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Novel Compounds Design of Acertannin, Hamamelitannin, and Petunidin-3-Glucoside Typical Compounds of African Leaves (*Vernonia amygdalina* Del) as Antibacterial Based on QSAR and Molecular Docking

Desain Senyawa Novel Acertannin, Hamamelitannin, dan Petunidin-3-Glukosida Daun Afrika (*Vernonia amygdalina* Del) sebagai Antibakteri Berbasis QSAR dan Molecular Docking

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ABSTRACT

Antibacterial secondary metabolites such as tannins and their derivatives are found in the *Vernonia amygdalina* Del. Antibiotic resistance can develop due to overuse, reducing the efficacy of drugs to prevent and treat infections. This research aims to use the Quantitative Structure-Activity Relationship (QSAR) and the semi-empirical method Austin Model 1 (AM1) to design a modified novel compound from African leaves that has improved antibacterial activity. This research includes a descriptor calculation of QSAR using AM1 MOE on typical compounds from African leaves, and calculation results are chosen based on a multilinear regression statistical analysis. The model equation represents the three primary parameters of QSAR, which are electronic, hydrophobic, and steric parameters, which will be used to measure modified compounds. Molecular docking using Autodock Tools (The Scripps Research Institute, USA), and analysis of results of docking Autodock Tools using Discovery Studio 3.5 Client. The best QSAR model obtained is $LogEC_{50}$ = (0.829 x LogP) - (1,302 x AM1_HOMO) - (0.339 x AM1_dipole) - (5,128 x mr) + (0.145 x vol) - (11,355). The results showed that EC_{50} prediction of modified hamamelitannin has the best activity with the lowest ΔGbind -9.0 kcal/mol and inhibition constant of 0.249 μM. In summary, the novel compound's design calculation has better antibacterial activity, as indicated by a lower $EC_{50,}$ than fosfomycin or compounds without modification. The modified hamamelitannin compound was found to have better antibacterial activity (prediction EC_{50} = 0.1933 μM) than the original (experimental EC_{50} = 145.50 μM).

Keywords: antibacterial, antibacterial resistance, QSAR, *Vernonia amygdalina* Del

ABSTRAK

Metabolit sekunder antibakteri seperti tanin dan turunannya telah ditemukan di *Vernonia amygdalina* Del. Resistensi antibiotik dapat terjadi karena penggunaan berlebihan, sehingga mengurangi efektivitas obat yang digunakan untuk mencegah dan mengobati infeksi. Penelitian ini menggunakan Quantitative Structure Activity Relationship (QSAR) dan metode semi empiris Austin Model 1 (AM1) untuk merancang senyawa baru yang dimodifikasi dari daun Afrika yang berguna untuk meningkatkan aktivitas antibakteri. Penelitian ini meliputi perhitungan deskriptor QSAR menggunakan AM1 MOE pada senyawa khas dari daun Afrika dan hasil perhitungan dipilih berdasarkan analisis statistik regresi multilinear. Persamaan model mewakili tiga parameter utama QSAR yaitu parameter elektronik, hidrofobik, dan sterik, yang akan digunakan untuk mengukur senyawa termodifikasi. *Molecular docking* menggunakan Autodock Tools (The Scripps Research Institute, USA), kemudian analisis hasil docking menggunakan Discovery Studio 3.5 Client. Hasil penelitian menunjukkan model QSAR terbaik yang diperoleh adalah

$LogEC_{50}$ = (0.829 x LogP) - (1,302 x AM1_HOMO) - (0.339 x AM1_dipole) - (5,128 x mr) + (0.145 x vol) - (11,355). Hasil penelitian menunjukkan prediksi EC_{50} hamamelitannin termodifikasi mempunyai aktivitas terbaik dengan ΔGbind terendah -9,0 kkal/mol dan konstanta inhibisi sebesar 0,249 μM. Berdasarkan hasil penelitian, perhitungan desain senyawa baru memiliki aktivitas antibakteri yang lebih baik, ditunjukkan dengan EC_{50} yang lebih rendah dibandingkan fosfomisin atau senyawa tanpa modifikasi. Senyawa hamamelitannin yang dimodifikasi ternyata memiliki aktivitas antibakteri yang lebih baik (EC_{50} prediksi = 0,1933 μM) dibandingkan aslinya (EC_{50} eksperimental = 145,50 μM).

