Emergency antiviral drug discovery during a pandemic

-a case study on the application of natural compounds to treat COVID-19

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Abstract:

The spreading COVID-19 pandemic has brought the world to a halt in 2020. One of the major challenges is the lack of effective antiviral drugs. Drug and vaccine development typically takes years; a practical approach to formulate knowledge-based prescriptions is to conduct *in silico* screening for drugs and compounds that has the potential to disrupt viral protein functions. By evaluating the dataset from the "Shennong project", an *in silico* screening of the DrugBank library against SARS-CoV-2 proteins, we identified chlorogenic acid and rutin displayed a strong affinity with diverse viral proteins. Chlorogenic acid is naturally present in coffee in large quantity, and rutin is available as nutraceutical products, both compounds are considered safe to consume, hence could potentially aid the recovery or treatment for COVID-19 patients at low health risk. We emphasise that the results require further clinical clarification, the impact of this work shall be examined by professionals carefully.

Introduction:

Control of novel contagious diseases remains a great challenge to human society. One of the key problems is that the outbreak window is typically too short for drug and vaccine development, and yet when the epidemic ends, the lack of both the patients and demand further compromise the drug development process. Taking the SARS (Severe Acute Respiration Syndrome) epidemic as an example, the epidemic between November 2002 and June 2003, caused 774 deaths with a fatality rate of 1% (Chan-Yeung & Xu, 2003). The pharmaceutical industry was unable to deliver effective drugs during the epidemic, and post-epidemic drug development has been slow, possibly due to the lack of patients for clinical studies (Kumar, Jung, & Liang, 2013). As the result, after 17 years of development, no anti-SARS drug or vaccine was available for use in the COVID-19 outbreak in 2020, which was caused by a virus sharing ~80% sequence identity to that of SARS (Wu et al., 2020; Zhou et al., 2020).

On the other hand, the research on SARS-CoV did yield invaluable structural and pathological insights on coronavirus (Almeida, Johnson, Herrmann, Geralt, & Wuthrich, 2007; Yu Chen et al., 2011; Huang et al., 2004; Jia et al., 2019; Joseph et al., 2007; Kirchdoerfer & Ward, 2019; Ma et al., 2015; Su et al., 2006; Sutton et al., 2004; Yu, Oldham, Zhang, & Chen, 2006). The SARS-CoV genome contains a large open reading frame, which encodes polyproteins pp1a/1ab, that are latter cleaved into 16 non-

structural proteins (Brierley, Digard, & Inglis, 1989; te Velthuis, Arnold, Cameron, van den Worm, & Snijder, 2009). The non-structrual proteins, designated as nsp1-16, are responsible for the RNA replication. The rest of the genome encodes 4 structural proteins, namely Spike(S), Membrane (M), Nucleocapsid (N) and Envelope (E) and several putative accessory proteins. The structural proteins are responsible for the encapsulation and the internal organisation of viral RNA. Among which, the sequence identity of S protein determines the host compatibility of the virus (F. Li, 2016). The knowledge of coronavirus is an invaluable guide to the research community to prepare countermeasures in the COVID-19 pandemic.

Due to the lack of effective drugs, many traditional Chinese herbal medicines were also tested both during SARS and COVID-19 epidemics. The joint administration of herbal extracts was reported to help the prevention and recovery of patients (Yang, Islam, Wang, Li, & Chen, 2020), and some showed *in vitro* inhibitory activities to both SARS and SARS-CoV-2 (F. Chen et al., 2004). However, the clinical effects of herbal medicines were not conclusive due to the lack of rigorous population studies (Luo et al., 2020).

Natural compounds are an important source of medicine, for example, quinine and artemisinin for malaria treatment (Cui & Su, 2009; White, 2005). However, one of the major challenges of using herbal medicine is that the active ingredients, if any, are in a mixture of compounds. That comes with two problems, first, how to identify the "active" compounds and quantify their doses; and second, how to evaluate the toxicity of other compounds and minimise their presence. In both SARS and COVID-19 treatments, the prescriptions of Chinese medicine were formulated according to the principles described in works of traditional herbal literature, which typically contain several to dozens of herbs, and the underlying biochemistry is unclear. Therefore, bridging the knowledge gap between Chinese medicine and biochemistry is crucial for further drug development, and one approach to take is via *in silico* drug screening to search for active ingredients.

The "Shennong project" is an industry-academia collaboration aimed to computationally screen drugs for COVID-19 treatment (Link: https://shennongproject.com:11443/#/home). The structure of 14 target proteins were generated by modelling the sequence of SARS-CoV-2 onto the atomic-resolution structures of their SARS counterparts as template. Then, the potential drug-targeting sites, 19 in total, were designated according to disruption of either the catalytic site or functional protein complex assembly (Xu et al., 2020). Compounds from the DrugBank library, 8498 in total, were screened against selected targeting sites on viral proteins, and 2 targeting sites of the human ACE2 structure which serves as the entry point for the viral infection (Yan et al., 2020). The drug-protein interactions were ranked according to the affinity strength, and the respective spatial interaction models are available online.

We analysed the *in silico* docking dataset and identified a range of natural compounds widely used by food, nutraceutical and Chinese medicine industry. Although their pharmaceutical properties are under investigation, the toxicity of these compounds has been thoroughly examined, and many of them are being produced at the industrial scale. Therefore, they are important candidates to be investigated for COVID-19 treatment. Among which, chlorogenic acid (DrugBank designation: DB12029) and rutin (DB01698) achieved a high affinity ranking to the viral proteins in the simulation, and they are present in *Lonicera japonica* and *Forsythia suspensa*, both are listed as herbal drugs to treat SARS and COVID-19 (Yang et al., 2020).

Here we present our analysis on the potential molecular mechanism of chlorogenic acid and rutin for SARS-CoV-2 inhibition, and we investigated the potential dose of these compounds in the Chinese herbal medicines, and nutraceutical products. It is important to emphases that the work we present is based on, and strictly limited to, the *in silico* simulation framework of the "Shennong project". This work aims to provide threads for rationalised drug selection and therapeutic design to treat COVID-19, and the conclusions from this work require carefully experimental validations.

Results:

The affinity of chlorogenic acid and rutin to listed drug targets.

We selected the drug with the strongest affinity to each drug target from the dataset for comparison and listed the ranks of chlorogenic acid and rutin (Table 1). Both chlorogenic acid and rutin are ranked within the top 500 examined compounds for targeting human ACE2 receptor. Chlorogenic acid ranked 157th for affinity to nsp16; rutin is ranked 123rd for affinity to nsp5, and 123rd for nsp10_2. Among the 8498 compounds in the dataset, these ranks are within the top 2% choices.

Table 1: List of binding energy and ranking of chlorogenic acid and rutin. The key molecular interactions analysed in this article are lighlighted yellow.

