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Molecular docking study and molecular dynamics simulation of spice metabolites against main protease enzymes and NSP3 macrodomain SARS CoV-2

Purwaniati[1]*, Asep Roni[2]
[1] Pharmaceutical Analysis and Medicinal Chemistry, Pharmacy Faculty, Universitas Bhakti Kencana
Jl. Soekarno-Hatta 754 Bandung, Indonesia
[2]Pharmaceutical Biology, Pharmacy Faculty, Universitas Bhakti Kencana
Jl. Soekarno-Hatta 754 Bandung, Indonesia

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ABSTRACT

COVID-19 is still a global pandemic. The transmission is very fast and wide. Its prevalence continues to increase. There is no specific antiviral drug for SARS CoV 2 yet. This study aims to find lead compounds from compounds derived from spices that can work as multitarget SARS CoV-2 antivirals. The target of drug action chosen in this study is the main protease enzyme and non-structural protein 3 (NSP3) macrodomain. Antiviral compounds that work on both targets are expected to be more potent. This antivirus will work to inhibit virus replication through main protease inhibition and increase innate immunity through NSP3 macrodomain inhibition. Molecular docking and molecular dynamics simulation were chosen as the methods in this study. Based on the results of molecular docking, it was found that the compound of dauricine, tomentin A, daurisoline, xhantoangelol, rutin and myricetin gave good affinity to both targets. These compounds provide an inhibition constant below 10000 nM or 10 micromolar. Meanwhile, in the molecular dynamics simulation test, it was found that dauricine, rutin, myricetin, and xhantoangelol have good interaction stability with both targets. So from this study, it can be concluded that dauricine, rutin, myricetin and xhantoangelol are potential compounds as lead compounds for SARS CoV-2 antivirals that act on the main protease and the NSP3 macrodomain.

Keywords: COVID-19, main protease, molecular docking, NSP3 macrodomain, SARS CoV-2

Corresponding author:
Purwaniati
Universitas Bhakti Kencana
Jl. Soekarno-Hatta 754 Bandung, Indonesia
Email: Purwaniati@bku.ac.id

INTRODUCTION

COVID-19 is a disease that resembles pneumonia which was discovered at the end of 2019. SARS CoV 2 (severe acute respiratory syndrome coronavirus 2) is the causative agent of the disease. Its prevalence continues to increase. This virus is a member of the Coronaviridae family. Viruses that have this unique phylogenetic position, belong to the order Sarbecovirus (Wu et al., 2020 ; Belouzard S. et al, 2012). There is no specific drug to cure this disease. Finding new effective drugs with good safety profiles is a challenge in itself. Indonesia is a country that is already known to be rich in spices, which are not only used as a seasoning but also as traditional medicines. This research was conducted as an effort to screen for active compounds from various spices as COVID-19 drugs. The research was conducted in silico with a molecular docking and molecular dynamics simulation. The main protease and NSP3 macrodomain enzymes were chosen as their target of action. From this research, it is hoped that a lead compound will be found that is effective at both targets.

Garlic and many other spices have been known to contain several active compounds that are useful in various treatments. These materials were used either in their whole form or as isolates. The potential of some metabolites in spices, such as turmeric, garlic, white pepper, and others as an antiviral for SARS CoV 2 has also been widely studied. Most of the research is conducted in silico by targeting a specific target. SARS CoV 2 has many structural and non-structural protein. These proteins are needed for the survival of the virus. Spike (S), membrane (M), envelope (E), nucleocapsid (N), and hemagglutinin esterase (HE) glycoprotein are structural proteins that function to maintain the structure of the virus. HE is not possessed by all types of coronaviruses. The viral membrane contains at least 3 types of proteins, namely: S, M, and E (Belouzard et al., 2012). Currently, there are at least 21 non-structural protein (NSP) that are possible to be selected as targets SARS CoV 2 antiviral drugs (Purwaniati, 2020; Ibrahim et al., 2020). However, based on literature searches in the Science Direct database, the most studied targets were: main protease, S, M, helicase, RNA-dependent RNA -polymerase (RdRp), and E. The data search was carried out in March 2021.

