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Virtual screening of Flavonoids in search of a potential drug candidate for COVID- 19: Applications of DFT study and molecular docking

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The COVID-19 pandemic brought on by SARS-CoV-2 had a significant impact on and altered the state of the planet. Here, we used computational models to assess certain Flavonoids bioactivity as SARS-CoV-2 M provirus (6LU7) inhibitors. We described the optimization of Flavonoids using B3LYP/6-311G+(d,p) level theory and density functional theory (DFT). The free energy, dipole moment, and chemical reactivity descriptors were determined using DFT simulations. The SARS-CoV-2 M provirus was subjected to moleculardocking to investigate the interactions and binding affinities of every Flavonoid with that protein. To assess its binding affinity, the well-known medication Chloroquine of the SARS- CoV-2 major protease was also docked. In addition to the DFT results, docking studies suggested that the Flavonoids- Flavone (**1**), Flavonol (**2**), Isoflavone (**3**), Flavanone (**4**), Flavanonol (**5**), Flavan-3-ol (**6**), Epicatechin (**7**), Chalcone (**8**), Apigenin (**9**) have the lowest binding affinities and may be as effective SARS-CoV-2 inhibitors as Chloroquine. Thepresence of hydrogen bonds and various hydrophobic interactions between the flavonoid and the essential amino acid residues of the receptor were both suggested as explanations for the high binding affinity of flavonoids. Flavonoids have a high-lying HOMO, electrophilicity index, dipole moment and the highest chemical softness according to the DFT calculations. All of these factors could interact to varying degrees and have a significant impact on how well these Flavonoids bind to the active protein locations.

Keywords: Chloroquine, DFT, electrophilicity index, Flavonoids, hydrophobic interactions, molecular docking, SARS-CoV-2.

1.Introduction

A significant cluster of unusual pneumonia cases caused by a brand-new coronavirus was identified in Wuhan, China, in 2019 [1]. Despite initiatives to contain the pandemic in its initial location, SARS-CoV-2 has persisted in spreading broadly to several nations. As a result, the WHO declared the unchecked spread of SARS-CoV-2 to be a pandemic in March 2020 [2]. This disease still poses a threat to human life more than two years after the infectionstarted [3].

Controlling SARS-CoV-2 is urgently needed not just for human health but also for the welfare of nations and the advancement of civilization, and in order to accomplish thisgoal, international cooperation is crucial [4].

The virus that caused COVID-19, the coronavirus (CoV) is a member of the coronaviridae family. They contribute to a significant group of viruses with RNA genomes and have spikes on their surfaces [5]. Coronaviruses are typically divided into four primary categories based on their genetic and serological relationships: Alpha, Beta, Gamma, and Delta [6]. TheseRNA viruses have a very high potential to infect the human respiratory system [7, 8] and are primarily found in birds and mammals [7]. A few coronaviruses that infect people are HCoV-OC43, -229E, NL63, HKU1, SARS-CoV (Severe Acute Respiratory Syndrome- Coronavirus), MERS-CoV (Middle East Respiratory Syndrome-Coronavirus), and as of recently, SARS-CoV-2 [6, 9].

A Complete understanding of the problem is crucial while looking for a remedy. SARS-CoV-2 interacts with the cell membrane of ACE2 (Angiotensin-converting enzyme 2), according to numerous investigations including those conducted using cryogenic electron microscopy [10, 11] is shown in **Figure1.**

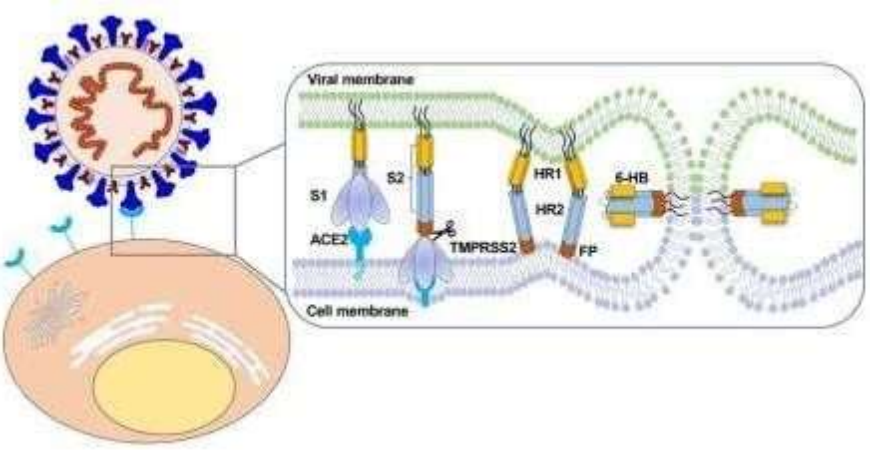


Figure 1: Diagram of SARS-CoV-2 entry into host cells and S protein binding to ACE2receptor and virus attachment to the cell

RdRp, 3CL-Mpro, Papain-like proteinase (PL2pro) and a superfamily 1-like helicase (HEL1)are examples of mature NSP (non-structural proteins) that are involved in viral replication

and are carried in the coronavirus genome by two replicase polyproteins [12-14]. The structure indicates that numerous crucial proteins, including M (membrane protein), S (spike protein), N (nucleocapsid protein), E (envelop protein), and Coronavirus major protease, are encoded by the SARS-CoV-2 genome. These proteins cleave polyproteins into replication- related proteins, which are essential for gene expression. Three domains, I, II, and III, makeup the 3CL-Mpro structure; I and II are N-terminal domains, and III is a C-terminal domain [15].

Many Flavonoids have developed into bioactive substances with pharmacological, antibacterial, and insecticidal capabilities that interact with proteins or nucleic acids. Therefore, Flavonoids are of interest in both medicines as treatments and in agriculture as pesticides [16, 17]. Their ability to influence important cellular enzyme functions in addition to their anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic [18, 19] is shown in **Figure 2**.