Kata Kunci: antibakteri, resistensi antibakteri, QSAR, *Vernonia amygdalina* Del

INTRODUCTION

As a tropical country, Indonesia is ideal for growing microorganisms that are both pathogenic and beneficial to humans. Microorganisms are microscopic organisms that can live and reproduce rapidly (Madigan et al., 2016). Infectious diseases can be spread by the growth of pathogenic or harmful microorganisms such as viruses, bacteria, fungi, or parasites (Kementerian Kesehatan Republik Indonesia, 2011; Kong & Ryu, 2015). Infections that occur can cause organ function disorders so that they cannot function properly, which results in death (Guyton, 2007). Antibacterial drugs are commonly used to treat bacterial infections.

By interfering with bacteria's metabolism, antibacterial compounds can inhibit, control, and even kill their growth. Antibacterials used to prevent and treat infectious diseases for an extended period can lead to antibacterial resistance. Antibiotic-resistant bacteria, such as multidrug-resistant *Escherichia coli* (MREC), multidrug-resistant *Staphylococcus aureus* (MRSA), multidrug-resistant *Acinetobacter baumannii,* and *Salmonella sp.*, are becoming more common (Farha et al., 2021; Kurniawan & Zahra, 2021). Alternative drugs that are effective and do not because of antibacterial resistance are still being researched and developed.

Vernonia amygdalina Del., also known as the African plant, is a biopharmaceutical commodity not well-known to the public. African plants can thrive in both tropical and subtropical climates. Plant parts such as leaves, flowers, seeds or fruit, tree bark, and roots can be used as traditional medicine (Ma'rufah & Aziz, 2019). Flavonoids, tannins, saponins, and terpenoids are secondary metabolites found in African plants with antiamoeba, antitumor, and antimicrobial properties (Pratiwi & Gunawan, 2018). Typical tannin compounds from African plants are acertannin, hamamelitannin, and petunidin-3-glucoside (Oko et al., 2018).

Antibacterial activity against the UDP-N acetylglucosamine enolpyruvyl transferase (MurA) enzyme receptor has been demonstrated *in silico* using compounds found in African leaves. Acertannin, hamamelitannin, and petunidin 3-glucoside are three active compounds found in African leaves, with affinity energies of -7.4, -5.6, and -6.1 kcal/mol and inhibition constants of 3.713, 77.735, and 33,397 μM, respectively. This value indicates that the ligand fosfomycin, with an affinity energy of -4.5 kcal/mol and an inhibition constant of 498,673 μM, has better activity. These active compounds are thought to prevent bacteria from making cell walls, resulting in morphological changes, agglutination, cytolysis due to osmotic pressure imbalance, and autolysis (Kurniawan, 2021).

The bacterial cell wall is made up of β1→4 glycosidic bonds connecting linear copolymers of N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (Mur2Ac) and cross-linked with peptides attached to Mur2Ac (Nelson & Cox, 2017). The MurA enzyme, also known as UDP-N-acetylglucosamine enolpyruvyl transferase, is involved in peptidoglycan biosynthesis and influences bacterial survival. MurA is found in Gram-positive and Gram-negative bacteria. Gram-positive bacteria have two copies of the MurA gene, while Gram-negative bacteria have one (Du et al., 2000; Eschenburg et al., 2005).

Long experimental stages are required to design and discover new compounds with high efficacy, including design, synthesis, identification, purification, and activity testing. A computational modeling approach can be used as one of the first steps in overcoming the experimental stage (Ananto et al., 2020). The Quantitative Structure Activity Relationship (QSAR) approach is used to find a model with structural, electronic, and geometric relationships of a compound that is thought to have a specific biological activity using computational compound modeling (Beale & Block, 2010). The QSAR method is a mathematical method for describing the relationship between biological activity and chemical compound structural properties. In the pharmaceutical industry and drug

development, QSAR modeling is an unavoidable process. The QSAR principle is based on several structural properties that result in various biological activities. Structural properties refer to the physicochemical structure, biological activity, and pharmacokinetic properties such as absorption, distribution, metabolism, excretion, and toxicity (Kwon et al., 2019). The effect of acertannin, hamamelitannin, and petunidin-3-glucoside from *Vernonia amygdalina* Del has never been tested on MurA through QSAR and molecular docking analysis. As a result, the antibacterial biological activity of common African leaves compounds such as acertannin, hamamelitannin, and petunidin-3-glucoside must be further investigated *in silico* using the QSAR in structural, chemical compounds from African leaves. Antibacterial bacterial cells can be used to combat antibacterial resistance.

