In silico phytochemical repurposing of natural molecules as entry inhibitors against RBD of the spike protein of SARS-CoV-2 using molecular docking studies

Pawan Gupta*

Department of Pharmaceutical Chemistry,
Shri Vile Parle Kelavani Mandals Institute of Pharmacy,
Dhule, Maharashtra-424001, India
Email: pawanpharma79@gmail.com
Email: pawan.gupta@svkm.ac.in

Swati Gupta

Centre of Biotechnology,
University of Allahabad,
Prayagraj, U.P-211002, India
Email: swatigupta737979@gmail.com

Sukrat Sinha

Department of Zoology,
Nehru Gram Bharati (Deemed to be) University,
Prayagraj-221505, India

and

Department of Zoology,
Sri Venkateshwara College,
University of Delhi, India
Email: sukratsinha@gmail.com

Shanthy Sundaram*

Centre of Biotechnology,
University of Allahabad,
Prayagraj, U.P-211002, India
Email: shanthy.cbt@gmail.com
*Corresponding authors

Vishnu K. Sharma

Department of Pharmacoinformatics,
National Institute of Pharmaceutical Education and Research (NIPER),
Mohali, Punjab-160062, India
Email: vishnushashi@gmail.com

Anjana Munshi

Department of Human Genetics and Molecular Medicine,
Central University of Punjab,
Bathinda, Punjab-151001, India
Email: anjana.munshi@cup.edu.in

Abstract: The receptor binding domain (RBD) of Spike-protein (S-protein) is responsible for virus entry via interaction with host protein ACE2 (angiotensin-converting enzyme 2), present on the cell surface of humans. Therefore, S-protein is an important target to block the entry of the SARS-CoV-2 into the cell for further growth. In the present study, phytochemical repurposing of natural molecules: narirutin, naringin, neohesperidin and hesperidin were performed against the RBD S-protein/ACE2 interface as well as the RBD of the S-protein using molecular docking. These natural molecules were found to have structural similarity to each other and had binding potential against the viral infections. It is first time reported here that the naringin and narirutin are having binding potential against both RBD S-protein/ACE2 interface and active site of RBD of S-protein using binding mode analysis. Hence, this study will open avenues for multitargeting similar natural molecules binding against the SARS-CoV-2 proteins as all reports are made in this single study.

Keywords: SARS-CoV-2; spike protein; ACE2; natural molecules; binding mode analysis; docking.

Reference to this paper should be made as follows: Gupta, P., Gupta, S., Sinha, S., Sundaram, S., Sharma, V.K. and Munshi, A. (2023) '*In silico* phytochemical repurposing of natural molecules as entry inhibitors against RBD of the spike protein of SARS-CoV-2 using molecular docking studies', *Int. J. Computational Biology and Drug Design*, Vol. 15, No. 4, pp.267–288.

Biographical notes: Pawan Gupta is currently working as an Associate Professor at the Department of Pharmaceutical Chemistry, SVKMs Institute of Pharmacy, Dhule, Maharastra. He has more than 12 years research in the field of computer aided drug design and discovery in India and abroad. His current research interests are identification of novel small molecules/leads/hits against biological targets of the life-threatening diseases using computer aided drug design and discovery methods. He has published more than 32 papers in the journals of international repute. He has delivered the 18 talks in different institutions in India on the topic of his expertise.

Swati Gupta has completed her BSc and MSc in Biotechnology from Chhatrapati Shahu Jee Maharaj University, Kanpur, India. She has been a throughout first class student. She has submitted her PhD under supervision of Prof. Shanthy Sundaram.

Sukrat Sinha completed his BSc from University of Allahabad, MSc from Jawaharlal Nehru University, New Delhi, PhD. from University of Allahabad,

India. He worked as a Dr. D.S Kothari Fellow at Centre of Biotechnology, University of Allahabad further he worked as a Young Scientist in the Department of Science and Technology Fast Track scheme of Govt. of India. He has worked as Fulbright Nehru Postdoctoral Fellow at Emory Vaccine Center, Emory University, Atlanta. His area of specialisation is immunology and computational biology of infectious diseases. He has won several national and international awards for his scientific work presentations. He has 25 publications, two books and two book chapters to his credit.

Shanthy Sundaram is serving as a Faculty in Centre of Biotechnology, University of Allahabad. She had supervised 20 PhD students. 5 Postdoctoral fellows have worked under her supervision. She has worked as Head of the Department, Centre of Biotechnology. She has been a Common Wealth fellow and British council fellow to pursue her research work. She has published around 130 research papers, 23 books and book chapters, presented in 125 national and international conferences, 4 gene bank submissions and 2 patents. She has collaborated with several national and international scientists.

Vishnu K. Sharma is a researcher cum technical expert in the Department of Pharmacoinformatics, National Institute of Pharmaceutical Education and Research (NIPER), SAS Nagar, India. He studied B. Sc. (Bio.) followed by DPharm from University of Rajasthan, Jaipur. He completed his MSc Chemistry from MPBOU, Bhopal, MP, India. During his Doctoral research (2014–2018), he got accustomed with various Pharmacoinformatics techniques utilised for molecular modelling, which would enable him to work towards the goal of working on rational design of antileishmanial inhibitors and to understand the catalytic mechanisms of the enzymes. His current research interests encompass computer-aided drug design targeting various communicable diseases. He has published ~10 research articles in international journals. He has more than 10 year's research experience in the field of pharmacoinformatics.

Anjana Munshi is currently working as a Professor in the Department of Human Genetics and Molecular Medicine, School of Health Sciences, Central University of Punjab, Bathinda, Punjab, India. She is having more than 20 years of experience in the field of pharmacogenomics and bioinformatics studies of genes involved in stroke, cancer, epilepsy, diabetes and rare genetic diseases. She has published the more than 145 research as well as review papers in the journal of international repute. The 30 book chapters and 3 books published with international publishers are also under her credit. She has delivered the various talks in the national and international events as per her expertise in the field.

1 Introduction

The Spike-protein (S-protein) which has the receptor-binding domain (RBD), is helpful to bind with residues of ACE2 protein and initiate the entry process of the SARS-CoV-2 virus for further reproduction in human cell. The RBD of the S-protein is crucial for binding of SARS-CoV-2 virus with host cell angiotensin-converting enzyme 2 (ACE2) receptor. Without binding with ACE2 protein, SARS-CoV-2 cannot enter into the cell for proliferation and growth. Therefore, S-protein is an important target to block the entry of the SARS-CoV-2 into the host. Recent reports suggested that the residues of the ACE2

and RBD are important sites for drug design and discovery of inhibitors preventing entry (Du et al., 2009; Du et al., 2017; Lan et al., 2020; Yan et al., 2020).

In-silico binding mode analysis has been attractive method for decades to identify the binding potential of small molecules against disease targets for drug design and discovery. For this purpose, molecular docking is method of choice and widely used to understand the binding interactions and mechanisms of binding to the active site of the target (Alakhdar et al., 2020; Botelho et al., 2020; Coro-Bermello et al., 2021; Eweas et al., 2022; Gupta et al., 2010). In addition, the structural similarity concept was also found very useful in drug design and discovery to identify structurally similar molecules with a similar type of activity (Sharma et al., 2019; Singh et al., 2020). On the other hand, phytochemical repurposing is an useful method to identify the new therapeutic category or use of the old phytochemicals against the new diseases or new drug targets (Chadha et al., 2022; Sharma et al., 2019; Zhang et al., 2021). There are lot of reports about successful use of repurposing concept in drug design and discovery (Chadha et al., 2022; Eweas et al., 2022; Zhang et al., 2021). This in-silico methodology is time-saving and cost-effective for the identification of potential molecules against biological targets for drug design.