The nucleotide sequence of genes SARS CoV 2 and SARS CoV are very similar. The similarity is more than 80%. This virus has 2 polyproteins (pp) namely pp1a and pp1ab (Oerlemans et al., 2021; Zhou et al., 2020). Both will divide to form non-structural proteins (NSPs). pp1a will generate 11 NSPs, while pp1ab will generate 16 NSPs. All NSPs play a very important role in the viral replication process (Anand et al., 2003). The cleavage of polyproteins to form NSPs is facilitated by protease enzymes. The main protease enzymes involved in this pp cleavage are main protease (M^{pro}) or 3C-like proteinase (3CLpro) and papain-like protease (PLpro). M^{pro} is localized on NSP5, while PLpro is on NSP3 (Mielech et al., 2014).

M^{pro} plays a very important role in the viral replication process. Therefore, this enzyme becomes a promising target molecule in the SARS CoV 2 antiviral drug discovery effort. M^{pro} consists of 3 domains. The catalytic site on the cysteine-histidine residue. The substrate will be catalyzed in the region between the domains. The structure of M^{pro} is quite enduring (not easy to mutate), so it becomes a promising target for drug design and development (Banerjee et al., 2021). All amino acid residues at the binding site have the potential to bind to the substrate. These residues include Thr24, Thr25, Cys44, Met49, Tyr54, Phe140, Asn142, Gly143, Cys145, His163, His164, Met165, Glu166, Leu167, Pro168, Asp187, Arg188, Gln189, and Thr190 (Morse et al., 2020). On the other hand, NSP3 which has a macrodomain is a protein that largely determines the pathogenesis of the coronavirus. This NSP3 macrodomain acts against the interferon (INF) response of the host. This INF response is needed by the host as a component of innate immunity and prevention of viral infections (Ivashkiv & Donlin, 2014;Schoggins, 2019). So the presence of this response is very important, especially if the virus has already replicated in the host cell (Russo et al., 2021). Based on the interest in these 2 targets, this research was carried out. Research is to find lead compounds that can act on the main protease so that it inhibits viral replication, and work on the NSP3 macrodomain to increase the innate immune response of the host.

METHODOLOGY

Materials and instruments

Materials used in this study included: test compounds and target protein molecules. The test compounds are compounds derived from spices as summarized in the publication Prasansuklab et al. (Prasansuklab et al., 2021). The target proteins used are the main protease with PDB ID 6ZRT (Oerlemans et al., 2021) and NSP3 macrodomain with PDB ID 7BF6 (Ni et al., 2021).

The instruments used include hardware and software. The software used included: Pyrx, Autodock 4.2.6, and Amber. While the hardware used is a computer with Processor Intel® XEON®CPU E5-2620 v4@2.10GHz x 16, VGA: Graphics GeForce GTX 1080/PCIe/SSE2, RAM 64 GB, OS: Linux Ubuntu 16.04 LTS, HDD: 2 TB.

Preparation of test ligands and target proteins

The test ligand structure was modeled using the ChemDraw Ultra 2014 application and then optimized geometry using the Gaussian09 application. The target proteins were downloaded from the Protein Data Bank (PDB) database with IDs 6ZRT and 7BF6. The protein is then separated from its ligands and water molecules. The proteins that have been separated are then the target molecules.

Molecular docking

Molecular docking was first performed with the PyRx Autodock Vina application (Trott & Olson, 2009). The molecules that provide low binding affinity were then further studied with Autodock 4.2.6 (Morris & Dallakyan, 2013). Further data analysis was carried out with the Discovery Studio Visualizer application.