METHODS

1. Equipment/ Tools/ Materials

This research has been done through the QSAR analysis and molecular docking simulation as a form of computational drug delivery by employing computer with specifications for AMD Ryzen 5 RAM 24 GB, NVIDIA GeForce GTX 1050 graphics cards, and Radeon Vega 8 Graphics with the Windows 10 Professional 64-Bit operating system, Discovery Studio 3.5 Client, Auto Dock Vina Tools (The Scripps Research Institute, USA), Marvin View, Molecular Operating Environment (MOE) 2015, and SPSS version 25. The 3D structure used in this study are files with the extension Fasta, PDB, and PDBQT. The 3D structure of the MurA enzyme with PBD code 1UAE was acquired from The Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB). The chemical structure of ligands such as Acertannin PubChem code 275075606, Hamamelitannin PubChem 44584241, 10315050, Petunidin 3-glucoside PubChem 443651, and the chemical structure of fosfomycin. The database of the ligand was obtained from PubChem Substance and Compound Database.

2. Calculation of typical compound descriptors from African leaves

The QSAR modeling process includes the following steps: structure optimization using the MMFF94 force field; evaluation of chemical structure descriptors, descriptor selection, structural diversity analysis of the database based on the selected descriptor set, and evaluation of the model's significance level and each of the specified descriptors. The descriptors used in this study represent the three main parameters of QSAR, namely hydrophobic parameters, including the n-octanol/water partition coefficient or lipophilicity (LogP) and solubility level (LogS). The electronic parameters include the highest energy-occupied molecular orbital (AM1_HOMO), the lowest energy-occupied molecular orbital (AM1_LUMO), and the dipole moment or dipole moment (AM1_dipole). And steric parameters in the form of Van der Waals volume (vol), total hydrophobic surface area (ASA_H), and molar reactivity (mr) (Yeni et al., 2018).

3. Statistical analysis and QSAR equations validation

SPSS 25 was used to perform multilinear regression analysis with Log EC50 as the dependent variable and the value of the independent variable as the descriptor. The regression results yielded several equations that explained the relationship between the structure and activity of tannin compounds found in African leaves. The equation model chosen for further testing in this study has a value of $r > 0.9$. Validation aims to produce a reliable and tested QSAR equation. Validation using the cross-validation Leave One Out (LOO) method, with each compound's predicted activity data being removed from the regression analysis. The LOO (q^2) cross-validation square measures the model's performance and stability. One QSAR equation will be obtained from LOO validation. The criteria for a good QSAR equation have a value of $r^2 \geq 0.8$ and $q^2 \geq 0.5$ (Yeni et al., 2018).

4. Modification of chemical structure and molecular docking

Designing modified acertannin, hamamelitannin, and petunidin-3-glucoside compounds by changing, reducing or adding new substituents is predicted to increase the antibacterial activity. The modified compound design considered the QSAR formula and then performed geometry optimization and descriptor calculations to obtain the predicted EC_{50} value. Molecular anchoring studies were carried out using free software, Auto Dock Tools 1.5.7, on the MurA protein or enzyme, whose structure was downloaded from the Protein

Data Bank with the access code 1UAE (Skarzynski et al., 1996). The active protein site was created using a grid of 16 x 16 x 18 Å on the XYZ side with a grid point spacing of 1.0 Å, following the binding site of the co-crystal ligand (Fosfomycin). Molecular docking was repeated 20 times. Other parameters are used following the default. Visualization of molecular docking results was carried out using the Discovery Studio Visualizer 2016 (Yeni et al., 2018).

RESULTS & DISCUSSION

1. Quantitative Structure-Activity Relationships (QSAR)

QSAR prediction using all compounds derived from African leaves was used as a training set. Parameter or descriptor calculations will be performed on acertannin, hamamelitannin, and modified petunidin-3-glucoside compounds to obtain the equation and value of biological activity predicted by QSAR. The descriptors used in this study represent the three main QSAR parameters, namely hydrophobic parameters, including the n-octanol/water partition coefficient or lipophilicity (LogP) and solubility level (LogS). Then, the electronic parameters include the highest energy-occupied molecular orbital (AM1_HOMO), the lowest energy-occupied molecular orbital (AM1_LUMO), and the dipole moment or dipole moment (AM1_dipole). Furthermore, the steric parameters in the form of Van der Waals volume (vol), total hydrophobic surface area (ASA_H), and molar reactivity (mr) are listed in **Table 1**.

Table 1. Descriptors used in QSAR analysis.

