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Original Article

IN SILICO STUDY OF YODIUM LEAF (*JATROPHA MULTIFIDA* LINN) ACTIVE COMPOUND AS ANTIBIOTIC FOR DIABETIC WOUNDS

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ABSTRACT

Objective: In this study, an *in silico* test of 13 active compounds of leaf *Jatropha multifida* Linn. was carried out against the gyrase receptor (PDB ID: 2XCT).

Methods: The methods include molecular docking, ADMET prediction, and a review of Lipinski's Rule of Five.

Results: Molecular docking simulation results obtained three test compounds with free energy of binding (ΔG) and inhibition constants (Ki) at active site A, which are lower than the comparison compound, ciprofloxacin (ΔG-5.41 kcal/mol). The three compounds are C2 (multidione), C5 (citlalitrione), and C6 (cleomiscosin A) which have ΔG of-6.00,-6.90, and-5.56 kcal/mol. Based on ADMET prediction, compound C5 has better pharmacokinetics, pharmacodynamics, and toxic activities compared to ciprofloxacin.

Conclusion: Therefore, C5 is the best active compound from *J. multifida,* which can be used as a candidate for new antibiotics in the treatment of diabetic wounds.

Keywords: *Jatropha multifida* Linn, Diabetic wounds, Molecular docking, Antibiotic

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INTRODUCTION

Diabetes mellitus is a non-communicable disease that causes many complications and death. Complications of diabetic wounds in diabetics are the main cause of lower limb amputations. Estimated in 2045, the number of people with diabetes will rise to 700 million worldwide [1, 2]. Indonesia is ranked 7th position of the country with the highest number of diabetes sufferers in the world in 2019 [2]. The magnitude of this number is directly proportional to the incidence of complications related to diabetes mellitus, including lower limb amputations [3].

The main cause of lower limb amputation is diabetic wounds, namely wounds or lesions in Diabetes Mellitus (DM) patients, with about 85% of diabetic wound patients undergoing amputation. According to research that has been done previously, pathogenic bacteria that cause infections that are commonly found in diabetic foot wounds include *S. aureus*, followed by *P. aeruginosa*, and *Proteus mirabilis* [4]. Management of these infections is done by using broad-spectrum topical antibiotics, such as quinolones. However, like other classes of antibiotics, resistance to these antibiotic agents has developed. Therefore, research on new antibiotics needs to be pursued [4, 5].

The wealth of biological resources in Indonesia has enormous potential to be used as a treatment, one of which is to be used as antibiotics. One of them is the *Jatropha multifida* which is empirically used by the community as a natural antibiotic to treat wounds and prevent infection dan several studies have proven that this plant extract has antibacterial activity [6]. Based on this background, it is necessary to carry out further investigations regarding the active compounds in the *J. multifida* to obtain the compounds that have the most potent antibiotics to heal diabetic wounds [7].

One of the ways to find out which compounds have the most potent antibiotics is to do an *in silico* study. This study is carried out with the help of software and computers so that it can save time and costs. In this study, we need to have a prominent target for the treatment of diabetic wounds. DNA gyrase is one of the most prominent targets encoded by bacteria. Inhibition of DNA gyrase as one of the class from type IIA topoisomerase produces a cytotoxic effect in bacteria. Therefore, DNA gyrase becomes an attractive target for antibacterial agents [8, 9].

MATERIALS AND METHODS

There were several tools used in the research, i.e., Windows 10 64 bit Laptop, Chemdraw Pro 12.0, Biovia Discovery Studio 2021, AutoDock Tools 1.5.6, PreADMET program (https://preadmet.bmdrc.kr/), and Lipinski's rule of five (http:///www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp). There were also several compounds used in this research, i.e., gyrase receptor obtained from the Protein Data Bank (PDB) with PDB ID of 2XCT (resolution: 3.35 Å), ciprofloxacin as comparison compound, active compounds: multifidone, multidione, multifolone, multifidol glucoside, citlalitrione, cleomiscosin A, pictolinarigenin, jatrophone, multifidanol, fraxidine, jatrothrin, jatrophenone, and japodagrone obtained from PubChem.

