Journal of Spectroscopy and Molecular Sciences

Journal Homepage: https://dergipark.org.tr/tr/pub/jsms

Rutin, luteolin, and myricetin as potential inhibitors of SARS-CoV-2 Main Protease (M^{pro}): A virtual screening study

Tayfun Gencsoy[1], Naim Peker[1], Hasan Tugra Yavas[1], Ozan Unsalan[2,*]

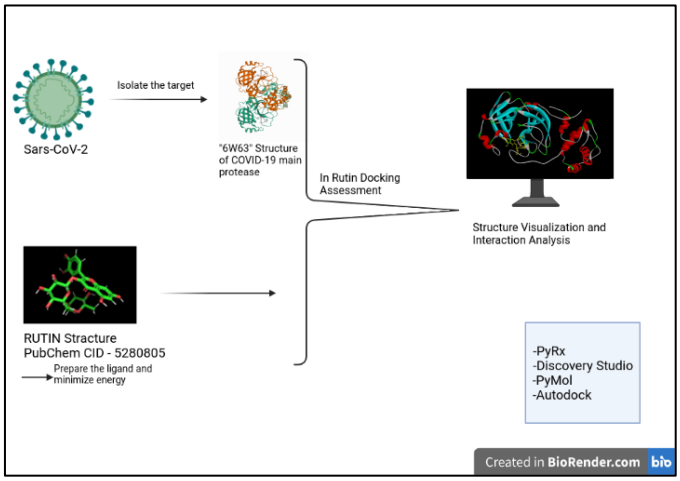
[1]Department of Biochemistry, Faculty of Science, Ege University, Izmir, Turkey

[2]Department of Physics, Faculty of Science, Ege University, Izmir, Turkey

*Correspondence: physicistozan@gmail.com (Ozan Unsalan)
ORCIDs Tayfun Gencsoy: https://orcid.org/0000-0001-7893-280X
Naim Peker: https://orcid.org/0000-0002-6446-1258
Hasan Tugra Yavas: https://orcid.org/0000-0002-6604-8731
Ozan Unsalan: https://orcid.org/0000-0001-5736-7530

Abstract: The COVID-19 pandemic appeared in China on November 17, 2019. As of 11 August 2022, after the first case was seen, 587,502,315 cases were observed and 6,427,422 deaths were reported (Johns Hopkins University, 2022). After the recognition of COVID-19 as pandemic, a mobilization for vaccine development started all over the world. While the vaccine development studies continue, there are some drugs recommended for the treatment but not with the most effective results. Since drug design, development and testing procedures are time consuming, virtual screening studies with the help of existing drug databases take the initiative and save time at this point. Moreover, drug repurposing strategies promise to identify new potential agents for such diseases in a time-critical manner. Here, we report structure-based virtual screening method to reveal the docking profiles of three flavonoids, rutin, luteolin, and myricetin on one of the COVID-19 main protease (6W63) of SARS-CoV-2.

Keywords: Docking; Rutin; Luteolin; Myricetin; SARS-CoV-2; COVID-19; 6W63.



Graphical abstract

Received 27 December 2021, Accepted 03 September 2022, Available online 23 October 2022.

1. Introduction

The current coronavirus disease 2019 (COVID-19) pandemic is of global emergency, with its fast expansion and high fatality rate. The number of persons infected with COVID-19 is rapidly increasing globally. Pneumonia, severe symptoms of acute respiratory distress syndrome (ARDS), and multiple organ failure can all occur in COVID-19 patients (N. Chen et al., 2020; C. Huang et al., 2020; D. Wang et al., 2020; N. Zhu et al., 2020). Coronaviruses are enclosed, positive single-stranded big RNA viruses that may infect humans as well as a variety of other animals. Tyrell and Bynoe, who cultivated the viruses from patients with common colds, first described coronaviruses in 1966(Tyrrell & Bynoe, 1966) . They named coronaviruses (Latin: corona = crown) because of their shape as spherical virions with a core shell and surface projections resembling a solar corona. Coronaviruses are divided into four subfamilies: alpha, beta, gamma, delta, and omicron. While alpha and beta coronaviruses are thought to have originated in mammals, particularly bats, gamma and delta viruses are thought to have originated in pigs and birds. Beta-coronaviruses, one of seven coronavirus subtypes that may infect humans, can cause serious sickness and death, whereas alpha-coronaviruses induce asymptomatic or slightly symptomatic infections.SARS-CoV-2 belongs to the B lineage of the beta-coronaviruses and is closely related to the SARS-CoV virus (Karim & Karim, 2021; Tyrrell & Bynoe, 1966; Zhou et al., 2020) (Shu & McCauley, 2017). The nucleocapsid protein (N), spike protein (S), small membrane protein (SM), and membrane glycoprotein (M) are the four primary structural genes, with an extra membrane glycoprotein (HE) found in the HCoVOC43 and HKU1 beta coronaviruses. SARSCoV2 is 96 percent similar to a bat coronavirus at the complete genome level (Chan et al., 2020; Rottier, 1995).

Drug repurposing is a technique of identifying new indications for existing medications that is regarded to be a cost-effective and efficient method. It is estimated that 75% of currently available drugs could be repurposed to treat a variety of diseases(F. Huang et al., 2020). The value of medication reuse has been proven in previous investigations. Applying computer-aided drug design approaches to swiftly find viable options is extremely efficient, especially after the full 3D structures of critical viral proteins have been determined. Using the crystal structure of the SARS-CoV-2 primary protease enzyme (M^{pro}) in association with its natural inhibitor(Fig. 1)was recently published (F. Huang et al., 2020).

Rutin (3, 3′, 4′, 5, 7-pentahydrohyflavone-3-rhamnoglucoside) (RTN) is a pigment found in a variety of fruits and vegetables. Rutin may be found in buckwheat, Japanese pagoda trees, and Eucalyptus. Rutin is a multifunctional phenolic natural substance that is essential in diets and of significant interest owing to its multiple stated health benefits (Pawan K. Agrawal, Agrawal, & Blunden, 2021).

Luteolin 3′,4′,5,7-tetrahydroxyflavone (LTN), is a flavonoid found in a variety of plants such as fruits, vegetables, and medicinal herbs. Plants high in luteolin have been used in Chinese traditional medicine to treat hypertension, inflammatory disorders, and cancer. LTN operates biochemically as either an antioxidant or a pro-oxidant, and has many biological effects such as anti-inflammation, anti-allergy, and anticancer (Lin, Shi, Wang, & Shen, 2008).

Myricetin (MYR) is a flavonoid found in a variety of natural plants. MYR has been shown to have a variety of biological roles, and it is a natural substance with considerable research and development potential. The molecule has a wide variety of actions, including anti-oxidant, anti-cancer, anti-diabetic, and anti-inflammatory effects (Song et al., 2021).

Here, we report the docking profiles of three selected flavonoids on 6W63 M^{Pro} of SARS-CoV-2. To the best of our knowledge there are no docking studies of these flavonoids into 6W63 specifically.

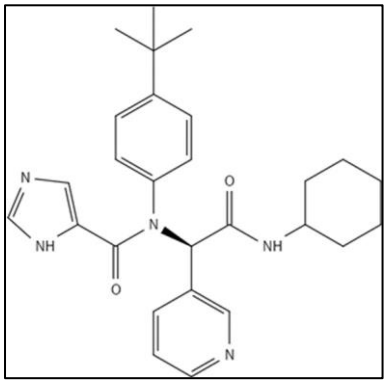


Figure 1. Structure of natural ligand (X77) of SARS-CoV-2 M^{Pro} (PDB ID: 6W63).

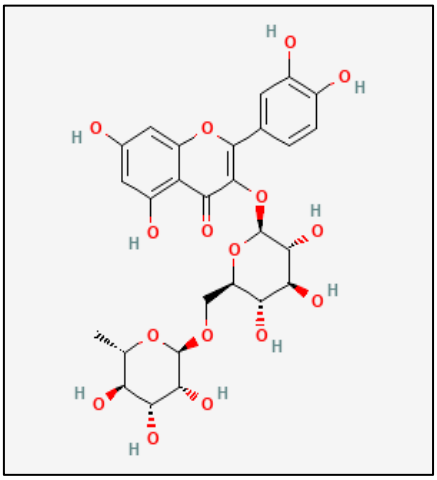


Figure 2. Chemical structure of rutin.

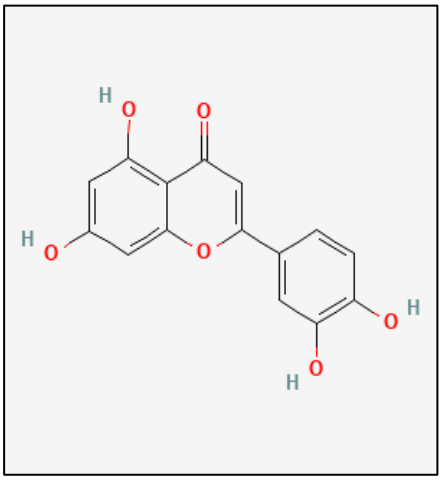


Figure 3. Chemical structure of luteolin.

