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Molecular docking study involving bioactive natural compounds against SARS-CoV-2 proteins

Rodolfo Cabral Marcelino^{1,*}, Leonardo Pereira de Araújo¹, João Ricardo Bueno de Moraes Borba¹, Nelson José Freitas da Silveira¹

¹Laboratory of Molecular Modeling and Computer Simulation/MolMod-CS, Institute of Chemistry, Federal University of Alfenas, Alfenas, MG, 37130-001, Brazil

ABSTRACT: At the end of 2019, atypical cases of pneumonia accompanied by particular symptoms started being reported in the Chinese city of Wuhan. After a few weeks had passed, the aforementioned disease spread throughout China and became a global pandemic some months later. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the etiological agent of that atypical pneumonia, which has been named Coronavirus Disease 2019 (COVID-19). In an attempt to find an efficient therapy to either fight the spread of COVID-19 or to cure infected people from its tissue damages, researchers around the world have been studying several plants, herbs and natural products. These potential medicines have been used for therapeutic purposes throughout the years due to the great variety of bioactive compounds characterizing them. The present work aimed to perform virtual screenings by using AutoDock Vina software to execute molecular docking studies of tridimensional (3D) structures of well-known bioactive natural compounds against 24 SARS-CoV-2 proteins. We have analyzed energy scores (provided in -Kcal/mol) as well as ligand-protein intermolecular binding interactions. *In vitro* and *in vivo* assays should be furtherly conducted to corroborate with our findings and therefore validate chemical substances derived from plants and herbs, such as *Punica granatum*, *Centella asiatica* and *Solanum spp.*, as promising drug candidates to either treat or prevent COVID-19.

1. INTRODUCTION

In December of 2019, the world was struck by a new type of pneumonia with symptoms varying from fever to dry cough and dyspnea. The emergence of the virus causing this disease took place in Wuhan, China. Initially, that illness was named Wuhan pneumonia since the number of cases was restricted to the Wuhan region. However, due to the rapid virus spread and its associated disease's high infection rate in other countries, the World Health Organization (WHO) considered this pathogen a global threat and declared its resulting disease a pandemic on March 11, 2020. Through sequencing of the virus genome, it was possible to uncover that the causative agent of this newly discovered pneumonia belongs to the coronavirus family, which is knowingly capable of infecting humans (Liu et al., 2020).

Coronaviruses are RNA-type microorganisms widely spread among humans, other mammals and birds, and they can cause respiratory, enteric and neurological disorders. The etiological agent of the aforementioned atypical pneumonia has been named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), and its consequent disease has been named Coronavirus Disease 2019 (COVID-19). Initially, the

SARS-CoV-2 spike glycoprotein binds to human angiotensin-converting enzyme 2 (ACE2) in order to infect cells. Then, its transcription machinery produces four structural proteins, namely envelope small membrane (E), spike (S), nucleocapsid (N) and membrane (M), but also a myriad of accessory proteins, such as open reading frame (ORF) 3a, 8 and 9b, as well as replicase polyproteins that are proteolytically cleaved into sixteen nonstructural proteins (Nsps), including papain-like protease/PL^{pro} (Nsp3), 3C-like protease (Nsp5), Nsp9, RNA-directed RNA polymerase/RdRp (Nsp12) and helicase (Nsp13) (Liu et al., 2020).

After the pathophysiology of SARS-CoV-2 was discovered, an incessant search began for a drug or compound capable of fighting its replication and consequent infectious disease process. Different drugs and active ingredients have been tested at the same time that several countries have started a race to produce an effective vaccine against COVID-19 (Wu et al., 2020). Numerous steps are necessary to verify both the efficacy and the safety of potential new drugs when it comes to validating studies involving SARS-CoV-2. Techniques pertaining to areas of Molecular Biology, Bioinformatics,

* Corresponding author.

E-mail address: rodolfo.marcelino@sou.unifal-mg.edu.br (Rodolfo Cabral Marcelino)

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Immunology, Pharmacodynamics and Pharmacokinetics are imperative for the development of compounds that can fight COVID-19 (Pan et al., 2013). In this context, molecular docking is a computational experiment that aims to search for the leading interactions between a target (receptor/protein) and a given ligand. This bioinformatic technique takes into account the best fit as well as the finest interaction comprising a target, e.g., protein binding sites, and the specific geometry of a ligand, so that an associated binding energy score can be generated. In this type of experiment, specific *in silico* techniques are used to analyze protein-ligand interactions as well as different ligand conformations that are obtained as a result of a complex formed with a protein's active or allosteric site (Azevedo & Walter, 2019).

Nowadays, one of the most widely used computer programs on the molecular docking realm is AutoDock Vina, a software developed by Trott and Olson (2010) and whose main application is to perform rigid-flexible molecular docking screenings. In this type of *in silico* methodology, the chosen target remains rigid (with no rotation, no translation and no torsion), while its associated ligand has enough flexibility to generate distinct conformations (positions), out of which a favorable interaction can be established (Trott & Olson, 2010).

Medicinal plants have been utilized to combat a multitude of pathogens. This important fact may be due to the existence of various bioactive compounds found in natural plants (Figure 1). As a result, a great variety of drug classes originated from natural plants has emerged. Teas, poultices, decoctions and a myriad of other different forms of both extracting herbs' medicinal constituents and administering them have been used for centuries. Additionally, in the last decades, evaluations have been conducted to establish their promising applications on a therapeutic scope (Atanasov et al., 2015).

Natural compounds have a remarkable advantage over synthetic substances. Although both of these chemical entities have the same structure and therefore equal physicochemical and biological properties, compounds obtained from medicinal plants do not need to be synthesized. As a positive outcome, they do less harm to the environment since extraction techniques may use smaller quantities of organic solvents, which are, for the most part, knowingly toxic (Fuzimoto & Isidoro, 2020). For this reason, we analyzed several natural chemical compounds with the aim of verifying their efficacy against SARS-CoV-2 proteins through *in silico* investigations.

2. MATERIAL AND METHODS

All natural compounds that comprise the Food and Drug Administration (FDA) library were chosen as ligands and then downloaded. Subsequently, all selected FDA structures were converted to *.pdbqt format using AutoDock Tools (ADT) v1.5.6, which was used as a graphical interface (GUI) to edit and generate all converted files.

With regard to the quest for potential targets in SARS-CoV-2, the majority of proteins that we chose participate in the initial infection as well as in the subsequent viral replication

process that characterizes COVID-19. A total of 24 proteins were chosen from PDB (Protein Data Bank) website (<https://www.rcsb.org>), and the most adequate files (the ones exhibiting the highest resolution) were downloaded in *.pdb format, which contains all information relating to the elected structures. Proteins were also prepared using ADT GUI. Each 3D protein structure file in *.pdb format was separately inserted and read into ADT GUI. Then, polar hydrogen atoms were added, and water molecules as well as other unwanted ligand molecules were removed so that there was no bias due to the presence of these chemical entities on the binding sites. Finally, all files were saved in *.pdbqt format.

Molecular docking assays were performed using AutoDock Vina v1.2.3. To ensure that ligands would interact with the regions where they exhibit the best fit in each of the 24 chosen proteins, molecular docking calculations were delimited by a 3D space called grid box, in which Vina's search algorithms test different positions with an eye to find the first-rate ligand conformation that complexes with the given target. The aforementioned grid box delimitations encompassed the whole proteins, for we wanted to extend our search to cover potential allosteric sites.

Results for all proteins and their respective complexed ligands were arranged in an increasing energy order (from most negative to most positive) for the purpose of determining the finest interaction profiles. Then, once the most favorable results had been obtained, the best ranked conformations of every ligand associated with each macromolecule were analyzed in order to dissect interaction profiles (Figure 2). In this context, we used PyMOL v2.5 as a molecular visualization system.

We analyzed all results from a scoring function perspective, also called score, which performs variation in rotational, translational, and conformational positions as well as in motions to find the most stable target-ligand complex. Aside from that, hydrogen bonds (H-bonds) were also determined. To this end, we utilized Maestro v13.0. Finally, to validate our *in silico* results, we conducted redocking, and Root-Mean-Square Deviation (RMSD) values were calculated using AutoDock Tools (ADT) v1.5.7 (Figure 3).

3. RESULTS AND DISCUSSION

In an attempt to find leading drugs and bioactive substances to either prevent or treat COVID-19, we noticed that green tea contains catechins and polyphenols, both of which may be effective in combating this disease. Additionally, these chemical entities are promising drug candidates capable of impairing viral replication, and their activities against different types of viruses have inspired us to search for auspicious compounds capable of inhibiting SARS-CoV-2 initial infection and/or replication (Calland et al., 2012; Carneiro et al., 2016; Ismail & Jusoh, 2017).

