Received: 9 January 2021 | Revised: 22 February 2021 | Accepted: 8 March 2021

DOI: 10.1002/ddr.21815

RESEARCH ARTICLE

DRUG DEVELOPMENT RESEARCH WILEY

Potential antiviral activity of isorhamnetin against SARS-CoV-2 spike pseudotyped virus in vitro

Yingzhuan Zhan | Wenjing Ta | Wenjuan Tang | Ruochen Hua | Jue Wang | Cheng Wang | Wen Lu

School of Pharmacy, Xi'an Jiaotong University, Xi'an, China

Correspondence
Wen Lu, School of Pharmacy, Xi'an Jiaotong University, Yanta West road, Xi'an 710061, China.
Email: lvlu2004@xjtu.edu.cn

Funding information
Basic Research Project in Shaanxi Administration of traditional Chinese Medicine, Grant/Award Number: JCMS028; Fundamental Research Funds for the Central Universities, Grant/Award Number: xjj2018170; National Natural Science Foundation of China, Grant/Award Number: 81930096; Natural Science Basic Research Plan in Shaanxi Province of China, Grant/Award Number: 2020JM-023

Abstract

Coronavirus Disease 2019 (COVID-19) cases and deaths are still rising worldwide, there is currently no effective treatment for severe inflammation and acute lung injury caused by new coronavirus (SARS-COV-2) infection. Therapies to prevent or treat COVID-19, including antiviral drug and several vaccines, are still being development. Human angiotensin-converting enzyme 2 (ACE2), expressing in lung, has been confirmed to be a receptor for SARS-COV-2 infection, interventions for attachment of spike protein of SARS-CoV-2 to ACE2 may be a potential approach to prevent viral infections and it is considered as a potential target for drug development. In this study, we observed that seabuckthorn and its flavonoid compounds quercetin and isorhamnetin were shown strong retention to ACE2 overexpression HEK293 ($ACE2^h$) cells by CMC analysis. Based on drug receptor interaction analysis and viral entry studies in vitro, we evaluated the interaction of two flavonoid compounds and ACE2 as well as the inhibitory effect of the two compounds on viral entry. Surface plasmon resonance assay proved the effect that isorhamnetin bound to the ACE2, and its affinity (KD value) was at the micromolar level, that was, 2.51 ± 0.68 μM. Viral entry studies in vitro indicated that isorhamnetin inhibited SARS-CoV-2 spike pseudotyped virus entering $ACE2^h$ cells. Based on promising in vitro results, we proposed isorhamnetin to be a potential therapeutic candidate compound against COVID-19.

KEYWORDS
antiviral activity, isorhamnetin, SARS-CoV-2

1 | INTRODUCTION

The disease caused by SARS-CoV-2 virus infection has been named Coronavirus Disease 2019 (COVID-19). Its pandemic is one of the most serious global public health crises to date. It produces clinical symptoms that include fever, dry cough, dyspnea, headache, pneumonia with potentially progressive respiratory failure owing to alveolar damage, and even death (Nishiga et al., 2020; Patel et al., 2020; Scimeca et al., 2020).

Currently, at the end of 2020, the death toll from the COVID-19 pandemic worldwide reached 1.6 million, as the total number of infections exceeded 72 million. The treatment options for COVID-19 are very limited, and several vaccines can be prevented SARS-CoV-2 virus is under development (Flanagan et al., 2020; Jeyanathan et al., 2020; Tregoning et al., 2020). Drug repurposing has been used to deal with emerging infectious diseases so as to quickly discover potential treatments. Unfortunately, remdesivir, an Ebola virus (EBOV) inhibitor, that were once considered the most promising therapeutic drug has also been confirmed to be ineffective in clinical studies (Pruijssers et al., 2020; Spinner et al., 2020; Wang, Zhang, et al., 2020). To date, there is no effective prevention and treatment strategy for this disease, the main treatment strategy is limited to symptomatic treatment and organ support for patients with COVID-19 and severe or critical illness, such as corticosteroid therapy and antiviral therapy, oxygen therapy (Zumla et al., 2020).

The therapeutic target of COVID-19 can be directed against the SARS-CoV-2 virus and its protein or host cell targets. Angiotensin-converting enzyme 2 (ACE2), an important metalloprotease, is a negative regulator of the renin-angiotensin system, which balances the function of ACE to maintain blood pressure homeostasis (South et al., 2020). Evidence shows that ACE2 is expressed in tissues such as heart, lung, kidney, liver, intestine, and testis (Imai et al., 2010; Tipnis et al., 2000), and it has been confirmed to mediate COVID-19 virus infection (Bourgonje et al., 2020; Devaux et al., 2020). It is known that one approach of SARS-CoV2 entering the recipient host cell is interaction with angiotensin-converting enzyme (ACE) 2 on the cell membrane of pulmonary epithelial cells (Hassan et al., 2020; Lu et al., 2020; Shang, Wan, et al., 2020; Walls et al., 2020). Structural analysis shows that the spike protein of SARS-CoV-2 attaches to ACE2 by contacting the apex of subunit I of the ACE2 catalytic domain (Seyran et al., 2020). Once attached by SARS-CoV-2, the ectodomain of ACE2 will be cleaved and the transmembrane region or whole molecule of ACE2 along with the virus enters the cell by endocytosis (Wrapp et al., 2020). Therefore, interventions for ACE2 have aroused great interest in reducing viral infections. Interruption of interaction between spike protein and ACE2, thereby preventing SARS-CoV-2 receptor binding and subsequent spread of infection might be a potential approach to treat COVID-19 patients (Barlow et al., 2020; Wu et al., 2020).

