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RESEARCH PAPER

In Vitro and Silico Antibacterial Activity Evaluation Jackfruit Parasite Plant *Macrosolencochinchinensis* (Lour.) van Tiegh

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Abstract: In traditional Melayu Riau medicine, the jackfruit parasite plant, also known as Macrosolen cochinchinensis (Lour.) van Tiegh, has been utilized as a remedy for coughs, a treatment for cancer, a diuretic, and to speed up the recovery process after giving birth. Through in vitro and in silico testing, this investigation aims to learn more about the bioactive components and antibacterial activity of n-hexane, ethyl acetate, and ethanol obtained from the extracts of the jackfruit parasite plant (Macrosolen cochinchinensis (Lour.) van Tiegh. The disc diffusion method was used for the in vitro testing, and the molecular docking method was used for the in silico testing. Both were conducted against Staphylococcus aureus ATCC 12600 and Escherichia coli ATCC 25922. Each sample extract was prepared in three different concentrations (10%, 30%, and 50%), while chloramphenicol was used as the positive control. Inhibition zones of the extract against E. coli bacteria were found to be as follows: 6 mm, 8.4 mm, and 10.6 mm for the n-hexane extract; 8.2 mm, 10.7 mm, and 15.6 mm for the ethyl acetate extract; and 6 mm, 7.1 mm, and 14.1 mm for the methanol extract. The results of the test to determine the extract's antibacterial activity were as follows: The following is a list of the zones of inhibition that the extract has against S. aureus bacteria: 6 mm, 7.5 mm, and 13.7 mm in diameter for the extract of n-hexane; 12.8 mm, 14.2 mm, and 19.2 mm in diameter for the extract of ethyl acetate. Test results of 7.2 mm, 9.3 mm, and 15.3 mm were obtained for the methanol extract. In accordance with the findings of the study, the ethyl acetate extract of jackfruit parasite leaves exhibited the highest level of antibacterial activity, as measured by an inhibition zone diameter of 19.2 mm, when tested on S. aureus bacteria. In the meantime, the results of molecular docking of punicalin and rutin, which have been reported to have activity against proteins 6GOS.pdb and 1OJZ.pdb, have potential as antibacterials because they form 5 and 9 hydrogen bonds with important amino acids of the target protein, with cDOCKER values of -57.9239 and -88.3993, respectively. It suggests that punicalin and rutin can inhibit the growth of bacteria.

Keywords: Antibacterial, Diffusion, the jackfruit parasite, Molecular Docking

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INTRODUCTION

The use of antimicrobial agents as a mainstay in handling infectious cases causes their use to increase. The widespread and irrational use of antimicrobials leads to new problems of resistance. Antibacterial resistance is becoming a significant health problem at this time. Resistance occurs when a microorganism changes in some way that reduces or diminishes the effectiveness of a drug, chemical, or other agent designed to cure or prevent infectious disease [1]. Uncontrolled use of antibiotics will tend to increase the resistance of originally sensitive bacteria. Bacteria that can produce enzymes will inactivate the action of antibiotics. Several surveys found that Beta-lactam type antibiotics were the most widely prescribed antibiotics, so bacteria were resistant to these antibiotics. The emergence of antibiotic resistance has caused certain groups of antibiotics to be unusable, so it is necessary to search for natural-based antibiotics

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to reduce the number of antibiotic resistance [2]. One of the plants reported to have much activity is the parasite plant.

Parasites are a group of plants that grow wild, attached to, and parasitic on other tree branches. Parasites that were initially thought to be of no use turned out to have various properties for treatment. Empirically, the parasite has been used to treat several diseases, including inflammation of the uterus, whooping cough, tonsils, measles and chickenpox, diarrhea, hookworms, and gabag. In addition, the parasite is used as a medicine for liver disease and cancer. Tea, mango, and duku fruit parasites are often used as medicinal plants. The part used is the leaves or all parts of the plant in a fresh state or after being dried [3].

