Original Article: Study of the Interaction Between Some Flavonoids of Plant Origin with COVID-19 Main Protease by Molecular Docking Method

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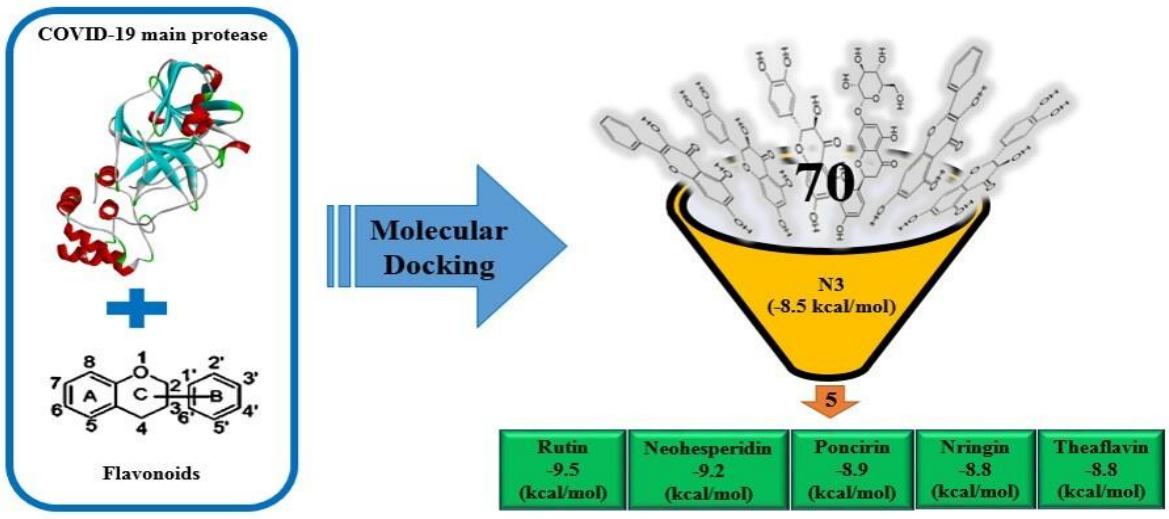
Abstract

The spread of coronavirus disease 2019 (COVID-19) pandemic in all countries causing a global effort to discovered rugs or vaccines. The potential of 70 plant origin-flavonoids for inhibition of the main protease of COVID-19 was investigated the molecular docking approach. COVID-19 Docking Server was applied to check the inhibitor activity of the flavonoids in comparison to the native protease inhibitor of the main protease. Results suggest five compounds namely rutin, neohesperidin, poncirin, naringin, and theaflavin. For these compounds, the predicted binding energy was respectively -9.5, -9.2, -8.9, -8.8, and -8.8 kcal/mol in comparison to the -8.5 kcal/mol value for the native inhibitor. The hydrogen bond with residues Ser46, Gly143, His163 and hydrophobic interaction with residues Asn142, Gly143, Cys145, and Met165 are the major interaction between selected flavonoids with the main protease. Anti-GRP78 flavonoids naringin and poncirin are repurposing flavonoids for inhibition of Mpro of COVID-19 that previously their inhibitory effects were appeared by our using invitro analysis for GRP78. Also, rutin was proposed based on the consensus decision-making along with other studies for the inhibitory effect of flavonoids against the main protease of COVID-19.

Keywords: COVID-19, Flavonoid, Main protease, GRP78, Molecular Docking

Graphical Abstract



Introduction

The coronavirus disease 2019 (COVID-19) is a new infectious disease that has been named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Zhou *et al*., 2020). The virus was firstly appeared in Wuhan, China at the end of 2019 (Paraskevis *et al*., 2020) and rapidly characterized as a pandemic coronavirus with spread worldwide. This pandemic was declared a global public health emergency by the

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world health organization (WHO) due to the quick outbreak, high mortality rate, and lack of effective treatment (Lai *et al*., 2020; Li and De Clercq, 2020). It appeared the main protease (Mpro) of COVID-19 is a key enzyme of this coronavirus that plays a pivotal role in mediating viral replication and transcription which makes it an attractive drug target (Jin *et al*., 2020).

