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In silico Study of Essential Oil of *Bambusa vulgaris* Leaves as an Anti Beta-lactamase Compound

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Background: *Klebsiella pneumoniae* is known as an extended spectrum beta (β)-lactamases (ESBLs)-producing bacteria, which produces enzymes that cause resistance to β-lactam antibiotics by degrading β-lactam ring. A solution is needed to prevent the degradation of the β-lactam ring. In this *in silico* study, combining β-lactam antibiotics with secondary metabolites has the possibility to inhibit the active site of the β-lactamase enzyme. This study aimed to explore the potential of the essential oil of yellow bamboo (*Bambusa vulgaris*) leaves as inhibitors of β-lactamase.
Materials and methods: This research was conducted by simulating molecular docking to determine the interaction of ligands with proteins, pharmacological tests of compounds based on the Lipinski's rule of five, and ligand toxicity tests with pkCSM.
Results: The free bond energy values (ΔG) were in the range of -4.3 to -8.0 kcal/mol. The ligands with the best ΔG value were sulfur pentafluoride (-8.0 kcal/mol), squalene (-7.3 kcal/mol), 3-aminodibenzofuran (-7.1 kcal/mol), and 2- monolaurin (-5.5 kcal/mol). Secondary metabolites from the essential oil of *B. vulgaris* leaves fulfilled Lipinski's rule of five, so that oral use can be carried out except for squalene and tridecane.
Conclusion: Secondary metabolite compounds in the essential oil that have potential as oral drugs based on the Lipinski pharmacological test and the pkCSM toxicity test are dipivaloylmethane, β-ocimene, 2-monolaurin, and undecane.

Keywords*: β-lactamase, Bambusa vulgaris, essential oil, Klebsiella pneumoniae*

Introduction

In 2019, infections caused by multidrug resistance result in the death of 44,000 people in the United States. One of the causes of multidrug resistance is Gram-negative bacteria that produce extended spectrum beta (β)-lactamases (ESBLs).[1,2] This is evidenced by 50% of ESBL cases in the United States is caused by Enterobacteriaceae.[3] A study based on data from Indonesia, Thailand, the Philippines, Malaysia and Singapore shows the prevalence of *Klebsiella* spp. which produce β-lactamase reaches 46.7% with Indonesia having the highest prevalence (64%).[4]

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Klebsiella pneumoniae is known as one of the pathogenic bacteria that causes nosocomial infections due to the virulence and antibiotic resistance of these bacteria.[5] *K. pneumoniae* is a Gram-negative bacterium classified in Enterobacteriaceae family and commonly found in urinary tract, lower respiratory tract and bloodstream infection.[6,7] A research conducted at Dr. Soeradji Tirtonegoro Hospital, Klaten, Central Java shows that around 52.98% of clinical isolates identified in the hospital are ESBL-producing *K. pneumoniae*.[4] A study conducted by Sanglah Hospital, Denpasar shows that the prevalence of β-lactam antibiotics resistance in the *K. pneumoniae* group reaches 69.2%.[8] Another study shows that *K. pneumoniae* isolates are resistant to ampicillin (78.3%), cefalotin (75%), ceftriaxone (32%), and cefotaxime (24%) from 75 samples.[9] *K. pneumoniae* is also discovered to be resistant against ceftazidime, another β-lactam drug.[10]

β-lactam antibiotics are a class of antibiotics that have the β-lactam ring and are generally used to treat bacterial infections.[11] These antibiotics have a mechanism as bactericidal by inhibiting bacterial cell wall synthesis. The antibiotic binds to penicillin-binding proteins (PBPs), leading to incomplete transpeptidation reaction, although cell wall formation is continued.[12] There is no cross-link formation in the cell wall synthesis, and the peptidoglycan is inadequately formed, hence it is weaker and easily degraded. The result is the activation of lytic enzymes that causes bacterial cell death.[13]

K. pneumoniae has the ability to produce extended spectrum β-lactamases (ESBLs), which are encoded by a gene on the conjugative plasmid determining resistance to β-lactam antibiotics.[9] β-lactamases cause antibiotic resistance by hydrolyzing the β-lactam ring and changing the structure of the drug when binding to PBPs. Changes in the structure of the drug cause inactivation of the drug.[14] Therefore, a solution is needed to prevent the degradation of the β-lactam ring. One of them is by combining β-lactam antibiotics with secondary metabolites to inhibit the active site of the β-lactamase. Previous studies have mentioned that clavulanic acid, one of the metabolites produced by *Streptomyces clavuligerus* is an effective treatment for many diseases caused by pathogens, such as *Klebsiella* spp, when combined with amoxicillin, a β-lactam antibiotic.[15] In addition, polyphenolic compounds, such as epigallocatechin-3-gallate and caffeic acid can synergize with gentamicin, ciprofloxacin and tetracycline in inhibiting the growth of ESBL-producing *K. pneumoniae*.[16]

