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RESEARCH ARTICLE

In silico screening of *Allium cepa* phytochemicals for their binding abilities to SARS and SARS-CoV-2 3C-like protease and COVID-19 human receptor ACE-2

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ABSTRACT

Corona virus SARS-CoV-2-induced viral disease (COVID-19) is a zoonotic disease that was initially transmitted from animals to humans. The virus surfaced towards the end of December 2019 in Wuhan, China where earlier SARS (Severe Acute Respiratory Syndrome) had also surfaced in 2003. Unlike SARS, SARS-CoV-2 (a close relative of the SARS virus) created a pandemic, and as of February 24 2021, caused 112,778,672 infections and 2,499,252 deaths world-wide. Despite the best efforts of scientists, no drugs against COVID-19 are yet in sight; five vaccines have received emergency approval in various countries, but it would be a difficult task to vaccinate twice the world population of 8 billion. The objective of the present study was to evaluate through *in silico* screening a number of phytochemicals in *Allium cepa* (onion) regarding their ability to bind to the main protease of COVID-19 known as the 3C-like protease or $3CL^{pro}$, (PDB ID: 6LU7), $3CL^{pro}$ of SARS (PDB ID: 3M3V), and human angiotensin converting enzyme-2 (ACE-2), [PDB ID: 1R42], which functions as a receptor for entry of the virus into humans. Molecular docking (blind docking, that is docking not only against any target pocket) were done with the help of AutoDockVina. It was observed that of the twenty-two phytochemicals screened, twelve showed good binding affinities to the main protease of SARS-CoV-2. Surprisingly, the compounds also demonstrated good binding affinities to ACE-2. It is therefore very likely that the binding affinities shown by these compounds against both $3CL^{pro}$ and ACE-2 merit further study for their potential use as therapeutic agents.

Keywords: *Allium cepa*; *in silico* screening; phytochemicals; 3C-like protease; ACE-2 receptor.

INTRODUCTION

Corona viruses belong to the Family *Coronaviridae* and are so named because of the spike like protein on their surface resembling a corona. Some corona viruses are quite common. They cause mild fever and bronchitis in humans, which goes away by itself within a week or so with minimal fatalities. Seven known human corona viruses (HCoVs), that cause diseases in humans have been identified so far, namely HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, severe acute respiratory syndrome corona virus (SARS-CoV), Middle East Respiratory Syndrome coronavirus (MERS-CoV), and SARS-CoV-2 or COVID-19, which emerged in late December 2019 in Wuhan, China. SARS and SARS-CoV-2 have a sequence identity of 79% (Park *et al*., 2020). Four HCoVs (HCoV-229E, HCoV-NL63, HCoV-OC43 and HCoV-HKU1) are more commonly found circulating in the global human population and contribute to approximately one-third of common cold infections in humans every year (Van der Hoek, 2007).

The human receptor for both SARS and SARS-CoV-2 is the angiotensin converting enzyme-2 (ACE-2). The spike (S) protein on these two very much identical viruses contains two subunits – S1 and S2. Binding to the receptor and then fusion to the host cell is mediated by the S1 subunit through its receptor binding domain (RBD). The S2 domain contributes to the membrane fusion and internalization of the virus. Molecular docking analysis have indicated that several flavonoids and non-flavonoid compounds bind to two of the 3 domains of the spike protein, namely the C-terminus of the S1 domain (but not the N-terminus) and the S2 domain of the spike protein, which may then hinder binding to ACE-

2 leading to subsequent internalization and intracellular replication of the virus (Rane *et al*., 2020).

Most of the corona virus genome (in SARS) encodes two large polyproteins, pp1a and pp1ab. Once inside the host cell, cleavage and transformation of these two polyproteins into mature non-structural proteins (NSPs) are achieved by a chymotrypsin-like protease (3C-like protease or $3CL^{pro}$) and a papain-like protease, PL^{pro}. The Cys145-His41 dyad is necessary for cleavage by $3CL^{pro}$ (Anand *et al.*, 2003). The 3C-like proteases of SARS-CoV-2 differ from that of SARS by only 12 amino acids. A high level of alignment of the 3C-like proteases has been reported for SARS and SARS-CoV-2 of the key amino acids involved in substrate binding, which includes the Cys145-His41 dyad and His163/His172/Glu166, the latter residues providing an opening gate for the substrate. The 3C-like protease monomer contains 3 domains of which the first two domains comprise of residues 8-101 and residues 102-184, respectively. These two domains are responsible for the chymotrypsin-like catalysis. Studies are ongoing to find compounds which can bind to the 3C-like protease and so inhibit the virus.