Parasites are reported to be active because they contain a marker compound of the Loranthaceae family in the form of the flavonoid quercetin. Quercetin is a flavonoid aglycone with a polyphenol group, so its highly reactive phenolic component can stabilize the compound and act as an antioxidant. In addition to the active compound quercetin, parasite leaves also contain alkaloids, saponins, flavonoids, tannins, rutin, quercetin, and punicalin compounds [4]. In this study, phytochemical tests and antimicrobial activity of n-hexane, ethyl acetate, and methanol extracts from the leaves of the jackfruit parasite (M. cochinchinensis (Lour.) van Tiegh) were carried out in vitro. To ensure the activity resulting from the compounds in the parasite, an In silico activity test using molecular docking [5-7], so that the interaction produced by the compound to the amino acids of the target protein used is known. Based on the above background, a study was conducted to test the activity of jackfruit parasite leaves (M. cochinchinensis (Lour) van Tiegh) against pathogenic bacteria Staphylococcus aureus and Escherichia coli.

MATERIALS AND METHODS

Tools and Chemicals

The tools used in this study were: Discovery Studio® 3.1 software (Accelrys, San Diego, USA), a digital balance, a distillation apparatus, a rotary evaporator Rotavapor R-3 (BUCHI), a set of gravity column chromatography tools with a diameter of 5 cm and a height of 20 cm, chamber, Fisher Jones brand Melting Point Apparatus, TLC plate, capillary pipette, vial, desiccator, micropipette, volume pipette, caliper, loop needle, spiritus, and glassware commonly used in the laboratory.

The materials used in this study were: n-hexane extract, ethyl acetate, methanol, jackfruit parasite (Macrosolen cochinchinensis (Lour.) van Tiegh), Nutrient Agar (NA), Nutrient Broth (NB), S. aureus ATCC 25922, E. coli ATCC 12600, dimethylsulfoxida (DMSO), chloramphenicol (antibacterial positive control), H_2SO_4 2 N, Dragendorff reagent, Mayer reagent, anhydrous acetic acid, concentrated H_2SO_4, metal Mg, concentrated HCl, 1% $FeCl_3$, distilled water, chloroform. Protein crystals 1OJZ.pdb and 6GOS.pdb were obtained from the Protein Data Bank (http://www.rscb.org), rutin, quercetin, and punicalin compounds.

Procedures

Sample Extraction

Extraction was carried out using cold extraction with a multilevel maceration technique. The solvents used were n-hexane, ethyl acetate, and ethanol. As much as 1 kg of simplicia powder was put into a dark bottle, then n-hexane solvent was put into a dark bottle containing simplicia until submerged ± 2 cm above the simplicia surface. The total volume of n-hexane used for maceration was 8 L. The maceration was carried out for 3x24 hours with stirring several times until the solvent was clear. The maceration results were filtered using filter paper to separate the filtrate from the residue. The filtrate obtained was then evaporated using a vacuum rotary evaporator to obtain a thick n-hexane extract.

The remaining residue was macerated using ethyl acetate solvent. The ethyl acetate solvent was put into a dark bottle and then macerated for 3x24 hours with stirring several times until the solvent was clear. The total volume of ethyl acetate used for maceration was 10 L. The maceration results were filtered using filter paper to separate the filtrate from the residue. The filtrate obtained was then evaporated using a vacuum rotary evaporator to obtain a viscous ethyl acetate extract.

The remaining residue was macerated using ethanol solvent. The methanol solvent was put into a dark bottle and then macerated for 3x24 hours with stirring several times until the solvent was clear. The maceration results were filtered using filter paper to separate the filtrate from the residue. Then the filtrate was evaporated with a vacuum rotary evaporator to obtain a viscous ethanol extract.

Phytochemistry test

Phytochemical testing of each extract followed the method [8]

Antibacterial Activity Test In Vitro by Agar Diffusion Method

Sterilization

Heat-resistant tools, materials, and medium to be used for research were sterilized using an autoclave for 15 minutes at 121°C and 1 atm pressure. Previously, tools were thoroughly washed, dried, and wrapped in paper. Tools that cannot stand heat are sterilized using alcohol.

Preparation of Nutrient Agar (NA) and Nutrient Broth (NB) Media

As much as 5 grams of Nutrien Agar (NA) medium powder was dissolved in 250 mL of distilled water in an Erlenmeyer and sterilized in an autoclave at 121°C for 15 minutes. Then put the media into the test tube, tilt and let it stand [9].

