RESEARCH ARTICLE

Phytochemical 6-Gingerol – A promising Drug of choice for COVID-19

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ABSTRACT: Recently, a novel corona virus (COVID-19), identified as one of the acute respiratory syndrome corona virus (SARS CoV-2) and emerged as a pandemic disease in Asia and European countries in 2020. The World Health Organization (WHO) has declared the current outbreak as a global public health crisis. Due to the variability in the amino acid and amino acid sequences, it does not develop suitable vaccines against the viral proteins. Hence, the inhibitor to be developed against the viral proteins of the corona virus is a promising idea to develop structure-based drugs from the photochemical compounds. Here, the novel drug was identified and well studied against the viral receptors by using the molecular docking technique. Phytocompound 6-gingerol possesses excellent drug likeliness with zero violations and very good pharmacokinetic properties with the highest binding affinity ranging from -2.8764 KJ/mol to -15.7591 KJ/mol with various COVID-19 viral protein targets. Our study reveals that 6-gingerol from ginger could act as a promising drug of choice to treat COVID-19.

KEYWORDS: SARS-CoV-2; COVID-19; 6-Gingerol, corona virus; respiratory infection.

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1. INTRODUCTION

Corona virus is the group of viruses that have a crown-like appearance when viewed under the electronic microscope. Corona viruses cause respiratory tract infections in humans, which can cause a wide range of illnesses from the mild common cold to lethal SARS and MERS. There are no vaccines and anti-viral drugs are available yet to treat corona viral infections. Corona virus possesses positive sense single-stranded enveloped RNA as their genetic material. The genome of corona virus is the largest group among the viruses is host specific which is based on the receptor specificity of their S-Protein. It is an enveloped virus that is made up of glycoprotein.

In December 2019, the novel corona virus (2019-nCoV or SARS CoV-2), cause corona virus disease 2019 (COVID-19) in humans, was an outbreak in Wuhan, China. The epidemic disease of SARS CoV-2 in Wuhan has occurred human to human transmission among close contacts, which becomes emerged as a pandemic disease from January 2020 spread through international travelers across the different countries and enters almost all the countries except few of them. As of 10th April 2020, there have been over 1.6 million cases with over 0.1 million deaths for the COVID-19 outbreak worldwide [1] However, there are currently no effective medications against SARS CoV-2. Several national and international research groups are working on the development of vaccines to prevent and treat the SARS CoV-2, but effective vaccines are not available yet. There is an urgent need for the development of effective prevention and treatment strategies for SARS CoV-2 outbreak. Indian people are consuming Indian traditional medicinal herb extract and Indian spices to boost the immune system to fight COVID -19.

Ginger (*Zingiber officinale*) is the herbaceous plant native to South Asia belonging to the family of Zingiberaceae. The characteristic pungent flavor of the ginger rhizome is used extensively in foods and beverages [2]. Ginger is a common Indian spice and traditional medicinal plants have important pharmacologic activities such as antibacterial, antiviral, anti-hypertensive, antioxidant, analgesic and antipyretic properties [3]. Ginger has been proved to be effective on various viruses [4-8]. Ginger rhizome possesses several outstanding bioactive non-volatile phenolic compounds such as gingerols, paradols, shogaols, and zingerones [9]. Ginger is one of the best choices of bioactive Phyto-compound in traditional medicine in Ayurvedic, Chinese and Unani systems to treat different diseases in ancient times.

6-gingerol is a bioactive phenolic phytocompound found in the fresh ginger rhizome. 6-gingerol is a promising drug candidate to treat various diseases associated with inflammation,

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cancer, and viral disease. Fresh ginger possesses anti-viral activity against human respiratory syncytial virus due to the presence of bioactive phenolic phytocompound 6-gingerol [10]. Hence, the present study aims to examine phytocompound 6-gingerol from the ginger plant (*Zingiber officinale*) that could act as a promising drug against COVID-19 proteins and screened through *in silico* approach.

MATERIALS AND METHOD

1.1. Ligand generation

The 2D structure of *Zingiber officinale* phytocompound 6-gingerol (CID 442793) was retrieved from Pubchem, a database of chemical molecules [11]. The retrieved 2D SDF file format of 6-gingerol was submitted to "Online SMILES convertor and Structure file generator" [12] and converted into 3D SDF format.