Research studies show that several ligands are able to interact with various SARS-CoV-2 proteins, and their results exhibit a first-rate binding affinity between a given ligand and its associated protein (Senger et al., 2020). This type of

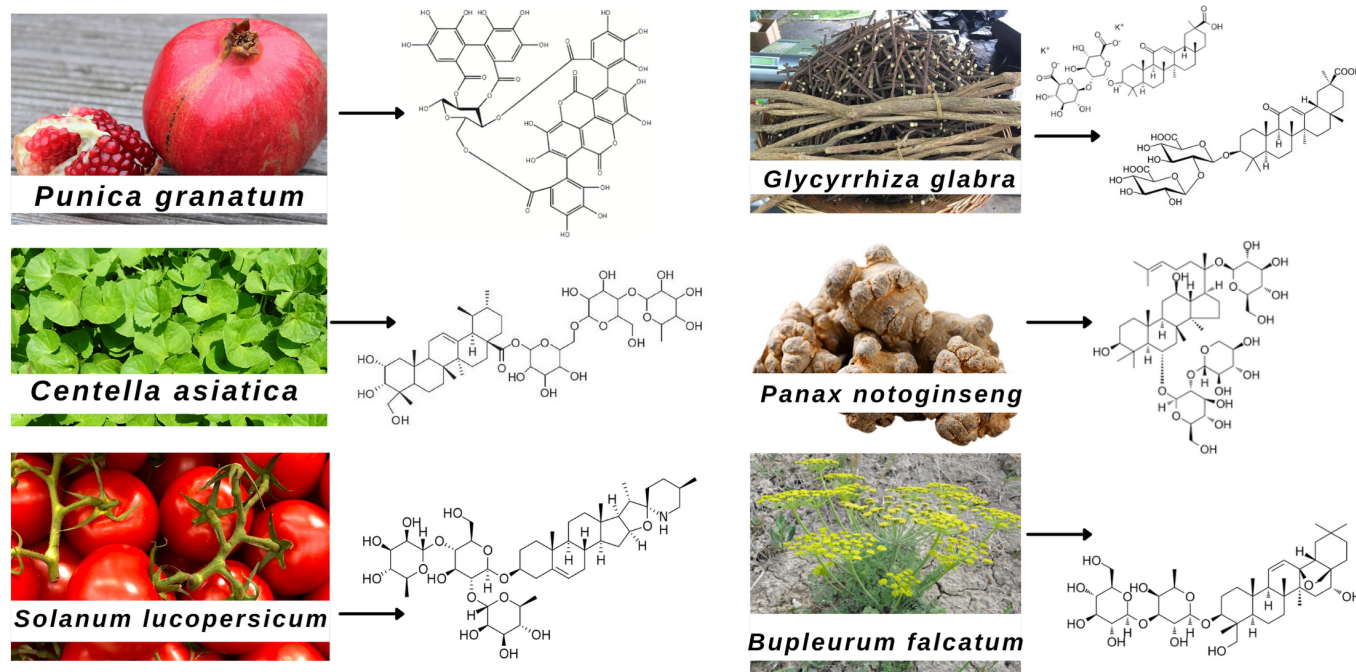
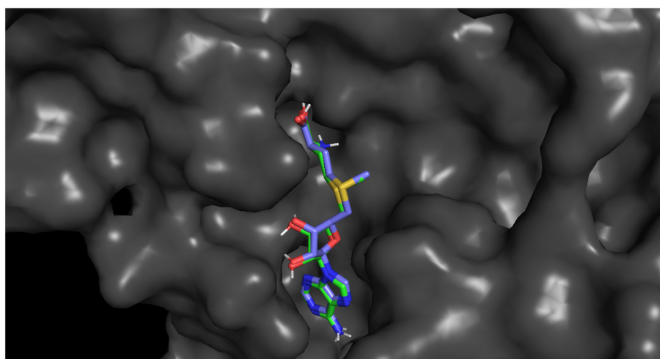


Figure 1. Plants and chemical structures of their bioactive natural compounds

Random seed: -985198944
RMSD: 1.008 Å



Random seed: 318503200
RMSD: 1.182 Å

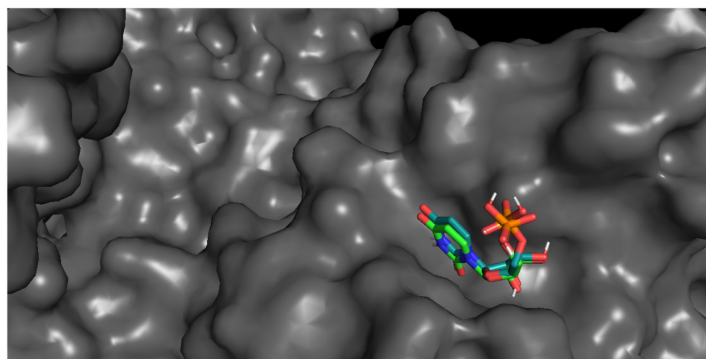


Figure 2. Visualization of 3D Molecules

binding energy between two molecules is based on the "lock and key" scheme, a well-known strategy in computational drug design. The protein-ligand or even protein-protein perfect fit is mainly based on this concept. Specific interactions between two structures occur through H-bonds, van der Waals forces as well as other interacting bonds that result in a complex between two given molecules. In this context, researchers look for the lowest possible energy to generate stability, which takes place when two substances that form a complex come together, as previously described by the "lock and key" scheme (G. Chen et al., 2020). Molecular docking screenings using AutoDock Vina v1.2.3 enabled us to find first-rate positions defined by binding energy scores between given ligands and their respective SARS-

CoV-2 macromolecules (Table 1).

Various bioactive substances, well-known for treating both neuroinflammation and neurodegenerative diseases, have demonstrated great interaction profiles with SARS-CoV-2 proteins. Punicalagin is a natural compound present in pomegranate (*Punica granatum*) and consumed worldwide. This fruit is used in popular medicine because it helps treat chronic diseases, such as diabetes, atherosclerosis and inflammatory processes (Akhtar et al., 2015; Banihani et al., 2013; Danesi & Ferguson, 2017; Igdoura et al., 2013). The majority of bioactive compounds found in pomegranate, namely phytochemicals, flavonoids and anthocyanins, are from its peel. These substances possess therapeutic properties,

Table 1

General characteristics of all SARS-CoV-2 proteins selected for molecular docking studies.

Authors	Country of origin	PDB code	DOI	Expression system
Shi et al. (2020)	China	7C01	https://doi.org/10.1038/s41586-020-2381-y	<i>Homo sapiens</i>
Xia et al. (2020)	China	6LXT	https://doi.org/10.1038/s41422-020-0305-x	<i>Escherichia coli</i>
Benton et al. (2020)	United Kingdom	6ZGE	https://doi.org/10.1038/s41586-020-2772-0	<i>Homo sapiens</i>
Mandala et al. (2020)	USA	7K3G	https://doi.org/10.1038/s41594-020-00536-8	<i>Escherichia coli</i>
Zinzula et al. (2021)	German	6ZCO	https://dx.doi.org/10.1016%2Fj.bbrc.2020.09.131	<i>Escherichia coli</i>
Semper et al. (2021)	Canada	7K3N	https://dx.doi.org/10.1016%2Fj.isci.2020.101903	<i>Escherichia coli</i>
Frick et al. (n.d.)	USA	6WEY	https://dx.doi.org/10.1021/acs.biochem.0c00309	<i>Escherichia coli</i>
Shin et al. (2020)	German	6W9C	https://doi.org/10.1038/s41586-020-2601-5	<i>Escherichia coli</i>
Jin et al. (2020)	China	6LU7	https://doi.org/10.1038/s41586-020-2223-y	<i>Escherichia coli</i>
Yin et al. (2020)	China	7BV1, 7BV2	https://doi.org/10.1126/science.abc1560	<i>Spodoptera frugiperda</i>
Littler et al. (2020)	Australia	6WXD	https://doi.org/10.1016/j.isci.2020.101258	<i>Escherichia coli</i>
Krafcikova et al. (2020)	Czech Republic	6W4H	https://doi.org/10.1038/s41467-020-17495-9	<i>Escherichia coli</i>
Kandwal and Fayne (2020)	Ireland	6WLC	https://doi.org/10.1080/07391102.2020.1825232	<i>Escherichia coli</i>
Kern et al. (2021)	USA	6XDC	https://dx.doi.org/10.1101%2F2020.06.17.156554	<i>Spodoptera frugiperda</i>
Flower et al. (2021)	USA	7JTL	https://dx.doi.org/10.1101%2F2020.08.27.270637	<i>Escherichia coli</i>

including anti-inflammatory, antioxidant, hypoglycemic and antimicrobial effects ([Grabež et al., 2020](#); [Lansky & Newman, 2007](#); [Saeed et al., 2018](#); [Xu et al., 2022](#)).

As showed in [Table 2](#), punicalagin interacted with several macromolecules, mostly proteins related to SARS-CoV-2 replication process, such as RdRp-nsp7-nsp8 complex (7C2K, 7BV1 and 7BV2), 3CL^{pro}, also named main protease – M^{pro} (7C2Q and 6LU7, in both immature and mature states, respectively), PL^{pro} (6WX4), Nsp-16-Nsp10 complex proteins (6W4H and 6WKS), ORF3a protein (6XDC), Nsp7-Nsp8 replicase complex (6XIP) and Nsp-1 protein (7K3N). In addition, punicalagin also interacted with spike glycoproteins (6LXT and 6ZGE). The best energy score results are the ones from the punicalagin-RdRp-nsp7-nsp8 complex. The interaction profile between punicalagin and 6ZGE is characterized by four H-bonds established with amino acid

residues PRO 2222, HIS 2232, SER 3115 and SER 3123, all of which are disclosed in the scheme that represents ([Figure 4](#)).