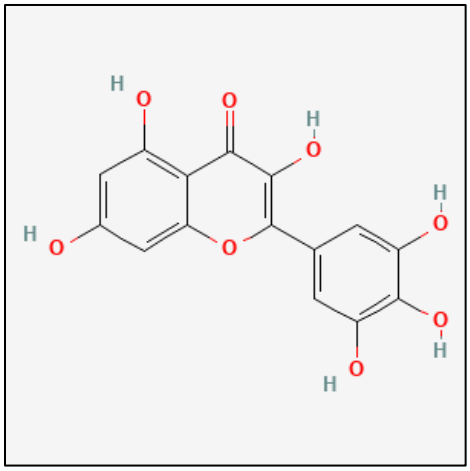


Figure 4. Chemical structure of myricetin.

2. Materials and methods

2.1. Preparation of protein structure

The protein data bank (PDB) (https://www.rcsb.org/) was used to get the 3D crystal structure of SARS-CoV-2 (PDB ID: 6W63). Discovery Studio (DS) Visualizer 3.5 was used to visualize and prepare the protein. Water molecules in the protein structure were eliminated from the system prior to docking in order to embed the candidate ligands into the pocket where the nearby receptor sites are located.

2.2. Ligand selection and ligand file preparation

The ligand's Standard Database Format (.sdf) file was acquired from the PubChem Database (Kim et al., 2016) at https://pubchem.ncbi.nlm.nih.gov/ and converted to a PDB file type using the PyRx (version 0.8) software's Open Babel (O'Boyle et al., 2011) plugin.

2.3. Energy minimization and model validation

The PyRx virtual screening program was employed to prepare the ligands and minimize their energy and finally dock the ligands to selected protein and its selected residues. The goal of employing Universal Force Field (UFF) was to reduce the amount of energy associated with ligands. Following that, the considered ligand was optimized first and then converted to a mol2 format using PyRx.

2.4. Prediction of the active or binding site

Discovery Studio Visualizer 2020 was used to visualize the ligands (BIOVIA). The photos were also created with Discovery Studio Visualizer 2020 (BIOVIA). After the removal of water molecules from the protein, related residues were determined where natural ligand (X77) of the 6W63 M^{Pro} is in interaction, particularly with hydrogen bonds. Then these residues were used for our docking purposes with the flavonoids under investigation in our work.

2.5. Molecular docking and interaction analysis

Molecular interactions of three flavonoids were analyzed by the Auto Dock wizard with the help of PyRx software. Auto Dock wizard panel was used to create macromolecules. Grid parameters were created via the Auto Grid engine in PyRx. The active site of the protein containing amino acids interacting with this ligand was predicted using this grid box. Embedding of the compounds were performed by following the PyRx's wizard's step.

2.6. Structure Visualization through DS

Discovery Studio Visualizer (BIOVIA, 2020) was used to visualize the docking results in 2D, and 3D space by using BIOVIA Discovery Studio Client 2020 software. Protein and ligand structures were created as pdbqt file to properly open in the docking preparation interface in the software.

3. Results

Based on our docking work, docking scores of all three compounds were found between –8.4 and –7.8 kcal/mol. Binding scores of these ligands are –8.4, –7.9, and –7.8 kcal/mol for rutin, luteolin, and myricetin, respectively and the findings were presented in Table 1. The interactions in both 3D and 2D of the ligands studied in this work with 6W63 were illustrated between Figs. 5-10. After the determination of the residues (His41, Cys44, Met49, Glu166, Gln 192) of M^{Pro} where hydrogen bonds, pi-sulfur bonds, pi-pi bonds are mainly in interaction with the natural ligand, we then evaluated our docking results for our flavonoids and found residues for the M^{Pro} inhibition by the flavonoids studied. Our results suggested that top three modes of rutin interacts via H-bonds towards Glu166, Gln192, pi-sulfur bonds towards Cys44, Met49, and pi-pi bond for His41, whereas fourth mode (rutin 3) is in interaction with additional residues (hydrogen bonded: Asn142, Phe140, Ser144, His163, Glu166, pi-sulfur: Met165) compared to the first three modes. However, luteolin showed hydrogen bonded interactions between Leu141, Glu 166, Met165, Phe140. Moreover, myricetin was observed to exhibit only hydrogen bonded with the surrounding residues (Phe140, His163, Glu166).

Table 1. Docking scores of three flavonoids.

Ligand[a]	**Binding affinity (kcal/mol)**[b]	**Mode**	**RMSD*/UB**	**RMSD/LB**
Rutin	–8.4	0	0.000	0.000
	–8.1	1	4.999	1.937
	–8.0	2	6.355	2.576
	–8.0	3	8.842	4.266
Luteolin	–7.9	0	0.000	0.000
Myricetin	–7.8	0	0.000	0.000

[a]Flavonoids by virtual screening study we used in this work. [b] Binding affinities were given in kcal/mol.*RMSD: Root mean square definition.

Table 2. Interactions of the selected flavonoids with their surrounding residues.

Ligand (Mode)	Interactions*
Rutin (0)	**Glu166, Gln192,** Cys:44[a] , Met:49[a], His:41[b]
Rutin (1)	**Glu166, Gln192,** Cys:44[a] , Met:49[a], His:41[b]
Rutin (2)	**Glu166, Gln192,** Cys:44[a] , Met:49[a], His:41[b]
Rutin (3)	**Asn142, Phe140, Ser144, His163, Glu166,** Met165[a]
Luteolin (0)	**Leu141, Glu166,** Met165[a]
Myricetin (0)	**Phe140, His163, Glu166**

***Bold**: conventional hydrogen bond, [a]pi-sulfur bond , [b]pi-pi stacked bond.

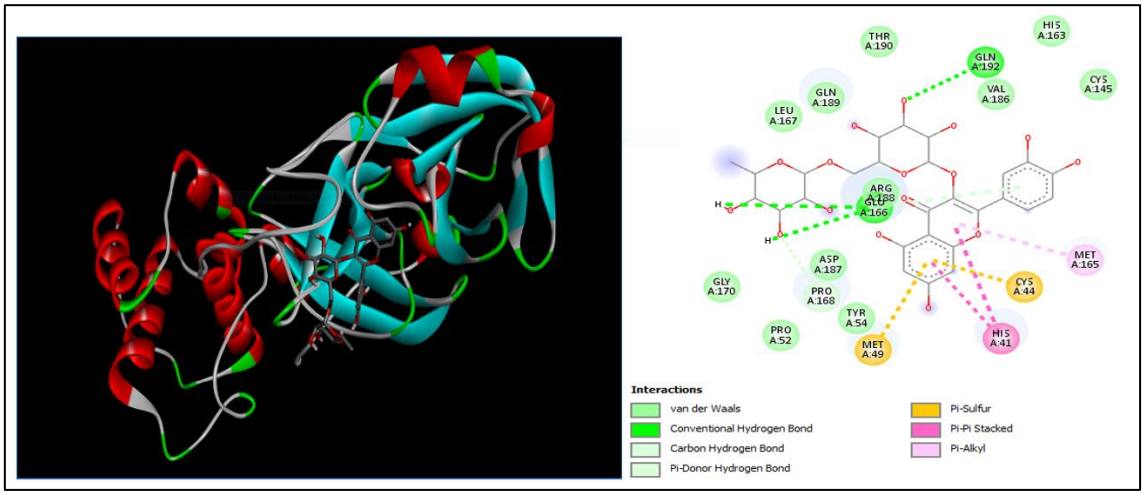


Figure 5. Rutin (mode 0).

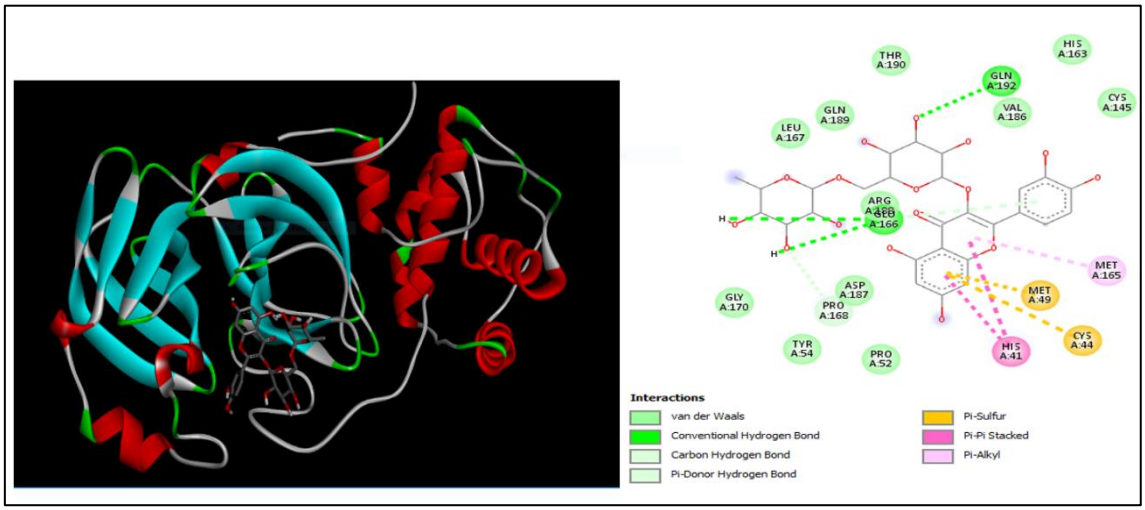


Figure 6. Rutin (mode 1).

