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In silico Molecular Docking and Quantum Chemical Calculations of Flavonoid-Derived Compounds as Potential Inhibitors of SARS-CoV-2 RNA-dependent RNA Polymerase

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Abstract: The highly contagious respiratory virus COVID-19 has profoundly influenced the global economy and public health. It has been discovered that the RNA-dependent RNA polymerase catalyzes the synthesis of viral RNA and plays an important role in the replication cycle of the COVID-19 virus. The current study focused on the virtual screening of selected isoflavones, flavonols, and chalcones, which inhibit the enzyme RNA-dependent RNA polymerase. Ligand molecules were evaluated for ADMET activity using SwissADME. Docking studies were performed using AutoDock Vina. The optimized structures and molecular electrostatic potential surfaces were predicted by DFT analysis using B3LYP. The docking scores ranged from −7.0 to -8.7 kcal/mol. Malonyldaidzin had the highest binding affinity (−8.7 kcal/mol) compared to the control Remdisivir (−7.0 kcal/mol). DFT analysis showed that the band energy gaps and ionization potentials of the chosen flavonoids ranged from 0.14 to 0.16 eV and 0.20 to 0.21 eV, respectively, compared to remdesivir, which exhibited an energy gap of 0.17 eV and ionization potential of 0.22 eV, indicating better reactivity of the molecules. The results show that the chosen flavonoids may inhibit or block other protein pathways in SARS-CoV-2 and could capitalize on improved targeted delivery approaches.

Keywords: COVID-19; RNA-dependent RNA polymerase; molecular docking; virtual screening; DFT; flavonoids.

1. Introduction

The COVID-19 pandemic was caused by the outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is genetically related to severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV). Amazingly, the epidemiology of COVID-19 is also similar to that of SARS-CoV [1]. Studies have suggested that the natural hosts for SARS-CoV maybe bats and civets as intermediate hosts. Bats may be the main route of human transmission of the virus [2]. The signs and symptoms of this infection range from asymptomatic to acute respiratory. Like the Nipah virus, the incubation period ranged from 4 to 14 days [3]. The symptoms of this disease are high fever, dry cough, chest pain, headache, dizziness, shortness of breath, nausea,

vomiting, and diarrhea in humans [4]. COVID-19 can be detected using RT–PCR, a qualitative test [5].

RNA-dependent RNA polymerase (RdRp) is an important drug target for SARS-CoV-2 because it is vital for the virus's replication cycle by generating multiple copies of viral RNA [6]. However, no sequence or structural homolog of coronavirus RdRp has been found in humans; therefore, developing potent inhibitors of coronavirus RdRp could be a potential therapeutic strategy without the risk of affecting human polymerases [7].

Several antiviral drugs have been developed to target human immunodeficiency virus (HIV), Ebola virus, hepatitis C virus (HCV), and Marburg virus, and they also target SARS-CoV-2 RdRp [8]. Recent studies have suggested that two known antiviral drugs, remdesivir and favipiravir, are effective alternatives for treating COVID-19, but their safety and effects are yet to be understood [9,10].

Plant bioactive compounds such as isoflavones, flavonols, and chalcones, which are prevalent in plant tissues and have antioxidant and antiviral effects, can potentially inhibit the replication of viruses. Flavonoids and their derivatives may be prospective chemicals for subsequent clinical investigations to improve treatment efficacy against coronavirus infection because of their pleiotropic properties and lack of systemic toxicity [11].

In structure-based drug design, molecular docking has become an important tool and is most frequently used to predict the binding conformation of small ligands to desired target molecules [12,13]. The present study discusses the molecular interactions between flavonoid-derived compounds [14] and the RdRp of coronavirus, providing better insights into drug mechanisms and disease pathology.

2. Materials and Methods

2.1. Preparation of the protein.

The three-dimensional (3D) structure of SARS-CoV-2 RNA-dependent RNA polymerase [RdRp] [PDB ID: 7BV2] was retrieved from the Protein Data Bank (http://www.rcsb.org) [15]. The protein structure was prepared by removing water molecules and adding polar hydrogens and Kollmann charges using AutoDock Tools. The protein was then saved in PDBQT format for molecular docking.

