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Anti-HIV-drug and phyto-flavonoid combination against SARS-CoV-2: a molecular docking-simulation base assessment

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ABSTRACT

At the health emergence, no such potent prophylactic therapy is available to control the deadly emerged Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). However, existing antiviral, anti-inflammatory, antimalarial drugs is the only option against SARS-CoV-2, but it may be harmful to patients without more clinical evidence. As an alternative solution, we proposed a newer hypothesis using the selective 10 potent anti-HIV drugs and flavonoid class of phytochemicals from previous reports to use in combination against SARS-CoV-2. Primarily, 10 anti-HIV protease inhibitor drugs and 10 phyto-flavonoids as ligands in molecular docking study against the putative target, the SARS-CoV-2-main protease (M^{pro}) ID: 6Y2E), as an essential enzyme in viral genome replication. According to molecular docking and drug-ability scores of each ligand, the anti-HIV drug, the darunavir (with a docking score, −10.25 kcal/mol and drug-likeness rating, 0.60) and the quercetin-3-rhamnoside (with a docking score, −10.90 kcal/mol and drug-likeness rating, 0.82) were selected for further analysis in combined effect. Perceptibly, the combined 'anti-HIV drug and phyto-flavonoid' docking complex has actively interacted with eight strong H-bonds with stability, briefly elucidated through RMRD-, RMSF-Rg-plots and MM/PBSA-binding energy calculation during 100 ns than the individual against SARS-CoV-2-M^{pro}. Thus, the 'anti-HIV-drug-phyto-flavonoid' combination therapy could be used against SARS-CoV-2 after some experimental validation.

Abbreviations: ADME/T: absorption, distribution, metabolism, excretion/toxicity; CADD: computer-aided drug development; COVID-19: coronavirus-2019 disease; DCGI: drug controller general of India; FDA: food and drug administration; GROMACS: Groningen machine for chemical simulations; HIV: human Immunodeficiency virus; mAb: monoclonal antibody; MD: molecular dynamics; MERS: middle east respiratory syndrome; MM/PBSA: molecular mechanics/Poisson-Boltzmann surface area; NK: natural killer; NO: nitric oxide; NPT: number of particles, pressure, temperature; NVT: number of particles, volume and temperature; PDB: protein data bank; Rg: radius of gyration, RMSD, root mean square deviation; RMSF: root mean square fluctuation; (+) ssRNA: positive-sense single-strand RNA; SARS-CoV-2: severe acute respiratory syndrome coronavirus-2; SMILES: simplified molecular-input line-entry system; TMC: traditional Chinese medicine; WHO: world health organization.

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1. Introduction

The pandemic of coronavirus-2019 (COVID-19) disease from the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) has created a health emergency from the end of 2019 (Munster et al., 2020; Wang, Horby et al., 2020a; WHO, 2020). The outbreak and quick transmission of the COVID-19 is close to the severe pandemic like Spanish flu in 1918–1919. However, > 78–85% of SARS-CoV-2 infected patients were found asymptomatic (Pan et al., 2020). On the other hand, the discriminating graph of infection, genomic diversity and mortality cases from different geographical areas/communities are still unclear. As per the source, severe pneumonia and multiorgan dysfunction have been associated with a higher mortality rate from SARS-CoV-2 in different age groups (Huang et al., 2020; Islam, Ahmed et al., 2020a; Lin et al., 2020). With the unavailability of any recommended-cum-potential drug or vaccine against the deadly disease, a considerable challenge is going on for every nation-state and the World Health Organization (WHO) to control the global health system from the unknown viral infection (WHO-Health systems respond to COVID-19, 2020).

According to current reports from the WHO, several existing drugs have been approved for human trials and in parallel several vaccine candidates also got permission for human

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testing (Caly et al., 2020; Sanders et al., 2020; Thanh Le et al., 2020). However, fighting against the aggressive positive-sense single-strand RNA or (+) ssRNA viral infection without any effective drug/vaccine has created the most unexpected global health crisis in the 21st century (Caly et al., 2020; Sanders et al., 2020). Physicians are still using several alternative combinations of existing antiviral, antiparasitic and anti-inflammatory drugs on a hit-and-trial basis to tackle the situation (Ahn et al., 2020; Iyer et al., 2020). Mainly, existing Food and Drug Administration (FDA) approved antiviral drugs, baloxavir marboxil, darunavir, favipiravir, lopinavir, oseltamivir, remdesivir, ritonavir, etc., several immune-modulatory, antiinflammatory medications, fingolimod, sarilumab, tocilizumab, etc., and antiparasitic drug, chloroquine, hydroxychloroquine, ivermectin are using individually and/or combination against SARS-CoV-2 in repurposing approach (Ahn et al., 2020; Gautret et al., 2020; Iyer et al., 2020). Fortunately, applied drug combinations seem good towards controlling SARS-CoV-2 from several clinical reports. As a result, several promising anti-COVID drug and vaccine candidates got the approval from Drug Controller General of India (DCGI) for more human testing, manufacturing and marketing. However, the effectivity is still unclear due to discriminate results according to geographical region and the patient to patient variation (Ahn et al., 2020; Gautret et al., 2020; Sanders et al., 2020).

From the vaccination point of view, among all proposed >140 vaccine candidates, only 10–12 candidates showed promising results at the final stage of the clinical validation stage. However, as per the expert source, it needs a minimum of another 5–6 months for final reports, manufacture and marketing against SARS-CoV-2. Simultaneously, several alternative non-targeting treatment therapies and regimens such as nitric oxide (NO) inhalation, human natural killer (NK) cells, or innate lymphocytes, monoclonal antibody (mAb)-based formulation and several active traditional regimens are under clinical validation according to the FDA guidelines (Lu, Hwang et al., 2020a; Swain, Panda et al., 2020; Zhang et al., 2020). Therefore, we cannot switch-off the drug combinations ordinarily until the development of any substituted-recommended vaccine candidates. However, the nonstandardized recommended drug combinations without any proper clinical evidence create severe side effects unknowingly (FitzGerald, 2020; Harrison, 2020). Thus, the development of well-tolerated treatment therapies with lesser side effects is the call of the day.

As an alternative solution, using these screened-out antioxidants, anti-inflammatory and antiviral phytochemicals, individually or in combination with any anti-viral/anti-HIV (Human Immunodeficiency Virus) medicines expected to be a prudent option to combat the SARS-CoV-2 with diminishing post-treatment side effects. From Indian ancient Ayurvedic and Traditional Chinese Medicine (TMC), several natural products/regimens have also been active against viral diseases (Luo et al., 2020; Rastogi et al., 2020; Rege and Chowdhary, 2013; Swain, Panda et al., 2020; Yang et al., 2020). The SARS-CoV-2 activity of such secondary metabolites has been reported continuously from several computational and experimental studies (Aanouz et al., 2020; Islam, Parves et al., 2020b; Ul Qamar et al., 2020; Wang, Horby et al., 2020a). The polyphenol or flavonoid class of phytochemicals is well verified and reported to have potential immune-stimulant and antiviral properties (Choi et al., 2012; Huang et al., 2014; Lalani and Poh, 2020; Pasetto et al., 2014). In parallel, some TMC regimens have already been tested and several formulations are also under clinical validation for FDA approval after a positive response at primary assessment against SARS-CoV-2 (Gentile et al., 2020; Jin et al., 2020).

The present work hypothesized smartly with selective anti-HIV drugs and phyto-flavonoids to combat SARS-CoV-2 with an iron hand. As per the hypothesis, an advanced, cost-effective and time-saving molecular-docking-simulation approach was used to evaluate the proposed combination's potency, toxicity and drug-ability. Accordingly, the crystallographic structure of the main protease (M^{pro}, also known as $3CL^{pro}$) was used as a drug target during the anti-SARS-CoV-2 activity validation. The viral protease is a prudent drug target, as the M^{pro} plays a key enzyme for viral genome replication (Gentile et al., 2020; Jin et al., 2020; Khan et al., 2020; Mohammad et al., 2020; Ul Qamar et al., 2020). Thus, this might be a novel idea in current anti-CoV drug development modules by utilizing the potent natural products along with existing anti-HIV drugs feasibly and cost-effectively in computational-intelligence platforms.