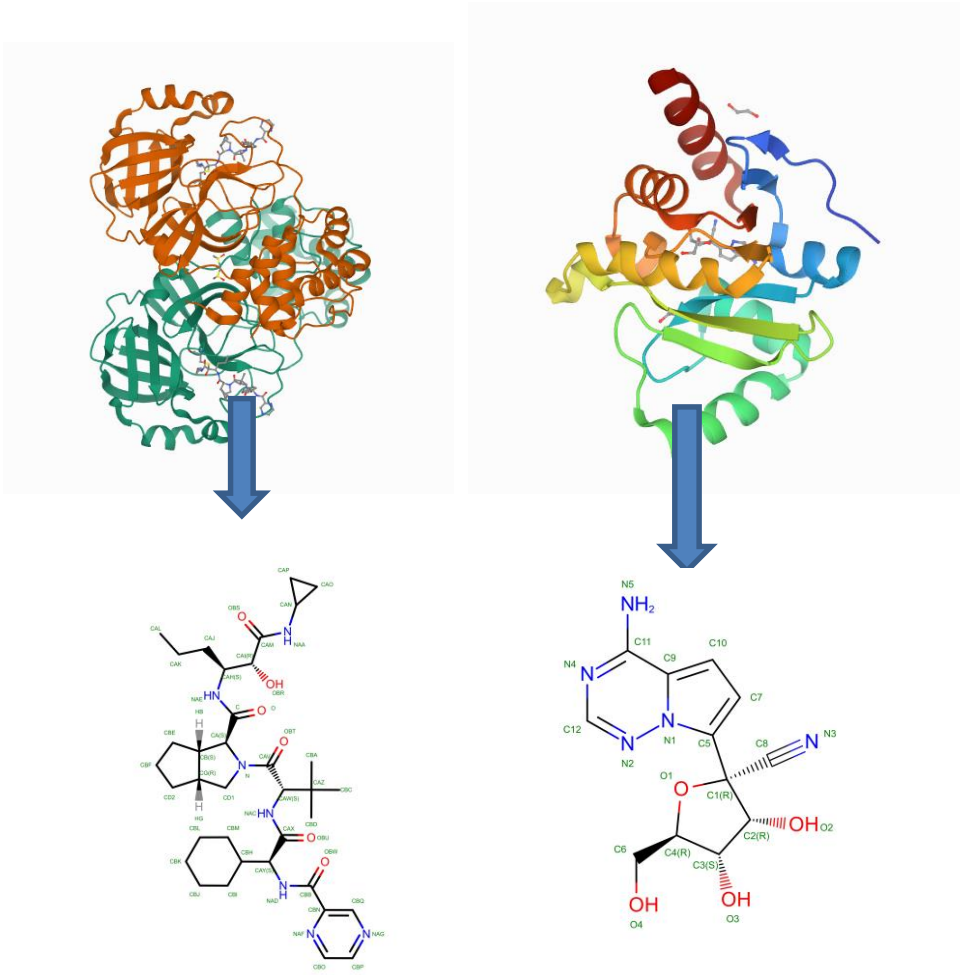
Molecular dynamic simulation

The compounds which gave low binding affinity were continued in molecular dynamics simulations. The application used is Amber18. RMSD and RMSF are used as parameters in this simulation.

RESULT AND DISCUSSION

Preparation of test ligands and target proteins

The 71 test compounds were used in this study. The target protein was downloaded from the Protein Data Bank (PDB) database with ID 6ZRT for the main protease. The protein structure is then separated between the protein and its natural ligand. The natural ligand is the molecule *(1S,3aR,6aS)-2-[(2S)-2-({(2S)-2-cyclohexyl-2-[(pyrazine-2-ylcarbonyl)amino]acetyl}amino)-3, 3-dimethylbutanoyl]-N-[(2R,3S)-1-(cyclopropylamino)-2-hydroxy-1-oxohexan-3-yl]octahydrocyclopenta[c]pyrrole-1-carboxamide* (boceprevir). As for NSP3 macrodomain taken from PDB with ID 7BF6, whose natural ligand is *(2~{R},3~{R},4~{S},5~{R})-2-(4-azanylpyrrolo[2, 1-f][1,2,4]triazine-7-yl)-5-(hydroxymethyl)-3,4-bis(oxidanyl)oxolane-2-carbonitrile* (GS-441524, a metabolite of remdesivir). The protein structure and natural ligands of the two targets can be seen in Figure 1.



a) b)

Figure 1. A complex structure of proteins and their natural ligands, a) Main protease protein structure and its natural ligands; b) NSP3 macrodomain protein structure and its natural ligands

Molecular docking

The results of molecular docking with PyRx Autodock Vina on the 6ZRT target showed that all the tested ligands had a fairly good binding affinity. Where the value of the binding affinity ranges from -3.9 to -7.2 Kcal/mol. While the target protein 7BF6 ranged from -4.4 to -9.9 Kcal/mol. The data for the 20 compounds with the best binding affinity for the main protease can be seen in Table 1, and the NSP3 macrodomain protein can be seen in Table 2. Based on the data in Tables 1 and 2, it is known that the test compounds gave different affinities for the main protease and NSP3 macrodomain. However, several test compounds showed a good affinity for both targets. 10 test ligands with the best binding affinity values were chosen to be docked again with Autodock 4.2.6. Molecular docking with Autodock 4.2.6 is done with the aim of getting more accurate docking results. The first stage molecular docking with Pyrx Autodock Vina is a rapid docking as an initial screening. The ten selected compounds are *dauricine, tomentin A, daurisoline, xhantoangelol, rutin, kuromanin, quercetin rhamnoside, emodin, quercetin rutinoside,* and *myricetin*. These compounds have a good affinity for the main protease and the NSP3 macrodomain. So it has the potential to be a candidate for drugs that work on both.