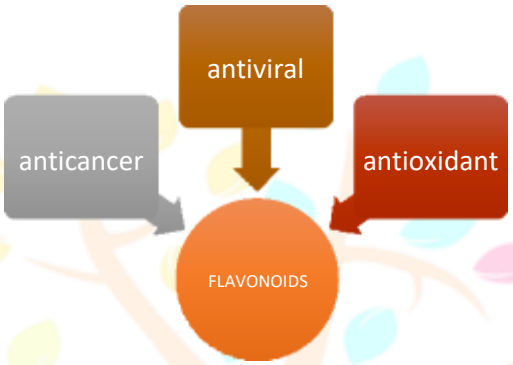


Figure 2: Effects of Flavonoids on the human body

Currently, it is advised to consume fruits, vegetables, and beverages with flavonoids [20-22]. Drug development and vaccine creation are essential for treating and controlling SARS-CoV-2 infection. Today, several scientists from all around the world are investigating substances that have antiviral capabilities. Numerous phytochemicals that are found in nature have pharmacological and antiviral effects [23, 24, 25]. The capacity of phytochemicals to prevent the virus from adhering to the 3CL-Mpro is connected to their inhibitory property [26, 27]. Through DFT calculations and molecular docking, we investigated a number of flavonoids Flavone (**1**), Flavonol (**2**), Isoflavone (**3**), Flavanone (**4**), Flavanonol (**5**), Flavan-3-ol (**6**), Epicatechin (**7**), Chalcone (**8**), Apigenin (**9**) and to comprehend their use in medication development and effectiveness against SARS-CoV-2. Comparing molecular docking to conventional drug discovery techniques, molecular docking is more reliable, efficient, affordable, and time-efficient [28]. For this investigation, Chloroquine is used as the reference chemical.

2.Materials and Methods

This section includes the materials and research methodology employed in the present study. Implementation of theories related to computational method i.e. Density Functional Theory (DFT). In this study, Original Flavonoid geometry was collected from the internet chemical database PubChem and edited using Gaussian 09 software and GaussView5 [29, 30]. Finally, the chapter is concluded with the docking studies of the phytochemicals with the help of AUTODOCK and LIGPLOT.

2.1.DFT calculations

In this investigation, computational computations were carried out utilising the 6-311G+(d, p)basis set in the gas phase of the Gaussian09 programme package [29, 30] and the hybrid functional B3LYP at the DFT level of theory. In order to calculate the energy of the HOMO (Highest Occupied Molecular Orbital), LUMO (Lowest Unoccupied Molecular Orbital), energy gap (E), dipole moment (μ) and free energy, the study first optimises the geometry of all the phytochemicals. Initial phytochemicals geometry was collected from the online chemical database PubChem and transformed using Gaussian 09 software and GaussView 5 [31]. The energies of frontier HOMOs and LUMOs were also used to compute the chemical reactivity descriptors of all the phytochemicals including hardness (η), softness (δ), electronegativity (χ) and electrophilicity index (ω) were also calculated from the energies of frontier HOMOs and LUMOs [32, 33]. The following equations are used for the calculation of hardness (η), electronegativity (χ), electrophilicity index (ω) and softness (δ): [32, 33]

$$\eta = -1/2(E_{HOMO} - E_{LUMO}) \quad (1)$$

$$\chi = -1/2(E_{HOMO} + E_{LUMO}) \quad (2)$$

$$\omega = \chi^2/2\eta \quad (3)$$

$$\delta = 1/\eta \quad (4)$$

2.2.Molecular docking

From the online Protein Data Bank (PDB), the three-dimensional crystal structure of the SARS-CoV-2 M provirus was downloaded in PDB format [33, 34]. The protein's energy minimization was carried out by Auto Dock 4.2 program after all of the existing hetero atoms and water molecules were eliminated [35]. Then, SARS-CoV-2 M provirus (6LU7) was tested using molecular docking with optimized phytochemicals is shown in **Figure 3**.

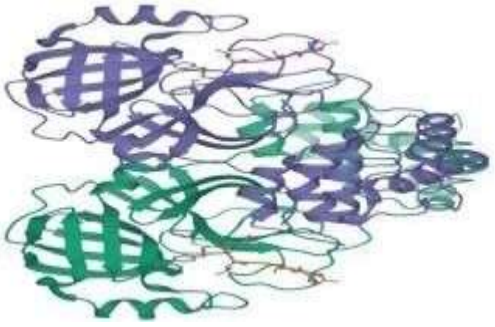


Figure 3: Crystal structure of COVID-19 main protease PDB ID (6LU7)

In computer-aided drug design, molecular docking simulation can be used to predict the binding affinity and mode(s) of the ligand with the target protein. Auto Dock Tools was used to simulate molecular docking using the protein as the macromolecule and the phytochemical as the ligand. In this work, stiff docking was used, with the centre grid box size being - 26.346, 12.605, and 58.919 along the x, y, and z axes, respectively. All rotatable links were changed into non-rotatable bonds. There are 126, 126, and 126 points in the x, y, and z directions, respectively. The protein and ligand structures were both stored after docking, which was necessary to analyze and visualize the docking result and look for interactions between ligands and receptor protein amino acid residues. The outcomes were shown using Ligplot+.

2.3 DERIVATIVES OF FLAVONOIDS FOR STUDY

Herein we have selected a few derivatives of flavonoids for the study as - Chalcones,Epicatechin, Flavones, Flavonols, Flavanones, Isoflavones, Flavan-3-ol, Apigenin, and Flavanonols (**Figure 4**).

Figure 4: Derivatives of Flavonoids under study

Density functional theory (DFT) was used in this inquiry to accomplish the QM calculation. For all Flavonoids, it combined Lee, Yang, and Parr (LYP) correlation functional with Becke
(B) exchanges functional B3LYP [36, 37, 38]. The Flavonoids were optimized, as well as other calculations, using Pople 6-311G+(d, p) basis set. The 2D Structures of the Flavonoids under study were adopted from PubChem as shown in **Figure 5**.

