Supporting Information for

Scutellaria baicalensis extract and baicalein inhibit replication of SARS-CoV-2 and its 3C-like protease *in vitro*

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This Support Information contains Supplementary Material and Methods, Supplementary Figures S1-S3, and Supplementary Tables S1-S2.

Supplementary Material and Methods

Enzyme inhibition assay in cell lysate

HEK293T cells were plated into 6-well plates (Corning) at a density of 5×10^5 cells/well in 2 ml DMEM containing 10% Fetal Bovine Serum. The cells were cultured for 12h at 37 ° C in a 5% CO_2 incubator. The transfection was performed by using FuGENE® HD reagent (Promega) with 3.3 μg/well of FRET substrate construct according to the manufacturer's instructions. The transfected cells were incubated at 37° C in a 5% CO_2 incubator for 48 h. At 48 h, the medium was removed from cells and washed with PBS. The cells were digested by 0.05% trypsin-EDTA and resuspended by cell culture medium. The cells were spun down and resuspended by PBS. Count the transfected cell density and collect the same number of untransfected 293T cells. The cells were lysed by sonication in assay buffer. The lysate was clarified by centrifugation at 14,000 × g for 15 min. The supernatant from untransfected 293T was collected and added purified SARS-CoV-2 $3CL^{pro}$ (final concentration 5μM), crude extract (final concentration 1 mg/ml and 500 μg/ml) or Baicalein (final concentration 1 mM and 500 μM). $3CL^{pro}$ and inhibitors in cell lysis were preincubated for 10 min. And then added the same volume of lysate containing FRET substrate from transfected cells to react 30 min and 50 min with final concentration of 2.5 μM $3CL^{pro}$ and 500 μg/ml and 250 μg/ml crude extract or 500 μg/ml and 250 μM Baicalein. The FRET fluorescence variation was analyzed by excitation at 433 nm and reading emission from 460 nm to 560 nm.

Determination of the ratio of baicalein in the crude extract by HPLC

HPLC method was performed on water as the mobile phase A and methanol as the mobile phase B. The chromatographic column was SB-C18. The flow rate was 1.0 mL/min and the detection wavelength was 280 nm.

Supplementary Figures

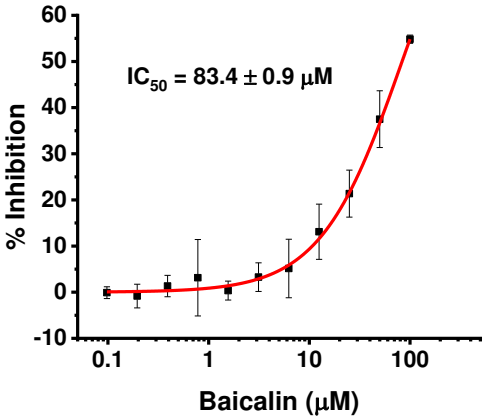

Figure S1. The *in vitro* anti-SARS-CoV-2 3CL^{pro} activity of baicalin.

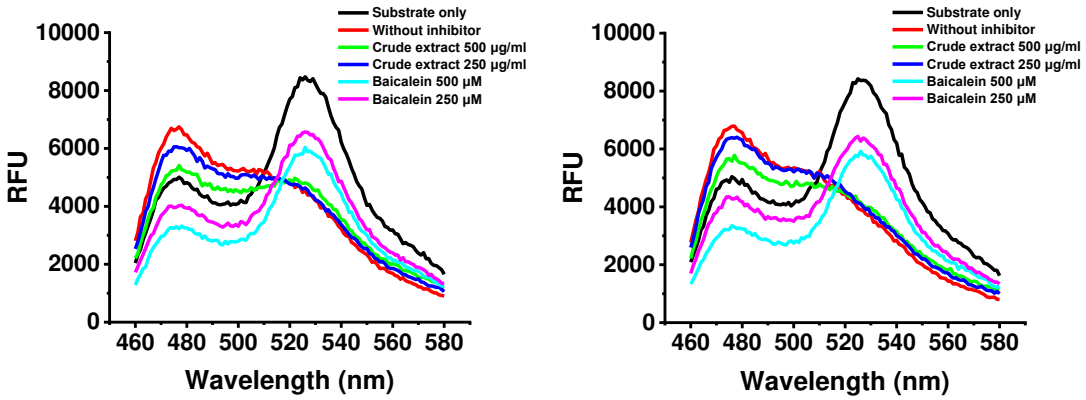

Figure S2. The anti-SARS-CoV-2 3CL^{pro} activity of *S. baicalensis* ethanol extract and baicalein in cell lysate for reaction (A) 30 min and (B) 50 min.

