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## COVID-19 *In Silico* Drug with *Zingiber officinale* Natural Product Compound Library Targeting the M<sup>pro</sup> Protein

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### Abstract

Coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has become a worldwide pandemic. Ginger (*Zingiber officinale*) is a rhizome, which is commonly used for culinary and medicinal purposes. In Indonesia, ginger is taken as traditional medicine by processing it into a drink known as *jamu*. The present study aimed to assess and evaluate the bioactive compounds in ginger that can be used in drug design for treating COVID-19. The crystal structure of the SARS-CoV-2 main protease (M<sup>pro</sup>) was generated from a protein sequence database, i.e., Protein Data Bank, and the bioactive compounds in ginger were derived from the existing compounds library. M<sup>pro</sup> is involved in polyprotein synthesis, including viral maturation and nonstructural protein gluing, making it a potential antiviral target. Furthermore, the bioactive compounds in ginger were analyzed using Lipinski's rule of five to determine their drug-like molecular properties. Moreover, molecular docking analysis was conducted using the Python Prescription 0.8 (Virtual Screening Tool) software, and the interaction between SARS-CoV-2 M<sup>pro</sup> and the bioactive compounds in ginger was extensively examined using the PyMOL software. Out all of the 16 bioactive compounds that were docked successfully, 4-gingerol, which has the lowest binding energy against SARS-CoV-2 M<sup>pro</sup>, as per the virtual screening results, was proven to have the most potential as a viral inhibitor of SARS-CoV-2.

**Keywords:** COVID-19, M<sup>pro</sup>, molecular docking, SARS-CoV-2, *Zingiber officinale*

### Introduction

Since the first case reported in Wuhan, Hubei Province, Central China, coronavirus disease 2019 (COVID-19) has spread rapidly to over 200 countries around the world. The latest category of coronavirus, which is called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [initially recognized as 2019 novel coronavirus (2019-nCoV)], is responsible for this pandemic [1]. At present, the newly mutated SARS-CoV-2 has caused more than 1.5 million deaths, with more than 67 million declared cases around the world, representing a severe danger to general well-being [2].

Coronavirus polyproteins encode two proteases, namely, the papain-like protease (P1<sup>pro</sup>) and the main protease called 3C-like protease (M<sup>pro</sup>), which correlate while

processing and releasing the translated nonstructural proteins. Both M<sup>pro</sup> and P1<sup>pro</sup> are essentially the focus of drug design and development against the ongoing COVID pestilences, including MERS-CoV and SARS-CoV. The foremost accessible crystal structure of the COVID-19 protein is M<sup>pro</sup>, which was identified and published in February 2020 [3]. In contrast to MERS-CoV, coronavirus M<sup>pro</sup> forms a phylogenetic group with SARS-CoV.

During the aforementioned virtual screening study, several medications consisting of an expansive range of antivirals (e.g., ribavirin and telbivudine), vitamins, and drugs that act systemically, were selected. Significantly, ribavirin was utilized in treating the different stages of SARS-CoV. An earlier investigation conducted by Kandeel and Al-Nazawi [3] extensively focused on the COVID-19 M<sup>pro</sup> structure and discovered the stored

medications that are appropriate for reuse against the M<sup>Pro</sup> virus along with the provision of a medication list, which can be consolidated and utilized for the treatment of COVID-19.

In Indonesia, treating oneself with traditional herbal medicines is a part of the belief-system that has given adequate results sometimes. Indonesians have handed down the culture of consuming traditional concoctions from generation to generation; this is particularly true for those who live in or come from Java [4]. A popular example of a traditional herbal remedy in Indonesia is *jamu*, which is the herbal medicine made from natural materials. Indonesians consume herbs to improve their stamina, energy, and endurance to prevent several diseases [5]. Ginger (*Zingiber officinale*), which is a typical and broadly utilized spice, is one of the ingredients used to make herbal medicines. Ginger has many different chemical components, including poly-saccharides, organic acids, raw fiber, phenolic compounds, terpenes, and lipids. The medicinal advantages of ginger are fundamentally credited to its phenolic compounds, e.g., gingerols and shogaols. The conducted analyses have indicated that ginger has numerous medicinal properties, such as antimicrobial, cardiovascular protection, antidiabetic, antioxidant, anti-inflammatory, anticancer, neuroprotective, and respiratory protection [6].

Accordingly, this study employed the SARS-CoV-2 protease structure, obtained from Protein Data Bank (PDB), and ligands, obtained from the PubChem database, along with some computer simulations, such as molecular docking analysis and molecular dynamics simulations, which would illustrate how M<sup>Pro</sup> in the SARS-CoV-2 virus behaved when exposed to different chemical compounds known for their 3C-like protease inhibitor action and antiviral activity. This research is expected to improve the scientific aspects of *jamu*, particularly ginger, in Indonesia.

