Study of The Potential Compound on Leaves of *Syzygium samarangense* as An Antibacterial to *Streptococcus pneumoniae*

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Abstract. Pneumonia is one of the respiratory infections caused by *Streptococcus pneumoniae*. Bacterial resistance problems regarding the treatment of pneumonia can be overcome by exploring active compounds in plants that have potential as antibacterials, one of which is water guava (*Syzygium samarangense*). This study aims to discover active compounds with the antibacterial potential of *S. pneumoniae* from water guava leaves (*S. samarangense*). This study was conducted in September 2022 at Universitas Negeri Surabaya with in silico method (blind molecular docking). The method used in this study was in silico approach, including the collection of proteins and compounds, preparation of proteins and ligands, minimization of compounds, blind molecular docking, and visualization of the results of molecular docking. The results showed that the value of binding affinity and RMSD of PBP-2X protein (PDB ID: 1QME) which binds to 1-methyl ethyl acetate, valeraldehyde, and dibutyl phthalate was -5.6 KJ/mol and 2.575 Å, -5 KJ/mol and 1.373 Å, and -6.7 KJ/mol and 1.637 Å, respectively. The result indicates that the selected compounds have the potential as antibacterial to *S. pneumoniae*. In the near future, this study requires further in vitro and in vivo testing as a step to validate the activity of the active compound of guava leaves as an antibacterial of *S. pneumoniae*.

1. Introduction

Acute Respiratory Infection (ARI) is one of the infectious diseases that cause morbidity and mortality. In 2016, as many as 2.3 million people of all ages and >600 thousand children aged <5 years died from ARI [1]. The recorded pneumonia cases nationally are estimated at 3.55% [2]. Pneumonia can be caused by pathogenic bacteria, namely *Streptococcus pneumoniae*. These pathogens are easily transmitted through direct contact with sputum and through droplets originating from coughs or sneezes of pneumonia patients [3].

Intervention efforts against *S. pneumoniae* infection can be carried out with antibiotics treatment. However, the relatively high-intensity dosage of antibiotics can cause problems and constitute a global threat to health, especially in the form of bacterial resistance to antibiotics [4]. In addition, the inappropriate use of antibiotics can lead to these pathogens' resistance. Emerging problems regarding the treatment of *S. pneumoniae* infection become the background for the need to do research related to other alternatives.

One alternative that can be used to treat bacterial infectious diseases is medicinal plants. In various countries, medicinal plants are widely used because they represent a source of antibacterial agents and a source of many potential drugs [5]. One example of a plant that can be used as medicine and antibiotics is guava (*S. samarangense*). Phytochemical compounds found in guava leaves (*S. samarangense*) are flavonoids, phenolics, and tannins that have potential as antimicrobials [6]. As a medicine, the guava leaves extract is efficacious in treating astringent, fever, diarrhea, diabetes, cough, and headache [7]. These symptoms are usually caused by infection of pathogenic bacteria such as *S. pneumoniae* [8].

To determine the potential of this plant to be used as an antibiotic candidate against S. *pneumoniae* infection, it can be done using the in silico method, namely molecular docking, which is a computationally based in silico method. Molecular docking is a method that can be used to find the most appropriate and involving interaction patterns between two molecules, namely receptors and ligands [9]. Currently, research using computational methods is very important in various fields, especially biology and medicine. One of the benefits of using this method is in various drug discovery and manufacturing processes [10]. Therefore, this study aimed to find compounds in guava leaves (*S. samarangense*) that have the most potential as an antibacterial against *S. pneumoniae*.

2. Methods

This research is an in silico observational research conducted in September 2022 at the Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, State University of

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Surabaya. The tools used in this study include hardware in the form of a laptop with a minimum specification of two processor cores, 2 GHz, 2 GB RAM, Windows 10 operating system type, Autodoc software, PyRx, LigPlot, NotePad++, PyMol, Discovery Studio 2016 Client, PubChem database (https://pubchem.ncbi.mlm.nih.gov/), Protein Data Bank (https://www.rcsb.org), the Swiss Target Prediction web server (www.swisstargetprediction.ch), and the drug-like properties test web server, namely SWISS ADME (http://www.swissadme.ch/).

