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Original Article

Active Ingredients of Reduning Injection Maintain High Potency against SARS-CoV-2 Variants*

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ABSTRACT **Objective:** To investigate the anti-coronavirus potential and the corresponding mechanisms of the two ingredients of Reduning Injection: quercetin and luteolin. **Methods:** A pseudovirus system was designed to test the efficacy of quercetin and luteolin to inhibit SARS-CoV-2 infection and the corresponding cellular toxicity. Luteolin was tested for its activities against the pseudoviruses of SARS-CoV-2 and its variants. Virtual screening was performed to predict the binding sites by Autodock Vina 1.1.230 and PyMol. To validate docking results, surface plasmon resonance (SPR) was used to measure the binding affinity of the compounds with various proteins of the coronaviruses. Quercetin and luteolin were further tested for their inhibitory effects on other coronaviruses by indirect immunofluorescence assay on rhabdomyosarcoma cells infected with HCoV-OC43. **Results:** The inhibition of SARS-CoV-2 pseudovirus by luteolin and quercetin were strongly dose-dependent, with concentration for 50% of maximal effect (EC_{50}) of 8.817 and 52.98 μmol/L, respectively. Their cytotoxicity to BHK21-hACE2 were 177.6 and 405.1 μmol/L, respectively. In addition, luetolin significantly blocked the entry of 4 pseudoviruses of SARS-CoV-2 variants, with EC_{50} lower than 7 μmol/L. Virtual screening and SPR confirmed that luteolin binds to the S-proteins and quercetin binds to the active center of the 3CLpro, PLpro, and helicase proteins. Quercetin and luteolin showed over 99% inhibition against HCoV-OC43. **Conclusions:** The mechanisms were revealed of quercetin and luteolin inhibiting the infection of SARS-CoV-2 and its variants. Reduning Injection is a promising drug for COVID-19.

KEYWORDS Reduning injection, Chinese medicine, luteolin, quercetin, SARS-CoV-2

The corona virus disease 2019 (COVID-19) pandemic has become a global health emergency. The spike protein (S-protein) of SARS-CoV-2, consisting of S1 and S2 subunits, is critical to receptor recognition and virus-cell fusion. SARS-CoV-2 binds to host cell receptors via the receptor-binding domain (RBD) of S1, which induces the formation of 6-helical bundle (6-HB) between the two heptad repeats (HRs) in S2, namely HR1 and HR2, and the consequential conformational change of S2, which facilitates the virus-membrane fusion. In December 2020, several effective SARS-CoV-2 vaccines were authorized. However, the emergence of new SARS-CoV-2 strains attenuates the efficacy of vaccines and neutralization antibodies.[1,2]

Reduning Injection (热毒宁注射液, RDN), approved by the Chinese Food and Drug Administration in 2005, is a Chinese medicine (CM) prepared from *Lonicerae Japonicae Flos*, *Artemisiae Annuae Herba*, and *Gardeniae Fructus*. CM has

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been very helpful to control the COVID-19 epidemic. We recently found CM ingredients effective to emerging coronaviruses, such as thymoquinone,[3] resveratrol, and polydatin.[4] We suspected that RDN would be effective to COVID-19 because it evidently has antibacterial, antiviral, antipyretic,[5,6] and anti-inflammatory effects,[7-9] and it has been used to treat a variety of infectious diseases. Ma, et al[10] tested RDN in COVID-19 patients and characterized its antiviral activity. They found that RDN markedly inhibited SARS-CoV-2 proliferation and viral plaque formation *in vitro* and reduced inflammatory cytokine production in the infected cells. Gao, et al[11] reported the molecular mechanisms and network pharmacology of RDN inhibiting SARS-CoV-2. Fifty effective components, corresponding to 208 targets, were identified. Network topology analysis revealed that quercetin, kaempferol, luteolin, isohamnetin, and chryseriol are the compounds that have the highest degree value. Based on these *in vitro* tests and clinical evaluations,[10] RDN was incorporated into the Diagnosis and Treatment Programs of 2019 New Coronavirus Pneumonia, which was formulated by the National Health Commission of China. It has also been highly recommended by the National Health Council and National Administration of Chinese Medicine (Trial 7th edition, 2020) for the diagnosis and treatment of pneumonia caused by SARS-CoV-2.

