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Supplementary Materials

Quantitative 1H NMR Spectroscopy Method for Determination of Anthraquinone Derivatives in Extracts from Rubia tinctorum L. Roots and Rhizomes

Vasilii Vasil'ev *, Anzhelika Sheremeta , Vasilii Ivlev , Sergey Goriainov , Fadi Hajjar , Cesar Esparza, Evgeniy Platonov, Arkadiy Khromov, Alexandr Kolesnov , Victoria Romashchenko and Gennady Kalabin

RERC Pharmacy, Peoples' Friendship University of Russia (RUDN University), 6 Miklukho-Maklaya Street, Moscow 117198, Russia; goryainovs@list.ru (S.G.); phfadyhajj@gmail.com (F.H.); arkadiy18@yandex.ru (A.K.)
* Correspondence: vasyavasilyev@mail.ru

Supplementary Material A

^{1}H NMR spectral parameters of ruberythric acid, lucidin-3-primeveroside, alizarin, purpurin, lucidin internal standards

Ruberythric acid. ^{1}H NMR (600 MHz, DMSO-D$_6$, δ, ppm): 12.66 (s, 1H, OH-1), 8.27 – 8.24 (m, 1H, arom. H), 8.21 – 8.18 (m, 1H, arom. H), 7.97 – 7.92 (m, 2H, arom. H), 7.74 (d, J = 8.5 Hz, 1H, arom. H), 7.62 (d, J = 8.5 Hz, 1H, arom. H), 5.51 (d, J = 5.3 Hz, 1H, OH-3'), 5.28 – 5.23 (m, 2H, OH-4', 5'), 5.07 (d, 1H, J = 7.6 Hz, anomeric H-2' glucose), 4.98 (d, J = 4.6 Hz, 2H, OH-4'', 5''), 4.14 (d, J = 7.6 Hz, 1H, anomeric H-2'' xylose).

Lucidin-3-primeveroside. ^{1}H NMR (600 MHz, DMSO-D$_6$, δ, ppm) δ 13.04 (s, 1H, OH- 1), 8.26 – 8.24 (m, 1H, arom. H), 8.20 – 8.18 (m, 1H, arom. H), 7.98 – 7.92 (m, 2H, arom. H), 7.48 (s, 1H, H-4), 5.12 (d, J = 7.3 Hz, 1H, anomeric H-2' glucose), 4.66 (d, J = 11.4 Hz, 1H, - CH2-), 4.58 (d, J = 11.4 Hz, 1H, -CH$_2$-), 4.14 (d, J = 7.4 Hz, 1H, anomeric H-2'' xylose).

Alizarin. ^{1}H NMR (600 MHz, DMSO-D$_6$, ppm) δ 12.62 (s, 1H, OH-1), 10.88 (s, 1H, OH- 2), 8.25 – 8.23 (m, 1H, arom. H), 8.19 – 8.17 (m, 1H, arom. H), 7.96 – 7.90 (m, 2H, arom. H), 7.67 (d, J = 8.4 Hz, 1H, arom. H), 7.24 (d, J = 8.3 Hz, 1H, arom. H).

Purpurin. ^{1}H NMR (600 MHz, DMSO-D$_6$, ppm) δ 13.40 (s, 1H,OH-1), 13.12 (s, 1H, OH-4), 8.28 – 8.22 (m, 3H, arom. H), 8.01 – 7.89 (m, 3H, arom. H), 6.70 (s, 1H, arom. H).

Lucidin. ^{1}H NMR (600 MHz, DMSO-D6, ppm) δ 13.19 (s, 1H, OH-1), 8.21 (dd, J = 7.5, 1.5 Hz, 1H, arom. H), 8.15 (dd, J = 7.4, 1.6 Hz, 1H, arom. H), 7.91 (dqd, J = 14.6, 7.3, 1.4 Hz, 2H, arom. H), 7.25 (s, 1H, arom. H), 4.54 (s, 2H, -CH$_2$-).

Supplementary Material B

Table S1. RA and LP content in the untreated sample and the samples either heated at 60 °C for 15 min or exposed to the ultrasound for 30 min.

Sample	C (RA), %	C (LP), %	C (total), %
Normal conditions	1,72±0,02	2,07±0,03	3,79±0,04
Heating (60 ºC)	1,74±0,03	2,10±0,04	3,84±0,03
Ultrasonic treatment (30 min)	1,86±0,02	2,15±0,3	4,01±0,04

Table S2. RA and LP and their total content after the ultrasound treatment for 0, 15, 30, 45, and 60 minutes.

