REVISTA
CIENCIAS NATURALES
Facultad de Ciencias Naturales · Universidad Nacional de Salta

e-ISSN 2953-5441
Vol. 2(1): 10-21

ARTÍCULO

Antiviral Activity in Silico of Benzylisoquinoline Alkaloids existing in *Berberis lilloana* and *B. commutata* against key Proteins of SARS-Cov-2

Actividad Antiviral in Silico de Alcaloides Bencilisoquinolinas Presentes en *Berberis lilloana* y *B. commutata* Contra Proteínas Claves Del SARS-Cov-2

María A. Zígolo

Instituto de Investigaciones para la Industria Química (INIQUI), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Facultad de Ciencias Naturales, Universidad Nacional de Salta, Av. Bolivia 5150, (4400) Salta, Argentina.
maz130685@gmail.com

Abstract

SARS-CoV-2, a new strain of coronavirus (CoV), was identified in Wuhan, China, in 2019, and has been threatening public health worldwide. The aim of this work was to evaluate protoberberine alkaloids compounds of vegetal origin as potential SARS-CoV-2 inhibitors through docking studies. Three key proteins of SARS-CoV-2, recently crystallized, were used as molecular targets: the spike glycoprotein (S), the main protease (Mpro) and the RNA-dependent RNA polymerase (RdRp). Molecular docking was performed using AutoDock, with the Lamarckian Genetic Algorithm, to analyse the probability of docking. The best energy binding values for S protein were, in kcal/mol: -10.67 for Jatrorrhizine, -9.65 for berberine, -9.22 for 5, 6-dihydroconstrictosine. For Mpro, they were, in kcal/mol: -10.15 for 5,6-dihydroconstrictosine, -9.86 for jatrorrhizine, -8.48 for berberubine. Finally, the best binding values for RdRp were, in kcal/mol: -9.04 for belambine, -8.99 for canadine and -8.90 for berberine. Key hydrogen bonds and hydrophobic interactions between protoberberine alkaloids and the respective viral proteins were identified. These results suggest that these alkaloids could potentially be useful as drugs to be experimentally evaluated against COVID-19.

Keywords: Binding affinity; Docking; Protoberberine alkaloids; SARS-CoV-2

Resumen

El SARS-CoV-2, una nueva cepa de coronavirus identificada en Wuhan, China, en 2019, ha estado amenazando la salud pública en todo el mundo. El objetivo de este trabajo fue estudiar compuestos alcaloides de protoberberina de origen vegetal como potenciales inhibidores del SARS-CoV-2 mediante estudios de acoplamiento. Se emplearon como dianas moleculares tres proteínas claves del SARS-CoV-2, recientemente cristalizadas: la glicoproteína de pico (S), la proteasa principal (Mpro) y la ARN polimerasa dependiente de ARN (RdRp). El acoplamiento molecular se realizó mediante AutoDock, con el Algoritmo Genético Lamarckiano, para analizar la probabilidad de acoplamiento. Los mejores valores de energías de unión para la proteína S fueron, en kcal / mol: -10,67 para jatrorrizina, -9,65 para berberina y -9,22 para 5,6-dihidroconstrictosina. Para Mpro fueron, en kcal/mol: -10,15 para 5,6-dihidroconstrictosina, -9,86 para jatrorrizina y -8,48 para berberubina. Para RdRp fueron, en kcal / mol: -9.04 para berlambina, -8.99 para canadina, y -8.90 para berberina. Se identificaron enlaces de hidrógeno claves e interacciones hidrofóbicas entre algunos de los alcaloides de protoberberina y las respectivas proteínas virales. De estos resultados surge que estos alcaloides podrían ser potencialmente útiles como fármacos antivirales a ser evaluados experimentalmente contra COVID-19.

Palabras clave: Afinidad de unión; Alcaloides de protoberberina; Docking; SARS-CoV-2

Zigolo, M. A. (2024). In Silico Antiviral Activity from *Berberis lilloana* and *B. commutata*. Protoberberine Alcaloids Against Key Proteins SARS-Cov-2. Revista Ciencias Naturales, 2(1), 10–21.

Recibido: 26/7/2023 Aceptado: 21/2/2024 Publicado: 15/3/2024 Editora: María Victoria García

INTRODUCTION

In 2019, a new coronavirus caused an outbreak of pulmonary disease in the city of Wuhan, the capital of Hubei province in China, and has since spread globally and represent a challenge to public health. This virus has been named SARS-CoV-2, because the RNA genome is about 82% identical to the SARS coronavirus (SARS-CoV). The disease caused by SARS-CoV-2 is called COVID-19 (Wu *et al.*, 2020; Zhou *et al.*, 2020). Human-to-human transmission is very fast. (Li, 2020). However, waiting for a vaccine, there are currently no effective medications against 2019-nCoV/ SARS-CoV-2.There is an urgent need for the development of effective prevention and treatment strategies for COVID-19 disease. Taking into account that although many of the vaccines developed promise to be very effective, the period of immunity they will generate in the population is unknown. (Detoc *et al.*, 2020; Kaur & Gupta, 2020; Malika *et al.*, 2020).

Many drugs known for their effectiveness against other viruses are being investigated in search of a solution to the rapid and lethal advance of the new SARS-CoV-2. Some protease inhibitors used for treatment of patients with Human Immunodeficiency Virus infection and acquired immune deficiency syndrome (HIV/AIDS) or neuraminidase inhibitors or nucleotide analogues like Remdesivir (GS-5734™) used for other pathogenic CoVs (Khaerunnisa *et al.*, 2020). Too the chloroquine/hydroxychloroquine extensively used against malaria and previously described as a potent inhibitor of most coronaviruses in vitro, including SARS-CoV-19 (White *et al.*, 2014; Devaux *et al.*, 2020). Plants are great producers of secondary metabolites with biological activity very interesting (Tallei *et al*., 2020; Yang *et al.*, 2018). The antiviral activitys of Many of these secundary metabolites have been described in numerous medicinal plants and associated with compounds like flavonoids, heterosides, terpenes, and triterpenes, organic acids, alkaloids, saponins, and quaternary ammonium salts, among others (Roy & Saraf, 2006; Cecil *et al.*, 2011; Wang *et al.*, 2017; Khaerunnisa *et al.*, 2020).

Many species containing protoberberin alkaloids have long been used in traditional medicine in India, China, Tibet, and Japan primarily as antimicrobial remedies (Leitao Da-Cunha *et al.*, 2005). The antiviral activity of berberine-related alkaloids was demonstrated and many of the infective mechanisms have been established. In respiratory syncytial virus infection (RSV), (Shin *et al.*, 2015) in several strains of influenza A H1N1 (Cecil *et al.*, 2011; Enkhtaivan *et al.*, 2018) and in the hepatitis C virus (Hung *et al.*, 2018).

