

Phytochemicals of Citrus Fruits: The *in-silico* Investigation against Sars-CoV-2 Proteins

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Received: 31 March 2022, Revised: 9 June 2022, Accepted: 16 June 2022, Published: 26 November 2022

Abstract

The current pandemic Covid-19 brought about by a newly emerged and highly infectious virus named as Sars-CoV-2 as a worldwide danger, has infected more than 600 million people and number of deaths are continuously rising day by day. Till date there are no medications accessible for treatment. All over the world scientists and researchers are involved in the study of this emerged virus and its lifecycle. Structures of proteins in the life cycle of virus has been revealed in RCSB PDB (Research Collaboratory for Structural Bioinformatics Protein Data Bank) by researchers. Citrus fruits are used to treat many distresses of humans. Literature survey shows that it has various activities. Our research work is meant to identify the phytoconstituents which are having phenolic composition and good antiviral and antioxidant properties from citrus fruits against Covid-19 proteins (spike binding domain with ACE2 receptor and spike binding domain with Main protease) and to know its *in-silico* molecular basis. In this study, about 25 compounds from citrus fruits which is having a good antiviral and antioxidant properties and also phenolic composition were employed for molecular docking analysis, molecular dynamic simulation studies and ADME studies. Based on present study 2 compounds from Citrus fruits acted well against the Covid-19 proteins. The MD simulations were employed to identify Hesperidin and Procyanidin B₂ as hit compounds. Further ADME analysis were studied for top 2 compounds, these compounds can be further taken for *in-vitro* studies to know the effective activity against Covid-19.

Keywords: Corona virus, Citrus fruits, Phytochemicals, Molecular docking, Molecular dynamic simulations, ADME

Introduction

In the last few decades identifications, extraction and characterization of natural products has created interest in the pharmaceutical industry to study natural bioactive active compounds as drugs. Natural products have played crucial role in modern drug development and constitutes major source of novel lead compounds for the drug discovery.

Citrus fruits have nutritional and health benefits which is well documented in literature [1,2]. They have good levels of phytonutrients such as alkaloids, flavonoids, tannins, phenols, saponins including essential oils and vitamins. There are varieties of citrus fruits and some of the important types are oranges, mandarins, lemons, limes, grapefruits, pummelos, and citrons which are shown in **Figure 1**. They are classified in the genus Citrus, which has about 16 species [3]. A review of therapeutic potential of *C. sinensis* as a source of natural compounds with important activities that are beneficial for human health which can be used to develop drugs is discussed by Favela-Hernández *et al.* [4].

Flavonoids are an important group of low molecular weight secondary metabolites produced by plants. They are aromatic molecules with variable phenolic structures. They are found in vegetables, fruits, flowers, seeds, grains, bark, stems, roots and beverages such as tea and wine. Flavonoids are divided into different structural classes like flavanones, isoflavones, flavones, flavonols, flavanols and anthocyanins [5]. Flavonoids have high antioxidant potential and contribute in human health mainly due to their estrogenic, antiviral, antibacterial, antiparasitic and antimicrobial properties [6]. The flavonoid constituents of citrus plants have been analysed during various developments of the plant and the citrus fruit. Both qualitative and quantitative analysis of citrus flavonoids have been done and it is observed citrus plants contain a wide range of flavonoid constituents and many of them are unique to citrus plants [7-12]. More than 40 types of flavonoids have been identified in the citrus plant [13]. The beneficial flavonoids found are flavanone (as naringin, hesperidin and neohesperidin), flavone (as diosmin, luteolin and sinensetin) and flavonol (as quercetin and kaempferol) in different varieties of citrus fruit and peel [3,11,14,15].

They are used as folk medicine to treat bronchitis, tuberculosis, cough, cold, menstrual disorder, hypertension, anxiety, and depression. As citrus fruits are used as folk medicines, a number of investigations have been done to characterize to know the effect of citrus components as antimicrobial substances. Kawaguchi *et al.* [16] studied the effect of citrus flavonoid Hesperidin (HES) on the endotoxic shock produced by the lipopolysaccharide (LPS) of Gram-negative bacteria with *Salmonella typhimurium*. They observed that there was reduction of bacterial numbers in liver and spleen and LPS level decreased with pre-treatment with HES. The antibacterial activity of Citrus compounds and citrus oils with different concentration was studied against several gram positive and gram-negative bacteria including potential pathogens like Methicillin resistant *Staphylococcus aureus* (MRSA), *Listeria monocytogenes* and *Salmonella typhimurium* [17-19].

Inforecite of above findings, citrus fruits are taken into consideration for the treatment of Covid-19 disease. Therefore, in the current investigation, we applied a computational strategy aimed to identify the potential of the phytoconstituents against the SARS-CoV2 virus proteins.



Figure 1 Varieties of citrus fruits.

Therapeutics of flavonoids against Covid-19 targets

Flavonoids are divided into many subgroups, including flavanes, flavanols, flavanones, flavanonols, flavones, flavonols, isoflavones, and procyanidins, which all have a flavan (2-phenylchroman) basic form. In the human body, polyphenolic substrates serve a number of antioxidant roles. Many of them are bioactive molecules that can interfere with nucleic acids or proteins, giving them a wide range of pharmacological properties. Flavones are prove to be the most effective antioxidants, preventing the effects of reactive

oxygen species (ROS) in the body [20]. Flavonoids have been shown to have multiple functions including anti-cancer [21], antimicrobial [22,23], antioxidant [24], antiviral [25] and anti-inflammatory activities [26,27]. Hesperidin, a flavanone with a higher binding affinity towards 3CLpro and S2RBD has been shown to inhibit SARS CoV-2 infection [28]. Moreover, hesperidin was also reported as an ACE2 inhibitor since it can interact with the RBD of the S protein Sars-CoV-2 and ACE2 interface [29]. Hesperidin is considered safe to be administered as nutraceuticals. Recently, this is under clinical investigation for the management of Covid-19 [30,31], flavone and flavanone such as naringenin and naringin interact with 3CLpro and ACE2 activity of Sars-CoV-2 According to Clementi *et al.* [32], naringenin may be considered a stable anti-Sars-CoV-2 agent with Sars-CoV-2 inhibitory action. Quercetin, a flavonol, inhibits SARS virus entry by attacking ACE2 and has antiviral properties [33,34]. Derosa *et al.* [36] demonstrated the function of quercetin in Sars-CoV-2, due to its ability to inhibit main protease as well as its anti-inflammatory and thrombin inhibitory activities [35-37]. Rutin, a flavone, has been used to treat the secretion of IL-6, IL-1, and TNF in LPS-induced acute lung damage in *in-vivo* ICR mice by inhibiting oxidative stress and the MAPK-NF- κ B pathway [38]. Luteolin has been stated to interact with the spike, key protease, and nucleocapsid protein of Sars-CoV-2 to suppress viral infection [39]. These are some of the flavonoids which target the different targets (proteins) of Sars-CoV-2 and can be used as potent therapeutic agent against Sars-CoV-2.