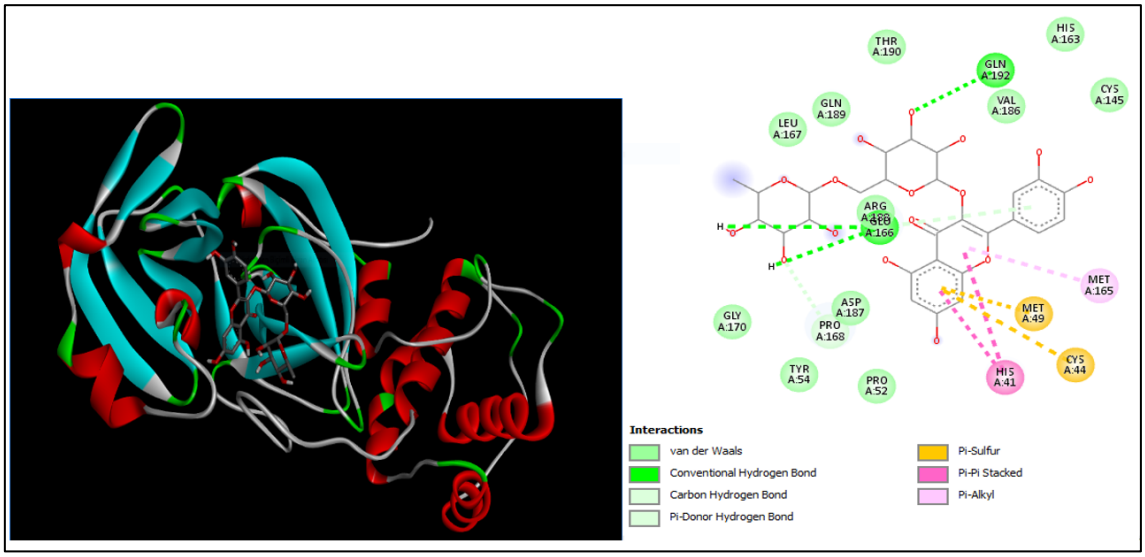


Figure 7. Rutin (mode 2).

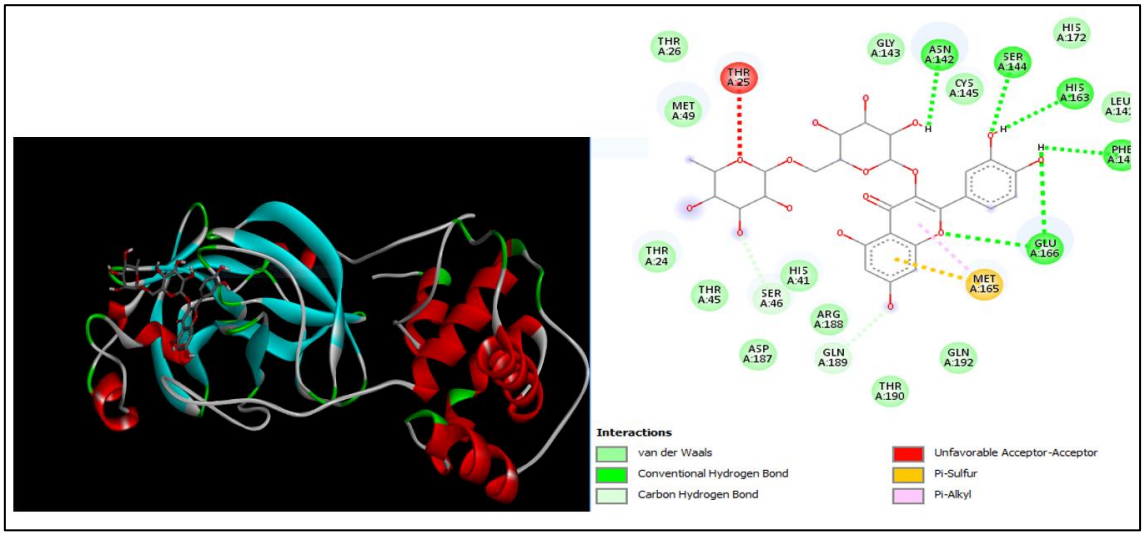


Figure 8. Rutin (mode 3).

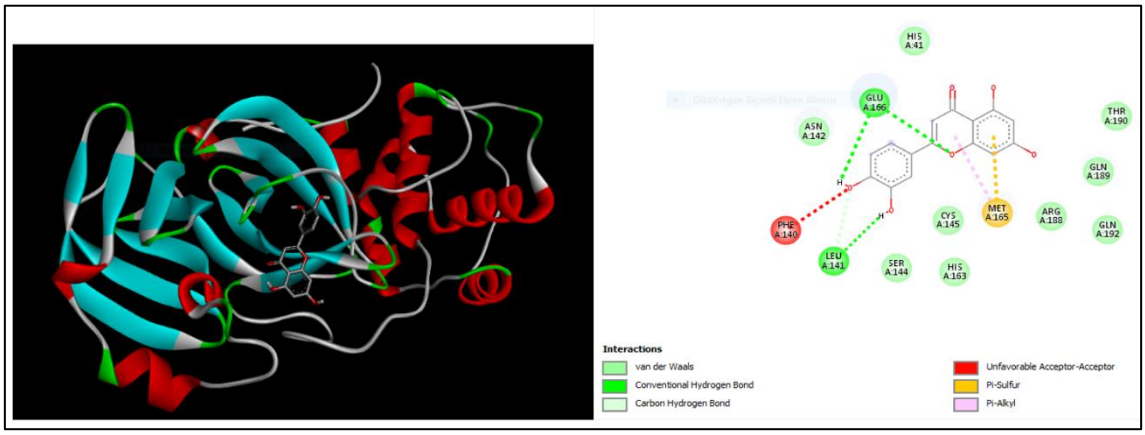


Figure 9. Luteolin (mode 0).

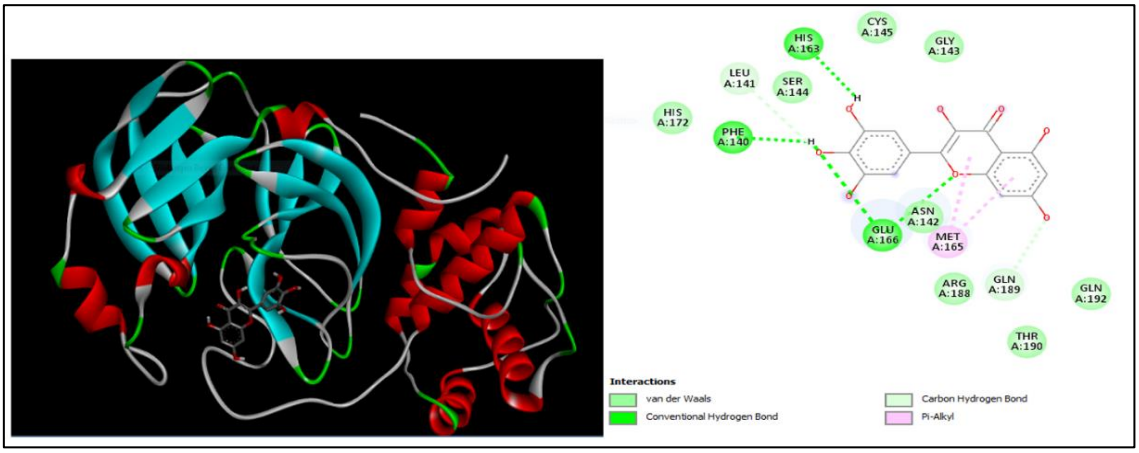


Figure 10. Myricetin (mode 0).

Table 3. ADME/Tox evaluation and molecular descriptors for rutin (*:for human).

Absorption	**Unit**	**Value**	**Distribution**	**Unit**	**Value**	**Toxicity**	**Unit**	**Value**
Water solubility	log mol/L	–2.89	VDss*	log L/kg	1.66	AMES toxicity	(Yes/No)	No
Caco2 permeability	log Papp (μcm/s)	–0.95	Fraction unbound*	Fu	0.19	Max. tolerated dose*	log mg/kg/day	0.45
Intestinal absorption*	(% Absorbed)	23.45	BBB permeability	log BB	–1.90	hERG I inhibitor	(Yes/No)	No
Skin Permeability	(log Kp)	–2.23	CNS permeability	log PS	–5.18	hERG II inhibitor	(Yes/No)	Yes
P-glycoprotein substrate	(Yes/No)	Yes	**Metabolism**	**Unit**	**Value**	Oral Rat Acute Toxicity (LD50)	mol/kg	2.49
P-glycoprotein I inhibitor	(Yes/No)	No	CYP2D6 substrate	(Yes/No)	No	Oral Rat Chronic Toxicity (LOAEL)	log mg/kg	3.67
P-glycoprotein II inhibitor	(Yes/No)	No	CYP3A4 substrate	(Yes/No)	No	Hepatotoxicity	(Yes/No)	No
Excretion	**Unit**	**Value**	CYP1A2 inhibitor	(Yes/No)	No	Skin Sensitization	(Yes/No)	No
Total Clearance	log ml/min/kg	–0.37	CYP2C19 inhibitor	(Yes/No)	No	*T.Pyriformis* toxicity	log μg/L	0.28
			CYP2C9 inhibitor	(Yes/No)	No			
Renal OCT2 substrate	(Yes/No)	No	CYP2D6 inhibitor	(Yes/No)	No	Minnow toxicity	log mM	7.67
			CYP3A4 inhibitor	(Yes/No)	No			
Descriptors	**Unit**	**Value**	**Descriptors**	**Unit**	**Value**	**Lipophilicity**	**Unit**	**Value**
Total molecular weight	g/mol	610.52	Irritant	(Yes/No)	No	log $P_{o/w}$ (iLOGP)	Number	1.58
clogP	Number	–1.26	Shape index	Number	0.42	log $P_{o/w}$ (xLOGP3)	Number	–0.33
clogS	Number	–2.40	Molecular flexibility	Number	0.39	log $P_{o/w}$ (WLOGP)	Number	–1.69
H-acceptors	Count	16	Molecular complexity	Number	0.97	log $P_{o/w}$ (MLOGP)	Number	–3.89
H-donors	Count	10	Rotatable bonds	Number	6	log $P_{o/w}$ (SILICOS-IT)	Number	–2.11
Total surface area	$Å^2$	397.70	Aromatic rings	Number	2	Consensus log $P_{o/w}$	Number	–1.29
Polar surface area	$Å^2$	265.52	Globularity SVD	Number	0.42	**Druglikeliness**	**Unit**	**Value**
Druglikeliness	Number	1.93	Globularity volume	Number	0.67	Lipinski rule of five violation	(Yes/No)	Yes
Mutagenic	(Yes/No)	No	vdW surface	Number	478.42			
Tumorigenic	(Yes/No)	No	vdW volume	Number	547.57			

