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A novel antiviral candidate from *Moringa oleifera* through dual targeting mechanism of SARS-CoV-2 protease: computational screening

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Abstract

COVID-19 is triggered by SARS-CoV-2 which is related in a similar way to SARS-CoV and MERS-CoV. RdRp is an essential component of the virus in replication and transcription. RdRp triggers polymerase activity through binding to cofactors such as nsp7 and nsp8. M^{pro} plays an important role in viral protease activity for the assembly process. RdRp and M^{pro} can be used as targets to inhibit the replicative activity of SARS-CoV-2. *Moringa oleifera* is used by people around the world as a traditional medicine because it has antioxidant, anti-inflammatory, and antiviral properties. This study reveals the molecular mechanism of *Moringa oleifera* as an inhibitor of key proteins in SARS-CoV-2 replication through a computational approach. Additionally, the *in silico* method in this study consists of sample preparation in the database, druglikeness prediction, antiviral probability, virtual screening, chemical bond interaction, and 3D visualization. *Moringa oleifera* may have potential as an antiviral candidate through Ellagic Acid activity as a dual inhibitor through inhibition of SARS-CoV-2 replication and assembly. The candidate compound can generate weak bonding interactions such as hydrogen and hydrophobic to trigger binding stability at specific domains.

Keywords: COVID-19, SARS-CoV-2, RdRp, Moringa oleifera

Introduction

COVID-19 is triggered by SARS-CoV-2 which is closely related to SARS-CoV and MERS-CoV. SARS-CoV-2 genome (27-32 kbp) consists of six ORFs, the first ORFs consist of ORF1a/b at the 5' end encodes non-structural proteins (nsp) such as polyprotein a and b[1]. ORFs located at the 3' end encode four proteins such as spike (for attachment to host cell receptors), membrane protein (M) is a form of virion, RNA wrapping such as nucleocapsid protein (N), and viral interferon inhibitors such as ORF3a,

ORF3d, ORF6, ORF7a, ORF7b, ORF8, ORF9b, ORF14, and ORF10[2]. Several proteins are important for SARS-CoV-2 drug development with inhibitor mechanisms such as RNA-dependent RNA polymerase (RdRp) or nsp12 and SARS-CoV-2 chymotrypsin-like main protease (M^{pro})[3].

RdRp is an essential component of viruses in replication and transcription. RdRp triggers polymerase activity through binding to cofactors such as nsp7 and nsp8. The molecular complex consisting of RdRp-nsp7-nsp8 can be the core of viral RNA replication. The structure of the SARS-CoV-2 RdRp consists of the catalytic core nsp12 and nsp7-nsp8 with heterodimer and subunit structures[4]. A point mutation with sequence number 14408C > T was reported to occur in RdRp which triggered an increase in viral infection activity[5]. RdRp is known to have conserved points in catalytic motifs such as motif A with residue positions from T611 to M626. Antiviral candidates such as remdesivir are known to be RdRp inhibitors to prevent the formation of RdRp-RNA complexes in the SARS-CoV-2 replication process[6]. However, some studies have shown side effects of remdesivir, indicating the need to screen for new antiviral agents[7].

SARS-CoV-2 M^{pro} acts as a dimer consisting of two catalytic domains such as beta sheets with a substrate part at the N-terminal[8]. M^{pro} has a conserved region for nsp8-nsp9 binding or cleavage site. Mpro activation occurs through the catalytic dyad (C145 and H41) and the final stage cleavage on Gln-Ser/Ala/Asn residues. When the COVID-19 outbreak occurred, researchers around the world screened for M^{pro} inhibitors with the aim of preventing virus assembly and many previous synthetic-based drugs failed[9]. These can be replaced from natural ingredients that are predicted to be obtainable and reduce the presence of side effects. *Moringa oleifera* is used by people around the world as a traditional medicine for antioxidant, anti-inflammatory, and antiviral properties. Previous studies reported *Moringa oleifera* can be a virucidal agent for Influenza A through inhibitory activity of proinflammatory agents and lowering cytopathic effects[10]. Antiviral activity of compounds from *Moringa oleifera* has not been identified as SARS-CoV-2 antiviral, this study reveals the molecular mechanism of *Moringa oleifera* as an inhibitor of key proteins in SARS-CoV-2 replication through a computational approach.