Receptor and ligand preparation

The receptor was prepared using Biovia Discovery Studio 2021 software. Water molecules were removed and the natural ligand and receptor were separated. Editing the hydrogen with polar only, adding the Kollman charge, and saving the file in pdbqt format. Meanwhile, the 2D ligand structure (table 1) was created using ChemDraw. Then, the 3D structure is created and its energy minimization (MM2) is made using the Chem3D application.

Process validation

Validation process is done by re-docking natural ligands with their receptors which aims to ensure that the molecular docking parameters are valid so that they can be used for further simulation of the test compounds. Process re-docking is carried out by docking the natural ligand compound to the receptor using the AutoDock 4.2.6 program with the Command Prompt. The grid parameters can be seen in table 2. The molecular docking method is assessed to be valid if obtained RMSD value (less than 2).

Molecular docking simulation

Procedure for molecular docking of the test compound is the same as the validation procedure, but in the grid preparation process, the interaction coordinates are adjusted according to the interaction coordinates of the receptor with native ligands (table 2). All the test compounds were selected based on the literature review of the compounds contained in *Jatropha multifida.*

Interpretation of the results is carried out by analyzing the molecular docking results file (. dlg format) using notepad software, autodock 4.2.6, and Biovia Discovery Studio 2021. The values of the inhibition constants (Ki) and bond-free energy (ΔG) can be seen through the notepad and autodock 4.2.6. The ligand conformation of the best molecular bonding results was seen with autodock 4.2.6. Then, the interactions between ligand and receptor can be seen with Biovia Discovery Studio 2021 in 2D.

Pre-ADMET testing

Pre-ADMET testing was conducted using the site https://preadmet.bmdrc.kr/. First, open the site and select the ADME section, then draw the structure of the compound. Furthermore, for toxicity testing, use the site https://preadmet.webservice.bmdrc.org/toxicity/and select the *toxicity* and then redraw the structure of the compound. The result of the ADME profile and toxicity can be seen within minutes after submitting the structure of the compounds.

Overview of lipinski's rule of five

Overview of Lipinski's rule of five was conducted using the site http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp. To review Lipinski's rule of five first go to the site. Enter the file in. pdb format and submit. The results of the review will come out and can be observed.

RESULTS

Validation method have been done by redocking the native ligand (ciprofloxacin) to the receptor (DNA gyrase) with the result on table 1.

Table 1: Molecular docking validation results

Native-ligand	Grid address (x,y,z)	ΔG (kcal/mol)	RMSD	Interaction
Ciprofloxacin A	(29.162, 35.489,-18.005)	-5.41	0.520 Å	Arg1122, Gly459, Ser1084*
Ciprofloxacin B	(45.061, 42.802,-18.273)	-7.02	0.707 Å	DG X: 9, DA Y: 13, Ser S: 1084

*Van der Waals interactions

Several parameters of the molecular docking results, including the value of ΔG, Ki, and the interaction between the ligand and the receptor. The interaction is predicted through hydrogen bonds and van der Waals bonds. All the results can be seen on table 2 and 3.

Table 2: Molecular docking parameters on active site A

Ligand	ΔG (kcal/mol)	Ki (μM)	Receptor-ligand interactions	
			Hydrogen bond	Van der Waals bond
Ciprofloxacin A	-5.41	108.56	-	Arg S: 1122, Ser U: 1084, Gly S: 459
C1 (Multifidone)	-4.63	403.81	-	Ser U: 1084, Gly S: 459, Lys S: 460
C2 (Multidione)	-6.00	40.13	Arg S: 459	Ser U: 1094, MN W: 2000
C3 (Multifolone)	-1.87	42410	-	Gly S: 459, Lys S: 460, Ser U: 1084, MN W: 2000
C4 (Multifidol Glucoside)	-1.40	94130	DC X: 12	Ser U: 1084, Arg S: 458, Glu S: 477, Gly S: 459, Arg S: 1122
C5 (Citlalitrione)	-6.90	8.73	DG Y: 9, DC X: 12	Gly S: 459, Arg S: 1122
C6 (Cleomiscosin A)	-5.56	84.19	DA W: 7	DA X: 11, DT Y: 10, DC X: 12, DC X: 13, DT W: 6, DC X: 14, Ser U: 1084, Asp S: 437, Gly S: 459, Arg S: 1122
C7 (Pictolinarigenin)	-3.67	2.04	-	Gly S: 459, Phe S: 1123, Arg S: 1122
C8 (Jatrophone)	+6610.33	-	-	MN W: 2000, DA X: 11, Ser U: 1084, Arg S: 1122, Gly S: 459
C9 (Multifidanol)	+3215.07	-	-	Lys S: 460, MN W: 2000
C10 (Fraxidin)	-5.13	173.05	Arg S: 1122	MN W: 2000, Arg S: 458, Gly S: 459
C11 (Jatrothrin)	+16.11	-	-	Ser U: 1048, Arg S: 1122, Gly S: 459, Lys S: 460
C12 (Jatrophenone)	-0.24	664260	DG Y: 9	Gly U: 459, Ser S: 1048, Arg U: 1122, Phe S: 1123, Arg S: 458, Lys S: 460
C13 (Japodagrone)	+8.10	-	DG W: 8	Gly U: 459, Lys U: 460, DA X: 11