Symbol	Unit	Definition
AM1_HOMO	eV	The highest energy level of a molecular orbital filled with electrons
AM1_LUMO	eV	The lowest energy level of a molecular orbital that is not filled with electrons
AM1_dipole	Debye	Dipole moment or dipole moment
LogP	-	Partition coefficient of n-octanol/air
LogS	-	Solubility level
vol	$Å^3$	Van der Waals Volumes
mr	$Å^3$	Molar refractivity
ASA_H	-	Total hydrophobic surface area

The MOE 2015.10 software was used to calculate the descriptor value of each compound, which was then used to construct the QSAR equation. The descriptor was used as the independent variable, and the biological activity of the research target was used as the dependent variable in the form of the median effective concentration (EC_{50}). **Table 2** shows the results of the multilinear regression statistical analysis calculation. Four models of the QSAR equation were obtained as a result of this analysis, and they were chosen based on the best equation validation. The equation model was created using regression analysis and the backward method, resulting in a set of equations and statistical parameters, as shown in **Table 2**. The best QSAR model obtained is LogEC50 = (0.829 x LogP) - (1.302 x AM1 HOMO) - (0.339 x AM1 dipole) - (5.128 x mr) + (0.145 x vol) - (11.355).

Table 2. QSAR equation model based on multilinear regression analysis.

Model	Descriptor/parameter	r	r^2	SE	F_{hit}/F_{tab}
1	AM1_LUMO, mr, LogP, AM1_dipole, LogS, AM1_HOMO, ASA_H, vol	0.999	0.997	0.079	0.199
2	AM1_LUMO, mr, LogP, AM1_dipole, LogS, Am1_HOMO, vol	0.986	0.972	0.182	0.517
3	Mr, LogP, AM1_dipole, LogS, AM1_HOMO, vol	0.973	0.946	0.207	0.976
4	Mr, LogP, AM1_dipole, AM1_HOMO, vol	0.955	0.913	0.227	1.34

Note: r = correlation coefficient; r^2 = coefficient of determination; SE = standard error; F = Fisher's criterion results of ANOVA analysis

Quantitative Structure Activity Relationships (QSAR) is one of the medicinal chemistry disciplines that work in tandem with the development of new drugs to make them more effective and efficient. Based on the similarity of chemical structures to other similar chemicals/drugs and research or experimental data, QSAR aims to predict the biological activity or toxicity of chemicals/pharmaceutical agents that are not yet known or studied (Basant et al., 2015; Demchuk et

al., 2011; Naven & Louise-May, 2015). In the pharmaceutical industry and drug development, QSAR modeling is an unavoidable process. The QSAR principle is based on several structural properties corresponding to various biological activities. Structural properties refer to the physicochemical structure, biological activity, and pharmacokinetic properties in absorption, distribution, metabolism, excretion, and toxicity (Kwon et al., 2019). Using the QSAR approach through information on the molecular structure of compounds can describe the relationship between the structure and biological activity of a compound (Ananto, 2019). The Hansch method is one of the methods used in QSAR. The Hansch method was developed based on the concept of a relationship between a compound's chemical structure and biological activity (log X), which can be quantified using the hydrophobic (π), electronic (σ), and steric (Es) parameters of the substituents (Beale & Block, 2010).

The equation model was created using regression analysis and the backward method, resulting in a set of equations and statistical parameters, as shown in **Table 2**. At the 95% confidence level, not all QSAR equation models obtained were significant. **Table 2** shows that the price of the F_{count}/F_{table} ratio is less than 1, indicating that the F_{count}/F_{table} equation model is not acceptable statistically (Yeni et al., 2018). The equation 4 model, with an R-value of 0.955, is said to meet the requirements of R> 0.9, the closer the value to 1; the value of r^2 0.913 meets the requirements of r^2 >0.6; and F_{count}/F_{table} is 1.34, which is greater than 1 (Adeniji et al., 2018; Yeni et al., 2018).

The model 4 equation was chosen because fewer descriptors had a value that satisfied the statistical parameter, namely ≥ 0.9. The descriptors in the equation represent the three main QSAR parameters, electronic, hydrophobic, and steric, which will be used to measure the modified molecular QSAR. LogEC50 = (0.829 x LogP) - (1.302 x AM1 HOMO) - (0.339 x AM1 dipole) - (5.128 x mr) + (0.145 x vol) - (11.355) is the QSAR model 4 formula, which will be validated.

