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Chemical system biology approach to identify multi-targeting FDA inhibitors for treating COVID-19 and associated health complications

Biswajit Naik[a], Venkata Satish Kumar Mattaparthi[b], Nidhi Gupta[a], Rupal Ojha[a], Pundarikaksha Das[b], Satyendra Singh[a], Vijay Kumar Prajapati[a] and Dhaneswar Prusty[a]

[a]Department of Biochemistry, School of Life Sciences, Central University of Rajasthan, Ajmer, India; [b]Department of Molecular Biology and Biotechnology, Tezpur University, Assam, India

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ABSTRACT

In view of many European countries and the USA leading to the second wave of COVID-19 pandemic, winter season, the evolution of new mutations in the spike protein, and no registered drugs and vaccines for COVID-19 treatment, the discovery of effective and novel therapeutic agents is urgently required. The degrees and frequencies of COVID-19 clinical complications are related to uncontrolled immune responses, secondary bacterial infections, diabetes, cardiovascular disease, hypertension, and chronic pulmonary diseases. It is essential to recognize that the drug repurposing strategy so far remains the only means to manage the disease burden of COVID-19. Despite some success of using single-target drugs in treating the disease, it is beyond suspicion that the virus will acquire drug resistance by acquiring mutations in the drug target. The possible synergistic inhibition of drug efficacy due to drug-drug interaction cannot be avoided while treating COVID-19 and allied clinical complications. Hence, to avoid the unintended development drug resistance and loss of efficacy due to drug-drug interaction, multi-target drugs can be promising tools for the most challenging disease. In the present work, we have carried out molecular docking studies of compounds from the FDA approved drug library, and the FDA approved and passed phase −1 drug libraries with ten therapeutic targets of COVID-19. Results showed that known drugs, including nine anti-inflammatory compounds, four antibiotics, six antidiabetic compounds, and one cardioprotective compound, could effectively inhibit multiple therapeutic targets of COVID-19. Further *in-vitro*, *in vivo*, and clinical studies will guide these drugs' proper allocation to treat COVID-19.

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Introduction

On March 11, 2020, WHO has announced coronavirus disease 2019 (COVID-19) as a "global pandemic". The disease, caused by SARS-CoV-2, has been unceasingly spreading and becoming the most devastating health crisis of the century (Akriti et al., 2021; Ozkendir et al., 2020). As of November 14 2020, COVID-19 claimed 53,164,803 infections and 1,300,576 deaths worldwide. It has been predicted that the SARS-CoV-2 spread could gain further momentum, in low temperatures and dry weather conditions, during the coming winter months (Mandal & Panwar, 2020; Sarkodie & Owusu, 2020). Instantaneously, recent UK and USA studies have identified a new mutation, known as D614G, in the virus's spike protein that could make it more transmissible with increasing viral load (B Korber et al.; Bette Korber et al., 2020). Altogether, it can be assumed that the second wave of COVID-19 will be riskier than the earlier one. Like all other viruses, it is well within the realm of possibility that SARS-CoV-2 will acquire some new mutations in the drug targets to get around our existing therapeutics. Dealing with the evolutionary trend of drug resistance, drugs interfering with multiple viral proteins' functions all together will have substantial implications in controlling the disease.

Accumulating evidence suggests that COVID-19 patients have varied clinical manifestations, extending from no symptoms and mild symptoms to severe respiratory failure, septic shock, and multiple organ dysfunction (Di Gennaro et al., 2020). The damages caused by uncontrolled immune responses are majorly responsible for the disease's clinical deterioration (Felsenstein et al., 2020). Moreover, some of the severe COVID-19 patients admitted to ICU have been reported to have bacterial co-infections caused by *Staphylococcus aureus*, *Haemophilus influenza, Enterobacteriaceae,* and *Streptococcus pneumonia* (Contou et al., 2020). A meta-analysis of literature studies showed that comorbidities, including hypertension, diabetes, cardiovascular disease, chronic pulmonary disease, and chronic kidney disease, are major risk factors for severe patients (Yang et al., 2020).

As of now, we do not have any clinically approved and targeted therapy for treating COVID-19 (Kheirandish, 2021). However, many repurposed drugs, including antivirals, antimalarials, antibacterial, and immunomodulators, are in different stages of clinical trials for treatment (Lam et al., 2020).

CONTACT Dhaneswar Prusty dhaneswar08@gmail.com, dhaneswarprusty@curaj.ac.in Department of Biochemistry, School of Life Sciences, Central University of Rajasthan, NH-8, Bandarsindri, Kishangarh, Ajmer, Rajasthan 305817, India

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Many computational efforts by employing various *in silico* tools have been conducted searching for drug candidates against COVID-19 (Harismah & Mirzaei, 2020; Khalid et al., 2020; Mirzaei et al., 2020; Mohamed et al., 2021). We also have done a high throughput virtual screening of 3963 natural compounds (NPASS database- http://bidd.group/NPASS/index.php) to identify possible drug candidates for COVID-19, which might inhibit multiple crucial proteins of SARS-CoV-2 (Biswajit Naik et al., 2020).

The current interdisciplinary chemical system biology approach connects chemical biology and system biology (Networking Chemical Biology, 2008) is focused on identifying polypharmacological compounds from FDA approved drug library and FDA approved and passed phase −1 drug library to expedite the discovery of specific drugs for the treatment of COVID-19 and associated health complications. We have selected essential viral and host proteins for our high-throughput virtual screening approach. The proteins include spike protein, spike protein complex with ACE2, PL protein (PLpro), 3 C-like proteinase (3CLpro), RNA dependent RNA polymerase (RDRP), helicase, endoRNAse, 2′-O-ribo methyltransferase (Methyltransferase), 3′-5′ exoribonuclease, and nucleocapsid, which have been reported as prime targets for developing drugs against COVID-19 (Saxena, 2020). High throughput virtual screening of the above-mentioned libraries resulted in twenty best-docked multi-targeting molecules with known anti-inflammatory, antibacterial, cardioprotective, or antidiabetic properties were reported in this work. Further, obtained results were analyzed, and only five potential compounds were selected based on multi-target inhibitory activity. Later, molecular simulation dynamics also confirmed the microscopic interaction between identified SARS-CoV-2 inhibitors and their target proteins. Thus, five specific inhibitors identified under this investigation could be used to treat COVID-19 in combination with associated clinical complications.

Material and methods

Proteins as therapeutic targets, homology modeling, refinement and validation

Ten proteins of SARS-CoV-2 were studied as therapeutic targets for the identification of antagonists. At the time of the experiment, the crystal structure was available for the eight proteins, namely 3 C-like proteinase (PDB ID:6LU7), RNA dependent RNA polymerase (PDB ID: 6M71), EndoRNase (PDB ID: 6VWW), Methyltransferase (PDB ID: 6W61), Spike glycoprotein (PDB ID: 6VXX), Spikeglycoprotein-ACE2 complex (PDB ID: 6LZG), Papain like proteinase (PDB ID: 6W9C) and Nucleocapsid (PDB ID: 6M3M). However, for two target proteins, i.e. helicase (accession no: YP_009725308.1) and exoribonuclease (accession no: YP_009725309.1), homology structures were modeled using SWISS-model server (https://swissmodel.expasy.org/). These models were taken from the previous report published by Naik et al. (2020). In brief, the templates for the homology modeling were selected on two criteria, their sequence identity and sequence coverage, which is estimated by Global Model Quality Estimate (GMQE) and Quaternary Structure Quality Estimate (QSQE) score (Cardoso et al., 2018). Although homology modeling is a prevalent technique to model the tertiary structure of a protein sequence, an accurate estimation of each atom's three-dimensional coordinates in a protein sequence is a challenge (Cheng, 2008). Therefore, the obtained models are needed to be refined to attain their nearest native structure. Here, the modelled proteins were refined using a 3 D-refine server (http://sysbio.rnet.missouri.edu/3Drefine/). Further, a Ramachandran plot was developed for both refined protein models to estimate the number of amino acids present in the allowed, disallowed, and favorable region (Lovell et al., 2003) using RAMPAGE server (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php).

Selection of antagonist library (ligand selection)

Two libraries, namely FDA approved drug library (https://www.selleckchem.com/screening/fda-approved-drug-library.html) and FDA approved and passed Phase I library (https://www.selleckchem.com/screening/fda-approved-passed-phase-i-drug-library.html) from a Bioactive compounds expert company, Sellekchem were used as ligands.

Molecular docking

Protein preparation

Crystallographic protein structures of eight targets were retrieved using their PDBIDs from the RCSB Protein Data Bank (https://www.rcsb.org/). However, in the case of helicase and exoribonucleases targets, refined homology models were used for docking. All the structures were pre-processed and optimized at neutral physiological condition (pH 7) for the proper protonations of the residues in protein preparation wizard of maestro v11.9 module of Schrodinger suite 2019-1 (Release, 2016) to correct the bond information by adding hydrogen bonds, filling in missing side chains, and deleting water molecules and hetero groups to escape from hindrance in the binding zone.

Ligand preparation

A set of 2,683 compounds from FDA approved drug library, and 2820 compounds from FDA approved and passed phase I library were retrieved in .sdf format for their screening and identification of antagonists against SARS-CoV-2 targets. The compounds were processed by removing salts, adding hydrogens, deprotonation, and neutralization at the physiologically relevant pH 7 to obtain their possible isomers, tautomer, and stereoisomeric forms of three-dimensional conformation. All these actions were performed by the Ligprep v4.7 tool of Schrodinger 2019-1.

Grid generation

Generation of the grid around a receptor/protein is a very important step in a molecular docking study as it allows the ligands to bind with the specific binding region of the

receptor or the target proteins. For this study, a three-dimensional grid box was generated of size 20 Å around the active site amino acid residues of each target protein using the glide module v8.2 of Schrodinger. The active site residues were retrieved from the previously reported literature studies (Supplementary Table 1).

Virtual screening

All the ligands were docked against each target protein for the virtual screening purpose using the Glide tool v8.2 of Schrodinger 2019-1 (Friesner et al., 2004). A total of 6647 ligands and positive controls of respective proteins were screened in a 3-step rotational workflow for virtual screening i.e. High Throughput Virtual Screening (HTVS) to Standard Precision (SP) to Extra Precision (XP). For instance, the ligands were first screened with HTVS, output screened ligands from HTVS filter were put into the filter of SP and again the successfully screened ligands from SP filter were put forwarded to XP docking. The HTVS and SP mode of screening utilize the same scoring function to eliminate false positives with a difference of speed and accuracy, whereas XP utilizes extensive sampling protocols and advanced scoring to give higher enrichment (Pandey et al., 2015).

MM-GBSA: Estimation of binding free energy

Prime Molecular Mechanics Generalized Born model and Solvent Accessibility (MM-GBSA) module of Schrodinger 2019-1 was used to calculate the binding free energy (ΔG bind) between the target proteins and ligands. For this action, glide pose viewer complexes are taken as input files with the SVGB solvation model, and the OPLS3 force field was set in the setting tab of prime MM-GBSA module of Schrodinger suite (Lyne et al., 2006).

Pharmacokinetics and physiochemical properties of ligands

To study the druggable characteristics of ligands, we used the Qikprop v5.9 tool of Schrodinger, which reveals the absorption, distribution, metabolism, and excretion properties of individual ligands for the evaluation of suitable drugs. Furthermore, we have also analyzed the toxicity profile of compounds using the most reliable webserver pkCSM (http://biosig.unimelb.edu.au/pkcsm/).

ROC plot analysis

For the validation of molecular docking protocol, here we performed the enrichment study that estimates the Receiver Operating Characteristics (ROC), Area Under Curve (AUC) and Boltzmann-Enhanced Discrimination of Receiver Operating Characteristic (BEDROC) in the form of different metric values (Truchon & Bayly, 2007). In this study, the effectiveness of SP and XP mode of virtual screening protocol was observed through a statistical receiver operating characteristics curve that shows a significant difference between the active compounds and the decoy (false positive/inactive) molecules (Fawcett, 2006). The ROC plot was represented by 1-specificity on the X-axis representing the false-positive molecules and sensitivity on Y-axis representing the active compounds. For this action, an active-decoy docked file in pose viewer (pv. maegz) format for SP and XP mode of docking was given as input, and all these processes were done by the enrichment calculator tool provided by Schrodinger suite. Furthermore, AUC and BEDROC values were also calculated for enrichment study of the SP and XP docking.

