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Exploiting In Silico Methods to Determine the Anti-viral Activities of some Phytochemicals against the SARS-CoV-2

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Abstract

SARS-CoV-2 virus which has become a serious health concern, is a highly transmittable and pathogenic virus. Though several drugs like chloroquine, arbidol, remdesivir, and favipiravir are showing positive results in clinical trials, there is still no specific drug identified to fight against this virus. This paper is mainly focused on this to find a novel drug using different tools of bioinformatics and computational biology like molecular docking, molecular dynamics simulation, PASS prediction, P450 site of metabolism prediction, drug likeness properties analysis and ADMET analysis. Through these prediction tools, Quercetin was identified as a promising candidate to inhibit the SARS-CoV-2 virus. Apart from this compound's high binding score and high molecular stability with viral receptors, it also showed good solubility, pharmacodynamics properties and drug likeness properties. This study contributes to search new drugs against this virus and help researcher in designing specific drug for the treatment of COVID-19 patients.

Keywords: SARS-CoV-2; COVID-19; Phytochemicals; Molecular Dynamics Simulation; Anti- viral agents

INTRODUCTION

The newest coronavirus strain, SARS-CoV-2 has created a pandemic threat to the world. People infected with this virus can transmit it without showing specific symptoms. The virus can stay undetected in the body for several days and even weeks because its genome expresses proteins that delay the immune system from sending caution (Fischetti et al.,2020). COVID-19 patients show different symptoms like fever, cough, fatigue, headache, haemoptysis, acute cardiac injury, hypoxemia, dyspnoea, diarrhea. However, it also shows some unique clinical features involving lower airway that is conspicuous in respiratory symptoms like rhinorrhoea, sneezing, and sore throat (Rothan et al.,2020).

Coronavirus are positive-sense single stranded RNA viruses of Coronaviridae family. SARS-CoV- 2 is a sphere of protein with a diameter of 100 nm. The SARS-CoV-2 genome is about 29,900 bases long that surpass the genome size of influenza (13,500 bases) and rhinoviruses (8,000 bases). The genome of this virus is so long that it can produce plethora of protein enabling it to carry a lot of information and perhaps some sophisticated replication (Fischetti et al.,2020).

The first step of viral infection is to bind to receptor expressed within the host cell followed by fusion. Lung epithelial cells are the main targets of SARS-CoV-2 virus. ACE2 (angiotensin- converting enzyme 2) has been identified as the binding domain for spike protein of the virus (Rothan et al.,2020). When SARS-CoV-2 particle enters human body, it floats in airway until attaching to ACE2 receptor of lung. The virus uses host's machinery to increase its population. After binding to ACE2 receptor, virus-lung cell fusion creates channel to facilitate the entry of N proteins and RNA into lung cell. Inside the cell, this virus creates vesicle around it stretching out hosts endoplasmic reticulum and replicates with its polymerase, making viral protein like spike proteins. The newly formed viruses leaving from cell may go back into air or get killed by immune response. After being affected, both innate and adaptive immune system gets activated to handle the situation. SARS-CoV-2 uses several strategies to evade our immune system (Fischetti et al., 2020, Islam et al., 2020).

Different labs and universities around the world are searching for a potential drug to fight against this virus. Though maximum of these drugs may not destroy the whole virus, antiviral drugs or vaccines may interfere with the viral attachment or prepare immune response for future virus attack respectively (Fischetti et al.,2020).

Again, still now no vaccine is available in the market which can effectively fight the COVID-19 (Sarkar et al., 2020, Ullah et al., 2020a). Several targets are identified to make drugs or vaccines against this virus. For replication process of many of positive stranded RNA viruses, proteolytic processing of viral polyproteins is a major step. The replicase gene incorporates the sequence motifs of both papain-like protease and 3-chymotrypsin like protease. Their vital role in the activities of replication complexes has made them one of the main targets of drug discovery. RdRp is the RNA polymerase that helps the virus in translational process and have been used as potential target. Similarly, viral S protein is also being used as target for drug discovery. This virus can infect the individuals through interaction between human ACE2 receptor and Spike protein. The process of viral cell fusion involves binding between ACE2 receptor and spike protein and proteolytic processing of spike proteins (Liu et al,.2020).

As SARS-CoV-2 is a newer pathogen, there have been no definite drugs. However, scientists are considering existing drugs to treat COVID-19 patient (Liu et al,.2020). Among the existing drugs, Remdesivir is vital candidate that had been produced for the treatment of Ebola virus. It helps to block RNA production mechanism of the virus by evading viral exonuclease activity. There are two similar drugs Hydroxychloroquine and Chloroquine which are commonly used in treating lupus erythematosus, rheumatoid arthritis, and malaria. Chloroquine is suggestive to treat patient in the early stage of infection as it blocks fusion of virus cell and ACE2 receptor. Hydroxychloroquine maintain similar activity with less toxicity for having hydroxyl group. Effect of combining Chloroquine and zinc is positive. The combined drug Lopinavir-Ritonavir is being used as approved drug for HIV infection by inhibiting HIV-1 protease. Lopinavir acts as inhibitor that may inhibit the protease (3CLpro or PLpro). Umifenovir had been associated with prophylaxis and h influenza A and B treatment. Now it has been used as antiviral agent in the treatment of Ebola virus, human herpesvirus 8 (HHV-8), hepatitis C virus (HCV), and Tacaribe arenavirus. Several reports predicted its efficacy against the SARS-CoV-2. Favipiravir is another drug which had been used for treatment of avian influenza or novel influenza. It is a purine nucleoside that acts as an alternate substrate leading to inaccurate viral RNA synthesis. Recent study also suggested its validity to work against SARS-CoV-2 (Wu et al.,2020). Arbidol can treat the patient with its inhibitory activity against S protein that may block viral entry into host cells (Liu et al,.2020).

