The Potential of Several alkaloids of *Rhizophora stylosa* To Enhance Destruction Cell Wall Mechanism Against Bacterial Resistance

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ABSTRACT

The resistance of the beta-lactams became a major factor that contributes to mortality caused by bacterial infection. The combination of clavulanate acid and penicillin groups has been used widely as a solution against bacterial resistance. However, several cases reported that clavulanate acid caused an allergy response. The Rhizophora stylosa (R.Stylosa); is one of the mangrove subspecies widely investigated due to its empirical use in traditional medicine. This study aimed to predict the potential of alkaloid compounds of these plants as a candidate for co-antibacterial agents against bacterial resistance. This study used several alkaloids that have been found in R.Stylosa *(sanguinarine, muscimol, glycosin, ergobasine, mescaline, pilosine, and nordihydrocapsaicin) as a subject of this study. The beta-lactamases (PDB ID: 6NVU) and penicillin-binding proteins (PDB ID: 3UY6) were used as targeted proteins. The drug-likeness and pharmacokinetics of compounds were analyzed using Swiss ADME. Meanwhile, the affinity of ligands was predicted using Autodock Vina. The root means square distance (RMSD) value (<2 A) and the binding cavity of drugs (amoxicillin and clavulanate acid) were used as parameters of validation. This study resulted that these alkaloids have good drug-likeness and high gastrointestinal absorption. However, prediction of other parameters (BBB permeant, Pgp substrate, and inhibition of cytochrome enzyme) show that only muscimol, mescaline, and pilosine didn't affect these parameters. The affinity value of four compounds (sanguinarine, glycosin, ergobasine, and mescaline) indicated better affinity in both proteins. Moreover, the combination use of amoxicillin with these four compounds increases the affinity of amoxicillin. These conclude that some alkaloids of R. Stylosa could potentially be used as co-drugs with amoxicillin to both increase activity and prevent bacterial resistance*

Keywords: *In-silico, R.Stylosa, alkaloid, bacterial resistance*

INTRODUCTION

Infection is a condition caused by the invasion of pathogens in the body such as bacteria and fungi. Antibiotics are used as first-line treatment to eradicate the pathogen, hence preventing severe infection in the body. However, the continuous use of antibiotics leads to the mechanism of resistance to some bacterial pathogens (1). One of the antibiotics that has been reported to experience many cases of resistance is the beta-lactam group.

Beta-lactam antibiotics are commonly prescribed antibiotics that have been reported resistant to some bacteria. These groups are characterized by the beta-lactam ring, which is acted as a pharmacophore of bactericide activity of drugs (2). Several clinical reports, that the continuous use of beta-lactam groups could cause resistance. Some bacteria could exert beta-lactamase that disrupts beta-lactam rings which caused loss of bactericide activity of antibiotics. Clavulanic acid is a compound with a potent inhibitory action against beta-lactamases; the protein known to damage beta-lactam rings. It has a poor antibacterial effect but is usually used in combination with beta-lactam groups. Some case reported a few cases of clavulanat acid allergies with either delayed or immediate reactions (3,4).

The *Rhizophora Stylosa* is the most common species of mangrove which can be found in the coastal area of java. Some

folklore describes these plants as traditional medicine, especially for cough, infection, antipyretic and antiasthmatic (5). Empirically, the different parts of mangroves could be used for several common sicknesses. Many studies used to explore the medicinal properties of these plants. The bioactive compounds extracted from these plants exhibit pharmacological activities such as antioxidant, antimicrobial, antidiarrhea, cytotoxic on various cancer cells and hepatoprotective (6,7). The ethanolic extract of the Rhizophora sp and Avicennia marina have antifungal effects against some Penicillium family. Some bioactive compunds reported to have potential activity as antibiotics with bacteria nucleic acid mechanism (8,9)

This study aims to investigate the potential of alkaloid compounds of R. Stylosa to be used as an enhancement of antibiotic agents against bacteria resistance. A preliminary study was needed to investigate the pharmacological effects that could potentially be developed. Besides, this study also provides information on potential combination of amoxicillin and these compounds in humans.

