

# Coroless, Effective Ingredient Isolated from Traditional Chinese Medicine for the Prevention and Treatment of COVID-19

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## ABSTRACT

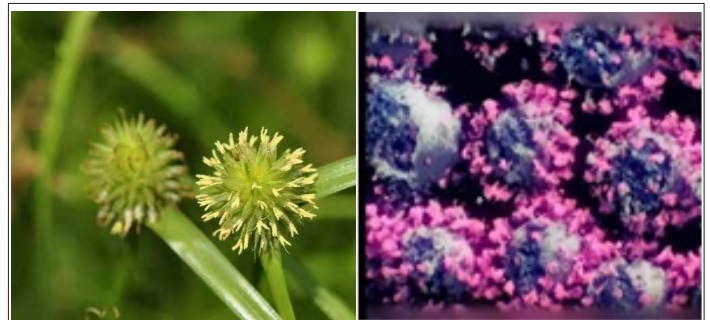
We believe that a good medicine for the prevention and treatment of COVID-19 requires three points. 1) Effectively inhibit the cells from being infected by COVID-19. 2) Quickly repair lung damage. 3) Non-toxic and harmless. Fortunately, from the treasure depot of Chinese traditional medicine, we found *Kyllinga brevifolia* Rottb, which is sufficient for three points and can be used for prevention, treatment and rehabilitation. Not only that, we have isolated the active ingredient of the *Kyllinga brevifolia* Rottb, named it Coroless, and verified that Coroless inhibits the COVID-19 from infecting cells through *in vitro* cell experiments.

**Keywords:** Coroless; Coronavirus; Traditional medicine; Prevention; Treatment and rehabilitation

## INTRODUCTION

As of January 31, 2021, more than 100 million patients have been diagnosed with COVID-19 infections globally and more than 2 million people have died. The new corona virus has damages to the lung, kidney, liver, and cardiovascular system [1]. There is an urgent need for COVID-19 prevention, treatment and rehabilitation drugs. We believe that a good medicine for the prevention and treatment of new corona virus requires three points. 1) Effectively inhibit the cells from being infected by COVID-19. 2) Quickly repair lung damage. 3) Non-toxic. Fortunately, from the treasure depot of Chinese traditional medicine, we found the *Kyllinga brevifolia* Rottb, which is sufficient for three points and can be used for prevention, treatment and rehabilitation [2,3]. *Kyllinga brevifolia* Rottb can cure flu, colds, headaches, muscle pain, coughs, malaria, jaundice, dysentery, sores, swelling, and bruises [4]. Coincidentally, the cluster-shaped seed ball of the herb is similar to the COVID-19 (Figure 1) [5]. *Kyllinga* is distributed in East Asia, Southeast Asia, South Asia, West Africa, Central Africa, South Africa, North America, South America, and Oceania. *Kyllinga* is non-toxic and harmless, and it is food for cattle, sheep and fish in Central South China. For developing countries in Asia, Africa and Latin America, for COVID-19 prevention and treatment, *Kyllinga* is an available emergency option. However, the origins of medicinal materials are different, thus the efficacy of this very medicine varies greatly. Fortunately, we isolated the active ingredient from the volatile oil of the herb and named it Coroless. Cell experiments confirmed

that it effectively inhibits COVID-19 infection.



**Figure 1:** *Kyllinga* seed balls and Cryo-electron micrographs of Covid-19 virus.

## MATERIALS AND METHODS

### COVID-19 pseudo-virus cell infection experiment

Coroless (5 µl/ml) is diluted serially. After the drug is diluted, and prepare the COVID-19 pseudo-virus (Beijing Sino Biological Inc. Each take 50 µl and mix in equal volume, incubate at 37°C for 1 h, and add to the 293T-ACE2 cells plated in advance, with 2 replicate wells for each dosage. After culturing for 24 h, 48 h, and 72 h, the cell status was observed and photographed, and the Luciferase luminescence value of the samples in the 96-well plate was detected with a chemiluminescence instrument. The positive

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control is COVID-19 pseudo-virus infection experiment. The negative control is a normal cell growth experiment. To evaluate the effect of Coroless on cell growth, 50  $\mu$ l of Coroless was diluted and added to the pre-plated 293T-ACE2 cells in 2 replicated wells; the cells were cultured for 24 h, 48 h, and 72 h, and then the cell status was observed and photographed. The experimental process of COVID-19 pseudo-virus cell with Kyllinga is the same as that of Coroless.

