Brief Report

Anti-SARS coronavirus 3C-like protease effects of *Rheum palmatum* L. extracts

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Summary　　**The present study aims to clarify the inhibitive effect of the compounds from *Rheum palmatum* L. on the SARS-3CL protease. The SARS-CoV 3CL gene was amplified from RNA of the SARS virus by PCR. The SARS-CoV 3CL protease was purified from a colon bacillus recombinant. Drugs and 3CL protease were incubated together. The inhibition rate and IC_{50} were calculated based on absorbance. Components from the *Rheum palmatum* L. had a high level of anti-SARS-CoV 3CL protease activity. The IC_{50} was 13.76 ± 0.03 μg/mL and the inhibition rate was up to 96%. In conclusion, extracts from *Rheum palmatum* L. have a high level of inhibitory activity against 3CL protease, suggesting that extracts from *Rheum palmatum* L. may represent a potential therapeutic for SARS.**

Keywords: Rheum palmatum L., SARS-3CL protease

1. Introduction

Chinese rhubarb, a Chinese medicinal herb, includes roots and rhizomes of *Rheum palmatum* L., *Rheum tanguticum* Maxim ex Reg., and *Rheum officinale* Baill. Its main active components are anthraquinones. Chinese rhubarb has an anti-virus effect on both DNA and RNA viruses such as the Coxsackie virus, epidemic hemorrhagic fever virus, rubella virus, simple herpes virus, varicella virus, varicella zoster virus, AIDS virus, hepatitis B virus, influenza virus A, and influenza virus B (*1,2*). Anti-SARS coronavirus effects of *Rheum palmatum* L. extracts were recently reported (*2*). We have used a SARS-3C-l protease inhibition test to screen compounds from *Taxus celebica*, Radix *Sophorae flavescentis*, Radix Glycyrrhiza, *Uvaria microcarpa*, *Rubus suavissimus* S. Lee, *Auricularia auricula* (L.) Underw, Java Brucea Fruit, male silkworm

moths, leaf of *Mangifera indica* Linn, Rhizoma Cyrtomii Fortunei, *Scutellaria baicalensis* Georgi, and Artesunate. The present study reveals that among these compounds Chinese rhubarb extracts had the highest level of anti-SARS-3CL protease activity.

2. Materials and Methods

2.1. *Drugs and reagents*

To obtain extracts, *Rheum palmatum* L. (identified by Ze-Xiang Du, Guilin Medical College) was powdered, sieved, drip filtered, and then combined with 75% alcohol. The filtrate was dried, extracted, and then chromatographed as described below.

　　Taxus celebica, *Uvaria microcarpa*, Java Brucea Fruit, male silkworm moths, Rhizoma Cyrtomii Fortunei were extracted with 75% ethanol. *Rubus suavissimus* S. Lee, *Auricularia auricula* (L.) Underw, Radix Glycyrrhiza, leaf of *Mangifera indica* Linn, *Scutellaria baicalensis* Georgi, Radix *Sophorae flavescentis* were extracted with water. These extracts were concentrated and dried. The sample of Artesunate

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was provided by China National Institute for the Control of Pharmaceutical and Biological Products.

SARS-3CL protease was provided by China National Center for Drug Screening. Substrate of SARS-3CL protease, Thr-Ser-Ala-Val-Leu-Gln-pNA (TQ-6pNA), was provided by GL Biochem (Shanghai) Ltd.. Chen312-5 as a positive control was provided by China National Center for Drug Screening.

2.2. *Separation of the various components of Rheum palmatum L.*

RH10 was separated from *Rheum palmatum* L. extract through concentration and drying. Massive particles, RH11, were also separated during concentration. RH10 was suspended with water and treated with petroleum ether and chloroform (Figure 1). The remaining material, RH12, was extracted with ethyl acetate and then RH121, RH122, RH123, RH124, and RH125 were separated by silica gel column chromatography with a gradient of chloroform/methanol (10:0-0:10, v/v).

