In Silico Assay of Antibacterial Activity of Binahong (*Anredera cordifolia*) Leaf Extract to *Streptococcus pneumoniae*

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Abstract. Pneumonia is a disease that frequently infects toddlers. Pneumonia cases in 2021 caused 9.4% of deaths in children aged 12-59 months. The effectiveness of pneumonia treatment is decreasing due to the increase of *Streptococcus pneumoniae*'s resistance to antibiotics. The application of natural plant extracts such as binahong (*Anredera cordifolia*) leaf extract is chosen as an alternative treatment to pneumonia. The purpose of this study was to disclose the active compound of binahong leaf extract (*A. cordifolia*) that has the potential as an antibacterial to *S. pneumoniae*. This study was conducted in September 2022 at Universitas Negeri Surabaya with in silico method (blind docking) using PyRx software. The results of the drug-likeness assay showed that the three selected compounds (Linoleic Acid, Phytol, and Hexadecanoid Acid) met the criteria as drugs. The molecular docking assay that has been carried out showed that Linoleic Acid has the lowest binding affinity value (-5.4 kcal/mol) when compared to Phytol and Hexadecanoid Acid indicating its higher potential. This study shows that the selected compounds of the binahong leaf extract have the potential to be antibacterial to *S. pneumoniae* with PBP2 as the target protein. It is nevertheless necessary to conduct further research to determine the effectiveness and bioavailability of these compounds.

1 Introduction

Streptococcus pneumoniae is a gram-positive microflora in the human body. These bacteria may become pathogenic and cause pneumonia when the immunity level is low [1]. Pneumonia is an acute inflammatory infectious disease that attacks the lungs, leading to breathing difficulty because of the low exchange of CO_2 and O_2 between the blood and the lungs. The inability of the body's immune system to attack pathogens that infect the respiratory tract causes the alveoli to be filled with inflammatory fluid and leukocyte exudate [2]. Pneumonia can be transmitted through aspiration, inhalation, or bloodstream infection with pathogenic bacteria [3].

Pneumonia cases in Indonesia are one of the highest causes of death. Accordingly, 14.4% of post-neonatal deaths in 2021 were attributed to pneumonia, according to data from the Ministry of Health of the Republic of Indonesia. Pneumonia is also the most common cause of death in toddlers aged 12-59 months with a mortality percentage of 9. 4% in 2021 [4]. Toddlers infected with pneumonia show symptoms such as coughing and difficulty breathing.

Pneumonia treatment is carried out by giving antibiotics. However, misuse of antibiotics is the causality of the resistance problem. *S. pneumoniae* has developed multidrug resistance (MDR) due to the increasing use of antibiotics [6]. From 1997 to 2012, *S. pneumoniae* had increased resistance to penicillin from 0% to 28% and sulfamethoxazole from 9% to 62% [7]. In the mid-1990s was reported that *S. pneumoniae* were resistant to penicillin (21%) and erythromycin (36%) in Jakarta, Indonesia [8]. A recent study in 2010 reported that *S. pneumoniae* was resistant to penicillin by 24% and cotrimoxazole by 45% [9].

Traditional herbs using plants with potential medicinal compounds can be an alternative in the treatment of pneumonia. Binahong leaf (*Anredera cordifolia*) is a traditional medicinal herb widely used by the community. The ethanol extract of binahong leaves contains various potential active compounds including triterpenoids, saponins, alkaloids, flavonoids, phenols, and tannins [10]. These compounds have the potential to be antibacterial, antidiabetic, antiobesity, antihyperlipidemic, vasodilator, and accelerate wound healing [11].

Various in vitro studies have proven that binahong leaves have antibacterial activity. Binahong leaves were able to inhibit the growth of *Propionibacterium acnes* with a low concentration of 20% and a high concentration of 100% [12]. Another study also reported that binahong leaves can inhibit the growth of *Staphylococcus aureus* at concentrations of 5%, 10%, and 15% [13]. A recent study reported that the ethanol extract of binahong leaves had an antibacterial activity on *S. pneumoniae* with the highest inhibitory concentration of 75% [5]. However, the study did not define the specific compounds that inhibit the growth of *S. pneumoniae* significantly.

The most effective active compounds in binahong leaves as antibacterial can be determined through in

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silico studies. Molecular docking is an in silico technique that plays an important role in drug design and development [14]. Molecular docking can predict the interaction between the active compound and the treatment target protein through computational scoring. This scoring is used to predict the binding affinity of active compounds and receptors based on their preferences [15]. This study aimed to determine the most significant active compound of binahong leaf extract (*A. cordifolia*) in inhibiting the growth of *S. pneumoniae.*

2 Methods

This research employed web databases (PubChem, RCSB PDB), web servers (SwissADME), and software such as AutoDock Tools, PyRx, eduPyMOL, and LigPlot. Figure 1 showed the material used includes a 3D structure of the target protein *S. pneumoniae* PBP2 obtained from the RCSB Protein Data Bank web database (https://www.rcsb.org/). The 3D structure of the active compound binahong leaf (*A. cordifolia*) and the antibiotic Benzylpenicillin as a comparison was obtained from the PubChem web database (https://pubchem.ncbi.mlm.nih.gov/).

