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Effectiveness of Quercetin and Its Derivatives Against SARS CoV2 -*In silico* Approach

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ABSTRACT

The COVID-19 pandemic that erupted in November 2019 is continuing, with no effective antiviral agent to date. Synthetic antiviral agents have limitations such as a narrow range of therapeutic effectiveness of the activity, toxicity, and resistant viral strains and traditional antiviral medicines at large seem not to have these limitations. Here, some of the existing phytochemicals are cherry-picked for repurposing against the enzyme or protein targets of SARS CoV2, by the principles of structure-based drug design based on molecular docking studies. The most important drug targets of SARS CoV2 namely, Mpro protease (6LU7), RdRp polymerase (7BTF), and Spike glycoprotein of SARS CoV2(6VSB) were employed for docking analysis with chosen phytochemicals and binding affinity was calculated using PRODIGY software and docking sites determined using Chimera software. For docking studies, 160 phytochemicals were selected from a large pool of phytochemicals. Based on the binding affinity values, 61 phytoconstituents were selected for further in-silico screening which resulted in 15 phytochemicals, with higher binding affinity to spike glycoprotein of SARS CoV2. Moreover, Guaijaverin, Quercetin, Quercitrin, Quinic acid, and spiraeoside binds both to the spike glycoprotein of SARS Cov2 and the host receptor of human ACE2. Hence these compounds may serve as two-pronged drug candidates for SARS CoV2. In nutshell, we present a few phytochemical candidates with higher binding affinity to the Spike protein of SARS CoV2, which needs to be further optimized by in vitro studies to minimize the cytotoxicity and increase or retain the binding affinity, towards an effective antiviral drug against COVID 19.

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1 Introduction

The COVID-19 pandemic was recorded to have originated in the Chinese city of Wuhan around November 2019, caused by a novel zoonotic beta-coronavirus closely related to bat coronaviruses. The virus was named as Novel Corona Virus and later renamed SARS CoV2 due to its similarities with SARS (Severe Acute Respiratory Ryndrome) coronavirus first discovered in 2003 (Chatterjee et al. 2020; Dhama et al. 2020; Zheng, 2020). Globally now, more than 261 million people were affected by this pandemic and the death toll is more than 5 million. Unusual characteristics of SARS CoV2 infections are comparable to that of SARS and MERS and they share symptoms such as fever, fatigue, dry cough, and dyspnoea, leading to acute respiratory distress syndrome (ARDS) (Sharma et al. 2020). SARS CoV2 is highly transmissible and spreads through fomites, cough, and cold droplets, and human contact. Globally accepted prevention strategies such as hand sanitizing, wearing a mask, and keeping social distance are still counted as crucial while vaccine and drug research are proceeding at war footing (Chiu et al. 2020).

By now, many prophylactic vaccines are available against COVID 19 and a few chemical compounds such as chloroquine, remdesivir, lopinavir, molnupiravir, paxlovid, and ritonavir have shown favorable results *Invitro* and clinical studies. The SARS CoV2 genome has about ten ORFs (open reading frames). ORF-1 which covers a major region of viral RNA encodes 16 NSPs (non-structural proteins). RNA-dependent RNA Polymerase (RdRp) main protease (M pro) and Papain-like protease (PLpro) are the major NSPs while spike (S), envelope (E), nucleo-capsid (N), and membrane (M) proteins are the four main structural proteins (Luk et al. 2019; Fahmi et al. 2020). The SARS CoV2 replication cycle mainly includes virus entry, germination of virions, genome replication, and assembly. M-pro, RdRp, and spike glycoproteins are the most important viral proteins aiding the spreading of the virus. The Spike glycoprotein region of Covid-19 is considered as the region responsible for transmission by binding with angiotensin-converting enzyme 2 (ACE2) (Cagiliani et al. 2020; Letko et al. 2020; Lu et al. 2020; Luan et al. 2020). M-pro cleaves polyprotein towards forming of replication-transcriptase complex. The NSP12 region which is otherwise known as the RdRp region binds with NSP-7 and NSP-8 regions of the whole genome for the initiation of the replication of the virus (Romano et al. 2020). Interruption of any stage of viral entry or replication cycle is expected to be a potential strategy for the development of antiviral agents (V'Kovski et al. 2021).

An effective strategy for developing antiviral agents will be by interrupting any stage of viral entry or replication cycle against SARS CoV2. We screened a vast number of phytochemicals, reported to have significant antiviral activity and or extensively used as traditional medicines of plant origin. Synthetic antiviral agents have limitations such as a narrow spectrum of activity, limited therapeutic usefulness, toxicity, and resistant viral strains, and traditional antiviral medicines at large seem not to have these limitations. Whereas, phytochemicals from traditional medicine represent a vast repertoire of pharmacologically active substances with less toxicity and some of them are well researched lately. Here, some of the existing phytochemicals are cherry-picked for repurposing against the enzyme or protein targets of SARS CoV2, by the principles of structure-based drug design based on molecular docking studies. Such a computation-based approach will not only hasten drug discovery but will also lead toward specific antiviral agents. The current study aimed to deduce a library of potential antiviral agents against SARS CoV2 by *in silico* approach.