Different classes of flavonoids such as flavone, flavanone and flavonol are targeting the different viral proteins of Sars-CoV-2, to inhibit the activity of Covid-19. From the literature survey the different sources and classes of compounds shows a very good inhibiting activity against Sars-CoV-2 have been depicted in **Table 1**.

Table 1 Flavonoids against Covid-19.

Sl. No	Compounds	Class of flavonoids	Sources	Target protein (PDB ID)	Binding energy kcal/mol (Tool)	Reference
1	Quercetin	Flavonol	apples, red grapes, citrus fruits, onions	6LU7	-7.5 (Autodock Vina)	[40]
2	Kaempferol	Flavonol	apples, oranges, grapes	6LU7	-9.82 (Autodock Vina)	[41]
3	Hesperidin	Flavanone	citrus fruits, green tea	6LU7	-8.84 (Swiss dock)	[42]
4	Rutin	Flavone	apple peels, green tea, onions, citrus fruits	6Y2E	-5.4 (Autodock Vina)	[43]
5	Luteolin	Flavone	oranges, lemons, grape fruits, green peppers	6WX4	-7.1 (Autodock Vina)	[44]
6	Hesperetin	Flavanone	lemons, oranges, vegetables	6M0J	-7.7 (Autodock Vina)	[45]
7	Naringin	Flavanone	citrus fruits	6LU7	-8.3 (Autodock Vina)	[46]
8	Nobiletin	Flavone	oranges, lemons and other citrus fruits	6LU7	-6.5 (Autodock Vina)	
9	Tangeretin	Flavone	citrus fruits	6LXT	-8.18 (MOE tool)	[47]

Materials and methods

In-silico prediction of phytochemicals against Sars-CoV-2 proteins

In order to search for a possible candidate to control Covid-19, *in-silico* computational docking study was performed for all phytoconstituents acquired from different species of citrus fruits on the binding pocket of enzyme of Sars-CoV-2. Molecular docking analysis were performed for the selected phytochemicals properties of phenolic groups in citrus fruits. The results of phytochemicals from the molecular docking were very promising and also analyzed molecular dynamic simulations to give the potential hits against Sars-CoV-2. Further ADME studies were carried out for top candidates to prove its potential are showed in **Figure 2**.

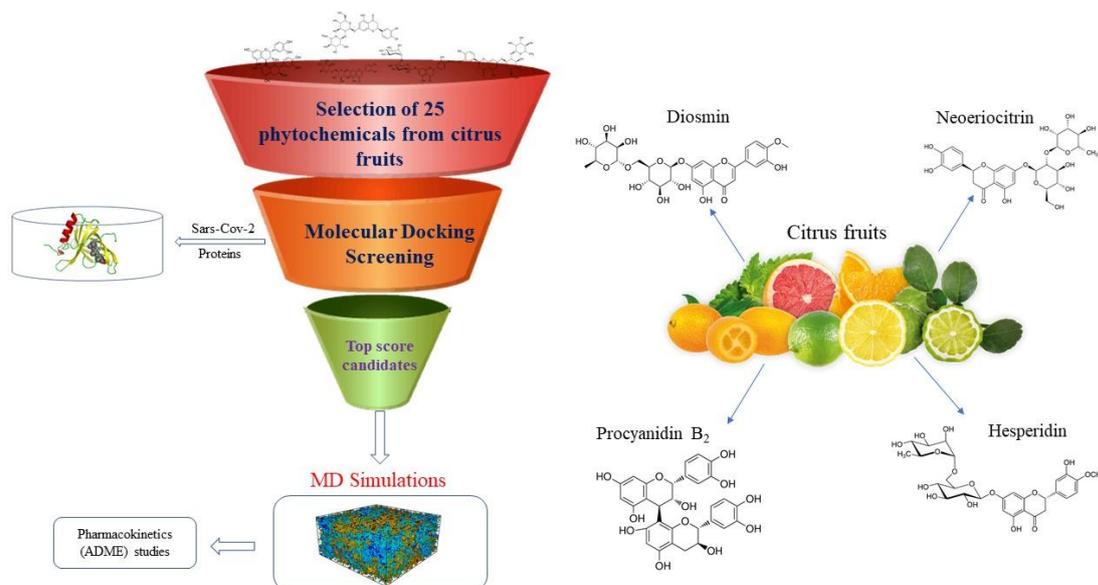


Figure 2 Schematic representation for screening of flavonoids in citrus fruits.

Molecular docking

Docking was carried out to evaluate the real time interaction between the phytochemical and target protein of interest SARS-CoV-2 (6LU7 & 6M0J) by measuring the binding energy of the complex (drug with target protein). AutoDock 4.0 (AD) AutoDock Vina 1.5.6 (ADV) and CB-Dock web tool was used for this process and results were visualized using Discovery studio visualization tool.

Protein preparation

To know the effect of ligand with protein, molecular docking analysis were performed to appraise the binding by using AutoDock 4.0 (AD) AutoDock Vina 1.5.6 (ADV) and CB-Dock web tool [48]. The X-ray crystal structure of spike binding domain with ACE2 receptor (PDB ID: 6M0J) and spike binding domain with main protease (PDB ID: 6LU7) of Sars-CoV-2 were downloaded from RCSB protein data bank website (<https://www.rcsb.org/>). The AutoDock tool was used to add the hydrogen atoms to the receptor for a protein preparation and was utilised to run and analyse the docking simulations. Finally, the structure of the receptor was saved in PDBQT format.

Ligand preparation

The 3D chemical structure of selected compounds from citrus fruits were retrieved from PubChem database website (<https://pubchem.ncbi.nlm.nih.gov/>) as SDF format, later on the ligand with SDF format was converted into PDB format using Marvin JS software [49]. The PubChem CID's for selected compounds is given as, Diosmin (5281613), Neohesperidin (114627), Hesperidin (1062), Procyanidin B₂ (122738), Limocitrol (12311234), Sinensetin (145659), Isolimocitrol (15290461), Isosakuranetin (160481), Heptamethoxyflavone (389001), Naringin (442428), Narirutin (442431), Neohesperidin (442439), Poncirin (442456), Luteolin (5280445), Kaempferol (5280863), Rhoifolin (5282150), Limocitrin (5489485), Isosinensetin (632135), Tangeretin (68077), Hesperetin (72281), Nobiletin (72344), Eriocitrin (83489), Naringenin (932), Isorhoifolin (9851181) and Rutin (5280805). All the compounds were optimized and converted from PDB to PDBQT format by using AutoDock tools 4.2.6.

