

Supporting Information

Isolation and Determination of Phenolic Glycosides and Anthraquinones from Rhizomes of Various *Reynoutria* Species

Izabela Nawrot-Hadzik¹, Sebastian Granica², Krzysztof Domaradzki³, Łukasz Pecio⁴, Adam Matkowski^{1,5}

Affiliation

¹ Department of Pharmaceutical Biology and Botany, Medical University, Wrocław, Poland

² Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University, Warsaw, Poland

³ Department of Weed Science and Soil Tillage Systems, IUNG-Institute of Soil Science and Plant Cultivation State Research Institute and Soil Science, Wrocław, Poland

⁴ Department of Crop Biochemistry and Crop Quality, IUNG-Institute of Soil Science and Plant Cultivation State Research Institute, Puławy, Poland

⁵ Botanical Garden of Medicinal Plants, Wrocław Medical University, Poland

Correspondence

Prof. Dr. Adam Matkowski

ul. Borowska 211

50556 Wrocław

Poland

Phone: +48717840497

Fax +48717840498

pharmaceutical.biology@wp.eu

Material and Methods

Development and validation of analytical HPLC method

In the preliminary experiments, we used different reversed phase HPLC columns (Agilent Zorbax-SB₁₈, XDB-C₁₈, Phenomenex Kinetex C₁₈, XB-C₁₈ and C₈) and checked different mobile phases (MeOH:H₂O, CH₃CN:H₂O with or without formic or acetic acids) using flow rates from 0.2 to 0.4 mL/min. In order to obtain better chromatographic separation, we used gradient elution at different temperatures (20, 25, 30, and 40°C). Finally, the best separation was obtained using the set up described in Material and Methods section of the main article. To achieve optimal UV absorbance for all quantified compounds, we acquired their UV spectra and determined maximum absorption. We confirmed that 290 nm was the maximum absorbance wavelength for emodin and physcion, 318 nm for resveratrol and piceid, and 314 nm for vanicosides A and B. Since there were no significant differences in the UV absorbance of compounds between their maximum absorbance and 298 nm, we selected 298 nm for all compounds and demonstrated a high degree of sensitivity and precision.

Selectivity was evaluated by comparison of the chromatograms at 298 nm. Based on the UV-Vis and mass spectra, we showed that none of the quantified compounds interfere with other substances. Both positive and negative mode were analyzed, and despite that the positive ion mode often produced adducts, it was helpful for identification of physcion (compound **C8**).

The linearity for each compound was investigated over the range of the concentration presented in Table 4S. Resveratrol, vanicoside B, vanicoside A, emodin, and physcion were prepared as a mixture (as described above), while piceid was prepared separately because dissolving of piceid in 100% MeOH followed by dilution in the mixture resulted in peak blurring in the chromatogram, which was not observed when it was dissolved in 80% MeOH and analyzed separately. The regression equation was plotted as the peak area versus the corresponding concentration of the analyte. The values of correlation coefficient factors for

analyzed substances were in the range of 0.9995–0.9999. The LOD and LOQ for all of the quantified constituents were determined to be within 0.23–5.29 µg/mL (LOD) and 0.71–16.02 µg/mL (LOQ) (Table 3S). For establishment of linearity, at least five concentrations of each compound were analyzed above the LOQ.

Repeatability was calculated from nine replications covering the specified range for extract of *R. x bohemica* (three concentrations with three injections at each concentration). ICH also allows study of repeatability by measured at least six replications at 100% of the tested target concentration. However, we analyzed more than one extract of each species which contain different levels of target substances, so it was impossible to establish 100% of concentration for each reference compound.

Instead, using a range of concentrations was more reliable. Extract from *R. x bohemica* was chosen for analysis because it contained intermediate concentrations of each of the isolated compounds. Repeatability varied between 0.11 and 0.59%. Intra-day precision, similarly to repeatability, was calculated from nine replications injected to the HPLC system within the same day, and was in the range of 0.34–2.97%, while inter-day precision was determined in two different days and ranged from 1.26 to 3.78% (Table 4S.). The accuracy of the method was established by determining the recovery of piceid, resveratrol, vanicoside B, vanicoside A, emodin, and physcion spiked to the freshly prepared extract of *R. x bohemica* in six known concentrations covering investigated linearity range. The proposed method had high recovery rates, from 95.17% to 106.88% (Table 4S.)