Because of its contagious nature and absence of any drugs or vaccines for effective treatment, SARS-CoV-2 has become a global pandemic. As of February 24 2021, the virus has caused 112,778,672 infections and 2,499,252 deaths. In USA, the corresponding figures are 28,901,327 infections and 515,211 deaths; in India, the figures are 11,034,000 infections and 156,622 deaths; and in Bangladesh, the figures are 544,544 infections and 8,379 deaths (https://www.worldometers.info/coronavirus/?utm_campaign=homeAdvegas1). Current allopathic drugs that have been tried on COVID-19 patients with varying degrees of success include chloroquine/hydroxychloroquine with or without azithromycin, favipiravir, lopinavir plus ritonavir, and remdesivir (the list of drugs given is not all inclusive) (Rabbi, 2020). The drug ivermectin is also used and has been found to inhibit the replication of SARS-CoV-2 *in vitro* (Caly *et al*., 2020). In hospitalized patients with COVID-19, dexamethasone appears to reduce 28-day mortality among patients receiving invasive mechanical ventilation but not among patients not receiving respiratory support (Recovery Collaborative Group, 2020).

Herbal treatment is another option being tried mostly in China. Twenty-six herbs have been found in Traditional Chinese Medicine for treatment of breathing respiratory infections caused by the virus (Dudani & Saraogi, 2020). *In silico* screening of various plant-derived compounds (phytochemicals) particularly against ACE-2 and 3C-like protease is another line of approach taken by scientists in their efforts to discover an anti-COVID drug (Joshi *et al*., 2020). The objective of this study was to use molecular docking as the *in-silico* screening tool to find out the activity of some major phytochemicals of *Allium cepa* L. (onion) against 3C-like protease of both SARS and SARS-CoV-2 and their human ACE-2 receptor. The antiviral property of onion has been reported and reviewed (Sharma, 2019), which makes it an effective target to study against COVID-19.

MATERIALS AND METHODS

We have used the pdb file (6LU7) of the main protease of SARS-CoV-2 3C-like protease or SARS-CoV-2 $3CL^{pro}$ as published before (Liu *et al*., 2020). Inhibitor (N3) was removed from the pdb file before using the protein's structure in our molecular docking studies. The interacting residues of N3 with the protease amino acids included His41, Met49, Phe140, Leu141, Asn142, Gly143, His163, His164, Glu166, Leu167, Pro168, Gln189, Thr190, and Ala191. The active residues of SARS-CoV-2 3C-like protease are His41 and Cys145. Monomeric form of protein was used for molecular docking. The same protease from SARS (pdb: 3M3V) was used for docking studies with the same phytochemicals. The two proteases (SARS-CoV $3CL^{pro}$ and SARS-CoV-2 $3CL^{pro}$) share a 96% sequence identity and have a highly similar three-dimensional structure (Zhang *et al*., 2020).

Compounds used in docking studies

We have studied twenty-two major phytochemicals present in *Allium cepa*. Ligand molecules were downloaded from Pubchem (Ihlenfeldt, 2018) in sdf format. They were optimized with the force field type MMFF94 using Openbable software and saved as pdbqt format. Besides the twenty-two phytochemicals of *Allium cepa*, we have also used two experimental antiviral compounds lopinavir and nelfinavir as controls. Lopinavir and nelfinavir are antiviral drugs currently under testing in various clinical trials against COVID-19 (Bolcato *et al*., 2020; Meini *et al*., 2020).

Ligand molecular docking studies

Blind molecular docking was done using AutoDock Vina (Trott & Olson, 2010). In this form of docking, the target site is not taken into consideration or unknown and the ligand is docked to the whole surface of a protein. As such, a favorable protein-ligand complex pose needs several runs and energy calculations. The predicted binding affinity values were taken as an average of values from five independent runs of the docking program. The figures (Figures 1-6) show the docked poses of two phytochemicals bound to SARS, SARS-CoV-2 3C-like protease, and ACE-2 as obtained from PyMOL and displayed in Discovery Studio (Studio, 2015).

Three-dimensional structure of human ACE-2

The pdb structure of human ACE-2 (PDB ID: 1R42) was used as published elsewhere (Towler *et al*., 2004). Two domains comprise the extracellular region of human ACE-2. The first domain (residues 19-611) is a zinc metallopeptidase domain and is contained in the N-terminus region. Residues 612-740 form the second domain located at the C-terminus. The metallopeptidase domain can be further divided into catalytic sub-domains I and II; they form the two sides of a cleft, which is long and deep in the shape of a canyon. Residues 511-531 connect the two sub-domains and can thus be said to form the floor of the canyon. When an inhibitor binds to ACE-2, the movement of the sub-domains causes the cleft to close around the inhibitor (Towler *et al*., 2004).

Phytochemicals

Phytochemicals present in *Allium cepa* were obtained from a number of publications (Bora & Sharma, 2009; Bystricá *et al*., 2013; Uapadhyay, 2016; Marrelli *et al*., 2019) and their 3D structures were obtained from PubChem. It was not possible to cover all phytochemicals of *Allium cepa*; we concentrated essentially on the major phytochemicals of the plant.

Lipinski's rule of five

Lipinski's rule of 5 or Ro5 (Lipinski *et al*., 2001) was followed to evaluate any drug like properties of the twenty-two phytochemicals of *Allium cepa*. According to the rule, poorly absorbed molecules by intestinal wall, that is not showing drug-like behavior would show at least two or more violations of these physico-chemical properties: molecular weight over than 500, lipophilicity (log P >5), hydrogen-bond (HB) donor groups (expressed as the sum of OHs and NHs groups) more than 5, more than 10 HB acceptor groups (expressed as the sum of Os and Ns atoms), and molar refractivity outside a range of 40-130.