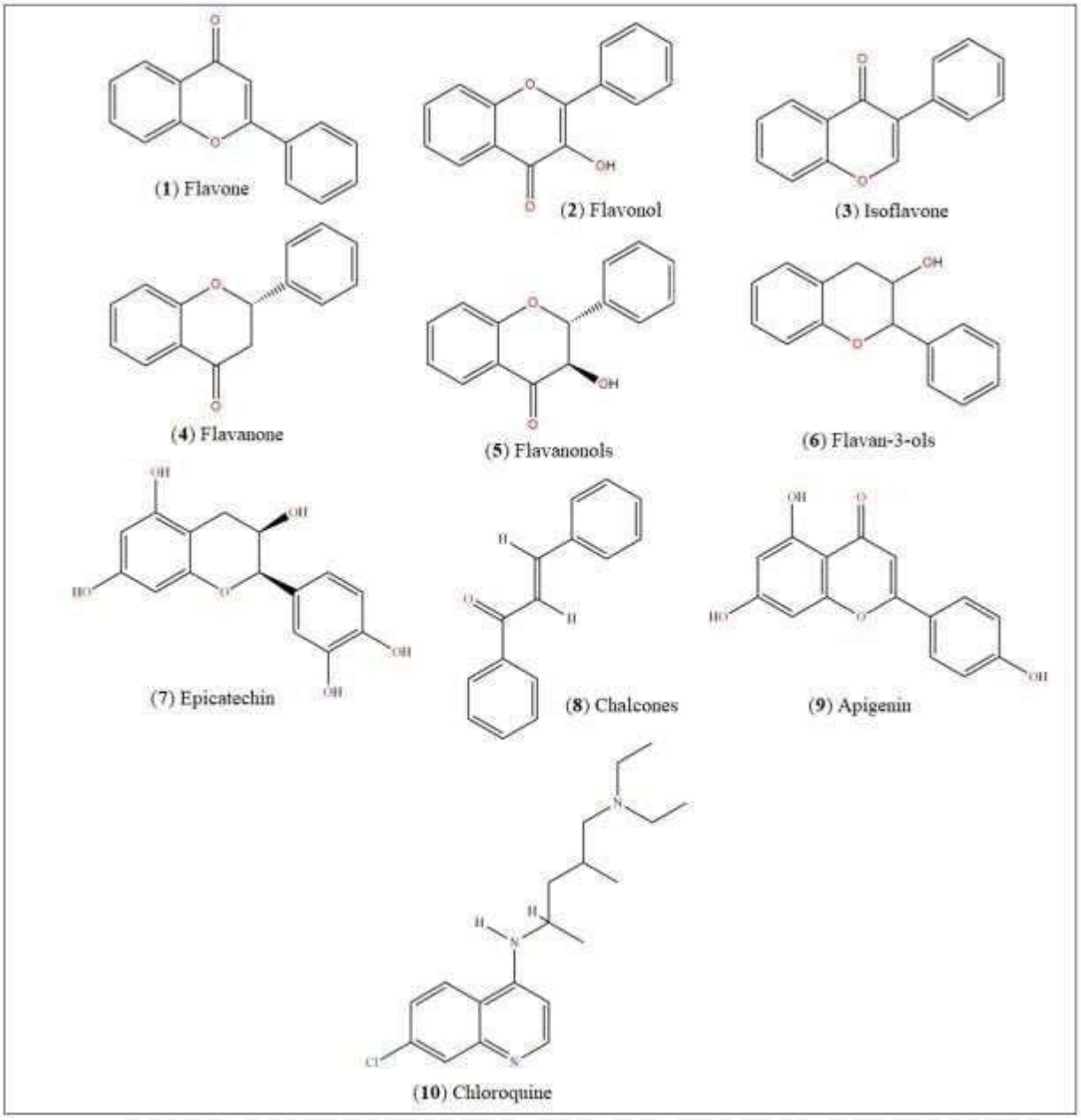


Figure 5: 2D structure of the Flavonoids

3.Result and Discussion

3.1.DFT calculation studies

The DFT calculations were determined using Gaussian09 software at B3LYP 6-311G+(d,p) basis set. The optimized structures (**Figure 6**) of the Flavonoids were drawn with GaussView 5.

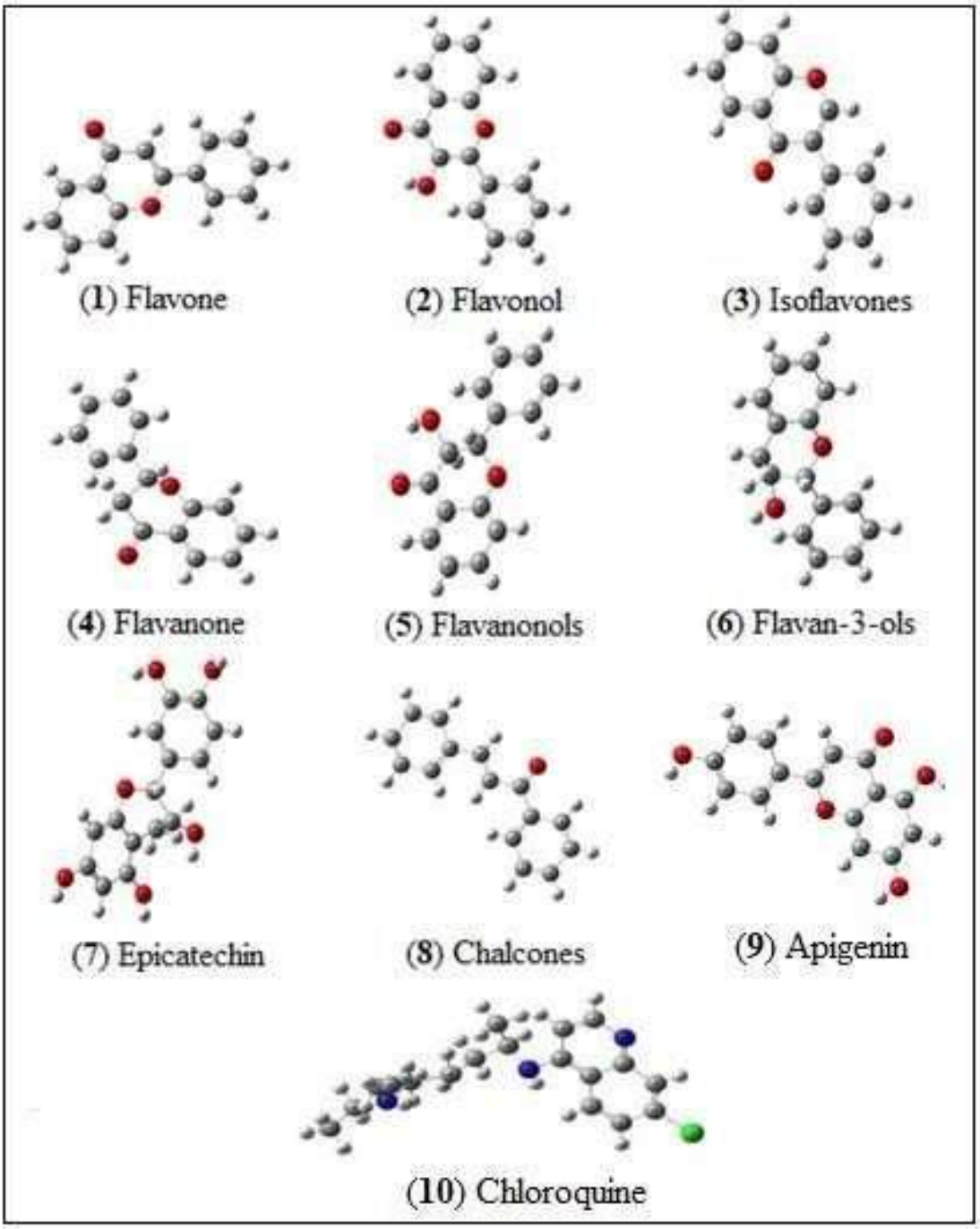


Figure 6: The optimized structure of investigated Flavonoids and Chloroquine.

