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Epigallocatechin-gallate and Theaflavin-gallate interaction in SARS CoV-2 spike-protein central-channel with reference to the hydroxychloroquine interaction: Bioinformatics and Molecular Docking Study.

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Short title: Tea-flavonoids bind COVID-19 spike-proteins

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Abstract

SARS CoV-2 or COVID-19 pandemic global-outbreak created the most unstable situation of human health-economy. Last two decades different parts of the word experienced smaller or bigger outbreak related to human-coronaviruses. The spike-glycoproteins of the COVID-19 (similar to SARS-CoV) attach to the angiotensin-converting-enzyme (ACE-2) and transit over a stabilized open-state for the viral-internalization to the host-cells and propagate with great efficacy. Higher rate of mutability makes this virus unpredictable/less-sensitive to the protein/nucleic-acid based-drugs. In this emergent situation, drug-induced destabilization of spike-binding to RBD could be a good strategy. In the current study we demonstrated by Bioinformatics (CASTp: Computed-Atlas-of-Surface-Topography, PyMol: molecular-visualization) and Molecular docking (PatchDock) experiments that tea flavonoids catechin-products mainly EGCG or other like theaflavin gallate demonstrated higher Atomic Contact Energy (ACE), surface area and more amino-acid interactions than hydroxychloroquine (HCQ) during binding in the central channel of the spike-protein. Moreover, out of three distinct binding-sites (I, II and III) of spike core when HCQ binds only with site III (farthest from the nCoV-RBD of ACE2 contact), EGCG and TG bind all three sites. As because site I and II is in closer contact with open state location and viral-host contact area so these drugs might have significant effects. Taking into account the toxicity/side-effects by CQ/HCQ, present drugs may be important. Our laboratory is working on tea flavonoids and other phytochemicals in the protection from toxicity, DNA/mitochondrial damage, inflammation etc. The present data might be helpful for further analysis of flavonoids in this emergent pandemic situation.

Key Words: SARS CoV-2 or COVID 19, pandemic global-outbreak, Spike glycoprotein, ACE2, PatchDock, hydroxychloroquine

Introduction

SARS CoV-2 (COVID-19) has created a global health crisis. This pandemic-threatening closely resembles the SARS CoV outbreak occurring in 2003 [1]. But the present one is highly spreading with extremely higher degree of virulence. As of today, it killed more than 96 thousand people from a total 16,000,00 infected people in 210 countries [2]. Global economy has been in a jerk under a 2 months or even more period of lockdown in all most all places of globe. So the therapeutic/preventive intervention is an immediate requirement and challenging act against this highly stable and frequently mutable viral strain. There are no established preventive/ therapeutic measures against this infection. Some of the old drugs are used on the basis of previous experiences from similar kind of infections. Some tests have been done in some in vitro model with inclusive results. As for example, one recent report suggests that remdesivir and Chloroquine effectively inhibit this infection in an in vitro experimental model [3-5]. But it is now considered that chloroquine has a high level of toxicity and hydroxychloroquine is predicted to be less toxic than it [6]. In a trial and survey type experiment with a very small sample size it is shown, hydroxychloroquine treatment is significantly associated with viral load reduction/disappearance in COVID-19 patients and its effect is reinforced by azithromycin [7]. Out of few combination of medication, presently HCQ is being used in COVID-19 cases [3]. But this drug also has a varied range of toxicity [4]. Apart from that, this drug is also used in systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), so, strategies should be taken to resolve the issue of sufficient supply of this medicine for these patients also [8]. When there has almost nothing to do, chloroquine group of drugs are of use, but alarming call has been raised by scientists and clinicians for the wide range of side effects of this drug [8]. Beside lung, liver and the kidney tissues also face a significant degree of injuries and in this situation use of drugs needs critical analysis and careful approach [9,10]. Other drugs like remdesivir, lopinavir, ribavirin and ritonavir have shown efficacy to inhibit coronavirus in vitro. Teicoplanin, an antibiotic used to treat staphylococcal infections has been shown to inhibit MERS-CoV in human cells. So, this drug may be rechecked in the present situation also [11]. Molecular docking results suggest that Sofosbuvir, Galidesivir, and Tenofovir; few drugs can be used against SARS-CoV-2 RNA Dependent RNA Polymerase inactivation [12].