RESULTS AND DISCUSSION

Of the twenty-two phytochemicals of *Allium cepa* screened *in silico* for their binding affinities to 3C-like protease of SARS and SARS-CoV-2, and the human receptor for SARS and SARS-CoV-2, the angiotensin converting enzyme-2 or ACE-2, twelve compounds showed in molecular docking studies high predicted binding energies for all three proteins compared to the rest of the phytochemicals. The results are shown in Table 1. That the same compounds would show similar binding affinities to the 3C-like proteases of SARS and SARS-CoV-2 is not surprising, for the two proteases share a 96% sequence identity and are very similar in structure (Chen *et al*., 2020). However, the high binding energies of these twelve phytochemicals to ACE-2 were a surprise. Nevertheless, binding to 3C-like protease and ACE-2 can confer a sort of polypharmacological advantage to these phytochemicals. Binding of these phytochemicals to ACE-2 blocks the viral entry into the cell and also has the advantage of stopping the formation of non-structural proteins even if the virus has entered the cell.

The best binding energy (ΔG = kcal/mol) compared to the other phytochemicals and the two control antiviral drugs lopinavir and nelfinavir was demonstrated by the pentacyclic triterpenoid, oleanolic acid, which gave predicted binding energies, respectively, of -8.7, -9.3, and -9.8 kcal/mol to 3C-like protease of SARS-CoV-2 and SARS, and ACE-2. Other than this compound, the rest of the phytochemicals demonstrating predicted high binding energies belonged to the broad group of flavonoid class of compounds. Among the flavonoid compounds, cyanidin was relatively the most potent followed by (2*S*)-dihydrotricetin, a flavanone compound, and ampelopsin A, a flavonoid. The antiviral compounds used as controls, lopinavir and nelfinavir, also demonstrated high binding energies, particularly for ACE-2 with binding energies of -9.3 and -8.7 kcal/mol, respectively. The comparable binding energies of several *Allium cepa* phytochemicals (like oleanolic acid, cyanidin, (2*S*)-dihydrotricetin, and ampelopsin A) and the two antiviral drugs lopinavir and nelfinavir would suggest that a number of the phytochemicals of *Allium cepa* has potential therapeutic values against COVID-19. This is also borne out by the physico-chemical properties and number of violations of Lipinski's rule as shown in Table 2.

Figures 1-3 show the 2D and 3D interactions of oleanolic acid to amino acids of 3C-like protease of SARS-CoV-2 and SARS, and ACE-2, respectively. The non-bonding interactions of oleanolic acid and quercetin-4-*O*-β-glucoside to 3C-like proteases of SARS-CoV-2 and SARS, and ACE-2, respectively, are shown in Table 3. Like N3, oleanolic acid interacts with Met49 and Cys145 of SARS-CoV-2 3C-like protease. Additionally, it interacts with Ser144. Thus, it interacts with the first two domains of the protease comprising of residues 8-101 and residues 102-184, respectively. However, very surprisingly, despite the similarity between SARS and SARS-CoV-2 3C-like protease structures, the binding pattern of oleanolic acid to the SARS 3C-like protease is very different. With oleanolic acid, the interacting amino acids of this SARS 3C-like protease are Pro9, Ile152, Tyr154, Arg298 and Val303. These amino acids are predominantly hydrophobic such as

Table 1. Binding energy of *Allium cepa* phytochemicals to SARS and SARS-CoV-2 3C-like protease and SARS and SARS-CoV-2 human receptor ACE-2

Phytochemical	Binding energy (ΔG = kcal/mol)		
	SARS-CoV-2	SARS	ACE2
cis-3,4-Leucopelargonidin	-7.1	-7.0	-6.9
Cyanidin	-8.1	-8.5	-7.9
Diallyl disulfide	-3.4	-3.4	-2.9
Dimethyl trisulfide	-2.4	-2.5	-2.3
Flavon-3-ol	-6.8	-7.1	-7.4
Glycolic acid	-3.7	-3.8	-3.5
Isoalliin	-4.6	-4.7	-4.1
Leucocyanidin	-7.5	-7.2	-7.7
Leucodelphinidin	-7.4	-7.7	-7.7
(2*S*)-Dihydrotricetin	-7.4	-7.2	-8.5
Acetal	-4.0	-4.0	-3.5
Allicin	-3.4	-4.2	-3.3
Alliin	-4.4	-4.6	-4.3
Allylpropyl_disulfide	-3.6	-3.2	-3.2
Ampelopsin A	-7.5	-8.5	-8.2
Aromadendrin	-7.2	-7.2	-7.7
Oleanolic acid	-8.7	-9.3	-9.8
Pelargonidin	-8.0	-7.0	-7.0
Quercetin 3,4'-diglucoside	-7.9	-7.8	-7.8
Quercetin 4-*O*-β-glucoside	-7.5	-7.6	-7.6
Taxifolin	-7.5	-7.0	-7.5
Tricetin	-7.5	-7.1	-7.7
Lopinavir	-8.2	-8.1	-9.3
Nelfinavir	-8.1	-8.2	-8.7