Docking tools give information from protein-ligand interactions. This is critical when rushing in the search of metabolites that can cure or ease symptoms of diseases when time and resources for extensive experimental work are scarce. Recently, in the last months, many SARS-CoV-2 macromolecules have been crystallized which has been repositioned in the Protein Data Bank (PDB) and is accessible by the public. This allow more effective simulations may be performed than if model proteins from other closely related viruses have to be used (Jin *et al.*, 2020; Wrapp *et al.*, 2020; Zhang *et al.*, 2020). One of the best characterized drug targets among coronaviruses is the main protease (Mpro, also called 3CLpro) (Anand *et al.*, 2003; Joshi *et al*., 2020, 2021).

This enzyme is essential for processing the polyproteins that are translated from the viral RNA (Hilgenfeld, 2014). Cleavage by 3CLpro and others viral proteases occurs at a conserved glutamine catalitic residue via the protease CYS-HIS dyad in which the cysteine thiol functions as the nucleophile in the proteolytic process (Anand *et al.*, 2003). In the initial stage of the SARS-CoV-2 replication cycle, the attachment of the virion to the host cell is initiated by interactions between the S protein (spike) and human angiotensin-converting enzyme (ACE2 receptor) (Hoffmann *et al.*, 2020). The S protein-receptor interaction is the primary determinant for a coronavirus to infect a host species and governs the

tissue tropism of the virus. Structurally, the coronavirus spicule is a trimer arranged asymmetrically. Each monomeric unit has three segments: an extensive ectodomain, a single-pass transmembrane segment, and a small intracellular tail (Walls *et al.*, 2016). The ectodomain contains two subunits; the S1 subunit allows binding to the ACE2 receptor while the S2 allows the fusion of viral and host membranes. Two major domains in S1, N-terminal domain (S1-NTD) and C-terminal domain (S1-CTD), have been identified. The S1-CTD, also known as the receptor-binding domain (RBD), is responsible for recognizing protein receptors ACE2 (Liu *et al.*, 2015). Also, the spike protein exists in two structurally distinct conformations, prefusion and postfusion.

All RNA viruses encode an RNA-dependent RNA polymerase (RdRp) that catalyzes the synthesis of their RNAs. The polymerase RdRp is a crucial viral enzyme in the life cycle of RNA viruses; due this, it has been targeted in various viral infections, who the hepatitis C virus (HCV), the Zika virus (ZIKV), and coronaviruses (CoVs) (Ganesan & Barakat, 2017; Elfiky *et al.*, 2018, 2019). The RdRp active site is highly conserved, with two successive and surface-accessible aspartates in a beta-turn structure (Doublie & Ellenberger, 1998). This protein is an important target for new antiviral drugs for SARS-CoV2.

In the present study, we investigated commun protoberberine alcaloids from various vegetal species as potential inhibitor candidates for COVID-19 protease Mpro, the glycoprotein S and the RNA polymerase RdRp. These proteins are essential to the transmission and virulence of the virus. By inhibiting anyone of these proteins or all, for a higher active therapy, the severity of the infection will be reduced. Our efforts have been placed in competitively inhibiting the binding of its natural substrates. The findings of the present study will provide other researchers information to identify the right drug to combat COVID-19.

MATERIALS AND METHODS

Viral proteins

Main protease (Mpro)

SARS-CoV-2 Mpro was crystallized in a complex with N3 inhibitor and coordinates and structure factors were deposited in Protein Data Bank (PDB ID: 6LU7) (Jin *et al.*, 2020). The main protease monomer contains three domains. Domains I and II (residues 8-101 and 102-184) are made of antiparallel -barrel structures in a chymotrypsin-like fold responsible for catalysis. Binding pocket with the main residues: THR24, THR26, HIS41, PHE140, ASN142, GLY143, CYS145, HIS163, HIS164, GLU166, and HIS172. Contain the CYS-HIS catalitic dyad.

S protein

Due to the crucial importance of SARS-CoV-2- S spike protein, the characterization at the prefusion structure has been deposited in the Protein Data Bank (PDB ID: 6VSB) (Wrapp *et al.*, 2020).

RNA polymerase (RdRp)

RNA polymerase (RdRp) bound to its essential co-factors, nsp7 and nsp8 was crystallized and coordinates and structure factors were deposited in Protein Data Bank (PDB ID: 6NUR) (Kirchdoerfer & Ward, 2019).

Antiviral compounds

Different protoberberine alkaloids, of plant origin, with known antimicrobial activity, were selected and evaluated for their interaction with the two viral proteins. In addition, synthetic drugs at the present time used for the treatment of COVID-19 were used as controls. The alkaloids three-dimensional structure were obtained from database PubChem (https://pubchem.ncbi.nlm.nih.gov/): canadine (CID 21171), artavenustine (CID 181939), berberubine(CID 72704), berlambine (CID 11066), berberine (CID 2353), jatrorrhizine (CID 72323), lambertine (CID 10217), protopine (CID 4970), cryptopine (CID 72616), quinolizinium,

2,10 dihydroxy-13-oxidodibenzo (CID 148262), constrictosine (CID 10016432), pallimamine (CID 132542920), orientalidine (CID 185550), 5,6-dihydroconstrictosine (CID 10401464). The synthetic nelfinavir (CID 64143) and atazanavir (CID 148192), were used as controls for the interaction with Mpro and acyclovir (CID 135398513) and emtricitabine (CID 60877) to RdRp protein and and umifenovir (CID 131411) to S protein. The ivermectin (CID 9812710), chloroquine (CID 2719), hydroxychloroquine (CID 3652), were used like a control for the three proteins.

Docking analysis

Docking calculations were carried out with AutoDock software (consist of two generations of software: AutoDock 4 and AutoDock Vina). This software uses Lamarckian genetic algorithm (LGA) for calculations (Morris *et al.*, 2009). Number of genetic algorithm (GA) runs was set to 200 for each case analyzed. The Autodock 4 program was applied considering all rotatable bonds for ligands and the whole protein as a rigid structure. For the location and extent of the 3D area the search space was defined by specifying a center, the number of points at each dimension and the points between spaces to focus the search space in the enzyme active site or putative binding region.