## Methods

**Sample retrieval.** The three-dimensional (3D) crystal structure of the SARS-CoV-2 (COVID-19) main protease with inhibitor GC-376 was derived in.pdb format from PDB with Protein ID: 6WTT. It was used as it is well annotated in the database and has a good resolution (2.15 Å) and no mutation. This model was employed because it has the largest number of modeled non-hydrogen atoms in the deposited model (7,430) and the most recent release date (May 2, 2020). Moreover, the 3D structure of the target protein was sterilized using the PyMOL software to remove the original ligand. Information about the content of bioactive compounds in *Zingiber officinale* was obtained from Sonale and Kadimi [7], Kharisma, Ansori, and Nugraha [8], and Mao *et al.* [9]. Approximately 16 compounds of *Zingiber officinale*, including remdesivir and inhibitor

GC-376 as standards, were compiled from the PubChem database (Table 1) to obtain samples in the structure data format (.sdf). Subsequently, energy minimization was conducted and .sdf samples were converted into a .pdb format. In Python Prescription 0.8 (PyRx; Virtual Screening Tool) software, energy minimization was performed for all molecules and completed by utilizing OpenBabel with the default parameters. PyRx is a free software used to perform virtual screening from any platform, and it is effective in each process.

**Virtual screening.** The identification of small molecules in SARS-CoV-2 M<sup>Pro</sup> was performed via molecular docking analysis by inputting 16 chemical compounds in ginger and using the AutoDock Vina 1.1.2 program on PyRx. This experiment was conducted by applying a standard docking validation method because it aimed to identify the best ligands to bind the target protein with a default exhaustiveness value of 8. Comparing the binding energy of the standard ligand with that of the lead compounds was a good protocol that could show the correctness of the docking method [10]. However, other parameters, such as the X-ray crystal structure of the protein, LogP, hydrogen bond acceptors, hydrogen bond donors, number of rotational bonds, and volume of the molecule, should also be considered. Furthermore, Lipinski's rule of five was utilized to prove the potency of compounds with low binding affinities and drug-like molecular properties.

**Table 1. List of Compounds Derived from *Zingiber officinale***

Compound Name	Compound ID	Citation
Remdesivir (standard)	121304016	[3]
Inhibitor GC-376 (standard)	71481119	[3]
4-Gingerol	46901319	[8]
6-Gingediol	101660275	[8]
6-Gingerol	442793	[9]
6-Shogaol	5281794	[9]
8-Gingediol	101941698	[7]
8-Gingerol	168114	[8]
10-Gingerol	168115	[9]
$\alpha$ -Curcumene	92139	[9]
$\alpha$ -Farnese	5281516	[9]
$\beta$ -Bisabolene	10104370	[9]
$\beta$ -Sesquiphellandrene	12315492	[9]
Gingerdione	162952	[8]
Methyl-6-Gingerol	70697235	[7]
Methyl-6-Shogaol	91721066	[7]
Zingerone	31211	[8]
Zingiberene	92776	[9]

**Molecular visualization.** The PyMOL software, which is a molecular graphic tool that has been broadly applied to show the 3D images of molecules, nucleic acids, proteins, and surfaces, was employed to visualize the docking results and the 3D structure of the target protein [11]. This software was used to edit molecules data annotation and make motion pictures. Molecular visualization was accomplished by showing the 3D protein structure in animated and surface forms, and staining selection was performed to differentiate the domains between the chains on the target protein and the type of ligands attached.

**Molecular dynamics simulations.** The simulation method that mimicked biological processes was provided by molecular dynamics simulations (i.e., virtual cells). Through this simulation, the stability of an interaction could be assessed under particular conditions, such as temperature and solvent type. One of the most difficult challenges to molecular modeling is the proper handling of ligands, and for this reason, automated tools were highly favored. Several methodologies or software programs for estimating molecular dynamics force fields claim to have parameters consistent with different force fields. One of them is CABS-flex 2.0 (<http://212.87.3.12/CABSflex2/index>), in which the online simulation of molecular dynamics was performed for the best leads as it provided a rapid modeling approach for simulating the flexibility of protein structures. CABS-flex 2.0 is based on the CABS model, a well-known coarse-grained protein modeling tool—CABS design and applications, which can produce protein dynamics at a low computational cost (3 orders of magnitude), albeit with a significant resolution reduction. The purpose of utilizing this tool was to calculate the root mean square fluctuation (RMSF) indication of protein flexibility. During molecular dynamics simulations, the amplitude of atomic motions was monitored by this tool. The aforementioned factors should be documented in this manner, particularly the primary factor that yields the RMSF of 1–3 Å, which indicates the stability of peptides. The distance restraint generator, additional distance restraints, and advanced simulation settings will be set to the default values.