Molecular dynamics simulation

We performed Molecular dynamics simulation for the five complexes A) RDRP-Rutinhydrate B) Exo-Silymarin C) Helicase-Luteolin D) Methyltransferase-Amikacin hydrate E) Methyltransferase-Geneticin using the Particle Mesh Ewald Molecular Dynamics (PMEMD) (Kaus et al., 2013) module of AMBER12 (Case et al., 2010) software package. The partial charges and force field parameters for the ligands were generated automatically using the antechamber program (Wang et al., 2006) in AMBER12. Force field parameters were given for the ligand and the protein using the xleap module in AMBER12. General Amber force field (GAFF) (Wang et al., 2004) and AM1-BCC (Jakalian et al., 2002) charges were used for ligands, while AMBERff99SB force field was used for the protein. Using xleap and antechamber module, we obtained the initial coordinate and the topology files for all the complex systems. The resultant initial structure of the individual complex system was solvated using TIP3P (Jorgensen et al., 1983) water model, the water box size of 10 Å from the solute in the x, y, and z coordinates, and then counter-ions were added to neutralize the complex. The potential energy of initial structures was minimized using the 1000 steps of steepest descents followed by 2000 steps of the conjugate gradient method. We fixed the complex molecule using harmonic constraints (excluding the water molecules) with force constant of 30 kcal $mol^{-1}Å^2$ to overcome the bad contacts between water molecules and the complex. Again minimization was carried out without any constraints for 3000 cycles (1000 cycles of steepest descents and 2000 cycles of the conjugate gradient). The complex systems were then slowly heated from 0 to 300 K with weak 20 kcal $mol^{-1}Å^{-2}$ constraints at 40 ps in NVT condition. This allows the structure to undergo slow relaxation. In MD simulation, using geometrical tolerances of $5 \times 10\text{-}4$ Å, the SHAKE constraints were imposed on all covalent bonds involving hydrogen atoms, with a time set to 2 fs. Then, equilibration and long production steps were performed in NPT condition ($T = 300$ K and P = 1atm) and temperature regulation was achieved with the help of Berendsen weak coupling method (0.5 ps time constant for heat bath coupling and 0.2 ps pressure relaxation time) (Berendsen et al., 1984) (Ryckaert & Bellemans, 1978). Finally, we carried out 100 ns long NPT MD simulation using a heat bath coupling time constant of 1 ns to analyze the conformational dynamics of five complexes. The Analysis of structural convergence properties such as Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), the radius of gyration (Rg) were carried out using cpptraj (Roe &

Cheatham III, 2013) in AMBER12. For inspecting the 3 D structure of the molecule, we used UCSF Chimera (Pettersen et al., 2004) and VMD (Humphrey et al., 1996). And for the solvation free energy calculations of the complexes, we have used 3 D RISM theory (Hirata, 2003).

Results

SARS-CoV-2 therapeutic target modelling & retrieval of crystal structure and antagonists library compounds

The models for two therapeutic targets, i.e. helicase and exoribonuclease of SARS-CoV-2, were modelled using template sequences of 6jyt.2.A & 5nfy.1.A respectively. The targets were sharing 97.95% and 94.07% sequence identity with the chosen templates respectively. Indeed, it is known that the homology model is considered to be excellent and reliable if the template is chosen, and the target shares minimum of 30% sequence identity (Xiang, 2006).

Further, the homology model's quality was estimated by the QMEAN score (Benkert et al., 2009). The GMQE score ranges between 0 to 1, which means the higher the score, the higher the model's reliability and accuracy for the used template. In our case, the value for GMQE score was 0.98 for both targets, which denotes higher accuracy. Next, the models for both target enzymes were validated before and after refinement using the Ramachandran plot. Where Ramachandran plot confirmed that models in .pdb format after refinement are stable and better suited for docking with ligands, i.e. for helicase (non-refined % of favored residues 84.34% whereas refined % of favored residues 88.60%) and exoribonuclease (non-refined % of favored residues 89.44% whereas refined % of favored residues 93.10%) both, rest eight therapeutic targets were retrieved using their PDBIDs from the RCSB Protein Data Bank (https://www.rcsb.org/). Structural based drug screening is a method based upon the libraries of small compounds and targets active residues. Selleckchem has a collection of numerous libraries, which also includes FDA approved drug library (2683 compounds) and FDA approved and passed Phase I library (2820 compounds). Repurposing or already FDA approved drugs is the only secure and rapid way to meet the urgent requirement for ongoing pandemic treatment. We selected both FDA approved drug library, and FDA approved and passed Phase I library for our experiments from Selleckchem.

Computational virtual screening of FDA compounds against potential therapeutic targets of SARS-CoV-2

To find out molecules of therapeutic effects for inflammation, bacterial infection, diabetes, cardiovascular diseases, and kidney-related disorders and that can hit multiple therapeutic targets of SARS-CoV-2, molecular docking studies of compounds from FDA approved drug library, and FDA approved and passed phase -I drug library was performed against the target proteins including spike protein, spike protein complex with ACE2, PL protein, 3CLpro, RdRp, helicase, endoRNAse, methyltransferase, exoribonucleases, and nucleocapsid. The top 20 molecules showing better binding interactions with multiple proteins of SARS-CoV-2 compared to control compounds depicted in Table 1B and are listed in Table 1A. These compounds include nine anti-inflammatory compounds, four antibiotics, six antidiabetic compounds, and one cardioprotective compound. Among those compounds, four anti-inflammatory compounds, two antibacterial compounds, four antidiabetic compounds, and one therapeutic compound related to cardiovascular diseases are discussed in detail. Moreover, based on their docking scores with multiple therapeutic targets, three anti-inflammatory agents and two antibiotics are selected for molecular dynamic analysis.

Anti-inflammatory compounds against multiple therapeutic targets of SARS-CoV-2

Rutin hydrate

Rutin is a water-soluble flavone glycoside converted into quercetin in the bloodstream (Morand et al., 2000). It is widely found in buckwheat, apple skin, red wine, tea, and coffee (Kim et al., 2005; Kreft et al., 1999). It is reputed for its potent antioxidant capacity and has been reported effective against oxidative-stress-mediated diseases (López-Revuelta et al., 2006). Besides, rutin possesses several pharmacological properties, including analgesic, anticarcinogenic, cardioprotective, neuroprotective, vasoprotective, and cytoprotective activities (Ganeshpurkar & Saluja, 2017). Rutin works as a potential anti-inflammatory agent in many ways. It increases prostaglandins production, decreases histamine synthesis (Hussain et al., 2009; Kaithwas & Majumdar, 2010), inhibits the migration and/or degranulation of neutrophils (Selloum et al., 2003), blocks lipoxygenase pathway (Kurisawa et al., 2003), and suppresses proinflammatory cytokines production (S.-w. Wang et al., 2012). The antimicrobial and antiviral activities of rutin have been extensively studied against various bacteria, fungi, and viruses (Ganeshpurkar & Saluja, 2017). Computational studies of different research groups have revealed helicase (Wu et al., 2020), spike protein (Kadioglu et al., 2021), main protease (Mpro) (Das et al., 2020; Huynh et al., 2020), RdRp (Altayeb et al., 2020), envelope protein (E) (Bhowmik et al., 2020), methyltransferase (Kadioglu et al., 2021) as the therapeutic targets of rutin for SARS-CoV-2. In our molecular docking studies with ten therapeutic targets of SARS-CoV-2, rutin occupied the first rank by displaying better docking scores such as −11.37, −9.57, −9.83, −11.44, −12.66, −10.13, −9.07 and −13.76 for RdRp, Helicase, Spike protein, PL protein, 3CLpro, EndoRNAse, Methyltransferase, and Nucleocapsid respectively (Table 1A) than the reported positive inhibitors (Table 1B). The ligand-binding patterns of the docked complexes are displayed in Figure 1A, showing Rutin Hydrate strongly binds with the active site pocket of each target protein. For instance, it interacts through strong hydrogen bonds with the active site pocket residues of 3CLpro (Thr26, His41, Asn142, His164, Gln189 and Thr190), helicase (Lys288, Glu375, Asp534, Ser535 and Gly538), PLpro (chainA:Asp108, Asp286 and Ala288; chainC:Asp164 and Glu167), RDRP (Ser549, Arg553, Thr556, Tyr619, Lys621, Asp624 and Asp760) and Pi-cation inetractin with Arg555 of

Table 1A. List of selected compounds (along with their role) which showed interaction with therapeutics targets of SARS-Cov-2.

Name of the Compound	Drug used for the purpose	References	Therapeutic targets of SARS-CoV-2	Docking Score	MMGBSA Score
		Anti-inflammatory			
Rutin Hydrate	Anti-inflammatory, Antioxidant, Inhibitor of platelet aggregation, reduced heart risks	(Ojha et al., 2016)	**RDRP**	**−11.37**	**−65.34**
			Helicase	−9.57	−87.32
			Spike Protein	−9.83	−71.83
			PLprotein	−11.44	−72.29
			3CLpro	−12.66	−101.17
			EndoRNAse	−10.13	−92.21
			Methyltransferase	−9.07	−70.4
			Nucleocapsid	−13.76	−100.03
Silymarin	Anti-inflammatory Hepatoprotective, antioxidant, anticancer, and cardioprotective activities	(Baliga et al., 2014)	Exoribonucleases	−6.14	−77.75
			Spike Protein	−5.88	−58.7
			Spike protein complex with ACE2	−5.61	−64.41
			RDRP	−5.9	−41.01
			EndoRNAse	−7.08	−62.4
			Helicase	−7.82	−113.86
			Nucleocapsid	−10.02	−60.65
Quercetin	Antioxidant and anti-aging agent and anti-carcinogenic	(Li et al., 2016)	3CLpro	−9.07	−70.4
			Helicase	−13.76	−100.03
			Spike Protein	−12.66	−101.17
			Spike protein complex with ACE2	−9.57	−87.32
			RDRP	−5.35	−48.75
			EndoRNAse	−6.7	−50.99
			Exoribonucleases	−6.55	−40.73
Mitoxantrone 2Hcl	Antineoplastic agent used to treat multiple sclerosis, Immunosuppressant	(Fox, 2004)	Helicase	−8.35	−98.48
			Spike Protein	−6.15	−48.46
			Spike protein complex with ACE2	−8.11	−68.28
			RDRP	−8.13	−77.04
			EndoRNAse	−6.66	−71.5
			Methyltransferase	−6.69	−65.44
			Nucleocapsid	−9.5	−63.22
Luteolin	Anti-inflammatory and Neuroprotective agent	(Nabavi et al., 2015)	Helicase	−4.52	−73.99
			3CLpro	−7.50	−61.63
			Spike protein complex with ACE2	−5.84	−46.31
			PLprotein	−4.80	−33.54
			EndoRNAse	−7.18	−61.06
			Methyltransferase	−3.87	−42.55
Aloin	Anti-inflammatory used to treat acute Influenza. Antiarrhythmic, Anti-carcinogenic	(Huang et al., 2019)	Spike Protein	−7.52	−46.36
			Spike protein complex with ACE2	−6.19	−52.55
			RDRP	−7.47	−43.78
			EndoRNAse	−10.25	−50.69
			Methyltransferase	−7.13	−27.61
			Nucleocapsid	−8.24	−50.03
Morin Hydrate	Anti-inflammatory, antiproliferative	(Choudhury et al., 2017)	3CLpro	−6.92	−58.7
			Helicase	−4.95	−75.72
			Spike Protein	−3.13	−37.39
			Spike protein complex with ACE2	−6.15	−45.3
			EndoRNAse	−6.32	−44.79
			Exoribonucleases	−6.56	−52
Kaempferol	Anti-colorectal cancer (CRC) drug	(Riahi-Chebbi et al., 2019)	3CLpro	−7.48	−43.88
			Spike Protein	−3.58	−40.92
			Spike protein complex with ACE2	−5.49	−43.44
			Exoribonucleases	−5.23	−41.17
			Nucleocapsid	−6.1	−34.75
Madecassoside	Anti-inflammatory, wound healing, and antioxidant	(Li et al., 2007)	Helicase	−9.62	−131.31
			RDRP	−10.05	−36.38
		Antibiotics			
Amikacin Hydrate	Antibiotic	(Torres et al., 2018)	Helicase	−7.71	−53.43
			Spike Protein	−9.28	−66.72
			Spike protein complex with ACE2	−13.08	−71.28
			EndoRNAse	−10.37	−82.08
			Methyltransferase	−7.73	−71.19
			Exoribonucleases	−9.61	−88.19
Geneticin	Aminoglycoside antibiotic	(Zufferey et al., 2012)	**Methyltransferase**	**−5.37**	**−59.72**
			Spike protein complex with ACE2	−7.19	−63.84
			RDRP	−10.25	−61.95
			EndoRNAse	−6.91	−76.69
			Spike Protein	−4.4	−44.03
Netilmicin Sulfate	Antibiotic	(Craig et al., 1983)	Spike Protein	−2.96	−50.37
			Spike protein complex with ACE2	−9.85	−86.57
			Methyltransferase	−6.86	−71.81
Hygromycin B	Antibiotic (kills bacteria, fungi and	(Kirst & Allen, 2007)	**EndoRNAse**	**−8.12**	**−94.09**
			Spike protein complex with ACE2	−10.97	−68.83

(*continued*)

Table 1A. Continued.

