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A molecular docking study of EGCG and theaflavin digallate with the druggable targets of SARS-CoV-2

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ABSTRACT

Background: COVID-19 is an infectious disease caused by a novel positive-sense single-stranded RNA coronavirus called as SARS-CoV-2. This viral disease is known to infect the respiratory system, eventually leading to pneumonia. Crystallographic studies of the viral structure reveal its mechanism of infection as well as active binding sites and the druggable targets as scope for treatment of COVID-19.

Hypothesis: The role of tea polyphenols in prophylaxis and treatment of COVID-19 was established in this study.

Study design: Molecular docking interactions of tea polyphenols with some of the possible binding sites of SARS-CoV-2 were performed.

Materials and methods: From various studies on the SARS-CoV-2 reported in the literature, we chose possible drug targets (Chymotrypsin-like protease, RNA dependant RNA polymerase, Papain like protease, Spike RBD and ACE2 receptor with spike RBD) which are vital proteins. These receptors were docked against two tea polyphenols, Epigallocatechin gallate (EGCG) from green tea and Theaflavin digallate (TF3) from black tea. These polyphenols have been previously reviewed for their antiviral activities, especially against single-stranded RNA viruses. Two antiviral drugs, Remdesivir and Favipiravir were studied for comparative docking results.

Results: A comparative study of docking scores and the type of interactions of EGCG, TF3 with the possible targets of COVID-19 showed that the tea polyphenols had good docking scores with significant *in-silico* activity.

Conclusion: These results can provide a lead in exploring both the tea polyphenols in prophylaxis as well as treatment of COVID-19.

1. Introduction

The crystallographic structure of SARS-CoV-2 reveals that the virus is a positive-sense single-stranded RNA virus with four major structural proteins, the spike protein, envelope protein, membrane protein, nucleocapsid protein and some non-structural proteins (Nsp). A glycoprotein known as hemagglutinin esterase exists on the structure of β -strands [1]. The S protein further has two subunits S1 and S2. S1 helps the virus in binding to the host cell with its receptor-binding domain (RBD) whereas the S2 subunit is known to perform membrane fusion to complete the infection process [2]. The S protein is known to target specifically the angiotensin-converting enzyme 2 (ACE2) receptor on the host cell with the RBD. Various studies performed on the structural features of SARS-CoV-2 help us in identifying the major druggable targets. The Main protease or Chymotrypsin-like protease (3CLpro)

performs a vital function in the viral maturation step. It cleaves the non-structural proteins (Nsp) at 11 different sites which results in the formation of Nsp4-Nsp16. Since these Nsps perform important roles at some step in the viral replication cycle, 3CLpro is the most important drug target for COVID-19. Papain-like protease (PLpro) is another important enzyme, and hence a drug target that performs a function similar to 3CLpro. It generates Nsp1-Nsp3 by cleaving at different sites [3]. It is also known to suppress the immune system of the host cell, facilitating the infection process. RNA dependant RNA polymerase (RdRp) has a crucial role in the replication and transcription of the viral genome. Looking at the indispensable role of RdRp, it is yet another target for antiviral drugs. The spike RBD is the part that attaches the ACE2 receptor and initiates the infection. An alteration with the protein structure is, therefore, a possible mechanism of inhibition, making ACE2 receptor another drug target. Other binding sites of SARS-CoV-2 have also been explored in literature and attempts to discover their inhibitors

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List of abbreviations

3CLpro	Chymotrypsin-like protease
ACE2	Angiotensin-converting enzyme 2
EC	Epicatechin
ECG	Epicatechin-3-gallate
EGC	Epigallocatechin
EGCG	Epigallocatechin Gallate
GC	Gallocatechin
H-bond	Hydrogen Bond
Mpro	Main protease
NAMD	Nanoscale Molecular Dynamics Simulation
Nsp	Non-structural proteins
PDB	Protein Data Bank
PLpro	Papain like protease
RBD	Receptor-binding domain
RdRp	RNA dependant RNA polymerase
TF	Theaflavin
TF2A	Theaflavin-3-gallate
TF2B	Theaflavin-3'-gallate
TF3	Theaflavin-3,3'-digallate
VMD	Visual Molecular Dynamics

have been made [4,5]

Management of COVID-19 is still a challenge for healthcare workers around the world. Although techniques have been developed for effective diagnosis [6], no drug has been yet approved for treatment. Tea polyphenols are bioactive dietary nutraceuticals. Catechins, the most abundant phytoconstituents found in green tea and theaflavins, the polyphenols found in black tea are active phytochemicals. They display a vast array of bioactive properties like antitumorogenic, anti-inflammatory, antibacterial, antioxidative, antiproliferative apart from their antiviral effects [7,8]. Their antiviral activity has already been reported in numerous single-stranded RNA viruses hinting at the application of tea polyphenols as potential treatment methods for COVID-19 [9]. There are also some reports of these molecules showing activity in SARS-CoV-2 [10–12]. Bhardwaj et al. performed molecular docking studies of tea polyphenols on the main protease of SARS-CoV-2 and discussed their potential as inhibitors of 3CLpro [13]. The catechins