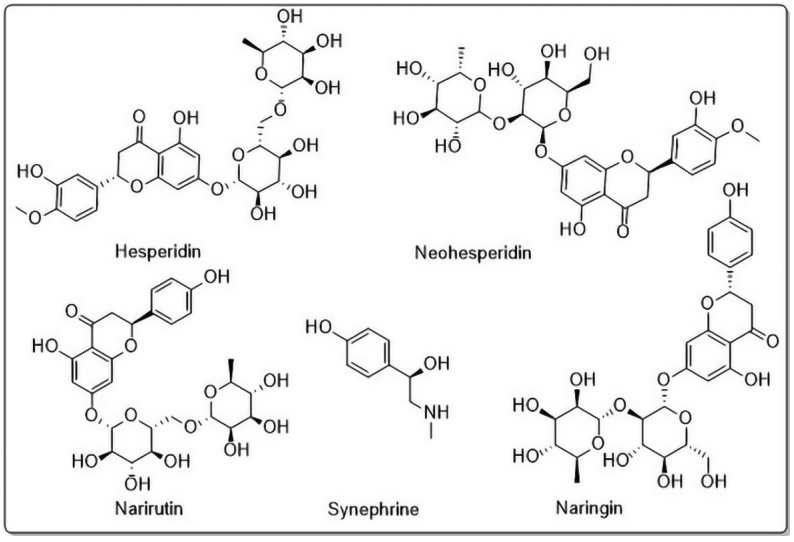
The natural molecules/herbs with reported antiviral activities can help to slow down the transmission and progression of the coronavirus disease. These plants are very useful raw materials available in abundance in developing countries and economically very feasible for harvesting and growing. The natural molecules (narirutin, naringin, neohesperidin and hesperidin) exhibited structural similarity (Figure 1). However, these natural molecules were also reported to have different activities for treatment of various diseases such as cancer, diabetes, TB, wound/skin disease, obesity, neuroprotective action, hepatic steatosis, cardiovascular diseases, etc. (Bagher et al., 2020; Chakraborty et al., 2021; Gollavilli et al., 2020; Heidary Moghaddam et al., 2020; Lu et al., 2020; Morita et al., 2020; Niu et al., 2021; Patel et al., 2020; Sahu et al., 2020; Shokoohi et al., 2020; Syed et al., 2020; Wang et al., 2020). These natural molecules were found to have binding potential against different SARS-CoV-2 targets (Bellavite and Donzelli, 2020; Cheng et al., 2021; Haggag et al., 2020; Haridas et al., 2021; Mahdian et al., 2020; Puttaswamy et al., 2020; Wu et al., 2020). Only, hesperidin and neohesperidin were reported to have binding potential against RBD-S-protein/ACE2 interface (Bellavite and Donzelli, 2020; Cheng et al., 2021; Haggag et al., 2020; Haridas et al., 2021; Mahdian et al., 2020; Puttaswamy et al., 2020; Wu et al., 2020). In addition, hesperidin was also reported against papain like protease (PL^{pro}), and main protease (M^{pro}) proteins of the SARS-CoV-2 which are responsible for replication of the SARS-CoV-2 (Agrawal et al., 2021; Laksmiani et al., 2020). Recently, neohesperidin, naringin and narirutin were found to have potential against RNA dependent RNA polymerase (RdRp) and Mpro of SARS-CoV-2 (Bousta et al., 2021; Ghosh et al., 2021; Kandeel et al., 2020; Puttaswamy et al., 2020; Saric et al., 2021). Due to structural similarity among these natural molecules, they were found to exhibit the biological activities against the same SARS-CoV-2 proteins (as mention above). Such similarity concept is already proved that structurally similar molecule may have similar type of the biological activity (Boström et al., 2006; Sharma et al., 2019).

The natural molecules: narirutin and naringin are never reported till date against RBD-S-protein/ACE2 interface as entry inhibitors for SARS-CoV-2 infection. Hence, *in silico* phytochemical repurposing was performed to evaluate the binding affinity of

these phytochemicals against the RBD-S-protein/ACE2 proteins required for SARS-CoV-2 entry into the host cell as entry inhibitor.

Figure 1 shows all the natural molecules (narirutin, naringin, neohesperidin and hesperidin) showing structural similarity with each other (Figure 1). Like hesperidin and neohesperidin, narirutin and naringin may also have the binding ability with the RBD-S-protein/ACE2 interface and/or RBD of S-protein. Therefore, in the present studies, *in silico* phytochemicals repurposing of narirutin and naringin were carried out to evaluate the binding potential against RBD-S-protein/ACE2 interface as well as RBD-S-protein using binding mode analysis approach. These results were compared with neohesperidin and hesperidin. These studies will give ideas about how narirutin and naringin can bind to the RBD-S-protein/ACE2 interface and RBD of S-protein alone. These natural molecules could interfere with the virus binding to the ACE2 protein and disrupt the virus cycle, thereby reducing the spread of viral infection. Hence, it may be useful to design further new entry inhibitor molecules against SARS-CoV-2 and thereby open new avenues for multitargeted drug binding with different proteins of the SARS-CoV-2.

Figure 1 Chemical structure of the molecules reported against SARS-CoV-2 infections



2 Material and methods

2.1 Ligand and protein structure preparation

The natural molecules (naringin, neohesperidin, narirutin, and hesperidin) were obtained from different literatures and found to have significant inhibitory potential against viral infections. All these natural molecules were collected from the PubChem and ZINC databases and their structures were prepared in the Ligprep in Schrödinger Maestro program.

To study the binding mode analysis, molecular docking method was utilised. For docking studies, first SARS-CoV-2/ACE2 complex (PDB 6M17 (Yan et al., 2020)) and SARS-CoV-2 spike (PDB: 6XR8) proteins were collected from Protein databank

(www.rcsb.org) and prepared for docking in the protein preparation wizard of Schrödinger platform. During the protein preparation, water molecules were removed, atomic clashes were optimised, only polar hydrogen were added and geometry of the hydrogen was also optimised. In SARS-CoV-2/ACE2 complex (PDB 6M17) only B (ACE2 residues) and E chain (SARS-CoV-2 spike residues) were kept for grid preparation, while rest of the chains and ligands were removed.

Table 1 Binding mode analysis of the natural ligands against SARS-CoV2-ACE2 and SARS-CoV2 protein active site

Name	*Entry*	*Score* (kcal/mol)	*Protein*	*H-bonding*	*Other interactions*
Neohesperidin	118	–8.956	6M17_BE	Lys26B, *Asp30B*, Thr92B, Gln96B, **Tyr505E**	**Hydrophobic:** Leu29B, Val93B, **Ala386B**, Ala387B, Pro389B, Leu392B, **Tyr505E**
Hesperidin	119	–8.919	6M17_BE	*His34B*, *Glu37B*, Arg403E, Gln409E, **Lys417E**, Tyr421E	**Hydrophobic:** Pro389B, *Phe390B*, Phe456E, Tyr473E
Narirutin	120	–7.785	6M17_BE	*Asp30B*, *Glu37B*, Gln96B, **Lys417E**	**Hydrophobic:** Leu29B, Val93B, Ala387B, Pro389B, *Phe390B*, Lys421E
Naringin	121	–6.798	6M17_BE	Lys26B, *Asp30B*, *Glu37B*, Tyr421E, **Lys417E**	**Hydrophobic:** *Phe390B*, Pro389B, Ala387B, Phe456E, Tyr421E **Pi-pi: Lys417E**
Naringin	64	–8.432	6XR8_A	**Tyr449**, Glu484, Cys488, Phe490, Leu492, [c]Gln493	**Hydrophobic: Tyr449**, Leu452, *Leu455*, Phe456, Cys488, Tyr489, Phe490, Pro491, Leu492
Hesperidin	66	–7.321	6XR8_A	Glu484, Gly485, Phe490, Leu492, [c]Gln493	**Hydrophobic:** Phe456, *Leu455*, *Phe486*, Cys488, Tyr489, Phe490, Pro491, Leu492,
Neohesperidin	68	–7.088	6XR8_A	Glu484, Gly485, Phe490, ***Ser494***	**Hydrophobic: Tyr449**, Leu452, ***Leu455***, Phe456, *Phe486*, Cys488, Tyr489, Phe490, Pro491, Leu492
Narirutin	70	–7.049	6XR8_A	Glu484, Gly485, Phe490, Leu492, [c]Gln493	**Hydrophobic:** *Leu455*, Phe456, *Phe486*, Cys488, Tyr489, Phe490, Pro491, Leu492 **Pi-pi:** *Phe486*, Tyr489

PDB: 6M17-Chain-B: ACE2; PDB: 6M17-Chain-E-SARS-CoV-2-RBD; 6XR8_A: SARS-CoV2.

[a, b, and c] indicated the important interactions reported in the literature for binding with RBD-S-proein/ACE2 interface and RBD of S-protein: [a]Bold text in interactions (Yepes-Pérez et al., 2020); [b]Bold-italic text in interactions (Choudhary et al., 2020); [c]Common in both the references *(Choudhary et al., 2020; Yepes-Pérez et al., 2020)* italics and underlined.

Gln493 critical for binding with ACE2 (Yuan et al., 2020).

2.2 Binding mode analysis

After protein preparation, grid was generated around the ACE2 residues (Gln24, Thr27, Asp30, Lys31, His34, Glu35, Asp38, Tyr41, Gln42, Glu37, Phe390, Gln388; Met82,

Lys353, Gly354, Asp355 and Arg357) which are used by S-protein of SARS-CoV-2 for binding and entering in to the host cell (Choudhary et al., 2020; Wan et al., 2020; Wu et al., 2009; Yepes-Pérez et al., 2020). The grid centre was X = 176.59, Y = 113.45, Z = 238.72. The docking studies were performed around the ACE2 residues which are responsible for binding with RBD of the S-protein. Similarly, SARS-CoV-2 S-protein structure (PDB: 6XR8) was also used to check the binding potential of natural molecules against the RBD domain. The RBD of S-protein was defined from residues 333 to 527. The RBD binds with the ACE2 protein by interacting with the key residues of it (Yuan et al., 2020). The grid was generated around the residues of RBD domain [333-527 (Yuan et al., 2020)] of the SARS-CoV-2 protein [Lys417, Tyr49, Tyr453, Leu455, Gln474, Phe486, Gln493, Ser494, Gly496, Gln498, Thr500, Asn501, Tyr505]. These residues were found to be important for binding of the ACE2 with RBD of S-protein (Lan et al., 2020; Wan et al., 2020; Yan et al., 2020; Yepes-Pérez et al., 2020). The centre of the grid X = 213.82, Y = 173.69, Z = 138.69 was calculated. Each grid files were used to dock the selected natural molecules against each protein (PDB IDs: 1M17 and 6XRB) using Glide module of Schrödinger platform at different levels: Standard precision (SP) and Extra precision (XP) to validate the docking results with subsequent refinement of the docking poses. All the natural molecules were used to check the binding modes against these proteins and to study their binding potential as an entry inhibitor. This would be useful for the treatment of the SARS-CoV-2 infection. The final output of the docking studies was analysed (Table 1).

