Current Research in Pharmacology and Drug Discovery 2 (2021) 100065

Contents lists available at ScienceDirect

Current Research in Pharmacology and Drug Discovery

journal homepage: www.journals.elsevier.com/current-research-in-pharmacology-and-drug-discovery

Potential inhibitors of SARS-CoV-2 (COVID 19) spike protein of the delta and delta plus variant: In silico studies of medicinal plants of North-East India

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ARTICLE INFO

Keywords:
SARS-CoV-2
Delta variant
Delta plus variant
Phytochemicals
Molecular docking

ABSTRACT

Phytochemicals of 38 Medicinal plants of North-East India, with anti-viral, anti-oxidant or anti-bacterial properties were screened for properties of drug likeness. 231 phytochemicals were screened with LIPINSKI rule of five to obtain 131 candidates, which were further screened with SWISS-ADME, to obtain 50 phytochemicals. These phytochemicals were docked with the spike protein of the Delta variant (B.1.617.2) and Delta-Plus (AY.1) variant of SARS-CoV-2 using Autodock Vina and MOE 09. The target proteins were constructed by homology modeling using Swiss-Model. Hydroxychloroquine, taken as a standard in docking analysis, exhibited a binding energy of −6.5 kcal/mol and −6.1 kcal/mol with respect to the Delta variant and Delta-Plus variant respectively. Among the 50 docked results most flavones showed very good docking scores. 3,5,8-Trimethoxy-6,7,4,5-bis(methylenedioxy)flavone, a Poly-Methoxyflavone, produced a highest docking score of −8.7 kcal/mol with respect to both the spike protein targets. Poly-Methoxyflavones and Poly-Ethoxyflavones exhibited good binding affinity for the target spike protein of SARS-CoV-2, and can be potential anti-viral drug candidates against the existing Delta variant of the SARS-CoV-2.

1. Introduction

The current outbreak of severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) has now undergone multiple significant mutations since its detection in 2019 in Wuhan, China. The spread of the Delta variant, which originated in India (Callaway, 2021), has caused concern all over the world, with cases detected in over 96 countries (WHO, 2021). The Delta variant has been denoted by the World Health Organisation as a "variant of concern". At present, the variant possess great threats to many countries like, the United States, Africa, Brazil Australia and Europe. India is still fighting a resurgence of the delta variant which appeared in the early part of 2021.

The Delta variant (B.1.617.2) has reported to be 60% more transmissible than the already highly infectious Alpha variant (B.1.1.7) (Callaway, 2021), and is believed to spread faster than any other variants (Planas et al., 2021). The recent studies on the variant has ignited fresh attention into how SARS-CoV-2 is able to adapt and mutate with the existing environment (Salvatore et al., 2021). Another variant which is very similar to the Delta variant is the Delta plus variant (AY.1) which was first detected in Europe and was declared as a "variant of concern" by the U.K. governmental agency Health England. The delta plus variant is a sub lineage of the delta variant, with a notable difference of possessing K417N mutation in the spike protein. Most significant mutations in these variants have been occurring in the RBD region of the spike protein (Shu and McCauley, 2017: Khateeb et al., 2021) and these mutations corresponds to the increased transmissibility (Zhang et al., 2020; Volz et al., 2021), increased immune evasiveness of the virus (Weisblum et al., 2020; Verma et al., 2021) and more flexibility to the spike protein to interact with the host receptors (Teruel et al., 2021).

With decrease in vaccine efficacy due to mutations (Noh et al., 2021) and the absence of strong anti-viral drug candidate against SARS-CoV-2, the world is still battling to overcome the Pandemic. In this situation, one can look into nature for a cure and a solution. Most traditionally used medicinal plants have phyto-constituents that are anti-viral, anti-inflammatory, anti-oxidant and anti-microbial. These traditional plants can be investigated for potential anti-viral drug against SARS-CoV-2. A number of Insilco studies have been done with phytochemicals in pursuit of developing anti-viral drugs for SARS-CoV-2 (Pandey et al., 2020;

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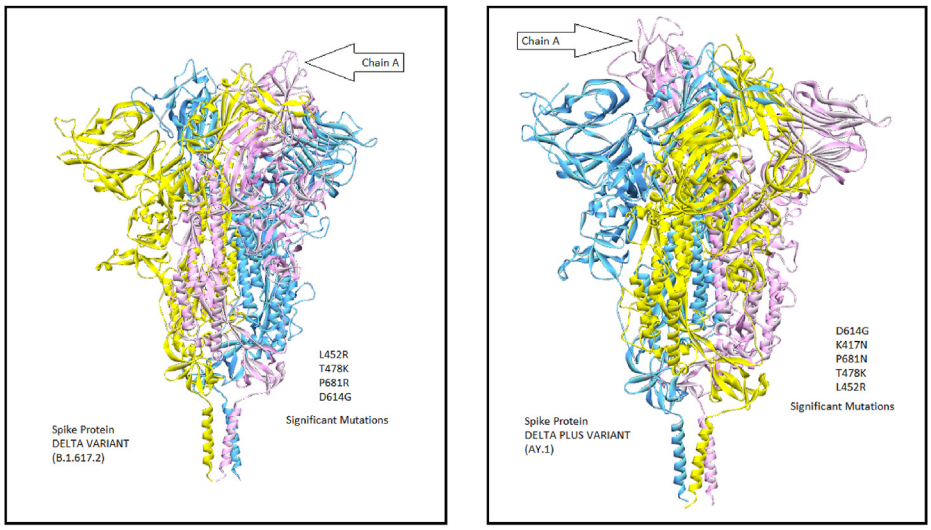
https://doi.org/10.1016/j.crphar.2021.100065
Received 27 July 2021; Received in revised form 10 October 2021; Accepted 17 October 2021



(a) (b)

Fig. 1. (a) Spike protein of the Delta variant of SARS-CoV-2, (b) Spike protein of the Delta Plus variant of SARS-CoV-2.

Table 1
Templates description for the Sars CoV-2 spike glycoprotein variants.

SARS- CoV-2 Variant	GenBank IDs	Template	Seq identity	Method	Resolution	Seq similarity	coverage	Description
Delta B.1.617.2	QWE52264.1	7krs.1.A	99.61%	EM	3.50 Å	0.62	1.00	Spike glycoprotein
Delta-Plus AY.1	QVO78843.1	7krs.1.A	99.37%	EM	3.20 Å	0.62	1.00	Spike glycoprotein

Chojnacka et al., 2020; Basu et al., 2020; Kothandan et al., 2021; Singh et al., 2021).

The North-east India is part of the 'biodiversity hotspot' in the world; it shares both the Himalaya and the Indo-Burma region. The region constitutes a rich reservoir of medicinal plants (Roy et al., 2015) and has a rich variety of flora and fauna. The present research tries to explore the Phytochemicals of some of these medicinal plants of this regions in search of a potential anti-viral drug against SARS-CoV-2 using in silico studies.

Table 2
List of top 15 hits from 50 phytochemical docked with the target spike protein of SARS-CoV-2 (Delta and Delta-Plus variant).