MATERIAL AND METHODS

MATERIAL

The molecular docking used specifications: Windows 10 OS, AMD A8 7410 processor (Quad-core; 2.2 GHz), and 4 GB RAM. The structure design tools used were Marvin sketch (ChemAxon), Discovery Studio Visualizer (Biovia), Preparation docking Autodock tools (ADT), and Autodock vina.

The materials used for in silico study were the penicillin binding protein (PDB ID: 3UY6) and beta-lactamase protein (PDB ID: 6NVU); obtained from the proteins database website (RCSB.org). The ligands (Sanguinarine, muscimol, glycosin, ergobasine, mescaline, pilosine, nordihydrocapsaicin) were obtained from the chemical web library database (Pubchem.org).

METHODS

1. Analysis of Drug-likeness and Pharmacokinetics of Ligands

The proteins and ligands both prepared under optimal energy possible. The proteins was cleaned from water, ions, and other native ligands that affect the process of dockings. The druglikeness and pharmacokinetics were performed using the web analysis Swissadme.com several parameters of the compounds tested. The druglikeness will processed based on the Lipinsky's rule of five which includes lipophilicity parameters, H-bond acceptors (HBA), H-bond donors (HBD), and molecular weight (MW) (10,11).

2. Molecular Docking of Alkaloids

Protein were prepared using autodock tools (ADT) to remove impure components and the addition of hydrogen atoms to the polar groups of proteins to minimize errors due to compounds tautomerization (12,13). Visualization of 2D and 3D bonds was carried out to clarify the possible bonds formed between ligand compounds and proteins. The Ds Visualizer was used to visualize form of bonds between amino acid and the functional groups of ligands that affect the pharmacological effects of compounds (14).

3. Data Analysis

This study uses pre and post docking validation between ligand and both standard compounds (clavulanate acid and amoxicillin). The validation used the specific binding site of the comparison drug compound (penicillin and clavulanic acid). The affinity will be chosed based on specific criteria of root mean square deviance (RMSD) with less than 2 A (15).

RESULT

1. The Drug-likeness and Pharmacokinetics Properties of Alkaloids

This study uses in silico experiment to predict the properties of several alkaloids of *R. Stylosa* namely sanguinarine, muscimol, glycosin, ergobasine, mescaline, pilosine, and nordihydrocapsaicin. Investigation of drug-likeness and pharmacokinetics properties was carried out using web-based analytics of ADME. The Lipinsky rule is used as a parameter of prediction following lipophilicity, molecular weight, donor, and acceptor of hydrogen (Lipinsky rule of five). Meanwhile, the prediction of pharmacokinetics targeted Gastrointestinal absorption, Blood-brain barrier permeant, and several types of cytochrome enzymes(10). The results show no violation of the Lipinsky rule, though only three compounds; muscimol, mescaline, and pilosine that has high gastrointestinal absorption but didn't affect other pharmacokinetics parameters (Table 1 and Table 2).

Table 1. The drug-likeness of alkaloids analyzed by Swiss Admet to determine the potentiall to orally used of compounds based on lipinsky rule of five.

Ligands	MW	H-bond acceptors	H-bond donors	Log-P	Violation of rule
Sanguinarine ($C_{20}H_{14}NO_4$)	332.3	4	0	2.88	0
Muscimol ($C_4H_6N_2O_2$)	114.1	3	2	-0.54	0
Glycosin ($C_{16}H_{14}N_2O$)	250.29	2	0	2.69	0
Ergobasine ($C_{19}H_{23}N_3O_2$)	325.4	3	3	1.85	0
Mescaline ($C_{11}H_{17}NO_3$)	211.26	4	1	1.43	0
Pilosine ($C_{16}H_{18}N_2O_3$)	286.33	4	1	1.36	0
Nordihydrocapsaicin ($C_{17}H_{27}NO_3$)	293.4	3	2	3.18	0

Table 2. The pharmacokinetics prediction of alkaloids on gastrointestinal, blood brain barrier permeability, Pgp Substrate and several metabolism enzym

