can inhibit the growth of *S. aureus*. However, the study did not define certain compounds that play an important role in inhibiting bacterial growth.

The active compounds in cherry leaf extract that have the ability as antibacterial agents can be identified using molecular docking. Molecular docking is one of the structure-based in silico methods widely used to predict interactions between molecules [12]. The advantage of this method is that the properties and behavior of bioactive compounds of drug candidates can be more easily estimated at an affordable cost and in a short time, and more new compound models are obtained [13]. This study aims to determine the active compound of cherry leaf ethanol extract which has the most promising potential as an inhibitor of *S. aureus* growth compared to benzylpenicillin.

2 Methods

The materials used were shown in Fig. 1. The 3D structure of the target protein *S. aureus* PBP2a with PDB ID: 5m18 was obtained from the Protein Data Bank (https://www.rcsb.org/) database page and stored in *PDB format. The 3D structure of the active compound cherry leaves (*M. calabura*) and the antibiotic

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Benzylpenicillin was obtained from the PubChem database page (https://pubchem.ncbi.nlm.nih.gov/).

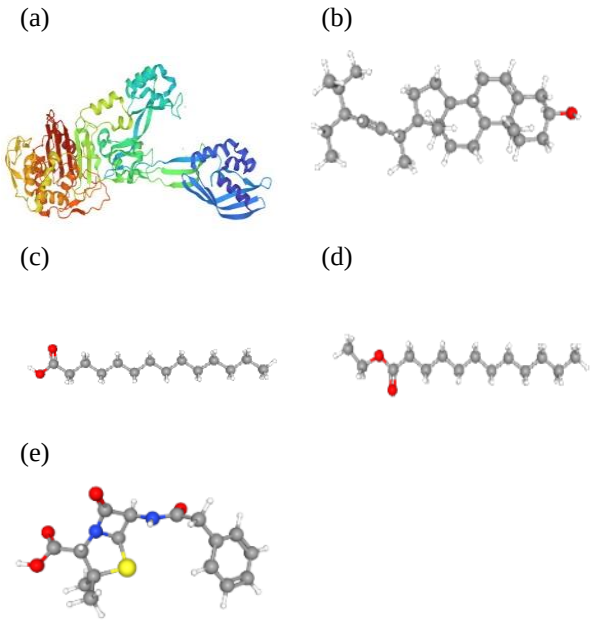

Fig. 1. 3D structure of protein targets and selected active compound of *M. calabura.* a) Target protein *S. aureus* PBP2a (PDB ID: 5m18), b) Stigmasterol, (c) Tetradecanoic acid, (d) Dodecanoic acid, ethyl ester, (e) Antibiotic Benzylpenicillin.

Then a drug similarity test was carried out to identify drug-like properties of the active compound selected based on the results of the analysis of Lipinski's rule of five. The SwissADME website (http://www.swissadme.ch/index.php) was used to input canonical SMILES data from selected active compounds collected from PubChem. There are five parameters in Lipinski's rule including molecular weight (MW) ranging from 150-500 g/mol, number of bond donors -H < 5, number of bond acceptors -H < 10, and lipophilic value (Mlog P) < 4.15 [14] [15].

Preparations before conducting molecular docking analysis were, (1). Collecting the target protein's 3D structure from the protein data bank as well as the control antibiotic (benzylpenicillin) and cherry leaf extract active compounds 3D structure from PubChem. (2). Sterilize the target protein using AutoDockTools software to separate protein molecules from the native ligand as well as unwanted molecules. The target protein *S. aureus* PBP2a (PDB ID: 5m18) is classified as a Penicillin-binding protein obtained using an X-ray diffraction method with a resolution of 1.98 Å. It has a sequence length of 642 and native ligand MUR 703(A). (3). Minimize the structure of active compounds and antibiotics to make them more flexible so that they can produce a low binding affinity with the target protein [16].

A molecular docking assay was carried out using PyRx software. The results of the analysis obtained were in the form of binding affinity values and Root Mean Square Deviation (RMSD) values [17]. Visualization of docking results was done using PyMol and Ligplot software. At this stage, the type of bond and amino acid residues at the binding site were obtained.