S. No	Top 15 hits	Binding Energy with Delta Variant (B.1.617.2)		Binding Energy with Delta-Plus Variant (AY.2)	
		Kcal/mol		Kcal/mol	
		Autodock Vina	MOE	Autodock Vina	MOE
1	3,5,3′-Trimethoxy-6,7,4′,5′-bis(methylenedioxy)flavone	−8.7	−6.6	−8.7	−7.3
2	Afzelechin	−7.5	−5.5	−7.3	−5.3
3	5,7-Dihydroxy-2-(4-hydroxyphenyl)chroman-4-one	−7.7	−5.4	−7.6	−5.5
4	Dihydrokaempferol	−7.9	−5.7	−7.7	−6.3
5	Kaempferol	−8.0	−5.2	−7.8	−6.2
6	Apigenin	−7.8	−5.9	−7.7	−5.5
7	Isorhamnetin	−7.8	−5.6	−7.7	−6.5
8	Chrysosplenetin	−7.6	−6.4	−7.4	−7.2
9	Ferreirin	−7.8	−5.8	−7.1	−6.2
10	Retusin	−7.5	−6.6	−7.6	−7.4
11	Rhamnocitrin	−8.0	−6.1	−7.9	−6.4
12	1,5-Dihydroxy-3-methoxy-7-methylanthracene-9,10-dione	−8.0	−5.6	−7.4	−6.6
13	Oxyberberine	−8.4	−5.7	−8.1	−7.1
14	Rugosinone	−8.1	−6.3	−7.8	−6.5
15	Jaceosidin	−7.8	−5.7	−7.7	−6.9
	Hydroxychloroquine	−6.5	−6.1	−6.1	−6.2

2. Materials and methods

2.1. Selection of medicinal plants and phytochemicals

Thirty-Eight indigenous medicinal plants, listed in Supplementary Table 1, of North-East India were selected for the study as they possessed anti-oxidant, anti-viral and anti-microbial properties based on researches carried out in literature (Chase and Singh, 2013; Bora et al., 2007; Panda

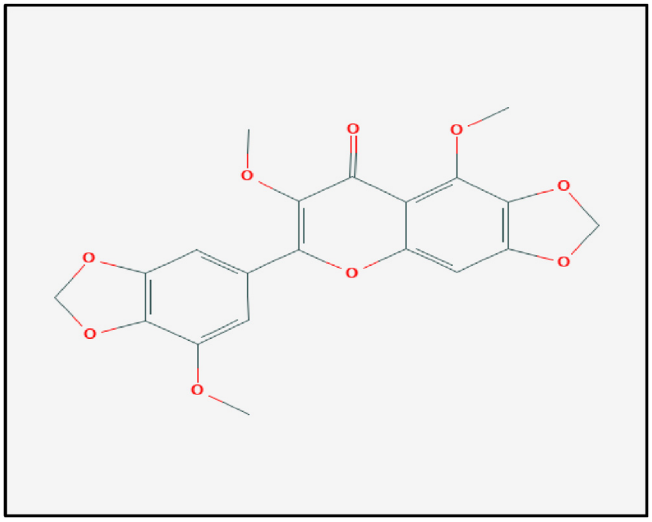
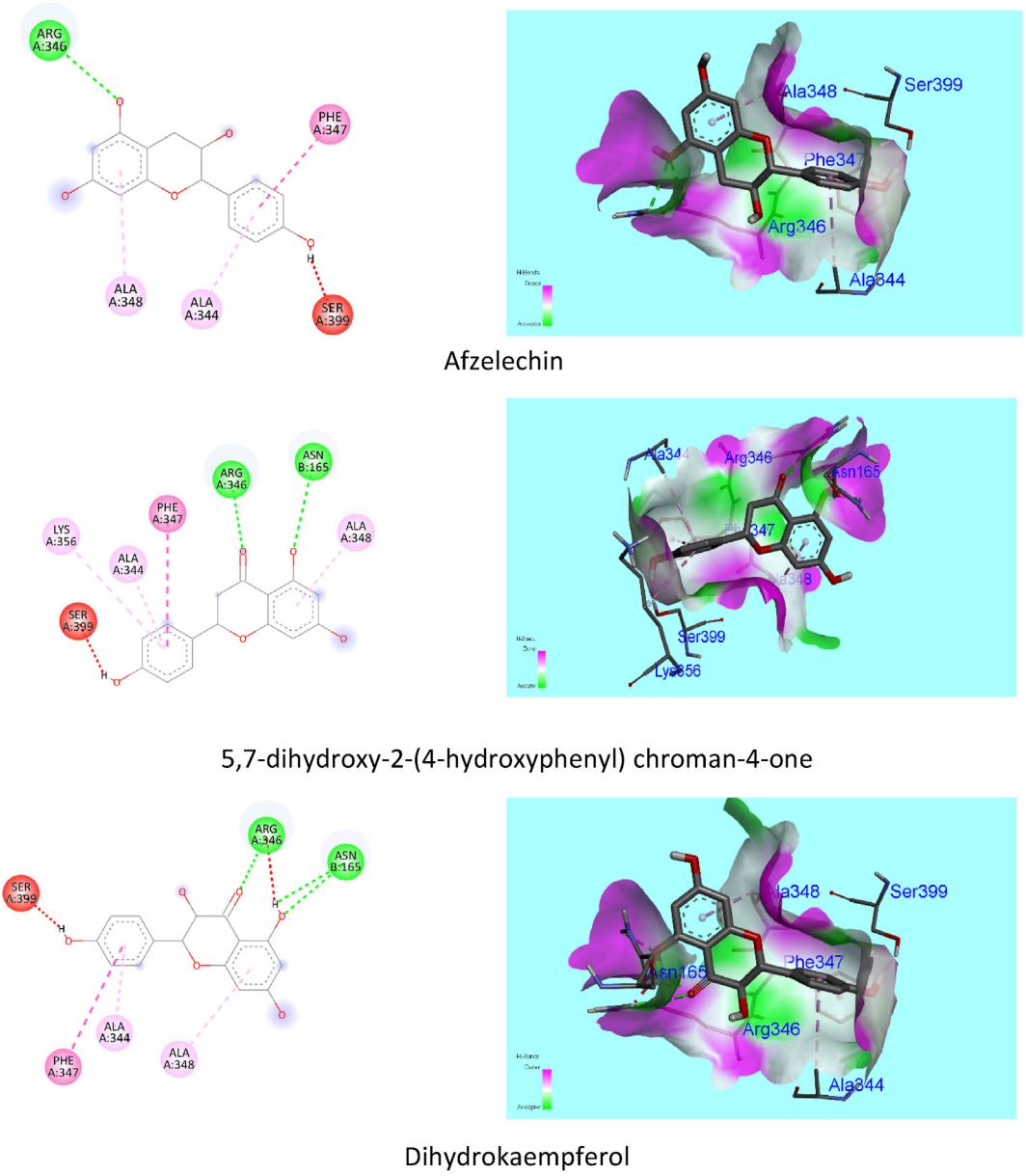


Fig. 2. 3,5,3′-Trimethoxy-6,7:4′,5′-bis(methylenedioxy)flavone: Ligand with best docking score.



Figs. 3. 2D and 3D structure of the docking result top 15 hits and Hydroxychloroquine with the target spikeprotein (B.1.617.2) of SARS-CoV-2.

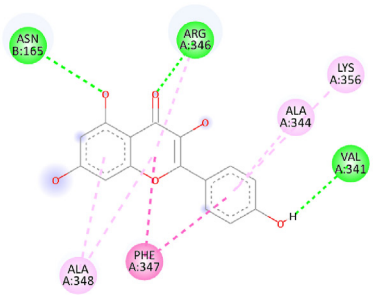
et al., 2018). 231 phytochemicals, listed in Supplementary Table 1, of theses medicinal plants were identified from literature and their sdf-structures were downloaded from Pubchem database, www.pubchem.ncbi.nlm.nih.gov (Kim et al., 2021).

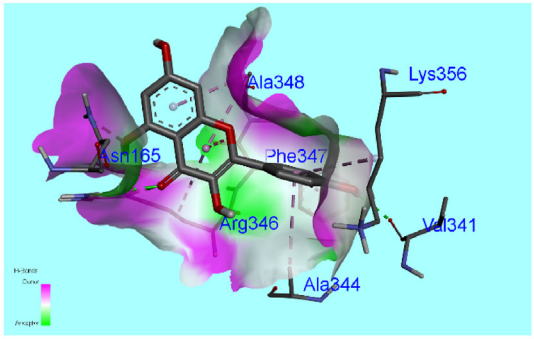
2.2. Screening of phytochemicals for druglikeness

Two levels of screening for drug-likeness were performed on the phytochemicals that were selected. All the 231 compounds were screened with LIPINSKI rule of five (RO5) (Lipinski, 2004; Jayaram et al., 2012) and the results are listed in Supplementary Table 2. The selected compounds obtained after screening with Lipinski's rule were further screened with SWISS-ADME server, which analyses many important aspects of the drug, like; lipophilicity, water solubility, pharmacokinetics, druglikeness and the chemical properties (Daina et al., 2017). The SDF structures of the compounds, downloaded from PUBCHEM, were fed into the SWISS-ADME server and were converted to SMILES format. 50 phytochemical compounds, listed in Supplementary Table 3, were finally selected as suitable candidates for docking with the spike protein of SARS-CoV-2 and their structures were converted into pdb format using PyMOL (Schrödinger and Delano, 2020).

