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Inhibition of Antibacterial Bioactive Compounds from Awar-Awar (*Ficus septica* Burm, F.) Leaves for Methicillin Resistant *Staphylococcus aureus* (MRSA) Infection: an *In Silico* Study

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ABSTRACT. *Methicillin resistant Staphylococcus aureus (MRSA) is a type of Staphylococcus aureus infection that can increase the patients' number with prevalence variation in each country. Methicillin resistant Staphylococcus aureus (MRSA) can be treated by inhibiting the activity of the BlaR1 transducer protein as an MRSA sensor against transmembrane antibiotics using bioactive compounds. Ficus septica is a local variety that has potential as an antibacterial. In silico study of the bioactive compounds found in Ficus septica can inhibit the synthesis of Staphylococcus aureus cell wall based on molecular docking tests with the control compound is Linezolid as antibiotics. In addition, ADME prediction, binding affinity, and binding site analysis were also performed. The results showed that there were 9 active compounds from Ficus septica leaves which had antibacterial potential and fulfilled Lipinski's rules criteria. Analysis of the binding site indicated that Coumarin compound could bind to the most BlaR1 protein active sites than other bioactive compounds in Ficus septica, including on the Lys526 and Thr527 active sites. Based on interaction analysis, it can be concluded that the Coumarin compounds of Ficus septica leaves can provide a therapeutic effect that can fight MRSA infection which can be proven by in vitro tests.*

Keywords: *Ficus septica, Methicillin resistant Staphylococcus aureus (MRSA), in silico study*

INTRODUCTION

Staphylococcus aureus is one of the main pathogenic bacteria that has the potential to cause various clinical infections. *Staphylococcus aureus* is part of the normal flora on the skin and respiratory tract, but this bacterial species can be one of the main agents causing bacteremia, infective endocarditis, osteoarticular, osteomyelitis, skin infections, urinary tract infections, pneumonia, meningitis, and several other serious infections [1]. The World *Health Organization* (WHO), as a world health institution, reported that *Staphylococcus aureus* infections in 2002 reached 8.7% of all nosocomial bacterial species and continued to increase every year. This makes *Staphylococcus aureus* a bacterium with a high infection rate compared to other types of nosocomial bacteria [2-3].

Treatment and management of infections due to *Staphylococcus aureus* can be treated with antibiotics. Antibiotics given to patients must be carried out specifically and thoroughly so as not to cause antibiotic resistance [4]. Based on WHO data from 2014, cases of antibiotic resistance to *Staphylococcus aureus* show a prevalence and mortality rate that continue to increase every year. Several studies also state that *Staphylococcus aureus* is resistant to several types of antibiotics or has *multi-drug resistance* (MDR) [5]. The incidence of *Staphylococcus aureus* infection in parts of the world reports that many cases of *Staphylococcus aureus* resistance occur to a type of *methicillin* antibiotic, which is also called *Methicillin resistant Staphylococcus aureus* (MRSA). The prevalence of MRSA infection has varied in each country in the last few decades, MRSA infection reached 70% with a rate of 23.5% in Indonesia in 2006, whereas in 2014 the prevalence of MRSA had decreased to 0.9%. Even though it has decreased, there are still 7 out of 29 countries in Asia and Europe that have a high enough MRSA prevalence rate of up to 25%, including Indonesia [5].

Methicillin-resistant Staphylococcus aureus (MRSA) has a *protein cohort* is a transmembrane antibiotic sensor or transducer protein that confers resistance to β-lactam antibiotics known as BlaR1.

BlaR1 has four transmembrane helices, a *cytoplasmic zinc-protease domain,* and *a soluble C-terminal extracellular sensor domain*. This protein plays a role in regulating β-lactam expression, which can hydrolytically destroy β-lactam antibiotics. The resistance phenotype begins with acylation of β-lactam antibiotics from the extracellular domain of BlaR1. Acylation events activate the cytoplasmic protease domain through a signal transduction mechanism [6]. The activity of BlaR1 protein has not been directly targeted for the MRSA treatment. However, protein BlaR1 can be targeted by several natural compounds or plant bioactive compounds.

