Indonesian Journal of Pharmacy

VOL 34 (4) 2023: 651–664 | RESEARCH ARTICLE

Molecular Docking and ADMET Prediction Studies of Flavonoids as Multi-Target Agents In COVID-19 Therapy: Anti-Inflammatory and Antiviral Approaches

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Article Info

Submitted: 26-02-2022
Revised: 26-08-2023
Accepted: 28-08-2023

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ABSTRACT

Hyper-inflammatory reactions due to cytokine storm lead to acute respiratory distress syndrome and are responsible for COVID-19 death toll. Thus, the pathways involved in inflammation and SARS-COV-2 replication represent promising therapeutic targets. By employing a computational model, we investigated the effect of plant flavonoids on pro-inflammatory proteins (glucocorticoid receptor [GR], cyclooxygenase-2 [COX-2], and 5-lipoxygenase [LOX] enzymes) and proteins involved in virus replication (main protease [Mpro] and papain-like protease [PLpro]). This in silico study aimed to identify promising flavonoids with anti-inflammatory and antiviral activities (multi-target) for combating COVID-19. The selected target proteins were Mpro (PDB ID: 6LU7), PLpro (PDBID: 6WX4), COX-2 (PDBID: 6COX), LOX (PDBID: 6N2W), and GR (PDBID: 1P93). We conducted molecular docking using PLANTS software and obtained Lipinski's "Rule-of-Five" parameters and the predicted pharmacokinetic and toxicity profiles using the pkCSM online platform. Results showed that two flavonoids, diosmin and hesperidin, exhibited a low binding score and higher strength than the reference ligands for the target proteins Mpro, PLpro, and LOX. Both compounds interacted with the amino acid residues of the protein targets through hydrogen bonds and showed similar binding patterns to approved drugs and native ligands. The prediction of ADMET and drug-likeness profiles indicated that these two compounds have low toxicity and good pharmacokinetic properties, except for the absorption profile. Therefore, hesperidin and diosmin are promising multi-target agents for COVID-19 treatment through inhibition of inflammatory progression and virus replication.

Keywords: SARS-Cov-2, medicinal plant, anti-virus, anti-inflammation, in silico

INTRODUCTION

Coronavirus disease (COVID-19) is an infectious disease caused by the SARS-COV-2 that attacks the respiratory system. The symptoms include cough, fever, fatigue, headache, diarrhea, difficulty in breathing, and bloody cough (Rothan & Byrareddy, 2020). The SARS-CoV2 genome structure is similar to that of other beta coronaviruses, such as MERS-COV and SARS-COV (Zehra *et al*., 2020). Genes S, M, and N encode structural, membrane, and nucleocapsid proteins, respectively, and are responsible for the formation of spike proteins. Structural and accessory proteins are essential for virus life cycle and account for up to 33% of the total genome. The remaining 67% of the genome is divided into 16 different non-

structural proteins (NSPs), such as main protease (Mpro), helicase, and RNA polymerase). These proteins are required for virus' interactions with the host cells during infection (Wu *et al*., 2020). Orf1ab is the largest gene in SARS-CoV-2 that encodes the overlapping polyproteins PP1ab and PP1a, which are cut into 15 NSPs by chymotrypsin-like cysteine protease (3CLpro), Mpro, and papain-like protease (PLPpro) (Ullrich & Nitsche, 2020).

COVID-19 pathophysiology is initiated by the infection of SARS-COV-2 (Yuki *et al*., 2020; Shereen *et al*., 2020). The life cycle of SARS-COV-2, especially in the transcription stage, is highly dependent on protease. Mpro, also known as 3C-like protease, is the most important protease because it digests viral polyprotein complexes into functional polypeptides required for replication (Anand *et al*., 2003; Jin *et al*., 2020). In SARS-COV-2, PLpro recognizes the LXGG tetrapeptide motif that is found between virus proteins nsp1 and nsp2, nsp2 and nsp3, and nsp3 and nsp4, and induces viral replication. Mpro and PLpro inhibition disrupts virus replication and transcription. Therefore, these two proteins show potential as antiviral targets.

SARS-COV-2 is recognized by antigen-presenting cells (APCs, e.g., macrophages and dendritic cells) (Fujimoto *et al*., 2000) whose activation produces pro-inflammatory proteins, such as IL-6, TNF-alpha, IL-1, IL-8, IL-12, IP10, MIP1A, and MCP1. The high production of pro-inflammatory proteins in plasma triggers a cytokine storm, an uncontrolled or excessive inflammatory response that aggravates clinical condition (Cascella *et al*., 2020; Coperchini *et al*., 2020) leading to an acute respiratory distress syndrome (ARDS) (Alunno *et al*., 2020; Mehta *et al*., 2020; Panigrahy *et al*., 2020). Furthermore, ARDS complication could lead to multi-organ failure and death (Funk & Ardakani, 2020). Other important proteins in inflammation are cyclooxygenase (COX) and 5-lipoxygenase (LOX) enzymes. Both enzymes regulate the production of prostaglandins and leukotriene that contribute to the pathophysiology of hyper-inflammation in COVID-19 (Hoxha, 2020; Smeitink *et al*., 2020). Thus, COX-2 and LOX represent promising therapeutic targets to combat inflammatory progressions such as ARDS in COVID-19.

