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In silico study of the essential oil compounds of ginger and thyme on Coronavirus-2 receptors

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ABSTRACT

Coronavirus-2 (SARS-Cov-2) is a virus that attacks the respiratory system and causes the Covid-19 pandemic. After the pandemic, prevention and appropriate therapy research continue to be carried out to anticipate the emergence of more dangerous viruses. In line with the culture of consuming herbs that has arisen due to the effects of the pandemic, in this study, an *in silico* study was carried out for essential oil compounds produced by ginger and thyme herbs which have been widely consumed by the public. The research aim to find the essential oil content that has the most potential as an antiviral against coronavirus-2. The moleculer docking method was carried out, including ligand preparation, receptor and method validation, and analysis of ligand-receptor binding interactions using the AutoDoc 4.2.6. As comparisons, remdesivir and favipiravir were used. The three components with the most potential based on the calculation of the free energy value, were determined by the ADMET parameters using the ADMETlab 2.0. The results showed that the three components in the essential oil exhibited better interactions when compared to remdesivir and favipiravir at the 3CL protease and spike glycoprotein receptors. The results of the *in silico* study and ADMET prediction test showed that of the three most potent compounds, α-farnesen was the most potent and safe to use

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Keywords: *moleculer docking, ginger and thyme,* 3CL protease

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INTRODUCTION

The COVID-19 pandemic, which was developing rapidly due to coronavirus-2, required an effective therapeutic strategy so that it could easily go through clinical trials to reduce human, social, and economic impacts. The reuse of previously approved drugs such as remdesivir and favipiravir allowed antiviral drugs to be used safely immediately (Grein et al., 2020). However, studies on some of these drugs were incomplete because they only approached classic viral targets (Singh et al., 2020). In addition, most of the drugs that have been approved were not clinically proven to be effective against coronavirus-2 because they showed marginal effectiveness and conflicted clinical trials (Bellera et al., 2021). In some cases, many people did not receive the vaccine. On the other hand, some virus variants may be resistant to the vaccines that have been used. Therefore, it was still necessary to develop new compounds that were effective against coronavirus-2.

Essential oils have long been developed and used as antivirals (Ma & Yao, 2020). Due to their lipophilic property, essential oils could easily penetrate viral cell membranes and cause membrane disruption, then synergistically affect replication and produce effects on the host, namely bronchodilation and mucus secretion (Asif et al., 2020). da Silva et al. 2020 conducted a molecular docking study of 171 essential oil compounds of various herba plants against various types of receptors. It had been known that thyme (*Thymus vulgaris*) and ginger (*Zingiber officinale*) were estimated to have the potential to produce essential oils that act synergistically with anti-virals, improved anti-viral performance and relieved some of the symptoms that appear. However, da Silva et al. 2020 had not described the interactions of these compounds against protease-derived receptors such as 3-Chromotrypsin like protease 3CL protease or $3CL^{Pro}$ and spike glycoprotein transmembrane receptors. On the other hand, da Silva et al. (2020) did not analyze the results by comparing the anti-viral drugs used. Therefore, in this research, an *in silico* study was carried out on essential oil components in thyme and ginger using the AutoDock 4.2.6 and observations of anti-viral drugs, namely favipiravir and remdesivir on 3-Cl protease receptors and spike glycoproteins. We examined the three components that had the best interactions; their adsorption, distribution, metabolism, excretion, and toxicity properties would be examined. It was expected that the results of this research could explain the effectiveness of the essential oils contained in thyme and ginger herbs when compared to the anti-virals that have been used previously, as well as explain the pharmacokinetic properties and toxicity of related compounds.

MATERIALS AND METHOD

Materials

The hardware tools were a set of Toshiba Portege Z30-C series Ultrabook with an Intel™ Core i7-6600U@2.6 GHZ and Windowws 10 pro operating system. The software tools were AutoDock 4.2.6 (AutoDock Tools 1.5.6), Discovery Studio Visualizer 20.1.0.19295, VMD 1.9.3, ADMETlab 2.0 (https://admetmesh.scbdd.com/), and internet connection. 3.2.2.

The materials used in this study were the structure of the ginger components, namely α-zingiberene, β-sesquifelandren, ar-curcumene, α-phellandrene α-farnesene and the thyme components, namely thymol, carvacrol, ρ-cymene, β-caryofilen and γ –terpinene. All the structure were downloaded from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/). The receptor material used was a three-dimensional structure of 6M2N (3-Cl protease in complex with an inhibitor) and 6VXX (SARS-CoV-2 spike glycoprotein (closed)), which were downloaded from the Protein Data Bank (www.rscb.org) (Myler et al., 2009; Zhao et al., 2022). The antiviral drugs used were Remdesivir and Favipiravir, which were downloaded from the Pubchem website (https://pubchem.ncbi.nlm.nih.gov/).