Name of the Compound	Drug used for the purpose	References	Therapeutic targets of SARS-CoV-2	Docking Score	MMGBSA Score
	higher eukaryotic cells)		RDRP	−9.98	−63.5
			Spike Protein	−3.71	−37.87
			Nucleocapsid	−10.1	−55.66
		Antidiabetics			
Acarbose	Antidiabetic (Type 2 Diabetes Mellitus)	(Chiasson et al., 2002)	Spike protein complex with ACE2	−11.07	−74.88
			PLprotein	−9.74	−55.2
			RDRP	−10.99	−60.78
			EndoRNAse	−10.67	−73.16
			Exoribonucleases	−10.67	−104.11
			Nucleocapsid	−14.58	−81.08
Apigenin	Anti-diabetes, amnesia and Alzheimer's disease, depression and insomnia, Anti-cancer, etc.	(Salehi et al., 2019)	3CLpro	−6.86	−49.99
			Spike protein complex with ACE2	−4.38	−34.62
			Methyltransferase	−3.98	−31.76
			Exoribonucleases	−4.07	−48.02
			Nucleocapsid	−5.37	−40.02
Hesperidin	Antihyperlipidemic, Cardioprotective, Antihypertensive, Antidiabetic a	(Zanwar et al., 2014)	3CLpro	−10.68	−91.32
			Helicase	−7.71	−67.17
			PL protein	−8.47	−52.6
			Nucleocapsid	−11.68	−84.31
Neohesperidin	Diabetes Mellitus	(Wu et al., 2017)	Spike Protein	−6.83	−65.7
			RDRP	−9.95	−87.01
			EndoRNAse	−8.69	−70.83
			Nucleocapsid	−10.84	−75.55
Troxerutin	Antidiabetic, Chronic Venous Insufficiency	(Zhang et al., 2013)	RDRP	−10.01	−72.04
			EndoRNAse	−10.31	−88.41
			Exoribonucleases	−10.75	−71
			Nucleocapsid	−13.0	−91.87
Notoginsenoside R1	Diabetic nephropathy, cardiovascular disease, cerebral vascular disease, and liver dysfunction	(Zhang et al., 2018)	RDRP	−9.73	−56.44
			EndoRNAse	−11.27	−93.95
		Cardiovascular Disease			
Salvianolic Acid B	Anti-diabetic, Angina pectoris, and other heart diseases, anti-inflammatory, antioxidant, hepatoprotective, neuroprotective, antidepressant	(Ma et al., 2019; Zhuang et al., 2012)	Helicase	−6.6	−119.88
			RDRP	−8.83	−61.9
			EndoRNAse	−10.08	−78.14
			Nucleocapsid	−11.24	−86.25

RDRP. Our findings have shown strong agreement with molecular docking studies reported by various research groups, besides, our studies have revealed the binding affinity of rutin with two more therapeutic targets including Nucleocapsid and EndoRNAse of SARS-CoV-2. In this sense, it can be predicted that the multi targeting property of rutin will eventually potentiate its synergistic antiviral effect against SARS-CoV-2.

Silymarin

Silymarin is a flavonolignan, commonly extracted from milk thistle fruits and seeds (*Silybum marianum*) (Shibano et al., 2007). It possesses widespread therapeutic applications for diseases including cancers, cardiotoxicity, nephrotoxicity, diabetes, Parkinson's disease, Alzheimer's disease, hepatic and lung disease, and prostate disease (Karimi et al., 2011; Milić et al., 2013). The anti-inflammatory effect of silymarin is mediated in several ways, including suppression of the signaling pathway of nuclear factor-kB (NF-kB) (Kang et al., 2004), cytokine production, and T cell proliferation (Gharagozloo et al., 2013). The potent antiviral activities against a wide range of viruses, including hepatitis B virus, hepatitis C virus, influenza virus, dengue virus, Chikungunya virus, and human immunodeficiency virus, have been reported (Liu et al., 2019). Recently, *in silico* studies have revealed that the compound has an inhibitory effect on S-glycoprotein and Mpro of SARS-CoV-2 (Ubani et al., 2020). Consequently, in our search of compounds having the multi-target ability for SARS-CoV-2, we have found that silymarin possesses better docking scores such as −7.82, −5.9, −5.88, −7.08, −6.14, and −10.02 for Helicase, RdRp, Spike protein, EndoRNAse, Exoribonucleases, and Nucleocapsid respectively (Table 1A) compared to the reported positive inhibitors (Table 1B). The ligand-binding patterns of the docked complexes are displayed in Figure 1B. Silymarin interacts by making hydrogen bond with the active site residues of EndoRNAse (His235, Lys290, Val292 and Ser 294), Exoribnuclease (Val91 and Asp273), nucleocapsid (chainB:Phe67, Gly70, Gln71, Asn127 and Ala135; chainD:Asn155), Spike glycoprotein (chainA:Ser494, Gly496 and Gly502; chainB:Asn437) and through Pi-cation interaction with Lys290 of EndoRNAse.

This study suggests the antiviral activity of silymarin against multiple essential viral proteins of SARS-CoV-2. It further supports the recently reviewed hypothesis of silymarin's dual targeting ability, i.e. against both essential viral proteins and host cytokine storm (Bosch-Barrera et al., 2020).

Table 1B. List of compounds used as positive control against the therapeutic targets of SARS-CoV-2.

Name of the compound used as control	References	Therapeutic targets of SARS-CoV-2	Docking Score	MMGBSA Score
TG-0205221-102285029	(Jin et al., 2020)	3CLpro	−6.918	−71.39
Tideglusib			−4.349	−50.28
Iodobananin	(Tanner et al., 2005)	Helicase	−4.877	−40.25
MLS001181552			−2.248	−38.09
Zorubicin	(Vincent et al., 2005)	Spike Protein	−5.339	−67
SSAA09E2			−2.718	−47.49
Chloroquine			−1.179	−31.2
Hydroxychloroquine	(Samarth & Kirk, 2020)	Spike protein complex with ACE2	−7.693	−67.56
Azithromycin			−6.808	−62.72
Cinacalcet	(Rimanshee et al., 2020)	PL protein	−2.007	−36.63
Biltricide			−1.572	−41.41
IDX-144	(Elfiky, 2020)	RDRP	−8.225	−63.65
Remdesivir			−5.068	−41.77
Setrobuvir			−2.85	−45.81
Idarubicin	(Chandra et al., 2020)	EndoRNAse	−4.9	−71.14
Glisoxepide			−1.79	−56.6
Raltegravir	(Khan et al., 2020)	Methyltransferase	−3.14	−39.82
Dolutegravir			−3.117	−46.74
Ritonavir	(Narayanan & Nair)	Exoribonucleases	−4.98	−67.52
Favipiravir			−4.062	−17.85
Gallocatechin	(Roh, 2012)	Nucleocapsid	−9.302	−77.23
Catechin			−8.933	−74.46

Luteolin

Luteolin is a flavone compound present as glycosides in many vegetables and fruits, including celery, pepper, thyme, and broccoli (Lopez-Lazaro, 2009). It possesses a broad range of pharmacological properties such as anticancer, neuroprotective, antioxidant, and immunomodulatory capacities (Imran et al., 2019; Park & Song, 2019; Xagorari et al., 2001). The current advancements of *in silico*, *in vitro*, and *in vivo* studies reveal that luteolin exhibit strong anti-inflammatory activities via regulation of MAPK in the AP-1 (activator protein −1) pathway, Src in the NF-κB pathway, and SOCS3 in the STAT3 (signal transducer and activator of transcription 3) pathway (Aziz et al., 2018). Because of these well-studied profiles of actions, it has been proposed that luteolin may have a therapeutic role in COVID-19 treatment by attenuating the secretion of proinflammatory cytokine and chemokines from pulmonary mast cells (Theoharides, 2020). In addition to that, luteolin has been shown to have potent antiviral activity against the Japanese encephalitis virus (Fan et al., 2016), influenza A virus (Yan et al., 2019), and HIV-1 (Mehla et al., 2011). Relevant computational docking studies on the binding affinity of luteolin against therapeutic targets of SARS-CoV-2, including 3CL protease, PLpro, RdRp, and spike proteins, have been reported (Pandey et al., 2020; Yu et al., 2020). In our SARS-CoV-2 multi-target based drug discovery approach, luteolin has shown binding affinities with 3CLpro, Helicase, Spike protein complex with ACE2, PLprotein, EndoRNAse, and Methyltransferase with better docking scores −7.50, −4.52, −5.84, −4.80, −7.18, and −3.87 respectively (Table 1A) as compared to the control molecules (Table 1B). The ligand-binding patterns of the docked complexes are displayed in Figure 1C. For example, Luteolin get binds with the active site pocket residue Thr190 of 3CLPro; Gly248 and Ser294 of EndoRNAse; D374 and Gln537 of Helicase; chainA:Asp6900, chainB:His4333 and Gly4341 of methyltransferase through strong hydrogen bonds and by making Pi-Pi skacking with Tyr343 of EndoRNAse.

Aloin

Aloin is abundantly found in the leaf exudates of various Aloe species such as Aloe vera. Aloin and its semisynthetic derivatives have been reported to possess invaluable medicinal characteristics, including anti-inflammatory (Park et al., 2011), antiproliferative (Nićiforović et al., 2007), antioxidant (Tian & Hua, 2005), and anticancer properties (Kumar et al., 2010). Nevertheless, it is an anthraquinone glycoside, and its antiviral activity has recently been documented (Huang et al., 2019). In the ongoing COVID-19 pandemic situation, the binding affinity of aloin for therapeutic targets such as transmembrane serine protease 2 (Tmprss2)(Coban, 2020), ACE2(da Silva Antonio et al., 2020), Mpro (Das & Singha Roy, 2020) is evaluated. Here, our results yielded the higher binding affinity represented by docking scores of aloin as −7.52, −6.19, −7.47, −10.25, −7.13, and −8.24 for Spike Protein, Spike protein complex with ACE2, RDRP, EndoRNAse, Methyltransferase, and Nucleocapsid respectively (Table 1A) compared to the positive controls (Table 1B).

Antibiotics against multiple therapeutic targets of SARS-CoV-2

Amikacin hydrate

Amikacin hydrate is a semisynthetic aminoglycoside antibiotic derived from Kanamycin A. It is broad specific, and effectively used in treating a wide range of infections caused by gram-negative bacteria, including pneumonia, meningitis, sepsis, intra-abdominal infections, and urinary tract infections. It is also used to treat mycobacterial, Nocardial, and neonatal infections (Ramirez & Tolmasky, 2017). It inhibits the translocation process of bacterial translation by binding to the A site of the16S RNA of the 30S ribosomal subunits (Kondo et al., 2006). In our high throughput virtual screening experiment against multiple therapeutic targets of SARS-CoV2, we have identified amikacin hydrate has higher affinities signified by docking scores −7.71, −9.28, −13.08,

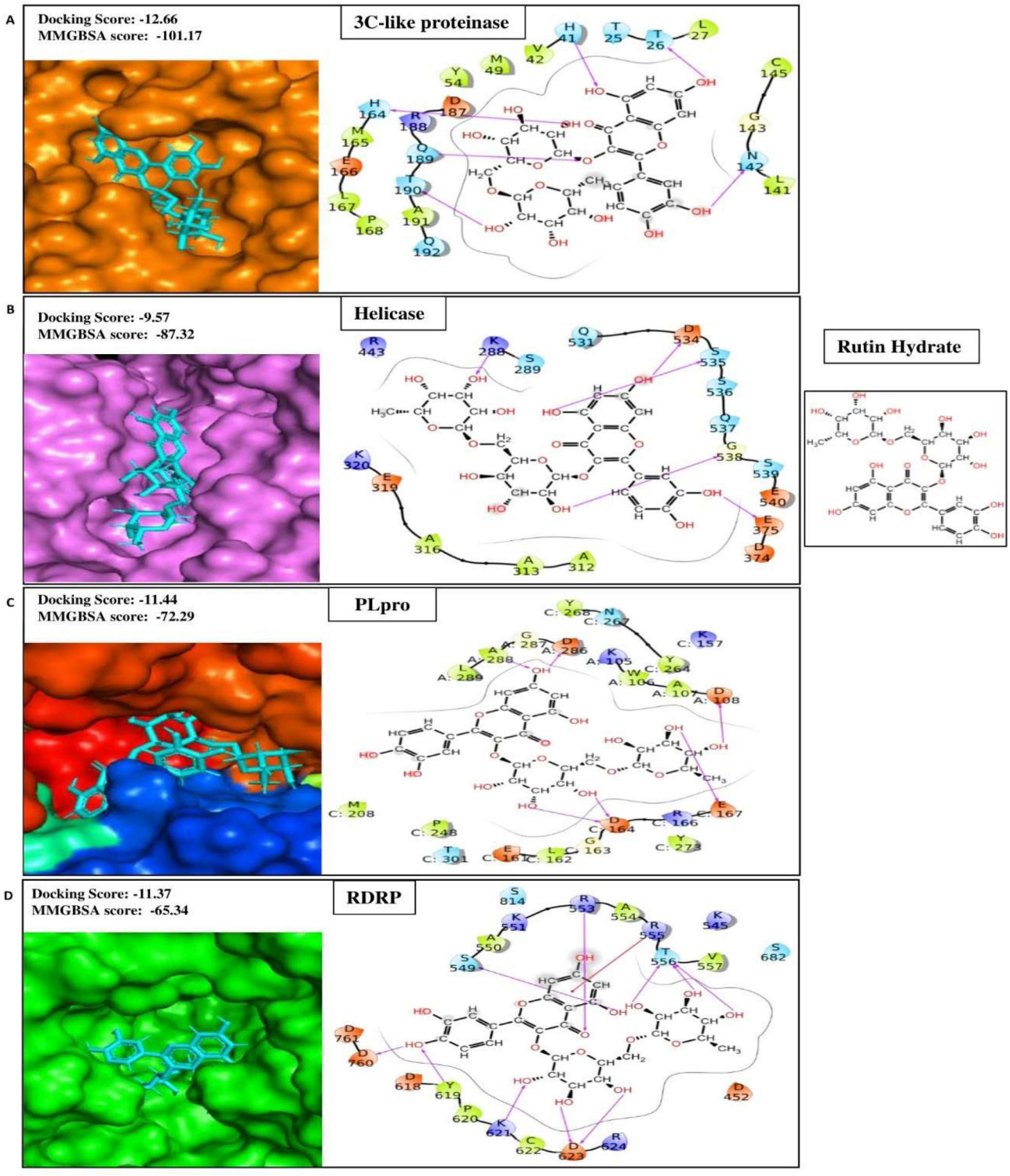

Figure 1A. Representation of protein-ligand interacting residues of compound Rutin hydrate with its best four protein targets i.e. (A) 3 C-like proteinase, (B) Helicase, (C) Papine-like proteinase (PLpro) and (D) RNA Dependent RNA Polymerase (RDRP).

