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Full Length Article

Berries anthocyanins as potential SARS-CoV–2 inhibitors targeting the viral attachment and replication; molecular docking simulation ☆

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ABSTRACT

The viral respiratory disease, severe acute respiratory syndrome (SARS), has turned into a global health concern. Till now, there is no drug or vaccine has yet been specifically approved for SARS-CoV-2. One of the urgent solutions against the recent COVID-19 disease is the use of dietary molecules, which can be found abundantly in functional food. In the current study, we have conducted a molecular docking approach for eighteen dietary molecules belong to the subclass of anthocyanins, as potential inhibitors of the main protease and spike glycoprotein of SARS-CoV-2. Both selected targets, playing a vital role in attachment and replication of the virus. The results indicated that cyanidin-3-arabinoside exhibited the lowest binding energy and located onto the pocket through a sufficient number of hydrogen bonds with the main protease virus. However, pelargonidin-3-glucoside and pelargonidin 3-rhamnoside display significant binding energy with the spike glycoprotein of SARS-CoV-2. All compounds mentioned above shown high drug-likeness and fulfils the Lipinski's rule of five, as well as confer favorable toxicity parameters, in addition to ADME values. Considering the obtained results, regular consumption of berry fruits, which are rich in anthocyanin compounds, should be supportive to inhibit viral infectious by reducing of propagation and pathogenicity of SARS-CoV–2.

1. Introduction

The viral respiratory disease, severe acute respiratory syndrome (SARS), has turned into a global health problem [1]. The infected cases increasing daily, at the times of writing, this number has now exceeded 12,5 million, with 560 k confirmed deaths. The COVID-19 pandemic is related to the coronavirus named SARS-CoV-2, which is an enveloped, crowned (+) single-stranded RNA viruses, belongs to the *Coronaviridae* family [2]. Several protein molecules encoded by the viral genome, exhibit a vital role in the virulence and replication of SARS-CoV-2, among them the main protease known as 3CLpro (Mpro), which have a crucial role in the replication of the polyproteins that translated from the viral RNA [3]. Another protein that plays a key role in viral attachment, fusion, and entry of SARS-CoV-2, is the spike glycoprotein (S-protein). The receptor-binding domain (RBD) of S-protein binds strongly to the human angiotensin-converting enzyme 2 (ACE2) receptors of lung cells [4]. These two proteins, main protease, and spike protein may be a promising drug target to build up vaccines and therapeutic agents to control the new coronavirus (COVID 19) infection. Till now, no vaccine or drug has yet been specifically approved for SARS-CoV-2. The conventional approaches for the development of a new effective therapies and vaccines against the COVID-19 are very costly and require at least one year before the first vaccine is approved and available for widespread use. One of the urgent solutions that have a great global interest among researchers for the treatment and prevention of recent COVID-19 disease is the use of dietary molecules, which can be found abundantly in functional food [5]. Numerous dietary molecules such as epigallocatechin gallate (EGCG), kaempferol,

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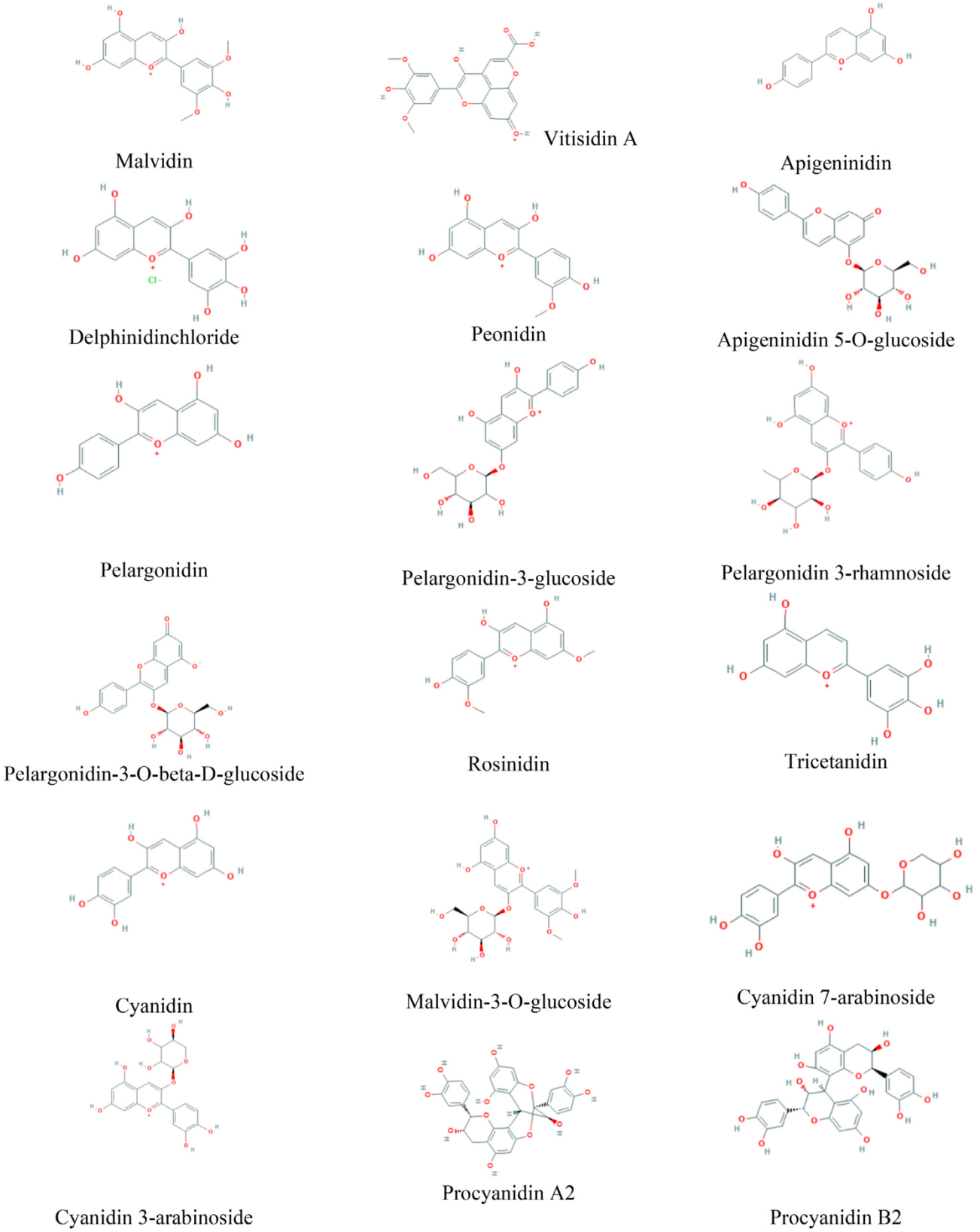


