Antiviral Activity of *Cinchona officinalis*, a Homeopathic Medicine, against COVID-19

Somasundaram Arumugam[1] Kumaravel Vadivel[2] Devendra Kumar Dhaked[3] Vani Sai Prasanna[1] Manoj Limbraj Yellurkar[1] Pamelika Das[1] Raja Manoharan[4] Austin Jose Thomas[5] Lalith Singh[5] Subhas Singh[6] Ravichandiran Velayutham[7]

[1] Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research (NIPER), Chunilal Bhawan, Kolkata, West Bengal, India
[2] Department of Pathology and Microbiology, National Institute of Homeopathy (NIH), Kolkata, West Bengal, India
[3] Department of Pharmacoinformatics, National Institute of Pharmaceutical Education and Research (NIPER), Chunilal Bhawan, Kolkata, West Bengal, India
[4] Department of Homeopathic Pharmacy, National Institute of Homeopathy (NIH), Kolkata, West Bengal, India
[5] National Institute of Homeopathy (NIH), Kolkata, West Bengal, India
[6] Department of Organon of Medicine, National Institute of Homeopathy (NIH), Kolkata, West Bengal, India
[7] National Institute of Pharmaceutical Education and Research (NIPER), Chunilal Bhawan, 168 Maniktala Main Road, Kolkata, West Bengal, India

Address for correspondence Ravichandiran Velayutham, PhD, Department of Natural Products, National Institute of Pharmaceutical Education and Research (NIPER), Chunilal Bhawan, 168 Maniktala Main Road, Kolkata 700054, West Bengal, India (e-mail: director@niperkolkata.edu.in).

Somasundaram Arumugam, PhD, Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research (NIPER), Chunilal Bhawan, 168 Maniktala Main Road, Kolkata 700054, West Bengal, India (e-mail: somasundaram.niperk@nic.in).

Homeopathy 2024;113:16–24.

Abstract

Background Coronavirus disease 2019 (COVID-19) is a potentially fatal disease caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Several studies have shown that hydroxychloroquine (HCQ) significantly inhibits SARS-CoV-2 infections *in vitro*.

Objective Since the phytoconstituents of *Cinchona officinalis* (CO) are similar to those of HCQ, the objective of this study was to test the antiviral potential of different homeopathic formulations of CO.

Methods An analysis of the molecular composition of CO was carried out using ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry, followed by a detailed docking study. The constituents of CO were docked against various targets of SARS-CoV-2, and the binding potential of the phyto-constituents was compared and quantified. The ligand with the lowest Glide docking score is considered to have the best binding affinity. The cytotoxicity of several homeopathic formulations, including CO mother tincture (CO-MT), was also checked on VeroE6 cells. A known antiviral, remdesivir, was used as a positive control for the *in vitro* assays to evaluate the effects of CO-MT against SARS-CoV-2-infected VeroE6 cells.

Results Molecular docking studies showed that constituents of CO exhibited binding potential to various targets of SARS-CoV-2, including Mpro, PLpro, RdRp, nucleocapsid

Keywords
- Homeopathy
- *Cinchona officinalis*
- COVID-19
- antiviral
- toxicity
- docking

received
February 6, 2023
accepted after revision
April 20, 2023
article published online
July 6, 2023

DOI https://doi.org/10.1055/s-0043-1770339.
ISSN 1475-4916.

protein, ACE2 (in host) and spike protein. Quinoline, one of the constituents of CO, can potentially bind the spike protein of SARS-CoV-2. Quinic acid showed better binding capabilities with Mpro, PLpro RdRp, nucleocapsid protein and ACE2 (allosteric site) than other constituents. Quinidine exhibited better binding to ACE2. Compared to HCQ, other phytoconstituents of CO had the equivalent potential to bind the RNA-dependent RNA polymerase, nucleocapsid protein, Mpro, PLpro and spike protein of SARS-CoV-2. *In vitro* assays showed that homeopathic CO-MT was not cytotoxic and that CO-MT and remdesivir respectively caused 89% and 99% inhibition of SARS-CoV-2 infection in VeroE6 cells.

Conclusion Based on this *in silico* and *in vitro* evidence, we propose CO-MT as a promising antiviral medicine candidate for treating COVID-19. *In vivo* investigation is required to clarify the therapeutic potential of CO-MT in COVID-19.

Introduction

Coronavirus disease 2019 (COVID-19), caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a potentially fatal viral illness that emerged in late December 2019 in Wuhan, China, and swiftly spread to become a pandemic.[1,2] Its symptoms range from mild, self-limiting, respiratory disease to severe progressive pneumonia, multi-organ failure, and death. Despite considerable effort and ongoing clinical trials, no particular therapeutic medicines have been available to treat or cure a coronavirus infection.[3–5] However, as per the guidelines and management strategies published by the National Institutes of Health, corticosteroids such as hydrocortisone, dexamethasone and prednisolone are recommended for severe and critical cases of COVID-19. But in non-severe cases, they reduce the protective immune response, leading to bacterial and fungal infections, delayed recovery and hypokalemia, compromising the health of the patient.[6]