Materials and methods

Sample Retrieval

SARS-CoV-2 main protease (M^{Pro}/3CLpro) (7ALH) and RNA-dependent RNA polymerase (RdRp) were obtained from the PDB database (https://www.rcsb.org/). These target proteins were pretreated by removing contaminants such as water and ligand molecules using PyMOL software. A total of 25 chemical compounds from Moringa oleifera that have been selected (table 1) are called ligands. The 3D structures with sdf and canonical SMILE formats of each compound can be downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). All ligands were energy conformational minimized using PyRx v0.8.8 software and saved in pdb form[11,12,13].

Drug-likeness Analysis

Drug-likeness analysis was performed on ligands using Lipinski's Rule of five on the SCFBIO web server (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp). The prediction is positive if at least 2 out of 5 Lipinski's Rule are met. Lipinski's Rule contains 1) molecular mass no more than 500 Daltons; 2) high lipophilicity (LogP) no more than 5; 3) Hydrogen Bond Donor (HBD) no more than 5; 4) Hydrogen bond acceptor (HBA) no more than 10; 5) Molar refractivity between 40-130. This analysis serves to determine the pharmacokinetic properties of a molecule (such as absorption, distribution, metabolism and excretion (ADME))[14,15,16].

Antiviral Probability Prediction

Prediction of 25 ligands derived from *Moringa oleifera* as antiviral agents was carried out with the PASS Online web server (http://way2drug.com/PassOnline/). The standard used is that the Pa (probability activity) score is more than 0.3 and the Pi (probability inactivation) value must be lower than the Pa value. This prediction identifies the probability of antivirus potential with the medium confidence method (computationally proven only)[17,18,19].

Virtual Screening

Virtual screening using molecular docking method was conducted between each ligand and target protein with AutoDock Vina software integrated in PyRx 0.8. The binding affinity of the docking results can be read as the binding affinity value (kcal/mol) between the logan-protein complex. Three ligands with the most negative binding affinity to each target protein were taken for further analysis. The more negative binding affinity, it can be predicted that the ligand is classified as a dual inhibitor that can trigger biological responses[20,21,22].

Chemical Interaction and 3D Molecular Visualization

Compounds with the most negative affinity from each protein were analyzed for the position and type of chemical bond interactions formed using BIOVIA Discovery Studio 2019 and 3D surface visualization, structural selection and coloring using PyMol v.2.5[23,24,25].

Results

Drug-likeness & antiviral probability prediction

Analysis of 25 *Moringa oleifera* compounds as drug candidates found that all of them fulfill at least 2 of Lipinski's rule of five and can be classified as drug-like molecules. Based on the analysis of compounds as antiviral candidates with PASS web server, all compounds except Echitamine (not recorded) have a Pa value of more than 0.3. Asiaticoside has the highest Pa value (0.762), and Hydroxypandutarin A with the lowest Pa value (0.339). These results need to be analyzed in vitro and in vivo because this prediction is medium confidence (**Table 1**).