Drug Target	Top Candidate	DrugBank Designation	Binding Energy (kcal/mol)	Chlorogenic acid (Rank Position)	Binding Energy (kcal/mol)	Rutin (Rank Position)	Binding Energy (kcal/mol)
ACE2_H34	Tariquidar	DB06240	-7.1	433	-5.8	1712	-5.2
ACE2_K353	Galicaftor	DB14894	-7.2	479	-5.6	318	-5.7
Spike	ITI-214	DB15039	-8.1	3560	-5.3	1073	-6.1
Nucleocapsid	R-428	DB12411	-9.2	2220	-6.6	828	-7.1
Envelope	Phthalocyanine	DB12983	-9.1	4502	-4.8	3026	-5.3
nsp1	Pimelic Acid	DB01856	-4.9				
nsp3	5-(2-chlorophenyl)furan-2-carbohydrazide	DB08757	-4.6				
nsp5 (Mpro)	Phthalocyanine	DB12983	-10.9	1520	-7.6	123	-8.6
nsp7	N-anthracen-2-yl-5-methyl[1,2,4]triazolo[1,5-a]pyrimidin-7-amine	DB08006	-5.6	3668	-3		
nsp8_D50/D5	3-(1h-Indol-3-Yl)-2-[4-(4-Phenyl-Piperidin-1-Yl)-Benzenesulfonylamino]-Propionic Acid	DB02449	-7.5	3169	-4.9	3390	-4.8
nsp8_K58	2-(4-fluorophenyl)-N-{[3-fluoro-4-(1H-pyrrolo[2,3-b]pyridin-4-yloxy)phenyl]carbamoyl} acetamide	DB06997	-6.8	3924	-4.6	1343	-5.2
nsp10	Rebamipide	DB11656	-9	933	-7	4120	-6
nsp10_1	LY-2090314	DB11913	-8.6	1554	-6.2	914	-6.4
nsp10_2	Zoliflodacin	DB12817	-9	512	-7.2	123	-7.6
nsp10_3	LY-2090314	DB11913	-8.6	1104	-6.2	1273	-6.1
nsp12	Genz-10850	DB04289	-9.9	2666	-6.8	1640	-7.1
nsp13	Phthalocyanine	DB12983	-11.2			619	-8.2
nsp14_1	Tirilazad	DB13050	-8.7	1656	-6.5	5175	-5.4
nsp14_2	2-[3-({Methyl[1-(2-Naphthoyl)Piperidin-4-Yl]Amino}Carbonyl)-2-Naphthyl]-1-(1-Naphthyl)-2-Oxoethylphosphonic Acid	DB04016	-12.3	3386	-8	822	-9.4
nsp15	Saquinavir	DB01232	-9.9	3539	-6.2	246	-7.6
nsp16	Amcinonide	DB00288	-9.5	157	-8.3	7004	-5.1

To further evaluate the significance of the ranking, we plotted the distribution of the ranked drugs for nsp5, nsp10_2 and nsp16 following the ascending order of binding energy required (Figure 1). The logarithmic correlation between free energy and drug potency is that a 1.36 kcal/mol reduction in free energy leads to a 10-fold increase in potency (Taylor & Triggle, 2006). We show that the binding energy for chlorogenic acid and nsp16 is 1 kcal/mol lower than 82% of tested compounds, and 2 kcal/mol lower than that of 46%. The energy required for rutin and nsp10_2 site interaction is 1 kcal/mol less than 78%, and 2 kcal/mol less than 37% of all screened candidates. Similarly, the affinity between rutin and nsp5, is 1 kcal/mol less than 84% of all screened compounds; and 2 kcal/mol less than that of 49%. Therefore, the interactions of chlorogenic acid with nsp16, and rutin with nsp10_2 and nsp5, are substantially superior than most compounds in the DrugBank.

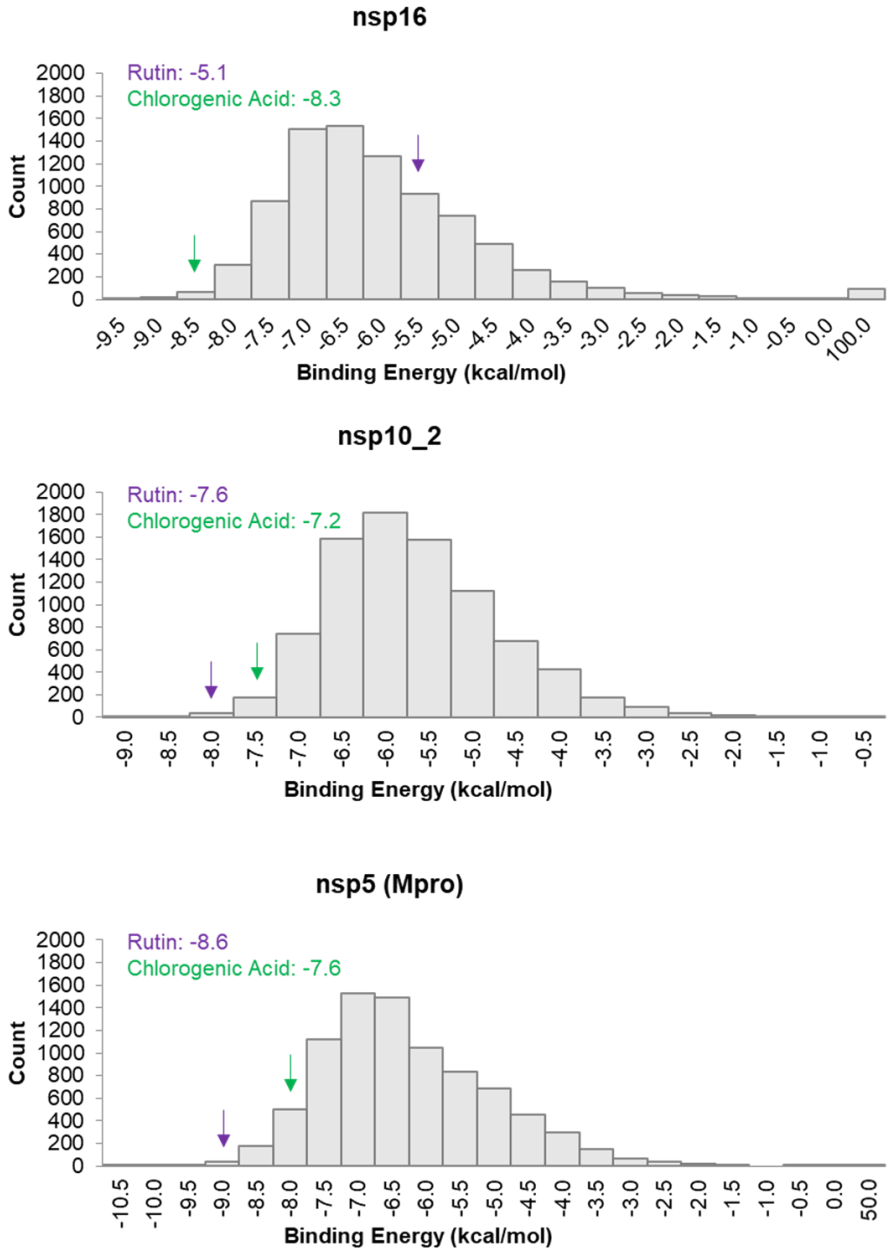

Figure 1: Distribution maps of the screened compounds against nsp16, nsp10_2 and nsp5 according to affinity. The number of compounds within each 0.5 kcal/mol affinity range are mapped in the figure. The position of rutin and the respective free energy are labelled in purple; that of chlorogenic acid in green.