Table 4. ADME/Tox evaluation and molecular descriptors for luteolin (*:for human).

Absorption	**Unit**	**Value**	**Distribution**	**Unit**	**Value**	**Toxicity**	**Unit**	**Value**
Water solubility	log mol/L	–3.09	VDss*	log L/kg	1.15	AMES toxicity	(Yes/No)	No
Caco2 permeability	log Papp (µcm/s)	0.09	Fraction unbound*	Fu	0.17	Max. tolerated dose*	log mg/kg/day	0.50
Intestinal absorption*	(% Absorbed)	81.12	BBB permeability	log BB	–0.91	hERG I inhibitor	(Yes/No)	No
Skin Permeability	(log Kp)	–2.73	CNS permeability	log PS	–2.25	hERG II inhibitor	(Yes/No)	No
P-glycoprotein substrate	(Yes/No)	Yes	**Metabolism**	**Unit**	**Value**	Oral Rat Acute Toxicity (LD50)	mol/kg	2.45
P-glycoprotein I inhibitor	(Yes/No)	No	CYP2D6 substrate	(Yes/No)	No	Oral Rat Chronic Toxicity (LOAEL)	log mg/kg	2.41
P-glycoprotein II inhibitor	(Yes/No)	No	CYP3A4 substrate	(Yes/No)	No	Hepatotoxicity	(Yes/No)	No
Excretion	**Unit**	**Value**	CYP1A2 inhibitor	(Yes/No)	Yes	Skin Sensitization	(Yes/No)	No
Total Clearance	log ml/min/kg	0.49	CYP2C19 inhibitor	(Yes/No)	No	*T.Pyriformis* toxicity	log µg/L	0.32
			CYP2C9 inhibitor	(Yes/No)	Yes			
Renal OCT2 substrate	(Yes/No)	No	CYP2D6 inhibitor	(Yes/No)	No	Minnow toxicity	log mM	3.17
			CYP3A4 inhibitor	(Yes/No)	No			
Descriptors	**Unit**	**Value**	**Descriptors**	**Unit**	**Value**	**Lipophilicity**	**Unit**	**Value**
Total molecular weight	g/mol	286.24	Irritant	(Yes/No)	No	log $P_{o/w}$ (iLOGP)	Number	1.86
clogP	Number	1.99	Shape index	Number	0.52	log $P_{o/w}$ (xLOGP3)	Number	2.53
clogS	Number	–2.56	Molecular flexibility	Number	0.26	log $P_{o/w}$ (WLOGP)	Number	2.28
H-acceptors	Count	6	Molecular complexity	Number	0.82	log $P_{o/w}$ (MLOGP)	Number	–0.03
H-donors	Count	4	Rotatable bonds	Number	1	log $P_{o/w}$ (SILICOS-IT)	Number	–2.03
Total surface area	$Å^2$	197.38	Aromatic rings	Number	2	Consensus log $P_{o/w}$	Number	1.73
Polar surface area	$Å^2$	107.22	Globularity SVD	Number	2.96	**Druglikeliness**	**Unit**	**Value**
Druglikeliness	Number	0.28	Globularity volume	Number	0.75	Lipinski rule of five violation	(Yes/No)	No
Mutagenic	(Yes/No)	No	vdW surface	Number	253.69			
Tumorigenic	(Yes/No)	No	vdW volume	Number	245.50			

Table 5. ADME/Tox evaluation and molecular descriptors for myricetin (*:for human).

Absorption	**Unit**	**Value**	**Distribution**	**Unit**	**Value**	**Toxicity**	**Unit**	**Value**
Water solubility	log mol/L	–3.07	VDss*	log L/kg	0.12	AMES toxicity	(Yes/No)	No
Caco2 permeability	log Papp (μcm/s)	0.32	Fraction unbound*	Fu	0.05	Max. tolerated dose*	log mg/kg/day	1.08
Intestinal absorption*	(% Absorbed)	66.13	BBB permeability	log BB	–1.70	hERG I inhibitor	(Yes/No)	No
Skin Permeability	(log Kp)	–2.73	CNS permeability	log PS	–3.76	hERG II inhibitor	(Yes/No)	No
P-glycoprotein substrate	(Yes/No)	Yes	**Metabolism**	**Unit**	**Value**	Oral Rat Acute Toxicity (LD50)	mol/kg	2.30
P-glycoprotein I inhibitor	(Yes/No)	No	CYP2D6 substrate	(Yes/No)	No	Oral Rat Chronic Toxicity (LOAEL)	log mg/kg	3.86
P-glycoprotein II inhibitor	(Yes/No)	No	CYP3A4 substrate	(Yes/No)	No	Hepatotoxicity	(Yes/No)	No
Excretion	**Unit**	**Value**	CYP1A2 inhibitor	(Yes/No)	Yes	Skin Sensitization	(Yes/No)	No
Total Clearance	log ml/min/kg	0.61	CYP2C19 inhibitor	(Yes/No)	No	*T.Pyriformis* toxicity	log μg/L	0.28
			CYP2C9 inhibitor	(Yes/No)	No			
Renal OCT2 substrate	(Yes/No)	No	CYP2D6 inhibitor	(Yes/No)	No	Minnow toxicity	log mM	1.25
			CYP3A4 inhibitor	(Yes/No)	No			
Descriptors	**Unit**	**Value**	**Descriptors**	**Unit**	**Value**	**Lipophilicity**	**Unit**	**Value**
Total molecular weight	g/mol	318.24	Irritant	(Yes/No)	No	log $P_{o/w}$ (iLOGP)	Number	1.08
clogP	Number	1.14	Shape index	Number	0.48	log $P_{o/w}$ (xLOGP3)	Number	1.18
clogS	Number	–2.19	Molecular flexibility	Number	0.30	log $P_{o/w}$ (WLOGP)	Number	1.69
H-acceptors	Count	8	Molecular complexity	Number	0.85	log $P_{o/w}$ (MLOGP)	Number	–1.08
H-donors	Count	6	Rotatable bonds	Number	1	log $P_{o/w}$ (SILICOS-IT)	Number	1.06
Total surface area	$Å^2$	208.29	Aromatic rings	Number	2	Consensus log $P_{o/w}$	Number	0.79
Polar surface area	$Å^2$	147.68	Globularity SVD	Number	0.30	**Druglikeliness**	**Unit**	**Value**
Druglikeliness	Number	–0.08	Globularity volume	Number	0.74	Lipinski rule of five violation	(Yes/No)	Yes
Mutagenic	(Yes/No)	High	vdW surface	Number	267.96			
Tumorigenic	(Yes/No)	No	vdW volume	Number	260.99			