For Mpro, the grid box center coordinates corresponded to the C atom of the histidine residue 163: x (-19.055), y (16.637), and z (64.100). Dimensions and extention of the grid box were 90 x 90 x 90 and point spacing was 0.492 Å. For the S protein, the grid box center coordinates corresponded to the oxygen atom of the alanine 419 residue for chain B: x (232.020), y (245.760), and z (265.659). Dimensions of the grid box were 124 x 124 x 124 and point spacing was 0.869 Å. Finally for the RNA polymerase RdRp, the grid box center coordinates corresponded to the nitrogen atom of the lysine 533 residue: x (142.000), y (139.000) and z (150.000). Dimensions of the grid box were 114x 114x 114 and point spacing was 0.469Å.

Prior to docking Mpro, the inhibitor N3 and water molecules were removed from the protein structure. Polar hydrogen atoms were added, and Gasteiger atom charges were assigned to protein atoms. Other parameters were set to default values.

The 200 conformers found for each compound with the Autodock program were grouped in clusters that were ordered according to a ranking, which was determined by the stability of the enzyme-ligand complexes within each cluster. Clustering of the 200 conformers was done according to the similarity with the conformation adopted inside the enzyme. The criterion used to evaluate such similarity was the residue mean quadratic square root deviation (RMSD). The residues were obtained through the difference between the atom coordinates of a given conformer respect to the cluster to which the most stable conformer belongs. Each cluster grouped conformers with RMSD lower or equal to 2.0 Å.

For visualization of protein-ligand complexes the software Visual Molecular Dynamics 1.9.1 (VMD) was used (Theoretical and Computational Biophysics Group, University of Illinois).

RESULTS

Selection of conformers

From the 200 docking runs for all the evaluated compounds, both from natural origin and the synthetic controls, the binding energies for each conformer were obtained which, in turn, were grouped into clusters. Each compound was represented by the most populated clusters, which in some cases were also those with the lowest binding energies. The binding energies for the interactions of each compound with the corresponding protein are shown in Table 1.

When Mpro protein interacted with natural compounds, similar values than those observed with the synthetic antivirals atazanavir and nelfinavir, were found. The strongest affinities for 5,6-dihydroconstrictosine and Jatrorrhizine, with ΔG of -10.15 and -9.86 kcal/mol, were obtained, respectively. Other

Alcaloid	Skeleton type	Mpro ΔG (kcal/mol)	S Protein ΔG (kcal/mol)	RpRp ΔG (kcal/mol)
Canadine	I	-7.69	-7.36	-8.99
Artavenustine	I	-7.33	-7.36	-7.76
Berberubine	II	-8.48	-7.67	-8.89
Berlambine	II	-7.38	-7.12	-9.04
Berberine	III	-7.35	-9.65	-8.90
Jatrorrhizine	III	-9.86	-10.67	-7.57
Lambertine	IV	-7.82	-9.20	-8.65
Protopine	V	-7.66	-7.40	-8.04
Criptopine	V	-7.94	-7.92	-7.80
Constrictosine	VII	-7.19	-7.20	-8.75
Pallimamine	VIII	-7.41	-9.11	-8.35
Orientalidine	IX	-7.36	-8.03	-7.41
5,6-dihydroconstrictosine	X	-10.15	-9.22	-7.57
Atazanavir		-9.47	-	
Nelfinavir		-8.25	-	
Umifenovir		-	-7.47	
Acyclovir		-	-	-5.88
Emtricitabine		-	-	-4.60
Chloroquine		-5.11	-7.25	-4.10
Hydroxychloroquine		-4.20	- 5.30	-5.10
Ivermectine		-6.70	-8.71	-6.03

Table 1. Gibbs free energy (ΔG, in kcal/mol) calculated for the interaction between SARS-CoV-2 and different molecular targets Mpro, S protein, RNA polymerase RdRp and selected alkaloids. Synthetic antivirals used as controls are in bold.

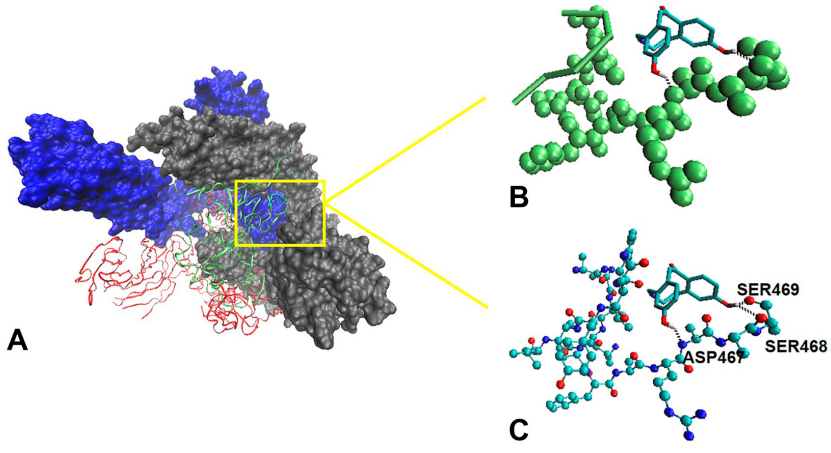

Figure 1. Three-dimensional (3D) molecular interaction of 5, 6 -dihydroconstrictosine with S glycoprotein. **A**. Structure of spike protein S: chain A (blue), chain B (red) and chain C (grey). Domain RBD of chain B (green ribbons). **B**. Residues 420 to 425 from domain RBD of chain B (green trace) and residues 460 to 461 (green spheres). The 5, 6- dihydroconstrictosine is represented in licorice form (cyan carbon atoms,

compounds showed interesting affinities for Mpro, for example: berberubine, canadine and cryptopine (Table 1). On the other hand, the plant-derived natural compounds evaluated for S protein, yielded very high affinity and slightly higher values compared to controls. The highest affinities were observed for jatrorrhizine, berberine, and 5,6-dihydroconstrictosine. The most interesting results were observed for jatrorrhizine and berberine (-10.67and -9.65 kcal/mol, respectively), which presented more affinity for the viral S protein compared to the synthetic antivirals umifenovir, ivermectin, chloroquine, and hydroxychloroquine (-7.47, -8.71, -7.25, and -5.30 kcal/mol, respectively) (Table 1).

Analysis of the molecular interactions of candidate compounds with the proteins evaluated.

The 5, 6- dihydroconstrictosine, berberine and jatrorrhizine showed the best interactions with the spike protein with the most favorable Gibbs free energies (-9.22, 9.65 and 10.67 kcal/mol respectively) (Table 1). 5, 6-dihydroconstrictosine interacted with a great number of residues from RBD domain (Fig. 1A). Whit residues 420 to 425 and 460 to 463 of chain B (Fig. 1B). There was a total of three hydrogen bonds with residues ASP467, SER469 and SER468 of the RBD domain of B chain (Fig. 1C).

