Antiviral activity of *Deschampsia antarctica* plant extracts *in vitro*

Svitlana Rybalko[1], Oksana Poronnik[2,3*], Ganna Myryuta[2,3], Anatoliy Balanda[2], Maryna Arkhypova[1], Daria Starosyla[1], Oleg Deryabin[1], Anton Puhovkin[3,4], Ivan Parnikoza[2,3], Viktor Kunakh[2]

[1]*L.V. Gromashevsky Institute of Epidemiology and Infectious Diseases of the NAMSU, Kyiv, 03038, Ukraine*
[2]*Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, Kyiv, 03143, Ukraine*
[3]*State Institution National Antarctic Scientific Center, Ministry of Education and Science of Ukraine, Kyiv, 01601, Ukraine*
[4]*Masaryk University, Faculty of Science, Department of Experimental Biology, Kamenice 5, A13–119, Brno 62500, Czech Republic*

Abstract

Main objective of research to study the *D. antarctica* extracts antiviral activity which was grown *in vitro* and propagated by cloning. The *D. antarctica* aqueous ethanolic extracts was tested on *in vitro* models of MDCK – Madin-Darby Canine Kidney cells and PEK – Porcine Embryonic Kidney cells and influenza virus, A/FM/1/47(H1N1) strain and transmissible gastroenteritis virus – porcine coronavirus (TGEV). The antiviral activity of *D. antarctica* plant extracts (G/D9-1 genotype) on experimental models of influenza viruses and Coronavirus TGEV *in vitro* was conducted. *D. antarctica* plant extracts high antiviral activity on influenza viruses and Coronavirus TGEV *in vitro* was shown.

Key words: *Deschampsia antarctica* extracts, antiviral activity, polyphenolic compounds, flavonoids

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Introduction

Antarctic vascular plants, in particular *Deschampsia antarctica* É. Desv., growing in most extreme environmental conditions, can be a promising source of polyphenolic compounds with a wide spectrum of biological activity(Köhler et al. 2017, Kunakh et al. 2023). Medicinal plants have an almost infinite variety of chemical compounds, among them it is possible to find active substances that can be used to in-

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*Corresponding author: O. Poronnik <oksana_poronnik@ukr.net>
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hibit the replication of both DNA and RNA viruses (Akram et al. 2018). So polyphenols block the entry into the host cells, inhibit the multiplication of the virus, seal blood vessels and protect against superinfection (Chojnacka et al. 2021). Some of the flavonoids showed more potent antiviral activity than the market available drugs used to treat viral infections (Badshah et al. 2021).

Analysis of *D. antarctica* plant extracts (G/D9-1 genotype) by MALDI MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) showed the presence of polyphenolic substances: simple phenols/hydroxybenzoic acids and their derivatives, hydroxycinnamic acids and their derivatives, flavonoids and their derivatives (coumestrol, tricin, luteolin trimethyl ether, isoswertisin, orientin, isoswertiajaponin, tetrahydroxy-methoxyflavone glucuronide, isoswertisin 2"-O-beta-arabinoside (apigenin derivatives), isoswertiajaponin 2"-O-beta-arabinopyranoside) and fatty acids (Ivannikov et al. 2021). Previously, *D. antarctica* extracts were shown to have antitumor and antioxidant activity (Gidekel et al. 2011, Ivannikov et al. 2021).

In this research, we studied the antiviral activity of extracts from *D. antarctica*, which was grown *in vitro* and propagated by cloning.

Material and Methods

Only two indigenous flowering plant species: *Deschampsia antarctica* É. Desv. (Poaceae) and *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae) are found in the maritime Antarctic. The plants of *D. antarctica* used in this study were sampled from the field and then cultivated on agar medium under greenhouse conditions. To study antiviral activity, an extract of *in vitro* cultivated *D. antarctica* (genotype G/D9-1) was taken as described in (Kunakh et al. 2023). The extracts were prepared from dry plant leaves of a 1,5-month-old plant (Fig. 1).

The extracts were prepared from dry plant leaves of a 1,5-month-old plant. Dried leaves of plants of this genotype were ground into powder and extracted with ethanol at a concentration of 45% at the rate 1 g of dry plant material per of 10 ml, for 7 days at room temperature. To prepare the reconstituted extract, 5 ml of the original extract was evaporated in a rotary evaporator and the resulting dry residue was dissolved in sterile distilled water to the volume of the original extract.