Solamargine is a substance found in tomatoes (*Solanum lycopersicum*), potatoes (*Solanum tuberosum*), aubergine/eggplant (*Solanum melongena*) as well as in other plants belonging to *Solanum* genus species. That natural compound was described by [Tang et al. \(2022\)](#) as an anticancer substance that can effectively treat various types of malignant tumors, such as hepatocellular carcinoma (HCC). Its alpha form is a knowingly bioactive alkaloid found in *Solanum surattense*, which previously showed great binding affinity with SARS-CoV-2 M^{pro} [Hasan et al. \(2020\)](#). Our results, on the other hand, exhibit that alpha-solamargine interacted through better binding energy scores with a spike-ACE2 complex (6M0J) as well as with PL^{pro} macrodomain (6WEY). H-bond interactions between solamargine and 6M0J are shown on [Figure 5](#).

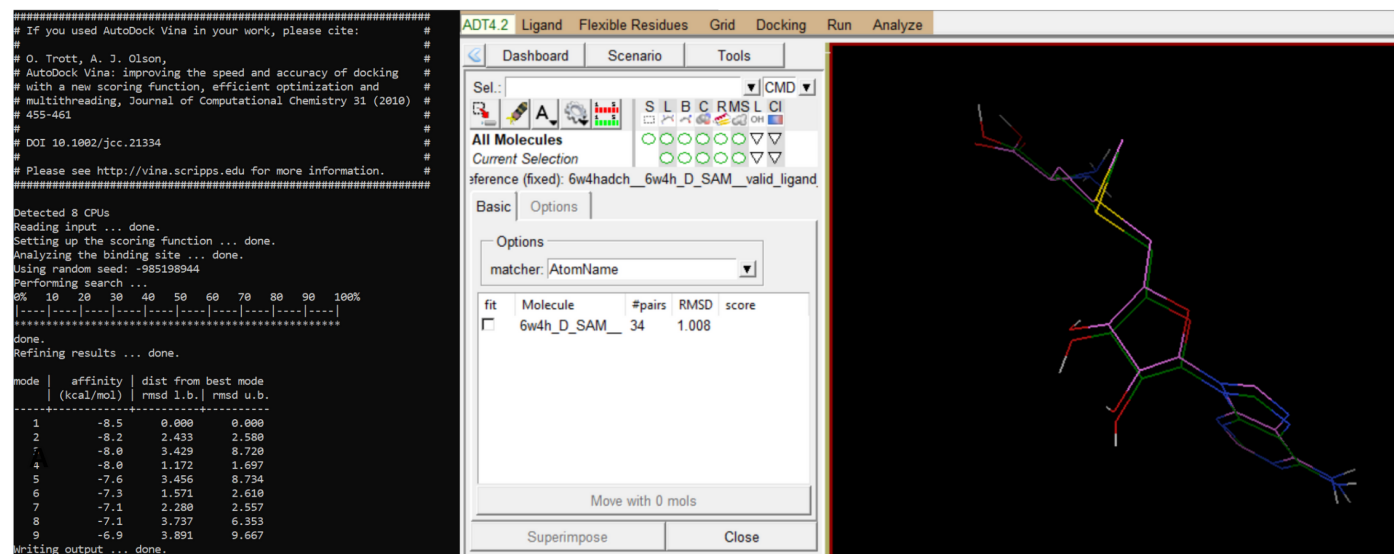


Figure 3. Docking Validation using AutoDock Vina

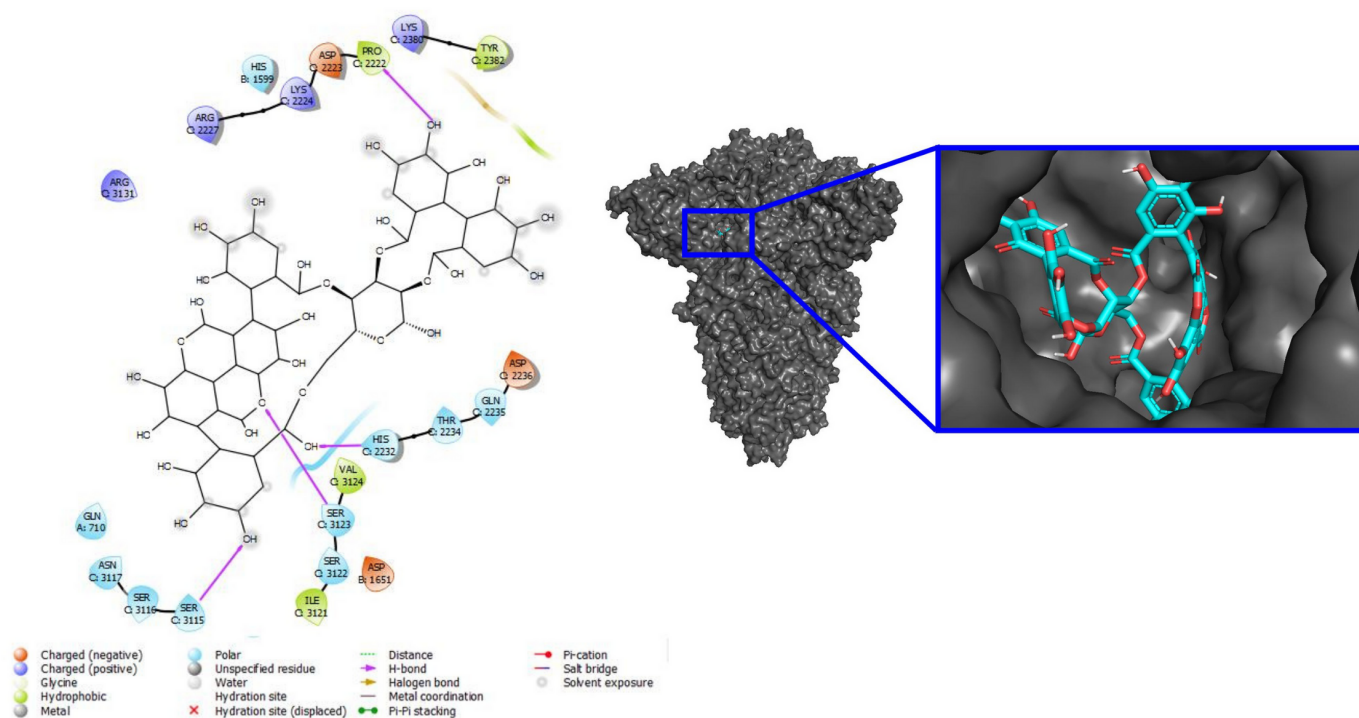


Figure 4. Interactions between Punicalagin and SARS-CoV-2 spike glycoprotein (6ZGE)

Licorice (*Glycyrrhiza glabra*) is a plant whose roots' chemical substances have been used as antioxidants to prevent cancer as well as natural compounds to inhibit the growth of *Leishmania major* promastigotes and amastigotes in infected mice. Additionally, these chemical entities have been utilized to treat bacterial, fungal and viral infections (Chrzanowski et al., 2020; Sheikhi et al., 2022). In 2005, Hoefer and collaborators discovered through molecular docking studies that glycyrrhizinate dipotassium and ammonium glycyrrhizinate, bioactive molecules found in *Glycyrrhiza glabra*, could increase

antiviral activity of glycyrrhizin derivatives against SARS-CoV (Hoefer et al., 2005). In 2020, Chrzanowski et al. (2020) disclosed that the aforementioned licorice chemical compounds enhanced glycyrrhizin potential binding to ACE2 (Chrzanowski et al., 2020). Our results show a probable multitarget function of licorice as glycyrrhizinate dipotassium and ammonium glycyrrhizinate interacted through extremely favorable binding energy scores of -10.7 kcal/mol and -10.5 kcal/mol with SARS-CoV-2 helicase/Nsp13 (5RL9) and nucleoprotein (6ZCO), respectively. In regard to 5RL9, glycyrrhizinate dipotassium

Table 2

Descriptions of all SARS-CoV-2 proteins selected for molecular docking studies.

Protein name	PDB code	Resolution (Å)	Characterization Method
Helicase/Nsp13	5RL9	1.79	1
3CL ^{pro} /M ^{pro} /Nsp5	6LU7	2.16	2
Spike glycoprotein (S2 subunit)	6LXT	2.90	2
Spike-ACE2 complex	6M0J	2.45	2
Nsp16-Nsp10 complex	6W4H	1.80	2
PL ^{pro} /Nsp3	6W9C	2.70	2
PL ^{pro} /Nsp3 (macrodomain)	6WEY	0.95	2
Nsp16-Nsp10 complex	6WKS	1.80	2
Nsp15	6WLC	1.82	2
PL ^{pro} /Nsp3	6WX4	1.66	2
Nsp9	6WXD	2.00	2
ORF3a	6XDC	2.90	3
Nsp7-Nsp8 replicase	6XIP	1.50	2
ORF9b	6Z4U	1.95	2
Nucleoprotein	6ZCO	1.36	2
Spike glycoprotein	6ZGE	2.60	3
RdRp-nsp7-nsp8	7BV1	2.80	3
RdRp-nsp7-nsp8	7BV2	2.50	3
Spike glycoprotein (S1 subunit)	7C01	2.88	2
RdRp-nsp7-nsp8	7C2K	2.93	3
3CL ^{pro} /M ^{pro} /Nsp5	7C2Q	1.93	2
ORF8	7JTL	2.04	2
Envelope small membrane	7K3G	NA	4
Nsp1	7K3N	1.65	2

1 – X-ray Crystallography; 2 – X-Ray Diffraction (XRD); 3 – Electron Microscopy (EM); 4 – Nuclear Magnetic Resonance (NMR); NA – not applicable.

formed three H-bonds with amino acid residues VAL 752, THR 770 and THR 974. All binding interactions established between glycyrrhizinate dipotassium and 5RL9 as well as between ammonium glycyrrhizinate and 6ZCO can be seen in detail on [Figure 6](#) and [7](#), respectively.