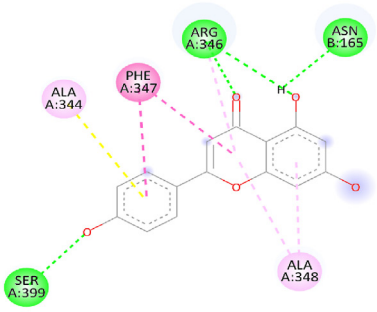
2.3. SARS CoV-2 protein target selection and homology modelling

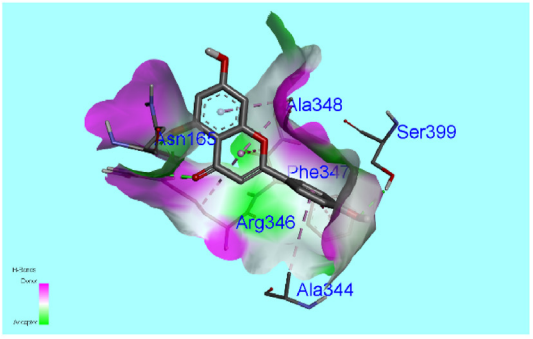
The 3D structure of the spike glycoprotein of the Delta (B.1.617.2) and Delta plus (AY.1) variants were not available at RCSB protein data bank, but were constructed with Swiss Model server (Waterhouse et al., 2018). The target sequence of the variants with GenBank IDs QWE52264.1 (https://www.ncbi.nlm.nih.gov/protein/QWE52264.1) and QVO78843.1 (https://www.ncbi.nlm.nih.gov/protein/QVO78843.1) were downloaded in FASTA format from NCBI (https://www.ncbi.nlm.nih.gov/) website for the Delta and Delta-plus variants respectively. The selected Gen sequence for the delta variant (QWE52264.1) contains all the significant mutations like; L452R, T478K, P681R and D614G, Fig. 1(a). The Gene sequence for



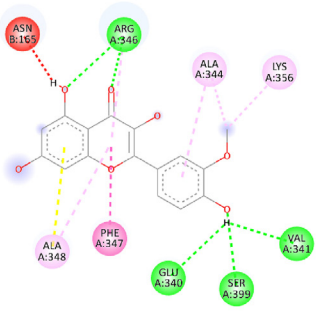


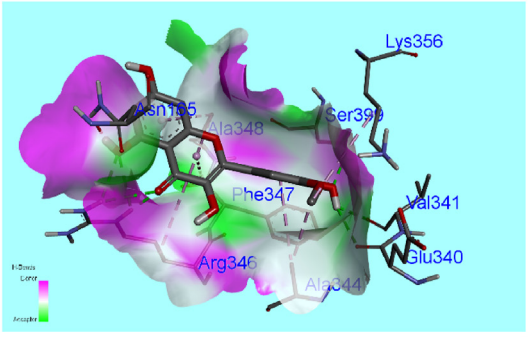
Kaempferol



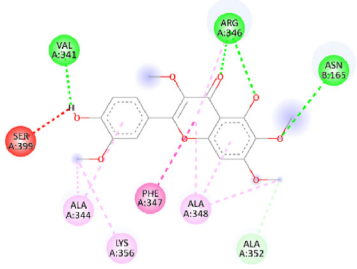


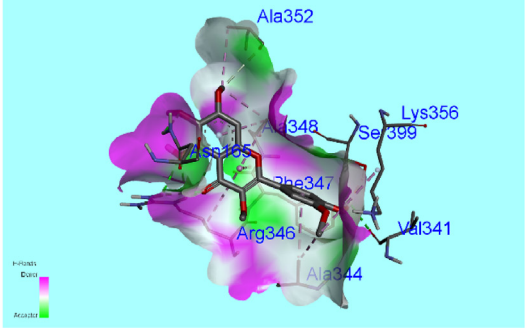
Apigenin





Isorhamnetin

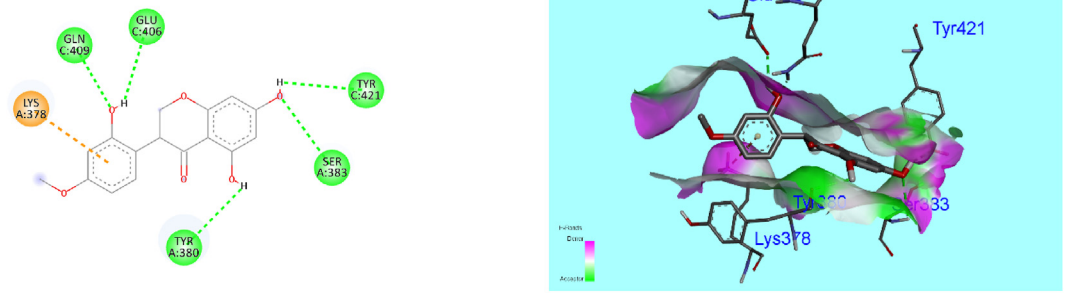




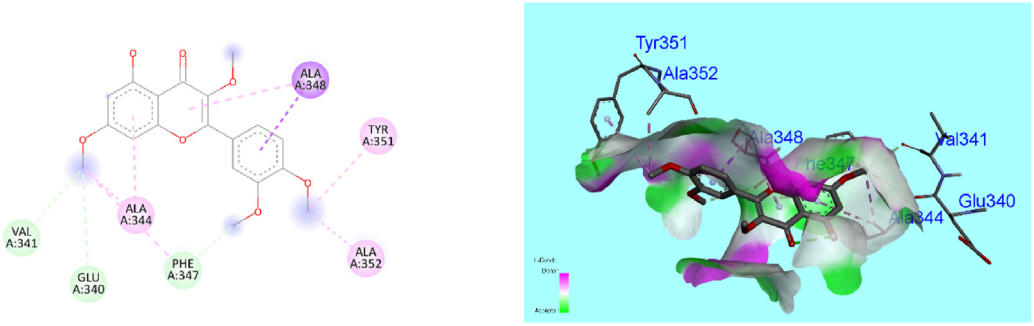
Chrysosplenetin

Figs. 3. (*continued*).

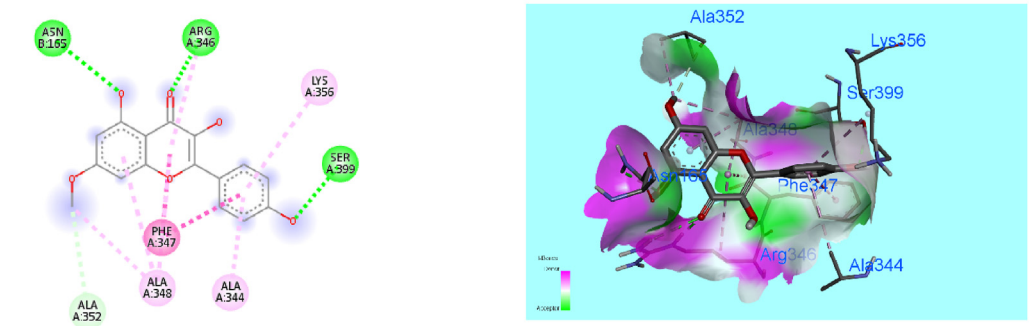
the Delta plus (QVO78843.1) also has the significant mutations such; D614G, K417N, P681N, T478K and L452R, Fig. 1(b). The Amino acid sequences of the surface glycoproteins were pasted in the Swiss Model server for construction of 3D model. BLAST and HHblits methods were used to search for template structure against the SWISS-MODEL template library. The same template with PDB ID 7krs.1.A was selected for both the



