Battle against Coronavirus: Repurposing old friends (Food borne polyphenols) for new enemy (COVID-19)

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Short running Title: Identification of Polyphenol based Structural Leads against COVID 19 virus

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Abstract

In the era of extreme scientific development where the scientific community have reached beyond moon, the entire world today is facing an immense problem due to deadly effect of COVID-19 (coronavirus disease), originated in Wuhan. Coronavirus is having dexterity to target immune compromised people very easily and swiftly get transmitted to healthy individuals from infected ones. Coronavirus infections are spreading very rapidly, and almost all the countries around the world are having corona positive people and asymptomatic carriers. This pandemic has created havoc both to human health and economy in lack of an effective treatment against this disease. Due to time limitations and urgency to find cure for COVID 19 we have undertaken the help of structure assisted drug design approach which mainly involves virtual screening program which identifies the structural leads which can target COVID-19 main protease (M^{pro}). This protease is the key enzyme of coronavirus which plays crucial role in virus replication and transcription, which can be targeted to retard the growth of virus inside the host. In the present work, the Phenol explorer database (version 3.6) containing 751 different food borne polyphenols were screened against the (M^{pro}) to identify suitable structural leads with potential to inhibit this protease though High throughput modelling and molecular docking approach. We identified six potential polyphenols belonging to Sanguiin, Theaflavin gallate, Theaflavin digallate, Kaempferol, Punicalagin and Protocatechuic acid chemical classes. All the six polyphenols have much higher docking scores ≥ -9.8 kcal/mol as compared to peptidomimetic inhibitor (N3) of COVID 19 virus M^{pro}. Pharmacokinetic and Drug likeness predictions of these polyphenols were done using SwissADME web tool where Protocatechuic acid shown fairly good results (1 Lipinski violation). The studies suggest the dietary intake of **"*black tea***" can improve the resistance to fight against COVID 19 virus in early stages of human infection. Importantly though, the enriched subset of six compounds identified from the larger library has to be validated experimentally.

Keywords: Human coronavirus, Virtual Screening, Molecular Docking, Polyphenols, M^{pro}, Phenol Explorer, ADMET, Pandemic

1.0 Introduction:

Coronavirus belongs to the family of respiratory human viruses. There are various strains of coronavirus among which SARS-CO is the extremely dreadful causing enormous mortalities once spread among human populations.[1] The outbreak of Coronavirus disease 2019 (COVID 19) was in the form of contagious pneumonia spreading in the regions of central China with Wuhan as epicentre in December, 2019.[2-5] In the last 20 years, two additional coronavirus epidemics have occurred where SARS-CoV was proven to be a large-scale epidemic beginning in China and involving two dozen countries with approximately 8000 cases and 800 deaths. Similarly, MERS-CoV that began in Saudi Arabia and has approximately 2,500 cases and 800 deaths and still causes sporadic cases worldwide.[6] The main symptoms of COVID-19 involve respiratory problems, dry cough, fever, malaise, muscle pain, etc.[7] The Director journal of WHO (World Health Organization) on March, 11, 2020 declares this disease as pandemic which is devastating for human population of a globe.[8] As of April, 10, 2020 there has been 1,596,493 infected cases worldwide, with more than 95,395 deaths across several countries.[9] This condition is even much worse in developed nations (USA, Italy, Spain) and death rate are also surging at an alarming rate in these countries.[9] The spread of this virus among human population of different countries can be categorize in four stages, where the community transmission stage is extremely dreadful and leads to failure of healthcare system due to sharp and sudden increase in the no. of infected cases which become untreatable, mainly due to lack of an effective drug/vaccine against this virus.[10]

Coronaviruses (CoVs) infect humans and other animal species, and cause variety of highly contagious and severe diseases, including Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS).[6] The virus belonging to coronavirus family

and are positive-sense, single-stranded RNA viruses featuring the largest viral RNA genomes[11] known till date, containing spike, membrane and envelope proteins (Figure 1). The genomic analysis of COVID 19 suggest that it comprised of ~30,000 nucleotides in its genome; which contains replicase gene which encodes for two overlapping polyproteins, pp1a (~450 kDa) and pp1ab (molecular mass of ~750 kDa.),[4, 5] which are pivotal for the replication and transcription of this family of viruses. One of the major protein of COVID 19 is M^{pro} (main protease), also referred to as the "3C-like protease" belonging to the proteases class of hydrolytic enzymes. This enzyme plays a key role in the processing of pp1a (responsible for generating copies of viral genome) and pp1ab (responsible for generating viral genome) as involved in their proteolytic cleavage at the conserved residues among COVID 19 genome.[12] Once, cleaved by M^{pro} , it translates into 16 non-structural proteins which is important for further synthesis of STR protein and non STR protein, causing translation of viral capsid and genome. These can assemble to give rise to virions inside the host cell and thus, replicate to produce multiple copies as shown in Figure 2. M^{pro} can act as potential target for structure based drug discovery as this enzyme not only involved in autocatalytic cleavage of itself and key viral enzymes, as well as lacks any close homologues among human host. Targeting this enzyme using suitable protease small molecule inhibitor holds immense potential to curb virus replication and transcription which are critical steps in virus life cycle.