Saikosaponin A (SSA) A, saikosaponin B2 (SSB2) and saikosaponin D (SSD) are compounds present in sickle-leaved hare's-ear (*Bupleurum falcatum*), an endemic European and Western Asian plant that has anti-ulcer properties as well as the capacity to inhibit the growth of colon tumor cells *in vitro* ([Sun et al., 1991](#); [X. Zhang et al., 2022](#)). Our molecular docking studies evince that SSA, SSB2 and SSD favorably interacted with SARS-CoV-2 ORF9b protein (6Z4U), envelope small membrane protein (7K3G) and PL^{pro} (6W9C), respectively, exhibiting favorable binding energy scores of -8.2 kcal/mol, -8.9 kcal/mol and -9.7 kcal/mol, one by one. All H-bond interactions established between SSA and 6Z4U, SSB2 and 7K3G as well as between SSD and 6W9C can be visualized in detail on [Figure 8](#), [9](#) and [10](#), respectively.

Table 3

Molecular Docking Results of Bioactive Natural Compounds against SARS-CoV-2 proteins.

PDB code	Compounds	Energy ΔG - Kcal/mol
7C2K	Punicalagin	-12.7
7BV2	Punicalagin	-12.1
6ZGE	Punicalagin	-11.7
5RL9	Glycyrrhizinate Dipotassium	-10.7
6ZCO	Ammonium Glycyrrhizinate	-10.5
6LXT	Punicalagin	-10.2
7BV1	Punicalagin	-10.1
6W9C	Saikosaponin D	-9.7
6W4H	Punicalagin	-9.6
7C2Q	Punicalagin	-9.5
6LU7	Punicalagin	-9.5
7K3G	Saikosaponin B2	-8.9
6XIP	Punicalagin	-8.9
6LGZ	Solamargine	-8.8
6WX4	Punicalagin	-8.7
6WEY	Solamargine	-8.6
6WKS	Punicalagin	-8.2
6XDC	Punicalagin	-8.2
6Z4U	Saikosaponin A	-8.2
6M0J	Solamargine	-7.9
7C01	Asiaticoside	-7.4
7K3N	Punicalagin	-7.0
6WXD	Notoginsenoside R1	-6.1
7JTL	-	-

Notoginsenoside R1 and asiaticoside are bioactive compounds present in two traditional Chinese medicinal herbs, namely *Panax notoginseng* and Gotu Kola (*Centella asiatica*), respectively. Immune stimulation effects ([H.Z. Zhang et al., 2016](#)) and alleviation of chronic hypoxic pulmonary hypertension are two *Panax notoginseng* therapeutic properties in mice ([Y. Chen et al., 2017](#); [Wang et al., 2015](#)). Pertaining to SARS-CoV-2, our results show that notoginsenoside R1 favorably interacted with Nsp9 replicase (6WXD), having exhibited two H-bonds with amino acid residues ARG 10 and ARG 221 ([Figure 11](#)). On the other hand, Gotu Kola is commonly used to improve scarring and to treat skin injuries, such as lupus, ulcers and psoriasis, as well as to help combat diarrhea and fever [Y. Chen et al. \(2017\)](#). In regard to the new coronavirus, asiaticoside exhibited a great binding interaction with spike glycoprotein S1 subunit (7C01). All H-bonds established between notoginsenoside R1 and 6WXD as well as between asiaticoside and 7C01 can be seen in detail on [Figure 11](#) and [12](#), respectively.

Only the ORF8 protein (7JTL) out of 24 SARS-CoV-2 macromolecules did not demonstrate a favorable binding affinity with any of all chemical substances tested in our molecular docking screenings. The remarkable energy score values found in our research study involving bioactive natural compounds may be explained by means of ligand-protein stabilization through hydrogen bonds observed from our *in*

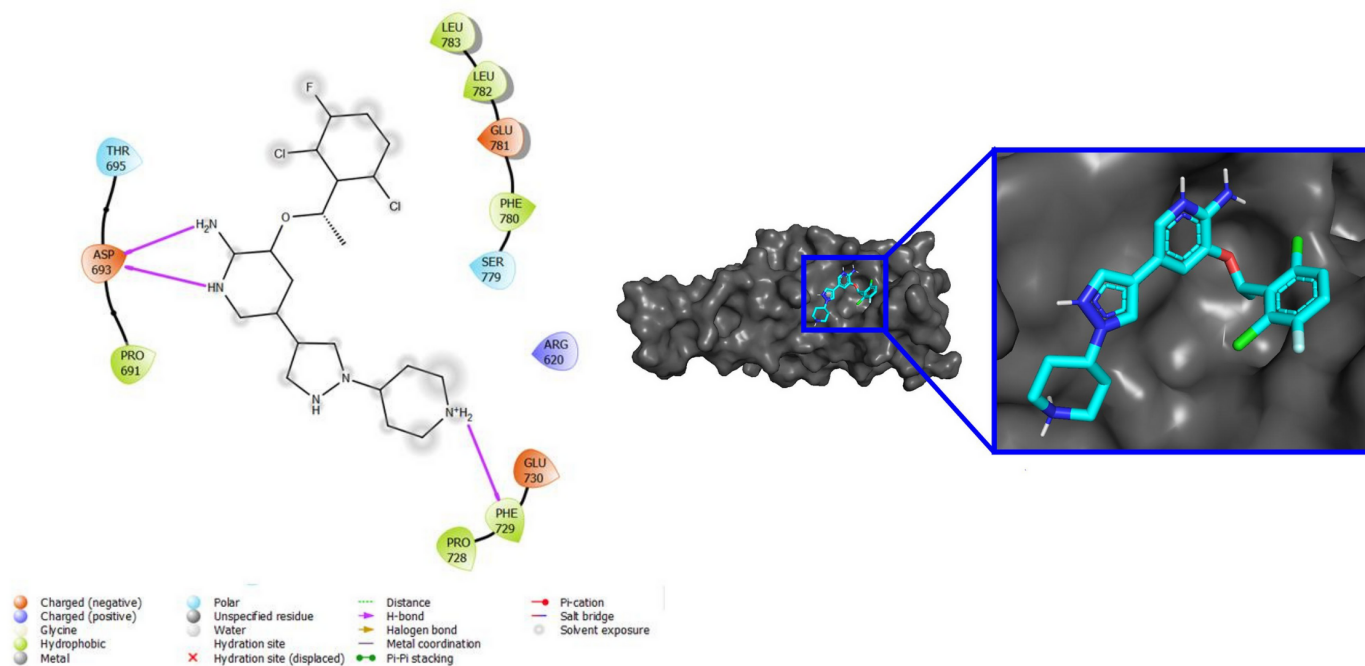


Figure 5. Interactions between Solamargine and spike-ACE2 complex (6M0J)

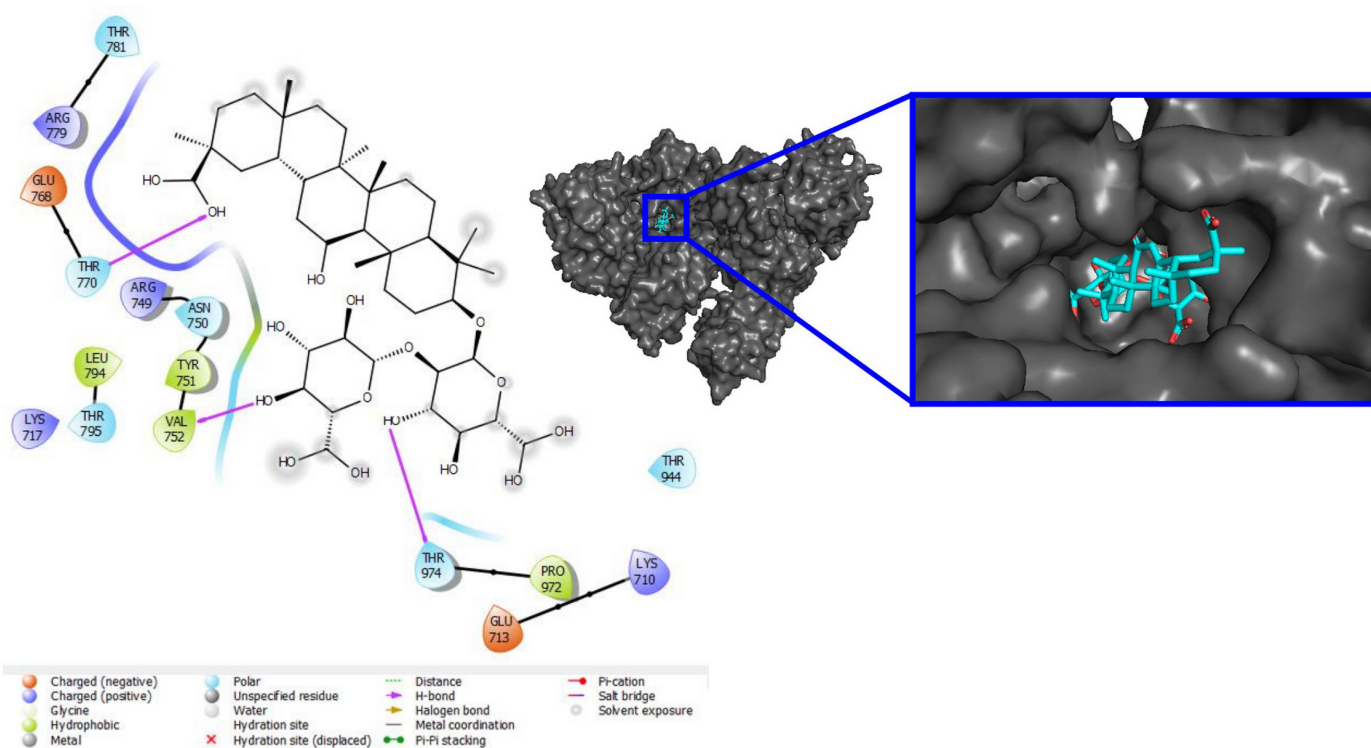


Figure 6. Interactions between Glycyrrhizinate Dipotassium and SARS-CoV-2 helicase (5RL9)