Aqueous ethanolic extracts of *D. antarctica* leaves were used for *in vitro* experiments.

The following continuous cell lines were used: MDCK – Madin-Darby Canine Kidney cells and PEK – Porcine Embryonic Kidney cells.

The viruses used in the study:

1. Influenza virus, A/FM/1/47(H1N1) strain, was obtained from the Depository of Viruses at L.V. Gromashevsky Institute of Epidemiology and Infectious Diseases, Academy of Medical Sciences of Ukraine. Infectious titer in MDCK cells - 3 – 10.0 lg ID_{50}/0.2 mL, hemagglutinin titer – 1:512 hemagglutination units/0.2 mL.

2. TGEV (Transmissible GastroEnteritis Virus) – porcine coronavirus, etiologic agents of porcine transmissible gastroenteritis. $D_{52\text{-}5}$ (BRE_{79}) strain is highly pathogenic for the pigs of any age. The virus used in the study was obtained after 5 passages in ST cells (fibroblast-like cells isolated from testicle of a pig). The strain of TGEV was kindly provided by Dr. Hubert Laude from Molecular Virology and Immunology Laboratory of INRA Biotechnological Center, Jouy-en-Josas, France.

Fig. 1. *Deschampsia antarctica in vitro,* G/D9-1 genotype (1,5-month-old plant).

The infectivity of virus was assessed by two methods. The technique of the end-point dilution was based on estimating cytopathic effect (CPE) in cell culture. The infectious titer was calculated by Kerber-Ashmarin and expressed as tissue cytopathic dose $(TCD)_{50}$/mL. The analysis of the negative plaques (S marker) was performed in 1.35% agar (Difco-Bacto) and the infectious titer was expressed in plaque-forming units (PFU)/mL. The results were registered following 120-h culture at 38°C.

50% cytotoxic concentration (CC_{50}) of the extracts under study was assessed by CPE in MDCK and PEK cells. The cells were seeded in a 96-well plate and various dissolution of the assayed extracts were added in triplicate. The cells were incubated for 5 days at 37°C in a 5% CO_2-humidified incubator. The plates were viewed daily for detecting CPE presence or absence. CPE (cell rounding, degeneration, cell detachment from the surface of the wells) was assessed by scoring the cell layer by 5-point scale such as follows:

"–" – degeneration is absent

"+" – damage of cell layer by not more than 25%

"++" – damage of cell layer by not more than 50%

"+++" – damage of cell layer by not more than 75%

"++++" – complete degeneration of cell layer

50% cytotoxic concentration (CC_{50}) was then calculated as the compound concentration required for the reduction of cell viability by 50%.

For assaying antiviral activity of the substances, 50% effective concentration (EC_{50}) was estimated as the compound concentration exerting half-maximal protective effect assessed by CPE inhibition and reduction of the infectious titer by not less than 2 lg. For assessing EC_{50}, test virus was added to cells grown in 96-well plate in a dose of 100 TCD_{50}/0.1 mL. Upon absorption of virus (60 min, 37°C), the cells were washed and the maintenance medium (RPMI-1640 with 2% of fetal calf serum) was added. Then the assayed substances were added in different concentrations. The protection against virus-induced CPE with the reduction of infectious titer by not less than 2 lg allowed calculating EC_{50} of the substance under study.

The selectivity index (SI) was calculated as CC_{50} to EC_{50} ratio.

The anti-influenza activity of the substances under study *in vitro* was assessed in MDCK cells. The cell monolayer in microplates was washed with trypsin-TPCK (50 µL per well). Then influenza virus was added at a dose of 100 TCD_{50}. Upon absorption of virus, the cells were washed and the assayed substances were added in different concentrations. The cells were cultured for 3 days; the state of cells was checked daily under microscope. In 72 h, the culture medium was collected for the assessment of the infectious titer of influenza virus.

Anti-coronavirus activity was assayed in PEK cells infected with TGEV at a dose of 100 TCD_{50}. The infected cells treated with the assayed substances were cultured for 3 days, and then the culture medium was collected for the assessment of TGEV infectious titer (Stefanov 2001[1]).

Statistical analysis

The data obtained were statistically processed by Microsoft Excel software. The significance of the difference was calculated by Student's t-test. The difference was considered as significant at $p < 0.05$.