The binding affinity and RMSD values of each active compound of *M. calabura* to its native ligand and the benzylpenicillin were then analyzed to determine its potential. The lower the affinity energy, the stronger the bond formed and, vice versa [18]. The lowest affinity binding value with the RMSD value indicated the optimum conformation [16]. The RMSD value is declared valid if the value ≤ 2 Å and vice versa [19].

3 Results and Discussion

Cherry leaf extract (*M. calabura*) contains active compounds that have antimicrobial properties such as stigmasterol, tetradecanoic acid, and dodecanoic acid, ethyl ester [20]. A drug-likeness test was conducted to determine the similarity properties of the drug in the three selected active compounds of *M. calabura.* Through this test, the potential of a compound as a drug candidate can be identified [21].

The results of the drug-likeness test in Table 2. showed that three compounds of *M. calabura* extract have a molecular weight (MW) less than 500g/mol indicating all compounds comply with Lipinski's first rule which means it has the potential to be a drug candidate [15]. Compounds with a small molecular weight will be easier to pass through the cell membrane. Conversely, if the molecular weight is more than 500 g/mol, it will cause molecular absorption to fail or the body will have difficulty being able to absorb it completely [22].

Table 1. Drug-likeness properties of selected active compound ethanol extract of *M. calabura*.

Molecules	PubChem ID	Formula	Canonical SMILES
Stigmasterol	5280794	$C_{29}H_{48}$ O	CCC(C=C C(C)C1CC C2C1(CC C3C2CC= C4C3(CC C(C4)O)C) C)C(C)C
Tetradecanoic acid	11005	C_{14} $H_{28}O_2$	CCCCCC CCCCCC CC(=O)O
Dodecanoic acid, ethyl ester	7800	$C_{14}H_{28}O_2$	CCCCCC CCCCCC(=O)OCC

Table 2. Drug-likeness properties of selected active compound ethanol extract of *M. calabura*.

Molecules	MW (g/mol)	HBA	HBD	MLogP
Stigmasterol	412.7	1	1	0,29
Tetradecanoic acid	228,37	2	1	3,69
Dodecanoic acid, ethyl ester	228.37	2	0	3,52

Stigmasterol, tetradecanoic acid, and dodecanoic acid, ethyl ester have H-bond acceptors (HBA) <10 and H-bond donors (HBD) <5, both of which met Lipinski's rules. The low number of H-bond acceptors and H-bond donors indicated that the energy required in the absorption of molecules is also low and vice versa [23].

Log P indicates the lipophilicity properties of a molecule, this property indicated the ability of a molecule to dissolve in fat. Molecules are categorized as having good lipophilicity if the MLOG P value < 4.15 [15]. The lower the Log P value, the higher the hydrophilic properties of a molecule and inversely proportional to the low hydrophobic properties [24]. Table 1. showed that all three active compounds of *M. calabura* comply with the Lipinski rule. Then it can be concluded that the three compounds have good lipophilicity properties as drug candidates. Andriani [25] stated that a drug molecule can function properly if its hydrophobicity is not too high so that it can be widely distributed in the body. On the other hand, high Log P values are more hydrophobic. This causes drug compounds to tend to be toxic because they will be retained longer in the lipid bilayer so that they are less distributed in the body which results in the bond to the target enzyme being reduced [26].

Molecular docking is one of the in silico methods applied at several levels in drug development for three main purposes, namely, the search for new ligands, predicting the bond model of active ligands, and predicting their affinity and conformation [27]. This method is one of the more concise and simple methods of approach. The results of molecular docking analysis were presented in Table 3.

Table 3. Binding affinity and RMSD values of ligands and target protein *S. aureus* PBP2a (PDB ID: 5m18).