Corticosteroids reduce the risk of death (Veronese *et al*., 2020) but are accompanied by major side effects and minor benefits for the COVID-19 patients complicated by ARDS (A. Kumar *et al*., 2020; Ling *et al*., 2020; C. Wu *et al*., 2020). One alternative approach is to develop a single agent with dual therapeutic targets: combating SARS-COV-2 infection and inflammation. A dual-target therapeutic agent is a promising treatment that is suitable for COVID-19 pathological complexity involving immune systems and is also in line with SARS-COV-2 mutating nature and drug resistance susceptibility (Talevi, 2015). Anticancer drugs sunitinib (targeting PDGFR and VEGFR), dasatinib (targeting Abl and Src), and lapatinib (targeting EGFR and HER2) are examples of clinically successful multi-target compounds discovered using the in silico method (Ma *et al*., 2010). Several plants, including black tea, turmeric, mangosteen, and aloe vera, have been studied for their potential as antiviral or anti-inflammatory agents for COVID-19 (Septiana, 2020). The flavonoids found in these plants have antiviral and anti-inflammatory properties (Lago *et al*., 2014; Yi *et al*., 2004; You *et al*., 1999). This study aimed to identify flavonoids with anti-inflammatory and antiviral activities for targeting COVID-19 using an in silico approach.

MATERIAL AND METHODS

Ligand and Protein Preparation

The ligands used were flavonoid compounds with antiviral and anti-inflammatory activities. All these compounds were collected through literature study. The ligand components (flavonoid compounds) and antagonist ligands were obtained from Pubchem database (pubchem.ncbi.nlm.nih.gov). The ligands were prepared using the MarvinSketch. Compounds were added with hydrogen atoms, assigned a charge, and generated into 10 conformations in the 3D conformer menu. The ligands were then saved as a mol2 file. Mpro in complex with an inhibitor N3 (PDB: 6LU7), PLpro in complex with peptide inhibitor VIR251 (PDB: 6WX4), LOX bound to NDGA (PDB: 6N2W), COX-2 complexed with selective inhibitor SC-558 (PDB: 6COX), and GR in complex with dexamethasone (PDB: 1P93) from PDB (rcsb.org) were selected as protein targets and native ligand using YASARA and prepared by checking for missing residues, adding hydrogen atoms, and adding charge to the receptor. Finally, the proteins and ligands were ready to use as materials in molecular docking simulations.

Molecular Docking

Molecular docking was accomplished using PLANTS. The binding side of the protein target is calculated on the basis of binding site radius and binding site center. The scoring function was

determined by $PLANTS_{CHEMPLP}$ and $PLANTS_{PLP}$. The generated ligands were docked with the proteins. The conformational results of the antagonist ligands with the best docking score were compared with the crystal form's conformation. Root mean square deviation (RMSD), a parameter used in pose validation, was obtained as the outcome. The method was validated when RMSD < 2. Among the ligands docked in the protein, the top 10 with the best docking score parameter in each receptor were selected. Furthermore, the interaction of ligands and essential protein amino acid residues was analyzed using BIOVIA Discovery Studio Visualizer. The results will indicate whether the potential flavonoids can be used as a multi-target drug (antiviral and anti-inflammatory) for patients with COVID-19.

Lipinski's Rule of Five (Ro5) Profile and Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) Predictions

Lipinski's Ro5 profile and ADMET predictions were used to forecast the bioavailability, pharmacokinetic profile, and toxic effects of a specific compound (in silico). The aspects included in Ro5 for drug-like molecules were log P ≤ 5 (P represents the partition coefficient in the octanol/water system that will determine its lipophilicity), molecular weight (BM) ≤ 500 Da., number of hydrogen bond acceptor ≤ 10, and number of hydrogen bond donor ≤ 5 (Lipinski, 2004). If more than two aspects are violated, then the test compound may have bioavailability problems. ADMET profile includes absorption, distribution, metabolism, excretion, and toxicity phases. After the top 10 compounds with the highest binding affinity scores were selected, Ro5 analysis and ADMET profiles were carried out on the pkCSM website (http://biosig.unimelb.edu.au/pkcsm/prediction) by inputting Canonical SMILES and then running predictions to generate data in the form of molecular properties, pharmacokinetic profile, and toxicity.

RESULT AND DISCUSSION

The pathophysiology of Covid-19 involves many pathways and proteins, especially related with virus multiplication and inflammatory pathways (Figure 1). In this study, we focused on protease enzymes (MPro and PLpro) and inflammatory-related enzymes (COX-2 and LOX). The effects of plant flavonoids on those proteins are discussed in the in silico context.