3.2.Thermodynamic Properties

Free energy and other thermodynamic variables play a significant role in predicting the stability of any given chemical process. Improved thermodynamic characteristics are indicated by higher negative values. In this investigation, it was discovered that the values arenegative (**Table 1**), indicating that the binding will take place naturally without requiring

extra energy. Chloroquine has a free energy of -1325.246 Hartree, but flavonoids have a similar free energy, indicating that the molecules are more favourable from an energetic and configurational standpoint. Chloroquine has a dipole moment of 4.281 Debye, and flavonoidsexhibit a dipole moment that is relatively close to that of Chloroquine. The creation of Hydrogen bonds, nonbonding interactions, binding affinity, and polarity of a molecule are all improved by increased levels of dipole moment.

Table 1: Free energy and dipole moment of chloroquine and Flavonoids

	Ligands	Free Energy (Hartree)	Dipole moment (Debye)
(**1**)	Flavone	-728.258	4.486
(**2**)	Flavonol	-803.509	3.404
(**3**)	Isoflavones	-728.252	3.119
(**4**)	Flavanone	-729.465	2.715
(**5**)	Flavanonols	-804.708	2.727
(**6**)	Flavan-3-ols	-730.667	1.183
(**7**)	Epicatechin	-1031.654	5.144
(**8**)	Chalcones	-654.207	3.198
(**9**)	Apigenin	-948.459	6.290
(**10**)	Chloroquine	-1325.246	4.2813

3.3.Molecular Orbital Properties

A molecule's energy gap, or HOMO-LUMO gap, can be used to determine the hardness and softness of the molecule. Because adding electrons to a higher LUMO and taking them away from a lower HOMO are both energetically advantageous in some reactions, big energy gaps are associated with high kinetic stability and poor chemical reactivity, while small energy gaps reflect low chemical stability. In this analysis, Chloroquine has a HOMO-LUMO gap 4.30eV (**Figure 7**) where Flavonoids- Flavonol (3.84eV), Chalcone (4.13ev) and Flavanonols(4.56eV) show the lower energy gap.

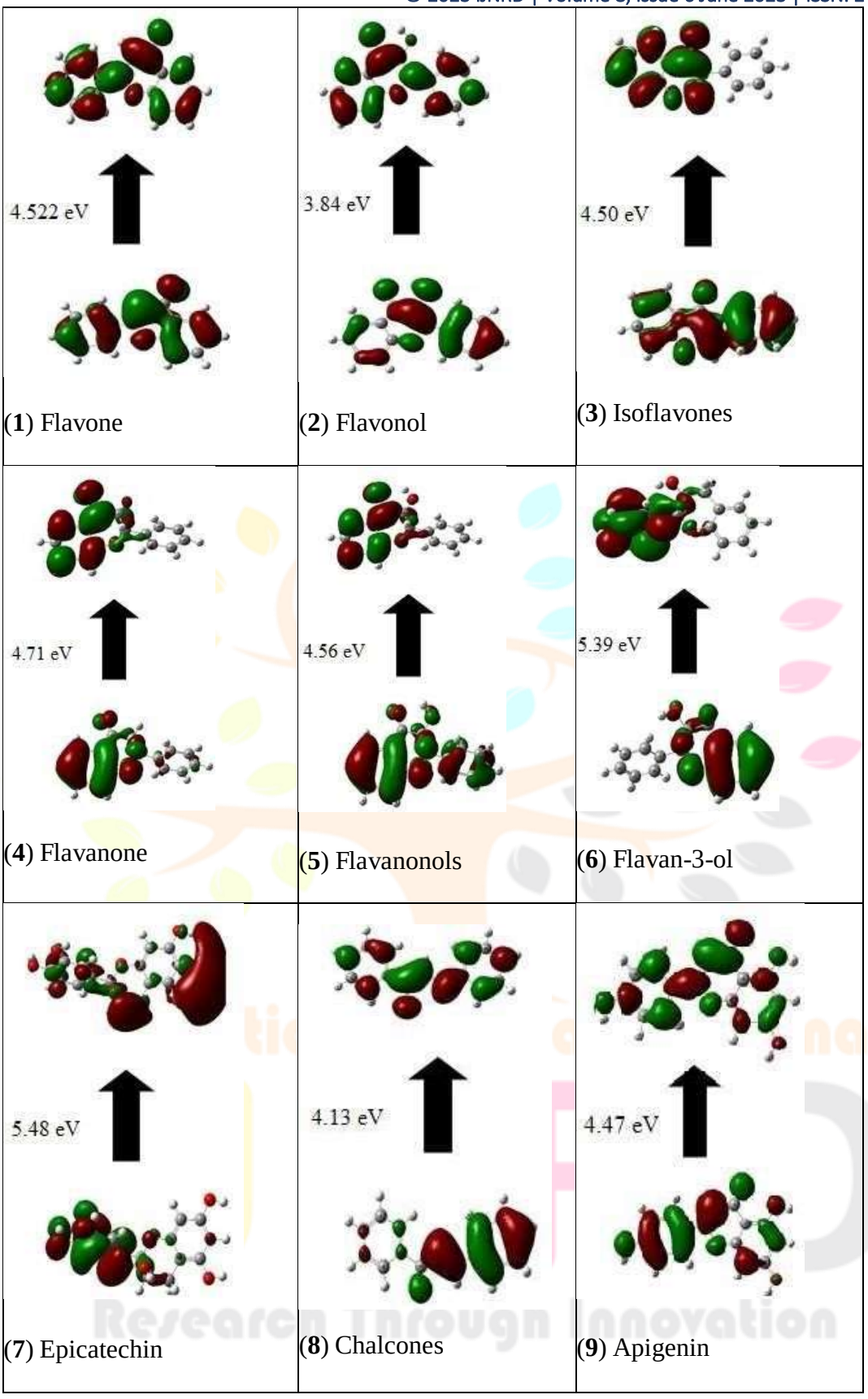


Figure 7: Showing HOMO-LUMO Gap in the Flavonoids under study.

Flavonol shows the lower chemical potential (-4.31eV) with the highest chemical softness (0.52eV) values as compared to Chloroquine which may contribute the higher chemical reactivity than Chloroquine (**Table 2**).