EC50, CC50 and SI were calculated using nonlinear regression analysis (Stefanov 2001[1]).

Results and Discussion

The study of the cytotoxic effects of two types of *D. antarctica* G/D9-1extracts of genotype in MDCK and PEK cells demonstrated significant difference (Table 1).

Extract	**Cell culture**	
	MDCK	PEK
	Extract dilution corresponding to CC_{50}	
Primary	1\128	1\128
Reconstituted	1\32	1\16

Table 1. CC_{50} of two types of extracts of *D. antarctica in vitro,* G/D9-1 genotype in MDCK and PEK cells.

The primary extract of *D. antarctica in vitro* turned out to be more cytotoxic as compared to the reconstituted one.

The data on the assessment of the EC_{50} of the substances in the models of influenza virus A/FM/1/47(H1N1) and TGEV are demonstrated in Figs. 2-5.

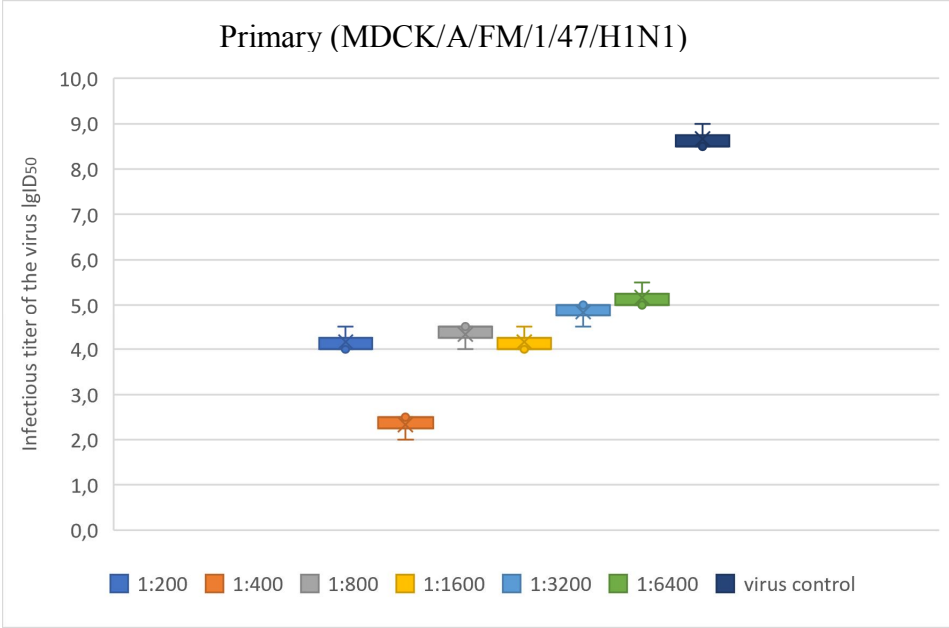

Fig. 2. Infectious titer of influenza virus A/FM/1/47(H1N1) in MDCK cells treated with the primary extract of *D. antarctica in vitro,* G/D9-1 genotype.