Ligands	Binding affinity (kcal/mol)	RMSD (Å)
Stigmasterol	-7.8	1.482
Tetradecanoic acid	-5.2	1.833
Dodecanoic acid, ethyl ester	-4.8	8.38
Benzylpenicillin	-6.6	2.447
Ligand MUR 703 (A)	-5.2	1.801

Stigmasterol has the lowest binding affinity energy (-7.8 kcal/mol) compared to ligands of other test compounds including penicillin antibiotics and the native ligand MUR 703(A). In addition, stigmasterol also has an RMSD value of 1,482 Å. According to Manna *et al* [28] the more negative the affinity energy indicates a more stable bond. Supported by the statement of Rena *et al* [29], good affinity has the lowest value (negative). It can be claimed that low-affinity energy suggests a molecule has a stronger ability to interact with the target protein because low affinity signifies a molecule takes less energy to bind. Likewise, the RMSD value was declared valid to use if the value < 2Å. The greater the RMSD value, the greater the deviation that occurs. The low RMSD value indicates that the docking ligand poses closer to the natural ligand pose [30]. In line with Muttaqin [31] the lower the RMSD value the closer the position of the native ligand of the docking result to the native ligand of the crystallographic result. Thus, stigmasterol has a high potential as an antibacterial compound against *S. aureus* compared to tetradecanoic acid and dodecanoic acid, ethyl ester.

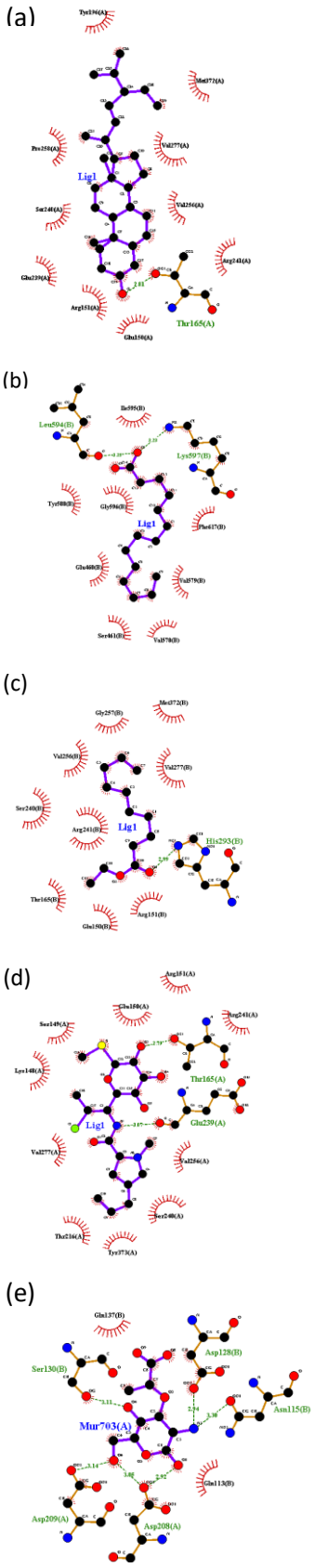

Figure 2. Visualization of the docking results with the target protein of *S. aureus* PBP2a (PDB ID: 5m18); (a) Stigmasterol, (b) Tetradecanoic acid, (c) Dodecanoic acid, ethyl ester, (d) Benzylpenicillin, (e) Native ligand MUR 703 (A).

Important parameters in predicting the potential of a compound to the interacting target proteins (receptors) can be seen from the type of bond and amino acid residues at the binding site. Through the visualization stage, the type of bond and amino acid residue can be determined (Fig. 2.). The type of bond and amino acid residue at the binding site are shown in Table 4.

Table 4. Type of bond and amino acid residues at the binding site with target protein *S. aureus* PBP2a (PDB: 5m18)