One of the local plant biodiversity known as awar-awar (*Ficus septica* Burm, F.) has the potential as an effective antibacterial agent. Research conducted by Mujiwahyuni (2013) regarding the antibacterial activity test of the ethanol extract of awar-awar leaves against the *Staphylococcus epidermidis* bacteria that causes skin infections shows that this plant provides moderate inhibition [7]. Awar-awar is a plant that can be found in Indonesia, especially in Java and Madura. Awar-awar has long been known to be widely used as a medicinal plant by the community. Awar-awar is found in many ecosystems, but is often found on roadsides, shrubs, and open forest at an altitude of 1,200 meters above sea level [8]. The part that is often used as medicine is the leaf because of its abundant availability and short harvest time. Awar-awar leaves have been widely used as a medicine for skin diseases, boils, antidotes for venomous animals, asthma, ulcers, and appendicitis [9].

The fast growth and spread of the awar-awar leaf species on every island in Indonesia are known as pioneer species, providing great opportunities for research related to the development of antibiotics as new innovations to overcome MRSA cases. Based on the explanation, awar-awar leaves have never been used as an antibiotic for *S. aureus*. In addition, there are indications that secondary metabolites in awar-awar leaves contain sterols, alkaloids, and flavonoids that have high antimicrobial antimicrobial power which is the basis for the need for further research on the effectiveness of antibacterial compounds from awar-awar (*Ficus septica* Burm, F.) leaves in handling MRSA cases with in silico studies.

RESEARCH METHODS

Screening ADME Prediction and Antivirus Ability of Bioactive Compounds from Awar-Awar (*Ficus septica* Burm, F.) Leaves

The active compounds contained in the leaves of Awar-awar (*Ficus septica*, Burm. F) were obtained from the KnapSack database (http://www.knapsackfamily.com/KNApSAcK/) and several literatures were tested on Way2Drug website with the AntiBac-Pred service (https://www.way2drug.com/antibac/) to determine their antibacterial activity against Staphylococcus aureus .

The active compounds in *Ficus septica Burm, F.* were collected from PubChen database (https://pubchem.ncbi.nlm.nih.gov/) and the Canonical SMILES data of the selected compounds were recorded and tested on SwissADME (https://www.swissadme.ch/). The parameters used are based on Lipinski's five rule's, which include molecular weight (MW) ≤500g/mol, number of hydrogen bond acceptors (Hbond acceptors) ≤10, number of hydrogen bond donors (H-bond donors) ≤5, and water partition coefficient (log P) ≤5 [9] and two additional drug-likeness criteria in the form of GI absorption and Bioavailability score. These ADME studies help determine the viability of drug candidates (Table 1).

Table 1. Compound Screening

Compound	CID
Genistin	5281377
Kaempferitrin	5486199
Coumarin	323
Stigmasterol	5280794
Tylophorine	92114
Aesculin	5281417
Sterol	1107
β-Sitosterol	222284
β-Amyrin	73145
α-Amyrin fatty acid ester	73170

Molecular Docking

Docking is carried out using active compounds that have been selected according to predetermined criteria. The 3D structure of the ligand was downloaded on the PubChem server (https://pubchem.ncbi.nlm.nih.gov/). Then the 3D structure of the BlaR1 protein, which acts as a receptor, is downloaded to RCSB (https://www.rcsb.org/) with PDB ID : 3Q7Z in PDB format. The control compound used in the form of Linezolid was also downloaded from the RCSB database with PDB ID: 441401. Receptors were prepared with Autodock Tools to remove native ligands and water molecules. Furthermore, the prepared receptors are stored in pdbqt format. The ligands were prepared with Pyrx to minimize

the energy of the ligands. Furthermore, the prepared ligands were stored in PDB format. docking is done with Autodock Vina contained in the PyRx application. After docking is complete, the value of binding affinity and bonds interaction formed will be known.

Docking Results Visualization

Docking results are visualized with Discovery Studio. All compounds including controls are visualized by changing the color, background and also displaying some features, such as linked amino acids, bond locations, poses, and bond types displayed in 2D. This visualization aims to determine the location of the bond between the ligand and the receptor and the type of bond formed so that the bond strength can be analyzed and interpreted for each compound which will then be compared with the control.

RESULTS AND DISCUSSIONS

Screening ADME Prediction and Antivirus Ability of Bioactive Compounds from Awar-Awar (Ficus septica Burm, F.) Leaves

Nine compounds indicated to be present in *Ficus septica* leaves have been analyzed with ADME predictions showing that there are eight compounds that fulfilled five Lipinski's rules consists of molecular weight (MW) ≤ 500 g/mol, hydrogen bond acceptor (HBA) ≤ 10, hydrogen bond donor (HBD) ≤ 5, lipophilicity (LogP) ≤ 5, and number of violation ≤ 2 (Table 2). These rules explain that the compounds have a high probability of being drug-likeness molecules [10]. Meanwhile, based on these rules, the Kaempferitrin compound is excluded in the category of drug-likeness molecules because it has a number of violations more than 2 and the identified sterol compound does not have a 3D structure from search results in the PubChem database.