Homeopathy is one of the most popular complementary and integrative health practices. It is based on the principle of "like heals like", which means that sickness can be healed by a substance that causes identical symptoms in healthy persons. Homeopathic remedies have been said to strengthen the natural defensive system.[7] They may play an immunomodulatory role, which affects the body's vulnerability to pathogens.[8] *Cinchona officinalis* (CO), a homeopathic medication, has been claimed to heal low-grade fever (remittent, intermittent, or malarial) and destroy parasites. Quinine ($C_{20}H_{24}N_2O_2$), quinidine, cinchonine and cinchonidine are the active components of CO bark.[9,10] Synthetic varieties such as chloroquine (CQ, $C_{18}H_{26}ClN_3$) and hydroxychloroquine (HCQ, $C_{18}H_{26}ClN_3O$) are similar to natural quinine in terms of their chemical structure and in treating malaria. The antimalarial drugs CQ and HCQ have been suggested as promising agents against COVID-19.[11] Several *in vitro* studies showed that HCQ has significant antiviral activity against SARS-CoV-2.[12] Since the active components of CO are chemically similar to CQ and HCQ, we hypothesized that CO formulations would also have antiviral potential against SARS-CoV-2.

The present study was planned to evaluate the potential of homeopathic CO formulations against SARS-CoV-2 and to assess their safety *in vitro*.

Materials and Methods

Materials

All the reagents and chemicals used in the study are of analytical grade from Sigma, until mentioned otherwise. CO mother tincture (MT) and potencies were procured from Hahnemann Publishing Co. Pvt. Ltd., Kolkata, a GMP-certified company.

Analysis by Ultra-High Performance Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry (UHPLC/Q-TOF-MS)

Identification of the phytoconstituents of CO-MT and potencies 3c, 6c and 12c were performed by UHPLC/Q-TOF-MS using the Agilent 1290 Infinity LC system coupled to an Agilent 6545 Q-TOF mass spectrometer with Agilent Jet Stream Thermal Gradient Technology. The UHPLC system was assembled with a diode array detector and auto-sampler. Raw data were deconvoluted into individual chemical peaks with Agilent Mass Hunter Qualitative Analysis (Mass Hunter Qual, Agilent Technologies, Santa Clara, CA, United States).[13] Data acquisition on the LC-Q-TOF was performed using Agilent Mass Hunter Acquisition software which contains validated profiles of various phytoconstituents. Accordingly, the phytoconstituents of CO-MT were identified on the basis of m/z values of the individual chemical peaks.

Molecular Docking

Crystallized three-dimensional structures of targets ACE2 (6LZG), Mpro (6LU7), PLpro (3E9S), RdRp (7BV2), Nucleocapsid Protein (6VYO) and Spike Protein (7DWY), with their co-crystallized ligands if available, were retrieved from Protein Data Bank (https://www.rcsb.org). All the ligand structures were subjected to the generation of multiple conformations and, finally, prepared using a LigPrep module in Schrodinger at a pH of 7.4. The SiteMap program was used to identify the suitable binding site(s) in the targets which do not contain co-crystalized ligands. SiteMap generated 5, 5 and 3 sites, for ACE2, nucleocapsid protein and spike protein, respectively. The program produced probable binding sites based on cavity size, volume, and scores for

suitability as a drug target. Site 1 was chosen for ACE2 and nucleocapsid protein, while site 2 was selected for spike protein based on their respective druggable scores. In addition, site 5 was considered for docking in the case of ACE2, which represents an allosteric site. Glide grids were generated using SiteMap output for the above-mentioned targets and co-crystallized ligands in the case of other proteins. Glide was employed to perform the molecular docking, using the ExtraPrecision (XP) mode to generate the binding poses. The best-docked pose with the lowest Glide docking score (DS; negative number) was recorded for each ligand[14,15] and is expressed as the DS, which measures binding affinity.[16]

In vitro Antiviral Studies against Severe Acute Respiratory Syndrome Coronavirus 2

Cytotoxicity Assay on VeroE6 Cells

The assay was performed in a 96-well plate format in three wells for each sample. VeroE6 cells ($1 \times 10e4$ per well) were incubated overnight at 37°C in a humidified incubator with 5% CO_2 for monolayer formation. The next day, VeroE6 cells were incubated with the test substance at the indicated concentration (4 µL of the sample [original, 1:10 or 1:20 dilution in ethanol] in a 200 µL reaction).[17] Control cells were incubated with the solvent for CO-MT (1.40% ethanol). After 30 hours incubation, all live and dead cells were stained with Hoechst 33342 (a cell-permeant nuclear counterstain that emits blue fluorescence when bound to dsDNA) and Sytox orange dye, which stains dead cells only. Using Image Xpress Microconfocal microscopy (Molecular Devices), 16 images per well (10X magnification) were taken to cover 90% of each well's area. The software counts the total number of cells in the Hoechst-stained images and the number of Sytox-stained dead cells.