The initial stage of this research was to test the similarity of the drug-likeness by accessing the Swiss ADME web server (http://www.swissadme.ch/index.php) and then entering the canonical SMILES data of selected active compounds from *S. samarangense* obtained from PubChem. The drug-likeness test was carried out to evaluate the drug-like properties of the selected active compounds according to Lipinski's five rules, namely molecular weight (MW) 150-500 g/mol, log P < 5 partition coefficient value, number of H-bond donors < 5, number of acceptors. H-bond < 10, and Lipinski's value is 0 [11] [12].

The next step was sample preparation, which includes the collection of the target protein molecule sample and the collection of the ligand compound molecule sample. The target protein molecule sample in the form of PBP-2X protein can be obtained by accessing the Protein Data Bank database (https://www.rcsb.org) with the PDB ID code 1QME and then downloaded in three dimensions and then saved in *.pdb format. After that, the sterilization process was continued using Autodock and Notepad++ software. The molecular samples of the ligand compounds, namely 1-methylethyl acetate, valeraldehyde, and dibutyl phthalate, can be obtained by accessing the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and then downloaded in 2D and then saved in *.sdf format (Figure 2). The structure 2D was then converted to the structure 3D in *.of format by using Discovery Studio software. After that, the minimization process was continued using PyRx software.

The next stage was molecular docking. The docking between the target protein (PBP-2X) with the native ligand (SO4) and the ligand compound (1-methylethyl acetate, valeraldehyde, and dibutyl phthalate) was carried out simultaneously using PyRx software. The docking results were then stored in two forms, namely *.csv format to see the value of the binding affinity energy and see the score from the RMSD, and *.pdb format to view the docking visualization to make it more representative. Docking results visualization was done using LigPlot software to view three-dimensional view. The visualized docking results were then saved in *.jpg format.

The sample preparation process includes four stages, namely the collection of samples of the target protein molecules (to find out the name of the target protein to be used by conducting a scientific search first), sterilization (the molecular structure of the protein needs to be separated from the original ligand structure and other unwanted molecules), collection ligand sampling (ligand sample information obtained from the compound database), and minimization (to make the ligand more flexible and produce the lowest energy when it binds to the target protein binding site) [13].

The sterilization stage aims to separate the molecular structure of the target protein from the native ligand that is still attached, as well as to remove other unwanted molecules such as the presence of contaminant molecules in the form of water, ligands, and even other foreign proteins. This stage was carried out using AutoDock and Notepad++ software. The minimization stage aims to convert the compound from .sdf to .pdb format through the PyRx software in order to make the ligand more flexible and produce the lowest binding energy when docked [13]

Structure preparation of active compounds and drugs was carried out to stabilize the 3D structure because a stable structure will minimize the energy involved in pharmacokinetic and pharmacodynamic testing, where penicillin-type antibiotic drugs are used as comparators [14]. The drug effect can be determined by the concentration of the drug on its target receptor and the pharmacodynamic effect of the interaction of the receptor with the drug [15].

Data collection techniques in this study were in the form of test data for drug-like compounds and in silico tests with docking and visualization between compounds and target proteins. Analysis of the results of the in silico test was carried out qualitatively descriptively by comparing the value of binding affinity, RMSD, the type of bond between the compound and the receptor on the target protein, and the amino acid in the binding site of the compound.

3. Results and Discussion

The materials used are the 3-dimensional structure of the target protein PBP-2X (Penicillin-Binding Protein 2X) with the code pdb 1QME (Figure 1) and the 3-dimensional structure of compounds 1-methylethyl acetate, valeraldehyde, and dibutyl phthalate (Figure 2). These three selected active compounds are contained in guava leaves and have the potential as antibacterials through binding to the PBP-2X protein.