2. Materials and methods

2.1. Preparation of ligand and target structure

Based on previous ethnomedicinal evidence, scientific literature and drug bank information, we have selected 10 FDA approved anti-HIV protease inhibitor drugs and 10 flavonoid class of phytochemicals for drug-combination approach against SARS-CoV-2-M^{pro} (Supporting information Figure S1). The anti-HIV drugs, amprenavir, ASC09 (TMC310911), atazanavir, darunavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, tipranavir and the phyto-flavonoid, apigenin, catechin, di-hydroquercetin, epi-gallocatechin gallate, hesperidin, hesperidin, LPRP-Et-97543, quercetin, quercetin-3-rhamnoside and rutin were retrieved as ligands from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Further, the three-dimensional crystal structure of the SARS-CoV-2-M^{pro} enzyme contains 306 amino acids from protein data bank (PDB) with an ID: 6Y2E, was retrieved for use as a target in the docking-simulation study. Concomitantly, the structure- and sequence-level analysis was carried out between the SARS-CoV-2-M^{pro} with the reported crystal structure of SARS-CoV-M^{pro} (PDB ID: 2DUC) using tools, Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) and the protein fold recognition server, Phyre2 (http://www.sbg.bio.ic.ac.uk/phyre2/).

2.2. Molecular docking study

We have used AutoDock 4.1 for molecular docking studies and the BIOVIA Discovery Studio (BIOVIA DSB v4.5) for

target–ligand interaction visualization study. Saved the retrieved target structure, SARS-CoV-2-M^{pro} and selected ligands in dot (.) pdb format before single- and double-blind docking study (Aanouz et al., 2020; Mishra et al., 2019; Swain et al., 2018; Swain, Paidesetty et al. 2020). Then, the energy minimized structure of the SARS-CoV-2-M^{pro} was produced through simulation at 100 nanoseconds (ns) and cleaned the geometry of each ligand to get reliability-cum-stability of the docking complexes. A default AutoDock parameter as Kollman charges-polar hydrogen bonds for the target protein and Geister partial charges-polar hydrogen for ligands were added before the docking study. Then, followed blind docking study for single and double docking with selected phyto-flavonoids and anti-HIV drugs (Swain et al., 2018; Swain, Paidesetty et al. 2020). Out of generated 10 interaction poses for each ligand, the most effective pose was selected based on its binding energy/docking score (Aanouz et al., 2020; Mishra et al., 2019; Swain, Paidesetty et al., 2020). Notably, the inter-changeable double docking study between most potential phyto-flavonoids, quercetin-3-rhamnoside and LPRP-Et-97543 were re-docked against SARS-CoV-2-M^{pro}-darunavir and SARS-CoV-2-M^{pro}-tipranavir complexes, individually. Additionally, a ligand–ligand docking study was performed between darunavir-quercetin-3-rhamnoside, darunavir-LPRP-Et-97543, tipranavir-quercetin-3-rhamnoside and tipranavir-LPRP-Et-97543 (Aanouz et al., 2020; Mishra et al., 2019; Swain, Paidesetty et al., 2020). Finally, the most effective protein with double ligand docking complexes was selected for a molecular dynamic (MD) simulation study to explore the solidity of protein–ligand binding.

2.3. Molecular dynamic simulation study

The native/apo-enzyme SARS-CoV-2-M^{pro} structure before docking, two single docking and double ligand docking complexes were selected for MD-simulation with the software GROMACS 5.1.4 (Groningen Machine for Chemical Simulations) in the GROMOS force field for 100 ns (Aanouz et al., 2020; Swain, Paidesetty et al., 2020). Parameters and topologies files for each ligand were generated using the PRODRG server (http://prodrg1.dyndns.org/submit.html). Native protein and selected three most effective docking complexes with the SPC-E water-cubic box model (whose volume was about 976.40, 976.50, 977.48, 977.48 nm^3, respectively) were simulated. A total of 30,576, 30,564, 30,573 and 30,563 numbers of solvent molecules were added to each system from native protein structure to double docking complexes. Neutralized the system by adding four Na + ions and the energy minimization using 50,000 steepest descent steps, was carried out for each docking complex. After minimizing the system, the NVT (number of particles, volume and temperature) and NPT (number of particles, pressure, temperature) equilibrations were performed for equilibrating the system for the 100 ps time scale for each. The final MD step was performed for the protein system for a 100 ns time scale (Aanouz et al., 2020; Swain, Paidesetty et al., 2020).

2.4. MM/PBSA binding free energy calculation

The binding free energy calculation is another advanced computational analysis to understand the contribution of each amino acid's binding energy and dynamic behaviors in the individual protein–ligand complex (Wang, 2020; Aanouz et al., 2020; Swain et al., 2018; Wang et al., 2019). The Molecular Mechanics/Poisson-Boltzmann Surface Area (MM/PBSA) based binding free energy (ΔG_{bind}) of each simulated docking complexes was calculated with the g_mmpbsav5.12 package in the GROMACS platform (Swain et al., 2018; Swain, Paidesetty et al. 2020). The Gibb's binding free energy (ΔG_{bind}) was calculated using the following equations:

$$\begin{aligned}\Delta G_{bind} &= G_{complex} - (G_{protein} - G_{ligand}) \\ &= \Delta E_{MM} + \Delta G_{sol} - T\Delta S \end{aligned} \quad (1)$$

$$\Delta E_{MM} = \Delta E_{bonded} + \Delta E_{nonbonded} = \Delta E_{bonded} + (\Delta E_{vdw} + \Delta E_{elec}) \quad (2)$$

$$\Delta G_{sol} = \Delta G_{polar} + \Delta G_{nonpolar} \quad (3)$$

where ΔG is the free energy and $\Delta G_{complex}$, $\Delta G_{protein}$ and ΔG_{ligand} define the total free energy of an individual and the protein–ligand docking complexes in the solvent. $T\Delta S$ is the entropic contribution to the free energy in a vacuum where T and S represent the temperature and entropy. ΔG_{solv} is the free energy of solvation, where G_{polar} and $G_{nonpolar}$ are the electrostatic and nonelectrostatic contributions to the solvation free energy. ΔE_{MM} is the vacuum potential energy of both bonded as well as nonbonded interactions.

2.5. Possible toxicity and drug-ability prediction

The possible toxicity profiles of anti-HIV drugs and phyto-flavonoids using the generated simplified molecular-input line-entry system (SMILES) code for each chemical structure were investigated (Swain et al., 2017; Swain, Paidesetty et al. 2020). The toxicity profiles include hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity, toxicity class and possible lethal dose (LD_{50}) value using the tool, ProTox (http://tox.charite.de/protox_II/) was recorded for each candidate. Furthermore, the overall drug-likeness or possible drug-ability score for each ligand/candidate was measured using the tool, Molsoft (http://molsoft.com/mprop/).

2.6. Possible pharmacokinetics profiles prediction

In addition to pharmacokinetics profile also play a crucial role in drug dose recommendation at a late clinical trial stage. Thus, the possible pharmacokinetic profiles such as Absorption, Distribution, Metabolism, Excretion and Toxicity (ADME/T) for all anti-HIV drugs and phyto-flavonoids were recorded using the tool, SwissADME (http://www.swissadme.ch/).

Table 1. Docking scores against SARS-CoV-2-M^{pro} (PDB ID: 6Y2E) and predicted drug-likeness, lethal doses and bioavailability scores of individual anti-HIV drugs.

Anti-HIV protease inhibitor drug	Docking score (kcal/mol)	Drug likeness	Lethal dose (kg/mg)	Bioavailability score
Amprenavir	−8.43	1.14	300	0.55
ASC09/ TMC-310911	−9.56	1.09	150	0.17
Atazanavir	−7.37	0.11	200	0.17
Darunavir	−10.25	0.60	245	0.55
Indinavir	−7.80	1.86	5000	0.55
Lopinavir	−9.00	1.10	5000	0.55
Nelfinavir	−9.97	1.41	600	0.55
Ritonavir	−8.75	0.11	1000	0.17
Saquinavir	−8.97	0.69	500	0.17
Tipranavir	−10.14	0.72	333	0.56

Table 2. Docking scores against SARS-CoV-2-M^{pro} (PDB ID: 6Y2E) and predicted drug-likeness, lethal doses and bioavailability scores of individual phyto-flavonoids.

Flavonoid class phytochemical	Docking score (kcal/ mol)	Drug likeness	Lethal dose (kg/mg)	Bioavailability score
Apigenin	−8.28	0.39	2500	0.55
Catechin	−8.86	0.64	10000	0.55
Dihydroquercetin	−7.98	0.50	159	0.55
Epigallocatechingallate	−7.69	0.23	1000	0.17
Hesperidin	−8.48	0.94	12000	0.17
Kaempferol	−7.91	0.50	3919	0.55
LPRP-Et-97543	−10.11	0.82	2000	0.55
Quercetin	−8.33	0.52	159	0.55
Quercetin-3-rhamnoside	−10.90	0.82	2300	0.17
Rutin	−5.48	0.91	5000	0.17

3. Results and discussion

3.1. Preparation of ligand and target structure

The physicochemical properties such as molecular weight (g/mol), number of H-bond acceptors, H-bond donors, octanol/water partition coefficient (XlogP), topological polar surface area (tPSA in Å), molar refractivity (mol/m^3), number of rotatable bonds of all anti-HIV drugs and phyto-flavonoids with PubChem IDs were recorded (Supporting information Tables S1 and S2). In current drug development modules, cited physicochemical parameters are collectively known as the Lipinski rule five (RO5), a standard practice to select the possible drug-able candidate in the early stage. However, all anti-HIV drugs do not follow the standard RO5 regulations, while a maximum number of phyto-flavonoids follow the RO5 rules. Nevertheless, there is a mutual negotiation in both biological activity and physicochemical property during best drug selection.