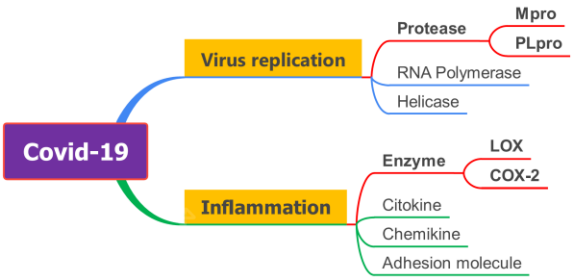

Figure 1. Two main mechanisms involve in the pathophysiology of Covid-19. The red-color branches are the investigated target in this study.

Binding Affinity

Several validation methods have been developed to validate molecular docking protocols. In this study, pose selection with re-docking was conducted between the native ligand and the protein. The pose of docked ligands was compared with native ligands in a co-crystallized form using RMSD to describe the capability of producing ligand conformations that are close to a co-crystallization form. If RMSD is <2 Å, then the docking simulation is valid (reliable) (Cole *et al*., 2005). Table 1 shows that our docking protocol was reliable with RMSD values fulfilling the threshold requirement. The docked ligand showed pose similarity to the co-crystallized ligand. This finding suggested that the protocols used in this study have a predictive capability for docking simulation (Marcou & Rognan, 2007).

Table I. RMSD of Molecular Docking

No	Protein	PDB id	RMSD	Docking Score
1	Mpro	6LU7	1.9000	−131.6730
2	PLpro	6WX4	1.2181	−116.2160
3	COX-2	6COX	1.2190	−61.4922
4	LOX	6N2W	1.6757	−70.4172
5	GR	1P93	0.8444	−83.2285

Docking simulation showed different results for each protein target (Table 2). Tiliroside, Diosmin, Sennoside A, and NDGA were the top (strongest) compounds interacting with the protein targets (Mpro, PLpro, LOX, COX-2 and GR, respectively). Diosmin, hesperidin and sennoside A were the most potential compound targeting antiviral-related proteins (Mpro and PLpro) and anti-inflammatory related proteins (LOX). (-)-Epigallocatechin gallate, liquiritin apioside, pectolinarin, theaflavin and tiliroside were able to interact with LOX, Mpro or PLpro; whereas aloe-

emodin-glucoside interacted with Mpro and glucocorticoid receptors. These results indicated that the flavonoids can exhibit a dual activity of interacting with anti-inflammatory- and antiviral-related targets.

The top 10 hits of flavonoids with the highest binding scores were compared with reference ligands. In Mpro binding, tiliroside, hesperidin, theaflavin, diosmin, sennoside A, astragalin, and naringin showed stronger binding scores than the reference ligand (boceprevir). Meanwhile, in PLpro binding, diosmin, pectolinarin, hesperidin, sennoside A, and naringin had lower binding scores than the reference ligand (lopinavir). In addition, LOX was the most potential target for inflammation because all the top 10 compounds showed lower binding scores than the reference ligand (setileuton). However, in COX binding, only two ligands had lower binding scores than the reference ligand (celecoxib). In GR binding, no ligand had a lower binding score than the reference ligand (methylprednisolone). We identified that hesperidin and diosmin were the compounds with the most targets, including the antiviral-related proteins Mpro and PLpro and the anti-inflammatory enzyme LOX. Hesperidin and diosmin showed a higher binding affinity than the reference ligands, boceprevir, lopinavir, and setileuton on Mpro, PLpro, and LOX, respectively. This finding suggested that hesperidin and diosmin bind to their respective protein targets stronger than the reference ligands and therefore could interact with the receptors involved in inflammation (LOX) and virus infection (Mpro and Plpro).

Citrus plants, such as lemons and oranges, are rich in flavonoid glycosides, including diosmin and hesperidin (Feldo *et al*., 2018; Haggag, 2020). Hesperidin and diosmin have antiviral and anti-inflammatory activities. Recent studies showed that hesperidin and diosmin exerted antiviral activity by inhibiting the cellular entry of the SARS-CoV-2 in VeroE6 cells. It also reduced the expression of ACE2 receptor and inhibited the interaction between ACE2 receptor and virus S protein (Cheng *et al*., 2021; Bae *et al*, 2002). Hesperidin inhibited inflammation in carrageenan-induced mice, inhibited pleurisy, and reduced leukocyte migration (Emim *et al*., 1994). This compound also inhibited the production of pro-inflammatory cytokines, prostaglandin E2, nitric oxide synthase, COX-2, and NF-κB-related proteins (Fu *et al*., 2018; Homayouni *et al*., 2018; Xiao *et al*., 2018; Yeh *et al*., 2007). Diosmin inhibited inflammation by inhibiting the synthesis of leukotriene B4 indicating that it functions through the lipoxygenase pathway (Crespo *et al*., 1999; Gimeno *et al*., 1996). This compound also inhibited NF-κB activation and pro-inflammatory cytokines expression (Imam *et al*., 2015; Islam *et al*., 2020; Tahir *et al*., 2013). The aforementioned studies of diosmin and hesperidin are in line with our results obtained using in silico approach. However, this molecular docking simulation needs to be further enhanced at ligand–amino acid residue interaction to provide insights into the mechanism.