The *in silico* interaction of chlorogenic acid and nsp16

The nsp16 protein is a 2'-O-methyltransferase (2'-O-MTase), which methylates the cap-0 structure to cap-1 structure (m7GpppNm-RNA) of the viral RNA (Bouvet et al., 2010; Yu Chen et al., 2011). The methylated cap structure is crucial for viral nucleotide to disguise as the eukaryotic host mRNA and evade from the innate immune response (Furuichi & Shatkin, 2000; Liu & Kiledjian, 2006). For SARS-CoV, the methylation is catalysed in two steps, first, the formation of cap-0 structure by nsp14, an N7-MTase (Y. Chen et al., 2013; Yu Chen et al., 2009); and then the nsp10/16 complex, of which, nsp16 is the catalytic subunit, and nsp10 is the stimulation subunit that stabilises the substrate-binding pocket of nsp16 (Yu Chen et al., 2011).

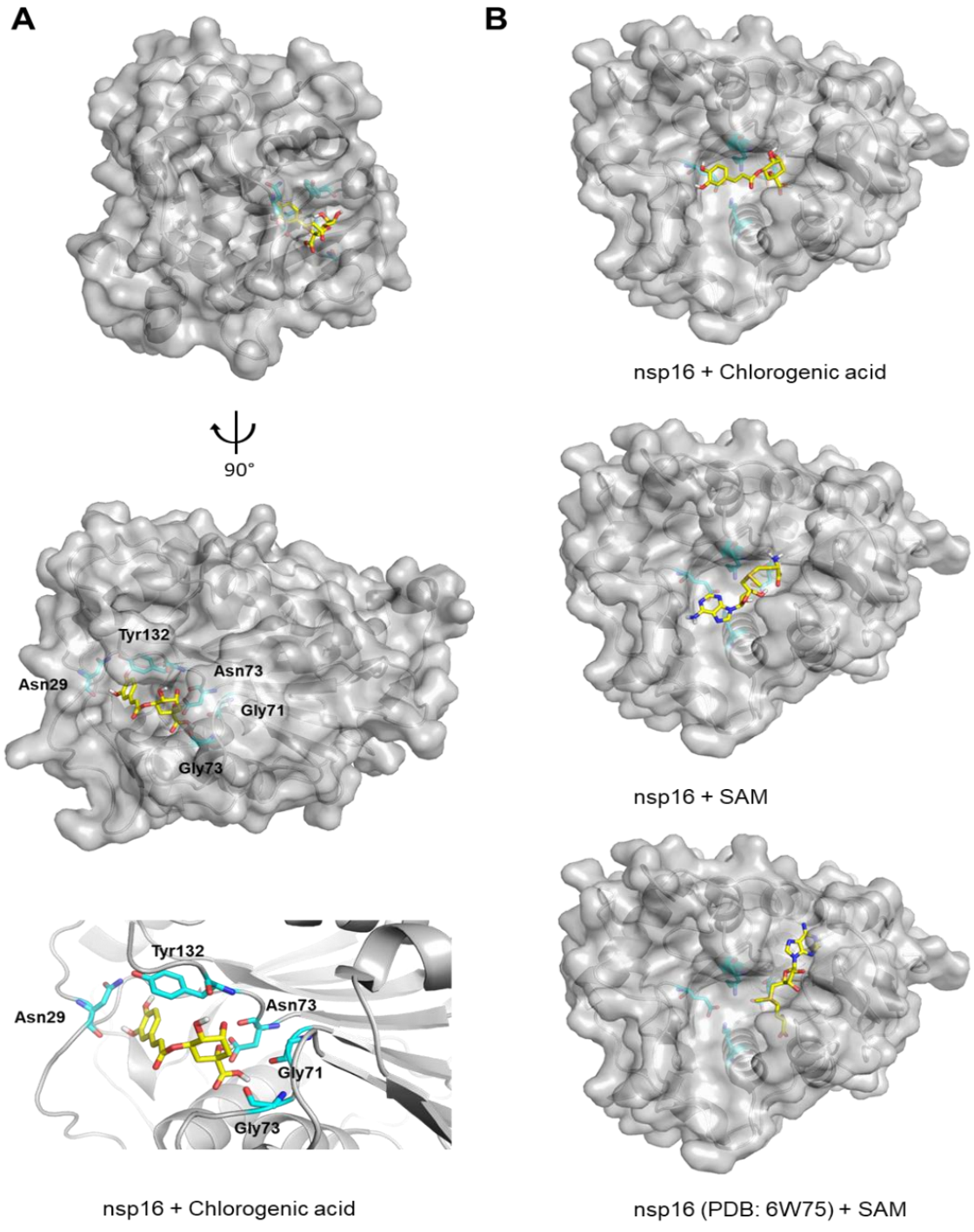

Figure 2: The structure of nsp16 with chlorogenic acid or SAM. (A) *in silico* interaction between nsp16 and chlorogenic acid, the interaction details are illustrated in the bottom section; (B) *in silico* interaction between nsp16 and chlorogenic acid (top and middle section), and the crystal structure of nsp16 with a bound SAM (PDB: 6W75). The nsp16 molecule is illustrated in gray; the residues interacting with chlorogenic acid molecule (in panel A), and the residues define the catalytic pocket of nsp16 (Leu100, Asn101, Asp130 and Met131 in panel B) are coloured in blue sticks, and the bound substrates are coloured in yellow sticks.

The catalytic pocket of nsp16, in which where the methyl group is transferred from donor S-adenosyl-l-methionine (SAM) to RNA, was selected as the drug target (Xu et al., 2020). *In silico* docking showed that the chlorogenic acid forms hydrogen bonding with residue Asn29, Gly71, Asn73, Asp130 and Tyr132, and pi-interaction with Tyr132 with the aromatic component, the residues participating in the interaction with chlorogenic acid would not be able to bind with RNA. (Figure 2A). The molecular interaction of SAM (DrugBank designation: DB00118) with nsp16 was also examined, the binding energy of the SAM-nsp16 interaction is -7.1 kcal/mol, hence the affinity of chlorogenic acid to nsp16 is substantially stronger (Figure 2B). However, the docking position of SAM in the simulation is inconsistent to that of a recent crystal structure of SARS-CoV-2 nsp10/nsp16 complex (PDB: 6W75). In the crystal structure, the SAM molecule is "flipped" along a side-channel, with the "tail" spanning towards the catalytic centre. The presence of chlorogenic acid molecule would spatially compete with SAM, but the efficacy requires experimental validation.

