Jurnal Kesehatan dr. Soebandi
ISSN : 2302-7932 (Print)
ISSN : 2527-7529 (OnLine)

In Silico Study of Bioactive Compounds of Betel (Piper betle) as Antidiarrhea Assisted by Liquid Chromatography – Mass Spectroscopy

Khrisna Agung Cendekiawan[1*], Kintoko[2], Sapto Yuliani[3], Linda Suci Anggraeni[4], Lailatul Fitria Ningrum[5], Siti Nur Hasana[6], Firdha Aprillia Wardhani[7]

[1,2,3] Doctoral Pharmacy Program, Faculty of Pharmacy, Ahmad Dahlan University,
[4,5,6]Pharmacy Study Program, Universitas dr. Soebandi,
[7]Pharmacy Study Program, Universitas Jember,

***Corresponding Author**.kintoko@pharm.uad.ac.id

Submited : 17th July 2025 , Accepted : 22th October 2025
Doi : https://doi.org/10.36858/jkds.v13i2.975

ABSTRACT

Diarrhea is a pathological condition characterized by abnormal frequency and consistency of fecal excretion. The betel plant (Piper betle L.) is a perennial climbing plant with woody stems at its base, capable of reaching lengths of up to 15 m. This study aimed to identify and analyze the bioactive compounds present in the methanolic extract of Piper betle leaves. The principal constituents reported to exhibit antidiarrheal activity include flavonoids, tannins, essential oils, and alkaloids. Among these, flavonoids, particularly quercetin, are known to inhibit acetylcholine release and reduce intestinal motility, thereby contributing to antidiarrheal effects. The in silico investigation was conducted through several computational stages, including ligand and receptor preparations, Lipinski's rule prediction, and analysis of amino acid interactions. The tools and software utilized in this study included PubChem, the RCSB Protein Data Bank, AutoDock Tools, and Discovery Studio Client 4.1. Docking analysis revealed that quercetin exhibited a favorable binding energy of –9.72 kcal/mol, indicating a potentially strong interaction with the target receptor. Furthermore, amino acid interaction analysis demonstrated a similarity between quercetin and the native ligand at residue VAL:209, with an RMSD of 1.83 Å. Nevertheless, the present findings cannot be fully validated, and the available computational data are insufficient to draw definitive conclusions regarding quercetin's antidiarrheal efficacy. Therefore, further in vitro and in vivo experimental studies are required to substantiate these in silico predictions and to confirm the pharmacological potential of quercetin derived from Piper betle leaves.

Keyword: In Silico, Quercetin, Antidiarrheal

ABSTRAK

Diare adalah kondisi patologis yang ditandai dengan frekuensi dan konsistensi feses yang abnormal. Tanaman sirih (Piper betle L.) merupakan tanaman merambat menahun dengan batang berkayu di pangkalnya, yang dapat mencapai panjang hingga 15 m. Penelitian ini bertujuan untuk mengidentifikasi dan menganalisis senyawa bioaktif yang terdapat dalam ekstrak metanol daun sirih. Konstituen utama yang dilaporkan menunjukkan aktivitas antidiare meliputi flavonoid, tanin, minyak atsiri, dan alkaloid. Di antara senyawa-senyawa tersebut, flavonoid, terutama kuersetin, diketahui dapat menghambat pelepasan asetilkolin dan mengurangi motilitas usus, sehingga berkontribusi terhadap efek antidiare. Investigasi in silico dilakukan melalui beberapa tahap komputasi, termasuk persiapan ligan dan reseptor, prediksi aturan Lipinski, dan analisis interaksi asam amino. Alat dan perangkat lunak yang digunakan dalam studi ini meliputi PubChem, RCSB Protein Data Bank, AutoDock Tools, dan Discovery Studio Client 4.1. Analisis docking mengungkapkan bahwa quercetin menunjukkan energi ikat yang menguntungkan sebesar -9,72 kkal/mol, yang menunjukkan potensi interaksi yang kuat dengan reseptor target. Lebih lanjut, analisis interaksi asam amino menunjukkan kesamaan antara quercetin dan ligan asli pada residu VAL:209, dengan RMSD sebesar 1,83 Å. Namun demikian, temuan ini tidak dapat sepenuhnya divalidasi, dan data komputasi yang tersedia tidak cukup untuk menarik kesimpulan definitif mengenai efikasi antidiare quercetin. Oleh karena itu, studi eksperimental in vitro dan in vivo lebih lanjut diperlukan untuk mendukung prediksi in silico ini dan untuk mengonfirmasi potensi farmakologis quercetin yang berasal dari daun sirih.

