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In-Silicon Test of *SARS-CoV-2* Ammunizer Potential in Active Compounds of Herbal Plants

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Abstract
Severe Acute Respiratory Syndrome *(SARS-CoV-2)* is a new type of coronavirus that has never been identified in history. *SARS-CoV-2* is the virus that caused the COVID-19 pandemic. The disease caused by *SARS-CoV-2* can be prevented through vaccines and antivirals, but the effort in providing and purchasing products from vaccines is quite expensive and time consuming. Herbal plants have long been used by local communities as a way to prevent a disease, one of which is caused by a virus. The purpose of this study is to determine the bioactive compounds of compounds in three herbal plants (*Curcuma xanthorrhiza, Zingiber officinale,* and *Cinnamomum burmanii*) that can be used as an ammunizer in preventing *SARS-CoV-2* in silico. The research method is in silico, with computational techniques using computer applications by testing through analysis of physicochemical properties, pharmacokinetics, toxicity, and molecular docking. The results obtained from physicochemical properties, pharmacokinetic properties and toxicity that six compounds have met the requirements of safe as oral drugs and potential as new drug candidates. The results of docking between six ligand compounds and ACE2 protein have a good interaction bond in inhibiting receptor activity and produce interaction visualization results in the form of amino acid residues. A study on predicting the activity of bioactive compounds conducted on six herbal compounds showed that the compounds had moderate or good activity as bioactives capable of fighting viruses.

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1. Introduction

In recent years, Indonesia and the global community have been affected by the COVID-19 pandemic caused by the *SARS-CoV-2* virus. The virus is transmitted both directly, through droplets and human-to-human contact, and indirectly, through contaminated objects and airborne transmission (Liu et al., 2020) [13]. *SARS-CoV-2* is a novel type of coronavirus that had not been previously identified. Among coronaviruses, two types are known to cause severe diseases, namely Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS). COVID-19 infection presents various symptoms, with the most prominent being shortness of breath and pneumonia. The average incubation period ranges from 5 to 6 days, with a maximum incubation period of up to 14 days (Wang, Zhou, 2020) [26].

The World Health Organization (WHO) has declared COVID-19 a global public health emergency and a pandemic. Both the WHO and the Centers for Disease Control and Prevention (CDC) recommend preventive measures such as maintaining physical distancing, wearing masks, and using antiseptics to reduce virus transmission (WHO, 2021). However, vaccine development requires a long period of time due to limited availability and the need to pass several stages of standardized testing, which also involves high costs (Nugroho & Hidayat, 2021) [17].

In emergency conditions, in addition to following WHO recommendations, the government encourages the public to consume nutritious food and beverages to help prevent COVID-19. One alternative is the use of herbal ingredients that are easily found in the surrounding environment as immune-supporting supplements. An immunizer is a natural herbal formulation that helps maintain and strengthen the immune system. It is commonly known as a supplement containing a combination of herbal extracts, minerals, vitamins, and active plant compounds, which function as protection against various diseases, particularly those affecting the immune system (Enesis Group, 2014) [4].

In Indonesia, herbal plants, commonly referred to as spices, are widely used as food ingredients and traditional medicine (Fikri et al., 2022) [6]. Herbal plants are believed to enhance the body's immune response. The bioactive compounds contained in herbs provide strong potential for identifying candidate drugs to combat various diseases, including *SARS-CoV-2* (Illian et al., 2021) [8]. In addition, the Quran frequently mentions plants as signs of God's power and as metaphors to convey wisdom. Certain plants, including fruits, are explicitly mentioned in the Quran, indicating that these references are meaningful and purposeful (Purwanto, 2008) [20].

The Quran, Surah Al-Ihsan, verse 17, mentions that one of the examples of heaven is a glass of drink that will be given to its inhabitants, namely a drink mixed with ginger, as stated in Surah Al-Ihsan, number 76, verse 17, which reads:

وَيُسْقَوْنَ فِيهَا كَأْسًا كَانَ مِزَاجُهَا زَنجَبِيلًا

"In Paradise they will be given to drink a glass (drink) mixed with ginger." (QS. Al Ihsan) (76): 17).

Herbal remedies can play an important role in combating viral infections (Singh, 2021) [24].

However, some people remain skeptical about their benefits because much of the information is based on traditional knowledge passed down through generations and is not always supported by scientific evidence. In the digital era, computational experiments are increasingly conducted in a scientific field known as bioinformatics, using a drug discovery approach called in silico analysis (Knapen et al., 2015) [12].

In silico testing is widely used as an initial step in the discovery of new drug compounds and to improve the efficiency of optimizing parent compounds before proceeding to *in vitro* and *in vivo* testing. This approach has several advantages, including predicting biological activity, generating hypotheses, and contributing to new discoveries or advancements in treatment and therapy (Kesuma et al., 2018) [11]. Recent studies using bioinformatics-based methods have demonstrated strong potential for identifying complex interactions among drugs, biological targets, and specific diseases (Liu et al., 2020) [13].

In this study, three types of herbal plants were selected: Javanese ginger (*Curcuma xanthorrhiza*), which contains two active compounds, xanthorrhizol and curcumin; ginger (*Zingiber officinale*), which contains shogaol, gingerol, and zingerone; and cinnamon (*Cinnamomum burmanii*), which contains cinnamaldehyde. Each compound from these herbal plants was evaluated for its biological activity to determine its potential as a candidate drug or immunizer in maintaining the body's immune system. Previous studies have shown that Javanese ginger contains essential oils, particularly xanthorrhizol, which exhibit anti-inflammatory, antibacterial, antiparasitic, and antiviral properties (Afriani Arif et al., 2022) [1].

