Original Research

Effective inhibition of coronavirus replication by *Polygonum cuspidatum*

Huan Xu[1,2,†], Jing Li[3,†], Shidong Song[4,†], Zhen Xiao[3,4,5], Xuanqing Chen[6], Boxuan Huang[7], Mengsi Sun[1], Guoqing Su[1], Demin Zhou[1,6], Guanyu Wang[5,8,9,]*, Ran Hao[10,]*, Na Wang[11,]*

[1]*Institute of Chemical Biology, Shenzhen Bay Laboratories, 518132 Shenzhen, Guangdong, China,* [2]*New Drug R&D Center, North China Pharmaceutical Corporation, 050015 Shijiazhuang, Hebei, China,* [3]*School of Pharmaceutical Sciences, South-Central University for Nationalities, 430000 Wuhan, Hubei, China,* [4]*Nanjing Gemni Biotechnology Co., Ltd, 210023 Nanjing, Jiangsu, China,* [5]*Guangdong Provincial Key Laboratory of Cell Microenviroment and Disease Research, 518055 Shenzhen, Guangdong, China,* [6]*State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, 100191 Beijing, China,* [7]*Institute for Advanced Study, Shenzhen University, 518052 Shenzhen, Guangdong, China,* [8]*Department of Biology, Southern University of Science and Technology, 518055 Shenzhen, Guangdong, China,* [9]*Guangdong Provincial Key Laboratory of Computational Science and Material Design, 518055 Shenzhen, Guangdong, China,* [10]*School of Nursing, Hebei Medical University, 050000 Shijiazhuang, Hebei, China,* [11]*Department of Pharmacognosy, Pharmaceutical College, Hebei Medical University, 050017 Shijiazhuang, Hebei, China*

TABLE OF CONTENTS

1. Abstract

Background: The coronavirus disease 2019 pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has infected more than 210 million individuals globally and resulted in over 4 million deaths since the first report in December 2019. The early use of traditional Chinese medicine (TCM) for light and ordinary patients, can rapidly improve symptoms, shorten hospitalization days and reduce severe cases transformed from light and normal. Many TCM formulas and products have a wide application in treating infectious and

non-infectious diseases. *Polygonum cuspidatum* Sieb. et Zucc. *(P. cuspidatum)*, is an important Traditional Chinese Medicine with actions of clearing away heat and eliminating dampness, draining the gallbladder to relieve jaundice, removing blood stasis to alleviate pain, resolving phlegm and arrest cough. In the search for anti-SARS-CoV-2, *P. cuspidatum* was recommended as as a therapeutic drug of COVID-19 pneumonia.In this study, we aimed to identifies *P. cuspidatum* is the potential broad-spectrum inhibitor for the treatment of coronaviruses infections. **Methods**: In the present study , we infected human malignant embryonal rhabdomyoma (RD) cells with the OC43 strain of the coronavirus, which represent an alternative model for SARS-CoV-2 and then employed the cell viability assay kit for the antiviral activity. We combined computer aided virtual screening to predicte the binding site and employed Surface plasmon resonance analysis (SPR) to comfirm the interaction between drugs and coronavirus. We employed fluorescence resonance energy transfer technology to identify drug's inhibition in the proteolytic activity of 3CLpro and Plpro. **Results**: Based on our results, polydatin and resveratrol derived from *P. cuspidatum* significantly suppressed HCoV-OC43 replication. 50% inhibitory concentration (IC_{50}) values of polydatin inhibited SARS-CoV-2 Mpro and Plpro, MERS Mpro and Plpro were 18.66, 125, 14.6 and 25.42 μm, respectively. IC_{50} values of resveratrol inhibited SARS-CoV-2 Mpro and Plpro, MERS Mpro and Plpro were 29.81 ,60.86, 16.35 and19.04 μM, respectively. Finally, SPR assay confirmed that polydatin and resveratrol had high affinity to SARS-CoV-2, SARS-CoV 3Clpro, MERS-CoV 3Clpro and PLpro protein. **Conclusions**: we identified the antiviral activity of flavonoids polydatin and resveratrol on RD cells. Polydatin and resveratrol were found to be specific and selective inhibitors for SARS-CoV-2, 3CLpro and PLpro, viral cysteine proteases. In summary, this study identifies *P. cuspidatum* as the potential broad-spectrum inhibitor for the treatment of coronaviruses infections.

2. Introduction

An unusual pneumonia of unknown origin was reported in December 2019 [1]. Its clinical features are similar to those of severe acute respiratory virus (SARS) [2]. The viral genome isolated from patients clustered into a clade of betacoronaviruses was distinct from that of the severe acute respiratory syndrome coronavirus (SARS-CoV) [3]. Thus, this disease, named the coronavirus disease 2019 (COVID-19) was caused by a novel coronavirus (2019-nCoV), which was later renamed the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). On March 11, 2020, COVID-19 was declared a global pandemic by the World Health Organization (WHO) [4]. In December 2020, several vaccines shown to be highly effective were granted emergency use authorization. While vaccines will prevent disease occurrence, infected individuals still need treatment options, and repurposing drugs circumvents the lengthy and costly process of drug development. Therefore, it is urgent to excavate the efficacy of currently approved drugs for the treatment of COVID-19 [5–7].

Traditional Chinese medicine (TCM) is an important party of the world complementary and alternative medicine. TCM offers a wide variety of application of traditional medicines and herbs for treating human diseases. Treating epidemic diseases with TCM and the treatment scheme of integrated Chinese and western medicine have proven their effectiveness in clinical practice in the management of COVID-19 pneumonia [8–10]. Effective prescriptions for the treatment of COVID-19 pneumonia include Qingfei Paidu decoction, Pneumonia No.1 Formula, and Shufeng Jiedu capsule. *Polygonum cuspidatum* Sieb. et Zucc., commonly known as Huzhang in Chinese and Japanese knotweed in English, is a widely used medicinal plant. The roots of *Polygonum cuspidatum* are used as an important traditional Chinese medicine for centuries, which have the actions of clearing away heat and eliminating dampness, draining the gallbladder to relieve jaundice, removing blood stasis to alleviate pain, resolving phlegm and arrest cough [11]. Of the traditional medicines tested, *Polygonum cuspidatum* and its active components, resveratrol, were found to inhibit Middle East respiratory syndrome coronavirus (MERS-CoV) in Vero E6 cells [12]. Over 67 compounds have been identified in *P. cuspidatum*, including quinones, stilbenes, flavonoids, coumarins, and lignans [13]. *P. cuspidatum* and its active components, resveratrol and emodin, were also found to attenuate influenza viral replication in A549 cells [14]. During the fight against COVID-19 in China, Chinese scientists found some herbs to be symptomatic for COVID-19 through clinical practice and through component screening in the Chinese medicine component library. One of them is *P. cuspidatum*, in which Polydatin has the strongest inhibitory effect on coronaviruses [15]. Hence, *P. cuspidatum* was recommended by the academician Zhang boli in the treatment of COVID-19 pneumonia [16].