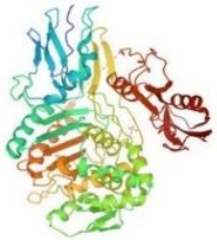

Fig. 1. The Three-Dimensional Structure of The PBP-2X Protein (PDB, 2022).

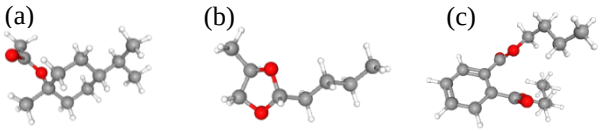

Fig. 2. Three-Dimensional Structure of Compounds a) 1-Methylethyl Acetate, b) Valeraldehyde, and (c) Dibutyl Phthalete (PubChem, 2022)

Based on the results of the analysis of drug-likeness tests on the active compounds of water guava leaves, it was obtained that variations in the intrinsic properties of the three compounds, namely variations in molecular weight were in the range of 144.21-278.34 g/mol, variations in proton acceptors were in the range of 2-4, did not have proton donors, and the iLOGP values of the three active compounds were in the range of 2.67-3.04 (Table 1).

Table 1. Characteristics and drug-likeness properties of selected active compound of *S. samarangense*

Molecules	Pubchem ID	Formulas	Canonical SMILES
1-Methylethyl Acetate	117304	$C_{12}H_{22}O$	CC(C)C1CCC(CC1)(C)OC(=O)C
Valeraldehyde	71587084	$C_8H_{16}O$	CCCCC1OCC(O1)C
Dibutyl Phthalete	3026	$C_{16}H_{22}O$	CCCCOC(=O)C1=CC=CC=C1C(=O)OCCCC

Table 2. Characteristics and drug-likeness properties of selected active compound of *S. samarangense*

Molecules	MW (g/mol)	HBA	HBD	iLOGP
1-Methylethyl Acetate	198.30	2	0	3.04
Valeraldehyde	144.21	2	0	2.67
Dibutyl Phthalete	278.34	4	0	2.97

Table 1 and Table 2 showed that the three compounds contained in guava leaves met the criteria of Lipinski's rule. According to Lipinski's rules, in developing and finding oral drug candidates, five conditions must be met, called the rule of five, namely molecular weight not exceeding five hundred grams per mole or dalton, high lipophilicity (expressed as log P not exceeding five), up to five hydrogen bond donors, and up to ten hydrogen bond acceptors [11]. The existence of the Lipinski Rule of Five is used to show the solubility of compounds in penetrating cells by passive diffusion [16], [17]. Based on the results of the drug-likeness analysis, it can be stated that the three molecules have met all the criteria for the five Lipinski rules.

Pharmacodynamic tests were carried out to see the physicochemical data and drug-likeness properties of a drug candidate. The pharmacodynamic data of the active compound in guava leaves obtained the value of proton donor and proton acceptor according to Lipinski's rule, which according to Lipinski's rule a maximum of five proton donors and ten proton recipients. Then the molecular weight was less than 500 and the drug-likeness properties of the three Log P values of the compounds were good based on the octanol/water partition coefficient [11]. According to [18], molecular weight affects the distribution process of drugs in the body through the diffusion process, where the smaller the value of the molecular weight, the easier the drug penetration process and the faster the absorption time.

The [11] stated that if a compound does not meet the Lipinski Rule of Five, it will disrupt the process of absorption of the drug orally. However, if a compound complies with the Lipinski Rule of Five it does not guarantee good activity because the law is not related to a particular chemical structure of the drug compound. Based on the statement of [19], states that almost 95% of drugs that have been clinically approved have physicochemical properties with the following conditions: Molecular Weight (130 to 725 g/mol), Hydrogen Donor Bonds (0 to 6), Hydrogen Bonds Acceptors (2 to 20), Log P (-2 to 6.5), and Rotatable Atom (0–15). Based on this statement, the three selected compounds contained in guava leaves still meet the requirements if they were to be used as new medicinal compounds [12], [20].