There is still no drug that directly targets SARS-CoV-2. Different antiviral drugs are being tested to examine their efficacy against this virus. So at this point, the concept of repurposing of drugs is going on to find a specific treatment. However, many drugs are under clinical trial to be viable. Because of this interest, this paper incorporates an in silico approach to find novel drug for this virus. In this study, we selected 20 phytochemicals for their antiviral activity. Two vital protease (PDB ID: 5r80, 6wcf) were selected because of their protease activity in the virus. Two screening methods were applied, Autodock-vina and Maestro Schrödinger suite, to assess the binding affinity and best interaction between the phytochemicals and proteins. Likewise, the phytochemicals were subjected to PASS and P450 Site of Metabolism (SOM) analysis to find their beneficial biological activities and possible sites of metabolism respectively. Furthermore, ligands were tested to Drug- Likeness and AdmetSAR analysis to examine their ADMET profiles. Then the best phytochemical with receptor complex were subjected to molecular dynamics (MD) simulations to test the thermodynamic properties.

SUBJECTS AND METHODS

Molecular docking

Initial Molecular Docking in Autodock-vina: The molecular docking of two receptor protein and 20 ligands were performed by Autodock-vina software (AutoDock 4.2). The crystal structures of two SARS-CoV-2 proteins i.e., the non- structural protein ADP ribose phosphatase of NSP-3 from SARS-CoV-2, in complex with MES (PDB ID: 6WCF) and SARS-CoV-2 main protease in complex with Z18197050 (PDB ID: 5R80), were retrieved from Protein Data Bank (https://www.rcsb.org/search). The ligands were retrieved from Pubchem (https://pubchem.ncbi.nlm.nih.gov/). At first, the structures were prepared using PyMOL tools (PyMOL) by removing the water molecules from the structure and then minimizing the structure using Swiss-PdbViewer(Guex, N. and Peitsch, M.C. et al ,1997). After that, for the Autodock-vina analysis, a grid box was made using active site predictor tool SCFBio (http://scfbio-iitd.res.in:8080/dock/ActiveSite.jsp) and PrankWeb server (http://prankweb.cz/). All docking calculation was executed using the Lamarckian Genetic Algorithm method with the spacing of 1 Å and the exhaustiveness of 10. All the other parameters were kept default. The ligands with docking score or bond energy with -7.5 kcal/mol or less than -7.5 kcal/mol were selected for MM-GBSA and IFD analysis.

MM-GBSA and IFD analysis: After the successful docking of all the ligands with their receptors, the selected ligands from the previous docking step were subjected to the molecular mechanics – generalized born

and surface area (MM-GBSA) study, where rescoring was conducted with the help of Prime module of Maestro Schrödinger suite (Prime, Schrödinger, 2018). For the MM-GBSA analysis, the two proteins were prepared using the Protein Preparation Wizard in the Maestro Schrödinger Suite 2018-4 (Schrödinger, 2018). During protein preparation, the bond orders were assigned and hydrogen molecules were added to heavy atoms as well as all the waters were deleted and the side chains were adjusted using Prime (Prime, Schrödinger, 2018). After that, the structure was optimized and minimized using force field OPLS_2005, which was conducted setting the maximum heavy atom RMSD (root-mean-square-deviation) to 30 Å and any remaining water less than 3 H- bonds to non-water was again deleted during the minimization step. Three dimensional structures of the selected ligand molecules were downloaded from the PubChem database (www.pubchem.ncbi.nlm.nih.gov). These structures were then prepared for MM-GBSA study using the LigPrep module of Maestro Schrödinger Suite (LigPrep, Schrödinger, 2018). Finally, after the MM-GBSA analysis, to carry out the Induced Fit Docking (IFD) study of the selected ligand molecules, again OPLS_2005 force field was applied after generating grid around the co-crystallized ligand of the receptor and Ligand Van Der Waals screening was set at 0.70 and 0.50 respectively. Three best ligands were selected from the MM-GBSA and IFD studies based on their lower MM-GBSA score, IFD score and XPGscore scores and the later analyses were conducted only on these three ligands.