The first genome sequence of SARS-CoV-2 has been entered into the database by Wu *et al*. [17] in February 2020. Coronavirus has six open reading frames (ORFs) and the first ORF (ORF1a/b), which comprises approximately 67% of the entire genome, encodes 16 non-structural proteins [18]. The remaining ORFs encode four major structural proteins, which are spike surface glycoprotein (S), small envelope protein (E), matrix protein (M), nucleocapsid protein (N), and accessory proteins.

The polypeptides are released from each polyprotein through extensive proteolytic processing, primarily performed by the virally encoded chymotrypsin-like protease 3CL protein (3CLpro, also called the main protease, Mpro) with additional cleavage performed by the viral papain-like protease Pl protein (Plpro) [19, 20]. Owing to

their essential roles in viral replication, both the proteases are recognized as attractive targets for the development of anti-SARS-CoV-2 therapeutics [21, 22]. While searching for a SARS-CoV-2 3CLpro and PLpro inhibitor from natural sources, we found that polydatin and resveratrol from *P. cuspidatum* possessed inhibitory activity against both the proteases. Herein, we identified the active ingredients from *P. cuspidatum* and their mechanism of inhibiting coronavirus.

3. Material and methods

3.1 Materials

Polydatin and resveratrol were purchased from solarbio company (Solarbio Science & Technology Company, Beijing, China). The Trans1-T1 strain, F-φ80(lacZ)$\triangle$M15$\triangle$*lacX74hsdR*(rk,m)$\triangle$recA 1398endAltonA, (TransGen Biotech, Beijing, China) was used to clone and propagate plasmid DNA. Miniprep and Maxiprep kits (Axygen, San Jose, CA, USA) were used to harvest and purify plasmid DNA. SARS-CoV-2, SARS-CoV, and MERS-CoV 3CLpro, and PLpro were purchased from novoprotein company (Novoprotein company, Beijing, China) or expressed and purified by genescript company (Genescript company, Nanjing, China). Biacore Sensor Chip CM5 and phosphate-buffered saline (PBS)-P were purchased from GE healthcare company (GE healthcare company, Uppsala, Sweden). The 12-mer fluorogenic peptide Dabcyl-KNSTLQSGLRKE-Edans substrate for the 3CLpro inhibition assay and fluorogenic peptide Dabcyl-KRLKGGAPIKGE-Edans substrate for the PLpro inhibition assay were sythesised by genescript company (Genescript company, Nanjing, China).

3.2 Viruses and Cell lines

HCoV-OC43(OC43) strain was purchased from American Type Culture Collection (Human Coronavirus OC43(ATCC VR-1558) and propagated using RD cells (ATCC CCL-136). OC43 stock virus was obtained in RD cells in DMEM supplemented with 2% FBS at 72 h post-infection (hpi). Viral titer (PFU/mL) was determined by plaque assay.

3.3 OC43 Viral infection inhibition assay

RD cells were infected OC43 at the multiplicity of infection (MOI = 0.01 IU) in the presence of indicated compound diluted in DMEM supplemented with 2% FBS [23]. The compounds and the virus were maintained with the cells during the 48 h incubation at 37 °C. OC43 infection efficiency in RD cell was evaluated by immunofluorescence staining against the Nucleoprotein. In general, RD cells were fixed by 100% methanol at 48 hpi, blocked by 1% BSA/PBS at 37 °C for 1 h, stained with anti-OC43 Np mAb (Sigma, Saint Louis, MO, USA) at 1:1000 at 37 °C for 1 h, and followed by secondary antibody incubation for an-

other 1 h. Nuclei were stained with Hoechst 33342 (Thermo Scientific, Waltham, MA, USA) at 1:5000 for 15 min. Images were captured by an Mshot fluorescence microscrope (Olympus, Tokyo, Japan).

3.4 Determination of the median cytotoxic concentration (CC_{50})

The median cytotoxic concentration (CC_{50}) of the compounds was determined by the CellTiter-Glo 2 (Promega Corporation, Madison, WI, USA) cell viability assay kit [24]. In general, 293T-hACE2 cells were seeded in 96-well plates with three-fold serial-diluted compounds (from 100 μM to 0.137 μM). After 24 h, the cell viability was detected by CellTiter-Glo 2 reagent by following the manufacture's instructions, and the luminescence was determined by a Spectra MaxiD3 multi-well Luminometer 458 (Molecular Devices, San Jose, CA, USA). The CC_{50} value was determined by nonlinear regression analysis [25].

3.5 Computer aided virtual screening

The SARS-CoV-2 3CLP (pdb:6lu7) and PLP (pdb:6w9c) program database (PDB) files were downloaded from the PDB website (http://www.rcsb.org/). All heterogeneous atoms and the 3CLP ligand were removed and 6lu7 chain A was selected for subsequent molecular docking. The 3CLP docking grid was maximized for polydatin docking. All heterogeneous atoms and the PLP ligand were removed and 6w9c chain A was selected for subsequent molecular docking. The PLP docking grid was maximized for polydatin docking.

PDB file (6lu7 chain A) and (6w9c chain A) were converted to the PDBQT format as macromolecules before virtual screening. The grid (ligand docking search space) was located as described above. Then, Autodock Vina 1.1.2 [26] was used for the subsequent molecular docking. Protein–ligand interactions were visualized using Pymol version 1.7.4.5 (Schrödinger, New York, NY, USA). The amino acid residues of 3CLP protein close to the hit ligands ($\leq$1 Å) were highlighted as potential interactive residues involved in the protein–ligand interaction.

