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IN SILICO STUDY OF SILVER NANOPARTICLE PREPARATION FROM *Clitoria ternatea* FLOWERS AS AN ANTIBACTERIAL AGENT

Nur An Nitta Rizki Fanani, Septian Dwi Mulyana*, Thiya Rahma Karina, Khoirun Niswatunnisa, Nadine Detta Rezza Nevanda, Septiara Dwi Ariyanti

Department of Pharmacy, Universitas Muhammadiyah Lamongan, Indonesia

*Corresponding Author: septiandemul@gmail.com

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ABSTRACT

Background: Antibacterials are compounds with the ability to slow down and inactivate pathogenic bacteria that can cause inflammation and abscess formation. Silver nanoparticles (AgNP) are metal particles measuring 1–100 nm that are known to have high antibacterial potential. In the formation process, a reducing agent is required to facilitate chemical interactions. Butterfly pea flower (*Clitoria ternatea*) is used as the active ingredient in the synthesis of silver nanoparticles based on green synthesis, where flavonoid compounds act as reducing agents. **Objective**: To determine the potential of butterfly pea flower quercetin as an antibacterial agent through in silico studies. **Methods**: The study was conducted in silico with protein 6J90 as the receptor and quercetin as the test ligand, with preparation through geometry optimization and molecular docking validation. Drug-likeness, pharmacokinetic, toxicity, and ligand–receptor interactions were analyzed using Lipinski's rule of five, PreADMET, Toxtree, AutoDock Tools, and Discovery Studio. **Results**: The study showed that the test compound met drug-likeness criteria, had a good pharmacokinetic profile, and was not carcinogenic with a safe exposure limit of ≤0.15 µg/day, with a binding affinity value of −5.96 kcal/mol that was more stable than the natural ligand. **Conclusion**: In silico studies show that quercetin from butterfly pea flower silver nanoparticles has potential as an antibacterial agent, with a binding affinity value of −5.96 kcal/mol to the 6J90 protein, which is more stable than natural ligands.

ABSTRAK

Latar belakang: Antibakteri adalah senyawa dengan kemampuan memperlambat dan menginaktivasi bakteri patogen yang dapat menyebabkan peradangan hingga pembentukan abses. Nanopartikel perak (AgNP) merupakan partikel logam dengan ukuran 1–100 nm yang dikenal memiliki potensi antibakteri tinggi. Dalam proses pembentukannya, diperlukan adanya agen pereduksi untuk membantu terjadinya interaksi kimia. Bunga telang (*Clitoria ternatea)* digunakan sebagai bahan aktif dalam sintesis nanopartikel perak berbasis *green synthesis* di mana senyawa flavonoid berperan sebagai agen reduktor. **Tujuan:** Mengetahui potensi senyawa quercetin bunga

telang sebagai agen antibakteri melalui studi *in silico*. **Metode:** Penelitian dilakukan secara *in silico* dengan protein 6J90 sebagai reseptor dan quercetin sebagai ligan uji, dengan preparasi melalui optimasi geometri dan validasi *molecular docking*. Analisis *drug-likeness*, farmakokinetik, toksisitas, serta interaksi ligan–reseptor dilakukan menggunakan *Lipinski's rule of five*, PreADMET, Toxtree, AutoDock Tools, dan Discovery Studio. **Hasil:** Studi menunjukkan bahwa senyawa uji memenuhi kriteria *drug-likeness*, memiliki profil farmakokinetik yang baik, serta tidak bersifat karsinogenik dengan batas paparan aman ≤0,15 µg/hari, dengan nilai *binding affinity* −5,96 kkal/mol yang lebih stabil dibandingkan ligan alami. **Kesimpulan:** Studi *in silico* menunjukkan bahwa quercetin dari nanopartikel perak bunga telang berpotensi sebagai agen antibakteri, dengan nilai *binding affinity* −5,96 kkal/mol terhadap protein 6J90 yang lebih stabil dibandingkan ligan alami.