Quercetin, an active ingredient of RDN, can prevent the entry of many viruses, including rhinovirus[12] and poliovirus,[13] and reduce their cytopathic effects. As a strong antioxidant[14] and scavenger flavonoid without any adverse effects, it is effective on the prophylaxis and treatment of coronavirus infections.[15] Quecetin can block SARS-CoV entry into Vero E6 cells with a half maximal effective concentration (EC_{50}) of 83.4 μmol/L and a half maximal cytotoxic concentration (CC_{50}) of 3.32 mmol/L.[16]

Luteolin, another active ingredient of RDN, has *in vitro* antiviral activities against a wide range of viruses.[17] The mechanism of luteolin inhibiting the fusion of SARS-CoV with the host cell membrane was revealed.[16,18] FAC/MS identified the high affinity of luteolin with the S2 subunit of the S-protein of SARS-CoV,[16] whose HR regions are crucial for the virus-membrane fusion. The interaction of HR1 and HR2 forms a 6-HB to bring viral and cellular membranes in close proximity. Hence, the S2 subunit of the S-protein of SARS-CoV-2 (SARS-CoV-2-S), as well as host proteases such as cathepsin L and TMPRSS2, are attractive therapeutic targets.[10,19] By bioinformatic analyses, Shawan, et al[20] found that luteolin belongs to the most effective flavonoids among a total of 43 flavonoids. Luteolin bound the target proteins with the lowest binding free energy and inhibition constant. The △G0 (Ki) values are –8.2 kcal/mol (927.87 nmol/L), –7.1 kcal/mol (5980.37 nmol/L), and –10.1 kcal/mol (37.13 nmol/L) for the main protease (3CLpro), Papain-like protease (PLpro), and angiotensin-converting enzyme 2 (ACE2), respectively.[20]

Here a pseudovirus system was used to test the efficacy of quercetin and luteolin to inhibit SARS-CoV-2 infection. We tested their effects on SARS-CoV-2 pseudovirus and found that luteolin significantly inhibited viral infection. We thus speculated that luteolin potently inhibits SARS-CoV-2 by blocking the formation of 6-HB. Surface plasmon resonance (SPR) confirmed that luteolin binds with S2, which was consistent with previous results on SARS-CoV.[16] We also tested luteolin against genetic variants of SARS-CoV-2, including alpha variant B.1.1.7, beta variant B.1.351, delta variant B.1.617, and omicron.

METHOD

Materials

Quercetin was purchased from MedChemExpress (Monmouth Junction, NJ, USA); luteolin was purchased from Targetmol (Shanghai, China). The Trans1-T1 strain, F-ϕ80(lacZ)△M15△lacX74hsdR rk,m) △recA 1398endAltonA (TransGen Biotech, Beijing, China) was used to clone and propagate plasmid DNA. Miniprep and Maxiprep kits (Axygen, USA) were used to harvest and purify plasmid DNA. 3CLpro and PLpro of SARS-CoV-2, SARS-CoV, and MERS-CoV were purchased from Novoprotein (Beijing, China) or expressed and purified by Genescript (Nanjing, China). The S2-proteins of SARS-CoV-2 and SARS-CoV, the S1+S2 ECD of MERS-CoV, HCoV-OC43, NL63-CoV, and HKU1-CoV were purchased from Sinobiologic (Beijing, China). CM5 chip and phosphate surfactant P20 (PBS-P) buffer were purchased from GE healthcare (Uppsala, Sweden). The 12-mer fluorogenic peptide substrates Dabcyl-KNSTLQSGLRKE-Edans and Dabcyl-KRLKGGAPIKGE-Edans, respectively for 3CLpro and PLpro inhibition assays, were synthesized by Genescript (Nanjing, China). BHK21-hACE2 cells were maintained in high glucose Dulbecco's modified

Eagle medium (DMEM) with 10% fetal bovine serum (FBS) and 1% antibiotic/antimycotic.