Antiviral Screening (Immunofluorescence Assay)

VeroE6 cells were cultured and treated with test substances, as explained in the previous section. Ethanol-treated VeroE6 cells served as control. Initially, VeroE6 cells were infected with SARS-CoV-2 at a multiplicity of infection (MOI) of 0.1 virions/cell.[18] After 30 hour incubation, cells were fixed in 4% paraformaldehyde and permeabilized with 0.3% Tween-20. Next, VeroE6 cells were stained sequentially with a primary antibody that explicitly detects SARS-CoV-2-infected cells (SARS-CoV-2 nucleocapsid mouse monoclonal antibody: Catalog Number: 40143-MM05) and a secondary anti-mouse antibody conjugated to Alexa fluor 568. Hoechst 33342 dye was used for nuclear staining. Images of cells stained for SARS-CoV-2 nucleocapsid (Alexa flour-568) and total nuclei (Hoechst) were captured at 10X with 16 images per well. Using a multi-wavelength cell scoring module in Meta Xpress software, nucleocapsid positive cells and total nuclei were counted and compared with the control. All experiments related to SARS-CoV-2 were performed in a biosafety level-3 laboratory, and the personnel involved were equipped properly to maintain safety precautions.[19]

Statistical Analysis

All data were statistically analyzed by Student's t-test using GraphPad Prism V5. Values are given as mean ± standard error of the mean.

Results

Identification of Constituents of *Cinchona Officinalis* Formulations

As per guidelines of the Homeopathic Pharmacopoeia of India (HPI), the CO (Batch 0752) for this study had the required specific gravity (0.8590 = 0.8556 g/mL; pH: 5.86), alcohol strength: 135o = 77.03% (v/v), and solid content: 0.201% (w/v). The Batch 0752 had no sediments and was near colorless. The identity and purity of the CO-MT were established by thin layer chromatography, which showed the presence of three expected spots with different Rf values (Rf: 0.07,0.13, 0.33, and lambda max [λ max]: 278.80 nm).

LC-MS analysis: The total ion chromatogram (TIC) of CO-MT and comparison of TICs of CO-MT, potencies 3c, 6c and 12c are given in ►**Fig 1A** and ►**Fig 1B**, respectively.

A comparison of the ion chromatograms of CO-MT and its magnified view (►**Fig. 1A** and **1A1**) show distinct peaks of phytoconstituents of MT. The list of identified phytoconstituents of CO-MT is given in ►**Table 1**. No phytoconstituents were identified in the three potencies (3c, 6c and 12c).

In silico Study of the Constituents of *Cinchona Officinalis* against Coronavirus Disease 2019 Target Proteins

Several reports suggest that specific SARS-CoV-2 proteins are targets for antiviral drugs. Therefore, we performed *in silico* analysis to determine if any of the phytoconstituents of CO (►**Fig. 1** and ►**Table 1**) showed significant binding with spike protein, RNA-dependent RNA polymerase (RdRp), nucleocapsid protein, ACE2 (site of entry), Mpro and PLpro of SARS-CoV-2. HCQ, CQ and remedesvir monophosphate (RMP) are the established inhibitors of SARS-CoV-2 and were used as positive controls in docking studies. Docking results for the selected constituents against the COVID-19 target proteins are given in ►**Table 2**. Though multiple conformations are possible for these phytoconstituents, the results are for the best conformation with the best DS. Three crucial inferences can be made from ►**Table 2**. First, remdesivir (RMP) shows good binding to RdRp, followed by ACE2 (site 1) and PLpro. The active form of remdesivir is remdesivir-monophosphate, and it may be present in mono- and dianionic forms, and binding scores of both forms are quite comparable. HCQ exhibits good binding to both sites of ACE2 including allosteric and active sites, and the binding score of CQ is quite comparable to HCQ at site 5. The cationic centers of both HCQ and CQ mostly participate in the formation of ionic interactions with Asp and Glu residues in most targets. However, in the case of the spike protein, the cationic center (tertiary amine group) of these drugs is involved in cation-pi interaction with Phe392 in the hydrophobic cavity.

