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STUDY OF VIRTUAL MOLECULAR DOCKING OF AVOCADOS COMPOUNDS AGAINST *Pseudomonas aeruginosa* (5N5H) BY Carbapenemase USING DOCK 6 ALGORITHM

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ABSTRACT

Antimicrobial resistance from bacteria is a global health problem that can cause death, and the cause is the emergence of carbapenem resistance Pseudomonas aeruginosa through VIM (Verona integron-encode metallo-β-lactamase), which causes the carbapenem class of antibiotics not to work properly. This species is a gram-negative bacteria which is the main cause of nosocomial pneumonia infection. This study aims to determine in silico inhibitory activity of 50 compounds obtained from avocado (*Persea Americana* Mill) on VIM, preventing carbapenem antibiotic resistance. The molecular docking process was carried out to test carbapenem's antibiotic resistance control activity by 50 compounds. Docking using DOCK 6 software with a flexible and rigid method, Molecular docking on a protein with PDB ID 5N5H, The target protein was prepared using the Chimera application. Visualization of ligand-protein interactions was carried out with PyMOL and PLIP. The results of the native ligand grid score obtained by each method are -63.013 kcal/mol (Flexible) and -64.032 kcal/mol (Rigid). The best test ligands in the flexible method are 44257090, 14282775 and 44257819, and the grid score are -77.474, -75.274 and -73.219 kcal/mol. The best test ligands in the rigid method are 5280637, 14282775 and 5490064; the grid score is -62.191, -61.714, and -60.453 kcal/mol. The results of the test ligands can provide a better grid score than native ligands, namely in the flexible method. However, the rigid method of grid score results is no better than the native ligand. A good result is that the test ligand grid score is smaller than native ligands, so it has less energy to bind to the active site.

Keywords: Carbapenem; avocado; VIM-1; resistance; molecular docking

INTRODUCTION

Antibiotics are chemical compounds produced by microorganisms (especially fungi) or produced synthetically to kill or inhibit bacteria and other organisms [1]. The discovery, commercialization, and routine administration of antimicrobial compounds to treat infections revolutionize modern medicine and change the therapeutic paradigm. Antibiotics have become one of the most important medical interventions needed to develop complex medical approaches such as cutting-edge surgical procedures, solid organ transplants, and the management of cancer patients. However, an increase in antimicrobial resistance among bacterial pathogens threatens the achievement of this therapy [2]. The World Health Organization has referred to antibiotic resistance as one of the three most

important public health threats in the 21st century [3].

Betalactam is the most widely used antibiotic worldwide and includes penicillins, cephalosporins, monobactams, and carbapenems. Unfortunately, carbapenem resistance (especially among Gram-negative bacteria) appears and spread rapidly throughout the continent [4]. Enzyme mediated resistance to carbapenem is caused by the production of β-lactamase, which can deactivate carbapenem together with other β-lactam antibiotics, so it is called carbapenemase [5, 6].

Carbapenemase consist of 3 classes, namely A, B, and D. Carbapenemase class B or Metallo-β-lactamases (MBLs) are clinically the most relevant carbapenemases. Metallo-β-lactamase or class B carbapenemases are further divided into subclasses (B1, B2 and B3). Still, the largest number of clinically relevant MBLs belong to subclass B1, including MBL (VIM), (IMP), and New Delhi MBL (NDM). VIM (Verona Integron-encode Metallo-β-lactamase) is the first enzyme in this family to be reported in the isolate Pseudomonas aeruginosa from Verona in 1997 [7]. The VIM-type metallo-beta lactamases (MBLs) are the most commonly reported carbapenemases in P. aeruginosa with a prevalence varying between 10-48% [8]. VIM-1 is a carbapenemase that we investigated for its interaction with the activity of 50 avocado compound isolates.

Studies on the antimicrobial activity of avocado seed extract show that it has an antibacterial effect on *Staphylococcus sp, Pseudomonas sp, Proteus sp, Escherichea sp, dan Bacillus sp*. The study found that the minimum inhibitory concentration (MIC) of P. *americana* methanol extract against the bacteria P. *aeruginosa*, K. *pneumoniae*, S. *aureus,* and E. *coli* were 40 mg/ml, respectively [9, 10]. The Minimum Bactericidal Concentration (MBC) test revealed 35 µg/mL for P. *aeruginosa* [11]. The peel of avocado have antimicrobial activity against Gram-positive, or Gram-negative bacteria included *Pseudomonas* spp, which encourage replacing synthetic medicines in the treatment of diseases caused by these bacteria. The antimicrobial activities of EEPA (Ethanolic extract of *Persea americana* peel) might be due to the high content of flavonoids and phenolic compounds [12].