Recently it was seen, Glucose-regulated protein 78kD a (GRP78) may act as a host receptor and play a critical role in the fusion of SARS-COV-2 to a host cell(Ha *et al*., 2020; Sabirli *et al*., 2021).GRP78is an endoplasmic reticulum (ER) chaperone protein that facilitates the folding and assembly of newly synthesized proteins(Ibrahim *et al.* 2019*)*. The stress protein generation is generally induced by environmental or physiological conditions such as hypoxia, low pH, and glucose deprivation which its overexpression happened in many tumors and cancer cells(Fernandez *et al*., 2000). A high GRP78 level was reported in Covid-19 infection(Sabirli *et al*., 2021). During replication and protein production of an active viral, ER-localized GRP78 assists in the proper folding and processing of virus proteins and maintaining ER homeostasis which provides a conducive cellular environment for assembly and maturation of viral. ER stress-induced by viral infection could also drive cell surface GRP78 translocation, further promoting viral entry. Also, during the final assembly and budding from the ER-Golgi intermediate compartment (ERGIC), GRP78 may be associated and released together with mature virions for enhancing the infectivity as the accessory host factor (Ha *et al*., 2020).In addition, the effect of the anti-GRP78 agentepig allocatechin gallate (EGCG) was identified in the production step of the Ebola and Zikaviral life cycle(Ha *et al*. 2020). Molecular docking analysis of with a ferin A, artemisinin, curcumin, and andrographolide was done with both GRP78 and Mpro of COVID-19 that the optimal interaction features of with a ferin A from *Withania somnifera*as proposed phytochemical was visualized with both receptors (Sudeep *et al*., 2020).

Various flavonoids were investigated for their antiviral activities and some of them exhibited significant antiviral properties(Akher *et al*., 2019; Karker and Ravikumar 2019; Lim *et al*., 2017; Zakaryan *et al.*, 2017). Flavonoids inhibited the main protease of a variety viral such as SARS(Nguyen *et al*., 2012; Yu *et al*., 2012), hepatitis C virus (HCV) (Bachmetov *et al*., 2012; Bose *et al*., 2017), human immunodeficiency viruses (HIV) (Critchfield *et* al., 1996; Mehla *et al*., 2011), avian influenza H5N1, influenza A virus, canine distemper virus (CDV), etc. Thus, they possess a wide range of antiviral activities. Accordingly, myricetin and scutella are in as novel chemical inhibitors were found for blocking the enzymatic activity of SARS main protease(Yu *et al*., 2012). The suppression of HCV with the quercetin flavonoid is mediated by inhibition of NS3 protease(Bachmetov *et al*., 2012), while a flavonoid isolated from plum (*Prunus domestica*) was also identified as a potent inhibitor of HCV (Bose *et al*. 2017). In addition, it was seen that luteolin, cripples HIV-1 an abrogation of the tat function (Mehla *et al*., 2011), and differential antiviral and anti-inflammatory mechanisms were reported for flavonoids of biochanin A and baicalein against H5N1 influenza A virus-infected cells(Sithisarn *et al*., 2013).

Molecular docking is the most important bioinformatics method for the prediction of the binding mode and energy of a protein-ligand complex(Dar and Mir 2017). This approach was applied to investigate the protease inhibition ability of flavonoids for Ebola (Nasution *et al*., 2018; Raj and Varadwaj 2016; Veljkovic *et al*., 2015) and HCV(Akher *et al*., 2019). Also, preliminary we investigated the inhibitory effect of 70 plant origin flavonoids against targetable cancer receptors GRP78 by molecular docking approach (Barzegar *et al*., 2021). Four flavonoids, namely, naringin, poncirin, prunin, and EGCG respectively were proposed for inhibition of GRP78.

This study aimed to conduct the In-vitro study of COVID-19 main protease inhibition by flavonoids of plant origin. The effect of 70 plant origin flavonoids on inhibition of Mpro function was investigated and theirinhibitory effect was evaluated against GRP78 by our previous work. COVID-19 Docking Server was applied for investigation of the inhibition effect of the natural compounds for COVID-19 therapy. The binding energy for best flavonoids was a greater value than the native inhibitor against Mpro function. Also, the major hydrogen bonding and hydrophobic interactions were identified between selected flavonoids with active site residues of Mpro of COVID-19.

Materials and Methods

Molecular docking was conducted to investigate the interaction between 70 non-synthesis flavonoids and the Mpro of COVID-19. These native flavonoids belong to the 8 subclasses which contain 18 flavones, 17 flavonols, 16 flavanones, 7 flavan-3-ols, 6 isoflavones, and 2 compounds from each subclass of flavan-4-ols, flavanonols, and neoflavonoids. The basic skeleton structure of the flavonoids and their 8 subgroups are shown in Figure 1.