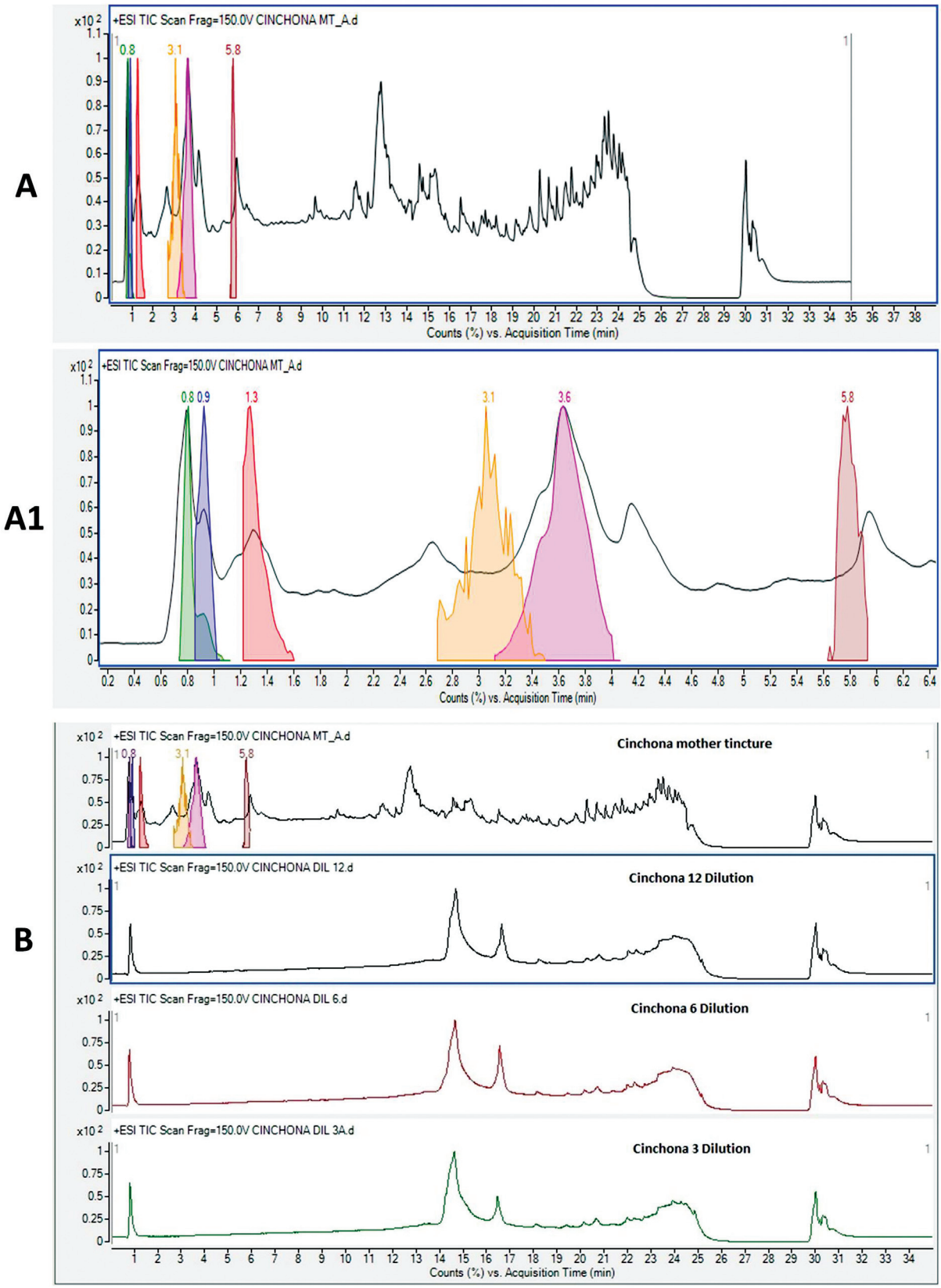

Fig. 1 LC-MS analysis of the *Cinchona officinalis* (CO) mother tincture and the potencies 3c, 6c and 12c. Identification of the components of CO-MT and potencies 3c, 6c and 12c were performed by UHPLC/Q-TOF-MS using the Agilent 1290 Infinity LC system coupled to an Agilent 6545 Q-TOF mass spectrometer with Agilent Jet Stream Thermal Gradient Technology. (**A**) Total ion chromatograms (TICs) of CO-MT. (**A1**) Magnified view of A showing the peaks of identified molecules. (**B**) Comparison of TICs of CO-MT with potencies 3c, 6c and 12c. No constituents are found in 3c, 6c and 12c.

Table 1 List of compounds identified in *Cinchona officinalis* MT by molecular feature analysis

Formula	m/z	Mass	RT (Min)	Height	Compound Name
$C_7H_{12}O_6$	215.0519	192.0628	0.8	21430	Quinic acid
$C_{19}H_{24}N_2O_2$	313.1903	312.1830	0.9	236358	Dihydroquinidine
C_9H_7N	130.0645	129.0573	1.3	48299	Quinoline
$C_{19}H_{22}N_2O$	295.1798	294.1732	3.1	971894	Cinchonidine/Cinchonine/ Epicinchonine/ Epicinchonidine
$C_{20}H_{24}N_2O_2$	325.1915	324.1846	3.6	6010683	Quinine/Quinidine/ Epiquinidine/Epiquinine
$C_{20}H_{24}N_2O_2$	325.1915	324.1846	5.8	90594	Quinine/Quinidine/ Epiquinidine/Epiquinine

Abbreviation: MT, mother tincture.

Table 2 Docking results for the constituents against COVID-19 target proteins

Molecule	ACE2 (6LZG)		Mpro (6LU7)	PLpro (3E9S)	RdRp (7BV2)	Nucleocapsid (6VY0)	Spike (7DWY)
	Site5	Site1				Site1	Site2
RMP_Monoanion	−5.35	−7.43	−5.45	−6.29	−5.72	−4.63	−5.38
RMP_Dianion	−5.78	−7.61	−5.36	−6.79	−7.11	−6.21	−4.56
Hydroxychloroquine	−8.78	−8.93	−4.98	−6.19	−5.46	−4.82	−5.93
Chloroquine	−8.50	−7.58	−4.04	−4.36	−3.66	−3.46	−7.00
Quinidine	−4.82	−6.11	−4.83	−2.53	−4.50	−4.50	−5.56
Cinchonine	−4.53	−3.46	−1.98	−5.69	−2.65	−3.39	−4.13
Dihydroquinidine	−5.37	−3.86	−3.89	−2.92	−2.37	−3.36	−3.94
Quinoline	−2.55	−3.26	−4.06	−3.57	−2.33	−2.36	−6.32
Quinic acid	−5.56	−5.59	−6.09	−6.00	−4.56	−6.24	−5.45

Abbreviations: COVID-19, coronavirus disease 2019; RMP, remdesivir monophosphate; PLpro, papain-like protease; RdRp, RNA-dependent RNA polymerase.