4. Discussion

Drug and vaccine studies for coronavirus, which is a great threat to humanity, continue to be carried out meticulously in most countries. While drug or vaccine development should be carried out both quickly and effectively, deaths and rapidly increasing cases slow down this process. Instead of Structure of COVID-19 main protease bound to potent broad-spectrum non-covalent inhibitor X77, according to our docking results rutin, luteolin and myricetin docking may be used to produce potential possible replacement/modified drugs against the virus. Our findings on three selected flavonoids and their potential to inhibit SARS-CoV-2 M^{Pro} are in line with previous flavonoids docking works that successfully demonstrate the effective use of flavonoids that could inhibit M^{Pro} (Abdul-Hammed et al., 2021; P K Agrawal, Agrawal, & Blunden, 2021; Alhadrami et al., 2021; Ali & Kunugi, 2021; Allam, Assaf, Hassan, Shimizu, & Elshaier, 2020; Babaeekhou, Ghane, & Abbas-Mohammadi, 2021; Batool et al., 2020; Bhati, Kaushik, & Singh, 2021; Bhati et al., 2021; Bhowmik, Nandi, Prakash, & Kumar, 2021; Biagioli et al., 2021; Bolelli, Ertan-Bolelli, Unsalan, & Altunayar-Unsalan, 2021; Chapman & Andurkar, 2021; C. N. Chen et al., 2005; da Silva et al., 2020; Dallakyan & Olson, 2015; Das, Majumder, Mandal, & Basak, 2021; K. Dubey & Dubey, 2020; R. Dubey & Dubey, 2021; Ebada et al., 2020; Fadaka et al., 2021, 2021; Fakhar, Faramarzi, Pacifico, & Faramarzi, 2021; Fayed et al., 2021; Glaab, Manoharan, & Abankwa, 2021; Glaab et al., 2021; Gogoi et al., 2021; Gomez et al., 2021; Goris et al., 2021; Gorla, Rao, Kulandaivelu, Alavala, & Panda, 2021; Guler, Sal, et al., 2021; Guler, Tatar, Yildiz, Belduz, & Kolayli, 2021; Gurung et al., 2021; Hassan et al., 2021; Hiremath et al., 2021; Hu et al., 2020; Ibrahim, Abdelrahman, et al., 2021; Ibrahim, Mohamed, et al., 2021; Irfan et al., 2021; Istifli et al., 2020; Jain et al., 2021; Jalmakhanbetova et al., 2021; Jannat et al., 2021; Jiménez-Avalos et al., 2021; S Jo, Kim, Kim, Kim, & Shin, 2020; Seri Jo, Kim, Shin, & Kim, 2020; Johns Hopkins, 2021; Khursheed et al., 2021, 2021; Kumar et al., 2021; Lee et al., 2021; Li et al., 2022; Liao et al., 2021; Liskova et al., 2021; Ma et al., 2021, 2021; Maddah et al., 2021; Maiti & Banerjee, 2021; Majumder & Mandal, 2020; Mandour, Zlotos, & Salem, 2020; Mangiavacchi et al., 2021; Maroli, Bhasuran, Natarajan, & Kolandaivel, 2020; Mathpal et al., 2021; Mohapatra, Chopdar, Dash, Mohanty, & Raval, 2021; Moradkhani, Farmani, Saidijam, & Taherkhani, 2021; Mosquera-Yuqui, Lopez-Guerra, & Moncayo-Palacio, 2020; Neves et al., 2021; Ngwa et al., 2020; Owis et al., 2020, 2021; Potshangbam, Nongdam, Kumar, & Rathore, 2021; Prasansuklab et al., 2021; Puttaswamy et al., 2020; Rahman et al., 2021; Rakshit, Muduli, Srivastav, & Mishra, 2021; Rakshit et al., 2021; Rameshkumar et al., 2021; Rehman, AlAjmi, & Hussain, 2021; Rizzuti et al., 2021; Rudrapal et al., 2021; Samy et al., 2021, 2021; Sen, Bhaumik, Debnath, & Debnath, 2021; Shaldam, Yahya, Mohamed, Abdel-Daim, & Al Naggar, 2021; J. Singh, Malik, & Raina, 2020; A. V Singh, 2021; Vijayakumar, Ramesh, Joji, Prakasan, & Kannan, 2020; J. Wang et al., 2021; Xiao, Cui, Zheng, Wang, et al., 2021; Xiao, Cui, Zheng, Zhang, et al., 2021; Xiong et al., 2021; Z. R. Xu et al., 2020; Z. Xu et al., 2020; Yosri et al., 2021; Yu, Chen, Lan, Shen, & Li, 2020; Zaki, Al-Karmalawy, El-Amier, & Ashour, 2020; Zhang, Yao, Yang, & Wu, 2021; Y. Zhu & Xie, 2020). Thus, it is crucial to perform much detailed and systematic investigations for the use of such compounds in drug design for future relevant studies for this disease. Here, we demonstrated how effective the rutin among other two flavonoids studied in this work could be replaced in the crystal structure of 6W63 instead of its natural ligand. We believe that our work could contribute to virtual screening studies on particularly this M^{Pro} of SARS-CoV-2.

Conflict of interest

Authors declare that there is no conflict of interest.

CRediT Author Statement

Tayfun Gencsoy: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Funding acquisition, Supervision, Writing - Original Draft, Writing – Review & Editing. **Naim Peker**: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Funding acquisition, Supervision, Writing - Original Draft, Writing – Review & Editing. **Hasan Tugra Yavas**: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Funding acquisition, Supervision, Writing - Original Draft, Writing – Review & Editing. **Ozan Unsalan:** Conceptualization, Supervision, Review & Editing, Methodology.

References

Abdul-Hammed, M., Adedotun, I. O., Olajide, M., Irabor, C. O., Afolabi, T. I., Gbadebo, I. O., … Ramasami, P. (2021). Virtual screening, ADMET profiling, PASS prediction, and bioactivity studies of potential inhibitory roles of alkaloids, phytosterols, and flavonoids against COVID-19 main protease (M-pro). *Natural Product Research*.

Agrawal, P K, Agrawal, C., & Blunden, G. (2021). Pharmacological Significance of Hesperidin and Hesperetin, Two Citrus Flavonoids, as Promising Antiviral Compounds for Prophylaxis Against and Combating COVID-19. *Natural Product Communications*, *16*(10).

Agrawal, Pawan K., Agrawal, C., & Blunden, G. (2021). Rutin: A Potential Antiviral for Repurposing as a SARS-CoV-2 Main Protease (Mpro) Inhibitor. *Natural Product Communications*, *16*(4). https://doi.org/10.1177/1934578X21991723

Alhadrami, H. A., Sayed, A. M., Hassan, H. M., Youssif, K. A., Gaber, Y., Moatasim, Y., … Gamaleldin, N. M. (2021). Cnicin as an Anti-SARS-CoV-2: An Integrated In Silico and In Vitro Approach for the Rapid Identification of Potential COVID-19 Therapeutics. *Antibiotics-Basel*, *10*(5).

Ali, A. M., & Kunugi, H. (2021). Propolis, Bee Honey, and Their Components Protect against Coronavirus Disease 2019 (COVID-19): A Review of In Silico, In Vitro, and Clinical Studies. *Molecules*, *26*(5).

Allam, A. E., Assaf, H. K., Hassan, H. A., Shimizu, K., & Elshaier, Y. A. M. M. (2020). Anin silicoperception for newly isolated flavonoids from peach fruit as privileged avenue for a countermeasure outbreak of COVID-19. *Rsc Advances*, *10*(50), 29983–29998.

Babaeekhou, L., Ghane, M., & Abbas-Mohammadi, M. (2021). In silico targeting SARS-CoV-2 spike protein and main protease by biochemical compounds. *Biologia*, *76*(11), 3547–3565.

Batool, F., Mughal, E. U., Zia, K., Sadiq, A., Naeem, N., Javid, A., … Saeed, M. (2020). Synthetic flavonoids as potential antiviral agents against SARS-CoV-2 main protease. *Journal of Biomolecular Structure & Dynamics*.

Bhati, S., Kaushik, V., & Singh, J. (2021). Rational design of flavonoid based potential inhibitors targeting SARS-CoV 3CL protease for the treatment of COVID-19. *Journal of Molecular Structure*, *1237*.

Bhowmik, D., Nandi, R., Prakash, A., & Kumar, D. (2021). Evaluation of flavonoids as 2019-nCoV cell entry inhibitor through molecular docking and pharmacological analysis. *Heliyon*, *7*(3).

Biagioli, M., Marchiano, S., Roselli, R., Di Giorgio, C., Bellini, R., Bordoni, M., … Fiorucci, S. (2021). Discovery of a AHR pelargonidin agonist that counter-regulates Ace2 expression and attenuates ACE2-SARS-CoV-2 interaction. *Biochemical Pharmacology*, *188*.

Bolelli, K., Ertan-Bolelli, T., Unsalan, O., & Altunayar-Unsalan, C. (2021). Fenoterol and dobutamine as SARS-CoV-2 main protease inhibitors: A virtual screening study. *Journal of Molecular Structure*, *1228*(xxxx), 129449. https://doi.org/10.1016/j.molstruc.2020.129449

Chan, J. F. W., Yuan, S., Kok, K. H., To, K. K. W., Chu, H., Yang, J., … Yuen, K. Y. (2020). A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *The Lancet*, *395*(10223), 514–523. https://doi.org/10.1016/S0140-6736(20)30154-9

Chapman, R. L., & Andurkar, S. V. (2021). A review of natural products, their effects on SARS-CoV-2 and their utility as lead compounds in the discovery of drugs for the treatment of COVID-19. *Medicinal Chemistry Research*.

Chen, C. N., Lin, C. P. C., Huang, K. K., Chen, W. C., Hsieh, H. P., Liang, P. H., & Hsu, J. T. A. (2005). Inhibition of SARS-CoV 3C-like protease activity by theaflavin-3,3′- digallate (TF3). *Evidence-Based Complementary and Alternative Medicine*, *2*(2), 209–215. https://doi.org/10.1093/ecam/neh081

Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., … Zhang, L. (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *The Lancet*, *395*(10223), 507–513. https://doi.org/10.1016/S0140-6736(20)30211-7

da Silva, F. M. A., da Silva, K. P. A., de Oliveira, L. P. M., Costa, E. V, Koolen, H. H. F., Pinheiro, M. L. B., … de Souza, A. D. L. (2020). Flavonoid glycosides and their putative human metabolites as potential inhibitors of the SARS-CoV-2 main protease (Mpro) and RNA-dependent RNA polymerase (RdRp). *Memorias Do Instituto Oswaldo Cruz*, *115*.