VSV-dG-fLuc are recombinant vesicular stomatitis virus variants with G protein deletion, expressing firefly luciferase (fLuc). The VSV-dG-fLuc pseudoviruses were generated through plasmid-based reverse genetics system. The pBluescript-N (pBS-N), pBS-P, pBS-L and pBS-G are helper vectors for the first round virus rescue. The plasmid pVSV-dG-fLuc, as well as the helper vectors, were purchased from Kerafast (Boston, MA, USA). The genes encoding the coronaviruses were synthesized by Genescript.

Expression and Purification of Recombinant Nsp13 of SARS-CoV-2 and SARS-CoV

The pET28a vectors for Nsp13 of SARS-CoV-2 and SARS-CoV were provided by Profs. AN Wen-lin and HUANG Jian-dong, respectively. The proteins were expressed in BL21(DE3) cells and purified using a Ni-affinity column and a FPLC Superdex 200 Increase column.[21,22]

Viruses and Cell Lines

HCoV-OC43 was purchased from American Type Culture Collection (ATCC VR-1558) and propagated using rhabdomyosarcoma (RD) cells (ATCC CCL-136). HCoV-OC43 stock virus was obtained from RD cells in DMEM supplemented with 2% FBS at 72 h post-infection (hpi). Viral titer (PFU/mL) was determined by plaque assay. BHK21-hACE2 was kindly provided by Prof. YAN Huan from Wuhan University.

VSV-Based Pseudovirus Production

VSV pseudotypes coated with the wide type (wt) and variant of SARS-CoV-2-S were generated based on a previously described protocol with slight modification.[23] HEK-293T or Vero-E6 cells were transfected with a plasmid overexpressing codon-optimized (i.e., the deletion of C-terminal 18 amino acids) SARS-CoV-2-S, which was able to significantly improve the pseudotypes packaging efficiency.[24,25] After 24 h, the cells were transduced with VSV-dG-fluc at multiplicity of infection (MOI) = 10 IU. After 1 h incubation at 37 ℃, the cells were washed by culture medium once and then replenished with fresh culture medium with anti-VSV rat serum to eliminate the infectivity of the residual input viruses. The SARS-CoV-2-S pseudotyped VSV viruses were harvested 24 h after transduction by clarification at 12,000 r/min for 2 min and then stored at –80 ℃. The virus titer was determined by a plaque assay on BHK21-hACE2 cells with serial-diluted inocula.

SARS-CoV-2 Pseudovirus Inhibition Assay

BHK21-hACE2 cells were seeded in 96-well plates. After 24 h, the cells were inoculated with SARS-CoV-2 pseudotyped VSV (MOI = 0.1 IU) expressing luciferase diluted using DMEM with 10% FBS in the presence of indicated chemicals of varying concentrations (100, 33, 11, 3.7, 1.23, 0.41, and 0.137 μmol/L for the tested compounds). About 16–20 h after infection, the cells infected with the pseudoviruses expressing firefly luciferase were lyzed by 1× passive lysis buffer (Promega, Madison, USA) at room temperate for 15 min, and the luciferase activity was evaluated by One-Glo luciferase assay kit (Promega) through a GloMax® 20/20 Luminometer. The cytotoxicity of the tested chemicals was evaluated by bright field images before fluorescence imaging or cell lysis. The median effective concentration (EC_{50}) was determined by nonlinear regression analysis.

Virtual Screening

The PDB files of SARS-CoV-2 3CLpro (PDB: 6lu7), SARS-CoV 3CLpro (pdb: 2duc), MERS-CoV 3CLpro (PDB: 4rsp), SARS-CoV-2 helicase (PDB: 6zsl), and SARS-CoV-2-S (PDB: 6vxx)[26,27] were downloaded from http://www.rcsb.org/.[28,29] All heterogeneous atoms and the bound ligands were removed. The chain A of 6lu7, 2duc, 4rsp, 6zsl, and 6vxx were selected for the subsequent molecular docking by Autodock Vina 1.1.2.[30] The protein–ligand interactions were visualized by using PyMol. For 3CLpro, helicase, and S-protein, the interactive residues were defined to be those whose distances to the hit ligand were ⩽1 Å, ⩽2.5 Å, and ⩽2.5 Å, respectively.[31] The ligands with binding energy lower than –6 kcal/mol were selected as candidates for the subsequent experimental verification.