These results for HCQ and CQ are in agreement with the literature[20] and therefore validate our docking approach. Second, quinoline showed a DS of −6.32 against the spike protein, which is the best score among all phytoconstituents of CO and COVID-19 target proteins. It forms mainly hydrophobic interactions in the active site of spike protein (►**Fig. 2**). Third, it is notable that RMP, HCQ, CQ, and three phytoconstituents of CO-MT (quinidine, quinoline and quinic acid) showed equivalent DSs, with a few exceptions. For example, quinic acid showed better binding capabilities with Mpro, PLpro RdRp, nucleocapsid protein and ACE2 (allosteric site) compared to other constituents. Quinic acid shows three to five hydrogen bonding interactions with different targets. Quinidine shows three hydrogen bonding interactions and one ionic interaction between the cationic center of ligand with ASP206 in site 1 of ACE2. In most targets, the dianion form of the phosphate group, the hydroxyl group of the sugar, and the amino group of RMP are involved in binding with residues of target proteins. Overall, these *in silico* data suggest that HCQ, CQ and specific phytoconstituents of CO-MT have equivalent potential to bind the spike and nucleocapsid proteins and could prevent SARS-CoV-2 entry into cells.

In vitro Antiviral Assay

In silico data suggested that HCQ, CQ and certain phytoconstituents of MT have equivalent binding potential for the spike and nucleocapsid proteins of SARS-CoV-2 (►**Table 2**). Therefore, an antiviral assay against SARS-CoV-2 was performed in VeroE6 cells, which are commonly used for virus culture.[21] Initially, an immunofluorescence cytotoxicity assay was performed to assess the safety of the CO formulations. The results confirmed that the CO-MT formulation and its potencies were not cytotoxic at the selected doses. Antiviral activity of CO-MT and potencies was tested in SARS-CoV-2 infected VeroE6 cells using the established antiviral drug remdesivir as a positive control. The total VeroE6 cell number was visualized with a nuclear stain (Hoechst blue color). Infected Vero cells were visualized with a dye conjugated to an antibody against nucleocapsid protein of SARS-CoV-2 (Alexa flour-568—orange color). Confocal microscopy images clearly show the presence of Vero cells in all panels (►**Fig 3C**). As expected, uninfected VeroE6 cells show a Hoechst stain but no orange stain.

Similarly, SARS-CoV-2-infected Vero cells treated with remdesivir or CO-MT also show Hoechst stain and little or no orange stain, since both these drugs potently inhibited