Table 3: Molecular docking parameters on active site B

Ligand	ΔG (kcal/mol)	Ki (μM)	Receptor-ligand interactions	
			Hydrogen bond	Van der Waals bond
Ciprofloxacin B	-7.02		DG X: 9, DA Y: 13, Ser S: 1084	Gly S: 1084, Arg U: 1122, MN W: 2001
C1 (Multifidone)	-4.60	424.16	DG X: 9	Ser S: 1084, Gly U: 459, Arg U: 1122, Lys U: 460
C2 (Multidione)	-4.19	853.93	DA Y: 13	Arg U: 1122, Gly S: 1082, Ser S: 1084
C3 (Multifolone)	+5.73	-	DG X: 9, DA Y: 13	Gly U: 459, Arg U: 1122
C4 (Multifidol Glucoside)	-4.63	401.38	DG X: 9, DC Y: 12, DA Y: 13	Gly U: 459, Arg U: 458, Gly S: 1082, Arg U: 1122, Ser S: 1084
C5 (Citlalitrione)	-5.04	203.78	-	Gly U: 459, Lys U: 460, Ser S: 1084
C6 (Cleomiscosin A)	+0.92	-	Arg U: 458, DG X: 9	Glu U: 477, Arg U: 1122, Ser S: 1084, MN W: 2001, Gly U: 459, Lys U: 460
C7 (Pictolinarigenin)	-5.08	189.25	DC Y: 12	Arg U: 1122, Ser S: 1084, MN W: 2001
C8 (Jatrophone)	+3208.05	-	-	Ser S: 1085, Arg U: 1122, Arg U: 458
C9 (Multifidanol)	+2969.92	-	-	Lys U 460, Arg U: 1122, Gly U: 459, MN W: 2001
C10 (Fraxidin)	-5.16	164.81	-	Gly U: 459, Ser S: 1048, MN W: 2001, DC Y: 12
C11 (Jatrothrin)	+54.65	-	-	Gly U: 459, Lys U: 460, Ser S: 1048
C12 (Jatrophenone)	-0.93	+206840	DA Y: 13	Gly U: 459, Ser S: 1048, Gly S: 1082, Arg U: 1122
C13 (Japodagrone)	+8.89	-	-	Gly U: 459, Lys U: 460, Ser S: 1048, DG V: 7, DC Y: 14, DT V: 8

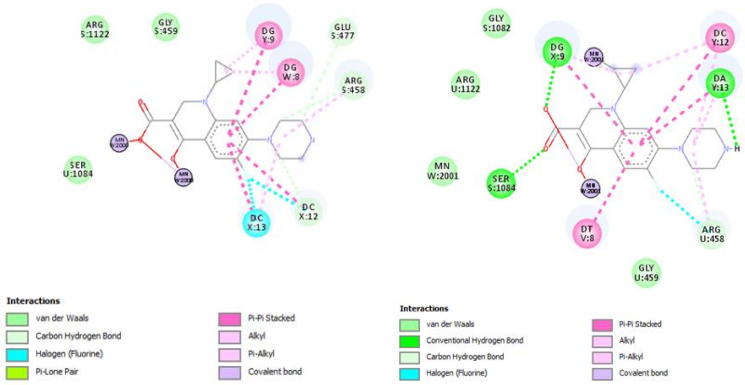

Fig. 1: The representative pose of ciprofloxacin as a native ligand in the gyrase receptor active site A (left) and active site B (right)