## 3.6 Surface plasmon resonance (SPR) analysis

To conform the binding affinities of polydatin and resveratrol to SARS-CoV-2, SARS-CoV, MERS-CoV 3CLpro and PLpro, SPR technology based Biacore T200 biosensor was used (Biacore AB, Uppsala, Sweden) [27]. At 25 °C, the 3CLpro and PLpro proteins were diluted to a final concentration of 20 μg/mL in 10 mM sodium acetate buffer (pH 4.5) and then immobilized to CM5 by using the standard primary amine coupling method. The final immobilization levels of 3CLpro of SARS-CoV-2, SARS-CoV, and MERS-CoV were 10,125, 10,452.5, and 9856 resonance units, respectively. The final immobilization levels of PLpro of SARS-CoV-2, SARS-CoV, and MERS-CoV were 15,847, 15,358, and 10,459 resonance units, respectively.
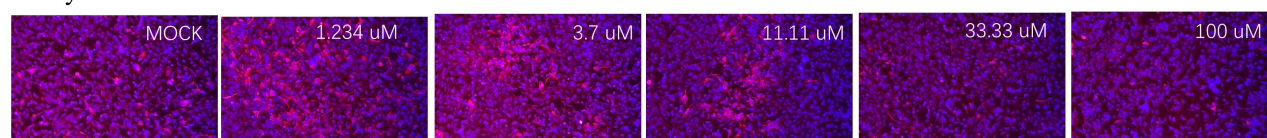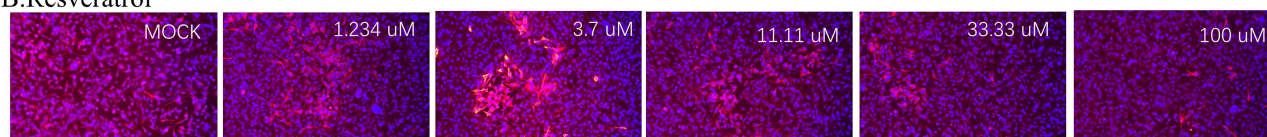
Fig. 1. Confirmation of anti-coronavirus activity by immunofluorescence assay (IFA) analysis. The IFA of the human coronavirus strain OC43 (HCoV-OC43) nucleocapsid (N) protein in inhibitor-treated RD cells. RD cells in 96-well plates were infected with HCoV-OC43-wild type (multiplicity of infection = 0.01 IU) in serial dilutions of tested compounds, with dimethyl sulfoxide used as the negative controls. At 48 h post infection, the cells were analyzed by IFA for N protein expression. Nuclei (blue) were stained with Hoechst 33342.
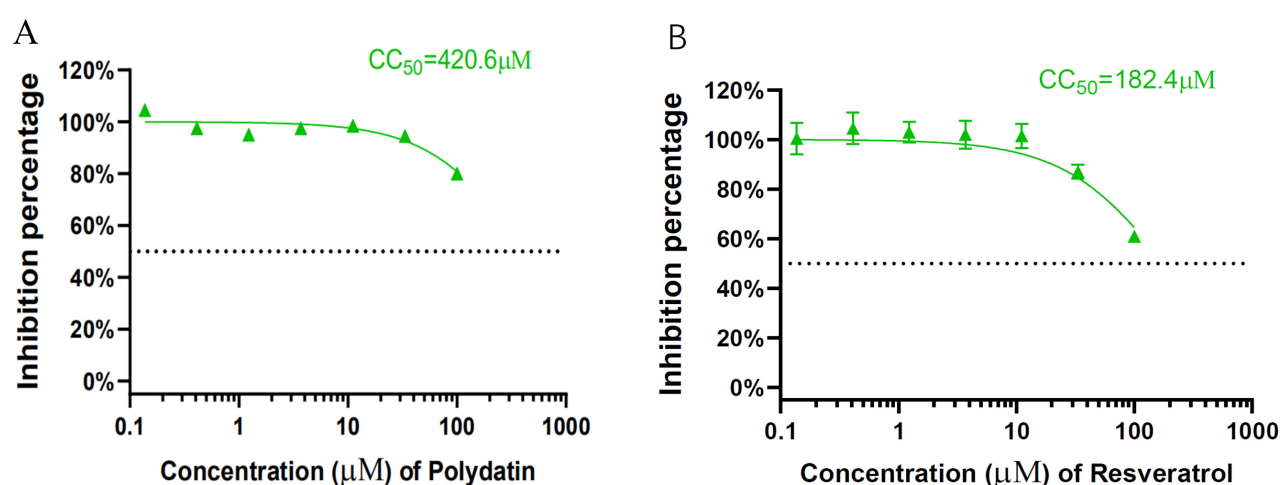
Fig. 2. Cytotoxicity of polydatin (A) and resveratrol (B) to HEK293T-ACE2 cells was measured by CellTiter-Blue assay. The median cytotoxic concentration value was determined by nonlinear regression analysis.

All the screening assays were performed over the unmodified dextran surface and the protein surface. During the 3CLpro and PLpro binder assay, the sample concentrations were set at 100 μM, 50 μM, 25 μM, 12.5 μM, 6.25 μM, 3.125 μM, 1.5625 μM, 0.78125 μM, 0 μM. For sample dilution, the pH and the concentration of both DMSO and buffer substances in samples and running buffer were carefully matched. Each sample assay consisted of a 180-s buffer injection and a 300-s dissociation phase and the blank injection was used to check the carryover effects. The signal was adjusted for nonspecific binding of the samples to dextran matrix by subtracting the signal in the reference channel from the signal in the active channel. The experimental data were fitted and analyzed using the BIA evaluation software by steady state analysis.

3.7 FRET technology-based IC$_{50}$ determination

To further investigate possible inhibitory activities of polydatin and resveratrol against SARS-CoV-2, SARS-CoV, and MERS-CoV 3CLpro, and PLpro, the inhibitory effects of the compounds were tested by the FRET method [28]. For this assay, the internally quenched fluorogenic substrate Dabcyl-KNSTLQSGLRKEE-Edans and Dabcyl-KRLKGGAPIKGE-Edans substrate (synthesized by genescript company) was applied and the compounds were diluted to 100 mM, 20 mM, 10 mM, 2 mM, 1 mM, 200 μM, 100 μM, and 20 μM. A 1.2 μL concentration of the solution of the compounds was taken and thoroughly mixed with 120 μL of 2 μM 3CL protease (final concentration of DMSO is 0.5%) and incubated at 4 °C for 2 h. Thereafter, 100 μL of each concentration sample was taken into a 96-well plate, and then added 100 μL of 20 μM fluorescent substrate solution to start the reaction. At this time, the final concentration of the 3CL or PL protease is 1 μM, the final concentration of the fluorescent substrate is 10 μM, and the final concentrations of the compounds are 500 μM, 100 μM, 50 μM, 10 μM, 5 μM, 1 μM, 0.5 μM, and 0.1 μM, respectively. The enhanced fluorescence emission upon substrate cleavage was monitored at the excitation and emission wave lengths of 340 and 488 nm. The reaction was measured

A:Polydatin&SARS-CoV-2 3CL   B:Polydatin&SARS-CoV-2 PLP
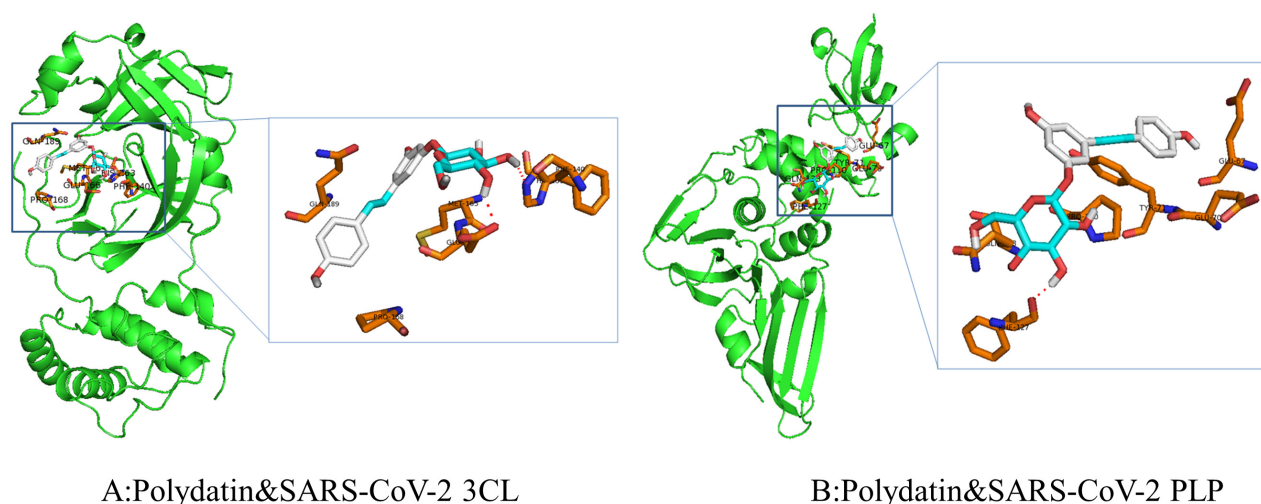
Fig. 3. Prediction of binding sites by virtual screening. (A) Polydatin binds to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) 3CLP (6lu7). Polydatin (gray stick) docked superposition on SARS-CoV-2 3CLP. Interactive amino acids (orange sticks) with polydatin (gray stick) on the SARS-CoV-2 3CLP (red dash line indicates the hydrogen bonds). (B) Polydatin binds to the SARS-CoV-2 PLP (6w9c). Polydatin (gray stick) docked superposition on the SARS-CoV-2 PLP, Interactive amino acids (orange sticks) with polydatin (gray stick) on the SARS-CoV-2 PLP (red dash line indicates the hydrogen bonds).