## Results and Discussion

The chemical compounds in ginger (*Zingiber officinale*), inhibitor GC-376 (naturally attached ligand to the M<sup>pro</sup> crystal structure), and remdesivir were obtained from the PubChem database. The sample data consisting of the target compound's 3D structure in .sdf were converted into the .pdb format. Therefore, the 3D structure could be used for further analysis. The structure selection of the target compound is displayed in a two-

dimensional (2D) representative form and colored based on the building blocks of the PyMOL software (Figure 1).

The chemical compounds in ginger are predicted to be drug-like molecules on the server ([scfbio-iitd.res.in/software/drugdesign/lipinski.jsp](http://scfbio-iitd.res.in/software/drugdesign/lipinski.jsp)) using Lipinski's rule of five, which aids in differentiating between the drug-like and non-drug-like particles based on their vital atomic characteristics [12]. Lipinski's rule of five foresees a high likelihood of success or failure due to the drug's similarity for molecules by meeting at least two of the five rules. The rule consists of the following items: atomic mass should be less than 500 Da, lipophilicity (LogP) must be less than 5, donor hydrogen bonds must be less than 5, hydrogen bond acceptors must be less than 5, and molar refractivity must be between 40 and 130. These rules can assist in early pre-clinical development and help evade the expansive pre-clinical and late-stage setbacks [13, 14]. In this study, the predictions show that all chemical compounds in ginger are drug-like molecules and Lipinski's rule of five is not violated (Table 2). Thus, these chemical compounds can enter the next stage of analysis to decide the binding affinity of the target protein.

In this investigation, the chemical compounds in ginger were taken as ligands, remdesivir and GC-376 as standards, and SARS-CoV-2 M<sup>pro</sup> as target. Docking simulations for all of these compounds were conducted by maximizing the Vina Search Space in the PyRx software with grid docking centers  $X = -15.905$ ,  $Y = 30.8490$ ,  $Z = 11.9730$  and dimensions (Å)  $X = 67.2865$ ,  $Y = 92.3788$ ,  $Z = 97.9204$ . The chemical compound in ginger with the lowest binding energy against SARS-CoV-2 M<sup>pro</sup>, as per the virtual screening results, was determined to be 4-Gingerol (Table 3). Molecular binding assay was conducted to identify molecules, structural parts that distinguish the possible binding modes, and energetic parts that predict the binding scores [15]. Proteins were prepared via the PyMOL software to remove water molecules and their original ligands because they can clash with the protein [16]. Ligands with the most negative binding energy can affect the biological activity of the target protein, which is in line with the purpose of this study. Henceforth, it aims to predict the compound with the most potential as a viral inhibitor of SARS-CoV-2 M<sup>pro</sup>. Given that atoms in nature will be commonly found in the structure with the least energy, their final configuration must likewise have low energy. Understanding these properties is essential in the judicious plan of potential inhibitors [17, 18].