Simultaneously, fold recognition analysis with the selected target SARS-CoV-2-M^{pro} (ID: 6Y2E) confirmed that $a > 92\%$ structural similarity was found with previously recognized SARS-CoV-M^{pro} (2DUC) protein (Supporting information Figure S2). Concomitantly, from sequence-level analysis, 12 substituted amino acids were found, from which nine variations/mutations were conservative in between both CoV-M^{pro}. Thus, the pandemic SARS-CoV-2-M^{pro} is structurally conserved with previously reported SARS-CoV-M^{pro}. The mutated amino acids may be associated with the development of severe pathogenesis and resistance to applied therapy compared to previous CoV-strain.

Since the last decade, a continuous outbreak starting from Dengue, Nipah, Ebola, Chikungunya, Zika and currently the SARS-CoV-2, an updated version of Middle East Respiratory Syndrome (MERS) and SARS from CoV family, threatening the human health and economy (Islam, Ahmed et al., 2020a; Wang, Li et al., 2020b). Like a higher generation of drug/antibiotic, the virus also reconstructed its genome through genetic or environmental pressure; as a result, the omnipotent human unable to protect from the massive storm of deadly SARS-CoV-2 infection at present (Huang et al., 2020; Islam, Ahmed et al., 2020a; Munster et al., 2020). The origin of SARS-CoV-2 is under investigation but it continues the unpredictable morbidity and mortality rates in wildfire gestures without any differentiation between age group, gender, race, poor, rich and geographical region (Islam, Ahmed et al., 2020a; Munster et al., 2020). However, the actual activities, route of transmission and especially symptoms of SARS-CoV-2 infection are still indistinguishable. Notably, the updated genomic information of SARS-CoV-2 is quite diversified from the previously isolated strain of the Co-V family; as a result, several newer serotypes continuously isolated from several geographic regions (Huang et al., 2020; Islam, Ahmed et al., 2020a; Lu, Zhao et al., 2020b; Naqvi et al., 2020; Wang, Horby et al., 2020a). For example, in late December 2020, a newer UK-based SARS-CoV-2 genetic variant (B.1.1.7) was identified. Nevertheless, it does not seem to make more ill or increase mortality risk, but more contagious/transmissible than the previous SARS-CoV-2 (Lauring and Hodcroft, 2021). The continuation in genomic information, clinical evidence and epidemiology analysis is stretching the scientific community in the form of prior background knowledge to develop potential therapeutic/vaccine candidates against SARS-CoV-2 (Lu, Hwang et al., 2020a; Lu, Zhao et al., 2020b; Wang, Li et al., 2020b; Wang, Zhang et al., 2020c).

3.2. Molecular docking study

The molecular docking score of selected 20 ligands (10 anti-HIV drugs and 10 phyto-flavonoids) against the energy minimized (by 100 ns MD simulation) against the allosteric target, SARS-CoV-2-M^{pro} were recorded (Tables 1 and 2; Figures 1–3). Among all anti-HIV drugs, darunavir with docking score, −10.25 kcal/mol and tipranavir with docking score, −10.14 kcal/mol were the two most potential re-purposing drugs against SARS-CoV-2-M^{pro} according to recorded docking scores (Table 1). Similarly, from the phyto-flavonoid side, quercetin-3-rhamnoside with docking score, −10.90 kcal/mol and LPRP-Et-97543 with docking score, −10.11 kcal/mol were recorded as most potential candidates (Table 2). From molecular interactions of docking complexes, the potent antiviral drug, darunavir formed three H-bond interactions with amino acid, LYS5 and LEU282 (Figure 3) and the potent phyto-flavonoid, quercetin-3-rhamnoside interacted with eight potent H-bond interactions with amino acid, LYS5, ALA7, GLN127, LYS137 and GLU290 (Figure 4) against SARS-CoV-2-M^{pro} were recorded. Thus, the phyto-flavonoid quercetin-3-rhamnoside was established as a more potent and stable interaction than anti-HIV drugs. As per the hypothesis of the combination drug approach, a total of four candidates ($n = 4$), from which two potent anti-HIV drugs, darunavir and tipranavir ($n = 2$) and phyto-flavonoids, LPRP-Et-97543 and

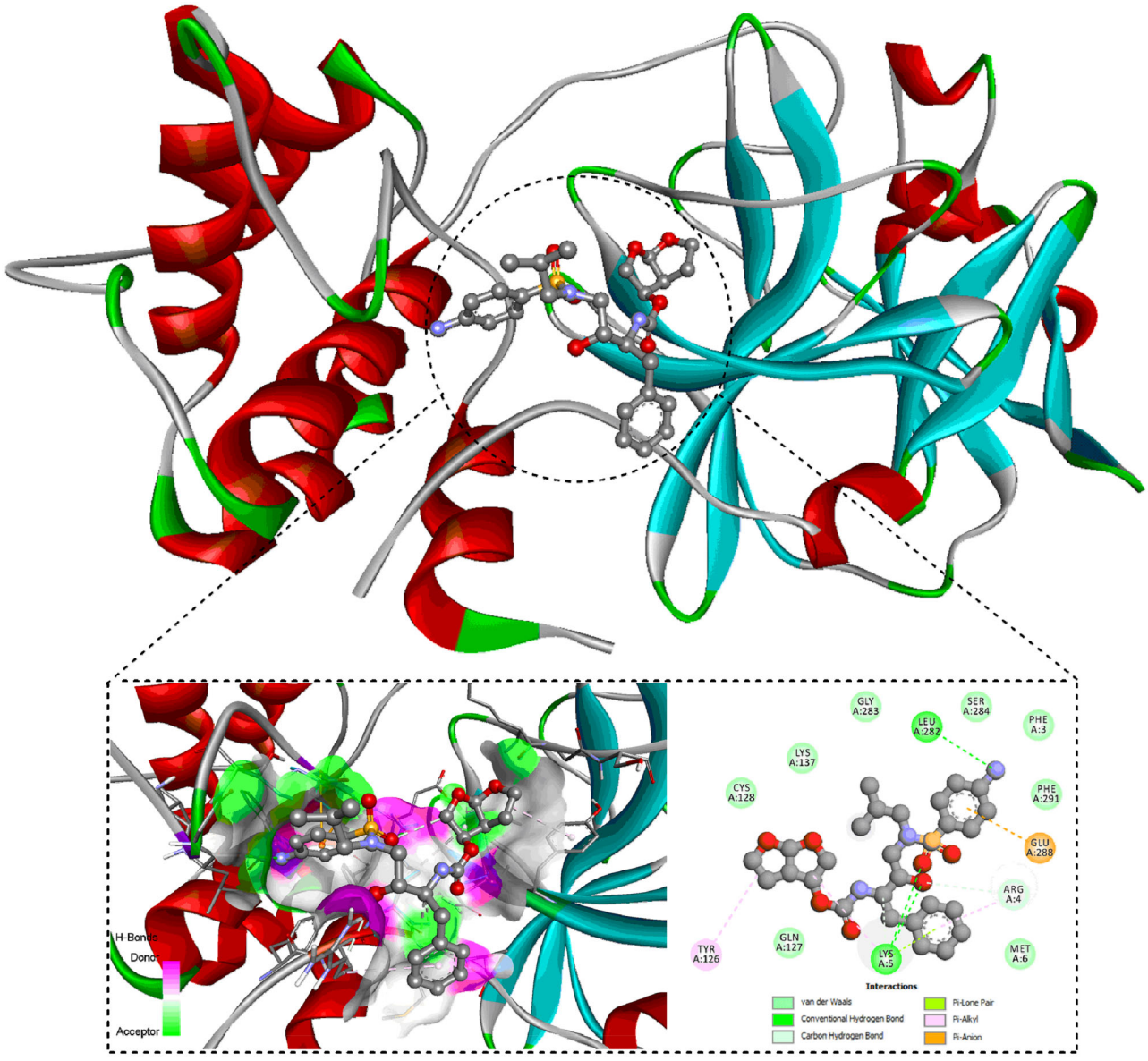

Figure 1. The molecular interaction of the potent antiviral drug, Darunavir with target enzyme, SARS-CoV-M^{pro} (PDB ID: 6Y2E), from docking study. The software, BIOVIA-DSV, was used to visualize in both 3 D & 2 D formats.

quercetin-3-rhamnoside ($n = 2$) were selected (Supporting information Figure S1). Hypothetically, the double-docking score is comparatively higher than the individual ligand docking score. Among four double-docking complexes, the darunavir-quercetin-3-rhamnoside ($n = 1$) combination was the most effective combination with a total docking score, −14.83 (double docking score, −10.95 kcal/mol and ligand–ligand docking score, −3.88 kcal/mol) than other double docking complexes (Table 3; Figure 3). Finally, from the above docking study, three docking complexes, SARS-CoV-2-M^{pro}-darunavir, SARS-CoV-2-M^{pro}-quercetin-3-rhamnoside and SARS-CoV-2-M^{pro}-darunavir-quercetin-3-rhamnoside, were selected for interaction stability analyses with MDS at 100 ns.