Dallakyan, S., & Olson, A. J. (2015). Small-molecule library screening by docking with PyRx. *Methods in Molecular Biology*, *1263*(January 2015), 243–250. https://doi.org/10.1007/978-1-4939-2269-7_19

Das, P., Majumder, R., Mandal, M., & Basak, P. (2021). In-Silico approach for identification of effective and stable inhibitors for COVID-19 main protease (M-pro) from flavonoid based phytochemical constituents ofCalendula officinalis. *Journal of Biomolecular Structure & Dynamics*, *39*(16), 6265–6280.

Dubey, K., & Dubey, R. (2020). Computation screening of narcissoside a glycosyloxyflavone for potential novel coronavirus 2019 (COVID-19) inhibitor. *Biomedical Journal*, *43*(4), 363–367. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7233213/pdf/main.pdf

Dubey, R., & Dubey, K. (2021). Molecular Docking Studies of Bioactive Nicotiflorin against 6W63 Novel Coronavirus 2019 (COVID-19). *Combinatorial Chemistry & High Throughput Screening*, *24*(6), 874–878.

Ebada, S. S., Al-Jawabri, N. A., Youssef, F. S., El-Kashef, D. H., Knedel, T. O., Albohy, A., … Proksch, P. (2020). Anti-inflammatory, antiallergic and COVID-19 protease inhibitory activities of phytochemicals from the Jordanian hawksbeard: identification, structure-activity relationships, molecular modeling and impact on its folk medicinal uses. *Rsc Advances*, *10*(62), 38128–38141.

Fadaka, A. O., Sibuyi, N. R. S., Martin, D. R., Klein, A., Madiehe, A., & Meyer, M. (2021). Development of Effective Therapeutic Molecule from Natural Sources against Coronavirus Protease. *International Journal of Molecular Sciences*, *22*(17).

Fakhar, Z., Faramarzi, B., Pacifico, S., & Faramarzi, S. (2021). Anthocyanin derivatives as potent inhibitors of SARS-CoV-2 main protease: An in-silico perspective of therapeutic targets against COVID-19 pandemic. *Journal of Biomolecular Structure & Dynamics*, *39*(16), 6171–6183.

Fayed, M. A. A., El-Behairy, M. F., Abdallah, I. A., Abdel-Bar, H. M., Elimam, H., Mostafa, A., … Elshaier, Y. A. M. M. (2021). Structure- and Ligand-Based in silico Studies towards the Repurposing of Marine Bioactive Compounds to Target SARS-CoV-2. *Arabian Journal of Chemistry*, *14*(4).

Glaab, E., Manoharan, G. B., & Abankwa, D. (2021). Pharmacophore Model for SARS-CoV-2 3CLpro Small-Molecule Inhibitors and in Vitro Experimental Validation of Computationally Screened Inhibitors. *Journal of Chemical Information and Modeling*, *61*(8), 4082–4096. Retrieved from https://pubs.acs.org/doi/pdf/10.1021/acs.jcim.1c00258

Gogoi, N., Chowdhury, P., Goswami, A. K., Das, A., Chetia, D., & Gogoi, B. (2021). Computational guided identification of a citrus flavonoid as potential inhibitor of SARS-CoV-2 main protease. *Molecular Diversity*, *25*(3), 1745–1759. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7685905/pdf/11030_2020_Article_10150.pdf

Gomez, C. R., Espinoza, I., Faruke, F. S., Hasan, M., Rahman, K. M., Walker, L. A., & Muhammad, I. (2021). Therapeutic Intervention of COVID-19 by Natural Products: A Population-Specific Survey Directed Approach. *Molecules*, *26*(4).

Goris, T., Perez-Valero, A., Martinez, I., Yi, D., Fernandez-Calleja, L., San Leon, D., … Nogales, J. (2021). Repositioning microbial biotechnology against COVID-19: the case of microbial production of flavonoids. *Microbial Biotechnology*, *14*(1), 94–110. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7675739/pdf/MBT2-14-94.pdf

Gorla, U. S., Rao, G. K., Kulandaivelu, U. S., Alavala, R. R., & Panda, S. P. (2021). Lead Finding from Selected Flavonoids with Antiviral (SARS-CoV-2) Potentials Against COVID-19: An In-silico Evaluation. *Combinatorial Chemistry & High Throughput Screening*, *24*(6), 879–890.

Guler, H. I., Sal, F. A. Y., Can, Z., Kara, Y., Yildiz, O., Belduz, A. O., … Kolayli, S. (2021). Targeting CoV-2 spike RBD and ACE-2 interaction with flavonoids of Anatolian propolis by in silico and in vitro studies in terms of possible COVID-19 therapeutics. *Turkish Journal of Biology*, *45*(4), 530–548. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8576337/pdf/turkjbio-45-530.pdf

Guler, H. I., Tatar, G., Yildiz, O., Belduz, A. O., & Kolayli, S. (2021). Investigation of potential inhibitor properties of ethanolic propolis extracts against ACE-II receptors for COVID-19 treatment by molecular docking study. *Archives of Microbiology*, *203*(6), 3557–3564. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8098016/pdf/203_2021_Article_2351.pdf

Gurung, A. B., Ali, M. A., Lee, J., Abul Farah, M., Al-Anazi, K. M., & Al-Hemaid, F. (2021). Identification of SARS-CoV-2 inhibitors from extracts of Houttuynia cordata Thunb. *Saudi Journal of Biological Sciences*, *28*(12), 7517–7527.

Hassan, A. R., Sanad, I. M., Allam, A. E., Abouelela, M. E., Sayed, A. M., Emam, S. S., … Shimizu, K. (2021). Chemical constituents from Limonium tubiflorum and their in silico evaluation as potential antiviral agents against SARS-CoV-2. *Rsc Advances*, *11*(51), 32346–32357.

Hiremath, S., Kumar, H. D. V, Nandan, M., Mantesh, M., Shankarappa, K. S., Venkataravanappa, V., … Reddy, C. N. L. (2021). In silico docking analysis revealed the potential of phytochemicals present in Phyllanthus amarus and Andrographis paniculata, used in Ayurveda medicine in inhibiting SARS-CoV-2. *3 Biotech*, *11*(2).

Hu, X. P., Cai, X., Song, X., Li, C. Y., Zhao, J., Luo, W. L., … He, Z. D. (2020). Possible SARS-coronavirus 2 inhibitor revealed by simulated molecular docking to viral main protease and host toll-like receptor. *Future Virology*, *15*(6), 359–368.

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., … Cao, B. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet*, *395*(10223), 497–506. https://doi.org/10.1016/S0140-6736(20)30183-5

Huang, F., Zhang, C., Liu, Q., Zhao, Y., Zhang, Y., Qin, Y., … Jiang, C. (2020). Identification of amitriptyline HCl, flavin adenine dinucleotide, azacitidine and calcitriol as repurposing drugs for influenza A H5N1 virus-induced lung injury. *PLoS Pathogens*, *16*(3), 1–16.

https://doi.org/10.1371/journal.ppat.1008341

Ibrahim, M. A. A., Abdelrahman, A. H. M., Atia, M. A. M., Mohamed, T. A., Moustafa, M. F., Hakami, A. R., … Hegazy, M. E. F. (2021). Blue Biotechnology: Computational Screening of Sarcophyton Cembranoid Diterpenes for SARS-CoV-2 Main Protease Inhibition. *Marine Drugs*, *19*(7).

Ibrahim, M. A. A., Mohamed, E. A. R., Abdelrahman, A. H. M., Allemailem, K. S., Moustafa, M. F., Shawky, A. M., … Atia, M. A. M. (2021). Rutin and flavone analogs as prospective SARS-CoV-2 main protease inhibitors: In silico drug discovery study. *Journal of Molecular Graphics & Modelling*, *105*.

Irfan, A., Imran, M., Khalid, N., Hussain, R., Basra, M. A. R., Khaliq, T., … Assiri, M. A. (2021). Isolation of phytochemicals from Malva neglecta Wallr and their quantum chemical, molecular docking exploration as active drugs against COVID-19. *Journal of Saudi Chemical Society*, *25*(12).

Istifli, E. S., Netz, P. A., Tepe, A. S., Husunet, M. T., Sarikurkcu, C., & Tepe, B. (2020). In silico analysis of the interactions of certain flavonoids with the receptor-binding domain of 2019 novel coronavirus and cellular proteases and their pharmacokinetic properties. *Journal of Biomolecular Structure & Dynamics*.

Jain, A. S., Sushma, P., Dharmashekar, C., Beelagi, M. S., Prasad, S. K., Shivamallu, C., … Prasad, K. S. (2021). In silico evaluation of flavonoids as effective antiviral agents on the spike glycoprotein of SARS-CoV-2. *Saudi Journal of Biological Sciences*, *28*(1), 1040–1051. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7783825/pdf/main.pdf

Jalmakhanbetova, R. I., Suleimen, Y. M., Oyama, M., Elkaeed, E. B., Eissa, I. H., Suleimen, R. N., … Ishmuratova, M. Y. (2021). Isolation and in Silico Anti-COVID-19 Main Protease (Mpro) Activities of Flavonoids and a Sesquiterpene Lactone from Artemisia sublessingiana. *Journal of Chemistry, 2021*. https://doi.org/10.1155/2021/5547013

Jannat, K., Paul, A. K., Bondhon, T. A., Hasan, A., Nawaz, M., Jahan, R., … Rahmatullah, M. (2021). Nanotechnology Applications of Flavonoids for Viral Diseases. *Pharmaceutics*, *13*(11).