2.2. Preparation of the ligands.

The structures of the isoflavone, flavonol, and chalcone ligands were retrieved from PubChem (www.pubchem.ncbi.nlm.nih.gov) in the Structure Data Format (SDF). The sequences were subsequently converted to PDB format with the help of Open Babel software [16] and prepared in docking format using AutoDock Tools.

2.3. ADMET analysis.

The SMILES notations of the ligand molecules were downloaded from the PubChem database and evaluated for their absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties and other pharmacokinetic and pharmacodynamic properties of the ligands using the online server SwissADME (http://www.swissadme.ch/).

2.4. Docking studies using AutoDock Vina.

Docking was performed using AutoDock Vina through the PyRx program. The RdRp of coronavirus was docked against isoflavone, flavonol, and chalcone ligands, and those with good docking scores were taken for further analysis. Based on the active site of the target, the grid centers were adjusted to 96.06, 93.00, and 93.92 Å for the X, Y, and Z axes, respectively, with 0.375 Å spacing. The X, Y, and Z dimensions of the grid were set to 43 × 45 × 44. The ligand was docked to the target protein, and the best-docked pose was saved. Discovery Studio Visualizer software [17] was used to visualize the docked results, and PyMol was used to analyze the protein-ligand interactions.

2.5. Density-functional theory analysis and reactivity study.

The phytochemicals' Lowest Unoccupied Molecular Orbital (LUMO) and Highest Occupied Molecular Orbital (HOMO) energies were determined using the ORCA 5.0 program, and the Becke3-Lee-Yang-Parr (B3LYP) hybrid functional exchange-correlation of DFT was used [18]. The molecular electrostatic potential map and energies of the compounds were obtained from the optimized geometry. Avogadro version 1.2 was used for visualization.

3. Results

3.1. ADMET properties of flavonoid-derived compounds screened against the RdRp of SARS-CoV-2.

In silico toxicity assessment, a compound is analyzed and predicted using computational methods. Determination of the toxicity of a ligand is important in drug design. Toxicity prediction is necessary to determine the harmful effects of ligands on humans [19]. The flavonoid-derived compounds were subjected to ADMET analysis, and the results for 11 molecules and controls were chosen for further study; the results are given in Table 1.

3.2. Virtual screening of flavonoid-derived compounds.

Molecular docking can be an efficient computational tool for understanding the role of intermolecular interactions[20]. Molecular docking investigations were carried out using AutoDock Vina here to understand the interaction and binding mode of isoflavone, flavonol, and chalcone with the active site of the CoV RdRp. Docking scores in Kcal/mole were obtained after docking the protein with the ligands. This value represents the affinity of the target protein RdRp for the ligands. Negative docking scores indicate stronger interactions within the receptor protein. Pi-alkyl interactions, along with conventional hydrogen bonds, were found in almost all the derivatives. Table 2 shows the docking scores of 11 molecules and controls for the RdRp and amino acids involved in the interactions.

The docking scores for all the compounds ranged from −7.0 to -8.7 kcal/mol (Table 2). Malonyldaidzin had the highest binding affinity (−8.7 kcal/mol), and all the other compounds exhibited greater binding affinities than did the control Remdisivir (−7.0 kcal/mol). The 2D and 3D representations of the best binding ligands generated using Discovery Studio Visualizer (Ver 2011) and PyMol are shown in Figure 1.

