Original article

Computational selection of flavonoid compounds as inhibitors against SARS-CoV-2 main protease, RNA-dependent RNA polymerase and spike proteins: A molecular docking study

Marimuthu Ragavan Rameshkumar [a,*], Purushothaman Indu [b], Narasingam Arunagirinathan [b,c], Babu Venkatadri [d], Hamed A. El-Serehy [e], Ajaz Ahmad [f]

[a] Laboratory Division, ICMR-National Institute of Epidemiology, Chennai, India
[b] Department of Microbiology and Biotechnology, Presidency College (Autonomous), Affiliated to University of Madras, Chennai, India
[c] Central Research Laboratory, Meenakshi Academy of Higher Research and Education (Deemed to be University), Chennai, India
[d] Department of Plant Biology and Biotechnology, Loyola College (Autonomous), Affiliated to University of Madras, Chennai, India
[e] Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia
[f] Department of Clinical Pharmacy, College of Pharmacy, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

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ABSTRACT

An outbreak of Coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 has been recognized as a global health concern. Since, no specific antiviral drug is proven effective for treatment against COVID-19, identification of new therapeutics is an urgent need. In this study, flavonoid compounds were analyzed for its inhibitory potential against important protein targets of SARS-CoV-2 using computational approaches. Virtual docking was performed for screening of flavonoid compounds retrieved from PubChem against the main protease of SARS-CoV-2 using COVID-19 docking server. The cut off of dock score was set to >−9 kcal/mol and screened compounds were individually docked against main protease, RNA-dependent RNA polymerase, and spike proteins using AutoDock 4.1 software. Finally, lead flavonoid compounds were subjected to ADMET analysis. A total of 458 flavonoid compounds were virtually screened against main protease target and 36 compounds were selected based on the interaction energy value >−9 kcal/mol. Furthermore, these compounds were individually docked against protein targets and top 10 lead compounds were identified. Among the lead compounds, agathisflavone showed highest binding energy value of −8.4 kcal/mol against main protease, Albireodelphin showed highest dock score of −9.8 kcal/mol and −11.2 kcal/mol against RdRp, and spike proteins, respectively. Based on the high dock score and ADMET properties, top 5 lead molecules such as Albireodelphin, Apigenin 7-(6″-malonylglucoside), Cyanidin-3-(p-coumaroyl)-rutinoside-5-glucoside, Delphinidin 3-O-beta-D-glucoside 5-O-(6-coumaroyl-beta-D-glucoside) and (-)-Maackiain-3-O-glucosyl-6″-O-malonate were identified as potent inhibitors against main protease, RdRp, and spike protein targets of SARS-CoV-2. These all compounds are having non-carcinogenic and non-mutagenic properties. This study finding suggests that the screened compounds include Albireodelphin, Apigenin 7-(6″-malonylglucoside), Cyanidin-3-(p-coumaroyl)-rutinoside-5-glucoside, Delphinidin 3-O-beta-D-glucoside 5-O-(6-coumaroyl-beta-D-glucoside) and (-)-Maackiain-3-O-glucosyl-6″-O-malonate could be the potent inhibitors of SARS-CoV-2 targets.

* Corresponding author.
E-mail addresses: rameshmicrobiologist@gmail.com, dmrrameshkumar@gmail.com (M.R. Rameshkumar).

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1. Introduction

Coronavirus disease 2019 (COVID-19) outbreak caused by SARS-CoV-2 has been raised as a global health concern. It was first reported in the Wuhan city, Hubei province of China in December 2019 (ul Qamar et al., 2020). As on 29th June 2020, 16,523,815 cases were confirmed for COVID-19 and 655,112 deaths occurred worldwide (World Health Organization. Coronavirus disease (COVID-19) outbreak situation. WHO, 2020). The fever, dry cough, body pain, and shortness of breath are the common symptoms of COVID-19 and pneumonia may occur in severe cases, sometimes leading to organ failure and death (World Health Organization (WHO)/. QA on coronaviruses (COVID-19), 2020). COVID-19 patients are treated using symptomatic therapy due to unavailability of disease specific approved drugs (Gabutti et al., 2020). Hence, there is an urgent need to discover and develop drugs for the clinical management of COVID-19 patients.

SARS-CoV-2 contains single-stranded RNA as a genome and produces approximately 800kDA polypeptides. The polypeptide encodes structural proteins such as Spike, nucleocapsid, matrix, and envelope, and non-structural proteins such as proteases and RNA-dependent RNA polymerase (RdRp) (Elfiky, 2020; Chen et al., 2020). The 3-chymotrypsin-like protease (3CL^{pro}) otherwise called as main protease (Mpro) cleaves the polypeptide in to various non-structural proteins which are involving in the viral replication (Anand et al., 2003; Needle et al., 2015). RdRp is a crucial viral enzyme in the life cycle of RNA viruses and it has been targeted in various viral infections including the hepatitis C virus (HCV), the Zika virus (ZIKV), and coronaviruses (CoVs) (Elfiky and Elshemey, 2016; Elfiky et al., 2017; Elfiky and Ismail, 2019). Spike glycoprotein is present on the surface of SARS-CoV-2 which helps in the viral initial attachment with human Angiotensin converting enzyme 2 (ACE 2) receptors. Targeting spike protein for inhibitor screening could be helpful for identifying the inhibitors that prevent viral attachment to ACE2 (Rane et al., 2020). These three protein targets are considering as potential targets for discovering drugs against SARS-CoV-2.