3 Results and discussion

3.1 Binding mode analysis

The recognition of RBD of S-protein of SARS-CoV-2 by ACE2 of human is achieved with the help of several residues present at the active site of both the proteins. The residues present in the ACE2 protein are Gln24, Thr27, Asp30, Lys31, His34, Glu35, Asp38, Tyr41, Gln42, Glu37, MET82, Lys353, Gly354, Asp355, Arg357 Phe390 and Gln388, while those found in the RBD of S-protein for ACE-2 binding, are Lys417, Tyr449, Tyr453, Gln474, Phe486, Gln493, Gly496, Gln498, Asn501, Thr500 and Tyr505 (Choudhary et al., 2020; Lan et al., 2020; Yan et al., 2020; Yepes-Pérez et al., 2020; Yuan et al., 2020). The analysis of the docking result was done using these important residues responsible for binding with RBD-S-protein/ACE2 interface of SARS-CoV-2.

In corona virus infection, virus enters in the human through interactions between RBD of SARS-CoV-2 and ACE2 of the host protein (human). ACE2 protein is mainly associated with cardiovascular diseases (like Heart attack, Chronic nephropathies, Hypertension, etc.). Directly hitting with ACE2 may lead to severe cardiac side effect. Blocking the binding of the RBD of S-protein with ACE2 protein could be the best strategies to inhibit the corona virus entry into the host cell. Thus, to study the effectiveness against S-protein/ACE2 complex, docking method was used against RBD-S-protein/ACE2 interface and RBD of the S-protein to understand the binding interactions of natural molecules. All the natural molecules were found to bind effectively into the active site of the RBD-S-protein/ACE2 complex as well as RBD of S-protein.

3.2 Binding modes with RBD S-protein/ACE2 interface

The results obtained from docking studies against RBD-S-protein/ACE2 interface exhibited that all the natural molecules (Hesperidin, Neohesperidin, Narirutin, Naringin) were fitted well into the RBD-S-protein/ACE2 interface (Figure 2) with good binding scores ranging from –8.95 to –6.79 kcal/mol (Table 1). All the natural molecules were found to be interacted with key residues responsible for recognition of RBD of S-protein to the ACE2 binding site. It was found that neohesperidin and hesperidin were shown strong binding to RBD-S-protein/ACE2 interface and had highest affinity (–8.956 and –8.919 kcal/mol respectively) as compared to other natural molecules (Table 1). The hesperidin and neohesperidin were able to interact with those critical residues (Choudhary et al., 2020; Yepes-Pérez et al., 2020) required for binding of RBD-spike to the human ACE2 receptor. The neohesperidin exhibited major H-bonding interactions with the residues Lys26B, *Asp30B*, Thr92B, Gln96B, and **Tyr505E** along with hydrophobic interactions with Leu29B, Val93B, **Ala386B**, Ala387B, Pro389B, Leu392B and **Tyr505E** (Figure S1 in supplementary data). Likewise, hesperidin showed H-bonding interactions with the residues *His34B*, *Glu37B*, Arg403E, Gln409E, **Lys417E**, and Tyr421E and hydrophobic interactions with Pro389B, *Phe390B*, Phe456E, Tyr473E (Figure S2 in supplementary data). Thus, these natural molecules (neohesperidin and hesperidin) exhibited multiple interactions with the critical residues of ACE2 which are essential for binding of RBD with ACE2 protein (Choudhary et al., 2020; Lan et al., 2020; Yan et al., 2020; Yepes-Pérez et al., 2020). On the other hand, narirutin and naringin were docked near to hesperidin and neohesperidin with the less binding affinity of –7.785 kcal/mol and –6.798 kcal/mol, respectively as compared to neohesperidin and hesperidin. The narirutin exhibited five H-bonding interactions with key residues *Asp30B*, *Glu37B*, Gln96B, **Lys417E** and hydrophobic interactions with Leu29B, Val93B, Ala387B, Pro389B, *Phe390B*, Lys421E (Figure 3). However, naringin established the H-bonding interactions with the residues Lys26B, *Asp30B*, *Glu37B*, Tyr421E, **Lys417E** and hydrophobic interactions with the residues *Phe390B*, Pro389B, Ala387B, Phe456E, Tyr421E (Table 1 and Figure 4). As evident from the literatures (Choudhary et al., 2020; Yepes-Pérez et al., 2020), all the natural molecules were exhibited satisfactory binding interactions against RBD S-protein/ACE2 interface of SARS-CoV-2. Highest binding affinities of the neohesperidin and hesperidin were observed at the contact interface of the RBD-spike-ACE2 protein.

3.3 Binding modes with RBD of S-protein

Recently, SARS-CoV-2 was resolved PDB ID: 6XR8 (Cai et al., 2020) by X-ray Crystallography. The RBD domain of the SARS-CoV-2 is crucial for binding to the ACE2 protein of the human cell, hence virus entry is possible into the human cell and leads to SARS-CoV-2 infection. Thus, the docking studies against the RBD of S-protein could help to understand the important binding interactions of the natural molecules against the RBD of S-protein which are responsible for recognition of ACE2 protein for SARS-CoV-2 entry.

The docking studies of natural molecules were focused against ACE2 binding site of RBD S-protein. All the natural molecules were fitted well into (Figure 5) the binding site of RBD of S-protein and shown good binding score ranging from –8.432 to –3.787 kcal/mol. All the natural molecules [naringin (–8.432 kcal/mol), hesperidin (–7.321 kcal/mol), neohesperidin (–7.088 kcal/mol) and narirutin (–7.049 kcal/mol)] exhibited good binding score and poses against RBD of S-protein (Table 1). It was found that naringin had highest affinity (–8.432 kcal/mol) against the RBD with respect to hesperidin, neohesperidin and narirutin. The key residues which shown major H-bonding interactions with naringin are **Tyr449**, Glu484, Cys488, Phe490, Leu492, and Gln493. While Leu452, *Leu455*, Phe456, Cys488, Tyr489, Phe490, Pro491 and Leu492 residues were shown hydrophobic interactions with naringin (Figure 6). Hesperidin established H-bonding interactions with Glu484, Gly485, Phe490, Leu492 and Gln493 and hydrophobic interactions with Phe456, *Leu455*, *Phe486*, Cys488, Tyr489, Phe490, Pro491 and Leu492 (Figure S3 in supplementary data). Similarly, neohesperidin (Figure S4 in supplementary data) and narirutin (Figure 7) were docked against RBD of S-protein, but exhibited slightly low binding affinities as compared to hesperidin (Table 1). All the natural molecules interacted with residue Gln493 which was reported to be crucial for binding with the ACE2 protein (Yuan et al., 2020). In addition, the critical residues (Choudhary et al., 2020; Yepes-Pérez et al., 2020; Yuan et al., 2020) which are required for binding of RBD to the human ACE2 receptor were also reflected in the docking results of these natural molecules (Table 1).

Figure 2 Docking poses of the natural products against RBD-S-protein/ACE2 interface (PDB ID: 6M17), yellow colour secondary structure represents the ACE2 protein and green colour secondary structure represents the RBD of S-protein. Hesperidin (in magenta), Neohesperidin (in Cyan), Narirutin (in brown) and Naringin (in blue) (see online version for colours)