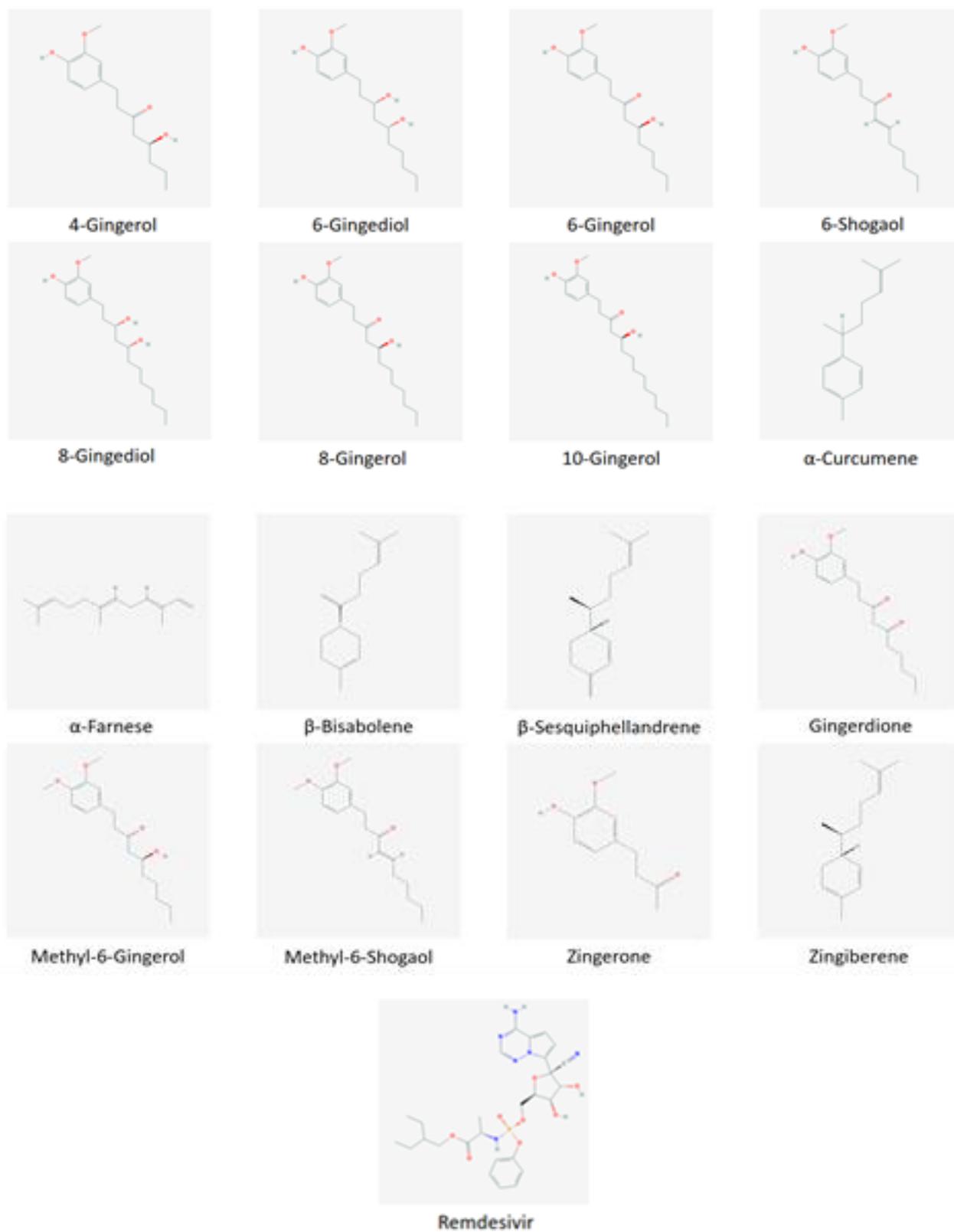


Figure 1. Two-dimensional (2D) Structures of Ginger and Remdesivir Chemical Compounds from the PubChem Database

**Table 2. Prediction Results of Drug-like Molecules Derived from the Chemical Compounds in Ginger**

Compound Name	Molecular Mass (Da)	High Lipophilicity (LogP)	Hydrogen Bond Donors	Hydrogen Bond Acceptors	Molar Refractivity
4-Gingerol	266	2.453599	2	4	73.518578
6-Gingediol	196	3.025599	3	4	83.752365
6-Gingerol	294	3.233799	2	4	82.752571
6-Shogaol	276	4.038999	1	3	81.268776
8-Gingediol	324	3.805798	3	4	92.986359
8-Gingerol	322	4.013999	2	4	91.986565
10-Gingerol	350	4.7942	2	4	101.220558
$\alpha$ -Curcumene	202	4.844920	0	0	68.258987
$\alpha$ -Farnese	204	5.201500	0	0	70.992981
$\beta$ -Bisabolene	204	5.035399	0	0	68.902977
$\beta$ -Sesquiphellandrene	204	4.891300	0	0	68.832977
Gingerdione	292	3.441999	1	4	81.752777
Methyl-6-Gingerol	308	3.536799	1	4	87.639771
Methyl-6-Shogaol	290	4.341999	0	3	86.155975
Zingerone	194	1.9224	1	3	53.660789
Zingiberene	204	4.891299	0	0	68.832977

**Table 3. Molecular Docking Results**

Ligand	Target	Binding Energy (kcal/mol)
Remdesivir (standard)	M <sup>pro</sup>	-8.3
Inhibitor GC-376 (standard)	M <sup>pro</sup>	-7.8
4-Gingerol	M <sup>pro</sup>	-7.3
$\beta$ -Bisabolene	M <sup>pro</sup>	-6.8
Gingerdione	M <sup>pro</sup>	-6.7
Methyl-6-Gingerol	M <sup>pro</sup>	-6.5
6-Shogaol	M <sup>pro</sup>	-6.4
6-Gingerol	M <sup>pro</sup>	-6.3
Methyl-6-Shogaol	M <sup>pro</sup>	-6.3
8-Gingerol	M <sup>pro</sup>	-6.2
6-Gingediol	M <sup>pro</sup>	-6
Zingerone	M <sup>pro</sup>	-6
10-Gingerol	M <sup>pro</sup>	-5.9
$\alpha$ -Farnese	M <sup>pro</sup>	-5.9
8-Gingediol	M <sup>pro</sup>	-5.7
$\alpha$ -Curcumene	M <sup>pro</sup>	-5.7
$\beta$ -Sesquiphellandrene	M <sup>pro</sup>	-5.6
Zingiberene	M <sup>pro</sup>	-5.6

In this investigation, M<sup>pro</sup> was utilized because it is an essential enzyme of coronaviruses and plays an important role in mediating viral replication and transcription. Furthermore, the main protease is important in the viral life cycle and has no close relation to homologous sequences in humans [19]. Molecular visualization of the docking results of this study was accomplished using the PyMOL software for staining and structural selection (Figure 2). All of the compounds were mostly docked at the same sites and located in Chain B of the target protein.

