

# Usnic Acid Isolated from *Usnea antarctica* (Du Rietz) Reduced In Vitro Angiogenesis in VEGF- and b-FGF- Stimulated HUVECs and Ex Ovo in Quail Chorioallantoic Membrane (CAM) Assay

Klaudia Petrová<sup>1\*</sup>, Miriam Bačkorová<sup>2</sup>, Zuzana Demčíšáková<sup>3</sup>, Eva Petrovová<sup>3</sup>, Richard Frenák<sup>4</sup>,  
Michal Goga<sup>4</sup>, Martin Bačkor<sup>4,5</sup>, Ján Mojžiš<sup>1</sup>, and Martin Kello<sup>1\*</sup>

- <sup>1</sup> Department of Pharmacology, Faculty of Medicine, Pavol Jozef Šafárik University, 040 01 Košice, Slovakia
- <sup>2</sup> Department of Pharmaceutical Technology, Pharmacognosy, and Botany, University of Veterinary Medicine and Pharmacy, 041 81, Košice, Slovakia
- <sup>3</sup> Department of Anatomy, Histology, and Physiology, University of Veterinary Medicine and Pharmacy, 041 81 Košice, Slovakia
- <sup>4</sup> Department of Botany, Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafárik University, Mánesova 23, 041 67, Košice, Slovakia
- <sup>5</sup> Institute of Biotechnology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Trieda Andreja Hlinku 2, 949 76, Nitra, Slovakia; \*  
Correspondence: claudy.petrova@gmail.com (K.P.); kellomartin@yahoo.com (M.K.)

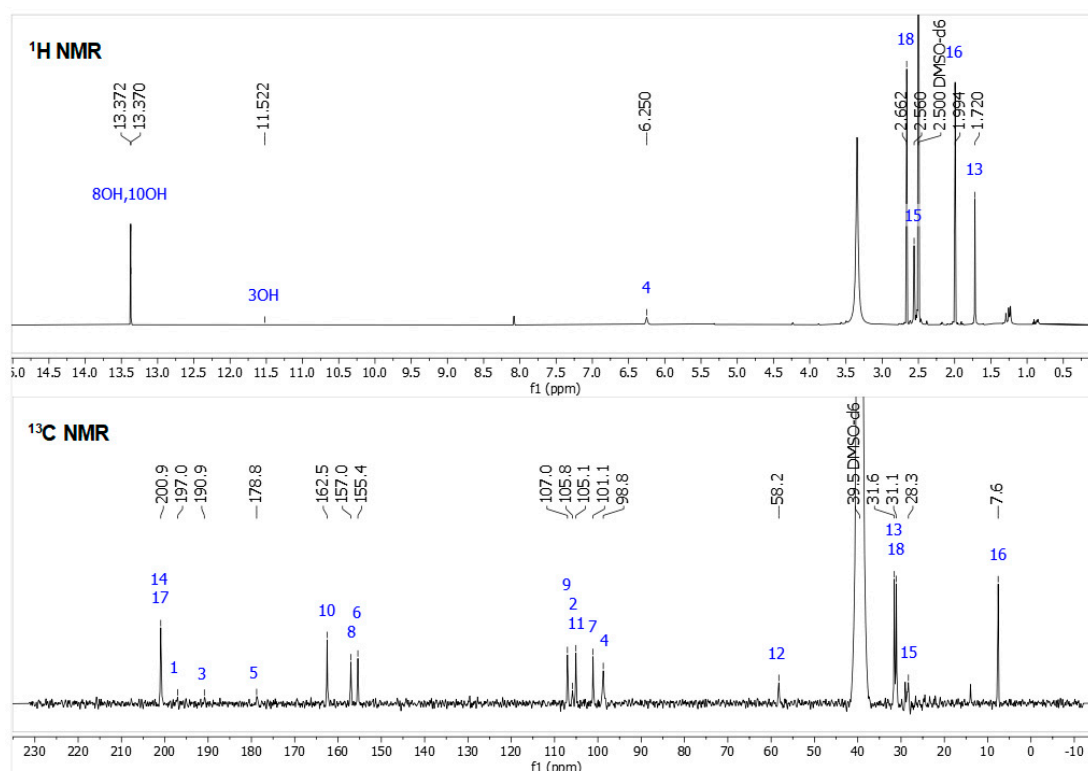
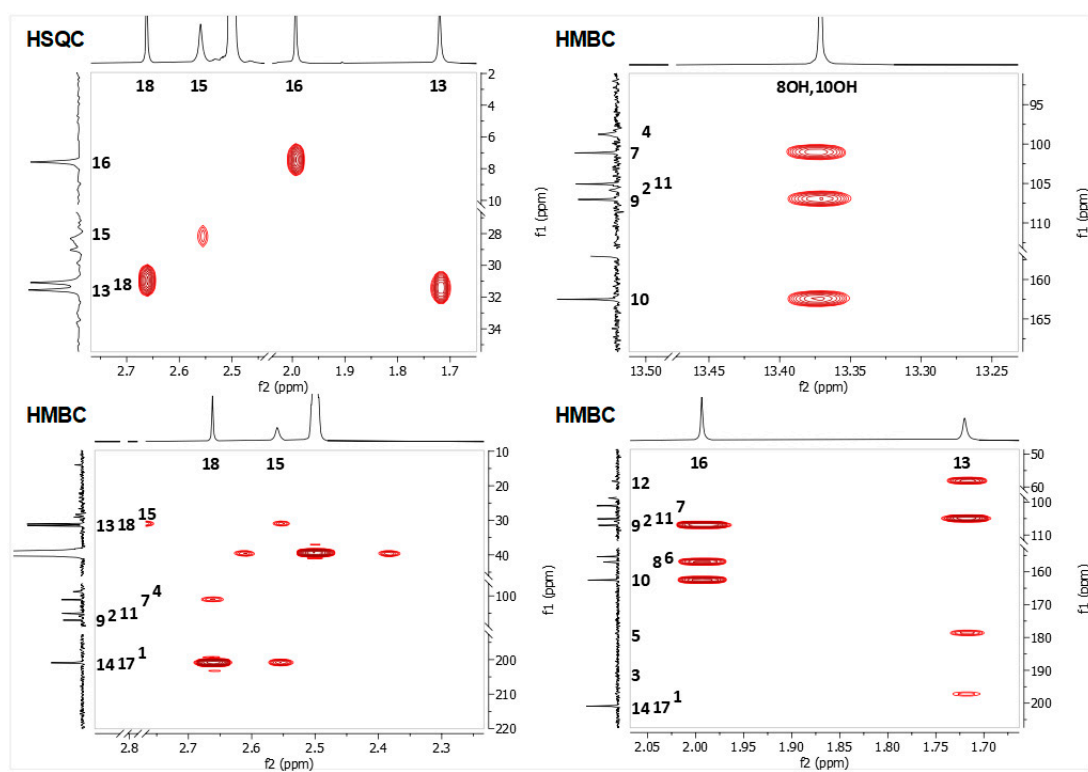
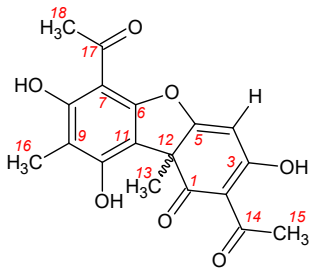