SPR Based Analysis

To measure the binding affinities of quercetin/luteolin with the 3CLpro and PLpro of SARS-CoV-2, SARS-CoV, MERS-CoV, the helicase of SARS-CoV-2 and SARS-CoV, and the S-proteins of SARS-CoV-2, SARS-CoV, MERS-CoV, HCoV-OC43, NL63-CoV and HKU1-CoV, a SPR based Biacore T200 biosensor (Biacore AB, Uppsala, Sweden) with CM5 was used as the sensor chip. At 25 ℃, the target proteins

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were diluted to a final concentration of 20 μg/mL in 10 mmol/L sodium acetate buffer (pH 4.5) and then immobilized to CM5 by the standard primary amine coupling method. The CM5 surface was first activated by injecting a 1:1 mixture containing 200 mmol/L 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride and 50 mmol/L N-hydroxysuccinimide for a target level of 10,000 RU at a flow rate of 20 μL/min. The target proteins were then injected to CM5 surface, which was then blocked by injecting 1 mol/L ethanolamine at pH 8.5 for 7 min. During the immobilization, HBS-EP running buffer (10 mmol/L HEPES, 150 mmol/L NaCl, 3.4 mmol/L EDTA, and 0.005% v/v surfactant P20, pH 7.4) was used. All the screening assays were performed over the unmodified dextran surface and the protein surface. For sample dilution, the pH and the concentration of both dimethyl sulfoxide (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 (Cytiva, Sweden) by steady state analysis.

HCoV-OC43 Inhibition Assay

RD cells were infected with HCoV-OC43 at MOI = 0.01 IU in the presence of the indicated compound diluted in DMEM supplemented with 2% FBS. The compounds and the virus were maintained with the cells during the 48 h incubation at 37 ℃. The efficiency of HCoV-OC43 infecting RD cells was evaluated by immunofluorescence assay (IFA) against the Nucleoprotein. In general, RD cells were fixed by 100% methanol at 48 hpi, blocked by 1% bovine serum albumin/phosphated buffered saline at 37 ℃ for 1 h, stained with anti-OC43 Np mAb (Sigma, MAb 9012, clone 541-8F) at 1:1000 at 37 ℃ for 1 h, and then incubated with the secondary antibody for another 1 h. nuclei were stained with Hoechst 33342 (Thermo Scientific H1399) at 1:5000 for 15 min. Images were captured by an fluorescence microscope (MI52-N, Mshot, China).

Determination of Half Maximal Cytotoxic Concentration

The CC_{50} of the compounds was determined by the CellTiter-Glo 2 (Promega) cell viability assay kit. In brief, 293T-hACE2 cells were seeded in 96-well plates with 3-fold serial-diluted compounds (from 0.137 to 100 μmol/L). Then 24 h later, the cell viability was detected by CellTiter-Glo 2 reagent following the manufacture's instructions, and the luminescence was determined with a Spectra MaxiD3 multi-well Luminometer 458 (Molecular Devices, USA). The CC_{50} value was determined by nonlinear regression analysis.

RESULTS

EC_{50} and CC_{50} of Quercetin and Luteolin for SARS-CoV-2 Pseudovirus

By adding different compound concentrations from 0.137 to 100 μmo/L, it was found that the inhibition of SARS-CoV-2 pseudovirus entry into the BHK21-hACE2 cells was strongly dose-dependent. For luteolin, EC_{50} = 8.817 μmo/L, CC_{50} = 177.6 μmo/L, selectivity index = 20.14. For quercetin, EC_{50} = 52.98 μmo/L, CC_{50} = 405.1 μmo/L, selectivity index = 7.65 (Figure 1). These data suggested that quercetin and luteolin are both effective against SARS-CoV-2 infection, with luteolin much stronger considering its small EC_{50}. Therefore, luetolin was chosen in the following as the compound to inhibit the pseudoviruses of SARS-CoV-2 variants.