Previous studies reported that plant secondary metabolites are helping to prevent viral infection, such as sea-buckthorn, green tea, honeysuckle, and so forth (Choi et al., 2012; Enkhtaivan et al., 2017; Patil et al., 2013). Seabuckthorn, also called *Hippophae rhamnoides* L., belonging to the family of Elaeagnaceae, is a berries plant native to the Asian and Europe. Berries of Seabuckthorn were used as traditional medicine in Asia (Bal et al., 2011; Enkhtaivan et al., 2017). Seabuckthorn berries contain lots of chemical compounds, nearly 200 bioactive ingredients have been found in seabuckthorn fruits, including flavonoids (isorhamnetin, quercetin, kaempferol, etc.), phenolic acid (gallic acid, vanillic acid, P-coumaric acid, etc.), vitamin C, vitamin E, carotenoids, amino acids and minerals (iron, calcium, phosphorus and potassium), and so forth (Kwon et al., 2017; Olas, 2016). Sea buckthorn berries extract is proved to have the activities of antioxidant, antiviral, anticancer, blood glucose and blood lipids regulation, immune regulation and anti-inflammation (Cho et al., 2017; Enkhtaivan et al., 2017; Larmo et al., 2013; Olas et al., 2018; Xing et al., 2002). In this study, we investigated the antiviral activity of active products of sea buckthorn berries against SARS-CoV-2 spike pseudotyped virus in vitro and found that isorhamnetin antagonized ACE2 and blocked the entry of 2019-nCoV spike pseudotyped virus into $ACE2^h$ cells. Some literatures reported flavanols including isorhamnetin and quercetin are considered as potential antiviral drugs targeting SARS-CoV-2 proteases, spike protein, RNA-dependent RNA polymerase (RdRp) or ACE2 receptor. However, these studies predicted targets of these compounds based on computer-based analysis, such as network pharmacological study (Huang et al., 2020), virtual screening or molecular docking (Derosa et al., 2020). Our studies investigated and confirmed experimentally the antiviral activity of isorhamnetin against SARS-CoV-2 spike pseudotyped virus, which might provide a new preliminary leading compound to be develop as therapeutics against COVID-19.

2 | MATERIALS AND METHODS

2.1 | Materials and reagents

Sea-buckthorn was purchased from Tongrentang Pharmacy (Xi'an, China), quercetin (Cat No. HR15522B1, purity ≥98%) and isorhamnetin (Cat No. HR14528B1, purity ≥98%) were obtained from Baoji Herbest Bio-Tech Co., Ltd. (Baoji, China). Fetal bovine serum, DMEM high glucose and pH 7.4 phosphate-buffered saline (PBS) were purchased from HyClone (Logan, Utah, USA). The penicillin–streptomycin solution was purchased from Servicebio. (Wuhan, China). Recombinant Human ACE-2 (C-6His) was synthesized by Novoprotein (Cat: DRA110, Shanghai, China). SPR chip was from Nicoya (Canada). The SARS-CoV-2 spike pseudotyped virus was obtained from Sino Biological (PSC001, Beijing, China). CCK8 were purchased from 7Sea Pharmatech Co., Ltd (Shanghai, China). Fluo-3 AM and Pluronic F-127 were from Biotium (Waltham, MA, USA).

2.2 | Cell culture

The $ACE2^h$ cell line was constructed by Genomeditech (Shanghai, China). $ACE2^h$ cells were maintained in DMEM high glucose medium, which contained 10% (vol/vol) fetal bovine serum (FBS), 1% penicillin–streptomycin and 4 μg/ml puromycin. All cell lines were cultured in a 5% CO_2 incubator at 37°C.

2.3 | Preparation of sea-buckthorn extract

The berries of Sea-buckthorn were commercially available as dry matter. The raw material was crushed and refluxed gently in 10 volumes of 95% ethanol (vol/wt) for 1 h for two times. The merged extracting solutions were filtered, and supernatant was evaporated under reduced pressure and dried in vacuum conditions overnight. The dry powder was stored at 4°C and dissolved in methanol before use.

2.4 | Preparation of sample solutions

The stock solutions of sea-buckthorn extract (SBE, 25 mg/ml), isorhamnetin (1 mg/ml) and quercetin (1 mg/ml) were prepared by separately dissolving the extract dry powder or the standard compounds in methanol. All chromatographic experiments were conducted at 37°C and PBS was used as the mobile phase.

2.5 | Preparation of cell membrane stationary phase

$ACE2^h$ cells (1×10^7) were harvested and washed three times with 5 mM PBS by centrifuging at 3000×*g* for 10 min, and the pellet was re-suspended with 50 mM Tris–HCl (pH 7.4), followed by ultrasonic destruction for 30 min. The homogenate was centrifuged at 1000×*g* for 10 min, and supernatant was collected and centrifuged at 12,000×*g* for 10 min. The precipitate was then suspended with 5 mM PBS. $ACE2^h$ CMSP was prepared by adsorption of the cell membrane suspension (5 ml) on the activated silica (0.05 g) under vacuum and with a gentle agitation. The CMSP was placed overnight and then washed five times with 5 mM PBS. Finally, the mixture obtained was packed into a column (10 × 2.0 mm I.D.) using a wet packing method (5 MPa, 20 min). All the procedure was performed at 4°C.

2.6 | Apparatus and conditions

CMC analysis was performed on a Shimadzu LC-2040C apparatus (Shimadzu, Kyoto, Japan), and the data acquired by the LCsolution software (Shimadzu, Kyoto, Japan) was processed by Graph-Pad Prism version 6.0 (San Diego, CA, USA). The detection wavelengths were 205 nm for SBE, 292 nm for isorhamnetin, 273 nm for quercetin. The chromatographic conditions were as follows: flow rate, 0.2 ml/min; column temperature, 37°C; mobile phase, ultrapure water.