2.3. *Anti-SARS-CoV 3CLprotease effects test*

2.3.1. *Experimental principle*

Virus RNA was extracted from SARS-CoV and SARS-CoV 3CL protease gene was amplified by RT-PCR. An expression vector of SARS-CoV 3CL protease gene was constructed. SARS-CoV 3CL protease was purified from an *E. coli* expression system. $(NH_4)_2SO_4$ precipitation and anion-exchange chromatography were used to prepare highly pure SARS-CoV 3CL protease. Thr-Ser-Ala-Val-Leu-Gln-pNA was spotted and the chemical bond between Gln and pNA was cleaved by

SARS-CoV 3CL protease. pNA released was detected at 405 nm by a microplate reader (SpectraMAX340, Molecular Devices, Sunnyvale, CA, USA).

2.3.2. *Experimental methods*

The drug test was done with 96-well plates. The total volume of the reaction system was 100 μL, which contained 2.7 μM 3CL protease, 2% DMSO, 50 mM Tris·HCl (pH 7.5), 1 mM DTT, 1 mM EDTA, and 250 μM TQ-6pNA. The reaction time was 3 h. Absorption intensities at 405 nm were detected at 0, 1, 2, and 3 h. Incremental absorption intensity per unit time representing the enzyme initial velocity (*v*) of 3CL protease was obtained by calculation. The initial concentration of drug screening was 100 μg/mL. Each sample was examined in triplicate, when the sample inhibition rate in initial screening was more than 50%, since this sample may be considered to have an effect on anti-SARS coronavirus 3C-like protease. The inhibition rate of the sample was tested again at doses of 100, 50, 25, 12.5, 6.25, 3.12, and 1.56 μg/mL, and the IC_{50} value was calculated as described below.

2.4. *Formula for the anti-SARS coronavirus 3C-like protease inhibition rate and IC_{50} of samples*

The anti-SARS coronavirus 3C-like protease inhibition rate (% inhibition) of samples was calculated by the following formula:

$$\% \textbf{\textit{Inhibition}} = \frac{\textbf{v}_{DMSO} - \textbf{v}_{Sample}}{\textbf{v}_{DMSO}} \times \textbf{100\%}$$
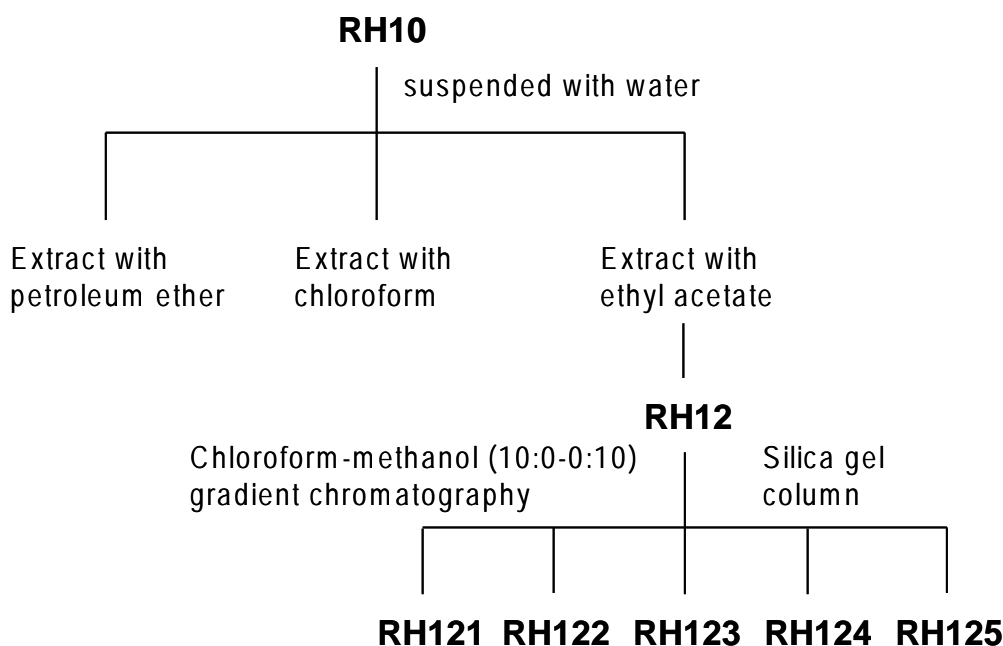

Figure 1. Flowchart for sample preparation.

where $\mathbf{v}_{DMSO}$ and $\mathbf{v}_{Sample}$ indicate the enzyme initial velocity of the DMSO group without the drug and the sample group, respectively.