The *in silico* interaction of rutin and chlorogenic acid with nsp10

The nsp10 protein is one of the accessory proteins that regulate the activity of nsp14 and nsp16 (Bouvet et al., 2014). The nsp10 protein does not contain catalytic domain, however, its interaction with nsp16 is crucial for efficient cap-0 to cap-1 conversion of the RNA of coronavirus, with one exception of that of the feline coronavirus genus *Alphacoronavirus* (Decroly et al., 2008). The nsp14 protein contains two catalytic domains, an N-terminal 3'-5' exoribonuclease (ExoN) domain responsible for proofreading during RNA replication (Minskaia et al., 2006; Snijder et al., 2003); and a C-terminal N7-MTase domain responsible for the initial cap-0 methylation of RNA (Y. Chen et al., 2013; Yu Chen et al., 2009). The nsp10 protein interacts with nsp14 using the same interface of that with nsp16 (Bouvet et al., 2014). It has been shown that the interaction of nsp 10 and nsp14 could increase the ExoN activity by over 35-fold (Eckerle, Lu, Sperry, Choi, & Denison, 2007), however, the N7-MTase activity remains unchanged (Bouvet et al., 2010). The results indicate that the interaction between nsp10 and nsp14 is specific to RNA replication, and potentially the cap-0 to cap-1 conversion is temporally inhibited during the RNA replication stage.

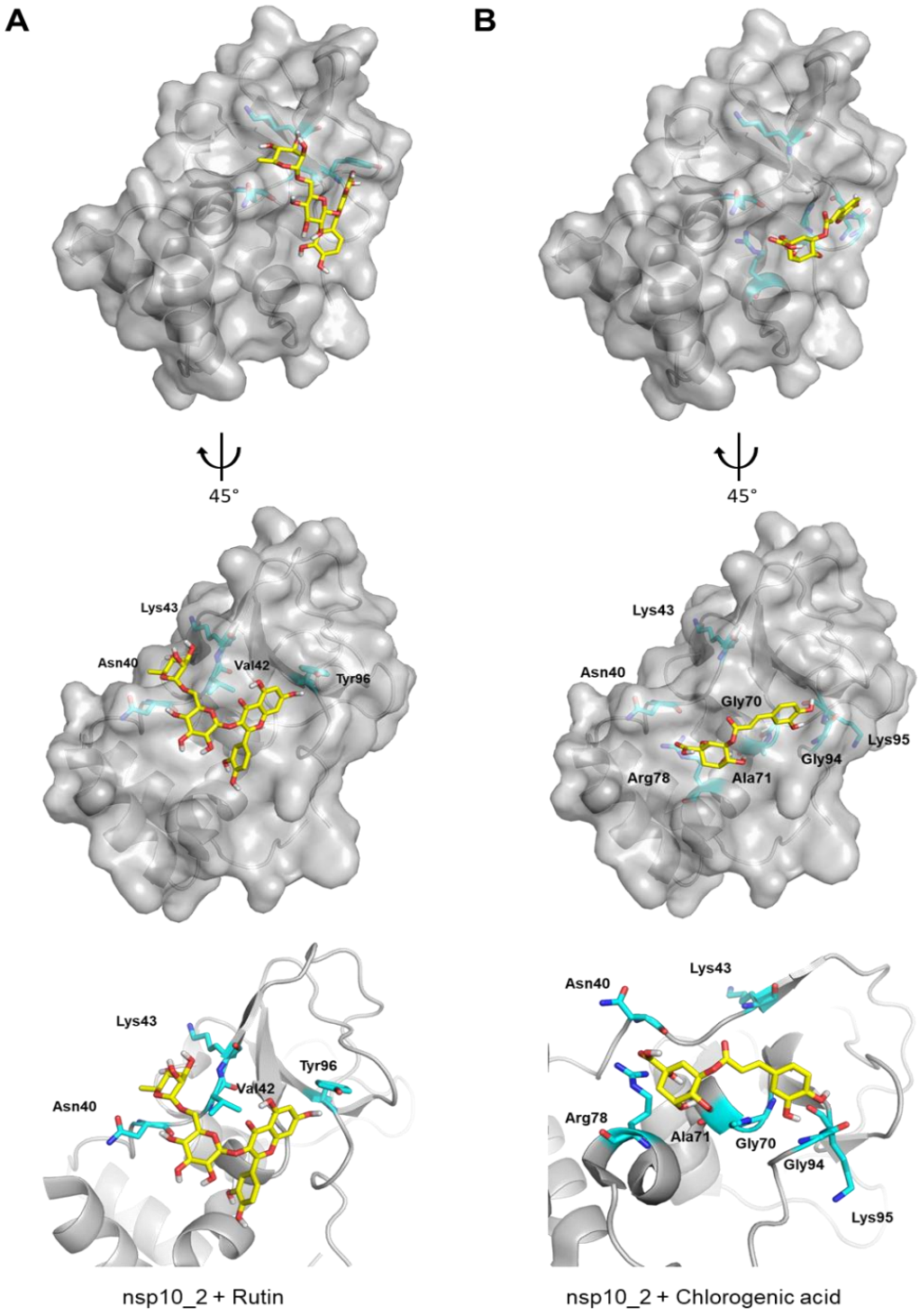

Figure 3: The in silico interaction between nsp10_2 and rutin (A) and chlorogenic acid (B). The chlorogenic acid and rutin molecules are coloured yellow, the key residues are coloured blue.

The selected drug-targeting interface of nsp10, which consists of residues Ala1, Asn3, Glu6 ,Phe16, Phe19, Val21 ,Asn40, Lys43, Leu45, Thr58, Ser72, Lys93, Tyr96, His80 and Cys90, is crucial for the interaction with nsp14 and nsp16, hence the drug molecule could potentially interfere both the replication and stabilisation of the viral RNA. The nsp10_2 site is defined by residues Asn40 and Lys43. Rutin forms hydrogen bond with side chains of residue Asn40 and the backbone of Lys43, as well as hydrophobic interactions with nearby apolar residues Val42 and Tyr96. In the dataset, the binding

energy of rutin with nsp10_2 is -7.6 kcal/mol. That of chlorogenic acid is -7.2 kcal/mol, whereas the median value of the whole library is >-6 kcal/mol. Therefore, the potency of rutin and chlorogenic acid to the nsp10_2 site could be 10-fold stronger than most screened compounds. The interaction between chlorogenic acid and nsp10_2 is established on the basis of forming hydorgen bonding with the side chain of Asn40 and Arg 78, and with the backbone of residue G70, A71, G94, K95. It is worth noting that using the nsp10_2 site as a drug target requires antagonising the protein-protein interaction between nsp10 and nsp14/nsp16. The interaction between proteins can be strong, hence the nsp10_2 site may not serve as an ideal target for small-molecule drugs.

The *in silico* interaction of rutin and nsp5

All nsp proteins from SARS-CoV-2 are initially expressed from a single open reading frame as a large polyprotein, which is latter separated by Papain-like protease (nsp3) and 3C-like protease (nsp5) (Thiel et al., 2003). The nsp5 is responsible of 11 cleavage between nsp4 and nsp16, hence also referred to as the main protease (designated as “Mpro” on the Shennong server). The proteolytic activity from of nsp5 is essential for the sequential assembly of the RNA replication and modification machinery (Stobart et al., 2013; Xia & Kang, 2011), making its catalytic site an important target for investigation.