continuously for 1 h. According to the logistic formula, the origin software was used to perform a non-linear fitting to calculate the IC_{50} value of compounds in the inhibition of 3CL protease activity. The formula is as follows:

$$A(I)/A_0 = 1 - \frac{1}{1 + (I/IC_{50})^p}$$

In the above formula, A_0 refers to the enzyme activity when 0.5% DMSO is added, A(I) refers to the enzyme activity under different concentrations of compounds, I refers to the concentration of the compound, and p refers to the u factor.

4. Results

### 4.1 Identification of anti-HCoV-OC43 activity

To verify the inhibitory effect of polydatin and resveratrol on coronavirus at the cellular level *in vitro*, we measured the antiviral activity of polydatin and resveratrol by indirect immunofluorescence assay (IFA) on RD cells infected with OC43-CoV which is an alternative model for SARS-CoV-2. As shown in Fig. 1A and 1B, polydatin and resveratrol significantly suppressed HCoV-OC43 replication, compared with that of the control (DMSO), and with over 90% inhibitory effect at a concentration of 100 μM, respectively. Cytotoxicity of these drugs to HEK293T cells was measured by CellTiter-Blue assay. As shown in Fig. 2A and 2B, cytotoxicity concentration 50% [CC_{50}] of polydatin and resveratrol was 420.6 μmol/L and 182.4 μmol/L, respectively.

4.2 The prediction of binding site

To elucidate the mechanism of polydatin and resveratrol on the inhibition of virus replication, we combined virtual screening with experimental studies. The chain A of 6lu7 (SARS-CoV-2 3CLP) was extracted to perform molecular docking with polydatin. For SARS-CoV-2 3CLP, polydatin binds to the PHE140, HIS163, MET165, GLU166, PRO168, and GLN189 amino acid sites (Fig. 3A). The chain A of 6w9c (SARS-CoV-2 PLP) was extracted to perform molecular docking with polydatin. For SARS-CoV-2 PLP, polydatin binds to the GLU67, GLU70, TYR71, PHE127, PRO130, and GLN133 amino acid sites (Fig. 3B).

4.3 FRET-based assay for the SARS-CoV-2 and MERS 3CLpro and PLpro

With the established FRET assay condition, we tested polydatin and resveratrol to identify its inhibition in the proteolytic activity of 3CLpro and PLpro (Table 1). Polydatin inhibited SARS-CoV-2 Mpro and PLpro with 50% inhibitory concentration (IC_{50}) values of 18.66 and 125 μM, respectively (Table 1, Fig. 4A,B), MERS Mpro and PLpro with 50% inhibitory concentration (IC_{50}) values of 14.6 and 25.42 μM (Table 1, Fig. 4C,D), respectively. resveratrol inhibited SARS-CoV-2 Mpro and PLpro with 50% inhibitory concentration (IC_{50}) values of 29.81 and 60.86 μM, respectively (Table 1, Fig. 4A,B), MERS Mpro and PLpro with 50% inhibitory concentration (IC_{50}) values of 16.35 and 19.04 μM (Table 1, Fig. 5A,B), respectively.

794
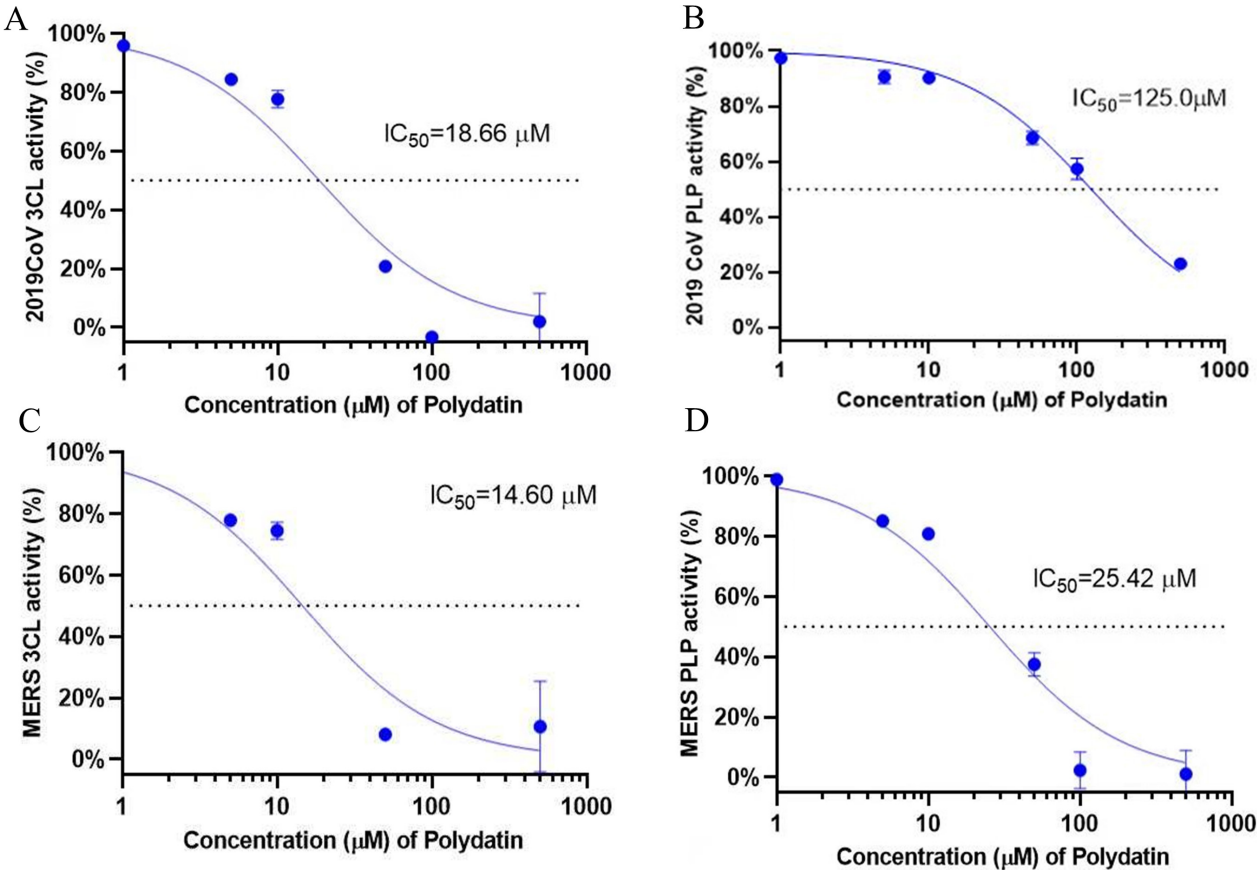
Fig. 4. Determination of the 50% inhibitory concentration (IC_{50}) of polydatin and resveratrol for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) 3CL(A), the Middle East respiratory syndrome coronavirus (MERS-CoV) 3CL(B), SARS-CoV-2 PLP(C), and MERS-CoV PLP(D) by fluorescence resonance energy transfer (FRET)-based assay.