Table 1. ADMET analysis of flavonoid-derived compounds

Molecule	Ali Class	Silicos-IT class	GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Leadlikeness #violations	Synthetic Accessibility
Remdesivir	Very soluble	Soluble	Low	No	No	No	No	No	No	No	2	5.04
Astragalin	Moderately soluble	Soluble	Low	No	No	No	No	No	No	No	1	5.29
Daidzin	Soluble	Soluble	Low	No	No	No	No	No	No	No	1	5.01
Dihydromyricetin	Soluble	Soluble	Low	No	No	No	No	No	No	No	0	3.55
Genistin	Moderately soluble	Soluble	Low	No	No	No	No	No	No	No	1	5.12
Hyperoside	Moderately soluble	Soluble	Low	No	No	No	No	No	No	No	1	5.32
Isoquercitrin	Moderately soluble	Soluble	Low	No	No	No	No	No	No	No	1	5.32
Isosalipurposide	Moderately soluble	Soluble	Low	No	No	No	No	No	No	No	1	5.08
Malonyldaidzin	Moderately soluble	Soluble	Low	No	No	No	No	No	No	No	2	5.29
Myricitrin	Moderately soluble	Soluble	Low	No	No	No	No	No	No	No	1	5.32
Puerarin	Soluble	Soluble	Low	No	No	No	No	No	No	No	1	4.98
Quercitrin	Moderately soluble	Soluble	Low	No	No	No	No	No	No	No	1	5.28

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Table 2. Docking scores of RNA-dependent RNA polymerase with flavonoid-derived compounds.

Sl. No.	Ligand	Docking score	Interacting amino acids			
			Conventional hydrogen bonds	Carbon hydrogen bonds	Pi-Pi Tshaped	Pi-alkyl
1	Remdisivir (Control)	-7	SER318			PRO461
2	Astragalin	-7.4	-	-	-	
3	Daidzin	-8.4	ARG349, ASN628, PRO677	SER318	PHE396	PRO323, PRO677
4	Dihydromyricetin	-7.6	-	-	-	
5	Genistin	-8.3	VAL315, ARG349, GLU350, ASN628	SER318	PHE396	PRO323, PRO677
6	Hyperoside	-7.4	SER501, ASN543, VAL560, THR565	VAL560		VAL557
7	Isoquercitrin	-8.3	SER318, THR319, ARG349, THR394			PRO461, PRO677
8	Isosalipurposide	-8	ARG457, PRO677			ARG349, PRO677
9	Malonyldaidzin	-8.7	VAL315, SER318, ARG349, PRO461, ASN628	THR319	PHE396	PRO323, PRO677
10	Myricitrin	-7.7	ARG553, LYS621, SER795	TYR619		PRO620, LYS798
11	Puerarin	-7.8	ARG249, ARG349			PRO323, ARG349, PRO461
12	Quercitrin	-7.8	ARG553, LYS621, SER795	TYR619		PRO620, LYS798