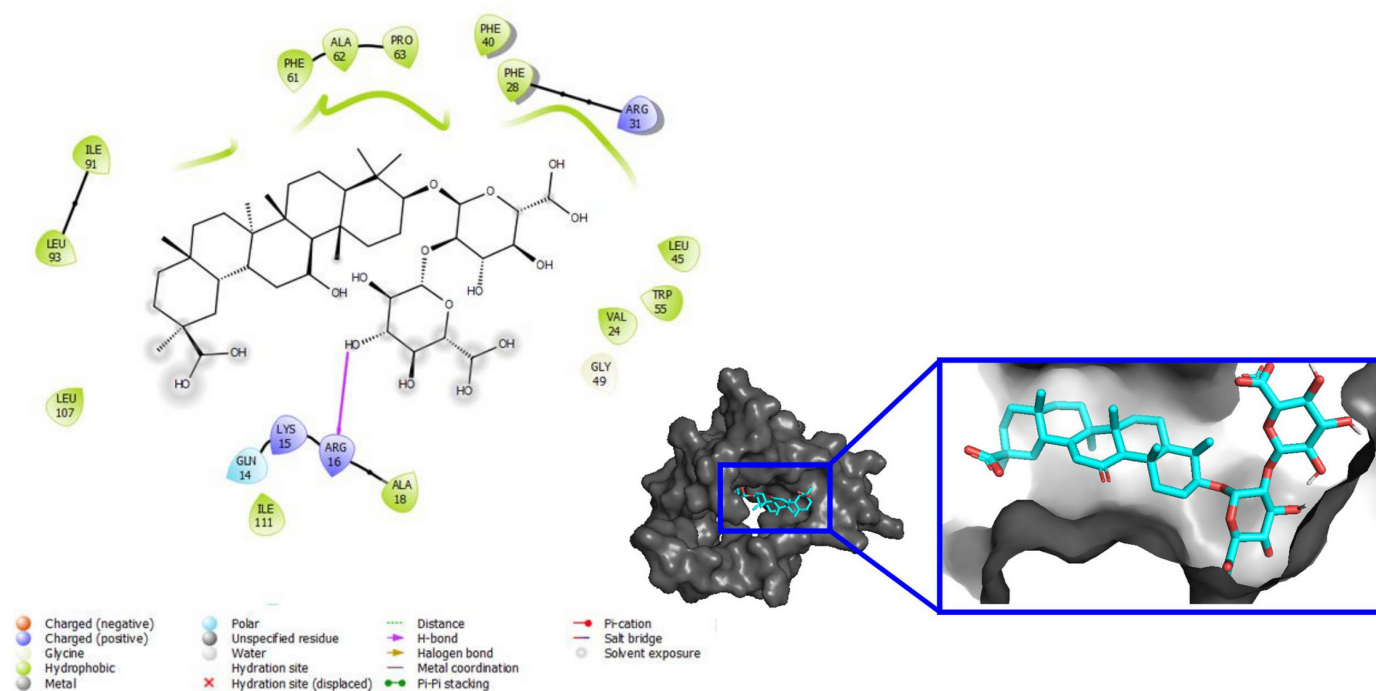


Figure 7. Interactions between Ammonium Glycyrrhizinate and SARS-CoV-2 nucleoprotein (6ZCO)

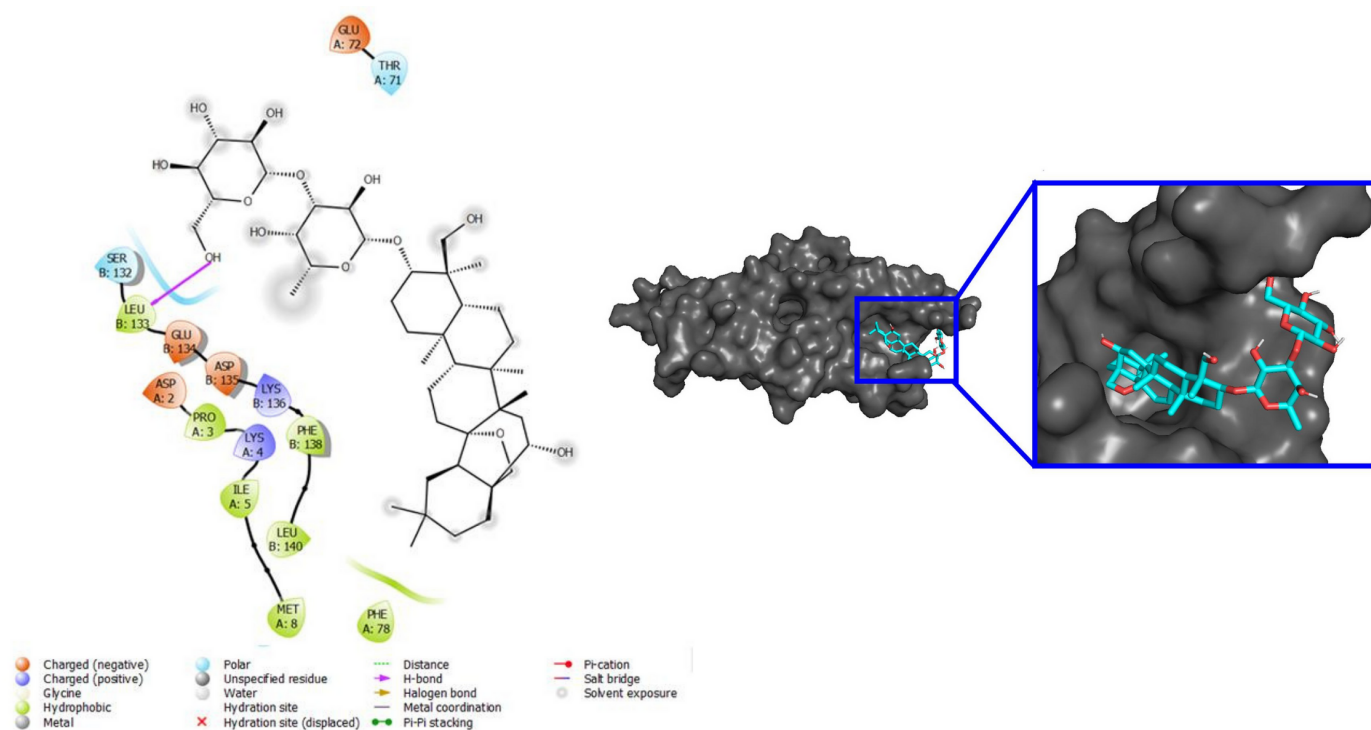


Figure 8. Interactions between Saikosaponin A and SARS-CoV-2 ORF9b protein (6Z4U)