Table 2: HOMO-LUMO, gap hardness (η), softness (δ), electronegativity (χ), electrophilicity index (ω), ionization potential (I) and electron affinity (E. A.) of the Flavonoids.

	Ligands	HOMO	LUMO	ΔE	χ	η	δ	ω	I.P	E. A.
(**1**)	Flavone	-6.74	-2.22	4.52	-4.48	2.26	0.44	4.45	6.74	2.22
(**2**)	Flavonol	-6.23	-2.38	3.84	-4.31	1.92	0.52	4.83	6.23	2.38
(**3**)	Isoflavones	-6.49	-1.99	4.50	-4.24	2.25	0.44	3.99	6.49	1.99
(**4**)	Flavanone	-6.69	-1.98	4.71	-4.34	2.35	0.42	4.00	6.69	1.98
(**5**)	Flavanonols	-6.85	-2.28	4.56	-4.57	2.28	0.43	4.57	6.85	2.28
(**6**)	Flavan-3-ols	-6.21	-0.81	5.39	-3.51	2.69	0.37	2.29	6.21	0.81
(**7**)	Epicatechin	-5.96	-0.48	5.48	-3.22	2.74	0.36	1.89	5.96	0.48
(**8**)	Chalcones	-6.66	-2.53	4.13	-4.60	2.06	0.48	5.11	6.66	2.53
(**9**)	Apigenin	-5.881	-1.404	4.476	-3.643	2.238	0.446	2.965	5.881	1.404
(**10**)	Chloroquine	-5.81	-1.51	4.30	-3.66	2.15	0.46	3.11	5.81	1.51

3.4 CHEMICAL REACTIVITY DESCRIPTORS

The HOMO- LUMO gap (E) is used to assess both the chemical hardness and softness of a molecule. Large HOMO-LUMO differences are associated with lower reactivity and greater stability, whereas small HOMO-LUMO gaps are associated with lower chemical stability. This is because it is energetically feasible to add electrons to a LUMO (high-lying) or removethem from a HOMO (low-lying) during a given reaction. The absolute hardness (η) and absolute softness (**δ**), taking into consideration Parr and Pearson's interpretation, were assessed using Koopman's theorem. A higher value of absolute hardness suggests a much superior base. The value of absolute softness is the strength of the chemical to attractelectrons, similar to Lewis acid.

Global softness (**δ**) denotes a molecule's capacity for absolutely receiving electrons, while global hardness (**η**) denotes a level of limitation on charge transfer. Soft molecules should

have a small energy gap between their molecular orbitals, making them highly reactive due to their ease in shifting electrons to acceptors. Utilizing electronegativity and hardness to determine electrophilicity (ω), which shows a reduction in energy variance due to electron movement between LUMO (acceptor) and HOMO (donor). The electrophilicity indexes are further ranked as follows: chalcones (5.11), Flavonol (4.83), Flavanonols (4.57), Flavone (4.45), Isoflavone (3.99), Apigenin (2.965), Flavan3ol (2.29) and Epicatechin (1.89) in **Table 2**. According to the study, Flavonol has the lowest energy difference (3.85eV) and the highest chemical softness (0.52eV) and electrophilicity index (4.83eV) values, which may explain why it is more reactive chemically than other degradants.

As we can see that among Flavonoids, Chalcone, Flavonol, Flavone and Flavonol showed the highest electrophilicity (5.11ev, 4.83ev, 4.57ev and 4.45ev) and high softness (0.48, 0.52, 0.44 and 0.0.43ev).

3.5 MOLECULAR ELECTROSTATIC POTENTIAL (MEP)

The MEP is a crucial factor to be established in order to confirm the reactive nature of any given molecule. It paints a representation of the magnitude and shape of the neutral, positive, and negative potential. MEP has proven to be a useful method for predicting the relationship between the physicochemical properties and molecular structure of compounds that are being studied. Additionally, this can be used to assess a compound's reactivity to various attacks (electrophilic and nucleophilic). The examined compounds' MEP is carried out using the same base sets as those shown in **Figure 8**. The MEP can be used to show that the majority of electron-rich regions are located near the oxygen atoms.