As much as 3.25 grams of Nutrien Broth (NB) medium powder, dissolved in 250 mL of distilled water in an Erlenmeyer and heated until dissolved until boiling. It is then sterilized in an autoclave at 121°C for 15 minutes [9]

Microbe regeneration

Bacterial rejuvenation was carried out using NA (Nutrient Agar) agar media. Microbes are taken in one loop using a sterile loop and then rubbed on the surface so that it is tilted in a zig-zag manner and incubated at 37 ºC for 24 hours [10].

Microbe suspension preparation

One ose of the bacterial cultures that had been rejuvenated for 24 hours was taken and put into a test tube containing 5 mL of physiological 0.9% NaCl, then 1 mL was taken and added to 10 mL of Nutrient Broth (NB) media. NB media containing bacteria was incubated in an incubator at 37 ºC.

Microbiological test

The inhibition test of n-hexane, ethyl acetate, and viscous methanol extracts from the leaves of the jackfruit parasite (M. cochinchinensis (Lour.) van Tiegh) on the growth of S. aureus, and E. coli bacteria were carried out by the diffusion method using paper discs. Put 1 mL of the tested microbial suspension solution into a sterilized petri dish, add 20 mL of NA, shake until the microbes are suspended evenly, and let it solidify. Place paper discs (6 mm in diameter) that have been dripped with the sample to be tested with variations in sample concentrations of 20, 30, and 50% (performed in duplicate), DMSO (negative control), and chloramphenicol (positive control) were used as comparison compounds. The Petri dishes were then incubated at 37°C for 24 hours. Furthermore, observations were made, measuring the diameter of the inhibition zone formed, which was marked by a clear zone using a shovel[11]

Data Analysis

The inhibition zone diameter data were calculated manually, then computerized using Microsoft Excel to find the average inhibition zone diameter using the formula:

$$\frac{(DT_1 + DT_2)}{2}$$

Where:

DT_1 = First inhibition zone diameter
DT_2 = Second inhibition zone diameter

In Silico Antimicrobial Activity Test

Insilico activity test was carried out using molecular docking. The docking process starts with preparing protein crystals with antibacterial activity. The 3-dimensional protein structure was taken from the protein data bank website (www.pdb.org). The protein taken is a crystal structure with the code 1OJZ.pdb for S. aureus and 6GOS.pdb for E. coli.

Target protein preparation

Before carrying out the docking process, the 1OJZ.pdb and 6GOS.pdb proteins must be prepared first. The first preparation is if there is more than one chain in the protein, one of the existing chains is selected, and then the protein is cleaned of residue. This process uses Chimera®1.10 software. Residues removed, such as H2O molecules and standard ligands found in the protein crystal. After the target protein is free from residue, the second preparation is continued using the Discovery studio® 3.1 software. Selecting "macromolecular" then "prepare." Proteins will be automatically prepared by adding hydrogen atoms, adding atomic charges, and repairing broken atomic bonds due to the release of standard ligands.

Ligand preparation

The test compounds to be carried out by the docking process are referred to as "ligands." Before carrying out the docking process, this ligand must also be prepared to obtain the most stable conformation of the ligand, adding charge to each atom making up the ligand, adding hydrogen atoms to the ligand, and minimizing energy. This ligand preparation was carried out using Discovery studio®3.1 software. Selecting "small molecule" then "prepare." The preparation results are then continued by selecting "minimize all."

Docking

Before carrying out the docking process, a coordinate (grid) is formed where the docking process occurs between the protein and the ligand. In the Discovery studio® 3.1 software, docking coordinates

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are made by copying standard ligands and pasting them into the protein that has been prepared, then selecting the grid menu. Then the docking coordinates will be formed automatically. Typically, the radii of these coordinates are 9–10 Å, depending on the size of the standard ligand. The docking process was first carried out with the standard ligand and had to obtain a small Root Mean Square Deviation (RMSD) value of 2 Å. It serves as a validation of the docking method to be used. The proposed compound can be docked with the target protein if these conditions are met. During the docking process, the target protein is made rigid, while the ligand is made flexible to bind to the protein's active site.

Docking analysis

The results of the docking process were opened using the Discovery studio 4.5 visualizer software. Then the ligand interaction menu was selected, and the energy and binding sites between the ligands and amino acid residues would automatically appear. The interactions that appear can be in the form of hydrogen bonds and phi bonds, with each bond distinguished by the color of the bond formed. Hydrogen bonds are usually colored green, while phi bonds are pink. Meanwhile, in the Discovery studio program, the binding energy between ligands and proteins can be seen as –cDOCKER energy.