Table 1. The molecular docking results of 20 test ligands with the best binding affinity with the Pyrx Autodock Vina application on the main protease

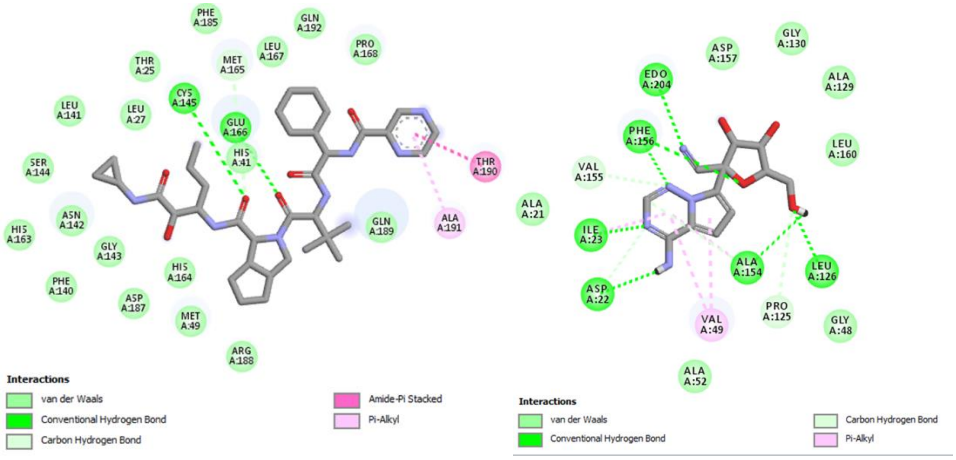
Compound Names	Binding Affinity (Kcal/mol)
Tomentin A	-7.2
Daurisoline	-7.1
Tomentin D	-7.1
Emodin	-6.9
Rutin	-6.9
Dauricine	-6.8
Dihydrotanshinone I	-6.8
Quercetin-3-O-rhamnoside	-6.8
Quercetin-3-O-rutinoside	-6.8
Tomentin B	-6.8
Xanthoangelol	-6.7
Natural ligan of 6ZRT	-6.6
Tomentin C	-6.6
Vicenin	-6.6
Xanthoangelol F	-6.6
Cryptotanshinone	-6.5
Tiliroside	-6.5
Kuromanin	-6.4
Afzelin	-6.3
Kazinol A	-6.3

Molecular docking with the Autodock 4.2.6 application begins with the validation process of the docking method for both targets. The docking method used is a grid point of 40x40x40 and numbers of GA runs 100. This method is declared valid because it gives an RMSD (root mean square deviation) value of less than 2 Å and the interaction of natural ligands and amino acid residues is confirmed. Natural ligands in the main protease form 2 hydrogen bonds with amino acid residues CYS A:145 and GLU A:166. Whereas in NSP3 macrodomain 7 hydrogen bonds are formed with amino acid residues ALA A:154, LEU A:126, EDO A:2014, PHE A:156, ILE A:123, and ASP A:22. PHE A:156 forms 2 hydrogen bonds with natural ligands. The hydrogen bond facilitates the interaction between the ligand and the target protein. This bonding also increases the binding affinity by displacing water molecules into the bulk of the solvent (Chen et al., 2016). So it can be concluded that the more hydrogen bonds formed, the stronger the interaction between the two molecules. The van der Waals interactions also determine the affinity. In the main protease, this interaction is very dominant, where there are at least 20 van der Waals interactions. While in the NSP3 macrodomain, there is not much van der Waals interaction. Where only 8 interactions occur. This means that the binding affinity for this protein is determined more by its hydrogen bonds because the strength of the van der Waals interaction is much weaker than that of hydrogen bonds. Visualization of the interaction can be seen in Figure 2.