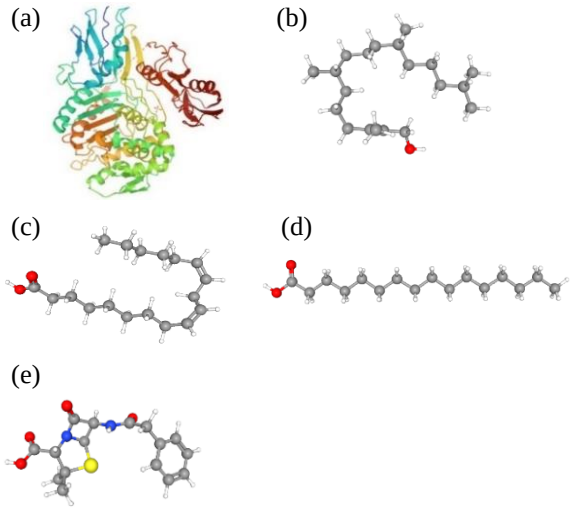

Fig. 1. 3D structure of protein targets and selected active compound of *A. cordifolia.* a) Target protein *S. pneumoniae* PBP2 (PDB ID: 1qme), b) 3D structure of Phytol (CID: 5280435), (c) Linoleic acid (CID: 5280450), (d) Hexadecanoic acid (CID: 16213579), (e) Antibiotic Benzylpenicillin (CID: 5904).

The drug-likeness test was carried out before molecular docking analysis to identify the drug-like properties of the active compound of binahong leaf (*A. cordifolia*) that had been determined. SwissADME web server (https://www.swissadme.ch/index.php) is used for the drug-likeness test by inputting canonical SMILES for each active compound and then analyzed according to Lipinski's rule of five. Lipinski's rule of five used as a reference includes several parameters including molecular weight (WM) ranging from 150-500 g/mol, the number of H-bond donors <5, the number of H-bond acceptors <10, and the MLog P coefficient 4.15 [16] [17].

The molecular docking assay began with sample preparation in four stages. The first stage began by collecting the target protein's 3D structure from the protein data bank. The PBP2 target protein belongs to the Penicillin-Binding Protein obtained by the X-ray diffraction method, it has a resolution of 2.40 Å, a sequence length of 702, and a native ligand SO4. The second stage was protein sterilization using AutoDock Tools software to separate the protein molecular structure from native ligands and contaminant molecules such as water, ligands, or other unwanted proteins. The third stage was the collection of the 3D structure of the active compound of the binahong leaf extract and the comparative antibiotic from PubChem. The fourth stage minimized the structure of active compounds and antibiotics to increase flexibility so that it will produce low binding affinity [18].

The molecular docking test was performed by using PyRx software to interact with the active chemical compound of binahong leaf extract (*A. cordifolia*) with the protein target of *S. pneumoniae* PBP2. The results obtained were RMSD (Root Mean Square Deviation) values and binding affinity values [18]. The docking results were visualized using PyMOL and LigPlot software and indicated the type of bond and the amino acid residues.

The analysis of the data involved comparing the RMSD value and binding affinities of each active component of binahong leaves with native ligands and benzylpenicillin. The valid RMSD value is 2Å [19]. The lower or more negative value of the binding affinity indicates the stronger the bond formed, and vice versa [20].

3 Results and Discussion

The use of antibiotics in the treatment of illnesses brought on by bacterial infections, from minor illnesses to fatal ones, has been widespread. Along with the use of various kinds of antibiotics, many cases of bacterial resistance to antibiotics are found. *S. pneumoniae* is reported to be resistant to various antibiotics, one of which is penicillin. Binahong leaves are one of the alternatives to antibiotics derived from natural ingredients because of their active compound contents.

Several selected active compounds contained in binahong leaves based on literature studies include Phytol, Linoleic Acid, and Hexadecanoic Acid [21]. The drug-likeness test was carried out to determine the similarity properties of the drug in each compound. The parameters used in this test provide a numerical value called a drug-like score. The score will be used to predict the potential of a compound as a new drug candidate [22].

The results of the drug-likeness test were presented in Table 1. The results showed that all compounds met the criteria for molecular weight (MW) according to Lipinski's rule, namely 150-500 g/mol. Molecular weight is an important physicochemical aspect in drug design because it relates to the ability of a molecule to penetrate the membrane (permeability) [23]. The

weightier a molecule, the more difficult it is to penetrate the biological membrane, and vice versa [23].