Ligand Preparation

Canonical SMILES of the flavonoids were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Then, the structure of flavonoids was drawn by ChemSketch (www.acdlabs.com) and was optimized with Austin Model 1 (AM1) semi-empirical method using HyperChem (version 8.0)(Release 2002)software. In the next step, the ligand structures were converted into SDF file format in Open Babel (version 2.3.2) software(O'Boyle *et al*., 2011) for serving to the COVID-19 Docking Server (https://ncov.schanglab.org.cn/) for the molecular docking study of the flavonoids with Mpro. In this server, the crystal structure of the input ligands was prepared for molecular docking and AutoDock Vina is used as the docking engine. The search grid of the active site identified by the server (x=-10.85, y= 12.58, and z=68.72) was located in the place of the native inhibitor N3 and the grid box was set to 30×30×30 Å. The server displays the 10 top conformations of each ligand in complex with Mpro which the best with the lowest binding energy was selected as the most stable conformation of the ligand-receptor complex. Results of the best binding energy for all 70 flavonoids were shown in Table 1. The structure of native inhibitor N3 in complex with Mpro COVID-19 (ID Code: 6LU7) is available in the protein data bank (PDB) that is n-[(5-methylisoxazol-3-yl) carbonyl] alanyl-l-valyl-n~1~-((1r,2z)-4-(benzyloxy) -4-oxo-1- {[(3r)-2-oxopyrrolidin-3-yl] methyl} but-2-enyl)-l-leucinamide. In the last step, the binding energy for N3 inhibitor identified by server as the threshold value of selection.

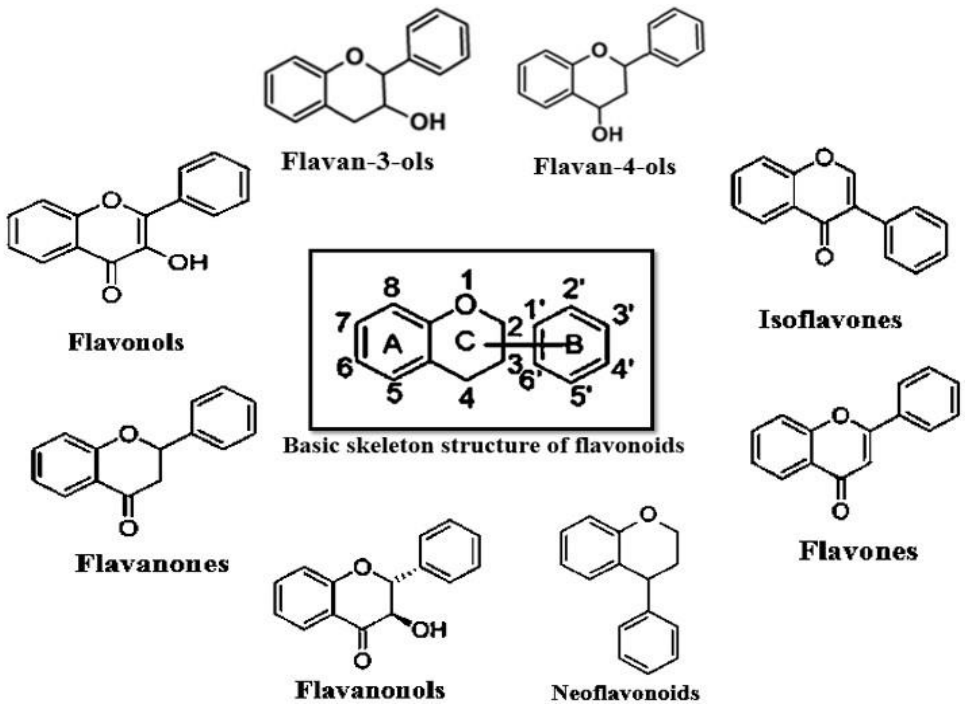

Fig. 1 Basic skeleton structure of flavonoids and their 8 subgroup.

Table 1 Name, SMILES notation, subgroup and predicted binding energy of 70 studied flavonoids.