2. Method

This study employed a quasi-experimental research design conducted in silico to predict the potential of candidate drug compounds. The testing techniques included analysis of physicochemical properties, pharmacokinetics, toxicity, and molecular docking. The research was conducted at the University of Muhammadiyah Malang from January to March 2024. The in silico procedures involved predicting compound biological activity, evaluating physicochemical characteristics, analyzing pharmacokinetic and toxicity profiles, and performing molecular docking analysis. Molecular docking was carried out to identify candidate compounds with potential as immunizers or antiviral agents against the Angiotensin-Converting Enzyme 2 (ACE2) receptor. Information regarding in silico studies on the antiviral activity of herbal plants such as temulawak (*Curcuma xanthorrhiza*), red ginger (*Zingiber officinale*), and cinnamon (*Cinnamomum burmanii*) in inhibiting the ACE2 receptor through molecular docking approaches remains limited. Therefore, this study is necessary to provide scientific evidence and contribute to the existing body of knowledge in this field.

The tools used in this study include a set of laptops with specifications of the xiaomi redmi book brand and an Intel® 3i .- 115GB processor (3.0 GHz, 2 Core, 4 Thread, 6MB Cache), OS Windows 10 and 8 GB RAM capacity, using software including Pyrx 0.8, Discovery Studio Visualizer, Pymol, Autodocktools-1.5.6, toxtree v2.6.13, PubChem, protein data bank, preAdmet, Swissadme, Lipinski's rule of five, and open knowledge maps. The materials used in this study are active compounds from the herbal temulawak (*Curcuma xanthorrhiza*) including xanthorrhizol and curcumin, herbal ginger (*Zingiber officinale*) including shogaol, gingerol, and zingerone, and herbal cinnamon (*Cinnamomum burmanii*) namely cinnamaldehyde which were downloaded 2d, 3d structures, and canonical smiles codes via PubChem. These compounds will be used as ligands or test compounds. While the target protein receptor uses angiotensin converting enzyme 2 (ace2) which was downloaded 3d crystal structure via the protein data bank (pdb) page with data code 7bnv.

Research Procedures

1. Analysis of Prediction of Bioactive Compounds of Javanese Turmeric (*Curcuma xanthorrhiza*), Red Ginger (*Zingiber officinale*), and Cinnamon (*Cinnamomum burmanii*)

Compounds from the herbal temulawak (*Curcuma xanthorrhiza*) include xanthorrhizol and curcumin, herbal ginger (*Zingiber officinale*) include shogaol, gingerol, and zingerone, and herbal cinnamon (*Cinnamomum burmanii*) namely cinnamaldehyde are predicted using the PASS Online software on the pagehttp://www.pharmaexpert.ru/passonline/. The initial procedure is to download the Canonical SMILES code via PubChem on the pagehttps://pubchem.ncbi.nml.nih.gov.Enter the SMILES code on the PASS Online page, predict the activity by clicking 'Get Prediction', and wait a few moments for the

results to appear. Results are obtained by comparing the Potential Activity (Pa) values of compounds with potential antiviral properties.

2. Physicochemical, Pharmacokinetic, and Toxicity Tests of Temulawak (*Curcuma xanthorrhiza*), Red Ginger (*Zingiber officinale*), and Cinnamon (*Cinnamomum burmanii*) Compounds

Physicochemical testing has predictive parameters such as molecular weight (MW), the logarithm of the octanol/water partition coefficient (Log P), donor hydrogen bonds, and receptor hydrogen bonds. Test predictions use Lipinski's Rule of Five software, which can be accessed through the website. http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp.Physicochemical test parameters aim to evaluate the similarity of compounds to oral drug characteristics that have biological activity in humans (Fikri et al., 2022) [6]. The procedure begins by entering the 2D structure in PDB format, then pressing submit to begin the prediction. After waiting a few moments, the test results will appear. The pharmacokinetic and toxicity tests have prediction parameters consisting of HIA (Human Intestinal Absorption), Human Colon Adenocarcinoma (Caco-2), and distribution (Plasma Protein Binding/PPB), while for toxicity through prediction of mutagenic and carcinogenic properties (Kelutur et al., 2020) [10]. The testing procedure begins by opening the PreADMET software on the page. https://preadmet.webservice.bmdrc.org/adme/,Then, draw the 2D structure of the compound to be tested, then press submit on the screen. Then, wait a few moments for the test results to appear, which can be downloaded in PDF format. Compound toxicity testing is conducted using a decision tree to estimate mutagenicity and carcinogenicity. Toxicity testing is performed using the Toxtree application, which can be downloaded for free from the website. https://toxtree.sourceforge.net/download.html.Enter the SMILES code retrieved from PubChem, then press enter on the keyboard. Then, click the "Method" tool in the application, then press "Select Decision Tree." Choose which test to perform. In this study, the tests performed were Chammer Rules, Kroes TTC Decision Tree, Carcinogeneity (Genotox and Nongenotox), and Mutagenecity Rules based by ISS (Amest test). The test results will appear on the application screen in a dominant color.