Methods

The major components (>4%) of essential oils components that have been screened, according to da Silva et al. (2020), were selected. The chemical structure of α-zingiberin, β-sesquiphelladrene, ar-

curcumene, α-felandren, α-farnesen thymol, carvacrol, ρ-simene, β-karyofilen, γ –terpinen, favipiravir, and remdesivir was obtained from the PubChem database. The collected Structure Data File (SDF) files of these compounds were converted into PDB format using Online SMILES Translator. All the ligands were optimized, and the lowest energy was determined.

The receptor target from the Protein Data Bank, 3 Cl-protease (PDB:6M2N) and spike glycoprotein (PDB:6VXX), were used in the molecular docking. Receptor was prepared by usin Autodock 4.2.6. programs (Forli et al., 2016). The active sites of ligand and protein were analyzed using Biovia Discovery Studio.

The validation of the molecular docking method was carried out by redocking the receptor and its native ligand and then determining the size and position of the grid box for the docking process. The receptor was separated from its native ligand and re-docked with its native ligand. The process was called valid if the value of RMSD was less than 2 Å.

Data Analysis

The docking process was carried out using the AutoDock 4.2.6 program, while Discovery Studio Visualizer 19.1.0 was used to obtain interaction. The results of docking the receptors with potential ligands (essential oils from thyme and ginger) were observed from the free energy value (ΔG) to determine the potential ligands that have interactions with the receptors. The interaction between the receptor and the potential ligand was compared to antiviral ligand. The physicochemical properties, ADMET, and toxicity studies were carried out on the three most potent substances and reference compounds. The results obtained were then compared and analyzed further with Admet lab 2.0. (Dong et al., 2018).

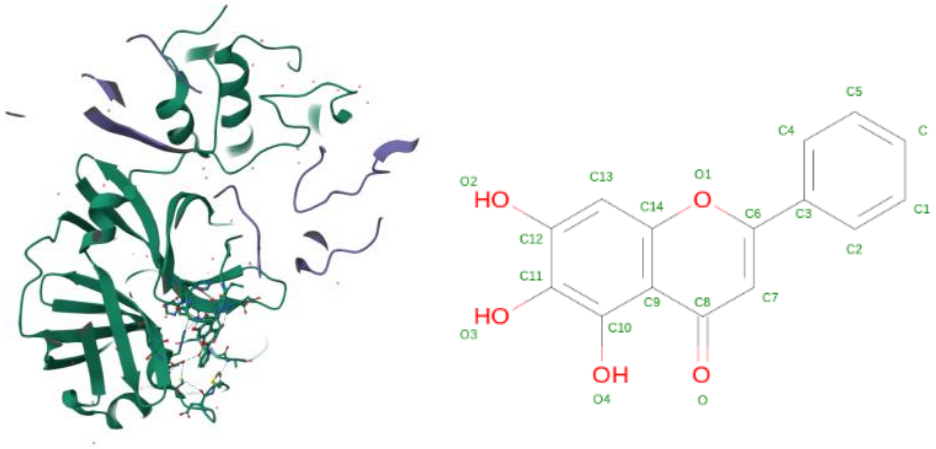
RESULT AND DISCUSSION

In this study, we used the compounds from ginger and thyme herbs. The selection of these two plants was based on the availability of herbs in Indonesia and their effectiveness as anti-viral drugs, which were tested in vitro on RC-37 cells using the plaque reduction method with IC 50 levels for ginger was 1 µg/mL, and for thyme was 7 µg/mL. The ligands used in this study were the structure of the ginger components, namely α-zingiberin (32.1%), β-sesquifelandren (10.9%), ar-curcumin (15.2%), α-felandren (4.4%). %) and α-farnesene (7.2%) and the thyme component, namely thymol (43.9%), carvacrol (14.4%), ρ-someone (10.5%), β-karyofilen (7%) and γ-terpinene (5.1%) (da Silva et al., 2020).