Our laboratory has been working for last several years on the tea *(Camelia sinensis)* flavonoids for their different therapeutic and disease protective roles. Tea flavonoids have been demonstrated as strong anti-toxicant, anti-oxidant and anti-inflammatory agents. Anti-tumerigenic role of catechin derivatives especially EGCG has been shown decisively by several laboratories [13,14,15]. Regarding the anti-viral role tea flavonoids have been shown to be strong agent [16]. Report demonstrates that catechin can prevent influenza A (H1N1) virus infection and gallic acid can inhibit influenza virus infection [17]. Last three decades several studies suggested that the regular consumption of green tea decreases influenza infection and some cold symptoms. The gargling with tea extracts and/or flavonoids may protect from influenza virus [18]. EGCG-fatty acid derivative is important because fatty acid on the phenolic hydroxyl group increase viral and cellular membrane permeability which protects from viral infection [19]. Preliminary attachment to cellular glycans is a critical step for entry of several human viruses. Some viruses, such as herpes simplex virus type 1 (HSV-1) and hepatitis C virus (HCV), bind to heparan sulfate, whereas others, such as influenza A virus (IAV), bind to sialic acid [20]. The SARS CoV-2 is also covered by a large number of NAG molecules.

In this background, we have been intended to test different flavonoid effects in the process of SARS CoV-2 spike glycoproteins binding to its host cell receptor ACE 2. In the present bioinformatics, cheminformatics and molecular modeling study we initially characterized the nature of nCoV and ACE-2 binding and surface contact areas. The role of specific amino acids and their binding energy have been evaluated. Further, different Flavonoids have been docked on two targets; nCoV spike proteins and host cell receptors and all the bindings were carefully characterized. The current study will help to screen some suitable drug that can block the viral binding and further host cell attachment/entry.

Materials and Methods

Protein Structure Retrieval

The electron microscopic structure of human coronavirus spike glycoprotein (PDB ID: 6vsb) and X-ray diffraction structure of human angiotensin converting enzyme 2 or ACE 2 (PDB ID: 4aph) were retrieved from RCSB Protein Data Bank (https://www.rcsb.org/) in PDB format. The structure 6vsb was selected because it was found with number of N-acetyl glucosamine (NAG) attached in different locations of the protein. On the other hand ACE 2 (PDB ID: 4aph) was selected as it was a complete tertiary structures with no such viral spike protein contaminations. Other two viral spike protein structures were also retrieved for analysis like 6VXX and 6VYB.

Ligand Structure Retrieval

The three dimensional (3D) structures of different selected molecules like, Hydroxychloroquine, Catechin, Catechin gallate, Epicatechin 3-O-gallate, Epigallocatechin, Epigallocatechin 3-gallate, Gallocatechin, Gallocatechin gallate, Theaflavin monogallate (TFMG) and Theaflavin digallate (TFDG) were retrieved in .sdf format from world's largest chemical information database, PubChem (https://pubchem.ncbi.nlm.nih.gov/).

Preparation of both Receptor and Ligand Molecules

Receptor molecules like human coronavirus (COVID19) spike glycoprotein (PDB ID: 6vsb) and human ACE 2 protein (PDB ID: 4aph) were found with numerous water molecules with their structures. The structures were individually dehydrated using PyMol molecular visualization system and edited structures were saved in .pdb format. Additionally angiotensin II molecule was removed from human ACE 2 protein also. Ligand molecules were retrieved in .sdf format then they were saved as .pdb format using PyMol and used for further analysis. The chemical structures, Compound CID, Molecular formula (MF) and Molecular Weight (MW) of selected ligands were listed in table 1.

Surface Topology Calculation of Receptor Protein Molecule

CASTp: Computed Atlas of Surface Topography of Protein (http://sts.bioe.uic.edu/castp/index.html?j_5e8c7bec25090)