Currently, the computer-aided drug development (CADD) program is a cost-effective/resource-saving and a preliminary decisive method in the ongoing drug discovery module. Mainly, the high-throughput computational screening to locate active most chemical moieties based on binding energy or docking scores before synthesis and expensive experimental validation are directly influencing the reduction of cost of medicine in the later stage (Swain, Paidesetty et al., 2020; Ul Qamar et al., 2020). Thus, the CADD program could be a promising endeavor in newer anti-CoV drug development using lead-drug candidates than the traditional hit-and-trial selection process (Hughes et al., 2011; Swain, Paidesetty et al., 2020; Ul Qamar et al., 2020).

3.3. Molecular dynamic simulation study

Towards understanding the structural stability of native protein with protein–ligand docking complexes through the root mean square deviation (RMSD)-protein backbone, root mean square fluctuation (RMSF)-C-alpha and Radius of gyration (Rg) of protein were analyzed by MD- simulation. The elucidated RMSD plot of a single protein determined that continuous unorthodoxy in backbone protein has appeared during 100 ns time. Mainly after 45 ns, found mere stability from the analyzed RMSD-plots (Figure 4). Similarly, the RMSF-

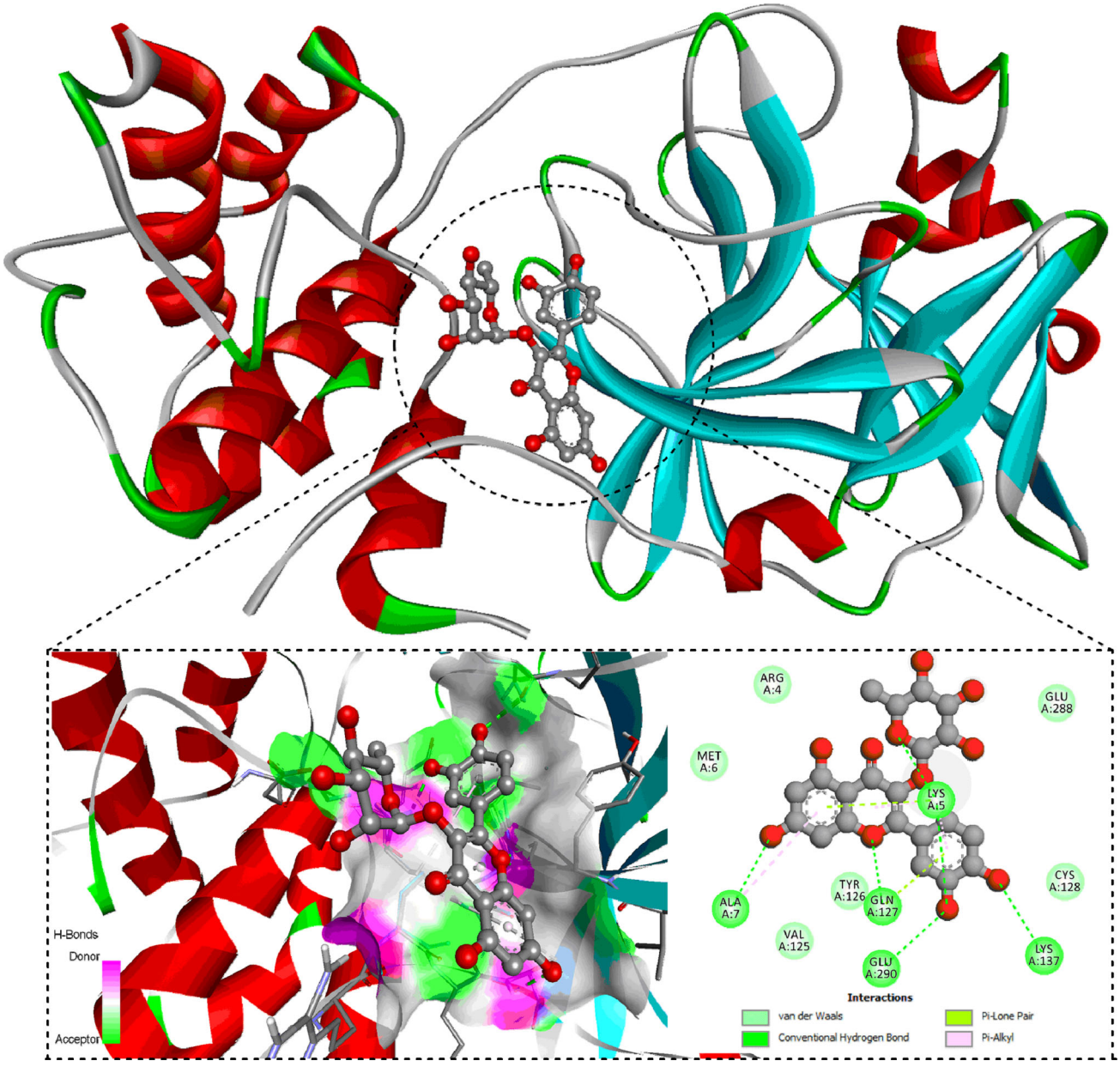

Figure 2. The molecular interaction of the potent phytochemical, quercetin-3-rhamnoside with target enzyme, SARS-CoV-M^{pro} (PDB ID: 6Y2E), from docking study. The software, BIOVIA-DSV, was used to visualize in both 3 D & 2 D formats.

plot describes the c-alpha and Rg-plot of the whole protein, including c-alpha, backbone and side-chain described the inconsistency to maintain the stability during 100 ns in extracted plots (Figures 5 and 6).

From the single docking complex of SARS-CoV-2-M^{pro}-darunavir, SARS-CoV-2-M^{pro}-quercetin-3-rhamnoside, the RMSD plot of quercetin-3-rhamnoside was comparatively more stable than the darunavir docking complex (Figure 4). The protein backbone of SARS-CoV-2-M^{pro}-quercetin-3-rhamnoside (green color bar in Figure 4) shows the least deviation throughout the 100 ns simulation period. In comparison, SARS-CoV-2-M^{pro}-darunavir (red color bar in Figure 4) displayed the variation for the first 45 ns and the rest 55 ns gradually less fluctuated in plotted RMSD. Similarly, the RMSD plot in the double docking case had comparatively higher fluctuation at an upper length, $>$0.4 nm between 60 and 85 ns (orange color bar in Figure 4). From the above RMSD plot analysis, quercetin-3-rhamnoside having the least fluctuation within 100 ns (Figure 4). Correspondingly, we have collected individual RMSF- and Rg-plots by overlaying plots and presented the variation with simulation time between three docking complexes (Figures 5 and 6). Briefly, the docking score in RMSF contest between individual quercetin-3-rhamnoside and darunavir complexes, a higher binding affinity with less fluctuation of c-alpha residues than darunavir complex was observed. Additionally, in the double docking complex, the higher in docking score than the individual with a moderate RMSF-fluctuation as the stabilization level was marked between each phyto- and anti-HIV drug. Thus, the potential quercetin-3-rhamnoside enhanced the anti-SARS-CoV-2 potency and maintained the stabilization phase in the double docking complex with darunavir (Figure 5). Based on the RMSF-analyses, both anti-HIV drug and double docking complex (SARS-CoV-2-M^{pro}-darunavir-quercetin-3-rhamnoside) exhibited the most diverged value in