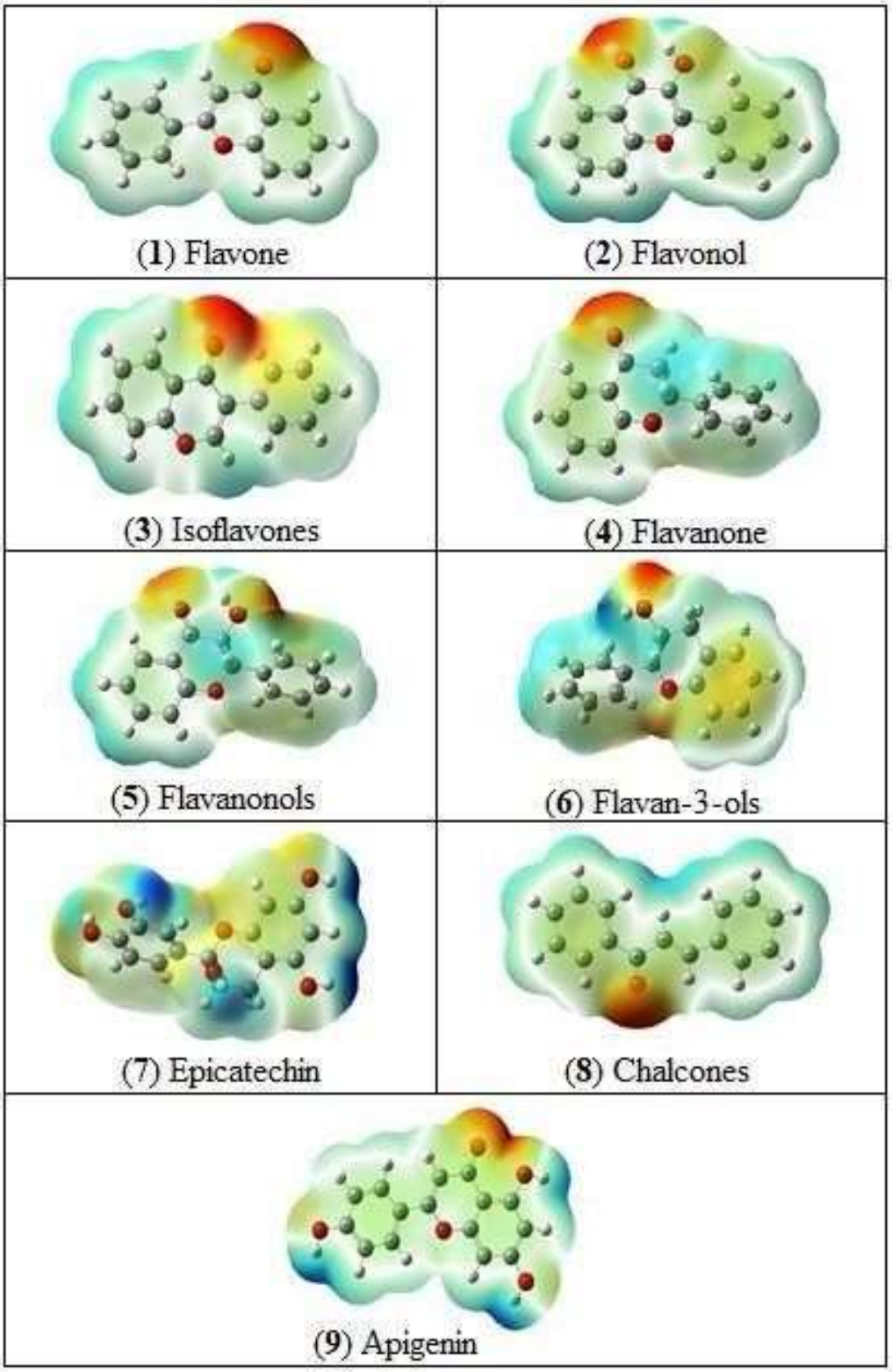


Figure 8: Showing MEP in the Flavonoids

This potential plot demonstrates that electrophile attacks are preferred at locations with thegreatest negative charges, which are highlighted in red. In contrast to the parts shown with

blue, this zone will draw an electrophile that is attacking. Because of the many types of atoms, including their electronic makeup, each molecule has a unique shape, size, and orientation of neutral, positive, and negative electrostatic potential. Therefore, the variation inthe MEP of the chemicals is what causes the variance in the interaction with the receptor.

3.6 MULLIKEN CHARGES

The Mulliken atomic charges of the estimated Flavonoids were calculated by the DFT using B3LYP as a method at 6-311G+(d,p) basis set, the data were tabulated in **Table 3**. Itdemonstrated that the greatest positive and negative charges for chalcone are C5 and C6, C25 respectively. On the other hand, it has been found that the most electrophilic susceptibility sites, C6 and C25, are also the most nucleophilic centers of SARS-CoV.However, the most negatively charged atoms in Epicatechin, Flavone, and Flavonols are C4, C3, and C2, while their corresponding positively charged atoms are C3, C4, and C1. The centers with the highest positive charge are the most vulnerable to nucleophilic assaults or electron donation.

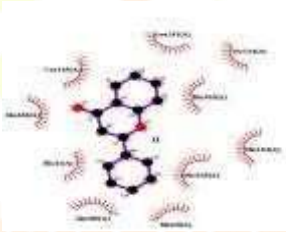
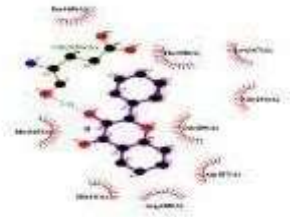
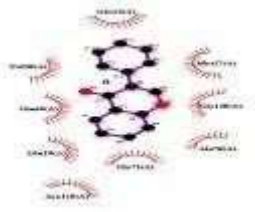
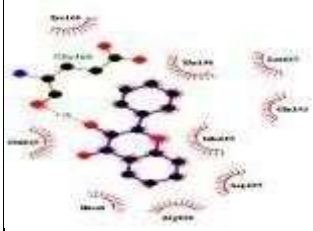
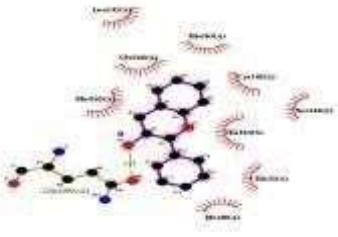
Table 3: Mulliken Charges of the corresponding Flavanoids

Flavones		Flavonol		Isoflavones		Flavanone		Flavanonols		Flavan-3-ols		Epicatechin		Chalcones		Apigenin	
1C	-0.236	1C	1.578	1C	0.298	1C	0.423	1C	-0.012	1C	1.158	1C	0.573	1C	-0.498	1C	-0.043
2C	0.133	2C	-0.925	2C	1.444	2C	-0.009	2C	-0.036	2C	-1.344	2C	-0.088	2C	-0.024	2C	0.299
3C	-1.028	3C	0.203	3C	-1.539	3C	-0.065	3C	0.449	3C	0.348	3C	1.559	3C	-0.151	3C	-0.014
4C	1.704	4C	-0.263	4C	-0.033	4C	0.351	4C	-1.564	4C	-0.056	4C	-1.826	4C	0.766	4C	0.315
5C	0.135	5C	0.091	5C	-0.122	5C	-1.827	5C	1.111	5C	0.025	5C	-0.259	5C	1.781	5C	-0.079
6C	-0.011	6C	0.068	6C	-0.007	6C	1.394	6C	0.367	6C	0.111	6C	-0.509	6C	-0.982	6C	0.312
11C	0.062	11C	-0.339	11C	-0.050	11C	0.358	11C	0.274	11C	-0.121	9C	0.190	12C	-0.311	9C	0.434
12C	-0.167	12C	-0.071	12C	-0.111	12C	0.264	12C	0.405	12C	-0.028	10C	-0.802	13C	-0.433	10C	-0.037
13C	0.013	13C	-0.256	13C	-0.092	13C	-0.297	13C	-0.196	13C	-0.191	11C	0.510	14C	0.932	11C	0.335
14C	-0.247	14C	0.393	14C	-0.453	14C	-0.276	14C	-0.014	14C	0.259	12C	-0.308	15C	-0.030	12O	-0.624
15C	-0.330	15C	1.563	15C	1.336	15C	0.012	15C	-0.260	15C	0.185	13C	-0.119	16C	-0.093	13O	-0.495
16C	1.115	16C	-0.647	16C	-0.470	16C	-0.162	16C	-0.339	16C	-0.366	14C	0.306	17C	0.053	14O	-0.238
22	-1.008	22C	-0.857	22C	-0.405	22C	-0.123	22C	-0.176	22C	-0.183	18C	0.898	23C	0.063	15O	-0.214

C 23 -0.113	23C -0.851	23C 0.839	23C -0.161	23C -0.161	23C -0.084	19C -0.192	24C 0.120	16C -0.066
C 24 0.274	24C 0.612	24C -0.294	24C 0.353	24C 0.387	24C 0.233	20C 0.097	25C -0.841	17C 0.012
C 25 0.021	25O 0.026	25O -0.061	25O 0.042	25C 0.045	25O 0.027	21C 0.064	26O -0.245	18C 0.034
O 26 -0.317	26O -0.362	26O -0.280	26O -0.279	26O -0.305	26O 0.028	22C 0.016		19C 0.295
O	27O 0.038			27O 0.023		23C -0.002		20C 0.040
						24C -0.066		21C -0.030
						25O -0.003		22O -0.237
						30O -0.039		22O -0.237

3.7. Binding Affinity and Binding Interactions Analysis

To forecast the phytochemicals manner of binding at the SARS-CoV-2 Mpro pocket, dockingmodelling studies were conducted (**Figure 9**).