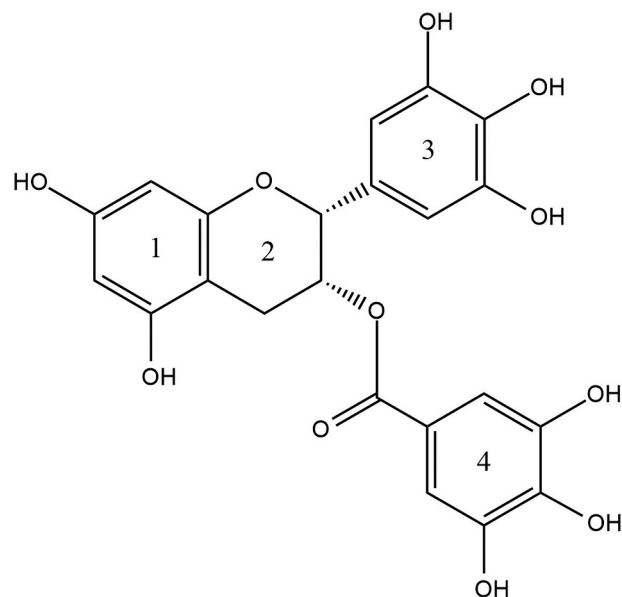


Fig. 1. Structure of epigallocatechin gallate (EGCG).

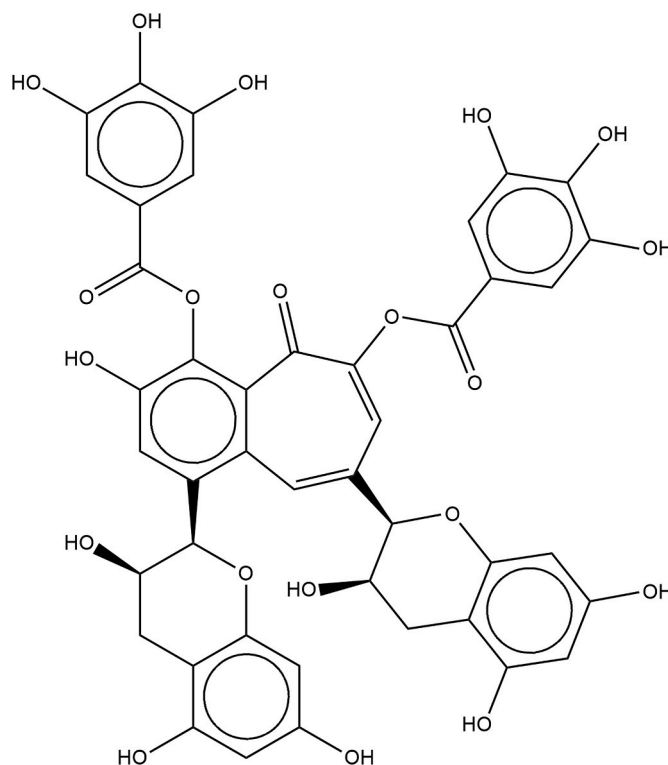


Fig. 2. Structure of theaflavin digallate (TF3).

found in green tea are catechin, gallocatechin (GC), epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC) and epigallocatechin-3-gallate (EGCG). Out of these, EGCG is found in the highest quantity in green tea, and also researched extensively as compared to the other catechins owing to its antiviral properties [14] (see Fig. 1). The theaflavins found in black tea are theaflavin (TF1), theaflavin-3-gallate (TF2a), theaflavin-3'-gallate (TF2b) and theaflavin-3,3'-digallate (TF3). TF3 (see Fig. 2) has particularly shown better antiviral activity than the other theaflavins [15]. In this study, we analysed interactions of two prime phytoconstituents present in tea, EGCG from green tea and TF3 from black tea with known binding sites of SARS-CoV-2 to anticipate the probable use of these polyphenols as a treatment method in COVID-19.

Both the tea polyphenols are generally regarded as safe by the FDA, hence there are no dose-dependent side effects. A cup of green tea of roughly 250 mL is expected to have around 50–100 mg of EGCG. Although no clear upper limit exists for the consumption of EGCG, a study estimated that a dose of 800 mg or higher per day can cause liver damage [16]. Similarly, there are no distinct dose restrictions for theaflavins. However, in one study 700 mg of Theaflavins showed side-effects, indicating that 700 mg is a safe amount, but not necessarily an upper limit [17]. A study performed in mice showed that toxic effects were visible at a dosage of 562 mg/kg [18].

To study the exact interaction of EGCG and TF3 with the possible binding sites of SARS-CoV-2, we performed molecular docking studies. Molecular docking is an *in-silico* method of predicting the most stable orientation of a particular ligand (which is generally a small molecule) in which it will bind to an active site of the receptor. There are two parameters to evaluate a better docking, docking score and the type of interaction. A better docking score is the one with more negative binding energy. Interactions can be non-covalent bonds like hydrogen bonding (H-bonding), Van der Waals interactions, pi-pi-stacking and in cases some ionic bonds. Remdesivir and Favipiravir were chosen as antiviral drugs to be tested specifically the RNA polymerase inhibiting activity. Molecular Dynamics Simulation was performed with the best-docked complex among all the receptors. The drug-likeness of these

molecules is also discussed.

2. Materials and methods

2.1. Obtaining protein structures and small molecules

The ligand 3-D structures were obtained from PubChem in the form of PubChem CIDs. The PubChem CIDs of the ligands were 65064, 136825043, 3652, 121304016, 492405 for EGCG, TF3, Remdesivir and Favipiravir respectively. The 3-D protein structures of the receptors were downloaded from the RCSB Protein Data Bank (PDB) as.pdb files. The PDB IDs of 3CLpro, Spike RBD, PLpro, RdRp and ACE2 receptor with spike RBD were 6LU7, 6XE1, 6W9C, 6M71, 6M0J respectively. These protein structures were imported in USCF Chimera version 1.14 [19].