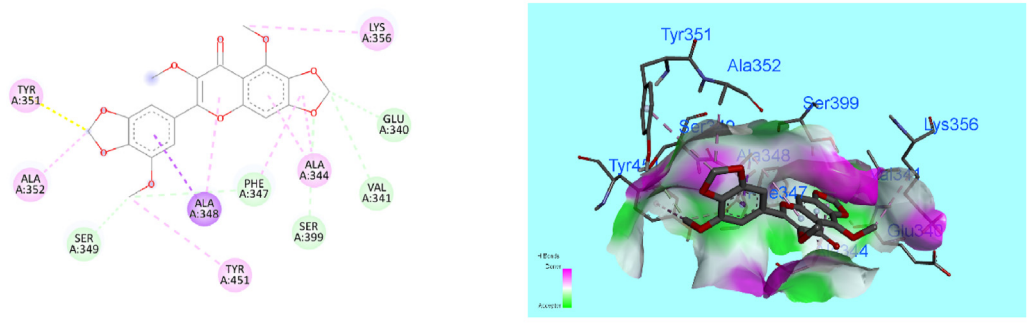
Ferreirin



Resutin



Rhamnocitrin



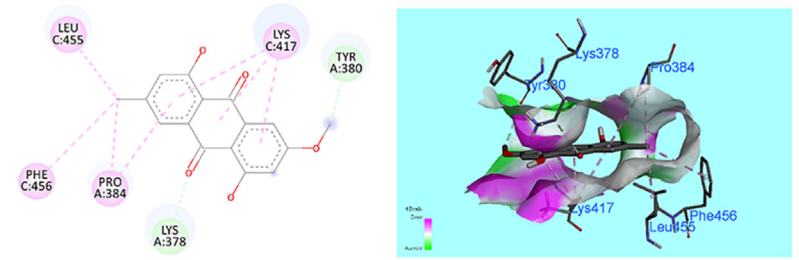
3,5,3-Trimethoxy-6,7,4,5-bis(methylenedioxy) flavone

Figs. 3. (*continued*).

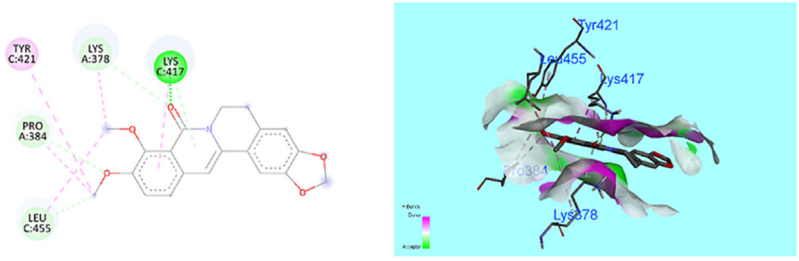
delta (B.1.617.2) and delta plus (AY.1) variants for building the 3D models. The description of the template used for building the 3D model is given in Table 1. The Structure obtained for the spike glycoprotein of the two variants has a QMEAN score of −1.61 and −1.57 for the Delta and Delta-plus variant respectively. In both the spike protein models chain A is slightly up compared to the other two chains which could be due to mutations (Akbulut, 2021), Fig. 1(a) and (b).

2.4. Protein and ligand preparation

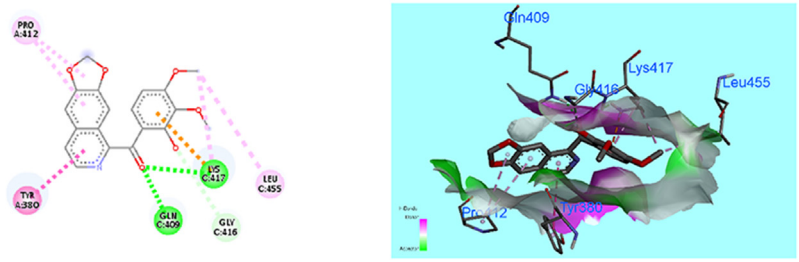
The modelled protein structures were prepared for docking using Autodock Vina (Trott and Olson, 2010). Ligands were removed, polar



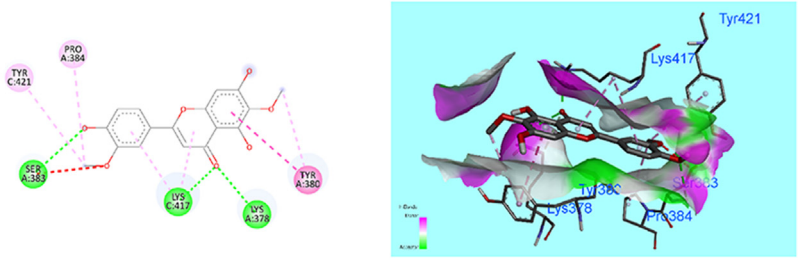
1,5-Dihydroxy-3-methoxy-7-methylanthracene-9,10-dione



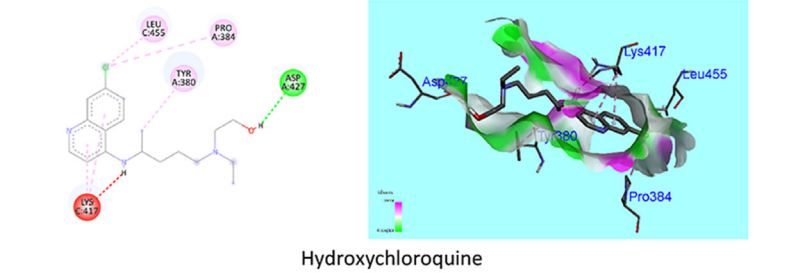
Oxyberberine



Rugosinone



Jaceosidin



Hydroxychloroquine

Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Donor Hydrogen Bond
Pi-Sigma
Amide-Pi Stacked
Pi-Pi T-shaped
Pi-Cation
Alkyl
Pi-Alkyl
Unfavorable Acceptor-Acceptor
Unfavorable Donor-Donor

Figs. 3. (*continued*).

hydrogen bonds and Gasteiger charges were added and the structures were saved in pdbqt format. The 50 phytochemical ligands were also prepared using Autodock Vina and their structures were saved in pdbqt formats. The binding site for the target spike protein was chosen by

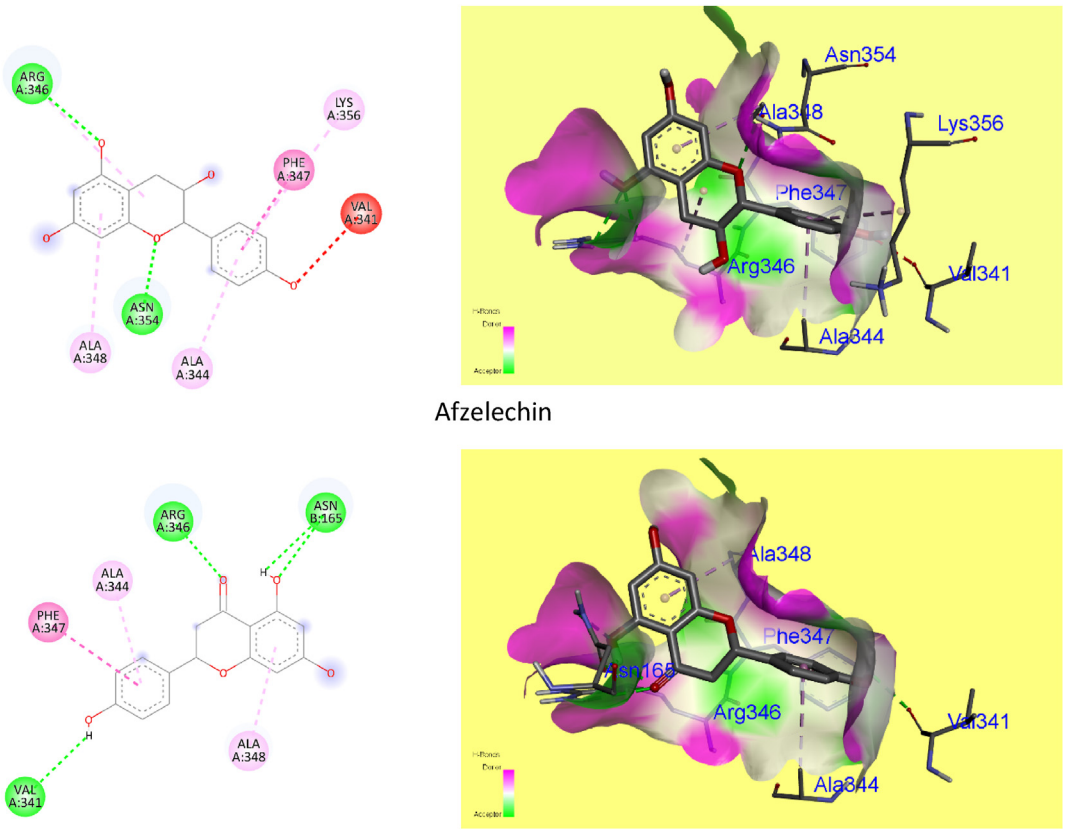


Fig. 4. 2D and 3D structure of the docking result of top 15 hits and Hydroxychloroquine with the target spike protein (AY.1) of SARS-CoV-2.