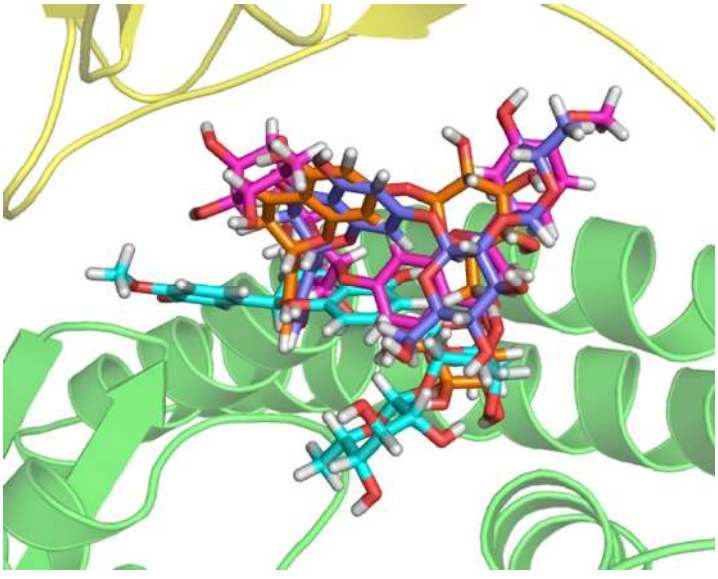
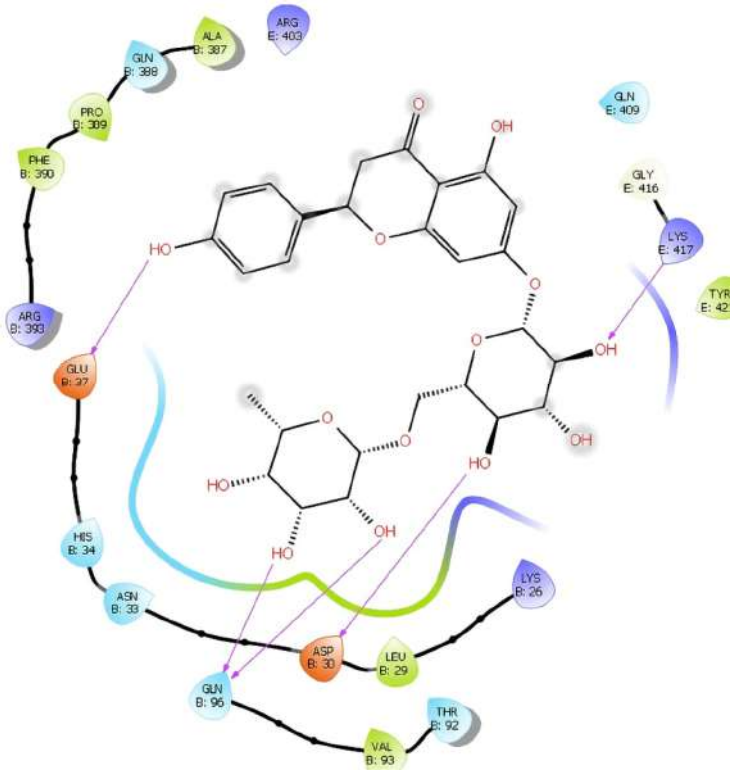
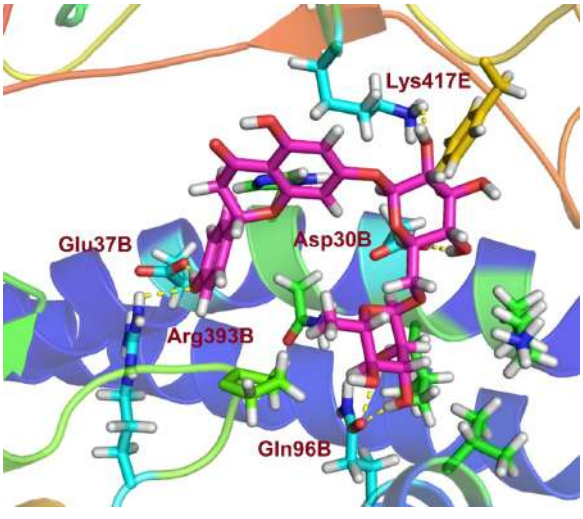


Figure 3 (a) 2D and (b) 3D docking poses of the natural product: **Narirutin** against RBD-spike (PDB ID: 6M17), Ribbon is secondary structure of RBD spike-ACE2 protein (PDB: 6M17). Magenta colour sticks represents Narirutin. Cyan and green colour sticks represent the binding residues having interactions with Narirutin. Yellow dots represent the H-bonding (see online version for colours)

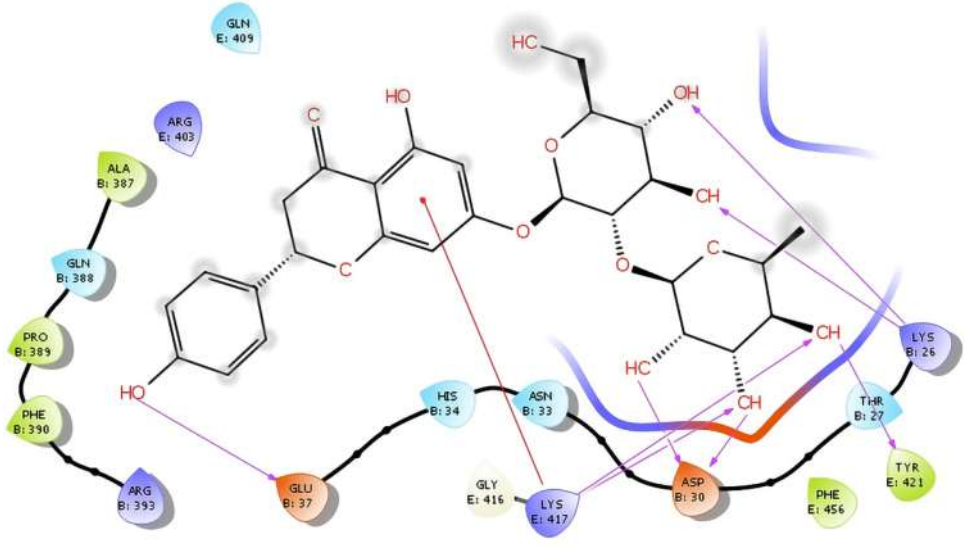


(a)

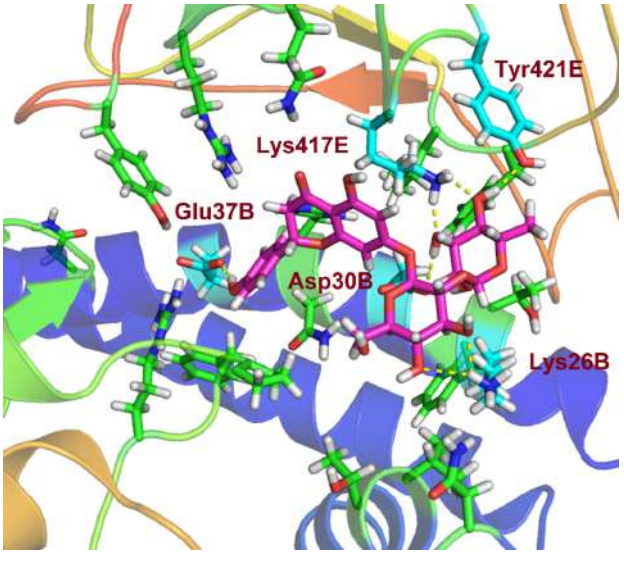


(b)

Figure 4 (a) 2D and (b) 3D docking poses of the natural product: **Naringin** against RBD-spike (PDB ID: 6M17), Ribbon is secondary structure of RBD spike-ACE2 protein (PDB: 6M17). Magenta colour sticks represents Naringin. Cyan and green colour sticks represent the binding residues having interactions with Naringin. Yellow dots represent the H-bonding (see online version for colours)



(a)



(b)

Figure 5 Docking poses of the natural products against RBD of S-protein (PDB ID: 6XR8), Green colour secondary structure represents the RBD-S-protein. Hesperidin (in magenta), Neohesperidin (in Cyan), Synephrine (in orange), Narirutin (in brown) and Naringin (in blue) (see online version for colours)