Figure S1. <sup>1</sup>H (600 MHz, DMSO-d<sub>6</sub>) and <sup>13</sup>C (150 MHz, DMSO-d<sub>6</sub>) NMR spectra of usnic acid.

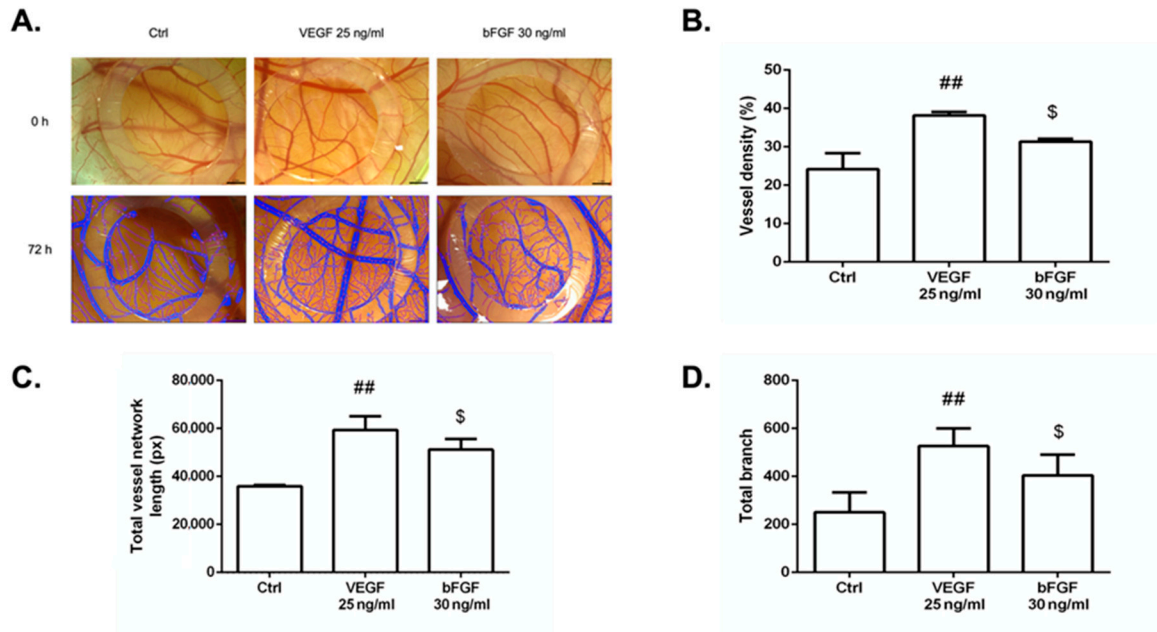


**Figure S2.** HSQC and HMBC spectra of usnic acid.

**Table S1.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR chemical shifts of isolated usnic acid in DMSO- $d_6$ .

			
No.	$\delta_{\text{H}}$ [ppm]	$\delta_{\text{C}}$ [ppm]	HMBC correlations
1		197.0	
2		105.8	
3	11.33*	190.9	
4	6.31	98.8	
5		178.8	
6		155.4	
7		101.1	
8	13.37*	157.0	101.1 (C-7), 107.0 (C-9)
9		107.0	
10	13.37*	162.5	
11		105.1	
12		58.2	
13	1.74	31.6	58.2 (C-12), 105.1 (C-11), 178.8 (C-5), 197.0 (C-1)
14		201.0	
15	2.59	28.3	105.8 (C-2), 201.0 (C-14)
16	2.00	7.6	107.0 (C-9), 157.0 (C-8), 162.5 (C-10)
17		201.0	
18	2.67	31.1	101.1 (C-7), 201.0 (C-17)

\*  $^1\text{H}$  chemical shift of OH proton



**Figure S3:** Proangiogenic effect of VEGF (25ng/ml) and bFGF (30ng/ml) on CAM vascularization (**A**). The results are summarized in the graphs as vessel density (**B**), total vessel network length (**C**), and total branch (**D**). Each group contained 10 CAMs and the experiment was repeated three times. Error bars represent  $\pm$  SD (<sup>##</sup> $p < 0.01$  versus VEGF; <sup>\$</sup> $p < 0.05$  versus bFGF).