3. Molecular Docking Analysis

Molecular docking testing has three important stages: ligand preparation, protein preparation, and compound docking. The results of the compound docking will display a visualization of the interaction between the ligand and the receptor. The process begins with ligand preparation, namely: The 3D structures of xanthorrhiza, curcumin, shogaol, gingerol, zingerone, and cinnamaldehyde compounds were downloaded from PubChem in SDF format. Then, minimize the structures using the Pyryx application, which can be downloaded for free from the website. https://sourceforge.net/projects/pyrx/. Enter the 3D structure compound in SDF format then perform minimization, and save the minimization results. The second process is the preparation of the protein receptor by downloading the 3D crystal structure of the Angiotensin Converting Enzyme 2 (ACE2) receptor which has the code 7BVN. The selection of the receptor structure code takes into account several criteria, namely ACE2 is carried out in humans (homo sapiens), the structure is obtained from X-ray diffraction research data and is not mutated. The initial step is to download the receptor through the Protein Data Bank (PDB) on the website page. https://www.rcsb.org/ in pdb format. Then open the Biovia Discovery Studio application, which can be downloaded for free from the website. https://discover.3ds.com/discovery-studio-visualizer-download. Enter the ACE2 crystal structure, prepare it by removing water molecules and ligands attached to the structure. Then save it in pdb file format. The third process is to perform molecular docking using the Pyryx application. Enter the minimized ligand compound and the prepared protein receptor alternately. Then click both simultaneously, then determine the correct Gridbox position so that the attached or bound ligand position can be done in any receptor area by pressing "Maximize" in the application. After that, press "Run Vina" to dock and wait a few moments until the results appear in the "Analyze Result" column. The docking results for the compound are in the form of Binding Affinity values. Then save the docked structure. The final process is to visualize the docking results between the ligand and the protein receptor using the Biovia Discovery Studio application. The results of the visualization can be in the form of 3D interactions between the ligand and the receptor, 2D structures of ligand interactions and interaction residues.

3. Results and Discussion

3.1. Prediction Analysis of Bioactive Compounds of Javanese Turmeric (*Curcuma xanthorrhiza*), Red Ginger (*Zingiber officinale*), and Cinnamon (*Cinnamomum burmanii*) as Antivirals

Immunostimulants are substances, drugs, or nutrients that aim to increase the immune system's ability to fight disease-causing viral infections by increasing components in the immune system (Wijaya & Yunita, 2023) [27]. The predictive parameters for a compound's antiviral activity can be determined through a Potential Activity (Pa) value that is higher than the Potential Inhibitors (Pi) value, namely if the Pa value> 0.7 indicates the compound has high potential to become a bioactive compound, a value of 0.5 <Pa <0.7 indicates the compound has moderate or sufficient potential to become a bioactive compound, and a Pa value <0.5 indicates the compound has low potential to become a bioactive compound. The biological activity of a compound indicates how important its role as a bioactive compound is in *in vitro* and *in vivo* testing and how much it has a high level of similarity to drug compounds (Chellia, 2008; Gul et al., 2017) [2, 7]. Based on the results of the compound activity prediction in Table 1.

Table 1: Results of Antiviral Activity Prediction Test of Herbal Plant Compounds

Plant	Bioactive Compounds	Pa	Pi	Biological Activity Prediction
Javanese Turmeric or Temulawak *(Curcuma xanthorriza)*	*Xanthorrhizol*	0,644	0,004	Antiviral (Rhinovirus)
		0,326	0,188	Antiviral (Picornavirus)
		0,314	0,074	Immunostimulant
	kurkumin	0,471	0,028	Antiviral (Influenza)
		0,418	0,013	Antiviral
		0,365	0,005	Antiviral (HIV)
		0,354	0,058	Antiviral (Herpes)
		0,326	0,201	Antiviral (Rhinovirus)
Red Ginger *(Zingiber officinale)*	*Shogaol*	0,477	0,043	Antiviral (Rhinovirus)
		0,424	0,039	Antiviral (Influenza)
		0,352	0,059	Antiviral (Herpes)
	Zingeron	0,384	0,052	Antiviral (Influenza)
		0,342	0,065	Antiviral (Herpes)
		0,350	0,159	Antiviral (Rhinovirus)
		0,332	0,180	Antiviral (Picornavirus)
	Gingerol	0,553	0,012	Antiviral (Rhinovirus)
		0,466	0,029	Antiviral (Influenza)
		0,363	0,061	Immunostimulant
		0,310	0,112	Simian Immunodeficiency virus proteinase inhibitor
Cinnamon *(Cinnamomum burmanii)*	*Sinnemaldehida*	0,516	0,018	Simian Immunodeficiency virus proteinase inhibitor
		0,446	0,078	Antiviral (Pirconavirus)
		0,403	0,046	Antiviral (Influenza)
		0,379	0,034	immunomodulator
		0,325	0,079	Antiviral (Adenovirus)
		0,321	0,077	Antiviral (Herpes)
		0,319	0,030	Antiviral (Influenza A)

The results of the activity test of the temulawak plant (*Curcuma xanthorrhiza*) xanthorrhizol compound has a Pa value of 0.644 which has moderate or sufficient potential as an antiviral bioactive in rhinovirus prediction, other Pa values were found to have low potential as an antiviral bioactive picornavirus and immunostimulant. The curcumin compound has low potential because the Pa value is <5 as a bioactive in the prediction of several antivirals such as influenza, HIV herpes rhinovirus. In the red ginger herbal plant (*Zingiber officinale*) produces a Pa value that is generally low potential as a bioactive compound, but there is one that has moderate or good potential as a bioactive, namely the gingerol compound which is predicted as a rhinovirus antiviral. While the cinnamon herbal plant (*Cinnamomum burmanii*) in the sinnemaldehyde compound has one Pa value that has moderate potential as a bioactive, namely the prediction as an antiviral Simian Immunodeficiency virus proteinase inhibitor (SIV). The compounds in three herbal plants, which have the best potential as antiviral bioactives are xanthorrhizol, gingerol, and cinnamaldehyde compounds which can also be tested *in vivo* and *in vitro* and these compounds have a moderate level of similarity to drug compounds. Based on previous *in vivo* research, that temulawak herbs have high activity as antivirals against hepatitis C (HCV), red ginger herbal plants have components such as ar-curcumene, gingerol, geraniol, shagaol, zingiberen, gingerenone, zingiberenol which have the ability to inhibit infection from *SARS-CoV-2*. Compounds in red ginger are used as ligands to determine the binding interaction between the S protein in the virus with ACE2 on the human receptor, through computational studies (Das, 2020; Kusuma Dewi and Amelia Riyandari, 2020). Meanwhile, previous research on cinnamon has antiviral properties that are quite effective in eradicating influenza, herpes, dengue type 2, junin virus, adenovirus type 3, poliovirus, and coxsackievirus (Tariq, 2019) [25].