Fig. 1. Structures of 18 anthocyanins phytoligands.

catechin, savinin, curcumin, and betulinic acid has been identified by molecular docking studies, as an effective anti-SARS-CoV-2 agent [6,7]. Several studies reported that anthocyanins are the main components in strawberry and black raspberry [8]. Mazza and Miniati (1993) have mentioned a range of 25 to 495 mg/100 g anthocyanins for high bush blueberries [9]. Antho-

Table 1
Binding energy values of 18 anthocyanins phytoligands against two SARS-CoV2 proteins.

Compounds	Targets proteins; binding energy (kcal/mol)	
	6LU7	RBD of S-Protein
Malvidin	−6.9	−6.4
Vitisidin A	−7.8	−6.5
Apigeninidin	−7.7	−6.5
Delphinidinchloride	−7.3	−6.5
Peonidin	−6.8	−6.4
Apigeninidin 5-O-glucoside	−8.2	−6.7
Pelargonidin	−7.6	−6.2
Pelargonidin-3-glucoside	−8.3	−6.7
Pelargonidin-3-rhamnoside	−8.6	−6.9
Pelargonidin-3-O-beta-D-glucoside	−8.2	−6.9
Rosinidin	−6.2	−6.3
Tricetanidin	−7.4	−6.5
Cyanidin	−7.3	−6.4
Malvidin-3-glucoside	−7.8	−6.4
Cyanidin 7-arabinoside	−8.00	−6.9
Cyanidin-3-arabinoside	−9.1	−6.7
Procyanidin A2	−9.2	−7.2
Procyanidin B2	−9.3	−7.0
Darunavir	−7.5	−7.7
Hydroxychloroquine	−5.6	−5.7

cyanins as dietary molecules are members of a large group of secondary metabolites secreted by plants, called polyphenols, which are categorized into four categories: flavonoids, phenolic acids, lignans, and stilbenes.

Flavonoids are subdivided into four subclasses, anthocyanins, flavonols, flavones, and flavanones [10]. This subclass was selected because several studies have shown that anthocyanins compounds display diverse beneficial effects, including anti-diabetes, anti-cancer, anti-allergy, anti-mutagenesis, cardioprotection, and antimicrobial effects. Several anthocyanins display also a good antiviral activity [8,11]. As far as we know, no available research on the evaluation of anthocyanins from berries using computational modeling against SARS-CoV-2 main protease (PDB: 6lu7) and SARS-CoV-2 spike glycoprotein (PDB: 6lzg). Molecular docking is promising tool for discovery of new therapeutic drug candidates against COVID-19, with this method we can analyze the orientation and conformation (stated together as the "pose") of molecules into the binding sites of a macromolecular targets [12]. Several literature reported about the use of molecular docking simulation to assess or predict the drug-target binding affinity with the viral protease [13–21]. Using this method, we have screened eighteen dietary molecules belong to the subclass of anthocyanin, which represent the main compounds in several berry fruits, as potent inhibiting agents of the main protease and targeting the receptor-binding domain (RBD) of S-protein for SARS-CoV2. Furthermore, molecular docking allocates swift recognition of the amino acid sequences throughout SARS-CoV-2 [22–24]. The docking outcomes were optimistic and suggested a probable inhibition against the SARS-CoV-2.

2. Materials and methods

2.1. Proteins and chemical compounds

As molecular targets, two important proteins for SARS-CoV2 infection were nominated. This including the Main Protease (Mpro) (also called 3C-like protease – 3CLpro), which represents a pivotal role in the propagation of SARS-CoV–2. The crystal structure of this enzyme in complex with the inhibitor N-[(5-Methylisoxazol-3-Yl) Carbonyl]Alanyl-Lvalyl-N1-((1r,2z)-4-(Benzyloxy)-4-Oxo-1-{[(3r)-2-Oxopyrrolidin-3- Yl]Methyl}But-2-Enyl)-L-Leucinamide, (N3), was obtained from the protein data bank (PDB), (PDB code: 6lu7), in PDB formats. However, the novel coronavirus spike