Compounds that are contained in the essential oil of yellow bamboo (*Bambusa vulgaris*) leaves were used in this study. Phytochemical screening of *B. vulgaris* shows the presence of alkaloids, flavonoids, saponins, and tannins, as well as several minerals, indicated by the presence of calcium and iron in *B. vulgaris* leaves.[17] This *in silico* study aimed to explore the potential of essential oil from *B. vulgaris* leaves in inhibiting the β-lactamase enzyme.

Materials and methods

Ligands and Target Protein Preparation

This research was conducted *in silico* by utilizing the protein and ligand database available on the website. The target protein used in the study was β-lactamase (PDB ID: 6M5P), which was downloaded via the Protein Data Bank (PDB) (www.rcsb.org) in .pdb format. This protein was prepared using PyMol software based on Autodock Vina 4.2 (Scripps Research, La Jolla, CA, USA). In the preparation of this molecule, the removal of the H_2O group, the separation of the native ligands contained in the protein molecule, and the addition of hydrogen atoms were conducted. Then, the file was saved in pdbqt format. The test ligands used in this study were secondary metabolites found in the essential oil of *B. vulgaris* leaves. The ligands were downloaded from PubChem (www.pubchem.ncbi.nlm.nih.gov) in SDF format and converted to PDB format by PyMol software.

Molecular Docking

The binding of ligands and protein molecules was carried out using the Autodock Vina-based PyRx program. The tethering results obtained were binding affinity (kcal/mol) and root mean square deviation (RMSD) value. The molecular docking test results require verification of the reliability of the method on the docking area used through RMSD value.[18,19] After validation, docking simulation between ligands and target protein was carried out.

Docking Visualization

Determination of the docking ligand conformation (the best pose) was done by selecting the ligand conformation that has the lowest bond energy. Parameters analyzed included amino acid residues, hydrogen bonds, predicted inhibition constants, and bond free energies. Binding sites and molecular interactions of secondary metabolites with target proteins were visualized in two-dimensional (2D) and three-dimensional (3D) structures using Biovia Discovery

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Studio 2020 (BIOVIA, California, USA). The visualization of the molecular docking results was used to determine the interaction of amino acid residues from β-lactamase with secondary metabolites in the essential oil extract of *B. vulgaris* leaves.

Pharmacological and Toxicity Test

The physicochemical and pharmacological properties of secondary metabolites in the essential oil extract of *B. vulgaris* were analyzed by Lipinski's rule of five. This law predicts the absorption, distribution, metabolism, and excretion performance of a compound as a drug.[20] The toxicity of secondary metabolites were tested using the pkCSM method.

Results

Compounds Found in B. vulgaris Leaves Essential Oil

A total of 8 compounds with the highest composition based on a previous study[21] were identified in *B. vulgaris* essential oil (Table 1).

Molecular Docking Results

Molecular docking results of the test ligands against β-lactamase showed that there were 9 tested ligands, consisting of 8 ligands from the essential oil of *B. vulgaris* leaves and 1 native ligand (Table 2). The native ligand used in this study was clavulanic acid (PubChem ID: 5280980). The docking results showed that the free bond energy values (ΔG) were in the range of -4.3 to -8.0 kcal/mol. The ligand with the best ΔG value was sulfur pentafluoride (-8.0 kcal/mol). The results of docking visualization were shown in Figure 1.

Pharmacological and Toxicity Analysis Results

Pharmacological properties prediction of secondary metabolites contained in the essential oil was analyzed by Lipinski's rule of five, while the toxicity of secondary metabolites was tested using the pkCSM method. Based on Lipinski's analysis, it was found that all test ligands complied with Lipinski's rule, except squalene and tridecane (Table 3). Prediction of secondary metabolite toxicity was carried out using the pkCSM test which included AMES toxicity test, maximum tolerated dose (MTD), human ether-a-go-go-related gene (hERG) I and II inhibitor, acute oral rat toxicity (median lethal dose; LD_{50}), hepatotoxicity, skin sensitization, and Minnow toxicity (Table 4).