Molecular Docking

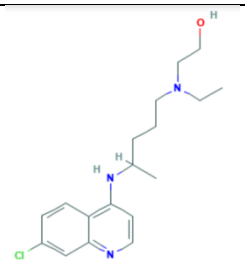
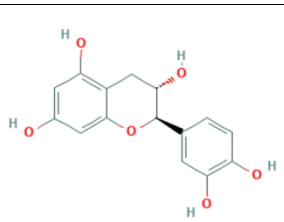
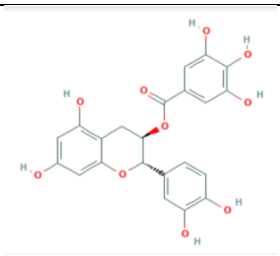
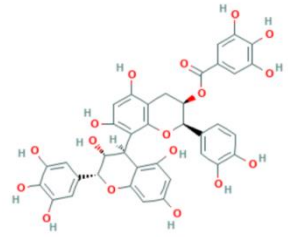
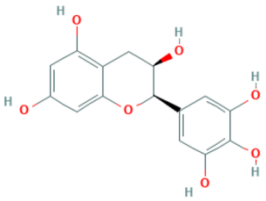
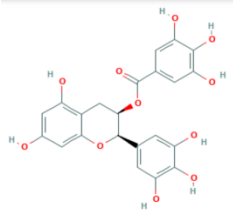
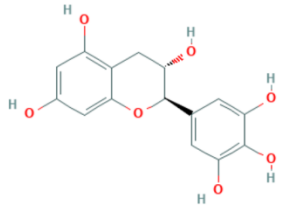
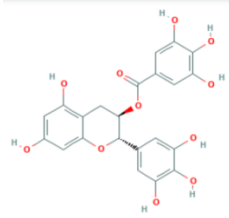
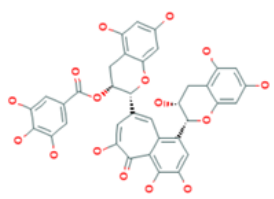
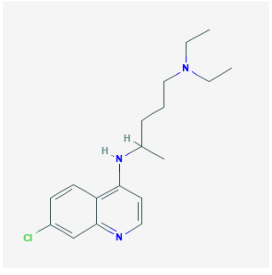
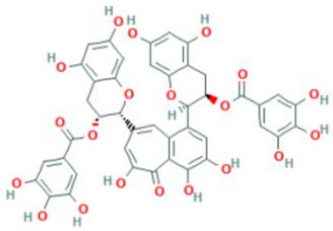
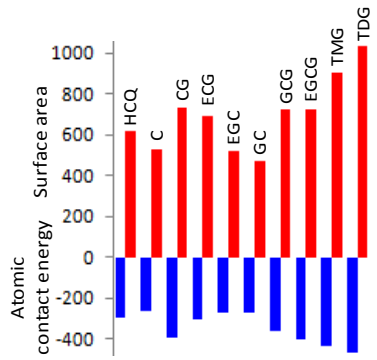
The molecular docking between human coronavirus spike protein – selected ligands and human ACE 2 protein – selected ligands were performed using PatchDock web server for interactive docking and simulation. PatchDock is developed as geometry-based molecular docking algorithm. It calculates the docking transformation between two molecules to get the best molecular interface complementarity which finds out the ligand posture in receptor with maximum interface area covered and minimum steric hindrance [21]. It also calculates the Atomic Contact Energy (ACE) Value of each docking positions to indicate the amount of required desolvation free energies to transfer the ligand molecule from water to protein (Receptor) interior.

Results and Discussions

A large body of evidences has clarified the health benefit of tea components catchin and theaflavin. Tea is a regular beverage that is consumed worldwide and specifically may be more in India, China and other parts of South East Asian countries. Moreover, daily food/ nutrients intake in these places are based on variety type of vegetable combinations, fruits and green leaves. These are highly enriched with phytochemicals, polyphenols like flavonoids. Earlier our laboratory has demonstrated the anti-carcinogenic role of green tea extract [13,14]. It decisively helps in DNA protections by scavenging free radicals and that efficacy was found to be even greater than the known specific radical-scavengers [14,15]. According to the relationships between structure and antiviral activity of catechin derivatives, the 3-galloyl and 5'-OH group of catechin derivatives appear critical to antiviral activities [22]. Preclinicalin vitroactivity of QR-435 green tea extract against influenza A virus as a virucide and in paper masks for prevention of viraltransmission. Masks impregnated with QR-435 (a flavonoid component) were highly effective in blocking the passage of live H3N2 virus [23]. In vivo prophylactic activity of QR-435 was demonstrated against H3N2 influenza virus infection [24].

The human coronavirus spike glycoprotein (PDB ID: 6vsb) and X-ray diffraction structure of human angiotensin converting enzyme 2 or ACE 2 (PDB ID: 4aph) were retrieved to analyze the attachment pattern, find out the types of amino acid involved in proper attachment, pre and post fusion posture of coronavirus spike

Table 1. *Chemical structure of selected ligands used for molecular docking study with Human ACE 2 and coronavirus spike protein.*

		
Hydroxychloroquine [Compound CID: 3652 ; MF: $C_{18}H_{26}ClN_3O$; MW: 335.9g/mol]	Catechin [Compound CID: 9064; MF: $C_{15}H_{14}O_6$; MW: 290.27g/mol]	Catechin gallate [Compound CID: 6419835 ; MF: $C_{22}H_{18}O_{10}$; MW: 442.4g/mol]
		
Epicatechin 3-O-gallate [Compound CID: 442678; MF: $C_{37}H_{30}O_{17}$; MW: 746.6g/mol]	Epigallocatechin [Compound CID: 72277; MF: $C_{15}H_{14}O_7$; MW: 306.27g/mol]	Epigallocatechin 3-gallate [Compound CID: 65064; MF: $C_{22}H_{18}O_{11}$; MW: 458.4g/mol]
		
Gallocatechin [Compound CID: 65084; MF: $C_{15}H_{14}O_7$; MW: 306.27g/mol]	Gallocatechin gallate [Compound CID: 199472; MF: $C_{22}H_{18}O_{11}$; MW: 458.4g/mol]	TFMG[Compound CID: 135458102]
		
Chloroquine [Compound CID 2719, MF: $C_{18}H_{26}ClN_3$, MW: 319.87g/mol	TFDG [Compound CID: 135403795; MF: $C_{43}H_{32}O_{20}$; MW: 868.7g/mol]	Number of galloyl group favors greater ACE, surface-area (table2), more contacting amino acids (fig4).