1.2. Drug Likeliness and Absorption, Distribution, Metabolism, Extraction and Toxicity [ADME] Calculations

Swiss ADME online server was used to calculate Drug Likeliness parameters. Drug likeliness of the phytocompounds gingerol was examined based on Violations of the following rules such as Lipinski, Ghose, Veber, Egan, and Muegge. Pharmacokinetic properties of phytocompounds gingerol were screened by preADMET is a web-based application to determine the pharmacological efficiency of phytocompounds. PreADMET predicts the various parameters associated with ADME and toxicity behavior of phytocompounds.

1.3. Preparation of Receptor and its Binding Site

Novel corona viral (SARS-CoV-2 or COVID-19) Proteases, Spike protein, RNA binding protein, N-terminal RNA binding domain are the key viral molecules involved in attachment and replication and reproduction of viral particle in the human host cells. These Protein target molecules served as a novel target to inhibit the viral lifecycle in human host cells. Three-dimensional structures of SARS CoV-2 nine molecules of main proteases (5R7Y, 5R80, 5R81, 5R83, 5R84, 6LU7, 6LVN, 6M03, 6Y84), one spike protein (6VSB), one RNA binding protein (6W4B) and one N-terminal binding domain (6M3M) was retrieved from RCSB PDB database (https://www.rcsb.org/) [13].To determine the binding affinities between the ligand and receptor, the amino acids with the binding pockets were predicted at the Q-site finder server [14].

1.4. Flexible docking

The generated gingerol SDF structures were docked with the predicted binding site of all selected protein target binding site by using teaching version of FlexX [15] with following parameters i) default general docking information ii) base placement using triangle matching, iii) scoring of full score contribution and threshold of 0.70 iv) Chemical parameters of clash handling values for protein-ligand clashes with maximum allowed overlap volume of 2.9 A03 and intra-ligand clashes with clash factor of 0.6 and considering the hydrogen in internal clash tests. v) Default docking details values of 200 for both the maximum number of solutions per iteration and a maximum number of solutions per fragmentation.

1.5. Prediction of ligand-receptor interactions

The interactions of phytocompound gingerol with twelve SARS CoV-2 proteins targets in the docked complex were analyzed by the pose-view of LeadIT [16]. 2D and 3D pose view of SARS COV-2 protein target-phytocompound gingerol was generated and analyzed using LeadIT.

1.6. Density Functional Theory DFT Analysis

DFT calculation for phytocompound gingerol was performed using Gaussian 09 software. DFT used to calculate HOMO–LUMO orbital providing energy gap between highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) providing high and low electron density regions on the compounds.

2. RESULT AND DISCUSSION

2.1. Drug Likeliness and ADME Calculation

Drug likeliness calculation for phytocompound gingerol was made in Swiss ADME server and revealed that 6-gingerol possess a molecular weight of 294.39 g/mol, the number of hydrogen bond acceptor and donor are 4 & 6 respectively. Gingerol also possess excellent TPSA(topological polar surface area), lipophilicities (iLog P) and water solubility (Log S ESOL) values of 66.76, 3.48 & -2.96 respectively which proved there are nil (zero) violations for drug likeliness rules such as Lipinski, Ghose, Veber, Egan, and Muegge essential for better drug likeliness properties

ADME property of gingerol calculated by preADMET web-based application revealed that very good pharmacokinetic properties such as absorption, bioavailability and distribution parameters of 6-gingerol such as HIA 86.75%, the pure water solubility of 0.3460 mg/ml, 100% plasma protein binding, which are tabulated and presented in Table 1.