The results of other studies showed that the ripe avocado skin extract had antimicrobial activity against all test organisms. The Minimum Inhibitory Concentration (MIC) revealed that P. *aeruginosa* had the highest MIC of 8.75 µg/mL. The spectrum of antimicrobial activity displayed by the extract can be related to the presence of phyto-chemicals identified and signify the potential of P. *americana* as a source of antimicrobial agents [13]. The inhibition test of P. *americana* Mill against *Pseudomonas* sp using the disk method with concentrations of 10%, 20%, 30%, 40%, and 50% obtained an inhibition zone of 16.6 mm; 21.6 mm; 26.0 mm; 28.4 mm; and 29.6 mm. Based on these results, it can be seen that the inhibition of *Pseudomonas* sp bacteria showed sensitive results [10].

Avocado seeds have potential as antibacterial drugs, as evidenced by the In Silico test using the molecular docking method with PyRx software between PBP2a receptors and phytochemical compounds in

avocado seeds as ligands. The ligand showed binding affinity: -18.2 kcal/mol, so avocado seeds were expected to increase the number of fibroblast cells caused by S. *aureus* [14].

The emergence of carbapenem resistance to P. *aeruginosa* through VIM (Verona integron-encode metallo-β-lactamase) causes the carbapenem class of antibiotics not to work properly. It becomes important to look for ways that can interfere with the activity of carbapenem resistance caused by VIM-1. This study aims to determine the activity between VIM-1 and 50 types of compounds obtained from avocado (*Persea americana mill*), preventing carbapenem antibiotic resistance. Antibacterial activity to test compounds can be carried out using the Dock-6 algorithm, the newest feature of molecular docking [15]. Dock-6 has advantages over previous versions: improved sampling and assessment capabilities, optimization and testing for compatibility with RNA, reduced sample conformation space, and molecular dynamics simulation [16]. The chosen ligand is S3C (2Z) -2- sulfanyl-3- (2, 3, 6- trichloro-phenyl) prop -2- enoic acid). Molecular docking was performed on a protein with GDP ID 5N5H on P. *aeruginosa*. The strength of the ligand binding will be tested, and then the results will be compared with the original protein-ligands and the strength of the comparison ligands.

METHODS

1. Materials

Research antibiotic resistance by carbapenemase (5N5H) with the DOCK 6 method using a Hp® Windows 10 computer device with Pro 64-bit Processor specifications, Intel® CoreTM i5-8250U @ 1.60 GHz, 4GB RAM. Equipped with Chimera version 1.13.1, Open Babel Portable 64-bit GUI, PyMOL 2.1.0, DOCK6, PLIP (Protein-Ligand Interaction Profiler), and Edit Plus version 5.0. The target protein, 5N5H, has 1.3 Å resolution of X-Ray crystallography with unique S3C ligand [17].

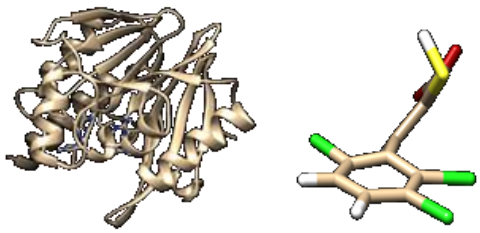
2. Methods

The 5N5H target protein of 1.3 Å resolution was obtained from www.rcsb.org for the P. *aeruginosa* organism. The 5N5H target protein was prepared using Chimera version 1.13.1 to remove nonstandard residues that leave only their ligands or amino acids with the addition of a charge and a hydrogen atom (H). Docking between the test ligand and protein is carried out using the DOCK6 application with the flexible and rigid method the docking results in a grid score that will be compared between test ligands and native ligand. The rigid and flex score grids used are selected from each of the three best. Ligands with the best grid scores are separated using the Edit Plus application version 5.0. The interaction between the target protein and the three best test ligands (rigid/flex) added by ligands without hydrogen is visualized in 3 dimensions (3D) using PyMOL and visualization in 2 dimensions (2D) using PLIP to see the residual amino acids involved and then analyzed [18, 19].