INTRODUCTION

Antibacterials are compounds with the ability to slow down and inactivate pathogenic bacteria that are the source of infection, which invade and replicate in body tissues. Some commonly found bacteria include *Salmonella spp*., *Klebsiella spp*. *Staphylococcus spp* and *Escherichia coli*. *Staphylococcus aureus* bacteria have the potential to cause infections in humans, characterized by inflammatory reactions, tissue damage (necrosis), and the formation of abscesses (Firdusy, 2024). Nanoparticle-based drug delivery system technology has emerged to address various problems related to the effectiveness and efficiency of pharmacological therapy.

Metal nanoparticles such as silver (Ag), zinc (Zn), gold (Au), and copper (Cu) are the primary focus due to their strong antibacterial properties. One type of nanoparticle that has attracted significant attention in research is silver nanoparticles (AgNP), known for their high antibacterial activity (Mareintika, 2021). Biosynthesis of AgNP can be performed using the Gram-positive bacterial isolate Bacillus firmus E65 as a bioreductive agent.This biosynthetic process is known to produce silver nanoparticles with effective antimicrobial activity against various pathogens, such as Escherichia coli and Xanthomonas oryzae pv. oryzae. The results of this biosynthesis are known to be capable of producing silver nanoparticles with effective antimicrobial activity against various pathogens, such as Escherichia coli, Xanthomonas oryzae pv. oryzae, and Colletotrichum gloeosporioides (Suryadi et al., 2022).

Synthesis by utilizing readily available plant extracts as sources of natural reducing agents, such as flavonoids, tannins, and steroids, which play a role in reducing Ag+ ions to Ag^{o} atoms while stabilizing nanoparticles. One potential plant is the butterfly pea flower (*Clitoria ternatea*), which is known to have antimicrobial, antioxidant, and anti-inflammatory properties (Fadhila *et al*., 2024). The flavonoid compounds in butterfly pea flower act as a reducer and stabilizing agent in AgNP synthesis, where the hydroxyl group (OH) interacts with silver particles (Ag^{+}) (Ruswanto *et al*., 2018). These compounds can donate electrons that reduce Ag^{+} ions

into silver atoms (Ag^0). Thus, the green synthesis method utilizes readily available plant extracts as reducing agents in the formation of silver nanoparticles (Nurfadia et al., 2024).

In drug development, in silico methods such as molecular docking are used to predict interactions between molecules so that the bonds that occur between active compounds and target proteins are known (Mboe et al., 2020). Research on the antibacterial activity of quercetin has been conducted extensively, both through in vitro and in silico tests. Quercetin has been proven to inhibit the growth of various pathogenic bacteria. However, most studies focus on single compounds in the form of extracts or pure isolates, not on plant-based green synthesis nanoparticles.

This research is novel in its use of butterfly pea flower extract as a green synthesis agent to produce AgNP, which has not been widely reported. In silico molecular docking analysis of the active compounds in butterfly pea flowers in the context of silver nanoparticles, not just single flavonoid compounds. The potential to discover new binding affinity profiles that differ from quercetin, which has been extensively studied, and contributions to the development of drug design based on green nanotechnology, which is more environmentally friendly than conventional chemical synthesis methods.

METHOD

Materials:

The structure of the DNA gyrase protein receptor (PDB ID: 6J90), which plays an important role in the mechanism of pathogenicity and bacterial survival. Quercetin was used as a test ligand, the chemical structure of which was obtained from the PubChem database. The selection of quercetin was based on its properties as a natural flavonoid compound that is widely found in butterfly pea flowers (*Clitoria ternatea*) and has been reported to have antibacterial activity.

Equipment:

The equipment used consists of computer hardware and software. The hardware includes a personal computer with specifications of an 11th Gen Intel(R) Core(TM) i3-1115G4 @3.00GHz processor, 8.00 GB RAM, and the Windows 11 Professional operating system Professional operating system, ChemDraw 3D software, MarvinSketch, AutoDock, Molegro Molecul Viewer, Command Prompt, Biovia Discovery Studio, Toxtree, and other online server-based support programs such as PreADMET and Lipinski's rule of five.