−10.37, −7.73, and −9.61 for Helicase, Spike Protein, Spike protein complex with ACE2, EndoRNAse, Methyltransferase, and Exoribonucleases respectively (Table 1A) than the positive controls (Table 1B). The ligand-binding patterns of the docked complexes are displayed in Figure 1D, in which Amikacin hydrate docked in the binding pocket of target proteins. It interacts through strong hydrogen bonds with Spike-ACE2 (chainA:Glu35, Asp38, Lys68, Glu75, Gln76; chainB:Gly485), EndoRNase (His235, Asp240, Gln245 and Glu340), methyltransferase (chainA:Asp6900; chainB:Cys4330, His4333, Ile4334, Asp4335, His4336 and Gly4341), Exoribinuclease (Asp90, Glu92, Glu191 and Asp273); by making salt bridge with Spike-ACE2 (chainA:Glu35, Asp38, Glu75; chainB:Glu484), EndoRNAse (Asp240 and Glu340), methyltransferase (chianA:Asp6900;

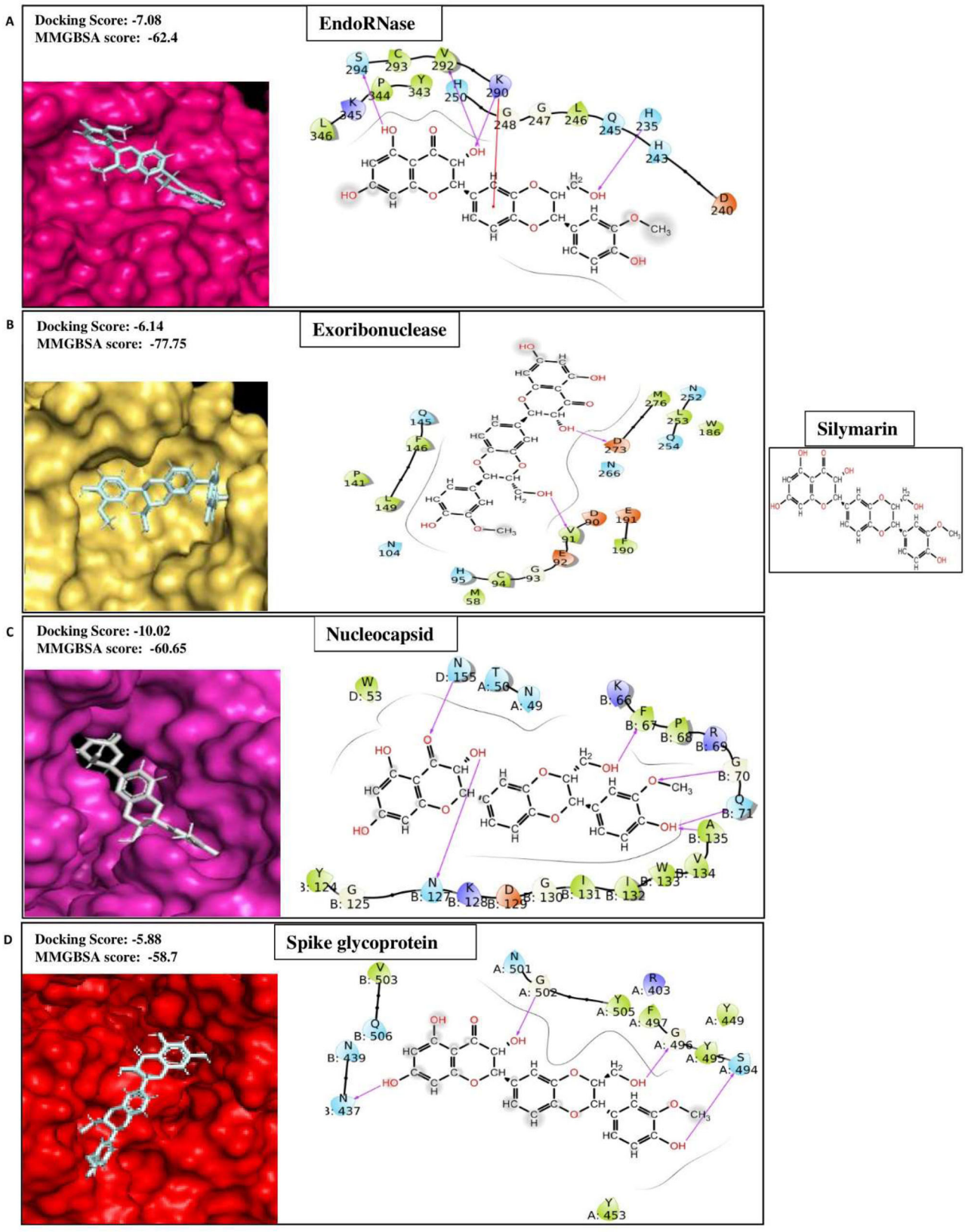

Figure 1B. Showing protein-ligand contacts between the compound Silymarin and its best four protein targets i.e. (A) EndoRNase, (B) Exoribonuclease, (C) Nucleocapsid and (D) Spike glycoprotein.

chainB:Cys4343), Exoribonuclease (Glu92 and Asp273); and through Pi-cation interaction with His243, Trp333 of EndoRNAse and chainA:Phe6901 of methyltransferase. Our results agree with the studies of Vijayan et al., 2021 and Shaankar et al., 2020 where they have shown the significant affinity of amikacin hydrate with EndoRNAse and

Methyltransferase, respectively (Shankar et al., 2020; Vijayan & Gourinath, 2021).

Geneticin

Geneticin, also known as gentamycin, is another aminoglycoside antibiotic. It induces non-functional or truncated proteins in bacteria by binding to 16 s rRNA of 30S ribosomal subunit and misreading genetic code (Beganovic et al., 2018). It is inexpensive and successfully used to treat a wide range of infections, including neonatal sepsis, gynecological infection, nasal infection, ear diseases, eye diseases, diabetic foot infections, and surgical site infection (Chen et al., 2014). Moreover, inhalational gentamycin therapy has been shown to be effective in treating lung infections such as pneumonic plague in the mouse model (Gur et al., 2018). Here, we have demonstrated that geneticin, along with its antibacterial property, retains binding affinities signified by docking scores −5.37, −7.19, −10.25, −6.91, and −4.4 for Methyltransferase, Spike protein complex with ACE2, RdRp, EndoRNAse and Spike Protein of SARS-CoV2 respectively (Table 1A). The ligand-binding patterns of the docked complexes are displayed in Figure 1E. Geneticin interacts within the binding pocket of EndoRNAse through strong hydrogen bond and salt bridge with Asp240 and Glu340. In the active site pocket of methyltransferase, geneticin binds with chianA:Asp6900, Phe6901 and chainB:His4333, Ile4334 and Asp4335 through hydrogen bond; chainA:Asp6900 and chainB:Asp4335 through salt bridges; and with chainB:His4333 by forming Pi-cation interaction. In RDRP binding site, geneticin forms hydrogen bonds with Tyr619, Lys621, Asp760, Glu811 and salt bridges with Asp761 and Glu811. Geneticin occupied inside the active site pocket of spike-ACE2 by forming hydrogen bonds with amino acids of chainA: Glu35 and Glu75, chainB: Glu484 and Gly485. Thus, our data suggest that aerosolized geneticin's inhalation will be clinically relevant in treating COVID-19 infection and associated respiratory bacterial infections.

Netilmicin sulfate

Netilmicin sulfate is another semisynthetic aminoglycoside antibiotic derived from sisomicin. It is used for the treatment of *P. aeruginosa* infections in cystic fibrosis (Prayle & Smyth, 2010), neonatal sepsis (Bacopoulou et al., 2009), and Septic Burn Patients (Munster, 1979). It has also been demonstrated that the entrapment of netilmicin sulfate in poly(DL-lactide-co-glycolide) (PLGA) hydrophobic matrix enhanced its bioavailability, reduce dosing frequency, and side effects (Kolate et al., 2015). In our molecular docking study designed to identify antibiotics with inhibitory actions against multiple therapeutic targets of SAR-CoV-2, we found that netilmicin sulfate has better docking scores such as −2.96, −9.85, and −6.86 for Spike Protein, Spike protein complex with ACE2, and Methyltransferase, respectively (Table 1A) as compared to the positive control (Table 1B).

Antidiabetic compounds against multiple therapeutic targets of SARS-CoV-2

Acarbose

Acarbose is the most commonly recommended oral drug for impaired glucose tolerance (IGT) treatment and is also useful for cardiovascular risk factors (Standl et al., 2014). Besides, it has been observed that acarbose exerted an anti-inflammatory effect in diabetic rats by inhibiting the expression of mRNA of IL-6 and TNF-α (Zhang et al., 2013). In silico studies further shows that the compound binds IL-6 and TNF-α, which could suppress the massive inflammatory response in COVID-19 patients (Xiaoqi et al., 2020). A recent study demonstrated that this compound retains a strong binding affinity for the main protease and spike protein of SARS CoV-2 (Tariq et al., 2020). Our molecular docking analysis showed that acarbose has significantly high binding affinities for six targets of SARS CoV-2 such as Nucleocapsid, Exoribonucleases, EndoRNAse, RDRP, PLprotein, and Spike protein complex with ACE2 with docking scores −14.58, −10.67, −10.67, −10.99, −9.74, and −11.07 respectively (Table 1A). The ligand-binding patterns of the docked complexes are displayed in Figure 1F, showing acarbose strongly binds with the active site pocket of each target protein. For instance, acarbose interacts through hydrogen bond with the active site pocket residues of Spike-ACE2 (chianA:Lys31, Glu35 and Gln76; chainB:Glu484, Gly485, Leu492 and Ser494); PLpro (chainA:Trp106 and Asp108; chainC:Pro248, Tyr262, Gly266 and Asn267); Exoribonuclease (Asp90, Glu92, Asn104, Ala187, Phe190, Glu191, Leu253 and Asp273); and Nucleocapsid (chainA:Thr50, Ser52, Arg89 and Tyr112; chainB:Asn127 and Lys128; chainD:Thr149, Asn151, Asn154 and Asn155).