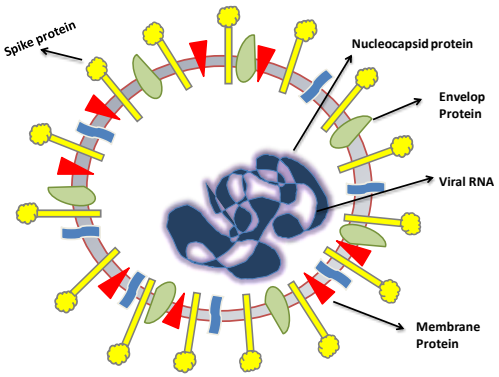

Figure 1. Structural components of coronavirus

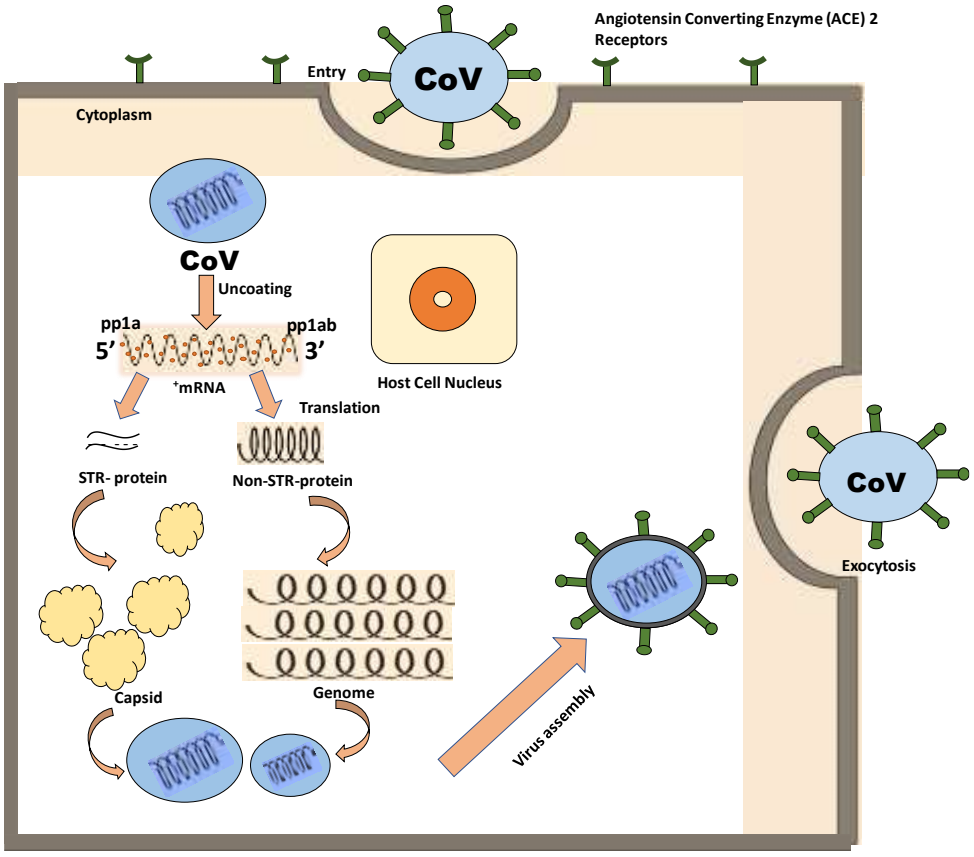

Figure 2. The mechanism of virus replication cycle inside the host cell. The entry of virus inside the cellular receptor angiotensin-converting enzyme 2 (ACE2) is assisted by its spike (S) proteins, which is followed by entry of the virus genomic material (positive single stranded RNA) into the host cell. The virus genome contains two overlapping polyproteins (pp1a and pp1ab) which are cleaved by M^{pro} (main proteases) into 16-non-structural protein which in turn translates structural (STR proteins) and non-structural (non-STR) proteins. This is followed by virus assembly and subsequently Virions are then released from the infected cell through exocytosis. S: spike, PP: polyproteins, CoV: coronavirus.

The need of the present hour is to develop potential inhibitors against COVID 19 as the no. of cases are increasing dramatically every hour. The impact of COVID 19 is so immense with varied mortality rates[9] among different nations with an average of above 3 % for the most developed nations of world. The drug discovery program usually takes about 10-15 years to bring a new drug candidate into market, so we have to repurpose either already known FDA approved drug candidates or look for small molecules obtained from natural consumable resources which are generally considered to have negligible toxicity.[13] Known FDA approved drugs like Hydroxychloroquine, Chloroquine, Ivermectin, Arbidol, Remedesvir, Cinanserin, etc[14],[15.] are already under application to treat COVID 19 patients but these drugs are having

their own toxic and pharmacokinetic implications. Vitamin B12 is reported to interfere with RNA polymerase activity of SARS-CoV 2(COVID 19 virus), which is a dietary supplement and natural in origin.[16] Hence, we have focus our attention on the alternative approach to screen the polyphenolic compounds found in consumable food products against the M^{pro} protease, which is the crucial enzyme for virus replication and transcription. Our approach has successfully identified six different polyphenols belonging to anthocyanins, flavanols and hydroxy benzoic acid subclass of flavonoids and phenolic acid that are found in commonly consumable food items.

2.0 Materials and Methodology

Data Set Preparation:

The Phenol explorer database (version 3.6) was utlized for present study.[17] The complete list of 751 polyphenols was downloaded in .xls fromat and the corresponding structures were exported from ZINC database in SDF format.[18] The database was then checked for redundancy and redundant molecules, in order to avoid any repetition. These structures were further refined in Chemdraw3D ultra by performing energy minimization using Molecular Mechanics 2 (MM2) force field method and saved in .mol2 format.[19] These 751 structures were further imported in AutoDock Vina 1.1.2 for ligand preparation.[20] Along with this, the SDF files of known 13 active drugs/leads reported against COVID 19 were also imported from ZINC database for comparative study.

Virtual Screening by Molecular Docking approach

Molecular docking is an important tool in computer-based drug design and drug discovery which helps to predict the small ligand conformation and orientation (Docking pose) within the active sites of the target receptor protein.[21] This technique has been highly useful in virtual screening of large libraries of ligands against suitable target proteins to identify potential

drug candidate based on scoring functions and intermolecular interactions. Following mentioned steps are undertaken for performing this study:

Protein Preparation

The three-dimensional crystal structure of M^{pro} (PDB ID: 6LU7) protease co-crystallized with its peptidomimetic inhibitor (**N3**) as reported by Yang *et al*. (2020)[15] was taken from the PDB (protein data bank) depository. The protein was prepared using the protein preparation wizard of Auto Dock Vina 1.1.2. This tool helps in adding hydrogens, Kollman charges, assigning AD4 type and repairing missing atoms. All water molecules were deleted from 6LU7, then the covalently bound peptidomimetic ligand was unbound from Cys145 and the α,β double bond of the ligand, that behave as a Michael acceptor, was restored. The protein was finally prepared and saved in .pdbqt format.