2.7 | Cytotoxicity assay

Cell viability was determined by CCK8 kit following the manufacturer's instructions. First, exponentially growing $ACE2^h$ cells were collected and seeded into 96-well plates at a density of 5×10^3 cells/well, and then the cells were treated with different concentrations of drugs (0, 0.1, 1, 10, 50, 100, 200, 300 and 400 μM) for 24 h, then CCK8 solution were added to each well and incubated at 37°C for 2 h. the absorbance at 450 nm was measured with a microplate reader (Bio-Rad, Carlsbad, California) to evaluate the relative cell viability. The formula for calculating the survival rate of $ACE2^h$ cells is as follows:

$$[(OD_{Treated} - OD_{Blank})/(OD_{Control} - OD_{Blank})] \times 100\%.$$

2.8 | Intracellular Ca^{2+} mobilization assay

ACE^h cells were seeded in 96-well plate at a density of 1×10^4 cells per well, culture medium was removed after 24 h of incubation, calcium imaging buffer (CIB: NaCl 125 mM, KCl 3 mM, $CaCl_2$ 2.5 mM, $MgCl_2$ 0.6 mM, HEPES 10 mM, glucose 20 mM, $NaHCO_3$ 1.2 mM, sucrose 20 mM, brought to pH 7.4 with NaOH) was added to the well, and cells were loaded with a fluorescent Ca^{2+} indicator, 0.1% Fluo-3 AM, along with 0.02% Pluronic F-127, for 45 min at 37°C. Then cells were washed three times with CIB and used immediately for imaging. Cells loaded Fluo-3 were observed at 488 nm excitation. Ten seconds after imaging starts, compounds were added to the wells and responses were monitored at 1 s intervals for an additional 120 s.

2.9 | Surface plasmon resonance assay

For assessment of interactions of small molecules and ACE2 protein, surface plasmon resonance (SPR) was adopted in this experiment. ACE2 protein with a 6-his tag (30 μg/ml) was covalently attached to the NTA sensor chip via capture coupling. Then, small molecules at different concentrations was injected into the chamber in sequence. The interaction of fixed ACE2 with the small molecules was detected using Open SPR™ (Nicoya Lifesciences, Waterloo, Canada) at 25°C. The binding time and disassociation time were both 250 s with the 20 μl/s of flow rate. A one-to-one diffusion-corrected model was fitted to the wavelength shifts corresponding to the varied drug concentration. The data were retrieved and analyzed using TraceDrawer.

2.10 | Detection of SARS-CoV-2 spike pseudotyped virus entry into $ACE2^h$ cells

Spike pseudotyped virus entry assay was conducted as described previously (Wang, Han, et al., 2020). $ACE2^h$ cells at the density of 5×10^4 in 50 μl medium per well were seeded into 96-well plates and cultured in 37°C for 2 h. After adherent, 25 μl of medium per well was discarded carefully, and same volume medium containing the tested drugs was added into plate for 2 h treatment. Subsequently, 5 μl of SARS-CoV-2 spike pseudotyped virus was added the plate and incubated at 37°C for 4 h, then 100 μl of complemented DMEM per well was added for 2 h incubation. Two hundred microliters per well of fresh DMEM medium was added to replace the culture medium containing the virus and incubated continuously at 37°C for 48 h. Twenty microliters of cell lysate was measured by the Luciferase Assay System (Promega, E1500), chemiluminescence was detected at 560 nm with a 1 s exposure time using a microplate reader.

2.11 | Molecular docking assays

Molecular docking assays were carried out using SYBYL-X 2.0 version. The small molecules and X-ray crystal structure of the protein (PDB code: 6M0J) were imported. Water molecules were removed and hydrogen was added. Tripos force field and Pullman charge were applied to minimize. Isorhamnetin were depicted by the Sybyl/Sketch module (Tripos Inc.), optimized by Powell's method with the Tripos force field with convergence criterion at 0.005 kcal/(Å mol), and assigned using Gasteiger–Hückel method.

2.12 | Statistical analysis

Data were shown as the mean ± *SEM*, and statistical analysis was performed by analysis of variance, two-tailed test was used for comparison between the two groups. The difference was statistically significant at $p < .05$.

3 | RESULTS

3.1 | Chromatographic analysis studies

The ACE2^h/CMC column was used to identify the active compound in SBE which could target ACE2. We found that SBE showed retention on the ACE2^h/CMC column, and retention time was 28.4 min (Figure 1a). Seabuckthorn berries contain a variety of flavonoids, and it is known that the main active flavonoids in sea buckthorn are quercetin and isorhamnetin, so we determined the binding of quercetin and isorhamnetin to ACE2. Quercetin and isorhamnetin injected into the ACE2/HEK293/CMC column. Figure 1b,c showed that the retention time of quercetin and isorhamnetin was 6.5 and 5.4 min respectively, that was, both quercetin and isorhamnetin bind to the ACE2/HEK293/CMC column. At the same time, all the tested samples had no retention on the negative control column (blank silica gel, data not shown), which indicated that quercetin and isorhamnetin had a strong affinity for ACE2.