This study also used molecular docking analysis to determine specific interactions between ligands, in this case, the active compounds of guava leaves, and the PBP-2X target protein. The docking results showed the binding affinity energy and Root Mean Square Deviation (RMSD) values of the three compound ligands in guava leaves (1-methylethyl acetate, valeraldehyde, and dibutyl phthalate), natural ligands (SO4), and antibiotics (penicillin) against the PBP-2X receptor/target protein (Table 2). While the types of bonds and interactions of amino acid residues with the active compounds of guava leaves were shown in Table 4 and Figure 3.

Table 3. Binding affinity energy and RMSD values of ligands and target protein *S. pneumoniae* PBP-2X (PDB Code: 1QME).

Molecules	Binding affinity (KJ/mol)	RMSD (Å)
1-Methylethyl Acetate	-5.6	2,575
Valeraldehyde	-5.0	1,373
Dibuthyl Phthalate	-6.7	1,637
Penicillin	-7.6	1,840
SO4 Liga ligands	-4.0	1857

Table 3 shows that the binding affinity energy of the three active compounds in guava leaves was lower than that of the natural SO4 ligand. Binding affinity is a value that indicates the strength of the bond between the protein and the ligand [21]. The more negative the value of the binding affinity, the stronger the bond, and vice versa [22].

A compound is said to be very potential as a drug candidate if it has hydrogen bonds, and the binding affinity value is less than 10. The binding affinity value is an indicator of the binding ability of the active compound to the target protein. Free energy is the enthalpy change required to break a certain bond in 1 mol of gas-inhibiting molecules. The active compound

is predicted to have the potential to have strong inhibitory and interaction properties if it has the same chemical interaction position on the target protein as the control [23].

According to [24], a compound can have a higher selectivity on the test receptor if the binding affinity of the test compound is lower than the control compound. The lower the binding affinity value, the stronger the binding force of the ligand to the receptor. The lower the binding affinity value, the stronger the bond between the compound and the receptor because of the stability and strength of the non-covalent interaction between the compound and the receptor. Therefore, it can be said that compounds in the binding site area interact more easily with native ligands compared to control compounds [25].

The smallest RMSD value was found in the compound valeraldehyde, and this value was smaller than the natural SO4 ligand and penicillin. RMSD is a comparison of the angle value of the docking ligand molecule with the initial ligand [26]. The RMSD value of the atomic weight of a valid docking compound used as a standard is less or equal to 2Å [27].

Table 4. Type of Bond and Amino Acid Residues at The Binding Site in Protein Target

Molecules	**Type of Bond**	**Amino Acid Residues**
1-Methylethyl Acetate	Hydrogen	Gly(A)664
	Hydro phobic	**Asn(A)417,**Arg(A)418, Lys(A)428, Val(A)662, Val(A)663, Val(A)696, Pro(A)697, Asp(A)698, Ala(A)734, Asn(A)735
Valeraldehyde	Hydrogen	**Lys(A)420,**Val(A)662
	Hydro phobic	**Thr(A)425, Arg(A)426,**Ile(A)498, Val(A)499, Arg(A)654, Pro(A)660, Ile(A)661, Tyr(A)702
Dibuthyl Phthalate	Hydro gen	Asn(A)397, Thr(A)550
	Hydro phobic	Ser(A)337, Try(A)374, Ser(A)395, Phe(A)450, Gln(A)452, The(A)526, Ser(A)548, Gly(A)549, Gln(A)552
Penicillin	Hydro gen	Pro(A)424, **Arg(426),** Arg(A)463
	Hydro phobic	**Asn(A)417, Lys(A)420,**Val(A)423, **Thr(A)425, Val(A)499, Arg(A)654, Val(A)662, Asp(A)698, Asn(A)735**
SO4 Native ligands	Hydro gen	GlyA)218, Ser(A)222
	Hydro phobic	Ser(A)217, Ser(A)221, Tyr(A)329, Thr(A)430, Leu(A)429