PASS and P450 Site of Metabolism (SOM) prediction

The PASS (Prediction of Activity Spectra for Substances) prediction of the three selected ligands was carried out by the PASS-Way2Drug server (http://www.pharmaexpert.ru/passonline/) (Filimonov et al., 2014). While carrying out PASS prediction, the Pa (probability to be active) was kept greater than 70%, since the Pa > 70% threshold generates highly reliable prediction (Geronikaki et al., 1999). In the PASS prediction study, 15 possible biological activities were predicted. The P450 Site of Metabolism (SOM) of the three best selected ligand molecules were determined by another online tool, RS-WebPredictor 1.0 (http://reccr.chem.rpi.edu/Software/RS- WebPredictor/) (Zaretzki et al., 2012).

Drug-likeness properties determination and ADMET analysis

Determination of the drug-likeness properties and ADMET analysis are two of the most important steps of in silico drug designing (Ullah et al., 2020). To predict drug-likeness properties of the three selected ligands from the previous docking step, the online tool, SwissADME (http://www.swissadme.ch/index.php) was used. Thereafter, another online tool, admetSAR (http://lmmd.ecust.edu.cn/admetsar2/) was utilized to predict the ADMET properties of the three selected ligand molecules. In both of these predictions, the canonical smiles of the ligands were used, which were obtained from the PubChem server (https://pubchem.ncbi.nlm.nih.gov/). The best ligand was selected from the three ligands according to their performances in the drug-likeness property analysis and ADMET test.

Molecular dynamics simulation

Finally, the best ligand was subjected to the molecular dynamics simulation study (MD study) against both the proteins, using the NAMD 2.8 dynamics simulation program (Phillips et al.,2005), taking the CHARMM 27 as force field (MacKerell et al.,1998). The complex was simulated using NAMD graphical interface embedded in VMD (visual molecular dynamics) (Humphrey et al.,1996). Molecular dynamics simulation was run in NVT ensemble for 1 ns followed by the NPT ensemble run for 70 ps (Martyna et al.,1994). Constant temperature of 310 k was kept by using a Langevin thermostat and constant pressure of 1.01325 bar was contained by an isotropic Langevin barostat. Periodic boundary conditions and time-step of 1 femto second were used. All the other parameters were kept default.

RESULTS

Molecular docking analysis

20 phytochemicals were initially docked using Autodock-vina tools. Best 10 ligands with score of -7.5 or less were chosen out of 20 ligands to run MM-GBSA and IFD analysis. Out of 10 ligands, 3 ligands were ranked based on lowest docking score of MM-GBSA and IFD analysis. Hesperidin, Myricetin, Quercetin were considered better candidate for COVID-19 target (PDB ID: 5R80 and 6WCF). Their binding pocket, interaction and interacting H-bonds were shown in Figure 1. Comparative analysis of this study was listed in Table 1 and Table 2.

PASS and P450 Site of Metabolism (SOM) prediction

15 biological activities were compared for our three selected phytochemicals Hesperidin, Myricetin, Quercetin. Three of them showed results under standard range. The results of PASS prediction experiment of three selected compounds were given in Table 3.

Table 4 depicted the possible sites of P450 metabolism. Possible metabolic sites of CYP (1A2, 2A6, 2B6, 2C19, 2C8, 2C9, 2D6, 2E1 and 3A4) of Hesperidin, Myricetin, Quercetin were identified. The metabolism sites were indicated by circle on the structure (Table 4).