Kata Kunci: *In Silico; Quercetin; Antidiare*

*Correspondence author: kintoko@pharm.uad.ac.id

How to Cite : Cendekiawan, K., Kintoko, K., Yuliani, S., Anggraeni, L. S., Ningrum, L. F., Hasana, S. N., & Wardhani, F. A. In Silico Study of Bioactive Compounds of Betel (Piper Betle) as Antidiarrhea Assisted by Liquid Chromatography – Mass Spectroscopy. *Jurnal Kesehatan Dr. Soebandi*, *13*(2). https://doi.org/10.36858/jkds.v13i2.975

Jurnal Kesehatan dr. Soebandi
ISSN : 2302-7932 (Print)
ISSN : 2527-7529 (OnLine)

Introduction:

Indonesia possesses vast potential for the development of natural-based medicines owing to its rich biodiversity. One promising herbal plant that has been traditionally used for medicinal purposes is the betel plant (*Piper betle* L.). This plant is easily recognized by the shape of its leaves and its distinctive aromatic scent released when the leaves are crushed or torn apart. *Piper betle* is a creeping, climbing, and woody shrub that can grow up to 15 m in length. It has long been used as a traditional herbal remedy because of its abundance of secondary metabolites and bioactive compounds (Hermanto *et al*., 2023).

Among its phytochemical constituents, flavonoids, tannins, essential oils, and alkaloids are believed to contribute to its antidiarrheal activity. Flavonoids, particularly quercetin, inhibit the release of acetylcholine and reduce intestinal contractions. Tannins have astringent effects that slow intestinal peristalsis, whereas essential oils and alkaloids act as antimicrobial agents that inhibit or eliminate intestinal pathogens (Sintia Manek *et al*., 2020).

Diarrhea is a pathological condition characterized by abnormal stool consistency and increased defecation frequency, typically more than three times a day, with or without mucus or blood (Sintya Septiani *et al*., 2025). The etiology of diarrhea includes infection, malabsorption, dietary factors and psychological stress. Transmission often occurs through contaminated food or water and is exacerbated by poor hygiene (Hartati *et al*., 2023). Infectious diseases, including diarrhea, continue to be a significant public health concern in developing countries, reducing the average life expectancy by approximately 1.97 years (RR Pratama *et al*., 2024).

Given the pharmacological potential of *Piper betle*, it is crucial to investigate its bioactive compounds as potential antidiarrheal agents. This study employed liquid chromatography-mass spectrometry (LC–MS) to identify phytochemical constituents and molecular docking to predict the biological activity of these compounds. Molecular docking is an essential *in silico* technique used in drug discovery and design, allowing researchers to simulate and visualize binding interactions between ligands and target receptors.

The use of *in silico* methods offers several advantages: it reduces the need for extensive laboratory experiments, minimizes animal testing, and saves time and resources (Putri *et al*. 2024). Furthermore, *in silico* analysis enables the prediction of pharmacokinetic properties, drug-like properties, and binding affinities before *in vitro* and *in vivo* validation. By integrating computational screening with analytical techniques such as LC–MS, researchers can identify and optimize bioactive compounds with high therapeutic potentials. Thus, *in silico* studies serve as a pivotal early-stage approach in exploring the pharmacological mechanisms of natural compounds like *Piper betle* and their potential as antidiarrheal agents.

Methods:

Tool

Candidate receptor structures were selected from the Protein Data Bank according to stringent quality criteria: (i) presence of a co-crystallized ligand in the orthosteric site; (ii) experimental resolution ≤ 3.0 Å; (iii) complete, well-ordered binding-site residues without chain breaks; (iv) human origin or closely homologous construct without distorting mutations; (v) retention of essential cofactors/ions; (vi) acceptable backbone geometry (>90% Ramachandran favored; <2% outliers); and (vii) chemically consistent protonation/charge states near pH 7.4. Structures were cleaned (nonessential waters were removed), hydrogens/charges were added, and the docking grid was centered on the native ligand to encompass the binding pocket. The receptors were validated by redocking their native ligands; only receptors achieving RMSD ≤ 2.0 Å for the top-ranked pose were carried forward.

Material

The materials used are six receptors including Cytochrome P450 1A1 (PDB code id: 4i8v), Ephrin Type-B Receptor 4 (PDB code id: 2VWY), Multidrug resistance protein 1 (PDB code id: 7A69), Dihydrofolate reductase (PDB code id: 5HT4), Extracellular calcium-sensing

Jurnal Kesehatan dr. Soebandi
ISSN : 2302-7932 (Print)
ISSN : 2527-7529 (OnLine

receptor (PDB code id: 5FBH), Rap guanine nucleotide exchange factor 4 (PDB code id: 4MGI), with the test organism being Homo sapiens downloaded from https://www.rcsb.org/pdb/home/home.do, the three-dimensional structures of the six receptors were created and then prepared using the MarvinSketch program version 24.1.0.