NO.	Compound name	Canonical SMILES	Subgroup name	Binding energy (kcal/mol)
1	Naringin	C1=CC=C(C=C1)C2=CC(=O)C3=C(O2)C=CC(=C3)O	Flavanones	-11.57
2	Poncirin	CC1(C=CC2=C(O1)C=CC(=C2)C3CC(=O)C4=C(O3)C=C(C=C4)O)C	Flavanones	-10.37
3	Prunin	COC1=CC=C(C=C1)C2=CC(=O)C3=C(C=C(C=C3O2)O)O	Flavanones	-10.19
4	Epicatechin gallate	COC1=CC(=CC2=C1C(=O)CC(O2)C3=CC=CC=C3)O	Flavan-3-ols	-10.04
5	EGCG	C1C(C2=C(C=C(C=C2OC1C3=CC=C(C=C3)O)O)O)O	Flavan-3-ols	-9.86
6	Luteolin-4'-glucoside	C1=CC(=CC=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O	Flavones	-9.41
7	Neohesperidin	C1=CC(=CC=C1C2C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O	Flavanones	-9.39
8	Sakuranin	COC1=CC(=CC2=C1C(=O)C(=C(O2)C3=CC(=C(C=C3)O)O)O)O	Flavanones	-9.37
9	Abyssinones	C1=CC=C(C=C1)C2=CC(=O)C3=C(O2)C=C(C(=C3O)O)O	Flavanones	-9.26
10	Theaflavin	C1=CC=C(C=C1)C2=CC(=O)C3=C(C(=C(C=C3O2)OC4C(C(C(C(O4)C(=O)O)O)O)O)O)O	Flavan-3-ols	-9.13
11	Spiraeoside	COC1=CC=C(C=C1)C2=COC3=CC(=CC(=C3C2=O)O)O	Flavonols	-8.82
12	Rutin	C1C(OC2=C(C1=O)C=CC(=C2)O)C3=CC(=C(C=C3)O)O	Flavonols	-8.78
13	wighteone	C1C(C(OC2=CC(=CC(=C21)O)O)C3=CC(=C(C=C3)O)O)O	Isoflavones	-8.52
14	Leucocyanidin	C1=CC=C(C=C1)C2=CC(=O)C3=C(C=C(C=C3O2)O)O	Flavan-3-ols	-8.37
15	Gossypetin	C1=CC(=CC=C1C2=COC3=C(C2=O)C=CC(=C3)O)O	Flavonols	-8.31
16	Diosmetin	COC1=C(C=C2C(=CCOC2=C1)C3=CC=CC=C3)O	Flavones	-8.28
17	Eupatorin	COC1=C(C=C2C(=CC(=O)OC2=C1)C3=CC=CC=C3)O	Flavones	-8.26
18	Tricin	COC1=C(C=C(C=C1)C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O	Flavones	-8.25
19	Oroxindin	C1C(C(OC2=CC(=CC(=C21)O)O)C3=CC(=C(C(=C3)O)O)O)OC(=O)C4=CC(=C(C(=C4)O)O)O	Flavones	-8.23
20	Luteoforol	C1C(C(OC2=CC(=CC(=C21)O)O)C3=CC(=C(C=C3)O)O)OC(=O)C4=CC(=C(C(=C4)O)O)O	Flavan-4-ols	-8.17
21	Rhamnetin	C1C(C(OC2=CC(=CC(=C21)O)O)C3=CC(=C(C(=C3)O)O)O)O	Flavonols	-8.17
22	Acacetin	C1C(OC2=CC(=CC(=C2C1=O)O)O)C3=CC(=C(C=C3)O)O	Flavones	-8.06
23	Rhamnazin	COC1=C(C=C(C=C1)C2=CC(=O)C3=C(C(=C(C=C3O2)OC)OC)O)O	Flavonols	-8.00
24	Scutellarin	C1=CC(=C(C=C1C2=C(C(=O)C3=C(O2)C=C(C=C3)O)O)O)O	Flavones	-7.97
25	Azaleatin	COC1=CC=C(C=C1)C2=COC3=C(C2=O)C=CC(=C3)O	Flavonols	-7.95
26	Alpinetin	C1=CC=C(C=C1)C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O	Flavanones	-7.93
27	Eriodictyol	C1C(C(OC2=CC(=CC(=C21)O)O)C3=CC(=C(C(=C3)O)O)O)O	Flavanones	-7.91
28	Butin	C1=CC(=CC=C1C2=COC3=CC(=CC(=C3C2=O)O)O)O	Flavanones	-7.90
29	Quercetagetin	COC1=C(C=C2C(=C1)C(=O)C(=CO2)C3=CC=C(C=C3)O)O	Flavonols	-7.89
30	Sterubin	C1=CC(=C(C=C1C2=C(C(=O)C3=C(O2)C(=C(C=C3O)O)O)O)O)O	Flavanones	-7.89
31	Apiforol	COC1=C(C=C(C=C1)C2CC(=O)C3=C(C=C(C=C3O2)O)O)O	Flavan-4-ols	-7.83
32	Myricetin	COC1=C(C=CC(=C1)C2CC(=O)C3=C(C=C(C=C3O2)O)O)O	Flavonols	-7.83
33	Dalbergin	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)OC4C(C(C(C(O4)CO)O)O)O)O)O	Neoflavonoids	-7.79
34	Epigallocatechin	COC1=C(C=CC(=C1)C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O	Flavan-3-ols	-7.79
35	Gallocatechin	COC1=CC=C(C=C1)C2CC(=O)C3=C(C=C(C=C3O2)O)O	Flavan-3-ols	-7.79
36	Luteolin	COC1=CC=C(C=C1)C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O	Flavones	-7.76
37	Sinensetin	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O	Flavones	-7.76
38	Taxifolin	C1=CC(=C(C=C1C2C(C(C3=C(C=C(C=C3O2)O)O)O)O)O)O	Flavanonols	-7.75
39	Fisetin	C1C(C2=C(C=C(C=C2OC1C3=CC(=C(C=C3)O)O)O)O)O	Flavonols	-7.73
40	Scutellarein	C1=CC(=C(C=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O)O	Flavones	-7.71
41	Naringenin	C1=CC(=C(C=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O)OC4C(C(C(C(O4)CO)O)O)O)O	Flavanones	-7.70
42	Glycitein	C1=CC(=C(C=C1O)O)C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O	Isoflavones	-7.69
43	Pachypodol	C1=C(C=C(C(=C1O)O)O)C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O	Flavonols	-7.69
44	Aromadendrin	C1C(OC2=CC(=CC(=C2C1=O)O)O)C3=CC=C(C=C3)O	Flavanonols	-7.68
45	Quercetin	CC1C(C(C(C(O1)OC2C(C(C(OC2OC3=CC(=C4C(=O)CC(OC4=C3)C5=CC=C(C=C5)O)O)CO)O)O)O)O)O	Flavonols	-7.68
46	Kaempferide	CC1C(C(C(C(O1)OC2C(C(C(OC2OC3=CC(=C4C(=O)CC(OC4=C3)C5=CC(=C(C=C5)OC)O)O)CO)O)O)O)O)O	Flavonols	-7.67
47	Apigenin	COC1=C(C=C(C2=C1OC(=CC2=O)C3=CC=CC=C3)O)OC4C(C(C(C(O4)C(=O)O)O)O)O)O	Flavones	-7.64
48	Catechin	COC1=C(C2=C(C=C1O)OC(=CC2=O)C3=CC=CC=C3)O	Flavan-3-ols	-7.64
49	Formononetin	COC1=CC(=C2C(=C1)OC(=C(C2=O)OC)C3=CC(=C(C=C3)O)OC)O	Isoflavones	-7.62
50	Oroxylin A	C1C(OC2=CC(=CC(=C2C1=O)O)O)C3=CC=CC=C3	Flavones	-7.60
51	Baicalein	CC1C(C(C(C(O1)OC2C(C(C(OC2OC3=CC(=C4C(=O)CC(OC4=C3)C5=CC=C(C=C5)OC)O)CO)O)O)O)O)O	Flavones	-7.52
52	Sakuranetin	C1C(OC2=CC(=CC(=C2C1=O)O)OC3C(C(C(C(O3)CO)O)O)O)C4=CC=C(C=C4)O	Flavanones	-7.52
53	Daidzein	C1=CC(=C(C=C1C2=C(C(=O)C3=C(O2)C=C(C(=C3O)O)O)O)O)O	Isoflavones	-7.45
54	Morin	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O	Flavonols	-7.45
55	Dalbergichromene	COC1=CC(=C2C(=C1)OC(=C(C2=O)O)C3=CC(=C(C=C3)O)OC)O	Neoflavonoids	-7.42