Discussion

Research related to the extraction and identification of compounds contained in *B. vulgaris* essential oil has been carried out previously, in which fresh and dry *B. vulgaris* leaves are distilled for 4 hours, and the oil obtained is dried using anhydrous sodium sulfate (Na_2SO_4). Then, gas chromatography-mass spectrometry (GC-MS) and the identification of chemical compounds contained in the *B. vulgaris* leaves essential oil based on the retention index of each compound are carried out.[21]

The molecular docking process was started with validation based on the RMSD value. The RMSD value is said to be good if <2 Å. The larger the deviation of the value, the greater the error in the prediction of ligand interactions with macromolecules. The best RMSD value is close to 0 Å.[22] Validation results indicated that the RMSD values of the molecular docking performed in this study were 0 Å. Based on the binding affinity value, ligand with

Table 1. Chemical compounds found in essential oil of *B. vulgaris* leaves.[21]

Identified Compound	Chemical Formula	PubChem ID
Sulfur pentafluoride	$C_7H_5ClF_5NOS$	9602898
Dipivaloylmethane	$C_{11}H_{20}O_2$	70700
3-aminodibenzofuran	$C_{12}H_9NO$	20061
β-ocimene	$C_{10}H_{16}$	18756
2-monolaurin	$C_{15}H_{30}O_4$	74297
Undecane	$C_{11}H_{24}$	14257
Squalene	$C_{30}H_{50}$	638072
Tridecane	$C_{13}H_{28}$	12388

Table 2. Molecular docking results between ligands and β-lactamase.

Ligand	Molecular Weight (g/mol)	Binding Affinity (kcal/mol)	RMSD (Å)	
			Lower Bound	Upper Bound
Sulfur pentafluoride	281.633	-8	0	0
Dipivaloylmethane	184.279	-5.1	0	0
3-aminodibenzofuran	183.21	-7.1	0	0
β-ocimene	136.238	-5	0	0
2-monolaurin	274.401	-5.5	0	0
Undecane	156.313	-4.3	0	0
Squalene	410.73	-7.3	0	0
Tridecane	184.367	-4.6	0	0
Clavulanic acid (native)	199.162	-5.7	0	0

the best value was sulfur pentafluoride (ΔG=-8.0 kcal/mol), squalene (ΔG=-7.3 kcal/mol), 3-aminodibenzofuran (ΔG=-7.1 kcal/mol), and 2-monolaurin (ΔG=-5.5 kcal/mol). Meanwhile, clavulanic acid had a ΔG value of -5.7 kcal/mol. Clavulanic acid, a commercial drug with the ability to inhibit β-lactamase, was used as a comparison ligand.[23] Sulfur pentafluoride, squalene, and 3-aminodibenzofuran had more potential, since these compounds have lower ΔG values compared to the native ligand. Lower ΔG value means that the bond between the ligand and macromolecule is more stable[24], since the stability and strength of noncovalent interactions can be analyzed based on the amount of free energy generated when the enzyme and the ligand interact.[25]

Research related to the potential of natural compounds as β-lactamase inhibitors has been carried out previously. The molecular docking of microalgae compounds shows