Table 4 shows that with the PBP-2X residue, the 1-methyl-ethyl acetate compound formed 1 hydrogen bond, namely Gly(A)664, the valeraldehyde compound formed 2 hydrogen bonds, namely Lys(A)420, Val(A)662, dibutyl phthalate compound. form 2 hydrogen bonds, namely Asn(A)397, Thr(A)550, penicillin as a control antibiotic forms 3 hydrogen bonds, namely Pro(A)424, Arg(426), Arg(A)463, and natural ligand SO4 forms 2 bonds hydrogen, namely GlyA)218, Ser(A)222. This proves that valeraldehyde and dibutyl phthalate have the same inhibitory potential as the natural SO4 ligand in PBP-2X when compared to 1 methyl acetate.

The amino acid residue bonds found in the PBP-2X protein between penicillin and 1-methylethyl acetate have the same 4 amino acid residues, namely Asn(A)417, Asp(A)698, Ala(A)734, Asn(A) 735. Penicillin and valeraldehyde have the same 5 amino acid residues, namely Lys(A)420, Thr(A)425, Arg(A)426, Val(A)499, Arg(A)654. Whereas penicillin with dibutyl phthalate and natural ligands do not have the same amino acid residues. These results indicate that the active compound valeraldehyde was similar to penicillin in the mechanism of its interaction with the target protein.

Visualization of the docking results can be seen at Figure 3, which showed the amino acids that were the binding site and the type of bond between the receptor or PBP2a target protein with the native ligand and the ligand compound produces a representative image.

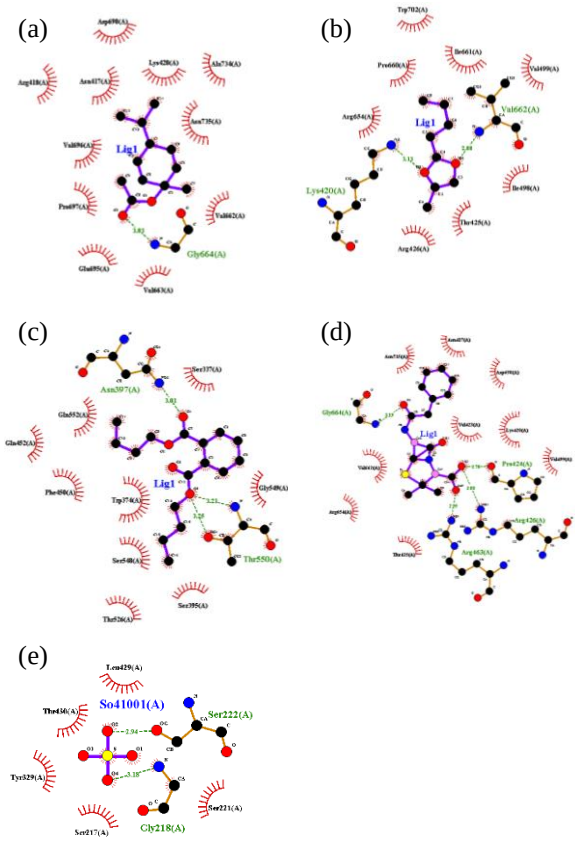

Fig. 3. PBP-2X amino acid residue docking interactions with (a) 1-Merhylethyl Acetate, (b) Valeraldehyde, (c) Dibutyl Phthalete, (d)) Penicillin, and (e) Natural SO4 Ligand.

The docking process is the event of a molecule forming a macromolecular complex due to interactions between one another. Docking simulation aims to determine the bond energy that occurs when one molecule binds to another molecule. The visualization process is a stage that aims to see a picture of the results of the docking to make it more representative. The visualization process can use LigPlot to see the visualization of the docking results in a three-dimensional view [13].