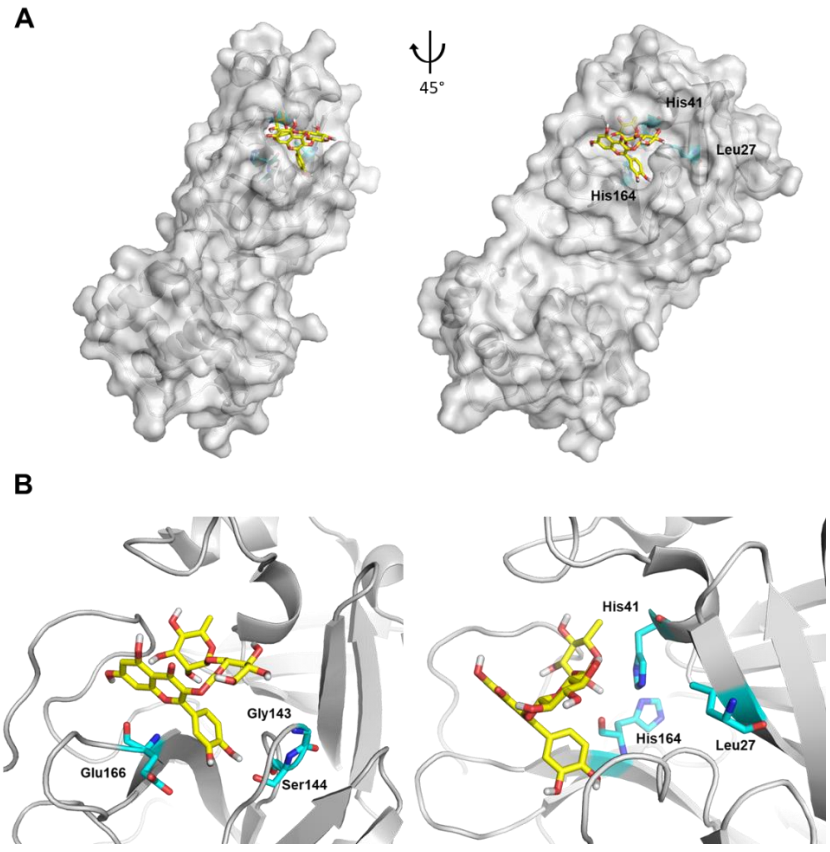

Figure 4: The *in silico* docking of rutin with nsp5. (A) The rutin-nsp5 docking model with 45o rotation view along the vertical axial. The residues define the catalytic pocket of nsp5 (Leu27, His41 and His164) are labelled in blue, whereas the rutin molecule is coloured in yellow. (B) The spatial coordination of rutin and the surrounding residues are illustrated on the left, with the three residues that forming hydrogen bonding coloured blue; on the right, the sparital relationship between the rutin molecule and the catalytic site of nsp5 is illustrated.

Although the proteolytic pocket of nsp5 is defined by key residues Leu27, His41 and His164, the rutin-nsp5 interaction is established by forming hydrogen bonding with residue Gly143, Ser144 and Glu166, and hydrophobic interactions with other nonpolar residues within the binding pocket. The binding energy required for the rutin-nsp5 interaction is -8.6 kcal/mol, which is superior than many drugs under clinical trials to treat COVID-19. For example, saquinavir which is an inhibitor to 3C-like protease of HIV, scored -7 kcal/mol; darunavir scored -7.3 kcal/mol, tipranavir scored -7.5 kcal/mol and indinavir scored best at -8 kcal/mol. Taken into account that the listed HIV inhibitors come with many side effects, rutin is perhaps a much safer choice. 3C-like protease is one of the major targets for antiviral drug development, hence the lead on rutin shall be examined closely.

Discussion:

Computer-aided drug selection

Due to the lack of effective drugs, many drugs are being tested for clinical treatments for COVID19 (Harrison, 2020), including traditional Chinese medicine (Ren, Zhang, & Wang, 2020). Due to the promising patient recovery rate, the Chinese National Health Commission included traditional Chinese medicine in the 7th version of "Chinese Clinical Guidance for COVID-19 Pneumonia Diagnosis and Treatment". The decision sparked controversies as the chemical composition of Chinese medicines is complex and inconsistent, so it is difficult to evaluate its clinical effects. On top of that, the toxicity of many ingredients in the Chinese medicine is unclear, e.g. *Rheum palmatum*, a commonly used herb can cause serial side effects (Steven Foster, 2006), which causes major public concerns. Therefore, it is vital to carefully evaluate the benefits versus risks on the application of herbal extracts.

The *in silico* molecular docking is a great tool for the rapid evaluation of the potential underlying molecular mechanism of drugs. With the assistance of simulation, compounds could be ranked within a standard matrix, which enables rationalised selection of drugs. However, it is important to highlight that many environmental elements *in situ*, such as pH, the ionic strength, membrane permeability and cellular metabolic activity, are not considered in the simulation, hence the information from a simulation is indicative but not conclusive (Xu et al., 2020). Another limitation of the simulation is the presence of competitive molecular interactions *in vivo*, for instance, the binding of rutin and nsp14_2 site is stronger than that of nsp5 and nsp10_2, despite the ranks for the latter interactions are much higher (Table 1). Therefore, the potency of a drug is not only influenced by the affinity strength but also the receptor competition, which is unclear for both chlorogenic acid and rutin. In addition, the absorption, cellular stability and metabolism of the compound are also important factors that define the efficacy of a drug (Rohrig, 2019). Hence the simulation data serves as a preliminary source of information, that requires further *in vitro* and clinical validation. The strength of simulation is to construct a knowledge-based framework for the application of drugs, especially to guide the administration of non-prescriptive drugs to patients and the public for prevention and supportive treatment during an epidemic.

Chlorogenic acid and the current medical evidence

Chlorogenic acid is listed as an investigational drug in the DrugBank library. It is the main active ingredient of *L. japonica*, which is widely used in the Chinese culture as an ingredient for herbal tea, soup and a classic medicine to treat flu-like symptom. In Europe, chlorogenic acid is mainly marketed as the main ingredient of the green coffee bean extract by the nutraceutical industry. Chlorogenic acid from green coffee extract is marketed as a weight-loss ingredient (Thom, 2007). However, its antioxidant, anti-carcinogenic, and anti-inflammatory properties were also exploited in several medicinal studies, including epidemiologic studies (Dos Santos, Almeida, Lopes, & De Souza, 2006; Tsuchiya, Suzuki, & Igarashi, 1996), Alzheimer's disease (Kwon et al., 2010), stroke (Lapchak, 2007) and blood pressure regulation (Suzuki et al., 2006; Suzuki, Kagawa, Ochiai, Tokimitsu, & Saito, 2002). Chlorogenic acid has been reported to inhibit SARS-CoV in laboratory testing (F. Chen et al., 2004). On the flipside, the absorption and metabolism of chlorogenic acid in human poses large variation among individuals, hence the efficacy of its medicinal application is difficult to evaluate (Monteiro, Farah, Perrone, Trugo, & Donangelo, 2007).

By analysing the "Shennong" database, we identify chlorogenic acid as a potential drug that interferes the viral replication by antagonising the catalytic site of nsp16 (Figure 1), which could block the cap-1 methylation of the viral RNA and enable the innate immune system to recognise and degrade the viral RNA (Bouvet et al., 2010; Furuichi & Shatkin, 2000). The hypothetical molecular mechanism requires further *in vitro* examination. Meanwhile, it is important to document the quantity of chlorogenic acid being administrated to patients taking Chinese medicines.