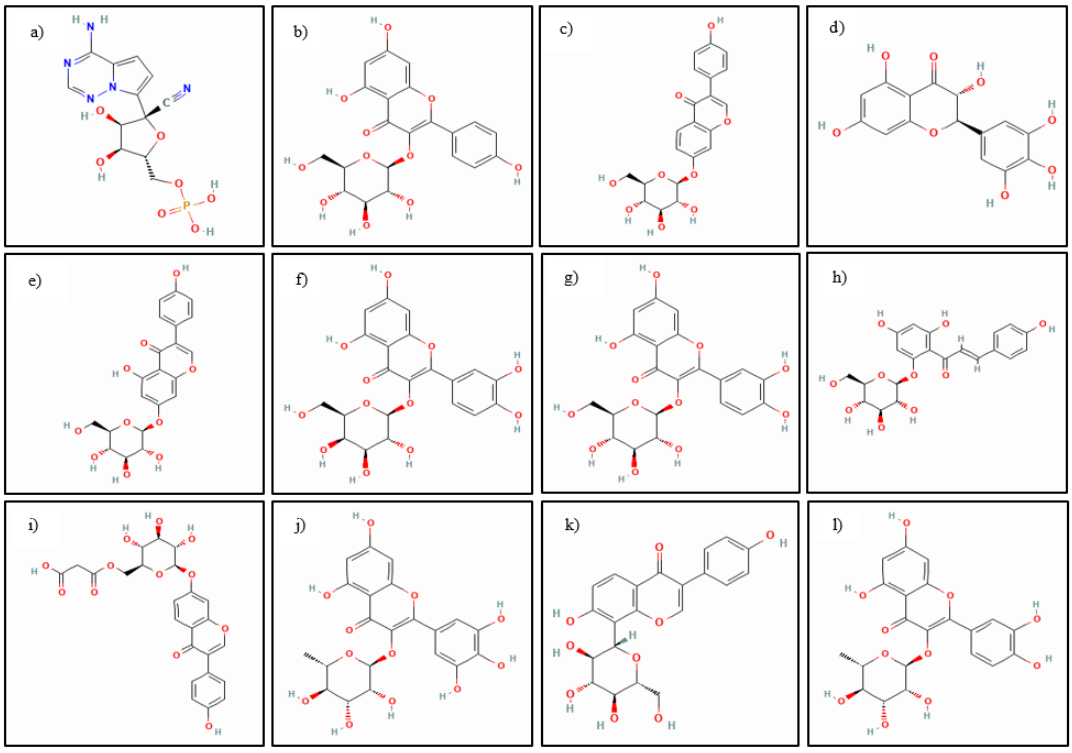

Figure 1. 2D representations of the compounds used in the present study **(a)** Remdisivir (control); **(b)** Astragalin; **(c)** daidzin; **(d)** dihydromyricetin; **(e)** genistin; **(f)** hyperoside; **(g)** isoquercitrin; **(h)** isosalipurposide; **(i)** malonyldaidzin; **(j)** myricitrin; **(k)** puerarin; **(l)** quercitrin.

Malonyldaidzin formed five hydrogen bonds with amino acids VAL315, SER318, ARG349, PRO461, and ASN628 of the target. Two Pi-alkyl interactions with amino acids PRO323 and PRO677 and one Pi-Pi T-shaped interaction with amino acid PHE396 were also observed, as shown in Figure 2.

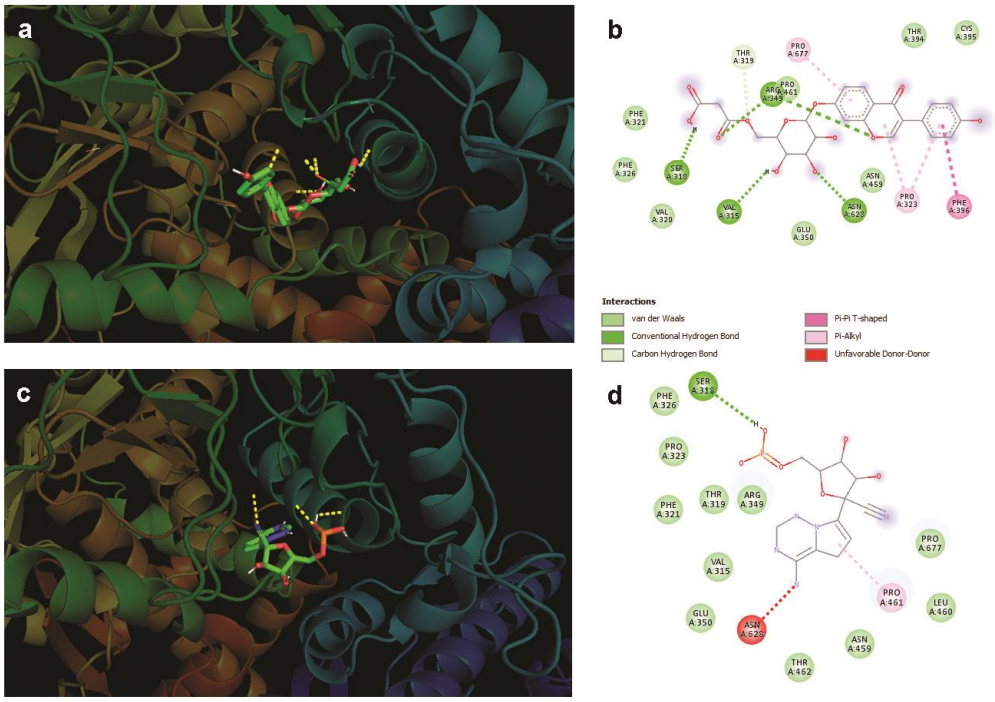

Figure 2. Docked conformations of the compounds in the active site of RdRp. **(a)** 3D representation of malonyldaidzin; **(b)** 2D representation of malonyldaidzin; **(c)** 3D representation of remdesivir; **(d)** 2D representation of remdesivir.