Table 1. Yielded IC_{50} values of polydatin and resveratrol against SARS-CoV-2 and MERS-CoV 3CLpro and PLP pro.

IC_{50}	2019 CoV 3CL	2019 CoV PLP	MERS 3CL	MERS PLP
Polydatin	18.66 μM	125 μM	14.6 μM	25.42 μM
Resveratrol	29.81 μM	60.86 μM	16.35 μM	19.04 μM

IC_{50}, 50% inhibitory concentration; SARS-CoV-2, severe acute respiratory syndrome coronavirus; MERS-CoV, Middle East respiratory syndrome coronavirus; PLP, papain-like protease; CL, chymotrypsin like protease.

Table 2. Affinity of polydatin binding to SARS-CoV-2, SARS-CoV, MERS-CoV 3CLpro, and PLpro protein at 25 °C.

Target	KD (M)	Rmax (RU)	Chi^2 (RU^2)	Chi
2019 CoV 3CL	1.601×10^{-5}	14.1	1.35	1.16
SARS 3CL	5.428×10^{-6}	9.251	0.313	0.56
MERS 3CL	1.644×10^{-5}	6.966	0.0164	0.128
2019 CoV PLP	3.953×10^{-5}	16.65	0.00983	0.0992
SARS PLP	1.976×10^{-6}	8.908	0.049	0.221
MERS PLP	7.745×10^{-6}	7.163	0.386	0.621

SARS-CoV-2, severe acute respiratory syndrome coronavirus; SARS-CoV, severe acute respiratory syndrome coronavirus; MERS-CoV, Middle East respiratory syndrome coronavirus; PLP, papain-like protease; CL, chymotrypsin like protease.

4.4 SPR technology–based binder identification

To confirm the interaction between polydatin and resveratrol and SARS-CoV-2, SARS-CoV, MERS-CoV 3CLpro, and PLpro protein, we tested whether polydatin and resveratrol had high affinity to target protein using the SPR assay. The SARS-CoV-2, SARS-CoV, and MERS-CoV 3CLpro and PLpro protein were immobilized separately on a CM5 chip with the tested compounds flowing across their surface. We found that polydatin bound to SARS-CoV-2, SARS-CoV, and MERS-CoV 3CLpro and PLpro protein exhibiting a strong dose-dependent response, with respective KD (equilibrium dissociation constant) val-