RESULTS AND DISCUSSION

Extraction of Jackfruit Parasite Leaves

The results of the jackfruit parasite leaf extraction process obtained yields of 0.45% n-hexane extract, 1.4% ethyl acetate extract, and 0.35% methanol extract, as shown in Table.1

Table 1. Extraction of Jackfruit Parasite Leaves

Sample	Weight (g)	Yield (%)
Jackfruit parasite leaves	2000	-
Dry parasite leaves powder	700	35
n-hexane extract	6.5	0.45
Ethyl acetate extract	20	1.4
Methanol extract	5	0.35

Phytochemistry test

Phytochemical profile tests were carried out on the extracts to determine the class of alkaloids, flavonoids, phenolics, saponins, steroids, tannins, and terpenoids, as shown in Table 2.

Table 2. Phytochemical profile of jackfruit parasite extracts

Compound groups	n-hexane	Ethyl acetate	Methanol
Alkaloid	+	+	-
Alkaloid	+	+	+
Flavonoid	-	+	+
Phenolic	-	-	-
Saponin	-	-	-
Steroid	+	-	-
Tannin	-	+	+
Terpenoid	-	-	-

In Vitro Antibacterial Activity Test

The results of the research conducted for the antibacterial test of the n-hexane, ethyl acetate, and methanol extracts of jackfruit parasite leaves are shown in Table 3.

Table 3. Antibacterial Test Results of n-hexane Extract on Jackfruit Benalu Leaves

No	Sample conc. (%)	Inhibition diameter (mm)	
		E. coli ATCC 25922	*S. aureus* ATCC 12600
1	10	6	6
2	30	8.6	7.5
3	50	10.6	13.7
4	Chloramphenicol (10 %)	23.6	25
5	DMSO	6	6

Note: Chloramphenicol is a positive control, and DMSO is a negative control. The disk diameter is 6 mm.

The n-hexane extract contains alkaloid and steroid bioactive compounds. Steroid compounds are antibacterial by damaging cell membranes. Damage to cell membranes causes important substances to come

out of cells and can also prevent important substances from entering cells (6).

Table 4. Antibacterial test results of ethyl acetate extract on jackfruit parasite leaves

No	Sample conc. (%)	Inhibition diameter (mm)	
		***E. coli* ATCC 25922**	***S. aureus* ATCC 12600**
1	10	8.2	12.8
2	30	10.7	14.2
3	50	15.6	19.2
4	Chloramphenicol (10 %)	23.3	25.6
5	DMSO	6	6

Note: Chloramphenicol is a positive control, and DMSO is a negative control. The disk diameter is 6 mm.

Ethyl acetate extract containing alkaloids and tannins gave an inhibition zone at each concentration to all tested bacteria with different inhibition zone diameters. Tannin compounds can act as antibacterial because they can interfere with peptidoglycan synthesis, making bacterial cell wall formation less than perfect. Tannin compounds act on the DNA synthesis system by inhibiting enzymes so bacterial cell proteins cannot be formed. Tannins in plants are used as protection from microorganisms that secrete enzymes that can lyse plant cell walls. These enzymes will be inactive when the tannins present in plants bind to enzymes secreted by these pathogenic microorganisms.

Table 5. Results of antibacterial testing of methanol extract on jackfruit parasite leaves

No.	Sample conc. (%)	Inhibition diameter (mm)	
		***E. coli* ATCC 25922**	***S. aureus* ATCC 12600**
1.	10	6	7.2
2.	30	7.1	9.3
3.	50	14.1	15.3
4.	Chloramphenicol (10 %)	24.5	24.8
5.	DMSO	6	6

Note: Chloramphenicol is a positive control, and DMSO is a negative control. The disk diameter is 6 mm.

