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Comprehensive In silico Analysis of Antibacterial Compounds in n-Hexane Fraction from Jeruju Leaf (*Acanthus ilicifolius*)

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Abstract

In an emergency, to identify the most promising Hesperidin, Kaempferol-3,4'-di-O-methyl ether (Ermanin); Myricetin-3-glucoside, Peonidine 3-(4'-arabinosylglucoside); Quercetin 3-(2G-rhamnosylrutinoside); and Rhamnetin 3-mannosyl-(1–2)-alloside as a lead compound from Jeruju to develop new drugs from flavonoid analog. The active compound's structure is derived from the PubChem database and improves the stability through ChemDraw v19. The Protein Data Bank website identified the protein macromolecule with PDB code 3VSL. Redocking was performed to ensure validation of 3VSL. The docking method was carried out using the IGEMDOCK software version v2.1. Also, the chimera-1.13.1 program is used to know the interaction profile. The web server pkCSM and Protox II online tool were used to determine the toxicity. 3,7,11,15-tetramethyl-2-hexadecen-1-ol is the juju leaf chemical with the most promise as an antibacterial and the one that shows the maximum binding affinity when taking into account toxicity.

Keywords: Antibacterial, Jeruju leaf, Toxicity, Docking, Toxicity

INTRODUCTION

Target identification is a crucial phase in the drug development process. Computer-aided drug target identification techniques generate more interest than traditional, time- and money-consuming approaches. Computer-aided drug target identity techniques can significantly lessen the scope of experimental goals and related charges by identifying the illnesses-related goals and their binding sites and evaluating the druggability of the anticipated energetic websites for medical trials (Liao, Wang, Wu, & Huang, 2022).

Molecular docking is one in silico method. The interactions between the active ingredient and the biological target are predicted with molecular anchoring (receptor). In this process, the molecular orientation of the ligand within the receptor is typically indicated in advance. The structure-based technique is obtained by looking at the required target 3-dimensional structure in specific software. Based on these findings, the molecular database can be ranked according to the structure and electronic complementarity of the ligand to a particular target (Pradani et al., 2021).

Jeruju is a mangrove plant with numerous advantages that still need to be appropriately researched, despite the fact that this plant may have biological effects such as antidiabetic, anti-osteoporosis, anti-microbial, anti-inflammatory, and others (Nurfitri, Widiastuti, & Cahyani, 2018). This necessitates the development of the Jeruju as a fresh therapeutic component. The *Acanthus ilicifolius* leaf sample underwent soxhlet extraction using solvent methanol (Sekaran, Janet, & Mercy, 2013), but the component has been predicting the docking interaction.

The n-hexane portion of Jeruju leaves (*Acanthus ilicifolius* L) contains chemicals such as sesquiterpene, (trans(.beta.)-caryophyllene, alpha humulene, naphthalene decahedron-4A.-methyl), terpenoid alcohol (3,7,11,15-tetramethyl-2-hexadecen-1-ol) and fatty acids (hexadecanoic acid methyl ester; hexadecanoic acid; octadecanoic acid methyl ester (CAS); 9,12-octadecadienoic acid (CAS); 9-octadecenoic acid), according to a study by (Ramadhani, 2020).

This study suggests that Jeruju leaves contain active compounds. It has yet to be investigated and researched; which of these components might be the most effective antibacterial? Since Gram-positive bacteria are resistant to vancomycin control, an in silico analysis of Jeruju leaves will be carried out in this study to discover more about the chemicals with

the most substantial potential as antibacterials. After that, the compounds will be examined for toxicity.