EC_{50} of Luetolin Inhibiting Pseudoviruses of SARS-CoV-2 Variants

Luetolin significantly blocked the entry of the pseudoviruses of SARS-CoV-2 alpha variant B.1.1.7, beta variant B.1.351, delta variant B.1.617, and the recent variant omicron, with EC_{50} being 6.44, 5.605, 4.635, and 3.489 μmol/L, respectively (Figure 2).

Quercetin and Luteolin Binding Site Prediction

Chain A of SARS-CoV-2-3CLpro (PDB: 6lu7), SARS-CoV-3CLpro (2duc), MERS-CoV-3CLpro (4rsp), and SARS-CoV-2-helicase (6zsl) were extracted to dock with quercetin, which was found to bind to the sites formed by MET49, LEU141, HIS163, MET165, GLU166, ASP187 GLN189 of 6lu7 (Figure 3A), THR25, CYS44, GLU47, ASP48, MET49, MET165 of 2duc (Figure 3B), HIS41, PHE143, HIS166, MET168, GLU169, LYS191 of 4rsp (Figure 3C), and GLY-287, GLY289, LYS290, GLN406, ARG569 of 6zsl (Figure 3D), respectively. Then, chain A of SARS-CoV-2-S (6vxx) was docked with luteolin, which forms hydrogen bonds with S2 residues SER749, THR797 and ALA1075; HIS1077 may also participate

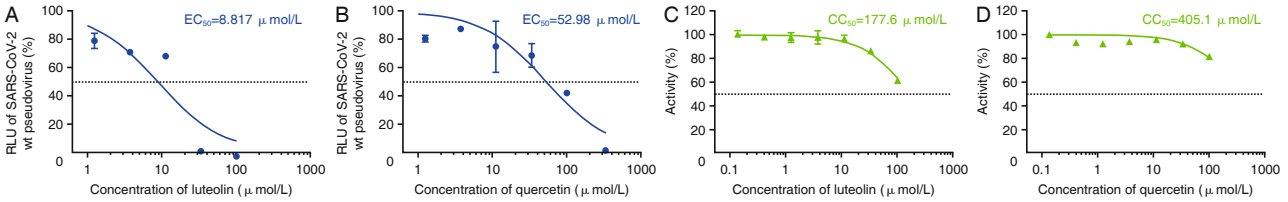

Figure 1. Determination of EC_{50} and CC_{50} of Luteolin/Quercetin against SARS-CoV-2 Pseudovirus

Notes: EC_{50}: maximal effective concentration; CC_{50}: half maximal cytotoxic concentration; the same below

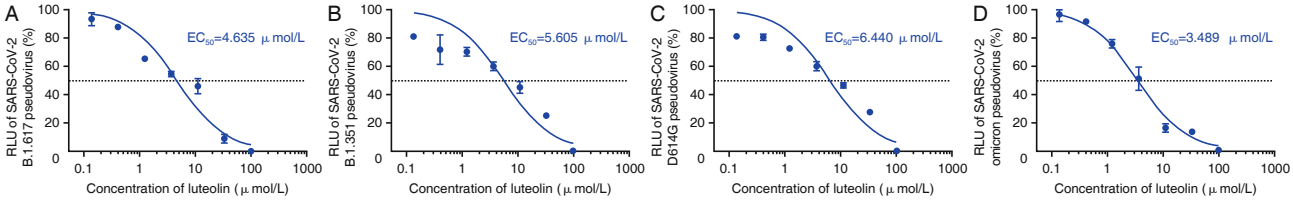

Figure 2. Determination of EC_{50} of Luteolin against SARS-CoV-2 Variant Pseudoviruses