Table 1S. ¹H NMR spectroscopic data for compounds **C1–C7** (CD₃OD, T = 25°C) measured at 500.20 MHz.

H	(C1)	(C2)	(C3)	(C4)	(C5)	(C6)	(C7)
1	3.61 (d, 11.8)	4.17 (d, 11.6)	4.34 (d, 11.9)	4.32 (d, 11.6)	4.34 (d, 11.8)	4.30 (d, 11.6)	
	3.42 (d, 11.8)	4.02 (d, 11.6)	4.31 (d, 11.9)	4.20 (d, 11.6)	4.30 (d, 11.8)	4.19 (d, 11.6)	
2							6.77 (d, 8.6)
3	5.58 (d, 8.5)	5.47 (d, 8.4)	5.59 (d, 8.5)	5.51 (d, 8.2)	5.64 (d, 8.7)	5.56 (d, 8.3)	7.37 (d, 8.6)
4	4.64 (dd, 8.6, 8.6)	4.60 (dd, 8.4, 8.4)	4.50 (dd, 8.5, 8.5)	4.44 (dd, 8.3, 8.3)	4.73 (dd, 8.7, 8.7)	4.61 (dd, 8.4, 8.4)	
5	4.19 (ddd, 9.1, 5.9, 3.6)	4.13 (ddd, 8.5, 6.2, 3.8)	4.18 (ddd, 8.5, 7.1, 3.4)	4.16 (ddd, 8.4, 6.0, 4.2)	4.19 (ddd, 9.3, 6.0, 3.8)	4.18 (ddd, 9.3, 6.3, 3.9)	7.37 (d, 8.6)
6	4.54 (dd, 12.1, 3.7)	4.53 (dd, 12.0, 3.8)	4.57 (dd, 12.0, 7.1)	4.49 (m)	4.58 (dd, 12.1, 3.9)	4.55 (dd, 12.0, 3.8)	6.77 (d, 8.6)
	4.48 (dd, 12.0, 6.0)	4.49 (dd, 11.9, 6.1)	4.53 (dd, 12.0, 7.1)		4.54 (dd, 12.1, 3.9)	4.50 (dd, 12.0, 6.3)	
OAc-1		2.11 (s)					
7							7.02 (d, 16.3)
8							6.85 (d, 16.3)
9							
10							6.79 (dd, 1.8, 1.8)
11							
12							6.45 (dd, 2.2, 2.2)
13							
14							6.61 (dd, 1.8, 1.8)
1'	5.68 (d, 3.8)	5.65 (d, 3.8)	5.50 (d, 3.7)	5.65 (d, 3.6)	5.56 (d, 3.9)	5.71 (d, 3.7)	4.89 (d, 7.3)
2'	4.61 (dd, 10.1, 3.8)	4.64 (dd, 10.1, 3.8)	3.42 (dd, 9.8, 3.8)	4.62 (dd, 10.1, 3.7)	3.46 (dd, 9.9, 3.9)	4.67 (dd, 10.1, 3.8)	3.45 (m)
3'	3.83 (dd, 10.2, 8.9)	3.82 (dd, 10.2, 8.9)	3.65 (dd, 9.8, 8.9)	3.87 (dd, 10.1, 9.1)	3.65 (dd, 9.6, 9.1)	3.88 (dd, 10.3, 9.1)	3.45 (m)
4'	3.38 (dd, 9.3, 9.3)	3.38 (t, 9.3)	3.41 (dd, 9.8, 9.0)	3.49 (dd, 9.9, 9.0)	3.30 (dd, 10.3, 9.2)	3.40 (dd, 9.9, 8.9)	3.38 (m)
5'	4.28 (ddd, 8.9, 7.4, 1.2)	4.24 (m)	3.98 (ddd, 10.0, 4.6, 2.5)	3.93 (ddd, 9.9, 4.5, 2.4)	4.32 (m)	4.28 (ddd, 9.1, 7.8, 1.3)	3.46 (m)
6'	4.68 (dd, 11.4, 1.2)	4.69 (d, 10.2)	3.91 (dd, 11.9, 2.5)	3.89 (dd, 12.0, 2.8)	4.72 (dd, 12.0, 2.4)	4.69 (dd, 11.5, 1.5)	3.93 (dd, 12.1, 2.2)
	4.24 (dd, 11.3, 7.2)	4.23 (m)	3.81 (dd, 11.9, 4.6)	3.78 (dd, 12.0, 4.5)	4.22 (dd, 11.9, 7.7)	4.23 (dd, 11.5, 7.4)	3.71 (dd, 12.1, 5.9)