Table 2. Physico-chemical properties of selected compounds *of Allium cepa*

Compounds name	Molecular weight	Number of H bond acceptors	Number of H bond donors	Log *P*	Molar refractivity	No. of violations
Cyanidin	287.24	6	5	-2.59	76.17	0
Leucocyanidin	306.27	7	6	1.19	75.50	1
Leucodelphinidin	322.27	8	7	1.29	77.52	1
(2*S*)-Dihydrotricetin	304.25	7	5	1.18	75.62	0
Ampelopsin A	470.47	7	6	2.07	128.34	1
Aromadendrin	288.25	6	4	1.42	72.73	0
Oleanolic acid	456.70	3	2	3.92	136.65	1
Pelargonidin	271.24	5	4	-2.29	74.15	0
Quercetin 3,4'-diglucoside	626.52	17	11	2.68	142.28	3
Quercetin 4-*O*-beta-glucoside	464.38	12	8	1.45	110.16	2
Taxifolin	304.25	7	5	1.30	74.76	0
Tricetin	302.24	7	5	1.33	78.04	0
Lopinavir	628.80	5	4	3.44	187.92	2
Nelfinavir	567.78	5	4	3.87	166.17	1

Table 3. Nonbonding interactions of oleanolic acid and quercetin-4-*O*-â-glucoside with 3C-like protease of SARS-CoV-2 **(**A**)**, SARS **(B)** and the human receptor ACE-2 **(C)**

Phytochemicals	Interacting amino acid residue(s)	Distance	Bond category	Bond type
A. SARS-CoV-2				
		Oleanolic acid		
	SER144	2.73	H	CH
	CYS145	2.33	H	CH
	CYS145	4.47	Hydrophobic	Alkyl
	CYS145	5.21	Hydrophobic	Alkyl
	MET49	4.67	Hydrophobic	Alkyl
		Quercetin 4-*O*-β-glucoside		
	ARG188	2.19	H	CH
	HIS163	2.14	H	CH
	GLN192	2.38	H	CH
	GLU166:O	3.26	H	C
	MET165	3.46	H	C
	GLU166	3.06	H	Pi-Donor Hydrogen Bond
	CYS145	5.93	Other	Pi-Sulfur
	CYS145	5.68	Other	Pi-Sulfur
	MET165	5.37	Other	Pi-Sulfur
	HIS41	5.12	Hydrophobic	Pi-Pi Stacked
	MET49	5.02	Hydrophobic	Pi-Alkyl
B. SARS				
		Oleanolic acid		
	ASN119	2.50	H	CH
	MET49	5.25	Hydrophobic	Alkyl
	CYS145	4.63	Hydrophobic	Alkyl
	MET165	4.69	Hydrophobic	Alkyl
	HIS41	4.99	Hydrophobic	Pi-Alkyl
	HIS41	4.34	Hydrophobic	Pi-Alkyl
		Quercetin 4-*O*-β-glucoside		
	ARG40	2.47	H	CH
	ARG40	2.37	H	CH
	GLU55	2.96	H	CH
	CYS85	2.99	H	CH
	PHE181	2.30	H	CH
	ASP187	2.49	H	CH
	ARG188	1.84	H	CH
	PRO52	2.81	H	CH
	PHE181	2.27	H	CH
	ASN84	3.47	H	C
	LYS180	3.37	H	C
	PRO184	3.52	H	C
	ARG188	3.19	H	C
	ASN84	3.41	H	C
	VAL186	3.64	Hydrophobic	Pi-Sigma
C. ACE-2				
		Oleanolic acid		
	ARG393	2.56	H	CH
	HIS378	3.57	H	C
	ALA348	3.12	H	C
	PHE40	3.64	Hydrophobic	Pi-Sigma
	PHE390	3.90	Hydrophobic	Pi-Sigma
	ALA348	4.36	Hydrophobic	Alkyl
	ARG393	3.43	Hydrophobic	Alkyl
	PHE40	5.00	Hydrophobic	Pi-Alkyl
	PHE40	4.12	Hydrophobic	Pi-Alkyl
	HIS378	4.77	Hydrophobic	Pi-Alkyl
	PHE390	4.92	Hydrophobic	Pi-Alkyl
	PHE390	4.73	Hydrophobic	Pi-Alkyl
	HIS401	4.50	Hydrophobic	Pi-Alkyl
	HIS401	4.08	Hydrophobic	Pi-Alkyl

Table 3 continued...