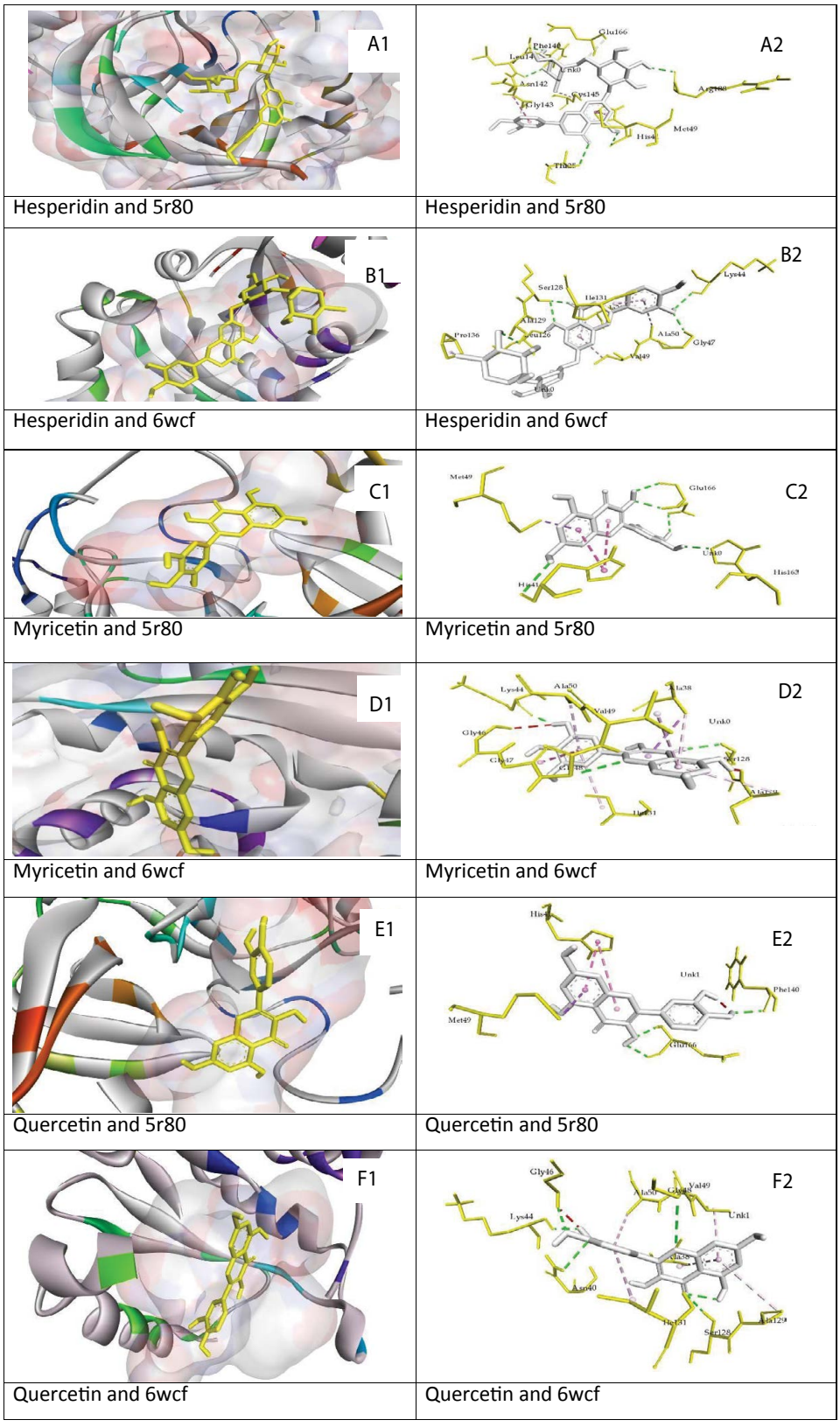


Figure 1. (A1, B1, C1, D1, E1, F1) Docked ligand in the pocket of receptor. The surface of the protein is in white and ligand is in yellow color. (A2, B2, C2, D2, E2, F2) interaction between ligand and interacting part of protein. The ligand is in white, interacting protein residue in yellow and hydrogen bond in green color.

Table 1. The results of AutoDock-Vina binding energy between 20 phytochemicals and two proteins (PDB ID: 5R80 and 6WCF), along with H-bond analysis

Antiviral phytochemical	AutoDock-Vina binding energy (Kcal/mol)		Number Of H-bonds		H-bonds lowest distance (A˚)	
-	5r80	6wcf	5r80	6wcf	5r80	6wcf
Hesperidin	-8.9	-9.6	7	11	2.16	1.94
Myricetin	-7.9	-8.6	5	4	2.18	1.81
Quercetin	-7.8	-8.6	3	7	2.12	2.07
Angelicin	-6.2	-7.4	1	4	2.60	2.06
Apigenin	-7.3	-8.3	4	3	2.06	2.18
Atropine	-6.6	-7.6	2	3	2.18	2.21
Camptothecin	-8.5	-9.2	6	2	2.11	2.44
Fisetin	-7.5	-8.9	3	6	2.01	2.21
Harman	-6.2	-7.3	1	6	2.99	2.21
Harmine	-6.6	-7.7	5	4	2.54	2.19
Harmol	-6.6	-7.5	2	3	2.44	2.22
Luteolin	-7.5	-8.9	2	8	2.34	2.15
Lycoricidine	-7.8	-8.7	4	7	2.29	2.17
Lycoricidinol	-7.5	-8.7	5	7	2.02	2.34
maslinic acid	-7.6	-8.1	4	1	2.20	2.74
Moronic acid	-7.9	-8.1	2	none	2.16	none
Naringenin	-7.3	-8.3	3	6	1.84	2.21
ursolic acid	-7.6	-7.6	2	2	2.49	2.15
Baicalin	-8.3	-9	4	9	1.95	1.83
berberine	-7.7	-9	5	2	2.63	2.79