Grid box generation and virtual molecular docking

By using an Autodock Autodock Vina software's and CB-Dock online tool, binding energies were reported and determined in kcal/mol unit for ligand-protein. The grid box was generated by targeting the active site with a size of ($x = 51.01, y = 59.65, z = 52.382$) and a centre of ($x = -11.83, y = 12.45, z = 69.92$) for main protease 6LU7 and grid box with a size of ($x = 72.32, y = 68.31, z = 100.85$) and centre of ($x = -24.66, y = 16.07, z = -10.64$) was set to cover the binding site for 6M0J protein [50]. Virtual molecular docking and analysis have been performed by using AutoDock Vina 1.5.6, AutoDock, CB-Dock tool. All the ligand molecules have been docked to active site of Sars-CoV-2 proteins 6LU7 and 6M0J. Finally, protein-ligand interactions were analysed by using Biovia discovery studio visualiser.

Molecular dynamics

By using Desmond modules of the Schrodinger 2020-2 suite software molecular dynamics simulation study of protein-ligand complex was carried to analyze the ligand consistency and the binding mode stability in the binding pocket of protein [51]. Top candidates with good docking results were carried for MD simulation to know the compound stability with spike binding domain with Main protease (6LU7) and spike binding domain with ACE2 receptor (6M0J) of Sars-CoV-2. The cubic box was overwhelming with simple point charge TIP3P water molecules the system was solvated and the OPLS3 (optimized potential for liquid simulations) force field were used to prepare whole complex and interactions was evaluated. The minimum quantity of Na⁺ ions along with salt atoms were added to maintain neutral conditions of the system [52]. The compound was inserted by the energy minimization on a convergence threshold of 1 Kcal mol⁻¹ Å by using conjugated algorithm. Once the compound attained energy minimization, it was put through equilibrium using NPT gathering for 2 ns. The relaxed whole system was subsequently taken to 20 ns MD simulations, which was setup under NPT ensemble using a Marlyna-Tobias-Klein barostats at 1 bar pressure and Nose-Hoover thermostat at 300 K. By using a root mean square deviation (RMSD), root mean square fluctuations (RMSF), protein ligand interaction and energy potential stability of the protein-ligand complexes system were studied [53].

ADME analysis

The standard pharmacokinetics (absorption, distribution, metabolism and excretion were determined to classify the ligand as a potential drug molecule in the field of drug discovery process [54]. The compounds structures which are drawn on Marvin JS software in 2-D structure format and all the structures are converted into SMILES in the Swiss-ADME online tool (<http://swissadme.ch/>). The SMILES of those compounds was inserted into pkCSM online tool. [55]. The pkCSM online tool were used for the prediction of ADME. By using pkCSM online tool the ADME mode was selected which includes Intestinal absorption, skin permeability, BBB permeability, CNS permeability, total clearance and renal OCT 2 substrate.

Results and discussion

Selection of phytochemicals from citrus fruits

Varieties of citrus fruits were listed and 45 active phytochemicals of citrus fruits was taken from ZINC database were included in the study. Different kinds of phytochemicals have been depicted in the table S1. The phytochemicals which are having phenolic composition were screened based on their anti-viral and anti-oxidant properties [56,57] and further taken for *in-silico* studies of molecular docking studies, molecular dynamic stimulation studies and ADME analysis. The 25 phenolic phytochemicals were analyzed for their ability to bind to the relevant targets (6LU7 and 6M0J). Compounds with the best binding energies were subjected to molecular dynamics simulation and then ADME analysis were predicted.

Molecular docking studies

The docking analysis of all the 25 phytochemicals were conducted successfully against the spike binding domain with main protease (PDB ID: 6LU7) and spike binding domain with ACE2 receptor (PDB ID: 6M0J) of Sars-CoV-2. Based on their dock score all 25 compounds were ranked. After docking studies, the list of active molecules with docking score were depicted in table S2. Prominent results were observed for 4 compounds such as Procyanidin B₂, Hesperidin, Neoeriocitrin and Diosmin. Based on their interactions with the viral proteins (PDB ID: 6M0J and 6LU7). The best 2 compounds were selected, the 2 compounds were Procyanidin B₂ and Hesperidin exhibited the best dock score with 6M0J and 6LU7 proteins and remaining 2 compounds Neoeriocitrin and Diosmin also exhibited good interaction with the 6LU7 and 6M0J proteins.

Targeting spike binding domain with ACE2 receptor (PDB ID: 6M0J)

The docking analysis of 4 best compounds (Diosmin, Neoeriocitrin, Hesperidin and Procyanidin B₂) with Sars-CoV-2 spike binding domain with ACE2 receptor were analyzed. Among these 4 compounds, hesperidin exhibited the best possible interaction with spike binding domain with ACE2 receptor of binding energy -9.1 kcal/mol (ADV), -6.32 kcal/mol (AD) and 9.0 kcal/mol (CB-Dock), suggesting high affinity for binding pocket. The phytoconstituent Procyanidin B₂ showed a good binding with spike binding domain with ACE2 receptor of Sars-CoV-2 at the active site with the binding energies of -8.9 kcal/mol (ADV), -5.20 kcal/mol (AD) and -8.8 kcal/mol (CB-Dock), respectively. All the binding modes of the compounds are in the active binding pocket. The hesperidin and Procyanidin B₂ binds with protein residues of ALA348, ASP350, TRP349, THR347, HIS345, ASN51, SER47, TYR385, GLU398, PHE390, ARG393, GLU375,

GLU398, PRO346, HIS401, ASP382 and TRP349 with various interaction. The other 2 compounds Neeriocitrin and diosmin are also interacted well with SARS-CoV-2 protein. The detailed interactions have been shown in (Figures 4 and 5) and bond length and docking energies are given in table S3. Thus, it clearly indicates that both the 2 phytochemicals show good inhibiting activity against spike binding domain with ACE2 receptor complex of Sars-CoV-2 and interacts well. Docking analysis have been performed for phytochemicals of citrus fruits with 6M0J protein using different docking tools (AutoDock, AutoDock Vina and CD-Dock) have been shown in Figure 3 for top 4 candidates.