The target protein was visualized on a transparent surface and an animated structure, with the red raspberry color denoting Chain A, blue marine color denoting Chain B, and pale yellow color denoting Chain C (Figure 3). The chemical combinations with the least binding energy were extensively examined to determine the position of their molecular interactions, and the types of bonds formed were visualized using the ProteinPlus web portal in the PoseView program. The PoseView program generates 2D diagrams of complexes with known 3D structures using chemical structure drawing rules [20]. The direct bonds between the protein and ligands were denoted by dashed lines, whereas the interplaying protein residues and ligands

were illustrated in the structural diagrams. Furthermore, the spline sections represented the hydrophobic portions of the ligands and the communicating amino acids represented the hydrophobic interactions. To derive the structural diagrams and modify their layouts, the 2D drawing package was utilized [21]. The estimations of the interactions between molecules were completed by applying the atom type and basic geometric-criteria-based built-in interaction model. 4-gingerol compounds interact in the SARS-CoV-2 M<sup>pro</sup> domain with hydrophobic bonds at the Phe294, Asn151, Gln110, and Val104 positions and hydrogen bonds at the Asn151, Thr111, and Asp153 positions (Figure 3). The atomically resolved structure at the ligand and protein interface is required to launch a novel strategy for drug design and development. The maintenance of atom sites in this approach was accomplished using diverse biophysical factors, e.g., molecular weight, hydrogen bonds, size, hydrophobic interactions, and shape. In the open conformational environment of protein structures, the reduced intermolecular interactions, including hydrogen bonding and hydrophobic interactions, stabilize the energetically-favored ligands. Prioritizing hydrophobic interactions over hydrogen bonding leads to tight binding [22].