2.2. Ligand preparation

The PubChem CIDs of EGCG, TF3, Remdesivir and Favipiravir were used as direct input to UCSF Chimera version 1.14 software. From their respective CID's the structure was built using the structure builder option of Chimera. The structures obtained in 3-D format were checked for the number of hydrogens and saved as separate.pdb files.

2.3. Receptor preparation

After importing the receptor structures as.pdb files, the non-standard residues, native ligands and the water molecules if present in the structure were removed. The protein structures were initially prepared by structural minimization with 200 steepest descent steps with a step size of 0.02 Å and 20 conjugate gradient steps with 0.02 Å step size. Hydrogen atoms and charges were added using the Dunbrack 2010 rotamer library [20]. The charges were computed with the help of ANTECHAMBER using AM1-BCC charge method [21]. This minimized structure was saved as a.pdb file.

2.4. Binding site prediction

Binding sites were predicted using AutoGridFR, a platform for the prediction of docking sites on protein receptors. It is part of the AutoDockFR suite [22]. Crucial amino acids participating in ligand interaction cited in the primary citation of the receptor and previous reports were considered in the selection of the docking site. The grid coordinates generated with AutoGridFR were used for molecular docking.

2.5. Molecular docking

EGCG, TF3 and HCQ were docked with all the receptors chosen. Remdesivir and Favipiravir are well known as RNA polymerase inhibitors, hence were docked only with RdRp. The protein structure and ligands which were prepared in USCF Chimera were imported as.pdb files. Molecular docking studies were performed in PyRx version 1.14 using AutoDock Vina web service by the National Biomedical Computation Resources [23]. The docking grid was created with the help of coordinates found by AutoGridFR. A total of 8 binding modes were generated. Out of these modes, the binding mode with the best docking score was saved as a.pdb file and was used for further evaluation. This ligand-receptor complex was then minimized again using the same parameters used to prepare the receptor. The interactions formed between the receptors and the ligands were visualized using Discovery Studio Visualizer v.20.

2.6. Mutagenesis analysis

To understand the importance of amino acids composing the binding pocket, the active site was mutated and the polyphenols were re-docked to the mutated protein structures. The three-dimensional structure was

visualized using PyMOL and point mutations at amino acids in the active sites were carried out using the mutagenesis wizard [24,25]. The stability of the mutant protein structure was evaluated using the DynaMut web server [26]. The ligands were docked with these mutated protein structures using the procedure described earlier for the wild protein structures.

2.7. Molecular dynamics simulation

To validate the docking results, molecular dynamics simulation of the complex was performed. The docked protein-ligand complex was simulated using the Nanoscale Molecular Dynamics Simulation (NAMD) software. The best-docked pose of the ligand was used to generate ligand topology files with the help of the CHARMM-GUI web-server using the input generator for the NAMD feature [27–29]. The server employs the CHARMM force field to the ligand. The ligand file was then imported into Visual Molecular Dynamics (VMD), a software supporting molecular visualization. The protein structure file was build using the automatic psf builder feature of VMD. The ligand and protein files were prepared using a VMD script to form a single system. The system was solvated in VMD using the solvate feature. The system was simulated for 1.2 ns at 310 K in the NPT ensemble.

2.8. Pharmacokinetic properties of tea polyphenols

The pharmacokinetic properties were evaluated using the Swiss ADME and the pkCSM web server [30,31]. The entire methodology is summarized in Fig. 3.

3. Results and discussion

3.1. Binding sites prediction

The amino acid residues composition of the binding sites and their grid coordinates for the receptors is summarized in Table S1 in the supplementary information. The binding site for 3CLpro was chosen based on the native ligand N3 which is established as a potential inhibitor of the protein. For all other receptors, there are no reported inhibitors and hence the docking site is not reported in the literature. The binding site with the highest active site score as reported by AutoGridFR was chosen for molecular docking.

3.2. Docking results of tea polyphenols and other drugs

As observed from Table 1, the docking scores for both the tea polyphenols with different receptors of SARS-CoV-2 are encouraging. The reported docking score for N3, the native ligand of 3CLpro is -7.0 kcal/mol [10], whereas the observed scores for tea polyphenols were -8.3 kcal/mol and -8.4 kcal/mol for EGCG and TF3 respectively. For the 3CLpro receptor, EGCG and TF3 exhibited a combination of hydrogen bonding, van der Waals and other hydrophobic interactions in the docked complex. EGCG formed 2 hydrogen bonds and a pi-anion bond with GLU166 residue of the A chain. GLU residues are generally functionally important in isomerases [32]. The same residue was found to form 2 hydrogen bonds with galloyl moieties of TF3. MET49 also was observed to form Pi-alkyl bond with EGCG and a Pi-sulphur bond with TF3. CYS145, which is usually important in transferases, was observed to form Pi-alkyl bonds with both the tea polyphenols. Unsurprisingly, these amino acid residues are also reported to form interactions with N3 in various reports [33–35]. Apart from them, a lot of other residues were observed to be forming van der Waals interactions with the polyphenols as mentioned in table no 2.