Table 1. Lipinski and PassOnline analysis results

Compound	MW	HBD	HBA	LOGP	MR	Antiviral Probability	
						Pa	Pi
14-deoxyandrographolide	334.000	2	4	2.99	92.17	0.617	0.012
Andrographolide	350.000	3	5	1.96	93.56	0.424	0.012
Ascorbic acid	176.000	4	6	-1.41	35.26	0.567	0.009
Asiaticoside	312.000	5	6	-0.06	77.15	0.762	0.004
Beta-Sitosterol	414.000	1	1	8.02	128.22	0.686	0.006
Caffeic acid	180.000	3	4	1.20	46.44	0.549	0.017
Curcumin	368.000	2	6	3.37	102.02	0.471	0.028
D-allose	180.000	5	6	-3.22	35.99	0.697	0.005
Echitamine	385.000	3	5	1.39	104.41	-	-
Ellagic acid	302.000	4	8	1.24	68.45	0.481	0.005
Eugenol	164.000	1	2	2.13	48.56	0.396	0.095
Gallic acid	170.000	4	5	0.50	38.40	0.654	0.009
Glucosinolates	448.000	6	10	0.46	103.47	0.461	0.042
Hydroxypanduratin A	392.000	3	4	5.71	114.35	0.339	0.177
Kaempferol	286.000	4	6	2.31	72.39	0.496	0.005
Linoleic acid	280.000	1	2	5.88	86.99	0.623	0.005
Linolenic acid	278.000	1	2	5.66	86.90	0.603	0.006
Methylripariochromene A	262.000	0	4	3.09	73.29	0.381	0.114
Moringin	311.000	3	6	0.50	78.16	0.446	0.033
Niazimicin	357.000	4	7	0.30	90.49	0.496	0.023
Oleic acid	282.000	1	2	6.11	87.09	0.652	0.009
Orthosiphol A	676.000	2	11	4.24	174.77	0.564	0.010
Orthosiphol B	676.000	2	11	4.24	174.77	0.564	0.010
Panduratin A	406.000	2	4	6.01	119.24	0.367	0.133
Quercetin	302.000	5	7	2.01	74.05	0.498	0.005

MW: Molecular Weight; HBD: Hydrogen Bond Donor; HBA: Hydrogen Bond Acceptor; LOGP: High lipophilicity; MR: Molar refractivity

Molecular docking simulation results and chemical bond interactions

M^{pro} docking grid using center coordinates (Å) X:-26.284 Y: 12,5976 Z: 58.9679; dimensions (Å) X:51.3737 Y:66.9738 Z:59.6069 and center (Å) X:119.717 Y:123.605 Z: 120.2977; dimensions (Å) X:79.2745 Y:84.5421 Z:85.7243 for SARS-CoV-2 RdRp protein. The molecular docking analysis performed can see the stability of the interaction between the ligand and the target protein which is read as binding affinity. The more negative the binding affinity value, the more stable the interaction, so based on computerized predictions it can be said that the ligand can affect the biological activity of the target protein, for example inhibiting the activity of the target protein[26,27,28].

M^{pro} functions as a proteolytic that produces non-structural proteins (NPS) such as RdRp and helicase, while RdRp has an important role in the replication and transcription process in

coronaviruses[29.30]. Inhibition of the activity of these two proteins can affect the reproduction process of SARS-CoV-2. Following the molecular docking simulation, three compounds that have the most negative binding energy with M^{pro} protein are Ellagic Acid (-8.4), Glucosinolates (-7.8), and Kaempferol (7.8). This value is still below Nirmatrelvir (-8.5) as the positive control. The RdRp proteins were Asiaticoside (-8.6), Ellagic Acid (-8.2), and Orthosiphol B (-8.8). This value exceeds Nirmatrelvir (-7.9) as a positive control. These five compounds are predicted to have potential as SARS-CoV-2 antivirals through a dual inhibitor mechanism (**Table 2**). Mpro functions as a proteolytic that produces non-structural proteins (NPS) such as RdRp and helicase, while RdRp has an important role in the replication and transcription process in coronaviruses[31].