METHODOLOGY

Data Set

Several test chemicals and target proteins were used as study materials. Nine test substances were employed, including sesquiterpene (trans(.beta.)-caryophyllene, alpha humulene, naphthalene decahedron-4A-methyl), terpenoid alcohol (3,7,11,15-tetramethyl-2-hexadecane-1-ol), and fatty acids (hexadecanoic acid methyl ester; hexadecanoic acid; octadecanoic acid methyl ester (CAS); 9,12-octadecadienoic acid (CAS); 9-octadecenoic acid (Ramadhani, 2020). The target protein is the PBP3 protein, which has a macromolecular crystal structure and is found in a compound with cefotaxime in staphylococcus aureus. The Protein Data Bank website (http://www.rcsb.org/pdb) identified the protein macromolecule with PDB code 3VSL. The hardware and software used in this study are the instruments. The support software contains Chemdraw 19 and Chimera-1.16 (Pettersen et al., 2004) from https://www.cgl.ucsf.edu/chimera/download.html. Igemdock v2 was used for the primary docking analysis. The complex's residual interactions were evaluated using software http://gemdock. life.nctu.edu.tw and Chimera-1.16. The computer hardware is a personal laptop manufactured by AsusTekComputer INC. with model number E402MA, Asus-NotebookSKU.

Procedure

Chemical Structure Design

The test ligand model is created using the structure from the PubChem database, which is then optimized for stability by ChemDraw version 15.1 and stored as *.mol2 or *.pdb files. The ChemDraw application's calculation feature must be utilized to determine the energy stability of the test ligands before docking. Nine substances used as test ligands have been discovered to have energy stability and display potential qualities as antibacterial activity inhibitors (Brogi, Ramalho, Kuca, Medina-Franco, & Valko, 2020)

Preparing the Ligand and Protein Structure

Before docking, nine compounds from juju leaves and the comparison vancomycin were downloaded from PubChem in 3D, optimized using ChemDraw v19.0, and then saved in the form of *.sdf or *.pdb files. By removing polar hydrogen, the ligand and protein are adjusted. The flexibility of the ligand is then saved in *.mol2 format. Macromolecules (PBP3 receptors) are optimized by bringing out polar hydrogen atoms and arranging them as rigid macromolecules with *.pdb format (Khalaf et al., 2020).

Protein validation (3VSL)

The protein crystals had a crystal resolution of 2.40Å, with 51% of the protein chain having a high fit residue to electron density and 3% indicating the fraction of residues that have a poor fit to the electron density (Kumar et al., 2020). Native ligand and dock ligand alignment were performed to ensure that the 3VSL protein was suitable for use.

Molecular Docking

RCSB can be contacted to obtain the PBP3 protein's 3VSL crystal structure. In both models, ligand and target protein, an extra atom was removed from water by adding hydrogen using the UCSF Chimera-1.13.1 program (Ruswanto, Siswandono, Richa, Tita, & Tresna, 2017). With optimal outcomes in minimal complex energy, Igemdock determines how a molecule's shape changes and how the ligand interacts with the target protein.

Toxicity Prediction

Here, the authors anticipate the toxic substances in jeruju leaves using the online Protox II tool, pkCSM (http://tox.charite.de/tox/). Testing chemical and comparator, vancomycin was translated into smiles format using online smiles translator (https://cactus.nci.nih.gov/translate/). These smiles are processed using the pkCSM online tool (http://biosig.unimelb.edu.au/pkcsm/prediction) to predict compound toxicity. To assess oral toxicity (LD50) in rodents and classification of substance toxicity based on the Globally Harmonized System (GHS), the Protox online tool (http://tox.charite.de/tox/) was utilized (A. A. Pratama, Rifai, & Marzuki, 2017).

RESULTS AND DISCUSSION

Preparation of the target proteins and test ligands

The 3VSL protein structure, a crystal structure of penicillin-binding protein 3 (PBP3) from *Staphylococcus aureus* in bound form with cefotaxime, was employed in this work as the target substance or target protein. The Protein Data Bank (PDB) was consulted to obtain the structure of the target chemical used in the investigation. The target compound and 3VSL code can be acquired from http://www.rscb.org/pdb.

Nine chemicals found in Jeruju extract that had been the subject of prior research underwent

molecular docking. Previous studies have demonstrated the presence of sesquiterpene compounds, alcohol terpenoids, and fatty acids with antibacterial activity in the n-hexane fraction of Jeruju.