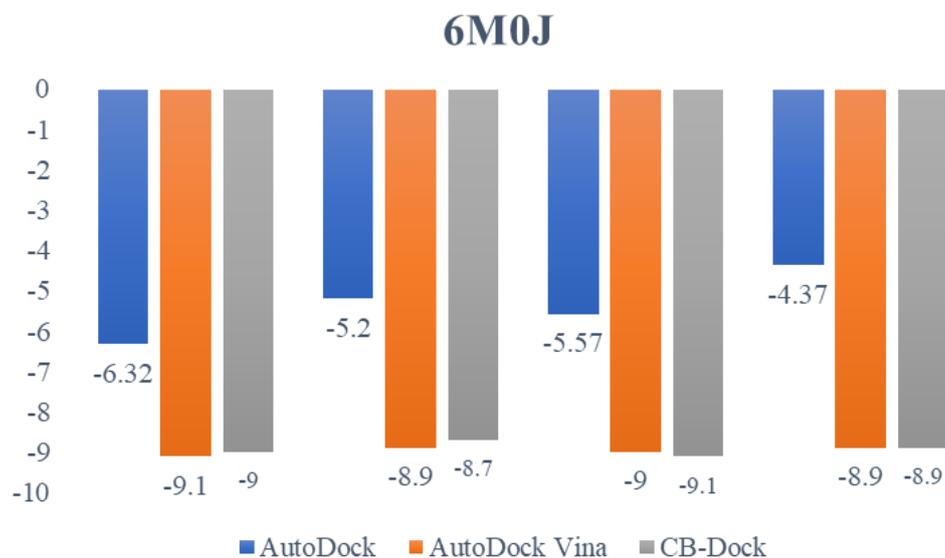


Figure 3 Results of docking score blue bar represents AutoDock results, orange bar represents AutoDock Vina results and grey bar represents CB-Dock results.

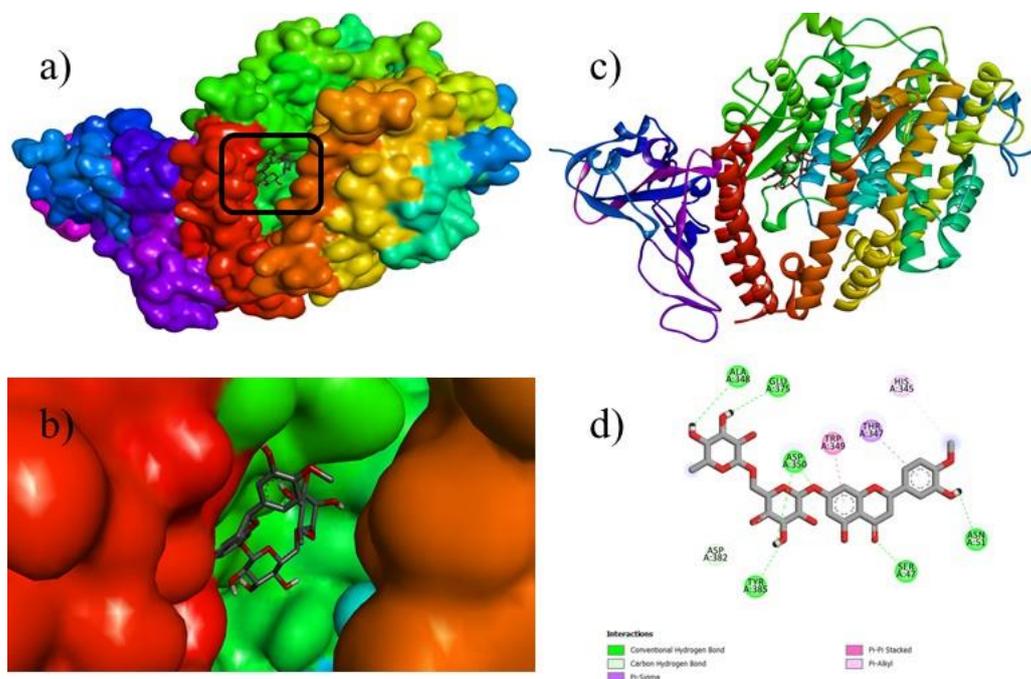


Figure 4 Molecular docking of hesperidin with 6M0J (a) Docking surface pocket pose, (b) docking interaction at surface, (c) 3-D 6M0J - hesperidin interaction and (d) active site amino acid residue interaction with hesperidin.

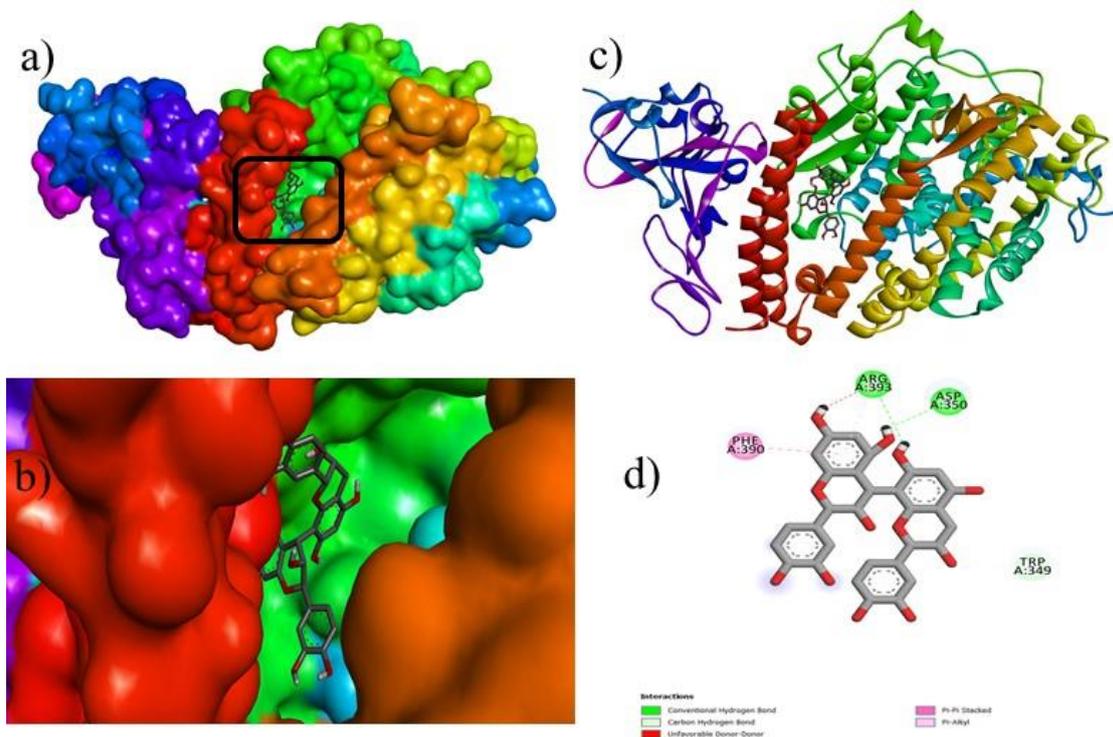


Figure 5 Molecular docking of Procyanidin B₂ with 6M0J (a) Docking surface pocket pose, (b) docking interaction at surface, (c) 3-D 6M0J - Procyanidin B₂ interaction and (d) active site amino acid residue interaction with Procyanidin B₂.