Ligand Preparation

The 3D structural files of 751 polyphenols and 13 drug molecules were fetched from ZINC-database in SDF format.[18] The ligands were then subjected to energy minimization using MM2 by keeping a check on the connection error in the bonds. The torsions for the ligands were set by detecting the roots in AutoDock Vina 1.1.2 followed by setting aromaticity criteria of 7.5.[20]

Ligand Docking

First of all, the bound ligand (**N3**) was extracted from M^{pro} and then re-docked to generate the same docking pose as found in its co-crystallized form (PDB: 6LU7) for validation of docking protocol. The prepared sets of 751 ligands were then docked against the M^{pro} protease using AutoDock Vina 1.1.2. Based upon docking score and docked pose the protocol was validated for docking of multiple polyphenolic ligands. All screenings were performed by using the ligand database prepared as stated before (no tautomers or alternative protonation states).

Visualization

The results obtained from AutoDock Vina (1.1.2) were visualized using the academic version of Pymol software.[22]

DRUG-LIKENESS PROPERTIES AND PHARMACOKINETIC STUDIES

Physicochemical and pharmacokinetic properties of the top 6 polyphenolic compounds based on their binding affinity scores (-10.3 to -9.8 kcal/mol) were studied using SwissADME, a free online web too developed by Diana *et al*.(http://www.swissadme.ch/index.php) by submitting their respective SMILES notations.[23]

3.0 Results and Discussion

Virtual Screening through Molecular Docking

The M^{pro} protease with its peptidomimetic inhibitor **N3** (Fig. 3a) contains only one polypeptide[15] which are divided into three different Domains (Domains I (residues 8–101) (shown in red) and II (residues 102–184) (shown in green) have an antiparallel β-barrel structure. Domain III (residues 201–303) (shown in cyan) in Fig. 3b. Domain III is connected with Domain II through connecting loop region (residues 185–200) shown in magenta in Fig 3b. COVID-19 virus M^{pro} has a Cys–His catalytic dyad in its substrate-binding site where **N3** is bound, which is mainly located in a cleft between Domain I and II.

The molecular docking based virtual screening was performed by validation of the docking protocol performed by redocking of Michael acceptor inhibitor (irreversible inhibitor) **N3**, this ligand is able to dock inside M^{pro} protease almost in identical binding pose (extended conformation) as shown in its co crystallized structure with M^{pro} (PDB: 6LU7) with the binding affinity value of -8.3 kcal/mol (Figure 4). Fig 4a, demonstrates the superimposition of co crystallized pose with the docked pose of **N3** inside the cleft between Domain I and Domain II of M^{pro} . This inhibitor forms hydrogen bonds with key amino acid residues Gly143, Gln189,

Thr190, Ser144, and an important covalent bond with Cys145 (characteristic feature of **N3,** extended binding mode),[15] and van der Waals contacts with His163, Met165, Phe140, Asn142, and Met165 residues of M^{pro} enzyme of COVID 19 virus, respectively (Fig 4b). This docking protocol was found to be acceptable and was further utilized to screen the library of 751 polyphenols (obtained from Phenol Explorer) through *in silico* screening. To achieve this, the database of polyphenols was docked on M^{pro} using AutoDock Vina (1.1.2).

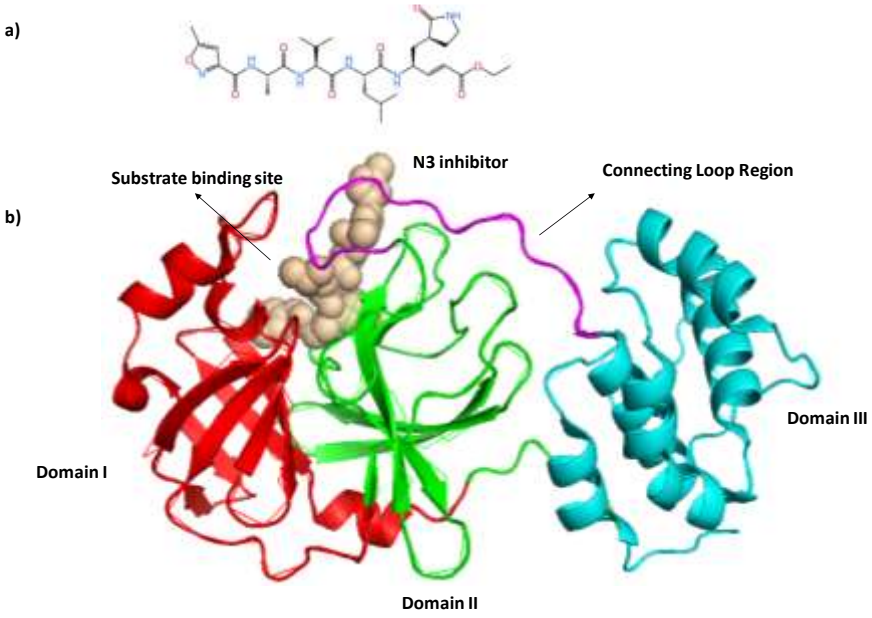

Figure 3. a) The 2D structural representation of **N3**; b) The 3D structure of M^{pro} is composed of three domains (Fig. 1b). Domains I (residues 8–101) (shown in red) and II (residues 102–184) (shown in green) have an antiparallel β-barrel structure. Domain III (residues 201–303) (shown in cyan) contains five α-helices arranged into a largely antiparallel globular cluster, and is connected to domain II by means of a long loop region (residues 185–200) (shown in magenta). COVID-19 virus M^{pro} has a Cys–His catalytic dyad, and the substrate-binding site where **N3** (shown in wheatish) is bound is located in a cleft between Domain I and II.