2 Materials and Methods

2.1 Selection of phytoconstituents and protein

From previous reports and traditional knowledge (Ethnopharmacology), based on reported anti-inflammatory as well as antiviral activities total of 160 phytoconstituents were selected (Mukhtar et al. 2008; Pushpa et al. 2013; Lin et al. 2014; Ma et al. 2015; Domitrovic and Potocnjak 2016; Bachar et al. 2021; Idrees et al. 2021; Ambrose et al. 2022; Sharma et al. 2022). The SDF files of the phytoconstituents were redeemed from the database of chemical molecules named PubChem (https://pubchem.ncbi.nlm.nih). Mol2 and PDB structures of selected phytoconstituents were deduced using Open babel software (O'Boyle et al. 2011). M protease, RdRp polymerase, spike glycoprotein, and ACE2 human receptor are the four targets selected for interaction studies. All the corresponding structures were redeemed from the databank of protein – RCSB PDB, 6LU7 for Mprotease, 7BTF for RdRP polymerase, 6VSB for SARS CoV2 Spike glycoprotein and 6M17 for Human ACE2 receptor (www.rcsb.org).

2.2 Docking analysis

The selected phytoconstituents were screened individually as in figure 1 by measuring the interactivity with targeted proteins using the software tool Auto Dock Vina (Trott and Olson 2010). For further analysis, the structures obtained using docking were redeemed in the PDB format and affinity values for binding were determined using PRODIGY software (Xue et al. 2016). The Chimera software (version.1.13.1) was used for the analysis of docked structures for calculating the possible bond distances obtained from intra as well as inter-hydrogen bonding (Pettersen et al. 2004). The chemical structures of selected phytoconstituents are redeemed from EMBL-EBI (www.ebi.ac.uk/chebi /).

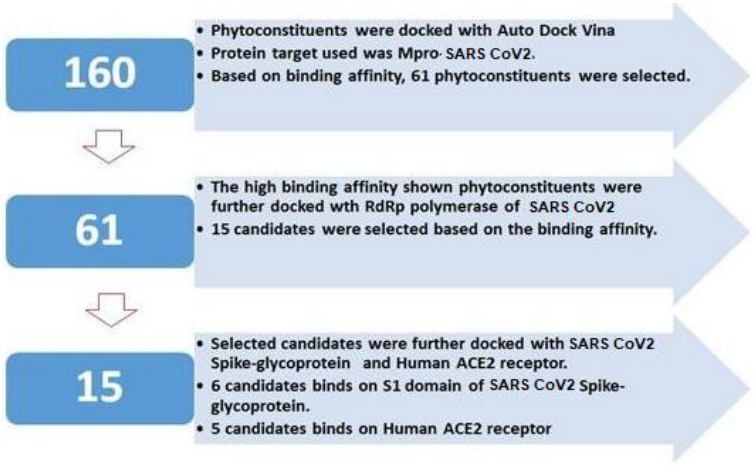

Figure 1 Flowchart represents the screening strategy for the selection of anti-SARS CoV2 phytoconstituents

2.3 ADMET and drug-likeness evaluation

The SMILE (canonical simplified molecular input line entry systems) of selected 15 phytoconstituents were considered for the molecular as well as pharmacokinetic evaluation- *in-silico* in the Swiss ADME tool. The adsorption, desorption, metabolism, and excretion (ADME) predictions were computed in the Swiss ADME tool for blood-brain barrier permeability, Log Kp of skin permeation value, gastro-intestine absorption, P-gp substrate and cytochrome-P inhibitors (Daina et al. 2017). For understanding the toxicological behavior of the selected phytoconstituents, the Pro Tox II was used for evaluating immunotoxicity, carcinogenicity, mutagenicity, cytotoxicity, LD50, etc. and Osiris property explorer was used for determining irritant properties (Banerjee et al. 2018).

3 Results and Discussion

3.1 Selection and screening of phytoconstituents

The most important drug targets of SARS CoV2 namely, Mpro protease (6LU7), RdRp polymerase (7BTF), and Spike glycoprotein of SARS CoV2 (6VSB) were employed for docking analysis with the chosen phytochemicals. From the docked structures, binding affinity was calculated using PRODIGY software and docking sites were determined using Chimera software. For docking studies, 160 phytochemicals were selected from a large pool of phytochemicals known to have ethnopharmacological indications and or reported antiviral activity and were docked with Mpro (6LU7) protease and the binding energy

Table 1 Comparison of binding affinities to some phytochemical constituents and control drug on Mpro protease (6LU7) and RdRp Polymerase (7BTF)

S. N.	Name of the Ligand	ChemID	Ligand Source	Binding affinity Mpro Protease: 6LU7 (KCal/Mol)	Binding affinity RdRp polymerase: 7BTF (KCal/Mol)
1	Apigenin	CHEBI:18388	*Ficus mucuso*	-7.8	-7.6
2	Ursolic acid	CHEBI:9908	*Malus domestica,* *Radermachera boniana* *Rubia yunnanensis* *Symplocos lancifolia* *Ficus mucuso* *Prunus domestica* *Terminalia catappa* *Rhododendron ferrugineum* *Rosa laevigata*	-7.6	-7.7
3	Oleanolic acid	CHEBI:37659	*Radermachera boniana* *Symplocos lancifolia* *Diospyros kaki* *Juglans sinensis* *Rhododendron ferrugineum*	-7.8	-7.6
4	Vasicinolone	CID: 13970119	*Adathoda vassica*	-6.15	-8.04
5	Vasicol	CID:92470596	*Adathoda vassica*	-6.27	-7.8