Targeting spike binding domain with main protease (PDB ID: 6LU7)

The molecular docking analysis of 4 best compounds (Diosmin, Neerocitrin, Hesperidin and Procyanidin B₂) with Sars-CoV-2 spike binding domain with main protease were analyzed. The best binding energy of -9.2 kcal/mol (ADV), -8.52 kcal/mol (AD) and -9.3 kcal/mol (CB-Dock) for Procyanidin B₂ shows a possible and good interaction with spike binding domain with main protease of Sars-CoV-2. The compound hesperidin also showed good binding with spike binding domain with main protease of Sars-CoV-2. The free energies at the active sites being -9.0 kcal/mol (ADV), -7.24 kcal/mol (AD) and -8.6 kcal/mol, respectively. These results suggest the high affinity of Procyanidin B₂ and Hesperidin towards different amino acids THR190, GLU166, THR26, HIS163, ARG188, HIS41, MET165 and CYS145 with various types of interactions. The other 2 compounds Neerocitrin and diosmin are also interacted well with SARS-CoV-2 protein. This clearly indicates that both the 2 phytochemicals interacted well at the active site of Sars-CoV-2 of spike binding domain with main protease complex and also binds more favorably. The detailed interactions have been shown in (Figures 7 and 8) and bond length and docking energies are given in table S4. Docking analysis have been performed for phytochemicals of citrus fruits with 6LU7 protein using different docking tools (AutoDock, AutoDock Vina and CD-Dock) have been shown in Figure 6 for top 4 candidates.

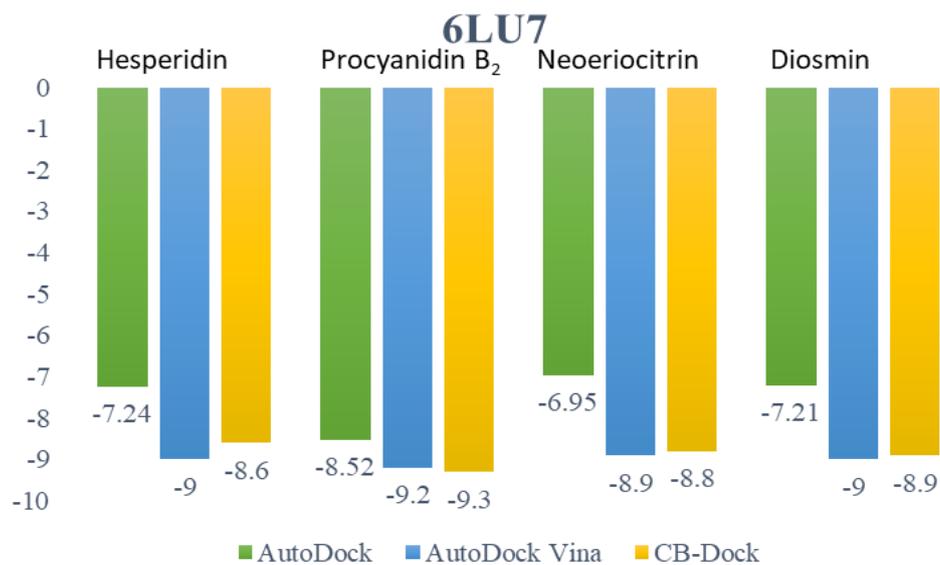


Figure 6 Results of docking score green bar represents Autodock results, blue bar represents AutoDock Vina results and yellow bar represents CB-Dock results.

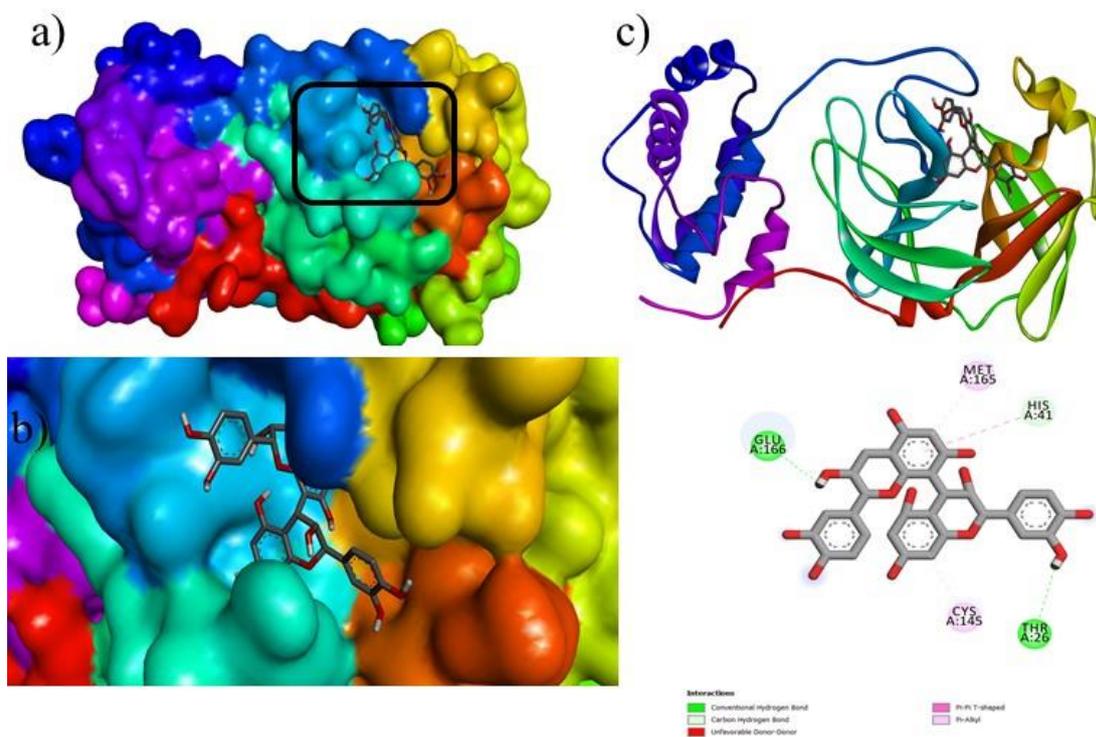


Figure 7 Molecular docking of Procyanidin B₂ with 6LU7 (a) Docking surface pocket pose, (b) docking interaction at surface, (c) 3-D 6LU7 - Procyanidin B₂ interaction and (d) active site amino acid residue interaction with Procyanidin B₂.