Apigenin

Apigenin, a flavone compound, usually is available in many vegetables, fruits, nuts, and tea. The mechanism of the antidiabetic effects of apigenin is related to inhibition of pancreatic stellate cell activity, oxidative damage of pancreatic β-cells, hyperglycemia condition, and modulation of GLUT4 translocation (Vinayagam & Xu, 2015). This compound has also been reported to possess anti-inflammatory, anti-mutagenic, antioxidant, and antiviral properties (Bahare Salehi et al., 2019). The docking based molecular interaction data of different research groups have indicated that apigenin can inhibit Mpro (Khaerunnisa et al., 2020) and ACE 2 (Preeti Pandey et al., 2020) of SARS CoV-2. Our study reveals that the compound has significantly high binding affinities for five targets of SARS CoV-2, such as 3CLpro, Spike protein complex with ACE2, Methyltransferase, Exoribonucleases, and Nucleocapsid with the docking scores of −6.86, −4.38, −3.98, −4.07, and −5.37 respectively (Table 1A). The ligand-binding patterns of the docked complexes are displayed in Figure 1G. Apigenin forms hydrogen bond and pi-pi stacking interactions with the binding pocket amino acid residues of target proteins. Hydrogen bond formation ocuurs between apigenin and amino acid residue of 3CLpro (His41, Gly143, Arg188 and Gln189); Methyltransferase (chainA:Phe6901; chainB:His4333); Exoribonuclease (Val91, Glu191, Leu253,

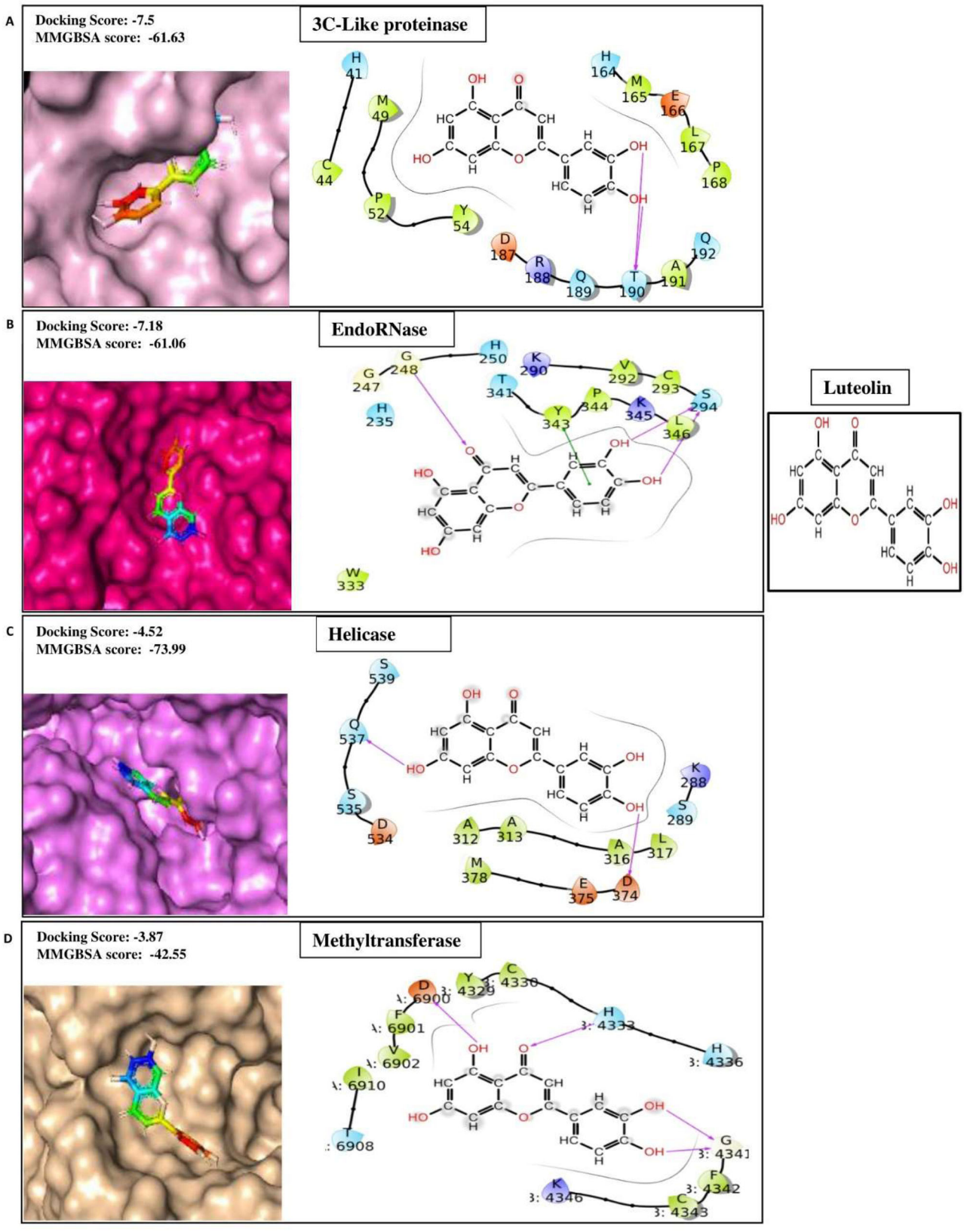

Figure 1C. 2 D-interaction diagram representing protein-ligand contacts of Luteolin with its best four targets i.e. (A)3c-like proteinase (B) EndoRNase (C) Helicase (D) Methyltransferase.

Gln254, Asn266); and Nucleocapsid (chainA:Asn49; chainB:Pro68, Asn127, Ile131). Apigenin forms pi-pi stacking interaction with the chainB:His4336 and chainB:Trp133 of Methyltransferase and Nucleocapsid respectively.

Hesperidin

The antidiabetic properties of hesperidin are documented in many *in vitro* and *in vivo* studies (Mahmoud & Hussein, 2016). This compound's antioxidant and anti-inflammatory

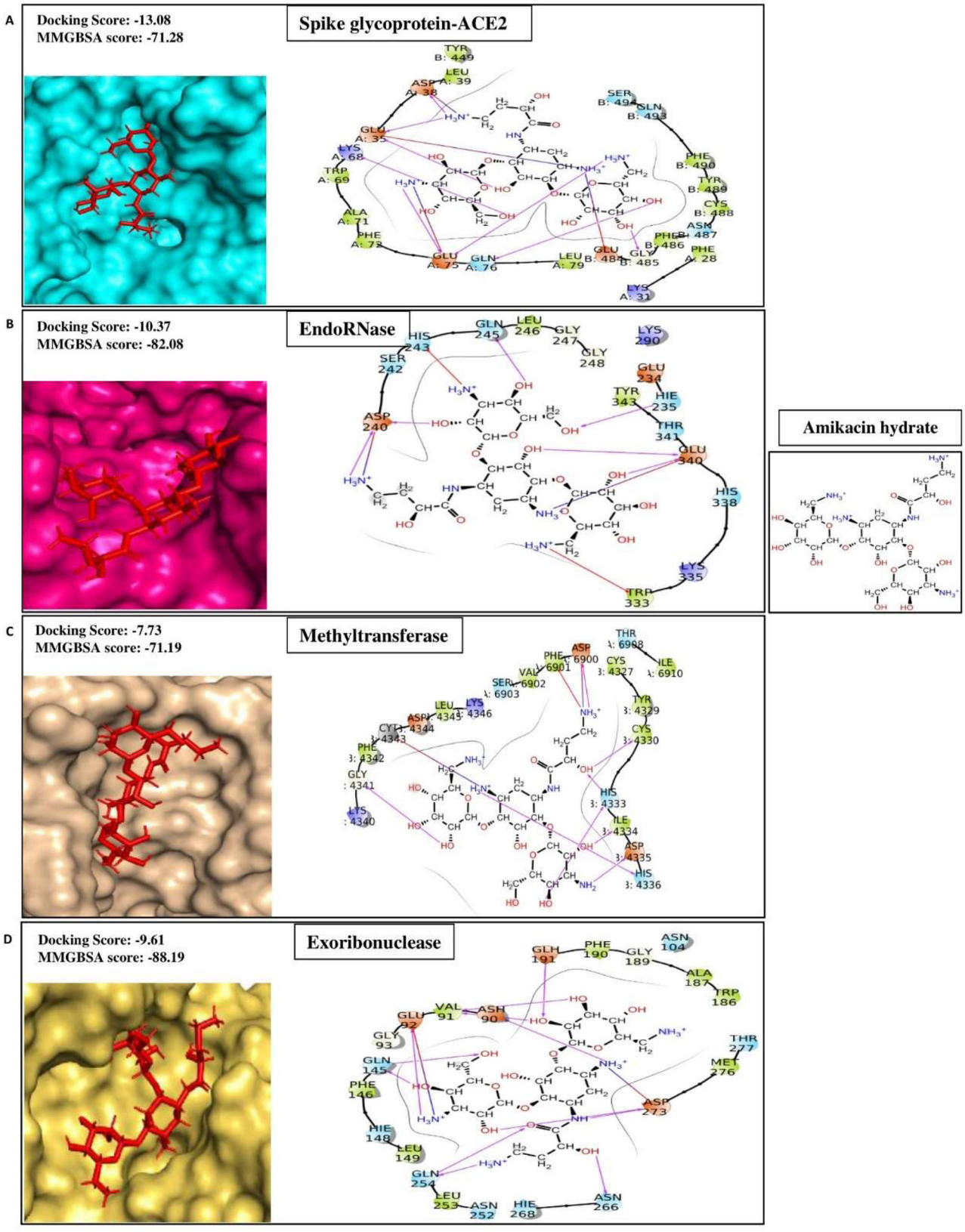

Figure 1D. Representing protein-ligand contacts of Amikacin hydrate with its best four protein targets i.e. (A) Spike glycoprotein-ACE2 (B) EndoRNase (C) Methyltransferase (D) Exoribonuclease.

activities are also documented (Barreca et al., 2020). On the other hand, virtual screening studies have shown the binding affinities of hesperidin for Mpro (Adem et al., 2020), ACE2 receptor (Cheng et al., 2020), and 3CLpro (Chen et al., 2020) of SARS CoV-2. In this context, our studies show that hesperidin has better binding affinities for the key target protein, including 3CLpro, helicase, PL protein, and nucleocapsid, with docking scores −10.68, −7.71, −8.47, and −11.68, respectively (Table 1A).

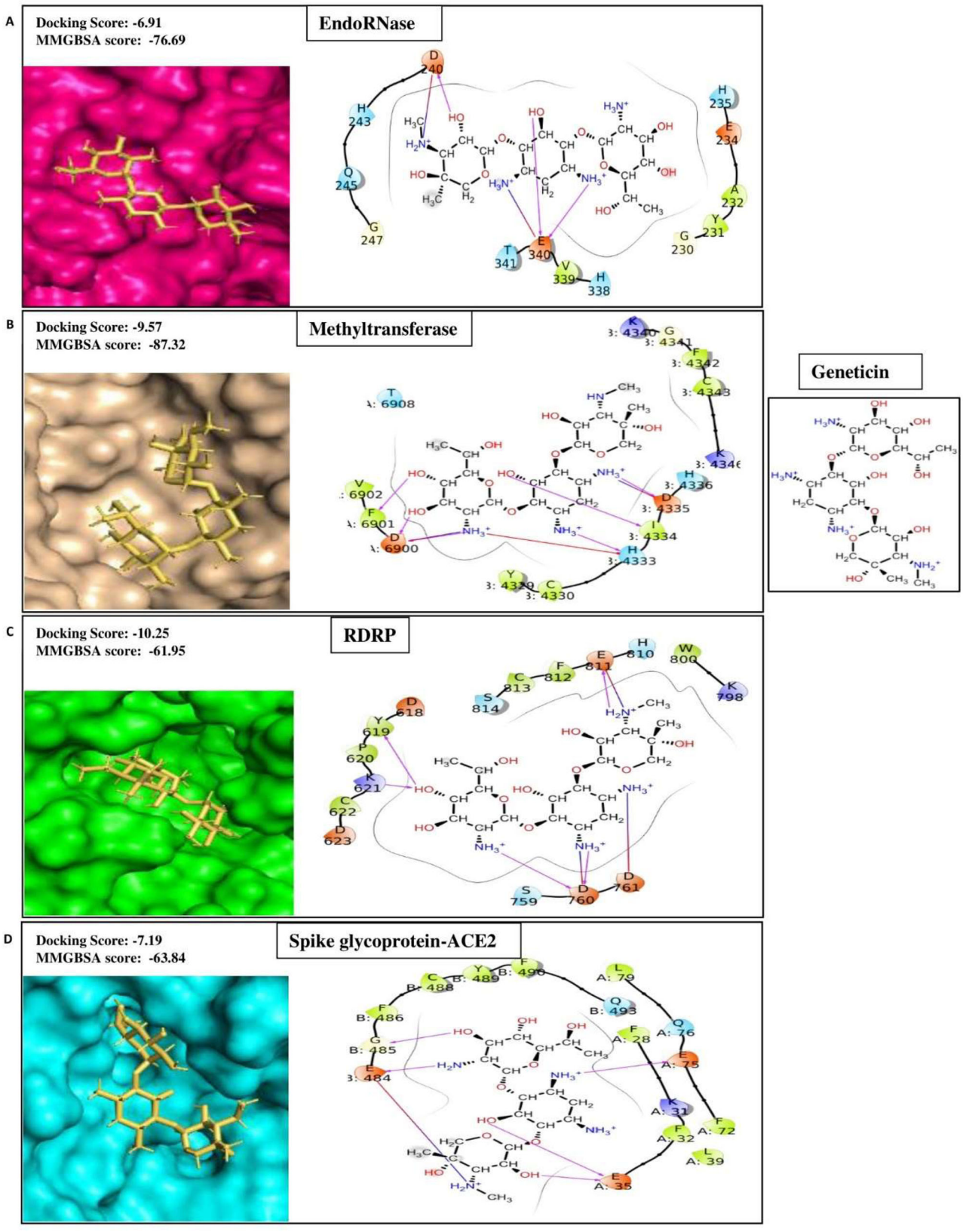

Figure 1E. Displaying protein-ligand contact residues of Geneticin with its best four protein targets i.e. (A) EndoRNase, (B) Methyltransferase, (C) RNA dependent RNA polymerase (RDRP) and (D) Spike glycoprotein-ACE2.