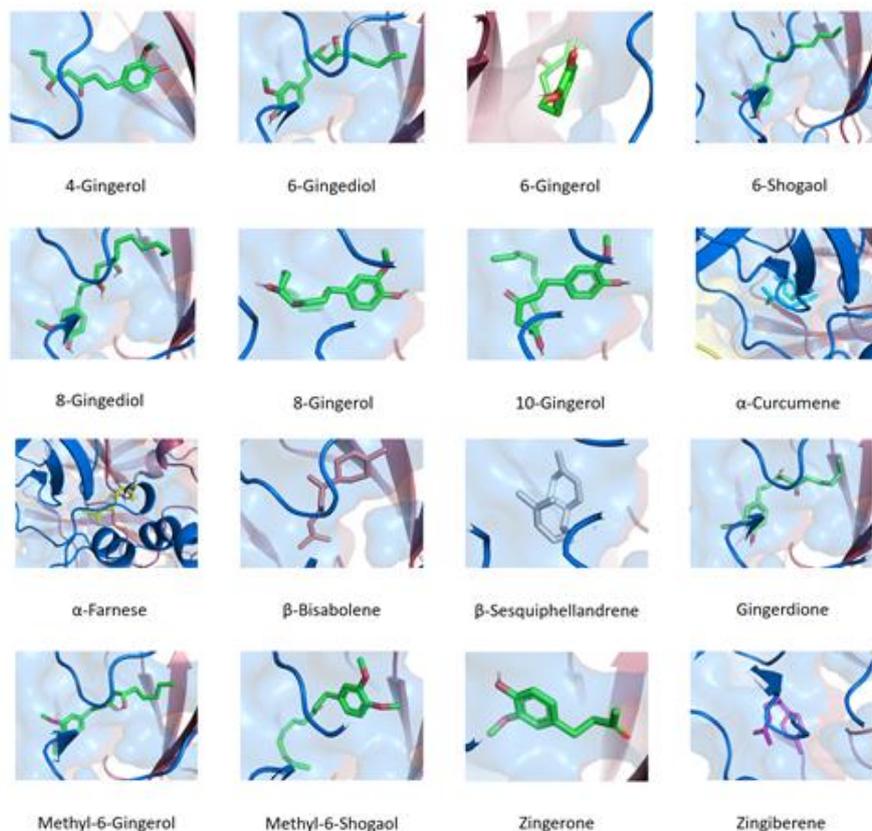
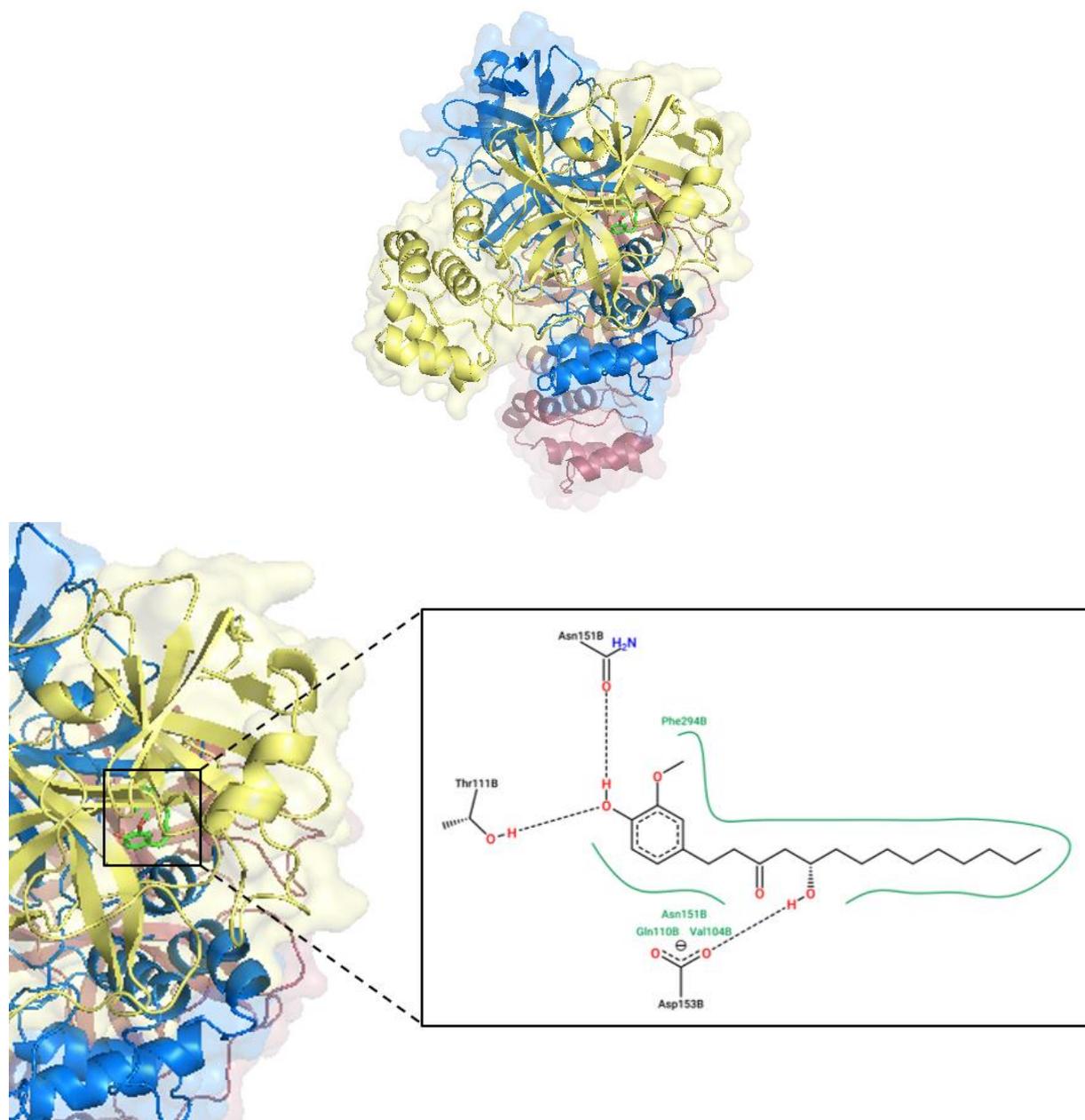


Figure 2. Molecular Visualization of Bioactive Compounds in Ginger Binding to M<sup>pro</sup> using the PyMOL Software



**Figure 3. Structure of the Target Protein (SARS-CoV-2 M<sup>pro</sup>) and its Chemical Interactions with 4-gingerol. Visualization of the SARS-CoV-2 M<sup>pro</sup> Target Protein using Transparent and Animated Surfaces. Staining Selection Performed using the PyMOL Software differentiates between the Chains (A, B, and C) of the Protein**