3.2. Results of Physicochemical, Pharmacokinetic, and Toxicity Tests of Temulawak (*Curcuma xanthorrhiza*), Red Ginger (*Zingiber officinale*), and Cinnamon (*Cinnamomum burmanii*) Compounds

Prediction of drug candidates in herbal plant compounds can be based on Lipinski's Rule of Five, a physicochemical property test. Physicochemical properties have several parameters for evaluating the similarity of compounds to oral drugs that have biological activity in humans (Fikri et al., 2022) [6]. The rules in Lipinski's five laws have parameters that must be met before proceeding to the docking simulation of the drug candidate molecule as follows: 1) no more than 5 hydrogen bond donors (HBD), 2) No more than 10 hydrogen bond acceptors (HBA) because a high number of hydrogen bonds can reduce the partition of molecules from the water-soluble phase into the lipid bilayer membrane for permeation by passive diffusion, 3) Molecular mass (BM) less than 500 Dalton because a high molecular mass reduces the concentration of compounds on the surface of the intestinal epithelium which reduces absorption, 4) Log P (octane water partition coefficient), because a high log P value can cause poor absorption, and 5) molar refractivity must be between 40-130 (Yasin et al., 2020) [28]. Based on the results of the physicochemical properties test in Table 2.

Table 2: Results of Physicochemical Properties Test of Herbal Plant Compounds (Lipinski's Law of Five)

Herbal Plants	Compound	BM	LogP	HBD	HBA	Molar Refractivity	Application of the Law of Five (Lipinski)
Japanese ginger (*Curcuma xanthorrhiza*)	Curcumin	368	3.10	2	6	102.80	Fulfilled
Japanese ginger (*Curcuma xanthorrhiza*)	Xanthorrhizol	218	3.48	1	1	71.57	Fulfilled
Red Ginger (*Zingiber officinale*)	Shogaol	276	3.66	1	3	82.91	Fulfilled
Red Ginger (*Zingiber officinale*)	Gingerol	194	2.03	1	3	84.55	Fulfilled
Red Ginger (*Zingiber officinale*)	Zingeron	294	3.58	2	4	54.54	Fulfilled
Cinnamon (*Cinnamomum burmannii*)	Cinnamaldehyde	132	1.51	0	1	41.54	Fulfilled

Prediction of pharmacokinetic and toxicity testing aims to develop a new drug candidate, while toxicity is used to estimate the extent of toxic or toxic hazards. Pharmacokinetic properties are based on several parameters, namely absorption capacity (Human Intestinal Absorption/HIA) with criteria that must be met by drug candidate compounds ranging from 70-100%. The Caco-2 (Human Colon Adenocarcinoma) parameter shows permeability with the Caco-2 parameter category being >70 nm/s as a high permeability compound, the value category of 4-70 nm/s as a medium permeability, and the value category <4 nm/s as a low permeability ability, by passing through the barrier between intestinal epithelial cells (Morris, 2008) [14]. Caco-2 is widely used as an *in vitro* model in predicting drug absorption in humans through intestinal epithelial cell barriers (Fikri et al., 2022; Prasetyawati, 2021) [6, 19]. The Plasma Protein Binding (PPB) parameter aims to determine the percentage of a drug bound to plasma proteins. PPB values are categorized into two categories: if the PPB value is >90%, the compound is categorized as having a strong bond with plasma proteins. If the PPB value is <90%, the bond is weak with plasma proteins (Sagitasa et al., 2021) [23]. The Blood Brain Barrier (BBB) parameter aims to measure the ability of a drug to penetrate the blood brain barrier. In addition, another goal is to help reduce side effects and toxicity and increase the efficacy of drugs that have pharmacological activity in the brain. The value in the BBB parameter has three categories: if the BBB value is >2.0, the compound is categorized as having good penetration (ability to penetrate) into the brain. If the BBB value is between 2-0.1, the compound is categorized as having sufficient penetration into the brain. Meanwhile, if the BBB value is <0.1, the compound is categorized as not having a good target in its penetration into the brain (Izzaturahmi, Ahda, 2023) [9]. Based on the results of pharmacokinetic properties testing using PreADMET in

Table 3: Results of Pharmacokinetic Properties Test of Herbal Plant Compounds

Absorption and Distribution Analysis	Japanese ginger (*Curcuma xanthorrhiza*)		Red Ginger (*Zingiber officinale*)			Cinnamon (*Cinnamomum burmannii*)
	Curcumin	Xanthorrhizol	Shogaol	Gingerol	Zingerone	Cinnamaldehyde
HIA (Human Intestinal Absorption)	94.403	100	96.181	91.964	88.612	100
Caco-2 (Human Colon Adenocarcinoma)	20.073	53.284	51.858	24.517	21.191	23.784
PPB (Plasma Protein Binding)	88.030	100	100	100	85.776	52.892
BBB (Blood–Brain Barrier)	0.091	12.873	3.986	1.474	0.774	1.261