1. **Ligand Preparation**

   The ligand was prepared by designing a 2D structure of the Quercetin compound using ChemDraw Professional software version 16.0. After that, it was copied using MarvinSketch software. Then, the geometry and protonation were optimized at pH 7,4.

2. Drug Scan

Drug screening was performed on the dye compounds derived from the Quercetin compound, considering the criteria outlined in Lipinski's rule of five (the rule of good medicine), namely molecular weight <500 g/mol, lipophilicity <5, hydrogen bond donors <5, hydrogen bond acceptors <10, and refractory molar between 40-130, as well as oral bioavailability of the ligand (Mulyana et al., 2021, Shofi, 2022).

3. PreADME

Pre-ADME testing was conducted via the website https://preadmet.bmdrc.kr/ The parameters used by PreADME are $CaCo_2$ values, Human Intestinal Absorption (HIA), and Plasma Protein Binding (PPB) (Mulyana et al., 2021).

4. Toxicity Testing

Toxicity testing was performed using the Toxtree software with the test compound Quercetin. The parameters examined in the toxicity test were the predicted parameters Cramer Rules, Kroes TTC decision tree, and Benigni/Bossa rulebase (Mulyana et al., 2021).

5. Receptor Preparation

The antibacterial receptor used was obtained from the Protein Data Bank (PDB). The receptor with code 6J90 was downloaded and optimized using Autodock software (Mulyana et al., 2021).

6. Docking Method Validation

Docking validation aims to assess valid ligands that can pass the docking process. The supporting software in this docking validation is Autodock Tools. Docking validation parameters can be seen from the Root Mean Square Deviation (RMSD) with a valid value of <2Å) (Astuty & Komari, 2022).

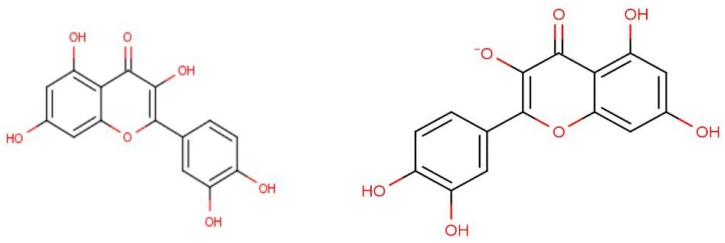
7. Docking of Test Ligands and Visualization of Results

The optimized quercetin compound was docked with the target protein, which had its natural ligands removed, using AutoDock Tools with the same procedure as in the method validation. Docking validity was determined based on the RMSD value (<2Å). Docking results were analyzed through free bond energy values and hydrogen bond interactions, then visualized in 2D and 3D formats using Discovery Studio to understand the ligand–protein interaction patterns.

RESULT

1. Ligand Preparation

From the Quercetin compound, the following Compound R3 test ligand sample was taken:



(a) *(b)*

Figure 1. Test ligand Compound R3 (a) before protonation, (b) after protonation

Protonation at pH 7.4 was carried out to adjust the pH of the ligand to the physiological pH of blood, followed by a conformational search to find the minimum energy that indicates high stability when the ligand binds to the active site of the receptor. Ligand preparation began with drawing a 2D structure using ChemDraw Professional 16.0, followed by geometry optimization and protonation at pH 7.4 using MarvinSketch to ensure the resulting molecular conformation matched conditions in a living human body and had optimal binding potential. After optimization, the ligand files are saved in .mol2 and .mrv formats for further analysis, ensuring the ligands are in the most stable and relevant form for biological interactions in docking simulations (Maghfiroh et al., 2025).

2. *Drug Scan*

This drug scan was conducted to assess the molecular characteristics of ligands based on Lipinski's rule of five. This refers to the similarity of a compound to an oral drug (Ferdian et al., 2021).This refers to the similarity of a compound to an oral drug. These parameters were viewed using MarvinSketch software. The following are the drug scan test result.