Ligands	Pharmacokinetics Parameters			
	GI abs	BBB	Pgp	Metabolism enzyme
Sanguinarine ($C_{20}H_{14}NO_4$)	High	Yes	Yes	CYP1A2, CYP2C19
Muscimol ($C_4H_6N_2O_2$)	High	No	No	-
Glycosin ($C_{16}H_{14}N_2O$)	High	Yes	No	CYP1A2, CYP2C19
Ergobasine ($C_{19}H_{23}N_3O_2$)	High	No	Yes	CYP2D6
Mescaline ($C_{11}H_{17}NO_3$)	High	Yes	No	-
Pilosine ($C_{16}H_{18}N_2O_3$)	High	Yes	No	-
Nordihydrocapsaicin ($C_{17}H_{27}NO_3$)	High	Yes	No	CYP1A2, CYP2D6

2. Molecular Docking of Compounds

This study were performed using Autodock vina. The targeted protein used were beta-lactamase and penicillin binding protein, while the alkaloids used as ligand. The comparative drugs used were amoxicillin as beta-lactam drugs that bind to penicillin binding proteins and clavulanate acid, a drugs known to bind and destroy beta-lactamase. The molecular docking predicted affinity between ligand and protein (13). The results show four compounds (Sanguinarine, glycosin, ergobasine and pilosine) has higher affinity on both proteins than standard (Table 3).

Table 3. The results of molecular docking of alkaloids using autodock vina. *) Higher affinity than standard drugs

Ligands	Affinity	
	Beta-lactamase (PDB ID: 6NVU)	*Penicillin-binding Proteins* (PDB ID : 3UY6)
Clavulanate acid	-5.3	-
Amoxicillin	-	-6.7
Sanguinarine	-5.6*	-7.5*
Muscimol	-4.5	-4.4
Glycosin	-7.6*	-6.9*
Ergobasine	-6.5*	-8.8*
Mescaline	-4.4	-5.7
Pilosine	-6.3*	-7.0*
Nordihydrocapsaicin	-5.2	-6.2

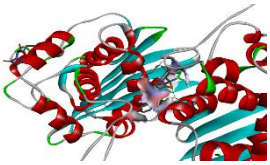
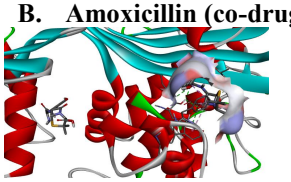
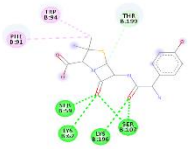
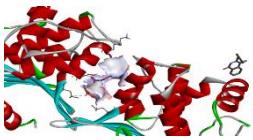
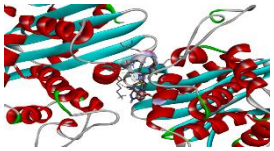
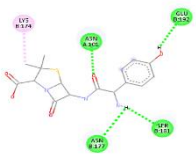
3. The Affinity of Amoxicillin Combined With Several Alkaloids of *R. Stylosa*

The selected alkaloids with higher affinity will be used as a combination of drugs with amoxicillin. This study was used to investigate the affinity of amoxicillin in presence of alkaloids. Based on the previous study, the four compounds were selected as candidates for co-drugs with amoxicillin (Sanguinarine, glycosin, ergobasine, and pilosine). Further investigation was also performed to identify the binding site of ligands and amino acid bonding between compounds and proteins. The results show all combination increase affinity of amoxicillin on penicillin-binding proteins. Moreover, there are some similarity in binding site of amoxicillin (Table 4 and Table 5).

Table 4. The affinity and amino acid bond of amoxicillin with combined with several alkaloids.