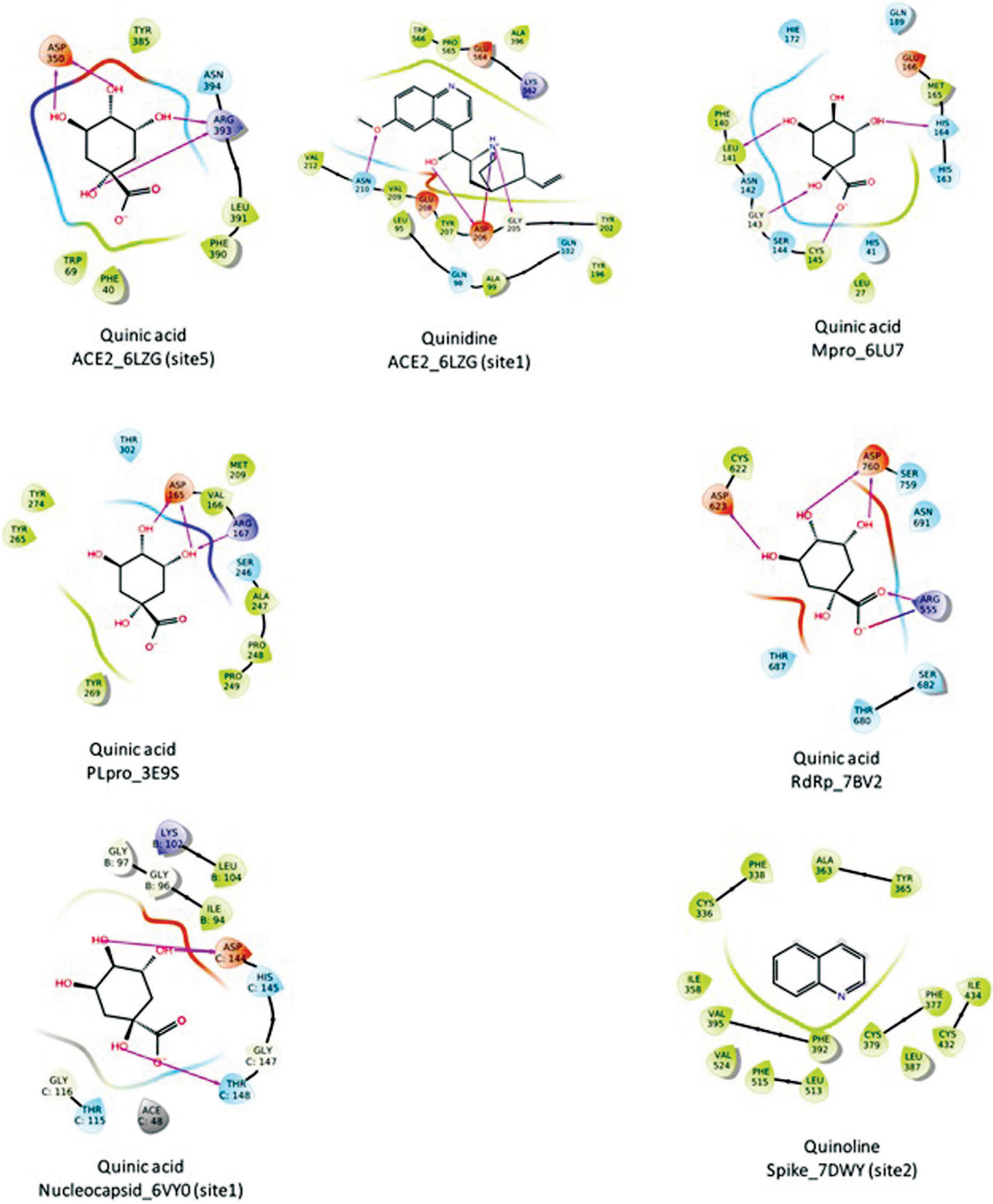

Fig. 2 Binding mode of phytoconstituents of MT against different targets of SARS-Cov-2.

intracellular replication of the virus. Indeed, calculations revealed that remdesivir (10 µM) and CO-MT (concentration of 4 µL/200 µL) showed respectively a 99% and 89% inhibition of SARS-CoV-2 infection (►**Fig 3B**). However, SARS-CoV-2-infected Vero cells treated with three potencies (CO 3c, CO 6c, and CO 12c) showed only 5% to 15% inhibition of SARS-CoV-2 infection (►**Fig 3B, –3C**). This is consistent with our observation that the active phytoconstituents of CO are absent in the three potencies (CO 3c, CO 6c, and CO 12c) (►**Fig 1**). Thus, ►**Fig 3B and –3C** indicate that the antiviral activity of CO-MT is comparable to that of remdesivir, an established antiviral drug for COVID-19.

Discussion

This study was performed to identify the antiviral potential of CO against the SARS-CoV-2 virus, which causes COVID-19. First, we found that the homeopathic formulations of CO in