The recommended drug remdesivir formed only one hydrogen bond with SER318, while one unfavorable donor–donor interaction with ASN628 was also observed, which accounts for its low binding affinity.

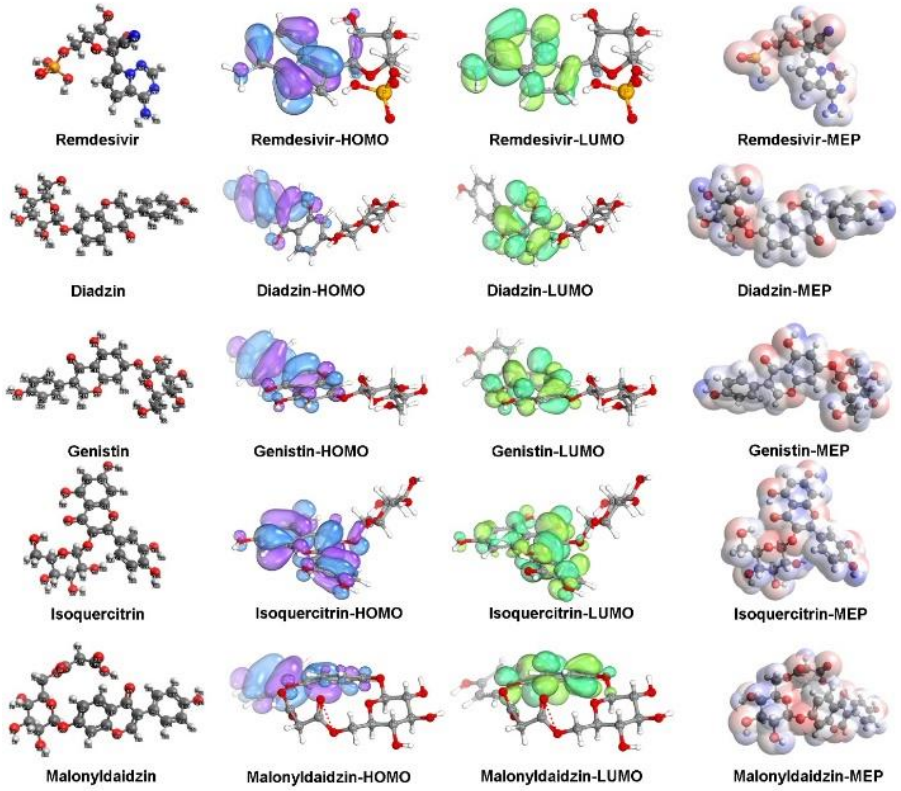

Figure 3. The optimized molecular structures, HOMOs, LUMOs, and molecular electrostatic potential (MEP) surfaces of the compounds.

3.3. Density-functional theory analysis for optimized structures.

Based on quantum mechanistic evaluation, the reactivity of the efficiently docked compounds was further examined utilizing molecular orbital descriptors, such as LUMO and HOMO energies. A DFT study was carried out to evaluate the reactivity parameters of malonyldaidzin, daidzin, genistin, isoquercitrin, and the control drug Remdisivir using bandgaps of molecular orbital energies. The obtained HOMO and LUMO orbitals for the selected compounds are presented in Figure 3, and their energy, EHOMO, and ELUMO values are listed in Table 3.

The following descriptors were calculated based on the energy of the HOMO and LUMO: ΔE_{gap} = (ELUMO - EHOMO); ionization potential; (I = - EHOMO); electron affinity; (A = - ELUMO); electronegativity; (χ = (I + A)/2)); global hardness; (η = (I - A)/2)); and softness (S = 1/η) [21].

Table 3. The various quantum chemical parameters of the isolated compounds.