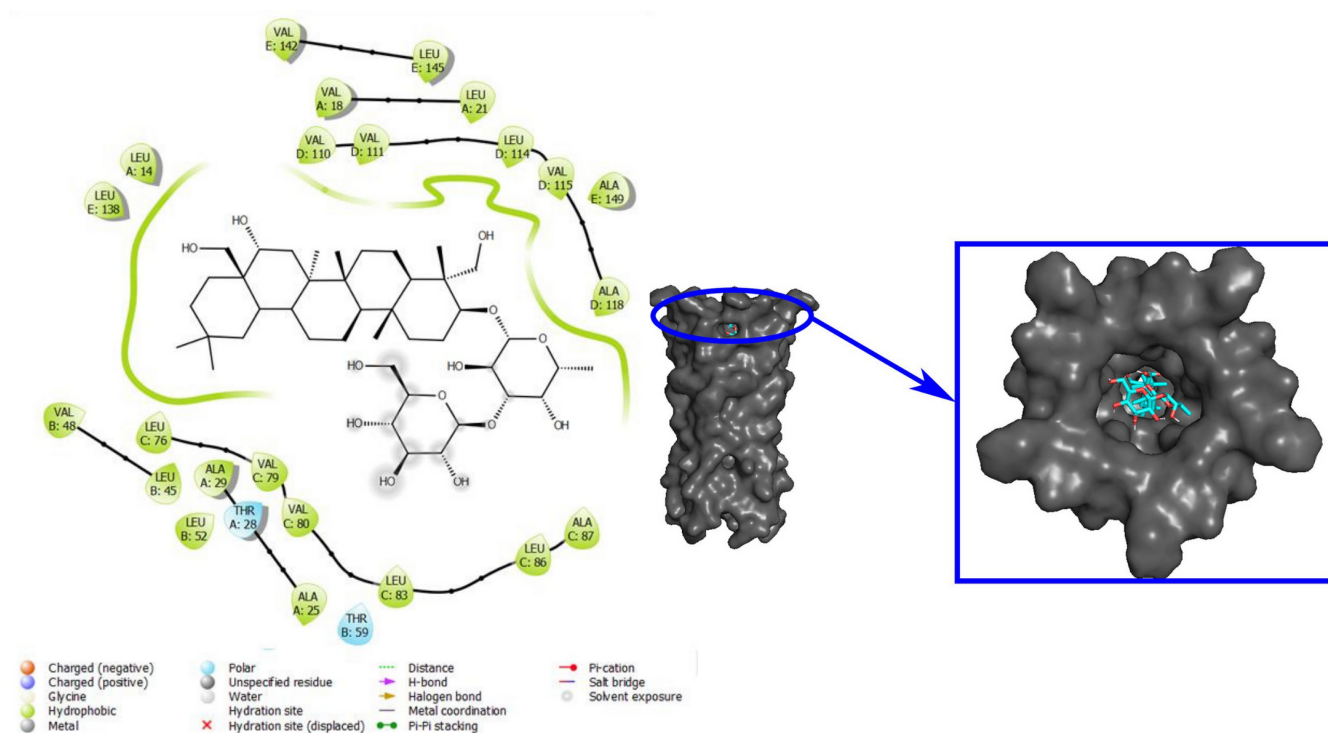


Figure 9. Interactions between Saikosaponin B2 and SARS-CoV-2 envelope small membrane protein (7K3G)

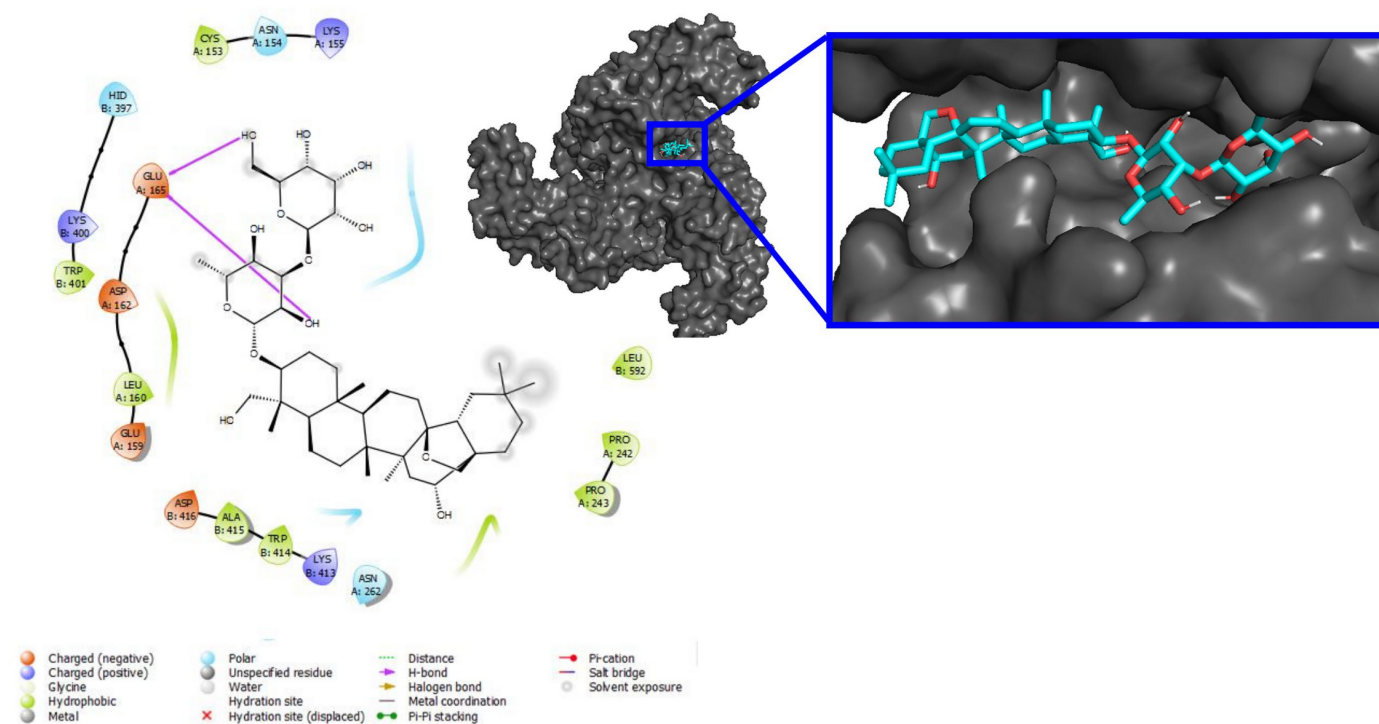


Figure 10. Interactions between Saikosaponin D and SARS-CoV-2 PLpro (6W9C)

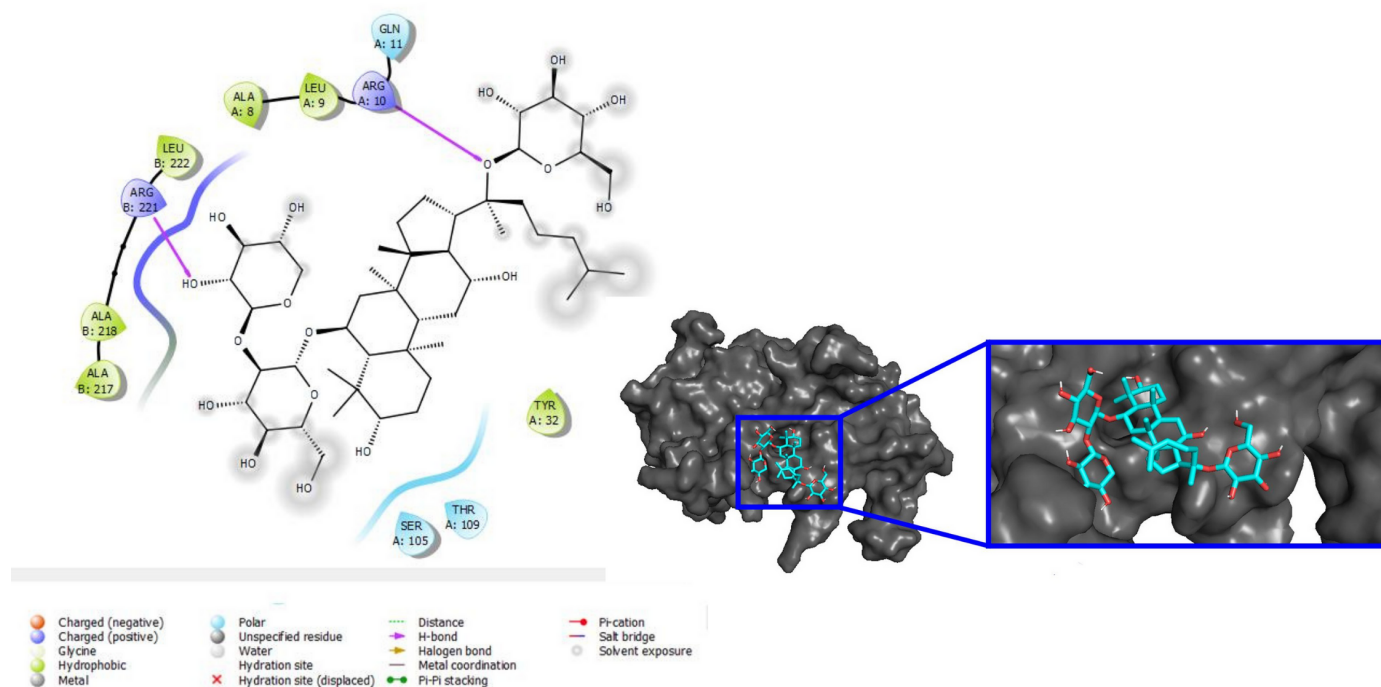


Figure 11. Interactions between Notoginsenoside R1 and SARS-CoV-2 Nsp9 replicase (6WXD)