Table 1. Drug-likeness properties of selected active compund ethanol extract of *A. cordifolia*.

Molecules	PubChem ID	Formula	Canonical SMILES
Phytol	5280435	$C_2OH_{40}O$	OCC=C(CCCC(CCCC(C)CCCC(C)C)C)C
Linoleic acid	5280450	$C_{18}H_{32}O_2$	CCCCCC=CCC=CCCCCCCC(=O)O
Hexadecanoic acid	16213579	$C_{16}H_{32}O_2$	CCCCCCCCCCCCCCCC(=O)O

Table 2. Drug-likeness properties of selected active compund ethanol extract of *A. cordifolia*.

Molecules	MW (g/mol)	HBA	HBD	MLogP
Phytol	296,53	1	1	5,24
Linoleic acid	280,45	2	1	4,47
Hexadecanoic acid	256,42	2	1	4,19

Phytol, linoleic acid, and hexadecanoic acid have H-bond acceptors (HBA) and H-bond donors (HBD) met Lipinski's rules, where HBA <10 and HBD <5. These results indicated the active compound of binahong leaves (*A. cordifolia*) has properties that were easily absorbed by the body. The low amount of HBA and HBD indicated that the absorption of drug-candidate molecules requires low energy and vice versa [24].

Log P is the partition coefficient in the solvent or water system which indicates the lipophilicity of a molecule. Lipophilicity is a basic property related to the physicochemistry and biochemistry of a molecule. A low Log P value indicates that the molecule has high polarity and low lipophilicity or the ability to penetrate the lipid bilayer membrane. Data presented in Table 1 showed that all compound's MLog P values did not meet Lipinski's rules because the values were more than 4.15. A high Log P value indicates that a molecule is more non-polar and has a high ability to penetrate the lipid bilayer [23]. However, a high Log P value indicated that the molecule is difficult to dissolve in water because it is more non-polar or hydrophobic [25]. The hydrophobic nature is related to the ability of the drug to penetrate the lipid bilayer. A drug molecule must not have highly hydrophobic properties, because once the molecule has penetrated the lipid bilayer, it will be difficult to penetrate again [19]. In addition, molecules that are too hydrophobic tend to be toxic [24]. This is because the molecules of these compounds cannot be widely distributed so they will be retained longer in the body.

Hydrophobic drug molecules (higher log p) are more compatible with hydrophobic compartments such as the lipid bilayer whereas hydrophilic drug molecules are more compatible through hydrophilic compartments such as blood serum [26]. Some efforts that can be made to increase the solubility of difficult molecules in the solution include molecular complexation techniques, emulsion formation, micelles, microemulsions, cosovalens, particle size reduction, soft-gel technology, nanocrystal solid-state replacement technology, solid dispersion technology, engineering crystals, and nanomorph technology [27].

Table 3. Binding affinity and RMSD values of ligands and target protein *S. pneumoniae* PBP2 (PDB ID: 1qme).

Ligands	Binding affinity (kcal/mol)	RMSD (Å)
Phytol	-5,3	2,7
Linoleic acid	-5,4	2,4
Hexadecanoid acid	-5	1,6
Benzylpenicillin	-7,6	1,5

Table 3 shows the results of the molecular docking assay for the active compound of binahong leaves with the target protein PBP2. Linoleic acid has more negative binding affinity values than phytol and hexadecanoic acid and native ligand (SO4), but higher than antibiotics (Benzylpenicillin). Phytol and hexadecanoic acid also have lower binding affinity values than native ligands but higher than antibiotics. These results indicated that linoleic acid, phytol, and hexadecanoic acid can bind to protein targets with lower binding energies compared to native ligands. The lower or negative bond affinity indicates the stronger the formed bond, and vice versa [20]. Based on the docking results, all compounds have the potential as antibacterial, with linoleic acid having the highest potential, but the potential was still lower than benzylpenicillin.

The visualization shows the type of the bond formed and the amino acid residues where the binding occurs between the active compound of binahong leaves and the target protein of *S. pneumoniae* PBP2. Phytol has activity with Thr526(A), Thr550(A), Trp374(A), Glu378(A), Ser548(A), Phe570(A), Tyr595(A) through hydrophobic bonds and Gly549(A), Ser571(A) through hydrogen bonds to bind to the target protein of *S. pneumoniae* (Figure 2a). Phytol (3,7,11,15-tetramethylhexadec-2-EN-1-OL) is a chlorophyll constituent compound and is classified as a diterpene essential oil. Studies have shown that phytol has very high antimicrobial resistance and low toxicity [28]. Studies report that phytol can inhibit the growth of *E. coli* [28], and *P. aeruginosa* [29]. The phytol inhibits the growth of *P. aeruginosa* through the oxidative stress response. This response causes a decrease in glutathione levels, DNA damage, cell filamentation, and depolarization of the bacterial cell membrane [29].