Betel Leaf Extract (*Piper betle*)

Betel leaves (*Piper betle*) were used as the sample. The betel leaf samples were dried by airing at room temperature, 25ºC-30ºC, for 3 × 24 h. The betel leaf samples were then ground until a fine powder was obtained. This research stage included sample collection, extraction with methanol using the total maceration method, and concentration using evaporation at a temperature of ±40°C (Putri *et al*., 2024).

Identification of Phytochemical Profiles Using LC-MS

The separation of compounds from betel leaves (*Piper betle*) was performed using an LC-MS/MS instrument with a QToF-MS detector (Xevo G2-S QToF, Waters, USA). The analysis began with four sample injections using a 5 µL microsyringe into a UPLC column (ACQUITY UPLC® H-Class System, Waters, USA) of type ACQUITY UPLC BEH C18, with a particle size of 1.8 µm and dimensions of 2.1 × 50 mm. The samples were ionized in the positive ESI mode (+) with a mass range of 50–1200 m/z. The operational conditions included an ion source temperature of 100°C and a desolvation temperature of 350°C. The separation of ions was performed using a Q-ToF analyzer before the detector read the signal. A gradient elution system was applied using eluents (A) water with formic acid and (B) acetonitrile with formic acid at a flow rate of 0.2 mL/min. Chromatogram peaks were interpreted using MassLynx software, which was used for data acquisition and analysis.(RR Pratama *et al*., 2024).

Ligand Preparation

The ligand to be tested was then prepared using Marvin sketch. The ligand was conditioned at pH 7.4 and saved in the MRV format. Subsequently, a conformational search was performed and saved as a file in pdb and mol2 formats. (Mulyati & Sutjiningtyas, 2022).

Protein Preparation

Protein structures were obtained from databases such as the Protein Data Bank (PDB). Docking was performed using AutoDock Tools. Docking results are then selected from those with low binding affinity values and saved in PDB format.(Agu *et al*., 2023).

Validation of Docking Method

The molecular docking method was validated using AutoDock Tools 1.5.6 by performing a redocking process for each native ligand and its corresponding receptor to evaluate the accuracy and reliability of the docking protocol. Each docking simulation was performed with 30–80 independent runs per receptor-ligand pair, following the Lamarckian Genetic Algorithm (LGA) protocol implemented in AutoDock. The number of runs was selected to ensure adequate conformational sampling and convergence of the docking poses.

For each receptor, the docking grid was centered on the coordinates of the native ligand, with the dimensions adjusted to fully encompass the active site. The best-ranked conformation was determined based on the lowest binding energy (ΔG) value and by visually inspecting the ligand orientation relative to the native pose.

The accuracy of the docking procedure was assessed by calculating the Root Mean Square Deviation (RMSD) between the docked pose and crystallographic conformation of the native ligand. A receptor was considered valid for subsequent docking analysis if the RMSD value was ≤ 2.0 Å, indicating a reliable reproduction of the experimental binding mode. (Astuty & Komari, 2022).

Docking Results Analysis

Molecular docking data analysis was performed based on Gibbs free binding energy (ΔG) values, RMSD values, amino acid interactions, visualization of docking results, and the Lipinski rule (Rule of Five). To see amino acid interactions, the Molegro Molecul Viewer

Jurnal Kesehatan dr. Soebandi
ISSN : 2302-7932 (Print)
ISSN : 2527-7529 (OnLine

(MMV) software was used in 2D and 3D forms.(Fadillah *et al*., 2023).

Ligand-Based Drug Likeness Screening (drug scan)

Ligand-Based Drugs Likeness Screening (Drug Scan) Observation of drug candidates is carried out on resveratrol ligands by paying attention to Lipinski's Rule of Five. (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp)which includes a molecular weight of <500 g/mol, hydrogen bond donor (<5), hydrogen bond acceptor (<10), molar refractivity between 40-130 (Cavin *et al*., 2024).

Results :

Identification of chemical compounds using LC-MS.

Compounds from the betel leaf extract are obtained through analysis using the Liquid Chromatography-Mass Spectrometry (LC-MS) instrument. The results of the LC/MS-MS data analysis will produce a chromatogram in the form of a peak height flow, and the molecular weight of the compounds in the extract will be obtained, allowing the amount of compounds in each sample to be determined. (Novia *et al*., 2023).

Figure 1. Total Ion Chromatogram of *Piper betle*

Subsequently, the spectra obtained from LC-MS were analyzed using the MassLynx software to identify the composition and classify the compounds corresponding to each peak in the spectra. The results are presented in Table 1.