Phytocompounds might be the most efficient drug candidate in the current need since they have high bioavailability and low toxicity. Flavonoids are a major group of phytocompounds produced by plants as secondary metabolites. Flavonoid compounds are already reported for various biological properties
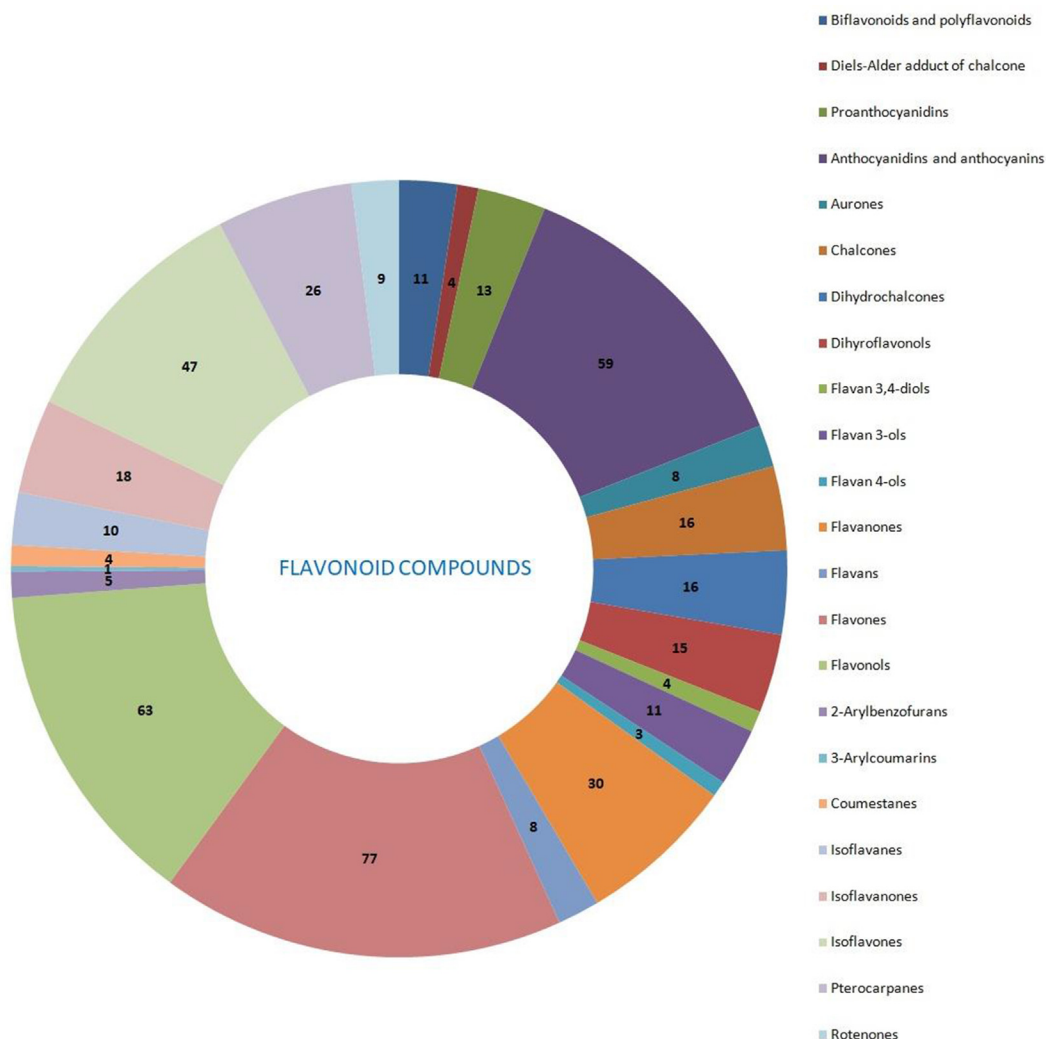

Fig. 1. Total flavonoid compounds used for docking analysis against protein targets of SARS-CoV-2.

Agathisflavone

Albireodelphin

Cyanidin 3-(6-p-caffeoyl)glucoside

Delphinidin 3-O-beta-D-glucoside 5-O-(6-coumaroyl-beta-D-glucoside)
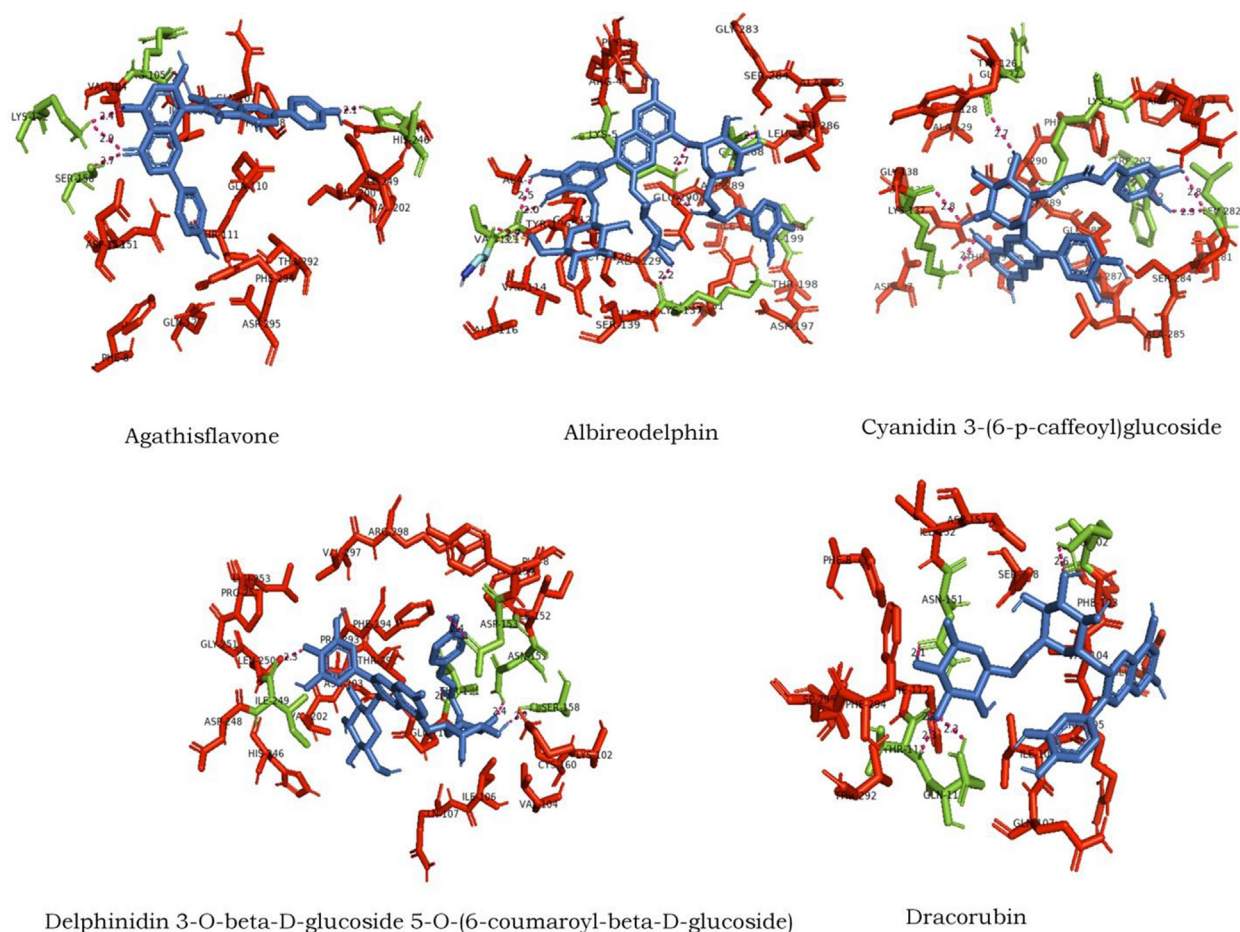
Dracorubin