Like caffeine, the chlorogenic acid in coffee beans is affected by roasting, hence the chlorogenic acid in coffee ranging between 70-350 mg for a 200 ml cup (Clifford, 2000; Renouf et al., 2010). The recommended dose for nutraceutical uses of green coffee extract is ~ 40-200 mg per day. The content of chlorogenic acid in dried floral of *L. japonica* for Chinese medicine is between 1.5-3% (w/w) (Duan et al., 2019); and typically 15-35 g of the herbs is required for each dose, hence ~ 0.2-1 g of chlorogenic acid could be present in the Chinese medicine prepared from herbal mixture. It is worth noting that the extraction procedure described in the literature was based on solvent extraction (Duan et al., 2019), whereas, the herbal medicine preparation only involves boiling, hence the concentration of chlorogenic acid in the remedies is unclear. Therefore, it is very important to keep a clear record and samples for further analysis. In addition, "Shuanghuanglian" granules, a well-known Chinese medicine premix manufactured by pharmaceutical companies also contain chlorogenic acid, however, its quantity is ~ 6.5 mg for a standard 5 g does (An et al., 2012). Shuanghuanglian has shown *in vitro* inhibition to SARS-CoV-2 (Wang, Wang, Ye, & Liu, 2020), but its chlorogenic acid content could be too little to be considered as an active ingredient.

Rutin and the current medical evidence

Together with the florals of *L. japonica*, the fruit of *F. suspensa* is often jointly used in Chinese medicine to treat flu-like symptoms. In this study, we identify rutin as a good drug candidate for COVID-19 treatment, not only because the potential molecular interaction between rutin and multiple viral proteins achieved high score in the simulation; also, as an approved nutraceutical product, rutin is available to the public and safe to consume. Rutin as an investigational drug has been reported to have anti-inflammatory (Bao et al., 2016), antibacterial (Ganeshpurkar & Saluja, 2017) and antiviral

effects (Wen Su, Huifu Xu, 2013). On the flipside, a thorough investigation on the safety of joint administration of rutin, chlorogenic acid and other medicines should be conducted.

The *F. suspensa* fruit contains a wide range of flavonoids, including forsythoneoside, rutin and baicalin (Dong et al., 2017). Rutin is one of the active ingredients under investigation, its quantity in dried fruits is ~ 2 mg/g (X. Li, Zhang, & Yuan, 2002). The use of *F. suspensa* for Chinese medicine is typically in the rang of 10-15 g, and the actual rutin concentration in the herbal medicine might subject to further reduction due to extraction efficiency. Hence the estimated presence of rutin in the herbal mixture is < 30 mg. Many commercial rutin products use *Styphnolobium japonicum* as source, possibly due to better yield or purity, and the recommended dose for well-being purpose is between 0.5-1 g per day.

Shennong project

Based on the Shennong simulation, both chlorogenic acid and rutin could potentially disrupt the viral pathogenesis at multiple stages; and more importantly, both compounds score better than the following drugs under clinical trials, remdesivir, chloroquine, favipiravir and tocilizumab. Remdesivir is the best-known drug to treat COVID-19 with high recovery rate, we are unable to pin its molecular mechanism from the simulation. In contrast, lopinavir and ritonavir, the anti-HIV drugs designed to target 3C-like protease, and used on COVID-19 patients in South Korea (Lim et al., 2020), are specifically among the top choices against nsp15 of SARS-CoV-2, not the 3C-like protease. Therefore, the simulation does provide clues but has its limitations.

Conclusion:

The COVID-19 epidemic marks the first, perhaps not the last, global threat in the third decade of the 21st century. Due to the shortage of effective drugs, it has become a matter of urgency to screen for substances that could inhibit the virus with low health risks. Computer-aided molecular docking simulations provided clues on the candidate drugs, from which, we analysed the molecular interaction of chlorogenic acid and rutin with the viral and human protein targets. We show that both compounds could potentially interact with the viral non-structural proteins that are required for the replication and stabilisation of the viral RNA. The affinity between chlorogenic acid and nsp16, and that of the rutin and nsp5 and nsp10 (site 2) are ranked among the top 2% of all screened drugs, with the potential of being 10 times as potent than approximately. half of the library. Both compounds are available as nutraceutical products in the US and EU market, and the source plants are used widely as traditional medicine and herbal drink in the oriental culture. Therefore, they could be excellent supplements for prevention and supportive treatments.

Through literature study, we concluded that the dose of chlorogenic acid in Chinese medicine should be under 1 g; coffee could be a suitable alternative source of chlorogenic acid. The rutin content in Chinese medicine, however, might be negligible. It might worth testing commercial rutin products on patients for efficacy. We want to highlight that it is very important to keep a clear record and samples of the remedy for future laboratory testing.

References:

Almeida, M. S., Johnson, M. A., Herrmann, T., Geralt, M., & Wuthrich, K. (2007). Novel-barrel fold in the nuclear magnetic resonance structure of the replicase nonstructural protein 1 from the Severe Acute Respiratory Syndrome coronavirus. *Journal of Virology*. https://doi.org/10.1128/jvi.01939-06

An, Y. Q., Tang, D. Q., Yin, X. X., Jiang, X. I., Wu, J., & Chen, H. (2012). Simultaneous determination of nine chemical components in Shuanghuanglian granule by HPLC. *Chinese Traditional and Herbal Drugs*.

Bao, J., DIng, R., Zou, L., Zhang, C., Wang, K., Liu, F., … He, C. (2016). *Forsythiae fructus* inhibits B16 melanoma growth involving MAPKs/Nrf2/HO-1 mediated anti-oxidation and anti-inflammation. *American Journal of Chinese Medicine*. https://doi.org/10.1142/S0192415X16500580

Bouvet, M., Debarnot, C., Imbert, I., Selisko, B., Snijder, E. J., Canard, B., & Decroly, E. (2010). *in vitro* reconstitution of SARS-coronavirus mRNA cap methylation. *PLoS Pathogens*. https://doi.org/10.1371/annotation/a0dde376-2eb1-4ce3-8887-d29f5ba6f162

Bouvet, M., Lugari, A., Posthuma, C. C., Zevenhoven, J. C., Bernard, S., Betzi, S., … Morelli, X. (2014). Coronavirus nsp10, a critical co-factor for activation of multiple replicative enzymes. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M114.577353

Brierley, I., Digard, P., & Inglis, S. C. (1989). Characterization of an efficient coronavirus ribosomal frameshifting signal: requirement for an RNA pseudoknot. *Cell*. https://doi.org/10.1016/0092-8674(89)90124-4

Chan-Yeung, M., & Xu, R. H. (2003). SARS: epidemiology. *Respirology*. https://doi.org/10.1046/j.1440-1843.2003.00518.x

Chen, F., Chan, K. H., Jiang, Y., Kao, R. Y. T., Lu, H. T., Fan, K. W., … Yuen, K. Y. (2004). *in vitro* susceptibility of 10 clinical isolates of SARS coronavirus to selected antiviral compounds. *Journal of Clinical Virology*. https://doi.org/10.1016/j.jcv.2004.03.003