Table 6 CDOCKER Energy Table and Binding Interactions of Secondary Metabolites from the Jackfruit Parasite Plant (M. cochinchinnis) (Lour) van Tiegh). Against Amino Acid Protein S. aureus (1OJZ.pdb)

Compound	CDOCKER Energy (kcal/mol)	Hydrogen Bonds
Rutin	-88.3993	**GLN140**, **LEU86,** ARG150, **ASN88**, **ASP90, ARG85,** TYR136, ASN44, TYR36
Quarantine	-69.9095	**ASP90, ASN88, SER138,** TYR36, SER40
Punicalin	-42.3552	THR45, SER40, **SER138,** ARG150, ASN86, **ASP90**
Chloramphenicol	-38.34	**GLN140**, THR139, ARG150, ARG48
Native ligand NAD	-90.9874	GLU133, ASP90, ASN88, ARG85, GLN140, LEU86, GLU180, GLN178, SER138, THR37

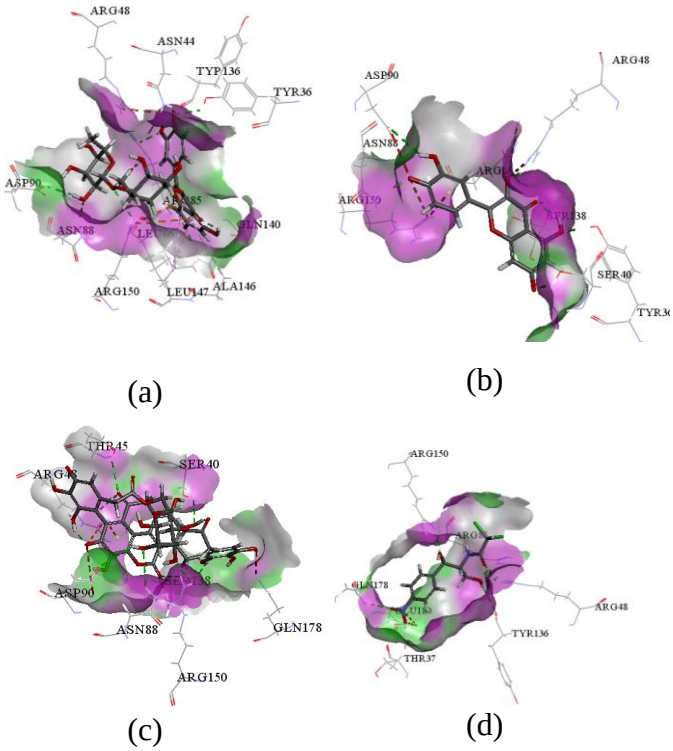

Figure 1. (a) Rutin 3D bond interaction, (b) Quercetin 3D bond interaction, (c) Punicalin compound 3D bond interaction, (d) Chloramphenicol 3D bond interaction with target protein 1OJZ.pdb

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The methanol extract contains polar bioactive compounds, namely flavonoids, alkaloids, and tannins which also provide an inhibition zone against the test bacteria. The content of methanol extract is flavonoid compounds, a group of phenolic compounds that play a role in binding proteins, thereby disrupting metabolic processes. The hydroxyl groups present in the structure of flavonoid compounds will cause toxic effects on bacteria. Flavonoid compounds can infiltrate and form complex combinations with the bacterial cell wall, which causes disruption or damage to the permeability of the cell membrane. The mechanism of action of alkaloids as antibacterials is by interfering with the constituent components of polypeptidoglycan in bacterial cells so that the cell wall layer is not formed entirely and causes cell death [12].

Molecular Docking

Based on the docking of antibacterial activity performed on routine test compounds, quercetin, [13] and punicalin [14], chloramphenicol and standard ligands against S. aureus protein (1OJZ.pdb) and E. coli (6GOS.pdb) then the results are obtained as in Table 6.

Table 7. CDOCKER Energy Table and Binding Interactions of Secondary Metabolites from the Jackfruit Parasite Plant (Macrosolen cochinchinnis) (Lour) van Tiegh). Against Amino Acid Protein E. coli **(6GOS.pdb)**

Compound	CROCKER Energy (kcal/mol)	Hydrogen Bonds
Rutin	-52.352	**TYR202, SER122,** PRO121, GLY124
Quarantine	-52.3668	**TYR202, SER122**
Punicalin	-57.9239	**PRO121,** TYR127, ALA123, **SER122, GLY123**
Chloramphenicol	-21,.433	MET209, **GLY123, GLY124, SER122**
Native ligand FMN	-47.7613	GLY123, GLY124, SER122, PRO121, TYR202

