

Exploring the potential of traditional Chinese medicines for antiviral therapy



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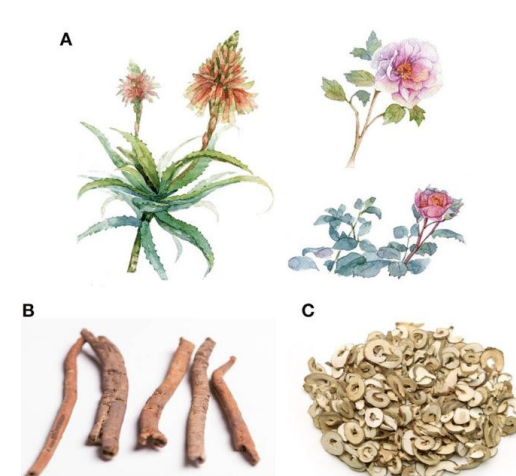
Acknowledgments

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Introduction

The SARS-CoV-2 virus has caused a devastating pandemic, resulting in the death of nearly 7 million people worldwide and causing significant socio-economic consequences.¹ The pandemic has highlighted the need for effective antiviral drugs, as current treatments have proven insufficient. Traditional plant-based formulas have been integrated into the treatment protocols for COVID-19 in Chinese hospitals, and many of these have shown beneficial effects.² Our preliminary study investigated the antiviral activity of plants commonly used in Chinese treatments, including *Paeonia suffruticosa* and the results demonstrated significant antiviral activities with no signs of cytotoxicity on the VERO E6 cells.³

Objective



Observing the phytochemical profile of *Paeonia suffruticosa* with an emphasis on the active molecules' antiviral activity, with the aim of inspiring the development of new antiviral drugs.

Methodology

Bioguided fractionation techniques were employed for the better understanding of the composition of *Paeonia suffruticosa* cortex such as preparatory HPLC and DCVC.

Furthermore, the fractions obtained from these techniques were tested on the SARS-CoV-2 virus using VERO E6 cells. The antiviral activity was observed through the RT-qPCR detection method and INCUCYTE® live-cell analysis system.

Results

The bioguided fractionation assay performed on *Paeonia suffruticosa* allowed us a better understanding of the plant composition.

Additionally, molecules were tested on the SARS-CoV-2 virus using VERO E6 cells. RT-qPCR results demonstrated an interesting activity from the fraction D which structure remains to be elucidated.

The results obtained by RT-qPCR were also corroborated by the INCUCYTE® live-cell analysis system.

Analysis

Preparative HPLC

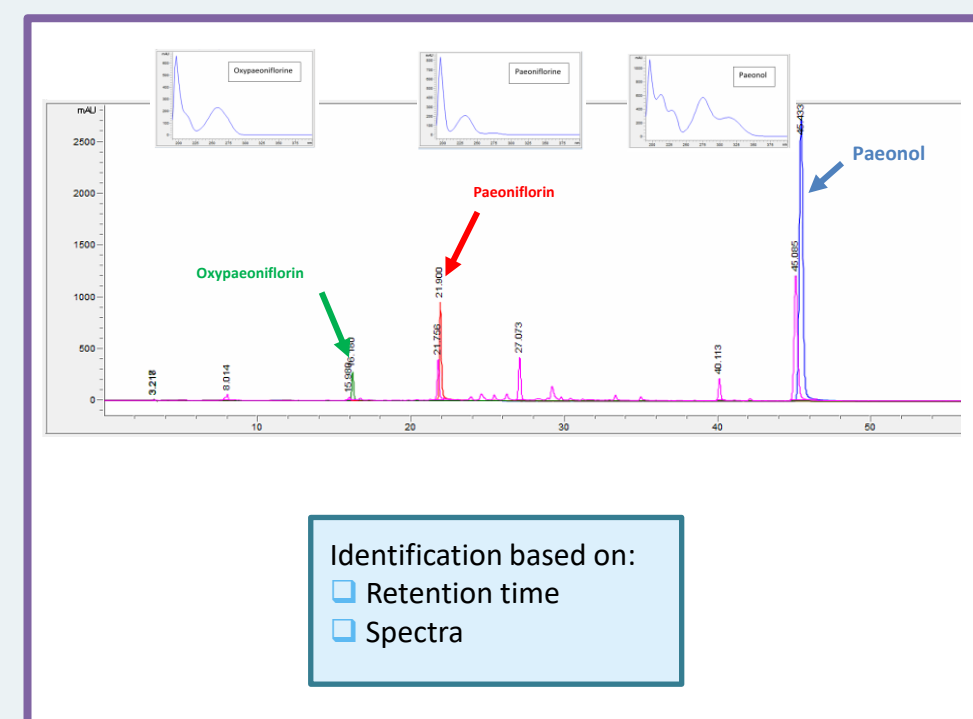
- Stationary phase: C₁₈ column (250 X 25 mm; 12µm)
- Mobile phase A: water + TFA (0,05%) (v/v)
- Mobile phase B: acetonitrile
- Detection: 254 and 350 nm