The herbal plant temulawak (*Curcuma xanthorrhiza*) in the xanthorrhizol compound and the cinnemaldehyde compound in the cinnamon herb (*Cinnamomum burmanii*) which has the highest HIA value of 100% so it is categorized as very good, because it has a very good ability in the absorption process in the intestine. The results of the HIA test on curcumin, shogaol, and gingerol compounds have an average percentage value that is also good, which is still around 96.2-91.9%. While the zingerone compound, although the percentage of the HIA value is the lowest at 88.6%, is still categorized as having a very good ability in the process of drug absorption in the human intestine. The results of the Caco-2 (Human Colon Adenocarcinoma) parameter test, it was found that the bioactive compounds from the three herbal plants have values between 4-70 nm / s, these results indicate that Caco-2 has a moderate absorption permeability ability in the intestine. Testing of the Plasma Protein Binding (PPB) parameters showed that the compounds xanthorrhizol, shogaol, and gingerol had PPB values of more than 90% with a percentage value of 100% which was categorized as having strong bonds in plasma proteins. However, curcumin, zingerone, and cinnamaldehyde compounds have PPB values of less than 90%, which are categorized as having weak bonds to plasma proteins. Meanwhile, the results of the Blood Brain Barrier (BBB) parameter test showed that xanthorrhizol compounds had a BBB value of 12.8 and shogaols had a BBB value of 3.9, so these compounds are categorized as having good brain penetration capabilities. Zingerone compounds have a BBB value of 1.47, gingerol has a BBB value of 0.77, and cinnamaldehyde has a BBB value of 1.3, so these compounds are categorized as having fairly good brain penetration capabilities. Meanwhile, curcumin compounds have a BBB value of 0.09, so it is categorized that the compound does not have a good brain

penetration target. Toxicity testing using the Toxtree V2.6.13 application in Table 5.

Table 4: Results of Toxicity Tests of Herbal Plant Compounds

The Herbs	Compounds	Cramer Rules	Kroes TTC Decision Tree	Toxicity Test Parameters (Toxtree Benign / Bossa Rules or Carcinogenic/Genotoxic and Nongenotoxic)	Mutagenicity Rules (Ames Test)
Japanese ginger (*Curcuma xanthorrhiza*)	Curcumin	High Class	The substance would not be expected to be a safety concern	• Negative for genotoxic carcinogenicity • Negative for nongenotoxic carcinogenicity	No alerts for *S. Typhimurium* mutagenicity
Japanese ginger (*Curcuma xanthorrhiza*)	Xanthorrhizol	Intermediate Class	The substance would not be expected to be a safety concern	• Negative for genotoxic carcinogenicity • Negative for nongenotoxic carcinogenicity	No alerts for *S. Typhimurium* mutagenicity
Red Ginger (*Zingiber officinale*)	Shogaol	Intermediate Class	The substance would not be expected to be a safety concern	• Structural alert for genotoxic carcinogenicity • Negative for nongenotoxic carcinogenicity	Structural alert for *S. Typhimurium* mutagenicity
Red Ginger (*Zingiber officinale*)	Gingerol	Intermediate Class	The substance would not be expected to be a safety concern	• Negative for genotoxic carcinogenicity • Negative for nongenotoxic carcinogenicity	No alerts for *S. Typhimurium* mutagenicity
Red Ginger (*Zingiber officinale*)	Zingerone	Low Class	The substance would not be expected to be a safety concern	• Negative for genotoxic carcinogenicity • Negative for nongenotoxic carcinogenicity	No alerts for *S. Typhimurium* mutagenicity
Cinnamon (*Cinnamomum burmannii*)	Cinnamaldehyde	Low Class	The substance would not be expected to be a safety concern	• Negative for genotoxic carcinogenicity • Negative for nongenotoxic carcinogenicity	Unlikely to be a *S. Typhimurium* TA100 mutagen based on QSAR

The results showed that in the chammer rules parameter, the curcumin compound contained in Javanese ginger (*Curcuma xanthorrhiza*) is in class III (high class), which indicates the compound has a high potential for toxicity. The xanthorrhizol, shogaol, and gingerol compounds are in class II (intermediate class), indicating that the three compounds have moderate potential for toxicity, while the test results for the zingerone and cinnamaldehyde compounds are in class I (low class), indicating that both compounds have low potential for toxicity. The Kroes TTC decision tree parameter was used to estimate the threshold for exposure to drug compounds in humans (Muhammad Fillah et al., 2022) [15]. The test results in Table 5 show that six compounds xanthorrhizol, curcumin, shogaol, gingerol, zingerone and cinnamaldehyde are written with the statement the substance would not be expected to be a safety concern which means the six compounds do not contain substances that exceed the threshold for drug compound exposure in humans. The Carcinogenecity test results show that the compounds xanthorrhizol, curcumin, gingerol, zingerone and cinnamaldehyde are included in Negative for genotoxic carcinogenicity and Negative for nongenotoxic carcinogenicity meaning these compounds are negative for genotoxic carcinogens and negative for non-genotoxic carcinogens, while one compound, namely shogaol, has test results that are included in the Structural Alert for genotoxic carcinogenicity which means it has a warning for genotoxic carcinogens. Furthermore, the Mutagenecity Rules base parameter by ISS or the Ames test is a widely used method that uses bacteria to test whether certain chemicals can cause mutations in the DNA of test organisms. The results of the mutagenicity test (Ames test) showed that the compounds xanthorrhizol, curcumin, gingerol, and zingeron showed no warnings against mutagenesis of S. Typhimurium, one compound, namely shogaol, showed a warning against mutagenesis of S. Typhimurium and one compound cinnamaldehyde showed that it was impossible to be a mutagen of S. Typhimurium TA100 based on QSAR (Qualitative or Quantitative Structure-Activity Relationship), which is a method that can be used in toxicology studies (Rahayu, 2022) [22].