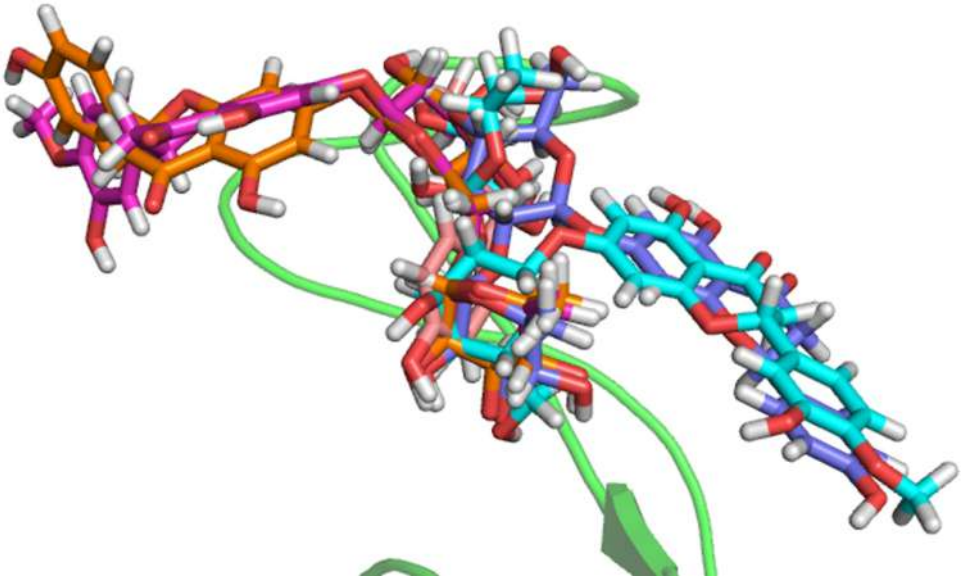
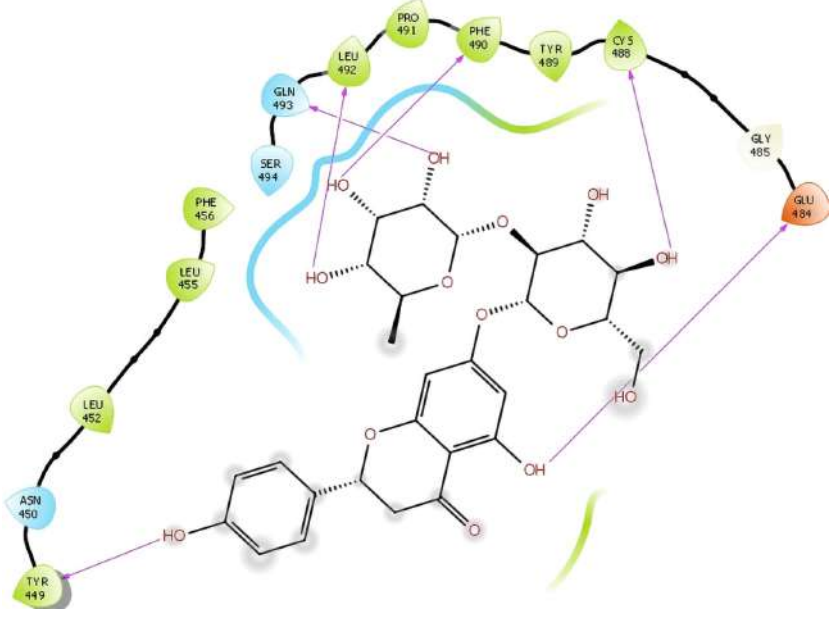
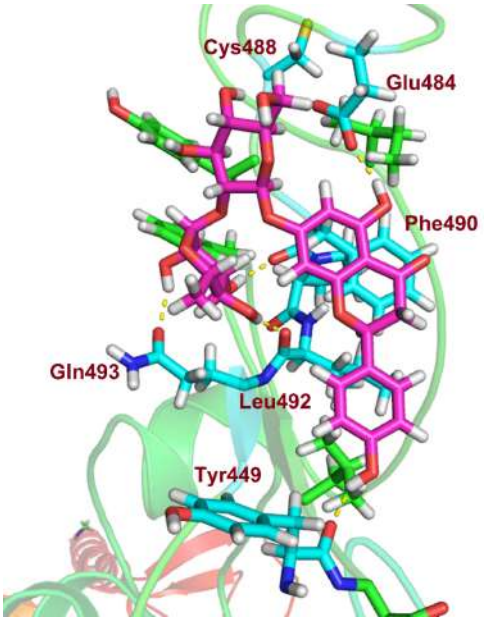


Figure 6 (a) 2D and (b) 3D docking poses of the natural product: **Naringin** against RBD of S-protein (PDB ID: 6XR8), Ribbon is secondary structure of RBD of S-protein (PDB: 6XR8). Magenta colour sticks represents naringin. Cyan and green colour sticks represent the binding residues having interactions with naringin. Yellow dots represent the H-bonding (see online version for colours)



(a)

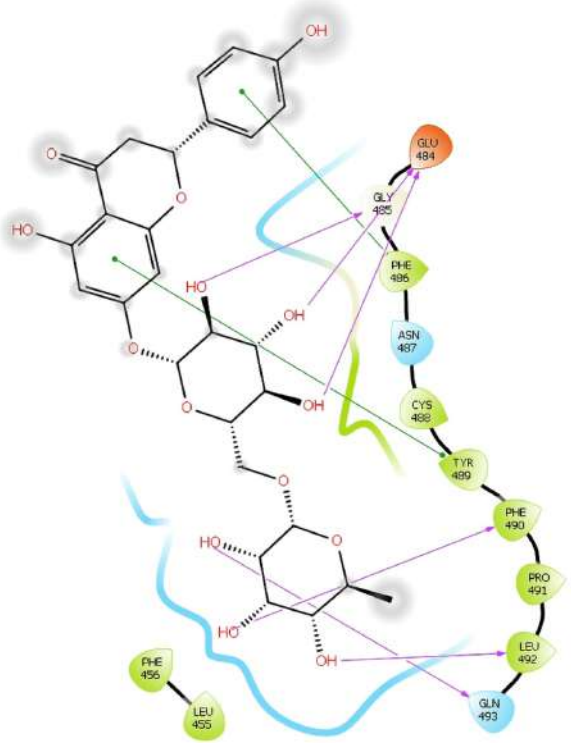
Figure 6 (a) 2D and (b) 3D docking poses of the natural product: **Naringin** against RBD of S-protein (PDB ID: 6XR8), Ribbon is secondary structure of RBD of S-protein (PDB: 6XR8). Magenta colour sticks represents naringin. Cyan and green colour sticks represent the binding residues having interactions with naringin. Yellow dots represent the H-bonding (see online version for colours) (continued)



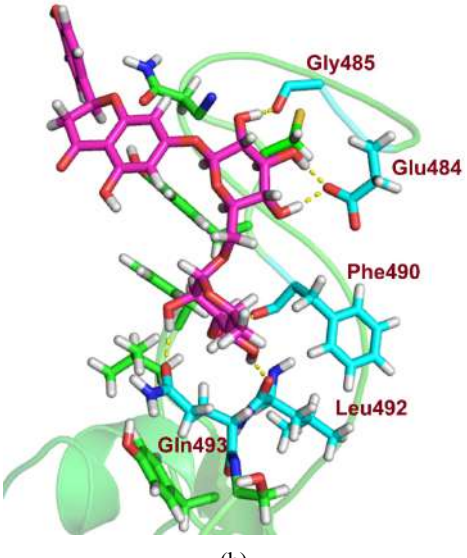
(b)

Above docking studies, revealed that the neohesperidin and hesperidin were found to have high binding potential against RBD S-protein/ACE2 interface as well as RBD of S-protein. However, the naringin was found to have high binding affinity with RBD of S-protein only. The narirutin also showed high binding potential with RBD S-protein/ACE2 interface as compared to RBD of S-protein. All the natural molecules exhibited the binding with residue Gln493 which is reported to be important for binding with ACE2 protein. In addition, H-bonding interactions with residue Lys417E was found to be important for forming salt bridges with Asp30B of ACE2 (Ali and Vijayan, 2020; Shah et al., 2020) protein. The final results of binding mode analysis of phytochemical repurposing using docking studies were reflected that naringin and narirutin had the potential to disrupt the interactions of RBD with ACE2 protein of human, i.e. as entry inhibitors against SARS-CoV-2 infection. However, hesperidin (Mahdian et al., 2020; Wu et al., 2020) and neohesperidin (Wu et al., 2020) are already reported in literatures to have binding potential against RBD of S-protein as well as RBD S-protein/ACE2 interface. The naringin and narirutin first time reported for binding potential against RBD S-protein/ACE2 interface as well as RBD of S-protein as entry inhibitors. This work concluded that naringin and narirutin may have multitargeted binding potential against SARS-CoV-2 proteins (RBD S-protein/ACE2 interface and RBD of S-protein).

Figure 7 (a) 2D and (b) 3D docking poses of the natural product: **Narirutin** against RBD of S-protein (PDB ID: 6XR8), Ribbon is secondary structure of RBD of S-protein (PDB: 6XR8). Magenta colour sticks represents naringin. Cyan and green colour sticks represent the binding residues having interactions with naringin. Yellow dots represent the H-bonding (see online version for colours)



(a)



(b)

4 Conclusions

SARS-CoV-2 infection has raised global health threat and millions of people are infected. Till date there is no specific treatment approved for this pandemic infection. In the SARS-CoV-2 life cycle, virus entry into the host cell is the crucial step to control the infection in the human. This step disrupts the spread of the viral infection. For SARS-CoV-2 entry into host, RBD of S-protein binds with host ACE2 protein, leading to viral entry. If interactions between both the proteins (RBD S-protein and ACE2 protein) is disrupted then viral entry into the host cell can be blocked. The naringin and narirutin were exhibited binding potential against both RBD of S-protein and RBD S-protein/ACE2 interface in docking studies. More importantly, these natural molecules were found to have interactions with key residues (Gln493 and Lys417E) which were found crucial for binding RBD S-protein with ACE2 protein. Thus, these natural molecules **(**naringin and narirutin**)** may disrupt the entry of the SARS-CoV-2 into the human cell, which need to be validated experimentally. However, the naringin and narirutin potential against other SARS-CoV-2 targets are opening the avenue for ***multitargeted binding with similar natural molecules*** against the SARS-CoV-2 proteins.

Acknowledgements

Prof. Anjana Munshi and Dr. Pawan Gupta are very much thankful to DST-SERB, New Delhi for providing a grant under TARE scheme (Ref. No: TAR/2019/0260). Dr. Vishnu is very much thankful to DBT for providing BIC-DBT grant (DBT project no BT/PR40164/BTIS/137/17/2021).

Ethics approval and consent to participate

Not applicable.

Human and animal rights

Not applicable.

Consent for publication

Not applicable.

Conflict of interest

None.

References

Agrawal, P.K., Agrawal, C. and Blunden, G. (2021) 'Pharmacological significance of hesperidin and hesperetin, two citrus flavonoids, as promising antiviral compounds for prophylaxis against and combating COVID-19', *Natural Product Communications*, Vol. 16, No. 10, p.1934578X211042540.