Fig. 2. Docking pose of lead flavonoid compounds against main protease of SARS-CoV-2.

such as antimicrobial, antioxidant, anti-inflammatory and anti-cancer properties (Forni et al., 2019; Panche et al., 2016; Kumar and Pandey, 2013). Different flavonoids including both flavones and flavonols are mainly investigated for their potential antiviral properties and many of them showed significant antiviral response in both *in vitro* and *in vivo* studies (Kumar et al., 2020). In this study, a library of flavonoid compounds was screened to identify the potent inhibitors against main protease, RNA-dependent RNA polymerase and spike proteins of SARS-CoV-2 using computational methods.

2. Materials and methods

2.1. Ligand and target preparation

In this study, 3D structures of phytocompounds belonging to flavonoid group were retrieved from PubChem database. All the 3D structures of phytocompounds were energy minimized using uff minimization algorithm and 3D structures were converted into PDBQT format before performing docking using Autodock 4.1. Similarly, the 3D structures of Mpro (6LU7) and RdRp (6M71) and spike (6VW1) proteins were downloaded from the Protein Data Bank. Water molecules, ions, and other ligands present in the pro-

tein targets were removed and the structures were converted into PDBQT format for molecular docking analysis.

2.2. Molecular docking

Preliminary virtual screening was performed for screening the flavonoid compounds against the main protease using virtual screening module COVID-19 Docking Server. COVID-19 Docking Server is generally used for predicting the binding modes between the targets and small molecules, peptides or antibodies by implementing Autodock Vina and CoDockPP as docking engines (Kong et al., 2020). The compounds having the dock score of >−9 kcal/mol was considered as the lead compounds for further analysis. The lead compounds screened through virtual screening were individually docked against Mpro, RdRp and spike proteins using Auto-Dock 4.1 (Morris et al., 2008). The *pdbqt* files of Mpro, RdRp and Spike proteins were used and the grid was placed to the center and x, y, and z coordinates were fixed as 40, 40, and 40, respectively. The docked receptor and ligand interactions were visualized using Pymol.

2.3. Pharmacokinetics and drug-likeness properties

Pharmacokinetics, drug-likeness and medicinal chemistry properties were predicted for the lead compounds using the Swis-
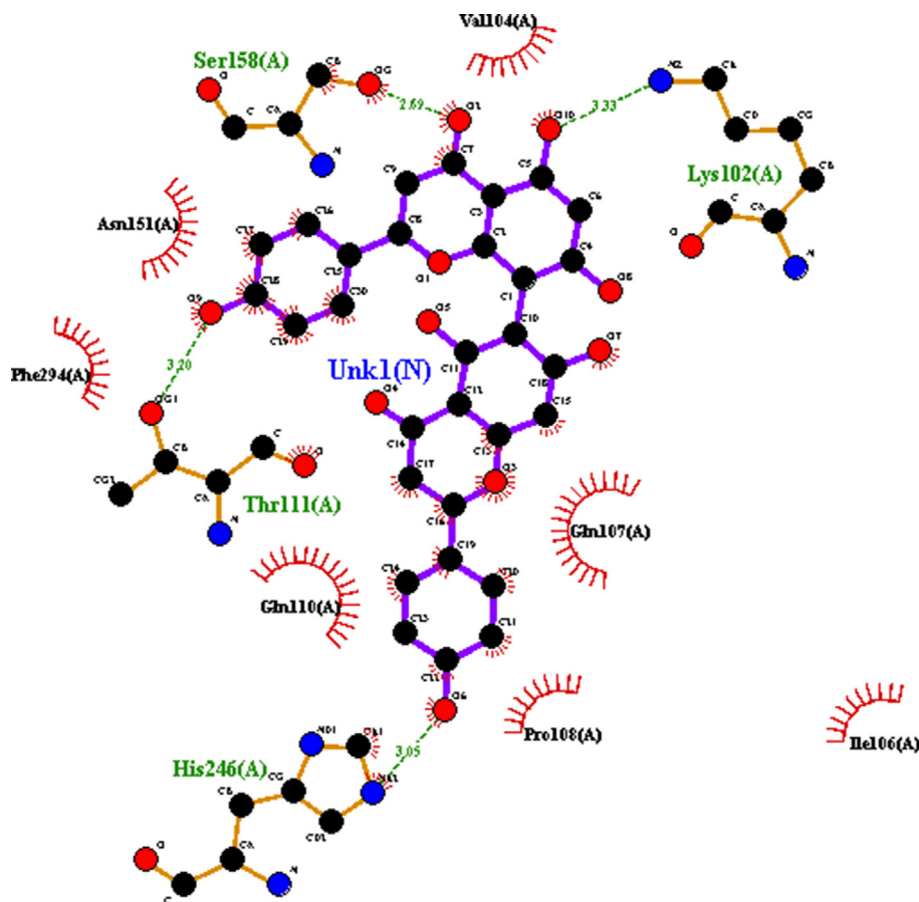
Fig. 3. Ligplot image of Agathisflavone compound against main protease of SARS-CoV-2.

sADME server. The SMILES format of the phytocompounds was used as input for the tool (Daina et al., 2017).

3. Results

3.1. Virtual screening of flavonoid compounds

In this study, a total of 458 flavonoid compounds obtained from various databases were virtually screened against the Mpro of SARS-CoV-2 (Fig. 1). The cut off of dock score was set to >−9 kcal/mol. Out of 458 flavonoids analysed, 36 lead compounds were selected which had docking score above the cut off value.