### ACE2: RBD molecule binding competition inhibition experiment

Coroless (5  $\mu$ l/ml) and Kyllinga are serially diluted. The inhibitor screening kit used was produced and prepared by Beijing Sino Biological Inc. (item number: KIT001, batch number: CW14MY1401). The experiment process was carried out according to the kit instructions.

## RESULTS AND DISCUSSION

In order to verify the cytotoxicity, Coroless was incubated with the cells for 72 hours. Figure 2 shows that the cells almost cover the culture plate, indicating that Coroless has no harm to cell growth. We have also observed that after the pseudo-virus is incubated with the cells, a large number of cells die. However, with the pseudo-virus and Coroless both incubated with the cells, the cells proliferate well.

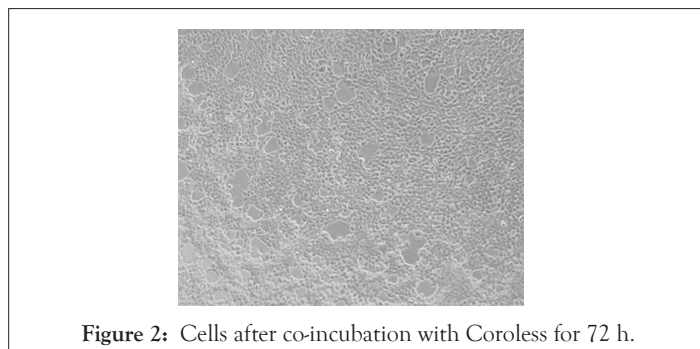


Figure 2: Cells after co-incubation with Coroless for 72 h.

Our previous work has shown that Kyllinga is effective in preventing, treating and recovering pneumonia caused by COVID-19 [2,3]. After the Coroless in Kyllinga was isolated, the *in vitro* cell efficacy was tested. Surprisingly, as shown in Table 1, Coroless's inhibition rate against COVID-19 pseudo-virus infection is as high as 98.89%, and the inhibition rate against COVID-19 pseudo-virus infection is still over 50% after 128-fold dilution. As shown in Table 2, the inhibition rate of Kyllinga against the COVID-19 pseudo-virus is as high as 87.71%. It shows that Coroless, like Kyllinga, effectively inhibits COVID-19 pseudo-virus infecting cells.

Table 1: COVID-19 pseudo-virus cell infection experiment with coroless.

Samples in serial dilutions	RLU1	RLU2	AVE	Inhibition rate/%
1	3971	12903	8.44E+03	98.89
2	439972	236293	3.38E+05	54.37
4	182580	279481	2.31E+05	68.84
8	211921	307354	2.60E+05	64.97
16	245116	363015	3.04E+05	58.97
32	277001	272255	2.75E+05	62.95
64	219713	491851	3.56E+05	51.95
128	198948	104996	1.52E+05	79.51

Negative control	205	286	2.46E+02	-
Positive control	939502	542045	7.41E+05	-

Table 2: COVID-19 pseudo-virus cell infection experiment.

Sample	RLU1	RLU2	AVE	Inhibition rate/%
Coroless	3971	12903	8.44E+03	98.89
Kyllinga	90116	92323	9.12E+04	87.71

In the high-concentration COVID-19 experiment, Kyllinga effectively inhibited the cell death caused by the new corona virus, and the half-inhibitory concentration  $IC_{50}$  was 3.76 mg/ml, which was equivalent to the actual clinical Kyllinga concentration [2,3]. The Coroless against COVID-19 experiment is being verified, and the preliminary experiments are also gratifying. Coroless also inhibits the infection of cells with high concentrations of the new corona virus. The research is still in progress.

In order to explore the mechanism of Coroless and Kyllinga inhibiting the new coronavirus infection, we have done Coroless and Kyllinga ACE2: RBD competitive inhibition experiment. The results of Table 3 and Figure 3 shows that the Kyllinga has a competitive inhibitory effect on the binding of ACE2: RBD. ACE2 is the cell indicating receptor of COVID-19, RBD is the receptor binding area of the new corona virus spike protein, and the ELISA experiment shows that the inhibition rate is 69%. However, Coroless has almost no effect on the binding competition inhibition of ACE2: RBD, as shown in Table 4. It shows that one possible way for the herb to inhibit COVID-19 infection is to interfere with the binding of spike protein to the ACE2 receptor, and Coroless does not function in this way. Although there are differences, the mechanism of Coroless and Kyllinga inhibiting the new corona virus infection should be related to GTP enzyme [6].