Alakhdar, A.A., Saleh, A.H. and Arafa, R.K. (2020) 'Targeting homologous recombination (HR) repair mechanism for cancer treatment: discovery of new potential UCHL-3 inhibitors via virtual screening, molecular dynamics and binding mode analysis', *J. Biomol. Struct. Dyn.*, Vol. 40, No. 1, pp.1–14.

Ali, A. and Vijayan, R. (2020) 'Dynamics of the ACE2–SARS-CoV-2/SARS-CoV spike protein interface reveal unique mechanisms', *Sci Rep*, Vol. 10, No. 1, p.14214.

Bagher, Z., Ehterami, A., Safdel, M.H., Khastar, H., Semiari, H., Asefnejad, A., Davachi, S.M., Mirzaii, M. and Salehi, M. (2020) 'Wound healing with alginate/chitosan hydrogel containing hesperidin in rat model', *J. Drug Deliv. Sci. Technol.*, Vol. 55, p.101379.

Bellavite, P. and Donzelli, A. (2020) 'Hesperidin and SARS-CoV-2: new light on the healthy function of citrus fruits', *Antioxidants*, Vol. 9, No. 8, p.742.

Boström, J., Hogner, A. and Schmitt, S. (2006) 'Do structurally similar ligands bind in a similar fashion?', *Journal of Medicinal Chemistry*, Vol. 49, No. 23, pp.6716–25.

Botelho, F.D., Dos Santos, M.C., Gonçalves, A.d.S., Kuca, K., Valis, M., LaPlante, S.R., França, T.C. and de Almeida, J.S. (2020) 'Ligand-based virtual screening, molecular docking, molecular dynamics, and MM-PBSA calculations towards the identification of potential novel ricin inhibitors', *Toxins*, Vol. 12, No. 12, p.746.

Bousta, D., Gonçalves, R., Hoummani, H. and Achour, S. (2021) *Medicinal Plants Against Coronavirus (SARS-COV-2) in Morocco Via Computational Virtual Screening Approach*, DOI: https://doi.org/10.21203/rs.3.rs-679827/v1

Cai, Y., Zhang, J., Xiao, T., Peng, H., Sterling, S.M., Walsh, R.M., Rawson, S., Rits-Volloch, S. and Chen, B. (2020) 'Distinct conformational states of SARS-CoV-2 spike protein', *Science*, Vol. 369, No. 6511, pp.1586–1592.

Chadha, J., Harjai, K. and Chhibber, S. (2022) 'Repurposing phytochemicals as anti-virulent agents to attenuate quorum sensing-regulated virulence factors and biofilm formation in pseudomonas aeruginosa', *Microbial Biotechnology*, Vol. 15, No. 6, pp.1695–718.

Chakraborty, S., Rakshit, J., Bandyopadhyay, J. and Basu, S. (2021) 'Multi-target inhibition ability of neohesperidin dictates its neuroprotective activity: implication in Alzheimer's disease therapeutics', *Int. J. Biol. Macromol*, Vol. 176, pp.315–324.

Cheng, F-J., Huynh, T-K., Yang, C-S., Hu, D-W., Shen, Y-C., Tu, C-Y., Wu, Y-C., Tang, C-H., Huang, W-C. and Chen, Y. (2021) 'Hesperidin is a potential inhibitor against SARS-CoV-2 infection', *Nutrients*, Vol. 13, No. 8, p.2800.

Choudhary, S., Malik, Y. S. and Tomar, S. (2020) 'Identification of SARS-CoV-2 cell entry inhibitors by drug repurposing using in silico structure-based virtual screening approach', *Front Immunol.*, Vol. 11, p.1664.

Coro-Bermello, J., López-Rodríguez, E.R., Alfonso-Ramos, J.E., Alonso, D., Ojeda-Carralero, G.M., Prado, G.A. and Moreno-Castillo, E. (2021) 'Identification of novel thiadiazin derivatives as potentially selective inhibitors towards trypanothione reductase from trypanosoma cruzi by molecular docking using the numerical index poses ratio pr and the binding mode analysis', *SN Applied Sciences*, Vol. 3, No. 3, pp.1–20.

Du, L., He, Y., Zhou, Y., Liu, S., Zheng, B-J. and Jiang, S. (2009) 'The spike protein of SARS-CoV – a target for vaccine and therapeutic development', *Nat. Rev. Microbiol.*, Vol. 7, No. 3, pp.226–236.

Du, L., Yang, Y., Zhou, Y., Lu, L., Li, F. and Jiang, S. (2017) 'MERS-CoV spike protein: a key target for antivirals', *Expert Opin. Ther. Targets*, Vol. 21, No. 2, pp.131–143.

Eweas, A.F., Osman, H-E.H., Naguib, I.A., Abourehab, M.A. and Abdel-Moneim, A.S. (2022) 'Virtual screening of repurposed drugs as potential spike protein inhibitors of different SARS-CoV-2 variants: molecular docking study', *Current Issues in Molecular Biology*, Vol. 44, No. 7, pp.3018–3029.

Ghosh, K., Amin, S.A., Gayen, S. and Jha, T. (2021) 'Chemical-informatics approach to COVID-19 drug discovery: exploration of important fragments and data mining based prediction of some hits from natural origins as main protease (Mpro) inhibitors', *Journal of Molecular Structure*, Vol. 1224, p.129026.

Gollavilli, H., Hegde, A.R., Managuli, R.S., Bhaskar, K.V., Dengale, S.J., Reddy, M.S., Kalthur, G. and Mutalik, S. (2020) 'Naringin nano-ethosomal novel sunscreen creams: development and performance evaluation', *Colloids Surf B Biointerfaces*, Vol. 193, p.111122.

Gupta, P., Kumar, R., Garg, P. and Singh, I.P. (2010) 'Active site binding modes of dimeric phloroglucinols for HIV-1 reverse transcriptase, protease and integrase', *Bioorg. Med. Chem. Lett.*, Vol. 20, No. 15, pp.4427–4431.

Haggag, Y.A., El-Ashmawy, N.E. and Okasha, K.M. (2020) 'Is hesperidin essential for prophylaxis and treatment of COVID-19 infection?', *Med. Hypotheses*, Vol. 144, p.109957.

Haridas, M., Sasidhar, V., Nath, P., Abhithaj, J., Sabu, A. and Rammanohar, P. (2021) 'Compounds of Citrus medica and Zingiber officinale for COVID-19 inhibition: in silico evidence for cues from ayurveda', *Future Journal of Pharmaceutical Sciences*, Vol. 7, No. 1, pp.1–9.

Heidary Moghaddam, R., Samimi, Z., Moradi, S.Z., Little, P.J., Xu, S. and Farzaei, M.H. (2020) 'Naringenin and naringin in cardiovascular disease prevention: a preclinical review', *Eur. J. Pharmacol.*, Vol. 887, p.173535.

Kandeel, M., Kitade, Y. and Almubarak, A. (2020) 'Repurposing FDA-approved phytomedicines, natural products, antivirals and cell protectives against SARS-CoV-2 (COVID-19) RNA-dependent RNA polymerase', *PeerJ*, Vol. 8, p.e10480.

Laksmiani, N.P.L., Larasanty, L.P.F., Santika, A.A.G.J., Prayoga, P.A.A., Dewi, A.A.I.K. and Dewi, N.P.A.K. (2020) 'Active compounds activity from the medicinal plants against SARS-CoV-2 using in silico assay', *Biomedical and Pharmacology Journal*, Vol. 13, No. 2, pp.873–881.

Lan, J., Ge, J., Yu, J., Shan, S., Zhou, H., Fan, S., Zhang, Q., Shi, X., Wang, Q. and Zhang, L. (2020) 'Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor', *Nature*, Vol. 581, No. 7807, pp.215–220.

Lu, J.F., Zhu, M.Q., Zhang, H., Liu, H., Xia, B., Wang, Y.L., Shi, X.E., Peng, L. and Wu, J.W. (2020) 'Neohesperidin attenuates obesity by altering the composition of the gut microbiota in high-fat diet-fed mice', *FASEB J.*, Vol. 34, No. 9, pp.12053–12071.

Mahdian, S., Ebrahim-Habibi, A. and Zarrabi, M. (2020) 'Drug repurposing using computational methods to identify therapeutic options for COVID-19', *J. Diabetes Metab. Disord.*, Vol. 19, pp.691–699.

Morita, T., Akiyoshi, T., Sato, R., Uekusa, Y., Katayama, K., Yajima, K., Imaoka, A., Sugimoto, Y., Kiuchi, F. and Ohtani, H. (2020) 'Citrus fruit-derived flavanone glycoside narirutin is a novel potent inhibitor of organic anion-Transporting polypeptides', *J. Agric. Food Chem.*, Vol. 68, No. 48, pp.14182–14191.

Niu, L., Wei, J., Li, X., Jin, Y. and Shi, X. (2021) 'Inhibitory activity of narirutin on RBL-2H3 cells degranulation', *Immunopharmacol. Immunotoxicol.*, Vol. 43, No. 1, pp.68–76.

Patel, P., Barve, K. and Bhatt, L.K. (2020) 'Narirutin-rich fraction from grape fruit peel protects against transient cerebral ischemia reperfusion injury in rats', *Nutr. Neurosci.*, Vol. 25, No. 5, pp.920–930.