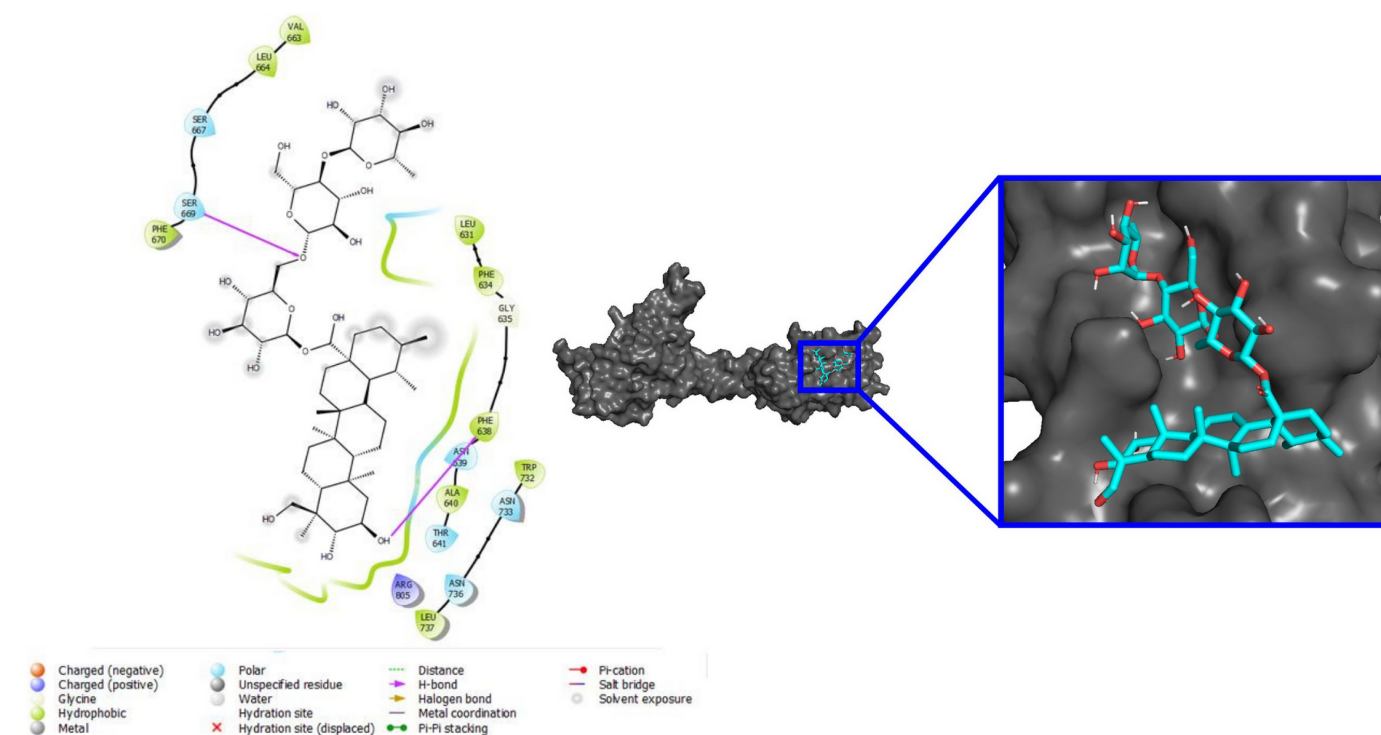


Figure 12. Interactions between Asiaticoside and SARS-CoV-2 spike glycoprotein S1 subunit (7C01)

silico point of view.

4. CONCLUSIONS

In essence, all aforementioned binding interaction profiles disclosed by our molecular docking study reveal that bioactive natural compounds derived from plants and herbs can negatively affect SARS-CoV-2 infection process. The capacity of these chemical entities to interact with essential proteins of the new coronavirus reaffirm researchers' perspective regarding the imperativeness of continuing to study phytochemicals against pathogens. Furthermore, natural compounds may be more environmentally friendly concerning the use of solvents, for there is no need of a synthetic reaction step when it comes to extracting chemical substances.

Hence, all results found in our research study indicate a valuable forthcoming application of promising bioactive natural compounds derived from plants and herbs, such as *Punica granatum*, *Centella asiatica* and *Solanum spp.*, in the realm of both *in vitro* and *in vivo* studies that will corroborate with our findings against COVID-19.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding this article.

ORCID

Rodolfo Cabral Marcelino	0000-0002-5320-3907
Leonardo Pereira de Araújo	0000-0003-0212-3365
João Ricardo Bueno de Moraes Borba	0000-0002-8356-2126
Nelson José Freitas da Silveira	0000-0001-9257-7322

AUTHOR CONTRIBUTIONS

RCM, LPA, NJFS - Research concept and design; RCA, LPA - Collection and/or assembly of data; RCA, LPA - Data analysis and interpretation; RCA - Writing the article; JRBMB, NJFS - Critical revision of the article; JRBMB, NJFS - Final approval of the article.

REFERENCES

- Akhtar, S., Ismail, T., Fraternali, D., Sestili, P., 2015. Pomegranate peel and peel extracts: chemistry and food features. *Food Chemistry*. 174, 417–425. <https://doi.org/10.1016/j.foodchem.2014.11.035>
- Atanasov, A.G., Waltenberger, B., Pferschy-Wenzig, E.M., Linder, T., Wawrosch, C., Uhrin, P., Temml, V., Wang, L., Schwaiger, S., Heiss, E.H., Rollinger, J.M., Schuster, D., Breuss, J.M., Bochkov, V., Mihovilovic, M.D., Kopp, B., Bauer, R., Dirsch, V.M., Stuppner, H., 2015. Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnology Advances*. 33(8), 1582–1614. <https://doi.org/10.1016/j.biotechadv.2015.08.001>
- Azevedo, D., Walter, 2019. Docking Screens for Drug Discovery, and others, (Eds.). Springer Protocols, pp. 978–979. <https://doi.org/10.1007/978-1-4939-9752-7>
- Banihani, S., Swedan, S., Alguraan, Z., 2013. Pomegranate and type 2 diabetes. *Nutrition Research*. 33, 341–348. <https://doi.org/10.1016/j.nutres.2013.03.003>

- Benton, D.J., Wrobel, A.G., Xu, P., 2020. Receptor binding and priming of the spike protein of SARS-CoV-2 for membrane fusion. *Nature*. 588, 327–330. <https://doi.org/10.1038/s41586-020-2772-0>
- Calland, N., Albecka, A., Belouard, S., Wychowski, C., Duverlie, G., Descamps, V., Hober, D., Dubuisson, J., Rouillé, Y., Séron, K., 2012. Epigallocatechin-3-gallate is a new inhibitor of hepatitis C virus entry. *Hepatology*. 55(3), 720–729. <https://doi.org/10.1002/hep.24803>
- Carneiro, B.M., Batista, M.N., Braga, A., Nogueira, M.L., Rahal, P., 2016. The green tea molecule EGCG inhibits Zika virus entry. *Virology*. 496, 215–218. <https://doi.org/10.1016/j.virol.2016.06.012>
- Chen, G., Seukep, A.J., Guo, M., 2020. Recent Advances in Molecular Docking for the Research and Discovery of Potential Marine Drugs. *Marine Drugs*. 18(11), 545. <https://doi.org/10.3390/md18110545>
- Chen, Y., Zeng, L., Lu, Y., 2017. Treatment effect of a flavonoid prescription on duck virus hepatitis by its hepatoprotective and antioxidative ability. *Pharmaceutical Biology*. 55, 198–205. <https://doi.org/10.1080/13880209.2016.1255977>
- Chrzanowski, J., Chrzanowska, A., Graboń, W., 2020. Glycyrrhizin: An old weapon against a novel coronavirus. *Phytotherapy Research*. 35, 629–636. <https://doi.org/10.1002/ptr.6852>
- Danesi, F., Ferguson, L.R., 2017. Could pomegranate juice help in the control of inflammatory diseases? *Nutrients*. 9(9), 958. <https://doi.org/10.3390/nu9090958>
- Flower, T.G., Buffalo, C.Z., Hooy, R.M., Allaire, M., Ren, X., Hurley, J.H., 2021. Structure of SARS-CoV-2 ORF8, a rapidly evolving immune evasion protein. *Biophysics and Computational Biology*. 118(2), e2021785118. <https://doi.org/10.1073/pnas.2021785118>
- Frick, D.N., Virdi, R.S., Vuksanovic, N., Dahal, N., Silvaggi, N.R., n.d.. Molecular Basis for ADP-Ribose Binding to the Mac1 Domain of SARS-CoV-2 nsp3. *Biochemistry*. 2020(28), 2608–2615. <https://doi.org/10.1021/acs.biochem.0c00309>
- Fuzimoto, A.D., Isidoro, C., 2020. The antiviral and coronavirus-host protein pathways inhibiting properties of herbs and natural compounds-Additional weapons in the fight against the COVID-19 pandemic? *Journal of Traditional and Complementary Medicine*. 10(4), 405–419. <https://doi.org/10.1016/j.jtcme.2020.05.003>
- Grabec, M., Škrbić, R., Stojiljković, M.P., Rudić-Grujić, V., Paunović, M., Arsić, A., Petrović, S., Vučić, V., Mirjanić-Azarić, B., Šavikin, K., Menković, N., Janković, T., Vasiljević, N., 2020. Beneficial effects of pomegranate peel extract on plasma lipid profile, fatty acids levels and blood pressure in patients with diabetes mellitus type-2: a randomized, double-blind, placebo-controlled study. *Journal of Functional Foods*. 64, 103692. <https://doi.org/10.1016/j.jff.2019.103692>
- Hasan, A., Mahamud, R.A., Bondhon, T.A., Jannat, K., nazir Farzana, B., Jahan, R., Rahmatullah, M., 2020. Molecular docking of quassinoid compounds javanicolides A-F and H with C3-like protease (or 3CLpro) of SARS and SARS-CoV-2 (COVID-19) and VP8* domain of the outer capsid protein VP4 of rotavirus A. *Journal of Medicinal Plants Studies*. 8(4), 14–19.
- Hoever, G., Baltina, L., Michaelis, M., Kondratenko, R., Baltina, L., Tolstikov, G.A., Cinatl, J., 2005. Antiviral activity of glycyrrhizic acid derivatives against SARS-coronavirus. *Journal of Medicinal Chemistry*. 48(4), 1256–1259. <https://doi.org/10.1021/jm0493008>
- Igdoura, A.-J.A., Zhang, F., Tenedero, Y., White, C.B., Macdonald, E.J., Igdoura, M.E., Trigatti, S.A., L, B., 2013. The effect of pomegranate extract on coronary artery atherosclerosis in SR-BI/ APOE double knockout mice. *Atherosclerosis*. 228, 80–89. <https://doi.org/10.1016/j.atherosclerosis.2013.02.025>
- Ismail, N.A., Jusoh, S.A., 2017. Molecular Docking and Molecular Dynamics Simulation Studies to Predict Flavonoid Binding on the