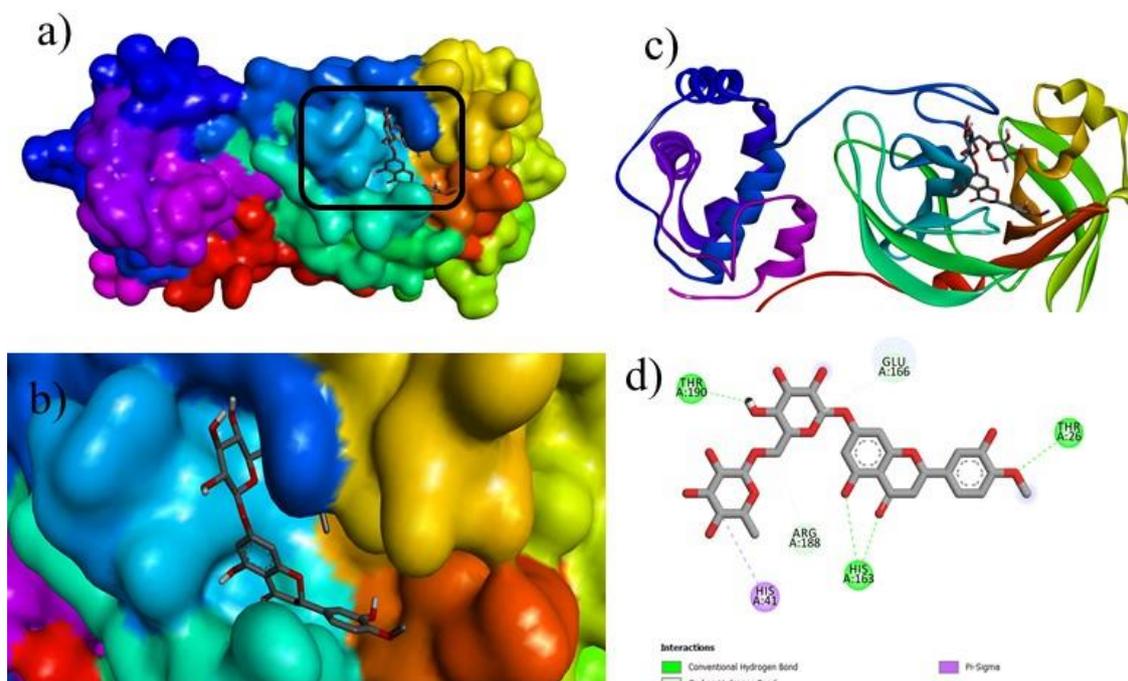


Figure 8 Molecular docking of Hesperidin with 6M0J (a) Docking surface pocket pose, (b) docking interaction at surface, (c) 3-D 6M0J - Hesperidin interaction and (d) active site amino acid residue interaction with Hesperidin.

Molecular dynamics simulation studies

To understand the conformational and most effective active analogues in the active site of the protein, molecular dynamics simulation is a vital method to discover the behavior of each system in real time. The selected top 2 compounds which showed a good docking score against Sars-CoV-2 proteins of 6LU7 and 6M0J were investigated. The root mean square deviation (RMSD) of the protein C α was calculated over 20 ns to know the stability of the protein ligand complex. The RMSD plot of backbone atoms were shown in (Figure 9) for the potent compounds (Hesperidin and Procyanidin B₂) complex with spike binding domain with Main protease (6LU7) and spike binding domain with ACE2 receptor (6M0J) of Sars-CoV-2.

RMSD analysis

The results show that all the 4 systems are almost stable in the average range of protein RMSD value which were in between 2 - 4 Å. In the case of Hesperidin-6LU7 complex system attains a stability by completion of 10 ns. The range of Protein RMSD value will be in between 2 - 2.4 Å. The system is stable upto 13.5 ns, after completion of 13.5 ns a slightly small divergence was observed for some period of time and remained stable during the period of the simulations. Similarly, in the case of Hesperidin-6M0J shows a high divergence upto 17 ns and after reaching 17 ns the system attains stability upto 19 ns. The protein RMSD range value will be in between 3.5 - 4 Å. There will be a small divergence after completion of 19 ns. For The complex system Procyanidin B₂-6LU7 shows a small deviation upto 12 ns and soon after system gains a stability between 12 - 14.5 ns. The protein RMSD value would be approximately in the range of 1.5 - 1.75 Å, after completion of 14.5 ns there will be acceptable small deviation for some period of time. On the other hand, complex system Procyanidin B₂-6M0J reported small deviation upto 7 ns, after completion of 7 ns the system attains stability and the acceptable protein RMSD range value would be in between 2.4 - 2.8 Å. The system shows a slight divergence after completion of 11 ns and remained stable during the period of the simulations. All the 4 complex system shows an approximate average range of RMSD value which are shown in Figure 9.

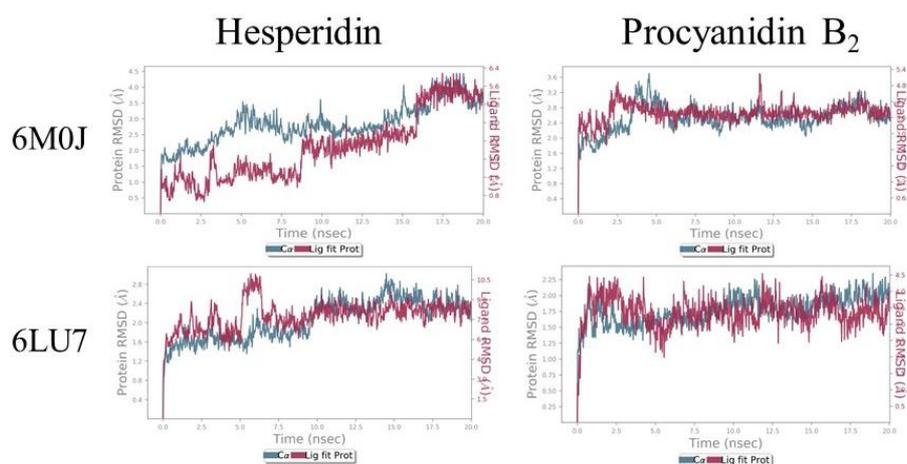


Figure 9 RMSD analysis of Hesperidin, Procyanidin B2 with 6M0J, 6LU7 proteins.