S. N.	Name of the Ligand	ChemID	Ligand Source	Binding affinity Mpro Protease: 6LU7 (KCal/Mol)	Binding affinity RdRp polymerase: 7BTF (KCal/Mol)
6	Anisotine	CHEBI:2738	*Adathoda vassica*	-6.87	-9.08
7	Quinic Acid	CHEBI:17521	*Klenia grandiflora* *Quercus pedunculata* *Arabidopsis thaliana*	-7.86	-8.81
8	Avicequinone C	CID:10563004	*Glycosmos pentaphylla*	-7.20	-7.00
9	Guaijaverin	CID:5481224	*Psidium guajava*	-8.60	-8.00
10	Marmeleide/ Imperatorin	CID:10212	*Aaglemarmelos*	-7.10	-7.50
11	Quercetin	CHEBI:16243	*Mimosa diplotricha* *Ophioglossum pedunculosum* *Lepisorususs uriensis*	-8.00	-7.90
12	Saponin	CHEBI:26605	*Asparagus racemosus* *Randia spinosa* *Gymnema sylestre* *Bacopa monnieri* *Ficus hispida* *Clerodendrum serratum* *Mimusops elengi* *Coscinium fenestratum* *Achyranthes aspera* *Putranjiva roxburghii* *Saraca asoca* *Symplocos racemosa* *Hemidesmus indicus* *Terminalia arjuna*	-8.10	-9.50
13	Isorhamnetin	CID:5281654	*Trigonella foenum graecum*	-7.30	-8.00
14	Kaempferol	CHEBI:28499	*Pittocaulon velatum* *Ficus mucuso*	-7.50	-8.20
15	Ellagic Acid	CHEBI:4775	*Myrciaria jaboticaba*	-8.30	-7.90
16	Carpaine	CHEBI:3433	*Carica papaya* *Trigonella foenum graecum*	-7.70	-8.80
17	Graecunin E	CID:156783	*Trigonella foenum graecum*	-8.40	8.80
18	Fenugreekine	CID:444170	*Trigonella foenum graecum*	-8.20	-8.80
19	Yamogenin	CHEBI:10086	*Trigonella foenum graecum*	-7.40	-7.10
20	Diosgenin	CHEBI:4629	*Dioscorea bulbifera* *Trigonella foenum graecum*	-7.40	-7.00
21	Smilagenin	CID:91439	*Trigonella foenum graecum*	-7.50	-7.00
22	Sarasaspogenin	CID:92095	*Trigonella foenum graecum*	-7.50	-7.00
23	Tigogenin	CHEBI:9595	*Trigonella foenum graecum*	-7.50	-7.00
24	Neotigogenin	CHEBI:80752	*Trigonella foenum graecum*	-7.30	-7.00
25	Gitogenin	CHEBI:5363	*Trigonella foenum graecum*	-7.50	-7.10
26	Neogitogenin	CHEBI:80854	*Trigonella foenum graecum*	-7.50	-7.00
27	Yuccagenin	CID:3083608	*Trigonella foenum graecum*	-7.40	-7.00

S. N.	Name of the Ligand	ChemID	Ligand Source	Binding affinity Mpro Protease: 6LU7 (KCal/Mol)	Binding affinity RdRp polymerase: 7BTF (KCal/Mol)
28	Rutin	CHEBI:28527	*Physalis longifolia* *Trigonella foenum graecum*	-8.10	-9.10
29	Vitexin	CHEBI:16954	*Eminium spiculatum* *Trigonella foenum graecum*	-8.20	-7.70
30	Isovitexin	CHEBI:18330	*Ficus deltoidea* *Trigonella foenum graecum*	-7.50	-7.10
31	Sigmastadienol	CID:129636643	*Vermonia anthelmitia*	-7.80	-7.00
32	Stigmasterol	CHEBI:28824	*Vermonia anthelmitia*	-7.60	-7.80
33	Luteolin	CHEBI:15864	*Cynaldon dactylon*	-7.90	-7.50
34	Orientin	CHEBI:7781	*Sonchus arvensis* *Cynaldon dactylon*	-8.20	-8.60
35	Neoxanthin	CHEBI:25501	*Cynaldon dactylon*	-7.80	-7.20
36	Violaxanthin	CHEBI:27295	*Cynaldon dactylon*	-7.60	-7.30
37	Asiaticoside	CHEBI:79928	*Centella asiatica*	-8.00	-9.90
38	Madecassoside	CHEBI:66651	*Centella asiatica*	-8.90	-8.50
39	Narcissin	CID:5481663	*Aerva lanatta*	-9.10	-8.50
40	Sitogluside	CID:5742590	*Aerva lanatta*	-7.40	-7.80
41	Solanine	CHEBI:9188	*Solanum tuberosum* *Solanum lycopersicum* *Solanum melongena* *Aerva lanatta*	-8.00	-9.50
42	Chaconine	CID:4115417	*Aerva lanatta*	-9.20	-9.30
43	Kaempferol-3-α-D Glucoside	CID:44258798	*Aerva lanatta*	-8.10	-8.00
44	Mangiferrin	CID:5281647	*Mangifera indica*	-7.50	-8.10
45	Artemisinin	CHEBI:223316	*Artemisia annua*	-6.80	-6.70
46	Hyperoside	CHEBI:67486	*Quercetin derivative*	-7.80	-8.20
47	Isoquercitrin	CID:5280804	*Quercetin derivative*	-8.50	-8.10
48	Spiraeoside	CID:5320844	*Quercetin derivative*	-7.90	-8.10
49	Quercitrin	CHEBI:17558	*Quercetin derivative*	-8.20	-8.10
50	Avicularin	CHEBI:65460	*Juglans regia* *Foeniculum vulgare* *Quercetin derivative*	-8.50	-7.60
51	Protocatechuic acid	CID:72	*Hibiscus sabdariffa*	-5.40	-6.20
52	Caffeic acid	CHE BI:36281	*Eucalyptus globulus*	-5.70	-5.60
53	Liquiritin	CHE BI:80845	*Polygonum aviculare* *Artemisia capillaris*	-8.00	-7.80
54	Hesperidin	CHEBI:28775	*Citrus aurantium*	-8.80	-9.50
55	Apigetrin	CHEBI:11595	*Teucrium gnaphalodes*	-7.80	-8.20