Chimera application was used for preparation. The preparation seeks to get rid of water molecules and non-residual amino acid complexes to get rid of these compounds. These compounds were improved by adding hydrogen atoms. Protonation is purposed to get pH closer to the complete compound. The format of the optimization results file is mol2 (A. B. Pratama, Herowati, & Ansory, 2021).

Chem3D application was then used to validate the improved protein. The basic idea behind docking validation is to replicate the docking of the original ligand to the target protein after first preparing the ligand and protein with parameters and methods that will be utilized for the test ligand docking study. The alignment of the native ligand and docked ligand is shown in Figure 1.

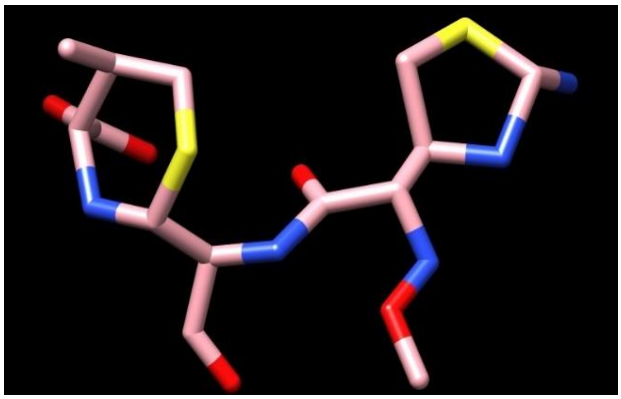

Figure 1. Alignment of native ligand (white) and docked ligand (red)

RMSD value is a validation parameter. The success of the binding mode prediction is evaluated using the RMSD value, which is crucial for the docking program's validation. Generally, if the RMSD value is less than 2 Å, it is considered good. The higher the divergence, the more inaccurate the forecast of the ligand-protein interaction will be (Brooijmans, 2009). The root means square deviation (RMSD) was calculated using the protein conformation route and was found to be 0 Å. This result, it may be safely assumed that the employed docking protocol is effective..

Molecular Docking

The research obtained (Ramadhani, 2020) showed that the compounds derived from the isolation of the n-hexane fraction of the leaf of the shrimp (Acanthus ilicifolius L) contained compound groups sesquiterpenes alcohol terpenoids and fatty acids that have antibacterial activity. Concentrations of 1%, 2%, and 4% of the n-hexane fractions of crab leaves have moderate to strong antibacterial activity. The best antibacterial is shown from the lowest docking energy value of -88,5 Kcal/mol, which is in the compound 3,7,11,15-etramethyl-2-hexadecen-1-ol. The optimum pose on the docking results was chosen based on the overall energy created and hydrogen bonds formed between the ligands and amino acids. A hydrogen bond is a connection between a H atom, which has a partial positive charge, and an electronegative atom, such as O, N, or F, which has a lone pair of electrons and a complete octet. Compounds with groups like OH-O, OH—N, OH—F, NH—O, NH—H, and NH—F can form hydrogen bonds.

There are two types of hydrogen bonding: intramolecular and intermolecular. (Maahury & Allo, 2021) said, the interaction that occurs between receptor and ligand is through hydrogen bonds. Hydrogen bonding interactions occur between carbonyl (C=O) of α, β, and γ-mangosteen, which act as hydrogen bond acceptors with hydroxyl groups (-OH) (Gaspersz & Sohilait, 2019). Intramolecular hydrogen connections take place within molecules (occurring between molecules). Intermolecular bonds are weaker than intramolecular ones in terms of strength. Even though van der Waals bonds are produced, they have little impact on the best pose outcomes. As a result of the van der Waals bond, which acts as an attractive force between molecules or atoms that are not charged, are close to one another, or are 4-6 Å. The polarization of the molecule or atom causes this bond to form. The results obtained from the research are shown in Table 1.