	Quercetin 4-*O*-β-glucoside		
TYR199	2.24	H	CH
SER511	2.52	H	CH
ARG514	2.95	H	CH
ARG514	2.94	H	CH
GLN102	1.76	H	CH
ASP206	2.25	H	CH
GLN98	2.12	H	CH
ASP206	2.26	H	CH
ASN394	2.25	H	CH
ASP509	2.06	H	CH
ASP509	2.51	H	CH
GLY205	3.25	H	C
ASP206	4.74	Electrostatic	Pi-Anion
TRP203	4.33	Hydrophobic	Pi-Pi T-shaped
TRP203	5.57	Hydrophobic	Pi-Pi T-shaped

CH = Conventional Hydrogen bond, H = hydrogen bond, C = carbon hydrogen bond.

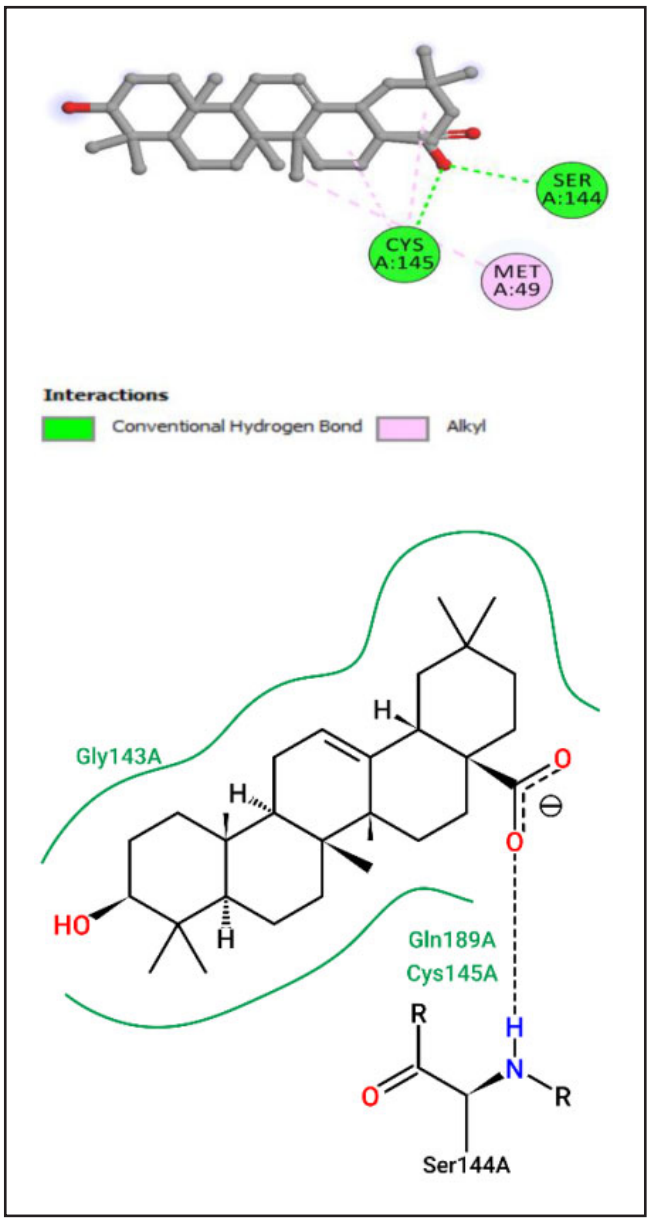

Figure 1. 2D, 3D depictions of interacting amino acids of SARS-CoV-2 3C-like protease with oleanolic acid.