The 3-Cl protease (PDB: 6M2N) receptor was a 3C-like proteinase construction composed of an A chain with 306 sequences (Figure 1a) and one native ligand 5,6,7-trihydroxy-2-phenyl-4H-chromen-4-one (C15H10O5) (Figure 1b) (Su et al., 2020). The receptor has a total weight of 136.38 kDa with a total number of atoms: 9544. The selection of the 3 chromotropin like-protease receptor was based on potential antiviral therapeutic targets that were currently being developed because these proteases were required for viral transcription and replication. The specificity of 3Cl-protease media was conserved compared to different coronaviruses and was similar to the main picornavirus protease, making it an ideal target for the development of broad-spectrum antiviral drugs (Pillaiyar et al., 2016). The 5 replicates receptor validation results showed a RMSD value of 1.98±0.0037 Å, which was less than 2 Å, so the method was valid. Conservation residue was found in the 3-Cl protease binding pocket and, in this study, was the main target for its antiviral activity.

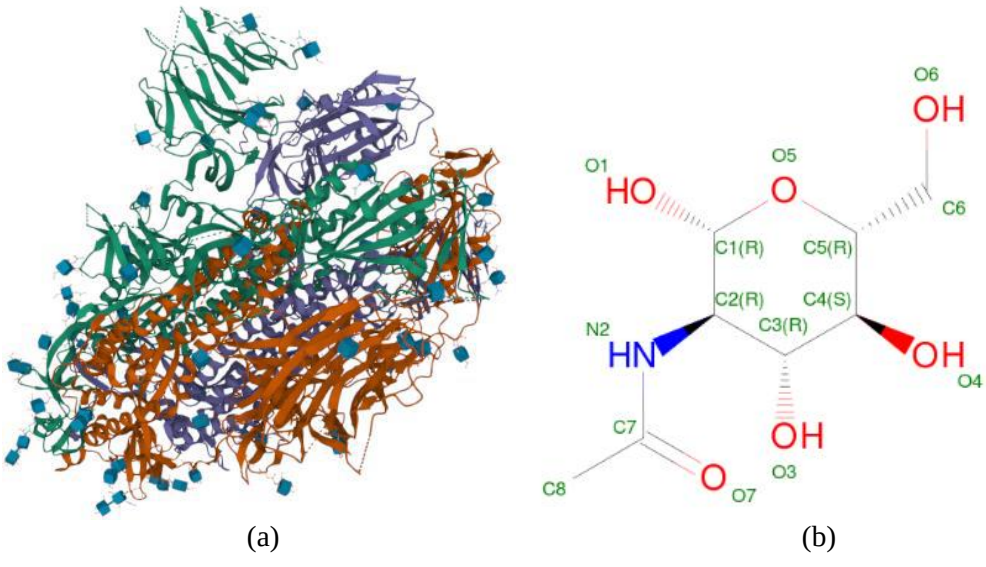
The spike glycoprotein receptor has a total weight of 438.26 kDa with a total atomic count of 23694, classified as a spike glycoprotein of severe acute respiratory syndrome coronavirus 2 virus expressed in humans. The receptor consists of chain of two protein entities with chains A, B, and C attached to 2-acetamido-2-deoxy-beta-D-glucopyranose-(1-4)-2-acetamido-2-deoxy-beta-D-glucopyranose and the D,E,F,G,H,I, J,K,L,M,N,O,P,Q and r chains attached to 2-acetamido-2-deoxy-beta-D-glucopiranose (Figure 2a). The ligand assigned was 2-acetamido-2-deoxy-beta-D-glucopyranose ($C_8H_{15}NO_6$) (Figure 2b) (Myler et al., 2009). The selection of the spike glycoprotein

receptor was based on its uniqueness in mediating coronavirus entry into cells through interaction with Angiotensin Converting Enzyme-2. Because of its location on the surface, it could be developed to become the main target for drug development and vaccine design (Myler et al., 2009). The 5 replicates validation showed a RMSD value of 1.88±0.0044 Å, which was less than 2 Å, so the method could be declared valid.



(a) (b)

Figure 1. (a) 3-Cl protease with inhibitor in 3D and (b) ligand 5,6,7-trihydroxi-2-phenil-4H-chromen-4-on (C15H10O5)



(a) (b)

Figure 2. (a) spike glycoprotein complex and (b) ligand 2-acetamido-2-deoxy-beta-D-glucopiranose ($C_8H_{15}NO_6$)

The results of docking the compounds with the two receptors were carried out 3 times and expressed in the mean free energy value (ΔG), which is shown in Table 1.