OAc-2'	2.07 (s)	2.07 (s)		2.08 (s)		2.10 (s)	
X'-1							
α''			7.66 (d, 15.9)	7.66 (d, 16.0)	7.66 (d, 15.9)	7.65 (d, 15.9)	
β''			6.35 (d, 16.0)	6.35 (d, 16.0)	6.36 (d, 15.9)	6.35 (d, 15.9)	
2''			7.42 (d, 8.6)	7.43 (d, 8.7)	7.43 (d, 8.6)	7.42 (d, 8.7)	
3''			6.75 (d, 8.6)	6.76 (d, 8.7)	6.76 (d, 8.4)	6.75 (d, 8.7)	
5''			6.75 (d, 8.6)	6.76 (d, 8.7)	6.76 (d, 8.4)	6.75 (d, 8.7)	
6''			7.42 (d, 8.6)	7.43 (d, 8.7)	7.43 (d, 8.6)	7.42 (d, 8.7)	
X'-3							
α'''	7.72 (d, 15.9)	7.72 (d, 15.9)	7.73 (d, 15.9)	7.73 (d, 15.9)	7.72 (d, 15.9)	7.73 (d, 15.9)	
β'''	6.45 (d, 15.9)	6.45 (d, 15.9)	6.44 (d, 15.9)	6.48 (d, 15.9)	6.45 (d, 15.9)	6.48 (d, 15.9)	
2'''	7.51 (d, 8.7)	7.52 (d, 8.7)	7.49 (d, 8.7)	7.51 (d, 8.6)	7.49 (d, 8.6)	7.50 (d, 8.7)	
3'''	6.80 (d, 8.6)	6.81 (d, 8.6)	6.80 (d, 8.7)	6.82 (d, 8.8)	6.80 (d, 8.7)	6.81 (d, 8.7)	
5'''	6.80 (d, 8.6)	6.81 (d, 8.6)	6.80 (d, 8.7)	6.82 (d, 8.8)	6.80 (d, 8.7)	6.81 (d, 8.7)	
6'''	7.51 (d, 8.7)	7.52 (d, 8.7)	7.49 (d, 8.7)	7.51 (d, 8.6)	7.49 (d, 8.6)	7.50 (d, 8.7)	
X'-6'							
α''''	7.62 (d, 15.9)	7.62 (d, 15.9)			7.62 (d, 16.2)	7.62 (d, 15.9)	
β''''	6.45 (d, 15.9)	6.46 (d, 15.9)			6.48 (d, 15.9)	6.46 (d, 15.9)	
2''''	7.20 (d, 2.0)	7.22 (d, 2.0)			7.21 (d, 1.9)	7.21 (d, 2.0)	
3''''							
5''''	6.76 (d, 8.2)	6.77 (d, 8.2)			6.76 (d, 8.4)	6.77 (d, 8.2)	
6''''	7.01 (dd, 8.2, 2.0)	7.03 (dd, 8.3, 1.9)			7.01 (dd, 8.3, 2.0)	7.03 (dd, 8.3, 1.9)	
OMe	3.87 (s)	3.86 (s)			3.85 (s)	3.85 (s)	
X'-6							
α'''''	7.61 (d, 15.9)	7.61 (d, 15.9)	7.66 (d, 15.9)	7.66 (d, 15.9)	7.59 (d, 16.2)	7.60 (d, 15.9)	
β'''''	6.28 (d, 16.0)	6.29 (d, 15.9)	6.39 (d, 16.0)	6.39 (d, 16.0)	6.28 (d, 16.0)	6.29 (d, 15.9)	
2'''''	7.38 (d, 8.6)	7.38 (d, 8.6)	7.48 (d, 8.7)	7.48 (d, 8.7)	7.35 (d, 8.7)	7.36 (d, 8.7)	
3'''''	6.78 (d, 8.7)	6.77 (d, 8.7)	6.80 (d, 8.7)	6.80 (d, 8.7)	6.77 (d, 8.6)	6.76 (d, 8.7)	
5'''''	6.78 (d, 8.7)	6.77 (d, 8.7)	6.80 (d, 8.7)	6.80 (d, 8.7)	6.77 (d, 8.6)	6.76 (d, 8.7)	
6'''''	7.38 (d, 8.6)	7.38 (d, 8.6)	7.48 (d, 8.7)	7.48 (d, 8.7)	7.35 (d, 8.7)	7.36 (d, 8.7)	