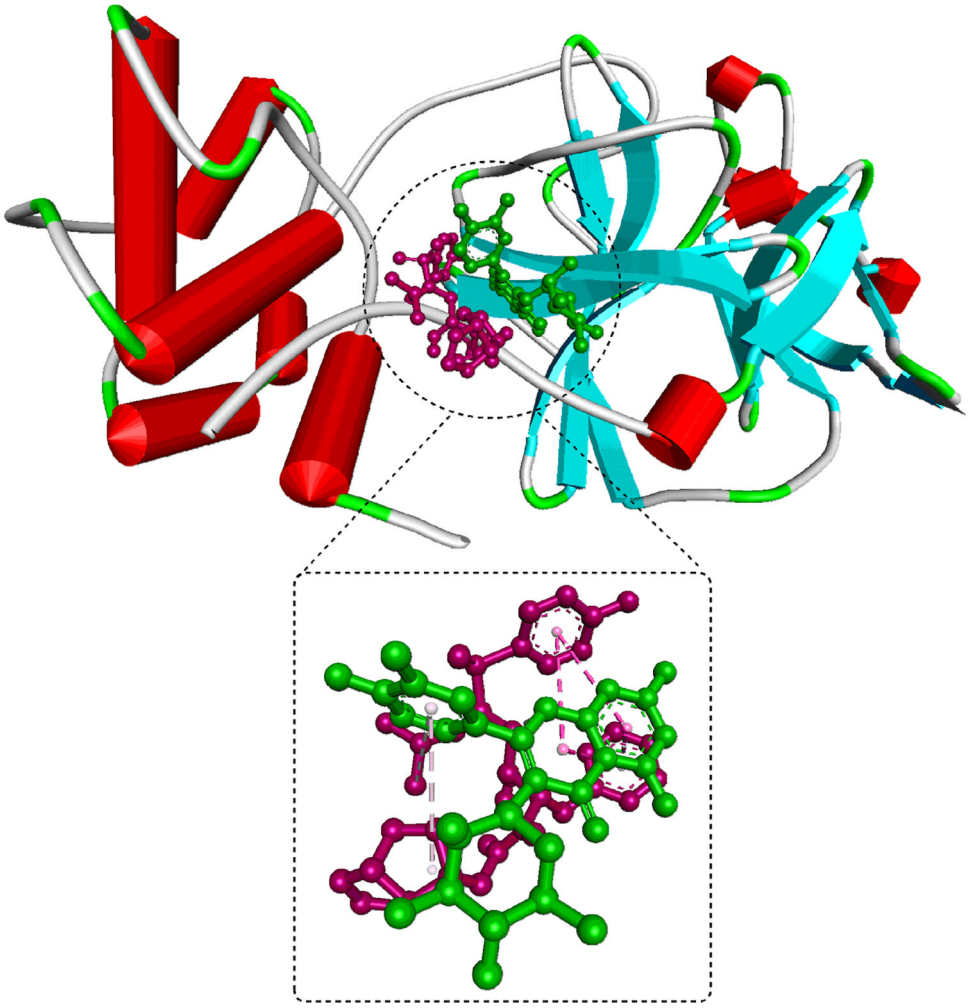

Figure 3. Molecular interactions of the potent antiviral drug, darunavir and the phytochemical, quercetin-3-rhamnoside phytochemical in the synergistic formulation against SARS-CoV-M^{pro} from docking study was visualized.

corresponding to C-alpha residues than phyto-flavonoids. Furthermore, Rg-plots showed the compactness or solidity of the quercetin-3-rhamnoside with more squeezed Rg-values than other complexes (Figure 6). Additionally, H-bond interaction analyses exposed that 8 and 10 strong H-bond interactions in both SARS-CoV-2-M^{pro}-quercetin-3-rhamnoside (green color bar in Figure 7) and SARS-CoV-2-M^{pro}-darunavir-quercetin-3-rhamnoside (in the orange color bar) were found after 100 ns MD simulation (Figure 7). Thus, in the form of activity based on strong H-bond interactions points of view, the quercetin-3-rhamnoside was a most active component than the anti-HIV drug darunavir; as a result, the flavonoid combined drug formulation exhibited the more therapeutic potency against SARS-CoV-2.

3.4. MM/PBSA binding free energy calculation

The advanced MM/PBSA calculation was performed for the above simulated three docking complexes to measure the binding energy. Before calculating the binding affinity between protein–-ligand docking complexes, other energy components (E, MM, G polar and G nonpolar) between 90 and 100 ns at a 0.1 ns time step were calculated. Among all complexes, the SARS-CoV-2-M^{pro}-darunavir-quercetin-3-rhamnoside showed the least binding energy that is −117.526 kJ/mol and the rest two complexes SARS-CoV-2-M^{pro}-quercetin-3-rhamnoside and SARS-CoV-2-M^{pro}-darunavir computed binding energies were −109.357 and −82.774 kJ/mol, respectively (Table 4). Along with binding energy, the van der Wall energy, electrostatic energy, polar solvation energy, SASA energy were also calculated for each complex, and mainly $\geq$ −0.5 kJ/mol consumed energy taken into account with a bar diagram arrangement for both individual and double docking complexes (Figure 8). Some common residues, such as MET6, GLU14, etc., were presented in all docking complexes.

Furthermore, the backbone RMSDs, Cα-RMSF, Rg-plot, H-bond-intermolecular analyses by all-atom MD-simulation and MM/PBSA binding energy calculation provide more productive results, static thermodynamic features and underlying kinetics behavior of protein–ligand docking complexes (Swain et al., 2018; Swain, Paidesetty et al. 2020; Ul Qamar et al., 2020; Wang et al., 2019). Notably, MD simulation suggested that SARS-CoV-2-M^{pro}-quercetin-3-rhamnoside is the 'most-active-cum-reliable' complex than SARS-CoV-2-M^{pro}-darunavir as per the variability observation within 100 ns. Additionally, the combination of darunavir-quercetin-3-rhamnoside makes strong interactions and possesses high binding

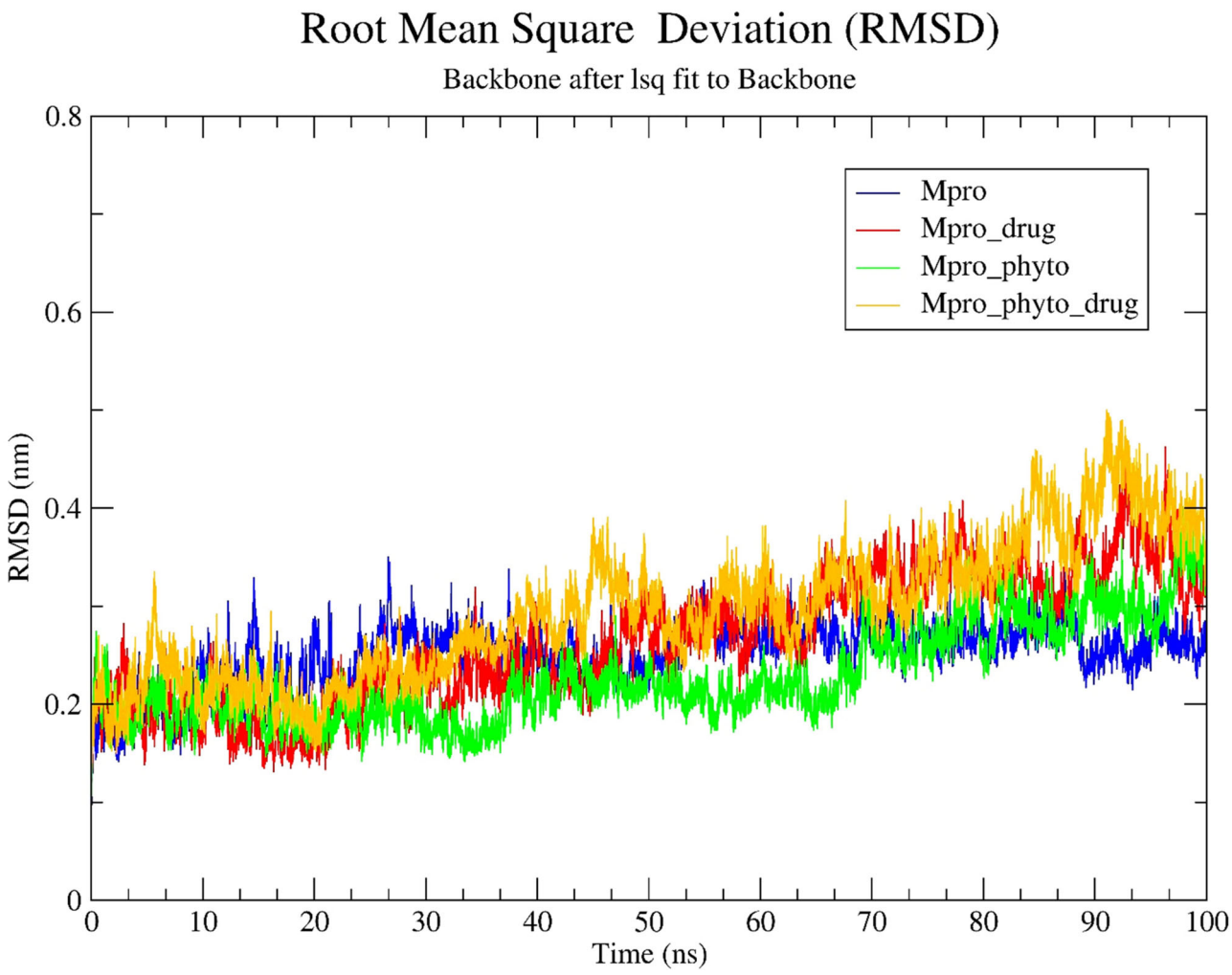

Figure 4. Conformational stability of M^{pro}-drug, M^{pro}-phyto and M^{pro}-phyto-drug docked complexes based on RMSD-score during 100 ns time period indicated in different color graphs.