performing a blind docking with Hydroxychloroquine as the ligand. The grid box contained both the S1 and S2 subunits of the spike protein. The most probable active site of the spike protein was identified in the receptor binding domain (319-541aa). For docking with MOE 09, the sdf-structure of the 50 phytochemical ligands were converted to pdb format using Pymol and the structures were optimized for docking using Amber12:EHT Forcefield. 3D-protonation and energy minimization using Amber12:EHT was used to prepare the pdb-structure of the target proteins that were constructed using homology modelling.

2.5. Molecular docking of phytochemicals with spike protein targets

Docking studies was carried out using Autodock Vina. A grid box of 62 × 68 × 40 Å was assigned in the active site of the spike glycoprotein for both delta and delta-plus variant. The set of 50 ligands were screened with the receptor using Autodock Vina. Hydroxychloroquine was used as a standard in comparing the docking scores. The docking results, listed in Table 2, were viewed and analysed with Discovery studio visualizer (Biovia, Dassault Systèmes, 2021). MOE docking tool was also used to dock the 50 ligands into the receptor binding domain of the spike proteins (319-541aa). The 5 finest docked postures were created applying a scoring job London dG. The docking scores are listed in (Supplementary Table 4) and the top hits are listed in Table 2.

3. Molecular dynamics (MD) simulation

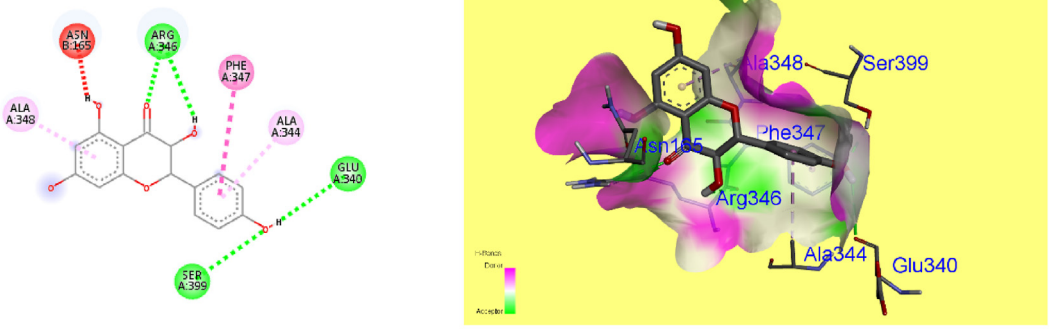
The ligand, 3,5,3′-Trimethoxy-6,7:4′,5′-bis(methylenedioxy)flavone, was taken to perform MD simulation with both the Target variants, B.1.617.2 and AY.1. VMD, NAMD and CHARMM-GUI was used to perform molecular dynamics simulation. CHARMM-GUI was used to obtain CGenFF topology file for the ligand (Jo et al., 2008). VMD was used to generate the topology file for the both the target proteins, which was then merged with the ligand and the complex was solvated (Humphrey et al., 1996). NAMD was then used to perform simulation for the Protein-Ligand complex with CHARMM36m force field (Phillips et al., 2020). All the Simulation analysis plots, i.e., RMSD, RMSF and H-bonds were prepared using QtGrace.

4. Results and discussion

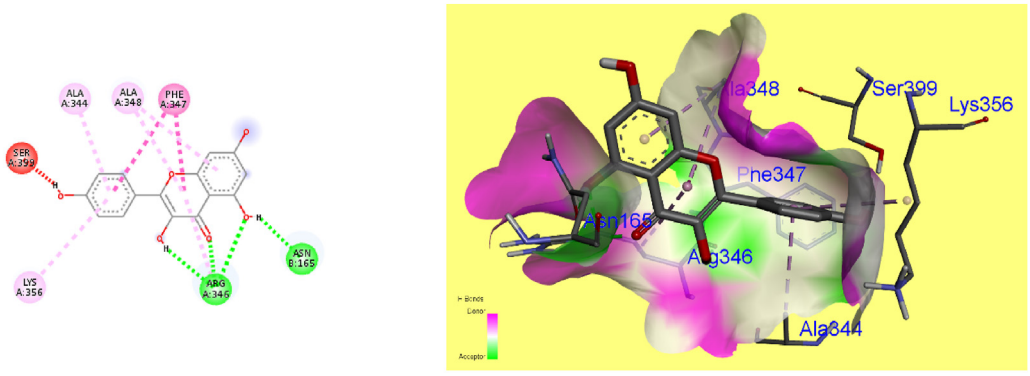
In this study 231 phytochemicals were initially selected from 38 medicinal plants, which were reported to exhibit anti-oxidant, anti-viral or anti-microbial properties. Screening of these phytochemicals for drug likeness was performed using LIPINSKI rule of five and SWISS-ADME. A list of 50 phytochemicals were finally selected for docking with the target spike glycoprotein of SAR-CoV-2 (Delta and Delta-plus variant). The target proteins, i.e., spike protein of the Delta and Delta-plus SARS-CoV-2 variant were constructed using homology modelling with SWISS MODEL. Hydroxychloroquine, with a binding affinity of −6.5 kcal/mol with the delta variant and −6.1 kcal/mol with the delta-plus variant, was used as a standard in the docking analysis. Most phytochemicals exhibited higher binding score than the standard. The top 15 phytochemicals in the binding score, listed in Table 2, are all flavones. The binding affinity of the phytochemicals towards the two targets are quite similar with only a few exceptions.

The docking score using MOE 09 were almost in correspondence with the scores using Autodock Vina, accept that the binding energy computed using MOE are generally lesser for all the 50 ligands (Supplementary Table 4).

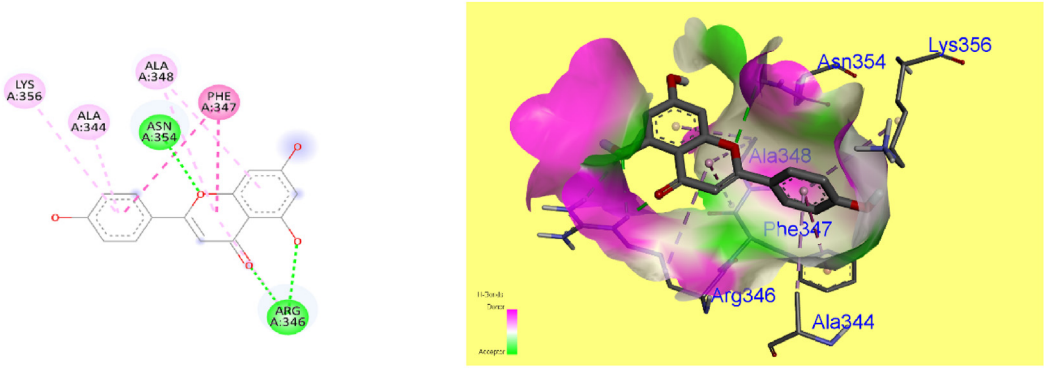
The docking analysis using Autodock Vina identified 3,5,3′-