3.3. Molecular Docking Analysis Results

Molecular dockingis a method process carried out with the aim of predicting the interaction between the test ligand and the target protein receptor that has the best affinity (binding power) (Muttaqin, 2019) [16]. Docking research is based on binding affinity and Root Mean Square Deviation (RMSD). Binding affinity is a parameter or measure of the drug's ability to bind to the receptor. While the RMSD parameter as a protein docking on the ligand is used to find the 3D conformation of the ligand to the receptor. The RMSD value <2 Å indicates that the smaller the error of the calculation, so it can be said that the calculation is more accurate. However, if the RMSD value> 2 Å indicates that the deviation from the calculation results is greater (Ferwadi, Rahmat G, 2017) [5]. Compound docking (molecular docking) is carried out in three preparations, namely ligand preparation, receptor protein preparation and docking (molecular docking). Based on the results of molecular docking tests between herbal plant compound ligands and the ACE2 receptor in Table 6.

Table 6: Molecular Docking Test Results between Ligands (Compounds) and ACE2 Receptors (Best Df from Best Mode)

Herbal Plants	Ligand Compounds	GDP Code	Affinity (kcal/mol)	RMSD Lower Bound	RMSD Upper Bound
Japanese ginger (*Curcuma xanthorrhiza*)	Curcumin	7BNV (ACE2)	−7.4	0.000	0.000
Japanese ginger (*Curcuma xanthorrhiza*)	Xanthorrhizol	7BNV (ACE2)	−7.2	0.000	0.000
Red Ginger (*Zingiber officinale*)	Shogaol	7BNV (ACE2)	−6.6	0.000	0.000
Red Ginger (*Zingiber officinale*)	Gingerol	7BNV (ACE2)	−7.0	0.000	0.000
Red Ginger (*Zingiber officinale*)	Zingerone	7BNV (ACE2)	−6.0	0.000	0.000
Cinnamon (*Cinnamomum burmannii*)	Cinnamaldehyde	7BNV (ACE2)	−5.7	0.000	0.000

The largest compound interaction is the cinemaldehyde compound which has a binding affinity value of -5.7 kcal/mol. Interactions of compounds that have small affinity values include the zingerone compound which has a binding affinity value of -6.0 kcal/mol and shogaol with a binding affinity value of -6.6 kcal/mol. Then the gingerol compound has a fairly small binding affinity of -7.0 kcal/mol, the xanthorrhizol compound with a binding affinity value of -7.2 kcal/mol, and the curcumin compound which has the smallest binding affinity value of -7.4 kcal/mol. The curcumin compound has the lowest binding affinity value among the other compounds, indicating that the binding affinity between the receptor and the ligand is getting higher. Meanwhile, the RMSD value of the docking results of six natural ligand compounds with the Angiotensin Converting Enzyme 2 (ACE2) protein receptor has an RMSD value of lb 0.000, and the RMSD value up to 0.000 indicates that the docked natural ligand compound has a high similarity to the natural (actual) structure. The docking results that have been carried out also show that the docking method is valid and meets the validity criteria, so it can be used as a basis for further studies *in vitro* and *in vivo*. The World Health Organization (WHO) has published research from Chakotiya and Sharma (2020), finding that the active compounds contained in ginger show effective binding affinity to ACE2 (Crystallography, 2016) [3]. Recent research by Sampath (2021), reporting the potential of bioactive compounds in cinnamon shows that glycoproteins in *SARS-CoV-2* have a role in binding to the ACE2 protein which can be inhibited by phenylpropanoids (cinnamonaldehyde and cinnamal acetate). Other studies have shown that cinnamon has various antiviral effects on beta coronavirus and *SARS-CoV-2* by inhibiting the entry of the virus into host cells and infecting cells, inhibiting replication and inhibiting proteases from the virus (Polansky & Lori, 2020; Purwitasari & Alamudi, 2023) [18, 21].

Based on the results of molecular docking visualization between the test ligands from compounds contained in the herbal plant temulawak (*Curcuma xanthorrhiza*), red ginger herbal (*Zingiber officinale*), and cinnamon herbal (*Cinnamomum burmanii*) which occurred in the grid box axis x: 24.0037, y: -17.353, and z: 9.1689. Molecular docking visualization in Figure 1 interaction between ACE2 and Curcumin, Figure 2 interaction between ACE2 and Xanthorrizol, Figure 3 interaction between ACE2 and Gingerol, Figure 4 interaction between ACE2 and Shogaol, Figure 5 interaction between ACE2 and Zingerone, and Figure 6 interaction between ACE2 and Cinnemaldehyde.