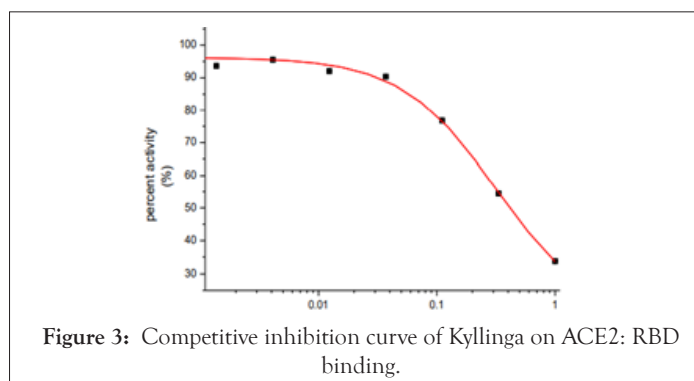


Figure 3: Competitive inhibition curve of Kyllinga on ACE2: RBD binding.

Table 3: Kyllinga's effect on ACE2: RBD binding competition inhibition experiment.

2nd Ab	Sino A5327-his scale up/HRP			
Coating	2019-n-CoV-RBD			
Sampling 2	ACE2-His(Cat:10108-H08B; Lot:MA14MA1401)-1: 40			
Sampling: 5-1	Sino A5327-his scale up/HRP			
Blank	0.811	0.782	0.797	
0.000051	0.872	0.745	0.809	101.443
0.000152	0.864	0.744	0.804	100.878
0.000457	0.737	0.686	0.712	89.272
0.001372	0.766	0.726	0.746	93.601

0.004115	0.74	0.782	0.761	95.483
0.012346	0.738	0.729	0.734	92.033
0.037037	0.721	0.719	0.72	90.339
0.111111	0.616	0.607	0.612	76.725
0.333	0.435	0.432	0.434	54.391
1	0.249	0.288	0.269	33.689

**Table 4:** Coroless's effect on ACE2: RBD binding competition inhibition experiment.

2nd Ab	Sino A5327-his Scale up/HRP			
Coating	2019-n-CoV-RBD			
Sampling 2	ACE2-His(Cat:10108-H08B; Lot:MA14MA1401)-1:40			
Sampling:6-1	OD450-1	OD450-2	Average	Percent activity
Blank	0.821	0.797	0.81	
0.000051	0.815	0.793	0.8	100.5
0.000152	0.843	0.836	0.84	104.938
0.000457	0.761	0.835	0.8	99.75
0.001372	0.746	0.857	0.8	100.188
0.004115	0.743	0.812	0.78	97.188
0.012346	0.73	0.822	0.78	97
0.037037	0.779	0.874	0.83	103.313
0.111111	0.75	0.849	0.8	99.938
0.333	0.777	0.796	0.79	98.313
1	0.685	0.751	0.72	89.75

## CONCLUSION AND SUGGESTIONS

We have confirmed through cell infection experiments that Coroless and Kyllinga can inhibit the infection of cells by COVID-19. In order to verify the safety of Coroless and the efficacy of damage repair, Dr. Nie self-administered Coroless (7 mg per day for three days) to treat oral ulcers, which has significant effects and no adverse reactions. The preliminary results of Coroless in clinic for COVID-19 are also encouraging. The new corona virus pandemic is severe. Especially in developing countries where medical resources are scarce, Kyllinga is one choice for persons in need. People in one world do things of one world. We hope that research institutions, pharmacies and hospitals in the world will cooperate with us to advance the follow-up research of Coroless, and provide good

medicine for the prevention, treatment and rehabilitation of new corona virus infection. Hand in hand, human being would be free of coronavirus fears. Harmless, Coroless!

## AUTHOR CONTRIBUTIONS

Dr. Nie Leng discovered the use of Kyllinga brevifolia Rottb and Coroless for COVID-19 prevention and treatment. Liang Youdong participated in the drug efficacy analysis. Yang Xinwei had clinical advice.

## ACKNOWLEDGMENT

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