Table 1. Drug Scan Results for the Compound Quercetin

Compound Name	Drug Scan				
	BM	**HBA**	**HBD**	**Log P**	**Refractory Molar**
Pubchem	**<500 g/ml**	**<5**	**<10**	**<5**	**40-130**
Quercetin	312	5	6	-0,053101	77.145782

Description: MW = Molecular Weight; Log P = Lipophilicity; HBA = Hydrogen Bond Acceptors; HBD = Hydrogen Bond Donors.

Based on physicochemical testing, the Quercetin compound in butterfly pea flowers meets the 5 Lipinski parameters, namely molecular weight, Hydrogen Bond Acceptors, and Hydrogen Bond Donors, Log P, and Refractory Molar.

3. PreADME

This ADME testing was conducted using an online server called PreADMET, which only refers to the absorption and distribution processes, in the

form of $CaCo_2$ values, Human Intestinal Absorption (HIA), and Plasma Protein Binding (PPB). The following are the results from PreADMET.

Table 2. PreADME Results For the Quercetin Compound

Compound Name	**$CaCo_2$ (nm/sec)**	**HIA (*Human Intestinal Absorption) %***	**PPB (*Protein Plasma Binding) %***
Quercetin	3,45 Moderate	67.911573% Moderate	81,55% Very Tightly Bound

4. Toxicity Test

One software that can be used to test potential toxicity in humans is Toxtree. Toxtree is an application designed to automatically predict the toxicity level of a molecule. This toxicity test was conducted on the compound Quercetin, which had previously been eliminated in the ADME test. The following are the results of the toxicity test using the Toxtree software:

Table 3. Results of Toxicity Testing Using Toxtree

Compound Name	**Parameter**		
	Cramer Rules	***Kroes TTC decision tree***	***Benigni / Bossa rulebase***
Quercetin	Class III	The substance is not expected to pose a safety risk.	The substance does not show potential as a non-genotoxic carcinogen.

Cramer Rules were used to exclude non-carcinogenic chemicals and to determine the TTC level. Quercetin derivatives were categorized into class three, indicating high toxicity potential, so their use at high concentrations does not guarantee safety. According to the Kroes TTC decision tree, the exposure threshold for quercetin is set at ≤0.15 µg/day based on a dose-response analysis of potentially carcinogenic compounds. This limit is estimated to minimize toxic risk by 86-97%, particularly in relation to genotoxic potential.

5. Receptor Preparation

Receptor preparation in this study was done by downloading the antibacterial receptor from the Protein Data Bank (PDB). There was one receptor with the code: 6J90 and the receptor with a good RMSD value was selected. Then the receptor was downloaded in .pdb format.

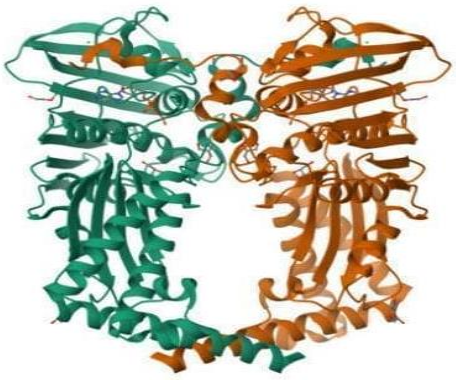


Figure 2. Protein 6J90

6. Method Validation

Method validation was performed by docking the original ligand to find the original ligand conformation. The macromolecules prepared previously underwent a redocking process with the original ligand. The docking results obtained were then compared with the original ligand conformation in the crystallographic structure expressed as the Root Mean Square Deviation (RMSD) value (9). RMSD is the deviation or difference that appears in the ligand due to differences in conformation before and after the docking process, with the condition that the RMSD value is <2Å (Elfita et al., 2023).

This validation aims to ensure that the method used is valid and suitable for testing other compounds, thereby reducing the possibility of errors (Nugroho & Fauzi, 2024). The following is the docking validation data in Table 4:

Table 4. Docking Validation Results

RECEPTOR	GRID BOX			RMSD
	X	Y	Z	
6J90	-12.062	36.909	23.761	1,75

Figure 3. Natural Ligand 6J90

Based on the validation results, the RMSD value for the viral antibacterial receptor with code 6J90 is 1.75, which means the value is less than 2. A smaller RMSD value indicates that the predicted ligand position is more accurate because it is closer to the original ligand position (Klara et al., 2023).

