FLAVONOID LIBRARY SCREENING REVEALS HIGHLY ANTIVIRAL MITIGANTS OF AFRICAN SWINE FEVER VIRUS

Charles C. Elrod,[1, 2] **Joshua A. Jackman,**[3] **Erik Arabyan,**[4] **and Hovakim Zakaryan**[4]

[1]*Natural Biologics Inc., Newfield, NY 14867, USA;* [2]*Department of Animal Science, Cornell University, Ithaca, NY 14853, USA;*
[3]*School of Chemical Engineering, Sungkyunkwan University, Suwon 16419, Republic of Korea;*
[4]*Group of Antiviral Defense Mechanisms, Institute of Molecular Biology of NAS, Yerevan, Armenia*

Funding For This Research Provided By:
Natural Biologics, Inc.,
Newfield, NY

natural biologics®
solutions by nature

INTRODUCTION:

Naturally occurring plant flavonoids are a promising class of antiviral agents that may be used to inhibit African swine fever virus (ASFV). Published studies of specific flavonoids have reported a wide range of antiviral mechanisms against ASFV and other viruses, which motivates a broader search for flavonoids with greater anti-ASFV potency and unique mechanisms of action.

OBJECTIVE:

To screen a library of 90 flavonoid compounds in a Vero cell-based antiviral assay against the ASFV BA71V strain.

METHODS:

Flavonoid Screening Assay

- Confluent Vero cells were infected with ASFV BA71V (0.2 $TCID_{50}$/well) and immediately treated with the flavonoids at 20 µg/ml or 10 µg/ml for 7,8-benzoflavone, diosmin, and khellin.
- ASFV-infected cells treated with DMSO (<0.5%) served as a negative control while ASFV-infected cells treated with 20 µg/ml apigenin served as a positive control.
- The infection was allowed to proceed for 72 hours when full cytopathic effect (CPE) was observed in the negative control wells.
- After incubation, the cells were washed with MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and incubated for two hours.
- Purple formazan was extracted with DMSO and the optical density of each well was read at 570 nm.
- The percentage of CPE inhibition was measured for each compound and triplicate measurements were performed.
- Positive hits exhibited more than 40% CPE inhibition with no apparent cell monolayer damage.

Cytotoxicity Assay

- The cytotoxicity of positive-hit flavonoids was studied in Vero cells by the crystal violet staining method.
- Confluent Vero cells were exposed to the flavonoids at concentrations ranging from 2.5 to 20 µg/ml for 24 hours.
- Crystal violet solution was then added and incubated for 40 minutes, followed by washing and methanol addition.
- The optical density of each well was measured at 570 nm and the 50% cell cytotoxicity level was calculated for each flavonoid.

Antiviral Assay

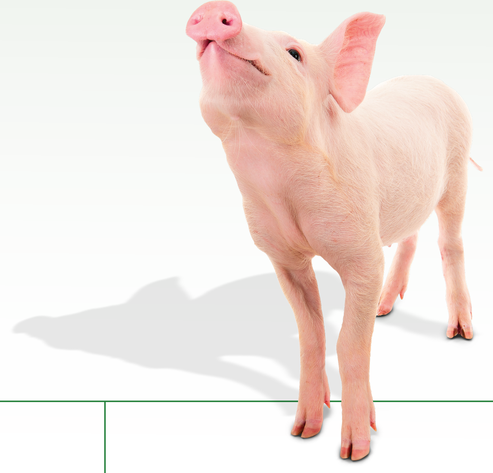
- Confluent Vero cells were infected with ASFV BA71V (0.2 $TCID_{50}$/well) and immediately treated with the selected flavonoids at 20 µg/ml or 10 µg/ml for 7,8-benzoflavone, diosmin, and khellin.
- After the first cycle of viral replication had occurred by 24 hours, the supernatant was harvested for virus titration.
- Serial dilutions of the supernatant were performed and the samples were added to confluent Vero cells. The infection was allowed to proceed for 72 hours until negative control wells showed complete cytopathic effects.
- The titer was calculated using the Spearman-Kärber endpoint method and expressed as log $TCID_{50}$/ml.

Virucidal Assay

- ASFV BA71V (2 x 10^5 $TCID_{50}$) was incubated with each flavonoid for one hour, and then diluted 20-fold and added to confluent Vero cells.
- The infection was allowed to proceed for 24 hours and the virus titer was titrated by CPE assay.