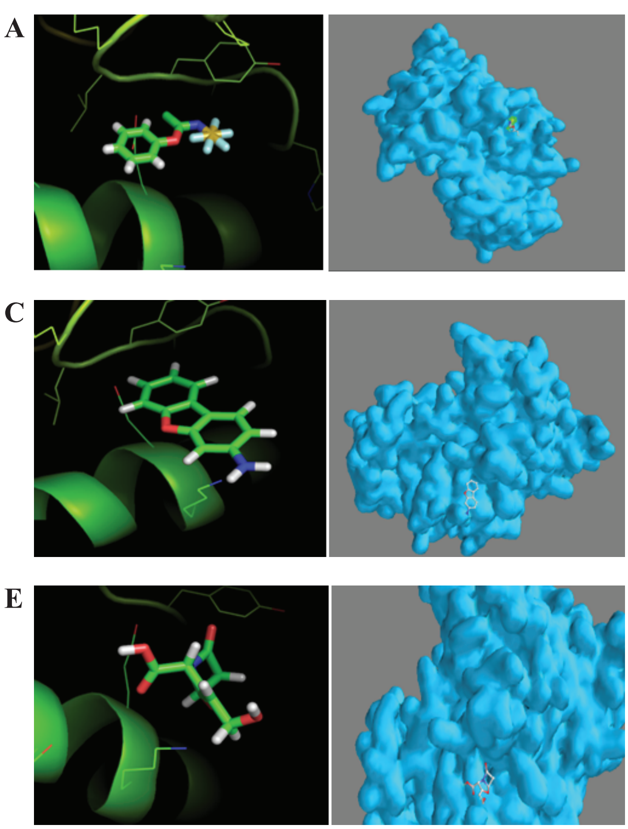

Figure 1. The 3D interaction between compounds from essential oil of *B. vulgaris* leaves and β-lactamase. A: sulfur pentafluoride; B: squalene; C: 3-aminodibenzofuran; D: 2-monolaurin; E: clavulanic acid (native).

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Table 3. Lipinski's rule of five analysis results.

Ligand	Molecular Mass (Dalton)	Hydrogen Bond Donor	Hydrogen Bond Acceptor	LogP	Molar Refractivity
Sulfur pentafluoride	281.5	0	1	3.48	47.64
Dipivaloylmethane	184	0	2	2.61	53.54
3-aminodibenzofuran	183	2	2	3.17	58.13
β-ocimene	136	0	0	3.47	48
2-monolaurin	274	2	4	2.8	75.9
Undecane	156	0	0	4.54	52.9
Squalene	410	0	0	10.61	140.06
Tridecane	184	0	0	5.32	62.13
Clavulanic acid (native)	199	2	6	-1.09	42.93

that phenylacridine (4-Ph), quercetin (Qn), and cryptophycin (Cryp) exhibit a better binding score and binding energy than commercial clinical medicine β-lactamase inhibitors, such as clavulanic acid, sulbactam, and tazobactam.[26] Moreover, the molecular docking of eight L2-β-lactamase inhibitors shows that these compounds possess ΔG values (lowest to the highest) as follows: relebactam -6.8 kcal/mol, meropenem -6.54 kcal/mol, nitrocefin -6.28 kcal/mol, avibactam -6.14 kcal/mol, imipenem -5.34 kcal/mol, carbapenem -5.24 kcal/mol, ceftazidime -4.83 kcal/mol, and aztreonam -4.6 kcal/mol, respectively.[27] Based on the binding affinity values obtained in the present study, compounds from *B. vulgaris*, namely sulfur pentafluoride, squalene, and 3-aminodibenzofuran were more potential as β-lactamase inhibitors than those compounds as indicated by lower ΔG values.

A good drug must follow Lipinski's rule. The drug administered to the patient will be eliminated from the body due to various factors that can eliminate the drug or prevent it from reaching the desired target site. These four factors, namely absorption, distribution, metabolism, and excretion, are called pharmacokinetics or the ability of the body to respond to drugs. Pharmacokinetic aspects are very important to be considered during drug design, since a drug cannot interact with the target if it does not reach the target. Therefore, Lipinski's rule of five is developed. Drugs capable of reaching the target when administered orally must meet the following requirements: molecular weight <500 Daltons, the number of hydrogen bond donor groups is no more than 5, the number of bond acceptor groups is no more than 10, has high lipophilicity (logP<5) and molar refractivity in the range of 40-130.[28]

Based on the results of Lipinski's rule analysis, secondary metabolites of *B. vulgaris* essential oil complied with Lipinski's rule, hence these compounds can be administered orally, except for squalene and tridecane. Squalene did not meet Lipinski's rule because it had a logP>5 and a molar refractivity >140. Meanwhile, tridecane did not meet Lipinski's rule because it had a logP>5. The logP value is related to the hydrophobicity of the drug

Table 4. pkCSM test results.

Ligand	LD_{50} (mol/kg)	Hepatotoxicity	Skin Sensitization	Minnow Toxicity (log mM)	AMES Toxicity	MTD (Human; log mg/kg/day)	hERG I Inhibitor	hERG II Inhibitor
Sulfur pentafluoride	2.762	No	Yes	0.886	Yes	0.49	No	No
Dipivaloylmethane	1.626	No	Yes	0.797	No	0.846	No	No
3-aminodibenzofuran	2.945	No	No	0.404	Yes	0.196	No	No
β-ocimene	1.636	No	No	0.784	No	0.636	No	No
2-monolaurin	1.405	No	Yes	0.511	No	0.651	No	No
Undecane	1.597	No	Yes	-0,134	No	0.389	No	No
Squalene	1.848	No	No	-3.485	No	-0.393	No	Yes
Tridecane	1.542	No	Yes	-0.674	No	0.269	No	No
Clavulanic acid (native)	1.546	Yes	No	3.966	No	1.35	No	No