Table 1. The docking compounds in ginger and thyme against Coronavirus receptors

Compound	Interaction with the 3-Cl protease		Interaction to the spike glycoprotein	
	ΔG (kcal/mol)	protein residue	ΔG (kcal/mol)	protein residue
zingiberin	-6.10	HIS41,MET49	-7.60	LEU226
α-farnesene	-7.24	HIS41,MET49,CYS44	-8.70	ILE119,VAL227,TYR170, LEU229,PHE168,ILE128
β-sesquiphellandrene	-6.90	HIS41,MET49,CYS44, MET165,PRO52	-8.34	ILE119,TYR170,ILE203, PHE192, TRP104
ar-curcumene	-7.10	HIS41,MET 49,CYS44	-8.28	ILE119,VAL126, TYR170
α -felandren	-4.70	HIS41,CYS44, MET165,PRO52	-5.50	ILE119,VAL126,ILE103, VAL227,PHE192,TRP104
Tymol	-4.60	HIS41,MET165	-5.50	VAL126,SER205
carvacrol	-4.70	HIS41,MET165	-5.50	VAL126,PHE192
p-cymene	-4.80	HIS41,MET49	-5.40	VAL126,PHE192
β- caryophyllene	-5.60	HIS41,	-5.90	VAL126
γ-terpinene	-4.70	HIS41,MET49,CYS44, MET165,PRO52	-5.40	VAL126,HIS207

Table 2. The docking compounds in favipiravir and remdesivir against Coronavirus receptors

Compound	Interaction with the 3-Cl protease		Interaction to the spike glycoprotein	
	ΔG (kcal/mol)	protein residue	ΔG (kcal/mol)	protein residue
Favipiravir	-5.10	HIS41,MET49,CYS44, HIS164,ARG188,ASP187, PRO52,ASP48	-4.64	ILE119,PRO225,GLN134, ASN137,LEU110,GLN239
Remdesivir	-6.24	HIS41,MET49,CYS44, ASN142,ASP187,GLU166, ARG188,THR190	-6.22	PRO225,LYS41, PHE43, GLY283, ASP40

The important role of 3-Cl protease in converting lipoproteins into functional proteins in viral replication made the receptor an attractive target for the development of compound inhibitors. The interactions that occur between the 10 compounds and the antiviral drugs with the receptor (Tables 1 and 2) show π-π interactions between compounds with the amino acid HIS41 and π-alkyl interactions with amino acids MET 4. In some compounds that have small ΔG values, namely α-farnesen (-7.24), β-sesquifelandren (-6.90), and Ar-curcumin (-6.10) as well as favipiravir (-5.10) and remdesivir (-6.24), the van der waals interaction with CYS44 also occured. However, this did not appear in γ-terpinen (-4.70), which also has interactions with CYS44 but has a large ΔG. It was suspected that this was due to the large space barrier effect on γ-terpinen. The results of this study were broadly in line with the research conducted by (Benhander & Abdusalam, 2022), who conducted an insulin Allium roseum study on the 3-Cl protease (6M2N) receptor.

The glycoprotein spike receptor interacts with ACE-2 in mediating the coronavirus to enter cells. The interactions that occurred between the 10 herbs compounds (Table 1) showed hydrophobic interactions between the compounds with the amino acid ILE119, and the three compounds with small ΔG also had hydrophobic interactions with TYR170. The results of this analysis are in line with research conducted by (Rolta et al., 2021). In the comparison compound favipiravir there was a

hydrophobic interaction with the amino acid ILE119 and a pi-sigma interaction with the amino acid PRO225 while in remdesivir there was an interaction of pi-sigma with the amino acid PRO225 and a π-π interaction with PHE43. The results of research on favipiravir and remdesivir were in line with research conducted by (Veerasamy & Karunakaran, 2022).

Favipiravir, as a purine analog compound that has been used as an antiviral drug, shows a less binding affinity to the 3-Cl protease receptor and spike glycoprotein receptor when compared to remdesivir. According to (Wang et al., 2020), favipiravir was effective in high doses to inhibit viral replication. On the other side, remdesivir, as an adenosine analogue that inhibits viral replication, was known to have a high affinity for proteases compared to spike glycoprotein receptors (Eweas et al., 2021). The results of this study showed that α-farnesen, β-sesquifelandren and Ar-curcumin were more potent than favipiravir and remdesivir because these three compounds showed better binding affinity at two different receptors because they had lower free energy values.

According to the potency of α-farnesen, β-sesquifelandren and Ar-curcumin compared to favipiravir and remdesivir, this study analyzed the physicochemical properties of these three compounds to be developed as new drug candidates following Lipinski's rules (Chen et al., 2020). This rule was used to predict the drug likeness of a chemical compound with its physico-chemical properties for the oral route of administration. According to the Lipinski rule, Role of 5 (RO5), a drug-like compound should have a molecular weight of not more than 500, log P value not more than 5, hydrogen bond donors not more than 5 and hydrogen bond acceptors not more than 10.