Ultrasonic treatment, min	C (RA), %	C (LP), %	C (total), %
0	1,53 ± 0,03	2,09 ± 0,03	3,63 ± 0,03

15	1,57 ± 0,02	2,13 ± 0,02	3,70 ± 0,02
30	1,68 ± 0,03	2,17 ± 0,03	3,86 ± 0,03
45	1,68 ± 0,02	2,17 ± 0,02	3,85 ± 0,02
60	1,68 ± 0,03	2,18 ± 0,03	3,86 ± 0,03

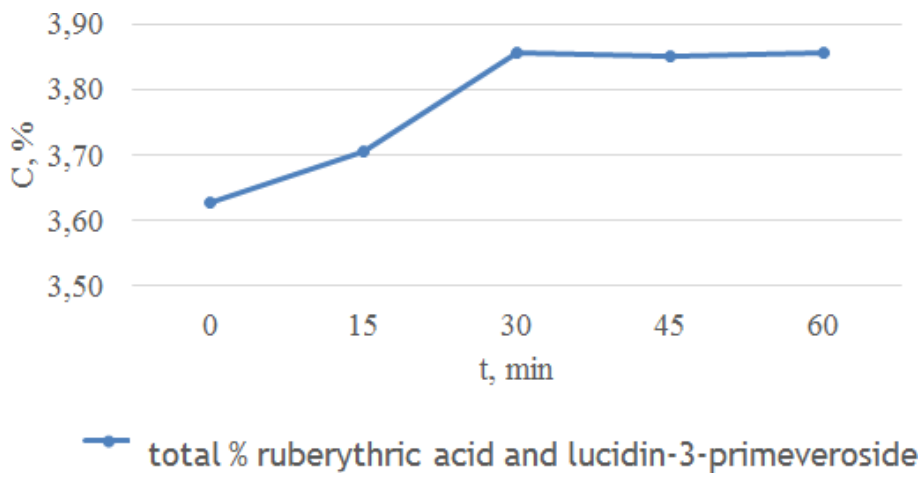

Figure S1. The dependence of the concentration of ruberythric acid and lucidin-3-primeveroside on ultrasound exposure time.

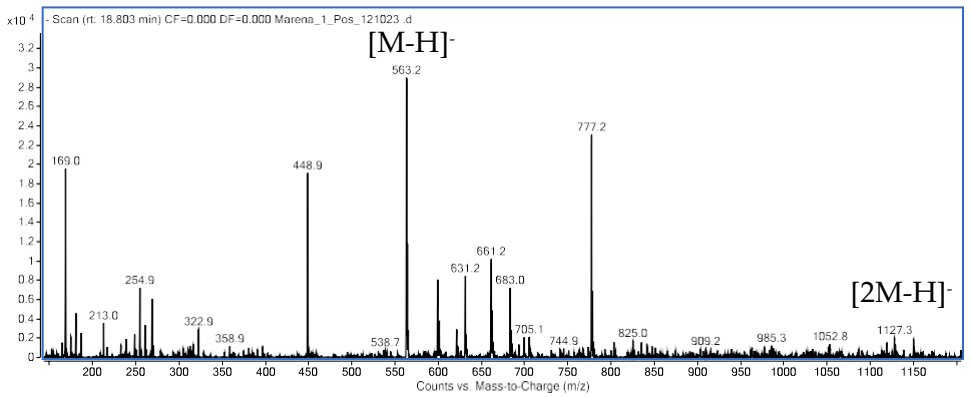

Figure S2. ESI (-) Mass spectrum of LP in the extract from the *Rubia tinctorum* L. roots and rhizomes.

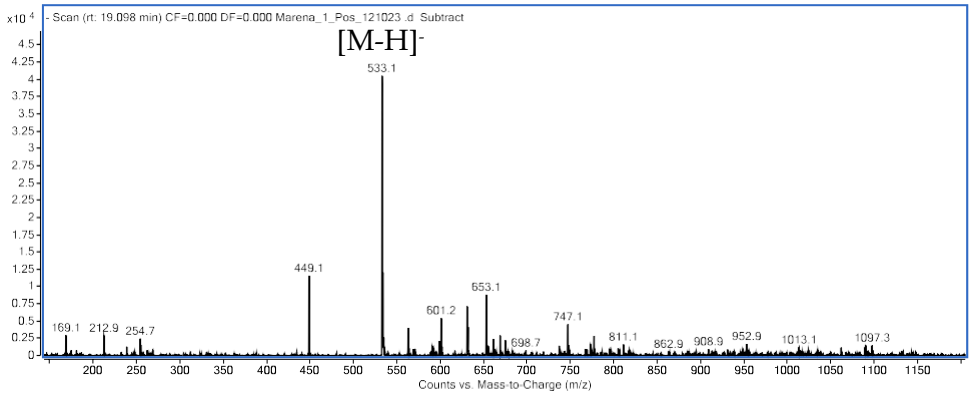

Figure S3. ESI (-) Mass spectrum of ruberythric acid in the extract from the *Rubia tinctorum* L. of roots and rhizomes, registration of negative ions.