glycoprotein and finally used to find out potential spike protein blocker. Here we have selected green tea components like Catechin (CID: 9064), Catechin gallate (CID: 6419835), Epicatechin 3-O-gallate (CID: 442678), Epigallocatechin (CID: 72277), Epigallocatechin 3-gallate (CID: 65064), Gallocatechin (CID: 65084), Gallocatechin gallate (CID: 199472), Theaflavin monogallate (TFMG; CID: 135458102) and Theaflavin digallate (TFDG, CID: 135403795) along with hydroxychloroquine (CID: 3652) as molecular docker (Table1). The surfaces of all selected ligand were also reported in Table 1. All the selected ligands have minimum one benzene ring. The ligands were retrieved in 2D as well as 3D forms. 2D forms were used in Table 1.

Table 2. *Comparative analysis of Hydroxychloroquine, Catechin, Catechin gallate, Epicatechin 3-O-gallate, Epigallocatechin, Epigallocatechin 3-gallate, Gallocatechin, Gallocatechin gallate, TFMG and TFDG binding with ACE2 and nCoV 2 through molecular docking.*

Sl. No.	Compound	ACE (Atomic contact energy) value with nCoV2 (Area in Å^2)
1	Hydroxychloroquine	-293.32 (616.90)
2	Catechin	-266.41 (525.20)
3	Catechin gallate	-393.05 (732.90)
4	Epicatechin 3-O-gallate	-308.25 (689.60)
5	Epigallocatechin	-270.01 (523.40)
6	Epigallocatechin 3-gallate	-407.58 (723.90)
7	Gallocatechin	-274.72 (471.40)
8	Gallocatechin gallate	-364.16 (722.70)
9	TFMG	-434.42 (906.20)
10	TFDG	-465.17 (1034.60)

ACE value: Atomic Contact Energy value

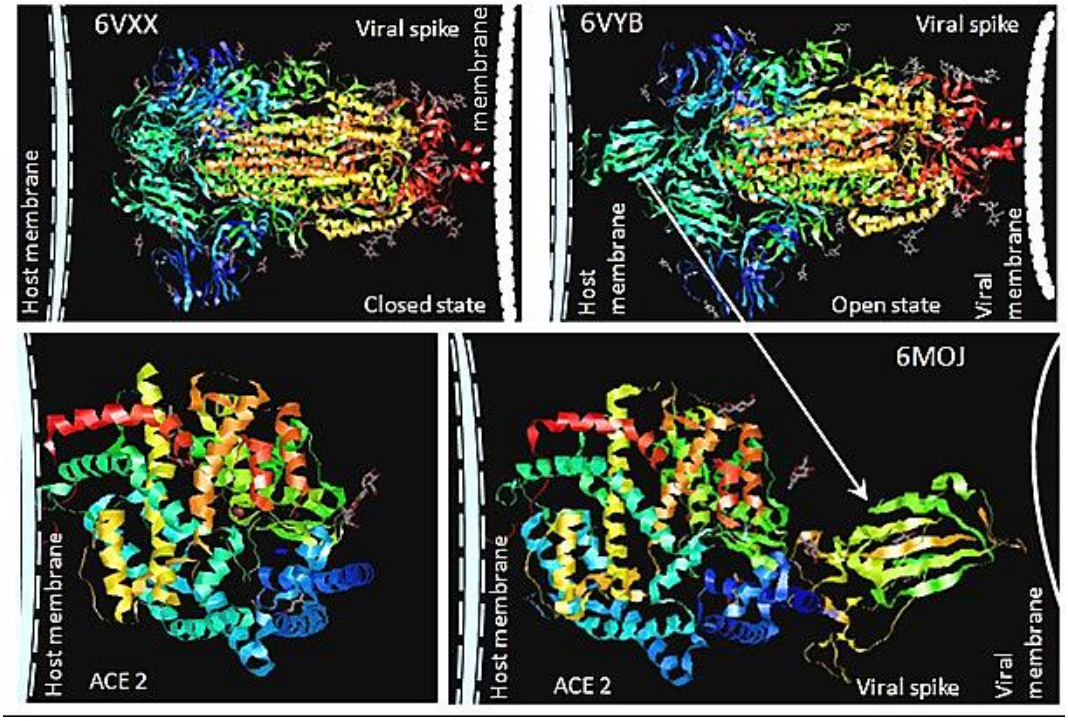


Figure 1. *Two different stages of coronavirus spike protein; closed state of receptor attachment site (a), open state of receptor attachment site (b). Structure of membrane bound viral receptor protein ACE 2 (c). Attachment of viral spike protein with membrane bound ACE 2 protein (d).*

According to literature human SERS COV-2 or COVID - 19 have 14-15 different conserved regions in their spike glycoproteins [25]. Among them S1 region is responsible for surface attachment and bond formation with human cell membrane receptor

ACE 2. During this attachment a significant conformational change was observed within the spike glycoprotein (Figure 1).