Neohesperidin

Neohesperidin, flavanone glycoside, exhibited hypolipidemic and antidiabetic effects in diabetic KK-A^{Y} mice (Jia et al., 2015). Furthermore, It has been shown that the compound could bind with high affinity with ACE2 (Cheng et al., 2020), Tmprss2 (Coban, 2020), and 3CLpro (Ghosh et al., 2020; Jannat et al., 2020). In our present study, the compound's strong binding affinities have been observed for four

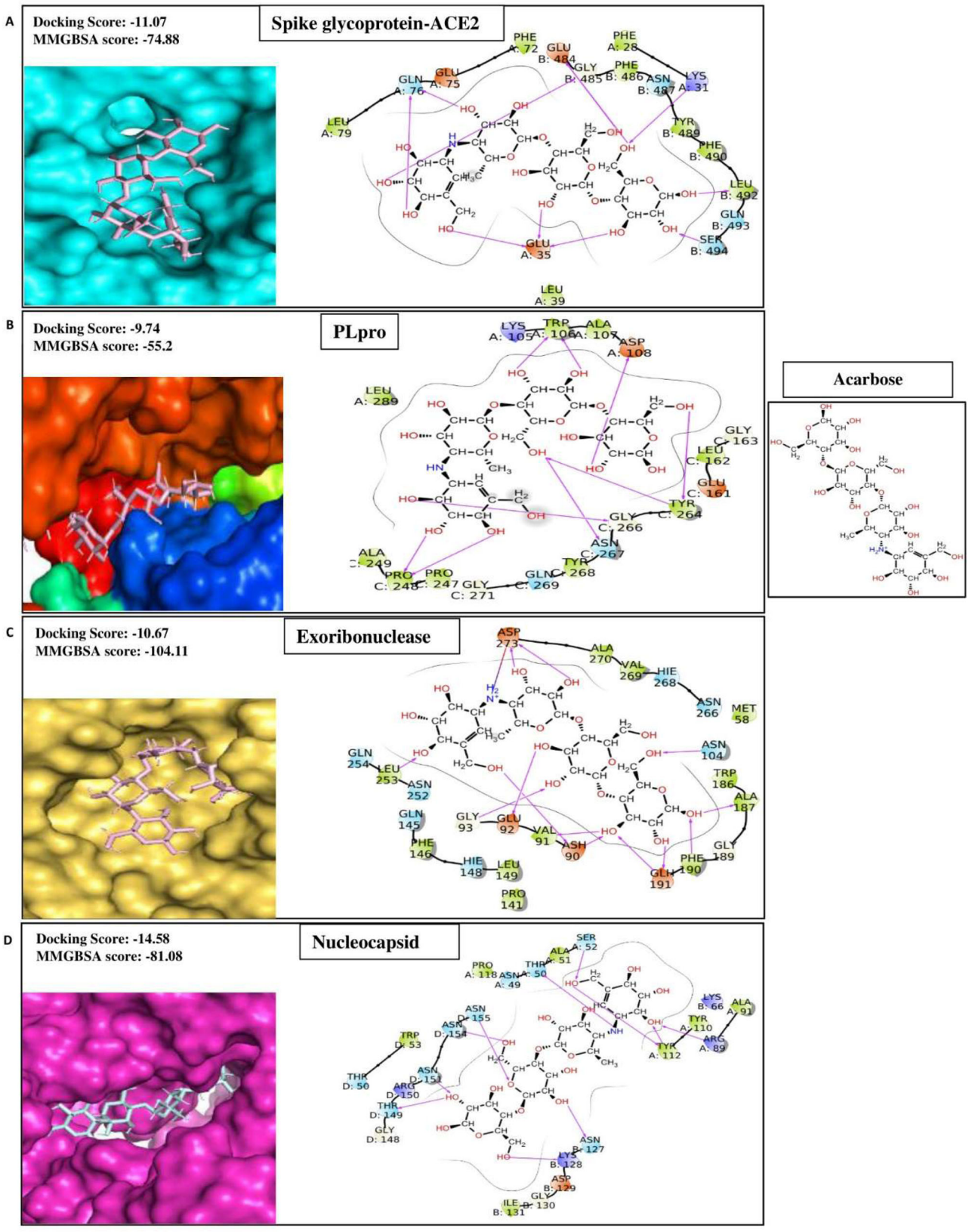

Figure 1F. 2 D-interaction diagram indicating protein-ligand contacts of acarbose with its best four targets i.e. (A) Spike glycoprotein-ACE2, (B) Papine like proteinase (PLpro), (C) Exoribonuclease and (D) Nucleocapsid.

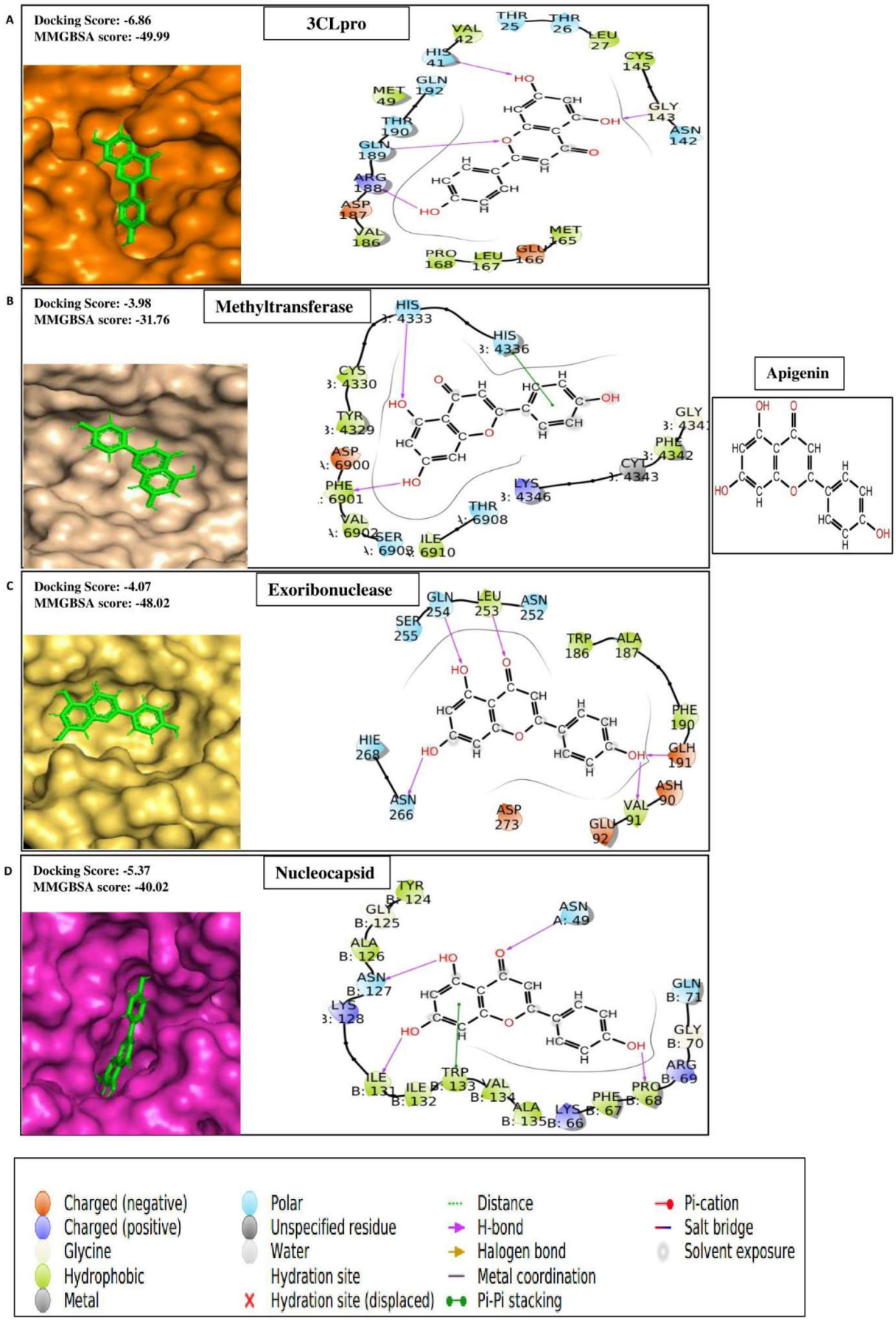

Figure 1G. Showing protein-ligand contacts between the compound apigenin and its best four protein targets i.e. (A) 3c-like proteinase (3CLpro), (B) Methyltransferase, (C) Exoribonuclease and (D) Nucleocapsid.

Table 2A. ADME properties of the selected twenty compounds.

Drug Name	QPlogPo/w (-2.5 to 6.5)	QPlogS (-6.5 to 0 .5)	QPlogHERG (below -5.0)	QPPCaco (<25%-poor,>500-great)	QPlogBB (-3 to 1.2)	QPPMDCK (<25%-poor,>500-great)	QPlogKp (-8.0 to-0.1)	QPlogKhsa (-1.5 to 1.5)
Anti-inflammatory								
Rutin Hydrate	−2.476	−2.624	−5.864	0.895	−4.915	0.251	−7.269	−1.358
Silymarin	1.672	−4.808	−5.924	26.684	−2.636	9.847	−4.911	0.009
Quercetin	0.384	−2.874	−5.088	20.005	−2.367	7.212	−5.457	−0.348
Mitoxantrone 2HCl	0.512	−1.919	−7.66	0.855	−3.088	0.292	−9.085	−0.327
Luteolin	0.963	−3.109	−5.061	41.618	−1.954	15.92	−4.86	−0.188
Aloin	−0.464	−2.896	−5.035	9.05	−3.05	3.06	−5.958	−0.652
Morin Hydrate	0.419	−2.67	−4.853	26.94	−2.158	9.949	−5.206	−0.361
Kaempferol	1.054	−3.056	−5.077	57.783	−1.805	22.699	−4.542	−0.197
Madecassoside	−2.561	−2.203	−5.001	0.586	−5.55	0.159	−7.596	−1.648
Antibiotics								
Amikacin Hydrate	−8.684	−0.237	−6.399	0.002	−5.175	0.001	−12.607	−2.555
Geneticin	−5.151	2	−7.209	0.208	−2.481	0.07	−9.781	−1.448
Netilmicin Sulfate	−3.598	2	−8.628	0.556	−2.199	0.203	−8.935	−1.097
Hygromycin B	−5.929	1.997	−6.901	0.047	−3.441	0.014	−10.945	−1.788
Antidiabetic								
Acarbose	−6.893	0.846	−4.921	0.128	−4.679	0.034	−9.797	−2.257
Apigenin	1.632	−3.341	−5.126	119.76	−1.428	49.902	−3.942	−0.035
Hesperidin	−1.437	−2.798	−5.425	4.802	−3.926	1.543	−6.139	−1.172
Neohesperidin	−1.128	−4.292	−6.533	3.283	−4.846	1.023	−6.447	−1.174
Troxerutin	−2.526	−2.119	−6.331	0.818	−6.05	0.228	−6.65	−1.939
Notoginsenoside R1	−1.195	−3.123	−5.482	1.138	−5.704	0.325	−6.847	−1.423
Cardiovascular Disease								
Salvianolic Acid B	2.25	−5.927	−4.095	0.004	−7.326	0.001	−8.372	−0.397

Table 2B. Principal drug-like properties of the selected twenty compounds.

Drug Name	MW (130-725)	SASA (300-1000)	FOSA (0-750)	FISA (7-330)	PISA (0-450)	Volume (500-2000)
Anti-inflammatory						
Rutin Hydrate	610.5	778.6	199.8	368.1	210.7	1523
Silymarin	482.4	739.7	187.4	252	300.3	1343
Quercetin	302.2	516.9	0	284.2	232.8	864.3
Mitoxatrone 2HCl	444.5	793.8	328.3	301.4	164.1	1392
Luteolin	286.2	504.4	0	250.6	253.8	844.8
Aloin	418.4	645.1	153	320.5	171.6	1174
Morin Hydrate	302.24	503.217	0	270.537	232.68	851.42
Kaempferol	286.2	501.1	0	235.6	265.5	839.9
Madecassoside	975.1	1135	680.8	445.9	8.085	2514
Antibiotics						
Amikacin Hydrate	585.6	835.1	342.7	492.4	0	1605
Geneticin	496.6	699.2	426.5	272.7	0	1363
Netilmicin Sulfate	475.6	754.9	501.5	220.4	33	1421
Hygromycin B	527.5	753.9	411.6	342.3	0	1417
Antidiabetic						
Acarbose	645.6	803	350.5	451.9	0.589	1634
Apigenin	270.2	490.7	0	202.2	288.5	819.3
Hesperidin	610.6	928.4	375.1	377.1	176.2	1701
Neohesperidin	612.6	904	351	381.5	171.5	1697
Troxerutin	742.7	1019	445.7	430.6	142.2	1967
Notoginsenoside R1	933.1	1188	765.6	415.4	7.188	2541
Cardiovascular Disease						
Salvianolic Acid B	718.6	921.7	70.32	502.6	348.8	1871

proteins, including Spike Protein, RDRP, EndoRNAse, and Nucleocapsid, with docking scores −6.83, −9.95, −8.69, and −10.84, respectively (Table 1A).