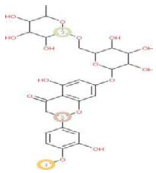
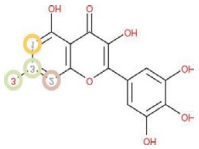
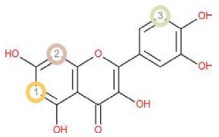
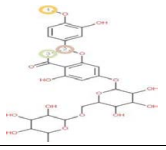
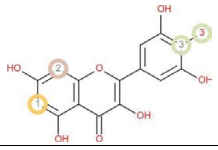
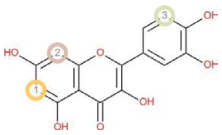
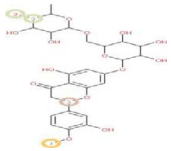
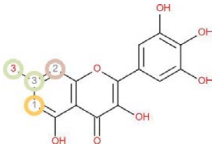
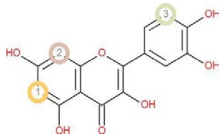
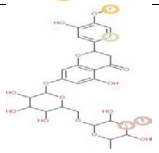
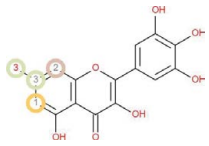
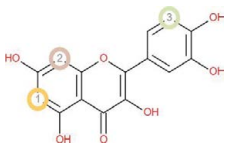
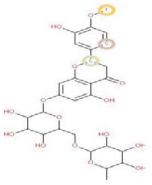
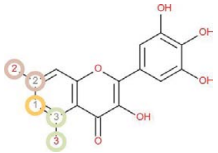
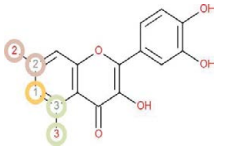
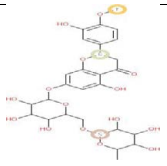
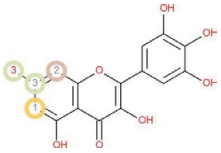
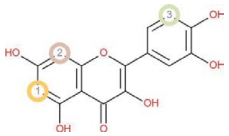
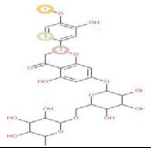
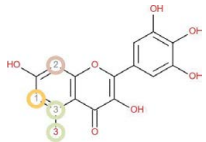
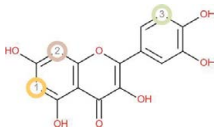
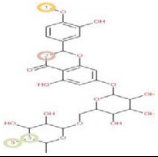
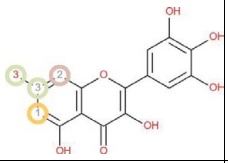
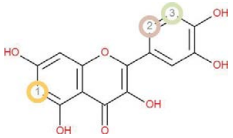
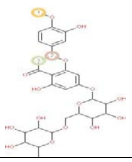
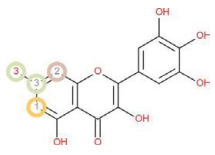
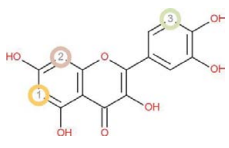
Table 2. The results of MM-GBSA and IFD analysis between 10 phytochemicals selected from the previous step and two proteins (PDB ID: 5R80 and 6WCF)

Name of the ligand	MM-GBSA ΔGbind score (in kcal/mol)		XP Gscore (in kcal/mol)		IFD score (in kcal/mol)	
-	5R80	6WCF	5R80	6WCF	5R80	6WCF
Fisetin	-41.20	-51.28	-11.64	-10.65	-1152.58	-1176.97
Hesperidin	-54.23	57.89	-14.39	-13.45	-1176.58	-1183.20
Lycoricidine	-38.93	-46.72	-9.29	-10.39	-1131.28	-1145.22
maslinic acid	-51.62	-53.25	-11.35	-9.19	-1172.71	-1171.32
Moronic acid	-49.71	-58.61	-12.10	-10.34	-1174.44	-1155.67
Myricetin	-61.56	-62.34	-14.13	-12.90	-1189.34	-1176.37
Ursolic acid	-36.41	-41.51	-8.93	-8.42	-1141.37	-1150.91
Baicalin	-32.90	-39.99	-9.91	-9.63	-1138.99	-1131.39
Berberine	-46.83	-47.85	-10.56	-11.57	-1132.93	-1165.82
Quercetin	-57.09	-60.36	-12.85	-13.11	-1169.22	-1178.19

Table 3. The PASS prediction results showing the 15 biological activities of the best three phytochemicals

Sl no	Biological activities	Hesperidin		Myricetin		Quercetin	
		Pa	Pi	Pa	Pi	Pa	Pi
01	Lipid peroxidase inhibitor	0.991	0.001	0.836	0.003	0.788	0.004
02	Antioxidant	0.846	0.003	0.924	0.003	0.872	0.003
03	Chemopreventive	0.975	0.001	0.734	0.005	0.717	0.006
04	Free radical scavenger	0.989	0.001	0.832	0.002	0.811	0.003
05	Anticarcinogenic	0.982	0.001	0.784	0.006	0.757	0.007
06	UDP-glucuronosyltransfer ase substrate	0.978	0.002	0.861	0.004	0.857	0.004
07	Hepatoprotectant	0.977	0.001	0.737	0.006	0.706	0.007
08	Membrane permeability inhibitor	0.976	0.001	0.959	0.002	0.938	0.003
09	Vasoprotector	0.974	0.001	0.800	0.005	0.824	0.004
10	Hemostatic	0.963	0.001	0.899	0.002	0.771	0.003
11	Membrane integrity agonist	0.960	0.003	0.968	0.002	0.973	0.002
12	Cardioprotectant	0.946	0.002	0.886	0.003	0.883	0.003
13	Monophenol monooxygenase inhibitor	0.965	0.001	0.803	0.003	0.792	0.003
14	Histamine release stimulant	0.906	0.001	0.713	0.004	0.751	0.003
15	Iodide peroxidase inhibitor	0.915	0.001	0.903	0.001	0.891	0.001