S. N.	Name of the Ligand	ChemID	Ligand Source	Binding affinity Mpro Protease: 6LU7 (KCal/Mol)	Binding affinity RdRp polymerase: 7BTF (KCal/Mol)
56	Rosmarininc acid	CID:5281792	*Salvia rosmarinus* *Perilla frutescens* *Salvia officinalis* *Mentha arvense* *Ocimum basilicum.*	-7.60	-7.50
57	Oxypeucedaninhydrate	CID:5281792	*Ferulago sylvatica*	-7.30	-7.30
58	Byakangelicin	CHEBI:3250	*Murraya koenigii* *Triphasia trifolia*	-7.00	-7.50
59	Glycyrrhizin	CID:128229	*Glycyrrhiza uralensis* *Glycyrrhiza inflata*	-7.10	-9.60
60	Nobiletin	CHEBI:7602	*Citrus tankan*	-6.60	-6.50
61	6-Gingerol	CID:442793	*Illicium verum* *Piper nigrum*	-5.80	-5.50
62	Ramdesivir	CID:121304016	*Control-Drug*	-7.20	
63	Saquinavir	CID:441243	*Control-drug*		-9.2

exhibited a range of -3.50 to 9.20 Kcal /Mol (Mukhtar et al. 2008; Pushpa et al. 2013; Lin et al.2014; Domitrovic and Potocnjak 2016; Ma et al. 2015;). As a standard for the analysis Mpro was also docked with the known antiviral agent Remdesivir and the corresponding binding affinity of -7.20Kcal/Mol, was considered as the cut-off value for the initial screening. Based on the binding affinity values, 61 phytoconstituents were selected for further in-silico screening.

RdRp polymerase (7BTF) is responsible for the replication of the virus and so the selected phytochemicals were docked with 7BTF and a binding affinity ranging from -5.7 to -10.20 Kcal/Mol was observed, while saquinavir, an accepted antiviral drug taken as control, had an affinity of -9.2 Kcal/Mol with RdRp polymerase. Table 1 represents the binding affinity of selected candidates towards Mpro and RdRp polymerase. Based on the binding affinity towards Mpro and RdRP polymerase, 15 phytochemicals were shortlisted for further analysis as mentioned in figure 1.

3.2 Protein-ligand interaction study: With spike glycoprotein of SARS CoV2

Spike, a class 1 fusion protein is the surface glycoprotein of SARS CoV2 and is responsible for viral attachment with human ACE2 (Angiotensin-converting enzyme 2) receptor and its consecutive fusion with the host cells, which follows the S1 subunit of the protein attached to the ACE2 receptor via its RBD region, the receptor binding domain and protein changes its conformation to a post-fusion form. The key amino acids of the S1 subunit, responsible for viral attachment, are reported as LEU455, PHE486, GLN493, SER494, ASP501, and TYR505, present in the ACE2 receptor binding region (333-527 residues) (Yuan et al. 2020). The S2 subunit is composed of a HR1, HR2, FP, TM domain, and cytoplasmic domain fusion (CT). S2 is responsible for viral fusion and entry but not actively involved in viral attachment and after binding, the protein changes to a post-fusion form. In addition to that the interacting regions of TMPRSS2- transmembrane protease serine 2 of the viral spike proteins at 685-ARG / 686-SER and 815-ARG / 816-SER.

Table 2 and figure 2 represent the protein-ligand interaction studies of 15 phytochemicals, among them higher binding affinity showing phytoconstituents are Asiaticoside (-12.04 Kcal/mol), Fenugreekine (-15.05 Kcal/mol) and Graecunin E (-18.81 kcal/mol), which were observed to bind with the S2 cavity region of spike glycoprotein. This could potentially contribute to the high binding affinities of these compounds. But Guaijaverin derived from *Psidium guajava* shows a binding affinity of -11.22 Kcal/mol and forms hydrogen bond in the S1 domain of spike glycoprotein, especially at the ACE2 receptor binding region, i.e., R466 and R355. In other words, other phytochemicals binding to the S1 subunit, such as Avicequinone C, hyperoside, quercetin, spiraeoside, 9 benzyl 2 fluoro 9 hpurin 6amine show considerably less binding affinity than Guaijaverin, but binds near RBD with binding amino acids positions such as avicequinone C-R355, hyperoside -N544, quercetin-S514 &R355, spiraeoside-I312, 9 benzyl 2 fluoro 9 hpurin 6 amine-T478. An interesting fact is that Guaijaverin, hyperoside and spiraeoside are quercetin derivatives. Another quercetin derivative, quercitrin binds to the R815 of the S2 domain, the TMPRSS2 binding site of spike glycoprotein. The interaction of TMPRSS2 to the spike protein is a crucial port for viral entry. The druggability will be more for the compound that interacts with the TMPRSS2 region that interacts with the C-terminal