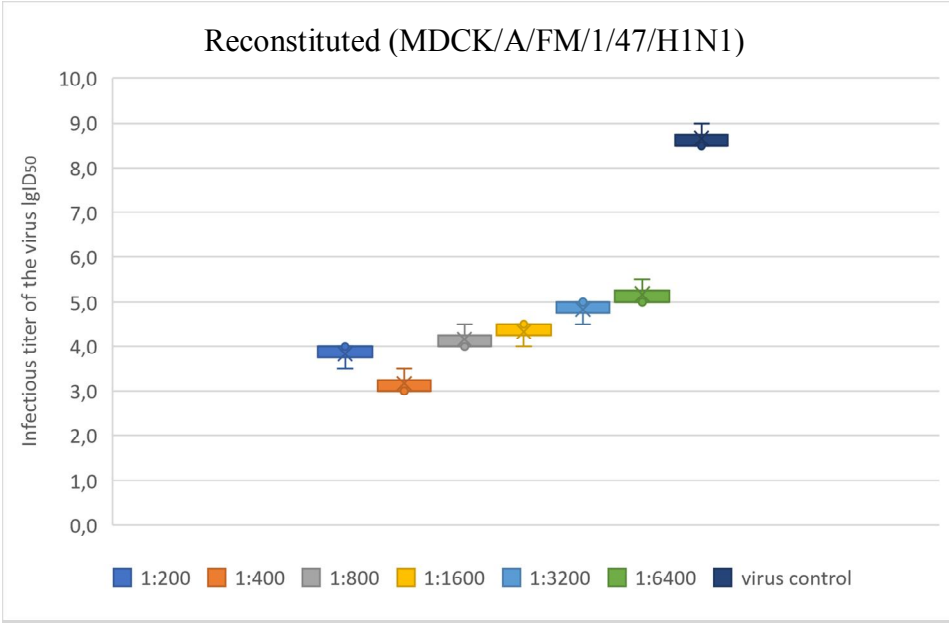

Fig. 3. Infectious titer of influenza virus A/FM/1/47(H1N1) in MDCK cells treated with the reconstituted extract of *D. antarctica in vitro,* G/D9-1 genotype.

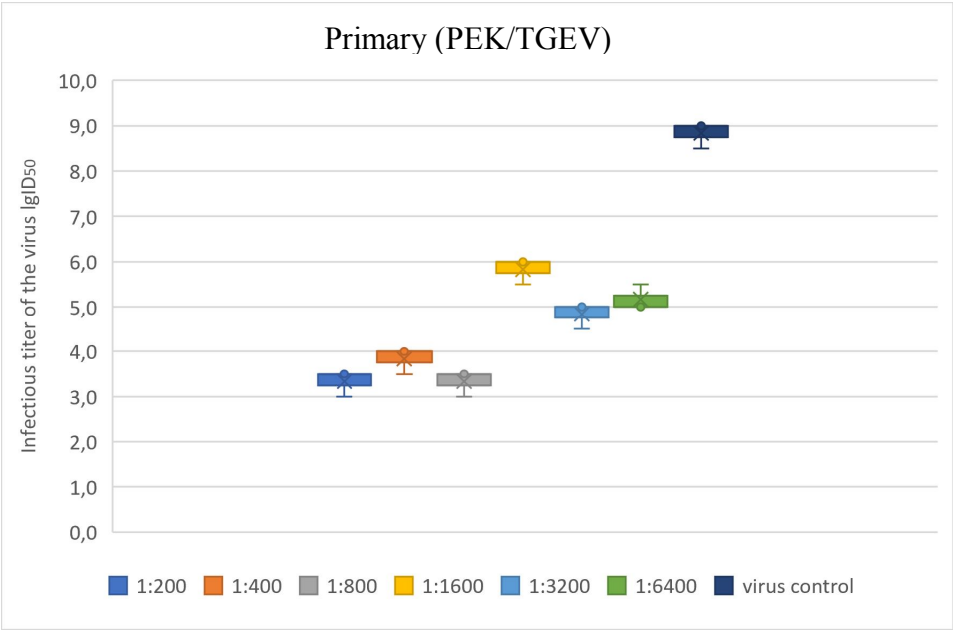

Fig. 4. Infectious titer of TGEV in PEK cells treated with the primary extract of *D. antarctica in vitro,* G/D9-1 genotype.