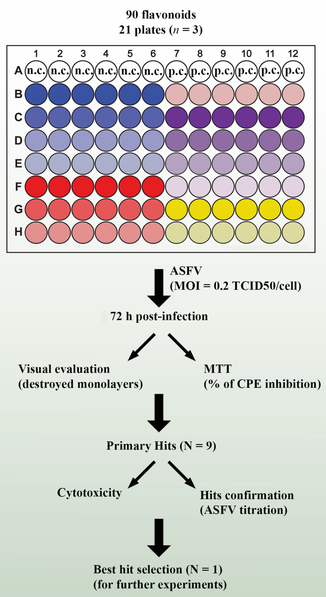
SUMMARY:

A wide range of plant extracts, known as flavonoids, have been shown to inhibit viral pathogens. Previous work from our team has demonstrated that certain flavonoids are effective at disrupting the infection and replication cycle of African Swine Fever virus, which causes nearly 100% mortality in infected pigs. In this study, we sought to broaden our knowledge of antiviral flavonoid compounds by screening a library of 90 compounds against the African Swine Fever virus. From that pool of candidates, we identified nine which effectively inhibited the virus. Of those, one directly inactivated the virus, while the other 8 flavonoids disrupted various stages of viral infection and replication. Kaempferol, an extract of saffron, cumin and capers, was observed to have the greatest inhibitory activity against the virus. More detailed analysis of Kaempferol's antiviral activity will be presented in an oral session at this meeting.



RESULTS:

FIGURE 1: Flow Chart of Experiments.

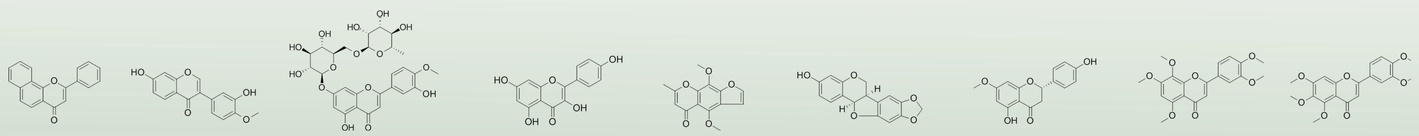


From the screening, flavonoids which exhibited >40% inhibition of ASFV and no apparent cell monolayer damage were selected as positive hits. Nine such flavonoids were then included in the antiviral and virucidal evaluations.

TABLE 1: Antiviral and virucidal activities of flavonoids against ASFV *in vitro* (log $TCID_{50}$/mL). * P<0.05

	Control *(virus only)*	7,8-benzo.	Calycosin	Diosmin	Isosinensetin	Kaempferol	Khellin	Maackiain	Sakuranetin	Sinensetin
Antiviral	5.14	4.47*	4.46	4.48*	4.25	3.98*	4.52	4.2*	4.3*	4.75*
Virucidal	5.24	4.64*	5.0	5.02	5.35	5.5	5.18	5.2	5.14	5.7

FIGURE 2: Molecular structures of the nine identified flavonoids that inhibited ≥40% ASFV-induced cell cytopathic effect *in vitro*.



7,8-benzoflavone
Formula: C19H12O2
M.w.: 272.3 g/mol

Calycosin
Formula: C16H12O5
M.w.: 284.26 g/mol

Diosmin
Formula: C28H32O15
M.w.: 608.54 g/mol

Kaempferol
Formula: C15H10O6
M.w.: 286.2 g/mol

Khellin
Formula: C14H12O5
M.w.: 260.2 g/mol

Maackiain
Formula: C16H12O5
M.w.: 284.27 g/mol

Sakuranetin
Formula: C16H14O5
M.w.: 286.3 g/mol

Isosinensetin
Formula: C20H20O7
M.w.: 372.4 g/mol

Sinensetin
Formula: C20H20O7
M.w.: 372.37 g/mol

FIGURE 3: Antiviral activity of the nine positive-hit flavonoids.

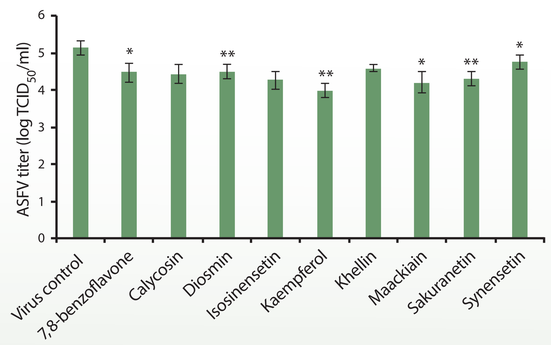


FIGURE 4: Virucidal activity of the nine positive-hit flavonoids.