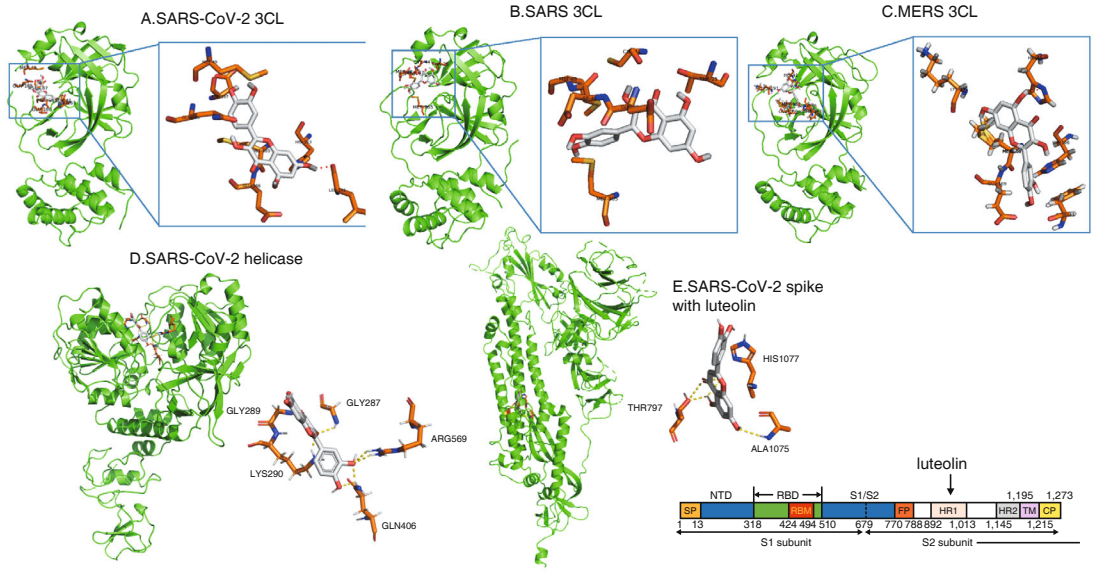

Figure 3. Binding Site Prediction

Notes: (A) Quercetin (gray stick) binds to some residues (orange sticks) of SARS-CoV-2 3CLpro (6lu7). (B) Quercetin binds to SARS 3CLpro (2duc). (C) Quercetin binds to MERS 3CLpro (4rsp). (D) Quercetin (gray stick) binds to SARS-CoV-2 helicase (6zsl, green cartoon), with the interacting residues indicated as orange sticks and the potential hydrogen bonds indicated as dashed lines. (E) Luteolin (gray stick) binds to SARS-CoV-2-S (6vxx, green cartoon), with the interacting residues indicated as orange sticks and the potential hydrogen bonds indicated as dashed lines.

in the interaction (Figure 3E). These results suggest that luteolin inhibits the infection of SARS-CoV-2 by disturbing the formation of the 6-HB fusion core.

SPR-Based Binder Identification

To validate our docking results, we used SPR to measure the affinity of luteolin and quercetin binding with various proteins of the coronaviruses (Appendix 1). The proteins were immobilized on CM5 chips. We let the compounds flow across the CM5 surface and found that the binding were strongly dose-dependent, including luteolin binding to the S-proteins and quercetin binding to the active center of the 3CLpro, PLpro, and helicase proteins (Table 1).

Anti-HCoV-OC43 Activity of Quercetin and Luteolin

The result of IFA on RD cells showed that quercetin and luteolin significantly suppressed HCoV-OC43 replication. Over 99% inhibition was achieved at the 11.11 μ mol/L concentration (Figure 4).

DISCUSSION

Since the outbreak of COVID-19, scientists have been assiduously finding effective methods to treat coronavirus infection. For example, Wei, et al[29] adopted an *in silico* drug repurposing strategy; by

Table 1. Affinity at 25℃ of Luteolin and Quercetin Binding to Proteins of Some Coronaviruses

Compound	Proteins	KD (μmol/L)	Rmax (RU)	Chi^2 (RU^2)	Chi
Luteolin	SARS-CoV-2 S2	25.57	6.50	0.0125	0.112
	SARS S1+S2 ECD	2.31	6.20	0.0508	0.225
	MERS S1+S2 ECD	38.62	2.16	0.0066	0.081
	OC43 S1+S2 ECD	7.88	6.72	0.3150	0.561
	NL63 S1+S2 ECD	83.44	33.83	0.0753	0.274
	HKU1 S1+S2 ECD	38.18	26.46	0.0135	0.116
Quercetin	SARS-CoV-2 3CLpro	0.92	4.22	0.0650	0.255
	SARS-CoV-2 PLpro	7.72	44.15	6.2300	2.500
	SARS 3CLpro	5.97	26.92	5.7800	2.404
	SARS PLpro	10.67	35.18	0.0096	0.098
	MERS 3CLpro	33.64	40.65	0.7030	0.839
	MERS PLpro	7.71	24.60	4.0000	2.000
	SARS-CoV-2 helicase	9.43	24.16	3.2300	1.800
	SARS-CoV helicase	7.37	36.93	4.8800	2.210