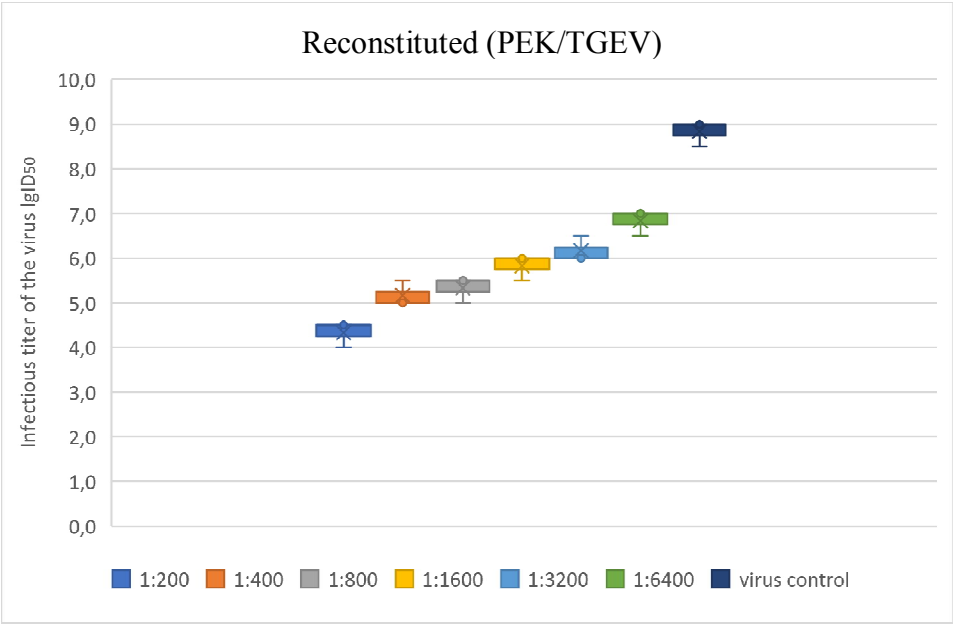

Fig. 5. Infectious titer of TGEV in PEK cells treated with the reconstituted extract of *D. antarctica in vitro,* G/D9-1 genotype.

The data demonstrated that the assayed extracts of *D. antarctica in vitro,* G/D9-1 genotype, inhibited reproduction of influenza virus A/FM/1/47(H1N1) and TGEV in dilutions up to 1:6400 effectively. The calculated selectivity index is presented in Table 2.

Virus	Extract *D. antarctica*	CC_{50}, mkg/ml	EC_{50}, mkg/ml	SI
Influenza A/FM/1/47(H1N1)	Primary	1:128	1:6400	50
	Reconstituted	1:32	1:6400	200
Coronavirus TGEV	Primary	1:128	1:6400	50
	Reconstituted	1:16	1:6400	400

Table 2. Selectivity index (SI) for the assayed extracts of *D. antarctica in vitro,* G/D9-1 genotype for influenza virus and coronavirus.

Therefore, selectivity index for the reconstituted extract for 4-fold higher for influenza virus and 8-fold higher for TGEV. Selectivity index for both extracts exceeds the value of 16 set as acceptance criterion for assessing prospecting substances with antiviral activity.

This result can be explained by fact that the *D. antarctica in vitro* water-ethanol extract contains apigenin and luteolin as part of the polyphenolic fraction.

Earlier, flavonoids-containing phytopreparation antiviral activity against human alphaherpesvirus 2, hepatitis C surrogate virus and transmissible gastroenteritis coronavirus was shown by this method (Galkin et al. 2023).

In addition, apigenine and luteolin studies have shown antiviral activity against viruses of SARS-CoV 3CL(pro), hepatitis C, enterovirus-71 and coxsackievirus A16 (Ryu et al. 2010, Shibata et al. 2014, Zhang et al. 2014, Dai et al. 2019, Xu et al. 2014).

We plan to continue researching the antiviral activity of plant extracts from the Antarctic flora.

Conclusion

For the first time, conducted studies of the antiviral activity of *D. antarctica*.

G/D9-1 genotype plant extracts on experimental models of influenza viruses and Coronavirus TGEV *in vitro* showed high antiviral activity of its extracts.

References

AKRAM, M., MAHMOOD, I., TAHIR, S., SHAH, M. A., MAHMOOD, Z., ALTAF, A., AHMAD, K., MUNIR, N., DANIYAL, M., NASIR, S. and MEHBOOB, H. (2018): Antiviral potential of medicinal plants against HIV, HSV, influenza, hepatitis, and coxsackievirus: A systematic review. *Phytotherapy Research*, 32(5): 811-822. doi: 10.1002/ptr.6024

BADSHAH, S. L., FAISAL, S., MUHAMMAD, A., POULSON, B. G., EMWAS, A. H. and JAREMKO, M. (2021): Antiviral activities of Flavonoids. *Biomedicine & Pharmacotherapy*, 140: 111596. doi: 10.1016/j.biopha.2021.111596

CHOJNACKA, K., SKRZYPCZAK, D., IZYDORCZYK, G., MIKULA, K., SZOPA, D. and WITEK-KROWIAK, A. (2021): Antiviral properties of polyphenols from plants. *Foods*, 10: 2277. doi: 10.3390/foods10102277

DAI, W., BI, J., LI, F., WANG, S., HUANG, X., MENG, X., SUN, B., WANG, D., KONG, W. and JIANG, C. J. V. (2019): Antiviral efficacy of flavonoids against enterovirus 71 infection *in vitro* and in newborn mice. *Viruses*, 11(7): 25. doi: 10.3390/v11070625