The spike RBD is a possible druggable target due to its role in viral attachment. The tea polyphenols showed significantly high docking scores of -9.7 kcal/mol and -11.6 kcal/mol. EGCG was observed to form 5 and TF3 was found to form as many as 8 hydrogen bonds. TYR91

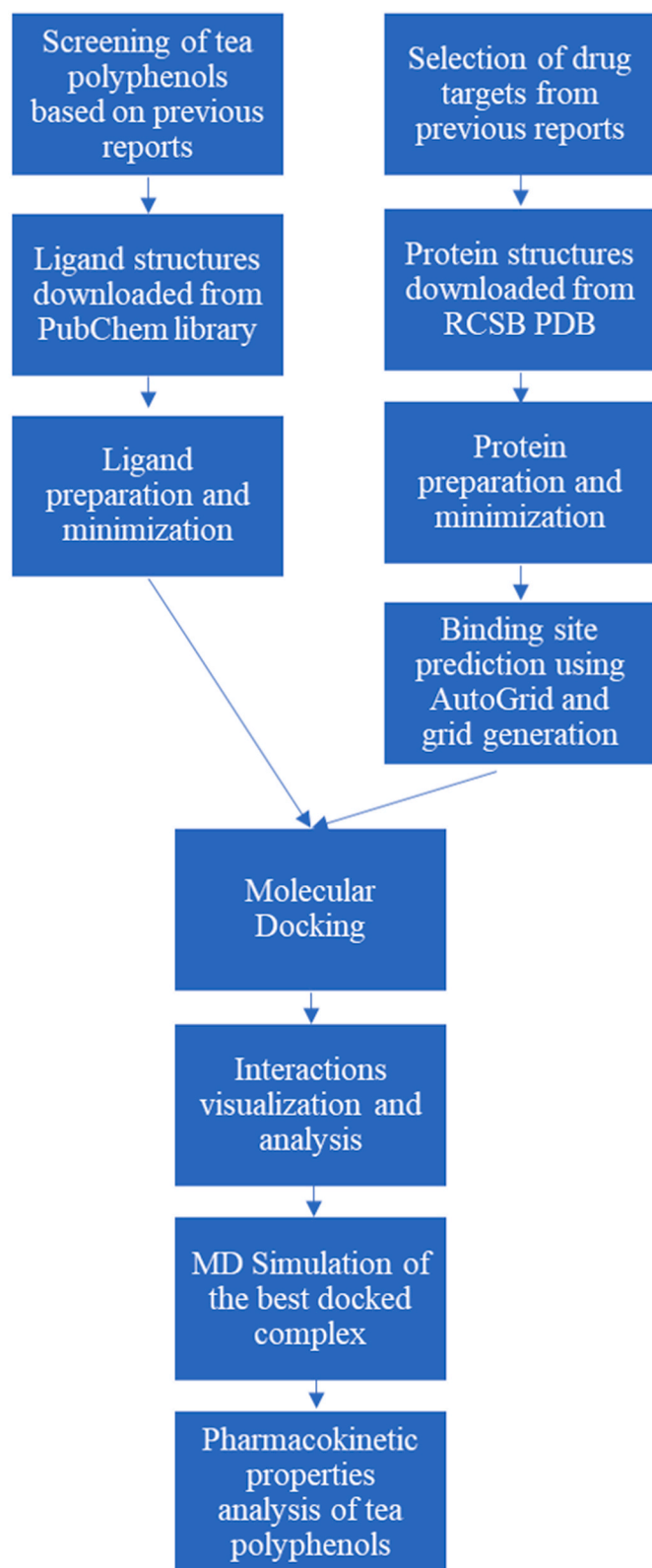


Fig. 3. Flowchart depicting the process of in-silico study.

amino acid residue of the L chain was observed to form hydrogen bonds with both the polyphenols. Pi-cation bonds were formed by residues LYS417 and ARG403 of the E chain with EGCG and TF3, VAL50 was another amino acid residue which was seen interaction with both polyphenols. It formed a Pi-Sigma bond with EGCG and a Pi-alkyl bond with TF3.

Table 1
Docking results with SARS-CoV-2 receptors.

Sr. no	Receptor with PDB IDs	Ligand	Docking score (kcal/mol)	No of H-bonds	Amino acid residues forming H-bond with their length in Å
1	3CLpro (6LU7)	EGCG	-8.3	3	GLU166 (3.86, 3.33), TYR54 (6.79)
		TF3	-8.4	8	THR25 (3.32, 3.28), PHE140 (5.91, 4.47), GLU166 (5.03), THR26 (4.44), THR24 (4.33)
2	Spike RBD (6XE1)	EGCG	-9.7	5	TYR91 (4.76), ARG408 (4.49), GLN96 (4.87), SER35 (5.19), TRP47 (5.26)
		TF3	-11.6	8	TYR91 (4.65), ASP95 (3.84), ASP97 (6.45, 5.05), THR415 (4.71), GLN414 (4.22), ASP61 (2.93), SER94 (4.22)
3	PLpro (6W9C)	EGCG	-8.9	4	LEU162 (3.27), B: GLN269 (3.03), ASN109 (5.24), GLY160 (4.15)
		TF3	-11.3	8	A:GLN269 (2.37), B:GLN269 (293), ASN109 (5.07), LEU162 (3.32), THR158 (4.52), GLU161 (4.91), VAL159 (4.45, 5.11)
4	RdRp (6M71)	EGCG	-5.7	3	GLU610 (5.32), VAL609 (5.25), SER607 (5.44)
		TF3	-6.0	4	SER607 (4.40), LYS603 (3.43), VAL609 (4.90, 4.75)
		Remdesivir	-5.0	2	LYS603(4.36), GLU610 (4.74)
		Favipiravir	-4.8	3	ARG750 (3.86, 4.63), ASP608 (4.39)
5	ACE2 receptor with spike RBD (6M0J)	EGCG	-8.5	2	LYS441 (5.36), ILE291 (4.17)
		TF3	-8.0	1	THR434 (4.09)