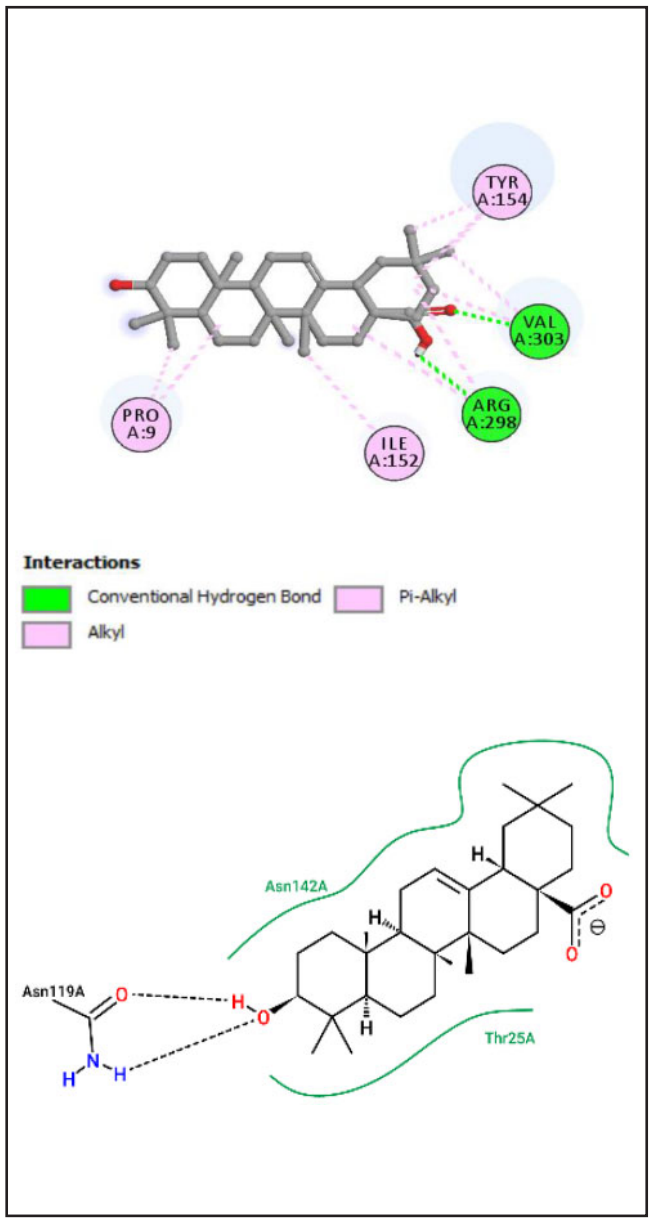

Figure 2. 2D, 3D depictions of interacting amino acids of SARS 3C-like protease with oleanolic acid.

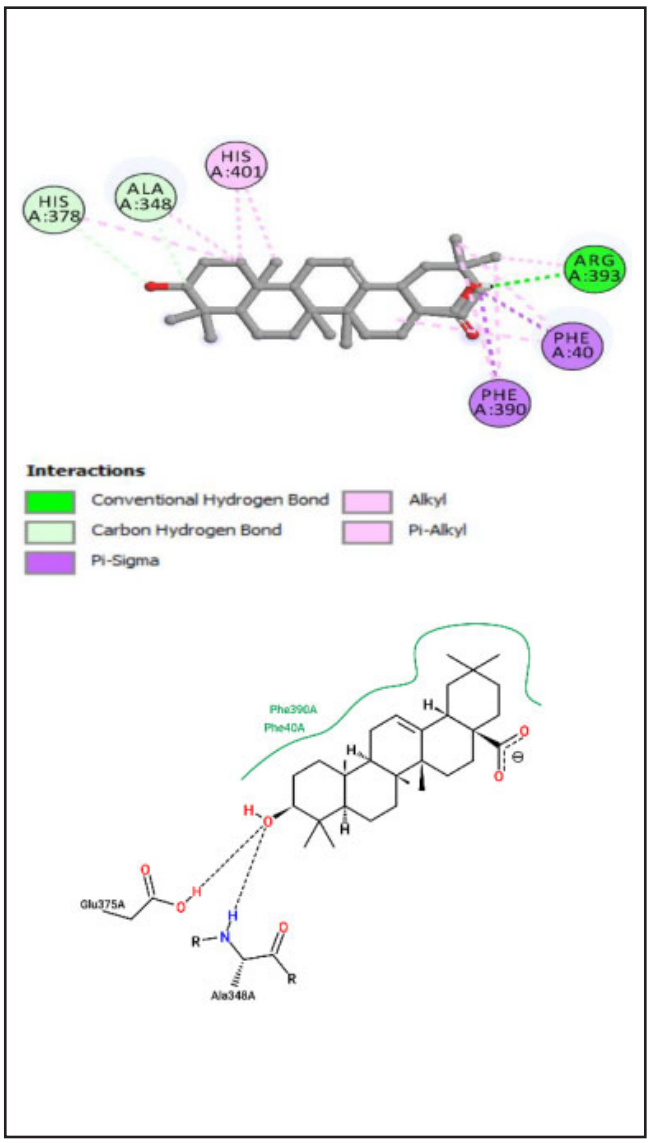

Figure 3. 2D, 3D depictions of interacting amino acids of ACE-2 with oleanolic acid.