Puttaswamy, H., Gowtham, H.G., Ojha, M.D., Yadav, A., Choudhir, G., Raguraman, V., Kongkham, B., Selvaraju, K., Shareef, S. and Gehlot, P. (2020) 'In silico studies evidenced the role of structurally diverse plant secondary metabolites in reducing SARS-CoV-2 pathogenesis', *Sci. Rep.*, Vol. 10, No. 1, p.20584.

Sahu, P.K., Mohapatra, P.K., Rajani, D.P. and Raval, M.K. (2020) 'Structure-based discovery of narirutin as a shikimate kinase inhibitor with anti-tubercular potency', *Curr. Comput. Aided Drug Des.*, Vol. 16, No. 5, pp.523–529.

Saric, B., Tomic, N., Kalajdzic, A., Pojskic, N. and Pojskic, L. (2021) 'analysis of selected components of grapefruit seed extract against SARS-CoV-2 main protease', *The EuroBiotech Journal*, Vol. 5, No. s1, pp.5–12.

Shah, M., Ahmad, B., Choi, S. and Woo, H.G. (2020) 'Mutations in the SARS-CoV-2 spike RBD are responsible for stronger ACE2 binding and poor anti-SARS-CoV mAbs cross-neutralization', *Comput Struct Biotechnol, J.*, Vol. 18, pp.3402–3414.

Sharma, S., Gupta, J., Prabhakar, P.K., Gupta, P., Solanki, P. and Rajput, A. (2019) 'Phytochemical repurposing of natural molecule: Sabinene for identification of novel therapeutic benefits using in silico and in vitro approaches', *Assay Drug Dev. Technol.*, Vol. 17, No. 8, pp.339–351.

Shokoohi, M., Khaki, A., Shoorei, H., Khaki, A., Moghimian, M. and Abtahi-Eivary, S.H. (2020) 'Hesperidin attenuated apoptotic-related genes in testicle of a male rat model of varicocoele', *Andrology*, Vol. 8, No. 1, pp.249–258.

Singh, S., Gupta, P. and Gupta, J. (2020) 'Virtual structural similarity elucidates bioactivity of fenchone: a phytochemical enriched in fennel essential oil', *Curr. Drug Discov. Technol.*, Vol. 17, No. 5, pp.619–630.

Syed, A.A., Reza, M.I., Shafiq, M., Kumariya, S., Singh, P., Husain, A., Hanif, K. and Gayen, J.R. (2020) 'Naringin ameliorates type 2 diabetes mellitus-induced steatohepatitis by inhibiting RAGE/NF-κB mediated mitochondrial apoptosis', *Life Sci.*, Vol. 257, p.118118.

Wan, Y., Shang, J., Graham, R., Baric, R.S. and Li, F. (2020) 'Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus', *J. Virol.*, Vol. 94, No. 7, pp.e00127–20.

Wang, S-w., Sheng, H., Bai, Y-f., Weng, Y-y., Fan, X-y., Lou, L-j. and Zhang, F. (2020) 'Neohesperidin enhances PGC-1α-mediated mitochondrial biogenesis and alleviates hepatic steatosis in high fat diet fed mice', *Nutr. Diabetes*, Vol. 10, No. 1, pp.1–11.

Wu, C., Liu, Y., Yang, Y., Zhang, P., Zhong, W., Wang, Y., Wang, Q., Xu, Y., Li, M. and Li, X. (2020) 'Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods', *Acta Pharm. Sin., B*, Vol. 10, No. 5, pp.766–788.

Wu, K., Li, W., Peng, G. and Li, F. (2009) 'Crystal structure of NL63 respiratory coronavirus receptor-binding domain complexed with its human receptor', *Proc. Natl. Acad. Sci., U.S.A.*, Vol. 106, No. 47, pp.19970–19974.

Yan, R., Zhang, Y., Li, Y., Xia, L., Guo, Y. and Zhou, Q. (2020) 'Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2', *Science*, Vol. 367, No. 6485, pp.1444–1448.

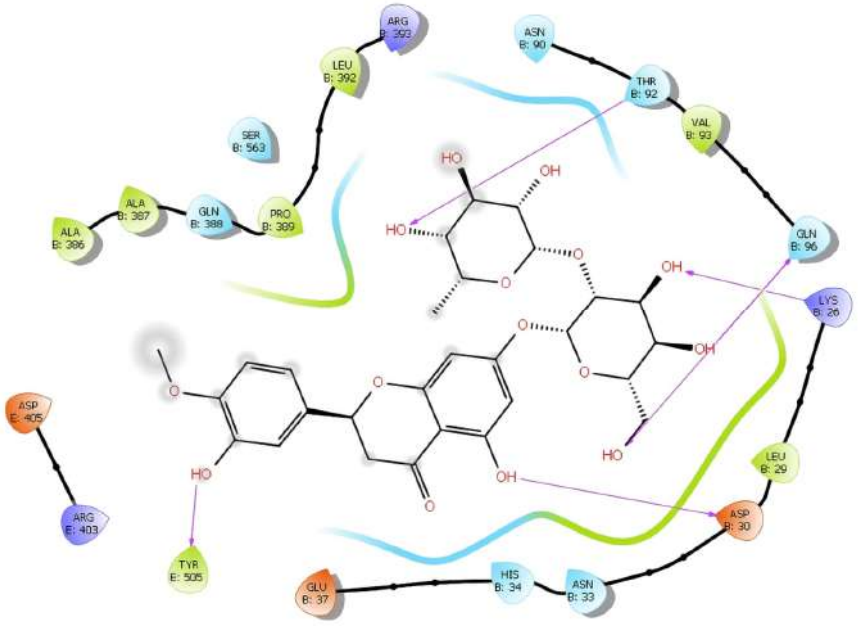
Yepes-Pérez, A.F., Herrera-Calderon, O. and Quintero-Saumeth, J. (2020) 'Uncaria tomentosa (cat's claw): a promising herbal medicine against SARS-CoV-2/ACE-2 junction and SARS-CoV-2 spike protein based on molecular modeling', *J. Biomol. Struct. Dyn.*, pp.1–17.

Yuan, M., Wu, N.C., Zhu, X., Lee, C-C.D., So, R.T., Lv, H., Mok, C.K. and Wilson, I.A. (2020) 'A highly conserved cryptic epitope in the receptor binding domains of SARS-CoV-2 and SARS-CoV', *Science*, Vol. 368, No. 6491, pp.630–633.

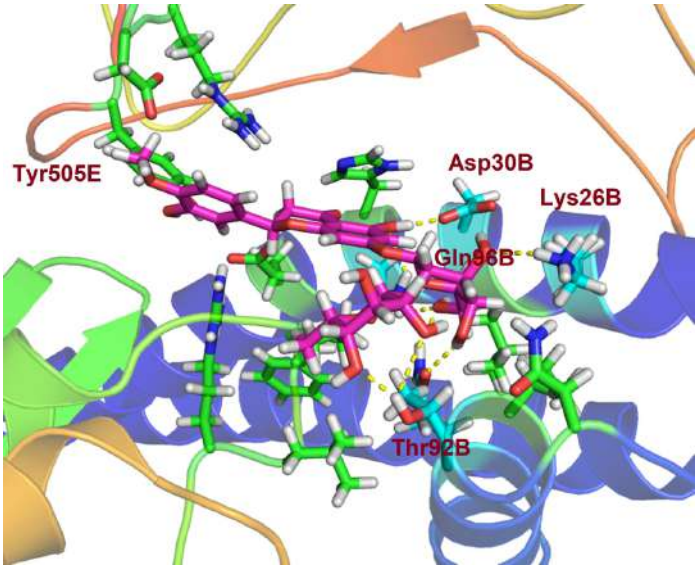
Zhang, M., Chen, X. and Radacsi, N. (2021) 'New tricks of old drugs: repurposing non-chemo drugs and dietary phytochemicals as adjuvants in anti-tumor therapies', *Journal of Controlled Release*, Vol. 329, pp.96–120.