EGCG and TF3 showed docking scores of -8.9 kcal/mol and -11.3 kcal/mol with the PLpro. 3 amino acid residues, namely LEU162 from chain A, GLN269 of chain B and ASN109 of chain C, were observed to form hydrogen bonds with both the polyphenols. GLU161 of chain B formed a hydrogen bond with TF3 whereas that of chain A formed a Pi-anion bond with EGCG. Several amino acid residues were observed to form van der Waals and hydrophobic interactions with both the polyphenols.

RdRp inhibitor drugs like Remdesivir and Favipiravir are some of the antiviral drugs being repurposed and are currently in clinical trials [36]. Remdesivir has also been approved by the US FDA for emergency use. A study of how the tea polyphenols performed as compared to these drugs is interesting. With RdRp, the docking scores of EGCG and TF3 were better than both the antiviral drugs. EGCG formed 3 hydrogen bonds with GLU610, VAL609 and SER607 of the chain A. GLU106 was also observed to form a hydrogen bond with Remdesivir whereas SER607 formed a hydrogen bond with TF3. A graphical representation of the

docking score is added in the supplementary section (Fig. S1). The interactions formed by tea polyphenols, especially EGCG were better than those formed by Remdesivir and Favipiravir as observed from the interaction diagrams in Fig. 4 and Fig. 5.

The polyphenols also showed a considerable binding affinity towards complex formed at the viral entry, consisting of ACE2 receptor and the spike RBD. ILE291 residue formed a hydrogen bond with EGCG and Pi-alkyl bond with TF3. LEU370 residue formed Pi-Sigma bond with EGCG and a Pi-alkyl bond with TF3. Several residues were involved in van der Waals interactions as mentioned in Table 2.

For all the receptors studied, both the polyphenols exhibited good docking scores with various types of interactions. Except for RdRp, the docking scores of EGCG and TF3 were consistently higher than -8.0 kcal/mol, in some cases even higher than -11 kcal/mol which is a good indicator. The interactions observed are a mix of hydrogen bonding, van der Waals interactions, Pi-Pi stacking, Pi-alkyl bonds and Pi-sigma bond with multiple amino acid residues interacting with the polyphenols and are represented in Fig. 4. For RdRp, although the absolute scores of

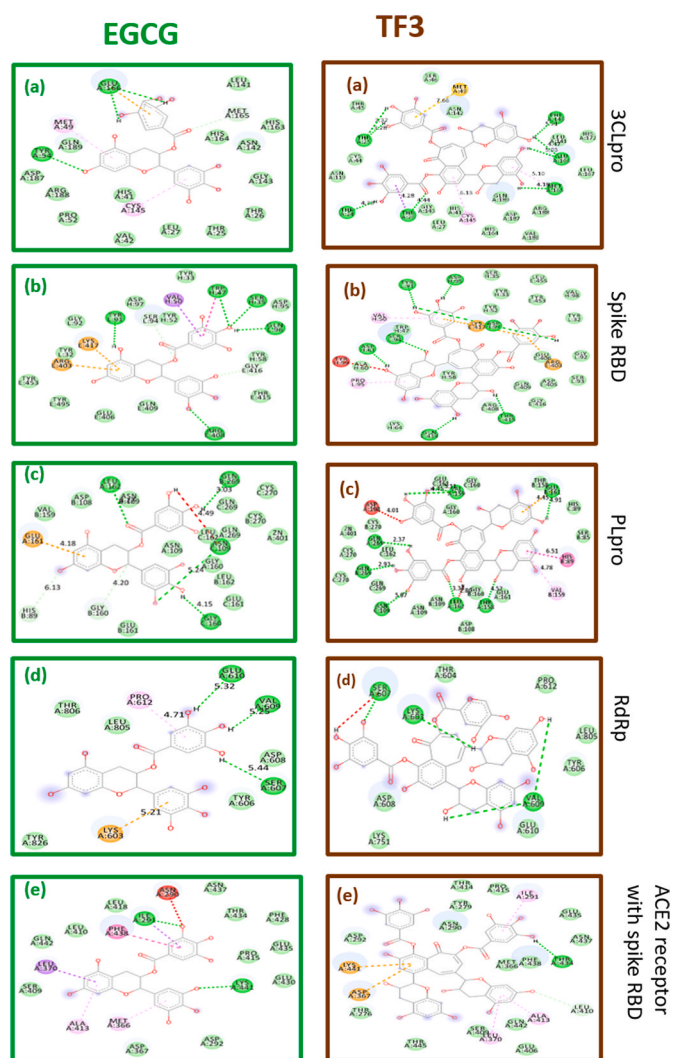


Fig. 4. Docking interactions of EGCG and TF3 with druggable targets of SARS-CoV-2. The first column represents EGCG and the second column represents TF3. The rows present: a) 3Clpro b) spike RBD 3) PLpro 4) RdRp 5) ACE2 receptor with spike RBD. The dotted green line indicates hydrogen bonding, amino acids in green colour indicate van der Waals interactions, orange dotted line represents pi-anion or pi-cation interaction, the pink dotted line represents a pi-alkyl bond, the purple dotted line indicates pi-sigma or pi-pi bond, yellow dotted line represent a pi-sulphur bond, a cyan dotted line indicates a halogen bond and red dotted line represents unfavourable interaction.