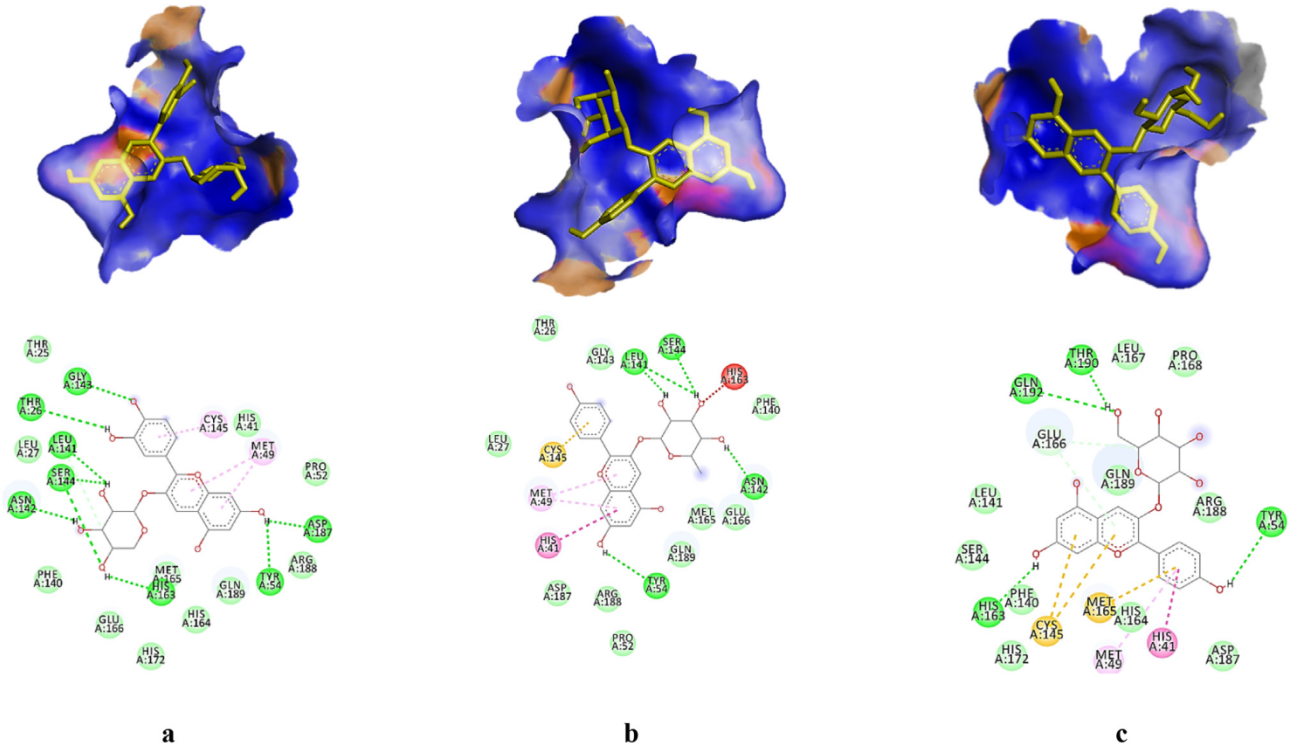


Fig. 2. Interaction of the compounds, cyanidin 3-arabinoside (a), pelargonidin 3-rhamnoside (b) and pelargonidin-3-glucoside (c) in the binding cleft of 6lu7 of COVID-19 shown in 3D and 2D representation.

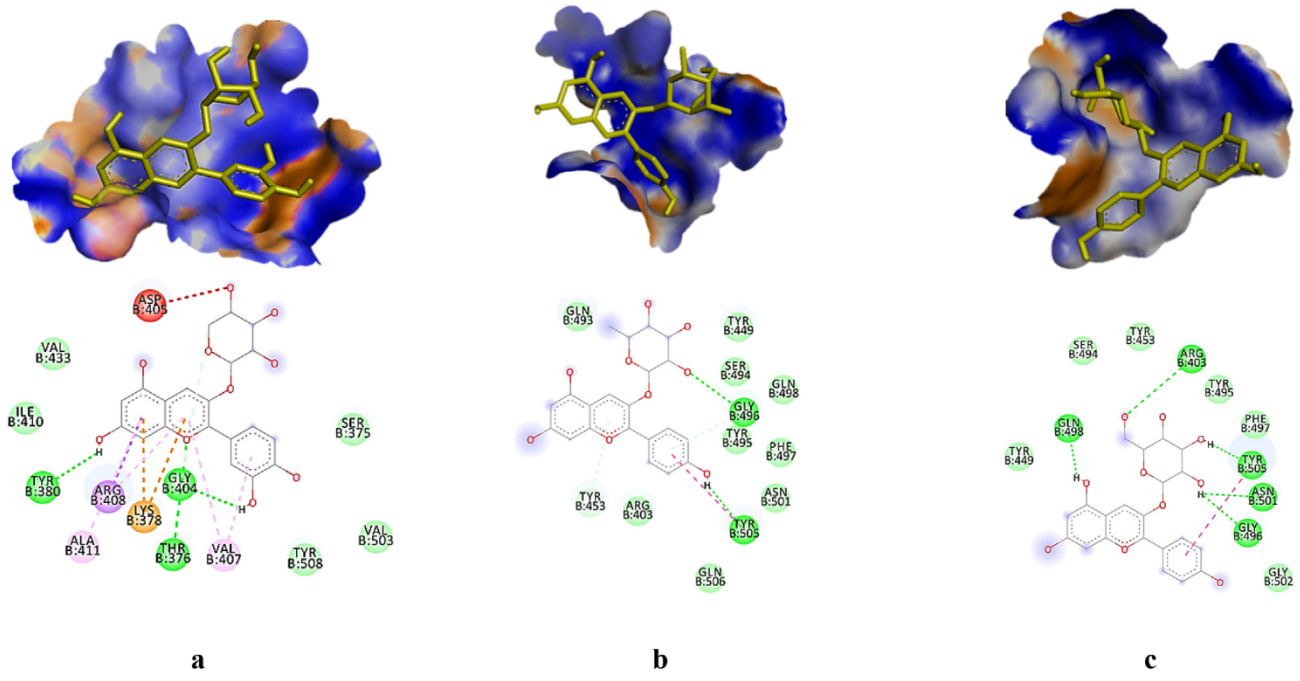


Fig. 3. Interaction of the compounds, cyanidin 3-arabinoside (a), pelargonidin 3-rhamnoside (b) and pelargonidin-3-glucoside (c) in the binding cleft of S-protein of COVID-19 shown in 3D and 2D representation.

receptor-binding domain complexed with its receptor ACE2 was gained from the Research Collaboratory for Structural Bioinformatics (RCSB) protein data bank (PDB ID: 6lzg). A library of eighteen compounds belongs to all the subclass of anthocyanins as displayed in Fig. 1 were selected and screened against both protein targets (main protease and spike protein).