Jiménez-Avalos, G., Vargas-Ruiz, A. P., Delgado-Pease, N. E., Olivos-Ramirez, G. E., Sheen, P., Fernández-Díaz, M., … Ygnacio-Aguirre, F. (2021). Comprehensive virtual screening of 4.8 k flavonoids reveals novel insights into allosteric inhibition of SARS-CoV-2 MPRO. *Scientific Reports*, *11*(1), 1–19. https://doi.org/10.1038/s41598-021-94951-6

Jo, S, Kim, S., Kim, D. Y., Kim, M. S., & Shin, D. H. (2020). Flavonoids with inhibitory activity against SARS-CoV-2 3CLpro. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *35*(1), 1539–1544. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7470085/pdf/IENZ_35_1801672.pdf

Jo, Seri, Kim, S., Shin, D. H., & Kim, M. S. (2020). Inhibition of SARS-CoV 3CL protease by

flavonoids. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *35*(1), 145–151. https://doi.org/10.1080/14756366.2019.1690480

Johns Hopkins. (2022). Covid 19 Map Channelnewsasis. Retrieved from https://coronavirus.jhu.edu/map.html

Johns Hopkins University. (n.d.). COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU).

Karim, S. S. A., & Karim, Q. A. (2021). Omicron SARS-CoV-2 variant: a new chapter in the COVID-19 pandemic. *The Lancet*, *398*(10317), 2126–2128. https://doi.org/10.1016/s0140-6736(21)02758-6

Khursheed, A., Jain, V., Rasool, A., Rather, M. A., Malik, N. A., & Shalla, A. H. (2021). Molecular scaffolds from mother nature as possible lead compounds in drug design and discovery against coronaviruses: A landscape analysis of published literature and molecular docking studies. *Microbial Pathogenesis*, *157*.

Kim, S., Thiessen, P. A., Bolton, E. E., Chen, J., Fu, G., Gindulyte, A., … Bryant, S. H. (2016). PubChem substance and compound databases. *Nucleic Acids Research*, *44*(D1), D1202–D1213. https://doi.org/10.1093/nar/gkv951

Kumar, B., Zaidi, S., Haque, S., Dasgupta, N., Hussain, A., Pramodh, S., … Mishra, B. N. (2021). In Silico Studies Reveal Antiviral Effects of Traditional Indian Spices on COVID-19. *Current Pharmaceutical Design*, *27*(32), 3462–3475. Retrieved from https://www.eurekaselect.net/article/112631

Lee, Y. G., Kang, K. W., Hong, W., Kim, Y. H., Oh, J. T., Park, D. W., … Kang, S. C. (2021). Potent antiviral activity of Agrimonia pilosa, Galla rhois, and their components against SARS-CoV-2. *Bioorganic & Medicinal Chemistry*, *45*.

Li, L. Y., Ma, L. Y., Hu, Y., Li, X. X., Yu, M., Shang, H., & Zou, Z. M. (2022). Natural biflavones are potent inhibitors against SARS-CoV-2 papain-like protease. *Phytochemistry*, *193*.

Liao, Q., Chen, Z. Y., Tao, Y. L., Zhang, B. B., Wu, X. J., Yang, L., … Wang, Z. T. (2021). An integrated method for optimized identification of effective natural inhibitors against SARS-CoV-2 3CLpro. *Scientific Reports*, *11*(1).

Lin, Y., Shi, R., Wang, X., & Shen, H.-M. (2008). Luteolin, a Flavonoid with Potential for Cancer Prevention and Therapy. *Current Cancer Drug Targets*, *8*(7), 634–646. https://doi.org/10.2174/156800908786241050

Liskova, A., Samec, M., Koklesova, L., Samuel, S. M., Zhai, K. V, Al-Ishaq, R. K., … Kubatka, P. (2021). Flavonoids against the SARS-CoV-2 induced inflammatory storm. *Biomedicine & Pharmacotherapy*, *138*.

Ma, L. L., Liu, H. M., Liu, X. M., Yuan, X. Y., Xu, C., Wang, F., … Zhang, D. K. (2021).

Screening S protein-ACE2 blockers from natural products: Strategies and advances in the discovery of potential inhibitors of COVID-19. *European Journal of Medicinal Chemistry*, *226*.

Maddah, M., Bahramsoltani, R., Yekta, N. H., Rahimi, R., Aliabadi, R., & Pourfath, M. (2021). Proposing high-affinity inhibitors from Glycyrrhiza glabra L. against SARS-CoV-2 infection: virtual screening and computational analysis. *New Journal of Chemistry*, *45*(35), 15977–15995.

Maiti, S., & Banerjee, A. (2021). Epigallocatechin gallate and theaflavin gallate interaction inSARS-CoV-2 spike-protein central channel with reference to the hydroxychloroquine interaction: Bioinformatics and molecular docking study. *Drug Development Research*, *82*(1), 86–96. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7436314/pdf/DDR-9999-na.pdf

Majumder, R., & Mandal, M. (2020). Screening of plant-based natural compounds as a potential COVID-19 main protease inhibitor: anin silicodocking and molecular dynamics simulation approach. *Journal of Biomolecular Structure & Dynamics*.

Mandour, Y. M., Zlotos, D. P., & Salem, M. A. (2020). A multi-stage virtual screening of FDA-approved drugs reveals potential inhibitors of SARS-CoV-2 main protease. *Journal of Biomolecular Structure & Dynamics*.

Mangiavacchi, F., Botwina, P., Menichetti, E., Bagnoli, L., Rosati, O., Marini, F., … Santi, C. (2021). Seleno-Functionalization of Quercetin Improves the Non-Covalent Inhibition of M-pro and Its Antiviral Activity in Cells against SARS-CoV-2. *International Journal of Molecular Sciences*, *22*(13).

Maroli, N., Bhasuran, B., Natarajan, J., & Kolandaivel, P. (2020). The potential role of procyanidin as a therapeutic agent against SARS-CoV-2: a text mining, molecular docking and molecular dynamics simulation approach. *Journal of Biomolecular Structure & Dynamics*.

Mathpal, S., Sharma, P., Joshi, T., Joshi, T., Pande, V., & Chandra, S. (2021). Screening of potential bio-molecules from Moringa olifera against SARS-CoV-2 main protease using computational approaches. *Journal of Biomolecular Structure & Dynamics*.

Mohapatra, P. K., Chopdar, K. S., Dash, G. C., Mohanty, A. K., & Raval, M. K. (2021). In silico screening and covalent binding of phytochemicals of Ocimum sanctum against SARS-CoV-2 (COVID 19) main protease. *Journal of Biomolecular Structure & Dynamics*.

Moradkhani, S., Farmani, A., Saidijam, M., & Taherkhani, A. (2021). COVID-19: docking-based virtual screening and molecular dynamics study to identify potential SARS-CoV-2 spike protein inhibitors from plant-based phenolic compounds. *Acta Virologica*, *65*(3), 288–302.

Mosquera-Yuqui, F., Lopez-Guerra, N., & Moncayo-Palacio, E. A. (2020). Targeting the 3CLpro and RdRp of SARS-CoV-2 with phytochemicals from medicinal plants of the Andean Region: molecular docking and molecular dynamics simulations. *Journal of Biomolecular Structure &*

Dynamics.

Neves, K. O. G., Ramos, A. S., Bruginski, E. R. D., Souza, A. D. L., Nunomura, R. D. S., Campos, F. R., … Machado, M. B. (2021). Lisboaeflavanonol A: A new flavonoid glycoside obtained from Amazonian Eugenia lisboae. *Phytochemistry Letters*, *43*, 65–69.

Ngwa, W., Kumar, R., Thompson, D., Lyerly, W., Moore, R., Reid, T., … Toyang, N. (2020). *against COVID-19*. 1–10.

O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., & Hutchison, G. R. (2011). Open Babel. *Journal of Cheminformatics*, *3*(33), 1–14.

Owis, A. I., El-Hawary, M. S., El Amir, D., Aly, O., Abdelmohsen, U. R., & Kamel, M. S. (2020). Molecular docking reveals the potential of Salvadora persica flavonoids to inhibit COVID-19 virus main protease. *Rsc Advances*, *10*(33), 19570–19575.

Owis, A. I., El-Hawary, M. S., El Amir, D., Refaat, H., Alaaeldin, E., Aly, O. M., … Kamel, M. S. (2021). Flavonoids of Salvadora persica L. (meswak) and its liposomal formulation as a potential inhibitor of SARS-CoV-2. *Rsc Advances*, *11*(22), 13537–13544.

Potshangbam, A. M., Nongdam, P., Kumar, A. K., & Rathore, R. S. (2021). Phenylbenzopyrone of Flavonoids as a Potential Scaffold to Prevent SARS-CoV-2 Replication by Inhibiting its M-PRO Main Protease. *Current Pharmaceutical Biotechnology*, *22*(15), 2054–2070. Retrieved from https://www.eurekaselect.net/article/113711

Prasansuklab, A., Theerasri, A., Rangsinth, P., Sillapachaiyaporn, C., Chuchawankul, S., & Tencomnao, T. (2021). Anti-COVID-19 drug candidates: A review on potential biological activities of natural products in the management of new coronavirus infection. *Journal of Traditional and Complementary Medicine*, *11*(2), 144–157.