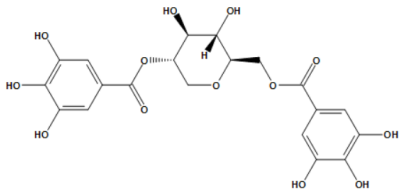
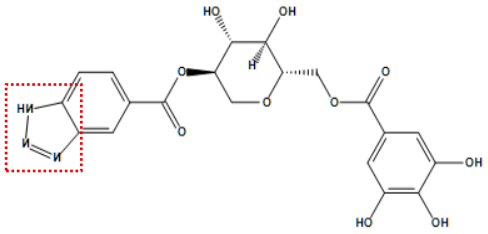
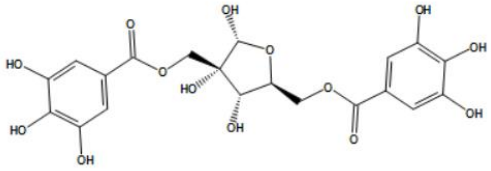
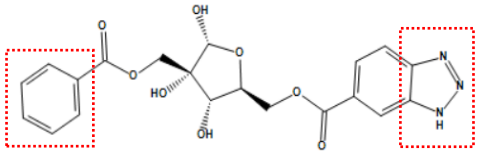
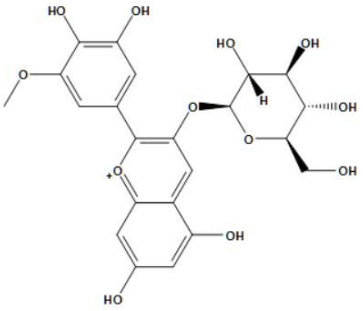
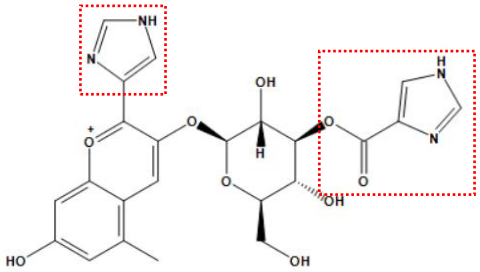
Validation aims to produce a good QSAR equation with proven repeatability. Cross-validation techniques are used for validation. The test method employs cross-validation with Leave One Out (LOO). The LOO approach omitted each predictable compound from experimental activity data in linear regression analysis. The LOO cross-validation square (q^2) measures the model's performance and stability. The value of q^2 must be greater than 0.5, indicating the method's repeatability (Yeni et al., 2018). The validation of the Leave One Out (LOO) method yields a q^2 value of 0.504 for the model 4 equation, indicating that it is valid and can be used in modification and QSAR calculations.

A modification process can be used to carry out the QSAR approach to develop and discover compounds with good biological activity potential. The goal of molecular modification is to increase the biological activity of the experimental compound by simplifying, combining, or adding other molecules to the experimental core molecule (Li et al., 2016). Compounds are molecularly modified to create new compounds with improved biological activity and effectiveness over previous compounds with lower toxicity and side effects (Cartika, 2016).

2. Structure Modification and Molecular Docking

A modification process can be used to carry out the QSAR approach to develop and discover compounds with good biological activity potential. The galloil group of the typical African leaves compound acertannin, hamamelitannin, and petunidin-3-glucoside was modified, as was the amount of hydroxyl (OH) in the structure, to increase the compound's biological activity as an antibacterial. Modifications were carried out to increase the bioactivity value and reduce the value of the median effective concentration (EC_{50}) of compounds typical of African leaves. Table 3 shows the design of the modified compounds.

Table 3. Modification of compounds typical of African leaves

Compound Name	Original Structure	Modified structure
Acertannin		
Hamameli-tannin		
Petunidin-3-Glucoside		

Description: Molecules inside the red dashed box are modified substituents from Oko et al., (2018)

The validated equation is used to calculate the descriptor for the modified compound that has been optimized. The results of the descriptor calculations for the three modified compounds are in Table 4. Table 4 shows that the modified compound can reduce the EC_{50} value of acertannin, hamamelitannin, and petunidin-3-glucoside compounds. The modified compound had an EC_{50} smaller than fosfomycin or the compound without modification.