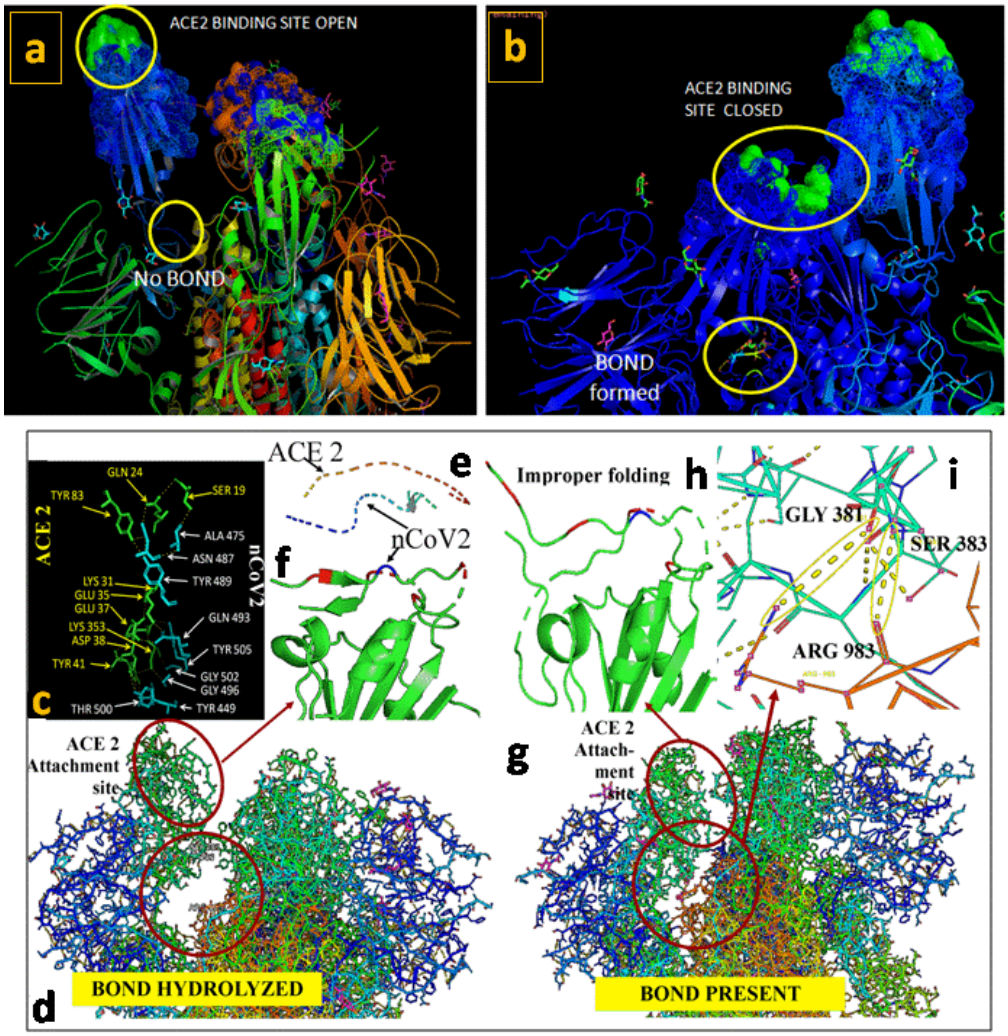


Figure 2. *Complete exposure of coronavirus spike protein (nCoV 2) attachment surface during opened state (a). Partial exposure of attachment surface during closed state (b). Attachment between ACE 2 and nCoV 2 stabilized by different stable and H-bond formation (c). Attachment site opened due to specific internal bond hydrolysis (d) which gives proper conformation to viral attachment site (e, f). Specific internal bonds stabilizes closed state (g) leading to improper folding of attachment site (h). The closed state is maintained by the H-bond formation among GLY 381, SER 383 and ARG 983 (i).*

PDB structure 6VXX showed a closed state structure unable to bind with host membrane. Whereas in PDB structure 6VYB a raised structure was observed that was an open state conformation of spike glycoprotein (Figure 1b). Viruses are host specific particulate. This specificity depends upon the presence of a particular receptor protein molecule with proper interactive site exposed out of the cell. This attachments lead to internalization of virus within the cell [26,27]. For COVID 19, ACE 2 plays that role through proper protein-protein interaction (Figure 1 c & d). The spike protein interaction is a result of internal hydrogen bond cleavage and formation of a hinge like structure (Figure 2a). With the cleavage of internal bonds, ACE 2 binding site is exposed at the top of the open conformation. Comparatively, in closed condition the interactive site remains embedded (Figure 2b). At the surface of ACE 2 and spike glycoprotein interaction, number of rigid as well as H-bonds forms (Figure c). So this attachment site demands a proper posture which is become ready after hinge region up conformation (Figure 2d, e, f). Whereas, specific bonds between ARG 983- GLY 3821 and ARG 983- SER 383 were observed in closed conformation (Figure 2g, h, i).