Cardio-protective compound against multiple therapeutic targets of SARS-CoV-2

Salvianolic acid B

Salvianolic acid B (SalB), the major component of *Salviae miltiorrhizae*, plays significant roles in cardio-protection in various ways, including antithrombotic and anticoagulant effects, inhibiting apoptosis of myocardial cells, inhibiting myocardial injury, and promoting cardiac angiogenesis (Jie Wang et al., 2013). In the virtual screening campaign against different drug targets of SARS CoV-2, SalB has been reported to have a significant binding affinity with 3CLpro (Contini, 2020) and Mpro (Ibrahim et al., 2020). Our result indicated that SalB has a potential binding affinity for nucleocapsid, endoRNAse, RDRP, and helicase with docking scores −11.24, −10.08, −8.83 and −6.6, respectively (Table 1A).

QikProp analysis of ADME and principal descriptor values

Lipinski's rule of five is beneficial for determining essential pharmacokinetics such as absorption, distribution, metabolism,

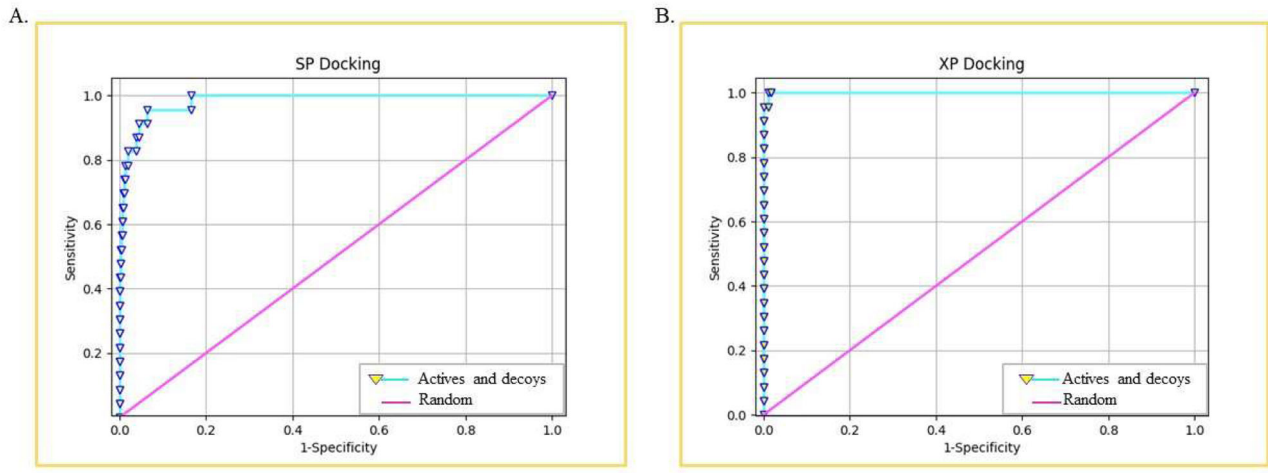

Figure 2. Protocol validation for SP and XP mode of docking through the depiction of both actives and decoys compounds by mapping the ROC curve.

Table 3. Table showing the validation of SP and XP docking based upon active counts and ROC.

Mode of docking	Percentage of results	1%	2%	5%	10%	20%	Enrichment Factors		Metric Value
SP docking	Active counts	9	14	18	21	22	ROC		0.94
	% of Actives	39.1	60.9	78.3	91.3	95.7	AUC		0.94
							BEDROC	160.9	0.858
								20.0	0.813
								8.0	0.870
XP Docking	Active counts	10	19	23	23	23	ROC		1.00
	% of Actives	43.5	82.6	100.0	100.0	100.0	AUC		0.99
							BEDROC	160.9	0.989
								20.0	0.983
								8.0	0.992

and excretion (ADME) of drug molecules (Lipinski, 2004). Therefore, ADME studies of selected 20 compounds using Schrödinger QikProp module have been performed. Among them Rutin hydrate, Silymarin, Quercetin, Luteolin, Aloin, Morin hydrate, Kaempferol and Apigenin were successfully passed through the maximum number of the physiochemical filters, including QPlogPo/w (Predicted octanol/water partition coefficient), QPlogS (Predicted aqueous solubility), QPlogHERG (Predicted IC_{50} value for the blockage of HERG K + channels), QPPCaco (Predicted apparent Caco-2 cell permeability for the gut blood barrier), QPlogBB (predicted brain/blood partition coefficient), QPPMDCK (Predicted apparent MDCK cell permeability in nm/s), QPlogKp (Predicted skin permeability) and QPlogKhsa (prediction of binding to human serum albumin) as shown in Table 2A. The drug-like properties of those compounds were further analyzed through the principal descriptor values, including Accept HB (Estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution), DonorHB (Estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution), PHOA (Percent human oral absorption), SASA (Total solvent accessible surface area), FOSA (Hydrophobic component of the SASA), FISA (Hydrophilic component of the SASA), PISA (π (carbon and attached hydrogen) component of the SASA), and MW (molecular weight of molecule). Importantly, we have found that the lead compound's scores lie within the acceptable range of most of the drug-like properties (Table 2B). Toxicity profile including AMES toxicity (prediction of mutagenicity of compounds) and hERG inhibition of selected 20 compounds were evaluated to give better insight for drug likeliness (Aishwarya et al., 2021). All the compounds shows negative value for mutagenicity (supplementary Table 12).

ROC curve plot and enrichment study

In computer-aided drug designing, the power to identify the true positive docked compounds against the false positive is a major challenge (Awuni & Mu, 2015). Therefore, a method that should have the ability to predict and identify true positive versus false positive in virtual screening mode can be used. In this study, the ROC plot and Area Under Curve (AUC) plot are used for this purpose. The SP and XP molecular docking output file was used for plotting the graphs, and it was observed that the ROC values are 0.94 and 1.00, respectively (Figure 2), revealing the specificity of docking. Further, the docking method's sensitivity and precision were validated by AUC plot, and the obtained values are 0.94 and 0.99 for SP and XP docking, respectively (Table 3). The value closer to 1 represents the higher chances of having true positive docked compounds. The BEDROCK at $\alpha = 160.9$ is calculated as 0.858 and 0.989 for SP and XP docking, respectively, which specifies the overall sound performance of XP docking (Table 3).

MD simulation of top drug-target protein complexes

Then we have carried out molecular dynamics simulation studies on the top five drug target protein complexes (RDRP-

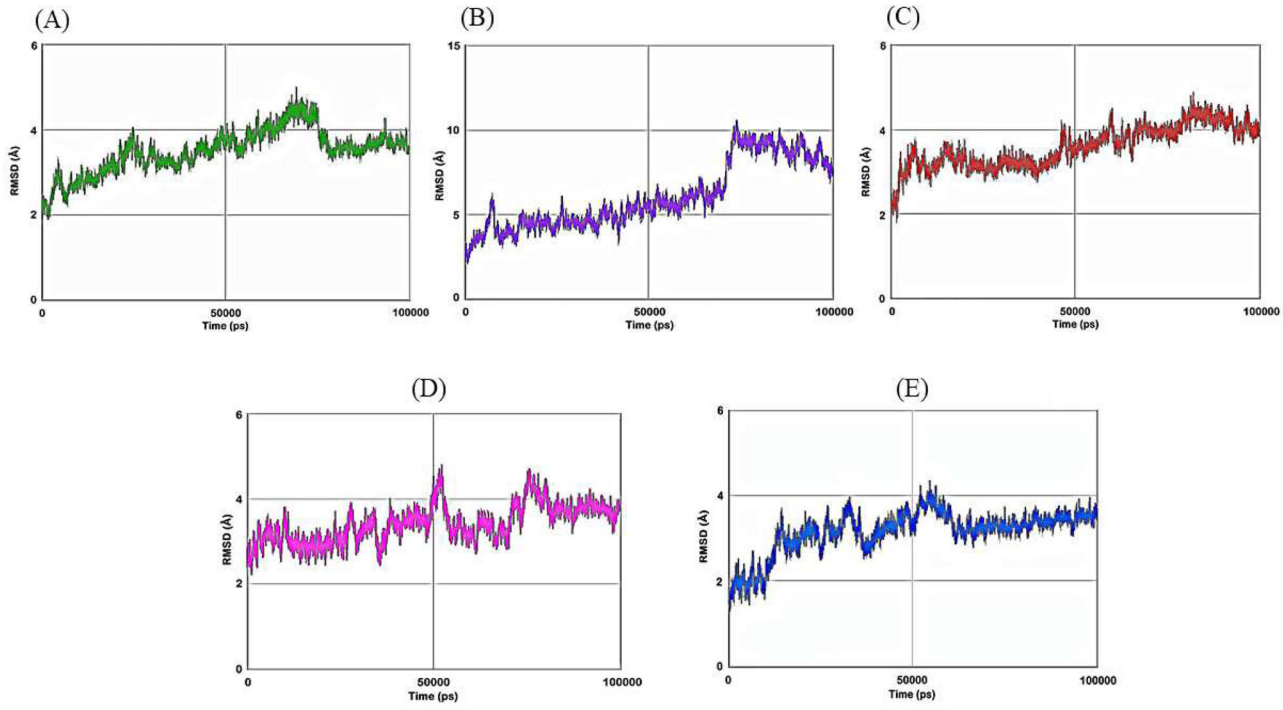

Figure 3. Plot of RMSD of Cα atoms of A) RDRP-Rutinhydrate B) Exo-Silymarin C) Helicase-Luteolin D) Methyltransferase-Amikacin hydrate E) Methyltransferase-Geneticin in angstroms as a function of simulation time in pico seconds.

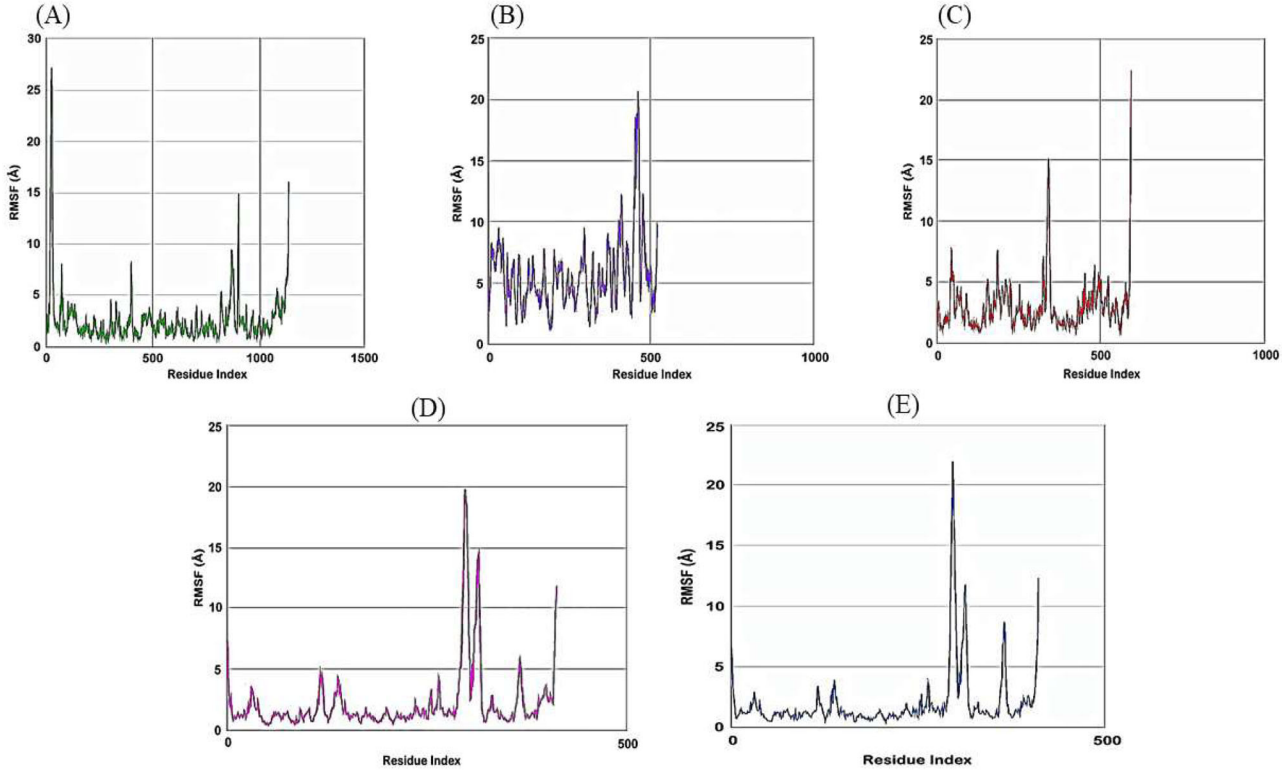

Figure 4. Plot of RMSF of Cα atoms of A) RDRP-Rutinhydrate B) Exo-Silymarin C) Helicase-Luteolin D) Methyltransferase-Amikacin hydrate E) Methyltransferase-Geneticin in angstroms as a function of Residue Index.