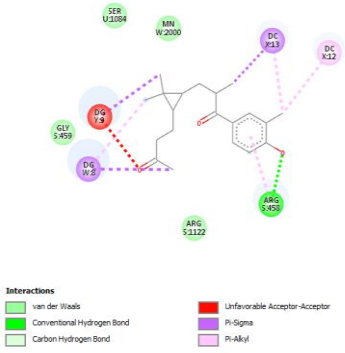

Fig. 2: The representative pose of multidione in the gyrase receptor active site A

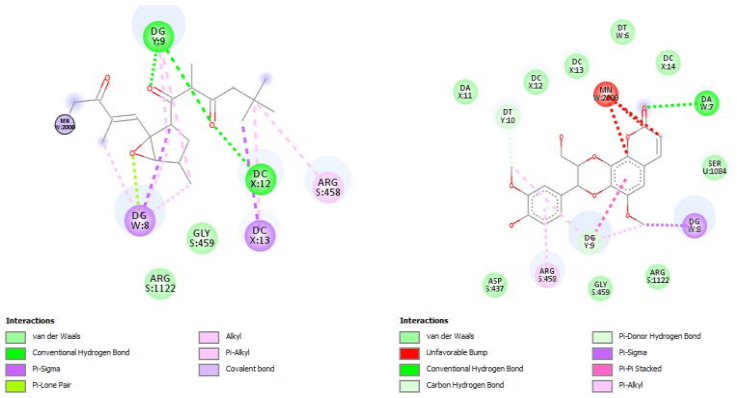

Fig. 3: The representative pose of citlalitrione (left) and cleomiscosin A (right) in the gyrase receptor active site

DISCUSSION

Based on the validation result, the native ligand has interaction to two active site (A and B). On active site A, the native ligand has van der Waals interactions with Gly S: 459, and Arg S: 1122 residue. Whereas on active site B, the native ligand has hydrogen bond interaction with DG X: 9, DA Y: 13, and Ser S: 1084 residue. Where A is alanine; D is Aspartate; G is glycine; Y is Tyrosine; and X is an unknown amino acid [11].

Molecular docking results show that the ΔG of the comparator at active site A is-5.41 kcal/mol while at active site B it is-7.02 kcal/mol. These enzymes probably contains two homologous domains and each bearing a functional, active site [12]. That is why gyrase receptor that we used in this research has two active sites.

The test compound has ΔG and Ki smaller than the comparison at active site A, which are C2, C5, and C6. Each value of ΔG was-6.00,-6.90, and-5.56 kcal/mol, respectively. Meanwhile, at active site B, none of the test compounds had a lower ΔG than the comparison. The ΔG indicates the bond strength and conformational stability between the test ligand and the receptor. The lower value indicates a more stable conformation [13].

This value is affected by various interactions that occur between the ligand and the receptor, such as hydrogen bonds, electrostatic interactions, and hydrophobic interactions. Compounds C2, C5, and C6 interact with active site A on key amino acid residues that are important in the process of inhibiting their activity, i.e., Gly S: 459, Arg S: 1122 fig. 1-3 illustrates the compounds' 2D interaction. The inhibition constant, which denotes the connection between the

ligand and its receptor, is another crucial statistic in this study [14]. The bond-free energy and the value of the inhibition constant are directly inversely correlated; the lower the value of the inhibition constant, the more stable the interaction. In the interaction with the active site A receptor, chemicals C2, C5, and C6 had the best inhibition constants.

When evaluating the pharmacokinetics of therapeutic candidate molecules, the prediction of ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity Analysis) is crucial. The absorption prediction consisted of HIA (Human Intestinal Absorption) and Caco-2 parameters. Parameters (HIA) can predict the permeability of compounds in the human gut in the route of administration and their effectiveness [15]. The range of categorization of these HIA parameters is 70-100% (good); 20-70% (moderate); and 0-20% (low). ADMET analysis identified that C2, C5, and C6 had HIA values above 90%. This indicates that the test compounds can be used as candidates for oral route drugs because they are well absorbed by the intestines. The Caco-2 parameter indicates the permeability of a compound to the intestinal epithelium which is used as a model for the selection of candidate drugs for oral administration [15]. The parameter categories of Caco-2 cells are>70 nm/sec (height); 4-70 nm/sec (medium);<4 nm/sec (low) [16]. The results of the analysis show that C2, C5, and C6 have moderate permeability.