Table 4. Results of descriptor value of modified compounds typical of African leaves

Compounds	AM1 dipole	AM1 HOMO	LogP	MR	Vol	EC_{50} Predictions	Bioactivity Value*
Unmodified Acertannin	4.810	-9.304	0.358	10.406	378.00	8.959	0.09
Unmodified Hamamelitannin	1.905	-9.261	0.124	10.501	386.63	144.695	0.33
Unmodified Petunidin-3-glucoside	8.802	-11.553	0.078	11.171	399.63	88.025	0.24
Modified Acertannin	4.2942	-9.369	0.835	10.843	379.62	0.3321	0.13
Modified Hamamelitannin	5,6028	-9,6512	1,138	10,595	368,00	0,1933	0,35
Modified Petunidin-3-Glucoside	5,6899	-11,590	-0,489	12,325	425,88	0,8940	0,41

Description: *Bioactivity value as an enzyme inhibitor; Value *: active = >0; moderate = -5.0 - 0; inactive = <-5.0

The galloil group of the typical African leaves compound acertannin, hamamelitannin, and petunidin-3-glucoside was modified, as was the amount of hydroxyl (OH) in the structure, to increase the compound's biological activity as an antibacterial (Farha et al., 2021; Kurniawan & Zahra, 2021; Wang et al., 2018). Modification of the structure of acertannin, hamamelitannin, and petunidin-3-glucoside compounds typical of African leaves is predicted to increase its biological activity as an antibacterial through inhibition or enzyme inhibitors, especially in inhibiting the activity of the MurA enzyme in the formation of cell walls so that the cell wall in bacteria is not formed which causes the bacteria to undergo morphological changes, agglutination, and lysis in bacteria (Kalaria et al., 2014). Modifications were carried out to increase the bioactivity value and reduce the value of the median effective concentration (EC_{50}) of compounds typical of African leaves.

The MMFF94 force field for small organic molecules in medicinal chemistry must be used to optimize the structure of the modified compounds (medicinal chemistry). The validated equation is used to calculate the descriptor for the modified compound that has been optimized. The results of the descriptor calculations for the three modified compounds are in **Table 4**. **Table 4** shows that the modified compound can reduce the EC_{50} value of acertannin, hamamelitannin, and petunidin-3-glucoside compounds. The modified compound had an EC_{50} smaller than fosfomycin or the compound without modification; the predicted EC_{50} for modified acertannin was 0.3321 µM, EC_{50} predicted for modified hamamelitannin was 0.1933 µM, and EC_{50} predicted for modified petunidin-3-glucoside was 0.8940 µM while original acertannin or unmodified EC_{50} predicted 8,959 m, original hamamelitannin or unmodified EC_{50} predicted 145.5 µM, and original petunidin-3-glucoside or unmodified EC_{50} predicted 90,315 µM in Table 4. Acertannin, hamamelitannin, and petunidin-3-glucoside had EC_{50} experimental values of 8,600, 145.50, and 90,315 µM, respectively (Richter et al., 2017; Watanabe & Devkota, 2017). The EC_{50} value of fosfomycin is 121.1 µM or 28.1 mg/L (Mao et al., 2021).

3. Molecular docking

The interaction of the ligand with the target protein was investigated using Molecular Docking Studies on MurA proteins or enzymes (Code: 1UAE). Redocking co-crystal ligands (Code FFQ) validated the molecular docking procedure, and the RMSD value of both structures was 0.379 Å, indicating that the procedure was correct. If the RMSD value is less than 2 Å, the ligand position is good; if it is more than 2 Å, a molecular shift in the tethering process has occurred, and the tethered ligand position is not relative to the X-ray conformation. RMSD values greater than 2 Å can be caused by various factors, including scoring, which occurs when the energy required to bind to molecules with low RMSD is insufficient (Baber et al., 2009).

By forming hydrogen bonds with amino acid residues Cys^{115}, Arg^{120}, Asn^{23}, Lys^{22}, and forming hydrophobic bonds with amino acid residues Gly^{114}, Arg^{91}, and Arg^{397}, the Fosfomycin-MurA interaction resulted in an affinity of -4.5 kcal/mol and inhibition constant of 498.67 µM (**Figure 1**).