Key interactions of possible candidate drugs with Mpro were analyzed: 5, 6-dihydroconstrictosine, jatrorrhizine, berberubine and nelfinavir (Fig. 2).

All the natural and semisynthetic active compounds were bounded to the enzyme active site, as well as the controls nelfinavir and atazanavir. The 5, 6-dihydroconstrictosine presented five hydrogen bond interactions (Fig. 2A): two with THR26 (2.51 Å and 2.73 Å), one whit GLY143 (1.33 Å), one with ASN142 (2.01 Å) and one with CYS145 (1.02 Å) implicated in CYS-HIS dyad. It also showed hydrophobic interactions with other residues from catalytic site. All these interactions increased the binding affinity with respect to the control compound nelfinavir, which presented only one hydrogen bond interactions with GLU166 (2.33 Å) and

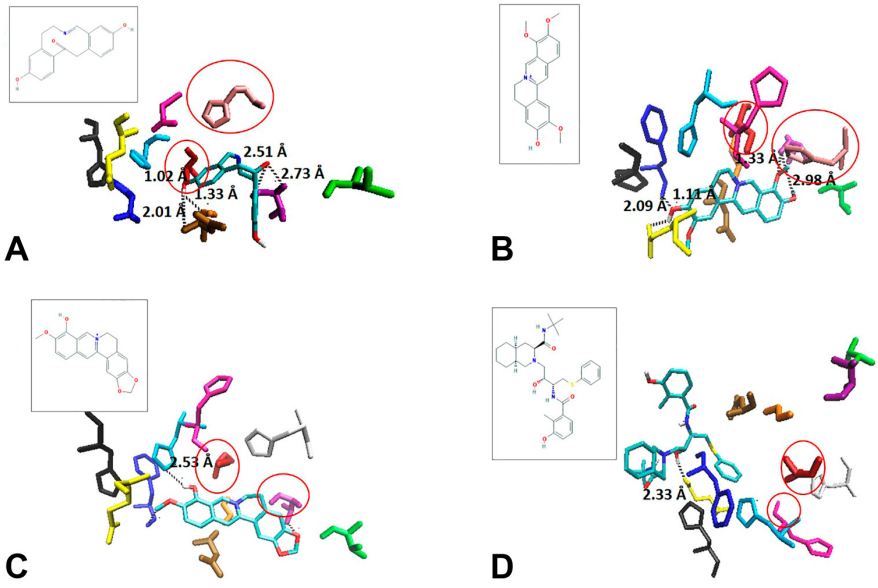

Figure 2. Three-dimensional (3D) molecular interaction of compounds: **A**. 5,6-dihydroconstrictosine. **B**. Jatrorrhizine. **C**. Berberubine. **D**. Nelfinavir with M^{pro}. Active site residues are represented with sticks: THR24 (green), THR26 (purple), HIS41 (pink), PHE140 (blue), ASN142 (brown), GLY143 (orange), CYS145 (red), HIS163 (cyan), HIS164 (magenta), GLU166 (yellow), and HIS172 (black). The black dotted lines, indicated Hydrogen bond. The residues CYS145 and HIS41 are indicated by red circle.

hydrophobic interactions with other protease catalytic residues (Fig. 2D). Jatrorrhizine, had four hydrogen bond interactions with catalytic residues HIS41 (implicad in CYS-HIS dyad), HIS164, GLU166 and PHE140 (2.98 Å, 1.33 Å, 2.09 and 1.11 Å respectively) and hydrophobic interactions with the other catalytic residues (Fig. 2B).

Berberubine interacted with the Mpro mainly by hydrophobic interactions, presenting only one hydrogen bond interaction with the HIS166 catalytic residue (Fig. 2C).

Jatrorrhizine and berberine interacted with residues 350 to 460 from RBD domain of B chain and the some residues of chain C. In contrast the control drug nelfinavir interacted only with residues of chain C (Fig. 3).

Berlambine, canadine and berberina binding to RdRp in NSP12, chain A (blue). All three alkaloids interact with the NSP12 chain through hydrophobic interactions with various residues of the polymerase including the ASP255 and ASP256 active site residues (Fig. 4).

DISCUSSION

Plants have a long evolutionary history of developing resistance against viruses. The ability to produce secondary metabolites, has generated a wide range of possible sources for antiviral drugs. Medicinal plants have been used ancestrally in different civilizations. The history have showed their low toxicity and their minimal adverse effects at the doses used. In this sense, representing an important advantage as compared with new synthetic drugs, which need to be evaluated for safety through strict clinical trials (Abbas *et al.*, 2016; Horden, 2016).

Phytocomplexes are mixtures of active compounds, formed by isomers and analogue compounds, with the capacity for synergistic activity and contains compounds that help mitigate the toxicity of the main active drugs. This help microorganisms are less likely to develop resistance to a phytocomplex than to a unique compound in a synthetic drug (Alonso, 2008; Bruneton, 2001; Camponovo & Bandoni, 1995). In this study, we analyzed variosus active compounds that are commonly found in the alkaloid extracts of various species of the Berberidaceae family and that have been used throughout history as medicinal plants.

It is important to highlight that all the evaluated protoberberine alkaloids bound with good affinities to the three evaluated SARS-CoV-2 targets. Therefore, we believe that they could act through different mechanisms of action.

The alkaloids 5, 6- dihydroconstrictosine and jatrorrhizine interact with key residues in main protease Mpro involved in substrate

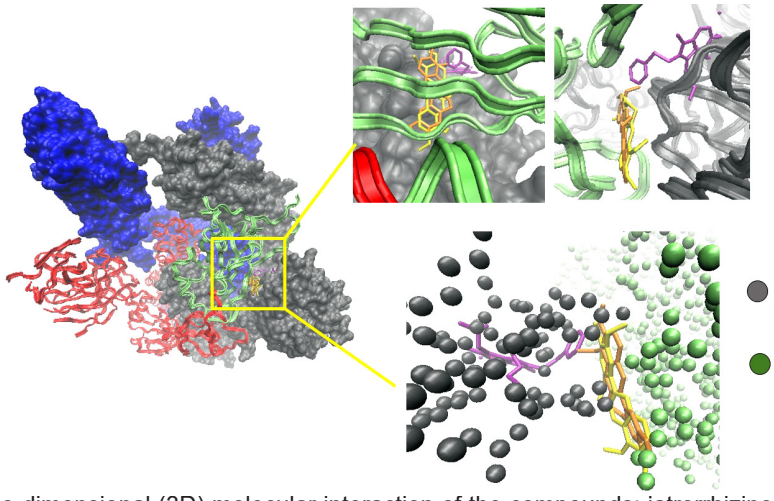

Figure 3. Three-dimensional (3D) molecular interaction of the compounds: jatrorrhizine (yellow), berberine (orange) and umifenovir (purple) with S glycoprotein. Structure of spike protein S: chain A (blue), chain B (red) and chain C (grey). Domain RBD of chain B (green ribbons). Residues 150 to 157 and 176 to 200 from chain C (grey spheres). RBD residues 350 to 460 from chain B (green spheres).