Table 1. Docking energy score

No	Compounds	Total Energy (Kcal/mol)	Types of bonds and amino acids involved
1	Octadecanoic acid methyl ester	-79,3	Hidrogen (SER) dan Van der Waals (THR, GLU, VAL, ARG, PRO)
2	9-Octadecenoic acid	-84,7	Hidrogen (THR, GLY, SER) dan Van der Waals (SER, THR, GLU, VAL, PRO)
3	9,12-Octadecanoic acid	-81,5	Hidrogen (SER) dan Van der Waals (TYR, THR, PRO)
4	Alpha Humulene	-60,5	Van der Waals (SER, TYR, ASN, THR)
5	Trans(.beta.)-caryophyllene	-58,6	Van der Waals (SER, THR)
6	Hexadecanoic acid methyl ester	-47,8	Van der Waals (SER, ASN)
7	Naphtalene decahydro-4A-methyl	-48	Van der Waals (SER)

8	Hexadecanoic acid	-83,9	Hidrogen (THR, GLY) dan Van der Waals (THR)
9	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	-88,5	Hidrogen (GLN, GLU) dan Van der Waals (SER, ASN, THR, GLU)

SER: Serine, THR: Threonine, GLU: Glutamine, VAL: Valine, ARG: Arginine, PRO: Proline, TYR: Tyrosine, ASN: Asparagine, GLY: Glysine

Three acids in jeruju leaf extract had the best gram-positive antibacterial performance 3,7,11,15-tetramethyl-2-hexadecane-1-ol, 9-octadecenoic acid, and hexadecanoic acid, according to the docking data that was acquired. The substance was picked because, when compared to the other eight substances, it had the least overall energy. The resulting bond is more stable, and the total energy required is lower. 3,7,11,15, tetramethyl-2-hexadecane-1-ol compound plaster had a total free energy of -88.5. In contrast to quercetin (flavonoid), which has the lowest energy when forming an H-bond with Gly143, van der Waals bonding Met165 is -8.88 (Wardani, 2022). Two amino acids involved are glutamine 623 and glycine 524, which have energy values of -3.5. The interaction between amino acid as protein (3VSL) and 3,7,11,15-tetramethyl-2-hexadecen-1-ol is shown in Figure 2.

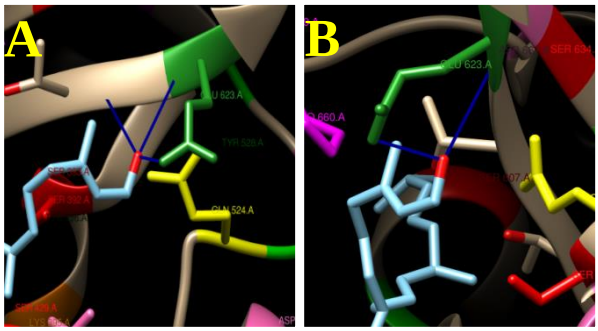

Figure 2. Interaction between amino acid as protein (3VSL) and 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (A) Glysin 524 (B) Glutamine 623

Substance 9-octadecenoic acid has a total free energy of -84.7. Three amino acids involved are threonine (619), which has an energy of -2.5; glycine (620), which has an energy of -2.9; and serine (634), which has an energy of -2.6. The interaction between amino acid as protein (3VSL) and 9-octadecenoic acid is shown in Figure 3. The total free energy of plaster on the hexadecanoic acid compound is -83.9. The amino acids involved are at three points: threonine 619 with an energy of -4.9; glycine 620 with an energy of -2.5; and threonine 621 with an energy of -3.5.

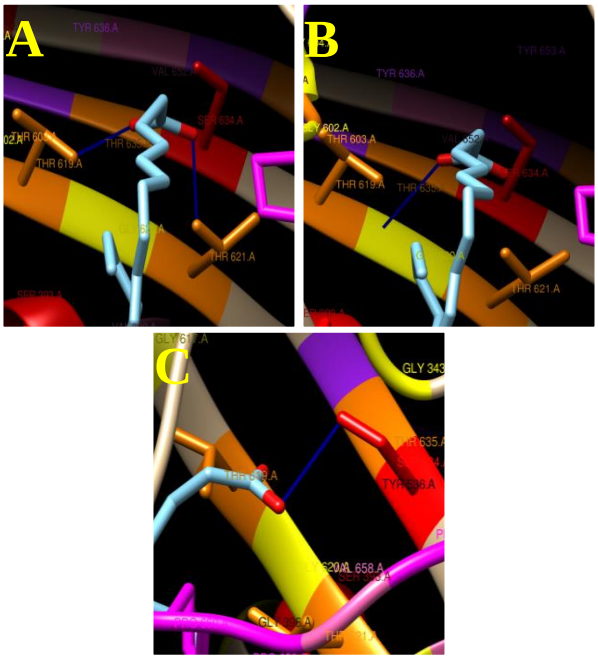

Figure 3. Interaction between amino acid as protein (3VSL) and 9-octadecenoic acid (A) Threonine 619 (B) Glysine 620 (C) Serine 634