Chen, Y., Tao, J., Sun, Y., Wu, A., Su, C., Gao, G., … Guo, D. (2013). Structure-function analysis of Severe Acute Respiratory Syndrome coronavirus RNA Cap guanine-N7-methyltransferase. *Journal of Virology*. https://doi.org/10.1128/jvi.00061-13

Chen, Yu, Cai, H., Pan, J., Xiang, N., Tien, P., Ahola, T., & Guo, D. (2009). Functional screen reveals SARS coronavirus nonstructural protein nsp14 as a novel Cap N7 methyltransferase. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.0808790106

Chen, Yu, Su, C., Ke, M., Jin, X., Xu, L., Zhang, Z., … Guo, D. (2011). Biochemical and structural insights into the mechanisms of sars coronavirus RNA ribose 2'-O-methylation by nsp16/nsp10 protein complex. *PLoS Pathogens*. https://doi.org/10.1371/journal.ppat.1002294

Clifford, M. N. (2000). Chlorogenic acids and other cinnamates - nature, occurrence, dietary burden, absorption and metabolism. *Journal of the Science of Food and Agriculture*. https://doi.org/10.1002/(SICI)1097-0010(20000515)80:7<1033::AID-JSFA595>3.0.CO;2-T

Cui, L., & Su, X. Z. (2009). Discovery, mechanisms of action and combination therapy of artemisinin. *Expert Review of Anti-Infective Therapy*. https://doi.org/10.1586/ERI.09.68

Decroly, E., Imbert, I., Coutard, B., Bouvet, M., Selisko, B., Alvarez, K., … Canard, B. (2008).

Coronavirus nonstructural protein 16 is a Cap-0 binding enzyme possessing (nucleoside-2'O)-methyltransferase activity. *Journal of Virology*. https://doi.org/10.1128/jvi.00407-08

Dong, Z., Lu, X., Tong, X., Dong, Y., Tang, L., & Liu, M. (2017). *Forsythiae fructus* : A review on its phytochemistry, quality control, pharmacology and pharmacokinetics. *Molecules*. https://doi.org/10.3390/molecules22091466

Dos Santos, M. D., Almeida, M. C., Lopes, N. P., & De Souza, G. E. P. (2006). Evaluation of the anti-inflammatory, analgesic and antipyretic activities of the natural polyphenol chlorogenic acid. *Biological and Pharmaceutical Bulletin*. https://doi.org/10.1248/bpb.29.2236

Duan, H. F., Wu, Q. N., Zhu, Y. Y., Liu, C. C., Huang, Z. H., Qian, D. W., … Shan, M. Q. (2019). Simultaneous determination of ten components in *Lonicerae Japonicae Flos* from different habitats by UPLC. *Chinese Traditional and Herbal Drugs*. https://doi.org/10.7501/j.issn.0253-2670.2020.23.029

Eckerle, L. D., Lu, X., Sperry, S. M., Choi, L., & Denison, M. R. (2007). High fidelity of murine hepatitis virus replication is decreased in nsp14 exoribonuclease mutants. *Journal of Virology*. https://doi.org/10.1128/jvi.01296-07

Furuichi, Y., & Shatkin, A. J. (2000). Viral and cellular mRNA capping: Past and prospects. *Advances in Virus Research*. https://doi.org/10.1016/s0065-3527(00)55003-9

Ganeshpurkar, A., & Saluja, A. K. (2017). The pharmacological potential of rutin. *Saudi Pharmaceutical Journal*. https://doi.org/10.1016/j.jsps.2016.04.025

Harrison, C. (2020). Coronavirus puts drug repurposing on the fast track. *Nature Biotechnology*. https://doi.org/10.1038/d41587-020-00003-1

Huang, Q., Yu, L., Petros, A. M., Gunasekera, A., Liu, Z., Xu, N., … Olejniczak, E. T. (2004). Structure of the N-terminal RNA-binding domain of the SARS CoV nucleocapsid protein. *Biochemistry*. https://doi.org/10.1021/bi036155b

Jia, Z., Yan, L., Ren, Z., Wu, L., Wang, J., Guo, J., … Rao, Z. (2019). Delicate structural coordination of the Severe Acute Respiratory Syndrome coronavirus nsp13 upon ATP hydrolysis. *Nucleic Acids Research*. https://doi.org/10.1093/nar/gkz409

Joseph, J. S., Saikatendu, K. S., Subramanian, V., Neuman, B. W., Buchmeier, M. J., Stevens, R. C., & Kuhn, P. (2007). Crystal structure of a monomeric form of Severe Acute Respiratory Syndrome Coronavirus endonuclease nsp15 suggests a role for hexamerization as an allosteric switch. *Journal of Virology*. https://doi.org/10.1128/jvi.02817-06

Kirchdoerfer, R. N., & Ward, A. B. (2019). Structure of the SARS-CoV nsp12 polymerase bound to nsp7 and nsp8 co-factors. *Nature Communications*. https://doi.org/10.1038/s41467-019-10280-3

Kumar, V., Jung, Y. S., & Liang, P. H. (2013). Anti-SARS coronavirus agents: a patent review (2008-present). *Expert Opinion on Therapeutic Patents*. https://doi.org/10.1517/13543776.2013.823159

Kwon, S. H., Lee, H. K., Kim, J. A., Hong, S. I., Kim, H. C., Jo, T. H., … Jang, C. G. (2010). Neuroprotective effects of chlorogenic acid on scopolamine-induced amnesia via anti-acetylcholinesterase and anti-oxidative activities in mice. *European Journal of Pharmacology*. https://doi.org/10.1016/j.ejphar.2010.09.001

Lapchak, P. A. (2007). The phenylpropanoid micronutrient chlorogenic acid improves clinical rating scores in rabbits following multiple infarct ischemic strokes: synergism with tissue plasminogen activator. *Experimental Neurology*. https://doi.org/10.1016/j.expneurol.2007.02.017

Li, F. (2016). Structure, function, and evolution of coronavirus Spike proteins. *Annual Review of Virology*. https://doi.org/10.1146/annurev-virology-110615-042301

Li, X., Zhang, Y., & Yuan, Z. (2002). Determination of rutin and forsythin in fruit of *Forsythia suspensa (Thunb.) Vahl* by capillary electrophoresis-electrochemical detection. *Chromatographia*. https://doi.org/10.1007/BF02493207

Lim, J., Jeon, S., Shin, H. Y., Kim, M. J., Seong, Y. M., Lee, W. J., … Park, S. J. (2020). Case of the index patient who caused tertiary transmission of coronavirus disease 2019 in Korea: the application of lopinavir/ritonavir for the treatment of COVID-19 pneumonia monitored by quantitative RT-PCR. *Journal of Korean Medical Science*. https://doi.org/10.3346/jkms.2020.35.e79

Liu, H., & Kiledjian, M. (2006). Decapping the message: a beginning or an end. In *Biochemical Society Transactions*. https://doi.org/10.1042/BST0340035

Luo, H., Tang, Q. ling, Shang, Y. xi, Liang, S. bing, Yang, M., Robinson, N., & Liu, J. ping. (2020). Can Chinese medicine be used for prevention of coronavirus disease 2019 (COVID-19): a review of historical classics, research evidence and current prevention programs. *Chinese Journal of Integrative Medicine*. https://doi.org/10.1007/s11655-020-3192-6