Table 1. Retention Time, Compound Name, Chemical Structure, and Molecular Weight Obtained from LC-MS Spectra

Retention Time (minutes)	Compound Name (prediction)	Chemical Structures	Molecular Weight (g/mol)
7.7	Unknown compound	---	206.23
2.65	Unknown compound	---	204.34
14.73	**Eugenol**	**$C_{12}H_{14}O_3$**	**206.23**
3.66	**Hydroxychavicol**	**$C_9H_{10}O_2$**	**150.17**
9.3	**Kaempferol**	**$C_{15}H_{10}O_6$**	**286.23**
19.22	Unknown compound	---	358.38

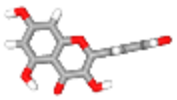
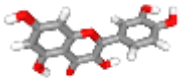
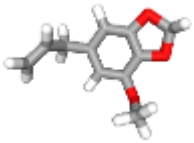
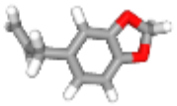
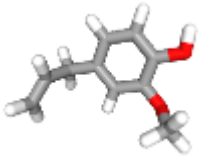
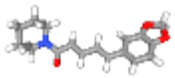
Jurnal Kesehatan dr. Soebandi
ISSN : 2302-7932 (Print)
ISSN : 2527-7529 (OnLine)

Retention Time (minutes)	Compound Name (prediction)	Chemical Structures	Molecular Weight (g/mol)
3.1	Unknown compound	---	178.22
5.76	**Myristicin**	**$C_{11}H_{12}O_3$**	**192.21**
13.48	Unknown compound	---	285.33
9.03	**Quercetin**	**$C_{15}H_{10}O_7$**	**302.23**
6.98	**Safrole**	**$C_{10}H_{10}O_2$**	**162.18**

Ligand preparation

The test ligand used in this in silico test is a compound from the betel leaf plant (*Piper betle*). Molecular docking simulations were carried out on the 6 compounds, in table 2, to determine their interactions with the 6 receptors.

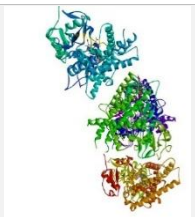
Table 2. Structure of test ligands from betel leaf (*Piper betle*).

Compound	Structure	Compound	Structure
Kaempferol		Quercetin	
Myristicin		Safrol	
Eugenol		Hydroxychavicol	

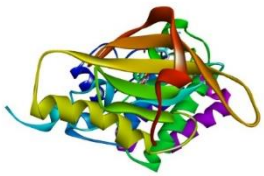
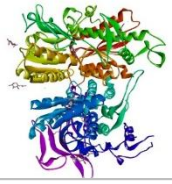
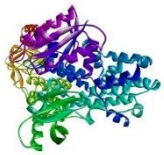
Protein preparation

The receptors used in this study were six receptors including *Cytochrome P450 1A1* (PDB code id: 4I8V), *Ephrin Type-B Receptor 4* (PDB code id: 2VWY), *Multidrug resistance protein 1* (PDB code id: 7A69), *Dihydrofolate reductase* (PDB code id: 5HT4), *Extracellular calcium-sensing receptor* (PDB code id: 5FBH), *Rap guanine nucleotide exchange factor 4* (PDB code id: 4MGI). The following is a 3D visualization of the antidiarrheal receptor:

Table 2. List of native ligands for each receptor

Receptor	Structure	Native ligand
4I8V		BHF (*2-phenyl-4h-benzo[h]chromen-4-one*)

Jurnal Kesehatan dr. Soebandi
ISSN : 2302-7932 (Print)
ISSN : 2527-7529 (OnLine

Receptor	Structure	Native ligand
2VWY		7X5N(*5-chloro-1,3-benzodioxol-4-yl)-n-(3 methylsulfonylphenyl)pyrimidine-2,4-diamine*)
7A69		R1Q (*vincristine*)
5HT4		65J(*7-ethyl-6-[(3 methoxyphenyl)sulfanyl] -5-methyl-7H-pyrrolo[2,3-d]pyrimidine-2,4-diamine*)
5FBH		CSO (*s-hydroxycysteine*)
4MGI		CMP (*adenosine-3',5'-cyclic-monophosphate*)

Validation of Docking Method

Validation of the molecular docking method was carried out by redocking between the native ligand, namely the target receptor downloaded from the protein data bank site using Autodock Tools software. The analysis used to evaluate the validation results is the RMSD value.

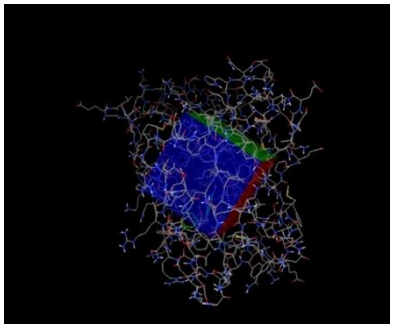


Figure 2. Protein Preparation Using Autodock Tools

Jurnal Kesehatan dr. Soebandi
ISSN : 2302-7932 (Print)
ISSN : 2527-7529 (OnLine

The conformation of the docking results obtained is then aligned with the conformation of the native ligand crystallography expressed in the RMSD value.