Ligand–protein Interactions

We found that the amino acid residues of each protein interacted with the ligands by hydrogen bonds (Table II). For hesperidin and diosmin, the binding involves several amino acid residues, and the hydrogen interaction is similar to the reference ligands (Figure 2) or has a shorter bond distance than the reference ligand (Figure 3). Short bond distance (low potential energy) stabilizes the interaction of diosmin and hesperidin with their protein targets (Becker & Cooper, 2014). However, a short bond could also generate repulsive force between the two atoms, resulting in high potential energy (Becker & Cooper, 2014).

Gly143, Cys145, His163, His164, Glu166, Gln189, and Thr190 were the seven amino acid residues in Mpro that were involved in the hydrogen bond (Jin *et al*., 2020). Hesperidin interacted with Gly143, His163, and Glu166, and diosmin interacted with Gly143, Cys145, His163 Glu166, and Thr190. Both interacted with Arg596 residue, which represents the active side of the LOX enzyme (Gilbert *et al*., 2020). Trp106, Asn109, Gly163, Tyr268, Gly271, and His272 were the other important amino acids in the interaction with PLpro inhibitors (Rut *et al*., 2020). Hesperidin and diosmin shared Gly163 and Tyr268 residues as a key in hydrogen bond formation and were predicted to have a similar activity to reference drugs due to their similar hydrogen bond profiles. Nevertheless, a previous study on the structure–activity relationship of flavonoids and their antiviral activity (HIV-1) suggested that the A and B rings of flavonoids and the atomic charges on C3 and C4 (carbonyl groups) have an important role in their activity (Olivero-Verbeal & Pacheco-Londoño, 2002).

Table II-a. Top 10 Hit Docking Score and Interacting Amino Acid Residues

Rank	Bioactive compounds	Docking score	Interacting Amino Acid Residues
		Mpro	
1	Tiliroside	−117.6520	TYR54,SER144, GLU166, ASP187, GLN192
2	Hesperidin	−108.8640	PHE140, LEU141, GLY143, HIS163, GLU166
3	Theaflavin	−106.4430	THR26, PHE140, GLU166, ARG188, GLN189
4	Diosmin	−104.1020	LEU141, GLY143, SER144, CYS145, HIS163, GLU166, THR190, ASN142, GLU166
5	Sennoside A	−103.3170	ASN142, HIS163, GLU166
6	Astragalin	−102.4630	LEU141, GLY143, SER144, GLU166, ASP187, GLN189, THR190, GLN192
7	Naringin	−102.2370	LEU141, ASN142, SER144, CYS145, GLU166, LEU141, LEU143
8	Aloe-emodin-glucoside	−101.6620	LEU141, SER144, CYS145, HIS164, GLU166, THR190, GLN192
9	Rutin	−101.2760	PHE140, GLY143, SER144, GLN192
10	Diacetylcurcumin	−101.2160	THR45, SER46, GLY143
		PLpro	
1	Diosmin	−108.4750	GLY163, ASP164, ARG166, GLY266, TYR268, TYR273
2	Pectolinarin	−108.0900	ARG166, THR301, GLY163, ASP164, TYR273, TYR264, TYR268
3	Hesperidin	−106.1130	TYR273
4	Sennoside A	−103.8550	PRO248
5	Naringin	−102.3317	GLY163, GLN250, ASN267
6	Apiin	−100.3190	ARG166, GLY266, ASP302
7	(−)-Epigallocatechin gallate	−99.7231	TYR264, GLY266, TYR268, TYR273, THR301
8	Liquiritin apioside	−97.1313	GLY163, PRO258, TYR268, TYR273, THR301
9	Rhoifolin	−96.3860	ASP164, ARG166, GLY266, THR301,
10	Diacetylcurcumin	−96.1860	TYR264, THR301
		COX-2	
1	NDGA	−98.8145	HIS90, LEU352, MET522
2	Curcumin	−94.7983	HIS90, ARG120, TYR385
3	Taxifolin	−91.1470	LEU352, SER353, MET522
4	Catechin	−89.8724	HIS90, GLN192, VAL349, LEU352, SER530
5	Alizarin complexone	−89.1087	TYR355
6	Sakuranetin	−89.0652	HIS90, SER353, TYR385
7	Naringenin	−88.9271	HIS90, TYR385
8	Homoeriodictyol	−88.8328	MET522, VAL523
9	Hesperetin dihydrochalcone	−88.7710	MET522, VAL523
10	Fustin	−88.1647	GLN192, TYR385, PHE518, GLY526
		LOX	
1	Sennoside A	−113.9480	GLN363, ARG596, HIS600
2	Theaflavin	−103.8750	HIS367, HIS432, GLY430
3	Curcumin	−103.0800	GLN363, ASN407, ARG596, HIS600
4	Tiliroside	−98.7756	GLN363, HIS367, GLY430, HIS432
5	Hesperidin	−98.0003	GLN363, HIS367, ALA410, ARG596, HIS600
6	Diosmin	−97.8722	GLN363, ARG596, HIS600, ILE673
7	Liquiritin apioside	−95.6045	GLN363, LYS409, GLN413, HIS432, HIS600
8	Pectolinarin	−94.2491	GLN363, HIS367, ARG596
9	Gericudranins B	−93.7114	GLN363, THR364, ASN407
10	(−)-Epigallocatechin gallate	−92.6418	ASN407, LYS409, ALA410, ILE673