The IC_{50} value is the inhibition rate (% inhibition) with respect to the sample concentration (I), which was calculated by the following formula:

$$\% \textbf{\textit{Inhibition}} = \frac{100}{1 + 10^{(Log\ IC_{50}-X)\times h}}$$

where h indicates Hill coefficient. IC_{50} of samples with a rate of inhibition of anti-SARS coronavirus 3C-like protease greater than 50% were further determined.

3. Results

At 100 μg/mL, the rate of sample inhibition of anti-SARS coronavirus 3C-like protease was 12% for Taxus celebica extracts, 13.5% for Radix Glycyrrhiza extracts, 21.4% for *Uvaria microcarpa* and Java Brucea Fruit extracts, 0% for *Rubus suavissimus* S. Lee extracts, 0% for *Auricularia auricula* (L.) Underw, 5.3% for Java Brucea Fruit, 32.1% for male silkworm moths extracts, 3.5% for leaf of *Mangifera indica* Linn, 25.7% for Rhizoma Cyrtomii Fortunei, 13.6% for *Scutellaria baicalensis*, 20.2% for Radix *Sophorae flavescentis*, and 11.0% for Artesunate (data not shown). The inhibition rates of these samples on anti-SARS coronavirus 3C-like protease were less than 50%. In contrast, the extracts of *Rheum palmatum* L. such as RH10, RH11, RH12, RH121, RH122, RH124, and RH125 significantly inhibited SARS coronavirus 3C-like protease (Table 1). Among these extracts, RH121 has the highest level of activity.

4. Discussion

Aqueous extracts of Radix et Rhizoma Rhei (the root tubers of *Rheum officinale* Baill.) are reported to have significant anti-SARS coronavirus activity (*3*). They inhibited the interaction of SARS-CoV S protein and ACE2 in a dose-dependent manner. Their IC_{50} values

ranged from 1 to 10 μg/mL. Emodin significantly blocked the S protein and ACE2 interaction with an IC_{50} of 200 μM. It also inhibited the infectivity of S protein-pseudotyped retrovirus with respect to Vero E6 cells. In the cell-based system, Aloe emodin had anti-SARS coronavirus activity with an IC_{50} of 99.1 ± 2.1 μg/mL (366 μM). In a non-cell-based system, the IC_{50} was 35.7 ± 1.5 μg/mL (132 μM) (*4*). 3C-like protease is known to be the target of anti-SARS-CoV virus drugs and a key enzyme for SARS coronavirus replication. When activity of 3C-like protease is inhibited, SARS-CoV virus replication in the host cell will be also inhibited. In 36% of papers on anti-SARS coronavirus drugs, 3C-Like protease served as the target (*6*). RH121 extracted from *Rheum palmatum* L. was highly active with respect to the anti-SARS-CoV virus since its rate of inhibition of SARS-3CL protease was as high as 96% and since its IC_{50} was 13.76 ± 0.03 μg/mL (Table 1). The ethanol extract from Rhubarb showed no cytotoxicity at a dose of 20 mg/mL, indicating that it could be a great tool for antivirus drug screening. Rhubarb is abundant in China and has long been used as a traditional Chinese medicine due to the high incidence of viral diseases today (*7*). More research should be conducted to yield specific chemical anti-virus compounds from Rhubarb and develop antiviral drugs with high potency and low toxicity.

References

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Table 1. Inhibitory effect of extracts from *R. palmatum* L. (100 μg/mL) on SARS-3CL protease

Samples	Rate of SARS-3CL protease inhibition (% inhibition)	IC_{50} (μg/mL)
RH10	98.12 ± 4.66	38.09 ± 1.70
RH11	87.67 ± 8.99	22.30 ± 1.26
RH12	72.17 ± 7.12	59.33 ± 6.52
RH121	96.20 ± 5.00	13.76 ± 0.03
RH122	69.60 ± 2.20	34.01 ± 5.68
RH123	32.80 ± 3.30	−
RH124	84.30 ± 1.80	52.43 ± 4.52
RH125	115.0 ± 3.00	20.53 ± 3.20
Chen312-5	88.3 ± 2.60[a]	2.25 ± 0.24

[a] % inhibition for Chen312-5 was examined at 20 μg/mL.