molecular docking, they searched Food and Drug Administration-approved drugs from DrugBank and natural compounds from Traditional Chinese Medicine Systems Pharmacology (TCMSP) for the compounds that can potentially interrupt the interaction between human ACE2 and SARS-CoV-2-S; they found more than 20 molecules binding to SARS-CoV-2-S with high affinity. CM has a long history to treat viral infection and proves to be effective in therapeutics via the direct inhibition of viral proliferation and the enhanced immune function.

RDN is a CM recommended for treating COVID-19. It has multiple pharmacological activities, including, antiviral,[5] antipyretic,[6] and anti-inflammatory,[7] antibacteria,[5,8] lung injury treatment,[9] etc. Among the ingredients of RDN, luetolin and quercetin were identified as key active ingredients to inhibit many viruses. Owing to pleiotropy and nontoxicity of quercetin, a clinical trial (NCT04377789) was started to evaluate quercetin against COVID-19. The synergism of quercetin with vitamins C/D may provide alternative or additional therapeutic and preventive options due to their overlapping antiviral and immunomodulatory properties. We designed SARS-CoV-2 pseudoparticles containing SARS-CoV-2-S assembled onto luciferase reporter gene-carrying VSV core particles based on the genome sequence of SARS-CoV-2 released on 01/12/2020.[32] These pseudotyped particles faithfully reflect key aspects of the entry of native SARS-CoV-2 into host cells via binding to the ACE2 receptors; they can thus be used to test entry inhibitors of SARS-CoV-2.[33] Luteolin inhibited SARS-CoV infection of Vero E6 cells with an EC_{50} of 10.6 μmol/L and CC_{50} of 155 μmol/L, while quercetin antagonized the entry of HIV-luc/SARS pseudo-types with EC_{50} of 83.4 μmol/L.[16] We found that luteolin and quercetin inhibit SARS-CoV-2 pseudovirus with EC_{50} of