HPLC

- Column: Hypersil ODS C₁₈ (250 mm x 4,6 mm; 5µm)
- Mobile phase A: Water + TFA (0,05 %) (v/v)
- Mobile phase B: Acetonitrile
- Detection: 254 and 350 nm

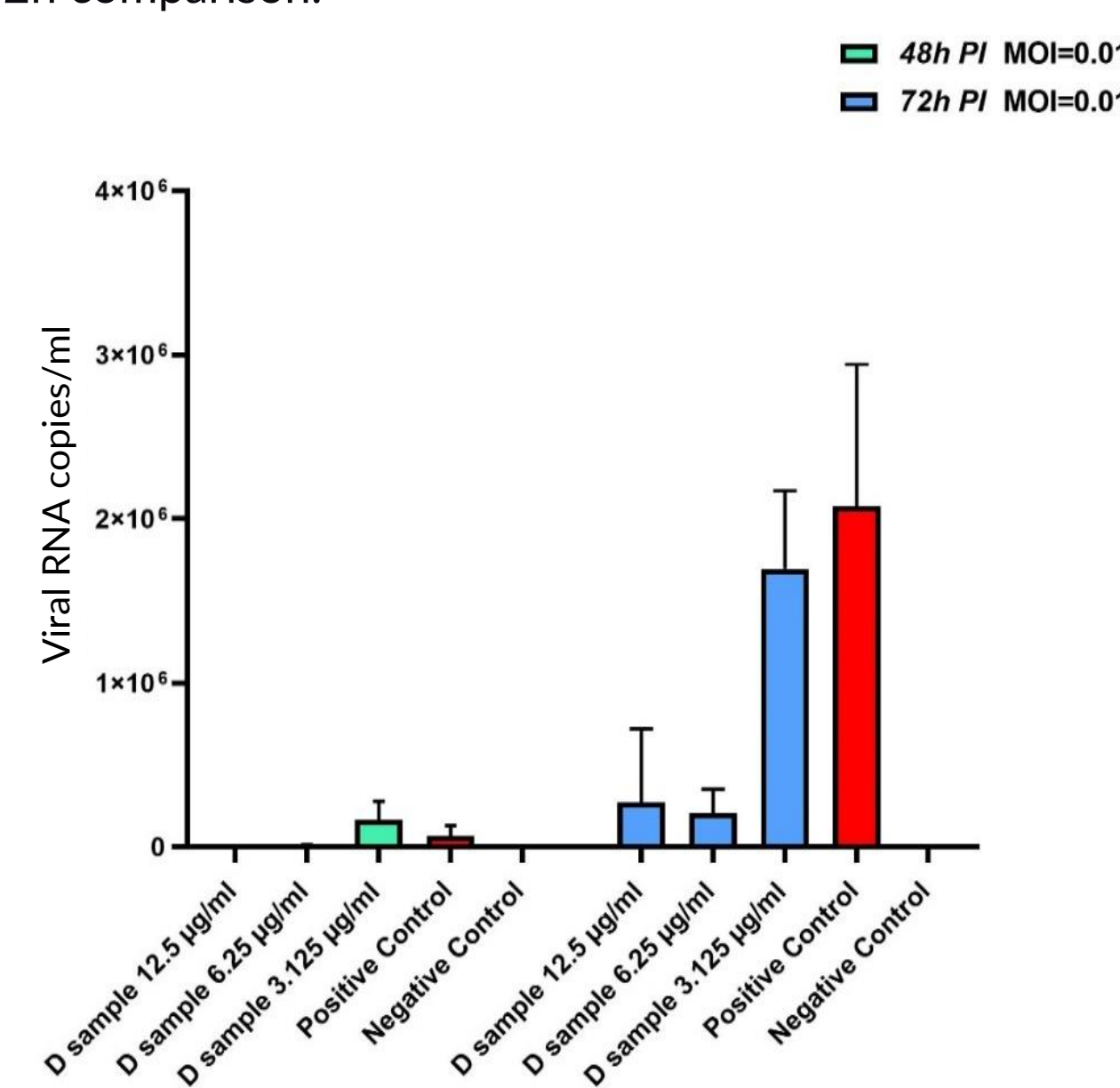
DCVC

- Column filled with silica gel
- Mobile phase: Ethyl acetate-water-formic acid-acetic acid (20:5:2:2:2:2 v/v/v/v/v)