Table II-b. Top 10 Hit Docking Score and Interacting Amino Acid Residues

		GR	
Rank	**Bioactive compounds**	**Docking score**	**Interacting Amino Acid Residues**
1	NDGA	−95.2073	GLN642, THR739, PHE623, GLN570, ARG611, MET604
2	Alizarin complexone	−89.9148	ASN564
3	Aloe-emodin-glucoside	−88.3437	LEU563, ASN564, MET604, ARG61, THR739
4	Gericudranins B	−88.1455	ASN564, GLN570, PHE623, LEU732
5	Eriodictyol	−84.2582	ASN564, ARG611, MET604, GLN570, PHE632
6	Catechin	−83.5423	MET604, ARG611,GLN642, ASN564, LEU563
7	Aloin	−83.4984	LEU563, ASN564, GLN570, MET604, ARG611, GLN642
8	Taxifolin	−81.7021	ASN564, GLN570, MET604, ARG611, PHE623
9	Sakuranetin	−81.4129	GLN570, ASN564
10	Homoeriodictyol 4′-isobutyrate	−81.1065	LEU563, THR739

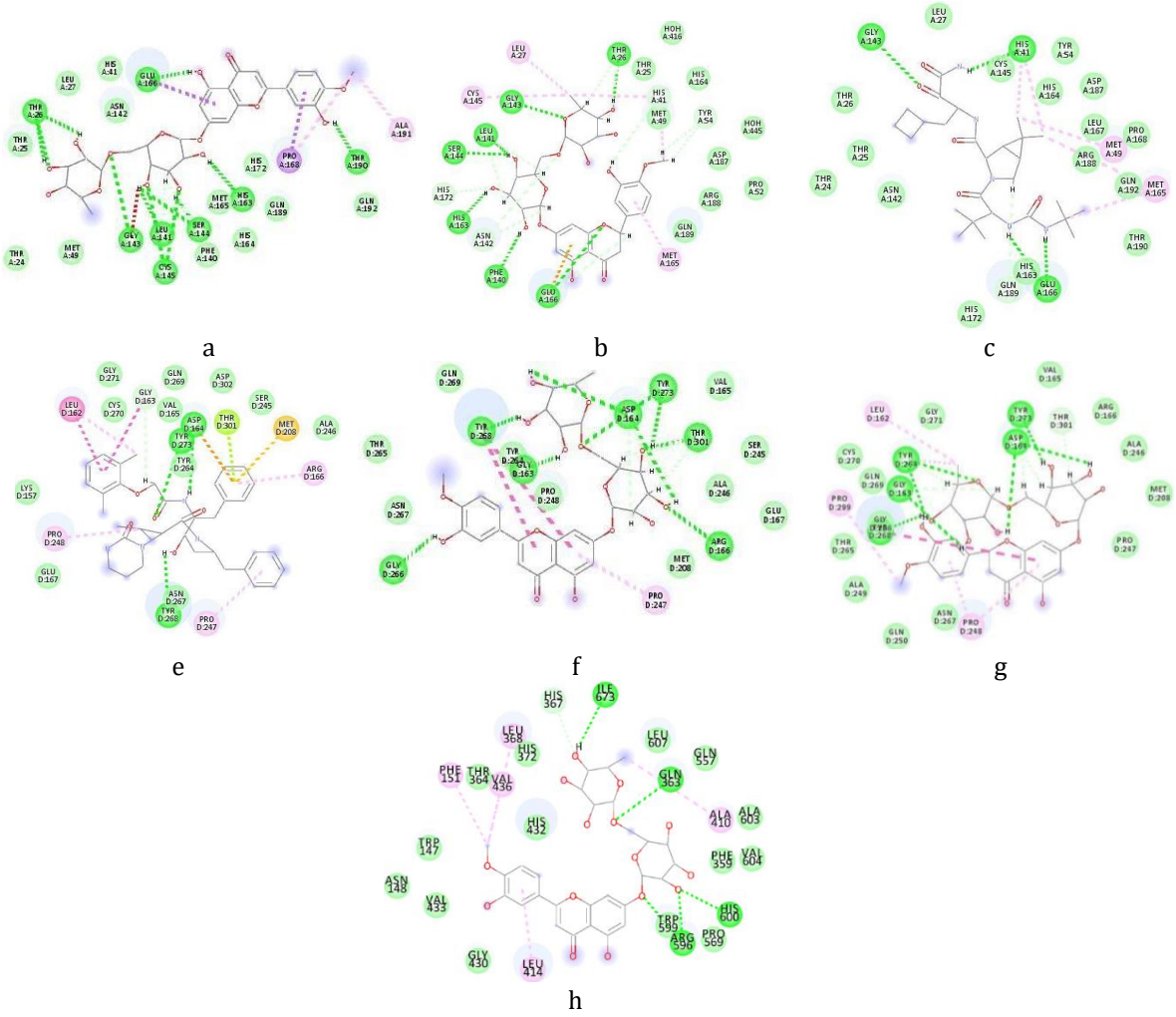

Figure 2: Visualization of ligand–protein interactions in two dimensions. (a) Diosmin interaction with Mpro amino acid residues, (b) Hesperidin interaction with Mpro amino acid residues, (c) Boceprovir interaction with Mpro amino acid residues, (d) Lopinavir interaction with PLpro amino acid residues, (e) Diosmin interaction with PLpro amino acid residues, (f) Hesperidin interaction with PLpro amino acid residues, and (g) Interaction of hesperidin with PLpro amino acid residues.

Table III. Top 10 Hit Docking Score and Interacting Amino Acid Residues

No.	Bioactive compounds	Ro5 Violation Count	Lipinski's Ro5				
			Molecular Weight	Log P	Donor	Acceptor	Rotatable bonds
1	(−)-Epigallocatechin gallate	2	458.4	2.23	8	11	3
2	Alizarin complexone	0	385.3	0.84	4	7	6
3	Aloin	1	418.4	−0.89	7	9	3
4	Aloe-emodin-glucoside	1	432.4	−0.81	6	10	4
5	Apiin	3	564.5	−1.49	8	14	7
6	Astragalin	1	448.4	−0.24	7	11	4
7	Catechin	0	290.3	1.55	5	6	1
8	Curcumin	0	368.4	3.37	2	6	8
9	Diacetylcurcumin	2	452.5	3.81	0	8	10
10	Diosmin	3	608.5	−1.11	8	15	7
11	Eriodictyol	0	288.3	2.22	4	6	1
12	Fustin	0	288.3	1.48	4	6	1
13	Gericudranins B	0	410.4	2.48	6	8	3