Supplementary material

Figure S1 (a) 2D and (b) 3D docking poses of the natural product: **Neohesperidin**against RBD of S-protein (PDB ID: 6M17), Ribbon is secondary structure of RBD-S-protein/ACE2 interface (PDB: 6M17). Magenta colour sticks represents neohesperidin. Cyan and green colour sticks represent the binding residues having interactions with naringin. Yellow dots represent the H-bonding (see online version for colours)

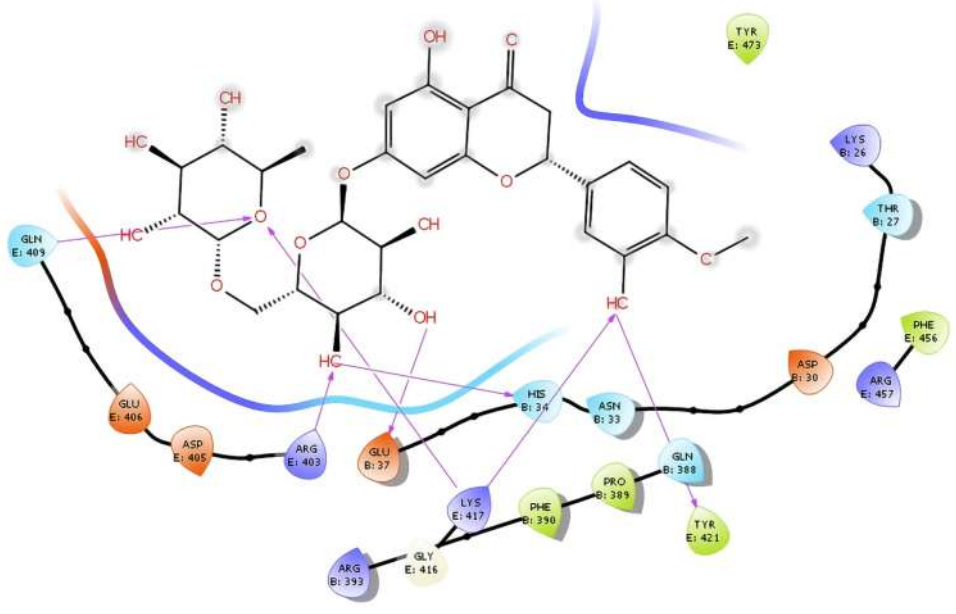


(a)

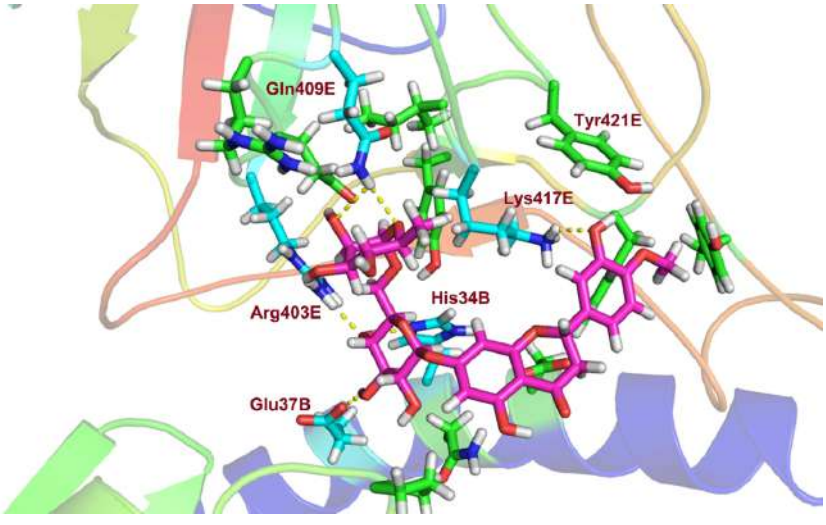


(b)

Figure S2 (a) 2D and (b) 3D docking poses of the natural product: **Hesperidin** against RBD of S-protein (PDB ID: 6M17), Ribbon is secondary structure of RBD-S-protein/ACE2 interface (PDB: 6M17). Magenta colour sticks represents hesperidin. Cyan and green colour sticks represent the binding residues having interactions with naringin. Yellow dots represent the H-bonding (see online version for colours)

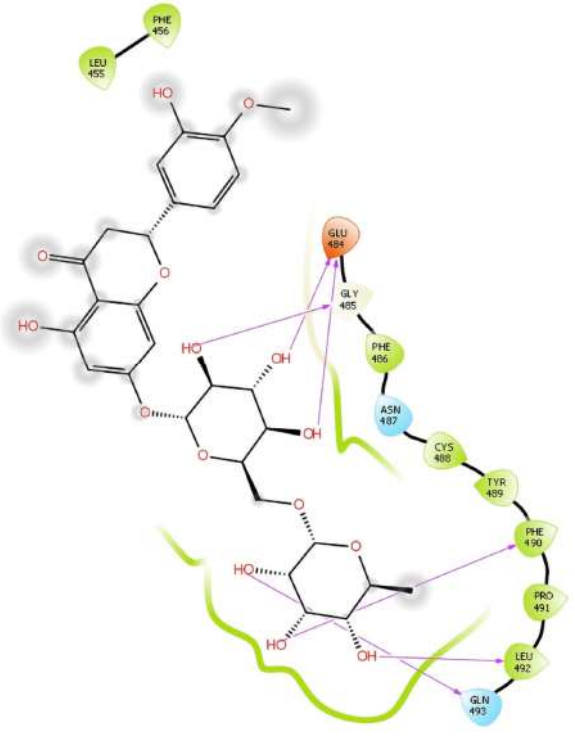


(a)

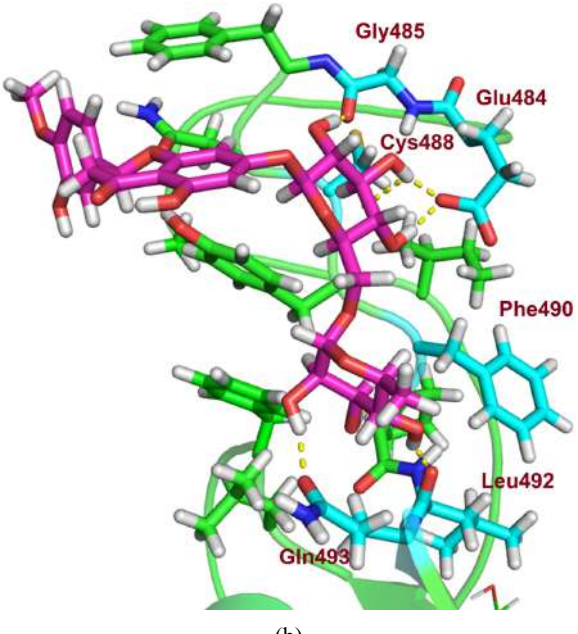


(b)

Figure S3 2D and 3D docking poses of **hesperidin** against PDB 6XR8: Magenta colour sticks represents hesperidin molecules. Cyan and green colour sticks represent the binding residues having interactions with hesperidin (see online version for colours)

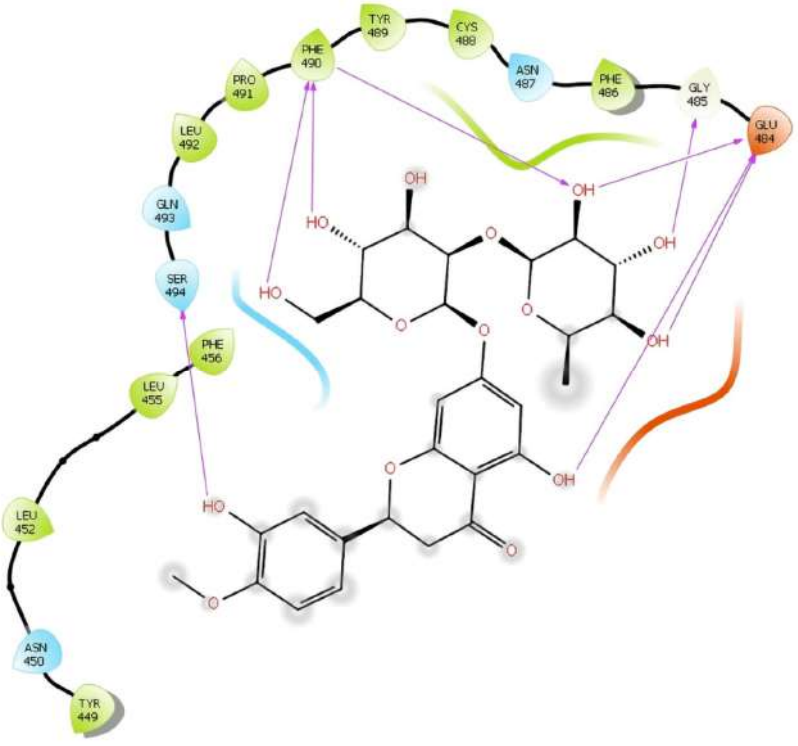


(a)

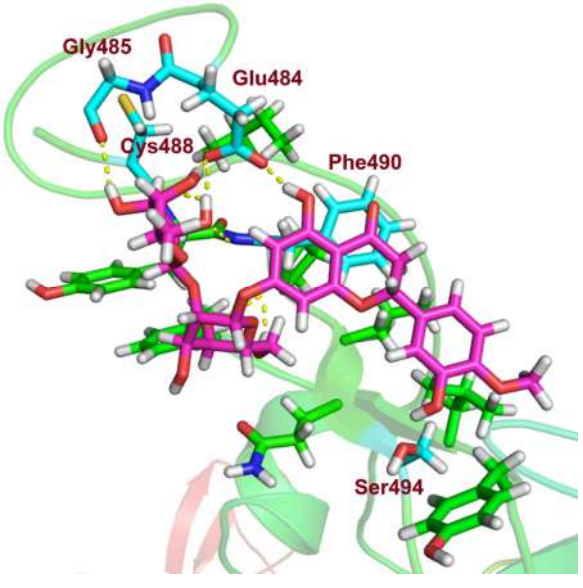


(b)

Figure S4 2D and 3D docking poses of **neohesperidin** against PDB 6XR8: Magenta colour sticks represents hesperidin molecules. Cyan and green colour sticks represent the binding residues having interactions with hesperidin (see online version for colours)



(a)



(b)