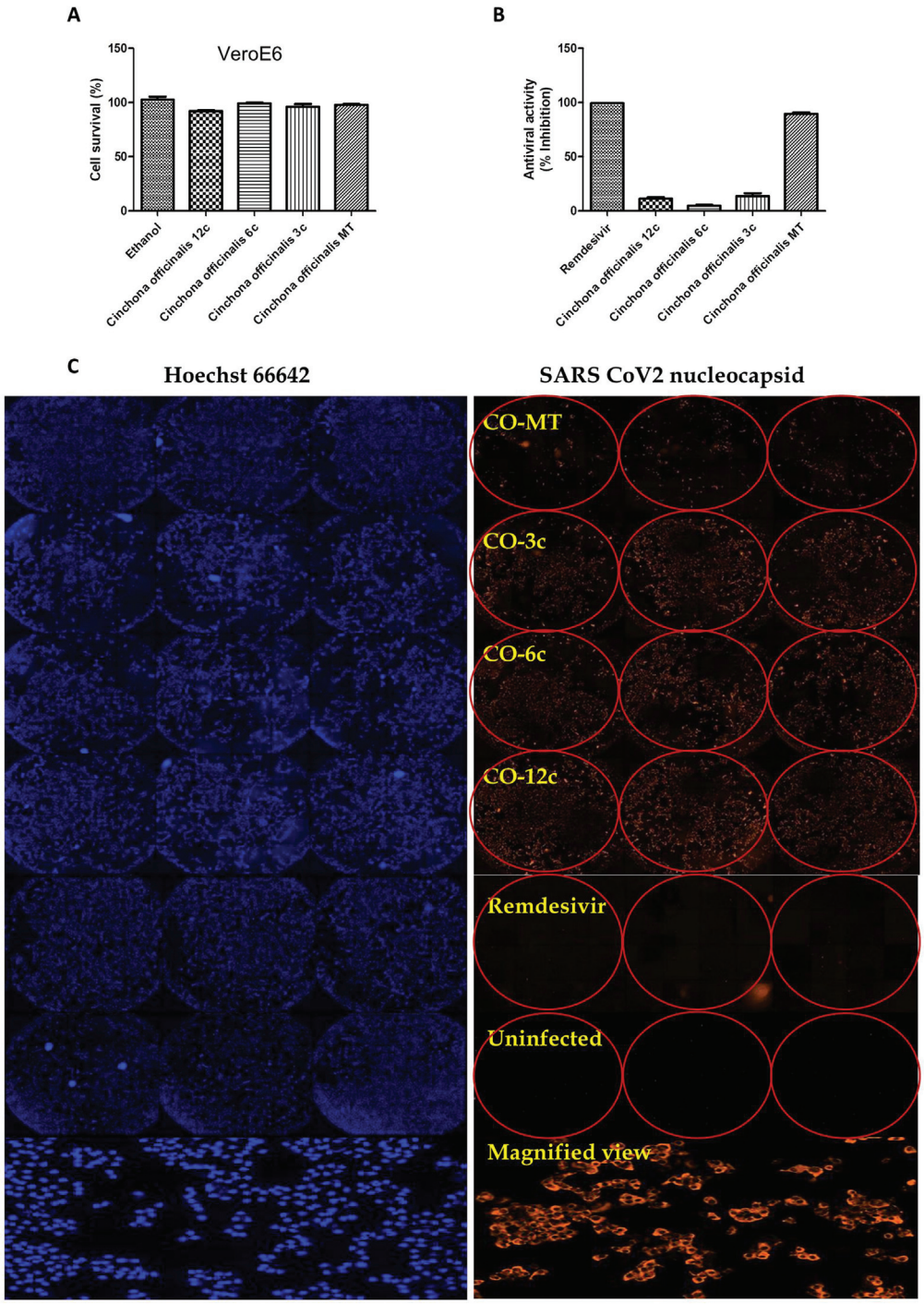

Fig. 3 (**A**) Effect of *Cinchona officinalis* (CO) on VeroE6 cells. Cells were incubated with the test substance at the indicated concentrations (4 µL of the sample [original, 1:10 or 1:20 dilution in ethanol] in 200 µl reaction). After 30 hours, cells were stained with Hoechst 33342 (live and dead cells) and sytox orange (dead cells). Cell viability was calculated by taking images (10X) of cells and total cell numbers from the software. (**B**) Antiviral activity of CO on SARS-CoV-2. The graph shows that SARS-CoV-2-infected cells treated with Remdesivir or CO-MT (4 µL/200 µL) show approximately 89% growth inhibition. Values are mean ± SEM. (**C**) Immunofluorescence images for the effect of CO on SARS-CoV-2 *in vitro.* Hoechst stained (*blue*) images (10X) indicate the total VeroE6 cell number. Alexa flour-568 stained cells (*orange*) indicate SARS-CoV-2-infected cells stained for viral nucleocapsid protein. Uninfected control cells lack an orange stain. SARS-CoV-2-infected cells treated with remdesivir or CO-MT have low orange stain since both drugs significantly inhibit viral replication. SARS-CoV-2-infected VeroE6 cells treated with CO-MT potencies show a bright orange stain because these potencies have weak antiviral activity. A magnified view of the cells stained with Hoechst and Alexa flour 568 is also provided for reference.

MT and three different potencies (3c, 6c and 12c) were not cytotoxic to VeroE6 cells (►**Fig. 3A**). Using state-of-the-art immunofluorescence methods to specifically identify SARS-CoV-2 infected cells and viable cells, we showed that CO-MT (dose of 4 µL/200 µL) and remdesivir caused >90% inhibition of the viral replication in VeroE6 cells. Therefore, the *in vitro* antiviral potential of CO-MT against SARS-CoV-2 is comparable to that of remdesivir. ►**Table 2** shows quinoline had a good DS against the spike protein SARS-CoV-2. Quinic acid showed better binding potential with Mpro, PLpro RdRp, nucleocapsid protein and ACE2 (allosteric site) compared to other constituents. Quinidine exhibited better binding to ACE2. Interestingly, docking scores of quinidine and quinic acid for the Mpro, PLpro, spike, nucleocapsid and RdRp proteins are very similar to those of HCQ and CQ for these proteins. Thus, compared to antivirals which exclusively target the spike protein, CO-MT could potentially be more effective against SARS-CoV-2 variants or mutants because five of its phytoconstituents can bind multiple SARS-CoV-2 target proteins. Therefore, the potent *in vitro* antiviral activity of CO-MT observed (►**Fig. 3C**) is strongly supported by the molecular docking data in ►**Table 2**.

A recent review examined the pathogenesis of systemic conditions of COVID-19 and compared them with the pathophysiological effects of various homeopathic medicines. The review concluded that the sphere of action of CO makes it a suitable medication for the relief of COVID-19 symptoms.[21] Our study supports this by establishing *in silico* and *in vitro* data, which provide clear scientific explanations and evidence for the antiviral potential of CO-MT against SARS-CoV-2.

Limitation of the Research

A key limitation of this research is the lack of a suitable experimental COVID-19 animal model at our laboratory to test the efficacy of the CO formulations *in vivo*.