ues of 16.01 μM, 5.428 μM, 16.44 μM, 39.53 μM, 1.976 μM, and 7.745 μM (Table 2, Fig. 6A–E); resveratrol bound to SARS-CoV-2, SARS-CoV, and MERS-CoV 3CLpro and PLpro protein exhibiting a strong dose-dependent response, with respective KD (equilibrium dissociation constant) values of 0.9812 μM, 92.51 μM, 7.312 μM, 12.35 μM, 1.555 μM, and 6.1 μM (Table 3, Fig. 7A–E). These results suggested that polydatin and resveratrol had a specific affinity
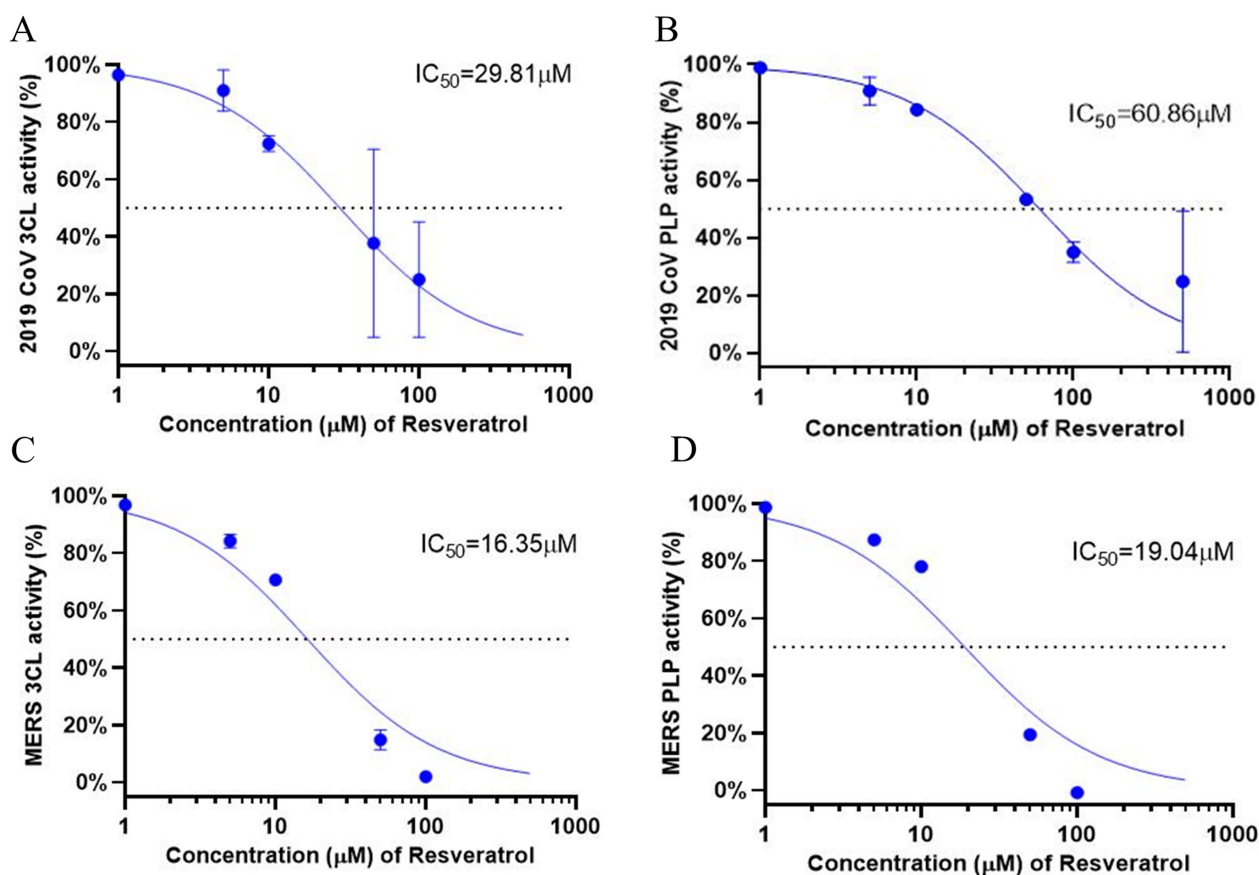
Fig. 5. Determination of the 50% inhibitory concentration (IC$_{50}$) of polydatin and resveratrol for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) 3CL (A), the Middle East respiratory syndrome coronavirus (MERS-CoV) 3CL (B), SARS-CoV-2 PLP (C), and MERS-CoV PLP (D) by fluorescence resonance energy transfer (FRET)-based assay.
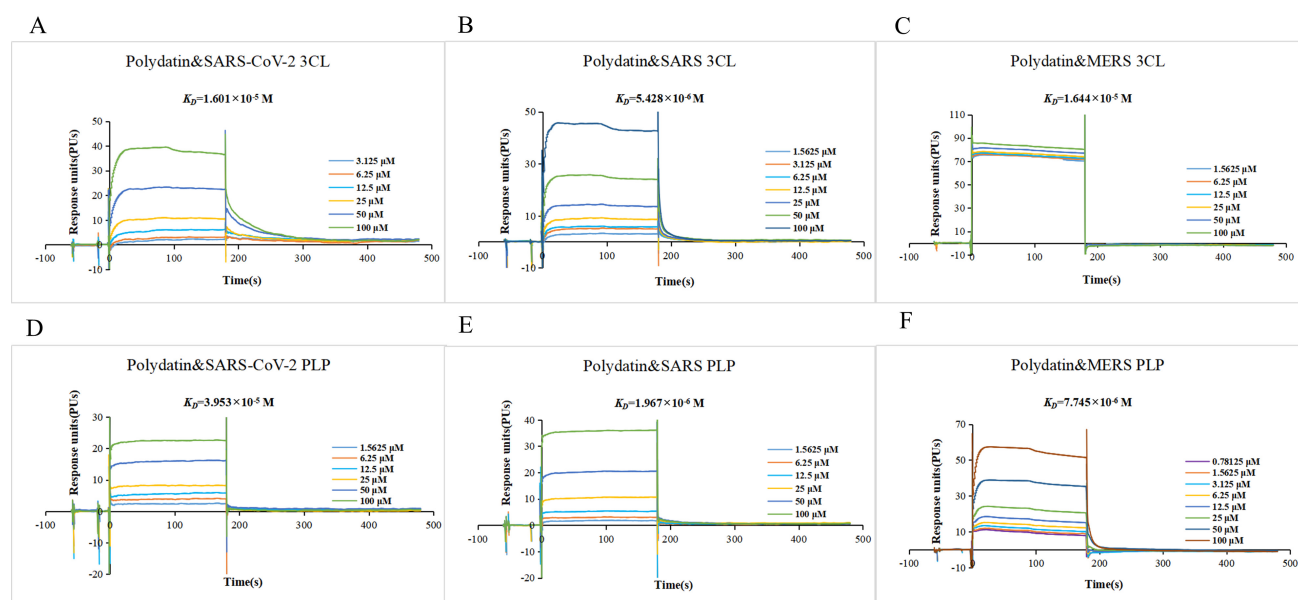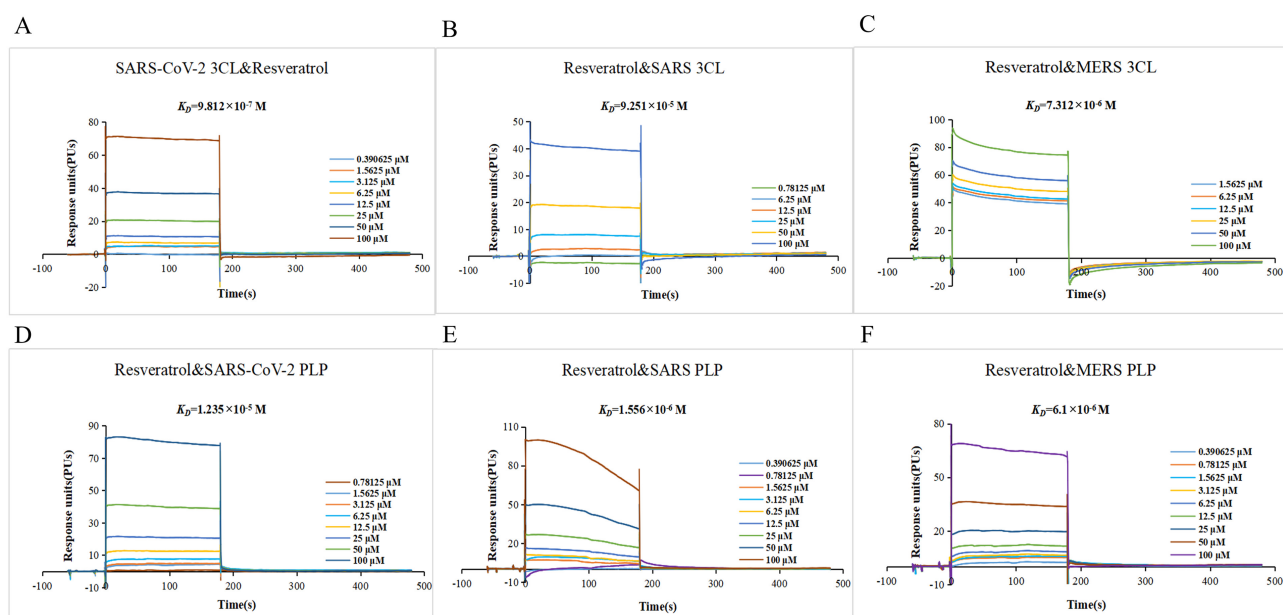

Fig. 6. Polydatin targets 3CLpro and PLpro of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), severe acute respiratory syndrome coronavirus (SARS-CoV), and Middle East respiratory syndrome coronavirus (MERS-CoV) (A–F). The sensor image of 3CLpro and PLpro of SARS-CoV-2, SARS-CoV, and MERS-CoV with polydatin.

Fig. 7. Resveratrol targets 3CLpro and PLpro of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), severe acute respiratory syndrome coronavirus (SARS-CoV), and Middle East respiratory syndrome coronavirus (MERS-CoV) (A–F). The sensor image of 3CLpro and PLpro of SARS-CoV-2, SARS-CoV, and MERS-CoV with resveratrol.