14	Hesperetin dihydrochalcone	0	304.3	2.33	4	6	5
15	Hesperidin	3	610.6	−1.16	8	15	7
16	Homoeriodictyol	0	302.3	2.52	3	6	2
17	Homoeriodictyol 4′-isobutyrate	0	372.4	3.37	2	7	4
18	Liquiritin apioside	3	550.5	−1.26	7	13	7
19	Naringenin	0	272.3	2.51	3	5	1
20	Naringin	3	580.5	−1.17	8	14	6
21	NDGA	0	302.4	3.57	4	4	5
22	pectolinarin	3	622.6	−0.79	7	15	8
23	Rhoifolin	3	578.5	−1.10	8	14	6
24	Rutin	3	610.5	−1.69	10	16	6
25	Sakuranetin	0	286.3	2.81	2	5	2
26	Scopolin	0	354.3	−1.02	4	9	4
27	Sennoside A	3	862.7	−1.10	12	18	9
28	Taxifolin	0	304.3	1.19	5	7	1
29	Theaflavin	3	564.5	2.21	9	12	2
30	Tiliroside	3	594.5	1.73	7	13	7
31	Boceprevir (Inhibitor Mpro)	1	519.7	1.71	4	5	8
32	Lopinavir (Inhibitor PLpro)	2	628.8	4.33	4	5	15
33	Celecoxib (Inhibitor COX-2)	0	381.4	3.40	1	7	3
34	Setileuton (Inhibitor LOX)	0	463.4	4.75	2	7	6
35	Methyl Prednisolon (Agonis GR)	0	374.5	1.80	3	5	2

As antiviral agents, hesperidin and diosmin are more effective against SARS-COV-2 than nelfinavir in terms of their ability to block the binding site of 3CL-pro/Mpro (Adem *et al*., 2020; Chen *et al*., 2020). In silico study predicted that hesperidin interferes with the interaction between the ACE2 of the host cell and the SARS-CoV-2 spike protein, thus preventing virus entry in lung cells. Hesperidin also showed a higher affinity for RBD-S, PD-ACE2, and Mpro SARS-CoV-2 proteins than lopinavir, penciclovir, indinavir, saquinavir, tipranavir, daclatasvir, hydroxychloroquine, and nafamostat (Das *et al*., 2021; Joshi *et al*., 2020; Wu *et al*., 2020; Utomo *et al*., 2020). This compound inhibited the 3CLpro enzyme of the SARS virus, which has a protease structure similar to SARS-CoV-2 (Bellavite & Donzelli, 2020). Compared with darunavir, indinavir, velpatasvir, and raltegravir, diosmin has more potent inhibitory activity against the Mpro of SARS-CoV-2 and should be further investigated in clinical trials (Bello *et al*., 2020).