Ligands	Penicillin binding Proteins (PDB ID : 3UY6)	
	Affinity	Amino acid bond
Amoxicillin (co-drugs: Clavulanat acid)	-7.1	1. Conventional hydrogen bond: Serine (181), Asparagine (177) 2. Carbonyl bond :Aspartic acid (77)
Amoxicillin (co-drugs: Sanguinarine)	-7.6	1. Conventional hydrogen bond: Serine (59), Lysine (62), Lysine (196), serine (107) 2. Carbon-hydrogen bond: Threonine (199) 3. Pi alkyl bond : Tryptophan (94), phenylalanine (91)
Amoxicillin (co-drugs: Glycosine)	-7.2	1. Conventional hydrogen bond: Serine (181), lysine (174) 2. Carbon-hydrogen bond: Serine (180) 3. Pi alkyl bond : Leusine (184)
Amoxicillin (co-drugs: Ergobasine)	-7.5	1. Conventional hydrogen bond: Glutamic acid (192), Serine (181), Asparagine (177), Asparagine (101) 2. Pi alkyl bond : Lysine (174)

Table 5. The visualization of binding cavity of penicillin binding proteins combined with alkaloids and clavulanat acid

A. Amoxicillin (co-drugs: Clavulanat acid)	
	
B. Amoxicillin (co-drugs: Sanguinarine)	
	
C. Amoxicillin (co-drugs: Glycosine)	
	
D. Amoxicillin (co-drugs: Ergobasine)	
	

DISCUSSION

The *Rhizophora stylosa* was one of the mangrove sub-species that have great potential as a phytotherapy source. Over years various bioactive compounds have been found, but yet developed from these plants (16,17). This study uses in silico experiment that determines the properties of several alkaloids compounds which has been found in *R. Stylosa* namely sanguinarine, muscimol, glycosin, ergobasine, mescaline, pilosine, and nordihydrocapsaicin.

These compounds have different physical-chemical properties that led to different pharmacokinetic activities in the body. The drug-likeness properties in this study were used to analyze the potential of orally used based on a parameter of lipophilicity, molecular weight, donor, and acceptor of hydrogen (Lipinsky rule of five) (10). The results showed that the alkaloids used have no violation of the rule of five. It indicates these alkaloids have good properties to be used as oral drugs (Table 1). Further investigation of some pharmacokinetics parameters showed that all compounds have high gastrointestinal absorption rate, while only three compounds (muscimol, mescaline, and pilosine) didn't affect other pharmacokinetics parameters (Pgp, blood-brain barrier, and metabolism enzyme) (Table 2).

The evaluation of pharmacokinetics provides a general prediction of compounds against some issues on bioavailability. It also gives information on the side effect of drugs. The blood-brain barrier cloud prevents xenobiotics from entering the brain thus avoiding the side effect of drugs (18). Some compounds show prediction of penetration on the brain barrier. Further investigation was needed to avoid the toxicity of compounds that could harm the body.

Molecular docking provides predicted affinity between compounds and proteins. These values are influenced by various factors like gibs energy to determine the efficacy of drugs caused by pharmacophore (13,19). The proteins used in this study were beta-lactamase; a protein that caused the breakdown of beta-lactam groups and bacterial resistance and penicillin-binding protein; a protein targeted by beta-lactam that destroyed cell walls in bacteria.

Several compounds namely sanguinarine, glycosin, ergobasine, and

mescaline show promising affinity against beta-lactamase higher than clavulanate acid, moreover these compounds also show higher affinity on penicillin-binding proteins than amoxicillin (Table 3). Interestingly, investigation on the combination of amoxicillin and these four compounds shows an increase in the affinity of amoxicillin on penicillin-binding proteins with similar amino acid bonding on serine (181) and asparagine (177) (Table 4 and Table 5).

The usage of amoxicillin has been limited because of the bacteria resistance, thus using combination with clavulanate acid became solution of this problems. Besides to break beta-lactamase, some study shows enhancement of antibacterial effect of amoxicillin (1). This study give opportunity to explore alkaloids of *R. Stylosa* as a substitute of clavulanate. The prediction of drugl-likeness, pharmacokinetics, and antibacterial properties show that some alkaloids (sanguinarine, glycosin, ergobasine, and mescaline) have potential to be developed as a combination with amoxicillin. However, further study of toxicity and formulation of compounds needed to ensure efficacy and safety of drugs.

CONCLUSION

Several alkaloids of *R. Stylosa* could potentially be used as co-drugs combined with amoxicillin to both increase efficacy and prevent bacterial resistance

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