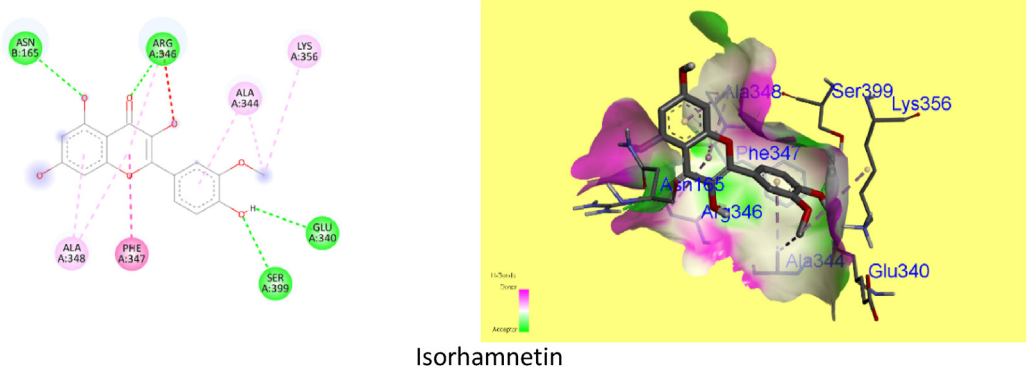
Dihydrokaempferol



Kaempferol



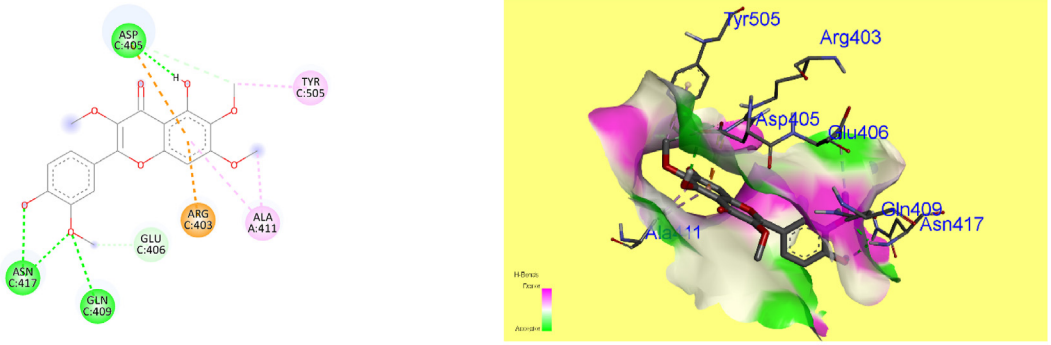
Apigenin



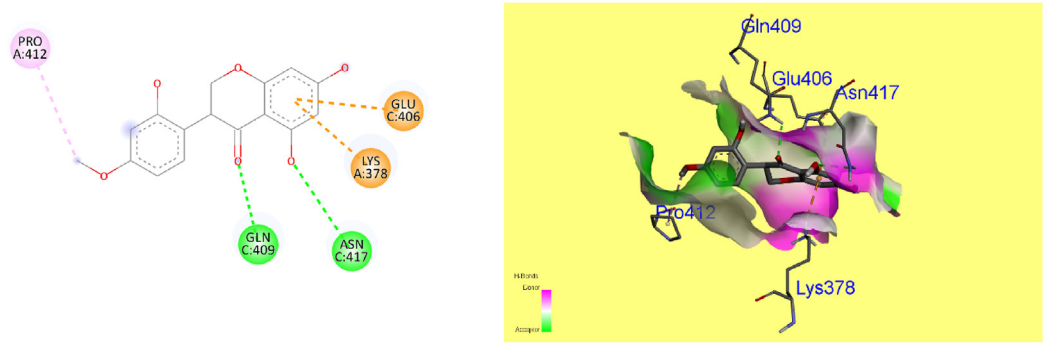
Isorhamnetin

Fig. 4. (*continued*).

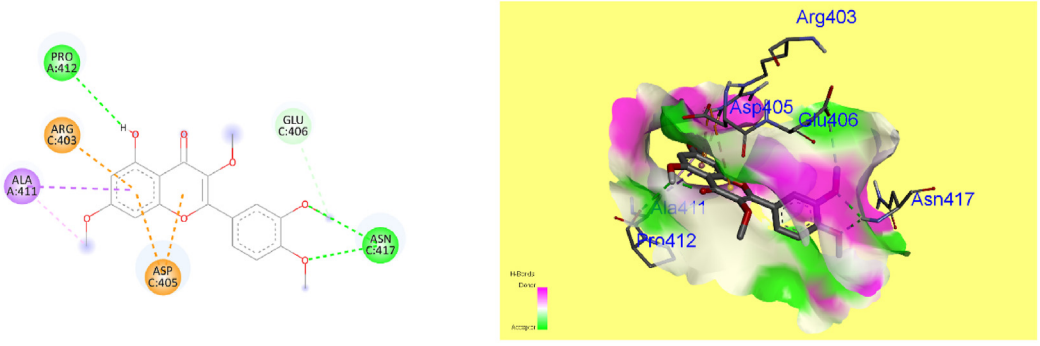
Trimethoxy-6,7:4′,5′-bis(methylenedioxy)flavone (Fig. 2) as having the highest binding affinity of −8.7 kcal/mol for both the target proteins, in comparison to the other 49 phytochemicals. This particular phytochemical is present in Nicotiana plumbaginifolia (Shajib et al., 2018;



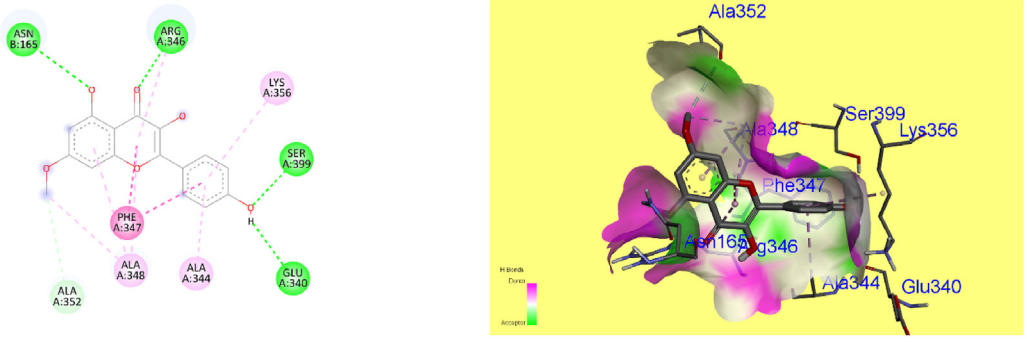
Chrysosplenetin



Ferreirin



Retusin

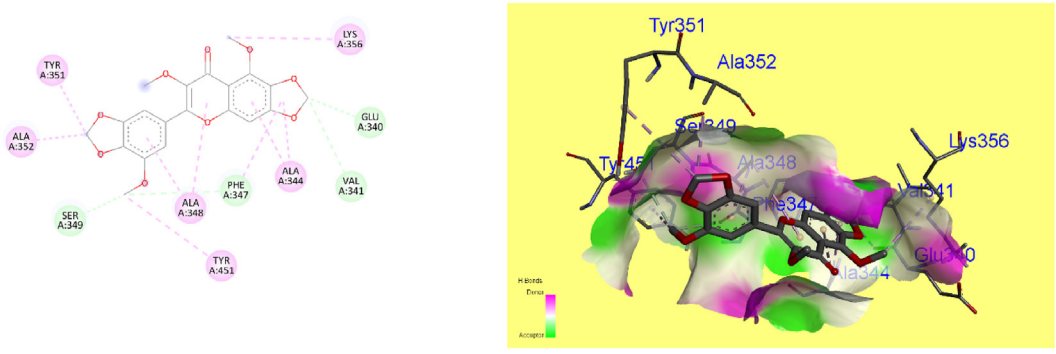


Rhamnocitrin

Fig. 4. (*continued*).