Table 2. The molecular docking results of 20 test ligands with the Pyrx Autodock Vina application on the NSP3 macrodomain

Compound Names	Binding Affinity (Kcal/mol)
Tomentin B	-9.9
Savinin	-9.0
Tomentin A	-8.9
Tomentin D	-8.9
TomentinC	-8.7
Xanthoangelol	-8.7
Broussochalcone A	-8.6
Quercetin-3-O-rutinoside	-8.5
Tomentin E	-8.5
Xanthoangelol B	-8.5
Cryptotanshinone	-8.4
Dauricine	-8.4
Daurisoline	-8.4
Decursidin	-8.4
Rutin	-8.4
Dihydrotanshinone	-8.3
Kazinol_A	-8.3
Hirsutenone	-8.2
Luteolin	-8.2
Tanshinone	-8.2

Binding affinity describes the strength of the interaction that occurs between two molecules. In this case between the ligand and the target protein. The more negative the binding affinity value, the stronger interaction between the two molecules. This binding affinity value is expressed in Gibbs free energy (ΔG) (Kairys et al., 2019). So it can be seen that the compounds in Tables 1 and 2 are compounds arranged from the one with the strongest interaction to the lowest.



A B

Figure 2. Visualization of natural ligand interactions with target proteins, a. main protease and its natural ligand (boceprevir), b. NSP3 macrodomain and its natural ligand (GS-441524, a metabolite of remdesivir)

The results of the docking of test molecules with the Autodock 4.2.6 application showed that the test molecules gave a fairly good affinity to both targets. Molecules that provide good affinity for both targets can be seen in Table 3.

Table 3. List of molecules with good affinity for the main protease and NSP3 macrodomain

Compounds Name's	6ZRT (Main protease)		7BF6 (NSP3 Macrodomain)	
	Binding Affnity (Kcal/mol)	Inhibition constants (nM)	Binding Affnity (Kcal/mol)	Inhibition constants (Nm)
Dauricine	-10.31	27.48	-10.29	28.85
Tomentin A	-9.79	66.58	-9.86	58.76
Daurisoline	-9.06	230.24	-9.06	227.14
Xhantoangelol	-8.37	727.28	-9.65	84.65
Rutin	-7.69	2290	-8.46	626.85
Curomanin	-7.68	2350	-8.89	306.71
Myricetin	-7.29	4540	-6.77	10950
Natural ligand 6ZRT	-10.79	12.27	Not tested	Not tested
Natural Ligand 7BF6	Not tested	Not tested	-7.72	2190

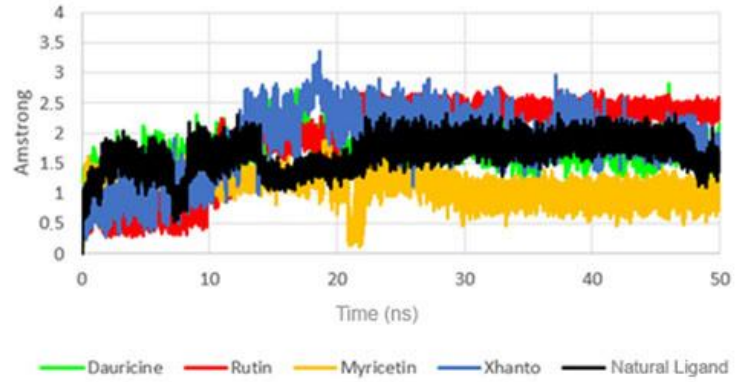
The data in Table 3 shows that the molecular docking results have been proven to be good because the natural ligand affinity for each target is confirmed. Dauricine, tomentin A, daurisoline, xhantoangelol, rutin, curomanin and myricetin gave good affinity values compared to their natural ligands. This shows that these compounds are potential inhibitors for both targets.

Molecular dynamic simulation

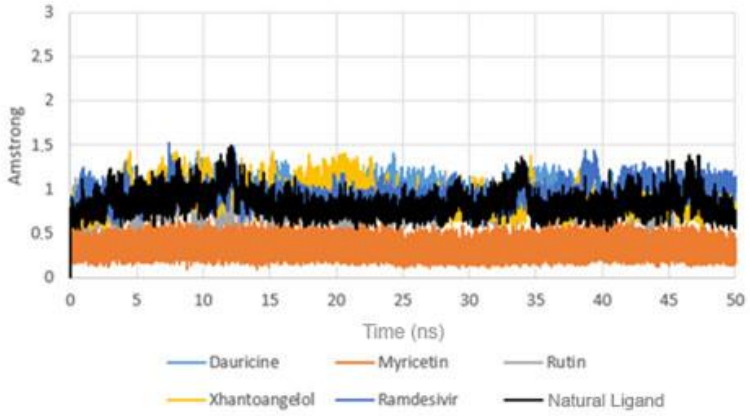
Molecular dynamics simulations were carried out to determine the stability of the complex formed between the ligand and the target. This stability is observed through the value of fluctuations, conformations, and changes in total energy (Sharif et al., 2018). This simulation was carried out within 50 ns. Simulations were carried out on each target's natural ligand, the test ligand, and remdesivir as a comparison on the NSP3 mscrodomain. The test molecules include; dauricine, tomentin A, daurisoline, xhantoangelol, rutin, curomanin and myricetin. The RMSD graph of molecular dynamics simulation on the main protease target and NSP3 macrodomain can be seen in Figure 2. The RMSD and RMSF graphs shown in this article are only for compounds that show good stability in both targets.