3.2. Molecular docking of screened flavonoid compounds with Mpro (6LU7)

All the 36 flavonoid compounds were individually docked to the active site of 6LU7 and it was observed that the compound agathisflavone had shown the highest binding energy value of −8.4 kcal/mol and key amino acids include Lys102, Thr111, Ser158, His246 were involved in the hydrogen bond (HB) interactions followed by Dracorubin and Cupressuflavone showed dock score of −8.2 kcal/mol. Amentoflavone and Cyanidin 3-(6-p-caffeoyl) glucoside exhibited a binding affinity of −8.1 kcal/mol. The compounds Delphinidin 3-O-beta-D-glucoside 5-O-(6-coumaroyl-

beta-D-glucoside), Apigenin 7-(6″-malonylglucoside), Albireodelphin, (-)-Maackiain-3-O-glucosyl-6″-O-malonate, and Cyanidin-3-(p-coumaroyl)-rutinoside-5-glucoside showed binding affinity of −7.9 kcal/mol, −7.7 kcal/mol, −7.6 kcal/mol,-7.6 kcal/mol and −7.6 kcal/mol, respectively (Figs. 2, 3, 8 & Table 1). These ten compounds were further selected and subjected for docking with RdRp and spike proteins.

3.3. Molecular docking of screened flavonoid compounds with RdRp (6M71)

The ten lead compounds were docked against the RdRp (6M71) of SARS Co-V-2 and found that the compound Albireodelphin had shown the highest binding affinity value of −9.8 kcal/mol with amino acids residues Arg348, Asp350, His378, Asp382, Phe390, Asn394, Asn397, Glu398, His401, Glu402, and Arg514 were contributed for the HB interactions. The compound Delphinidin 3-O-beta-D-glucoside 5-O-(6-coumaroyl-beta-D-glucoside) exhibited the high binding affinity value of −9.7 kcal/mol followed by Cyanidin-3-(p-coumaroyl)-rutinoside-5-glucoside (-8.9), (-)-Maackiain-3-O-glucosyl-6″-O-malonate (-8.7), Cyanidin 3-(6-p-caffeoyl)glucoside (-8.7), agathisflavone (-8.6), Apigenin 7-(6″-malonylglucoside) (-8.6), Cupressuflavone (-8.2), Amentoflavone (-8.1) and Dracorubin (-6). (Figs. 4, 5, 8 & Table 1).

Table 1
ADMET properties of lead flavonoid compounds screened against SARS-CoV-2 protein targets.

| Molecule	Formula	MW	Heavy atoms	Aromatic heavy atoms	Rotatable bonds	H-bond acceptors	H-bond donors	TPSA	ESOL Class	GI absorption	BBB permeant	Pgp substrate	Bio availability Score	PAINS alerts	Lead likeness violations	Synthetic Accessibility	Toxicity	Carcinognecity
Agathisflavone	C30H18O10	538.46	40	32	3	10	6	181.8	Poorly soluble	Low	No	No	0.17	0	2	4.17	No	No
Albireodelphin	C42H47O25	951.81	67	22	14	25	16	418.5	Soluble	Low	No	Yes	0.17	1	2	8.51	No	No
Amentoflavone	C30H18O10	538.46	40	32	3	10	6	181.8	Poorly soluble	Low	No	No	0.17	0	2	4.27	No	No
Apigenin 7-(6″-malonylglucoside)	C24H22O13	518.42	37	16	8	13	6	213.42	Soluble	Low	No	Yes	0.11	0	2	5.41	No	No
Cupressuflavone	C30H18O10	538.46	40	32	3	10	6	181.8	Poorly soluble	Low	No	No	0.17	0	2	4.2	No	No
Cyanidin 3-(6-p-caffeoyl)glucoside	C30H27O14	611.53	44	22	8	14	9	239.97	Soluble	Low	No	No	0.17	1	2	6	No	No
Cyanidin-3-(p-coumaroyl)-rutinoside-5-glucoside	C42H47O22	903.81	64	22	13	22	13	357.81	Moderately soluble	Low	No	Yes	0.17	1	2	8.36	No	No
Delphinidin 3-O-beta-D-glucoside 5-O-(6-coumaroyl-beta-D-glucoside)	C36H37O19	773.67	55	22	11	19	12	319.12	Soluble	Low	No	Yes	0.17	1	2	7.3	No	No
Dracorubin	C32H24O5	488.53	37	29	3	5	0	61.81	Poorly soluble	Low	No	No	0.55	0	2	4.64	No	No
(-)-Maackiain-3-O-glucosyl-6″-O-malonate	C25H24O13	532.45	38	12	7	13	4	179.67	Soluble	Low	No	Yes	0.11	0	1	5.58	No	No
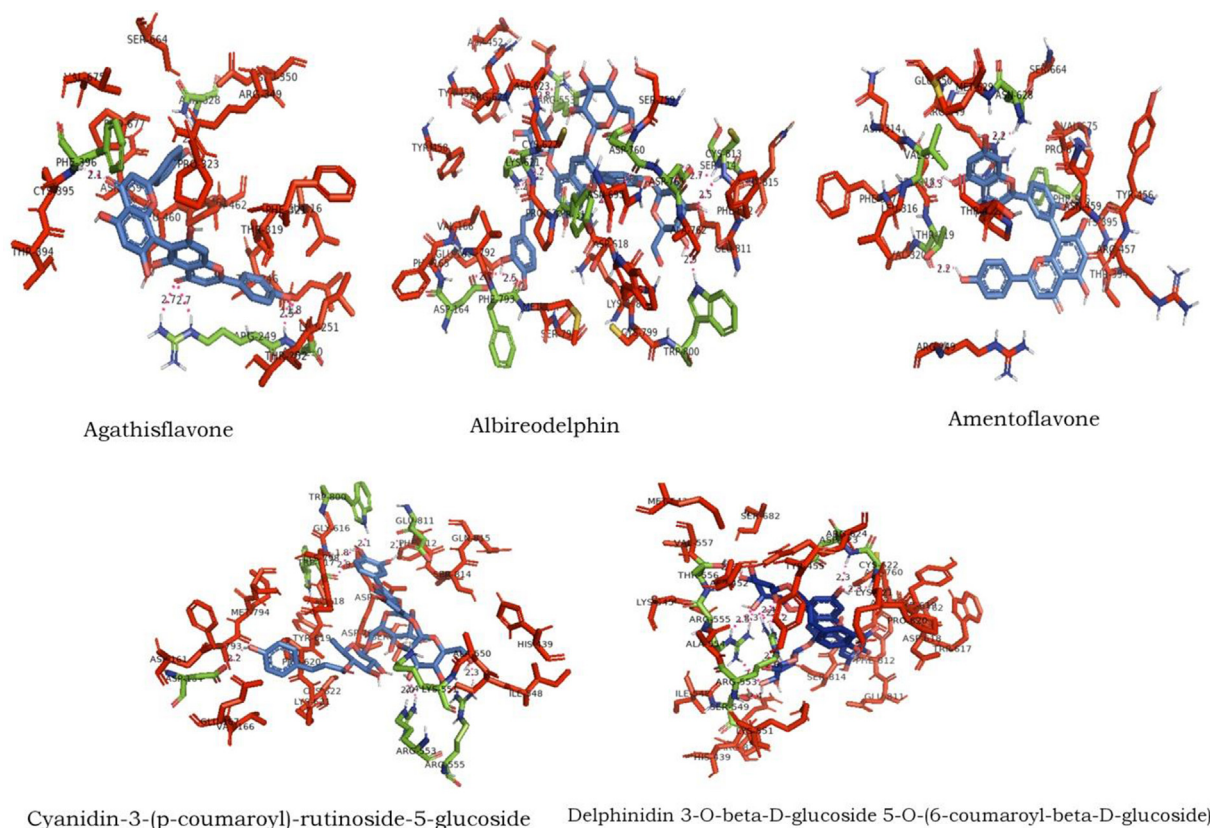
Fig. 4. Docking pose of lead flavonoid compounds against RNA-dependent RNA polymerase of SARS-CoV-2.