Table 3. The Physico chemical properties of the compound

Compound	Molecular weight	Hydrogen bond donor	Hydrogen bond acceptors	Log P
β-sesquifelandren	204.19	0	0	5.608
Ar-curcumin	202.17	0	0	5.9
α-farnesen	204.19	0	0	6.286
Favipiravir	157.03	3	5	-0.934
Remdesivir	602.23	5	14	1.961

The data in Table 3 showed that none of the potential herbal compounds followed Lipinski's rule regarding log P, which must be less than 5. The log P value indicated the distribution coefficient of compounds in fat and water, which plays an important role in drug adsorption. However, in several cases related to natural materials, the log P value may not suit Lipinski requirements. According to (Chen et al., 2020), if the log P value does not suit the requirements but the other parameters suit the requirements, then the compound could still be accepted, taking into account its other properties.

The results of the adsorption, distribution, metabolism, and excretion test of the three most potent compounds and comparators are shown in Table 4.

Table 4. ADMET calculation

Compound	CaCO-2 (log cm/ second)	VD (l/kg)	CYP3A4 inhibitor	Total clirens (mL/minute/ kg)	Rat Oral Toxicity (mg/kg)
β-sesquifelandren	-4.492	5.514	0.588	15.405	0.028
Ar-curcumin	-4.375	4.736	0.512	12.748	0.025
α-farnesen	-4.565	5.502	0.321	14.162	0.016
Favipiravir	-5.244	0.653	0.005	8.141	0.522
Remdesivir	-5.611	1.817	0.514	6.052	0.719

Notes :
CaCO-2: adenocarsinoma cell line, VD: volume distribution, CYP3A4; sitokrom isoenzim 3A4

Absorption of compounds in the intestine was an important parameter to determine the adsorption of compounds. The permeability of human colon adenocarcinoma cells was one of the most frequently used approaches to estimate drug in vitro in log cm/second units. A compound was said to have a good value if it had a unit value greater than -5.15 (Dong et al., 2018). From the results of the search for potential herbal compounds and comparators, it appears that the herbal compounds have good absorption in the intestine as indicated by a value of > - 5.15. The antivirus drug even has a value less than -5.15 and was declared not good. This was supported by in vivo test data on patients regarding the absorption of remdesivir and favipiravir. According to (Humeniuk et al., 2021), the bioavailability of remdesivir intravenously is 100%, and it was formulated for intravena uses. (Gülhan et al., 2022) found that the absorption of favipiravir in 52% of Covid-19 patients who consumed favipiravir at a loading dose of 3200 mg on the first day and a maintenance dose of 1200 mg on days 2 to 5 had not reached drug concentrations above 20µg/mL.

The distribution of a compound in the body was determined theoretically by measuring the volume of distribution expressed in the prediction of the compound binding to plasma proteins, the amount of distribution in the fluid, and the amount of absorption in the fluid. A compound was considered to have a good volume of distribution if it was in the range of 0.04-20 l/kg (Dong et al., 2018). From the calculation results it appears that the herbal compounds and antivirals drug had a good distribution in the body.

Drug metabolism in the body was generally divided into phase I (oxidative) and phase II (conjugative), with the contribution of cytochrome P450 enzymes in the liver. To predict ADMET, the approach of a compound could be used as a substrate or inhibitor of various isoenzymes, one of which was the CYP3A4 isoenzyme, which was most commonly found in the liver and was responsible for metabolism (Dong et al., 2018). The calculation results showed that all compounds were not potential as substrates, so it could be concluded that the compounds were not toxic to the liver.

Total clearance was an excretion parameter that was commonly determined as a pharmacokinetic parameter. According to (Dong et al., 2018), the prediction of total clearance was expressed in units of ml/minute/kg with a range of numbers, and if it was greater than 5, it was declared good. The results showed that all compounds had good total clearance values.

Rats acute toxicity was one of the toxicity parameters used to determine the safety of the test compound. In the ADMET lab2 prediction, it is expressed as a range of numbers with conditions 0-0.3 indicating good results, 0.3-0.7 indicating medium results, and 0.7-1 indicating poor results. From the data, it showed that the 3 potential compounds had good value when compared to favipiravir and remdesivir which tend to be toxic. Clinically, favipiravir and remdesivir had toxicity to the liver, gastrointestinal tract, respiratory tract, kidneys, and heart, which until now, according to (Fan et al., 2020), was still being investigated.

CONCLUSION

It can be concluded that α-farnesen, β-sesquiphellandrene and ar-curcumene in ginger in sufficient concentrations had the potential to be developed as oral corona antivirals. α-farnesene is the most potential compound because it has the best interaction among the other three compounds.

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