The nine compounds that have been identified were also analyzed for its antibacterial abilities with Way2Drug PassOnline. The potential compounds are Aesculin, Genistin, and Tylophorine as shown in (Table 3). Nine compounds showed that there were three compounds with the highest confidence value as antibacterial for resistant *Staphylococcus aureus* among the compounds found in *Ficus septica* leaves.

Table 2. Compound screening based on Five Lipinski's Rules for ADME Predictions

Compound	Pubchem CID	MW (g/mol)	LogP	HBA	HBD	Number of Violations
Genistin	5281377	432.38	-1.61	10	6	1
Kaempferitrin	5486199	578.52	-269	14	8	3
Coumarin	323	146.15	1.65	2	0	0
Stigmasterol	5280794	412.70	6.62	1	1	1
Tylophorine	92114	393.48	2.69	5	0	0
Aesculin	5281417	340.28	-1.48	9	5	0
Sterol	1107	248.40	4.31	1	1	1
β-Sitosterol	222284	414.71	6.73	1	1	1
β-Amyrin	73145	426.73	8.02	1	1	1
α-Amyrin fatty acid ester	73170	426.73	6.92	1	1	1

MW: molecular weight, MLogP: lipophilicity, HBA: hydrogen bond acceptor, HBD: hydrogen bond donor

Table 3. Compounds screening value

Compound	Confidence value
Aesculin	0.583
Genistin	0.493
Tylophorine	0.046

The results on another six compounds such as Coumarin, Stigmasterol, Sterol, β-Sitosterol, β-Amyrin, and α-Amyrin fatty acid ester have the antibacterial ability from several other bacterial species. This study, the eight compounds which included in the inclusion character will be used as ligands in the molecular docking test to prove the potential of the whole bioactive compounds contained in the leaves of *Ficus septica* to inhibit BlaR1 receptor for identifying the therapeutic effect against MRSA infection.

Binding Affinity of Awar-Awar (*Ficus septic*a Burm, F. Compound)

There are 10 bioactive compounds from the leaves of awar-awar (*Ficus septica* Burm, F.). However, there were only 9 compounds that met the criteria of the Lipinski rule when ADME screening was carried out. Then the 9 compounds that comply with the Lipinski rule were followed by molecular docking with specific docking. The docking results of the 9 compounds that meet Lipinski's criteria can be seen in (Table 4).

Based on the results of specific docking, it is known that the binding affinity values obtained range from -6.2 to -8. There were 4 active compounds that had better binding affinity values than the control, including β-amyrin, Stigmasterol, α-amyrin, and Genistin. Meanwhile, there were 4 other compounds that had lower binding affinity values than the control but were still in a range that was not much different from the control, including β-Sitosterol, Aesculin, Thyloporin, and Coumarin. Binding affinity is used to optimize screening results and find the ligand with the highest level of affinity for the target protein [11]. The smaller the binding affinity value of a compound, the more stable the compound will be when it binds to the receptor [12].

Table 4. Results of docking of active compounds from (*Ficus septica* Burm, F.) with BlaR1.

Compound	Binding Affinity
BlaR1_Genistin	-7,2
BlaR1_Coumarin	-6,2
BlaR1_Stigmasterol	-7,8
BlaR1_Tylophorine	-6,7
BlaR1_Aesculin	-6,8
BlaR1_Linezolid (Control)	-7
BlaR1_β-Sitosterol	-6,9
BlaR1_β-Amyrin	-8
BlaR1_α-Amyrin	-7,8