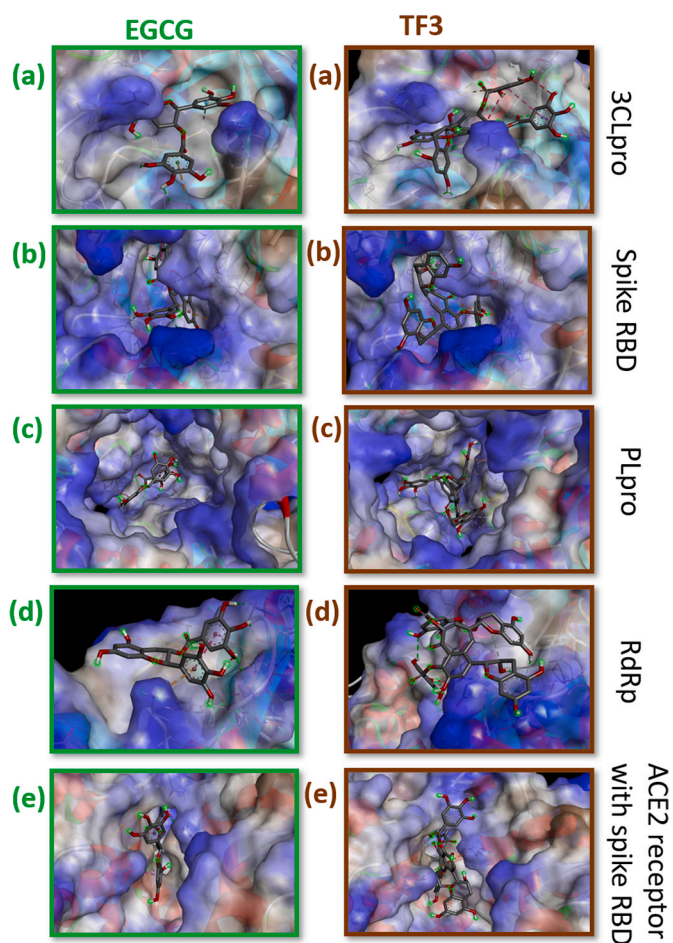


Fig. 5. 3-D docked poses of EGCG and TF3 with druggable targets of SARS-CoV-2. The first column represents EGCG and the second column represents TF3. The rows present: a) 3Clpro b) spike RBD 3) PLpro 4) RdRp 5) ACE2 receptor with spike RBD. The dotted green line indicates hydrogen bonding, amino acids in green colour indicate van der Waals interactions, orange dotted line represents pi-anion or pi-cation interaction, the pink dotted line represents a pi-alkyl bond, the purple dotted line indicates pi-sigma or pi-pi bond, yellow dotted line represent a pi-sulphur bond, a cyan dotted line indicates a halogen bond and red dotted line represents unfavourable interaction.

EGCG and TF3 were -5.7 kcal/mol and -6 kcal/mol respectively, these scores were better than those for Remdesivir and Favipiravir, which are RNA polymerase inhibitors already in clinical trials for repurposing in COVID-19. Both the polyphenols also showed a better binding affinity toward the receptors than HCQ. The broad-spectrum activity of naturally derived phytoconstituents is evident from the results in the present study.

3.3. Mutagenesis analysis

The amino acids that form the binding pocket and participate in interactions are crucial in terms of the binding affinity of the tea polyphenols. When these amino acids were mutated, the protein stability changed. Protein structure stability prediction results showed that the mutations were destabilizing with a marked shift in the $\Delta(\Delta G)$ value between the wild and mutated protein structures. On docking the tea polyphenols with mutated proteins, the docking score was significantly compromised. This could be because the polyphenols could not form interactions with the mutated active site as formed with wild structures. This also explains the significance of the amino acids comprising the wild-type active site in the activity of tea polyphenols. Moreover, the tea polyphenols also exhibit a targeted activity towards the amino acids and

Table 2
Interacting residues of SARS-CoV-2 receptors with tea polyphenols.