Table 3. Recorded interchanged double docking and ligand-ligand docking scores against SARS-CoV-2-M^{pro} with selected anti-HIV drugs ($n = 2$) and phyto-flavonoids ($n = 2$).

Docking types	Double docking (kcal/ mol)		Ligand-ligand docking (kcal/ mol)	
Flavonoids	SARS-CoV-2-M^{pro}-Darunavir	SARS-CoV-2-M^{pro}-Tipranavir	Darunavir	Tipranavir
Quercetin-3-rhamnoside	−10.95	−10.84	−3.88	−3.34
LPRP-Et-97543	−10.45	−10.34	−3.32	−2.77

energy against SARS-CoV-2-M^{pro} from H-bond and MM/PBSA analyses. The MD-simulation for an extended period with these complexes may share more high-volume results on stability or structural flexibility in different time intervals. Subsequently, computational tools can predict the possible toxicity and drug-ability profile of any proposed candidates and customarily make it easy to remove the unsuitable/toxic chemicals at the primary stage (Swain, Paidesetty et al., 2020). Thus, combined drug therapy with existing anti-HIV drugs could be an excellent and inspiring repurposing approach and the utilization of more phytochemicals in mainstream medicine to purpose an alternative treatment against SARS-CoV-2 at emergency.

3.5. Possible toxicity and drug-ability prediction

The toxicity or side effect is a significant concern before the recommendation of any therapeutic agents/drugs. A maximum number of lead-drug candidates are unable to express the safety profiles for human consumption and, later on, withdraw from the clinical trial/market. Herein, from the predicted toxicity profiles, phyto-flavonoids were comparatively safer than the anti-HIV drugs (Tables 5 and 6). However, except for hepatotoxicity and immunotoxicity, anti-HIV drugs have a safer profile like phytochemicals. The anti-HIV drug darunavir showed a moderate carcinogenicity, immunotoxicity and mutagenicity under class-III toxicity class with severe hepatotoxicity (Table 5). Similarly, the quercetin-3-rhamnoside was reasonably safer under toxicity class-V, as a more reliable-cum-inoffensive drug candidate (Table 6).

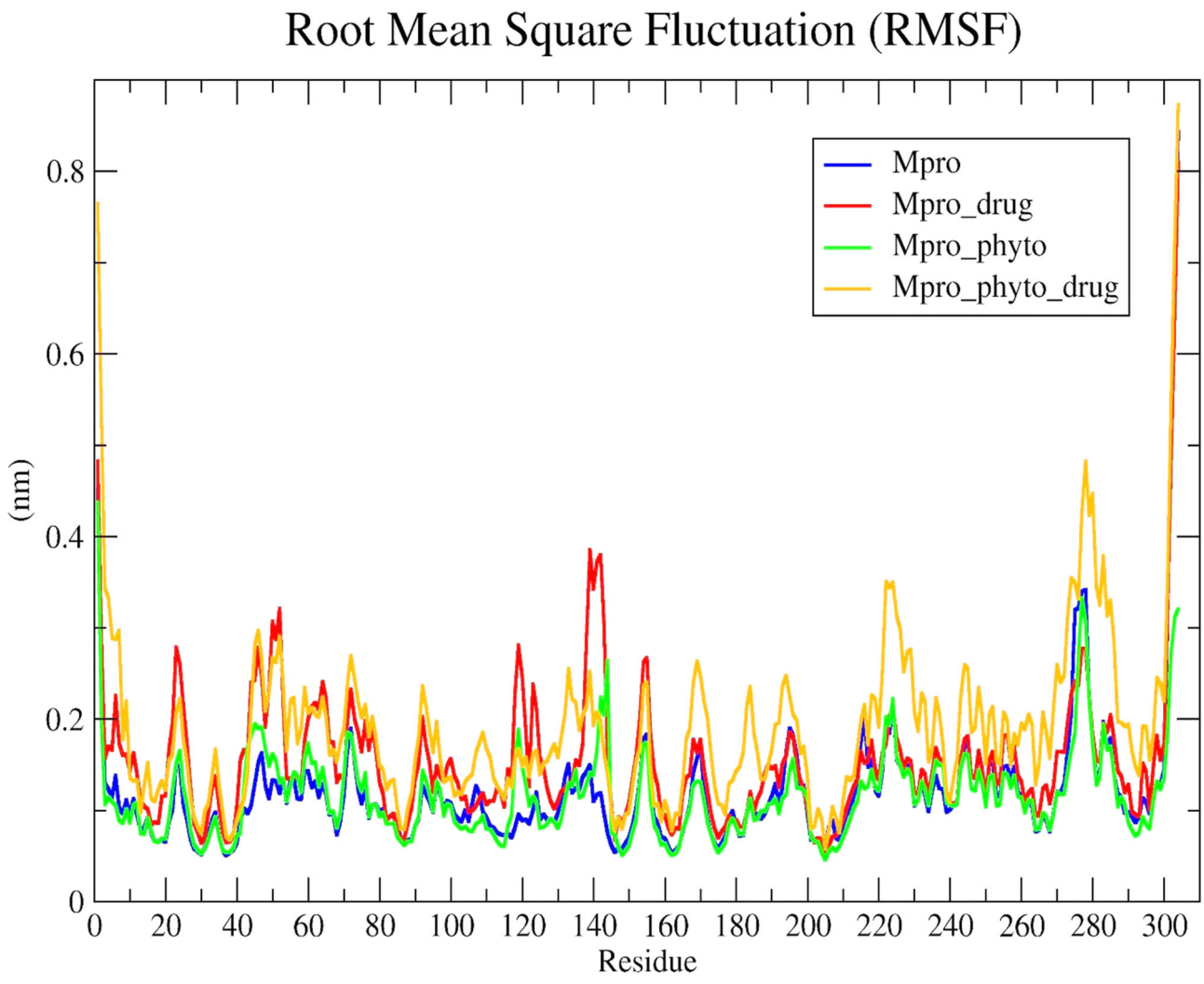

Figure 5. Conformational stability of M^{pro}-drug, M^{pro}-phyto and M^{pro}-phyto-drug docked complexes based on RMSF-score during 100 ns time period indicated in different color graphs.

Concurrently, the overall drug-likeness scorers for each ligand were recorded (Tables 1 and 2). As per the drug-ability plot, a positive drug-likeness value is good; a score between 0.60 and 1.20 is a perfect score for a successive drug molecule. The recorded predicted value concluded that all anti-HIV drugs showed positive drug-likeness scores. It was discernible that all phyto-flavonoids also exhibited positive drug-like scores similar to anti-HIV medicines. Significantly, the drug-ability score of the quercetin-3-rhamnoside had the most potential score, 0.82, while the anti-HIV drug darunavir score, 0.60 (Supporting information Figures S3 and S4). Thus, the predicted drug-likeness score evidenced and expected the success of the proposed phyto-drug combination against SARS-CoV-2.

3.6. Possible pharmacokinetics profile prediction

At the later stage of drug validation, the pharmacokinetics profile plays a crucial role during dose recommendation. Moreover, advanced computational tools can provide possible statistical-based reports for each candidate from the massive amount of training set documents at an early stage. Herein, both anti-HIV drugs and phyto-flavonoids pharmacokinetics profils were recorded (Supporting information Tables S3 and S4). As per reports, except for lopinavir and indinavir, the rest of the anti-HIV medicines have lower gastrointestinal absorption (GI-abs.), including darunavir (Supporting information Table S3). Similarly, all drugs are unable to cross the blood–brain barrier (BBB) report. On the other hand, pharmacokinetics profiles of phyto-flavonoids displayed in a variability manner of GI-abs, P-gp substrate, but quercetin-3-rhamnoside showed a similar pharmacokinetic profile to the anti-HIV drug. Similarly, all phytochemicals showed the same negative BBB cross report (Supporting information Table S4). The overall pharmacokinetics report of all ligands were presented, graphically (Supporting information Figures S5 and S6). The comprehensive reports showed all ligands/candidates recorded in deviate pharmacokinetics profile, mainly phytochemicals. Hopefully, the combination of the mainstream drug with phytochemicals may have enhanced the anti-SARS-CoV-2 activity and stability of the pharmacokinetics profiles in synergism during treatment.