Puttaswamy, H., Gowtham, H. G., Ojha, M. D., Yadav, A., Choudhir, G., Raguraman, V., … Chauhan, L. (2020). In silico studies evidenced the role of structurally diverse plant secondary metabolites in reducing SARS-CoV-2 pathogenesis. *Scientific Reports*, *10*(1).

Rahman, F., Tabrez, S., Ali, R., Alqahtani, A. S., Ahmed, M. Z., & Rub, A. (2021). Molecular docking analysis of rutin reveals possible inhibition of SARS-CoV-2 vital proteins. *Journal of Traditional and Complementary Medicine*, *11*(2), 173–179.

Rakshit, M., Muduli, S., Srivastav, P. P., & Mishra, S. (2021). Pomegranate peel polyphenols prophylaxis against SARS-CoV-2 main protease by in-silico docking and molecular dynamics study. *Journal of Biomolecular Structure & Dynamics*.

Rameshkumar, M. R., Indu, P., Arunagirinathan, N., Venkatadri, B., El-Serehy, H. A., & Ahmad, A. (2021). Computational selection of flavonoid compounds as inhibitors against SARS-CoV-2 main protease, RNA-dependent RNA polymerase and spike proteins: A molecular docking study. *Saudi Journal of Biological Sciences*, *28*(1), 448–458. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7581406/pdf/main.pdf

Rehman, M. T., AlAjmi, M. F., & Hussain, A. (2021). Natural Compounds as Inhibitors of SARS-CoV-2 Main Protease (3CLpro): A Mo-lecular Docking and Simulation Approach to Combat COVID-19. *Current Pharmaceutical Design*, *27*(33), 3577–3589. Retrieved from https://www.eurekaselect.net/article/111556

Rizzuti, B., Grande, F., Conforti, F., Jimenez-Alesanco, A., Ceballos-Laita, L., Ortega-Alarcon, D., … Velazquez-Campoy, A. (2021). Rutin Is a Low Micromolar Inhibitor of SARS-CoV-2 Main Protease 3CLpro: Implications for Drug Design of Quercetin Analogs. *Biomedicines*, *9*(4).

Rottier, P. J. M. (1995). The Coronavirus Membrane Glycoprotein. *The Coronaviridae*, 115–139. https://doi.org/10.1007/978-1-4899-1531-3_6

Rudrapal, M., Issahaku, A. R., Agoni, C., Bendale, A. R., Nagar, A., Soliman, M. E. S., & Lokwani, D. (2021). In silico screening of phytopolyphenolics for the identification of bioactive compounds as novel protease inhibitors effective against SARS-CoV-2. *Journal of Biomolecular Structure & Dynamics*.

Samy, M. N., Attia, E. Z., Shoman, M. E., Khalil, H. E., Sugimoto, S., Matsunami, K., & Fahim, J. R. (2021). Phytochemical investigation of Amphilophium paniculatum; an underexploredBignoniaceae species as a source of SARS-CoV-2 M-pro inhibitory metabolites: Isolation, identification, and molecular docking study. *South African Journal of Botany*, *141*, 421–430.

Sen, D., Bhaumik, S., Debnath, P., & Debnath, S. (2021). Potentiality of Moringa oleifera against SARS-CoV-2: identified by a rational computer aided drug design method. *Journal of Biomolecular Structure & Dynamics*.

Shaldam, M. A., Yahya, G., Mohamed, N. H., Abdel-Daim, M. M., & Al Naggar, Y. (2021). In silico screening of potent bioactive compounds from honeybee products against COVID-19 target enzymes. *Environmental Science and Pollution Research*, *28*(30), 40507–40514. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8088405/pdf/11356_2021_Article_14195.pdf

Shu, Y., & McCauley, J. (2017). GISAID: Global initiative on sharing all influenza data – from vision to reality. *Eurosurveillance*, *22*(13), 2–4. https://doi.org/10.2807/1560-7917.ES.2017.22.13.30494

Singh, J., Malik, D., & Raina, A. (2020). Computational investigation for identification of potential phytochemicals and antiviral drugs as potential inhibitors for RNA-dependent RNA polymerase of COVID-19. *Journal of Biomolecular Structure & Dynamics*.

Singh, A. V. (2021). Potential of amentoflavone with antiviral properties in COVID-19 treatment. *Asian Biomedicine*, *15*(4), 153–159.

Song, X., Tan, L., Wang, M., Ren, C., Guo, C., Yang, B., … Pei, J. (2021). Myricetin: A review of the most recent research. *Biomedicine and Pharmacotherapy*, *134*, 111017. https://doi.org/10.1016/j.biopha.2020.111017

Tyrrell, D. A., & Bynoe, M. L. (1966). Cultivation of viruses from a high proportion of patients with colds. https://doi.org/10.1016/s0140-6736(66)92364-6

Vijayakumar, B. G., Ramesh, D., Joji, A., Prakasan, J. J., & Kannan, T. (2020). In silico pharmacokinetic and molecular docking studies of natural flavonoids and synthetic indole chalcones against essential proteins of SARS-CoV-2. *European Journal of Pharmacology, 886*.

Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J., … Peng, Z. (2020). Clinical Characteristics of 138 Hospitalized Patients with 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA - Journal of the American Medical Association, 323*(11), 1061–1069. https://doi.org/10.1001/jama.2020.1585

Wang, J., Ge, W., Peng, X., Yuan, L. X., He, S. B., & Fu, X. Y. (2021). Investigating the active compounds and mechanism of HuaShi XuanFei formula for prevention and treatment of COVID-19 based on network pharmacology and molecular docking analysis. *Molecular Diversity*.

Xiao, T., Cui, M. Q., Zheng, C. J., Wang, M., Sun, R. H., Gao, D. D., … Zhou, H. G. (2021). Myricetin Inhibits SARS-CoV-2 Viral Replication by Targeting M-pro and Ameliorates Pulmonary Inflammation. *Frontiers in Pharmacology, 12*.

Xiao, T., Cui, M. Q., Zheng, C. J., Zhang, P. P., Ren, S. F., Bao, J. L., … Yang, C. (2021). Both Baicalein and Gallocatechin Gallate Effectively Inhibit SARS-CoV-2 Replication by Targeting M-pro and Sepsis in Mice. *Inflammation*.

Xiong, Y., Zhu, G. H., Zhang, Y. N., Hu, Q., Wang, H. N., Yu, H. N., … Ge, G. B. (2021). Flavonoids in Ampelopsis grossedentata as covalent inhibitors of SARS-CoV-2 3CL(pro): Inhibition potentials, covalent binding sites and inhibitory mechanisms. *International Journal of Biological Macromolecules, 187*, 976–987. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8322037/pdf/main.pdf

Xu, Z. R., Yang, L. X., Zhang, X. H., Zhang, Q. L., Yang, Z. B., Liu, Y. H., … Liu, W. K. (2020). Discovery of Potential Flavonoid Inhibitors Against COVID-19 3CL Proteinase Based on Virtual Screening Strategy. *Frontiers in Molecular Biosciences, 7*.

Xu, Z., Yang, L., Zhang, X., Zhang, Q., Yang, Z., Liu, Y., … Liu, W. (2020). Discovery of Potential Flavonoid Inhibitors Against COVID-19 3CL Proteinase Based on Virtual Screening Strategy. *Frontiers in Molecular Biosciences, 7*(September), 1–8. https://doi.org/10.3389/fmolb.2020.556481

Yosri, N., Abd El-Wahed, A. A., Ghonaim, R., Khattab, O. M., Sabry, A., Ibrahim, M. A. A., … El-Seedi, H. R. (2021). Anti-Viral and Immunomodulatory Properties of Propolis: Chemical Diversity, Pharmacological Properties, Preclinical and Clinical Applications, and In Silico Potential against SARS-CoV-2. *Foods, 10*(8).

Yu, R., Chen, L., Lan, R., Shen, R., & Li, P. (2020). Computational screening of antagonists against

the SARS-CoV-2 (COVID-19) coronavirus by molecular docking. *International Journal of Antimicrobial Agents*, *56*(2).

Zaki, A. A., Al-Karmalawy, A. A., El-Amier, Y. A., & Ashour, A. (2020). Molecular docking reveals the potential of Cleome amblyocarpa isolated compounds to inhibit COVID-19 virus main protease. *New Journal of Chemistry*, *44*(39), 16752–16758.

Zhang, Y., Yao, Y. F., Yang, Y. F., & Wu, H. Z. (2021). Investigation of Anti-SARS, MERS, and COVID-19 Effect of Jinhua Qinggan Granules Based on a Network Pharmacology and Molecular Docking Approach. *Natural Product Communications*, *16*(5).

Zhou, P., Yang, X. Lou, Wang, X. G., Hu, B., Zhang, L., Zhang, W., … Shi, Z. L. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*, *579*(7798), 270–273. https://doi.org/10.1038/s41586-020-2012-7

Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., … Tan, W. (2020). A Novel Coronavirus from Patients with Pneumonia in China, 2019. *New England Journal of Medicine*, *382*(8), 727–733. https://doi.org/10.1056/nejmoa2001017

Zhu, Y., & Xie, D. Y. (2020). Docking Characterization and in vitro Inhibitory Activity of Flavan-3-ols and Dimeric Proanthocyanidins Against the Main Protease Activity of SARS-Cov-2. *Frontiers in Plant Science*, *11*.