Optimization and grid box determination were performed using the Autodock application. Grid box determination, which is the binding area, aims to define the ligand binding space during the molecular docking process (Klara et al., 2023). For the receptor with code 6J90, the grid box position was set at coordinates X = -12.062, Y = 36.909, and Z = 23.761. Based on these validation results, the 6J90 receptor met the validation criteria for the docking method and was therefore suitable for ligand testing.

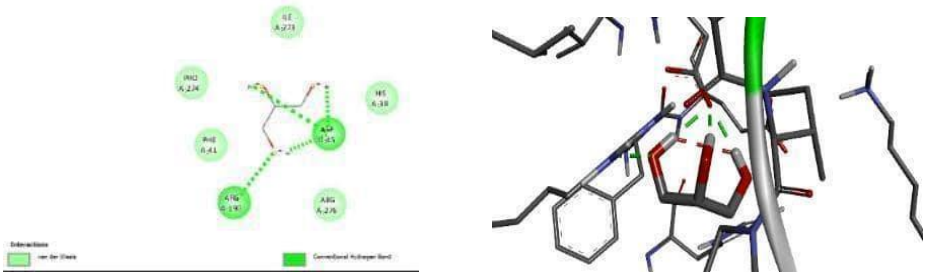
7. Test Ligand Docking and Visualization of Results

The bond affinity value (ΔG) is expressed in kcal/mol and is used to determine the most optimal conformation. This parameter indicates the strength of the interaction between the ligand and the target protein (Maghfiroh et al., 2025). The following are the results of Ligand Interaction with Protein Target.

Table 5. Results of Ligand Interaction with Test Target Protein

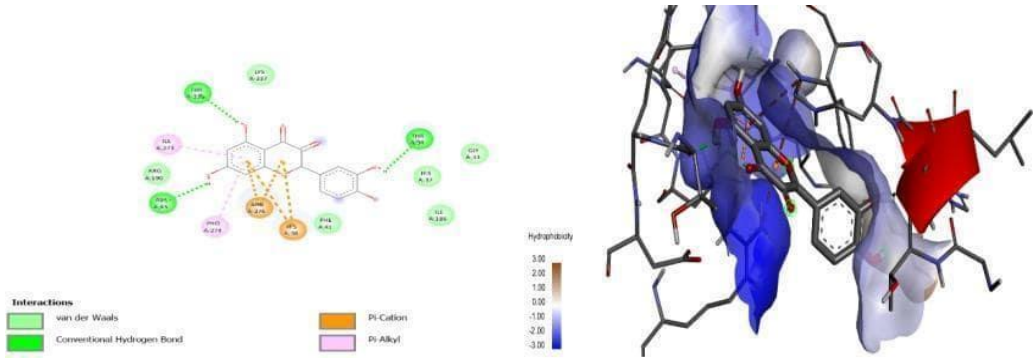
No	Compound Name	Binding Affinity Kkal/mol
		6J90
1	Ligan Alami	2.71
2	Quercetin	-5,96

After the docking process of the test compound is completed, the next step is to visualize the docking results in two-dimensional (2D) and three-dimensional (3D) formats. The visualized compound is Quercetin, which had good results in the preliminary test and had the best binding affinity value. Below are the results of the visualization of the interaction between the ligand and the target protein with code 6J90 in 2D and 3D formats using Discovery Studio software.