3.4. Molecular docking of screened flavonoid compounds with spike protein (6VW1)

The best ten lead compounds were also docked against the spike protein (6VW1) of SARS-CoV-2 and it was observed that the compounds exhibited substantial binding affinity with significant HB interactions. Out of the ten compounds, the compound Albireodelphin showed the highest binding energy value of −11.2 kcal/mol and HB interactions with amino acids namely Lys621, Asp623, Phe793, Lys798, Asp760, Asp761, Trp800, Glu811, Cys813, and Ser814. Delphinidin 3-O-beta-D-glucoside 5-O-(6-coumaroyl-beta-D-glucoside) exhibited a binding energy value of 11.1 kcal/mol, followed by Amentoflavone (10.2 kcal/mol), Cupressuflavone (−10 kcal/mol), agathisflavone (9.9 kcal/mol), Cyanidin-3-(p-coumaroyl)-rutinoside-5-glucoside (9.8 kcal/mol), Apigenin 7-(6″-malonylglucoside) and Cyanidin-3-(p-coumaroyl)-rutinoside-5-glucoside (9.4 kcal/mol), (-)-Maackiain-3-O-glucosyl-6″-O-malonate (−9 kcal/mol) and Dracorubin (−8) (Figs. 6–8 & Table 1).

3.5. Physiochemical, pharmacokinetic and drug likeness properties of lead flavonoid compounds

All the screened flavonoid compounds were found to have little violation in the Lipinski rule of 5 and it was believed to effective despite of few violations. All the ten compounds were not effective in crossing the blood brain barrier so that no toxic chemicals cross the blood brain barrier. All the compounds are poor in gastro intestinal adsorption. Out of the ten compounds five compounds are the substrate of P-glycoprotein namely Albireodelphin, Apigenin 7-(6″-malonylglucoside), Cyanidin-3-(p-coumaroyl)-rutino side-5-glucoside, Delphinidin 3-O-beta-D-glucoside 5-O-(6-coumaroyl-beta-D-glucoside) and (-)-Maackiain-3-O-glucosyl-6″-O-malonate and are water soluble. It was very convincing to note that all the ten compound screened were safe as it was non-carcinogenic and non-toxic by nature. Other properties like pharmacokinetic, physicochemical and drug-likeness properties are mentioned in Table 2.

4. Discussion

Computation docking is an effective strategy, and widely used technique for understanding the molecular aspects of proteins and protein-ligand interactions in the drug discovery process (Murgueitio et al., 2012). Rapid identification of potent drugs for treating COVID-19 patients is an urgent need in the current global pandemic of COVID-19. Phytochemicals especially flavonoids, terpenoids and alkaloids gained greater importance in the last few decades for its antiviral therapeutic applications (Naithani et al., 2008).
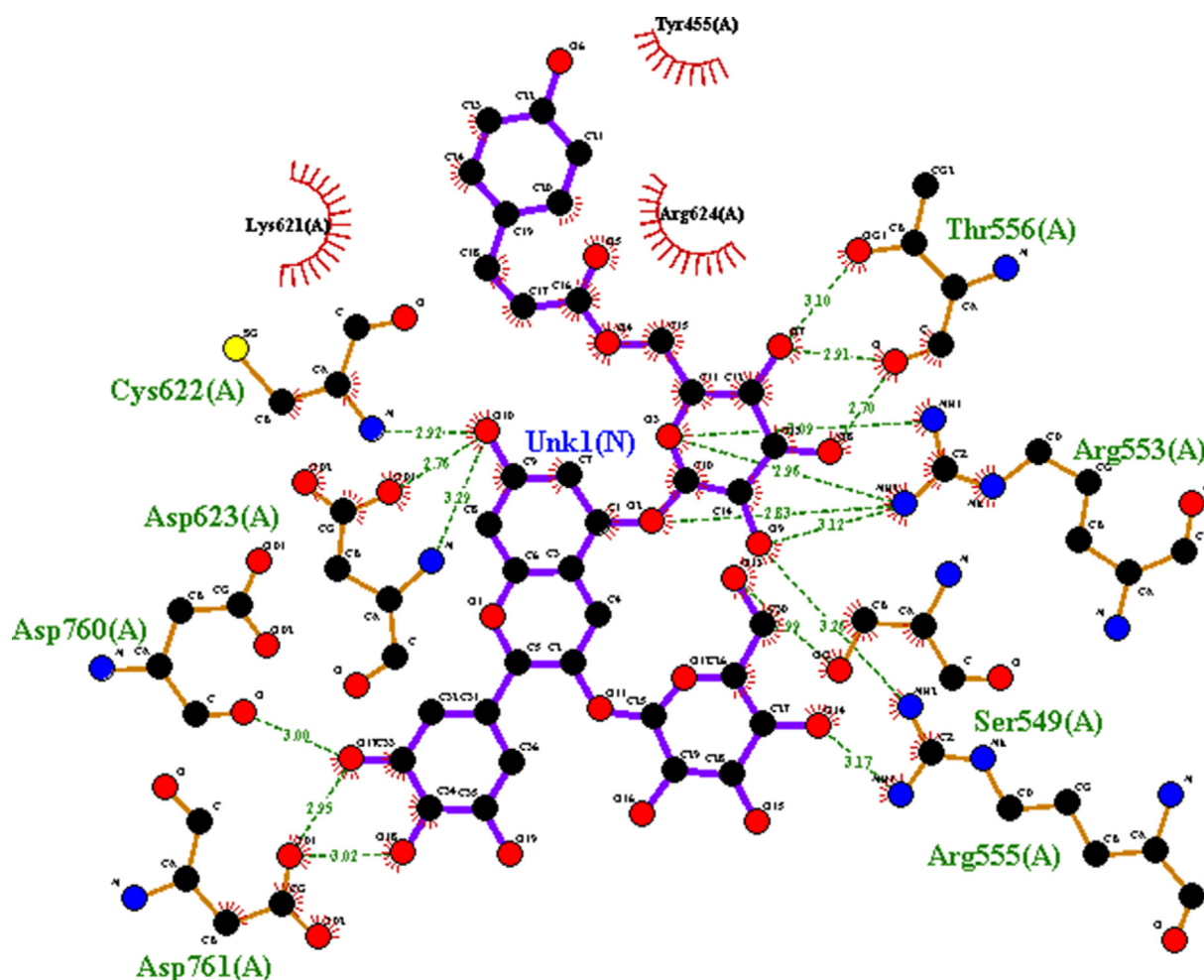
Fig. 5. Ligplot image of Delphinidin 3-O-beta-D-glucoside 5-O-(6-coumaroyl-beta-D-glucoside) compound against RNA-dependent RNA polymerase of SARS-CoV-2.