In Figure 2a it can be concluded that the stability of ligand-main protease complex was formed after the simulation lasted for 30 ns. This indicates that the equilibrium condition has been reached. All ligands in the main protease still showed stability of their interactions until the end of the simulation. Whereas in Figure 2b it can be seen that the interaction between all ligands and targets (NSP3 macrodomains) has been stable since the beginning. This means that the interaction of the ligand with the protein has reached its equilibrium since the start of the simulation (Yang et al., 2014).

Dauricine, xhantoangelol, rutin, and myricetin showed good stability during the simulation period. Myricetin appears to be very stable in both the main protease and the NSP3 macrodomain. So it can be predicted that myricetin will provide the best affinity for both targets. Until now there has been no research publication that focuses on the discovery of dual-target drugs, main proteases, and NSP3 macrodomains. Main protease is the most studied enzyme in SARS CoV-2 , which can be proven by search results in Science Direct. However, until now no drug is effective as an inhibitor of SARS CoV-2. No one has even reached the clinical trial stage yet (Cui et al., 2020). Efforts to find a COVID-19 drug targeting the NSP3 macrodomain are indeed not as popular as playing protease. There has not been much research on this work target. However, efforts to find inhibitors have begun, as was done by Russo et al (Russo et al., 2021).



a)



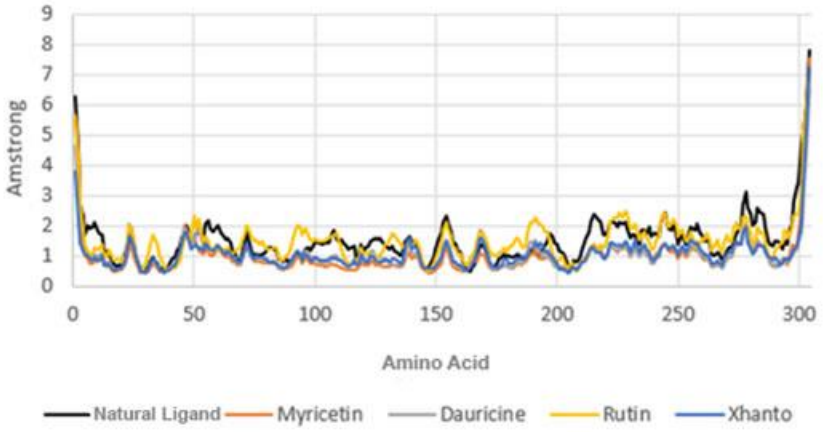
b)

Figure 3. RMSD graph of molecular dynamics simulation results; a) on Main Protease target proteins, and b) on the NSP3 macrodomain target protein

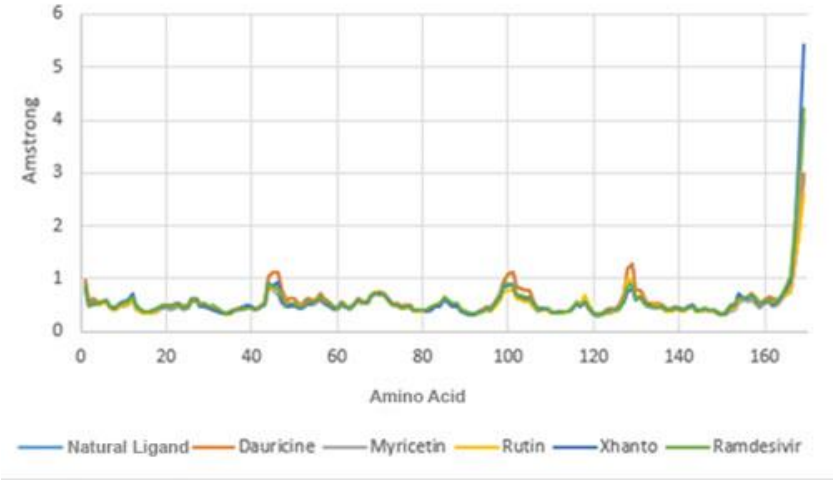
RMSF (root means square fluctuation) analysis was carried out to determine the stability of the target-ligand interaction at its binding site. RMSF indicates fluctuations in the amino acids of the target molecule (Dermawan et al., 2019). The RMSF graph can be seen in Figure 3.