Table 2. Binding affinity of ligand and protein molecular complexes

Compound	Pubchem ID	Binding Affinity (kcal/mol)	
		M^{pro}	RdRp
14-deoxyandrographolide	11624161	-6.6	-7.3
Andrographolide	5318517	-6.5	-7.4
Ascorbic acid	54670067	-5.3	-5.4
Asiaticoside	108062	-7.7	**-8.6**
Beta-Sitosterol	222284	-7.5	-7.5
Caffeic acid	689043	-5.9	-5.7
Curcumin	969516	-6.6	-6.9
D-allose	439507	-5.4	-5.6
Echitamine	11953926	-6.7	-7.2
Ellagic acid	5281855	**-8.4**	**-8.2**
Eugenol	3314	-5.3	-5.1
Gallic acid	370	-5.5	-5.8
Glucosinolates	6537198	**-7.8**	-7.5
Hydroxypanduratin A	636530	-6.9	-7.7
Kaempferol	5280863	**-7.8**	-7.2
Linoleic acid	5280450	-5.3	-5.5
Linolenic acid	5280934	-5.0	-5.2
Methylripariochromene A	177148	-5.9	-6.0
Moringin	153557	-5.7	-6.2
Niazimicin	10247749	-6.6	-7.0
Oleic acid	445639	-4.7	-5.6
Orthosiphol A	15385858	-6.3	-7.4
Orthosiphol B	15385859	-7.2	**-8.8**
Panduratin A	6483648	-6.5	-7.9
Quercetin	5280343	-7.4	-7.4

So that inhibition of the activity of these two proteins can affect the reproduction process of SARS-CoV-2. The results of molecular docking simulations, 3 compounds that have the most negative

binding energy with Mpro protein are Ellagic Acid (-8.4), Glucosinolates (-7.8) and Kaempferol (7.8). This value is still below Nirmatrelvir (-8.5) as the positive control. The RdRp proteins were Asiaticoside (-8.6), Ellagic Acid (-8.2), and Orthosiphol B (-8.8). This value exceeds Nirmatrelvir (-7.9) as a positive control. These five compounds are predicted to have potential as SARS-CoV-2 antivirals through a dual inhibitor mechanism (**Table 2**).

Table 3. Interaction of three ligands with the highest binding affinity with M^{pro} and RdRp proteins.

Protein Target	Compound	Binding Affinity (kcal/mol)	Position of Chemical Bond	
			Hydrogen	Hydrophobic
M^{pro}	Ellagic Acid	**-8,4**	Arg105, Gln110, Ser158	Val104, Asn151, Phe294
	Glucosinolates	-7,8	Lys137, Thr199, Tyr239, Leu287, Glu288	Lys137, Asp289, Glu290
	Kaempferol	-7,8	Tyr237, Leu287	Lys137, Tyr239
RdRp	Orthosiphol B	-8,8	-	Thr324, Leu329, Val330, Ala379, Ala382, Ala383, Phe396, Val398
	Asiaticoside	-8,6	Lys621, Thr687, Ser759, Asp760, Glu811	Asn691, Lys798
	Ellagic Acid	**-8,2**	Phe134, Asn781, Ser784	Tyr129, Ser709, Thr710

Table 3 shows the interaction of selected ligands with M^{pro} and RdRp proteins. For M^{pro} protein, the three ligands with the lowest binding affinity are Ellagic Acid, Glucosinolates and Kaempferol, respectively. Ellagic acid, which has a binding affinity of -8.4 kcal/mol, can bind to M^{pro} proteins through both hydrogen and hydrophobic bonds (**Figure 1A**). Through hydrogen bonds, Ellagic acid binds to amino acids Arg105, Gln110 and Ser158. While through hydrophobic bonds, this ligand can bind to amino acids Val104, Asn151 and Phe294. Then followed by Glucosinolates which binds to M^{pro} through hydrogen bonds (at positions Lys137, Thr199, Tyr239, Leu287 and Glu288) and hydrophobic (at positions Lys137, Asp289 and Glu290) to form the Mpro-Glucosinolates complex which has a binding affinity of -7.8 kcal/mol (**Figure 1B**). Similar to the Glucosinolates-M^{pro} complex, the M^{pro}-Kaempferol complex has the same binding affinity of -7.8 kcal/mol (**Figure 1C**). Through hydrogen bonding, this

ligand binds to amino acids Tyr237 and Leu287. While through hydrophobic bonds, the ligand forms bonds with amino acids Lys137 and Tyr239.