Based on the results of the docking of rutin, quercetin, and punicalin against S. aureus protein (1OJZ.pdb), it shows that rutin has the cDOCKER energy of -88.3993 kcal/mol. The cDOCKER energy produced by quercetin and punicalin is -69.9095 kcal/mol and -42.3552 kcal/mol, respectively. The cDOCKER energy produced by rutin, quercetin, and punicalin is lower when compared to the cDOCKER energy produced by chloramphenicol. It is -38.34 kcal/mol but higher when compared to the standard NAD ligand, which has cDOCKER energy of -90.9874 kcal/mol (Table 6). It shows that the compounds rutin, quercetin, and punicalin are more stable than chloramphenicol. However, the standard ligands are more stable when compared to rutin, quercetin, and punicalin, so it can be concluded that the tested compounds rutin, quercetin, and punicalin have potential as an antibacterial against S. aureus protein (1OJZ. pdb).

The hydrogen bonds formed by the standard ligand to the S. aureus protein (1OJZ.pdb) form 14 important amino acid bonds, including 10 hydrogen bonds with amino acid residues, GLU133, ASP90, ASN88, ARG85, THR37, SER138, GLN178, GLU180, LEU86, GLN140. 1 phi-phi bond, namely TYR91, 1 phi alkyl bond, namely LEU147, and 2 phi anion bonds ARG48 and ARG150. In routine compounds, 12 important amino acid bonds are formed, including 9 hydrogen bonds to amino acid residues, namely GLN140, LEU86, ARG85, TYR136, ASN44, TYR36, ARG150, ASP90, and ASN 88, and 2 phi alkyl bonds, namely ALA146, and LEU147. One phi anion bond, namely ARG48. In the quercetin compound, 8 important amino acid bonds are formed, including 5 hydrogen bonds, namely ASN68, ASP90, SER138, TYR36, SER40. 3 phi anion bonds namely ARG48, ARG85, ARG150. The punicalin compound forms 8 important amino acid bonds, including 6 hydrogen bonds, namely THR45, SER40, SER38, ARG150, ASN88, and ASP90. 1 phi anion bond, namely ARG48. Meanwhile, chloramphenicol forms 5 important amino acid bonds, including 4 hydrogen bonds, namely MET209, GLY1234, GLY124, and SER122. 1 phi-phi bond that is pro121. From the data that has been generated, it can be concluded that routine compounds, quercetin, and punicalin have the potential as antibacterials, judging from the cDOCKER energy and the type of interaction bond formed with the important amino acid residues of the target protein used.

Based on the results of the docking of rutin, quercetin, and punicalin against E. coli protein (6GOS.pdb), it shows that rutin has a cDOCKER energy of -52.352 kcal/mol, the cDOCKER energy produced by quercetin is -52.3668 kcal/mol, the cDOCKER energy that is produced by punicalin, namely -57.9239 kcal/mol. The cDOCKER energy produced by rutin, quercetin, and punicalin is lower than the cDOCKER energy produced by chloramphenicol which is -21.5433 kcal/mol, and the cDOCKER energy produced by the standard league is -47.7613 kcal/mol (Table 7). This shows that rutin, quercetin, and punicalin are more stable than chloramphenicol and the standard or original FMN ligands. So it can be concluded that the routine test compounds, quercetin, and punicalin have the

potential as an antibacterial against E. coli protein (6GOS.pdb).

The hydrogen bonds formed by standard ligands to E. coli protein (6GOS.pdb) form 8 important amino acid bonds, including 5 hydrogen bonds with amino acid residues, namely GLY124, TYR202, GLY123, SER122, and PRO121. 2 phi alkyl bonds namely MET2019 and MET212. 1 phi anion bond, namely ARG117. In routine compounds, 6 important amino acid bonds are formed, including 4 hydrogen bonds to amino acid residues, namely TYR202, GLY124, PRO121, and SER122. 1 phi anion bond, namely ARG117. In the quercetin compound, 7 amino acid bonds are formed, namely 2 hydrogen bonds, TYR202 and SER122. One carbon-hydrogen bond is GLY124. One phi alkyl bond, namely MET209. The punicalin compound forms 8 amino acid bonds, including 5 hydrogen bonds, namely PRO121, TYR127, ALA125, SER122, and GLY123. 1 phi alkyl bond MET209. 2 phi anion bonds SER208, ARF205. Meanwhile, chloramphenicol forms 5 important amino acid bonds, including 4 hydrogen bonds, namely MET209, GLY123, GLY124, and SER122. One phi-phi bond, i.e., PRO121. From the data that has been generated, it can be concluded that routine compounds, quercetin, and punicalin have in silico potential as antibacterials, judging from the cDOCKER energy and the type of interaction bond formed with the important amino acid residues of the target protein used.