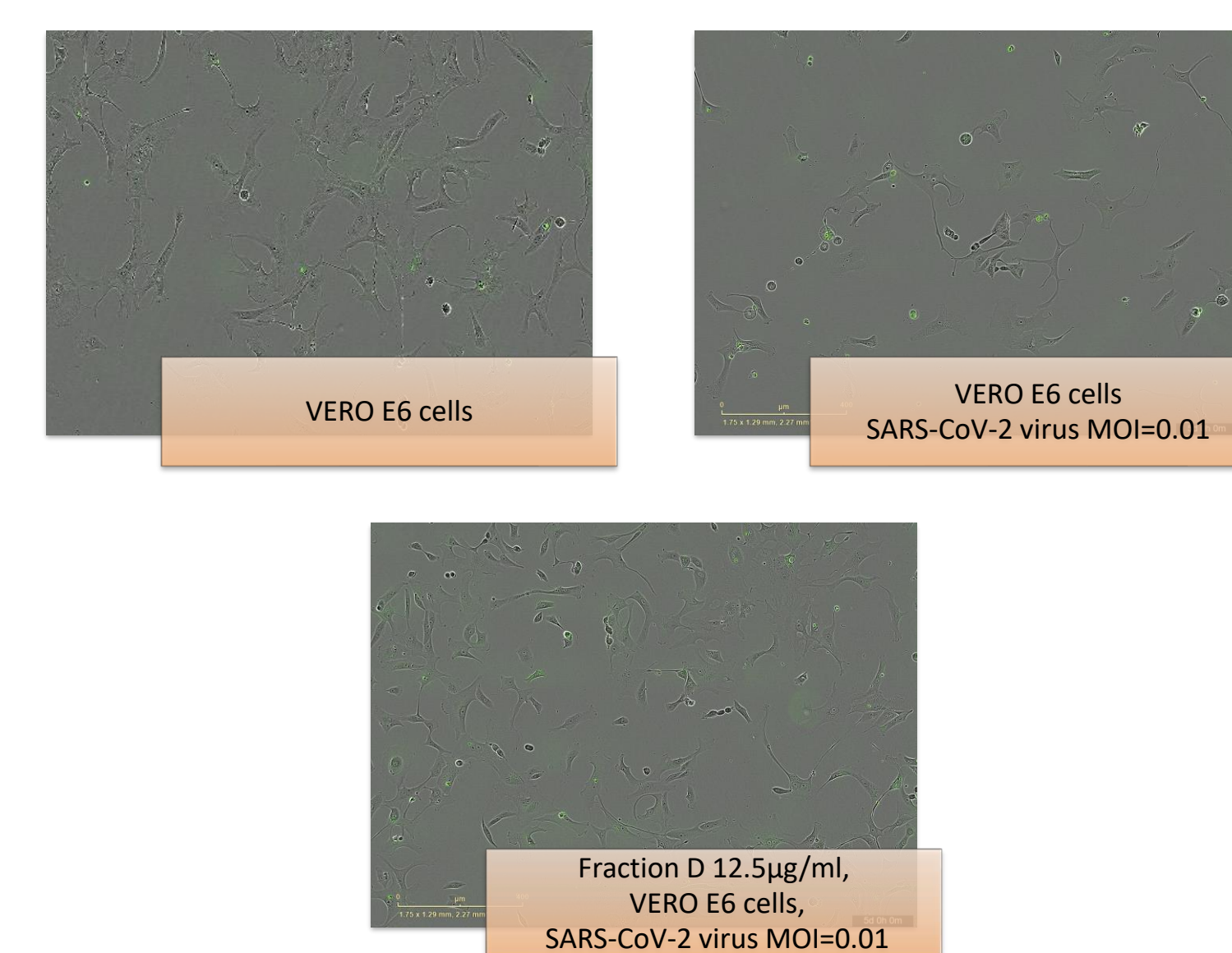
RT-qPCR

- The fractions collected during the previous steps were analyzed using VERO E6 cells and their antiviral activity was monitored against the SARS-CoV-2 virus over a period of 72h.
- Paeonol demonstrated overall a better activity than paeoniflorin and oxyapaeoniflorin at 50 µg/ml.
- However, the fraction nominated as D stood out from the rest of the other fractions, as it can be observed from the RT-qPCR graphic tested at 3 different concentrations during 48 and 72h comparison.



INCUCYTE®

- The 96-well plate was seeded at a concentration of 2.5 x 10⁴ cells/ml of VERO E6 cells.
- Three conditions were tested:
 - A. Only VERO E6 cells
 - B. VERO E6 cells plus SARS-CoV-2 virus at an MOI= 0.01
 - C. Fraction, VERO E6 cells plus SARS-CoV-2 virus at an MOI= 0.01
- All three conditions are treated with Cytotox Green Dye which emits fluorescence upon binding with the deoxyribonucleic acid (DNA) once the cells become unhealthy.
- The observation took place over a period of 5 days.



Conclusion

The molecule belonging to *Paeonia suffruticosa* (cortex) nominated as fraction D revealed interesting activity against the SARS-CoV-2 virus at an MOI of 0.01. The results were analyzed by RT-qPCR and later confirmed by INCUCYTE® live-cell analysis system thus reinforcing its traditional usage. Its structure remains to be elucidated as it can be a source of inspiration for the development of new antiviral drugs.

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