RMSF analysis

RMSF analysis were used to measure the fluctuations of each residue level. In the case of Hesperidin-6LU7 complex system, approximate RMSF value would be 3.2 Å and the residues fluctuated more and it shows more fluctuation in the range of 40 - 50 and 160 - 170. Similarly, Hesperidin-6M0J complex system shows an approximate RMSF value of 5.0 Å in the fluctuation region of 110 - 120 and 590 - 610. The fluctuations were also observed for Procyanidin B₂-6LU7 complex system, the residues were fluctuated less during the simulations the fluctuations will be in the region of 60 - 70 and 270 - 280. Where the approximate RMSF range will be 2.5 Å. Similarly, the Procyanidin B₂-6M0J shows a fluctuation in the region of 110 - 120 and 590 - 600, the approximate RMSF value will be 5.4 Å. All the 4 complex system shows an approximate average range of RMSF value would be in between 0 - 5.4 Å, which are shown in **Figure 10**. The overall RMSF values of the protein residues complexes showed that residue fluctuations are significantly affected by the binding of ligand. These results have shown that the target protein of Sars-CoV-2 (6LU7 and 6M0J) is stabilized by the binding of 2 compounds which shows good docking results.

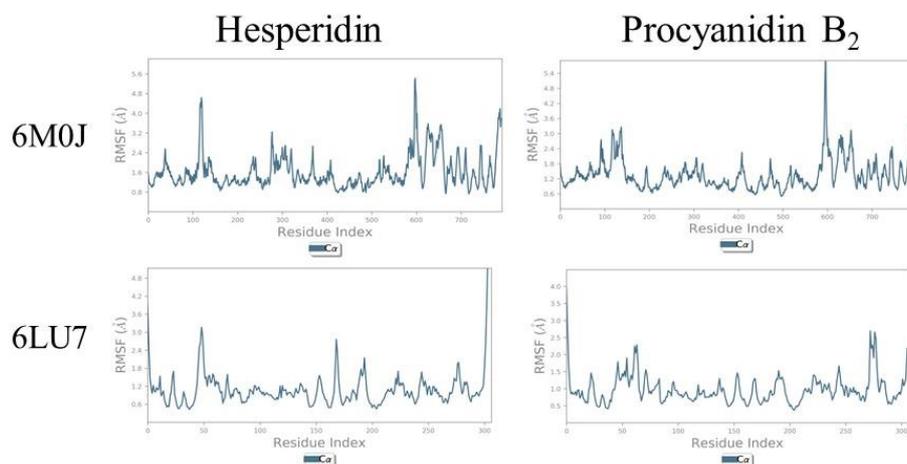


Figure 10 RMSF analysis of Hesperidin, Procyanidin B2 with 6M0J, 6LU7 proteins.

Occupancy of hydrogen bond

The Hesperidin-6LU7 complex system gives a hydrogen bond with GLU166 residue reported in 100 % of trajectories and ASP48 residue as observed in 70 % of trajectories. The Hesperidin-6M0J complex system shows a hydrogen bond with ASN350, GLU402 and ALA348 which was reported in more than 100 % of trajectories. Similarly, complex Procyanidin B₂-6LU7 system shows a hydrogen bond with GLY143 residue as observed in 100 % of trajectories and GLU166 residue was reported in more than 100 % of trajectories and the system Procyanidin B₂-6M0J complex shows a hydrogen bond with ASP382 residue as reported in more than 90 % of trajectories and the GLU398 residue was reported in 60 % of trajectories as shown in **Figure 11**. While the remaining ligand interaction with protein of amino acids in complex as shown in **Figure 12**.

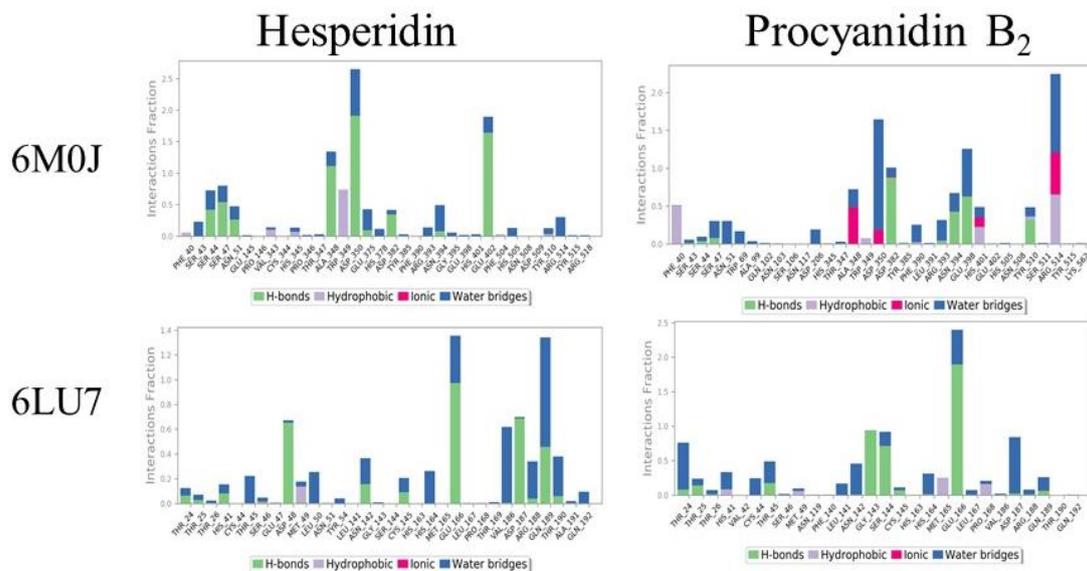


Figure 11 Protein-Ligand contacts of Hesperidin, Procyanidin B2 with 6M0J, 6LU7 proteins.

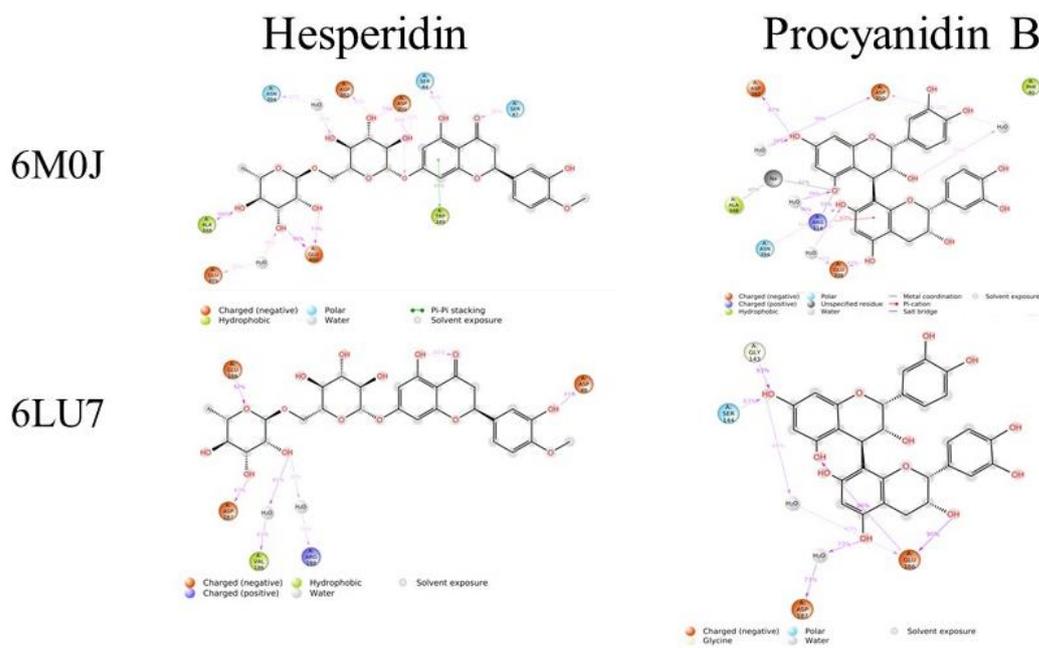


Figure 12 Ligand-protein contacts of Hesperidin, Procyanidin B2 with 6M0J, 6LU7 proteins.