Sr. no	Receptor with PDB IDs	Ligand	Interacting residues	
			Polar Interactions	Hydrophobic interactions
1	3CLpro	EGCG	GLN189, ASP187, ARG188, PRO52, HIS41, VAL42, LEU27, THR25, THR26, GLY142, ASN142, HIS164, HIS163, MET165, LEU141	MET49, CYS145, GLU166
		TF3	THR45, SER46, ASN142, LEU141, HIS172, LEU167, ARG188, GLN189, ASP187, VAL186, HIS164, HIS41, GLY143, LEU27, ASN119, CYS44	MET49, THR26, CYS145, MET165
2	Spike RBD	EGCG	GLY92, TYR32, TYR453, TYR495, GLU406, GLN409, THR415, TYR58, ASP95, TYR33, ASP97, TYR52	ARG403, LYS417, VAL50, TRP47, GLY416, SER94
		TF3	SER35, LEU455, TYR33, TYR52, TYR453, VAL98, TYR32, GLY92, GLU406, SER93, ASP405, GLN409, GLY416, ARG408, LYS64, TYR58, ALA60, TRP47	LYS417, ARG403, VAL50
3	PLpro	EGCG	VAL159, ASP108, ASN109, B:CYS270, C: CYS270, GLN269, LEU162, ASN109, GLN269, GLY160, LEU162, GLU161, GLU161,	GLU161, GLY160, HIS89
		TF3	CYS270, CYS270, GLN269, LEU162, CYS270, A:GLY160, C: GLY160, GLU161, THR158, HIS89, SER85, GLU161, GLY160, ASP108, ASN109, A:ASN109, B:ASN109, GLN269	HIS89, VAL159, GLU161
4	RdRp	EGCG	THR806, LEU805, TYR826, TYR606, ASP608	PRO612, LYS603
		TF3	THR604, PRO612, LEU805, TYR606, GLU610, LYS751, ASP608	-
		Remdesivir	TYR828, PRO612, VAL609, SER607, ASP608	TYR606, LEU805, LYS603
		Favipiravir	SER754, SER607, THR604, LYS751	ARG750, ASP608
5	ACE2 receptor with spike RBD	EGCG	LEU418, LEU410, GLN442, SER409, ASP367, ASP292, GLU430, PRO415, GLU435, PHE428, THR434, ASN437	PHE438, LEU370, ALA413, MET366, ILE291
		TF3	THR414, PRO415, TYR279, ASN290, ASP292, THR276, THR445, SER409, GLU406, GLN442, MET336, PHE438, ASN437, DLU4435	LYS441, ASP367, ILE291, ALA413, LEU370

any alteration can weaken the interactions reducing the binding affinity. The following figure no. 6 shows the variation between the docking scores.

3.4. Molecular dynamics simulation

To validate the docking scores, the best docking model was simulated. The complex of TF3 with spike RBD had the highest docking score (-11.6 kcal/mol) and multiple amino acid residues were observed to interact with TF3. The protein-ligand complex was simulated for 1.2 ns under NTP ensemble. RMSD and RMSF calculations were performed to analyse the trajectory generated after simulation and represented in Fig. 7. The RMSD of the protein-ligand complex attained a value of 1.5 Å at 40 ps and a constant value till 0.4 ns with an average of 1.72 Å. The average RMSD value after 0.4 ns until 1 ns was 2.38 Å remaining constant in that time frame. There was an increase in the RMSD to 3.1 Å after 1 ns but dropped down again to 2.8 Å at the end of the simulation. Calculations of RMSF of the backbone atoms to check the mobility of residues to allow conformational changes on forming the complex ligand were also performed. The average RMSF for the TF3-spike RBD complex was 1.11 Å and the fluctuations were not high, contained in the range of 0.5 – 2.5 Å.

On basis of RMSD and RMSF analyses, it can be concluded that the docked protein-ligand complex of TF3 with spike RBD was stable and the docking result could be validated.

3.5. Pharmacokinetic properties analysis

The physicochemical properties of these molecules were studied and summarized in Table 3.

The number of hydrogen bond donors and acceptors is high in both the polyphenols violating the Lipinski's rule of 5. TF3 also exceeds the ideal molecular weight of less than 500. The tea polyphenols need structural optimization to increase the drug likeliness to be considered as strong drug candidates. However, the absorption of these polyphenols is acceptably high, and these molecules also do not pass the Blood-brain barrier. These polyphenols show no hepatotoxicity. The LD₅₀ values of EGCG and TF3 are 2.634 and 2.482 respectively. The clearance of these polyphenols is also faster with a rate of 0.51 and 0.357 log mL/min/kg for EGCG and TF3 respectively.

4. Conclusions and future perspective

In the present study, we performed molecular docking studies of EGCG and TF3 with possible active binding sites of SARS-CoV-2. The docking scores and the interactions of tea polyphenols with these receptors are a good basis to further explore the application of these natural phytochemicals in COVID-19. Tea polyphenols showed good docking scores with all the receptors of SARS-CoV-2 studied here. The polyphenols also showed better docking scores than Remdesivir and Favipiravir when docked with RdRp. MD simulation studies also confirmed the stability of the TF3-spike RBD complex on basis of RMSD and RMSF values. These results suggest the potential activity of tea polyphenols in the treatment of COVID-19. The use of tea phytoconstituents over synthetic drugs is an exciting treatment option since they are safer, and a higher dose is feasible. However, the bulkiness of these polyphenols and violations of Lipinski's rules could be hindrances in their development. Oral bioavailability is a concern for these polyphenols for which to overcome their derivatives are being developed. EGCG derivatives are being tested for enhancement in physicochemical properties and better efficacy.

Among the derivatives, esters derivatives like palmitates and stearates have shown better antiviral activity [37,38]. These derivatives have to be further explored in the application of COVID-19. The *in-silico* results presented in the study should provide a lead in evaluating the broad-spectrum antiviral activity of the tea polyphenols in the treatment