	Malonyldaidzin	Daidzin	Genistin	Isoquercitrin	Remdesivir
E_{HOMO} (eV)	-0.2194	-0.212	-0.2055	-0.2227	-0.2285
E_{LUMO} (eV)	-0.0721	-0.0578	-0.0438	-0.0758	-0.0493
ΔE_{gap} (eV)	0.1473	0.1542	0.1617	0.1469	0.1792
I (eV)	0.2194	0.212	0.2055	0.2227	0.2285
A (eV)	0.0721	0.0578	0.0438	0.0758	0.0493
χ (eV)	0.14575	0.1349	0.12465	0.14925	0.1389
μ (eV)	-0.14575	-0.1349	-0.12465	-0.14925	-0.1389
η (eV)	0.07365	0.0771	0.08085	0.07345	0.0896
$S(eV)^{-1}$	13.58	12.97	12.37	13.61	11.16

4. Discussion

Despite modest progress in developing antiviral vaccines and widespread population immunization campaigns, the number of COVID-19 cases keeps rising due to the introduction of new SARS-CoV-2 mutations. The development of medications that can inhibit or halt the primary processes of coronavirus SARS-CoV-2 reproduction is critically needed [22].

RdRp catalyzes the replication of RNA with RNA as the template in all RNA viruses and some eukaryotes, and these RNAs are reported to encode this enzyme [23]. Being obligate intracellular parasites, viruses cannot survive independently outside cells, as they require live cells to translate mRNAs to produce proteins and replicate. Thus, any intervention in mRNA translation would likely inhibit viral replication, thereby spreading and evolving the virus [24].

Medicinal plants have been utilized as a source of natural drugs, including antiviral agents, for a long time despite the preoccupation with synthetic chemistry. Additionally, ethnopharmacological-based studies and traditional medicine serve as templates for the design and synthesis of novel substances [25].

Flavonoids are a class of safe phytochemicals commonly abundant in several fruits and vegetables. They offer a range of pharmacological activities, including antiviral effects, when consumed as a diet. These compounds have been demonstrated to target essential stages of the viral life cycle, thus inhibiting viral pathogenesis [26]. For this reason, flavonoids have attracted much attention in recent years because of their fruitful effects during COVID-19 infection. Flavonoids and their derivatives exhibit structural diversity that contributes to their versatile biological benefits, such as anti-inflammatory, neuroprotective, and antioxidative effects, as well as antiviral properties [27].

Several studies have even exploited the structure-activity relationship of natural flavonoids against SARS-CoV-2 proteins [28]. These compounds can also exert antiviral activity directly, where the virus is directly affected by flavonoids, or indirectly, where flavonoids improve host defense mechanisms against viral infections [29, 30].

An earlier study revealed that quercetin-3β-O-D-glucoside inhibited the envelope proteins of the Ebola virus and its replication [31]. Quercetin inhibited the replicon system of the Chikungunya virus by blocking its attachment to host cells [32]. Epigallocatechin gallate (EGCG) inhibited the entry of the Zika virus by blocking envelope proteins [33]. Silymarin inhibited replication by targeting the viral RNA synthesis process of influenza virus [34]. Naringenin inhibited secretion from cells infected with hepatitis C virus [35]. Dihydromyricetin targets H1N1 virus by blocking viral surface protein attachment to host cells [36]. Epigallocatechin gallate (EGCG) and theaflavin inhibited the main protease of SARS-CoV-2 [37].

The structure of SARS-CoV-2, having an overall arrangement similar to that of SARS-CoV, the apo RdRp complex, was reported to contain one nsp12, one nsp7, and two nsp8 proteins [38]. The nsp12 protein also contains an N-terminal β hairpin comprising residues 31 to 50 and an extended nidovirus RdRp-associated nucleotidyl transferase (NiRAN) domain comprising residues 115 to 250 [39]. This protein has seven helices and three β strands [38]. The NiRAN domain was observed as an interface domain (residues 251 to 365) with three helices and five β strands connecting the RdRp domain (residues 366 to 920). In our study, we found that the compound Malonyldaidzin formed hydrogen bonds with the amino acids VAL315, SER318, and ARG349 of the interface domain and PRO461 and ASN628 of the interface domain of the RdRp target.