(a) (b)

Figure 4. (a) Results of the docking of natural ligand compounds with 2D proteins and (b) 3D with 6J90 proteins



(a) (b)

Figure 5. (a) 2D Docking Results of the Quercetin Compound and (b) 3D with The 6J90 Proteins

Table 6. Conclusions on Binding Affinity, Hydrogen Bonds, Amino Acids of Natural Ligands and Test Ligands

Compound Name	Binding Affinity (Kca/mol)	Hydrogen Bonds	Amino Acids
		6J90 Code	
Nature Ligand	2.71	ARG190, ASP45	**PRO274**, **PHE41**, **ARG276**, **HIS38**, **ILE273**
Quercetin	-5,96	THR336, ASP45, THR34	ARG190, LYS337, **PHE41**, HIS37, ILE186, GLY, 33, **ILE273**, **PRO274**, **ARG276**, **HIS38**

Based on Table 6 and Figure 5 above, it is known that the binding affinity value of the active compound in butterfly pea flower is greater than that of natural ligands. The amino acids in the Quercetin compound that are similar to natural ligands are PHE41, ILE273, PRO274, ARG276, HIS38. This indicates that Quercetin interacts with the same binding pocket and forms similar hydrophobic and hydrogen bond patterns as the natural ligand, thereby mimicking the natural ligand's mechanism of action in inhibiting DNA gyrase enzyme activity. The similarity of these residues is supported by stronger binding affinity values, making the active compounds in butterfly pea flowers a potential antibacterial agent.

Docking Results Analysis

Based on the docking process between the ligand and the receptor, the lowest energy conformation of the ligand was obtained. Binding affinity is an indicator of a drug's ability to bind to a receptor. Affinity energy data (ΔG) indicates how strong the

bond formed between the receptor and the ligand is. The smaller (more negative) the value, the greater the potential for the ligand to form a strong bond with the receptor (Ferdian *et al*., 2021). Thus, the results can be analyzed as follows:

Table 7. Grid Box, Binding Affinity Values of Natural Ligands and Quercetin

PDB Code	Grid Box	RMSD	Natural Ligand Binding Affinity (Kca/mol)	Binding Affinity Quercetin (Kca/mol)
6J90	X= -12.062, Y= 36.909, Z= 23.761	1.75	2.71	-5.96

Based on the docking results, the binding affinity values in Table 3 for both test ligands have lower interactions than natural ligands because they have smaller binding affinity values with the 6J90 receptor code. Thus, it can be said that quercetin compounds have potential antibacterial activity due to their satisfactory (*fairly good*) affinity values.

DISCUSSION

1. Prediction of the Physicochemical Properties of Active Compounds in Blue Pea Flowers Using pkCSM

Based on the physicochemical drug scan results of the Quercetin compound contained in blue pea flowers, it was found that Quercetin meets Lipinski's rule of five, thus potentially having a good absorption and distribution profile in the body. Molecular weight is related to the distribution phase of drugs in the body, so that a drug with a molecular weight below 500 g/mol will easily enter the cell membrane for penetration, which is the initial stage for the compound to reach the molecular target in bacterial cells. Ligands with a molecular weight above 500 g/mol generally have difficulty penetrating biological membranes (Rani et al., 2023).

Lipophilicity/Log P is the main physicochemical determinant that plays a role in the level of absorption, ability to penetrate membranes, and toxicity risk of drugs. The lipophilicity value (Log P) of Quercetin falls within an appropriate range, allowing the compound to penetrate lipid membranes without causing excessive accumulation that could increase the risk of toxicity. The Log P value is related to the polarity of the ligand toward lipid solvents, oils, and non-polar solvents. A Log P value of 5 indicates that the ligand is highly lipophilic, meaning it remains longer in lipid membranes, spreads widely throughout body tissues, and risks losing specificity toward target enzymes, which could trigger toxic effects (Rani et al., 2023).

The hydrogen bond donor and acceptor parameters of Quercetin also play a crucial role in forming stable molecular interactions with biological targets, particularly proteins, through the formation of hydrogen bonds with polar amino

acid residues. The more hydrogen bonds formed, the higher the compound's polarity, which directly enhances its ability to the more hydrogen bonds formed, the higher the polarity of the compound, which directly enhances its ability to interact with water molecules and biological targets such as proteins.