56	Biochanin A	COC1=CC(=C2C(=C1)OC(=C(C2=O)O)C3=CC(=C(C=C3)O)O)O	Isoflavones	-7.41
57	Galangin	CC1C(C(C(C(O1)OCC2C(C(C(C(O2)OC3=C(OC4=CC(=CC(=C4C3=O)O)O)C5=CC(=C(C=C5)O)O)O)O)O)O)O)O	Flavonols	-7.41
58	Kaempferol	COC1=CC(=C2C(=O)CC(OC2=C1)C3=CC=C(C=C3)O)O	Flavonols	-7.41
59	Isorhamnetin	COC1=CC2=C(C(=O)CC(O2)C3=CC=C(C=C3)O)C(=C1)OC4C(C(C(C(O4)CO)O)O)O)O	Flavonols	-7.35
60	Isosakuranetin	C1=CC(=CC=C1C2=CC(=O)C3=C(O2)C=C(C(=C3O)O)O)O	Flavanones	-7.35
61	wogonin	C1=CC(=CC=C1C2=CC(=O)C3=C(C(=C(C=C3O2)OC4C(C(C(C(O4)C(=O)O)O)O)O)O)O)O	Flavones	-7.35
62	Pinocembrin	COC1=C(C=C(C=C1)C2=CC(=O)C3=C(C(=C(C=C3O2)OC)OC)OC)OC	Flavanones	-7.32
63	Genistein	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)OC4C(C(C(C(O4)CO)O)O)O	Isoflavones	-7.31
64	Chrysin	COC1=CC(=C2C(=O)CC(OC2=C1)C3=CC(=C(C=C3)O)O)O	Flavones	-7.30
65	6-Hydroxyflavone	C1=CC(=C(C=C1C2C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O	Flavones	-7.24
66	Homoeriodictyol	C1C(C(OC2=CC(=CC(=C21)O)O)C3=CC4=C(C(=C(C=C4C5C(CC6=C(C=C(C=C6O5)O)O)O)O)O)C(=O)C(=C3)O)O	Flavanones	-7.24
67	Hesperetin	COC1=CC(=CC(=C1O)OC)C2=CC(=O)C3=C(C=C(C=C3O2)O)O	Flavanones	-7.19
68	Baicalin	C1=CC(=CC=C1C2=CC(=O)C3=C(C(=C(C(=C3O2)C4C(C(C(C(O4)CO)O)O)O)O)C5C(C(C(C(O5)CO)O)O)O)O)O	Flavones	-7.04
69	Isoquercetin	CC(=CCC1=C(C2=C(C=C1O)OC=C(C2=O)C3=CC=C(C=C3)O)O)C	Flavonols	-6.42
70	Vicenin	COC1=C(C=C(C2=C1OC(=CC2=O)C3=CC=CC=C3)O)O	Flavones	-4.79