(**1**) Flavone 	(**2**) Flavonol 
(**3**) Isoflavones 	(**4**) Flavanone 
(**5**) Flavanonols 	(**6**) Flavan-3-ols 

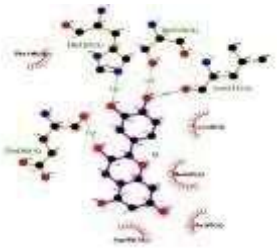
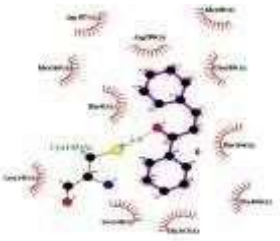
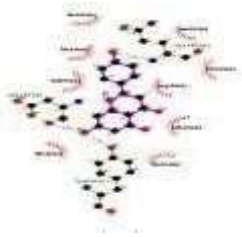
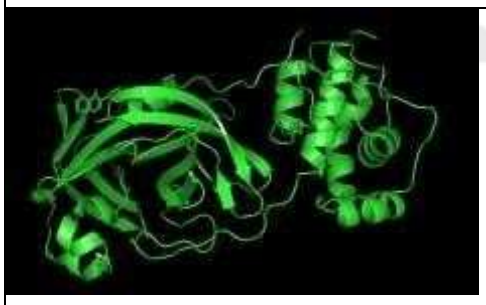
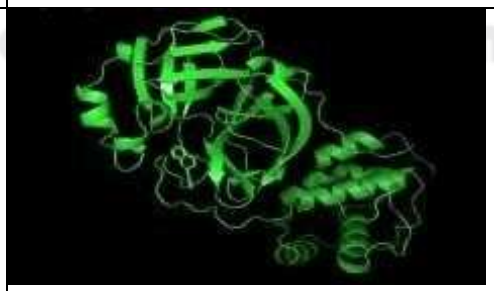
(**7**) Epicatechin 	(**8**) Chalcones 
(**9**) Apigenin 	(**10**) Chloroquine 

Figure 9: Amino acid residues in the SARS-CoV-2 M pro binding pocket

Similarly, Chloroquine was docked as a control in the protein pocket. After Docking ligand- protein complex were obtained and their structure was seen in the Python molecular viewer option of PyMOL 2.5.4 (http://www.pymol.org) as shown in **Figure 10**.

 (**1**) Flavone	 (**2**) Flavonol
	

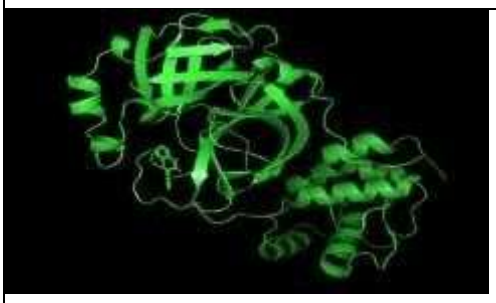
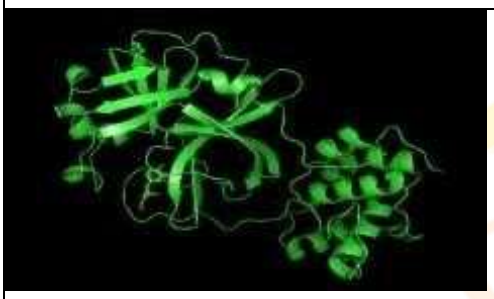
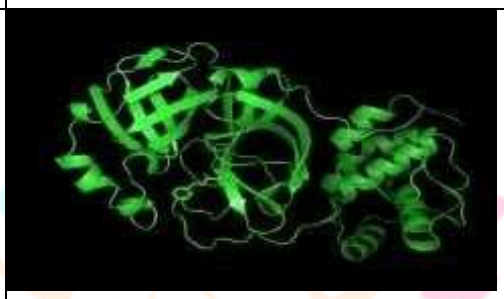
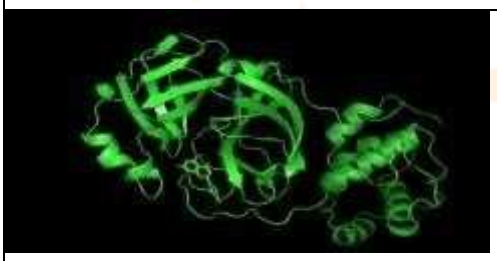
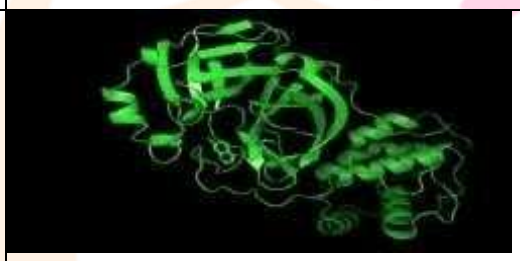
(**3**) Isoflavones	(**4**) Flavanone
	
(**5**) Flavanonols	(**6**) Flavan-3-ols
	
(**7**) Epicatechin	(**8**) Chalcones
	
(**9**) Apigenin	(**10**) Chloroquine

Figure 10: Flavonoids-Protein Binding as shown in PyMOL

Flavonoids binding affinities ranged from -5.00 to -6.69 Kcal/mol. The outcomesdemonstrated that Flavonoids are situated in the same active pocket as Chloroquine. Chloroquine displayed a binding energy of -4.79 kcal/mol. Compound Apigenin was also investigated computationally, however the outcomes were insignificant in comparison to the compounds that were reported, and the docking outcomes of the compounds with the highest binding energies were reported (**Table 4**).