Table 4. The P450 Site of Metabolism (SOM) prediction results of the best three phytochemicals

Names of P450 iso-enzymes	Hesperidin	Myricetin	Quercetin
1A2			
2A6			
2B6			
2C8			
2C9			
2C19			
2D6			
2E1			
3A4			

Drug-likeness and ADMET prediction

Drug-Likeness of our 3 compounds were compared following Lipinski's rule of 5. Among them Hesperidin violated 3 of the them, Myricetin violateed 1 of them and only Quercetin completely followed the Lipinski's rule of five. We also examined our 3 compounds to determine the Ghose, Veber, Egan, Muegge rules. Hesperidin violated all of the 4 rules and Myricetin followed only Ghose's rule. However, Quercetin obeyed all of them. In case of Quercetin, number of rotatable bonds, TPSA, Bioavailability scores were in the standard range (Table 5).

Table 6 depicted the relative ADMET profiles of screened ligand compounds. Pharmacokinetic data predicted that three of them have high Gastrointestinal absorption (GI) value and BBB value under ideal range. Three of them did not inhibit P-glycoprotein; Hesperidin, Quercetin acted as CYP3A4 substrate; three of them did not inhibit CYP2C9, CYP2C19, CYP2D6 cytochromes; did not show Carcinogenicity. Therefore, both Hesperidin and Quercetin qualified maximum criteria.

Molecular dynamics simulation

To determine the stability of docking complexes, these structures were subjected to molecular dynamics

Table 5. The Drug-Likeness properties of the best three phytochemicals

Drug Likeness Properties	Hesperidin	Myricetin	Quercetin
Molecular weight	610.56 g/mol	318.24 g/mol	302.24 g/mol
Concensus Log $P_{O/w}$	-0.72	0.79	1.23
Log *S*	-3.28	-3.96	-3.16
Num. H-bond acceptors	15	8	7
Num. H-bond donors	8	6	5
Molar Refractivity	141.41	80.06	78.03
Lipinski	No; 3 violations: MW>500, NorO>10, NHorOH>5	Yes; 1 violation: NHorOH>5	Yes; 0 violation
Ghose	No; 4 violations: MW>480, WLOGP<- 0.4, MR>130, #atoms>70	Yes	Yes
Veber	No; 1 violation: TPSA>140	No; 1 violation: TPSA>140	Yes
Egan	No; 1 violation: TPSA>131.6	No; 1 violation: TPSA>131.6	Yes
Muegge	No; 4 violations: MW>600, TPSA>150, H-acc>10, H-don>5	No; 2 violations: TPSA>150, H-don>5	Yes
Bioavailability score	0.17	0.55	0.55
TPSA (Å²)	234.29 Å²	151.59 Å²	131.36 Å²
No of rotatable bonds	7	1	1

Table 6. ADMET Prediction of the best three ligands

Properties	Hesperidin	Myricetin	Quercetin
Human Intestinal Absorption	positive(0.8161)	positive(0.9833)	positive(0.9833)
Blood Brain Barrier	negative(0.9570)	negative(0.4632)	negative(0.4632)
Caco-2	negative(0.8816)	negative(0.7367)	negative(0.6417)
Human oral bioavailability	negative(0.8286)	negative(0.5143)	negative (0.5429)
Subcellular localization	Mitochondria(0.6902)	Mitochondria(0.5892)	Mitochondria(0.5892)
P-glycoprotein inhibitor	negative(0.9166)	negative(0.9166)	negative(0.9191)
P-glycoprotein substrate	negative(0.5059)	negative(0.7944)	negative(0.8360)
CYP3A4 substrate	positive(0.6304)	negative(0.5148)	positive(0.5564)
CYP2C9 substrate	negative(1.0000)	negative(1.0000)	negative(1.0000)
CYP2D6 substrate	negative(0.8428)	negative(0.8553)	negative(0.8553)
CYP3A4 inhibition	negative(0.8619)	positive(0.6951)	positive(0.6951)
CYP2C9 inhibition	negative(0.9071)	negative(0.5823)	negative(0.5823)
CYP2C19 inhibition	negative(0.9025)	negative(0.9025)	negative(0.9025)
CYP2D6 inhibition	negative(0.9231)	negative(0.9287)	negative(0.9287)
CYP1A2 inhibition	negative(0.9045)	positive(0.9106)	positive(0.9106)
Ames mutagenesis	negative(0.6600)	positive(0.5300)	positive(0.9000)
Hepatotoxicity	positive(0.6500)	positive(0.6750)	positive(0.7500)
Carcinogenicity (binary)	negative(0.9714)	Negative(1.0000)	negative(1.0000)

simulation. In this process, the whole system was performed at 310 k and 1.01325 bar. There was no temperature fluctuation in this process for both complexes (Figure 2A,B). The RMSD value for 5R80-Quercetin complex was predicted around 1.5 Å (Figure 2C) and 0.8 Å for 6WCF-Quercetin complex(Figure 2D). The RMSF fluctuation was predicted approximately from 1.4 Å to 0.4 Å for 5R80-Quercetin complex(Figure 2E) and approximately 1 Å to 0.4 Å for 6WCF- Quercetin complex(Figure 2F). This results showed the stability of protein-ligand complex.