RESULTS AND DISCUSSION

The target protein on PDB ID VIM-1 is used, which is 5N5H with 1.3 Å resolution based on X-Ray crystallographic results [17]. The choice of 5N5H target protein, because it

has a unique ligand and resolution of less than 2.0 Å, means that the protein does not experience much movement, so the results of the in silico test are not much different from the in vivo and in vitro tests [20]. The results of X-ray crystallography also showed the most accurate protein photography. First, proteins and ligands are separated, then proteins without ligands are optimized using Chimera to obtain three molecules: charged protein molecules, surface protein molecules, and molecules without hydrogen. The optimized protein is used for calculations with the DOCK 6 docking application via dock prep. The hydrogen atom is removed from the surface protein molecule to return the ligand to its original form and added to the ligand to resemble the original shape. Interactions between ligands and proteins can be optimal by adding charge to the native l ligands [21].



(a) (b)

Figure 1. 5N5H Protein from the preparation: a. Original protein, b. Ligand with H.

Docking results required validation by considering chemical complementarity between ligands and proteins. The benefit of validation is used to see whether groups of charged ligands interact with side chains at opposite receptors. The validation parameter used is the RMSD (Root Mean Square Deviation) value to see the similarity of the test ligands (docking results) with crystallographic results [22]. The RMSD value in validation can be accepted if it is less than 5 [10]. The native ligand used was S3C (2Z) -2-sulfanyl-3- (2,3,6-trichlorophenyl) prop-2-enoic acid) (rcsb.org). The results showed that the RMSD obtained in the native ligand (S3C) was flexible and rigid, namely 5.3488 and 4.9446. The validation results of native ligands (flexible and rigid methods) are valid, although flexible ligands exceed number 5 but are still acceptable because they are close to number 5. The native ligand with the flexible method shows the possibility of the ligand in adjusting the structure to achieve a stable structure when it binds to the receptors [23].

Docking identifies low energy binding of either small molecules or ligands in active macromolecules or receptors whose structure is known. Compounds that can interact strongly with or bind to a receptor associated with a disease can inhibit its function and act as a drug [18]. The grid score (docking results) is the energy needed for ligands to bind to proteins. The bond between the ligand and protein gets bigger when the grid score gets smaller [24]. Table 1 shows the native ligand grid score (S3C) and the three best ligands grid scores of the test ligand on the 5N5H target protein by both flexible and rigid methods.

Table 1. The best grid scores for each method.

Flexible		Rigid	
Ligand Code	Grid Score (kcal/mol)	Ligand Code	Grid Score (kcal/mol)
native	-63.012669	native	-64.031792
14282775	-75.273967	**14282775**	-61.71418
44257090	-77.47377	5280637	-62.190956
44257819	-73.218758	5490064	-60.452637

The docking results between the 5N5H target protein and the test ligands (50 compounds from avocados) have the best three grid scores. The flexible method test ligands have the best three grid scores and are lower than native ligands (-63.012669 kcal/ mol), in code 44257090 of -77.47377 kcal/mol, code 14282775 of -75.273967 kcal/ mol, and code 44257819 of -73.218758 kcal/ mol, so that the test ligand has a higher activity compared to the native ligand. On the other hand, rigid method test ligands have the best three grid scores, but the results are still greater than native ligand (-64.031792 kcal/mol), namely in the code 5280637 (-62.190956 kcal/mol), 14282775 (-61.71418 kcal/mol), and 5490064 (-60.452637 kcal/mol) so that the test ligands has lower activity compared to the native ligand.

Although the rigid method test ligands grid score is greater than the native ligand, this research can find the interaction mechanism between the test ligands and the native ligand of the target protein that has something in common, code 14282775. The flexible and rigid method results are then compared to find residues the most involved [24].

Table 2. Ligand-protein interactions and the residues involved (test ligands) using PLIP.