ADME analysis

Different kinds of online computational tools were utilized to predict the ADME properties of the molecules in the field of drug discovery and development process. Among them by using an pkCSM tool we can predict the ADME properties of the molecules. The 2 phytochemicals were selected based on their molecular docking score with SARS-CoV-2 proteins and complex stability in dynamics simulation analysis. Both the 2 compounds are obeying the ADME limitations The ADME results of all the 2 compounds are interpreted based on standard values compared with resultant values as intestinal absorption less than 30 % is considered as poorly absorbed both the compounds are moderately absorbed, low caco-2 permeability value and as high skin permeability (log Kp > -2.5), whether the compounds is to be a P-glycoprotein substrate and inhibitor (I and II) are also shown in **Table 2**, compound procyanidin b₂ has low

human VDss is low and hesperidin has moderate value of VDss. In BBB and CNS permeability both compounds are poorly distributed and unable penetrate CNS. The compounds are likely to be a cytochrome P450 inhibitor or they are to be metabolized by P450 substrate were shown **Table 2**, whether the compounds are likely to be a renal OCT2 substrate are shown. The Bioavailability radar of the compound has been shown in **Figure 13**.

Table 2 ADME by using pkCSM tool.

Parameters	Predicted value		
	Hesperidin	Procyanidin B ₂	
Absorption	Water solubility (log mol/L)	-3.014	-2.892
	Caco2 permeability (log Papp in 10 ⁻⁶ cm/s)	0.505	-1.225
	Intestinal absorption (human) (% Absorbed)	31.481	66.749
	Skin Permeability (log Kp)	-2.735	-2.735
	P-glycoprotein substrate	Yes	Yes
	P-glycoprotein I inhibitor	No	Yes
	P-glycoprotein II inhibitor	No	Yes
Distribution	VDss (human) (log L/kg)	0.996	-0.158
	Fraction unbound (human) (Fu)	0.101	0.309
	BBB permeability (log BB)	-1.715	-1.94
	CNS permeability (log PS)	-4.807	-3.983
Metabolism	CYP2D6 substrate	No	No
	CYP3A4 substrate	No	No
	CYP1A2 inhibitor	No	No
	CYP2C19 inhibitor	No	No
	CYP2C9 inhibitor	No	No
	CYP2D6 inhibitor	No	No
	CYP3A4 inhibitor	No	No
Excretion	Total Clearance (log ml/min/kg)	0.211	-0.085
	Renal OCT2 substrate	No	Yes

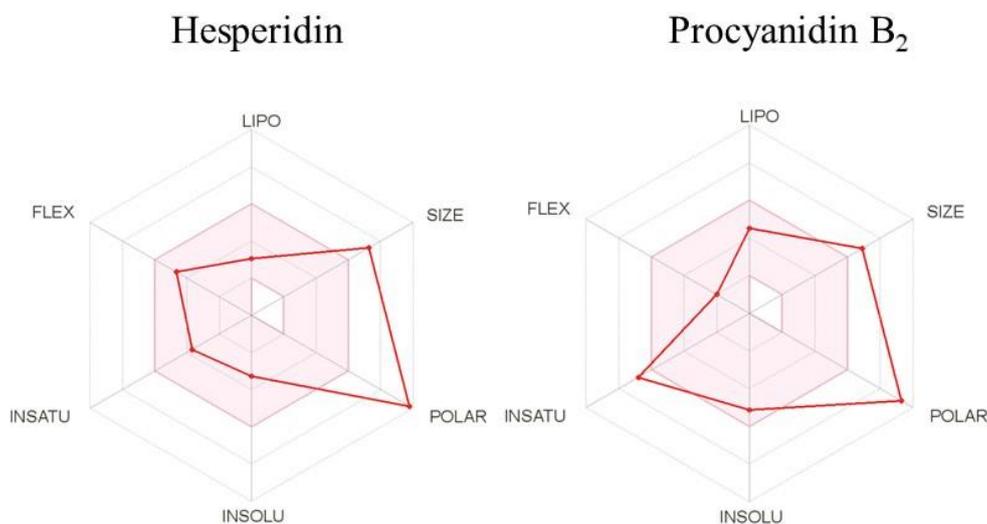


Figure 13 Bioavailability radar of Hesperidin and Procyanidin B₂.

Conclusions

In the present research work, potentially inhibiting molecules from citrus fruits against Covid-19 proteins were studied by *in-silico* techniques such as Molecular dynamic simulation, molecular docking analysis and ADME studies. All the molecules of citrus fruits (25 molecules) show a good interaction with the Covid-19 proteins. Thus, the *in-silico* properties of all these compounds are significant. The molecular docking studies predicts that 4 active compounds (Hesperidin, Procyanidin B₂ Diosmin and Neoeriocitrin) from Citrus fruits gives excellent binding affinity with the Sars-CoV-2 proteins (6LU7 and 6M0J). The top 2 hit compounds were subjected to MD simulations and among them Hesperidin and Procyanidin B₂ showed a highest occupancy of hydrogen bond and excellent range of RMSD and RMSF values for 6M0J and 6LU7. The overall results obtained from *in-silico* techniques for 2 phytochemicals (Hesperidin and Procyanidin B₂) is they have good binding affinity. From the MD analysis with RMSD, RMSF calculations and residue analysis exhibited a stable complex with Covid-19 proteins (6LU7 & 6M0J) and also both the compounds obey the limitations of ADME. Naturally obtained substances are key in drug discovery and design and lead enormously in drug discovery. Further, its potential can be proved by its *in-vivo*, *in-vitro* and clinical studies.

Acknowledgements

The authors are thankful to SJCE, JSS Science and Technology University for providing supercomputing facility and VGST-CISEE Project (GRD 647), GOK, Karnataka, India.

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