In an in silico study of silver nanoparticles based on butterfly pea flower extract (*Clitoria ternatea*), the flavonoid compound Quercetin, which acts as a reducing agent and stabilizer in AgNP synthesis, showed good antibacterial potential through molecular interaction with the antibacterial target protein encoded by 6J90. Molecular dockingshowed a binding affinity value for Quercetin of -5.96 kcal/mol, which is lower than that of natural ligands (2.71 kcal/mol), indicating a stronger and more stable bond. Additionally, the Quercetin compound has hydrogen bond donors and acceptors that comply with Lipinski's rule of five, namely 5 donors and 6 acceptors, which supports its ability to form stable hydrogen interactions with target protein residues, thereby strengthening the stability of the ligand–receptor complex and supporting its antibacterial activity.

Refractory molar is an indicator of the total polarizability of a compound, whose value is influenced by external factors such as refractive index, temperature, and pressure. It describes the molecule's ability to form a transient dipole when interacting with the target protein environment. Good polarizability allows for conformational adjustment of the ligand to the active site of the protein, thereby improving molecular interaction compatibility and strengthening bond affinity (Amin et al., 2023; Astuty & Komari, 2022). The physicochemical properties of Quercetin not only support the absorption and distribution processes, but also play an important role in the antibacterial mechanism through the inhibition of bacterial DNA gyrase.

2. Prediction of Pharmacokinetic Properties (ADME) of the Active Compound in Telang Flowers Using pkCSM

Using pkCSM ADME and toxicity prediction are pharmacokinetic predictions. Table 2 shows the results of PreADME using the PreADMET program for the quercetin compound, which had previously been eliminated in the drug scan test. Based on the CaCo2 values, it was found that the compound has low permeability because its value is below 4 nm/sec (3.45 nm/sec). This is because the permeability value considered good based on the absorption process in the human intestine ranges from 4 to 70 nm/sec. Therefore, the value (3.45 nm/sec) is classified as low (difficult to absorb by the intestine). The estimated drug absorption rate is based on the drug's absorption capacity by the intestinal wall. Therefore, the value (3.45 nm/sec) is classified as low (difficult to absorb by the intestine). The estimated drug absorption rate is based on the drug's capacity to be absorbed by the intestinal wall or can be estimated using the HIA (Human Intestinal Absorption) parameter. This is because oral administration requires effective absorption in the intestinal

wall. Compounds with low absorption will not effectively reach the target receptors. Therefore, the HIA parameter is important because it plays a role in determining the level of bioavailability (Yudha, 2022).

The toxicity parameters used include Cramer Rules, which are used to assess toxicity levels based on compound functional groups; Kroes TTC decision tree, which serves to estimate the exposure threshold of drug compounds in humans, and the Benigni or Bossa Rulebase, which is used to evaluate whether a compound has the potential to cause carcinogenic and mutagenic effects (Larasati *et al*., 2022). Cramer Rules are used to exclude non-carcinogenic chemicals and to determine the TTC level. Cramer Rules have an original questionnaire consisting of 33 Yes or No questions or rules. The answer to each question leads to another question until the final Cramer Rules classification for the chemical of interest is determined. The Cramer Rules classify substances into one of three classes (Class I-Low, Class II-Intermediate, Class III-High).

According to the Cramer Rules criteria, Quercetin derivative compounds are categorized into class three, which indicates that these compounds have a high level of toxicity. However, it should be emphasized that toxicity classification based on the Cramer Rules is predictive and conservative, whereby class three does not directly indicate that the compound is actually toxic, but rather indicates that the chemical structure of the compound requires further toxicological evaluation before it can be declared safe for biological or pharmacological use. This indicates that the chemical structure of Quercetin is assessed as having potential risks to safety, particularly due to the presence of heterocyclic compounds within it. However, the presence of heterocyclic structures does not always directly correlate with actual toxicity, but rather reflects the possibility of strong biological interactions with cellular systems, which in a pharmacological context can be therapeutic or harmful depending on the dose and route of exposure. Nevertheless, Quercetin does not contain functional groups that are directly associated with increased toxicity, so its potential hazards are more related to the dosage used. In other words, Quercetin has the potential to cause toxic effects when consumed in doses exceeding safe limits or outside its therapeutic index (Listyani et al., 2024). The results of the Cramer Rules classification for Quercetin further indicate the need for caution in determining dosage and formulation, rather than being an indicator that this compound is intrinsically toxic at therapeutic doses.