In this present study, a library of flavonoid compounds have been screened against the three major targets (Mpro, RdRp, and spike proteins) of SARS-CoV-2. Different categories of flavonoids have been already established for antiviral activity against Herpes Simplex Virus, Polio virus, respiratory syncytial virus, and Sindbis virus (Gerdin and Srensso, 1983; Zandi et al., 2011). In this study, flavonoid compounds such as Agathisflavone, Albireodelphin, Amentoflavone, Apigenin 7-(6″-malonylglucoside), Cupressu-flavone, Cyanidin 3-(6-p-caffeoyl)glucoside, Cyanidin-3-(p-coumar oyl)-rutinoside-5-glucoside, Delphinidin 3-O-beta-D-glucoside 5-O-(6-coumaroyl-beta-D-glucoside), Dracorubin and (-)-Maack iain-3-O-glucosyl-6″-O-malonate were identified as lead mole-cules based on the highest binding energy value against Mpro and ADMET properties. For identification of multiple target binding potential, these 10 compounds were further docked with RdRp an Spike protein targets. In a study by Iftikhar et al. (2020), a library of 4574 compounds including the FDA approved drugs were targeted against the three important targets such as Mpro, RdRp, and spike, and found that different set of compounds had shown high interac-tion profile against each protein target studied (Iftikhar et al., 2020). But in this study different approach was used that all the flavonoid compounds were initially screened against Mpro since this target is the crucial and highly potent target for inhibition of SARS-CoV-2 followed by individually docked against other two tar-gets proteins. Arriving of different set of phytocompounds against different targets could be minized using this approach and which easy the selection of compounds having inhibitory against all the targets. It was found in this study that agathisflavone exhibited the highest binding energy value of −8.4 kcal/mol against Mpro and observed key HB interactions with Lys102, Thr-111, Ser-158, and His-246. Kumar et al. (2020) reported that the compound Rhein had shown a high binding energy value of −8.1 kcal/mol and major HB bond interactions were observed with Lys102, Val104, Ile106 Gln110, Thr29, Thr111, Phe294, Asp295, Gln127, Phe8, Asn151, Ile152, Asp153, and Ser158. Amino acids involved in the HB interactions of Mpro found in this study were similar with the report of Kumar et al. Inhibitors were successfully identi-fied using virtual screening strategy against proteases of SARS-Co-
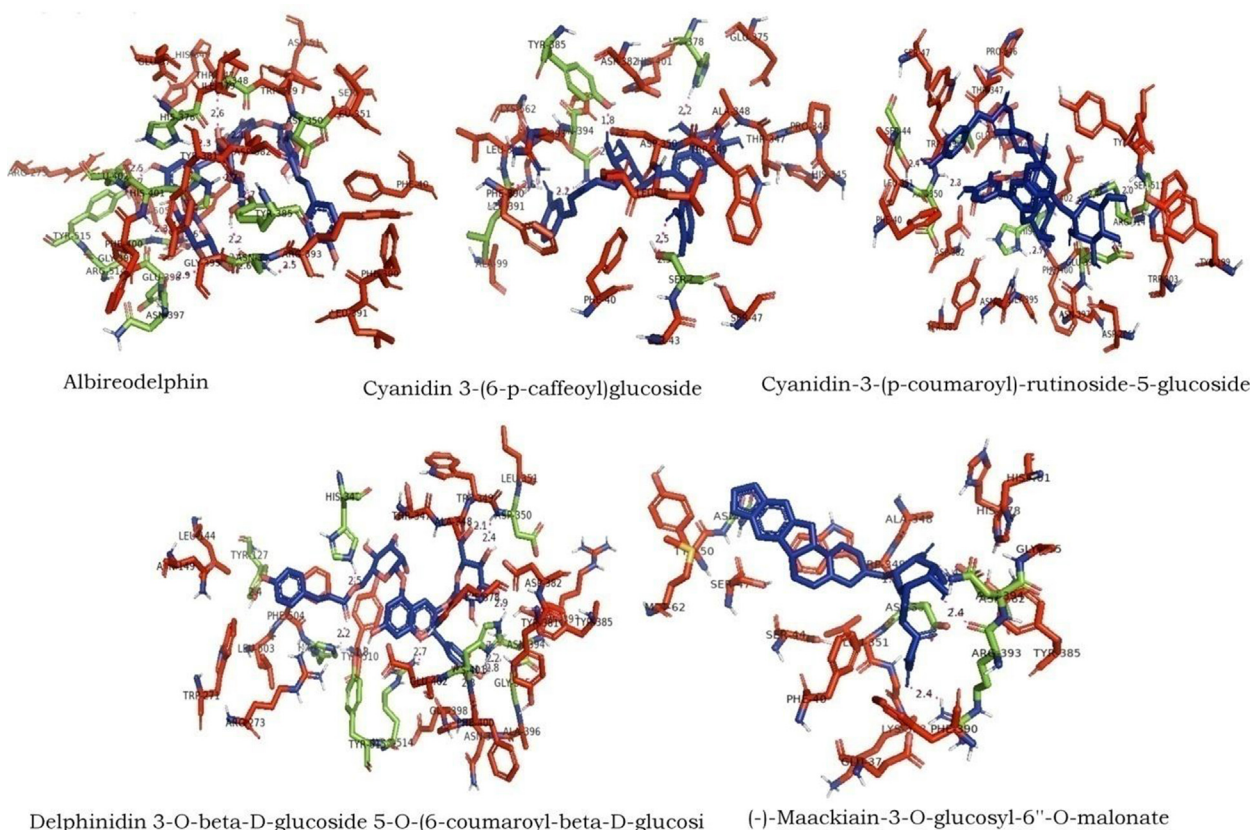
Fig.6. Docking pose of lead flavonoid compounds against spike protein of SARS-CoV-2.