2.2. Protein and ligands preparation

The preparation of the two targets proteins, 6lu7 and 6lzg, used in this study, was performed, through BIOVIA Discovery Studio Visualizer 2020. The water molecules were removed from the crystal structure of the two proteins, along with the original ligand that attached to the target protein 6lu7. However, to prepare the ligand, 6lzg, the hACE2 protein (part A) was removed from the complex PDB file, Spike Protein RBD-hACE2. Both receptors were stored in the (.PDB) format. The second step of preparation was performed on AutoDock Tools 1.5.6, by the addition of polar H- atoms and Kollman charges, then, the ligands were saved as PDBQT files. Ligands preparation was done by energy minimization using the Gasteiger algorithm, detecting root, and set the number of torsions.

2.3. Molecular docking

Molecular docking analysis was done using AutoDock Tools 1.5.4, to evaluate the lowest binding energy, between the ligands and the target proteins (6lu7 and RBD of S-protein). The same program was used also to obtain the three-dimensional grid box for docking simulation in which the box with a size of 40 × 40 × 40, at 0.742 Å was generated and centered at (x = 13.544, y = 12.198, z = 67.681) around hotspot residues of the active site of the target 6lu7. Furthermore, the grid box for RBD of S-protein was centered at (x = −36.921, y = 30.66, z = 2.967) with a spacing of 0.503 Å and dimensions of 40 × 84 × 40. The grid spacing value of 0.503 Å is employed instead of the default value of 0.375 Å, due to the grid box obtained when we use the spacing value of 0.375 Å is not a sufficient space to cover all residues of the active site of 6lu7. However, the grid spacing value of 0.503 allow to cover all amino acid exist in the active site of this enzyme.

After the successful execution of AutoGrid, default docking parameters were employed as follows: i) the number of GA runs: 10; ii) population size: 150; iii) the number of energy evaluations: 2.5 million (2.0 Å clustered tolerance); and iv) the number of generations: 27000. The Lamarckian genetic algorithm was used and the output was saved in docking parameter file (DPF) file format. The predicted binding poses for each compound were processed by the built-in clustering analysis (1.0 Å RMSD tolerance), and the lowest energy conformation from the largest cluster was selected as representative. Discovery Studio and PyMOL were implemented to visualize and scrutinize the interaction between the ligand fragments with the viral protein (3CLpro and RBD of S-protein).

2.4. Evaluation of drug-likeness, ADME/toxicity properties

To determine the effect of an active substance after its administration in the human body, it is essential to recognize their absorption, metabolism, excretion, and distribution (ADME), also called pharmacokinetic properties of the drug [25,26]. Early ADME estimation greatly decreases the fraction of pharmacokinetics-related failure in the clinical stages [27]. Five biochemical features have a great influence on ADME proprieties of the drug, this so-called Lipinski's rule of five, which outlined the relationship between physicochemical and pharmacokinetics properties. Lipinski's rule of five [28], along with other important parameter used to assess drug-likeness and toxicity of hit compounds, such as Veber's rule [29], bioactivity score, polar surface area (TPSA), solubility (logs), synthetic accessibility (SA) bioavailability score, inhibition of any CYP alleles and blood–brain barrier (BBB) permeant, has been evaluated using the free web tool SwissADME (Molecular Modeling Group, Swiss Institute of Bioinformatics) [27], and molinspiration tool.

3. Results and discussion

3.1. Molecular docking using AutoDock

The sequence of SARS-CoV-2 main protease (PDB: 6lu7) and spike glycoprotein (PDB: 6lzg) was obtained as a PDB format. The model validated before conducting the molecular docking simulation, then the ligands were subjected to energy minimization to obtain the optimized active geometries. Energy minimization was conducted with current forcefield charges using Amber10: EHT forcefield, cutoff (8.10 Å), R-field solvation, a distance-dependent dielectric constant of 4.0, and time scaling of 100 ns [24]. Docked complexes subjected to 500 iterative cycles with a radius offset of 0.4, and a gradient of 0.01[24]. The ligands were designated based on the obtained scores and their binding modes [30]. Virtual screening was carried from a library of eighteen Phyto ligands compounds, belong to the subclass of anthocyanins to distinguish the potential inhibitors of COVID-19 main protease (PDB ID: 6LU7) and the receptor-binding domain (RBD) of SARS-CoV-2 spike protein. Based on AutoDock results (Table 1), out of 18 compounds used in the present study, procyanidin B2 exhibited the lowest docking score with the main protease (3CLpro) (−9.3 kcal/-mol) active sites, followed by procyanidin A2 (−9.2 kcal/mol) and cyanidin-3-arabinoside (−9.1 kcal/mol). The three glycosylated pelargonidin display highest docking scores when compared with the aglycone compound pelargonidin, furthermore, the nature of sugar moieties attached to pelargonidin seems important pelargonidin substituted at position 3 with rhamnoside display high score (−9.1 kcal/mol) when compared to pelargonidin-3-glucoside (−8.3 kcal/mol) and pelargonidin-3-O-beta-D-glucoside (−8.2 kcal/mol). All the selected compounds (pelargonidin-3-glucoside, pelargonidin 3-rhamnoside, pelargonidin-3-*o*-beta-D-glucoside, cyanidin-3-arabinoside, procyanidin A2, procyanidin B2) shown docking binding energy higher than the recently used inhibitor (darunavir) for COVID-19 main protease. The molecular docking outputs, reveals the probable use of *Salvadora persica* flavonoids (kaempferol and isorhamnetin derivative), which are structurally closest to the class of anthocyanin, to inhibit SARS-CoV-2main protease [6]. The host cell receptor ACE2, interact with the SARS-CoV-2, via its Spike Glycoprotein. The inhibition of this interaction, using natural product sources, can prevent the virus infection [31]. Several studies selected the target, Spike glycoprotein, for virtual screening research rather than the ACE2 protein, since this latest is expressed in numerous human cells types, and consequently, targeting ACE2 results in various side effects, such as modulatory effect on blood pressure, and several other cardiovascular system-related side effects [32–34]. The values of binding affinity interaction, between the active target site, RBD of S-protein, and the 18 Phyto ligands used in this study, ranging from, −6.2 kcal/mol, for the ligand pelargonidin, to −7.2 kcal/mol, for the ligand procyanidin A2, followed by procyanidin B2 (−7.0 kcal/mol) as shown in Table 1. All anthocyanins ligands used in this study displayed Vina docking scores better than hydroxychloroquine. In the molecular docking, the least binding energies revealed the stronger docking between ligands and viral targets [35].The types of molecular interaction, particularly, H-bonds and hydrophobic interaction, involved between the ligands molecules and the residues of the active site of both target proteins (6lu7 and RBD of S-protein) have been studied, because they organize the shape and stabilization of the docking molecules [36]. With the Mpro target, the ligand cyanidin-3-arabinoside formed nine hydrogen bonds with eight different residues: Gly143, Thr 26, Leu 141, Ser 144,