- Surface of DENV2 E Protein. *Interdisciplinary Sciences: Computational Life Sciences*. 9, 499–511. <https://doi.org/10.1007/s12539-016-0157-8>
- Jin, Z., Du, X., Xu, Y., 2020. Structure of Mpro from SARS-CoV-2 and discovery of its inhibitors. *Nature*. 582, 289–293. <https://doi.org/10.1038/s41586-020-2223-y>
- Kandwal, S., Fayne, D., 2020. Repurposing drugs for treatment of SARS-CoV-2 infection: computational design insights into mechanisms of action. *Journal of Biomolecular Structure & Dynamics*. 40(3), 1316–1330. <https://doi.org/10.1080/07391102.2020.1825232>
- Kern, D.M., Sorum, B., Mali, S.S., 2021. Cryo-EM structure of SARS-CoV-2 ORF3a in lipid nanodiscs. *Nature Structural & Molecular Biology*. 28, 573–582. <https://doi.org/10.1038/s41594-021-00619-0>
- Krafcikova, P., Silhan, J., Nencka, R., 2020. Structural analysis of the SARS-CoV-2 methyltransferase complex involved in RNA cap creation bound to sinefungin. *Nature Communications*. 11, 3717–3717. <https://doi.org/10.1038/s41467-020-17495-9>
- Lansky, E.P., Newman, R.A., 2007. Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. *Journal of Ethnopharmacology*. 109, 177–206. <https://doi.org/10.1016/j.jep.2006.09.006>
- Littler, D.R., Gully, B.S., Colson, R.N., Rossjohn, J., 2020. Crystal Structure of the SARS-CoV-2 Non-structural Protein 9, Nsp9. *iScience*. 23(7), 101258. <https://doi.org/10.1016/j.isci.2020.101258>
- Liu, Y.C., Kuo, R.L., Shih, S.R., 2020. COVID-19: The first documented coronavirus pandemic in history. *Biomedical Journal*. 43, 328–333. <https://doi.org/10.1016/j.bj.2020.04.007>
- Mandala, V.S., McKay, M.J., Shcherbakov, A.A., 2020. Structure and drug binding of the SARS-CoV-2 envelope protein transmembrane domain in lipid bilayers. *Nature Structural & Molecular Biology*. 27, 1202–1208. <https://doi.org/10.1038/s41594-020-00536-8>
- Pan, S.-Y., Zhou, S.-H., Gao, Z.-L., Yu, S.-F., Zhang, M.-K., Tang, J.-N., Sun, D.-L., Ma, Y.-F., Han, W.-F., Fong, K.-M., Ko., 2013. New Perspectives on How to Discover Drugs from Herbal Medicines: CAM's Outstanding Contribution to Modern Therapeutics. *Evidence-Based Complementary and Alternative Medicine*. 2013, 627375. <https://doi.org/10.1155/2013/627375>
- Saeed, M., Naveed, M., Bibi, J., Kamboh, A.A., Arain, M.A., Shah, Q.A., Alagawany, M., El-Hack, M., Abdel-Latif, Yatoo, M.A., Tiwari, M.I., Chakraborty, R., Dhama, S., K., 2018. The promising pharmacological effects and therapeutic/medicinal applications of Punica granatum L. (pomegranate) as a functional food in humans and animals. *Recent Patents on Inflammation & Allergy Drug Discovery*. 12, 24–38. <https://doi.org/10.2174/1872213X12666180221154713>
- Semper, C., Watanabe, N., Savchenko, A., 2021. Structural characterization of nonstructural protein 1 from SARS-CoV-2. *iScience*. 24, 101903. <https://doi.org/10.1016/j.isci.2020.101903>
- Senger, M.R., Evangelista, T.C.S., Dantas, R.F., Santana, M.V.D.S., Gonçalves, L.C.S., Neto, L.R.D.S., Silva-Junior, .., P, F., 2020. COVID-19: molecular targets, drug repurposing and new avenues for drug discovery. *Memórias do Instituto Oswaldo Cruz*. 115. <https://doi.org/10.1590/0074-02760200254>
- Sheikhi, S., Khamesipour, A., Radjabian, T., Ghazanfari, T., & Miramin Mohammadi, A., 2022. Immunotherapeutic effects of Glycyrrhiza glabra and Glycyrrhizic Acid on Leishmania major-infection BALB/C mice. *Parasite Immunology*, 44–45. <https://doi.org/10.1111/pim.12879>
- Shi, R., Shan, C., Duan, X., 2020. A human neutralizing antibody targets the receptor-binding site of SARS-CoV-2. *Nature*. 584, 120–124. <https://doi.org/10.1038/s41586-020-2381-y>
- Shin, D., Mukherjee, R., Grewe, D., 2020. Papain-like protease regulates SARS-CoV-2 viral spread and innate immunity. *Nature*. 587, 657–662. <https://doi.org/10.1038/s41586-020-2601-5>
- Sun, X.B., Matsumoto, T., Yamada, H., 1991. Effects of a Polysaccharide Fraction from the Roots of Bupleurum falcatum L. on Experimental Gastric Ulcer Models in Rats and Mice. *Journal of Pharmacy and Pharmacology*. 43(10), 699–704. <https://doi.org/10.1111/j.2042-7158.1991.tb03461.x>
- Tang, Q., Li, X., Chen, Y., Long, S., Yu, Y., Sheng, H., Wu, .., W., 2022. Solamargine inhibits the growth of hepatocellular carcinoma and enhances the anticancer effect of sorafenib by regulating HOTTIP-TUG1/miR-4726-5p/MUC1 pathway. *Molecular Carcinogenesis*. 61, 417–432. <https://doi.org/10.1002/mc.23389>
- Trott, O., Olson, A.J., 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *Journal of Computational Chemistry*. 31, 455–461. <https://doi.org/10.1002/jcc.21334>
- Wang, T., Guo, R., Zhou, G., Zhou, X., Kou, Z., Sui, F., Li, C., Tang, L., Wang, Z., Weber, C., Sliva, K., Rhein, C.V., Kümmerer, B.M., Schnierle, B.S., 2015. Traditional uses, botany, phytochemistry, pharmacology and toxicology of Panax notoginseng (Burk.) F.H. Chen: A review. *Antiviral Research*. 188, 234–258. <https://doi.org/10.1016/j.jep.2016.05.005>
- Wu, Y., Kuan, Y., Chang, L., Lou, L.G., Edwards, Bly, K., Doma, Z.-R., Xie., 2020. In silico identification of drug candidates against COVID-19. *Informatics in Medicine Unlocked*. 21, 100461. <https://doi.org/10.1016/j.imu.2020.100461>
- Xia, S., Liu, M., Wang, C., 2020. Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion. *Cell Research*. 30, 343–355. <https://doi.org/10.1038/s41422-020-0305-x>
- Xu, P., Xu, L., Huang, S., Li, D., Liu, Y., Guo, H., Zhong, .., S., 2022. Analysis of the Molecular Mechanism of Punicagin in the Treatment of Alzheimer's Disease by Computer-Aided Drug Research Technology. *ACS Omega*. 7(7), 6121–6132. <https://doi.org/10.1021/acsomega.1c06565>
- Yin, C.W., Mao, X.D., Luan, D.D., Shen, Q., Shen, H., Su., 2020. Structural basis for inhibition of the RNA-dependent RNA polymerase from SARS-CoV-2 by remdesivir. *Science*. 368(6498), 1499–1504. <https://doi.org/10.1126/science.abc1560>
- Zhang, H.Z., Liu, D.H., Zhang, D.K., Wang, Y.H., Li, G., Yan, G.L., Cao, L.J., Xiao, X.H., Huang, L.Q., Wang, J.B., 2016. Quality assessment of Panax notoginseng from different regions through the analysis of marker chemicals, biological potency and ecological factors. *PLoS One*. 11, 2415–2422. <https://doi.org/10.1371/journal.pone.0164384>
- Zhang, X., Liu, Z., Chen, S., Li, H., Dong, L., Fu, X., 2022. A new discovery: Total Bupleurum saponin extracts can inhibit the proliferation and induce apoptosis of colon cancer cells by regulating the PI3K/Akt/mTOR pathway. *Journal of Ethnopharmacology*. 283, 114742. <https://doi.org/10.1016/j.jep.2021.114742>
- Zinzula, J., Basquin, S., Bohn, F., Beck, S., Klumpe, G., Pfeifer, I., Nagy, A., Bracher, F.U., Hartl, W., Baumeister, 2021. High-resolution structure and biophysical characterization of the nucleocapsid phosphoprotein dimerization domain from the Covid-19 severe acute respiratory syndrome coronavirus 2. *Biochemical and Biophysical Research Communications*. 538, 54–62. <https://doi.org/10.1016/j.bbrc.2020.09.131>