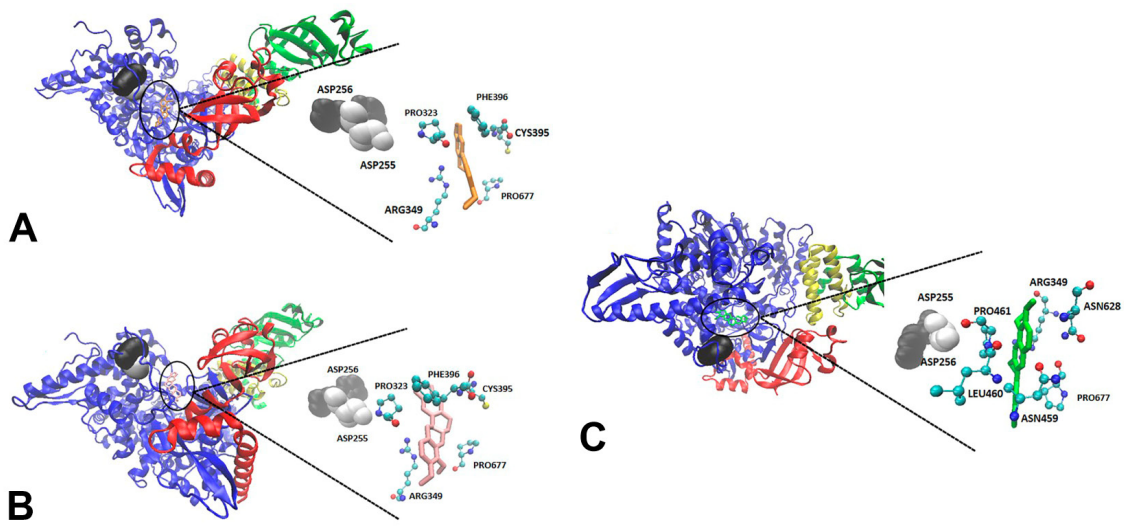

Figure 4. Three-dimensional (3D) molecular interaction of berlambine (**A**), canadine (**B**) and berberine (**C**) with polymerase RdRp. Black circles indicate the RdRp site where ligands bind. The two active site residues are shown as black (ASP256) and white (ASP255) spherical surface.

binding, including the CYS145-HIS41 dyad. This is an important factor for viral proteins sintesys inhibition. This factor was considered for the synthesis of potential inhibitors of the main protease Mpro structure-based ligand design. Macchiagodena *et al.* (2020), obtained a gibb-free energy value of -8.92 kcal/mol from to the most potent ligand for 3CLpro protease, which linked of the dyad CYS-HIS (Macchiagodena *et al.*, 2020). The compounds 5, 6-dihydroconstrictosine and jatrorrhizine showed higher free Gibbs energy (-10.15 and -9.61 kcal/mol).

The most studied protoberberine alkaloids is a Berberine. Berberine is marketed in various countries around the world as dietary supplements (BIOTICS RESEARCH®, DR. MEROLA®). It has antimalarial, antibacterial, and antiviral effects, among other biological activities (Wang *et al.*, 2017). Berberine can also inhibit the replication of influenza A in vitro in several different cell types and with two influenza A (H1N1) strains (Cecil *et al.*, 2011). As in this study, other researchers found that berberine displayed binding energy of -7.91 kcal/mol to Mpro (in this work -7.35 kcal/mol to Mpro and -9.65 kcal/mol to glycoprotein S) and had the ability to producing conformational changes in the target enzyme. (Agrawal *et al.*, 2020). In this work we show that it also binds to the spike protein S (-9.65 kcal / mol) and to the RdRp polymerase (-8.90) with higher affinity than in the Mpro protease. This makes it a very interesting compound to be clinically evaluated in COVID-19 considering that it is already commercially available and there are several laboratories that manufacture it.

Hydrophobic interactions played a particularly important role for the Mpro protein. In the case of berberubine and nelfinavir, which showed a good binding energy value, showed one only hydrogen bond and interactions of hydrophobic nature with the protease residues. Khaerunnisa *et al.* (2020) also showed the importance of hydrophobic interactions for a series of compounds of plant origin in the interaction with the viral protease Mpro (Malika *et al.*, 2020).

All of the compounds evaluated against S glycoprotein showed very favorable binding energies, higher than those for the synthetic

antiviral compounds, umifenovir (-7.47 kcal/mol), chloroquine (-7.25 kcal/mol), hydroxychloroquine (-5.30 kcal/mol) and ivermectin (-8.71 kcal/mol) directed to that viral target. The most favorable binding energies were obtained for jatrorrhizine (-10.67), berberine (-9.65kcal/mol), 5, 6-dihydroconstrictosine (-9.22 kcal/mol), lambertine (-9.20 kcal/mol) and pallimamine (-9.11 kcal/mol). These results are very promising. Others works, where the coupling of natural and synthetic compounds with viral glycoprotein S, were analyzed showed best binding energies obtained for S protein were: -11.55 kcal/mol to coenzyme A, -11.089 kcal/mol to flavin adenine dinucleotide, and - 9.36 kcal/mol to tiludronate (Hall & Ji, 2020).

In agreement with the low Gibbs free energy, for the 5, 6- dihydroconstrictosine, jatrorrhizine and brberine numerous molecular interactions were observed, all of them with the RBD region (recognition of ACE human receptors), chain B, were observed. Therefore, the three alkaloids would be a good candidate to be evaluated experimentally to learn how it would affect the glycoprotein activity.

The 5,6- dihydroconstrictosine is a arotopine alkaloids. This group of protoberberine alkaloids are apparently not very common. Their occurrence in Aristolochiaceae family was strictly confined to A. constricta. Protopine alkaloids are usually reported as C-2 substituted on the basis of biogenetic considerations. The unusual absence of C-2 substitution was noticed in the protopines of Aristolochia family (Rastrelli *et al.*, 1997).