Table 2
Lipinski's Rule of Five for ADME analysis of the top scoring phytoligands anthocyanins.

Compounds	Lipinski's rule of five		
	Properties	Value	violations
Pelargonidin-3-glucoside	Molecular weight (<500 Da)	433.39 g/mol	1
	LogP (<5)	−0.76	
	H-Bond donor (<5)	7	
	H-bond acceptor (<10)	10	
	Rotatable bonds (⩽5)	4	
Pelargonidin 3-rhamnoside	Molecular weight (<500 Da)	417.39 g/mol	1
	LogP (<5)	−0.21	
	H-Bond donor (<5)	6	
	H-bond acceptor (<10)	9	
	Rotatable bonds (⩽5)	3	
Pelargonidin-3-O-beta-D-glucoside	Molecular weight (<500 Da)	431.37 g/mol	1
	LogP (<5)	−0.11	
	H-Bond donor (<5)	5	
	H-bond acceptor (<10)	10	
	Rotatable bonds (⩽5)	4	
Cyanidin 7-arabinoside	Molecular weight (<500 Da)	419.36 g/mol	1
	LogP (<5)	−0.86	
	H-Bond donor (<5)	7	
	H-bond acceptor (<10)	10	
	Rotatable bonds (⩽5)	3	
Cyanidin 3-arabinoside	Molecular weight (<500 Da)	419.36 g/mol	1
	LogP (<5)	−0.73	
	H-Bond donor (<5)	7	
	H-bond acceptor (<10)	10	
	Rotatable bonds (⩽5)	3	
Procyanidin A2	Molecular weight (<500 Da)	576.50 g/mol	3
	LogP (<5)	1.60	
	H-Bond donor (<5)	9	
	H-bond acceptor (<10)	12	
	Rotatable bonds (⩽5)	2	
Procyanidin B2	Molecular weight (<500 Da)	578.52 g/mol	3
	LogP (<5)	1.39	
	H-Bond donor (5)	10	
	H-bond acceptor (<10)	12	
	Rotatable bonds (⩽5)	3	

Asn 142, His 163, Tyr 54, Asp 187, which indicate strong and stable interaction between this ligand and the active site of the main protease, however, two amino acid, Cys 145, Met 49, are involved in pi-alkyl interaction with the same compound as illustrated in Fig. 2.

The residues, Thr 26, Cys 145, Asn 142, Leu 141, at the protease active site form H- bonds with the ligand, procyanidin A2. Furthermore, six other residues are involved in forming unfavorable donor-donor (His 163), $\pi-\pi$ T-shaped (His 41), and π-Alkyl interaction (Met 165, Cys 145) withe the same ligand procyanidin A2. In addition, procyanidin B2 having a smaller number of hydrogen bond interaction as compared with, procyanidin A2 and cyanidin-3-arabinoside, in fact, only two hydrogen bond is observed between this compound and the two amino acid, Ser 144 and Leu 141 of Mpro. On the other hand, another interaction is observed between the compound procyanidin B2 and the target active site of 6lu7, this including unfavorable donor-donor (His 163, Glu 166), PI-PI T-Shaped (His 41), Pi-Alkyl (Met 165, Cys 145), and (π-σ interaction: Met 49).

The residue, Tyr 54, at the active site of the Mpro participates in the formation of hydrogen bonds with the three compounds, pelargonidin 3-rhamnoside, pelargonidin-3-glucoside, and pelargonidin-3-O-beta-D-glucoside. Four more hydrogen bond interaction were observed between the residues, Leu 141, Ser 144, Asn 142, and the monosaccharide, rhamnoside, of the compound pelargonidin 3-rhamnoside.

In addition to the amino acid Tyr 54, the compound pelargonidin-3-glucoside, interacted by two additional hydrogen bonds with the residues, Gln 192, Thr 190, of Mpro (Fig. 2). Rather than Tyr 54, no more hydrogen bond interaction was observed between the compound pelargonidin-3-*o*-beta-D-glucoside and the target 6lu7. The crystal structure of the main protease (Mpro), in complex with the peptide-like inhibitor (N3) was available in the Protein Data Bank (PDB: 6lu7). Several residues of Mpro is involved in the interaction with the target N3, including His41, Met49, Phe140, Leu141, Gly143, SER144, Cys145, His163-164, Met165, Glu166, Pro168, His172, Asp187, Arg188, Gln189, Thr190, Ala191 [3]. In our case, the top-ranked compounds were bound to 3CLpro through interaction with the suitable amino acids, which is in a great compliance with published scientific literature [37,38]. Previous studies show that COVID-19 virus Mpro, along with SARS 3CLpro, has a Cys–His catalytic dyad (His-41 and Cys-145) [39].