3.2 | Binding characteristics of quercetin and isorhamnetin with ACE2

To measure the direct binding of quercetin and isorhamnetin to the ACE2, the equilibrium dissociation constant (KD) of quercetin and isorhamnetin binding to ACE2 was performed with SPR technology. Increasing concentrations of compounds were injected on sensor chips with immobilized recombinant ACE2. As shown in Figure 2, quercetin bound to ACE2 protein with an KD (5.92 ± 0.92) μM, and isorhamnetin with an KD (2.51 ± 0.68) μM. That was, both quercetin and isorhamnetin showed affinity to ACE2 recombinant protein on SPR chips.

3.3 | The toxicity evaluation of quercetin and isorhamnetin on ACE2^h cells

The toxicity of quercetin and isorhamnetin on ACE2^h cells were assessed by the change in cell viability and intracellular Ca^{2+} flux. CCK8 assay was used to evaluate the effect of quercetin and isorhamnetin on cell viability. As shown in Figure 3a,b, compared with untreated group, there was no significant difference in absorbance of quercetin or isorhamnol treatment for 24 h at the concentration ranged from 0.4 to 200 μM, respectively, indicating that there was no significant cytotoxicity within the concentration range.

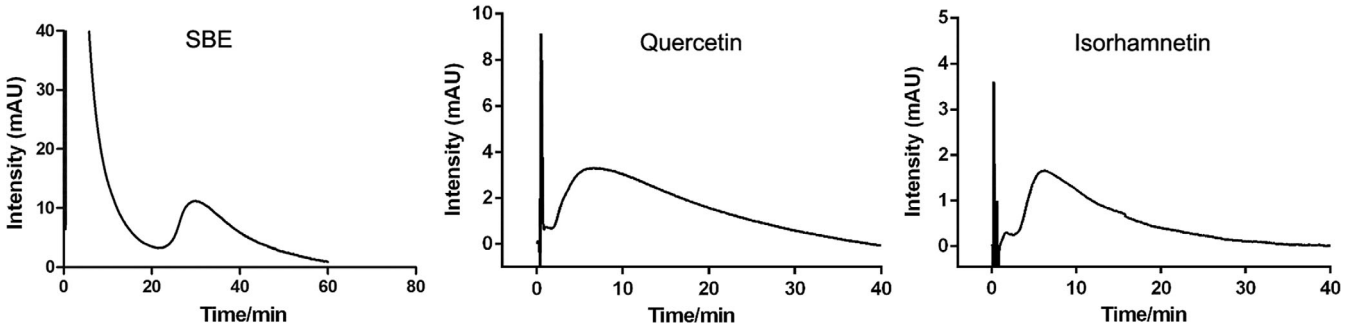

FIGURE 1 The chromatograms of sea-buckthorn extract (SBE), quercetin and isorhamnetin on ACE2/HEK293/CMC affinity column

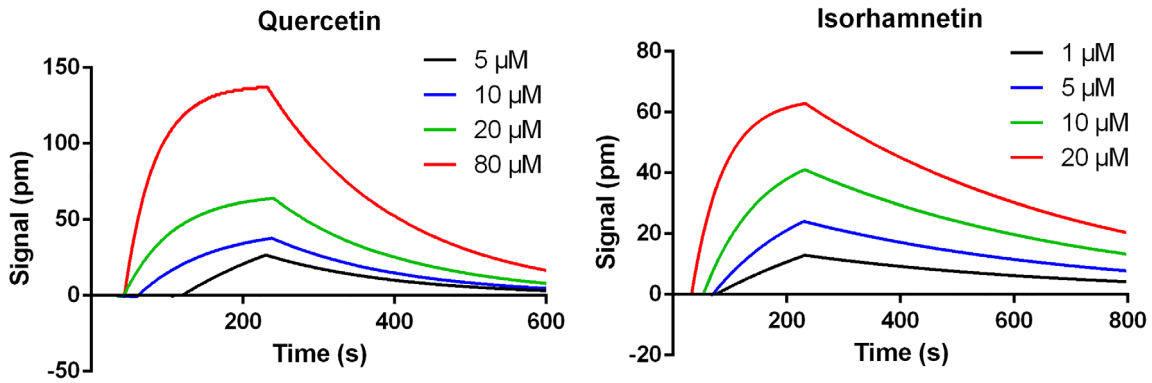

FIGURE 2 Determination of the equilibrium dissociation constant (KD) of quercetin and isorhamnetin with ACE2 by SPR analysis

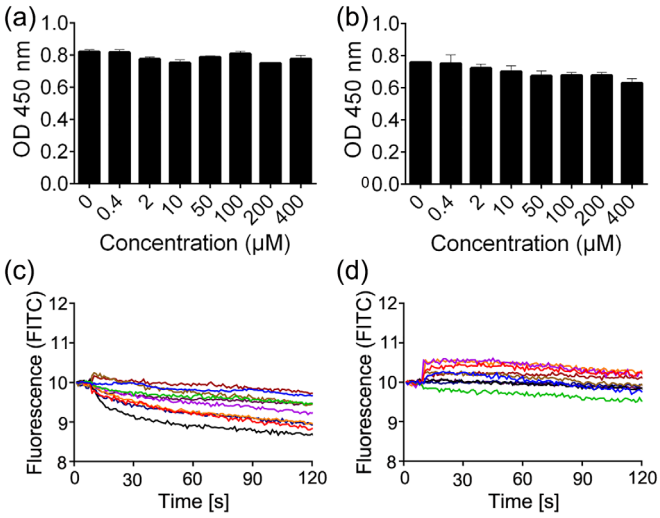

FIGURE 3 Evaluation of the toxicity of quercetin and isorhamnetin on ACE2^h cells by the change of cell viability and intracellular Ca^{2+} flux. (a and b) Effect of quercetin (a) and isorhamnetin (b) on the viability of ACE2^h cells. ACE2^h cells were treated with different concentrations of quercetin and isorhamnetin for 24 h, cells viability was tested by CCK8 assay. (c and d) Effect of quercetin (c) and isorhamnetin (d) on intracellular Ca^{2+} flux change in ACE2^h cells. ACE2^h cells were pretreated with 100 μM concentrations of quercetin and isorhamnetin, intracellular Ca^{2+} flux was tested by Ca^{2+} mobilization assay. The experiments were repeated three times. Data are presented as mean ± *SEM*