Table 3. Affinity of resveratrol binding to SARS-CoV-2, SARS-CoV, MERS-CoV 3CLpro, and PLpro protein at 25 °C.

Target	KD (M)	Rmax (RU)	Chi^2 (RU^2)	Chi
2019 CoV 3CL	9.812×10^{-7}	16.29	1.76	1.327
SARS 3CL	9.251×10^{-5}	42.87	1.63	1.276
MERS 3CL	7.312×10^{-6}	7.765	0.00146	0.0382
2019 CoV PLP	1.235×10^{-5}	26.02	0.217	0.466
SARS PLP	1.556×10^{-6}	12.34	0.654	0.809
MERS PLP	6.100×10^{-6}	13.78	0.739	0.86

SARS-CoV-2, severe acute respiratory syndrome coronavirus; SARS-CoV, severe acute respiratory syndrome coronavirus; MERS-CoV, Middle East respiratory syndrome coronavirus; PLP, papain-like protease; CL, chymotrypsin like protease.

to SARS-CoV-2, SARS-CoV 3CLpro and the PLpro protein, and thus can inhibit virus replication by binding to the active center of 3CLpro and the PLpro protein.

5. Discussion

Since the COVID-19 pandemic occurred in early 2020, scientists have been trying to find effective treatment methods and remedys. TCM has been proven effective for COVID-19 treatment [29–31]. *P. cuspidatum*, is a traditional Chinese medicine with actions of clearing away heat and eliminating dampness, draining the gallbladder to relieve jaundice, removing blood stasis to alleviate pain, resolving phlegm and arrest cough [32]. Polygonum cuspidatum and its active components were found to inhibit Mid-

dle East respiratory syndrome coronavirus (MERS-CoV) in Vero E6 cells [12] and attenuate influenza viral replication in A549 cells [14].

In this study, we focused on the SARS-CoV-2 3CL and PLP protein as target sites for the identification of possible Food and Drug Administration-approved drugs for repositioning in COVID-19 treatment.

As mentioned above, 3CLpro and PLpro of SARS-CoV-2 are good targets to design inhibitory chemicals since some flavonoids inhibit the proteolytic activity of SARS-CoV 3CLpro [33, 34]. The similar sequence between them [35, 36] together with their conserved active site suggests that similar flavonoids may work for SARS-CoV-2 3CLpro and PLpro. We found that polydatin and resveratrol revealed the prominent inhibitory activity against SARS-CoV-2 and MERS-CoV 3CLpro and PLpro. The measured IC$_{50}$ values against SARS-CoV-2 3CLpro were 18.66 and 29.81 μM, respectively. Polydatin and resveratrol also revealed the prominent inhibitory activity against OC43-CoV at the cellular level. The assay and virtual screening results indicate an important conclusion of *P. cuspidatum* as a broad-spectrum inhibitor of coronaviruses. Since the epithelial cells of the upper airways are the primary target site of SARS-CoV-2 infection. Nasal spray will be a suitable administration route of *P. cuspidatum*. Clinical trials and *in vitro* experiments supporting these findings would be of great importance towards overcoming COVID-19.

6. Conclusions

The COVID-19 pandemic wreaked havoc on human health from the beginning of 2020. In the search for anti-SARS-CoV-2 therapeutics, we identified the antiviral activity of flavonoids polydatin and resveratrol from *P. cuspidatum* on RD cells infected with the OC43 strain of the coronavirus. In order to elucidate the mechanism of polydatin and resveratrol on inhibition of virus replication, we combined virtual screening with experimental studies. Polydatin and resveratrol were found to be specific and selective inhibitors for SARS-CoV-2, 3CLpro and PLpro, viral cysteine proteases. In summary, this study identifies *P. cuspidatum* as the potential broad-spectrum inhibitor for the treatment of coronaviruses infections.

7. Author contributions

HX and NW wrote the manuscript, HX performed OC43 Viral infection inhibition assay and determination of the median cytotoxic concentration and computer aided virtual screening, JL and SS performed Surface plasmon resonance (SPR) analysis and FRET technology–based IC_{50} determination and provided comments. ZX, XC, BH, GS, DZ provided the comments and checked the manuscript. MS offered technical support for the analysis of surface plasmon resonance. NW, GW, RH designed study and offered professional advice. All authors read and approved the final manuscript.

8. Ethics approval and consent to participate

Not applicable.

9. Acknowledgment

Thanks to all the peer reviewers for their opinions and suggestions. We warmly appreciate professor Huan Yan (Wuhan University) for gifting OC43.

10. Funding

11. Conflict of interest

The authors declare no conflict of interest.

12. References

[1] Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, *et al*. A Novel Coronavirus from Patients with Pneumonia in China, 2019. New England Journal of Medicine. 2020; 382: 727–733.

[2] Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, *et al*. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet. 2020; 395: 497–506.

[3] Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, *et al*. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. the Lancet. 2020; 395: 565–574.

[4] WHO. WHO's COVID-19 response. 2020. Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/interactive-timeline (Accessed: 14 September 2021).

[5] Fan H, Wang L, Liu W, An X, Liu Z, He X, *et al*. Repurposing of clinically approved drugs for treatment of coronavirus disease 2019 in a 2019-novel coronavirus-related coronavirus model. Chinese Medical Journal. 2020; 133: 1051–1056.

[6] Drozdzal S, Rosik J, Lechowicz K, Machaj F, Kotfis K, Ghavami S, *et al*. FDA approved drugs with pharmacotherapeutic potential for SARS-CoV-2 (COVID-19) therapy. Drug resistance updates: reviews and commentaries in antimicrobial and anticancer chemotherapy. 2020; 53: 100719.

[7] Kang Y, Chou Y, Rothlauf PW, Liu Z, Soh TK, Cureton D, *et al*. Inhibition of PIKfyve kinase prevents infection by Zaire ebolavirus and SARS-CoV-2. Proceedings of the National Academy of Sciences. 2020; 117: 20803–20813.

[8] Ding Y, Zeng L, Li R, Chen Q, Zhou B, Chen Q, *et al*. The Chinese prescription lianhuaqingwen capsule exerts anti-influenza activity through the inhibition of viral propagation and impacts immune function. BMC Complementary and Alternative Medicine. 2017; 17: 130.

[9] Luo E, Zhang D, Luo H, Liu B, Zhao K, Zhao Y, *et al*. Treatment efficacy analysis of traditional Chinese medicine for novel coronavirus pneumonia (COVID-19): an empirical study from Wuhan, Hubei Province, China. Chinese Medicine. 2020; 15: 34.

[10] Runfeng L, Yunlong H, Jicheng H, Weiqi P, Qinhai M, Yongxia S, *et al*. Lianhuaqingwen exerts anti-viral and anti-inflammatory activity against novel coronavirus (SARS-CoV-2). Pharmacological Research. 2020; 156: 104761.

[11] Zhong HS. Science of Chinese materia medica. China Press of Traditional Chinese Medicine. 2016.