Linoleic acid (9-cis, 12-cis-octadecadienoate) is a group of polyunsaturated omega-6 fatty acids. Linoleic acid bioactivity with Glu378(A), Gly549(A), Trp374(A), Tyr548(A), Gln452(A), Asn377(A), Ser337(A), Phe450(A), Asn397(A) through hydrophobic bond and Thr550(A), Ser548(A), Ser395(A) through hydrogen bond to bind to the target protein *S. pneumoniae* PBP2 (Figure 2b). The antibacterial activity of linoleic acid has been widely studied. Recent studies have reported on the inhibition mechanism of linoleic acid on Lactobacillus, namely by disrupting cell membranes that affect bacterial metabolism leading to bacterial cell death [30]. Linoleic

acid is also able to inhibit the growth of *Bifidobacterium breve* DSM 20213 by causing metabolic stress leading to unbalanced redox status regulation that causes bacterial cell death [31].

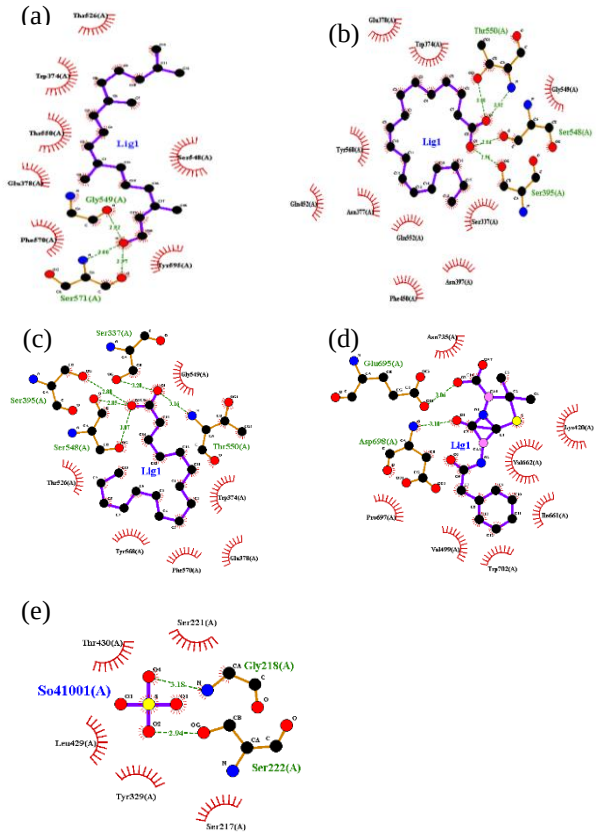

Figure 2. Visualization of the docking results with the target protein of *S. pneumoniae* PBP2 (PDB ID: 1qme); (a) Phytol, (b) Linoleic acid, (c) Hexadecanoic acid, (d) Benzylpenicillin, (e) Native ligand SO4.

Hexadecanoic acid is a derivative of a fatty acid commonly called palmitic. Hexadecanoic binds to the target protein *S. pneumoniae* PBP2 has bioactivity with Gly549(A), Thr526(A), Tyr568(A), Phe870(A), Glu378(A), Trp374(A) via hydrophobic bond, and Ser337(A), Ser395(A), Ser548(A), Thr550(A) via hydrogen bond (Figure 2c). Fatty acids can inhibit bacterial growth by disrupting cell membranes [32]. This inhibition will cause damage to cell membranes and inhibit biosynthetic enzymes so that cells will undergo lysis and cell death will occur [33].

Benzylpenicillin has bioactivity with Asn735(A), Lys420(A), Val662(A), Ile661(A), Trp702(A), Val499(A), Pro697(A) through hydrophobic bonds and the amino acid Glu695(A), Asp698(A). Native ligands have bioactivity with amino acids Ser221(A), Thr430(A), Leu429(A), Tyr329(A), Ser217(A) via hydrophobic bond, and Gly218(A), Ser222(A) via hydrogen bond (Figure 2e).

Based on the docking results, it is known that all active compounds in binahong leaves have potential as antibacterial *S. pneumoniae*, with linoleic acid having the greatest potential, but lower potency than benzylpenicillin.

4 Conclusion

The selected active compounds of ethanolic extract of binahong leaves (*A. cordifolia*) in this study are Phytol, Linoleic acid, and Hexadecanoic acid comply with the criteria as an antibacterial agent. The most significant compound as an antibacterial against *S. pneumoniae* was Linoleic acid.

Selected active compounds from binahong have the potential as antibacterial to *S. pneumoniae* with PBP2 target protein. The results of this study need to be further studied and clarified to deepen the specific mechanism, toxicity, and feasibility of each compound as a medicine for pneumonia.

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