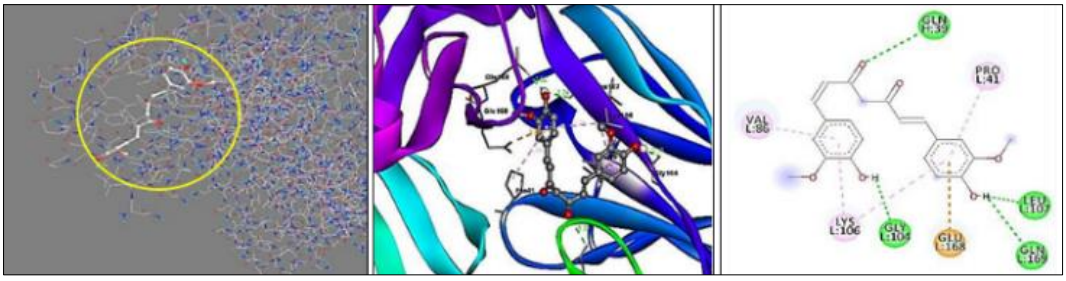

Fig 1: Interaction between ACE2 and Curcumin

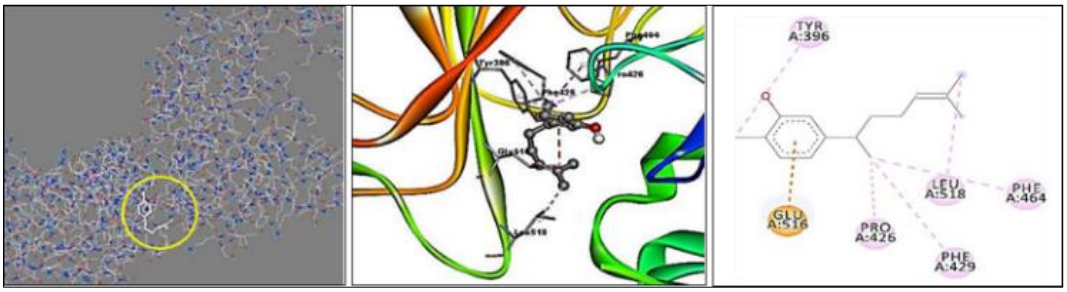

Fig 2: Interaction between ACE2 and Xanthorrizol

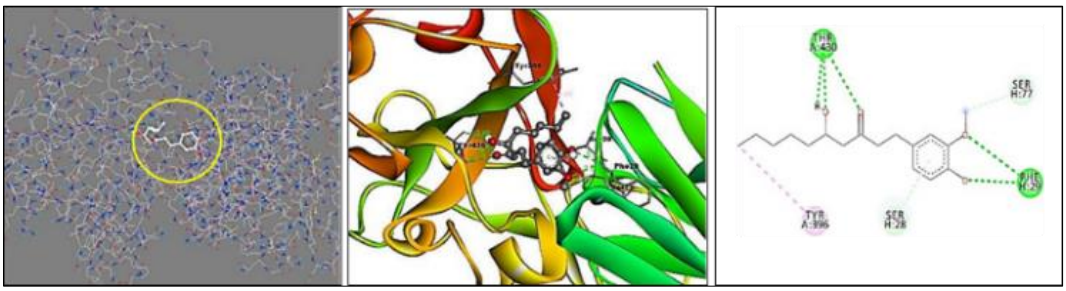

Fig 3: Interaction between ACE2 and Gingerol

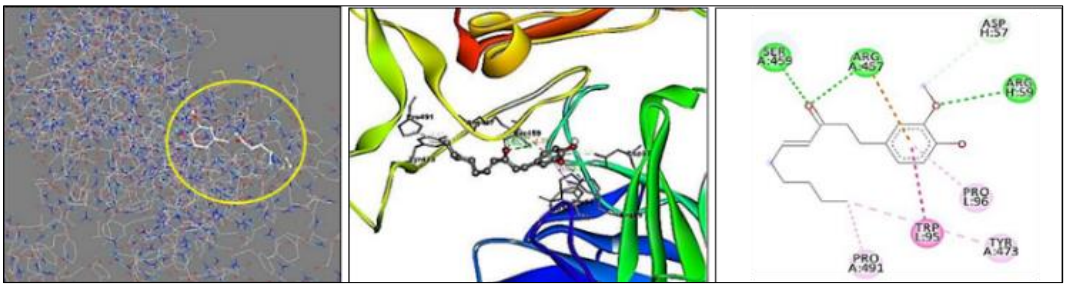

Fig 4: Interaction between ACE2 and Shogaol

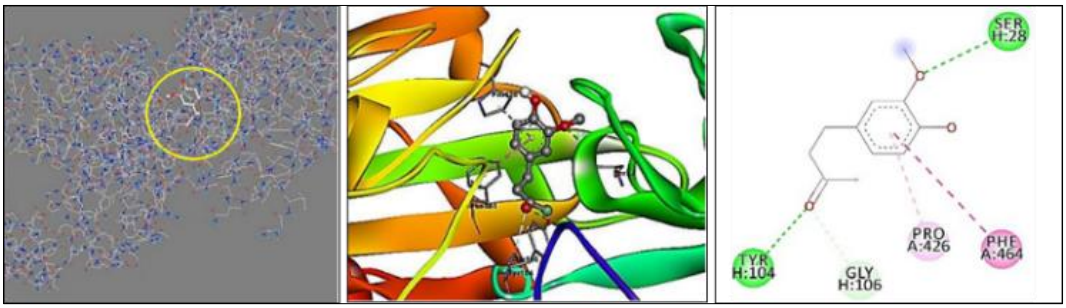

Fig 5: Interaction between ACE2 and Zingerone

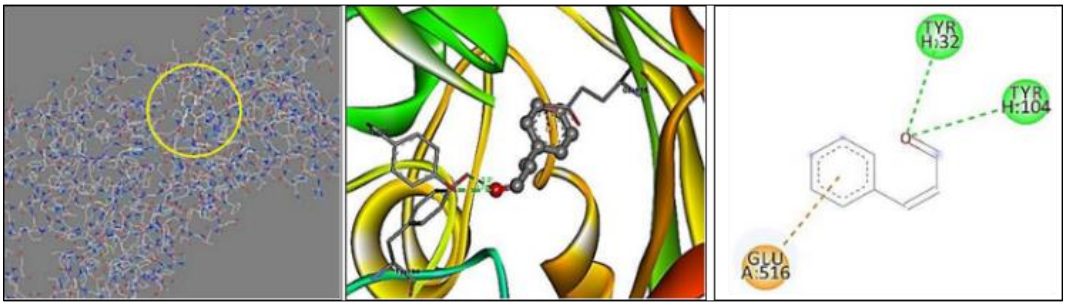

Fig 6: Interaction between ACE2 and Cinnemaldehyde

Figure 1 The binding position of the interaction between curcumin and ACE2 7BNV protein (in the yellow circle). displays the interaction in 3D representation, and displays the mapping of amino acids resulting from the interaction in 2D representation, resulting in residues GLNH: 39, GLNL: 169, GLYL: 104 and LEUL: 107 (conventional hydrogen bonds) as hydrogen bonds, PROL: 41, VALL: 86, and LYSL: 106 (Pi-Alkyl bonds) as bonds hydrophobic, and GLUL:168 (Pi-Anion) as an electrostatic bond. Figure 2 the position of the interaction between ACE2 and xanthorrizol (in the yellow circle),display displays interaction in 3D representation, and the mapping of amino acid resulting from the interaction in 2D representation resulting in residues TYRA:396 (alkyl bond), LEUA:518, PHEA:464, PHEA:429, PROA:426 (Pi-Alkyl bond) are hydrophobic bonds, and GLU516 (Pi-Anion) as an electrostatic bond. Figure 3 the position of the interaction between gingerol and ACE2 (in the yellow circle), displays the interaction in 3D representation, and the amino acid mapping resulting from the interaction in 2D representation which produces amino acid residues in the form of PHEH:29 and THRA:430 (Hydrocarbon Bond) Conventional), SERH:28 (Pi-Donor Hydrocarbon Bond), SERH:77 (Hydrocarbon Bond) and TYRA:396 which is a hydrophobic bond. Figure 4 the position of interaction between shogaol and ACE2 (in the yellow circle), displays the interaction in 3D representation, and displays the amino acid mapping resulting in 2D representation which produces amino acid residues in the form of SERA:459, ARGH:59, ARGA:457 (conventional hydrocarbon bond), ASPH:57 (hydrocarbon bond), PROA:491 (Alkyl bond), PROA:491, TYR473 (Pi-Alkyl bond), TRPL:95 (Pi-Pi T-shaped bond) is a hydrophobic bond, ARGA:457 (Pi-Cation bond) is an