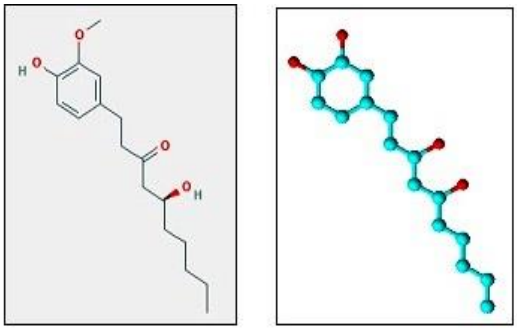


Figure 1- 2D & 3D structures of 6-Gingerol

Table 1 - ADME properties of 6-gingerol from ginger plant

ABSORPTION	
Human intestinal absorption (HIA, %)	86.75456
Caco-2 cell permeability (nm/sec)	13.5496
MDCK cell permeability (nm/sec)	0.627041
skin permeability (logKp, cm/hour)	-2.36594
BIOAVAILABILITY	
Buffer solubility (mg/ml)	0.7135
Pure water solubility (mg/ml)	0.3460
DISTRIBUTION	
Plasma protein binding (%)	100
Blood-brain barrier penetration	0.69481

2.2. Docking Study

Different COVID-19 target proteins (proteases, spike protein, RNA binding protein) and their docking score and 3D pose with 6-gingerol are presented in Table 2 and their detailed molecular interaction between them is tabulated and presented in Table 3 & Figure 2.

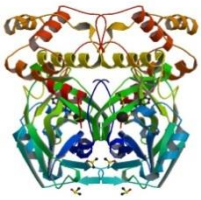
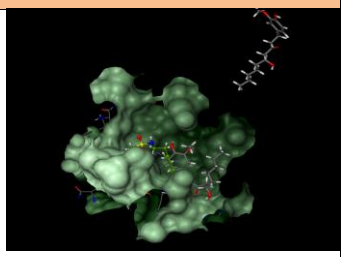
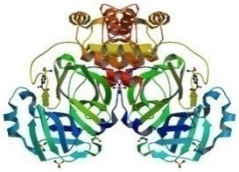
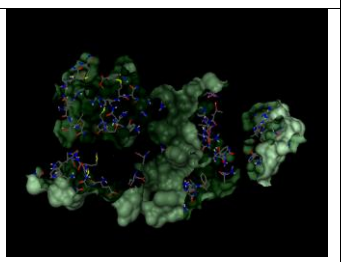
The results of a flexible docking study by flexX software between COVID-19 viral targets and gingerol was exhibit the binding affinity and docking score ranging from -2.8764 KJ/mol to -15.7591 KJ/mol. Gingerol exhibit the highest binding affinity (-15.7591 KJ/mol) with 5R7Y COVID-19 main protease essential for replication and reproduction of SARS Cov-2. Corona Viral protease 5R7Y residues such as His 164, Glu166, Thr190, Gln192 from hydrogen-bonded interaction with phytocompound gingerol, it also forms form non bonded interaction with the residues of His164, Met 165, Glu166, Leu167, Pro168, Arg188, Gln189, Thr190.