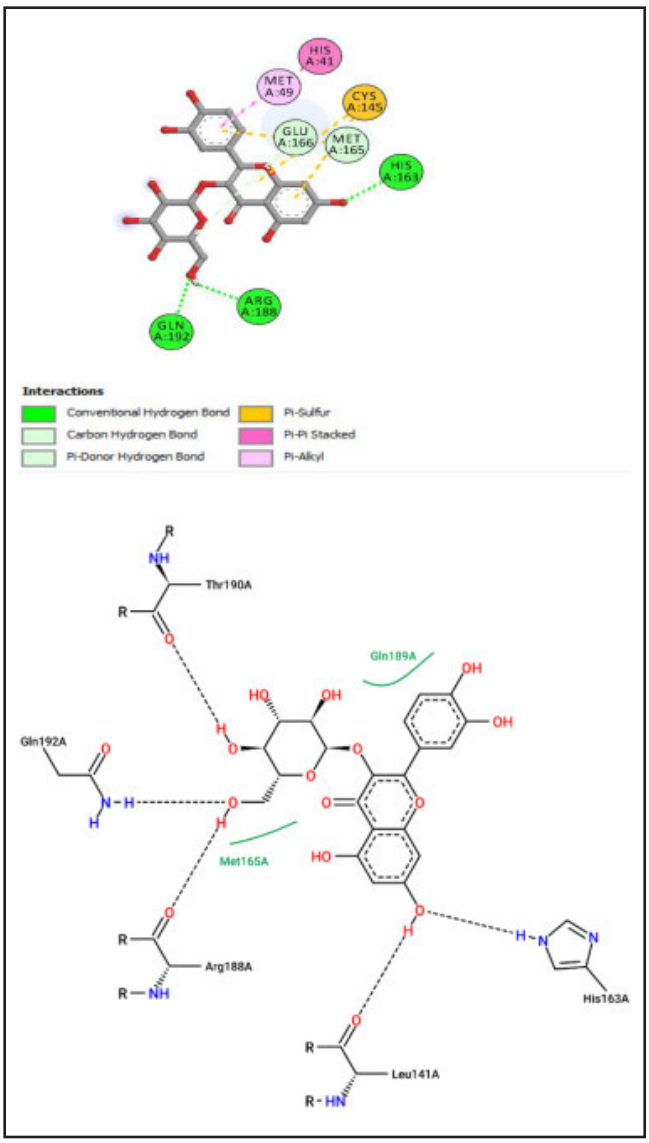

Figure 4. 2D, 3D depictions of interacting amino acids of SARS-CoV-2 3C-like protease with quercetin-4-*O*-β-glucoside.

Ile152, Tyr154 and Val303 and hold the hydrophobic oleanolic acid molecule more strongly (binding affinity = -9.3 kcal/mol) than the SARS-CoV-2 3C-like protease (binding affinity -8.7 kcal/mol). Binding of oleanolic acid to Pro9 of the first domain and also to the distal end of the second domain, additionally accounts for the higher binding energy of oleanolic acid to the SARS 3C-like protease than the SARS-CoV-2 3C-like protease. Regarding ACE-2, oleanolic acid binds to the N-terminus domain 1 amino acids, which are Phe40, Ala348, His378, Phe390, Arg393 and His401. The structure of the side chains of these amino acids reveals the strong role of amino groups. Arginine has a guanidinium side chain that forms a hydrogen bond with the carboxylic group while the imidazole ring of histidine forms hydrophobic interactions with the A ring of the phytosterol giving it a binding energy of -9.8kcal/mol. Taken together, the binding of oleanolic acid to 3C-like protease of SARS-CoV-2 and its receptor ACE-2 makes this compound a potentially good inhibitor of SARS-CoV-2 entry and replication and a therapeutic for COVID-19.

Figures 4-6 show the 2D and 3D of quercetin-4-*O*-β-glucoside to 3C-like proteases of SARS-CoV-2 and SARS, and ACE-2, respectively. Quercetin is a hydrophobic molecule that has several rings to contribute to the hydrophobic interactions and oxygen groups that can form electrophilic interactions. With the 3C-like protease of SARS-CoV-2, interactions were noted with amino acids His41, Met49, Cys145, His163, Met165, Glu166, Arg188 and Gln192. Similar to oleanolic acid, amino acid interaction patterns of SARS 3C-like protease with quercetin-4-*O*-β-glucoside were quite different from COVID-19. In the case of SARS, the interacting amino acids were polar-like with Gln110, Thr111, Asn151, and Thr292 forming the hydrogen bonds while the molecule was held hydrophobically by Ile249, Pro293 and Phe294. Interestingly, quercetin-4-*O*-β-glucoside interacts with both domains 1 and 2 of SARS-CoV-2 3C-like protease via hydrophobic and pi-interactions, and domain 2 of SARS 3C-like protease via hydrophobic and hydrogen bonding. The reason for the observed interactions could be the energetically stable docked pose of quercetin as the binding energy difference is almost similar (Table 1). With ACE-2, quercetin-4-*O*-β-glucoside interacts with the N-terminus residues, namely Ser105, Tyr199, Tyr202, Trp203, Ser511, and Arg514, the latter two amino acids being regarded as the floor amino acids between two clefts of the canyon.