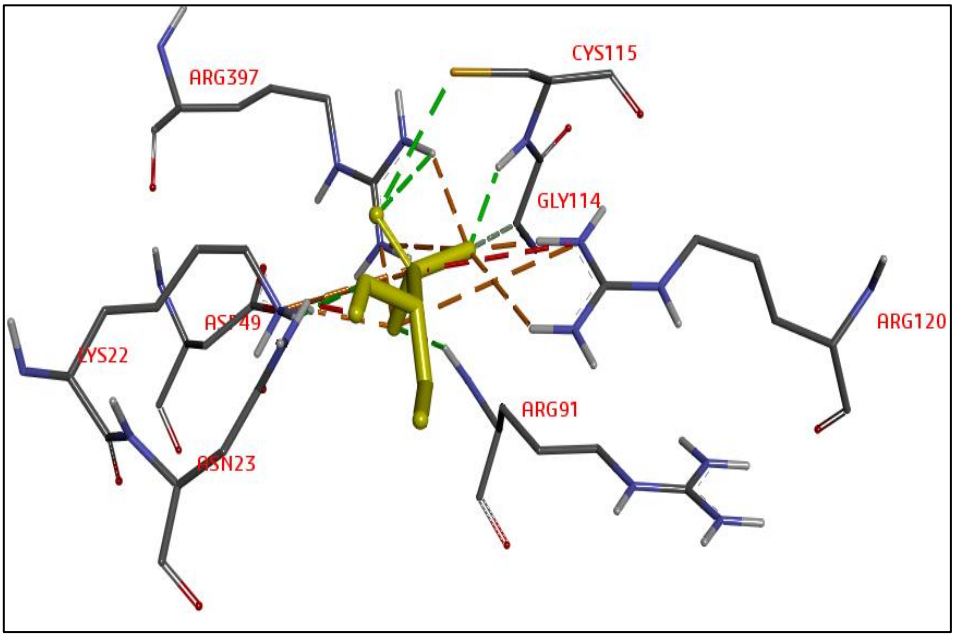


Figure 1. Co-crystal ligand interactions (fosfomycin) on the binding site of the MurA enzyme.

Acertannin yielded an affinity of -8.8 kcal/mol and an inhibition constant of 0.35 µM (**Figure 2A**). By forming hydrogen bonds with the amino acids Arg^{120} and Arg^{91}, and interacting hydrophobically with the amino acid residues Cys^{115}, Arg^{397}, Lys^{22}, and Asn^{23}, modified MurA-Hamamelitannin produced an affinity of -9.0 kcal/mol and inhibition constant of 0.249 M (**Figure 2B**). The amino acids Arg^{120} and Asn^{23} form hydrogen bonds with modified MurA-Petunidin-3-glucoside, which has an affinity energy of -5.6 kcal/mol and inhibition constant of 77.73 M and interact hydrophobically with the amino acid residues Cys^{115} and Arg^{397} (**Figure 2C**).