At present, from the experience of previously used combinations against MERS- and SARS-CoV strains were more

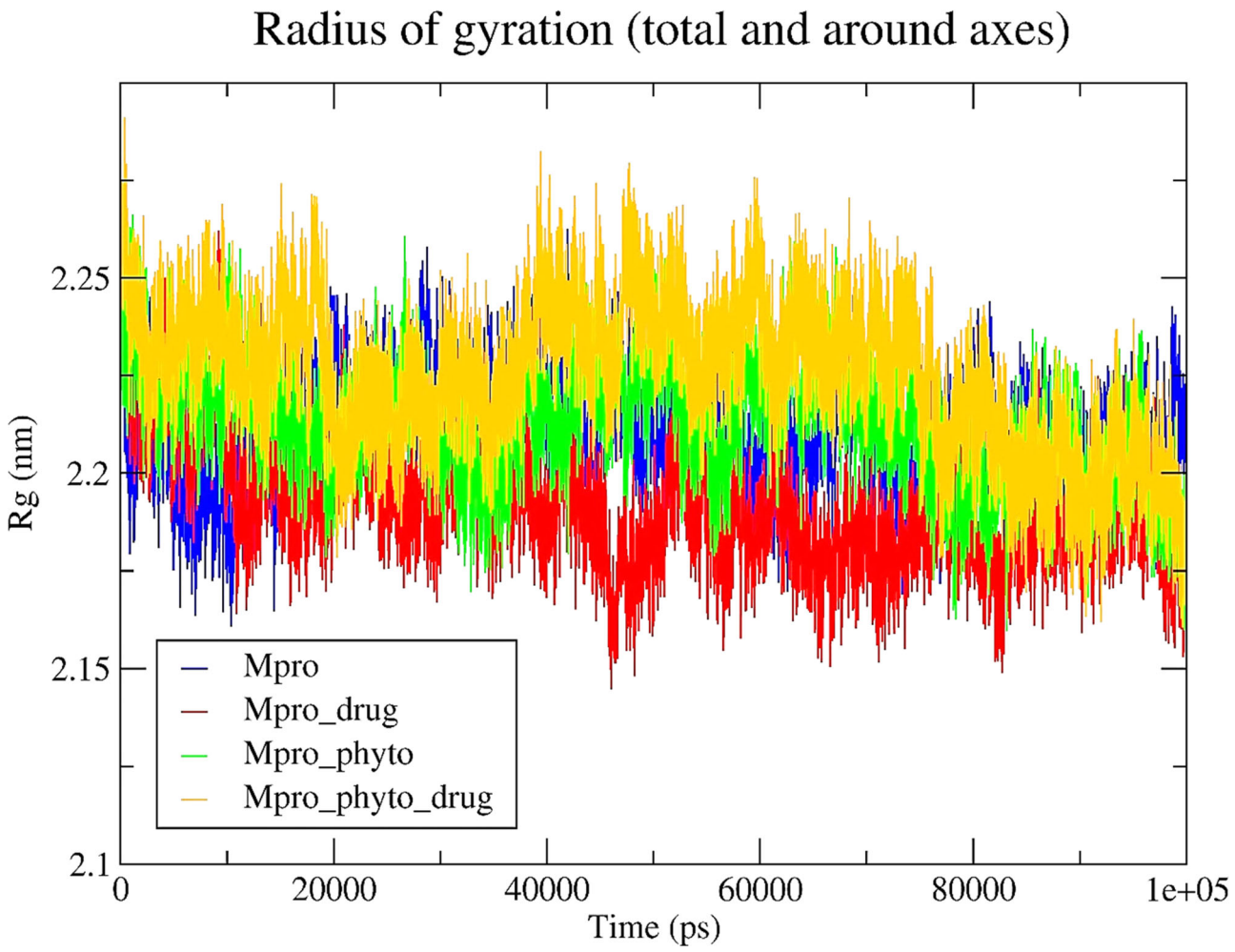

Figure 6. Conformational stability of M^{pro}-drug, M^{pro}-phyto and M^{pro}-phyto-drug docked complexes based on Rg-score during 100 ns time period indicated in different color graphs.

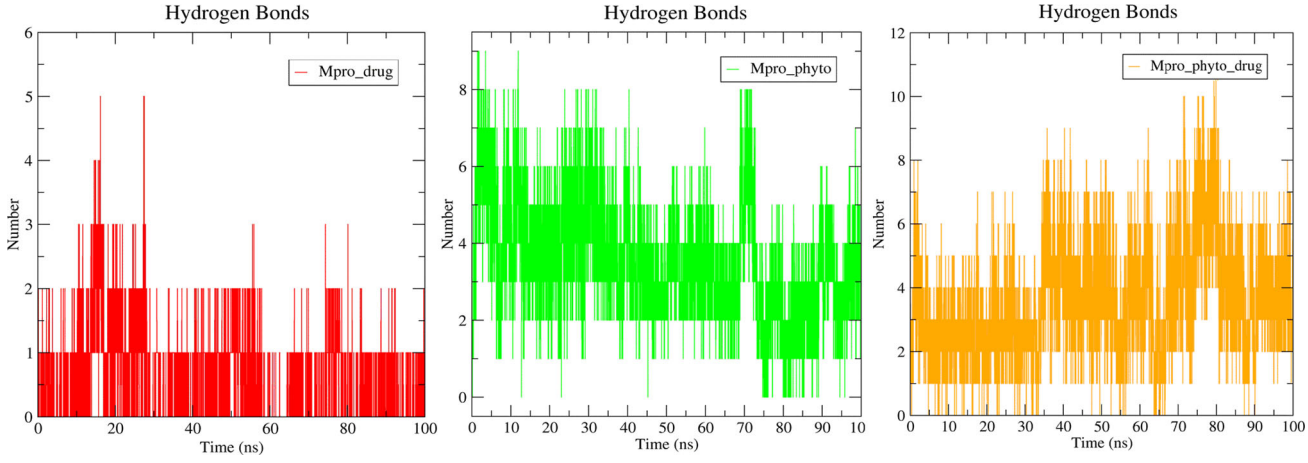

Figure 7. Stability/reliability analyses of M^{pro}-drug, M^{pro}-phyto and M^{pro}-phyto-drug docked complexes based on hydrogen-bond interaction during 100 ns.

Table 4. The binding free energy and other associated energy value of M^{pro}-drug, M^{pro}-phyto and M^{pro}-phyto-drug subtracted using MM/PBSA method.

Energy parameter (kJ/mol)	SARS-CoV-2-M^{pro}-darunavir	SARS-CoV-2-M^{pro}-quercetin-3-rhamnoside	SARS-CoV-2-M^{pro}-darunavir-quercetin-3-rhamnoside
Binding energy	−82.774 +/−41.82	−109.357 +/−16.82	−117.526 +/− 18.92
Electrostatic energy	−0.875+/− 11.47	−30.157 +/−16.80	−46.148 +/−12.70
Polar solvation energy	65.76+/− 44.38	160.75 +/− 26.05	169.17 +/− 21.09
Van der Waal energy	−135.300 +/− 12.273	−206.781+/− 14.972	−219.559 +/− 13.021
SASA energy	−12.364 +/− 2.624	−12.172 +/− 1.493	−20.990 +/− 1.676