The interaction between amino acid as protein (3VSL) and hexadecanoic acid is shown in Figure 4.

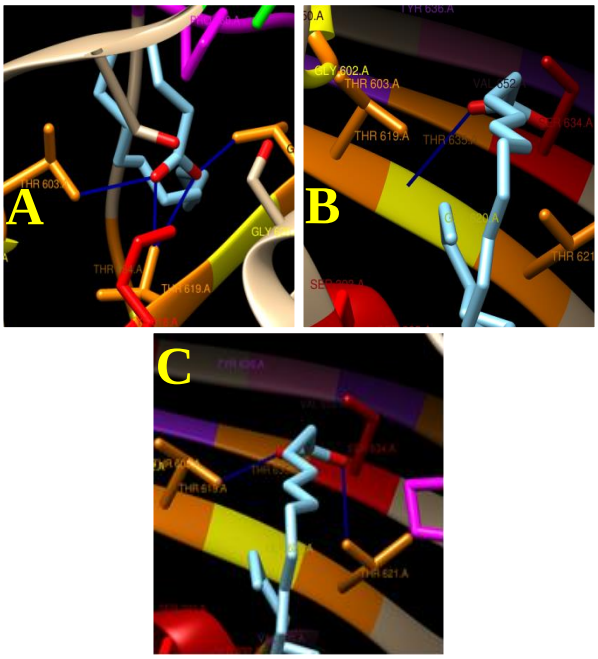

Figure 4. Interaction between amino acid as protein (3VSL) and Hexadecanoic acid (A) Threonine 619 (B) Glysine 620 (C) Threonine 621

Table 2. Vancomycin docking results

No	Compounds	Total Energy (Kcal/mol)	Types of bonds and amino acids involved
1	Vancomycin	-126.6	Hidrogen (SER, TYR, ASN, GLU. PRO) dan Van der Waals (ARG, TYR,THR,PRO)

Total free energy vancomycin as a control of the cast was -126.6 Kcal/mol. Amino acids are involved at five points: serine 429, tyrosine 430, asparagine 450, glutamine 623, and proline 661. Each energy formed at each point is -11.1, -3.5, -11,2, -3.5, -2.8, and -2.5 Kcal/mol. The bond between proteins (3VSL) and amino acid compounds Vancomycin is shown in Figure 5.