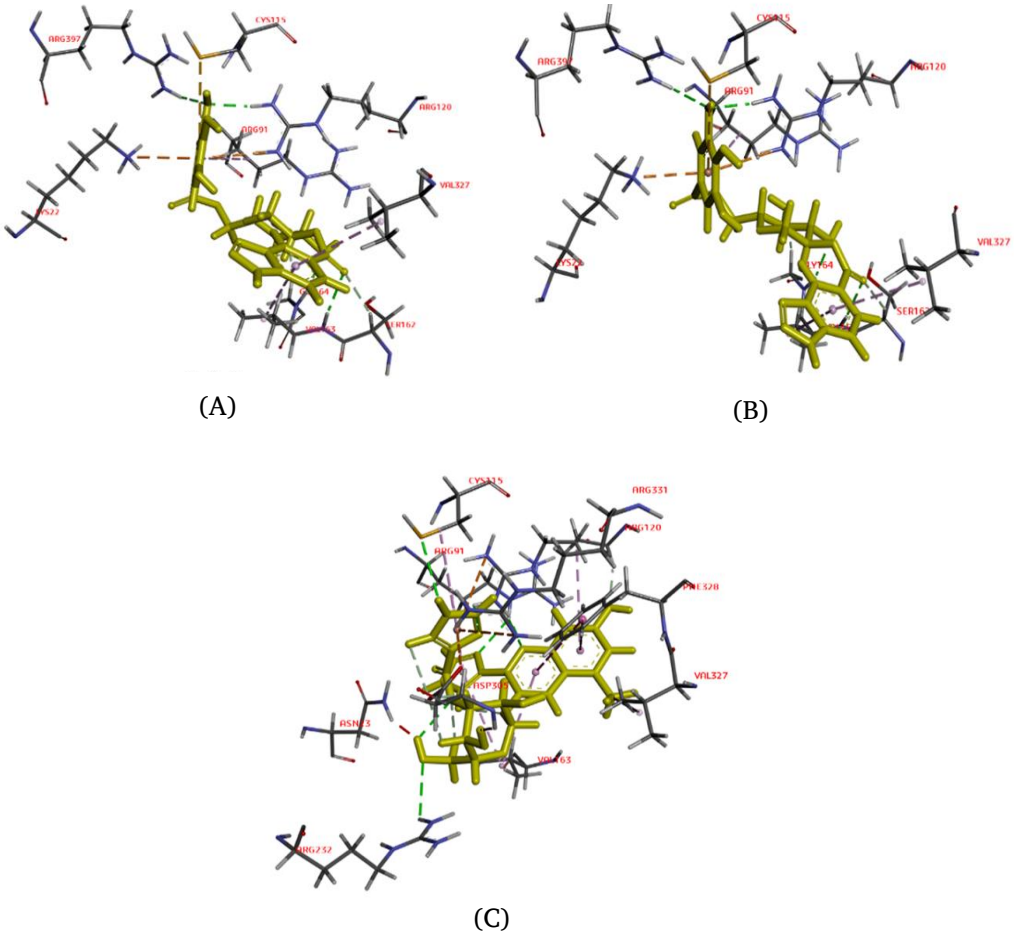


Figure 2. Ligand interactions (A) Acertannin; (B) Hamamelitannin; (C) Petunidin-3-Glucoside on the binding site of MurA enzyme

The beneficial or harmful effects of a drug on cell tissue or living matter as a targeted drug in the body are described as biological activity or pharmacological activity. Drug targets or biological targets can have specific effects, such as desired therapeutic effects or undesirable side effects (Mosby, 2016). Bioactive compounds as drug candidates must meet certain bioactivity values using Molinspiration, with an active compound value > 0.0, moderately active with a value of -5.0 - 0.0, and inactive with a value of < -5.0 against enzyme inhibitors (Khan et al., 2017). According to the findings, the bioactivity value as an enzyme inhibitor of the acertannin compound typical of modified African leaves showed an increase in bioactivity value as an enzyme inhibitor. The compound's bioactivity without modification was 0.09 for acertannin enzyme inhibitor, 0.33 for hamamelitannin, and 0.24 for petunidin-3-glucoside (Kurniawan, 2021).

Modified acertannin compounds increased by 0.13, modified hemamelitannin compounds increased by 0.35, and modified petunidin-3-glucoside compounds increased by 0.41. Table 4 shows that all the compounds found in modified African leaves are predicted to have active bioactivity as a MurA enzyme inhibitor in antibacterial formation. In the molecular docking study, the interaction of the ligand-binding site on the target enzyme's active site was demonstrated by acertannin, hamamelitannin, and modified petunidin-3-glucoside research, and the predicted energy affinity of each ligand showed that it had much lower energy than the co-crystal ligand.

An amino acid residue of Cys^{115} with approximately Arg^{397}, Arg^{120}, and Lys^{22} defines the active site of the MurA enzyme (Han et al., 2010). Other residues, such as Asp^{305}, which acts as a base in the initiation of UDP-N-acetylglucosamine deprotonation, Cys^{115}, which acts as an acid in the C-3 protonation process of phosphoenolpyruvate during the reaction, and Lys^{22}, which is involved in PEP binding and affects the conformational change of the enzyme, is involved in PEP binding and affect

the conformational change of the enzyme (Barreteau et al., 2008). The Gibbs free energy (G), which is used to determine the value of Ki, is a partial derivative of affinity energy. The concentration required to inhibit an enzyme is measured in Ki. As the Ki value decreases, the concentration required to produce an inhibitory effect decreases (Dermawan et al., 2019). According to the findings, the modified ligand had a much lower inhibition constant than the co-crystal ligand in the form of fosfomycin.

CONCLUSION

In the present study, acertannin, hamamelitannin, and modified petunidin-3-glucoside compounds, the EC_{50} of the QSAR approach are lower than fosfomycin or compounds without modification. The predicted EC_{50} for modified acertannin was 0.3321 µM, the predicted EC_{50} for modified hamamelitannin was 0.1933 µM, and the predicted EC_{50} for modified petunidin-3-glucoside was 0.8940 µM. While the original acertannin predicted 8,959 m without EC_{50} modification, the original hamamelitannin predicted 145.5 µM without EC_{50} modification, and the original petunidin-3-glucoside predicted 90,315 µM without EC_{50} modification. Acertannin, hamamelitannin, and petunidin-3-glucoside had experimental EC_{50} values of 8,600, 145.50, and 90,315 µM, respectively, and fosfomycin had an EC_{50} of 121.1 µM or 28.1 mg/L. Molecular docking studies showed that the designed compound was able to interact on the active site of the MurA enzyme with crucial amino acid residues or play an essential role through hydrogen or hydrophobic bonds. The active compounds acertannin, hamamelitannin, and modified petunidin-3-glucoside can be used as antibacterial activity by inhibiting cell wall synthesis in bacteria, causing morphological changes, agglutination, and cytolysis due to osmotic pressure imbalance and autolysis in bacteria.

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