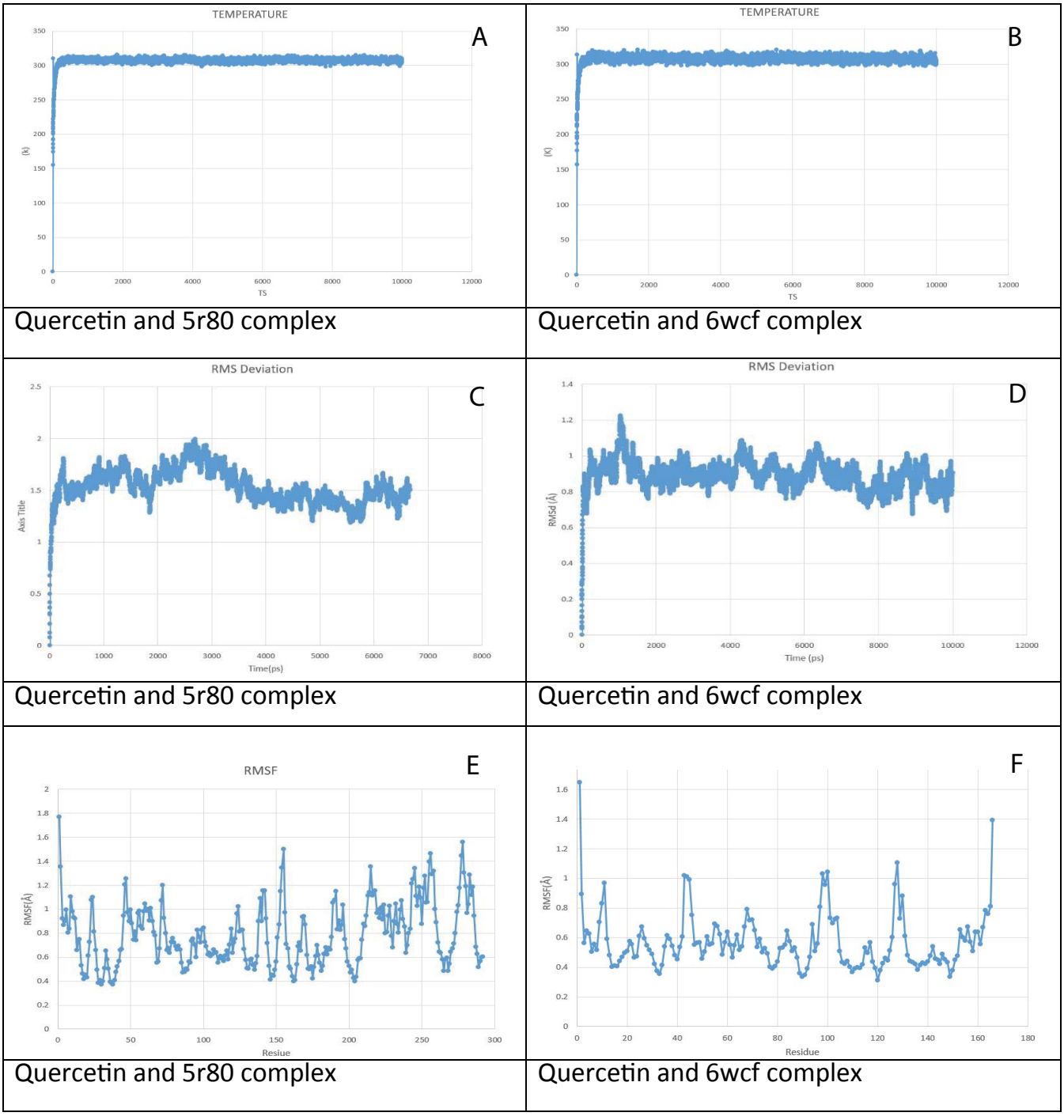


Figure 2. Molecular dynamics simulation: (A, B) showing temperature fluctuation for 5R80- Quercetin complex and 6WCF- Quercetin complex respectively; (C, D) showing RMS deviatio.

DISCUSSION

The identification of 5r80 as 3C-like protease and 6wcf as papain-like proteinase as potential targets for finding antiviral phytochemicals against SARS-CoV-2 was because of their vital roles in viral functionality. The reason to choose 3C-like protease is that it inhibits auto-cleavage process obstructing viral replication and reducing cytopathic effects of the host (Chuck et al., 2011). Next protein, papain-like proteinase was chosen because of its vital role in replication process (Barretto et al., 2005). So, targeting these two proteins should be quite effective in combating the virus.

At first, 10 best ligands were chosen from 20 ligands by autodock-vina, taking lowest binding score on consideration (Trott et al.,2010). Then through MM-GBSA and IFD analysis, Hesperidin, Myricetin, Quercetin were identified as the best three ligands. Quercetin had the third lowest IFD score for both recptors and second lowest MM-GBSA score. Myricetin had the lowest MM-GBSA score for both receptors and Hesperidin had lowest XP Gscore and IFD score. For this, three of the ligands were chosen as the best candidate to inhibit both receptor proteins of SARS COVID-19.