The six top-ranked compounds (cyanidin-3-arabinoside, procyanidin A2, procyanidin B2, pelargonidin 3-rhamnoside, pelargonidin-3-glucoside, and pelargonidin-3-O-beta-D-glucoside) showed higher docking scores and stronger binding energy with Cys-145 and His-41 residues, which represent the catalytic dyad of the active site of 3C-like protease.

Analyzing the interacting residues between the top-most binding compounds, which showed the lowest docking score with RBD of S-protein, Table 1, indicates that pelargonidin-3-glucoside, interact via five hydrogen bonds with the amino acids, Arg 403, Gln 498, Tyr 505, Asn 501, Gly 496, and one $\pi-\pi$ stacked bond with Tyr 505 as displayed in Fig. 3. However, the compound, procyanidin A2 interacted with RBDof S-protein through hydrogen bonding with the amino acids, Tyr 449 and Asn 450, and formed: unfavorable donor-donor bond with Phe 490, π-anion bond with Glu 484, pi-sigma bond with Leu 452, and $\pi-\pi$ stacked bond with Phe 490. Likewise, the ligand pelargonidin 3-rhamnoside, interacted by two hydrogen bonds with the amino acids (Tyr 505, Gly 496), and exhibited $\pi-\pi$ stacked bond with, Tyr 505, and π-donor hydrogen bond with Tyr 453, Gly 496 (Fig. 3).

Furthermore, the ligand cyanidin-7-arabinoside formed hydrogen bonds with, Glu 471, Ser 471, Ser 459, and π-cation/ anion bonds with, Asp 467 and Arg 457, along with one π-alkyl bond with Lys 458. Moreover, a single hydrogen bond was found between the residue Gly 496 and procyanidin B2. The same amino acid (Gly 496), along with the amino acid Gly 502, Arg 403, Asn 501, are involved in unfavorable donor-donor/acceptor-acceptor bond with the compound procyanidin B2, in addition to one $\pi-\pi$ t-shaped bond with the amino acid Tyr 505.

The SARS-CoV-2 spike glycoprotein uses many residues to contact the bottom side of the small ACE2 lobe, including: Try449, Tyr453, Asn487, Tyr489, Gly496, Thr500, Gly502, Tyr505, Leu455, Phe456, Phe486, Gln493, Asn501, Lys417, Gln474 and Gln498 [4]. The ligands, pelargonidin-3-glucoside, procyanidin B2, and pelargonidin 3-rhamnoside, by targeting the amino acids involved in the interaction between the S-protein and ACE2, they can disturb the attachment of the virus SARS-Cov-2 with the host's cell receptor ACE2. Consequently, these compounds can prevent the virus infection. Cyanidin-7-arabinoside did not exhibit any interaction with these key residues. For good interaction between the ligands and the S-protein, the presence of sugar moieties appears to be significant. The most scored-ligands (pelargonidin 3-rhamnoside, pelargonidin-3-O-beta-D-glucoside, and cyanidin-7-arabinoside) has the presence of monosaccharides group attached in their structures, consequently, it can attach with high affinity to the glycosylated part of the S-protein. On the other hand, the affinity is lower with the glycosylated anthocyanidins ligands (avidin, apigeninidin, peonidin, rosinidin, tricetanidin, cyanidin), the same remarque was raised by Oliveira et al. [40].

3.2. Drug-likeness analysis and cytotoxicity

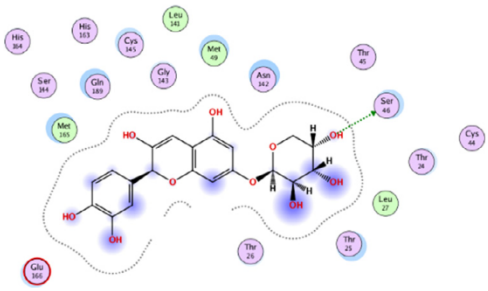
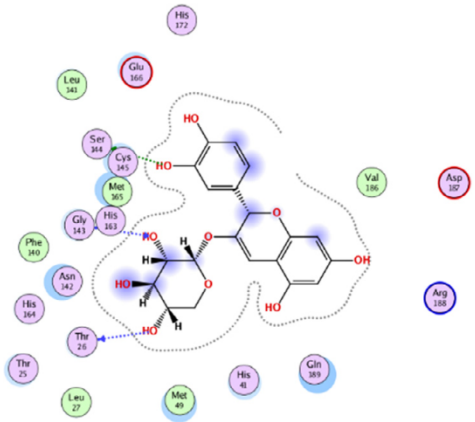
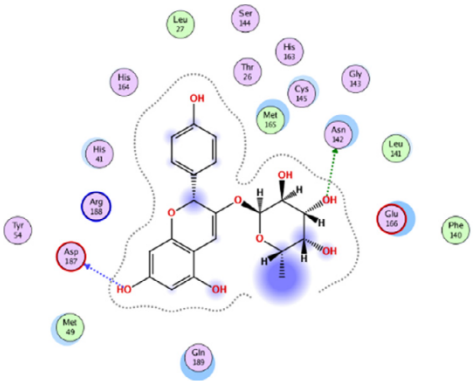
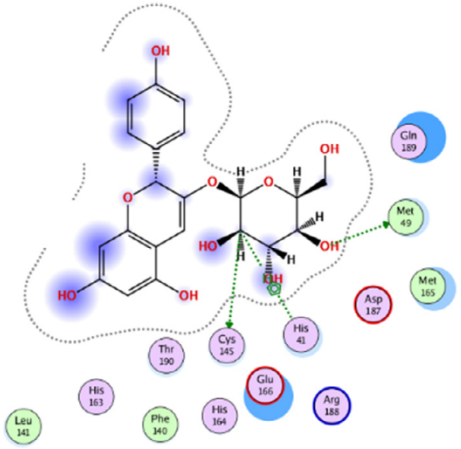
Drug-likeness evaluation of the best-scored anthocyanin compounds was carried out using Lipinski's Rule of Five that covers, lipophilicity (reported as octanol-water partition coefficient, and

Table 3
Drug-likeness properties and toxicity parameters of the best docking anthocyanins.