Conclusion

Cinchona officinalis, a homeopathic formulation, lacks toxicity and also has significant antiviral activity *in* VeroE6 cells. Molecular docking studies suggested that HCQ, CQ, and certain phytoconstituents of CO bind the major protein targets of SARS-CoV-2 (Mpro, PLpro, spike protein, nucleocapsid protein and RdRp) with similar affinity. Our study is a preliminary approach that validates the antiviral potential of CO suggested by others. It also provides a valuable foundation for designing future studies *in vivo* to investigate the therapeutic potential of homeopathic CO for the treatment of COVID-19 infections.

Highlights

- Quinine derivatives have shown promising results against SARS-CoV-2.
- Our *in silico* and *in vitro* findings identified *Cinchona officinalis*, a homeopathic formulation, as a potential remedy for COVID-19 infection.
- Further scientific evidence is necessary to identify the therapeutic potential of *Cinchona officinalis* in COVID-19.

Conflict of Interest

The authors have no conflict of interest, financial or otherwise. The study was approved by the Task Force of the Ministry of AYUSH, Government of India, and funded by the National Institute of Homeopathy, Kolkata.

Acknowledgements

We thank the National Institute of Homeopathy and Central Council for Research in Homeopathy (CCRH), Government of India, for providing financial support to the present study. The authors also thank the Regional Centre for Biology, Faridabad, for extending support for *in vitro* antiviral studies.

References

1 Zhu L, Xu X, Ma K, et al. Successful recovery of COVID-19 pneumonia in a renal transplant recipient with long-term immunosuppression. Am J Transplant 2020;20:1859–1863
2 Zhang H, Chen Y, Yuan Q, et al. Identification of kidney transplant recipients with coronavirus disease 2019. Eur Urol 2020; 77:742–747
3 Zhu N, Zhang D, Wang W, et al; China Novel Coronavirus Investigating and Research Team. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 2020;382:727–733
4 Liu K, Fang YY, Deng Y, et al. Clinical characteristics of novel coronavirus cases in tertiary hospitals in Hubei Province. Chin Med J (Engl) 2020;133:1025–1031
5 Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA 2020;323:1061–1069
6 Patel VK, Shirbhate E, Patel P, Veerasamy R, Sharma PC, Rajak H. Corticosteroids for treatment of COVID-19: effect, evidence, expectation and extent. Beni Suef Univ J Basic Appl Sci 2021;10:78
7 Manchanda RK, Miglani A, Gupta M, et al. Homeopathic remedies in COVID-19: prognostic factor research. Homeopathy 2021; 110:160–167
8 Remya V, Kuttan G. Homeopathic remedies with antineoplastic properties have immunomodulatory effects in experimental animals. Homeopathy 2015;104:211–219
9 Holmfred E, Cornett C, Maldonado C, Rønsted N, Hansen SH. An optimised method for routine separation and quantification of major alkaloids in cortex *Cinchona* by HPLC coupled with UV and fluorescence detection. Phytochem Anal 2017;28:374–380
10 Rajan A, Bagai U, Chandel S. Effect of artesunate based combination therapy with homeopathic medicine china on liver and kidney of Plasmodium berghei infected mice. J Parasit Dis 2013;37:62–67
11 Narożna M, Rubiś B Anti-SARS-CoV-2 strategies and the potential role of miRNA in the assessment of COVID-19 morbidity, recurrence, and therapy. Int J Mol Sci 2021;22:8663
12 Yao X, Ye F, Zhang M, et al. In vitro antiviral activity and projection of optimized dosing design of hydroxychloroquine for the treatment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Clin Infect Dis 2020;71:732–739
13 Panusa A, Multari G, Incarnato G, Gagliardi L, McCalley DV. Analysis of the Cinchona alkaloids by high-performance liquid chromatography and other separation techniques. J Pharm Biomed Anal 2007;43:1221–1227
14 Murugan NA, Kumar S, Jeyakanthan J, Srivastava V. Searching for target-specific and multi-targeting organics for Covid-19 in the Drugbank database with a double scoring approach. Sci Rep 2020; 10:19125

15 Gordon DE, Jang GM, Bouhaddou M, et al. A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. Nature 2020;583:459–468
16 Repasky MP, Shelley M, Friesner RA. Flexible ligand docking with Glide. Curr Protoc Bioinformatics 2007;Chapter 8:12
17 Sunila ES, Kuttan R, Preethi KC, Kuttan G. Dynamized preparations in cell culture. Evid Based Complement Alternat Med 2009; 6:257–263
18 Caldas LA, Carneiro FA, Higa LM, et al. Ultrastructural analysis of SARS-CoV-2 interactions with the host cell via high resolution scanning electron microscopy. Sci Rep 2020;10:16099
19 Runfeng L, Yunlong H, Jicheng H, et al. Lianhuaqingwen exerts anti-viral and anti-inflammatory activity against novel coronavirus (SARS-CoV-2). Pharmacol Res 2020;156:104761
20 Ogando NS, Dalebout TJ, Zevenhoven-Dobbe JC, et al. SARS-coronavirus-2 replication in Vero E6 cells: replication kinetics, rapid adaptation and cytopathology. J Gen Virol 2020;101: 925–940
21 Gosik MS, Mendes MFX, Werneck Dos Santos LMA, et al. Medicines for the new coronavirus in the view of classical systemic homeopathy. Complement Ther Clin Pract 2021; 45:101482