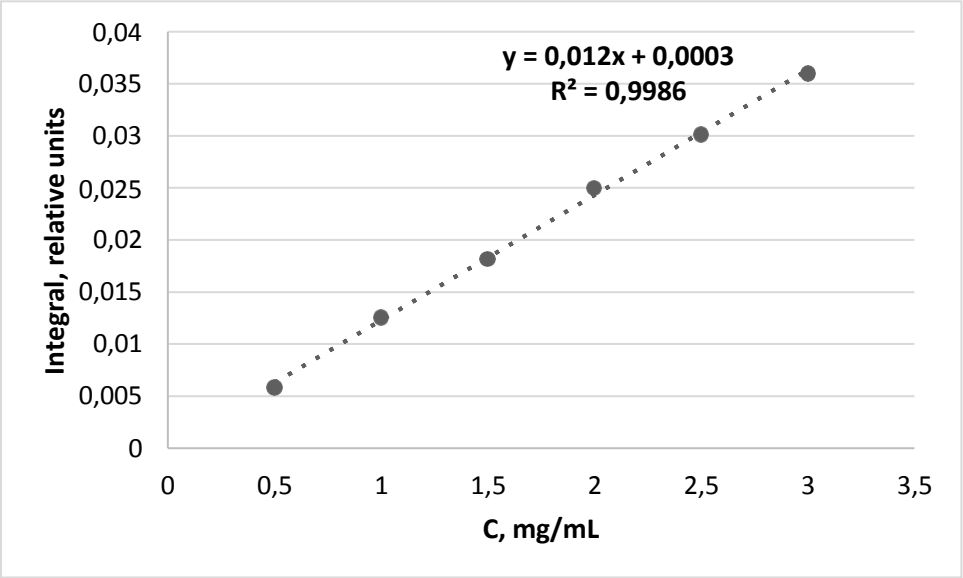

Figure S4. Linear dependence of lucedin-3-primeveroside proton signal integral on concentration.

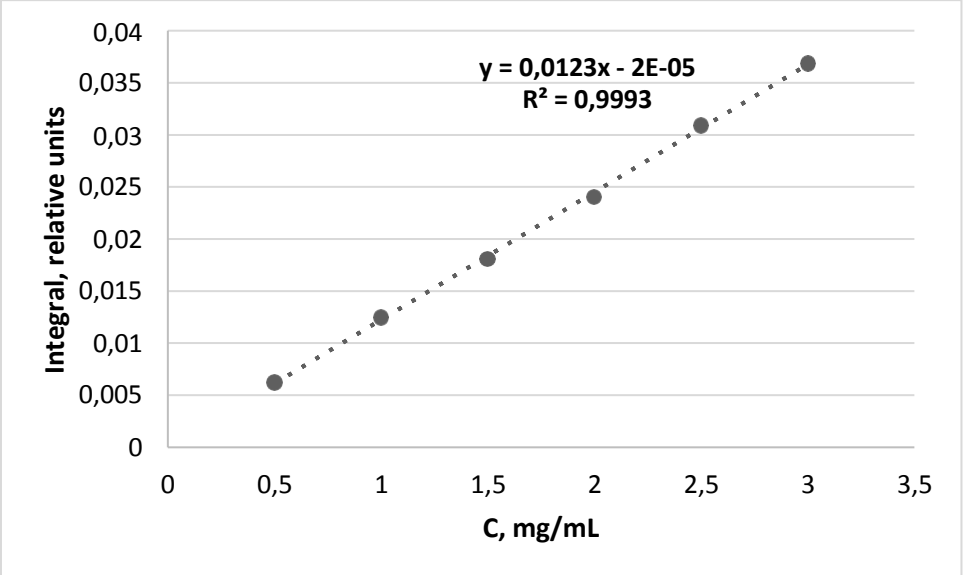

Figure S5. Linear dependence of ruberythric acid proton signal integral on concentration.