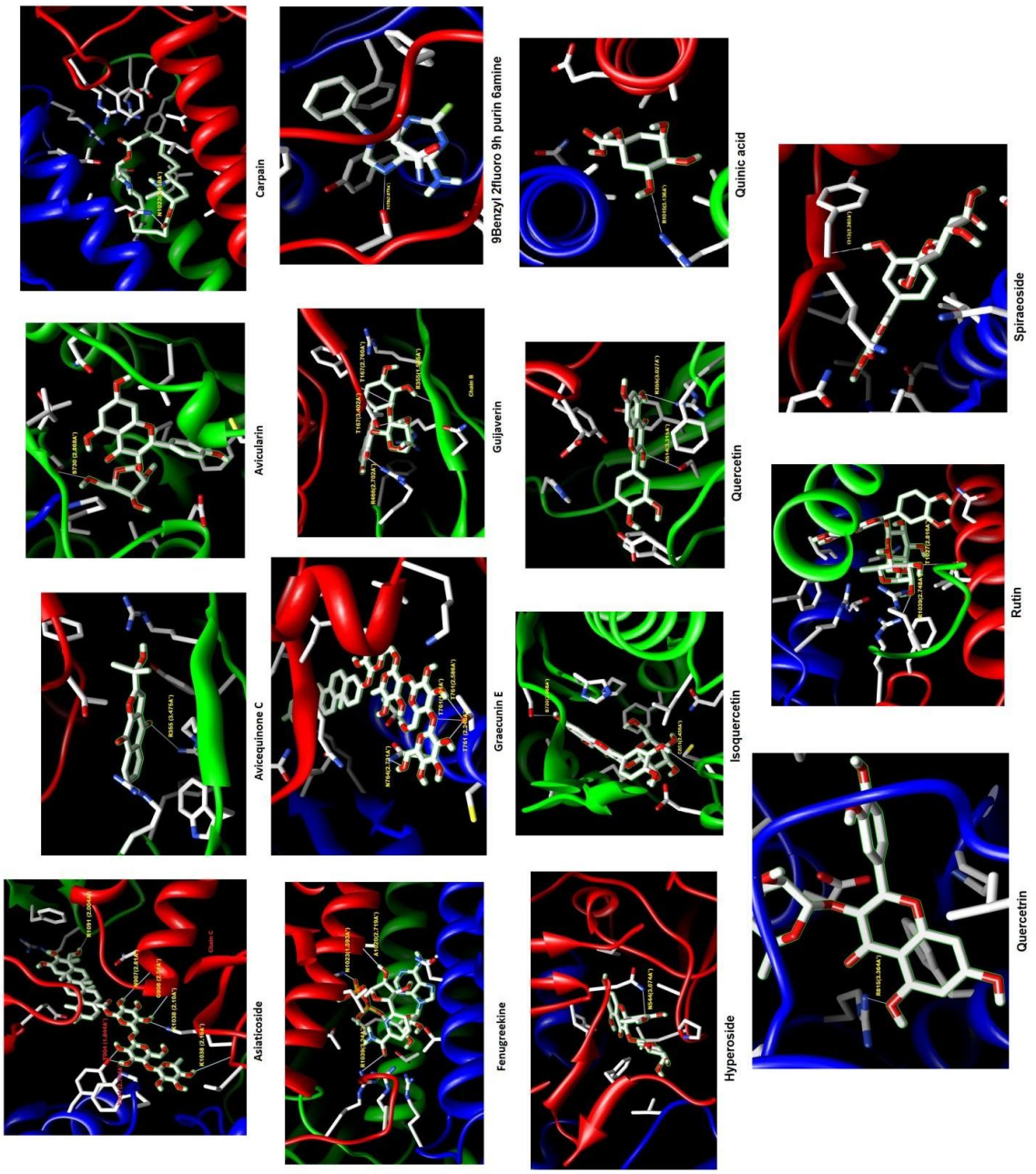

Figure 2 Binding regions of selected phytoconstituents with SARS CoV2spike glycoprotein

cleavage site of the S2 domain (Arg815/Ser816) of SARS-CoV-2 spike protein than in the N-terminal cleavage site observed in S2 domain (Arg685/Ser686) (Hussain et al. 2020).

3.3 Protein-ligand interaction study with ACE2, as the viral receptor

ACE2 receptor plays a pertinent role in the spreading of the disease by acting as a host receptor for the SARS CoV2. The residues 24-GLN, 82-MET, 79-ILE, 31-LYS, 34-HIS, 37-GLU, 354-GLY, 325-GLN, 38-ASP, 330-ASN, 329-GLU, 42-GLN and 45-LEU of ACE2 interact with RBD region of spike glycoprotein of SARS CoV2 (Vardhan and Sahoo 2020). So, from the present study, among the 15 phytochemicals that bind to the spike glycoprotein, Guaijaverin (ASN210), Quercetin (ASN210, GLU564), Quercitrin (ASN210), Quinic Acid (ALA296) and Spiraeoside (GLU495) binds to the ACE2 receptor.

Table 2 Binding affinities of the compounds on the Spike glycoprotein (6VSB) and their interaction with the binding site

Sl No	Name of the ligand	Binding region Spike protein	Binding Affinity with Spike glycoprotein-6VSB (Kcal/Mol)	Hydrogen bonding interactions with residues & Bond length (A°)
1	Asiaticoside	S2	-12.04	K1038 (2.16A°) Chain C K1038 (2.10A°) Chain C G908 (2.51A°) Chain C N907 (2.81A°) Chain C R1091 (2.004 A°) Chain B Y904 (2.764A°) Chain A Y904 (1.844 A°) Chain A
2	Avicequinone C	S1	-9.76	R355(3.475A°) Chain B
3	Avicularin	S2	-7.2	S730(2.868A°) Chain B
4	Carpaine	S2	-10.31	N1023 (3.158A°) Chain A
5	Fenugreekine	S2	-15.05	R1039 (3.24A°) Chain B A1020 (2.719A°) Chain C N1023 (1.593A°) Chain C
6	Graecunin E	S2	-18.81	N764 (2.731A°) Chain A T761 (2.246A°) Chain A T761 (1.914 A°) Chain A T761 (2.586 A°) Chain A
7	Guaijaverin	S1	-11.22	R466 (2.702A°) Chain B R355 (1.545 A°) Chain B T167 (3.402A°) Chain C T167 (2.760 A°) Chain C
8	Hyperoside	S1	-7.6	N544 (3.074A°) Chain B
9	Isoquercitrin	S2	-7.8	C851 (2.436A°) Chain B S730 (2.468A°) Chain B
10	Quercetin	S1	-7.91	S514 (3.315 A°) Chain B R355 (3.027A°) Chain B
11	Quercitrin	S2	-7.8	R815 (3.364A°) Chain B
12	Rutin	S2	-8.97	R1039 (2.748A°) Chain B R1029 (2.816A°) Chain B
13	Spiraeoside	S1	-7.6	I312 (2.203A°) Chain B
14	9Benzyl 2fluoro 9h purine 6amine	S1	-5.12	T478(2.875A°) Chain B
15	Quinic Acid	S2	-6.47	R1019 (3.138A°) Chain B