Jatrorrhizine is found in some plant species from the families Papaveraceae, Berberidaceae, Menispermaceae, Ranunculaceae, and Rutaceae (Cecil *et al.*, 2011). This compound has demonstrated inhibits mammary carcinoma cells (Sun *et al.*, 2019), in vitro and in vivo antitumor activities (Qin *et al.*, 2019), antihypercholesterolemic effect (Wu *et al.*, 2014). 5,6-dihydroconstrictosine, Jatrorrhi-zine and berberine interacts whit many hydrophobic interactions with the spike protein residues. It also formed hydrogen bonds. Many of these interactions of different nature were established in the RBD region of S glycoprotein. For this reason, we consider that they three compounds are the most promising compound with potential to inhibit spike protein.

For RNA polymerase RdRp the best values of binding energies were for Berlambine -9.04 kcal /mol, Canadine -8.99 kcal / mol and berberine -8.90 kcal / mol. Other studies evaluated the synthetic compounds rivavirin, tenofovir, sofosbuvir, IDX 184, setrobuvir and YAK as possible inhibitors of RNA polymerase RdRp with binding energy values of (-7.8, -6.9, -7.5, -9.0, -9.3 and - 8.4 kcal / mol respectively) (Elfiky, 2016) and azafluorene derivates (Venkateshan *et al.*, 2020).

The alkaloids with better binding energies berlambine, canadine and berberine binding to NSP12 in the active site (Fig. 4). This binding like and any union to its cofactors could affected the correct assembly of the polymerase, preventing its correct functioning. For this we thinking that berlambine, canadine and berberine would be good candidate drugs to be experimentally evaluated against this SARS-CoV-2 polymerase.

In fact, being derived from medicinal plants with ancestral use proven for oral consumption is an important advantage of some natural origin compounds. Should they be administered as part of phytocomplexes, there might be a synergistic effect of the desired activity while potential side effects of the main active compounds, if they were given pure, would be attenuated. This important advantage would facilitate the production and reduce the cost because fewer downstream operations would be needed as there is no need to obtain the high purity product. (Cortés *et al.*, 2004; Sharapin *et al.*, 2000).

CONCLUSIONS

There are already several drugs and vaccines being assessed clinically for SARS-CoV-2. However there is still a need to identify additional treatments as alternatives tha vaccines, since the period of immunity that they generate in the population is unknown and also if we take into account that there

are people who cannot be vaccinated for different reasons. Identifying antiviral drugs from medicinal plants and sintetic drugs with side-effect profiles known by clinicians are very important. In this sence, various protoberberine alkaloids proposed in this work has been from known ancestral uses and many of them are sold as supplements dietary for oral consumption.

Based on the binding energies obtained and that protoberberine alkaloids interact with the key catalytic residues, interaction conformations between the proposed natural compounds and protease Mpro, glycoprotein S and RNA polimerase RdRp proteins are highly possible. In addition, the tested natural compounds performed better than the synthetic antivirals used as controls: nelfinavir and atazanavir, for protease Mpro, umifenovir for S protein and acilovir and emtriacitabine for polymerase RdRp. Thus, the protoberberine alkaloids could be potential active agents for treatment of COVID-19, either alone or as adjunct therapies with other medications found to have antiviral activity in vivo.

This study also contains valuable information to increase the knowledge of certain protoberberine nucleus of compounds with best affinities towards these molecular targets. Furthermore, the present study provides molecular details that allow us to propose structural modifications of some compounds to make the interaction between them and proteins even more effective. Everyday we hope to be better prepared and we know that Effective antiviral treatments can save lives, and we need to have them ready in the future.

ACKNOWLEDGMENTS

The author expresses gratitude to CONICET and acknowledges the contributions of the editor, reviewers, and Cintia Avila for image processing. This work was funded by CONICET.

REFERENCES

Abbas Zaidi, S., Jameel, S., Jafri, K., Khan, S., & Ahmad, E. (2016). Ilaj bil hijamah (cupping therapy) in the Unani system of medicine: anecdotal practice to evidence-based therapy. *Acta Medico*-Historica Adriatica, 14(1) 81-94.

Agrawal, A., Jain N. K., Kumar, N., & Kulkarni, G. T. (2020). Molecular Docking Study to Identify Potential Inhibitor of COVID-19 Main Protease Enzyme: An In-Silico Approach. Chem *Rxiv*. https://doi.org/10.26434/chemrxiv.12170904.v1

Alonso, J. (2008). *Tratado de Fitofármacos y Nutracéuticos*. (1° Ed). Reimpr.Corpus Ediciones, Rosario, Argentina.

Anand, K., Ziebuhr, J, Wadhwani, P., Mesters, J. R., & Hilgenfeld, R. (2003). Coronavirus main proteinase (3CLpro) structure: Basis for design of anti-SARS drugs. *Science*, 300, 1763–1767. https://doi.org/10.1126/science.1085658

Bruneton, J. (2001). *Farmacognosia, fitoquímica, plantas medicinales*. (2° Ed). Editorial ACRIBIA S.A., Argentina.

Camponovo, L., & Bandoni, A. (1995). *Farmacología: Materia Médica y Terapéutica*. Buenos Aires: López Etchegoyen Libreros Editores, Argentina.

Cecil, C. E., Davis, J. M., Cech, N. B., & Laster, S. M. (2011). Inhibition of H1N1 influenza A virus growth and induction of inflammatory mediators by the isoquinoline alkaloid berberine and extracts of goldenseal (*Hydrastis canadensis*). *International Immunopharmacology*, 11(11), 1706-1714. https://doi.org/10.1016/j.intimp.2011.06.002

Cortés Gallardo, V., Macedo Ceja, J., Hernández Arroyo, M., Arteaga Aureoles, G., Espinoza Galván, D., & Rodriguez Landa, J. (2004). Farmacognosia: breve historia de sus orígenes y su relación con las ciencias médicas. *Revista Biomedica*, 15, 123-136. https://doi.org/10.32776/revbiomed.v15i2.381

Detoc, M., Bruel, S., Frappe, P., Tardy, T., Botelho-Nevers, E., & Gagneux-Brunon, A. (2020). Intention to participate in a COVID-19 vaccine clinical trial and to get vaccinated against COVID-19 in France during the pandemic. *Vaccine*, 38(45) 7002-7006. https://doi.org/10.1016/j.vaccine.2020.09.041

Devaux, C. A., Rolain, J. M., Colson, P., & Raoult, D. (2020). New insights on the antiviral effects of chloroquine against coronavirus: what to expect for COVID-19. *International Journal of Antimicrobial Agents*, 55(5):105938. https://doi.org/10.1016/j.ijantimicag.2020.105938