Drug Likeness properties	1	2	3	4
Lipinski	Yes	Yes	Yes	Yes
Veber	Yes	No	No	No
TPSA (Å^2)	173.21	152.98	172.88	173.21
Solubility (logs)	−1.52	−2.1	−2.69	−1.36
Synthetic accessibility	5.23	5.19	5.25	4.99
Bioavailability score	0.55	0.55	0.11	0.55
Inhibition of any CYP alleles	0	0	0	0
blood–brain barrier (BBB) permeant	No	No	No	No
Kinase inhibitor	No	No	No	No
Protease inhibitor	No	No	No	No
GPCR ligand	No	No	No	No
Ion channel modulator	No	No	No	No
Nuclear receptor ligand	No	No	No	No

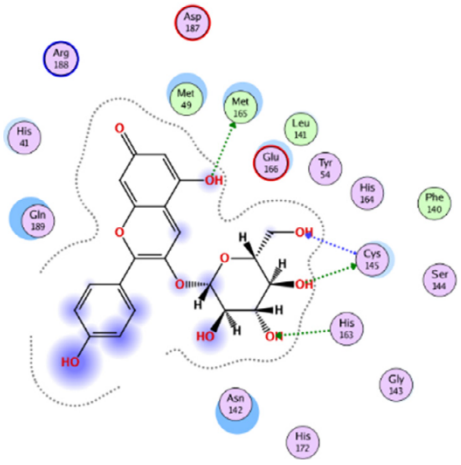
Pelargonidin-3-glucoside **(1)**, Pelargonidin 3-rhamnoside **(2)**, Pelargonidin-3-O-beta-D-glucoside **(3)**, Cyanidin-3-arabinoside **(4)**.

Table 4
Summary of docking interactions in the case of 6lu7.

#	Ligand	Ligand/receptor docking Interaction	Docking Score	Interpretation
1	Cyanidin 7-arabinoside		−6.5	H-bond and π−πinteraction with Ser46
2	Cyanidin-3-o-alpha-arabinoside		−7.5	H-bond and π−πinteraction with Thr 26, Ser 144, Cys 145, Gly 143, and His 163
3	Pelargonidin 3-rhamnoside		−7.4	H-bond and π−πinteraction withAsp 187, and Asn 142
4	Pelargonidin-3-glucoside		−7.2	H-bond and π−πinteraction with Met 46, Cys 145, and Arene-cation interaction withHis 41

(*continued on next page*)

Table 4 (*continued*)

#	Ligand	Ligand/receptor docking Interaction	Docking Score	Interpretation
5	pelargonidin-3-O-beta-D-glucoside		−7.3	H-bond and π−πinteraction withHis 163, Cys 145, and Met 165

known as LogP), the number of H- bond donors and acceptors, and the molecular weight. Compounds that conform to the Rule of Five, tend to have an increased chance of reaching the market [41].The results are displayed in Table 2.

The synthetic accessibility of the four compounds which show the best docking score and drug ability is less than 10 (range between 4.99 and 5.25), therefore they can be easily synthesized as displayed in Table 3.The ADME properties predicted using swiss ADME, suggested that the four compounds (pelargonidin 3-rhamnoside, pelargonidin-3-O-beta-D-glucoside, cyanidin-3-arabinoside, and pelargonidin-3-glucoside) did not show any hepatocellular toxicity since it was not inhibited any of the Human

Table 5
Summary of docking interactions in the case of 6lzg.

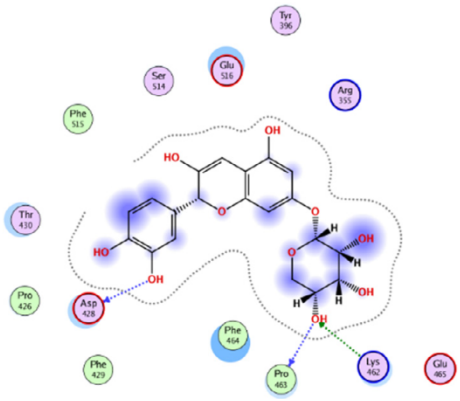
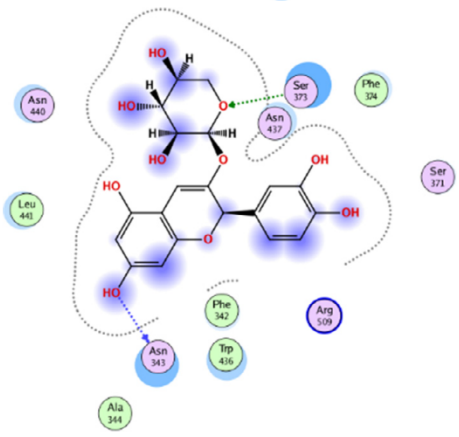
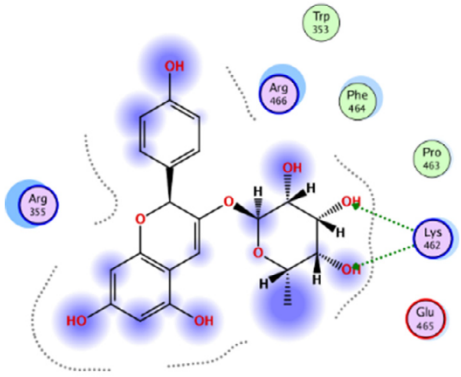
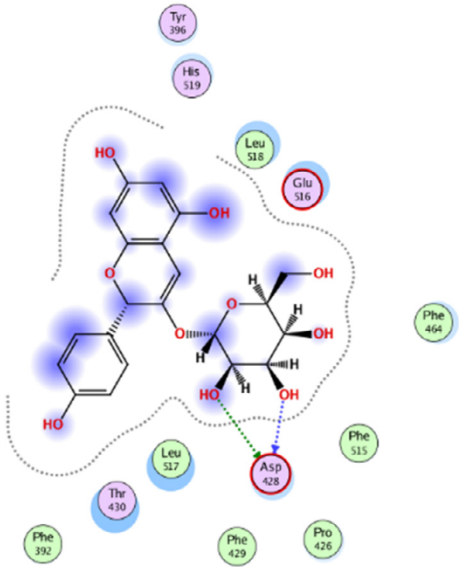
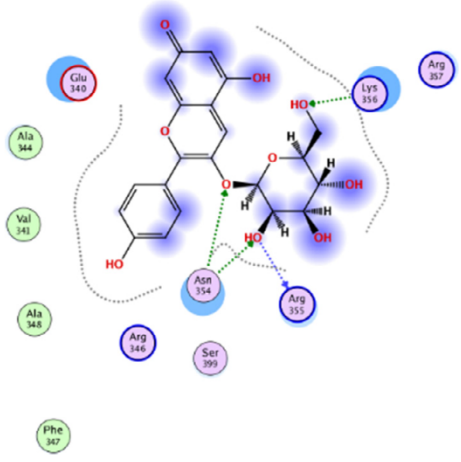
#	Ligand	Ligand/receptor docking Interaction	Docking Score	Interpretation
1	Cyanidin 7-arabinoside		−5.5	H-bond and π−π interaction with Lys 462 and Pro 463
2	Cyanidin-3-o-alpha-arabinoside		−5.5	H-bond and π−πinteraction withSer 373