Ligands with the lowest binding affinity to RdRp protein were Orthosiphol B, Asiaticoside and Ellagic Acid, respectively (**Table 3**). Unlike the previous ligands, although Orthosiphol B binds to the RdRp protein only through hydrophobic bonds (at positions Thr324, Leu329, Val330, Ala379, Ala382, Ala383, Phe396 and Val398) (**Figure 2A**), the protein-ligand complex has the highest binding affinity among other protein-ligand complexes at -8.8 kcal/mol. Furthermore, Asiaticoside can form a complex with RdRp through hydrogen and hydrophobic bonds and has a binding affinity of -8.6 kcal/mol (**Figure 2B**). Through hydrogen bonding, this ligand binds to amino acids Lys621, Thr687, Ser759, Asp760 and Glu811. While the amino acids Asn691 and Lys798 in the RdRp protein bind to this ligand through hydrophobic bonds. The next ligand is Ellagic acid and this ligand can form a complex with RdRp protein through hydrogen and hydrophobic bonds resulting in a binding affinity of -8.2 kcal/mol (**Figure 2C**). Amino acids Phe134, Asn781 and Ser784 on the RdRp protein bind to this ligand through hydrogen bonds. While the amino acids Tyr129, Ser709 and Thr710 on the RdRp protein bind through hydrophobic bonds.

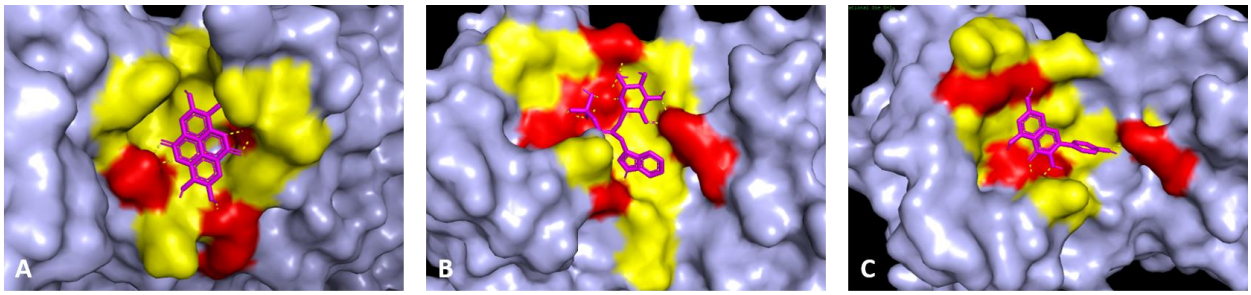

Figure 1. Visualization of Ellagic acid (A), Glucosinolates (B) and Kaempferol (C) binding to Mpro target protein. In order, proteins, ligands, hydrogen bonds and hydrophobic bonds are colored in light blue (light blue), purple (magenta), red (red), yellow (yellow).

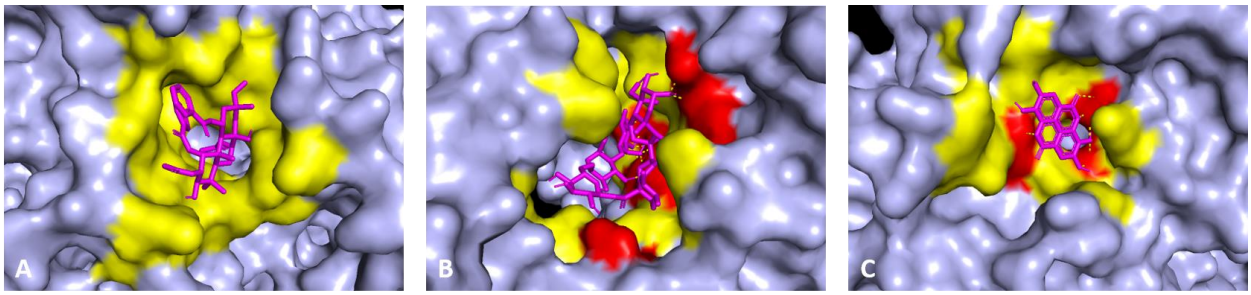

Figure 2. Visualization of the binding of Orthosiphol B (A), Asiaticoside (B) and Ellagic acid (C) to the target protein RdRp. In order, proteins, ligands, hydrogen bonds and hydrophobic bonds are colored in light blue, purple (magenta), red (red), yellow (yellow).