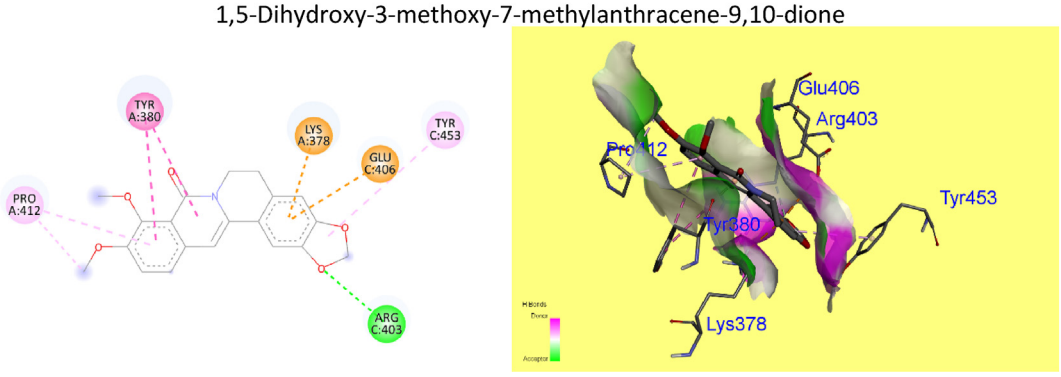
Nishtha et al., 2017). The same ligand performed very well when docked with MOE, producing the highest score with the delta variant and the second highest score with the delta plus variant. It is quite interesting to note that, all the hits produced using MOE docking tools are all present in the top 16 hits produced using Autodock Vina, accept for the ligand Reticuline, which did not perform well in Autodock Vina.

The interactions of the ligand (3,5,3′-Trimethoxy-6,7,4′,5′-bis(methylenedioxy)flavone) with the amino acids (Fig. 3 & Fig. 4) at the active site are very similar for both the target variants. GLU340, VAL341, PHE347, SER349, ALA352, LYS356, ALA344, TYR351, TYR451 and

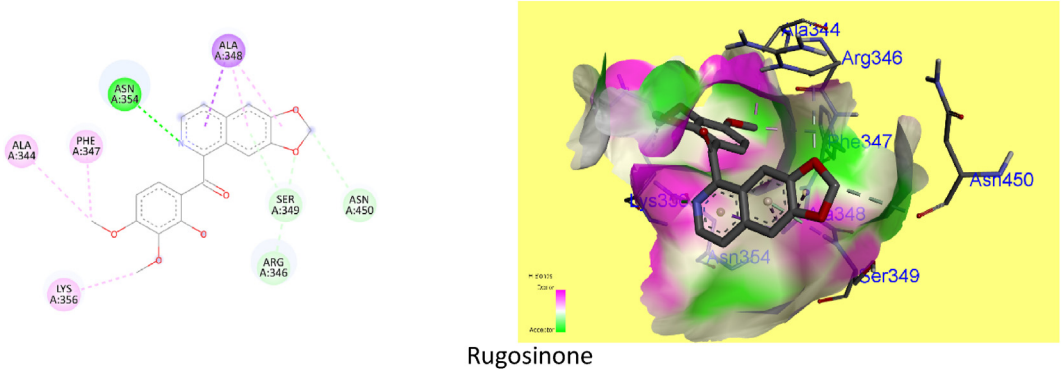


3,5,3-Trimethoxy-6,7,4,5-bis(methylenedioxy) flavone

1,5-Dihydroxy-3-methoxy-7-methylanthracene-9,10-dione



Oxyberberine



Rugosinone

Fig. 4. (*continued*).

ALA348 are the common Amino residues in both the docking sites. The interactions of 3,5,3′-Trimethoxy-6,7,4′,5′-bis(methylenedioxy)flavone with the delta variant spike protein has an additional amino acid residue (SER399) at the binding site (Fig. 3). This could indicate that there is very less structural difference between the two target proteins at the active site.

Among the top 15 hits, Afzelechin, 5,7-dihydroxy-2-(4-hydroxyphenyl) chroman-4-one, Dihydrokaempferol, Kaempferol, Apigenin, Isorhamnetin, Chrysosplenetin, Resutin, Rhamnocitrin, and 3,5,3′-Trimethoxy-6,7:4′,5′-bis(methylenedioxy)flavone have more structural

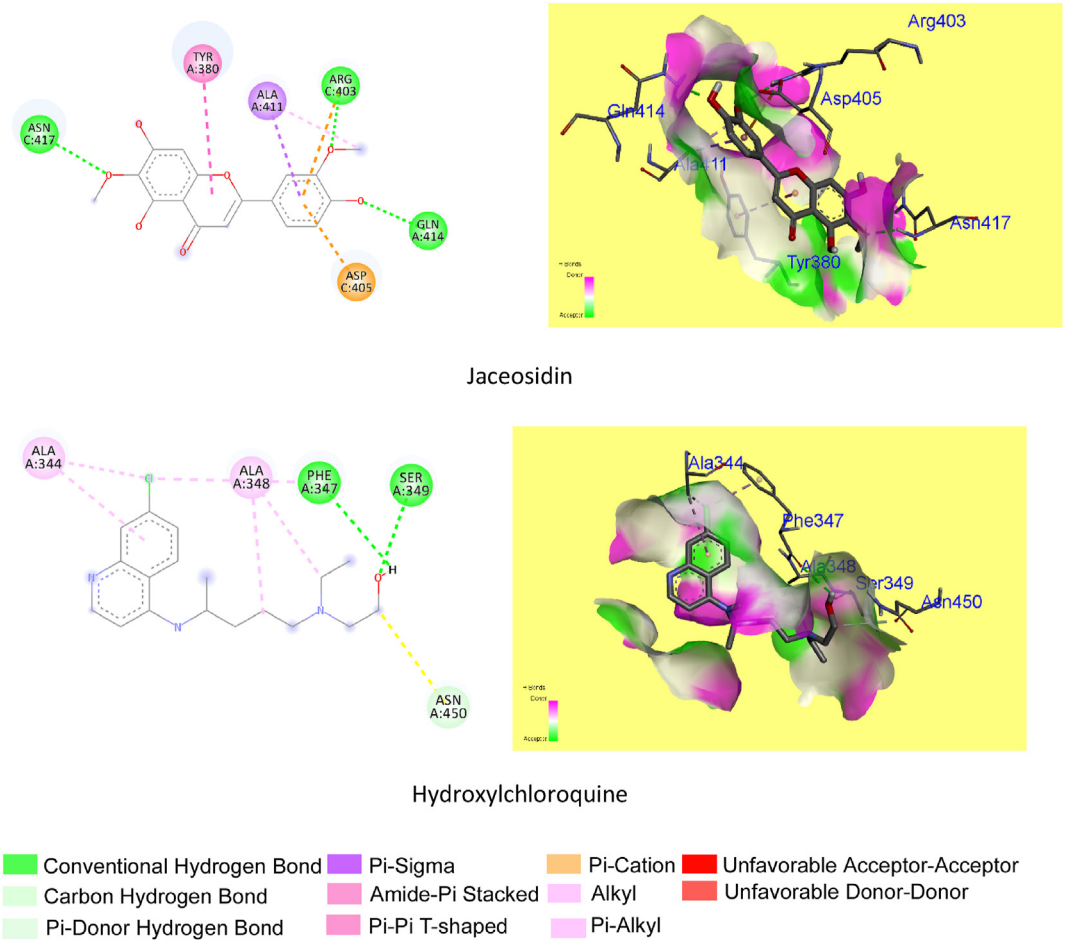


Fig. 4. (*continued*).

similarities and have better binding energy. In all these ligands, A344, A346, A347, A348 and B165 are the most common amino acid residues in the binding site. The Amino acid residues A344, A347 and A348 are involved in Pi-Pi T-shaped and Pi-alkyl interactions with the two aromatic rings of the ligands. The amino acid residue A346 is involved in conventional hydrogen bond with the carbonyl oxygen between the aromatic rings and B165 forms hydrogen bond with the aromatic hydroxyl group (Figs. 3 and 4).

The receptor binding domain (RBD) situated in the S1 subunit initiates viral entry into the host by binding to the cell receptor ACE2 in the aminopeptidase N (Huang et al., 2020). All the common amino acid residues actively involved in the interaction with the top hit ligands are in the RBD, which suggest that the ligands can disrupt the viral binding to ACE2 receptor.