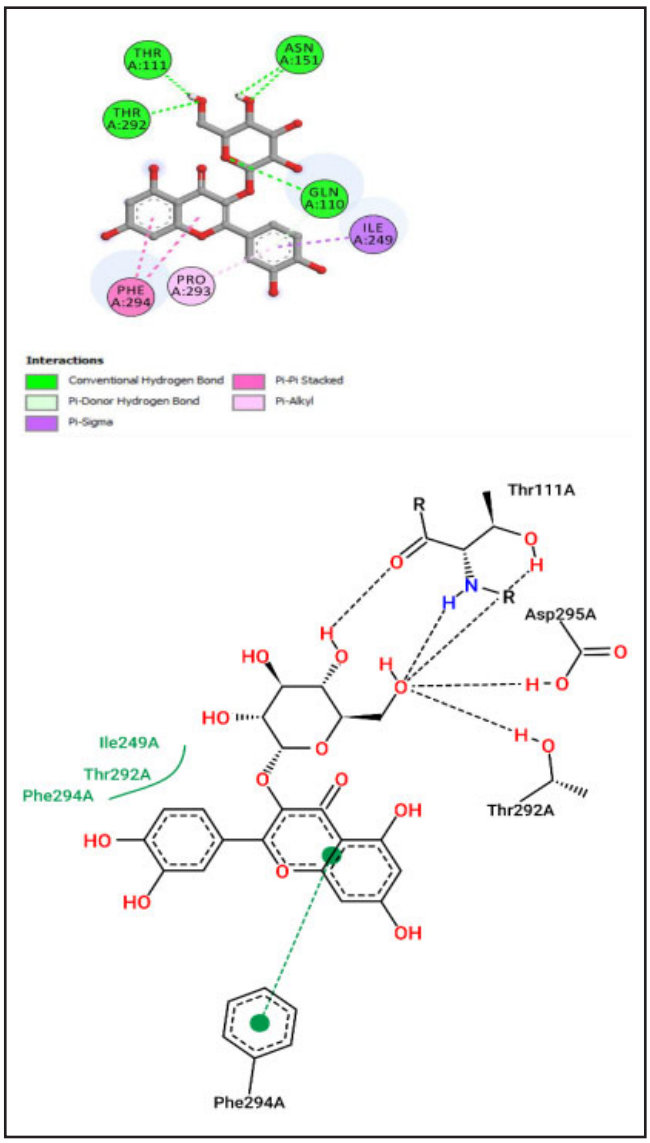

Figure 5. 2D, 3D depictions of interacting amino acids of SARS 3C-like protease with quercetin-4-*O*-β-glucoside.

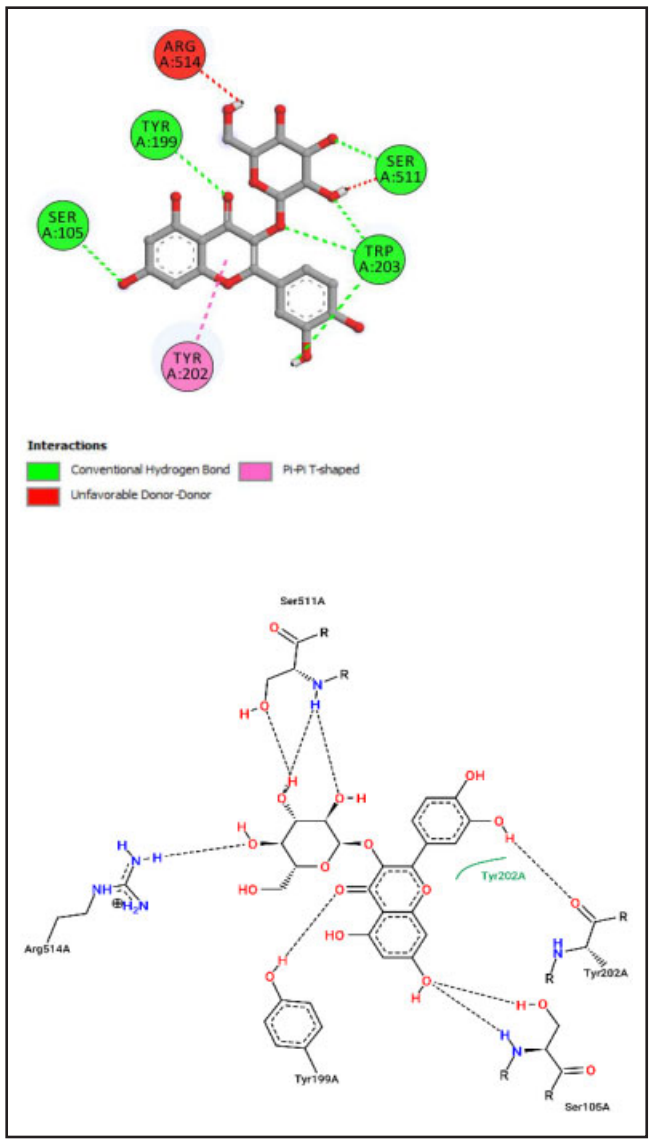

Figure 6. 2D, 3D depictions of interacting amino acids of ACE-2 with quercetin-4-*O*-β-glucoside.

CONCLUSION

In silico studies through molecular docking indicate that triterpenoid and flavonoid compounds present in *Allium cepa* have good and promising binding affinities for the 3C-like protease of SARS and SARS-CoV-2, and the receptor for the two viruses, namely the human ACE-2 protein. In fact, one of the phytochemicals, oleanolic acid gave better binding results than the two antiviral drugs used as controls, namely lopinavir and nelfinavir. An important aspect of the present study is the 'polypharmacology' nature of most of the high binding energy demonstrating phytochemicals (high binding energy compared to the other phytochemicals of *Allium cepa* evaluated in this study) in that they can bind to both the main protease of SARS and SARS-CoV-2, as well as ACE-2. This dual interaction is important for their probability as future therapeutics. Such polypharmacology effect is not uncommon; the drug metformin, besides being used for Type 2 diabetes mellitus, has anti-tumor, anti-aging, cardiovascular protective, and neuroprotective effects (Wang *et al*., 2017). This study has therefore its values, but molecular docking is a predictive work and the wet experimental laboratory antiviral studies need to be further investigate to validate these *in silico* findings.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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