Doublie, S., & Ellenberger, T. (1998). The mechanism of action of T7 DNA polymerase. *Current Opinion in Structural Biology*, 8(6):704-12. https://doi.org/10.1016/s0959-440x(98)80089-4

Elfiky, A. A., & Ismail A. (2019). Molecular dynamics and docking reveal the potency of novel GTP derivatives against RNA dependent RNA polymerase of genotype 4a HCV. *Life Sciences*, 238, 116958. https://doi.org/10.1016/j.lfs.2019.116958

Elfiky, A. A., & Elshemey, W. M. (2018). Molecular dynamics simulation revealed binding of nucleotide inhibitors to ZIKV polymerase over 444 nanoseconds, *Journal of Medical Virology*, 90(1), 13-18. https://doi.org/10.1002/jmv.24934

Elfiky, A. A., & Elshemey, W. M. (2016). IDX-184 is a superior HCV direct-acting antiviral drug: a QSAR study, *Medicinal Chemistry Research*, 25(5):1005-1008. https://doi.org/10.1007/s00044-016-1533-y

Enkhtaivan, G., Kim, D. H., Park, G. S., Pandurangan, M., Nicholas, D. A., Moon, S.H., Kadam, A.A., Patel, R.V., Shin, H. S., & Mistry, B.M. (2018). Berberine-piperazine conjugates as potent influenza neuraminidase blocker. *International Journal of Biological Macromolecules*, 119, 1204–1210. https://doi.org/10.1016/j.ijbiomac.2018.08.047

Ganesan, A., & Barakat, K. (2017). Applications of computer-aided approaches in the development of hepatitis C antiviral agents, *Expert Opin. Drug Discovery*, 12(4), 407-425. https://doi.org/10.1080/17460441.2017.1291628

Hall, D. C., & Ji, H. F. (2020). A search for medications to treat COVID-19 via in silico molecular docking models of the SARS-CoV-2 spike glycoprotein and 3CL protease. *Travel Medicine and Infectious Disease*, 35, 101646. https://doi.org/10.1016/j.tmaid.2020.101646

Hilgenfeld, R. (2014). From SARS to MERS: Crystallographic studies on coronaviral proteases enable antiviral drug design. *The FEBS Journal*., 281, 4085–4096. https://doi.org/10.1111/febs.12936

Hoffmann, M., Kleine-Weber, H., Krüger, N., Müller, M., Drosten, C., & Pöhlmann (2020). The novel coronavirus 2019 (2019-nCoV) uses the SARS-coronavirus receptor ACE2 and the cellular protease TMPRSS2 for entry into target cells. *BioRxiv*.The preprint server for biology. https://doi.org/10.1101/2020.01.31.929042

Horden, P. and Kukita Yoshikawa, Naoë (2016). *Medicine, Religion and Gender in Medieval Culture*., 60(3), 441-442.

Hung, T.-C., Jasse, A., Liu, C.- H., Lin, Ch.-J., Lin, Ch.- Ch., Wong, S. H., Wang, J. Y., Yen, M.- H., & Lin, L.- T. (2018). Berberine inhibits hepatitis C virus entry by targeting the viral E2 glycoprotein. *Phytomedicine*, 53, 62-69. https://doi.org/10.1016/j.phymed.2018.09.025

Jin, Z., Du, X., Xu, Y., Deng, Y., Liu, M., Zhao, Y., Zhang, B., Li, X., Zhang, L., Peng, C., Duan, Y., Yu, J., Wang, L., Yang, K., Liu, F., Jiang, R., Yang, X., You, T., Liu, X., Yang, X., Bai, F., Liu, H., Liu, X., Guddat, L.W., Xu, W., Xiao, G., Qin, Ch., Shi, Z., Jiang, H.,Rao, X., & Yang, H., (2020). Structure of Mpro from SARS-CoV-2and discovery of its inhibitors. *Nature*.582, 289-293. https://doi.org/10.1038/s41586-020-2223-y.

Joshi, T., Joshi, T., Sharma, P., Mathpal, S., Pundir, H., Bhatt, V., & Chandra, S. (2020). In silico screening of natural compounds against COVID-19 by targeting Mpro and ACE2 using molecular docking. *European Review for Medical and Pharmacological Sciences*, 24(8), 4529–4536. https://doi.org/10.26355/eurrev_202004_21036

Joshi, T., Bhat, S., Pundir, H., & Chandra, S. (2021). Identification of Berbamine, Oxyacanthine and Rutin from *Berberis asiatica* as anti-SARS-CoV-2 compounds: An in silico study. *Journal of Molecular Graphics & Modelli*ng, 109, 108028. https://doi.org/10.1016/j.jmgm.2021.108028

Kaur, S. P., & Gupta, V. (2020). COVID-19 Vaccine: A comprehensive status report. *Virus Research*, 288, 198114. https://doi.org/10.1016/j.virusres.2020.198114.

Khaerunnisa, S., Kurniawan, H., Awaluddin, R., Suhartati, S., & Soetjipto, S. (2020). Potential Inhibitor of COVID-19 Main Protease (Mpro) from Several Medicinal Plant Compounds by Molecular Docking Study. Preprints.org. https://doi.org/10.20944/preprints202003.0226.v1

Kirchdoerfer, R. N., & Ward, A. B. (2019). Structure of the SARS-CoV nsp12 polymerase bound to nsp7 and nsp8 co-factors. *Nature communication*, 10, 2342. https://doi.org/10.1038/s41467-019-10280-3

Leitao Da-Cunha, E. V., Fechine, I. M., Guedes, D. N., Barbosa-Filho, J. M., & Sobral Da Silva, M. (2005). *Protoberberine alkaloids*. The Alkaloids, Vol. 62. https://doi.org/10.1016/S1099-4831(05)62001-9

Liu, C., Tang, J., Ma, Y., Liang, X., Yang Y, Peng, G., Qi, Q., Jiang, S., Li, J., Du, L., & Li, F. (2015). Receptor usage and cell entry of porcine epidemic diarrhea coronavirus. *Journal of Virology*, 89(11), 6121–6125. https://doi.org/10.1128%2FJVI.00430-15

Macchiagodena, M., Pagliali, M., & Procacci, P. (2020). Identification of potential binders of the main protease 3CLpro of the COVID-19 via structure-based ligand design and molecular modeling. *Chemical Physics Letters*, 750, 137489. https://doi.org/10.1016/j.cplett.2020.137489.