V (Liu and Zhou, 2005), RdRp of Hepatitis C virus (ElHefnawi et al., 2012) and E protein of dengue virus (Zhou et al., 2008).

It was interesting to note that RdRp of SARS-CoV-2 and SARS-Co-V have 97% sequence identity, and it is believed that the active site of the RdRp of most CoVs and RNA viruses are highly conserved by nature. Elfiky (2020) had reported that the flavonoid compounds 3,5-dihydroxyphenyl)oxidanyl and 3-hydroxyphenyl)oxidanyl had shown the highest binding energy value of −9.3 kcal/mol and −8.9 kcal/mol, respectively against RdRp. In our study, the lead molecule Albireodelphin (−9.8 kcal/mol) exhibited the high dock score than the Elfiky et al. (2017) study and more favourable HB interactions were found with RdRp. In a study, nucleoside analogs include ribavirin, remdesivir, sofosbuvir, galidesivir, and tenofovir were reported as an effective inhibitors against RdRp of SARS-CoV-2 (Elfiky, 2020). Singh et al. (2020) suggested that natural polyphenols are having highest binding energy with RdRp target. In this current study, NTP entry site was used as the binding site in combination with the Mg^{2+} ion chelating site that includes amino acids Asp760, Asp761, Asp618, Asp623, Asn691, Thr680, Ser682, and Thr687. It was also found in our study that Albireodelphin showed the highest interaction energy value (−9.8) and formed eleven HB interactions with Arg348, Asp350, His378, Asp382, Phe390,

Asn394, Asn397, Glu398, His401, Glu402, and Arg514. In a study, Daidzein compound exhibited highest binding energy of −8.6 kcal/mol with no HB interactions (Elfiky, 2020). But the compounds screened against Spike protein in this study had many HB interactions which reveals that these compounds are having high possibility to be potent inhibitors of spike protein. Smith and Smith (2020) had found that pemirolast, isoniazid pyruvat, Nitrofurantoin, and Eriodictyol could be act as inhibitors against spike protein. The flavonoid compound Luteolin had shown significant antiviral effects on SARS-Co-V and Luercetin has been found to demonstrate an *in-vitro* dose-dependent antiviral activity against respiratory syncytial virus (Kadioglu et al., 2020) and Resveratrol has been reported for antiviral activity against Middle East Respiratory Syndrome coronavirus (MERS-CoV) and also reduced the expression of MERS-CoV nucleocapsid (N) protein (Raj and Varadwaj, 2016). Findings from the docking studies revealed that the screened compounds namely Albireodelphin, Apigenin 7-(6″-malonylglucoside), Cyanidin-3-(p-coumaroyl)-rutinoside-5-glucoside, Delphinidin 3-O-beta-D-glucoside 5-O-(6-coumaroyl-beta-D-glucoside) and (-)-Maackiain-3-O-glucosyl-6″-O-malonate are having high interaction profile with all the three protein targets studied. In These five compounds have more than 6 HB donors except (-)-Maack

Fig.7. Ligplot image of Albidoderphine compound against spike protein of SARS-CoV-2.

iain-3-O-glucosyl-6″-O-malonate and hydrogen bond acceptors more than 5. Surprisingly, these five compounds are also being a substrate of P-glycoprotein and water soluble. Drug-likeness analysis reveals that these flavonoid compounds are non carcinogenic and non toxic in nature.

5. Conclusion

The flavonoid compounds screened in this study such as Albireodelphin, Apigenin 7-(6″-malonylglucoside), Cyanidin-3-(p-cou

maroyl)-rutinoside-5-glucoside, Delphinidin 3-O-beta-D-glucoside 5-O-(6-coumaroyl-beta-D-glucoside) and (-)-Maack iain-3-O-glucosyl-6″-O-malonate had shown highest binding energy values against all the three protein targets include main protease, RNA-dependent RNA polymerase and spike proteins of SARS-CoV-2. These five compounds are also possessing *in silico* non-carcinogic and non-mutagenic properties. Due to the multiple targets binding potential, high dock score against all the three targets and supportive drug-likeness properties, this study suggests that the screened flavonoid pytocompounds could be the potent inhibitors of SARS-CoV-2 targets.