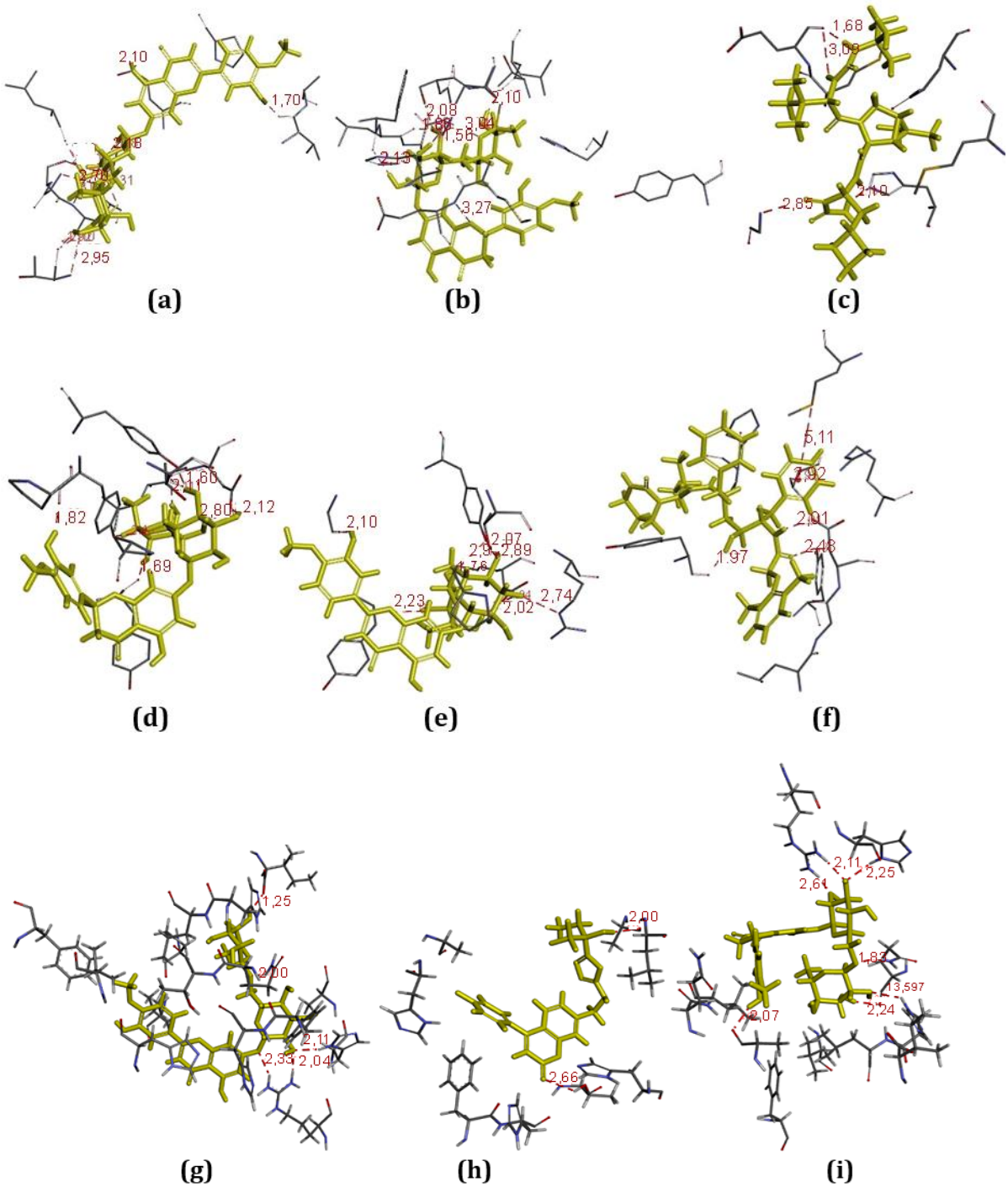

Figure 3. 3D visualization of ligand–protein interactions. (a) Diosmin interaction with Mpro amino acid residues, (b) Hesperidin interaction with Mpro amino acid residues, (c) Boceprovir interaction with Mpro amino acid residues, (d) Hesperidin interaction with PLpro amino acid residues, (e) Diosmin interaction with PLpro amino acid residues, (f) Lopinavir interaction with PLpro amino acid residues, (e) Diosmin interaction with PLpro amino acid residues, (g) Diosmin interaction with LOX amino acid residues, (h) Cetileutone interaction with LOX amino acid residues, and (i) Hesperidin interaction with LOX amino acid residues. Yellow = test ligand; Red = length of hydrogen interaction; gray = amino acid residues

A clinical trial on hesperidin and diosmin combination (Clinical trials: NCT04452799) for COVID-19 treatment is ongoing in Egypt. With this molecular docking approach, hesperidin and diosmin were predicted to be leading candidates for a multi-target agent with antiviral and anti-inflammatory properties.