The docking scores of most of the polyphenols found to be impressive as the calculated binding affinity for most of the polyphenols are found to be higher than >-8.3 kcal/mol (docking score of **N3** on M^{pro}). In Table 1, we have enlisted the top 14 compounds which show docking score in range of -10.3 to -9.5 kcal/mol, which are much higher in comparison to **N3** inhibitor (-8. 3 kcal/mol). Out of 14 high scoring polyphenols, 6 compounds belong to anthocyanins sub class, 5 compounds are from flavanols and 3 molecules are from hydroxy benzoic acid sub class of polyphenols (Table 1).[17] Sanguiin-H-6 (hydroxybenzoic acid

derivative) (**SH6**) is found to be highest scoring compound with the docking score of -10.3 kcal/mol. This is followed by two derivatives of theaflavin (anthocyanins sub class), theaflavin 3,3'-O-digallate (**TDG**) and theaflavin 3-O-gallate (**TG**) which are found to be present abundantly in black tea in 40 % and 18 % proportion of a total theaflavin content.[24]

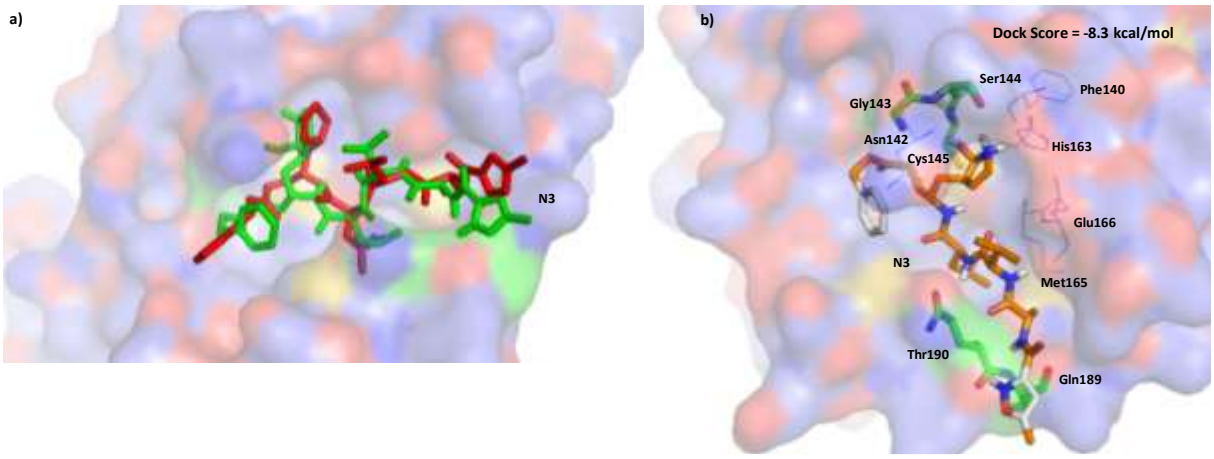

Figure 4. a) Superimposition of docked pose (shown in green) of **N3** with its co crystallized pose (shown in red) as found in M^{pro} (PDB: 6LU7); **b)** The binding mode of COVID-19 virus M^{pro} with N3 (sticks) shows that N3 forms hydrogen bonding with Gly143, Gln189, Thr190, Ser144, covalent bond with Cys145, van der Waal contacts with His163, Met165, Phe140, and Asn142.

The docking scores of theaflavin based compounds (**TDG** and **TG**) are also equally good (-10.0 kcal/mol and -9.8 kcal/mol) as compared to **SH6**. The ligand interaction diagram of **SH6** and **TDG** compounds are shown in Figure 5. These compounds have potential to bind in substrate binding site (cleft of Domain I and Domain II) with key amino acid residues Asn238, Lys137, Lys5 and Asn133 to form hydrogen bonds whereas their van der Waal contacts with back bone residues of Domain I and Domain II of M^{pro} are also evident from Figure 5. Subsequently, for further studies we have taken six compounds with docking scores ≥ -9.8 kcal/mol which are enlisted in Table 2 along with their ligand receptor interactions.

Table 1. Docking scores (in kcal/mol) of top 14 polyphenolic compounds against M^{pro} protease of COVID 19 virus

S. No	Polyphenol Class	Polyphenol Sub-Class	Compound Name	Docking Score on M^{pro} (kcal/mol)
1	Flavonoids	Anthocyanins	Cyanidin 3-O-galactoside	-9.5
2	Flavonoids	Anthocyanins	Peonidin 3-O-rutinoside	-9.5
3	Flavonoids	Anthocyanins	Peonidin 3-O-(6"-p-coumaroyl-glucoside)	-9.7
4	Flavonoids	Anthocyanins	Peonidin 3-O-sophoroside	-9.5
5	Flavonoids	Anthocyanins	Peonidin 3-O-sambubioside	-9.5
6	Flavonoids	Anthocyanins	Isopeonidin 3-O-sambubioside	-9.5
7	Flavonoids	Flavanols	Theaflavin 3-O-gallate	-9.8
8	**Flavonoids**	**Flavanols**	**Theaflavin 3,3'-O-digallate**	**-10.0**
9	Flavonoids	Flavanols	Procyanidin dimer B5	-9.5
10	Flavonoids	Flavonols	Kaempferol 3-O-glucuronide	-9.8
11	Flavonoids	Flavonols	Kaempferol 3-O-(2"-rhamnosyl-6"-acetyl-galactoside) 7-O-rhamnoside	-9.5
12	Phenolic acids	Hydroxybenzoic acids	Protocatechuic acid 4-O-glucoside	-9.8
13	**Phenolic acids**	**Hydroxybenzoic acids**	**Sanguiin H-6**	**-10.3**
14	Phenolic acids	Hydroxybenzoic acids	Punicalagin	-9.8