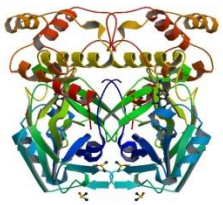
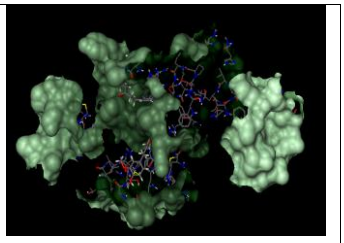
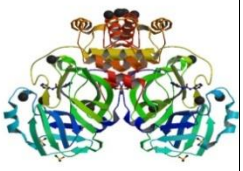
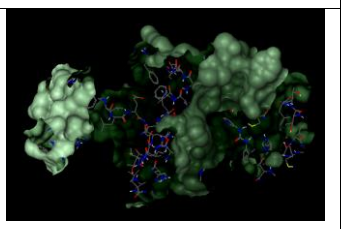
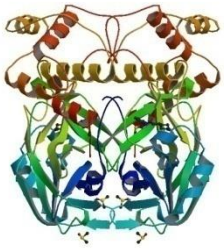
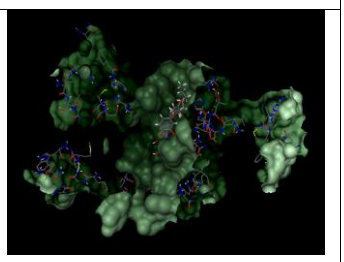
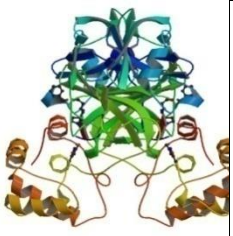
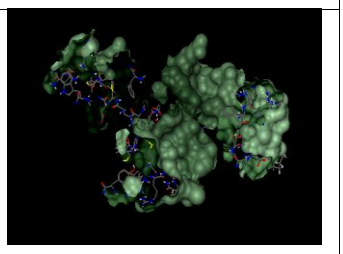
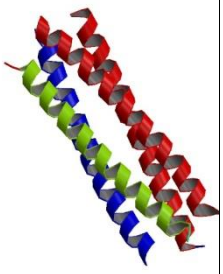
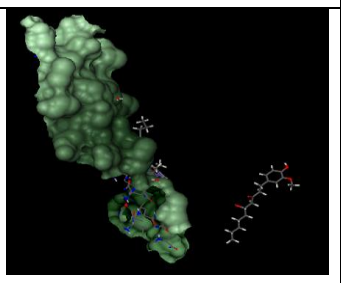
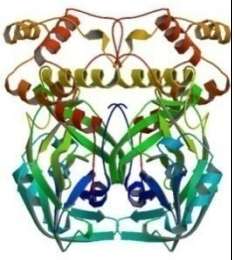
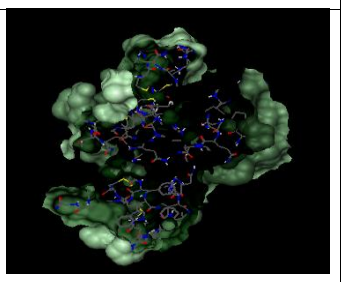
Gingerol exhibits binding affinity of -11.4082 KJ/mol, -12.9523 KJ/mol and -12.8835 KJ/mol with COVID-19 viral RNA binding protein (6W4B), N-Terminal RNA Binding Protein (6VSB), Spike glycoprotein (6M3M) respectively. Molecular interaction between COVID-19 viral spike glycoprotein with Gingerol forms hydrogen-bonded interaction with Glu63, Arg89, Thr92, Asp129 residues and form non bonded interaction with Glu63, Lys66, Arg89, Thr92, Leu168, Pro169 residues of COVID-19 spike glycol protein.

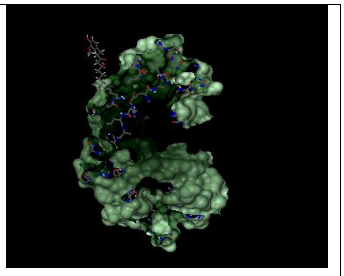
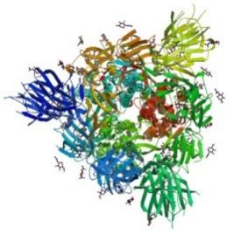
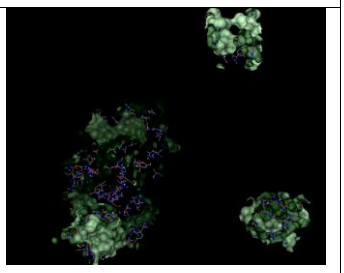
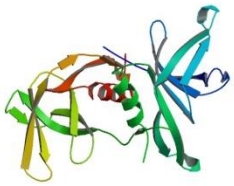
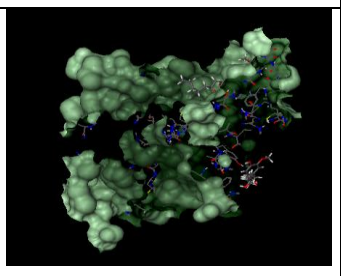
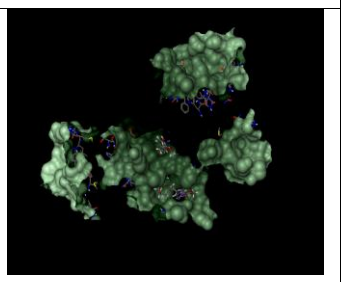
Molecular interaction between COVID-19 viral RNA binding protein with Gingerol makes hydrogen bonded interaction with Val42, Pro58, Ser60, Thr68 residues and form non bonded interaction with Arg40, Phe41, Val42, Phe57, Pro58, Lys59, Ser60, Ile66, Thr68, Ile92 residues of COVID-19 spike glycol protein.