According to the Benigni/Bossa Rulebase parameters, quercetin derivatives do not exhibit carcinogenic properties, as the analysis results were negative for both genotoxic and non-genotoxic carcinogenicity. However, these parameters do not provide information on the potential mutagenicity of the compound (Damayanti et al., 2021). These results reinforce the interpretation that although Quercetin is classified in the high toxicity class structurally according to Cramer Rules, this

compound does not show indications of carcinogenicity, so its potential risk is more conditional and depends on the level of exposure.

The Kroes TTC decision tree can be used to determine the toxicity level of a substance. The threshold of toxicological concern (TTC) is a principle that refers to the establishment of a threshold value for human exposure to all chemicals, below which there is no risk to human health. The parameters used in the Kroes TTC decision tree are the presence of heavy metals such as arsenic, cadmium, lead and mercury, the presence of compound structures that have the potential to be genotoxic, and the exposure dose (0.15 µg/day) (Gumiwang et al., 2024).

3. In silico Testing of Active Compounds in Butterfly Pea Flowers

The docking process between the ligand and the target protein produces various conformations of the analyzed compound. One important parameter in the docking results is the binding energy, known as binding affinity. Binding affinity indicates how strongly a compound can interact with a receptor. A lower binding affinity value indicates higher affinity between the ligand and receptor, while a higher value indicates lower affinity (Nugroho & Fauzi, 2024).

The data in Table 5 shows that both natural ligands and test ligands have the ability to bind to the target protein. Based on the analysis, natural ligands show higher binding affinity values compared to test ligands .The binding affinity value of the Quercetin compound is recorded at (-5.96), which indicates that this compound is able to interact with the target protein in a good and stable manner. This relatively low binding affinity value indicates that quercetin has the potential to inhibit the biological activity of the antibacterial target protein by occupying the active site or important sites on the protein, thereby disrupting the physiological function of the protein that plays a role in bacterial survival. Thus, these docking results support the antibacterial mechanism of Quercetin through the inhibition of the target protein at the molecular level.

This visualization aims to show the interaction between the test compound and the target protein, particularly at the amino acid residues in the antibacterial receptor. The interactions analyzed include hydrogen bonds and hydrophobic bonds. Hydrogen bonds play a crucial role in maintaining ligand stability within the receptor binding site (Sa'banah, Mardianingrum, and Endah, 2024). Meanwhile, strong hydrophobic interactions can enhance ligand affinity for the receptor and strengthen its pharmacological effects (Nugroho & Fauzi, 2024).

From the Structure–Activity Relationship (SAR) perspective, the presence of a hydroxyl group (–OH) in the quercetin structure plays an important role in the formation of hydrogen bonds with key amino acid residues in the target protein. The aromatic group and conjugated ring system in quercetin also contribute to stable hydrophobic interactions within the protein binding pocket. The combination of polar and nonpolar groups allows quercetin to have a good balance of affinity and

bond stability, thereby enhancing its biological activity as an antibacterial compound. Thus, the relationship between the chemical structure of quercetin and its biological activity indicates that the molecular configuration of quercetin supports optimal interactions with antibacterial target proteins. This strengthens the hypothesis that Quercetin's antibacterial mechanism occurs through specific inhibition of target proteins, which can subsequently disrupt bacterial cell metabolism and growth (Husen et al., 2025).

CONCLUSION

The results of the study show that the Quercetin compound from silver nanoparticle preparations of butterfly pea flower (*Clitoria ternatea*) exhibits good antibacterial activity potential based on in silico studies. From the docking results against the antibacterial receptor with the PDB code 6J90, the Quercetin compound has a binding affinity value of -5.96 kcal/mol, which is lower than its natural ligand with a value of 2.71 kcal/mol. This value indicates that Quercetin has a stronger binding affinity to the receptor. Thus, the Quercetin compound has the potential to be an effective antibacterial agent compared to its natural ligand based on the molecular docking approach.

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