Zandi *et al*. (2021) [40] evaluated the antiviral effect of the flavonoids baicalin and baicalein by targeting RdRp in Vero CCL-81 cells. *In silico* evaluations of these two compounds revealed that they had different interaction sites and exhibited greater affinity for RdRp than for remdesivir. In another study, MDCK cells infected with influenza viruses A and B were treated with quercetin-7-O-glucoside (Q7G) and oseltamivir as standards and molecular docking revealed that Q7G interacts effectively with the PB2 subunit of viral RNA polymerase [41].

In the present study, virtual screening of isoflavones, flavonols, and chalcones was carried out to identify compounds that interfere with the RNA replication of SARS-CoV-2 by targeting RdRp and could be used as possible prophylactic agents to prevent SARS-CoV-2 infection. Initially, flavonoid-derived compounds were retrieved and subjected to ADMET analysis, where their toxicity, carcinogenicity, and drug-like properties were analyzed. The pharmacokinetic profile determines the therapeutic actions of the drugs. Molecules' lipophilicity, hydrophilicity, and bioavailability play critical roles in being considered compounds as therapeutics [42]. Among the 11 compounds that passed the ADME, the compounds malonyldaidzin, daidzin, genistin, and isoquercitrin were found to interact better than the control Remdesivir. DFT analysis was carried out on these molecules to determine their reactivity with the protein RdRp.

The frontier molecular orbital (FMO) concept describes organic reaction processes and is especially relevant in investigating interactions between drugs and their receptors [43]. The band energy gap (ΔE) was calculated using the LUMO and HOMO energies, which represent the reactivity of a molecule. The band energy gaps were calculated with the objective of having a direct correlation with compound reactivity because lower band energy gaps indicate stronger

reactivity. The chemical reactivity of molecules is also characterized by a descriptor called the ionization energy, and lower values of this energy correspond to stronger chemical reactivity [44, 45]. The band energy gaps and ionization potentials of the chosen flavonoids ranged from 0.14 to 0.16 eV and 0.20 to 0.21 eV, respectively, compared to those of the well-known antiviral medication Remdisivir, which has an energy gap of 0.17 eV and ionization potential of 0.22 eV. These results show that the inhibitors have a considerable affinity for the target proteins and contribute to their high reactivity.

Global softness (S) is the inverse of a molecule's ability to take up electrons, whereas global hardness (η) indicates the degree of resistance to distortion of the electron cloud of molecules [43]. By definition, soft molecules have a low bandgap and may move electrons more readily than hard molecules, increasing their reactivity. A higher softness ranging from 12.3 to 13.6 (eV)-1 compared to 11.1 (eV)-1 for remdesivir indicates greater reactivity of the selected flavonoids.

5. Conclusions

With the increasing incidence of disease transmission, ethical and clinical trials are posing significant obstacles to COVID-19 treatment. Currently, antiviral drugs are recommended for patients to combat COVID-19 despite alternative treatment options being investigated. Recent studies have started using various natural compounds and computational methods to identify new drug targets. An in silico approach was used as a cost-effective approach, and flavonoids and their derivatives were found to target the binding sites of SARS-CoV viral proteins. Highly conserved domains and structurally significant binding sites within RdRp are expected to accomplish this goal.

Additionally, the DFT results revealed that, compared with remdesivir, selected flavonoids have better bioactivity and chemical reactivity and considerable intramolecular charge transfer between electron-donor and electron-acceptor groups and might be powerful candidates for inhibiting or blocking other protein pathways in SARS-CoV-2. A synergistic combination of flavonoids with conventional drugs would also be highly important. However, further in vitro and in vivo studies and clinical trials are needed for additional in-depth research. Collaborative studies across disciplines examining efficient and effective flavonoid-derived compounds could capitalize on improved targeted delivery approaches.

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Conflicts of Interest

The authors declare no conflict of interest.

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