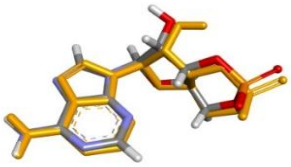


Figure 3. Conformation of native ligand structure with re-docking results.

Table 3 shows the RMSD value of each native ligand; all native ligands meet the validation criteria of the docking method, except the native ligand R1Q, which does not meet the requirements or has an RMSD value of 31.96.

Table 3. Redocking results

Native ligand	Run	Binding energy (kcal/mol)	Rmsd (Å)
BHF	77	-10.08	1.02
7X5N	30	-9.57	0.60
R1Q*	11	-2.78	31.96
CSO	35	-7.47	3.25
65J	26	-2.53	1.83
CMP	35	-9.01	0.55

out of criteria

Ligand Based Drug Likeness Screening (drug scan)

This analysis examines the similarity properties of drugs, which state that a compound has properties similar to a drug.

Table 4. Lipinski results for each compound

Compound	Molecular Weight (<500g/mol)	Hydrogen Donor (<5)	Hydrogen Acceptor (<10)	Log p (<5)
Kaempferol	286,239	4	6	2,2824
Quercetin	302,238	5	7	1,988
Eugenol	295,382	0	3	3,4559
Myristicin	322,269	7	8	0,7426
Safrol	409,073	0	2	2,2166
Hydroxychavicol	385,37	2	5	2,3767

Docking Results Analysis

This stage is done using PyRx software to run Autodock. After the Autodock run is complete, a list of compounds appears along with the docking results data, including the binding energy value.

Table 5. Molecular docking results of native ligand BHF with test ligands.

Compound	Binding energy (kcal/mol)				
	BHF	**7X5N**	**65J**	**CSO**	**CMP**
Kaempferol	-8.08	-6.64	-6.47	-4.67	83.91
Eugenol	-7.54	-7.18	-6.41	-4.88	95.38
Quercetin	**-9.72**	-6.92	**-6.87**	**-5.46**	150.38

Jurnal Kesehatan dr. Soebandi
ISSN : 2302-7932 (Print)
ISSN : 2527-7529 (OnLine

Myristicin	-3.65	-6.58	-5.64	-5.14	194.49
Safrol	5.08	-4.20	-4.96	-3.73	**5.79**
Hydroxychavicol	-9.4	**-7.37**	-5.48	-5.39	199.33

Based on the molecular docking above, Quercetin is the best test ligand.which binds to several receptors is characterized by the lowest binding energy value.

Amino Acid Residue Interactions

After molecular docking is done, it is important to see the amino acid interactions. In general, possible interactions include hydrogen bonds, hydrophobic interactions, van der Waals interactions and electrostatic interactions.

Table 6. Results of amino acid residue interactions

Compound	Hydrogen Bonds	Hydrophobic Bonds
BHF	ASP: 313, ASN :222	GLY:316, ALA:317, LEU:496, PHE:224, PHE:258, LUE:312, ILE:115
7X5N	ALA:700, MET:696, GLU:694, ALA:645	ILE:621, LEU:747, ILE:677, LYS:647, ILE:691
65J	GLY :117, TYR:121	ILE:16, VAL:8, ALA:9, THR:56, SER:59, ILE:60, PHE:34, LEU:22
CSO	VAL:209 (hydrogen)	-
CMP	LEU:449, ALA:416	ALA:415, VAL:386, CYS:395, ARG:414
Quercetin	VAL:209	ALA:230, TRP:208

Discussion:

The next stage is the molecular docking process, namely, by preparing the protein and the test ligand. The ligands to be tested were prepared using MarvinSketch software. Compound preparation was carried out using clean 2D and protonation methods, selecting those with the lowest energy value. After that, protein preparation was carried out, and the protein structure was first obtained from a database such as the Protein Data Bank (PDB). The downloaded file was opened using the MOLEGRO molecular viewer software to separate the native ligand from the protein. After that, preparation was carried out by removing water molecules using Discovery Studio. The removal of water molecules aimed to reduce the computational burden, which resulted in a relatively long simulation time. Therefore, the presence of water molecules will make the simulation impractical to use. After removing the water molecules, hydrogen atoms and charges were added to the system. After the receptor was prepared, it was stored in the receptor—pdbqt file.(Kalontong *et al*., 2022).