Punicalin showed better activity than rutin and quercetin of the three compounds that had been docked with E. coli protein (6GOS.pdb). It follows the phytochemical test, where the compound punicalin (tannin derivative) is active against ethyl acetate and methanol extracts. It is also in line with the in vitro test, where the ethyl acetate and methanol extracts were active against E. coli bacteria.

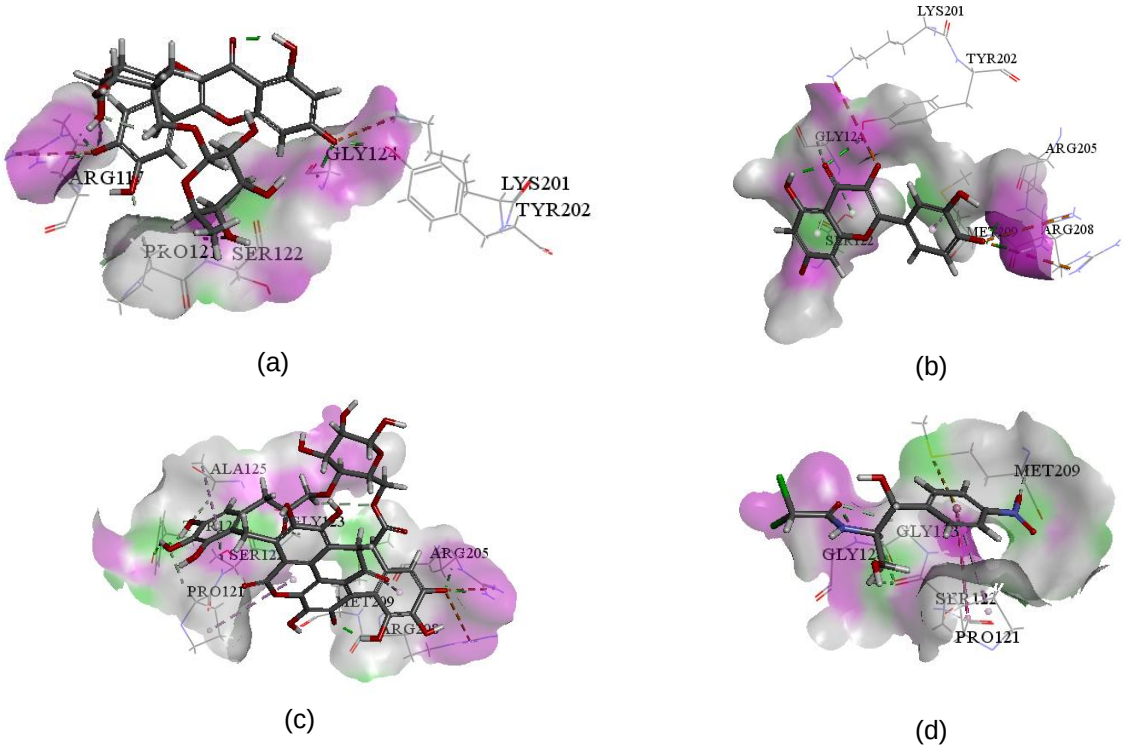

Figure 2. (a) 3D bond interaction of rutin, (b) 3D interaction of quercetin, (c) 3D interaction of punicalin, (c) 3D interaction of chloramphenicol with target protein 6GOS.pdb

CONCLUSION

Based on the research results, the secondary metabolites in the n-hexane extract are steroids and alkaloids, the ethyl acetate extract contains alkaloids and tannins, and the methanol extract contains flavonoids, alkaloids, and tannins.

The results of the in vitro antibacterial activity test showed that the inhibition zones of the n-hexane, ethyl acetate, and methanol extracts were included in the category with a strong inhibition zone for the test bacteria, with an inhibition zone of 19.2 mm for S. aureus bacteria and 15.6 mm for S. aureus bacteria. E. coli at a concentration of 50% of ethyl acetate extract.