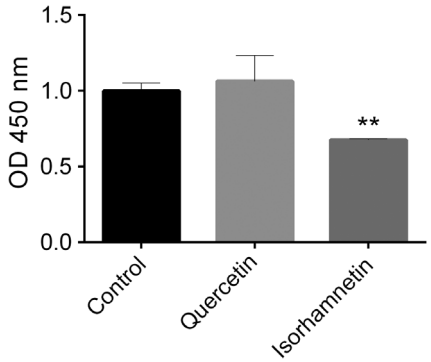

FIGURE 4 Effect of quercetin and isorhamnetin on the entrance of 2019-nCoV spike pseudotyped virus into ACE2^h cells. ACE^h cells infected with 2019-nCoV spike pseudotyped virus were considered as control, which luciferase luminescence value was defined as 1. The experiments were repeated three times. Data are presented as mean ± *SEM*. $^{**}p < .01$ compared with control

Ca^{2+} is an important second messenger involving in several cell pathways. The changes in Ca^{2+} concentration in ACE2^h cells were investigated through calcium imaging in ACE2^h cells probing with Fluo-3 AM. Intracellular Ca^{2+} mobilization assay results showed that no change was observed in Ca^{2+} fluorescence of ACE2^h cells after treatment with quercetin or isorhamnol at 100 μM, which indicated that quercetin or isorhamnol almost had no influence on Ca^{2+} influx in ACE2^h cells (Figure 3c,d).

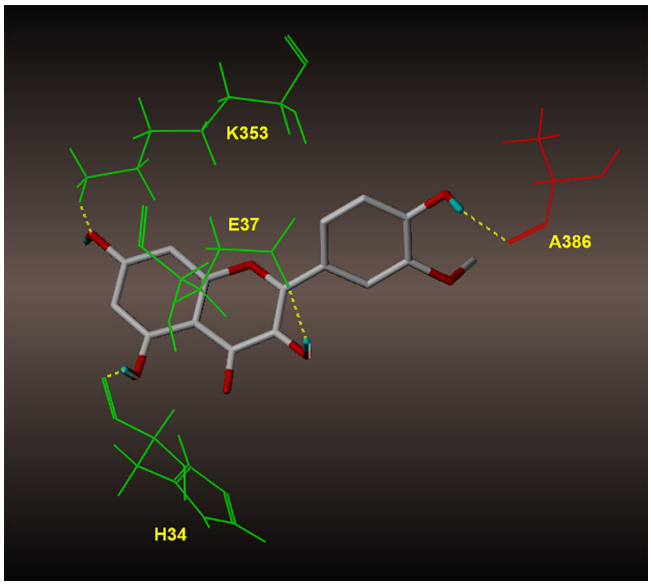

FIGURE 5 Molecular docking results of isorhamnetin

3.4 | The effect of quercetin and isorhamnetin on the entrance of SARS-CoV-2 spike pseudotyped virus into ACE^h cells

We performed SARS-CoV-2 spike pseudotyped virus assay to evaluate if quercetin and isorhamnetin inhibited viral entry. Based on cytotoxicity, at concentrations below 200 μM, no toxicity was observed, so we detected the inhibition of quercetin and isorhamnetin on viral entry at 50 μM. The results showed that the SARS-CoV-2 spike pseudotypes virus entrance ratio were reduced to 47.70 ± 0.72% after treatment of isorhamnol when compared to the control. In contrast of isorhamnetin, there was no significant reduction of viral entry after quercetin treatment (Figure 4). Taken together, there was evidence that isorhamnetin prevented viral entry, possibly by binding ACE2.

3.5 | Binding regions analysis of isorhamnetin and ACE2

Literature studies have shown that the RBD binding domain of spike protein can bind to ACE2 at R393, R357, K353, Y83, Q42, Y41, D38, E37, E35, H34, K31, D30, and Q24 locations (Lan et al., 2020; Shang, Ye, et al., 2020; Yan et al., 2020), thereby infecting cells, causing toxicity. Molecular docking was carried out to identify the critically binding regions of the isorhamnetin with ACE2. As shown in Figure 5, isorhamnetin bound with K353, E37, and H34 on ACE2. It was obvious that isorhamnetin shared three amine acids of ACE2 with SARS-CoV-2.

4 | DISCUSSION

Developing the antiviral therapy drug is an urgent need to suppress the spread of the epidemic SARS-CoV-2 virus. The application of botanicals in

the treatment of infectious diseases can be traced back thousands of years and they are potential sources of new drug candidates. This may be an option for new coronavirus treatment, some effective compounds from traditional Chinese medicine (TCM) are worthy of further verification through rigorous antiviral research and clinical trials. Sea buckthorn berries, a common TCM, was found to possess a very strong antiviral activity and wide range of action against avian influenza and herpes viruses (Enkhtaivan et al., 2017). In this study, we found that SBE exhibited strong interaction with ACE2 by using CMC analysis. Isorhamnetin and quercetin are the major compounds of flavonoid derived from Sea buckthorn berries, and they are proved to inhibit atherosclerotic plaque development, protect against cardiac hypertrophy, inhibit the production of reactive oxygen species, and exert antioxidant properties in vitro (Luo et al., 2015). Thus, we speculated quercetin and isorhamnetin might be the compounds binding to ACE2, so we further explored their interactions with ACE2, as well as their ability to prevent viral entry, thus might exerted potential antiviral in vitro.