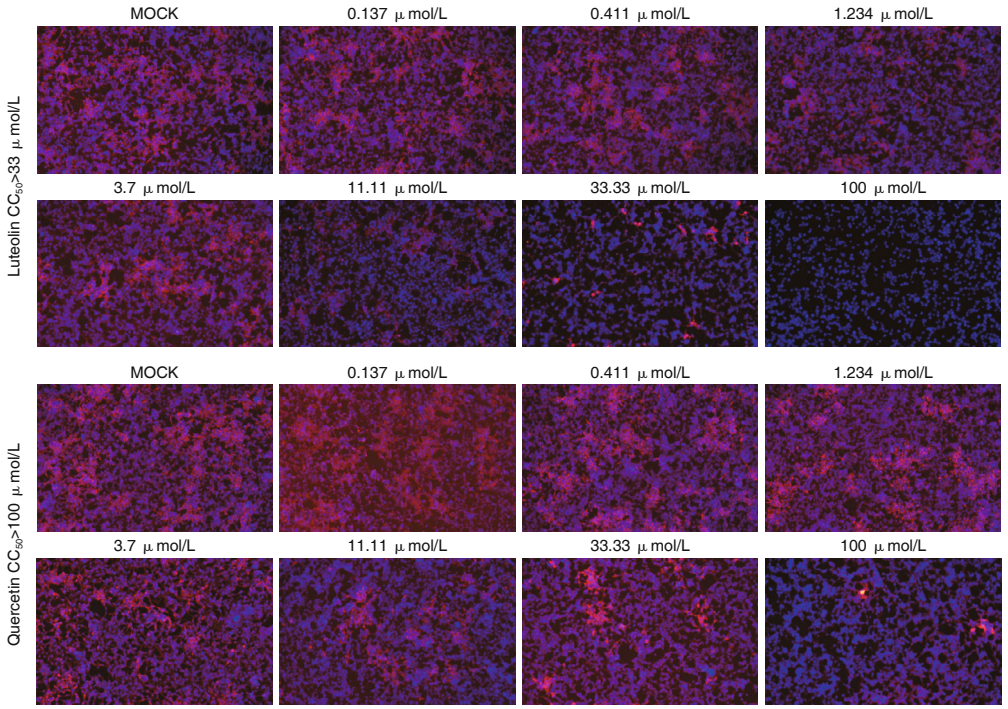

Figure 4. Luteolin and Quercetin on RD Cells Infected with HCoV-OC43 by Immunofluoresence Assay

8.817 and 52.98 μmol/L, respectively.

SARS-CoV-2 variants have been circulating around the world.[34] These variants were found to spread more easily than the original virus and to evade vaccine protections.[35] The majority of the entry inhibitors were designed to target the RBD-ACE2 interaction. However, targeting this domain show limited cross-reactivity because RBD is poorly conserved across human coronaviruses. The alpha, gamma, beta, delta, and omicron variants of SARS-CoV-2 have emerged, with some mutations identified in the RBD. In contrast, S2 is highly conserved and can potentially serve as a broad-spectrum target. To test luteolin, we designed the pseudoviruses of SARS-CoV-2 variants that contain the mutant S-protein assembled onto luciferase reporter gene-carrying VSV core particles. We have confirmed the broad-spectrum property of luteolin in targeting S2 of pseudoviruses of SARS-CoV-2 variants: alpha variant B.1.1.7, beta variant B.1.351, delta variant B.1.617, and omicron, with EC_{50} values of 6.44, 5.605, 4.635, and 3.489 μmol/L, respectively.

Our *in vitro* studies showed that luteolin bound to the S-protein of coronaviruses such as SARS-CoV-2, SARS-CoV, MERS-CoV, HCoV-OC43, NL63-CoV, and HKU1-CoV, with the respective dissociation constants (KD) being 25.57, 2.308, 38.62, 7.878, 83.44, and 38.18 μmol/L, respectively. The responses were strongly dose-dependent. Quercetin, although not a strong inhibitor of the S-protein, has high affinities with PLpro, 3CLpro, and NTPase/helicase, which are key proteins facilitating the coronavirus replication. It bound to 3CLpro of SARS-CoV-2, SARS-CoV, and MERS-CoV, with KD being 0.920, 5.972, and 33.64 μmol/L, respectively; it bound to PLpro of SARS-CoV-2, SARS-CoV, and MERS-CoV, with KD being 7.715, 10.67, and 7.712 μmol/L, respectively; it bound to the helicase of SARS-CoV-2 and SARS-CoV, with KD being 9.43 and 7.37 μmol/L, respectively. The above results are consistent with the molecular docking studies of both ourselves and others.[38]

The broad spectrum of luteolin and quercetin were also confirmed by targeting HCoV-OC43, a relative of SARS-CoV-2. For luteolin in particular, over 99% inhibition was achieved at 11.11 μmol/L concentration. Debiaggi, et al[36] found that 60 μg/mL quercetin reduced the infectivity of bovine coronaviruses NCDCV by 50%. Hence, quercetin and luteolin may be effective to several members of the *Coronaviridae* family.[37]

In conclusion, RDN is potentially effective against SARS-CoV-2, and we have tentatively revealed the underlying mechanisms upon which in-depth studies can be based.

Conflict of Interest

The authors declare no conflicts of interest.

Author Contributions

Xu H, Wang GY acquired funding and supervised the project. Xiao Z, Xu H, Sun MS performed the experiments. Xu H, Wang GY and Xiao Z were responsible for writing and revising the article. Qu ZY did the virtual screening. Ma XY, Huang BX and Wang BQ analyzed the data. Xiao Z, Xu H and Qu ZY contributed to this work equally. All the authors read and approved the final version of manuscript.

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