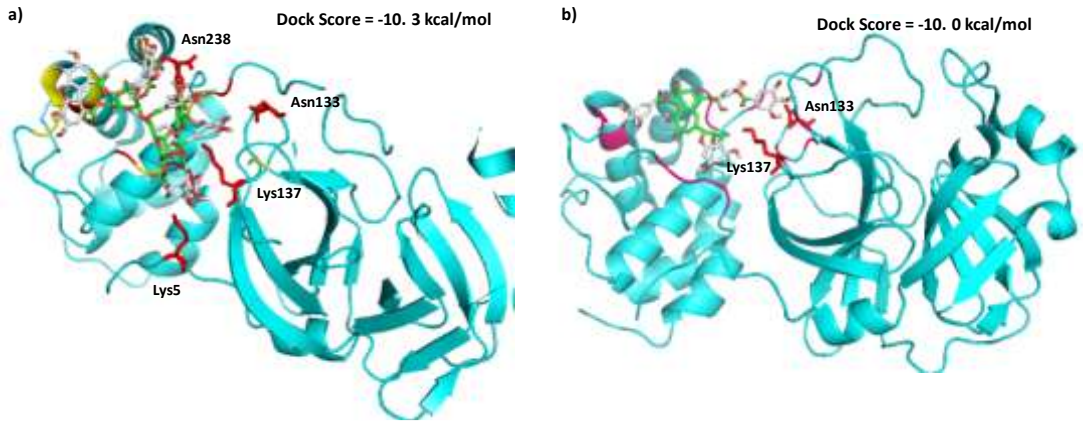

Figure 5. a) The binding mode of COVID-19 virus M^{pro} with **SH6** (sticks) shows that it forms hydrogen bonding with Asn238, Lys137, Lys5 (shown in sticks red) and van der Waal interactions with Glu288, Met276, Leu267, Gly275, Leu267, Gly275, Leu272, Tyr237, Asn274, Thr199, Asn238, Thr198, Lys137, Val171, Thr169; **b)** The binding mode of COVID-19 virus M^{pro} with **TDG** (sticks) shows that it forms hydrogen bonding with Asn133, Lys 137 (shown in sticks red) and van der Waal interactions with Asp197, Asn238, Ala194, Thr135, Val171, Arg131,Glu290, Asp289, Asn238, Tyr239, Glu288, Leu287, Leu271 and Gly275.

Table 2. Enlisting of Molecular interactions of high docked scoring compounds with M^{pro}

S.No.	Compound name	Hydrogen Bond	van der Waal interactions
1.	Sanguiin H-6	Asn238, Lys137, Lys5	Glu288, Met276, Leu267, Gly275, Leu267, Gly275, Leu272, Tyr237, Asn274, Thr199, Asn238, Thr198, Lys137, Val171, Thr169
2.	Theaflavin 3,3'-O-digallate	Asn133, Lys137	Asp197, Asn238, Ala194, Thr135, Val171, Arg131,Glu290, Asp289, Asn238, Tyr239, Glu288, Leu287, Leu271, Gly 275
3.	Theaflavin 3-O-gallate*	Gly143, Ser144, Asn142	Leu141, Thr25, Met165, Glu166, His164, His41, Met49, Gln189
4.	Kaempferol 3-O-glucuronide	His163	Ser144, thr25, phe140, gly143, asn142, met165, asp187, met49, gln189, tyr54, arg188
5.	Protocatechuic acid 4-O-glucoside	Ser144	Gln189, met165, glu166, his163, asn142, leu141, gly143, leu141, cys145, his41, asp187
6.	Punicalagin	Lys5, Phe140, Glu288	Glu288, Leu286, Glu290, Lys137, Gly170, Glu166, Phe140, Leu141, Ser139

*Compound shown covalent bonding with **Cys145** residue of M^{pro} protease of COVID19 virus.

As the compounds listed in Table 2 found to exist abundantly in daily consumable food items, so their natural sources can be utilized to consume these active polyphenols in order to improve the bodily resistance towards COVID 19 during early stages of infection. So, we have tabulated their food source along with respective quantities of these polyphenols present in them which are shown in Table 3 and Figure 6. As evident, from the published literature we have only mentioned the food source in which their presence was found to be highest in comparison to other sources. The daily consumption of red raspberry, black berry, pomegranate juice, and more importantly **"black tea"** (comparatively economical) might protect humans from the primary infection of COVID19. The two compounds from black tea[24] (**TDG** and **TG**) has found to be active in *in silico* studies, which suggest that theaflavin and its derivatives can play big role in the treatment of COVID 19 virus infection. Our study is also supported by the news reported by BBC on 25 march, 2020 stating the importance of black tea among COVID 19 patients in china.[25] We, further want to highlight the importance of Sanguiin and theaflavin

for treating COVID 19 patient as both of these compounds are well established bronchodilators[26,27] which may assist in improving the breathing discomfort among COVID 19 patients. In addition to this, theaflavin is also reported to have anti-oxidant property.[27]

Table 3. Potential Polyphenols (docking score ≥ -9.8 kcal/mol), along with their food source and quantity.