Table 3 The selected compounds interact with binding sites of ACE2 receptor molecules (6M17) and their bond distances

S. N.	Name of the ligand	Hydrogen bonding interactions with ACE2 receptor (6M17) residues & Bond length (A°)
1	Guaijaverin	N 210 (1.417 A°) Chain B
2	Quercetin	N 210 (2.884 A°) Chain B E 564 (2.134 A°) Chain B
3	Quercitrin	N 210 (3.434 A°) Chain B
4	Quinic acid	A 396 (2.696 A°) Chain B
5	Spiraeoside	E 495 (2.880 A°) Chain B

The phytochemical candidates which possess affinity towards spike binding regions of the ACE2 receptor may inhibit the interaction of spike glycoprotein to the ACE2 receptor (Table 3 and figure 3) (Vardhan and Sahoo 2020; Sahoo et al. 2020). Hence these compounds may serve as two-pronged drug candidates for SARS CoV2 and must be tested in cell culture models of SARS CoV2 infection.

3.4 Insilico Drug likeness and Toxicity analysis

The effectiveness of drug candidates was evaluated using ADME / Tox properties. Using the Swiss ADME, selected fifteen phytoconstituents were analyzed and the obtained results were compared with Ramdesivir and Saquinavir for establishing the drug-likeness nature of the candidates (Daina et al. 2017). Lipinski's rule

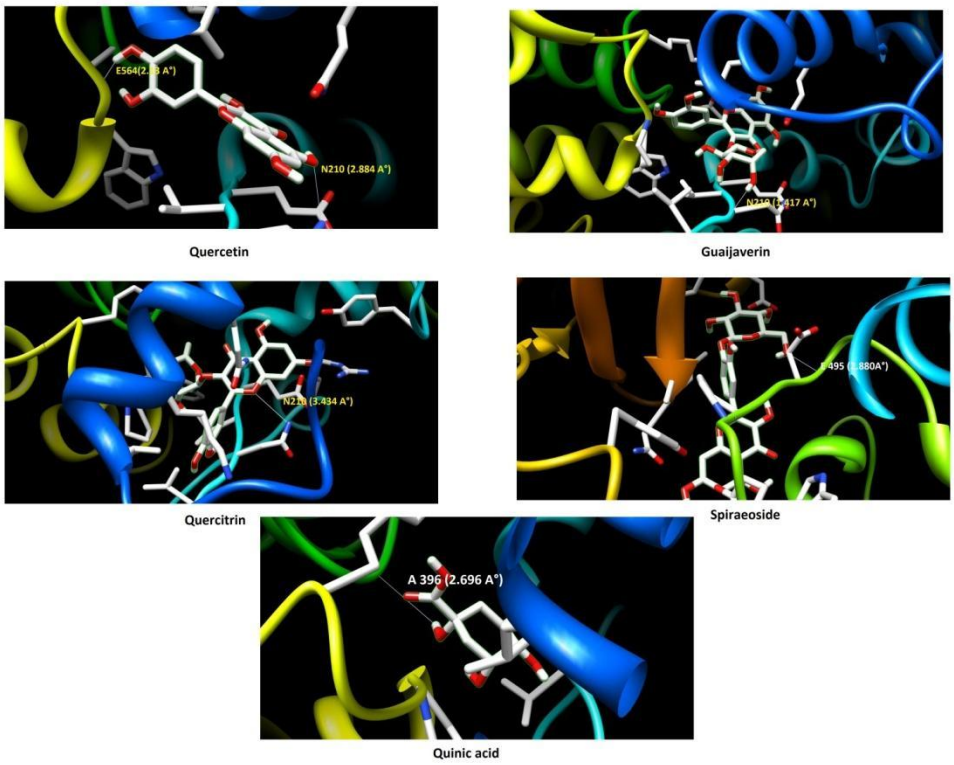

Figure 3 Binding regions of selected phytoconstituents with Human ACE2 receptor.

Table 4 Drug Likeness prediction of Compounds

Molecule	Formula	MW	NRB	NHA	NHD	TPSA	iLOGP	Lipinski rule of five violations
Asiaticoside	$C_{48}H_{78}O_{19}$	959.12	10	19	12	315.21	2.5	3
9 Benzyl 2 fluoro 9h purin 6 amine	$C_{12}H_{10}FN_5$	243.24	2	4	1	69.62	2.01	0
Avicularin	$C_{20}H_{18}O_{11}$	434.35	4	11	7	190.28	1.86	2
Avicequinone C	$C_{15}H_{12}O_4$	256.25	1	4	1	67.51	2.13	0
Carpaine	$C_{28}H_{50}N_2O_4$	478.71	0	6	2	76.66	4.4	0
Fenugreekine	$C_{21}H_{27}N_7O_{14}P_2$	663.43	11	18	8	346.89	0.24	3
GraecuninE	$C_{51}H_{82}O_{22}$	1047.18	11	22	12	335.06	3.78	3
Guaijaverin	$C_{20}H_{18}O_{11}$	434.35	3	11	7	190.28	1.61	2
Hyperoside	$C_{21}H_{20}O_{12}$	464.38	4	12	8	210.51	2.11	2
Isoquercitrin	$C_{21}H_{20}O_{12}$	464.38	4	12	8	210.51	0.94	2
Quercetin	$C_{15}H_{10}O_7$	302.24	1	7	5	131.36	1.63	0
Quercitrin	$C_{21}H_{20}O_{11}$	448.38	3	11	7	190.28	1.27	2
Quinic acid	$C_7H_{12}O_6$	192.17	1	6	5	118.22	-0.12	0
Rutin	$C_{27}H_{30}O_{16}$	610.52	6	16	10	269.43	2.43	3
Spiraeoside	$C_{21}H_{20}O_{12}$	464.38	4	12	8	210.51	1.45	2
Ramdesivir	$C_{27}H_{35}N_6O_8P$	602.58	14	12	4	213.36	3.24	2
Saquinavir	$C_{38}H_{50}N_6O_5$	670.84	16	7	5	166.75	3.66	2