Ligand	Type of bond	Amino acid residues
Stigmasterol	Hydrogen	Thr165(A)
	Hydrophobic	Met372(A), Val277(A), Val256(A), Arg241(A), Glu150(A), Arg151(A), Glu239(A), Ser240(A), Pro258(A), Tyr196(A)
Tetradecanoic acid	Hydrogen	Lys597(B), Leu594(B)
	Hydrophobic	Phe617(B), Val579(B), Val578(B), Ser461(B), Glu460(B), Tyr588(B), Gly569(B), Ile595(B)
Dodecanoic acid, ethyl ester	Hydrogen	His293(B)
	Hydrophobic	Arg151(B), Glu150(B), Thr165(B), Arg241(B), Ser240(B), Val256(B), Gly257(B), Met372(B), Val(B)
Benzylpenicillin	Hydrogen	Thr165(A), Glu239(A)
	Hydrophobic	Val256(A), Ser240(A), Tyr373(A), Thr216(A), Val277(A), Lys148(A), Ser149(A), Glu150(A), Arg151(A), Arg241(A)
Ligand MUR703 (A)	Hydrogen	Asp128(B), Asn115(B), Asp208(A), Asp209(A), Ser130(B)
	Hydrophobic	Gln113(B), Gin137(B)

Stigmasterol is a phytosterol group compound that belongs to the steroid group [32]. Phytochemical compounds such as steroids are one of the potential compounds and are widely isolated from various types of plants [33]. Stigmasterol is also known as stigmasterin, a group of plant sterols that are utilized in various chemical processes to produce synthetic and semisynthetic compounds in the pharmaceutical industry [34].

Stigmasterol was known to have a positive effect on health so it is widely used in the pharmaceutical field. Stigmasterol has the potential as anti-osteoarthritis, anti-hypercholesterolemia, antidiabetic, anticancer, antioxidant, antibacterial, antifungal, anti-parasitic, anti-inflammatory, and immunomodulatory [35].

Steroid group compounds can interfere with bacterial cell surface proteins so that will prevent transpeptidation [36]. Accordingly, Tamokou *et al* [37] state membrane disruption is one of the possible mechanisms of sterols in microbes.

Tetradecanoic acid or better known as myristic acid and dodecanoic acid, and ethyl ester which has other names laurate acid are included in the group of saturated fatty acids. Both types of fatty acids can be obtained from palm oil, coconut oil, palm kernels, and nutmeg. Tetradecanoic acid is slightly yellow or white, in the form of a shiny crystalline solid or yellowish-white or white powder [38].

Tetradecanoic acid and dodecanoic acid are also widely used in pharmaceuticals, one of which is as an antibacterial and antifungal [39]. Medium-chain fatty acids such as tetradecanoic acid and dodecanoic acid are effective in inhibiting the growth of *S. epidermidis* compared to short-chain fatty acids (carbon number <10). Tetradecanoic acid is more effective in inhibiting the growth of *S. epidermidis* at an inhibitory concentration of 80.6% compared to dodecanoic acid. These results show the potential of tetradecanoic acid as a candidate for the treatment of acne [40].

In line with previous studies, it is known that tetradecanoic acid can inhibit and control the expression of virulence factors in *P. aeruginosa* [41]. In a recent study conducted by Kim *et al* [42] tetradecanoic acid also had an inhibitory effect on the formation of *biofilms of S. aureus, E. coli,* and *C. albicans*.

Dodecanoic acid, ethyl ester can be converted into monolaurin which is antiviral, antibacterial, and antiprotozoal. Laurate acid is stated to be effective in disrupting the permeability of the cell membranes of *S. aureus* bacteria [43]. Nitbani *et al* [44] found lauric acid with a concentration of 5% was able to inhibit the growth of all test bacteria (S. *aureus, B. cereus, S. typhimurium,* and *E. coli*). The higher the concentration, the greater the antibacterial activity, indicated by the size of the diameter of the inhibition zone formed.

The mechanism of action of fatty acids as antibacterial, namely causing lysis of bacterial cells by disrupting the permeability of bacterial cell membranes to cause cytoplasm from the cells, this damage results in inhibition of growth to death in bacterial cells [45].

4 Conclusion

Stigmasterol has high potential as an inhibitor of *S. aureus* protein because it has a bond affinity of -7.8 kcal/mol, which is lower than other active compounds, benzylpenicillin antibiotics, and MUR 703(A) native

ligands. The findings of the drug-likeness test, which satisfy the Lipinski five rules and RMSD values, further support the possibility. Therefore, stigmasterol has the potential as a drug candidate in acne treatment therapy. Further research is needed to be revealed its bioavailability before it can be applied as an alternative medicine.

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