Next, the method was validated by redocking the native ligand using the AutoDock Tools software. The conformation of the docking results is then aligned with that of the native ligand crystallography, as expressed in the RMSD value. The grid box setting in the docking process is used to determine the binding space of the ligand to be docked.(AB Pratama *et al*., 2021). Table 3 shows the RMSD value of each native ligand; all native ligands meet the validation criteria of the docking method, except the native ligand R1Q, which does not meet the requirements or has an RMSD value of 31.96. So it can be said that the validation of the docking method on the native ligand R1Q is considered less accurate, or the protein used is unstable.

To see the physicochemical properties of a ligand when crossing the cell membrane in the body, the Lipinski test is carried out.(Kalontong *et al*., 2022). Drug molecules that do not meet Lipinski's rules are not recommended for oral use but are more recommended for injection. Molecular weight affects the ability of compounds to diffuse through cell membranes by passive diffusion. Ligands with a molecular weight of less than 500 g/mol penetrate cell membranes more easily than ligands with a molecular weight of more than 500 g/mol. Donor

and acceptor hydrogen bonds are parameters that describe the hydrogen bond capacity of a compound needed in the absorption process; thus, if the number of donor hydrogen bonds is ≥10 and acceptor ≥ 5, the energy required in the absorption process will be greater. The following parameter is the log P value, which is related to the polarity of the ligand in fats, oils, and nonpolar solvents. (Setyawati *et al*., 2023). Table 4 contains information on the Lipinski values of the test ligands; all six test ligands have met the Lipinski requirements.

The next stage is the analysis of the docking results, during which the binding energy values of the six test ligands are obtained. Binding energy is the energy needed to bind to the target receptor. The lower (negative) binding energy value indicates that the affinity of the compound to the target protein is stronger, so that the bond becomes more stable. (Prasiska Wulandari *et al*., 2023). Based on the results of the molecular docking above, Quercetin is the best test ligand, binding to several receptors marked by the lowest binding energy value.

Visualization of docking results is done to determine the interaction between compounds with amino acid residues on protein receptors. Observation of amino acid residue interactions aims to identify interactions that occur between ligands and receptors. The presence of amino acid interactions allows for contact between the ligand and the receptor, enabling inhibitory activity. (Sari *et al*., 2020). Hydrogen bonds are interactions that can stabilize ligand bonds with receptors. Other interactions between ligands that can increase conformational stability are electrostatic interactions and van der Waals interactions.(Harefa *et al*., 2021). So it is crucial to analyze what hydrogen bonds are formed. As listed in Table 6, in this test, Quercetin forms hydrogen bonds, namely VAL209, and hydrophobic bonds between ALA230 and TRP208. While the native ligand CSO forms hydrogen bonds in the form of VAL209, no hydrophobic bonds are formed. Based on these results, it can be observed that quercetin has similarities with the native ligand CSO, namely in the hydrogen bond in the form of VAL209. The results obtained indicate that quercetin may have the same biological activity as the native ligand CSO.

The receptor protein structure requires further analysis using the Ramachandran plot to assess its quality. This plot shows the amino acid residues of the protein or enzyme structure. It has four parts: most favored regions, additional allowed regions, generously allowed regions, and disallowed regions (Kalontong *et al*., 2022). The Ramachandran diagram, based on the protein's secondary structure, was used for protein analysis. A protein structure is considered good if over 90% of the amino acid residues are in the preferred area and less than 20% are in quadrant IV (Aziz *et al*., 2022). The image above shows the Ramachandran plot results for the target protein Rap guanine nucleotide exchange factor 4 with PDB code 4MGI. It has 92.0% in the most favored region and 0.1% in the disallowed region. This means the structure of this protein is good because it meets the Ramachandran plot standards.

This study identified several active compounds in *Piper betle* using LC–MS and computer simulations. However, there are some limitations. First, the simulations used fixed protein structures from the Protein Data Bank. These do not show how proteins move in real life so that the interactions might be different in actual biological conditions. Second, the accuracy of the predictions depends on the quality of the protein structures and the software settings. Although some validation was done, not all protein structures met the required standards, which could affect the results. In addition, the study did not use molecular dynamics (MD) simulations to check the stability of the compound-protein complexes over time. MD simulations can provide more information about the stability and adaptability of the binding. Additionally, no laboratory or animal tests were performed to confirm the predicted effects. Therefore, although quercetin and other compounds showed good binding and interactions, these results are still preliminary. Future research should include laboratory tests, studies on how the body processes the compounds, and safety tests to fully understand

the potential of *Piper betle* as a treatment for diarrhea.

Conclusion:

Based on molecular docking, one test compound was found to have the best potential: quercetin, with the lowest binding energy of -9.72. In addition, the interaction of amino acid residues between quercetin and the native ligand CSO (receptor code PDB ID 5FBH) was similar, specifically at VAL: 209. Therefore, quercetin meets the criteria for a new drug candidate as an antidiarrheal.