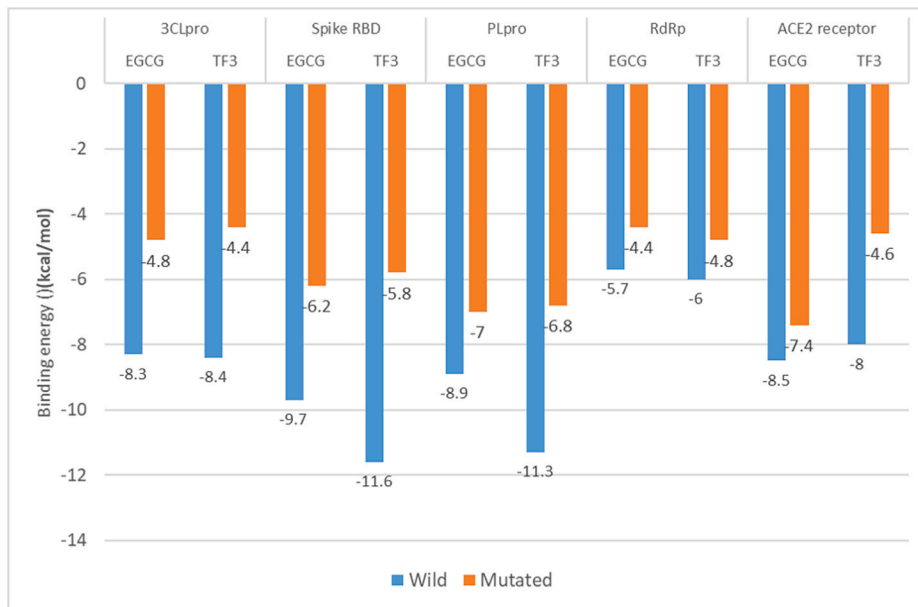


Fig. 6. Comparison of docking scores of wild and mutated structures.

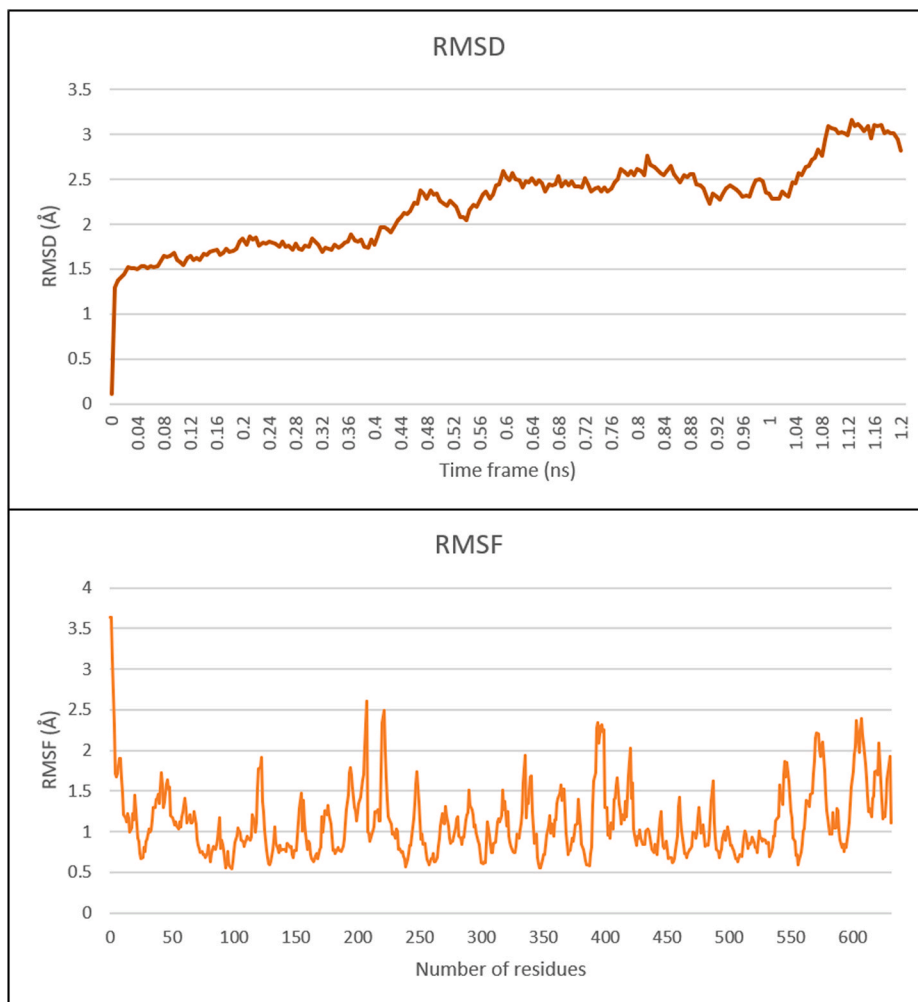


Fig. 7. RMSD And RMSF analysis of docked complex of TF3 and spike RBD.

Table 3

Physicochemical properties of tea polyphenols and the drugs evaluated in this study.

Ligand	EGCG	TF3
Molecular weight	458.37	868.8
Lipinski Rule Violations	2	3
Intestinal Absorption (%)	58.48	43.2
BBB permeability (log BB)	-2.238	-3.559
Fraction unbound (Fu)	0.314	0.388
Total clearance (log mL/min/kg)	0.51	0.357
Hepatotoxicity	No	No
Oral rat acute toxicity (mol/kg)	2.634	2.482
Oral rat chronic toxicity (log mg/kg of bw/day)	4.077	9.52

of COVID-19.

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Author contributions

Mr. Susmit Mhatre has performed all the docking studies. He analysed and interpreted the results. He has written the original manuscript and made all the tables and figures. Mr. Shivraj Naik designed the manuscript and revised the final draft. Dr. Vandana Patravale planned the manuscript as well as administered and supervised the entire work.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.compbiomed.2020.104137>.

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