

PROJECT #74908

The efficacy assessment of new compound against SARS-CoV-2 infection in *in vitro* models

The Test Article *Protefl* (alias Proteflazidum or "Compound-1_JVergar"). A Flavonoid Composition from plants *Deschampsia cespitosa* L. and *Calamagrostis epigeios* L. (both of the Poaceae = Gramineae Family), 1000 ug/ml in 74% Ethanol. Manufacturing Unit: Ecopharm (Ukraine).

Test article provided by Data Management Med High-Tech GmbH (Wiesbaden, Germany) was used to test antiviral properties against SARS-CoV-2 infection in two *in vitro* models.

SARS-CoV-2 inhibition by a cell-based assay

A slightly modified standard live virus-based microneutralization (MN) assay was used to rapidly evaluate the article efficacy against SARS-CoV-2 infection in *African green monkey* kidney (Vero E6) and adenocarcinomic human alveolar basal epithelial stably expressing human ACE2 receptor (A549/ACE2) cell cultures. Confluent Vero E6 and A549/ACE2 cells grown in 96-wells microtiter plates were pre-treated with serially 2-folds diluted article in duplicate over four concentrations for 2-, 0.5- or 0- hours before infection with ~100 and ~500, respectively, infectious SARS-CoV-2 (USA_WA-1/2020 isolate) particles in 100 µL EMEM supplemented with 2% fetal bovine serum (FBS). Vero E6 and A549 cells treated with parallelly diluted Vehicle with or without virus were included as positive and negative controls, respectively. After cultivation at 37 °C for 3- (Vero E6) or 4- (A549/ACE2) days, individual wells were observed

under the microcopy for the status of virus-induced formation of cytopathic effect (CPE). The efficacy of tested article was calculated and expressed as the lowest concentration capable of completely preventing virus-induced CPE in 100% of the wells. The toxicity to the treated cells was assessed by observing floating cells and altered morphology of adhered Vero E6 and A549 cells in wells under the microcopy. All work with SARS-CoV-2 was performed in the biosafety level 3 (BSL-3) facility of the Galveston National Laboratory.

Data interpretation

T – cytotoxic effect. Protection against SARS-CoV-2 infection cannot be assessed because of cytotoxic effect. To test the efficacy, the article’s formulation needs to be modified.

ND – no CPEs detected. The article at the tested concentration shows 100% protection against SARS-CoV-2 infection.

Single CPEs – only 1-3 CPEs detected. The article at the tested concentration shows partially protection against SARS-CoV-2 infection. There is a possibility that the higher concentration of tested article can increase protection level.

Slightly T/ ND - no CPEs detected, however, tested article produces slightly cytotoxic effect. The article at the tested concentration shows 100% protection against SARS-CoV-2 infection, however, slightly morphological changes in cell culture are observed which can affect infection.

Note: To confirm protection level of tested article:

1. Modify article formulation (using lower concentration of Ethanol or different Vehicle)
2. Test different *in vitro* model - Calu-3 (2B4)

Sincerely,



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 in vitro preliminary studies

ND - no CPE
 single: 1-3 CPE/well
 T - cytotoxicity

Article conc [ug/ml]	Vero E6						viral Ctrl	MOCK Ctrl
	2hrs		30min		0min			
10	T	T	T	T	T	T	CPE	T
5	T	T	T	T	T	T	CPE	T
2.5	T	T	slightly T/ND	slightly T/ND	single	single	CPE	T
1.25	slightly T/ND	slightly T/ND	CPE	CPE	CPE	CPE	CPE	ND

100 SARS-CoV-2 viral particles per sample
 2-fold dilution starting from 1:100
 data analyzed at 3 d.p.i.

Article conc [ug/ml]	A549/ACE2						viral Ctrl	MOCK Ctrl
	2hrs		30min		0min			
10	T	T	T	T	T	T	CPE	T
5	T	T	T	T	slightly T/ND	slightly T/ND	CPE	T
2.5	slightly T/ND	slightly T/ND	single	single	CPE	CPE	CPE	T
1.25	CPE	CPE	CPE	CPE	CPE	CPE	CPE	ND

500 SARS-CoV-2 viral particles per sample
 2-fold dilution starting from 1:100
 data analyzed at 4 d.p.i.