The results of molecular docking analysis of the compounds rutin, quercetin, and punicalin based on cDOCKER energy, number of bonds, and types of bonds to protein 1OJZ.pdb for S. aureus bacteria and protein 6GOS.pdb for E. coli bacteria have potential as antibacterials.

REFERENCES

1. Utami ER. Antibiotika, Resistensi, Dan Rasionalitas Terapi. Sainstis. 2012;1(4):191–8.
2. Isti'Azah, Nida dan Zuhrotun A. Potensi Theobroma Cacao L. Sebagai Antibiotik Alami. Farmaka. 2020;17(1):1–9.
3. Setiawan A, Tee SA. Aktivitas Antibakteri Ekstrak Etanol Daun Benalu Jati(Loranthus Spp) Terhadap Bakteri Staphylococcus aureus. War Farm. 2016;5(1):59–65.
4. Liu, C., Cai, D., Zhang, L., Tang, W., Yan, R., Guo, H., dan Chen, X. 2016. Identification of hydrolyzavle tannins (punicalagin, punicalin and geraniin) as novel inhibitors of hepatitis B virus covalently closed circular DNA. *Manuscript.* Chinese Academy of Sciences. 8(26) : 3-5.
5. Malau, N. D., & Azzahra, S. F. (2020). Analysis Docking Of Plasmodium Falciparum Enoyl Acyl Carrier Protein Reductase (Pfenr) With Organic Compunds From Virtual Screening Of Herbal Database. *Acta Chimica Asiana*, *3*(1), 127-134.
6. Rizma, B. R. P., Mubarok, Y. Z., Lestari, D. F. D., & Ananto, A. D. (2021). Molecular Study of Antiviral Compound of Indonesian Herbal Medicine as 3CLpro and PLpro Inhibitor in SARS-COV-2. *Acta Chimica Asiana*, *4*(2), 127-134.
7. Nasution, H., Enizan, N., Nurlaili, N., & Syahri, J. (2020). Design of trolox compounds as antioxidant and their analysis using Quantitative Structure Activity Relationship. *Acta Chimica Asiana*, *3*(2), 181-186.Yulian, M., dan Safrizal. 2018. Uji aktivitas antioksidan daun benalu kopi (*Loranthus Ferrugineus* Roxb.) dengan metode DPPH (1,1,-Difenil-2-Pikrihidrazil). Lantanida Journal. 6(2) : 193-199
8. Monalisa, D., Handayani, T., dan Sukmawati, D. 2011. Uji Daya Antibakteri Ekstrak daun Tapak Liman (*Elephantopus scaber* L.) Terhadap *Staphylococus aureus* dan *Salmonella typhi*. *Jurnal BIOMA*. 9(2) : 133-20.
9. Darsana, I. G. O. 2012. Potensi Daun Binahong (*Anredera cordifolia* (tenore) Steenis) dalam menghambat Pertumbuhan Bakteri *Escherichia coli* Secara In vitro. Indonesia Medicus Veterinus. 1(3) : 337-351.
10. Steinberg, D. A., & Lehrer, R. I. (1997). Designer assays for antimicrobial peptides. In *Antibacterial Peptide Protocols* (pp. 169-186). Humana Press.
11. Hossain, M., & Sultana, F. (2018). Methods for the characterization of plant-growth promoting rhizobacteria. In *Host-Pathogen Interactions* (pp. 307-328). Humana Press, New York, NY.
12. Ansari, M. A., & Asiri, S. M. M. (2021). Green synthesis, antimicrobial, antibiofilm and antitumor activities of superparamagnetic γ-Fe2O3 NPs and their molecular docking study with cell wall mannoproteins and peptidoglycan. *International Journal of Biological Macromolecules*, *171*, 44-58.
13. Jubair, N., Rajagopal, M., Chinnappan, S., Abdullah, N. B., & Fatima, A. (2021). Review on the antibacterial mechanism of plant-derived compounds against multidrug-resistant bacteria (MDR). *Evidence-Based Complementary and Alternative Medicine*, *2021*.
14. Álvarez-Martínez, F. J., Barrajón-Catalán, E., Encinar, J. A., Rodríguez-Díaz, J. C., & Micol, V. (2020). Antimicrobial capacity of plant polyphenols against gram-positive bacteria: A comprehensive review. *Current medicinal chemistry*, *27*(15), 2576-2606.