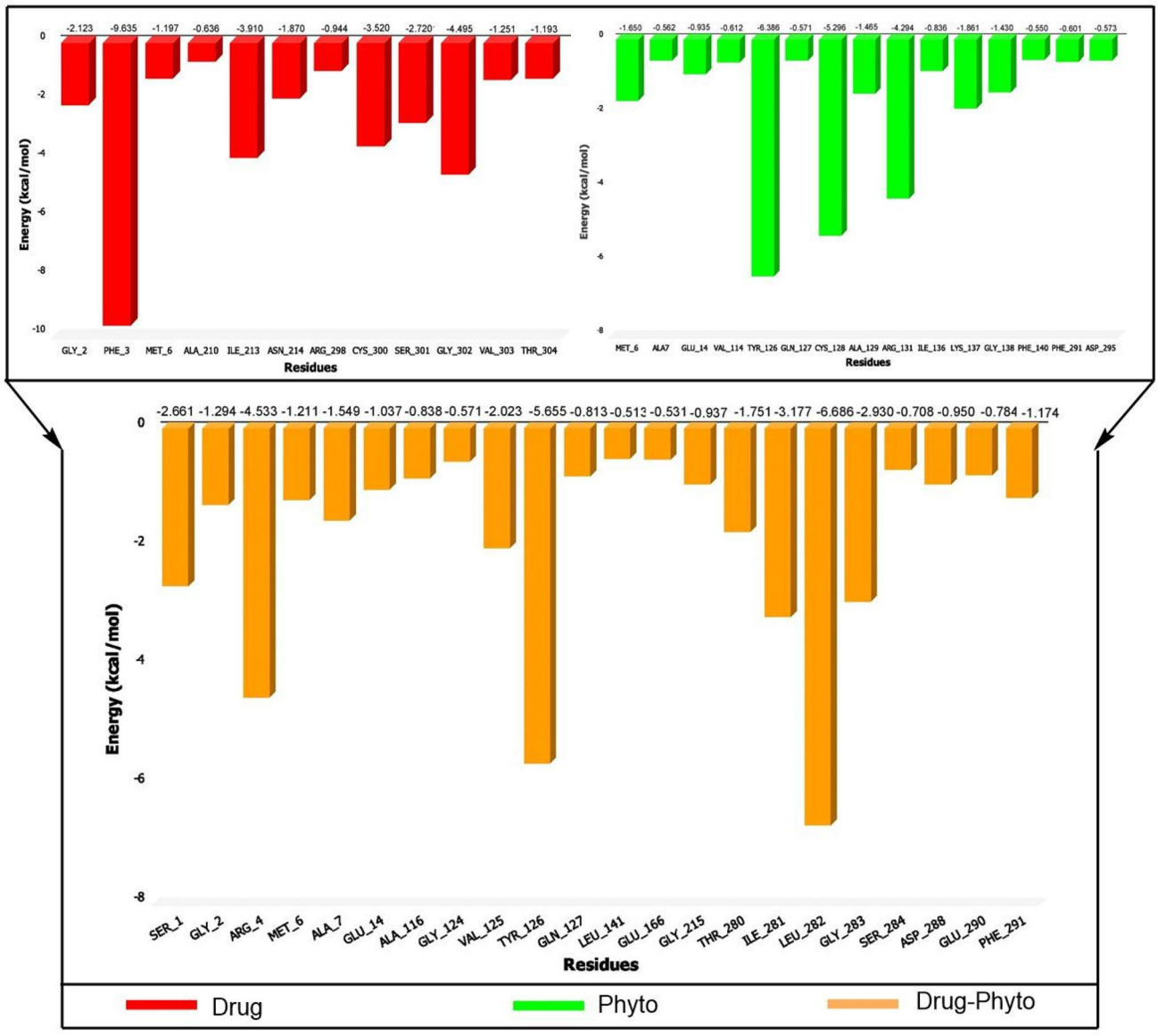

Figure 8. Energy per residue of M^{pro}-drug, M^{pro}-phyto and M^{pro}-drug-phyto docking complexes have been recorded through the MM/PBSA binding energy calculation method during 100 ns MD-simulation. Only energy $\geq$ -0.5 kJ/mol was taken into account for visualization in the above figures and displayed that the 'phyto-drug' combination has a prominent contribution in the form of effectivity than the individual.

viable, as members of similar taxa with a higher genomic match (Lu, Zhao et al., 2020b; Wang, Li et al., 2020b; Wang, Zhang et al., 2020c). The anti-HIV drugs, ASC09, darunavir, lopinavir, ritonavir as potent viral protease inhibitors, oseltamivir, a potent anti-H1N1 neuraminidase inhibitor, favipiravir and umifenovir as potent anti-influenza drugs targeting viral RNA-dependent RNA polymerase, anti-ebola drug, remdesivir targeting viral RNA polymerase, are most widely used drugs in repurposing basis with several combinations (Harrison, 2020; Sanders et al., 2020). However, the multidrug combinations are complicated in SARS-CoV-2 patients with multiple comorbidities such as diabetics, hypertension, respiratory disease and cardiac dysfunctions (Newman and Cragg, 2020; Tungmunnithum et al., 2018).

Similarly, several nontarget conventional treatment therapies with natural regimens/products are quite a classic for most infectious diseases (Newman and Cragg, 2020; Tungmunnithum et al., 2018). Ordinarily, phytochemicals have been suggested as a vibrant conservative resource, since individual plant crude extracts and isolated secondary metabolites curative potential against various health ailments (Choi et al., 2012; Huang et al., 2014; Newman and Cragg, 2020; Swain, Panda et al., 2020; Tungmunnithum et al., 2018). Natural plant products such as curcumin, quinine, taxol, vincristine, morphine, etc., have been playing a significant role in the contribution of several mainstream medicines (Choi et al., 2012; Huang et al., 2014; Newman and Cragg, 2020; Russo et al., 2010). Indeed, from the structural point of view, the flavonoid or polyphenol class of phytochemicals is more suitable and safer secondary metabolites for human administration with potent immune-stimulant and antioxidant as a lesser toxic/nontoxic class of drug candidates (Newman and Cragg, 2020; Russo et al., 2010; Swain, Panda et al., 2020). For example, the flavonoid class of

Table 5. Computationally predicted toxicity profiles including toxicity class of anti-HIV drug based on its chemical structure, using the tool, ProTox.

Anti-HIV protease inhibitor drug	Hepato-toxicity	Carcino-genicity	Immuno-toxicity	Muta-genicity	Cyto-toxicity	Toxicity class
Amprenavir						III
ASC09/ TMC-310911						III
Atazanavir						III
Darunavir						III
Lopinavir						V
Indinavir						V
Nelfinavir						IV
Ritonavir						IV
Saquinavir						IV
Tipranavir						IV

The green-color indicates safer and the light cyan-color indicate moderate safer chemical and on other hand, red color indicates the toxic/hazard class of chemical and light pink indicates moderate toxic in nature.

Table 6. Computationally predicted toxicity profiles including toxicity class of phyto-flavonoids based on its chemical structure, using the tool, ProTox.

Flavonoid class phytochemical	Hepato-toxicity	Carcino-genicity	Immuno-toxicity	Muta-genicity	Cyto-toxicity	Toxicity class
Apigenin						V
Catechin						VI
Dihydroquercetin						III
Epigallocatechingallate						IV
Hesperidin						VI
Kaempferol						V
LPRP-Et-97543						IV
Quercetin						III
Quercetin-3-rhamnoside						V
Rutin						V

See, Table 5 description of color indication.

derivatives, phenoxodiol, genistein and polyphenolic constituents from green tea and soyabeans, is now in a different clinical trial stage as a potent anticancer regimen (Newman and Cragg, 2020; Russo et al., 2010). Someway, nonconventional therapy using Ayurveda and TCM regimens gives some productive outputs against SARS-CoV-2 from several recent reports (Ali et al., 2020; Islam, Parves et al., 2020b; Luo et al., 2020; Swain, Panda et al., 2020; Yang et al., 2020).

Therefore, the proposed 'anti-HIV-drug-phyto-flavonoid' combination could be a potential and safer combination therapy against SARS-CoV-2. Strategically, computer-aided drug design could be a promising endeavor in newer anti-CoV drug development during possible lead-drug candidate(s) selection than the traditional hit-and-trial selection process in this challenging time.

5. Conclusion

Currently, the entire global health system is in the emergency and the whole community lives under a lack of self-confidence from the pandemic of SARS-CoV-2. Based on previous pharmacological evidence, the repurposing of existing antiviral, antiinflammatory, antiparasitic medicines are the only therapeutic options against SARS-CoV-2. However, clinical dose and a proper assessment of posttreatment side effects of repurposing drug therapies are essential guideline-

cum-challenge for any physician/clinician, as treatment has been linked with severe side effects/host-toxicity from several clinical reports. As an alternative solution, we proposed a newer hypothesis using potent an active flavonoid class of phytochemicals with anti-HIV drugs towards balancing the toxicity and biological activity to combat SARS-CoV-2. In parallel, flavonoids are a potent anti-oxidant, anti-inflammatory, immune-stimulant with potent antiviral activities with non-toxic action/safer therapeutic agents for human consumption. Briefly, based on advanced molecular docking-simulation (RMSD, RMSF, Rg and H-bond integration analyses), binding energy score, drug-likeness score, toxicity and pharmacokinetic profiles, the 'darunavir-quercetin-3-rhamnoside' was the most effective and less toxic pharmacological active mishmash against SARS-CoV-2-M^{pro}.

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Authors' contributions

S.S.S., S.R.S., A.S. conceptualized the research design; S.R.S., A.S, carried out the advanced computation assessments and organized the results according to S.S.S direction; S.S.S. drafted the MS and T.H. S.P. provided overall support.

DIsclosure statement

No potential conflict of interest was reported by the authors.

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