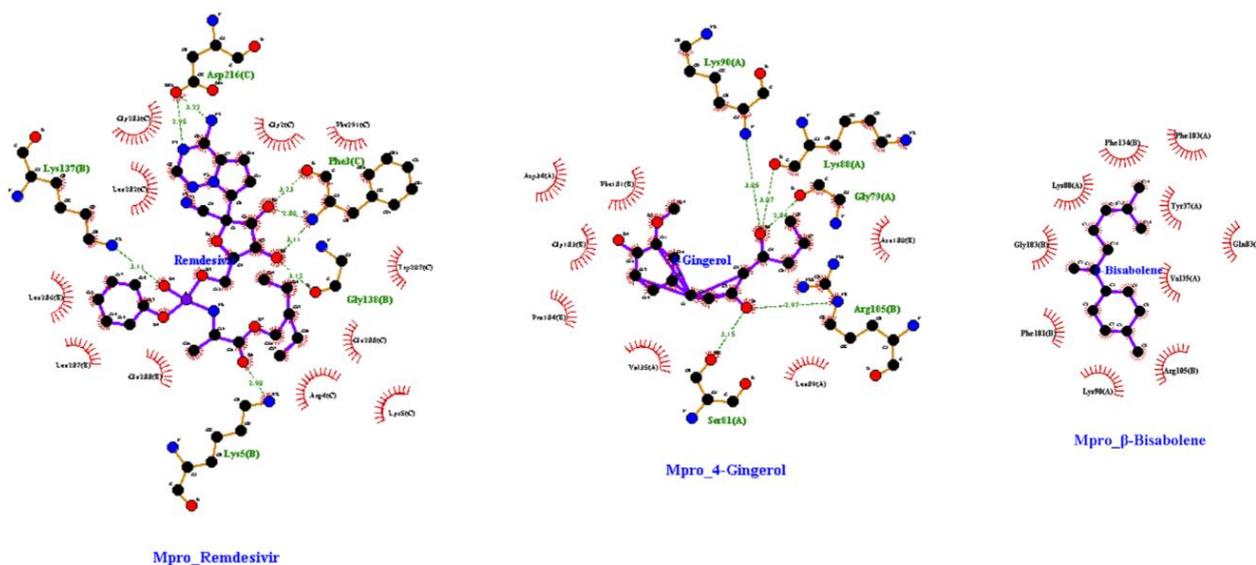
SARS-CoV-2 M<sup>pro</sup> interplays with the top three ligands identified by molecular docking analysis, namely, remdesivir, 4-gingerol, and  $\beta$ -bisabolene (Figure 4). No hydrogen bond was detected between  $\beta$ -bisabolene and the target protein. Remdesivir bonded with five different amino acid residues, i.e., Asp216, Phe3, Lys137, Gly138, and Lys5. Similarly, 4-gingerol bonded with five amino acids, i.e., Lys90, Lys88, Gly79, Arg105, and Ser81. In both remdesivir and 4-gingerol, hydrogen bonds occurred between the ligand and the target protein. Hydrogen bonds occur commonly in nature and are essential for protein–ligand interactions, protein folding,

and catalysis. By promoting molecular interactions, hydrogen bonds enhance a variety of biological activities; they are usually considered to facilitate protein–ligand binding [23].

Figure 5 shows the target protein with its ligands presented in a 3D structure and its 2D fluctuation plots. The fluctuation plot exhibits the residue-by-residue variations that were observed during the simulation. When dealing with multichain proteins, each chain is plotted individually. Because these ligands are bound on Chain B, only the plot from Chain B is presented. The

x-axis of the plot shows the RMSF values (Å), whereas the y-axis shows the amino acid residue index. Protein flexibility and dynamics will change depending on conformation, as shown in Figure 5. Molecular docking analysis using M<sup>PRO</sup> showed that 4-gingerol has the lowest free energy, and its protein fluctuation remains

below the RMSF criterion of 1–3 Å. Consequently, 4-gingerol is considered to have a stable conformation. Moreover, the M<sup>PRO</sup>-4-gingerol complex is detected in residues 49–51 and 191. Any discrepancy in RMSF indicates that the differences in ligand conformation will eventually affect the protein flexibility.



The meaning of the items on the plot is as follows:

- Ligand bond
- Non-ligand bond
- Hydrogen bond and its length
- His 53 Non-ligand residues involved in hydrophobic contact(s)
- Corresponding atoms involved in hydrophobic contact(s)

Figure 4. 2D Diagrams of the Interactions of SARS-CoV-2 M<sup>PRO</sup> with Remdesivir, 4-gingerol, and β-bisabolene, Visualized using the LigPlus Software [24]