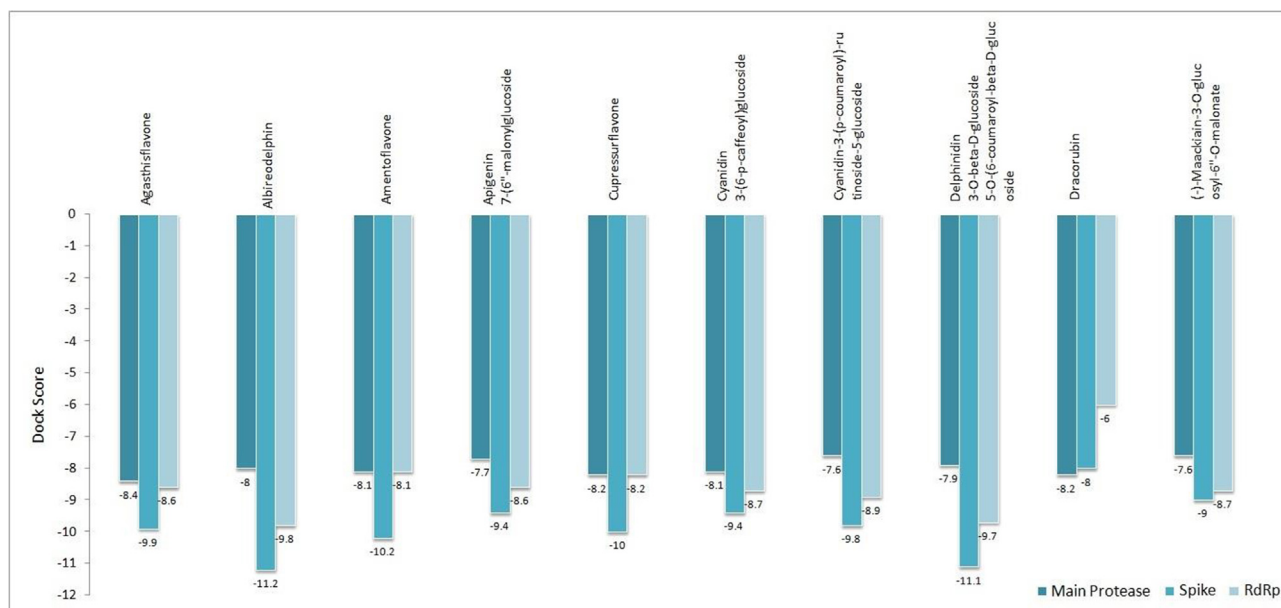*Marimuthu Ragavan Rameshkumar, P. Indu, N. Arunagirinathan et al.*

Fig. 8. Docking score of lead phytocompounds against main protease, RNA-dependent RNA polymerase and spike proteins of SARS-CoV-2.

Table 2
Amino acid interactions involved in the hydrogen bond formation between flavonoid compounds and protein targets of SARS-CoV-2.

Flavonoid Compounds	Main protease	Spike Protein	RNA-dependent RNA polymerase
Agathisflavone	Lys102 (3.3), Thr111(3.2), Ser158 (2.6), His246 (3.0)	Leu245 (3.2), Als250 (2.3), Leu251 (2.8), Thr394 (2.8), Phe396 (3.0), Asn628 (2.8)	Ser43 (3.2), Ala348 (2.7), Asp350 (3.0), Asp382 (3.1), Tyr385 (3.0)
Albireodelphin	Lys5 (4), Val125 (3.9), Lys137 (2.9), Ser139 (3.1), Thr199 (3.1), Glu288 (3.8)	Lys621(3.1), Asp623 (1.5), Phe793 (1.9), Lys798 (1.6), Asp760 (1.5), Asp761 (3.0), Trp800 (3.1), Glu811 (3.1), Cys813 (3.1), Ser814 (3.3)	Arg348 (1.3), Asp350 (3.0), His378 (3.1), Asp382 (3.0), Phe390 (1.9), Asn394 (3.0), Asn397 (3.1), Glu398 (3.1), His401 (3.1), Glu402 (3.1), Arg514(3.1)
Amentoflavone	Asn151 (2.9), His246 (3.1)	Val315 (2.8), Thr319 (2.8), Thr394 (3.1), Phe396 (3.2), Asn628 (3.0)	Ser43 (3.0), Asp350 (2.6), Tyr385 (2.6), Asn394 (3.1)
Apigenin 7-(6″-malonylglucoside)	Gln110 (3.0), Thr111 (3.9), Thr292 (2.7), Asp295 (2.9)	Asp452 (2.8), Trp617 (2.9), Tyr619 (3.4), Lys621 (3.2), Asp760 (3.2), Asp761 (2.8), Trp800 (2.9),	Als348 (3.2), Asp350 (3.2), His378 (3.2), Asp382 (3.0), Gly395 (3.1), Asn394 (2.8), His401 (3.1)
Cupressuflavone	Gln110(2.9), Thr111(2.7), Asn151 (3.0), Asp153 (2.8)	Thr246 (2.8), Arg349 (2.8), Thr349 (3.0)	Ser47 (3.3), Ala348 (3.0), His378 (3.2), Asp382 (3.0), Glu398 (2.7), His401 (2.9)
Cyanidin 3-(6-p-caffeoyl)glucoside	Phe3 (2.8), Lys5 (3.2), Gln127 (3.2), Lys137 (3.2), Trp207 (3.1), Glu290 (2.8), Leu382 (3.1)	Thr246 (2.8), Val315 (2.8), Thr319 (3.0), Asn459 (3.0), Asn628 (3.1)	Ser44 (3.3), Asp350 (2.8), His378 (3.1), Asp382 (3.0), Tyr385 (2.8), Arg393 (2.9), Asn394 (3.1)
Cyanidin-3-(p-coumaroyl)-rutinoside-5-glucoside	Thr111 (2.9), Gln110 (2.9), Asn151 (3.1)	Asp164 (1.8), Ile548 (3.0), Ser549 (3.0), Arg553 (3.1), Arg555 (3.0), Asp760 (3.0), Asp761 (1.9)	Ser44 (3.0), Asp206 (3.0), Als348 (3.1), Asp350 (1.9), Asn397 (3.1), Glu398 (3.3), Ser511 (1.9), Arg514 (3.1)
Delphinidin 3-O-beta-D-glucoside 5-O-(6-coumaroyl-beta-D-glucoside)	Asp153 (2.9), Asn151 (3.1), Ser158 (3.0), Thr111 (3.3), Ile249 (2.9)	Ser549 (2.9), Arg553 (3.1), Arg555 (3.1), Thr556 (3.1), Cys622 (2.9), Asp623 (3.2), Asp760 (3.0), Asp761 (3.0)	Try127 (1.9), His345 (1.3), Ala348 (1.9), Asp350 (2.9), Asp382 (2.8), Tyr385 (3.1), Asn394 (2.4), Asn397 (3.8), Arg401 (2.8), His505, Arg514 (1.1), Tyr515 (1.3)
Dracorubin	Gln110(2.9), Thr 111(2.9), Asn151 (3.0), Asp153 (2.8), Ser158 (3.0), Lys102 (3.1)	Arg249 (2.9), Phe396 (3.1)	–
(-)-Maackiain-3-O-glucosyl-6″-O-malonate	Lys5 (3.0), Asp289 (3.0), Glu288 (2.9), Lys137 (2.8)	Asp-760 (2.8), Asp-761 (2.9), Trp-800 (2.9), Glu-811 (3.0)	Asn51 (3.1), Ala348 (3.0), Asp350 (2.8), Asp382 (3.0), Tyr385 (3.0), Arg393 (2.9)

Declaration of Competing Interest

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

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