Result and Discussion

The present study sought to investigate the ability of some flavonoids to inhibition of COVID-19 main protease activity as protease inhibitors. The lowest binding energy for the best conformation of N3 was predicted to be -8.5 kcal/mol. Among the studied flavonoids, five compounds contain rutin, neohesperidin, poncirin, naringin, and theaflavin candidate based on their binding energy values. The minimum binding energy for these compounds was predicted respectively to be -9.5, -9.2, -8.9, -8.8, and -8.8 kcal/mol. These top compounds were among the three out of eight subgroups of investigated flavonoids including flavanones (neohesperidin, poncirin, naringin), flavan-3-ols (theaflavin), and flavonols (rutin).

Several studies such as virtual screening have documented that rutin flavonoids havea high efficacy as a potent inhibitor to treat COVID-19 infection (Chikhale *et al.*, 2021; Das *et al.*, 2021; Khaerunnisa *et a*l., 2020). Also, flavonoid inhibition potential for Mpro of COVID-19 was evaluated from several medicinal plant compounds by a molecular docking study. Kaempferol, quercetin, luteolin-7-glucoside, demethoxycurcumin, naringenin, apigenin-7-glucoside, oleuropein, curcumin, catechin and EGCG that found in the medicinal plants suggested acting as COVID-19 Mpro inhibitors (Eveleigh 2021).

Some of the natural sources of these flavonoids are as follows: Rutin is found in the seeds of tartary buckwheat (*Fagopyrum tataricum*), leaves, and petioles of rhubarb (*Rheum rhabarbarum*), fruits of orange (*Citrus aurantium*) and lemon (*C. limon*) (Zakaryan *et al*., 2017), etc. Neohesperidin is found in the seville or bitter oranges (*C. aurantium*) (Zhu *et a*l., 2013), etc. Poncirin is found in the edible citrus ougan (*C. reticulate* cv. Suavissima) (Yoon *et a*l., 2012), poncirus trifoliate (Wong *et al.,* 2013), etc. Naringin is found in the citrus fruits (Eveleigh 2021) and Chinese herbal medicines such as Drynaria fortunei (Kunze) J. Sm. (DF), *C. aurantium* L. (CA), and *C. medica* L. (CM) (Zhang *et al*., 2014), etc. Theaflavin is found in tea leaves, black tea, oolong tea (Leung *et al*., 2001), etc.

LigPlot software was used to show the hydrogen bonding and hydrophobic interactions between Mpro and native inhibitors and studied flavonoids in the binding site. The structure and 2D and 3D interaction diagram for the native inhibitor and five selected flavonoids at the active site of Mpro was shown in Figure 2. The best conformation of N3 forms the hydrogen bond interaction with the Phe 140, His 163, Glu 166, Gln 189, and Thr 190 amino acids of the active site. Also, it has a hydrophobic interaction with Thr 24, Thr 25, Leu 27, His 41, Met 49, Phe 140, Leu 141, Asn 142, Gly 143, Cys 145, His 163, His 164, Met 165, Glu 166, Leu 167, Pro168, Gln 189, Gln 192 residue.