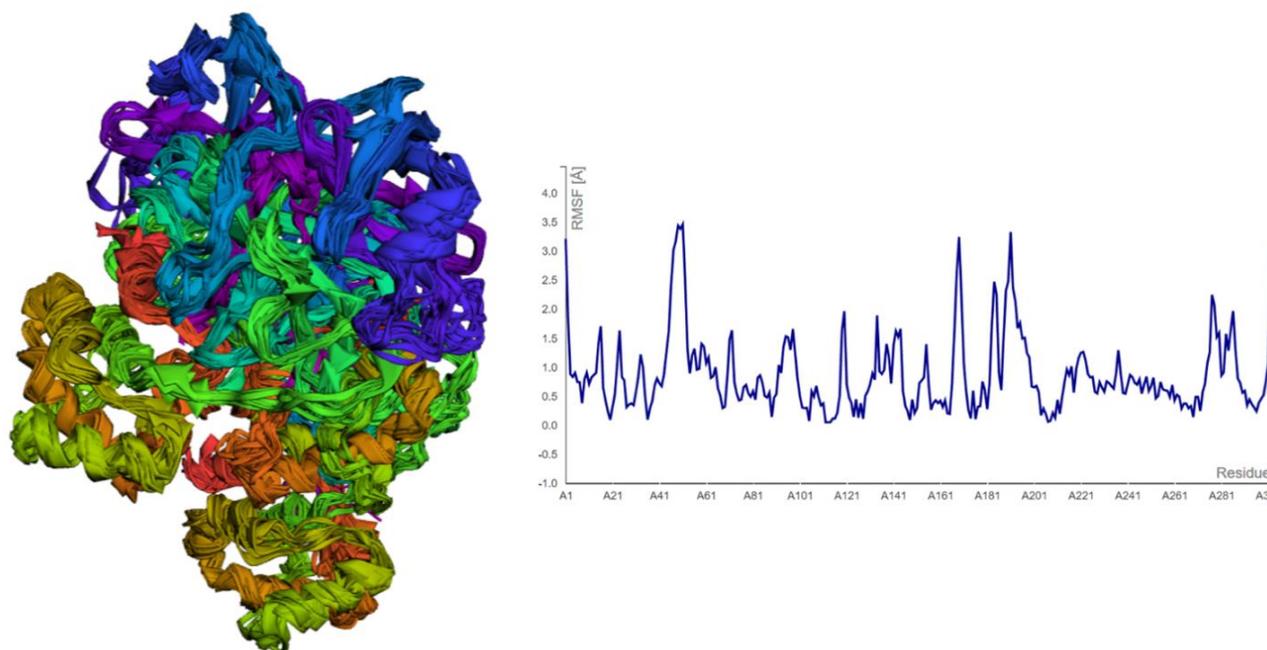


Figure 5. 3D structure and Fluctuation Plot of SARS-CoV-2 M<sup>PRO</sup> with 4-gingerol (CABS-flex)

As stated in FooDB, 4-gingerol belongs to the gingerols class of compounds. Gingerols are compounds that have a gingerol moiety with the structural definition “4-hydroxy-3-methoxyphenyl group” replaced at the C6 carbon atom by “5-hydroxy-alkane-3-one.” 4-Gingerol is insoluble in water and has low acidity. Ginger contains 4-Ggingerol, making it a possible biomarker for the intake of this dietary product.

Protein–ligand connections are essential for all cycles that occur in all life forms. The ligand mediates signal transmission through atomic complementarity, which is the basis for all life forms. These substance reactions include biological recognition at the subatomic level. Protein enhancement depends to a limited extent on the development of particular sites intended to tie the small molecular ligands with affinities tailored to the requirements of the cell [25]. 4-Gingerol can be a candidate for drug design and development for the treatment of COVID-19 because it can bind to SARS-CoV-2 M<sup>pro</sup> with the lowest binding energy and interact via hydrogen and hydrophobic bonds (Figure 3). 4-Gingerol also satisfies Lipinski’s rule of five and can be categorized as a drug-like molecule because it has an atomic mass of 266 Da, LogP of 2.453, two hydrogen bond donors, four hydrogen bond acceptors, and a molar refractivity of 73.518 (Table 2).

Molecular dynamics simulations showed that 4-gingerol could interact more effectively with SARS-CoV-2 M<sup>pro</sup>, indicating that it has therapeutic prospects for the treatment of SARS-CoV-2 infections by inhibiting M<sup>pro</sup>, leading to the inactivation and consequent failure of virion assembly. The comprehensive virtual screening yielded annotations indicating that compounds derived from ginger are the best leads. In this sense, the apparent reusability of bioactive compounds *Zingiber officinale* as SARS-CoV-2 M<sup>pro</sup> inhibitors has been highlighted. Before clinical trials, both in vivo and in vitro studies are strongly recommended to determine the therapeutic effectiveness of the aforementioned experimentally-created ligands.

## Conclusion

In this study, the bioactive compounds in ginger were subjected to several experiments, such as Lipinski’s rule of five, molecular analysis, molecular dynamics simulations, and chemical interaction evaluation, with M<sup>pro</sup> as the protein target of SARS-CoV-2. Among all the ligands, 4-gingerol was determined to be the best because it exhibited outstanding stability during molecular docking analysis and molecular dynamics simulations. Further investigation, particularly pathway prediction employing the Stitch database, is recommended, and the potential of 4-gingerol must be proven through in vitro and in vivo analyses to obtain the right formula. Thus, *jamu* made from ginger and turmeric has bioactive

compounds. To further validate this research, analyses could be performed to determine which atoms from the protein and ligand bonded.

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