Table 4: Binding affinity of the Flavonoids

	Ligands	BINDING ENERGY
(**1**)	Flavone	-6.35
(**2**)	Flavonol	-6.50
(**3**)	Isoflavones	-5.85
(**4**)	Flavanone	-6.50
(**5**)	Flavanonols	-6.69
(**6**)	Flavan-3-ols	-6.22
(**7**)	Epicatechin	-5.00
(**8**)	Chalcones	-6.12
(**9**)	Apigenin	-6.41
(**10**)	Chloroquine	-4.79

3.8 LIGAND– PROTEIN DOCKING (AUTODOCKING)

Molecular Docking illustrates the binding of the most suitable orientation of small molecules with the corresponding receptor protein. All of the examined Flavonoids compounds exhibit asimilar affinity to Chloroquine for the active site of SARS-CoV-2 according to the results of compound docking in the LIGPLOT+ viewer. Except for ordinary hydrogen bonding, non- bonding interactions have frequently used terms to determine the shape and behaviour of molecules. From molecular docking analysis, the major and common residues of 6LU7 activesites like HIS163, CYS145, MET165, THR190, HIS164, ASP187, ARG188, LEU167,
GLU166, GLN189, and PRO168 form different significant interactions with the ligands.

AutoDock results show that among all the Flavonoids (taken in this study), the Binding Affinities of Apigenin (-6.41kcal/mol), Flavonol (-6.63kcal/mol), Flavone (-6.35kcal/mol), Flavanone (-6.50kcal/mol), Isoflavone (-5.85kcal/mol), Flavanonols (-6.69kcal/mol) and Chalcones(-6.12kcal/mol) are comparable to a well-known ligand Chloroquine (-4.79 kcal) is shown in **Table 5**. Flavanonols showed the lowest binding affinity and interacted hydrophobically with the residues Gln189, Met165, Asp187, Arg188, His41, Pro168,Gln 192, Thr190 and Leu167. The polar interactions were found to be with His164 (3.28 Å).

Hydrogen bonding and different hydrophobic interactions between the inhibitor and the amino acid residues of the receptor are responsible for the binding affinity of Flavanonols. The result of the docking studies show that the amino acid residue Gln189, Met165, Asp187, Arg188, His41, Pro168, Gln 192, Thr190, and Leu167 interacted hydrophobically with the Flavanonol molecule whereas His164 (3.28Å) shows polar interactions. The Flavonoids Chalcone also shows good interaction with 3CL-Mpro. The hydrophobic interaction were found with residue Gln189, Met165, Arg188, His41, Pro168, Gln 192, Thr190, Glu166, and Leu167; and hydrogen bonding with Cys145 (3.08 Å). However, Epicatechin interacts hydrophobically with Met165, Pro168, Leu167, Arg188, Phe140, Cys145 and Gln189 and Glu166 (3.24 Å), His163 (3.34Å), Ser144 (3.21 Å), and Leu141 (3.09 Å) polar interactions. Apigenin interacts hydrophobically with Met165, Thr190, Leu167, Gln189, Pro168, Glu166, Arg188, His41 and Tyr54 (2.95 Å), Gln192 (3.34 Å) and Asp187 (3.08 Å) show polar interactions. Hence, these Flavonoids have potential to combat with SARS-CoV-2.

Table 5: Aminoacid residues involved in the interactions with the Flavonoids and Chloroquine.

	Ligands	Type of interactions		Number of bonds		
		H-bondresidues	Hydrophobic bond residues	H-bonds	Hydrophobic bonds	
(1)	Flavone	-	HIS163, CYS145, MET165, HIS164, LEU167, SER144, GLU166, GLN189, PRO168, ARG188, MET49	0	11	70%
(2)	Flavonol	GLU166 (3.28 Å)	MET165, THR190, HIS41, LEU167, GLU192, GLN189, PRO168, ASPP187, ARG188	1	9	50%
(3)	Isoflavones	-	MET17, GLU14, GLY120, GLY71, ALA70, GLU19, ASN119, GLU69, VA118	0	9	0%
(4)	Flavanone	-	MET165, THR190, GLU166, LEU167, GLN189, PRO168, GLN192, ASP187, ARG188,HIS41	0	10	
(5)	Flavanonols	GLU166 (3.28 Å)	ASP187, ARG188, HIS41, MET165, THR190, LEU167, GLN189, PRO168, GLN192	1	9	50%
(6)	Flavan-3-ols	GLN189 (3.11 Å)	MET49, HIS163, HIS164, CYS145, HIS41, MET165, GLU166, PRO168, SER144,	1	9	60%
(7)	Epicatechin	HIS163 (3.34 Å) GLU166 (3.24 Å) SER144(3.21 Å) LEU141(3.09 Å)	CYS145, MET165, GLN189, ARG188, PHE140	4	5	70%
(8)	Chalcones	-	HIS163, CYS145, MET165, HIS164, LEU167, SER144, GLU166, GLN189, PRO168, ASP187, ARG188, MET49	0	12	80%
(9)	Apigenin	TYR54 (2.95 Å) GLN192 (3.34 Å) ASP187 (3.08 Å)	MET165, THR190, LEU167, GLN189, PRO168, GLU166, ARG188, HIS41	3	8	50%
(10)	Chloroquine	-	HIS163, CYS145, HIS164, ASP187, HIS41, MET165, AG188, GLN189, PHE140, THR190, LEU167, GLU166, HIS172,	0	11	11%

4. Conclusion

The inhibitory impact of Flavonoids against SARS-CoV-2 M pro virus was investigated in this study. According to the DFT calculations, all of the compounds are thermally stable, and Flavonoids have higher chemical reactivity than the well-known medication Chloroquine. Flavonoids have high dipole moment with a small energy gap than Chloroquine. The docking results further revealed that Flavonoids with lower binding affinities than Chloroquine are more effective SARS-CoV-2 M pro-viral inhibitors. Herein, we came to the conclusion that of all the derivatives of Flavonoid, Flavonol with free energy -803.51 eV and dipole moment

3.40 db shows least HOMO-LUMO gap (3.84ev), highest softness 0.52 ev and least hardness

1.92 and high electrophilicity 4.83. The Flavonol with higher binding energy (6.63kcal/mole) than Chloroquine efficiently interacted hydrophobically with the protein, with the residues Gln189, Met165, Asp187, Arg188, His41, Pro168,Gln 189, Thr190 and Leu167. The polar interactions were found to be with Glu166 (3.34 Å). Thus establishing itself more potent inhibitor than Chloroquine. This research could help researchers better understand the physiochemical and biological characteristics of Flavonoids against this new coronavirus.

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