Lipinski's Rule of Five Profile and ADMET Predictions

A promising drug candidate must have potency, drug-like molecule profile, pharmacokinetics aspect, and toxicity data that all meet the established requirements. Therefore, the Lipinski's rule was created to facilitate drug candidate screening through physical and chemical parameters. In this study, pkCSM was used to determine whether the ligand complies with Lipinski's rule and has good predictive ADME properties. The results of drug-like screening for the top 10 flavonoids (hits) from molecular docking are presented in Table 3. The drug-like molecule properties of a compound were analyzed based on the violated Ro5. If a compound violates two or fewer aspects of Ro5, it is predicted to have good properties. We found that the following 11 flavonoids violated three aspects of Ro5: apiin, diosmin, hesperidin, liquiritin apioside, naringin, pectolinarin, rhoifolin, routine, sennoside A, theaflavin and tiliroside. There was no violation of Ro5 of more than two for the reference ligands.

ADMET predictions for the top 10 flavonoids are shown in Table 4. Absorption prediction was divided into Caco-2 permeability and intestinal absorption. We found that six compounds showed good permeability (>0.9) and 23 compounds were properly absorbed (>30%). Several compounds (diacetylcurcumin, naringenin, NDGA, sakuranetin, and taxifolin) were predicted to have good absorption (Caco-2 permeability and intestinal absorption). In terms of distribution, 20 compounds exhibited high-volume distribution (>0.45). We predicted that none of the compounds were able to cross the blood–brain barrier (>0.3) and the central nervous system (>−2). In the metabolic aspect, one compound (NDGA) inhibited the CYP2D6 enzyme, and three compounds (curcumin, diacetylcurcumin, and homoeriodictyol 4'-isobutyrate) inhibited the CYP3A4 enzyme. The majority of the flavonoids did not have a toxic effect, did not inhibit hERG, did not induce hepatotoxicity, and only have a minor toxic effect. Some flavonoids that were predicted to have toxic effects were fustine (mutagenic), alizarin complexone (hepatotoxic), and diacetylcurcumin (acute toxicity).

We found that diosmin and hesperidin violated two criteria of Ro5 (Table 3). Except for absorption, diosmin and hesperidin showed a good pharmacokinetic profile according to ADMET predictions (Table 4). Both have disaccharide rutinoside residue at the position of C7 which are mostly metabolized by β-glucosidase in the intestinal microflora to form their aglycones, hesperetin and diosmetin (Lyseng-Williamson, K. A., & Perry, C. M., 2003; Nielsen *et al*., 2006). A previous study showed that the use of micronized formulation can preserve the glycoside form of hesperidin and diosmin and boost their bioavailability in a clinical setting (Garner *et al*., 2002). The presence of glycosidic residues in hesperidin and diosmin improves their pharmacokinetic parameters and is also vital for their activity.

Multi-target agents may play an important role in the treatment of COVID-19 patients with ARDS conditions (Sorzano *et al*., 2020). Furthermore, the complex pathophysiology of COVID-19 including cytokine storms demands a therapeutic agent that could act as an immunomodulator (Gil *et al*., 2020). Since the SARS-COV outbreak in 2003, Mpro and PLpro, which are crucial for virus life cycle, have become an attractive targets in the search for new antiviral agents (Ullrich & Nitsche, 2020). The discovery of multi-target drugs targeting ARDS is a promising approach in combating COVID-19 with ARDS (Funk & Ardakani, 2020; Zhou *et al*., 2020). The incidence of multi-organ dysfunction due to ARDS could be prevented by inhibiting inflammatory mediators (England *et al*., 2021; Funk & Ardakani, 2020; Sasaki & Yokomizo, 2019).

CONCLUSION

With our *in silico* approach, diosmin and hesperidin were discovered as multi-target agents with the potential for the treatment of COVID-19 with ARDS complication. These compounds might inhibit SARS-CoV-2 pathogenesis by interfering with its replication cycle, thus reducing the severity of hyper-inflammatory reactions due to cytokine storm. Therefore, diosmin and hesperidin, the main flavonoids of citrus plants, show potential as a multi-target agent for the treatment of COVID-19 with ARDS complication. Further studies with in vitro and in vivo experimental models are required to confirm the efficacy of multi-target bioactive molecules for inflammation and virus infection.

ACKNOWLEDGEMENT

The authors thank to the Faculty of Pharmacy, Universitas Gadjah Mada for providing software and research facilities for this study.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest. The authors did not receive any grant for this research. This study was presented at the 2nd Bioinformatics and Biodiversity Conference (Virtual Conference) on 27–28 November 2021.

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