MW - Molecular weight, NHD - Number of Hydrogen Donor, NRB - Number of rotatable bonds, NHA - Number of Hydrogen Acceptor, TPSA - Total polar surface area

Table 5 ADME Predictions of selected phytoconstituents

Molecule	Formula	GI absorption	BBB permeability	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	log Kp (cm/s)
Asiaticoside	$C_{48}H_{78}O_{19}$	Low	No	Yes	No	No	No	No	No	-12.08
9Benzyl 2fluoro 9h purin 6amine	$C_{12}H_{10}FN_5$	High	Yes	No	Yes	No	No	Yes	Yes	-6.27
Avicularin	$C_{20}H_{18}O_{11}$	Low	No	No	No	No	No	No	No	-8.25
Avicequinone C	$C_{15}H_{12}O_4$	High	Yes	No	Yes	No	No	No	Yes	-6.44
Carpaine	$C_{28}H_{50}N_2O_4$	High	No	Yes	No	No	No	No	No	-4.75
Fenugreekine	$C_{21}H_{27}N_7O_{14}P_2$	Low	No	No	No	No	No	No	No	-14.55
GraecuninE	$C_{51}H_{82}O_{22}$	Low	No	Yes	No	No	No	No	No	-13.62
Guaijaverin	$C_{20}H_{18}O_{11}$	Low	No	No	No	No	No	No	No	-8.64
Hyperoside	$C_{21}H_{20}O_{12}$	Low	No	No	No	No	No	No	No	-8.88
Isoquercitrin	$C_{21}H_{20}O_{12}$	Low	No	No	No	No	No	No	No	-8.88
Quercetin	$C_{15}H_{10}O_7$	High	No	No	Yes	No	No	Yes	Yes	-7.05
Quercitrin	$C_{21}H_{20}O_{11}$	Low	No	No	No	No	No	No	No	-8.42
Quinic acid	$C_7H_{12}O_6$	Low	No	Yes	No	No	No	No	No	-9.15
Rutin	$C_{27}H_{30}O_{16}$	Low	No	Yes	No	No	No	No	No	-10.26
Spiraeoside	$C_{21}H_{20}O_{12}$	Low	No	Yes	No	No	No	No	No	-8.2
Ramdesivir	$C_{27}H_{35}N_6O_8P$	Low	No	Yes	No	No	No	No	Yes	-8.62
Saquinavir	$C_{38}H_{50}N_6O_5$	Low	No	Yes	No	No	No	No	Yes	-7.38

Log Kp - skin permeation value; GI - gastro-intestinal; BBB - blood–brain barrier; P-gp - P-glycoprotein; CYP - cytochrome-P

of five violations gives insight into recommending molecules as orally administrable drug candidates. Two or more violations lead to non-recommendation, and among fifteen candidates Asiaticoside, Fenugreekine, Graecunin E and Rutin show a violation of the rule and are considered to be non-recommendable for oral administration (Table 4). From the Swiss ADME analysis, 9 benzyl 2 fluoro 9H purin 6 amine, Avicequinone C, Carpaine, and quercetin shows high gastrointestinal (GI) absorption while 9 benzyl 2 chloro 9h purine 6 amine, Avicequinone C shows blood-barrier (BBB) permeation (Table 5). Like Ramdesivir and Saquinavir, Asiaticoside, Carpaine, Graecunin E, Quinic acid, Rutin, and Spiraeoside may act as substrates of permeability glycoprotein (Pgp). The CYP inhibition of candidates may lead to toxic or unwanted adverse effects. Asiaticoside, Avicularin, Carpaine, Fenugreekine, Graecunin E, Guaijaverin, Hyperoside, Isoquercitrin, Quercitrin, Quinic acid, Rutin and Spiraeoside are potential non-inhibitors for CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 while remdesivir and saquinavir are potential inhibitors of CYP3A4. In this sense, the above-mentioned phytochemical compounds are safer for human administration than remdesivir and saquinavir.

LD_{50} values indicate the acute toxicity and toxicity classification 1(toxic) and 6(non-toxic) of phytoconstituents. Based on LD_{50}, only Graecunin E (55mg/Kg) and quercetin (159 mg/Kg) have shown higher acute toxicity than Ramdesivir (1000 mg/Kg) which is class 4 (Table 6). From the toxicology prediction data obtained from Pro ToxII, Carpaine, Fenugreekine, and Quinic acid act as non-hepatotoxic, non-carcinogenic, non-Immunotoxic, non-mutagenic, non-cytotoxic and non-irritant along with Ramdesivir and Saquinavir (Banerjee et al. 2018). Whereas, Asiaticoside, Avicularin, Guaijaverin, Hyperoside, Isoquercitrin Rutin, and Spiraeoside have shown some degree of immunotoxicity.