Based on the RMSF graph, it can be concluded that the amino acids of the target molecule are very stable. The stable target-ligand interaction is expected to be able to produce an inhibitory effect on the main protease enzymatic activity. So the virus will not be able to replicate, and eventually the virus will die.

The amount of energy involved in the formation of ligand and target bonds can be predicted through the values of MM/PBSA (Molecular Mechanics Poisson-Boltzman Surface Area) and MM/GBSA (Molecular Mechanics Generalized Born Surface Area) (Yunta, 2016). The results of the analysis of MM/PBSA and MM/GBSA can be seen in Table 4 for the main protease and Table 5 for the NSP3 macrodomain.



a)



b)

Figure 4. RMSF graph of molecular dynamics simulation results; a). on the main protease target molecule, b). on the NSP3 macrodomain target protein

Table 4. MM/GBSA and MM/GBSA data on main protease

Ligand	ΔEvdw (kcal/mol)	ΔEele (kcal/mol)	ΔG Non-polar (kcal/mol)	ΔG Polar (kcal/mol)	ΔG total (kcal/mol)
Natural ligand	-63.7445	-40.4268	-104.1713	46.5055	-57.6658
Dauricine	-45.4764	-14.6237	-60.1002	23.9067	-36.1935
Rutin	-50.1740	-35.2188	-85.3928	50.3862	-35.0066
Xhantoangelol	-44.0362	-17.6144	-61.6506	27.3756	-34.2751
Myricetin	-26.8869	-46.1005	-72.9874	49.0354	-23.9520

Table 5. MM/GBSA and MM/GBSA data on NSP3 macrodomain

Ligand	ΔEvdw (kcal/mol)	ΔEele (kcal/mol)	ΔG Non polar (kcal/mol)	ΔG Polar (kcal/mol)	ΔG Binding (kcal/mol)
Natural ligand	-22.6805	-9.8197	-32.5002	13.1144	-19.3858
Ramdesivir	-37.5130	-22.2325	-59.7455	28.9768	-30.7688
Dauricine	-27.2543	-12.2725	-39.5268	15.3974	-24.1294
Rutin	-40.8922	-29.3942	-70.2864	41.4355	-28.8509
Xhantoangelol	-35.3342	-24.2683	-59.6025	29.1836	-30.4189
Myricetin	-30.3062	-20.1777	-50.4839	27.4827	-23.0012

Based on the data in Table 4 it can be predicted that the natural ligands in the main protease have the strongest affinity. This can be seen from the ΔG value which is the most negative compared to other ligands. Dauricine, rutin, xhantoangelol, and myricetin respectively have weaker affinities than natural ligands. Dauricine and xhantoangelol are two natural compounds that have the potential to be an antiviral for SARS CoV-2 (Prasansuklab et al., 2021). Amino acid residues that interact with natural ligands relative to the binding site are dominated by hydrophobic residues when compared to residues that interact with other ligands. This can be seen from the value of the van der Waals energy. The more negative the van der Waals energy value, the more hydrophobic the amino acid residues at the binding site are.

Meanwhile, based on the data in Table 5, it can be predicted that dauricine, rutin, xhantoangelol, and myricetin have stronger affinities than natural ligands in NSP3 macrodomain. Remdesivir has the highest affinity compared to natural and test ligands. Xhantoangelol has almost the same affinity as remdesivir. This supports previous research that supports that remdesivir works by inhibiting the NSP3 macrodomain (Jung et al., 2020).

CONCLUSION

Dauricine, rutin, xhantoangelol, and myricetin have the potential to be developed into 2 targets SARS CoV-2 antiviral. Namely through inhibition of the main protease and NSP3 macrodomain. Further testing of these compounds is needed to be used as a drug. Further testing can be done by designing more potent derivative compounds and testing their pharmacokinetics and toxicity.

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