S. No.	Polyphenol	Source	Quantity	Reference
1	Sanguiin H-6	Red raspberry, raw	76.10 mg/100 g FW	28
2	**Theaflavin 3,3'-O-digallate**	**Tea [Black], infusion**	**3.52 mg/100 ml**	29
3	**Theaflavin 3-O-gallate**	**Tea [Black], infusion**	**1.58 mg/100 ml**	29
4	Kaempferol 3-O-glucuronide	Red raspberry, pure juice	0.21 mg/100 ml	30
5	Protocatechuic acid 4-O-glucoside	Blackberry, raw	0.43 mg/100 g FW	31
6	Punicalagin	Pomegranate, pure juice	43.60 mg/100 ml	32

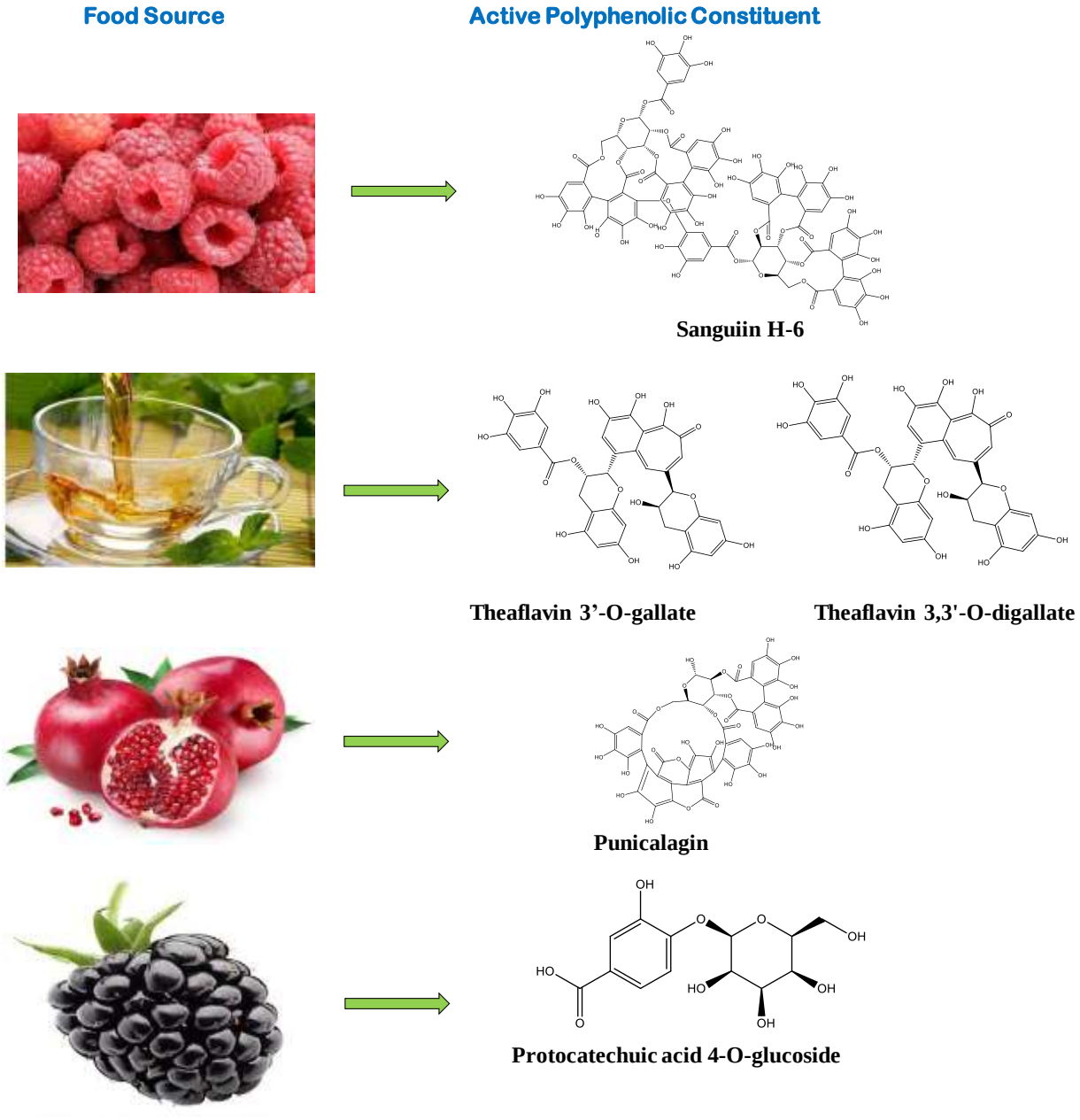

Figure 6. Polyphenolic constituents as potential inhibitors of M^{pro} found in commonly consumable food items.

Comparative study with known drugs/leads under treatment for COVID 19

In absence of any specific drug/vaccine for COVID 19 virus the scientist and clinicians around the world are repurposing FDA approved drugs for use in COVID 19 patients. Some of them like Hydroxychloroquine, Ivermectin and anti-HIV drugs, etc.[14,15, 33]. are found to be effective in decreasing the viral load. However, their mechanism of action on COVD 19 virus remain unknown. So, we carried out docking studies of 13 known drugs/compounds which are

being used for treatment of COVID 19 (or have shown activity in biological assays against COVID 19) on M^{pro} protease of COVID 19 virus. The results are mentioned in Table 4. The highest score was found to be for Ivermectin (-8. 2 kcal/mol) which is comparative to docking score of **N3**. This results on Ivermectin is also supported by study from Caly *et al.* where their group has shown that Ivermectin has potential to inhibit replication of COVID 19 virus and can reduce the viral load upto ~ 5000 fold in 48 hrs cell culture in *in vitro* studies.[33] Fig. 7a shows the binding mode of Ivermectin on M^{pro} where, it was found to bind in a substrate binding pocket with one hydrogen bonding interaction with Lys5. Similarly, binding pose of Ebselen with COVID-19 virus M^{pro} was shown in Fig 7b, where it has also shown to bind with Gly143 of substrate binding pocket of M^{pro}. Yang *et al.* reported[15] the activity of Ebselen with an IC_{50} of 0.67μM against M^{pro} inhibition in plaque-reduction assay. This molecule has found to display highest inhibition against M^{pro} in *in vitro* studies where the drugs from S. No. 6 to S. No. 13 (of Table 4) were tested in biological assay. Among, these 7 entries the docking score of Tideglusib is found to be highest (-7.4 kcal/mol), which is also in correlation with docking scores reported[15] by Yang *et al.* Hydroxychloroquine is a wonder drug now a days and used for the treatment of COVID 19 patient.[34] Fig. 7c, shows the deep penetration of this drug in the substrate binding pocket of M^{pro}, which might be one of the factor in its effective clinical use for COVID 19 patients.