The PASS computer tool was used to calculate the probable biological activities related to drug- like compounds. Two parameters Pa expresses probability of to be “active” and Pi expresses the probability of to be “inactive” (Filimonov et al.,2014). All of three ligands were run in pass prediction tool keeping Pa>7 threshold. They showed fifteen beneficial biological activities.

Cytochrome P450(CYP) plays vital role in drug metabolism. The oxidation phase of drug metabolism is catalyzed by the CYP system. Cytochrome P450 are differentiated by several isoform or individual isoform (McDonnell et al.,2013). Among them nine of the isoforms are widespread and we tested them on online tool RS-WebPredictor. Hesperidin showed three sites of metabolism for 1A2, 2A6, 2C9, 2C19, 2D6, 3A4 and four for 2B6, 2C8, 2E1. Myricetin showed four sites of metabolism for 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4. Quercetin showed three sites of metabolism for 1A2, 2A6, 2B6, 2C8, 2C9, 2D6, 2E1, 3A4 and four sites for 2C19. Therefore, three of them performed well in this assessment. Lipinski’s Rule of 5 was used to predict the drugability of compounds. In drug discovery system, this rule states: molecular weight of compound < 500 g/mol, number of H-bond donors ≤ 5, number of H-bond acceptors ≤ 10, molar refractivity = 40-130, lipophilicity < 5 (Benet et al.,2017). Quercetin obeyed five of them. However, Myricetin violated one of the rules and Hesperidin violated three of them. Hesperidin also violated four of Ghose and Muegge filter and one parameter of Veber and Egan filter. On the other hand, Quercetin violated none of them. Quercetin also had TPSA and bioavailability score which was 131.36 $Å^2$ and 0.55 respectively. However, Hesperidin violated both criteria and Myricetin one of the criteria. According to this examination Quercetin seemed better candidate as drug-like compound.

HIA (Human intestinal absorption) is one of the key elements of ADME analysis. It plays significant role during drugs transporting to targets and influences bioavailability of the compounds (Yan et al.,2008). Blood-brain barrier (BBB) separates the central nervous system (CNS) from the peripheral tissues to control transfer of material, nutrients from the blood to the brain and from the brain to the blood. It blocks entry of toxins, metabolites and other cellular impurities in CNS (Małkiewicz et al.,2019, Prottoy et al., 2019; Sarkar et al., 2019). Caco-2 cell monolayer model is used in predicting the in vitro human intestinal permeability of a drug and for this, its permeability shows the extent of absorption of drug in intestine. (Wang et al.,2019). P- glycoprotein (P-gp), an efflux membrane transporter being expressed throughout the body and is responsible for limiting cellular uptake and the distribution of xenobiotics and toxic substances (Amin et al.,2013). Our three phytochemicals Hesperidin, Myricetin, Quercetin showed positive results in HIA category. They shared negative BBB probability predicted that they did not cross blood brain barrier. However, they showed negative result in Caco-2 category depicting their poor absorption characteristics. They all had no P-glycoprotein inhibitor. Among them, Quercetin, Hesperidin performed as CYP3A4 substrate and showed no inhibition for CYP2C9, CYP2C19, CYP2D6.

Considering all data, we concluded that Quercetin performed better than other two ligands though at some point like ADMETSAR analysis, Hesperidin outperformed Quercetin. But in overall context, Quercetin showed promising results. The simulation results of both Quercetin-protein complexes were also promising. The fluctuation of their RMSD, RMSF values was less that ensured their proper stability. Also their nature of temperature fluctuation over time was very low that confirmed their stable structure. Molecular dynamics simulation further ensured their stable conformation. So it can be concluded that Quercetin was the best candidate against SARS-CoV-2 virus.

CONCLUSION

The SARS-CoV-2 virus has been posed a great threat to the human race. In spite of the severity of this deadly virus there is still no drug or vaccine available for us.

In this case, this study allowed us to find a novel drug against this virus. The concept of screening plant derived phytochemicals is based on their antiviral activity with fewer side effects within the body. The recent development in computational biology and bioinformatics paved the way to materialize the idea of drug discovery using these computational tools. The total procedure implied on freely accessible and open source tools to get all the data and process them. To find the best drug, we examined drug- likeness properties, ADMET analysis and PASS prediction with sites of metabolism analysis. At the end, molecular docking and dynamics simulation were performed to analyze the affinity and stability of the drugs. In our study, the proposed drug elicits satisfactory results against the SARS- CoV-2 virus and is a suitable candidate for in vitro and in vivo studies.

TRANSPARENCY

Declaration of funding

This was an independent non-sponsored trial.

Declaration of financial/other relationships

MM is an employee of Cantabria Lab, Difa Cooper. The other author (MP) reports no conflicts of interest.

Contribution statement

MP conducted the trial performing visits and instrumental evaluations. MM was involved in study protocol design. Both authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

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None reported.

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