References:

Agu, PC, Afiukwa, CA, Orji, OU, Ezeh, EM, Ofoke, IH, Ogbu, CO, Ugwuja, EI, & Aja, PM (2023). Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. Scientific Reports, 13(1). https://doi.org/10.1038/s41598-023-40160-2

Astuty, F., & Komari, N. (2022). Molecular Docking Study of Karwinaphthol B Compound from Dayak Onion Plant (Eleutherine palmifolia (L.) Merr) as Glucokinase Enzyme Inhibitor. In Molecular Docking Study | (Vol. 1, Issue 1). https://pubchem.ncbi.nlm.nih.gov/

Aziz, A., Andrianto, D., & Safithri, M. (2022). Molecular Docking of Bioactive Compounds from Wungu Leaves (Graptophyllum pictum (L) Griff) as Tyrosinase Inhibitors. In Indonesian Journal of Pharmaceutical Science and Technology Journal Homepage (Issue 2). http://jurnal.unpad.ac.id/ijpst/

Cavin, J., Sinaga, E., Kumaunang, M., & Tuther, P. (2024). In Silico Study: Antibacterial Activity of Resveratrol Compound Against Salmonella typhi Bacteria That Causes Typhoid Fever. Chem. Prog, 17(2), 138. https://doi.org/10.35799/cp.17.1.2024.55994

Frimayanti, N., Lukman, A., & Nathania, L. (2021). Molecular docking study of 1,5-benzothiazepine compound as an inhibitor of dengue DEN-2 NS2B/NS3 serine protease. Chempublish Journal, 6(1), 54–62. https://doi.org/10.22437/chp.v6i1.12980

Harefa, K., Hutauruk, D., Derliana Nainggolan, L., Pharmacy and Health Sciences, F., & Sari Mutiara Indonesia, U. (2021). Inhibitory Activity of 6lu7 Protease Enzyme of Sars-Cov-2 Virus by Flavonoid Compounds From Artocarpus Genus Plants By In-Silico Flavonoids Compound From Artocarpus Genus Inhibit 6lu7 Protease Enzyme Of Sars-Cov-2 Virus By In-Silico (Vol. 8, Issue 2). http://www.rscb.org/.protein

Hartati, S., Kamesyworo, & Elviani, Y. (2023). Risk Factors for Diarrhea Incidence in Toddlers in the Work Area of Pulau Pinang Health Center, Lahat Regency in 2022. Journal of Health and Medical Service, 2(1), 40–49. https://doi.org/10.55018/jakk.v2i1.15

Hermanto, LO, Nibenia, J., Sharon, K., & Rosa, D. (2023). As Traditional Medicine. In PHRASE (Pharmaceutical Science) Journal (Vol. 3, Issue 1). http://openjournal.wdh.ac.id/index.php/Phrase/index

Kalontong, P. K., Safithri, M., & Tarman, K. (2022). Molecular Docking of Active Compound of Spirulina platensis as TMPRSS2 Inhibitor to Prevent the SARS-COV-2 Infection. Indonesian Journal of Fisheries Processing, 25(2), 253–267. https://doi.org/10.17844/jphpi.v25i2.40645

Martha, RD, Danar, D., Safitri, YD, & Parbuntari, H. (2024). Identification of Flavonoid Compounds of Cumin Leaves (Plectranthus amboinicus) Using Liquid Chromatography-Mass Spectrometry (LC-MS) and Their Potential as Anticancer In-Vitro. Sainteks, 21(1), 67. https://doi.org/10.30595/sainteks.v21i1.21171

Melanie Ramadhina, F., Luthfiyatil, NF, & DIII Nursing Akper Dharma Wacana Metro, P. (2023). In Preschool Children (3-6 Years) In The Working Area Of Uptd Inspired Health Center North Metro Banjarsari. Jurnal Cendikia Muda, 3(3).

Mulyati, B., & Sutjiningtyas, S. (2022). In Silico Study Of Thiourea-Derived Compounds As Tyrosine Kinase Receptor Inhibitors. In Journal of Research Chemistry (Vol. 7, Issue 2). http://www.rscb.Org/

Literature Review: Molecular Docking of Natural Antioxidant Compound Activity in Several Plants in Indonesia. In Pharmaceutical Journal (Vol. 12, Issue 1).