The distribution prediction consists of BBB (Blood Brain Barrier) and PBB (Plasma Protein Binding) parameters. The BBB is a physiological barrier that restricts the flow of the majority of substances into the brain from the blood. The permeability of the BBB is an important parameter for compounds targeting the CNS (Central Nervous System). This drug is not targeted to penetrate the BBB in order to avoid psychotropic side effects. Therefore, compounds with high BBB values should be eliminated. BBB value categorization is>2.0 (high); 0.1-2.0 (medium); B<0.1 (low) [17]. Based on the prediction results of ADMET, C2 and C5 are classified as medium and low for C6. This makes the three compounds have low psychotropic side effects. The drug's interaction with albumin in the blood determines its half-life and is largely important for how the drug is distributed. The PBB value denotes this binding. The bioavailability of a substance and its dose are both influenced by its affinity for plasma proteins. Therefore, the drug's activity is reduced the more strongly the drug binds to plasma proteins [18]. When the PPB value is>90%, it means that the molecule is strongly bound to plasma proteins, and when it is below 90%, it is weakly bound [19]. Of the three compounds, C5 has the lowest PPB value. For the record, compounds with PPB>99% can still be tolerated because the concentration of free drugs can still be given.

Xenobiotics are metabolized by a group of microsomal enzymes known as cytochromes P450 (CYP450). The two most significant members of the CYP450 are CYP2C19 and CYP2D6, which are made up of a variety of distinct members involved in drug metabolism. In addition to the CYP substrate that this enzyme acts upon, CYP inhibitors raise drug concentration because they impair the action of the enzyme, allowing the drug to persist in the body and result in side effects. Therefore, predicting the ligand's interaction with CYP450 enzymes can help determine whether it will operate as a substrate, an inhibitor, or both [18]. Pre-ADMET analysis indicates that C2 and C5 are non-inhibitors of CYP2C19 but C6 is an inhibitor. The three compounds are non-inhibitors for CYP2D.

In order to forecast toxicity, the Ames-test parameters are examined. The Ames test is an easy procedure that evaluates a compound's mutagenic potential against strains of Salmonella typhimurium [20]. PreADMET study revealed that it was mutagenic for C5 and C6 but not for C2, according to the results.

Based on the prediction results of ADMET, C5 has the best results among other test compounds. In addition, C5 is also better than natural ligands and comparison drugs (ciprofloxacin). The ADMET prediction data results of all compounds available in the supplementary file (S3).

Molecular weight, hydrogen donor and acceptor, and log P value are the parameters in Lipinski's rule of five. Passive diffusion cannot allow substances with molecular weights greater than 500 to pass through cell membranes. The quantity of hydrogen bond providers and acceptors reveals a higher hydrogen bonding capacity, indicating a higher energy requirement for the absorption process. The compound's solubility in fat/water is shown by the log P value. The chemical is considered to be more hydrophobic the higher the log P value [21]. According to the analysis's findings, every test compound followed Lipinski's rule of five, and the complete data results are available in the supplementary file (S2).

CONCLUSION

The active compound C5 (citlalitrione) from *Jatropha multifida* leaf has a ΔG=-6.90 kcal/mol, good interaction through hydrogen bonding with the gyrase receptor on *S. aureus,* and fulfills RO5. Therefore, based on an *in silico*, compound C5 is the most potent active compound from *J. multifida* leaf to be used as a candidate for new antibiotics in the treatment of diabetic wound infections. Besides, Test compounds from *J. multifida* leaf have various ADME values. Based on the test results, C5 has better properties than the comparison compound (ciprofloxacin). Meanwhile, for Lipinski's rule of five, all the test compounds met the criteria. This *in silico* study still has several deficiencies. We could not do pharmacophore modeling and molecular dynamic due to researcher capability, cost limitation, and pandemic condition.

ASSOCIATED CONTENT

The supplementary data is available by request to the authors.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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