Malika, A. A., McFaddena, S. M., Elharakea, J., & Omera, S. B. (2020). Determinants of COVID-19 vaccine acceptance in the US. *EClinicalMedicine*, 26, ID 100495. https://doi.org/10.1016/j.eclinm.2020.100495

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). Software News and Updates. AutoDock4 and AutoDockTools4: Automated Docking with Selective Receptor Flexibility. *Journal of Computational Chemistry*, 30(16), 2785-2791. https://doi.org/10.1002/jcc.21256

Qin, Q.- P., Zou, B.-Q., Wang, Z.- F., Huang, X.- L., Zhang, Y., Tan, M.- X., Wang, S.- L., & Liang, H. (2019). High *in vitro* and in vivo antitumor activities of luminecent platinum (II) complexes with jatrorrhizine derivative. *European Journal of Medicinal Chemistry*, 183, 111727. https://doi.org/10.1016/j.ejmech.2019.111727

Rastrelli, L., Capasso, A., Pizza, C., De Tommasi, N., & Sorretino, L. (1997). New Protopine and Benzyltetrahydroprotoberberine Alkaloids from Aristolochia constricta and Their Activity on Isolated Guinea-Pig Ileum. *Natural Products* 60,11, 1065-1069. https://doi.org/10.1021/np960710b

Roy, A., & Saraf, S. (2006). Limonoids: overview of significant bioactive triterpenes distributed in plants kingdom. *Biological and Pharmaceutical Bulletin*, 29(2), 191-201.

Sharapin, N., Machado, L., Souza, E., de Albuquerque, E., Valverde, E., & López, J. M. (2000). Fundamentos de tecnología de productos fitoterapéuticos. Santa Fe de Bogotá, Convenio Andrés Bello y Red Iberoamericana de Productos Fitofarmacéuticos (RIPROFITO) del subprograma X de CYTED. Pp 248. España.

Shin, H.-B., Choi, M.-S., Yi, C.-M., Lee, J., Kim, N.-J., & Inn, K.-S. (2015). Inhibition of respiratory syncytial virus replication and virus-induced p38 kinase activity by berberine. *International Immunopharmacology*, 27, 65–68. http://dx.doi.org/10.1016/j.intimp.2015.04.045

Sun, Y., Gao, X., Wu, P., Wink, M., Li, J., Dian, L., & Lian, Z. (2019). Jatrorrhizine inhibits mammary carcinoma cells by targeting TNIK mediated Wnt/β-catenin signalling an depithelial-mesenchymal transition (EMT). *Pythomedicine*, 63,153015. https://doi.org/10.1016/j.phymed.2019.153015

Tallei, T. E., Tumilaar, S. G., Niode, N. J., Fatimawali, Kepel, B. J., Idroes, R., Effendi, Y., Sakib, S.A., & Emran, T. B. (2020). Potential of Plant Bioactive Compounds as SARS-CoV-2 Main Protease (Mpro) and Spike (S) Glycoprotein Inhibitors: A Molecular Docking Study. *Scientifica*, ID 6307457. https://doi.org/10.1155/2020/6307457

Venkateshan, M., Muthu, M., Suresh, J., & Ranjith Kumar, R. (2020). Azafluorene derivatives as inhibitors of SARS CoV-2 RdRp: Synthesis, physicochemical, quantum chemical, modeling and molecular docking analysis. *Journal of Molecular Structure*, 1220, 128741. https://doi.org/10.1016/j.molstruc.2020.128741

Walls, A. C., Tortorici, M. A., Bosch, B. J., Frenz, B., & Rottier, P. J. (2016). Cryo-electron microscopy structure of a coronavirus spike glycoprotein trimer. *Nature*, 531, 114–117. https://www.nature.com/articles/nature16988

Wang, W. J., Yang, T., Chen, H., Xu, Y. N., Yu, L. F., Liu, T., Tang, J., Yi, Z., Yang, C.G., Xue, W., & Yang, F. (2017). The synthesis and antistaphylococcal activity of 9, 13-disubtituted berberine derivatives. *European Journal of Medicinal Chemistry*, 127,424-433. https://doi.org/10.1016/j.ejmech.2017.01.012

White, N. J., Pukrittayakamee, S., Hien, T. T., Faiz, M. A., Mokuolu, O. A., & Dondorp, A. M. (2014) Malaria. *Lancet*, 383:723–35.

Wrapp, D., Wang, N., Corbett, K. S., Goldsmith, J. A., Hsieh, C. L., Abiona, O., Graham, B. S., & McLellan, J. S. (2020). Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science*, 367(6483), 1260-1263. http://dx.doi.org/10.1126/science.abb2507

Wu, F., Zhao, S., Yu, B., Chen, Y.-M., Wang, W., Song, Z.-G., Hu, Y., Tao, Z. W., Tian, J.-H., Pei, Y.-Y., Yuan, M.-L., Zhang, Y.-L., Dai, F.-H., Liu, Y., Wang, Q.-M., Zheng, J.-J., Xu, L., Holmes, E. C., & Zhang, Y.-Z. (2020). A New coronavirus associated with human respiratory disease in China. *Nature*, 579, 265–269. https://doi.org/10.1038/s41586-020-2008-3

Wu, H., Heb, K., Wang, Y. Z., Xue, D. F., Ning, N., Zou, Z., Ye, X., Li, X., Wang, D. Z., & Pan, J. (2014). The antihypercholesterolemic effect of jatrorrhizine is olated from Rhizoma Coptidis. Phytomedicine. https://doi.org/10.1016/j.phymed.2014.05.002

Yang, L., Wen, K.S., Ruan, X., Zhao, Y. X., Wei, F., & Wang, Q. (2018). Response of plant secondary metabolites to environmental factors. *Molecules*, 23(4), 762. https://doi.org/10.3390/molecules23040762

Zhang, L., Lin, D., Sun, X., Curth, U., Drosten, C., Sauerhering, L., Becker, S., Rox, K., & Hilgenfeld, R. (2020). Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. *Nature*. http://dx.doi.org/10.1038/s41586-020-2223

Zhou, P., Yang, X.L., Wang, X. G., Hu, B., Zhang, L., Zhang, W., Si, H. R., Zhu, Y., Li, B., Huang, C. L., Chen, H. D., Chen, J., Luo, Y., Guo, H., Jiang, R. D., Liu, M. Q., Chen, Y., Shen, X. R., Wang, W., Zheng, X. S., Zhao, K., Chen, Q. J., Deng, F., Liu, L. L., Yan, B., Zhan, F. X., Wang, Y. Y., Xiao, G. F., & Shi, Z. L. (2020). A. Pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*, 579, 270–273. https://doi.org/10.1038/s41586-020-2012-7