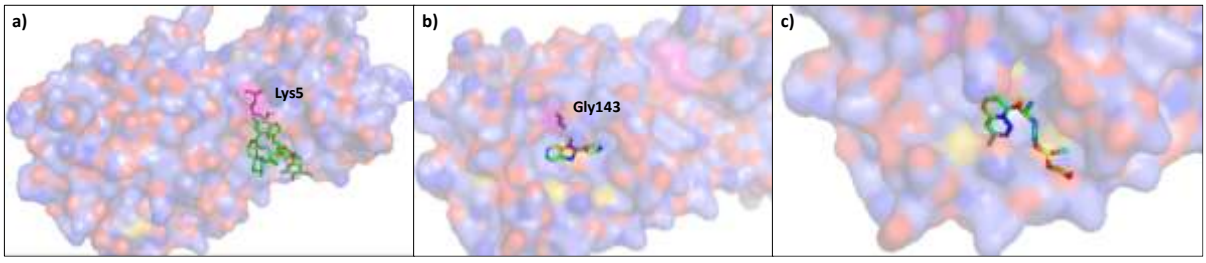

Figure 7. **a)** The binding mode of COVID-19 virus M^{pro} with **Ivermectin** (sticks) shows that it forms hydrogen bonding with Lys5, and van der Waal interactions with Glu290, Asp289, Lys137, Leu286, Arg131, Asn238; **b)** The binding mode of COVID-19 virus M^{pro} with **Ebselen** (sticks) shows that it forms hydrogen bonding with Gly143, covalent bond with Cys145, and van der Waal interactions with Thr25, Leu27, Ser 144, Gly143, Asn142, His163, Met49, Met165, Glu166; **c)** The binding mode of COVID-19 virus M^{pro} with **Hydroxychloroquine**

(sticks) shows that it forms van der Waal interactions with His41, Met165, Glu166, Leu167, Gln189, Met 49, Arg188 and Thr190.

Table 4. Docking scores of drugs/molecules in clinical trials which are reported to have activity against COVID-19 virus.

S. No.	**Drug**	**Docking Score on M^{pro} (kcal/mol)**	**US-FDA approved use**	**Reference**
1	Hydroxychloroquine	-6.1	Anti-malarial	35
2	Chloroquine	-5.7	Anti-malarial	36
3	Arbidol	-6.5	Anti-viral	37
4	Ivermectin	-8.2	Anti-parasitic	38
5	Remedesvir	-7.7	Anti-viral	39
6	Cinanserin	-6.3	A serotonin antagonist with limited antihistaminic, anticholinergic, and immunosuppressive activity	40-42
7	Carmofur	-6.1	Anti-cancer	43
8	Disulfiram	-5.7	Acetaldehyde dehydrogenase Inhibitor and Anti-cancer	44, 45
9	Shikonin[a]	-6.9	Anti-Inflammatory (Non-Steroidal)	46
10	TDZD_8 [a]	-5.0	GSK-3beta Inhibitor I	47
11	Tideglusib[a]	-7.4	NSAID and Neuroprotective agent	48
12	Ebselen[a]	-6.8	Anti-inflammatory, Anti-oxidant and Cytoprotective agent	49-50
13	PX_12[a]	-4.3	Anti-cancer	51
15	**Peptidomimetic inhibitor (N3)[b]**	**-8.3**	--	15

[a] Molecules are either in clinical trials or preclinical studies, [b]reported irreversible inhibitor of M^{pro}

Pharmacokinetics Prediction of Potential Polyphenolic Compounds (*in silico* ADME Studies):

Different classes of polyphenols are present in daily consumable food items, and helps in protecting our body against variety of communicable and non-communicable disease.[52] The blooming market for nutraceuticals products (majorly polyphenolic constituents) in recent time, is the result of growing awareness among the consumer to adopt preventive/precautionary approach rather than taking drugs (for cure) for various ailments.[53] The safety of polyphenolic based nutraceuticals is widely established owing to their dietary intake from more than1000 years. However, considering the single active constituent of food material for a particular therapeutic use needs evaluation in terms of its pharmacokinetics profiling to assess its drug likeness property. Hence, we carried out ADME studies of six active polyphenolic compounds, listed in Table 3, using SwissADME, a free web tool[23] to evaluate pharmacokinetic and drug likeness scores of medicinally important compounds. This software works on interpreting the molecular fingerprint (FP) of the submitted query structure, and searching the presence or absence of particular chemical features in a molecule to publish pharmacokinetic data. The *in silico* pharmacokinetic prediction of top six polyphenolic compounds are mentioned in Table 5 and 6. Except entry 4 and 5 (Table 5) which are derivatives of protocatechuic acid and kaempferol all the rest four, compounds violates Lipinski rule of 5, owing to their higher molecular weight beyond >500. These natural compounds (bioactive polyphenols) generally have low bioavailability owing to poor GI absorption.[54] This result is again reflected in Table 6 where most of the polyphenolic scaffolds show poor GI absorbtion and water solubility. However, protocatechuic acid derivatives shows comparatively better pharmacokinetic predictions and lead likeness, along with the ease of synthesis. The problem of poor bioavailability is being addressed now a day by formulating polyphenols in novel drug delivery vehicles like niosomes, liposomes, nanoparticles, polymer encapsulated formulations, etc.

which have solved the challenging issue of associated poor bioavailability with polyphenols to a larger extent.[55] Hence, we suggest that the best economical source of these polyphenols are from dietary intakes until the highly bioavailable formulations of these polyphenols have been sold in the market.