Ma, Y., Wu, L., Shaw, N., Gao, Y., Wang, J., Sun, Y., … Rao, Z. (2015). Structural basis and functional analysis of the SARS coronavirus nsp14-nsp10 complex. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.1508686112

Minskaia, E., Hertzig, T., Gorbalenya, A. E., Campanacci, V., Cambillau, C., Canard, B., & Ziebuhr, J. (2006). Discovery of an RNA virus 3′→5′ exoribonuclease that is critically involved in coronavirus RNA synthesis. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.0508200103

Monteiro, M., Farah, A., Perrone, D., Trugo, L. C., & Donangelo, C. (2007). Chlorogenic acid compounds from coffee are differentially absorbed and metabolized in humans. *The Journal of Nutrition*. https://doi.org/10.1093/jn/137.10.2196

Ren, J. ling, Zhang, A. H., & Wang, X. J. (2020). Traditional Chinese medicine for COVID-19 treatment. *Pharmacological Research*. https://doi.org/10.1016/j.phrs.2020.104743

Renouf, M., Guy, P. A., Marmet, C., Fraering, A. L., Longet, K., Moulin, J., … Steiling, H. (2010). Measurement of caffeic and ferulic acid equivalents in plasma after coffee consumption: small intestine and colon are key sites for coffee metabolism. *Molecular Nutrition and Food Research*. https://doi.org/10.1002/mnfr.200900056

Rohrig, T. P. (2019). Drug Stability. In *Postmortem Toxicology*. https://doi.org/10.1016/b978-0-12-815163-1.00008-3

Snijder, E. J., Bredenbeek, P. J., Dobbe, J. C., Thiel, V., Ziebuhr, J., Poon, L. L. M., … Gorbalenya, A. E. (2003). Unique and conserved features of genome and proteome of SARS-coronavirus, an early split-off from the coronavirus group 2 lineage. *Journal of Molecular Biology*. https://doi.org/10.1016/S0022-2836(03)00865-9

Steven Foster, R. L. J. (2006). Desk reference to nature's medicine. *Choice Reviews Online*. https://doi.org/10.5860/choice.44-1279

Stobart, C. C., Sexton, N. R., Munjal, H., Lu, X., Molland, K. L., Tomar, S., … Denison, M. R. (2013). Chimeric exchange of coronavirus nsp5 proteases (3CLpro) identifies common and divergent regulatory determinants of protease activity. *Journal of Virology*. https://doi.org/10.1128/jvi.02050-13

Su, D., Lou, Z., Sun, F., Zhai, Y., Yang, H., Zhang, R., … Rao, Z. (2006). Dodecamer structure of Severe Acute Respiratory Syndrome coronavirus nonstructural protein nsp10. *Journal of Virology*. https://doi.org/10.1128/jvi.00483-06

Sutton, G., Fry, E., Carter, L., Sainsbury, S., Walter, T., Nettleship, J., … Stuart, D. I. (2004). The nsp9 replicase protein of SARS-coronavirus, structure and functional insights. *Structure*. https://doi.org/10.1016/j.str.2004.01.016

Suzuki, A., Fujii, A., Yamamoto, N., Yamamoto, M., Ohminami, H., Kameyama, A., … Saito, I. (2006). Improvement of hypertension and vascular dysfunction by hydroxyhydroquinone-free coffee in a genetic model of hypertension. *FEBS Letters*. https://doi.org/10.1016/j.febslet.2006.03.047

Suzuki, A., Kagawa, D., Ochiai, R., Tokimitsu, I., & Saito, I. (2002). Green coffee bean extract and its metabolites have a hypotensive effect in spontaneously hypertensive rats. *Hypertension Research*. https://doi.org/10.1291/hypres.25.99

Taylor, J. B., & Triggle, D. J. (2006). *Comprehensive medicinal chemistry II*. *Comprehensive Medicinal Chemistry II*.

te Velthuis, A. J. W., Arnold, J. J., Cameron, C. E., van den Worm, S. H. E., & Snijder, E. J. (2009). The RNA polymerase activity of SARS-coronavirus nsp12 is primer dependent. *Nucleic Acids Research*. https://doi.org/10.1093/nar/gkp904

Thiel, V., Ivanov, K. A., Putics, Á., Hertzig, T., Schelle, B., Bayer, S., … Ziebuhr, J. (2003). Mechanisms and enzymes involved in SARS coronavirus genome expression. *Journal of General Virology*. https://doi.org/10.1099/vir.0.19424-0

Thom, E. (2007). The effect of chlorogenic acid enriched coffee on glucose absorption in healthy volunteers and its effect on body mass when used long-term in overweight and obese people. *Journal of International Medical Research*. https://doi.org/10.1177/147323000703500620

Tsuchiya, T., Suzuki, O., & Igarashi, K. (1996). Protective effects of chlorogenic acid on paraquat-induced oxidative stress in rats. *Bioscience, Biotechnology and Biochemistry*. https://doi.org/10.1271/bbb.60.765

Wang, L., Wang, Y., Ye, D., & Liu, Q. (2020). A review of the 2019 novel coronavirus (COVID-19) based on current evidence. *International Journal of Antimicrobial Agents*. https://doi.org/10.1016/j.ijantimicag.2020.105948

Wen Su, Huifu Xu, H. H. (2013). Effects of the extract of Forsythia suspensa on influenza A H1N1 infection in vitro. *Journal of Medicinal Plants Research*. https://doi.org/10.5897/JMPR10.320

White, N. J. (2005). Artesunate versus quinine for treatment of severe *falciparum* malaria: a randomised trial. *Lancet*. https://doi.org/10.1016/S0140-6736(05)67176-0

Wu, A., Peng, Y., Huang, B., Ding, X., Wang, X., Niu, P., … Jiang, T. (2020). Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. *Cell Host and Microbe*. https://doi.org/10.1016/j.chom.2020.02.001

Xia, B., & Kang, X. (2011). Activation and maturation of SARS-CoV main protease. *Protein and Cell*.

https://doi.org/10.1007/s13238-011-1034-1

Xu, C., Ke, Z., Liu, C., Wang, Z., Liu, D., Zhang, L., … Liu, B. (2020). Systemic in silico screening in drug discovery for coronavirus disease (COVID-19) with an online interactive web server. *ChemRxiv*.

Yan, R., Zhang, Y., Li, Y., Xia, L., Guo, Y., & Zhou, Q. (2020). Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science*. https://doi.org/10.1126/science.abb2762

Yang, Y., Islam, M. S., Wang, J., Li, Y., & Chen, X. (2020). Traditional Chinese medicine in the treatment of patients infected with 2019-new coronavirus (SARS-CoV-2): a review and perspective. *International Journal of Biological Sciences*. https://doi.org/10.7150/ijbs.45538

Yu, I. M., Oldham, M. L., Zhang, J., & Chen, J. (2006). Crystal structure of the severe acute respiratory syndrome (SARS) coronavirus nucleocapsid protein dimerization domain reveals evolutionary linkage between Corona- and Arteriviridae. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M602107200

Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., … Shi, Z.-L. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. https://doi.org/10.1038/s41586-020-2012-7

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