ACE2 is proved to be the receptor for SARS-CoV-2, theoretically, all organs with high expression of ACE2 are susceptible to SARS-CoV-2 infection. SPR analysis proved the two compounds could bind to ACE2 with the affinity at micromolar. The entry of viruses into host cells is a crucial step in the process of viral infection, blocking the entry process of the virus was considered to be the way to prevent infection. To further evaluate the effect of quercetin and isorhamnetin on viral entry, ACE2 overexpression HEK293 cells was constructed and used for in vitro screening and characterization of quercetin and isorhamnetin against SARS-CoV-2 in this study. Experiment studies performed on highly pathogenic viruses like the novel SARS-CoV-2 virus must be carried out in laboratories that satisfy strict biosafety level (BSL) requirements. Unfortunately, the requirements of high BSL, such as BSL-3 and BSL-4 labs often prevent more than a few specific institutions from handling these researches. The pseudovirus system is a useful alternative approach that can effectively screen drugs on pathogenic viruses outside of a BSL-3 or BSL-4 level laboratory (Yang et al., 2020). The SARS-CoV-2 pseudovirus was able to simulate the process of the virus entering the cells and then infect cells, so it was used for this study to detect the effect of the compounds on preventing the virus from entering the cells in vitro in BSL-2 laboratory. Unlike quercetin, isorhamnetin exhibited inhibition on the entrance of SARS-CoV-2 spike pseudotyped virus into ACE^h cells in in-vitro cell experiments at concentrations that are not toxic to host cells. Further molecular docking analysis explained potential binding domain shared with spike protein of SARS-CoV-2 by isorhamnetin. Isorhamnetin is an active constitute of sea-buckthorn, which indicates that sea-buckthorn may exert preventive effect that protect us against natural infections with SARS-CoV-2.

In summary, isorhamnetin could interact with ACE2, the functional receptor for SARS-CoV-2, thus prevent SARS-CoV-2 spike pseudotypes viral entry and infection of human cells expression ACE2, which suggesting that isorhamnetin might be a ACE2-spike protein interaction blocker and this study provided a new treatment for the control of COVID-19.

ACKNOWLEDGMENTS

This work was founded by National Natural Science Foundation of China (Grant number: 81930096), Basic Research Project in Shaanxi Administration of traditional Chinese Medicine (Grant No. JCMS028), the Fundamental Research Funds for the Central Universities (Grant No. xjj2018170), and the Natural Science Basic Research Plan in Shaanxi Province of China (Grant NOs. 2020JM-023).

CONFLICT OF INTEREST

The authors declare no competing financial interest.

DATA AVAILABILITY STATEMENT

The contents of the manuscript are shared upon request.

ORCID

Wen Lu https://orcid.org/0000-0001-8728-7099

REFERENCES

Bal, L. M., Meda, V., Naik, S. N., & Satya, S. (2011). Sea buckthorn berries: A potential source of valuable nutrients for nutraceuticals and cosmeceuticals. *Food Research International*, *44*(7), 1718–1727.

Barlow, A., Landolf, K. M., Barlow, B., Yeung, S., Heavner, J. J., Claassen, C. W., & Heavner, M. S. (2020). Review of emerging pharmacotherapy for the treatment of coronavirus disease 2019. *Pharmacotherapy*, *40*(5), 416–437.

Bourgonje, A. R., Abdulle, A. E., Timens, W., Hillebrands, J. L., Navis, G. J., Gordijn, S. J., Bolling, M. C., Dijkstra, G., Voors, A. A., Osterhaus, A. D., van der Voort, P. H., Mulder, D. J., & van Goor, H. (2020). Angiotensin-converting enzyme 2 (ACE2), SARS-CoV-2 and the pathophysiology of coronavirus disease 2019 (COVID-19). *The Journal of Pathology*, *251*(3), 228–248.

Cho, C. H., Jang, H., Lee, M., Kang, H., Heo, H. J., & Kim, D. O. (2017). Sea buckthorn (*Hippophae rhamnoides* L.) leaf extracts protect neuronal PC-12 cells from oxidative stress. *Journal of Microbiology and Biotechnology*, *27*(7), 1257–1265.

Choi, H. J., Song, J. H., & Kwon, D. H. (2012). Quercetin 3-rhamnoside exerts antiinfluenza A virus activity in mice. *Phytotherapy Research*, *26*(3), 462–464.

Derosa, G., Maffioli, P., D'Angelo, A., & Di Pierro, F. (2020). A role for quercetin in coronavirus disease 2019 (COVID-19). *Phytotherapy Research*, *35*(3), 1230–1236. https://doi.org/10.1002/ptr.6887

Devaux, C. A., Rolain, J. M., & Raoult, D. (2020). ACE2 receptor polymorphism: Susceptibility to SARS-CoV-2, hypertension, multi-organ failure, and COVID-19 disease outcome. *Journal of Microbiology, Immunology, and Infection*, *53*(3), 425–435.

Enkhtaivan, G., Maria John, K. M., Pandurangan, M., Hur, J. H., Leutou, A. S., & Kim, D. H. (2017). Extreme effects of Seabuckthorn extracts on influenza viruses and human cancer cells and correlation between flavonol glycosides and biological activities of extracts. *Saudi Journal of Biological Sciences*, *24*(7), 1646–1656.