The RMSD plots of the MD simulation in Fig. 5 (A, B) suggest that the protein-ligand complexes are stable within acceptable deviation. The systems after 70–80 ns, becomes more stable in the course of the simulation. 3,5,8-Trimethoxy-6,7,4,5-bis(methylenedioxy)flavone with the Delta variant spike protein exhibited an average RMSD of 1.348 Å, and the same ligand exhibited an average RMSD of 1.577 Å with the Delta Plus Spike protein. Thus, these results indicate that the protein-ligand complex system remains stable amidst internal motions and fluctuations during the course of the simulation.

Both protein-ligand complexes showed similar RMSF plot, Fig. 5(C, D), which also indicates their structural similarity. The stability of the complexes around the active RBD (319-541a) is noticeable in the plot, which could be attributed to the ligand recognition in the active site. Both the complexes showed an average RMSF of 1 Å with significant fluctuations in the region1000-1100. These results show that the target proteins are stabilized by the binding of the ligand, 3,5,8-Trimethoxy-6,7,4,5-bis(methylenedioxy)flavone, at the active site.

Based on the structural similarities of the tops hits, a ligand based pharmacophore model is generated by PharmaGist (Schneidman-Duhovny et al., 2008) which is shown in Fig. 6. The tops 15 hits were submitted to PharmaGist in mol2 format. The five acceptor groups and the three aromatic pockets are all involved in binding with the active sites of the spike proteins.

5. Conclusion

In conclusion, the study reports that many medicinal plants rich in Poly-Methoxyflavones and Poly-Ethoxyflavones, especially Nicotiana Plumbaginifolia, Bergenia Ciliata, Cardiocrinum Gigantum Wall, Cauletya Spicata Sm, Centella Asiatica Linn, Houttuynia Cordata Thunb, Mentha spicata, Paris Polyphylla Sm, Thalicrum foliolosum and Wedelia chinensis Osbeck, could be used to treat the outbreak of COVID-19. Further in vivo and in vitro studies on Poly-Methoxyflavones and Poly-Ethoxyflavones are needed to confirm the theory proposed by this paper. We assume that more Insilco studies on phytochemicals will reveal the potentials that are inbuilt in many traditional plants, to inhibit SARS-CoV-2.

Author contribution

Peter Solo: Methodology, Software, Investigation, Resources, Data curation, Visualization, Writing-Original draft preparation. M. Arockia doss: Conceptualization, Validation, Supervision, Writing-Reviewing and Editing.

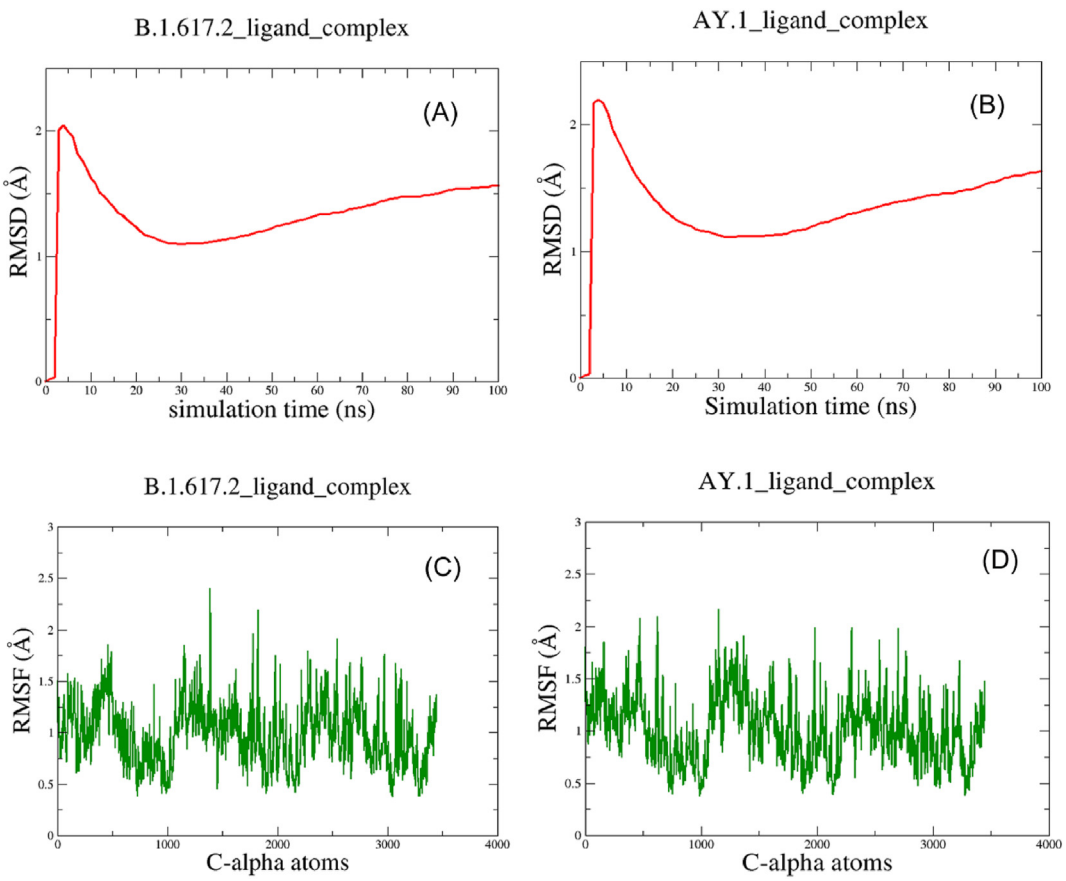


Fig. 5. (A, C) RMSD and RMSF of 3,5,8-Trimethoxy-6,7,4,5-bis(methylenedioxy)flavone with B.1.617.2. (B, D) RMSD and RMSF of 3,5,8-Trimethoxy-6,7,4,5-bis(methylenedioxy)flavone with AY.1.

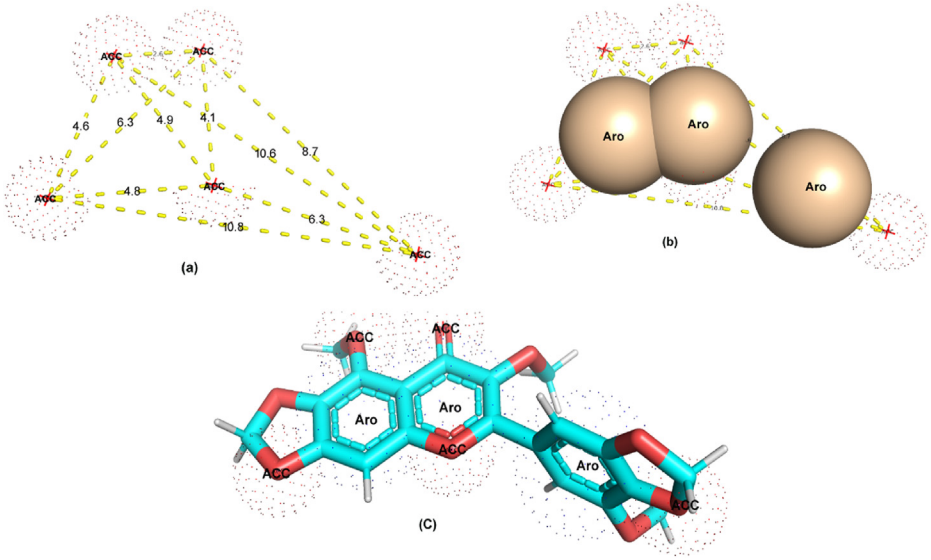


Fig. 6. (a) Pharmacophore model with five acceptors (b) Pharmacophore model with five acceptors & three aromatic centres (c) Pharmacophore model with the ligand (3,5,3′-Trimethoxy-6,7,4′,5′-bis(methylenedioxy)flavone).

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for –profit sectors.

CRediT author contribution statement

Peter Solo: The Author states that the individual contribution to the research are given bellow and that there is no conflict for authorship.

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.

Acknowledgments

The authors are grateful to the authorities of St. Joseph's College (autonomous) for providing the space and the equipment for performing computational analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crphar.2021.100065.

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