BlaR1 and Compound Interaction

The interaction of BlaR1 and awar-awar leaves compounds shows different types of bonds at specific amino acid sites (Fig. 1). Linezolid is a synthetic antibiotic that can prevent protein synthesis from bacteria through the binding of rRNA to both 30S and 50S ribosomal subunits. This can inhibit the formation of the initiation complex which can also reduce the length of the peptide chain and reduce the rate of the translation reaction. This linezolid can also prevent the expression of virulence elements which can cause a decrease in toxins produced from gram-positive pathogenic bacteria [13]. The visualization results carried out with the Discovery Studio 2019 software show that there are 5 interactions with amino acid residues. Based on the results of 3D and 2D visualization, the bond between Linezolid as a ligand was formed on the amino acid residues Trp424, Thr529, Ser389, Thr527 and Lys526 (Fig. 1a-b). Judging from the 5 interactions that occur, 2 of them are the active sites of BlaR1, namely Ser389, Thr527 and Lys526 [14]. The interactions formed on the five amino acid residues are hydrogen bonds on the amino acid residues Thr529, Ser389, Thr527, and Lys526 and Pi-Alkyl bonds on the amino acid residue Trp424. These results indicate that Linezolid has the potential to become an inhibitor of BlaR1 because it can bind to the active site which can increase antibiotic efflux and cause antibiotics to be pumped out of the bacterial cell. Linezolid also has a hydrogen bond at a site that would normally be a nucleophilic inhibitor on the beta-lactam ring [14].

Genistin 2D interaction shows that there are 3 interactions on the amino acid residues that were successfully formed, namely Pi-Pi-T-Shaped interaction in Trp424, hydrogen bond in Thr529 and Thr27 which is the active site from BlaR1 (Fig. 2b). Interactions between BlaR1 and Alpha-amyrin from the 2D shows Pi-Alkyl interaction in Phe421 (Fig. 3b). The 2D interaction of Aesculin shows that there are 4 interactions in the amino acid residues that were successfully formed, namely hydrogen bond in Thr529 and Ser389, Pi-Pi Stacked interaction in Phe421, and Pi-Alkyl interaction in Ala423 (Fig. 4b). Interactions between BlaR1 and Coumarin from the 2D shows that there are 3 interactions that were successfully formed, there are Pi-Alkyl interactions in Ile515, hydrogen bonds in lys526 and Thr527 which are the active sites from BlaR1 (Fig. 5b). Beta-sitosterol compounds form alkyl interactions in three amino acid residues, namely Ile515, Phe541, and Lys526 (Fig. 6b). The 2D interaction of Tylophorine shows that there are 2 interactions on the amino acid residues that were successfully formed, namely alkyl interaction in Ala432 and hydrogen bond in Gln435, Thr529 and Thr527. Two interactions included in the active site BlaR1 are Thr529 and Thr527 (Fig. 7b). Beta-amyrin has alkyl interactions in three amino acids, namely residues Ala423, Phe421, and Trp424 (Fig. 8b). The 2D interaction between BlaR1 and stigmasterol shows that there are 2 interactions on the amino acid residues that successfully formed, there are Pi-Alkyl and Alkyl interaction in Lys566, Ile515, Phe421 and Lys526 which is included as the active site BlaR1 (Fig. 9b).

Bioactive compounds from awar-awar (*Ficus septica* Burm, F.) leaves which have interactions at the crucial active site of BlaR1 are Genistin, Aesculin, Coumarin, Beta-sitosterol, Tylophorine, and Stigmasterol. Therefore, six compounds have strong potential as BlaR1 inhibitors. The use of Genistin compounds as an isoflavonoid derivative has been proven in previous in vitro studies which showed that Genistin has activity as a gram-negative and fastidious antibacterial compound, antifungal, and has anti-radiation properties that have the potential to inhibit cancer cell proliferation [15]. In vitro studies have proven that coumarin compounds have antimicrobial effects against various types of bacteria, including *Staphylococcus aureus*, *Escherichia coli*, and

Pseudomonas aeruginosa [16]. The interaction that occurs between the active site of the BlaR1 protein and the active compounds from awar-awar can be seen in (Figure 1).

In vitro studies prove that beta-sitosterol has antimicrobial activity against several pathogenic bacteria including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* [17]. The antibacterial activity of the Tylophorine compound has been proven in vitro by Maheswari & Vijayarengan (2020) with results showing that Tylophorine isolated from Tylophora indica has high antibacterial activity with an MIC of 10-20 mm on five types of test bacteria, namely *Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogens, Pseudomonas aeruginosa*, and *Salmonella typhi* [18]. The stigmasterol compound isolated from *Neocarya macrophylla* has been shown to have antibacterial activity against several test bacteria, such as *Salmonella typhimurium, Klebsiella. pneumoniae* and MRSA with the highest sensitivity [19].