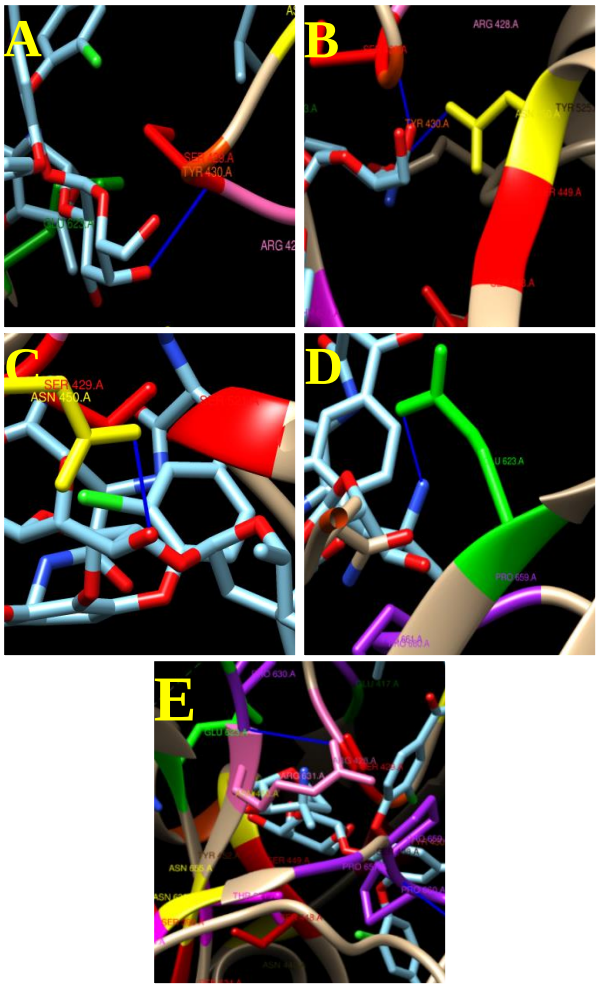

Figure 5. The bond between proteins (3VSL) and amino acids compounds Vancomycin (A) Serine 429 (B) Tyrosine 430 (C) Asparagine 450 (D) Glutamine 623 (E) Proline 661

Based on the docking results, the three compounds with the best docking energy were 3,7,11,15-tetramethyl-2-hexadecen-1-ol (-88,5); 9-octadecanoic acid (-84.7), and hexadecanoic acid (-83.9) show almost the same values to each other so that the binding affinity for the target receptor is nearly the same. The difference in the number of functional groups (pharmacophore) affects the bond affinity that occurs in compounds 3,7,11,15-tetramethyl-2-hexadecen-1-ol, and 9-octadecanoic acid has the same bond affinity because they have two functional groups. Meanwhile, hexadecanoic acid has lower bond affinity because it only has one functional group in the carboxylate, as shown in Figure 6.

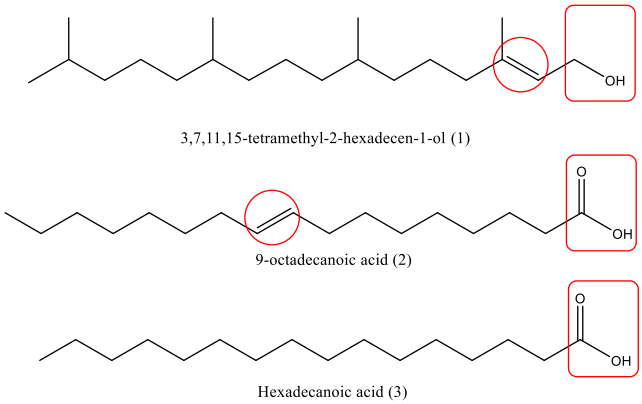

Figure 6. Differences of three best compound from docking

The difference in the number of residues bound to the ligand also affects the bond affinity. The more amino acid residues bound to the compound, the more stable it is. In the compound 3,7,11,15-tetramethyl-2-hexadecen-1-ol is bound to two residues, 9-octadecanoic acid is bound to three residues, and hexadecanoic acid is bound to two amino acid residues. 3,7,11,15-tetramethyl-2-hexadecen-1-ol has more potential for hydrogen bonds than three other compounds because the number of hydrogen atoms (Fitriana & Royani, 2022).

When seen from the docking energy and total hydrogen atoms, the compound 3,7,11,15-tetramethyl-2-hexadecen-1-ol is the best medication candidate of the three; however, when viewed from the number of residues bound by ligands, the molecule 9-octadecanoic acid is better. The data obtained will only be unimportant if the toxicity aspect is not covered. In terms of molecular structure, the three best compounds with the lowest docking energy values were in the order of 3,7,11,15-tetramethyl-2-hexadecen-1-ol, 9-octadecanoic acid, and hexadecanoic acid. These three compounds