The previous study reported that several phytocompounds of flavonoids and phenolic substances possess antiviral activities [17-19] especially 6-gingerol possess antiviral activities. Siti Khaerunnisa et al. [20] reported that 6-gingerol binds with the COVID-19 main protease active sites with the binding affinity of -5.40 K.Cal/mol.

Table 2 . COVID-19 Proteins Targets and its 3D Docking Pose with 6-gingerol

S.No	COVID-19 Protein Targets	Details	Protein 3D Structure	Docking Score (KJ/mol)	Docking
1	5R7Y	Crystal Structure of COVID-19 main protease in complex with Z45617795		-15.7591	
2	5R80	Crystal Structure of COVID-19 main protease in complex with Z18197050		-7.0885	

3	5R81	Crystal Structure of COVID-19 main protease in complex with Z1367324110		-8.2021	
4	5R83	Crystal Structure of COVID-19 main protease in complex with Z44592329		-7.4778	
5	5R84	Crystal Structure of COVID-19 main protease in complex with Z31792168		-9.5168	
6	6LU7	COVID-19 main protease in complex with an inhibitor N3		-2.8764	
7	6LVN	Structure of the 2019-nCoV HR2 Domain		-4.5961	
8	6M03	The crystal structure of COVID-19 main protease in apo form		-8.6837	

9	6M3M	SARS-CoV-2 nucleocapsid protein N-terminal RNA binding domain		-12.9523	
10	6VSB	Prefusion 2019-nCoV spike glycoprotein with a single receptor-binding domain up		-12.8835	
11	6W4B	Nsp9 RNA binding protein of SARS CoV-2		-11.4082	
12	6Y84	SARS-CoV-2 main protease with unliganded active site (2019-nCoV, coronavirus disease 2019, COVID-19)		-9.7625	

2.3. DFT Study

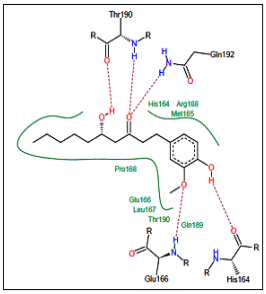
DFT study is used to explain the accurate structural and electronic properties of phytocompounds. The electronic distribution phytocompound 6-gingerol could provide a clear picture of SARS CoV-2 protein-gingerol interactions, which will be useful to explore the inhibition potentials of the phytocompound 6-gingerol. HOMO and LUMO orbital energies of 6-gingerol are shown in Figures 3 & 4. The calculated various HOMO-LUMO orbital energies are presented in Table 4.

The localization of HOMO and LUMO orbitals in the compound is very important in intermolecular interactions with SARS CoV-2 protein targets. 6-gingerol (-0.20606eV, 0.10303eV, and 9.3187eV) showed more stability and biological activity as it shows less energy gap, low hardness, and more softness. Thus the DFT calculations performed here better evidence highest binding affinity of 6-gingerol with SARS CoV-2 protein targets in molecular docking.

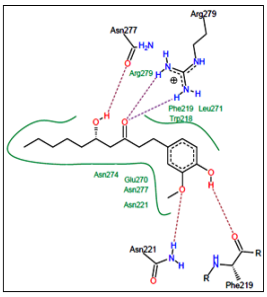
Similar comparative DFT study of phytochemical constituents of present in the bark extract of *Ficus racemosa* β-Amyrin (-0.06277eV, 0.031385eV and 31.86235eV) showed more stability and biological activity as it shows less energy gap, low hardness, and more softness followed by Betulinic acid (-0.23987eV, 0.119935eV and 8.33784eV) and Stigmasterol (-0.26925 eV, 0.134625 eV and 7.42804 eV) [21]. It was possible to characterize the compounds as well as their characteristics of electron donor/electron acceptor compared with other biological properties of the phytocompound by DFT analysis.