[12] Lin S, Ho C, Chuo W, Li S, Wang TT, Lin C. Effective inhibition of MERS-CoV infection by resveratrol. BMC Infectious Diseases. 2017; 17: 144.

[13] Zhang C, Zhang X, Zhang Y, Xu Q, Xiao H, Liang X. Analysis of estrogenic compounds in Polygonum cuspidatum by bioassay and high performance liquid chromatography. Journal of Ethnopharmacology. 2006; 105: 223–228.

[14] Lin CJ, Lin HJ, Chen TH, Hsu YA, Liu CS, Hwang GY, *et al*. Polygonum cuspidatum and its active components inhibit replication of the influenza virus through toll-like receptor 9-induced interferon beta expression. PLos ONE. 2015; 10: e0117602.

[15] Chinese scientists discover old drugs and herbal medicines

798

that may work against novel coronaviruses, including mon-telukast, *Polygonum cuspidatum*, Cinanserin. 2020. Available at: https://www.medsci.cn/article/show_article.do?ac=good&action=view_do&id=1535186e30d7 (Accessed: 14 September 2021).

[16] Zhang Boli on Tranditional Chinese medicine against epidemics: Polygonum cuspidatum and verbena inhibit COVID-19. 2020. Available at: https://finance.sina.com.cn/chanjing/cyxw/2020-04-03/doc-iimxyqwa4826348.shtml (Accessed: 14 September 2021).

[17] Wu F, Zhao S, Yu B, Chen Y, Wang W, Song Z, *et al*. A new coronavirus associated with human respiratory disease in China. Nature. 2020; 579: 265–269.

[18] Wu A, Peng Y, Huang B, Ding X, Wang X, Niu P, *et al*. Genome Composition and Divergence of the Novel Coronavirus (2019-nCoV) Originating in China. Cell Host & Microbe. 2020; 27: 325–328.

[19] Klemm T, Ebert G, Calleja DJ, Allison CC, Richardson LW, Bernardini JP, *et al*. Mechanism and inhibition of the papain-like protease, PLpro, of SARS-CoV-2. The EMBO Journal. 2020; 39: e106275.

[20] Zhang LL, Lin DZ, Sun XYY, Curth U, Drosten C, Sauerhering L, *et al*. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved alpha-ketoamide inhibitors. Science. 2020; 368: 409–412.

[21] Liu Y, Liang C, Xin L, Ren X, Tian L, Ju X, *et al*. The development of Coronavirus 3C-Like protease (3CLpro) inhibitors from 2010 to 2020. European Journal of Medicinal Chemistry. 2020; 206: 112711.

[22] Rathnayake AD, Zheng J, Kim Y, Perera KD, Mackin S, Meyerholz DK, *et al*. 3C-like protease inhibitors block coronavirus replication in vitro and improve survival in MERS-CoV–infected mice. Science Translational Medicine. 2020; 12: eabc5332.

[23] Kim DE, Min JS, Jang MS, Lee JY, Shin YS, Song JH, *et al*. Natural Bis-Benzylisoquinoline Alkaloids-Tetrandrine, Fangchinoline, and Cepharanthine, Inhibit Human Coronavirus OC43 Infection of MRC-5 Human Lung Cells. Biomolecules. 2019; 9: 696.

[24] Xu H, Liu B, Xiao Z, Zhou M, Ge L, Jia F, *et al*. Computational and Experimental Studies Reveal that Thymoquinone Blocks the Entry of Coronaviruses into in Vitro Cells. Infectious Diseases and Therapy. 2021; 10: 483–494.

[25] Douglas M.B, Donald G.W. Nonlinear Regression Analysis and Its Applications. John Wiley and Sons: Hoboken. 1988.

[26] Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of Computational Chemistry. 2010; 31: 455–461.

[27] Tang Y, Zeng X, Liang J. Surface Plasmon Resonance: an Introduction to a Surface Spectroscopy Technique. Journal of Chemical Education. 2010; 87: 742–746.

[28] Brown AS, Ackerley DF, Calcott MJ. High-Throughput Screening for Inhibitors of the SARS-CoV-2 Protease Using a FRET-Biosensor. Molecules. 2020; 25: 4666.

[29] Xian Y, Zhang J, Bian Z, Zhou H, Zhang Z, Lin Z, *et al*. Bioactive natural compounds against human coronaviruses: a review and perspective. Acta Pharmaceutica Sinica B. 2020; 10: 1163–1174.

[30] Ma QH, Pan WQ, Li RF, Liu B, Li CF, Xie YQ, *et al*. Liu Shen capsule shows antiviral and anti-inflammatory abilities against novel coronavirus SARS-CoV-2 via suppression of NF-kappa B signaling pathway. Pharmacological Research. 2020; 158: 104850.

[31] Huang Y, Bai C, He F, Xie Y, Zhou H. Review on the potential action mechanisms of Chinese medicines in treating Coronavirus Disease 2019 (COVID-19). Pharmacological Research. 2020; 158: 104939.

[32] Lu A. Theory of traditional Chinese medicine and therapeutic method of diseases. World Journal of Gastroenterology. 2004; 10: 1854.

[33] Cho JK, Curtis-Long MJ, Lee KH, Kim DW, Ryu HW, Yuk HJ, *et al*. Geranylated flavonoids displaying SARS-CoV papain-like protease inhibition from the fruits of Paulownia tomentosa. Bioorganic & Medicinal Chemistry. 2013; 21: 3051–3057.

[34] Jo S, Kim S, Shin DH, Kim M. Inhibition of SARS-CoV 3CL protease by flavonoids. Journal of Enzyme Inhibition and Medicinal Chemistry. 2020; 35: 145–151.

[35] Daczkowski CM, Dzimianski JV, Clasman JR, Goodwin O, Mesecar AD, Pegan SD. Structural Insights into the Interaction of Coronavirus Papain-Like Proteases and Interferon-Stimulated Gene Product 15 from Different Species. Journal of Molecular Biology. 2017; 429: 1661–1683.

[36] Hsu M, Kuo C, Chang K, Chang H, Chou C, Ko T, *et al*. Mechanism of the Maturation Process of SARS-CoV 3CL Protease. Journal of Biological Chemistry. 2005; 280: 31257–31266. .

Send correspondence to:

Na Wang, Department of Pharmacognosy, Pharmaceutical College, Hebei Medical University, 050017 Shijiazhuang, Hebei, China, E-mail: woshihuaxisha@163.com

Ran Hao, School of Nursing, Hebei Medical University, 050000 Shijiazhuang, Hebei, China, E-mail: haoran810720@sina.com

Guanyu Wang, Guangdong Provincial Key Laboratory of Cell Microenviroment and Disease Research, 518055 Shenzhen, Guangdong, China, Department of Biology, Southern University of Science and Technology, 518055 Shenzhen, Guangdong, China, Guangdong Provincial Key Laboratory of Computational Science and Material Design, 518055 Shenzhen, Guangdong, China, E-mail: wanggy@sustech.edu.cn

† These authors contributed equally.