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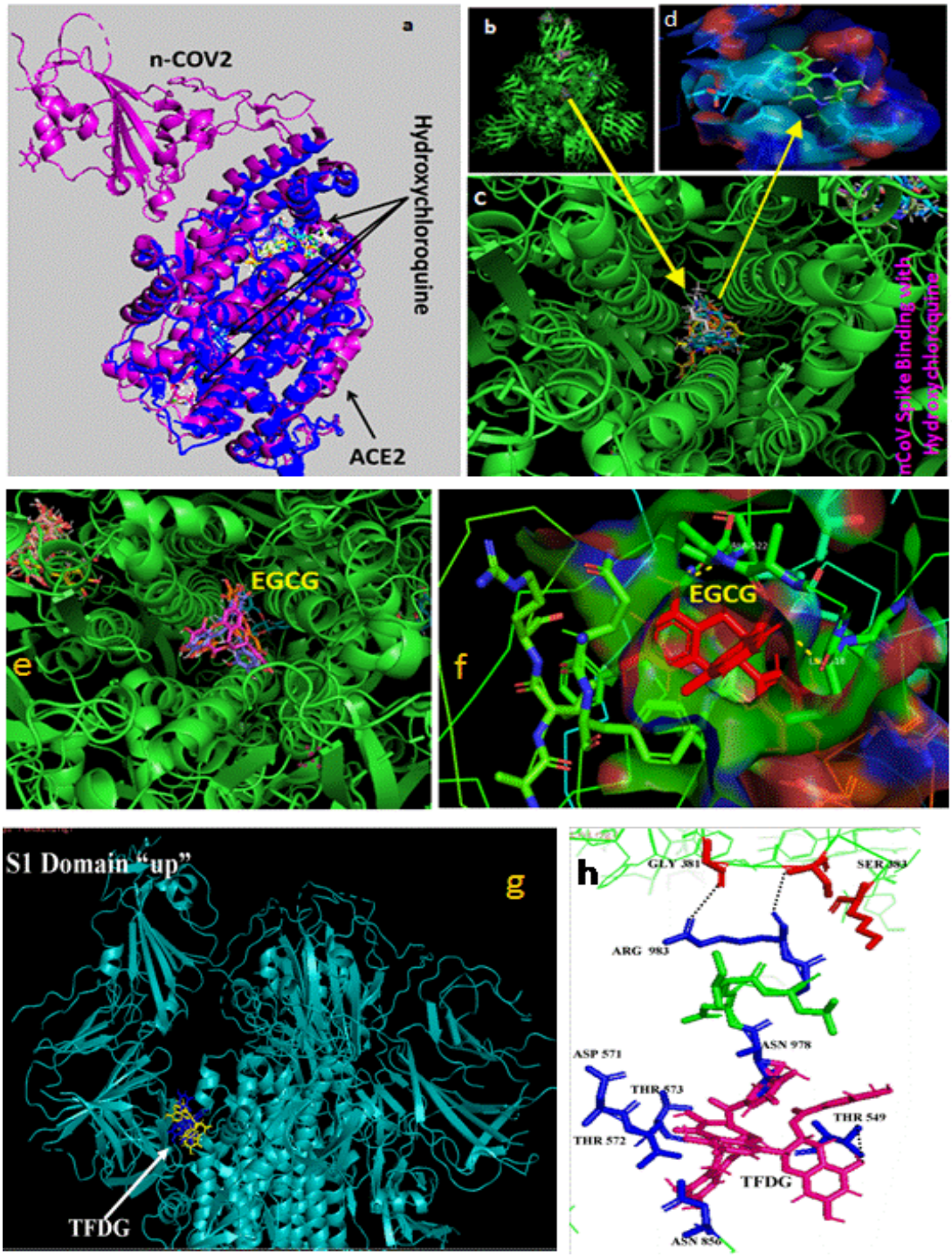

Figure 3. *Interaction of hydroxychloroquine with ACE 2 viral receptor protein (a). Best Docking Site of hydroxychloroquine within coronavirus spike protein central core region with ACE value -293.32 (b, c, d). Best docking site of TFDG at the hinge region of virus-receptor attachment site opening with ACE value -465.17 (g). TFDG forms rigid bonds with ASN 978, THR 549, ASN 856, THR 572, THR 573 and ASP578 near the ARG 983 (h).*