Ligand Code	Method	Bond Type	Residue
44257090	Flexible 1	*Hydrophobic Interaction*	87 TRP, 116 HIS, 210 ASN
		Hydrogen Bonds	67 TYR, 67 TYR, 118 ASP, 202 GLU, 210 ASN
		Π-Stacking	62 PHE, 116 HIS
		Salt Bridges	**116 HIS**,201 HIS, **240 HIS**
14282775	Flexible 2	*Hydrophobic Interaction*	116 HIS,117 ASP
		Hydrogen Bonds	118 ASP,210 ASN
		Π-Stacking	62 PHE, 116 HIS
	Rigid 2	*Hydrophobic Interaction*	62 PHE, 62 PHE, 210 ASN
		Hydrogen Bonds	67 TYR, 205 SER
		Π-Stacking	116 HIS
		Salt Bridges	201 HIS
44257819	Flexible 3	*Hydrogen Bonds*	179 HIS, 201 HIS, 202 GLU,240 HIS
		Π-Cation Interactions	240 HIS
		Salt Bridges	**179 HIS**
5280637	Rigid 1	*Hydrophobic Interaction*	62 PHE, 62 PHE, 62 PHE
		Hydrogen Bonds	87 TRP, 210 ASN
5490064	Rigid 3	*Hydrophobic Interaction*	62 PHE, 67 TYR,67 TYR, 240 HIS
		Hydrogen Bonds	118 ASP
		Π-Stacking	201 HIS
		Salt Bridges	**179 HIS, 240 HIS**

Table 3. Ligand-protein interactions and the residues involved (native ligand) using PLIP.

Ligand	Method	Bond Type	Residue
native ligand	*Flexible*	*Π-Stacking*	67 TYR
		Salt Bridges	114 HIS, **116 HIS, 179 HIS, 240 HIS**
	Rigid	*Salt Bridges*	114 HIS, **116 HIS, 179 HIS, 240 HIS**

The 2-dimensional (2D) visualization using PLIP aims to analyze the bonding between the native ligand and the test ligands used. The result of this visualization is the interaction of amino acid residues with ligands. The interaction that occurs allows the ligand to have inhibitory activity. The binding site is the area of the protein binding to the ligand that will affect the conformation and function of the protein. The binding site shows amino acid residues that play an important role in forming interactions between macromolecules and ligands [25]. The interactions between ligands and proteins can be in the form of hydrogen bonds, such as conventional hydrogen bonds, hydrogen carbon bonds, and pi-donor bonds. In addition, hydrophobic interactions such as pi-alkyl, alkyl, pi-sigma, pi-pi electrostatic interactions occur in the form of T, and pi-pi, such as salt bridges, pi-cations, and pi-anions, as well as other interactions, such as halogen interactions, And pi-lone pairs. Visualization results of the test ligand and native ligand were compared by looking at the same interactions at the same amino acid residues [26]. Based on the interaction results, it is known that the native ligand has five amino acid residues bound, 67 TYR through stacking II and 114 HIS, 116 HIS, 179 HIS, 240 HIS via salt bridge. This interaction becomes a reference for comparing amino acid residues bound to the test ligands. On the other hand, there are only two interactions in the native ligand. Therefore, the amino acid residues that are compared between the native ligand and the test ligands are also based on the two interactions shown.

The protein residues bind with the avocado test compound as ligands and proteins are shown in Table 2. The residues that interacted with the target protein were similar to those on the native ligand (Table 3) through salt bridges with 116 HIS, 240 HIS on test ligand code 44257090 flexible two and residues 179 HIS, 240 HIS on the test ligand code 5490064 rigid 3. The salt bridge is a salt bond between the oppositely charged groups on the amino acid side chain, and the salt bridge ligand group is included in the electrostatic interaction. These interactions include weak and non-covalent interactions so that they are easily separated. Still, because of the large number of electrostatic interactions, they contribute to protein conformations [27]. The similarity of residue interactions on the salt bridge further strengthens and supports that *Persea americana mill* can overcome antibiotic resistance with the similarity of residues on the native ligand. Furthermore, the interaction of native ligands with proteins dominated by salt bridges causes the salt bridge to determine the inhibitory potential of the test ligands. The same interaction indicates that the interaction of these ligands is at the same binding site on the protein so that the test ligand has the possibility of inhibitory activity.