The 1-methylethyl acetate is a monoterpene compound that has the potential as an antimicrobial [28]. Monoterpenes belong to the class of terpenes which have volatile properties. Terpenes are compounds composed of isoprene and carbon structures built by bonds between two or more C5 units [29]. Terpenes or terpenoids have various functions, including as antioxidants, anti-microorganisms (antimicrobials), and aromatherapy [30].

The mechanism of terpenoid compounds as antibacterial is by damaging the bacterial cell membrane. Terpenoids react with transmembrane proteins on the outer membrane of the bacterial cell wall and form strong polymeric bonds, resulting in damage to the transmembrane proteins. Damage to the transmembrane protein will reduce the permeability of the bacterial cell membrane resulting in a lack of nutrients in the bacterial cells so that the growth of the bacteria is inhibited. Cell membrane damage can occur when active antibacterial compounds react with the active site of the cell membrane or by dissolving lipid constituents and increasing their permeability so that antibacterial compounds can infiltrate cells easily or coagulate the cytoplasm of bacterial cells [31].

The valeraldehyde compound has a hydrazone bonding chain or group. There have been no reports of antibacterial activity for these compounds, but many of the compounds containing the hydrazone chain have antibacterial activity [32], and some antibacterial sources currently used in medicine are known to contain hydrazide-hydrazone moieties [33]. Valeraldehyde belongs to the flavonoid group [34]. Flavonoids are a phenolic hydroxy group that has high antioxidant activity, which has various bioactivities, including anti-bacterial, anti-cancer, anti-inflammatory, and boosting the immune system [35].

The mechanism of flavonoid compounds as antibacterial is by forming complex compounds with dissolved extracellular proteins so that they can damage the cell membrane of bacteria followed by the release of intercellular compounds. In addition, another mechanism that flavonoids have is to inhibit energy metabolism by inhibiting the use of oxygen by bacteria and inhibiting bacterial motility [36]. Meanwhile, according to [37], the mechanism of flavonoids as antimicrobials can be divided into 3 ways, namely inhibiting nucleic acid synthesis, inhibiting cell membrane function, and inhibiting metabolism energy.

Dibutyl phthalate is a part of the phthalate ester group which is widely used as a plasticizer. This is in line with research conducted by [38], which states that one of the chemicals that are widely used as plasticizers are phthalate compounds, for example, dibutyl phthalate (DBP). The Environmental Protection Agency (EPA) has stated that phthalate compounds are toxic to humans [39]. According to [40], dibutyl phthalate has the potential to have bioactive properties. dibutyl phthalate was reported as a highly bioactive (antioxidant and antibacterial) glucose-derived component of the shikimic acid pathway [41]. In addition, DBP has fairly low acute toxicity in experimental animals. However, several studies have reported that exposure to high doses of DBP can cause weight loss and decreased reproductive function [42]. Thus, it is necessary to further study the use of dibutyl phthalate as a drug.

Based on the description of the three compounds that have been docked, the three compounds have potential as antibacterials. These three compounds were active compounds that were very likely to inhibit the growth of *S. pneumoniae* bacteria during in silico testing. Overall, of the three compounds, based on the analytical parameters of the drug similarity test, binding affinity energy, RMSD value, number of hydrogen bonds, and the similarity of amino acid residues with the reference drug, the most effective compound to be used as a drug candidate was Valeraldehyde.

This study was the starting point of the process of finding drugs from natural compounds to treat diseases caused by the bacterium *S. pneumoniae*. The results of the selected compounds 1-methylethyl acetate, valeraldehyde, and dibutyl phthalate contained in guava leaves (*S. samarangense*) using the in silico technique can be used as a basis for conducting further research.

Each derivative of the compound present in guava leaves must be tested to determine its potential on a laboratory scale. Thus, this study requires further in vitro and in vivo testing as a step to validate the activity of the active compound of guava leaves as an antibacterial of *S. pneumoniae* in living cells.

4. Conclusion

The results showed that the three selected compounds in water guava leaves (*S. samarangense*) could potentially be antibacterial and the valeraldehyde compound was potentially the most effective as an antibacterial agent against *S. pneumoniae*.

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