Rutinhydrate, Exo-Silymarin, Helicase-Luteolin, Methyltransferase-Amikacin Hydrate, and Methyltransferase-Geneticin). For the study of the top five complexes' conformational dynamics, Root Mean Square Deviation (RMSD) analysis with respect to their corresponding initial reference structure was carried out. Figure 3 shows the RMSD for Cα atoms of the five complex systems as a function of time in pico-seconds. The RMSD values for the five complex systems were observed to be in the range 2 Å to 10 Å, and the values perhaps indicate the structural stability of all the complexes. The degree of flexibility of chain in the five complexes was depicted in terms of Root Mean Square Fluctuation (RMSF) of each residue from its time – average

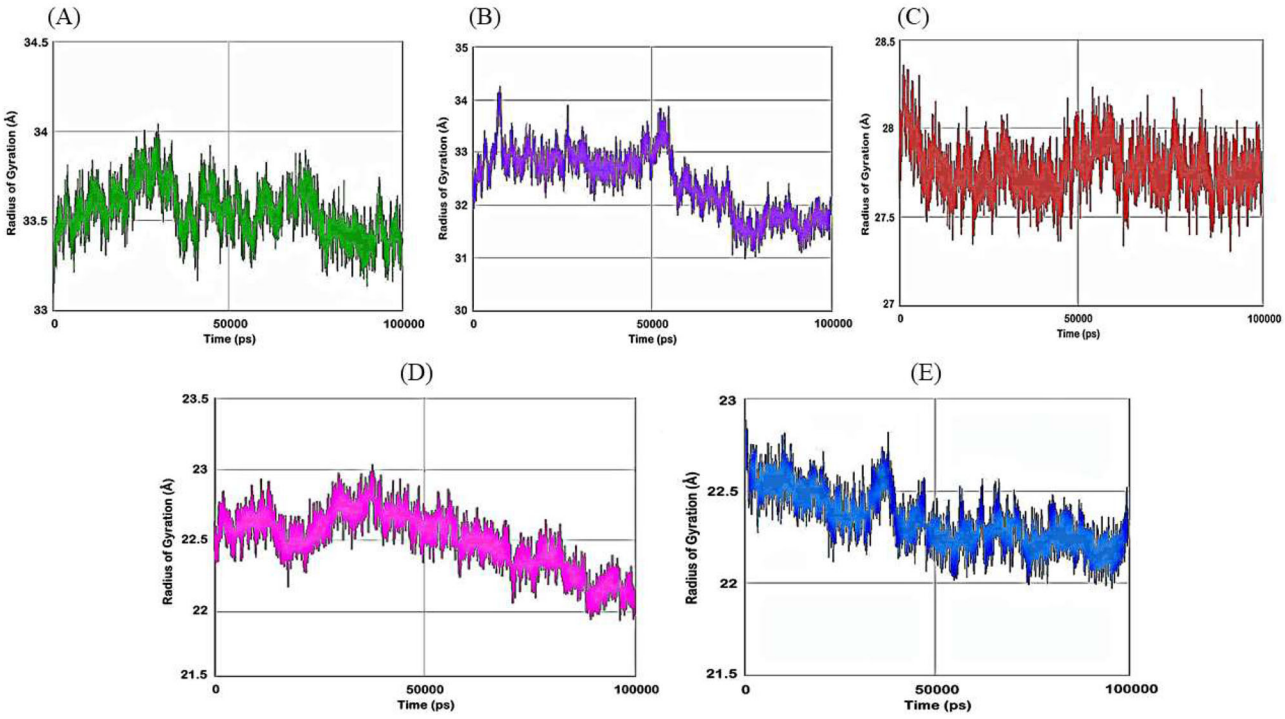

Figure 5. Plot of Radius of gyration of A) RDRP-Rutinhydrate B) Exo-Silymarin C) Helicase-Luteolin D) Methyltransferase-Amikacin hydrate E) Methyltransferase-Geneticin in angstroms as a function of simulation time in pico seconds.

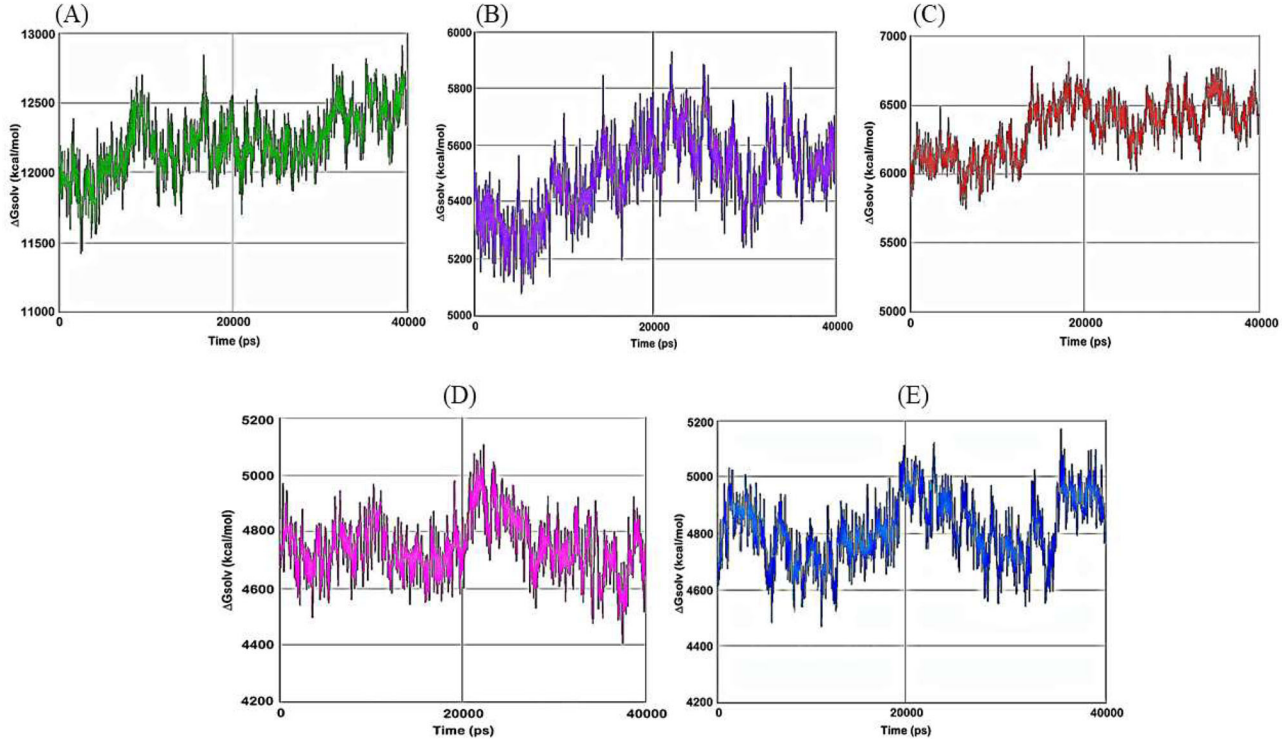

Figure 6. Plot of solvation free energy of A) RDRP-Rutinhydrate B) Exo-Silymarin C) Helicase-Luteolin D) Methyltransferase-Amikacin hydrate E) Methyltransferase-Geneticin in kcal/mol as a function of simulation time in pico seconds.

position in Figure 4. The RMSF plots show that the fluctuations were more in the Exo-Silymarin and Helicase-Luteolin complexes among the five complexes. We have also carried out the radius of gyration analysis to check the compactness of five complexes in a dynamic system (as shown in Figure 5). From Figure 5, we can infer that the five complexes' overall size remains altered, but subtle change we noticed in the Exo-Silymarin and Helicase-Luteolin complexes. In Figure 6, we have depicted the distribution of water molecules around the five complexes in terms of solvation free energy in kcal/mol. The five complexes' solvation-free energy shows different profiles because of the difference in the size of the protein molecule, which affects the protein-water interaction energy and solvent reorganization energy. We have also analyzed the different

intermolecular interactions between the protein and the ligand in the five complexes. The intermolecular interactions observed in the five complexes have been summarized in supplementary Tables 2–6. We see that the ligands display an appropriate number of hydrogen bonds in the dynamic system and are correlated with the molecular docking results from the intermolecular interactions. MMGBSA thermodynamics calculation for every 10000ps intervals was estimated for each protein-ligand complex during the course of simulation and standard deviations of binding free energy were tabulated in supplementary Tables 7–11.

Discussion

The global COVID-19 load has mounted to 53,164,803 confirmed cases and 1,300,576 deaths as of November 14, 2020. To break the rising trend of COVID-19 and related detrimental effects, drug repurposing therapeutic strategies have widely opted on a health emergency basis (Drożdżal et al., 2020). The unique advantages of using repurposed drugs in treating human diseases, including cancer, have already been well documented (Pushpakom et al., 2019). Numerous *in silico* incisive investigations of FDA approved drugs against the druggable targets of SARS-CoV-2 have been undertaken to boost the COVID-19 treatment strategies. Conventionally, compounds are selected to act on a single drug target with great potency. These compounds may turn to be unsuccessful in the long run, as the virus is evolving with new mutations. COVID-19 treatment difficulties are also deeply rooted in its complex association with uncontrolled immune response, secondary bacterial infection, diabetes, and cardiovascular diseases. The development of new inhibitors with known targets for different pathways of COVID-19 associated diseases and acting multiple targets of SARS CoV-2 will bring a paradigm change for COVID-19 therapy. Moreover, these polypharmacological compounds can avoid the possible adverse synergistic effects of drug-drug interactions during the treatment of COVID-19 and accompanying diseases.

Therefore, we investigated the capacity of compounds from FDA approved drug library, and FDA approved and passed phase −1 drug library towards polypharmacological drug identification through high throughput virtual screening. Our molecular docking studies assess how the two libraries' compounds can inhibit multiple SARS-CoV-2 essential proteins or host proteins that interact with the virus component to stop the virus's entry and proliferation. We found nine anti-inflammatory compounds such as Rutin Hydrate, Silymarin, Luteolin, Aloin, Madecassoside, Mitoxantrone 2Hcl, Kaempferol, Quercetin, and Morin hydrate; four antibacterial such as Amikacin Hydrate, Geneticin, Netilmicin Sulfate, and Hygromycin B; six antidiabetic compounds such as Hesperidin, Apigenin, Acarbose, Notoginsenoside R1, Neohesperidin, and troxerutin; and one cardioprotective compound such as Salvianolic Acid B, that could effectively interact with multiple therapeutic targets of SARS CoV-2. Some of these compounds are extensively discussed in the result section. For further validation of ligand-protein interactions, molecular dynamic studies for five compounds, including Rutin Hydrate, Silymarin, Luteolin, Amikacin Hydrate, and Geneticin, have also been performed.

As the two libraries have been thoroughly investigated for COVID-19 therapy, we found that these compounds' binding affinities with different therapeutic targets of SARS CoV-2 have been reported in many studies. However, our screening approach against ten validated drug targets of SARS CoV-2 together rather than individual targets provides us a unique opportunity to discover multi-target inhibitors. Remarkably, in many cases, our data agree with the earlier *in silico* studies of many computational groups. In some cases, we observe the differences in selecting best-docked molecules with earlier studies due to the difference in reference molecules used by various groups in their docking analysis.

Our studies are limited with a lack of experimental evidence that the compounds identified here can directly inhibit their target proteins or/and inhibit SARS-CoV-2 proliferation in patients. However, as per the list of clinical trials (clinicaltrial.gov website) on COVID-19 treatment to date, five of our best-docked compounds, including Quercetin (NCT04468139, phase-4), Silymarin (NCT04394208, phase-3), and Morin hydrate (NCT04348656, phase-3), Luteolin (NCT04404218, phase-2), and Apigenin (NCT04404218, phase-2) are in different phases of clinical studies. Hence, it is reasonable to assume that the best-scored compounds obtained by the present docking analysis may also retain the therapeutic capacity for COVID-19 treatment. Researchers can also focus prospectively on research and clinical trials of the remaining 15 best-docked molecules for developing multi-target drugs against the COVID-19 pandemic.

Finally, this drug repositioning method using the *in silico* screening method can be a novel computational strategy to explore single, multi-targeting, and multi-purposing therapeutic molecules. These drugs can treat COVID-19 and frequently associated co-morbid disorders such as uncontrolled immune response, secondary bacterial infection, diabetes, and cardiovascular diseases and can overcome the limitations of probable patient compliance out of drug interactions associated with combination therapy.

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Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Venkata Satish Kumar Mattaparthi http://orcid.org/0000-0002-5238-753X
Pundarikaksha Das http://orcid.org/0000-0002-0720-3578
Vijay Kumar Prajapati http://orcid.org/0000-0001-6510-0596
Dhaneswar Prusty http://orcid.org/0000-0003-0788-5473

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The authors have declared no competing interest.

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