Taken together, the results obtained from Table 1-3, it can be concluded that polyphenolic scaffolds have affinity to bind with substrate-binding pocket of COVID-19 virus M^{pro}, which is highly conserved among all CoV M^{pros}. This investigation intensely supports our hypothesis that small molecule inhibitors targeting M^{pro} or in combination with other adjuvant therapies could provide an effective therapeutic regime to fight against all coronavirus-associated diseases.

Table 5. *In silico* pharmacokinetics prediction (Lipinski parameters) for the six potential polyphenolic inhibitors of M^{pro}

S. No	Compound no.	MW[a]	n-rotb[b]	n-ON[c]	n-OH,NH[d]	MR[e]	TPSA[f]	[i]LOGP[g]	Lipinski #violations	Lead likeness #violations	Synthetic Accessibility
1.	Sanguiin H-6	1871.27	12	52	29	423.8	877.36	2.33	3	3	10
2.	Theaflavin 3,3'-O-digallate	868.7	8	20	13	214.87	351.12	1.77	3	2	6.35
3.	Theaflavin 3-O-gallate	716.6	5	16	11	179.69	284.36	0.64	3	1	5.8
4.	Protocatechuic acid 4-O-glucoside	316.26	4	9	6	69.57	156.91	-0.05	1	0	4.11
5.	Kaempferol 3-O-glucuronide	462.36	4	12	7	108.74	207.35	1.26	2	1	5.22
6.	Punicalagin	1084.72	0	30	17	250.86	518.76	0.77	3	1	8.18

[a]Molecular weight (MW), [b]Number of rotatable bonds (n-rotb), [c]Number of hydrogen bond acceptors (n-ON), [d]Number of hydrogen bond donors (n-OH,NH),[e]molar refractivity (MR), [f]Topological polar surface area (TPSA), [g]partition coefficient calculated between n-octanol and water by considering solvation energies ([i]Log P).

Table 6. Swiss ADME pharmacokinetics prediction for the six potential polyphenolic inhibitors of M^{pro}

S. No.	Zinc ID	Ali Class	Silicos-IT class	GI absorption	BBB permeation	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	Synthetic Accessibility
1.	Sanguiin H-6	Insoluble	Moderately soluble	Low	No	Yes	No	No	10
2.	Theaflavin 3,3'-O-digallate	Poorly soluble	Moderately soluble	Low	No	Yes	No	No	6.35
3.	Theaflavin 3-O-gallate	Poorly soluble	Moderately soluble	Low	No	No	No	No	5.8
4.	Protocatechuic acid 4-O-glucoside	Very soluble	Soluble	Low	No	No	No	No	4.11
5.	Kaempferol 3-O-glucuronide	Moderately soluble	Soluble	Low	No	Yes	No	No	5.22
6.	Punicalagin	Insoluble	Moderately soluble	Low	No	Yes	No	No	8.18

4.0 Conclusion

Drug discovery against the CoV is a challenging job owing to rapid transmission of virus among human host. The development of a vaccine is another important aspect, as it boosts host natural immune system to fight infection. But in case of COVID 19, it has been found that people who were recovered from the disease are acting as potential carriers and transmitting COVID 19 to healthy population thereby compromising the scope of potential vaccine for COVID 19 treatment. Subsequently, the recovered individuals are again testing positive for COVID 19 as per few reports from Wuhan which indicates virus eclipsing in host.[15] Hence, finding small molecule inhibitor is a prime necessitate which can potentially interfere with the virus replication cycle and decrease the viral load in the host to minimize chances of community transmission. Though, in state of such an urgency natural products based compounds hold immense potential owing to their safer toxic profiles and random availability in huge amounts among daily consumables. In this study, we have performed virtual screening of food borne polyphenols (a library of 751 compounds) against main protease (M^{pro}, PDB ID :6LU7), a key enzyme involved in virus replication and transmission. Out of 751 compounds, 14 polyphenols show docking scores in the range of -10.3 kcal/mol to -9.5 kcal/mol, respectively. Based on the molecular interactions and scoring values (-10.3 to -9.8 kcal/mol), we have identified top 6 docked polyphenols which are mainly derivatives of Sanguiin, Theaflavin, Kaempferol, Punicalagin and Protocatechuic acid based polyphenols. Both, of the highest scoring compounds (Sanguiin and Theaflavin) are already established bronchodilators, which can further be useful to overcome breathing difficulties in patients having severe COVID 19 disease. Hence, we suggest the use of **"black tea"** in routine diet as it is economical and easily available source of potential polyphenols belonging to theaflavins chemical class, which might stop the virus replication and transmission in current health emergency state. The Pharmacokinetics study pointed out the poor bioavailability of these polyphenols if taken

individually as active compound. However, protocatechuic acid (1 Lipinski violation) derivative among all have shown better pharmacokinetic profile.

We, hereby propose that people who are at early stage of COVID 19 with mild symptoms and are quarantined at homes during lockdown may very easily include these polyphenols in their daily diets to stay resistant against COVID 19. These identified polyphenols may be further tested using *in vitro* assays, to evaluate their efficacy to treat COVID 19. Again, as a preventive measure, we focus the attention of readers to increase the intake of tea, berries, grapes, etc. in their daily diet which can be useful for boosting their immunity against COVID 19.

Author contribution: S. Giri and A.F Lal carried out the computational analysis as guided and descripted by Dr. S. Bhatia. The original idea uprooted by Dr. S. Singh who is constantly consuming black tea enriched with cinnamon, liquorice, ginger, black pepper and clove extracts as immunity booster. All the data/results analysis, drafting and proof reading of the manuscript is thoroughly done by Dr. S. Singh and Dr. S. Bhatia.

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