Table 2S. ^{13}C NMR spectroscopic data for compounds 1–7 (CD_3OD , $T = 25^\circ\text{C}$). Compounds 1–7 were measured at 125.79 MHz.

C	(C1)	(C2)	(C3)	(C4)	(C5)	(C6)	(C7)
1	64.8	66.1	66.02	66.3	66.3	66.4	158.5
2	105.2	103.5	103.6	103.7	103.4	103.6	116.5
3	78.3	79.0	79.1	79.5	79.1	79.4	128.9
4	74.3	74.1	74.2	74.4	74.0	74.3	130.3
5	81.0	81.1	81.1	81.0	81.0	81.1	128.9
6	65.3	65.38	66.0	65.8	65.5	65.5	116.5
OAc-1		172.1					
		20.7					
7							130.0
8							126.7
9							141.4
10							107.0
11							160.5
12							104.1
13							159.6
14							108.3
1'	90.1	90.5	93.6	91.2	92.9	90.6	102.4
2'	74.4	74.2	73.0	74.50	72.9	74.3	75.0
3'	72.2	72.2	75.0	72.2	75.0	72.3	78.1
4'	72.13	72.1	71.6	71.3	72.3	72.1	71.5
5'	72.19	72.4	74.4	74.48	72.4	72.4	78.3
6'	65.5	65.4	62.7	62.3	65.8	65.4	62.6
OAc-2'	172.3	172.4		172.6		172.5	
	20.9	21.0		21.1		21.0	
X ² -1							
α''			147.2	147.2	147.17	147.2	
β''			114.8	114.6	114.7	114.6	
γ''			168.46	168.34	168.49	168.3	
1''			127.1	127.04	127.09	127.0	
2''			131.29	131.3	131.3	131.2	
3''			116.8	116.81	116.81	116.8	
4''			161.3	161.38	161.4	161.4	
5''			116.8	116.81	116.81	116.8	
6''			131.29	131.3	131.3	131.2	
X ² -3							
α'''	147.7	148.0	147.9	147.9	147.9	148.0	
β'''	114.4	114.1	114.4	114.3	114.4	114.2	
γ'''	168.5	168.4	168.43	168.33	168.48	168.4	
1'''	127.1	127.0	127.11	127.04	127.09	127.0	
2'''	131.5	131.6	131.5	131.5	131.2	131.6	
3'''	116.8	116.9	116.83	116.86	116.82	116.9	
4'''	161.5	161.6	161.5	161.6	161.5	161.6	
5'''	116.8	116.9	116.83	116.86	116.82	116.9	
6'''	131.5	131.6	131.5	131.5	131.2	131.6	
X ² -6'							
α''''	147.2	147.2			147.19	147.2	
β''''	115.3	115.3			115.4	115.3	
γ''''	169.2	169.2			169.3	169.2	
1''''	127.7	127.7			127.8	127.7	
2''''	111.6	111.6			111.5	111.6	
3''''	149.3	149.4			149.3	149.3	
4''''	150.6	150.7			150.6	150.6	
5''''	116.3	116.3			116.3	116.3	
6''''	124.5	124.6			124.6	124.6	
OMe	56.5	56.5			56.5	56.5	
X ² -6							
α''''''	146.8	146.9	147.0	147.0	146.8	146.9	
β''''''	114.9	114.8	114.9	114.8	114.9	114.8	
γ''''''	168.9	168.9	169.1	169.0	168.9	168.9	
1''''''	127.2	127.2	127.2	127.18	127.12	127.2	
2''''''	131.2	131.2	131.31	131.1	131.2	131.3	
3''''''	116.8	116.8	116.83	116.81	116.8	116.8	
4''''''	161.3	161.3	161.4	161.33	161.3	161.3	
5''''''	116.8	116.8	116.83	116.81	116.8	116.8	
6''''''	131.2	131.2	131.31	131.1	131.2	131.3	