Table 3 .Molecular interaction of 6-gingerol with different COVID-19 Protein targets

S.No.	COVID-19 Proteins Target PDB CODE	Gingerol and COVID-19 Target Protein Interactions	
		Hydrogen-Bonded Interactions	Non-Bonded Interactions
1	5R7Y	His 164, Glu166, Thr190, Gln192	His 164, Met 165, Glu166, Leu167, Pro168, Arg188, Gln 189, Thr190
2	5R80	Phe 219, Asn 221, Asn 277, Arg 279	Trp218,Trp219, Asn221, Glu270, Leu271, Asn274, Asn277, Arg279
3	5R81	Asp295,Arg298,Gln299,Thr304	Met6, Phe8,Pro9, Ile152, Tyr154, Arg298, Val 303, Thr304
4	5R83	Thr25, His41, Ser46, Gly143	-
5	5R84	Pro108,Gln110,His246	Pro108,Gly109,Gln110,Pro132, Ile200,Val202,Glu240,Ile249, Phe294
6	6LU7	Glu270,Gly275,Arg279	Phe223,Glu270,Leu271,Asn274
7	6LVN	Gln13,Asn20,Lys24	Gln13,Ile16,Asn20,Ala23,Lys24,Asn27
8	6M03	Gln110,Thr111,Phe294	Phe8,Gln110,Asn151,Phe294,Arg298,Val303
9	6M3M	Glu63, Arg89, Thr92, Asp129	Glu63, Lys66, Arg89, Thr92, Leu168, Pro169
10	6VSB	Gln773, Gln954, Ile1013, Arg1019	Glu773, Gln954, Ile1013, Arg1014, Glu1017, Arg1019
11	6W4B	Val42, Pro58, Ser60, Thr68	Arg40, Phe41, Val42, Phe57, Pro58, Lys59, Ser60, Ile66, Thr68, Ile92
12	6Y84	Met6, Ile152, Tyr154	Met6, Phe8, Pro9, Ile152, Tyr154, Arg298, Gln298, Val303, Thr304

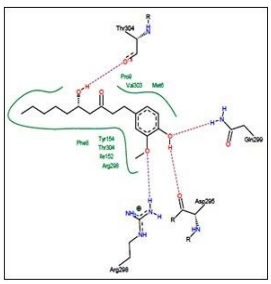


(a)

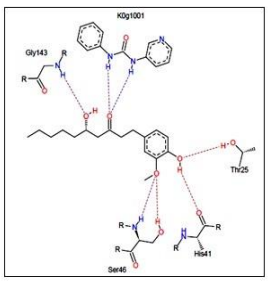


(b)

Figure 2 – Interaction plot for 6-Gingerol with COVID-19 Protein Targets (a-l)
a).Interaction of Gingerol with 5R7Y (-15.7591 KJ/mol) b).Interaction of Gingerol with 5R80 (-7.0885 KJ/mol)

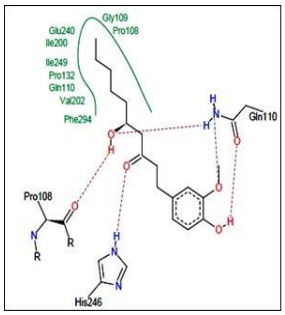


(C)

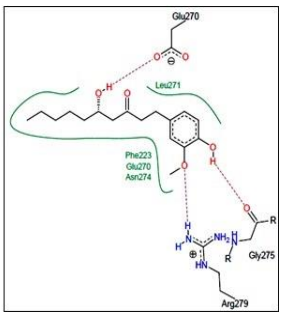


(d)

C).Interaction of Gingerol with with 5R81 (-8.2021KJ/mol)
d).Interaction of Gingerol with with 5R83 (-7.4778KJ/mol)

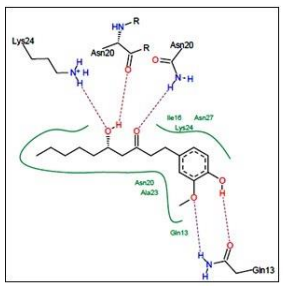


(e)

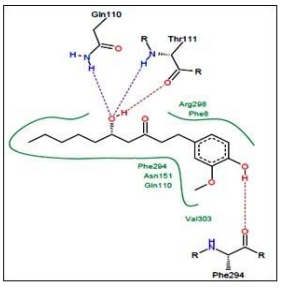


(f)

e).Interaction of Gingerol with 5R84 (9.5168KJ/mol)
f).Interaction of Gingerol with 6LU7 (-2.8764KJ/mol)