Table 5 (*continued*)

#	Ligand	Ligand/receptor docking Interaction	Docking Score	Interpretation
3	Pelargonidin 3-rhamnoside		−5.2	H-bond and π−πinteraction with Lys 462
4	Pelargonidin-3-glucoside		−5.4	H-bond and π−πinteraction withAsp 428
5	pelargonidin-3-O-beta-D-glucoside		−5.3	H-bond and π−πinteraction with Asn 354, Arg 355, and Lys 356

Cytochrome P450 (CYP) alleles, furthermore, they don't show any blood-brain barrier crossing suggesting low toxicity induced upon consumption as confirmed through Table 3. The prediction of the bioactivity score from molinspiration tools, indicates that the four compounds have a very low binding affinity towards key targets of our cell.

Thereby, they are inducing very low cellular toxicity, in fact, the compounds do not bind to nuclear receptors and the primary targets like GPCR (G-protein-coupled receptors), and do not act as inhibitors for kinase proteins, proteases, and critical enzymes in our cell.

3.3. Molecular docking simulation using MOE

The ability of the reported ligands to bind to SARS-CoV-2 main protease (PDB: 6lu7) and SARS-CoV-2 spike glycoprotein (PDB:

6lzg) depends on molecular docking scores [42]. The reported ligands exhibit $\pi-\pi$interactions and hydrogen bonding, with 6lu7 protease and 6lzg spike glycoprotein as summarized in Tables 4 and 5, respectively. These electrostatic interactions suggest that the reported ligands are powerful inhibitors for the SARS-CoV-2 protease [43], owing to the formation of complex geometries with higher binding energies [24]. Furthermore, H- bond formation with 6lu7 and 6lzg reveals the ability of the ligands to block the COVID-19 binding receptor [30]. By inspecting the molecular docking results, cyanidin-3-arabinoside exhibits the highest binding affinity with COVID-19 virus Mpro (PDB: 6lu7), and spike glycoprotein (PDB: 6lzg) with binding energies of −7.52, and −5.55 kcal/mol, respectively. The interaction of the ligand with their surface maps in case of SARS-CoV-2 main protease (PDB: 6lu7) and SARS-CoV-2 spike glycoprotein (PDB: 6lzg) are provided in supplementary material (Figs S1 and S2) respectively. The data of docking scores for all optimized ligands are provided in supplementary data (Tables S1 and S2).

4. Conclusion

Anthocyanins compounds are present in the highest concentration in berry fruits. This study evaluates the ability of eighteen compounds derived from anthocyaninin for the inhibition of the main protease (6lu7) and the receptor-binding domain of spike glycoprotein of SARS-CoV-2 through molecular docking simulation. Cyanidin-3-arabinoside exhibited significant binding stability and interaction, with key residues within the binding site of coronavirus main protease (6lu7). However, according to the molecular docking study, the three compounds, pelargonidin-3-glucoside, pelargonidin 3-rhamnoside, and cyanidin-7-arabinoside can inhibit the attachment of the SARS-CoV2 with the host cell receptor ACE2. These compounds have a high drug-likeness as per Lipinski's rule of five and confer favorable toxicity parameters and ADME values. According to our study, the ligand cyanidin-3-arabinoside, which displays potent inhibition of SARS-COV-2 *in silico,* is found in high concentration in black chokeberries, bilberries, lingonberries, gooseberries, rubus, blackcurrants, while pelargonidin-3-glucoside found in raspberry and strawberry. Consequently, they can prevent virus infection. The docking results demonstrated that the reported ligands display a high binding attraction with SARS-CoV-2 main protease (PDB: 6lu7), and spike glycoprotein (PDB: 6lzg) and could hinder its viral disease. Depending on the docking scores and binding energies, Cyanidin-3-arabinoside exhibits docking scores of −7.5 and −5.5 kcal/mol, so it is a potential inhibitor for SARS-CoV-2. Considering the obtained results, regular consumption of berry fruits should be crucial to inhibit and control COVID–19 disease by suppression of propagation and pathogenicity of SARS-CoV-2.

Declaration of competing interests

The authors declare that they have no known competing for financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpe.2021.01.001.

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