Table 3. Toxicity test results

Compound	Toxicity					
	Docking Energy (Kcal/mol)	Mutagenicity*	Hepato-toxicity*	Skin Sensitization*	LD50 acute** (mg/kg)	Class**
3,7,11,15-tetramethyl-2-hexadecen-1-ol	-88,5	No	No	Yes	5.000	5
9-octadecanoic acid	-84,7	No	No	Yes	48	2
9,12-octadecadienoic acid	-81,5	No	Yes	Yes	10.000	6
Alpha humulene	-60,5	No	No	Yes	3.650	5
Hexadecanoic acid methyl ester	-58,6	No	No	Yes	3000	5
Naphtalene	-47,8	No	No	Yes	316	4
Octadecanoic acid methyl ester	-79,3	No	No	Yes	3.000	5
Hexadecanoic acid	-83,9	No	No	Yes	900	4
Trans(.beta.)-caryophyllene	-58,6	No	No	Yes	5.300	5

*ProTox
**pkSCM

have a structure with similar stereochemistry, so the structure is more compact and relatively more stable. When docking energy compared to the positive control obtained, it is still below the vancomycin's (Wardani & Setyowati, 2020).

Toxicity Test

A virtual lab for predicting the toxicity of tiny molecules is called ProTox-II. The process of creating new medication designs includes the prediction of chemical toxicity. In addition to being quicker than calculating hazardous doses in animals, computational toxicity prediction can also help minimize the use of experimental animals. Table 3 displays outcomes based on the results of the toxicity prediction utilizing ProTox-II and pkcsm. It is known if nine compounds did not experience mutagenicity. When evaluating the mutagenic potential of chemicals using bacteria, mutagenicity is frequently used. Mutagenicity prediction indicates that the compound is not mutagenic, so it is not carcinogenic. Hepatotoxicity data showed that only one compound had an impact on liver damage, namely 9,12-octadecadienoic acid. To determine whether a chemical has the potential to induce allergic contact dermatitis, skin sensitivity is predicted (Strickland et al., 2019).

The prediction results virtually using a screening approach with a pkcsm web server; it was found that all compounds resulting from the fractionation of Jeruju are sensitive to the skin, making it possible to be the cause of an allergic reaction. Oral toxicity to rodents (LD50) of sesquiterpene compounds in Jeruju prediction tests was performed using the Protox online tool webserver and toxicity classification based on the Globally Harmonized System (GHS). LD50 is the amount of compound given that can cause the death of 50% of the experimental animal group. In Table two, it can be seen that most of the sesquiterpene group compounds are predicted to have LD50 values in rodents ranging from 300 to 10000 mg/kg and belong to the toxicity class above 3 GHS, which means compounds have a low acute toxicity effect. One of the compounds in this group, namely 9,12-octadecadienoic acid, has an LD50 value of 10,000 mg/Kg, which is relatively high. According to the toxicity class tabulation (Hodge & Sterner, 1949), it is included in toxicity class 6 at this dose, which means the toxicity is lowest.

Considering molecular docking and toxicity studies, one of the compounds that showed the best binding affinity is 3,7,11,15-tetramethyl-2-hexadecen-1-ol, chosen by considering the toxicity aspect of amino acid residues. Three compounds are negative for mutagenicity and hepatotoxicity but positive for skin sensitivity, which means it still requires structural modification so that it is not sensitive to the skin. Meanwhile, the LD50 value indicated that the best compound, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, had a relatively low docking energy of -88.5 Kcal/mol but higher than vancomycin as control. The LD50 value obtained is quite large, i.e. 5,000 mg/Kg. According to GHS, this dose is included in toxicity class 5, which means low toxicity, so this compound can be a drug candidate that can be developed.

CONCLUSION

After doing molecular docking experiments and toxicity tests, we found three of the best chemicals in Jeruju: 3,7,11,15-tetramethyl-2-hexadecen-1-ol, which has the most significant promise as an antibiotic. Compared to other chemicals, the docking energy produced is the lowest at -88.5 Kcal/mol; yet, based on the anticipated toxicity, it has low toxicity but is still harmful to skin sensitivity. It can be seen that threonine amino acids are equally well bound to 3,7,11,15-tetramethyl-2-hexadecen-1-ol and other compounds, including vancomycin as a control. Hence, these amino acids are vital in interactions with test compounds. In addition, judging from the toxicity tests, it is evident that these three compounds can be used as antibacterials because the results of the toxicity tests are inactive except for the skin sensitivity test.

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