Prasetiawati, R., Suherman, M., & Permana, B. (2021). Molecular Docking Study of Anthocyanidin Compounds Against Epidermal Growth Factor Receptor (EGFR) as Anti-Lung Cancer. In Indonesian Journal of Pharmaceutical Science and Technology Journal Homepage (Issue 1). http://jurnal.unpad.ac.id/ijpst/UNPAD8

Prasiska Wulandari, R., Gabriel, K., Aulia Nurdin, H., Hauna Pakhrul, DF, Salmandhiya Harits, S., Prameswari, N., Puteri Agustina Pribadi, A., Diah Lia Aulifa Department of Pharmacy, and, Pharmacy, F., & Padjadjaran Jalan Raya Bandung Sumedang, UK (2023). Indonesian Journal of Chemical Science In Silico Study of Secondary Metabolite Compounds in Parsley (Petroselinum crispum) as a Drug Terapy for Blood Cancer (Myeloproliferative Neoplasm (MPN)) targeting JAK-2. In J. Chem. Sci (Vol. 12, Issue 2). http://journal.unnes.ac.id/sju/index.php/ijcs

Pratama, AB, Herowati, R., & Ansory, HM (2021). Molecular Docking Study of Compounds in Nutmeg Essential Oil (Myristica fragrans H.) and Myristicin Derivative Compounds Against Skin Cancer Therapy Targets. Pharmaceutical Journal, 17(2), 233. https://doi.org/10.22146/farmaseutik.v17i2.59297

Pratama, RR, Septianto, A., Al-Hasyimi, SM, Herliana, H., Hasniah, H., & Muslikh, FA (2024). Exploration of Ethanol Extract Compounds of Belulang Grass (Eleusine indica (L.) Gaertn) Using LC-MS/MS-QTOF and Anti-Diarrhea Activity Test: In-Vivo. Bioscientist: Scientific Journal of Biology, 12(2), 2518. https://doi.org/10.33394/bioscientist.v12i2.14192

Putri, TZAD, Findrayani, RP, Isrul, M., & Lolok, N. (2024). Molecular Docking Study of Chemical Compounds from Mimosa pudica Herb on Inhibiting A-Glucosidase Enzyme as Antidiabetes Melitus. Jurnal Pharmacia Mandala Waluya, 3(4), 225–233. https://doi.org/10.54883/jpmw.v3i4.104

Ridhwan Anshor Alfauzi, Lilis Hartati, Danes Suhendra, Tri Puji Rahayu, & Hidayah, N. (2022). Extraction of Bioactive Compounds of Jengkol Skin (Archidendron jiringa) with Different Concentrations of Methanol Solvent as Additional Feed for Ruminant Livestock. Journal of Nutrition Science and Feed Technology, 20(3), 95–103. https://doi.org/10.29244/jintp.20.3.95-103

Ririn Setyawati, I., Pharmaceutical Studies, P., Pharmacy, F., National Science and Technology, I., Moh Kahfi, JI, Sawah, S., Jagakarsa, K., South Jakarta, K., & Khusus, D. (2023). In Silico Study: Active Compounds of Senggugu Roots (Clerodendrum serratum) on Inhibiting Human Chitotriosidase-1 (hCHIT1) Receptors as Antiasthma. 33(2). https://doi.org/10.37277/stch.v33i2

Santosa, A., Purnawarman, T., Mustika, AA, Rahma, A., & Lina Noviyanti Sutardi. (2023). Effectiveness of guava fruit infusion (Syzygium malaccense) as an antidiarrheal in mice (Mus musculus). Current Biomedicine, 2(1), 21–28. https://doi.org/10.29244/currbiomed.2.1.21-28

Sari, IW, Junaidin, J., & Pratiwi, D. (2020). Molecular Docking Study of Flavonoid Compounds of Cat's Whiskers Herb (Orthosiphon stamineus B.) on A-Glucosidase Receptors as Type 2 Antidiabetics. Farmagazine Journal, 7(2), 54. https://doi.org/10.47653/farm.v7i2.194

P., Syahrul Fadillah, Y., Fakih Kurniawan, M., & Rohmayanti, T. (2023). Molecular Docking Analysis of Compounds of Celery Extract (Apium Graveolens) For Angiotensin-Converting Enzyme 2 Inhibition Molecular Docking Analysis of Compounds of Celery Extract (Apium Graveolens) For Angiotensin-Converting Enzyme 2 Inhibition. http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp.

Silalahi, DK, & Wulandari, RA (2024). Impact of Environmental Factors on Diarrhea

Incidence in North Sumatra Province: Analysis of Indonesian Health Survey Data (Vol. 16, Issue 4).

Sintia Manek, M., Klau, ME, & Beama, CA (2020). Antidiarrheal Activity of Ethanol Extract of Betel Leaf (*Piper betle* L.) in Oleum Ricini-Induced Male Wistar Rats In the Chmk Pharmaceutical Scientific Journal (Vol. 3, Issue 2).

Sintya Septiani, R., Riwu, YR, & Takaeb, AEL (2025). Factors influencing the incidence of diarrhea in toddlers in the working area of the Rukun Lima Health Centre, Ende Regency.