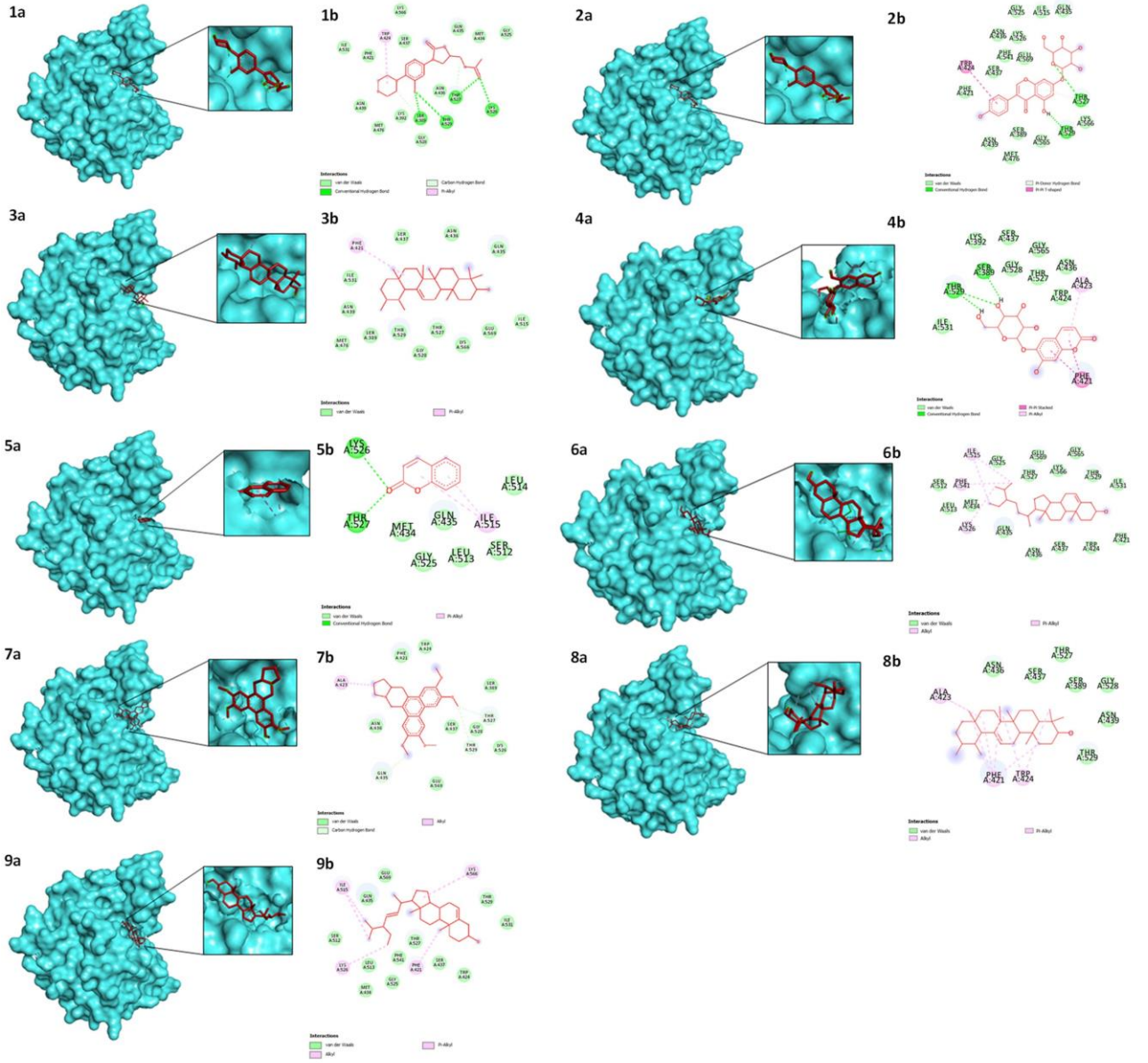

Figure 1. 3D and 2D interaction visualization between BlaR1 and compounds: 1a-b) linezolid (control); 2a-b) genistin; 3a-b) alpha-amyrin; 4a-b) aesculin; 5a-b) coumarin; 6a-b) beta-sitosterol; 7a-b) tylophorine; 8a-b) beta-amyrin; and 9a-b) stigmasterol

CONCLUSION

There were four compounds with better binding affinity values than Linezolid as control for nine interactions of the bioactive compounds of *Ficus septica* leaves inhibit the BlaR1 receptor of *Staphylococcus aureus* based on molecular binding screening, including the compounds β-Amyrin, Stigmasterol, α-Amyrin, and Genistin. We found that Coumarin compounds of *Ficus septica* leaf interacted at the most BlaR1 active sites than other biocompounds in *Ficus septica* leaf and potentially inhibited the activity of BlaR1.

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