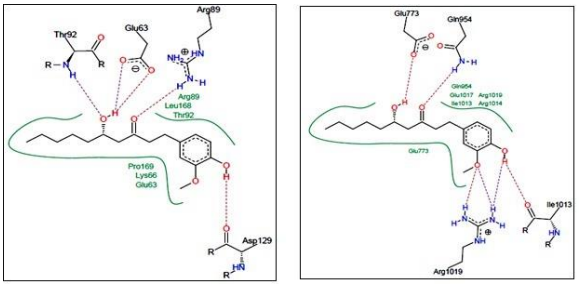


(g)



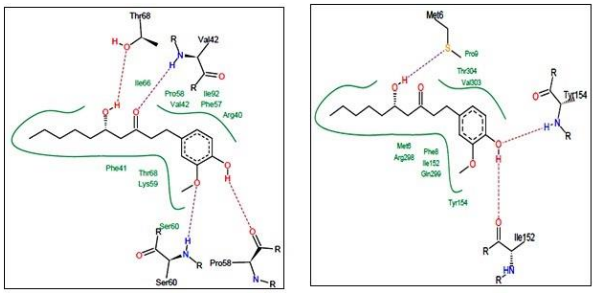
(h)

g).Interaction of Gingerol with 6LVN (-4.5961KJ/mol)
h).Interaction of Gingerol with 6M03 (-8.6837 KJ/mol)



(i) **(j)**

(i)Interaction of Gingerol with 6M3M (-12.9523 KJ/mol)
(j)Interaction of Gingerol with 6VSB (-12.8835KJ/mol)



(k) **(l)**

(k)Interaction of Gingerol with 6W4B (-11.4082 KJ/mol)
(l)Interaction of Gingerol with 6Y84 (-9.7625 KJ/mol

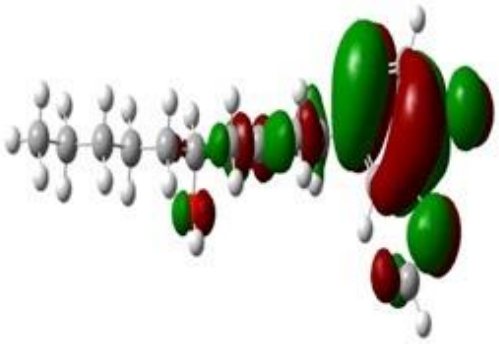


Figure 3 . HOMO orbital energy for 6-Gingerol

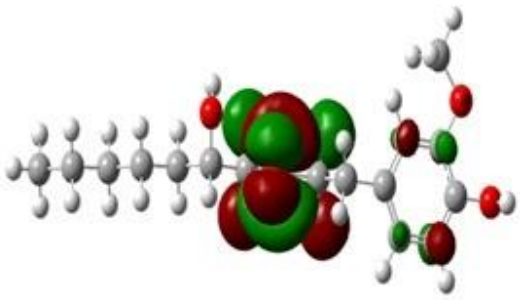


Figure 4.LUMO orbital energy for 6-Gingerol

3. CONCLUSION

The present study was attempted to prove that phytocompound 6-gingerol from *Zingiber officinale* acts as a promising drug to treat COVID-19. 6-Gingerol possesses excellent drug likeliness parameters with zero violations of different rules and very good ADME pharmacokinetic properties. Finally, 6-gingerol proves anti-viral efficiency against SARS CoV-2 by showing the highest binding affinity and interaction with multiple targets of COVID-19 including Viral proteases, RNA binding protein, Spike protein. DFT study finally evidences the reason behind the highest binding affinity between 6-gingerol and COVID-19 protein targets. The present study proves that 6-gingerol from the ginger plant could be served as a promising drug to treat the novel COVID-19.

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Compound	HOMO	LUMO	Energy Gap	Ionization potential(IE)(eV)	Electron affinity (EA(eV)	Electro negativity (χ)(eV)	Electro chemical potential (μ)(eV)	Hardness (η) (eV)	Softness (σ)(eV)
6-Gingerol	-0.21034	-0.00428	-0.20606	0.21034	0.00428	0.10731	-0.10731	0.10303	9.3187

Table.4. HOMO LUMO Orbital Energies for 6-Gingerol through DFT calculations

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