Flanagan, K. L., Best, E., Crawford, N. W., Giles, M., Koirala, A., Macartney, K., Russell, F., Teh, B. W., & Wen, S. C. (2020). Progress and pitfalls in the quest for effective SARS-CoV-2 (COVID-19) vaccines. *Frontiers in Immunology*, *11*, 579250.

Hassan, S. S., Ghosh, S., Attrish, D., Choudhury, P. P., Aljabali, A., Uhal, B. D., Lundstrom, K., Rezaei, N., Uversky, V. N., Seyran, M., Pizzol, D., Adadi, P., Soares, A., El-Aziz, T., Kandimalla, R., Tambuwala, M. M., Azad, G. K., Sherchan, S. P., Baetas-da-Cruz, W., … Brufsky, A. M. (2020). Possible transmission flow of SARS-CoV-2 based on ACE2 features. *Molecules*, *25*(24), 5906.

Huang, Y. F., Bai, C., He, F., Xie, Y., & Zhou, H. (2020). Review on the potential action mechanisms of Chinese medicines in treating coronavirus disease 2019 (COVID-19). *Pharmacological Research*, *158*, 104939.

Imai, Y., Kuba, K., Ohto-Nakanishi, T., & Penninger, J. M. (2010). Angiotensin-converting enzyme 2 (ACE2) in disease pathogenesis. *Circulation Journal*, *74*(3), 405–410.

Jeyanathan, M., Afkhami, S., Smaill, F., Miller, M. S., Lichty, B. D., & Xing, Z. (2020). Immunological considerations for COVID-19 vaccine strategies. Nature reviews. *Immunology*, *20*(10), 615–632.

Kwon, E. Y., Lee, J., Kim, Y. J., Do, A., Choi, J. Y., Cho, S. J., Jung, U. J., Lee, M. K., Park, Y. B., & Choi, M. S. (2017). Seabuckthorn leaves extract and flavonoid glycosides extract from seabuckthorn leaves ameliorates adiposity, hepatic steatosis, insulin resistance, and inflammation in diet-induced obesity. *Nutrients*, *9*(6), 569.

Lan, J., Ge, J., Yu, J., Shan, S., Zhou, H., Fan, S., Zhang, Q., Shi, X., Wang, Q., Zhang, L., & Wang, X. (2020). Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature*, *581* (7807), 215–220.

Larmo, P. S., Kangas, A. J., Soininen, P., Lehtonen, H. M., Suomela, J. P., Yang, B., Viikari, J., Ala-Korpela, M., & Kallio, H. P. (2013). Effects of sea buckthorn and bilberry on serum metabolites differ according to baseline metabolic profiles in overweight women: A randomized crossover trial. *The American Journal of Clinical Nutrition*, *98*(4), 941–951.

Lu, R., Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., Wang, W., Song, H., Huang, B., Zhu, N., Bi, Y., Ma, X., Zhan, F., Wang, L., Hu, T., Zhou, H., Hu, Z., Zhou, W., Zhao, L., … Tan, W. (2020). Genomic characterisation and epidemiology of 2019 novel coronavirus: Implications for virus origins and receptor binding. *Lancet*, *395*(10224), 565–574.

Luo, Y., Sun, G., Dong, X., Wang, M., Qin, M., Yu, Y., & Sun, X. (2015). Isorhamnetin attenuates atherosclerosis by inhibiting macrophage apoptosis via PI3K/AKT activation and HO-1 induction. *PLoS One*, *10*(3), e0120259.

Nishiga, M., Wang, D. W., Han, Y., Lewis, D. B., & Wu, J. C. (2020). COVID-19 and cardiovascular disease: From basic mechanisms to clinical perspectives. Nature reviews. *Cardiology*, *17*(9), 543–558.

Olas, B., Skalski, B., & Ulanowska, K. (2018). The anticancer activity of sea buckthorn [Elaeagnus rhamnoides (L.) A. Nelson]. *Frontiers in Pharmacology*, *9*, 232.

Olas, B. (2016). Sea buckthorn as a source of important bioactive compounds in cardiovascular diseases. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association*, *97*, 199–204.

Patel, M., Chaubey, A. K., Pittman, C. U., Jr., Mlsna, T., & Mohan, D. (2020). Coronavirus (SARS-CoV-2) in the environment: Occurrence, persistence, analysis in aquatic systems and possible management. *The Science of the Total Environment*, *765*, 142698.

Patil, D., Roy, S., Dahake, R., Rajopadhye, S., Kothari, S., Deshmukh, R., & Chowdhary, A. (2013). Evaluation of *Jatropha curcas* Linn. leaf extracts for its cytotoxicity and potential to inhibit hemagglutinin protein of influenza virus. *Indian Journal of Virology*, *24*(2), 220–226.

Pruijssers, A. J., George, A. S., Schäfer, A., Leist, S. R., Gralinksi, L. E., Dinnon, K. H., 3rd, Yount, B. L., Agostini, M. L., Stevens, L. J., Chappell, J. D., Lu, X., Hughes, T. M., Gully, K., Martinez, D. R., Brown, A. J., Graham, R. L., Perry, J. K., du Pont, V., Pitts, J., … Sheahan, T. P. (2020). Remdesivir inhibits SARS-CoV-2 in human lung cells and chimeric SARS-CoV expressing the SARS-CoV-2 RNA polymerase in mice. *Cell Reports*, *32*(3), 107940.

Scimeca, M., Urbano, N., Bonfiglio, R., Montanaro, M., Bonanno, E., Schillaci, O., & Mauriello, A. (2020). Imaging diagnostics and pathology in SARS-CoV-2-related diseases. *International Journal of Molecular Sciences*, *21*(18), 6960.