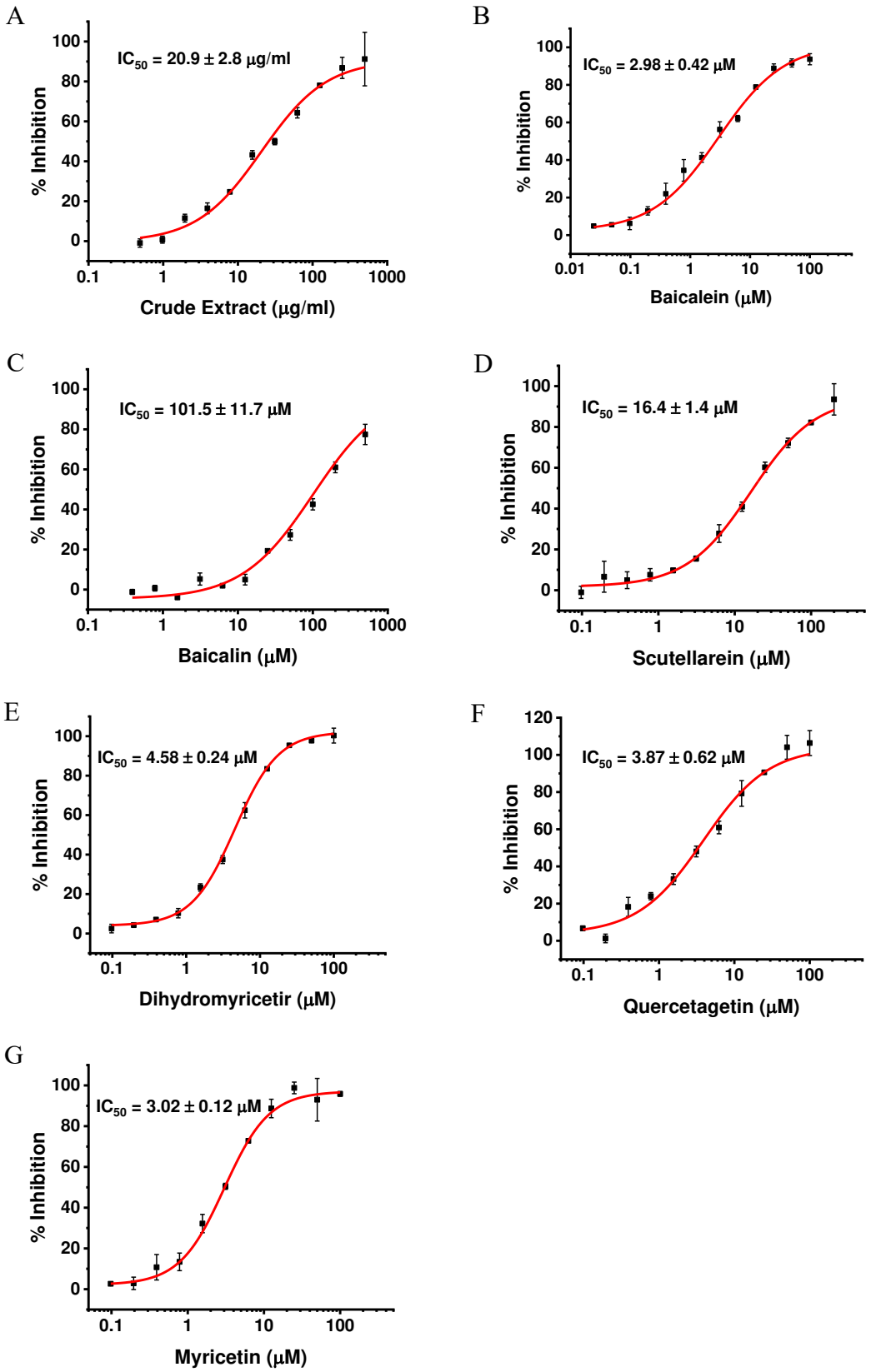

Figure S3. The *in vitro* anti-SARS-CoV 3CL^{pro} activity of (A) *S. baicalensis* ethanol extract, (B) baicalein, (C) baicalin, (D) scutellarein, (E) dihydromyricetin, (F) quercetagetin and (G) myricetin.

Supplementary Tables

Table S1. The docking scores of *S. baicalensis* ingredients and baicalein analogue flavonoids in the substrate binding site of SARS-CoV-2 $3CL^{pro}$

S. baicalensis ingredients	PubChem ID	Glide Docking Score
baicalein	5281605	-8.277
baicalin	64982	-8.458
wogonin	5281703	-7.929
wogonoside	3084961	-8.331
scutellarein	5281697	-8.823
scutellarin	185617	-8.810
myricetin	5281672	-8.364
myricetrin	5281673	-8.984
5,6-dihydroxyflavone	14349487	-6.832
6,7-dihydroxyflavone	5353357	-7.989
chrysin	5281607	-6.919
dihydromyricetin	161557	-7.966
herbacetin	5280544	-9.402
quercetagetin	5281680	-9.109

Table S2. The ratio of baicalein in the crude extract determined by HPLC.

Pure Baicalein (mg/mL)	Peak area (280 nm, mAU*s)	Crude extract	Baicalein peak area	The ratio of baicalein in the crude extract
0.5	17476.1	2 mg/mL	1237.3	2.07 %
0.25	8615.7			
0.125	4173.5			
0.0625	1936.1			
0.03125	845.3			
0.015625	405.3			