Table 3S. Linearity, LOD, and LOQ data for isolated compounds and resveratrol standard used for quantitation.

Diode array data							
Analyte	λ_{det} (nm)	Calibration equation	n	r	Linear range ($\mu\text{g/mL}$)	LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)
Piceid	298	$y = 32.83x - 7.9417$	6	0.9999	11.25–360	5.29	16.02
Resveratrol	298	$y = 60.021x - 0.4491$	6	0.9998	1.25–40	1.04	3.16
Vanicoside B	298	$y = 26.719x - 3.8342$	6	0.9999	4.375–140	1.41	4.28
Vanicoside A	298	$y = 34.161x - 2.9891$	6	0.9995	0.9375–30	0.23	0.71
Emodin	298	$y = 20.389x - 2.444$	6	0.9999	4.375–140	1.04	3.15
Physcion	298	$y = 7.1533x - 1.8287$	6	0.9999	3.75–120	1.02	3.08

Table 4S. Repeatability, precision, and accuracy of the analytical method for isolated compounds and resveratrol standard used for quantitation.

DAD data					
Analyte	Concentration ($\mu\text{g/mL}$)	Repeatability (%CV)	Intra-day precision (%CV)	Inter-day precision (%CV)	Recovery (%) \pm SD
Piceid	360				103.04 \pm 0.15
	180	0.59	0.87	1.98	99.18 \pm 0.32
	90	0.42	1.00	2.01	100.09 \pm 0.16
	45	0.20	0.78	1.34	102.67 \pm 0.16
	22.5				103.96 \pm 0.04
	11.25				103.17 \pm 0.07
Resveratrol	40				99.49 \pm 0.74
	20	0.16	0.67	1.89	95.17 \pm 0.34
	10	0.15	0.34	1.26	96.12 \pm 0.21
	5	0.21	0.98	1.57	98.85 \pm 0.21
	2.5				100.20 \pm 0.17
	1.25				99.36 \pm 0.08
Vanicoside B	140				104.19 \pm 0.66
	70				97.36 \pm 0.06
	35	0.15	1.08	2.66	103.33 \pm 0.30
	17.5	0.35	0.76	2.05	102.11 \pm 0.17
	8.75	0.29	0.35	1.36	103.22 \pm 0.17
	4.375				102.78 \pm 0.10
Vanicoside A	30				101.28 \pm 0.57
	15				97.39 \pm 0.44
	7.5	0.11	1.21	2.34	102.16 \pm 0.17
	3.75	0.18	2.37	2.71	100.82 \pm 0.16
	1.875	0.45	2.90	3.19	103.05 \pm 0.22
	0.9375				103.75 \pm 0.12
Emodin	140				99.85 \pm 0.63
	70	0.19	1.45	2.01	98.06 \pm 0.43
	35	0.15	0.99	3.19	98.97 \pm 0.29
	17.5	0.26	0.67	2.10	102.03 \pm 0.13
	8.75				103.56 \pm 0.19
	4.375				102.90 \pm 0.08
Physcion	120				95.24 \pm 0.59
	60	0.16	2.89	3.25	100.98 \pm 0.45
	30	0.11	2.73	2.81	102.89 \pm 0.32
	15	0.21	2.97	3.78	105.78 \pm 0.36
	7.5				106.88 \pm 0.16
	3.75				106.28 \pm 0.11