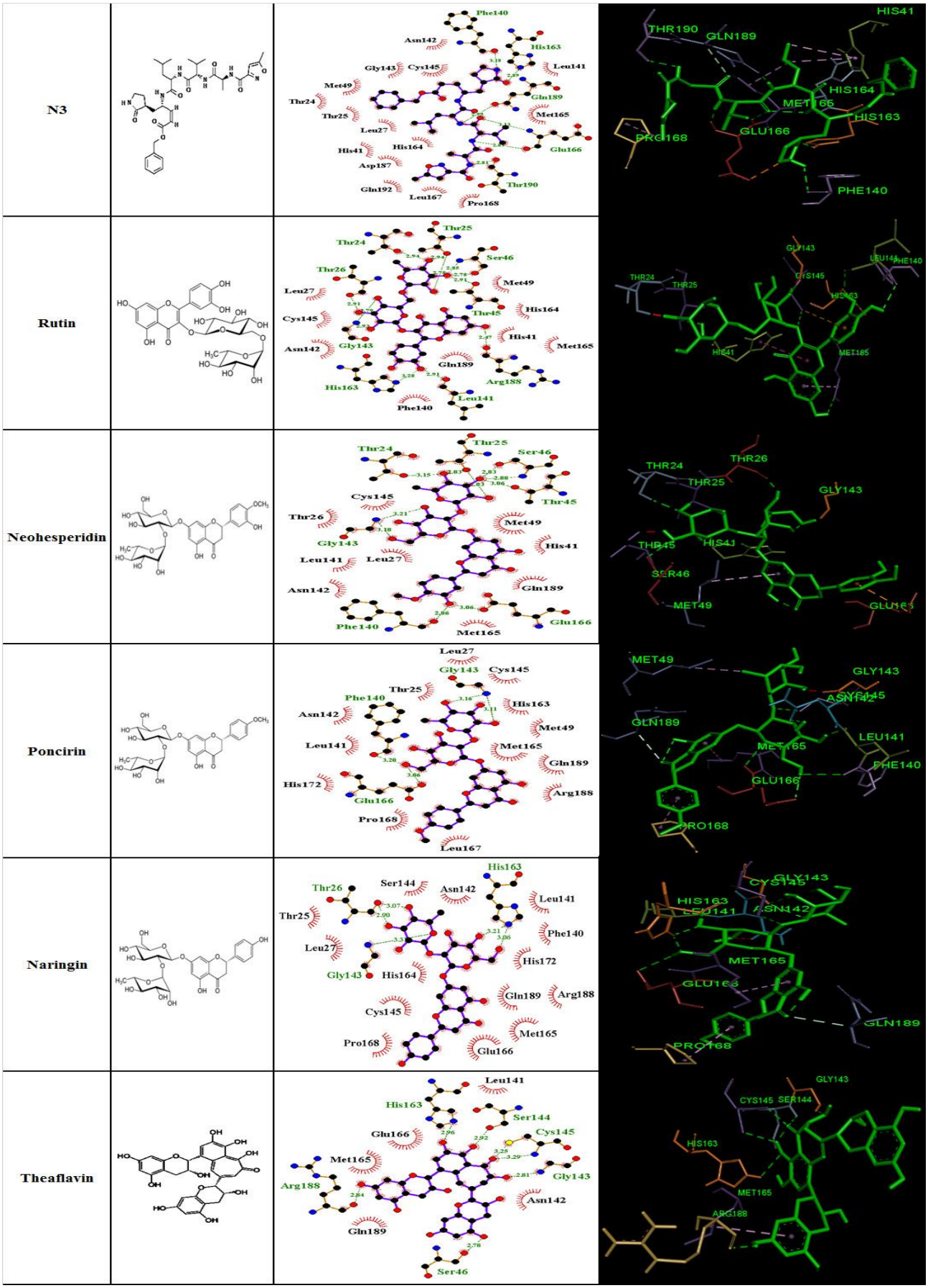

Fig. 2 2D and 3D interaction diagram for the native inhibitor and five selected flavonoids in active site of COVID-19; Hydrogen bonds (green dashed line), hydrophobic (spokes radiating).