Conclusion

To sum up, out of the fifteen chosen phytochemicals which have high binding efficiency to spike protein, Asiaticoside, Avicularin, Guaijaverin, Hyperoside, Isoquercitrin, Rutin and Spiraeoside exhibit higher toxicity values compared to remdesivir and saquinavir. Quercitrin, a phytochemical that binds to R 815 of S2, most likely has an implication in the activity of TMPRSS2 which is a crucial transmembrane molecule involved in viral entry to the cell. However, further *Invitro* studies are essential to analyze the extent of antiviral and toxicity effects of these phytochemicals. Appropriate chemical derivatization by retaining the binding affinity to the corresponding amino acid positions needs to be explored, to moderate the toxic effect. Guaijaverin (ASN210), Quercetin

Table 6 Prediction of Toxicity of phytoconstituents

Molecule	Formula	LD_{50} (mg/Kg)	Toxicity Class	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity	Irritant
Asiaticoside	$C_{48}H_{78}O_{19}$	4000	5	No	No	Yes	No	No	No
9Benzyl 2fluoro 9h purin 6amine	$C_{12}H_{10}FN_5$	1190	4	Yes	No	Yes	No	No	No
Avicularin	$C_{20}H_{18}O_{11}$	5000	5	No	No	Yes	No	No	No
Avicequinone C	$C_{15}H_{12}O_4$	1500	4	No	Yes	No	No	No	No
Carpaine	$C_{28}H_{50}N_2O_4$	500	4	No	No	No	No	No	No
Fenugreekine	$C_{21}H_{27}N_7O_{14}P_2$	7000	6	No	No	No	No	No	No
GraecuninE	$C_{51}H_{82}O_{22}$	55	3	No	No	Yes	No	Yes	No
Guaijaverin	$C_{20}H_{18}O_{11}$	5000	5	No	No	Yes	No	No	No
Hyperoside	$C_{21}H_{20}O_{12}$	5000	5	No	No	Yes	No	No	No
Isoquercitrin	$C_{21}H_{20}O_{12}$	5000	5	No	No	Yes	No	No	No
Quercetin	$C_{15}H_{10}O_7$	159	3	No	Yes	No	Yes	No	No
Quercitrin	$C_{21}H_{20}O_{11}$	5000	5	No	Yes	Yes	No	No	No
Quinic acid	$C_7H_{12}O_6$	9800	6	No	No	No	No	No	No
Rutin	$C_{27}H_{30}O_{16}$	5000	5	No	No	Yes	No	No	No
Spiraeoside	$C_{21}H_{20}O_{12}$	5000	5	No	No	Yes	No	No	No
Ramdesivir	$C_{27}H_{35}N_6O_8P$	1000	4	No	No	No	No	No	No
Saquinavir	$C_{38}H_{50}N_6O_5$	2000	4	No	No	No	No	No	No

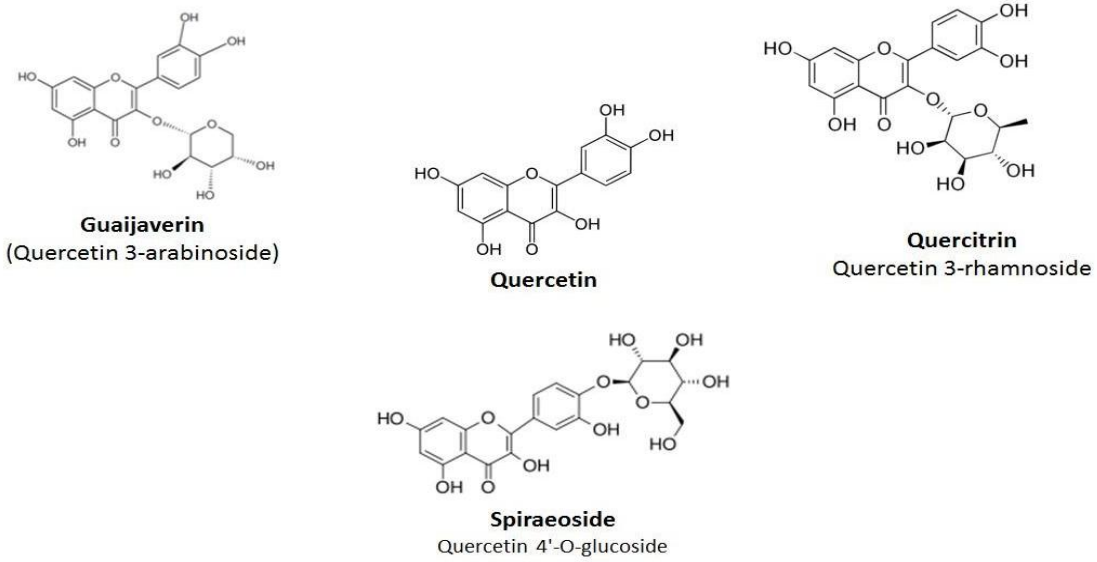

Figure 4 Chemical Structures of Quercetin and Quercetin Derivatives

(ASN210, GLU564), Quercitrin (ASN210), Quinic acid (ALA296), and spiraeoside (GLU495) can bind to the spike protein of the virus and ACE2 receptor of the host. Hence these compounds may serve as two-pronged drug candidates for SARS CoV2. Interestingly, the four among these are quercetin derivatives and are shown in figure 4. A few of the phytochemicals explored here such as Carpaine, Fenugreek, and Quinic acid, and their chemical modifications may increase the binding energy to spike protein. Further, synthetic combination molecules with high affinity and low toxicity moieties can be derived sooner than later which might inhibit the SARS CoV2 viral entry via spike protein to the human and animal hosts. We have a handful of very promising antiviral phytochemical moieties which may lead toward an effective antiviral drug against SARS CoV2.

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