molecule. High logP indicates higher hydrophobicity.[29] One of the requirements for a compound that can be used as a drug is it should not be too hydrophobic, which will affect the longer shelf-life in the lipid bilayer. This causes drug compounds to be retained for a long duration and are widely distributed in the body, causing the reduction of binding selectivity to the target protein. A logP value that is too negative is also not recommended because if the drug is too hydrophilic, the drug will not be able to pass through the lipid bilayer.[30]

AMES toxicity test is generally used to determine the mutagenic potential of a compound through bacteria testing. Positive results in this test indicate that the compound may be mutagenic and potentially carcinogenic.[31] Secondary metabolites of *B. vulgaris* essential oil did not cause mutagenic effects, except for sulfur pentafluoride and 3-aminodibenzofuran (Table 4). The MTD test is used as a recommendation for drug dosing. The MTD value of 0.477 log mg/kg/day is categorized as low MTD. MTD value will be categorized as high MTD if it is higher than 0.477 log (mg/kg/day).[32] The hERG I and II inhibitor test is used to test the inhibitory ability of a compound against hERG. Compounds that are inhibitors of hERG I or II may cause fatal ventricular arrhythmias. All compounds used in the present study did not act as inhibitors of hERG I and II, except for squalene.

LD_{50} is the amount of compounds that can cause the death of 50% of the experimental animals. Compounds that are predicted to have an LD_{50} ranging from 300–2,000 mg/kg are included in toxicity class 4 and 2,000–5,000 mg/kg are included in toxicity class 5 based on the Globally Harmonized System. Toxicity class 5 compounds have a low acute toxicity effect, while toxicity class 4 means their toxicity is relatively low.[33] Rat is selected as an animal model because there is a large amount of experimental data and it is often taken as representative of human LD_{50}. This species is an ideal choice for evaluating acute toxicity by the oral and inhalation routes according to Classifying, Labelling and Packaging (CLP) regulation (Regulation (EC) No. 1272/2008). The *in silico* mammalian acute toxicity prediction is developed using mathematical approaches, starting from large training sets, and sufficient explanations of the model. The models are based on the mathematical relationship between the chemical's quantitative molecular descriptors and its toxicological activities. However, when assessing the accuracy of a predictive model (*in silico* or *in vitro*), we must bear in mind that it is closely related to the uncertainty and variability of the original data of the standard model. If uncertainty and variability are high, we cannot expect high accuracy.[34]

Hepatotoxicity test was done to see the effect of a compound on normal liver function. It was predicted that compounds from *B. vulgaris* leaves essential oil will not interfere with liver function. Skin sensitization test was performed to predict the effect of a compound on the skin. In this test, 3-aminodibenzofuran, β-ocimene, and squalene showed negative results, which means that these compounds were not predicted to induce skin allergies and dermatitis. Skin sensitivity test is more commonly carried out on compounds that will be used as topical preparations, not oral preparations. Skin allergic reactions are not commonly observed in drugs administered via the oral route. Though they may share similar mechanisms, caution should be taken when extrapolating the compounds from skin sensitization potential for topically applied chemicals to predict "allergic potential" of drugs.[35] Moreover, research has been conducted to examine whether skin testing is necessary before re-administering penicillin to patients with nonimmediate reactions (NIR). The result of this research shows that administering penicillin orally without preceding skin testing is safe and sufficient to exclude penicillin allergy after NIR developing during penicillin treatment.[36] A small toxicity test showed a prediction of having high acute toxicity if the log median lethal concentration (LC_{50}) value <-0.3. In the present study, the test results showed that all compounds from *B. vulgaris* essential oil did not have high acute toxicity, except for squalene and tridecane.

Conclusion

The essential oil of *B. vulgaris* leaves has a potency as β-lactamase inhibitor based on docking simulations with ΔG values in the range of -4.3 to -8.0 kcal/mol. Secondary metabolite compounds in *B. vulgaris* essential oil that have potential as oral drugs based on the Lipinski pharmacological test and the pkCSM toxicity test are dipivaloylmethane, β-ocimene, 2-monolaurin, and undecane.

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