Seyran, M., Takayama, K., Uversky, V. N., Lundstrom, K., Palù, G., Sherchan, S. P., Attrish, D., Rezaei, N., Aljabali, A., Ghosh, S., Pizzol, D., Chauhan, G., Adadi, P., Mohamed Abd el-Aziz, T., Soares, A. G., Kandimalla, R., Tambuwala, M., Hassan, S. S., Azad, G. K., … Uhal, B. D. (2020). The structural basis of accelerated host cell entry by SARS-CoV-2. *The FEBS Journal*. https://doi.org/10.1111/febs.15651

Shang, J., Wan, Y., Luo, C., Ye, G., Geng, Q., Auerbach, A., & Li, F. (2020). Cell entry mechanisms of SARS-CoV-2. *Proceedings of the National Academy of Sciences of the United States of America*, *117* (21), 11727–11734.

Shang, J., Ye, G., Shi, K., Wan, Y., Luo, C., Aihara, H., Geng, Q., Auerbach, A., & Li, F. (2020). Structural basis of receptor recognition by SARS-CoV-2. *Nature*, *581*(7807), 221–224.

South, A. M., Diz, D. I., & Chappell, M. C. (2020). COVID-19, ACE2, and the cardiovascular consequences. *American Journal of Physiology. Heart and Circulatory Physiology*, *318*(5), H1084–H1090.

Spinner, C. D., Gottlieb, R. L., Criner, G. J., Arribas López, J. R., Cattelan, A. M., Soriano Viladomiu, A., Ogbuagu, O., Malhotra, P., Mullane, K. M., Castagna, A., Chai, L., Roestenberg, M., Tsang, O., Bernasconi, E., le Turnier, P., Chang, S. C., SenGupta, D., Hyland, R. H., Osinusi, A. O., … GS-US-540-5774 Investigators. (2020). Effect of Remdesivir vs standard care on clinical status at 11 days in patients with moderate COVID-19: A randomized clinical trial. *JAMA*, *324*(11), 1048–1057.

Tipnis, S. R., Hooper, N. M., Hyde, R., Karran, E., Christie, G., & Turner, A. J. (2000). A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *The Journal of Biological Chemistry*, *275*(43), 33238–33243.

Tregoning, J. S., Brown, E. S., Cheeseman, H. M., Flight, K. E., Higham, S. L., Lemm, N. M., Pierce, B. F., Stirling, D. C., Wang, Z., & Pollock, K. M. (2020). Vaccines for COVID-19. *Clinical and Experimental Immunology*, *202*(2), 162–192.

Walls, A. C., Park, Y. J., Tortorici, M. A., Wall, A., McGuire, A. T., & Veesler, D. (2020). Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell*, *181*(2), 281–292.e6.

Wang, N., Han, S., Liu, R., Meng, L., He, H., Zhang, Y., Wang, C., Lv, Y., Wang, J., Li, X., Ding, Y., Fu, J., Hou, Y., Lu, W., Ma, W., Zhan, Y., Dai, B., Zhang, J., Pan, X., … He, L. (2020). Chloroquine and hydroxychloroquine as ACE2 blockers to inhibit viropexis of 2019-nCoV spike pseudotyped virus. *Phytomedicine*, *79*, 153333.

Wang, Y., Zhang, D., du, G., du, R., Zhao, J., Jin, Y., Fu, S., Gao, L., Cheng, Z., Lu, Q., Hu, Y., Luo, G., Wang, K., Lu, Y., Li, H., Wang, S., Ruan, S., Yang, C., Mei, C., … Wang, C. (2020). Remdesivir in adults with severe COVID-19: A randomised, double-blind, placebo-controlled, multicentre trial. *Lancet*, *395*(10236), 1569–1578 Erratum in: Lancet. 2020;395(10238):1694.

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.

Wu, Y., Wang, F., Shen, C., Peng, W., Li, D., Zhao, C., Li, Z., Li, S., Bi, Y., Yang, Y., Gong, Y., Xiao, H., Fan, Z., Tan, S., Wu, G., Tan, W., Lu, X., Fan, C., Wang, Q., … Liu, L. (2020). A noncompeting pair of human neutralizing antibodies block COVID-19 virus binding to its receptor ACE2. *Science*, *368*(6496), 1274–1278.

Xing, J., Yang, B., Dong, Y., Wang, B., Wang, J., & Kallio, H. P. (2002). Effects of sea buckthorn (Hippophaë rhamnoides L.) seed and pulp oils on experimental models of gastric ulcer in rats. *Fitoterapia*, *73*(7–8), 644–650.

Yan, R., Zhang, Y., Li, Y., Xia, L., Guo, Y., & Zhou, Q. (2020). Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science*, *367*(6485), 1444–1448.

Yang, R., Huang, B., Ruhan, A., Li, W., Wang, W., Deng, Y., & Tan, W. (2020). Development and effectiveness of pseudotyped SARS-CoV-2 system as determined by neutralizing efficiency and entry inhibition test in vitro. *Biosafety and Health*, *2*(4), 226–231.

Zumla, A., Hui, D. S., Azhar, E. I., Memish, Z. A., & Maeurer, M. (2020). Reducing mortality from 2019-nCoV: Host-directed therapies should be an option. *Lancet*, *395*(10224), e35–e36.

How to cite this article: Zhan Y, Ta W, Tang W, et al. Potential antiviral activity of isorhamnetin against SARS-CoV-2 spike pseudotyped virus in vitro. *Drug Dev Res*. 2021;82: 1124–1130. https://doi.org/10.1002/ddr.21815