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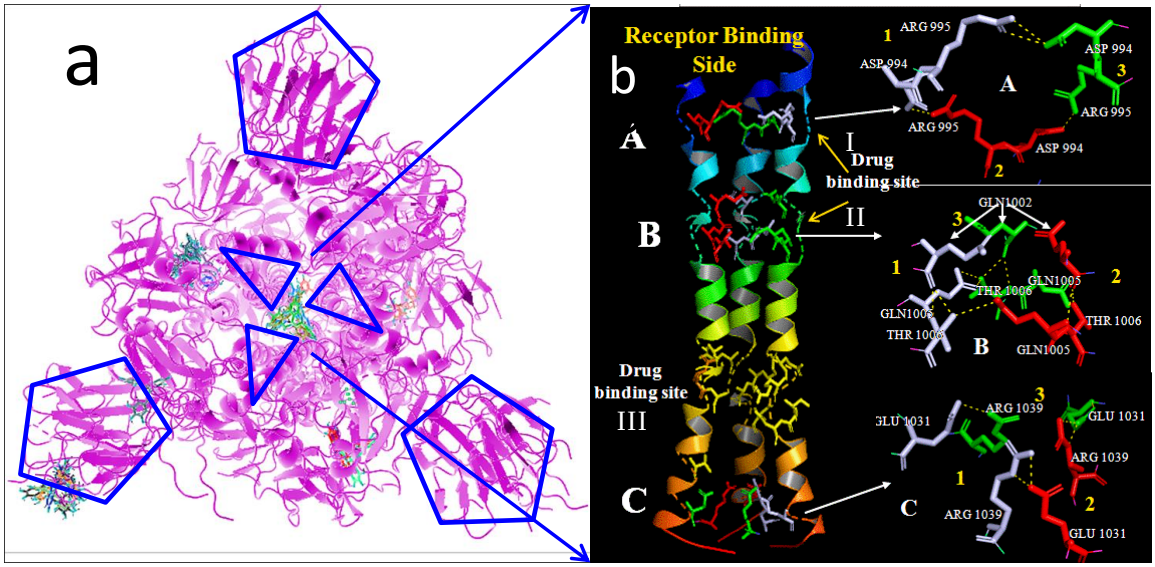
Molecular Docking Study

Before studying molecular docking (MD), surface area of ACE 2 receptor and Spike protein was calculated using CASTp: Computed Atlas of Surface Topography of Protein server [28]. ACE 2 was found with surface area: 2674.824; surface area volume: 2291.847 and spike protein was found with surface area : 33706.386; surface area volume: 63378.539 at their interior cavity.



Drug	Drug binding Site I	Drug binding Site II	Drug binding Site III
TFDG	**1**: GLN 1002 (NH2-O55; H), GLN 1002 (OE1-C44), THR 998 (H), ARG 995 (H), ASP 994 (3H), VAL 991 (H), TYR 756 (H); **2:** THR 998 (H), ARG 995 (H); **3:** THR 998 (H), ARG 995 (H), ASP 994 (3H), GLN 1002 (NH2-O55; H), GLN 1002 (OE1-C44) (ACE value -117.67)		**1**: THR 1027 (OG1-H7), ALA 1020 (CB-O50), ALA 1016 (C-O59); **2**: ASN 1023 (H), ALA 1020 (H), ARG 1019 (H); **3**: SER 1021 (H), ASN 1023 (H), ARG 1019 (H), ALA 1020 (H) (ACE value -417.62)
TFMG	1: ARG 995 (H), ASP 994 (H), TYR 756 (H), GLN 1002 (OE1-C14 & O33) 2: THR 1006 (H), GLN 1002 (OE1 & OE3- CD), THR 998 (C20- CB, C25-CG2), TYR 756 (H), ASP 994 (OD1, O41, OD2 - CG) 3: ASP 994 (H), TYR 756 (H), GLN 1005 (OE1-CD). (ACE value: -139)		1: GLU 725 (H), ASP 1041(H) 2. ALA 1020 (H), THR 1027(H), LEU 1024 (H), 3. LEU 1024 (H), ALA 1016 (H), ARG 1039 (H) (ACE value: -354)
Epigallo catechin 3-gallate	1: THR 998 (H), ARG 995 (H), TYR 756 (H); 2: THR 998 (CB – OG1, CG2- O), 2: PHE 970 (H) (ACE value: -122.95)		1: ARG 1039 (H), THR 1027 (H) 2: THR 1027 (CG2- BENZ-C), ARG 1034 (H) (ACE value: -151.90)
Hydroxy chloroquine	No such binding		1: ARG 1019 (2H) 2: ALA 1020 (C-CB) (ACE value: -285.65)

Figure 4. *Homotrimer coronavirus spike protein and its probable ligand binding site (a). Hydrogen bond stabilizing the central core of coronavirus spike protein (b). Different ligand-binding efficiencies in different drug binding-sites with atomic contact energy value. When ECGC and TG interact 3 different drug binding sites involving with more number of amino acids the HCQ interacts only on site 3 which is far from RBD involving with less number of amino acids.*