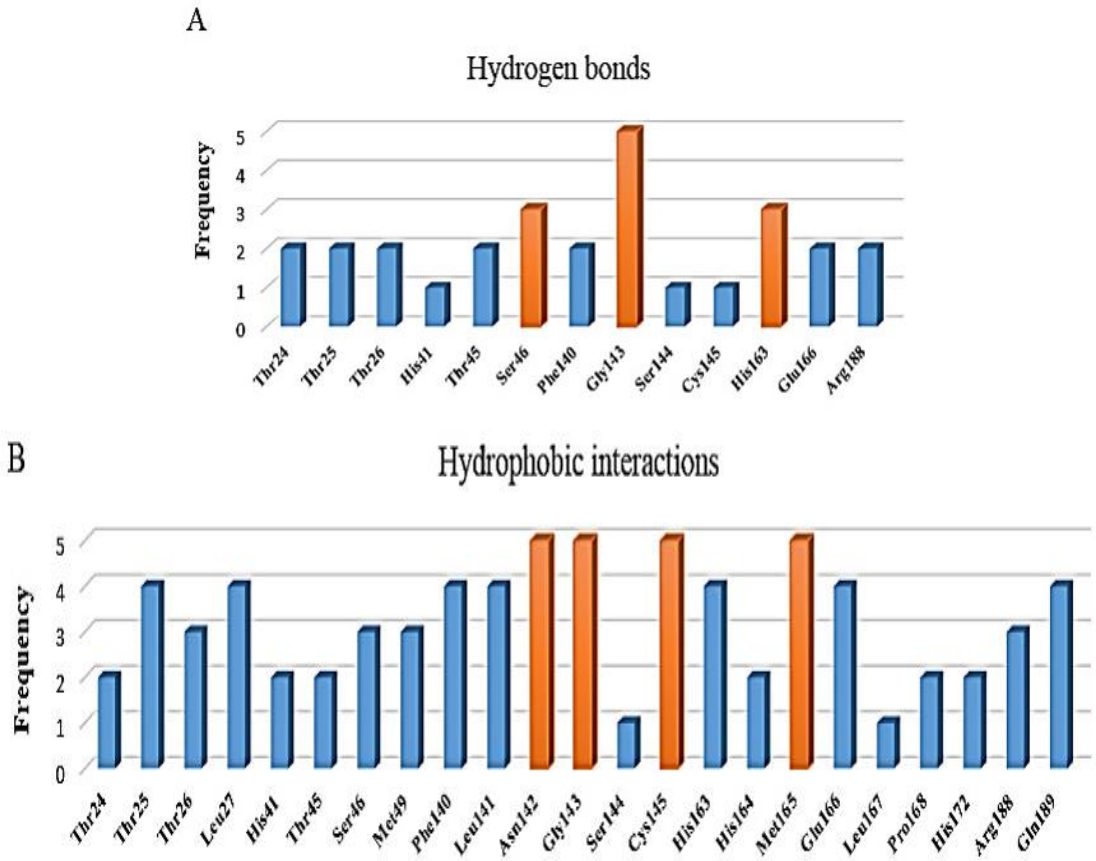

Fig. 3 Frequency of (A) hydrogen bond and (B) hydrophobic interactions of selected flavonoids with the residue of the active site of COVID-19 main protease.

The analysis of detailed interaction showed that five candidate flavonoids formed a hydrogen bond with 21 residues from the active site. Among these residues, Leu141, Ser144 and His163 were the most common. According to the analysis of the candidate flavonoids, residues play a key role in inhibiting Mpro by the formation of hydrogen bonds with inhibitors, including Ser46, Gly143, and His163. Additionally, residues that play a key role in inhibiting Mpro by the formation of hydrophobic interaction include Asn142, Gly143, Cys145, and Met165. The Frequency of hydrogen bond and hydrophobic interactions of selected flavonoids with the residue of the active site of Mpro respectively as shown in Figures 3A and B.

On the other hand, the presence of certain substitutions in some positions of studied flavonoids is important for inhibition of the active site. Naringin, poncirin, and neohesperidin have a $C_{12}H_{21}O_{10}$ substitution at position 7 of the A ring and major hydrogen bond interactions were observed between this substitution and the active site. Therefore, they have a good potential for Mpro inhibition. Theaflavin has a unique structure compared to other compounds. It seems that the presence of hydroxyl groups at different positions and forming hydrogen bond interactions with residues Ser46, Gly143, Ser144, Cys145, His163, and Arg188 increase the Mpro inhibitory effect. There was a $C_{12}H_{21}O_{10}$ substitution at position 3 in the rutin compound that formed effective links with the receptor. In brief, the findings of the present study suggest five active flavonoids have the potential for the development of Mpro inhibitors as a natural drug candidate for COVID-19. In addition, it should be cited that the two most phytochemical inhibitors of naringin and poncirin respectively with the predicted binding energy -11.60 and -10.40 (kcal/mol) against GRP78 (Barzegar *et al.*, 2021) was reappeared as selected inhibitors of Mpro of COVID-19 among the same 70 studied flavonoids from eight different subclasses. This correlation makes able to narrow down the scope of the suggestion by the proposition of naringin and poncirin. Regardless, this paper supplies precious insight about 70 studied flavonoids from eight different subclasses that are worth considering for the therapeutic aim of covid-19.

Conclusion

This study conducted an *in vitro* study of COVID-19 main protease inhibition by flavonoids of plant origin. The effect of 70 flavonoids on inhibition of Mpro function was investigated using COVID-19 Docking Server. In this study, five flavonoids namely rutin, neohesperidin, poncirin, naringin, and theaflavin were proposed as natural compounds for COVID-19 therapy. The binding energy for the best conformation of selected compounds respectively was -9.5, -9.2, -8.9, -8.8 and -8.8 kcal/mol in compare to the -8.5 kcal/mol value for the native inhibitor. It can be seen that the hydrogen bonding with residues Ser46, Gly143, His163 and hydrophobic interaction with residues Asn142, Gly143, Cys145, and Met165 are the major interaction between selected flavonoids with Mpro. However, naringin and poncirin flavonoids are more recommended due to an effective inhibitory feature against the both Mpro of COVID-19 and GRP78 receptor that previously was investigated by our work as well as rutin that was proposed based on a consumption decision along with other studies for the inhibitory effect of flavonoids against the main protease of COVID-19. Further clinical research is required to validate the obtained results for COVID-19 therapeutic.

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