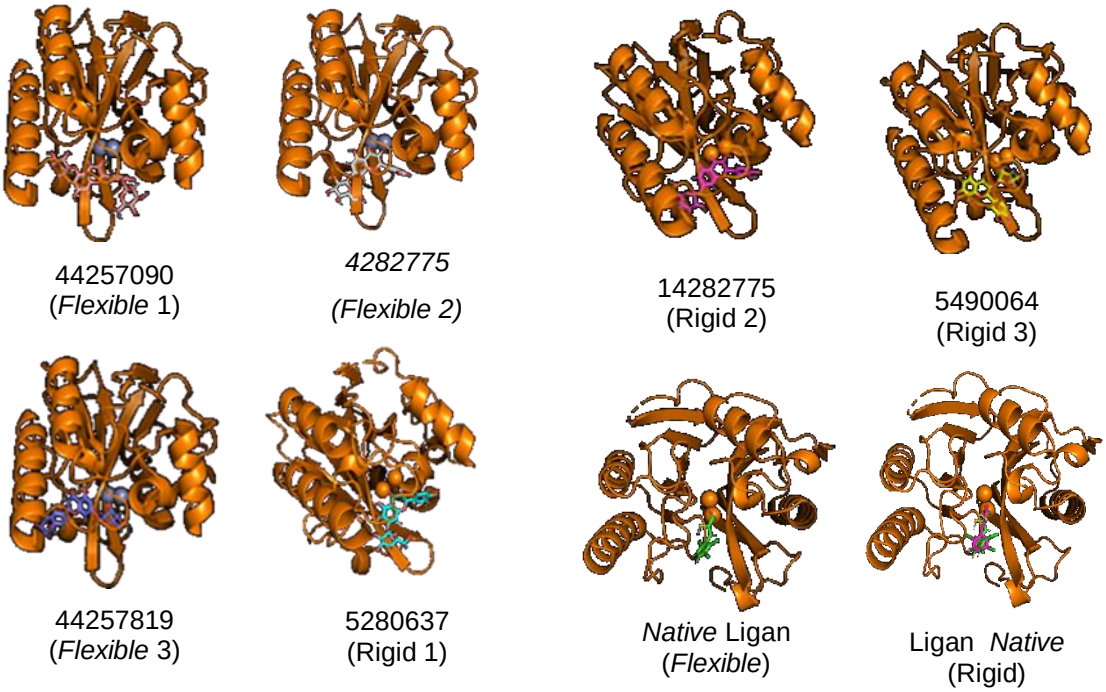


Figure 2. Visualization of Ligand-Protein using PyMol

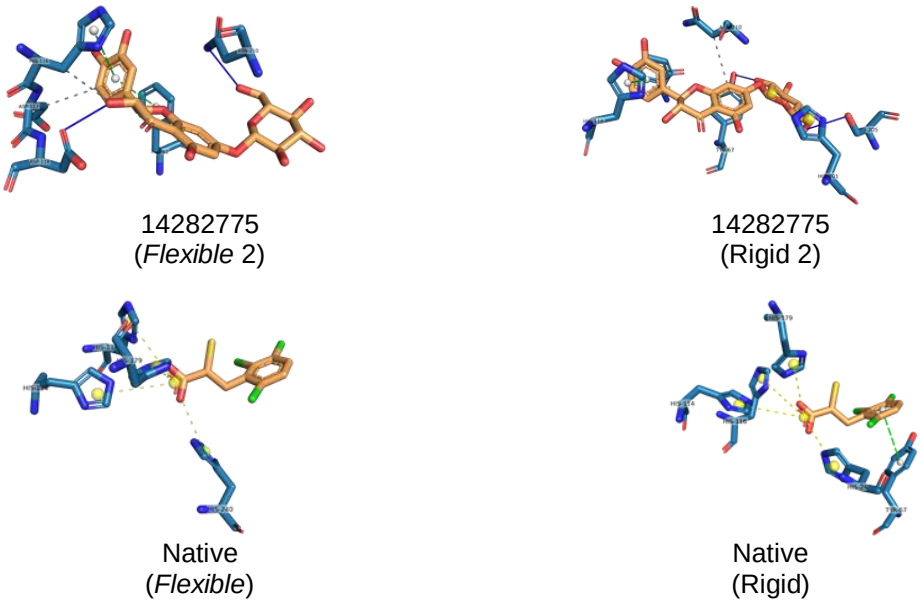


Figure 3. Visualization of Ligand-Proteins using PLIP.

CONCLUSION

The results of molecular docking activity of 50 compounds derived from avocados are better than native ligands as evidenced by the flexible method test ligand have the best 3 grid scores and lower than native ligands (-63.012669 kcal/mol), namely the code 44257090 for -77.47377 kcal/mol, code 14282775 of -75.273967 kcal/mol, and code 44257819 of -73.218758 kcal/mol; and so that the test ligand has activity and

potential for overcoming the problem of bacterial resistance. In addition, the similarity of residual interactions strengthens and supports that the *Persea americana* mill can control potential antibiotic resistance with similarities of residues in its native ligand. Although the results obtained are quite good, in silico studies still need further research to prove the truth of the results from molecular docking modelling.

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