Discussion

Moringa oleifera has compounds consisting of 14-deoxyandrographolide Andrographolide, Ascorbic acid, Asiaticoside, Beta-Sitosterol, Caffeic acid, Curcumin, D-allose, Echitamine, Ellagic acid, Eugenol, Gallic acid, Glucosinolates, Hydroxypanduratin A, Kaempferol, Linoleic acid, Linolenic acid, Methylripariochromene A, Moringin, Niazimicin, Oleic acid, Orthosiphol A, Orthosiphol B, Panduratin A, and Quercetin. Seeds of *Moringa oleifera* are reported to be a virucidal agent through replication inhibition, decreased cytopathic effects of influenza A virus, inhibition of regulation of proinflammatory cytokines such as TNF-α, IL-6, IL-1β, and IFN-β. Inhibition of foot and mouth disease virus was shown in *Moringa oleifera* leaf extract with increased host cell survival rate after administration[32,33].

Drug-likeness prediction aims to identify the similarity of query compounds with drugs based on physicochemical properties in the Lipinski rule. Lipinski Rule of Five consists of molecular mass < 500 Dalton, hydrogen bond donor <5, hydrogen bond acceptor < 10, high lipophilicity (LogP) < 5, and molar refractivity should be 40–130[34]. Lipinski Rule of Five analysis can show compounds that have potential as drugs and allow passing through cell membranes with targets in the cytoplasmic environment, query compounds with drug-like molecule properties can have activity similar to drugs[35]. Antiviral probability analysis aims to determine the antiviral properties referring to Pa and Pi. The results showed that all compounds from *Moringa oleifera* are drug-like molecule, twenty-four compounds are categorized as antiviral candidates but Echitamine cannot be probable as antiviral.

Docking aims to identify the interaction pattern and binding energy of the ligand on the target domain. Binding affinity is formed when intermolecular interactions are formed, binding affinity refers to the formation of stable complexes based on the laws of thermodynamics[36,37,38]. Negative values in binding affinity indicate the ability of the ligand to interact with the target. Ligands with more negative binding affinity can trigger target activities such as inhibition[39,40]. *Moringa oleifera* can have as antiviral through the formation of more negative binding energy with ligand-protein stable complexes for SARS-CoV-2 inhibitors. This was shown by the compounds Ellagic acid, Glucosinolates, and Kaempferol on M^{pro}, Asiaticoside, Ellagic acid, and Orthosiphol B on RdRp. Compounds with more negative binding affinity values on two targets are categorized as dual inhibitors, compounds from *Moringa oleifera* with dual inhibitor activity identified from the results of this study are Ellagic acid. Ellagic acid can form weak bond interactions that contribute to molecular stability, namely hydrogen and hydrophobic bonds.

Conclusions

Moringa oleifera may have potential as an antiviral candidate through Ellagic Acid activity as a dual inhibitor through inhibition of SARS-CoV-2 replication and assembly. The candidate compound can generate weak bonding interactions such as hydrogen and hydrophobic to trigger binding stability at specific domains. We recommend pocket binding domains Arg105, Gln110, Ser158, Val104, Asn151, & Phe294 on Mpro and Phe134, Asn781, Ser784, Tyr129, Ser709, & Thr710 RdRp as targets for SARS-CoV-2 antiviral agents. The results of this study must be proven or validated through in vitro and in vivo analysis for additional scientific evidence.

Acknowledgments

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Conflicts of Interest

The authors declare no conflict of interest in any capacity, including competing or financial.

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