electrostatic bond. Figure 5 the position of interaction between zingerone and ACE2 (in the yellow circle), displays the interaction in 3D representation, and shows the amino acid mapping resulting from the interaction in 2D representation, which produces amino acid residues in the form of SERH:28, TYRH:104 (conventional hydrocarbon bonds), and GLYH:106 (hydrocarbon bonds) which are hydrocarbon bonds. While PHEA:464 (T-shaped Pi-Pi bond) and PROH:426 (Pi-alkyl bond) are hydrophobic bonds. Figure 6 position of interaction between ACE2 and cinnamaldehyde (in the yellow circle), displays the interaction in 3D representation,and displays the amino acid mapping resulting from the interaction in a 2D representation that produces amino acid residues in the form of TYRH: 32, TYRH: 104 (conventional hydrogen bonds) and GLUA: 516 (Pi-Anion bonds) which are electrostatic bonds. Meanwhile, displays is a global structure of the molecular docking conformation Figure 7:

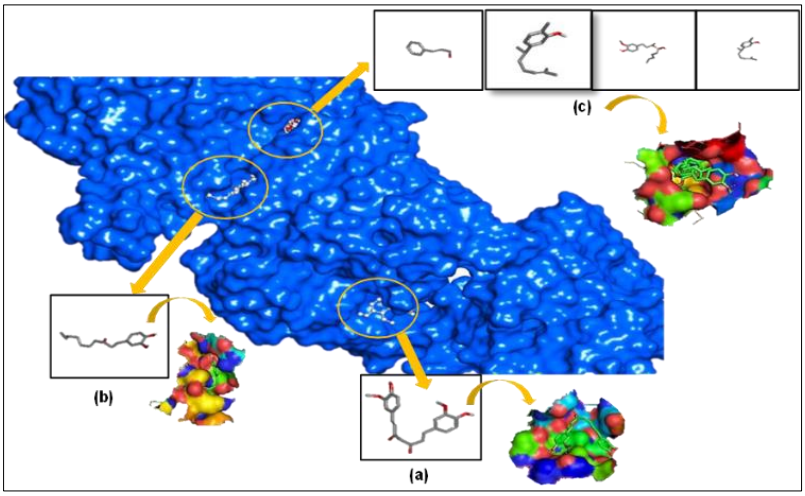

Fig 7: Global Structure of Molecular Docking Conformation with Angiotensine Converting Enzyme 2 (ACE2)

(a)Representation of the location of curcumin on the surface of the ACE2 receptor, (b) Representation of the location of shogaol on the surface of the ACE2 receptor, (c) Representation of the location (image structures in order from left to right) of cinnemaldehyde, xanthorrhizol, gingerol and zingerone. The interaction of the structures overlap or stack on each other on the surface of the ACE2 receptor.

5. Conclusion

A study on the prediction of bioactive compound activity involving six herbal compounds showed that these compounds exhibited moderate to good antiviral potential. In silico analysis revealed that the physicochemical properties of compounds derived from temulawak (*Curcuma xanthorrhiza*), red ginger (*Zingiber officinale*), and cinnamon (*Cinnamomum burmanii*) met the five criteria of Lipinski's Rule of Five, indicating their suitability as orally administered drugs. Furthermore, pharmacokinetic and toxicity analyses demonstrated that, in general, the tested herbal compounds showed favorable characteristics as potential drug candidates or novel immunizers based on Pre-ADMET parameters.

Molecular docking results indicated that the six herbal compounds used as ligands exhibited strong interactions in inhibiting the activity of the ACE2 protein receptor. Notably, compounds from temulawak showed the highest binding affinity, with curcumin exhibiting a binding energy of −7.4 kcal/mol and xanthorrhizol −7.2 kcal/mol. These values were lower than those observed for compounds from red ginger and cinnamon, suggesting stronger ligand–receptor interactions. In addition, molecular docking visualization is necessary to examine in detail the interactions formed between ligands and receptors, as well as to identify the specific amino acid residues involved in these interactions.

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