To find out potent viral spike protein destabilizing drug, MD study was performed using PatchDock where best 20 docking results for each interactions were considered for position specific docking posture analysis and selection of best one with lowest Atomic Contact Energy value calculation. All the selected drug molecules showed higher affinity to viral spike protein in comparison with ACE 2 receptor proteins (Table 2). Here hydroxychloroquine, the drug recently used for the treatment of COVID 19 on the basis of some previous inconsistent report showed ACE value of -154.06 with receptor molecule. But the highest affinity was observed for TFMG- ACE 2 interaction (-287.14). Epicatechin 3-O-gallate also showed affinity to receptor molecule (-265.13). Overall, the affinity of our several selected drugs towards the viral spike protein was observed to be very high. The highest value was observed for TFDG (-465.17) followed by TFMG (-434.42) and Epigallocatechin 3-gallate (-407.58). In contrast, Hydroxychloroquine was observed with ACE value of -293.32. ACE value indicated the energy required for the transfer of a molecule from water dissolve condition to desired protein's interior core [21]. From our result, lowest energy required for TFDG (-465.17) then gradually TFMG (-434.42) and Epigallocatechin 3-gallate (-407.58). Whereas, Hydroxychloroquine showed energy value of -293.32 for the binding to CoV spike. This indicated that transfer of Hydroxychloroquine to viral spike protein from dissolved state, requires more contact energy value.

The basic tendency of each 20 docking results for one combination was plotted in same receptor molecules. In this situation, hydroxychloroquine was observed at the interior core of ACE 2 receptor molecule. No influence was observed at or near spike protein binding site. Best Docking Site of hydroxychloroquine within coronavirus spike protein central core region with ACE value -293.32 (Figure 3b, c, d). Whereas, best docking site of TFDG at the hinge region of virus-receptor attachment site opening with ACE value -465.17 observed. The interior core region of viral spike protein was found to be occupied by hydroxychloroquine, EGCG and TFDG molecule. An attachment of TFDG molecule was observed at the hinge region of S1 up domain (Figure 3g) just beneath the ARG 983 binding site (Figure 3h). This ARG 983 is responsible for creating hydrogen bond with GLY 381 and SER 383 to maintain the close conformation of spike glycoprotein. In future TFDG could be used for the chemically fused therapeutic molecule synthesis for more strengthening the closed S1 conformation to avoid proper attachment of viral spike protein with receptor ACE2. Inhibitory effects of catechin-derivatives on the activities of HIV RT and cellular polymerases have been demonstrated [29]. Especially, EGCG significantly inhibits the HIV reverse transcription step [30].

A versatile role of EGCG has been demonstrated in the last decade regarding the antiviral effects. EGCG prevented neurotoxicity mediated by HIV-1 proteins gp120 and immune inflammatory responses via IFN-gamma where role JAK/STAT1 signaling has been found to be involved [31,32]. This suggests that beside the disfavoring role to the viral entry and propagation, EGCG also significantly restricted the cellular inflammatory burst. Role of overproduction of cytokines like IL1, IL-6 and TNF-α and cytokine storm may also be prevented by EGCG. The EGCG can play as inhibitor of hepatitis C and hepatitis B virus entry into the host [33,34]. Ability to block the several viral entries might indicate the interferences in the viral spike protein destabilization in the pre and post infusion events. These finding may be validated by the synthetic EGCG- palmitate efficiency against porcine reproductive/ respiratory syndrome virus [35]. The green tea catechin, EGCG has also been shown to inhibit chikungunya virus infection [36].

Finally, the attachment location of different selected drug molecules within the central core region of spike glycoprotein was analyzed. Different ligand binding sites were denoted in Figure 4a. Among them the central core region was focused elaborately. Where, in general the core region is being stabilized by inter and intra chain hydrogen bonding. Three locations were found which mainly stabilizes the channel like structure through intra chain H-bonding (Figure 4b). Here, four most potent molecules were analyzed figure 4. Amongst those, TFDG, TFMG and EGCG were found to interact with those amino acids at the central core regions which generally stabilize the core region through H-bonding. So, efficiency of these three molecules could have been better that hydroxychloroquine to destabilize the coronavirus spike proteins. These three molecules could be used for the COVID 19 treatment as such or after some chemical modification after making it more efficient in interfering the transition between open and closed state of the viral spike. It was proposed in a previous study gargling with tea catechin extracts may be practiced for the prevention of influenza infection in suspect/sensitive individuals. For the better bioavailability, and interaction with different viral-components i.e. spike-glycoprotein nanoparticulated catechin-derivatives may be used alone or in combination of other bioactive peptides/chitosan/lectins etc [37]. To serve promptly this bioinformatics, molecular-docking and virtual binding/inhibition study was finished with great pace. Further studies related to dry lab with other better software, or wet lab experiments are necessary to conclude decisively. We believe that our present work will add some new findings in the present emergent situation developed pandemic COVID-19 outbreak.

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