

Supplementary material

## ***Pulsatilla vulgaris* Inhibits Cancer Proliferation in Signaling Pathways of 12 Reporter Genes**

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**Supplementary Table S1.** Results of tests of biological activity of secondary metabolites isolated from methanolic extract of roots (mR) and leaves (mL) of *Pulsatilla vulgaris* Mill. and reference/control compound - resveratrol analogue, in the presence of various inducers on human cervical carcinoma HeLa cell lines with 13 different vectors (transcription factors) in volume 40 µg/mL (I assay)

Sample data	Transcription factors (vectors) <sup>1</sup> in the presence of various inducers <sup>2</sup> in the luciferase reporter gene assay													
	Stat3/ IL-6	Smad/ TGF-β	AP-1/ PMA	NF-κB/ PMA	E2F/ PMA	MYC/ PMA	Ets/ PMA	Notch (CSL- Luc)	FoxO in 10% FBS	Wnt/ wnt-3a	Hdghog/ PMA	pTK (4h)	miR-21	k-Ras
Resveratrol analog (6 µM)	92 <sup>3</sup>	68	78	28	64	47	62	65	66	44	57	95	78	86
<i>P. vulgaris</i> (mL 40 µg/mL) - I assay	98	154	96	57	115	96	106	109	134	179	120	141	156	126
<i>P. vulgaris</i> (mR 40 µg/mL) - I assay	-6	-4	-1	0	-11	-37	-7	-31	132	-13	-4	2	2	1

**Explanations to the table:**

<sup>1</sup> **Transcription factors, vectors in the signal transduction pathways of cancer processes** - Stat3, Smad, AP-1, NF-κB, E2F, MYC, Ets, Notch, FoxO, Wnt, Hdghog, miR-21, k-Ras and pTK - control

<sup>2</sup> **Inducer, promotor** of cancer processes - IL-6, TGF-β, PMA, wnt-3a

<sup>3</sup> Numerical value expressing the percentage ratio of the activity of the tested samples (metabolites) to the activity of the promoter based on the determination of the activity of the protein encoded by the luciferase reporter gene.

The lower the numerical value in the table, the greater the activity of the test sample is in inhibition than that of the tumor promoter.

Resveratrol analog – compounds with antitumor activity used in the research (explanations in the text)

*P. vulgaris* (mL 40 µg/mL) – is the volume of methanolic extract from the leaves of the *P. vulgaris* used for the -I assay - not biologically active

*P. vulgaris* (mR 40 µg/mL) – is the volume of methanolic extract from the root of the *P. vulgaris* used for the – I assay - biologically active antitumor

**Supplementary Table S2.** Results of tests of biological activity of secondary metabolites isolated from methanolic extract of roots (mR) of *Pulsatilla vulgaris* Mill. using their different volumes: 40 µg/mL (I assay) and 15 µg/mL and 10 µg/mL (II assay), in the presence of various inducers on human cervical carcinoma HeLa cell lines with 13 different vectors (transcription factors)

Sample data	Transcription factors (vectors) <sup>1</sup> in the presence of various inducers <sup>2</sup> in the luciferase reporter gene assay													
	Stat3/ IL-6	Smad/ TGF-β	AP-1/ PMA	NF-κB/ PMA	E2F/ PMA	MYC/ PMA	Ets/ PMA	Notch (CSL- Luc)	FoxO in 10% FBS	Wnt/ wnt-3a	Hdghog/ PMA	pTK (4h)	miR-21	k-Ras
Resveratrol analog (6 µM)	92 <sup>3</sup>	68	78	28	64	47	62	65	66	44	57	95	78	86
<i>P. vulgaris</i> (mR 40 µg/mL) - I assay	-6	-4	-1	0	-11	-37	-7	-31	132	-13	-4	2	2	1
<i>P. vulgaris</i> (mR 15 µg/mL) - II assay	-5	-4	-1	-9	-6	-33	-14	-31	129	-20	-4	4	8	2
<i>P. vulgaris</i> (mR 10 µg/mL) - II assay	36	9	95	11	99	17	23	120	126	34	88	105	168	113

**Explanations to the table:**

<sup>1</sup> **Transcription factors, vectors in the signal transduction pathways of cancer processes** - Stat3, Smad, AP-1, NF-κB, E2F, MYC, Ets, Notch, FoxO, Wnt, Hdghog, miR-21, k-Ras and pTK - control

<sup>2</sup> **Inducer, promotor** of cancer processes - IL-6, TGF-β, PMA, wnt-3a

<sup>3</sup> Numerical value expressing the percentage ratio of the activity of the tested samples (metabolites) to the activity of the promoter based on the determination of the activity of the protein encoded by the luciferase reporter gene.

The lower the numerical value in the table, the greater the activity of the test sample is in inhibition than that of the tumor promoter.

Resveratrol analog – compounds with antitumor activity used in the research (explanations in the text)

*P. vulgaris* (mR 40 µg/mL) – is the volume of methanolic extract from the root of the *P. vulgaris* used for the tests - test I - biologically active antitumor

*P. vulgaris* (mR 15 µg/mL) – is the volume of methanolic extract from the root of the *P. vulgaris* used for the tests - test II - biologically active antitumor

*P. vulgaris* (mR 10 µg/mL) – is the volume of methanolic extract from the root of the *P. vulgaris* used for the tests - test II - biologically active antitumor