GALKIN, N. A., ARKCYPOVA, M., STAROSYLA, D., DERIABIN, O., VASYLCHENKO, O., RYBALKO, S. and GOLEMBIOVSKA, O. (2023): Antiviral activity of original flavonoids-containing phytopreparation against human alphaherpesvirus2, hepatitis C surrogatevirus and transmissible gastroenteritis coronavirus. *Farmacia*, 71(5): 991-1000. doi: 10.31925/farmacia.2023.5.14

GIDEKEL, M., WEBER, H., CABRERA, G., GUTIERRES, A., OSORIO, J., BECERA, J., PODHAJCER, O., CAFFERATA, E., SUNKEL, C. and MIHOVILOVIC, I. (2011): Extracts of *Deschampsia antarctica* Desv. with antineoplastic activity. Pat. US 2011/0177178 A1, Appl. No. 12/928317

IVANNIKOV, R., LAGUTA, I., ANISHCHENKO, V., SKOROCHOD, I., KUZEMA, P., STAVINSKAYA, O., PARNIKOZA, I., PORONNIK, O., MYRYUTA, G. and KUNAKH, V. (2021): Composition and radical scavenging activity of the extracts from *Deschampsia antarctica* E. Desv. plants grown *in situ* and *in vitro*. *Chemistry Journal of Moldova*, 16: 105-114. doi: 10.19261/cjm.2021.841

KÖHLER, H., CONTRERAS, R. A., PIZARRO, M., CORTÉS ANTÍQUERA, R. and ZÚÑIGA, G. E. (2017): Antioxidant responses induced by UVB radiation in *Deschampsia antarctica* Desv. *Frontiers in Plant Science*, 8: 921. doi: 10.3389/fpls.2017.00921

KUNAKH, V. A., TWARDOVSKA, M. O., ANDREEV, I. O., DROBYK, N. V., NAVROTSKA, D. O., NUZHYNA, N., PORONNIK, O. O., KONVALYUK, I. I., IVANNIKOV, R.V. and PARNIKOZA, I. YU. (2023): Composition and antioxidant properties of plant extracts from Antarctica vascular plants (*Deschampsia antarctica* E. Desv.) *Polish Polar Research*, 44(1): 41-68. doi: 10.24425/ppr.2023.144537

RYU, Y. B., JEONG, H. J., KIM, J. H., KIM, Y. M., PARK, J.-Y., KIM, D., NAGUYEN, T. T. H., PARK, S.-J., CHANG, J. S. and PARK, K. H. J. B. (2010): Biflavonoids from *Torreya nucifera* displaying SARS-CoV 3CL(pro) inhibition. *Bioorganic & Medicinal Chemistry*, 18(22): 7940-7947. doi: 10.1016/j.bmc.2010.09.035

SHIBATA, C., OHNO, M., OTSUKA, M., KISHIKAWA, T., GOTO, K., MUROYAMA, R., KATO, N., YOSHIKAWA, T., TAKATA, A. and KOIKE, K. J. V. (2014): The flavonoid apigenin inhibits hepatitis C virus replication by decreasing mature microRNA122 levels. *Virology*, 462: 42-48. doi: 10.1016/j.virol.2014.05.024

XU, L., SU, W., JIN, J., CHEN, J., LI, X., ZHANG, X., SUN, M., SUN, S., FAN, P., AN, D., ZHANG, H., ZHANG, X., KONG, W., MA, T. and JIANG, C. (2014): Identification of luteolin as enterovirus 71 and coxsackievirus A16 inhibitors through reporter viruses and cell viability-based screening. *Viruses*, 6(7): 2778-2795. doi: 10.3390/v6072778

ZHANG, W., QIAO, H., LV, Y., WANG, J., CHEN, X., HOU, Y., TAN, R. and LI, E. (2014): Apigenin inhibits enterovirus-71 Infection by disrupting viral RNA association with transacting factors. *PLoS One*, 9(10): 110429. doi: 10.1371/journal.pone.0110429

Web sources / Other sources

[1] Preclinical studies of medicinal products (methodological recommendations), 2001. Edited by O.V. Stefanov. Kyiv: Avicenna. 528 p. (In Ukraine).