SUPPLEMENTARY MATERIAL

An optimized protocol for anthraquinones isolation from *Rhamnus frangula* L.

Renato Sonchini Gonçalves*[a], Expedito Leite Silva [a], Noboru Hioka [a], Celso Vataru Nakamura[b], Marcos Luciano Bruschi[b] and Wilker Caetano [a]

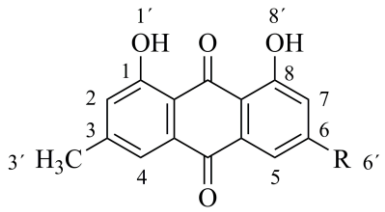
[a]*Department of Chemistry, State University of Maringá, Maringá, Brazil*

[b]*Department of Pharmacy, State University of Maringá, Maringá, Brazil*

*corresponding author. Tel.: (55) 44-3011-3680. Email: rsgoncalves2@uem.br

Abstract: Different from works described in the literature, which use expansive analytical methods to separation of anthraquinones derivatives (AQs), this communication reported a simple and inexpensive methodology to get them. In this way, the expensive commercial AQs: Chrysophanol, physcione, and emodine were extracted from plant material (*Rhamnus frangula* L) and isolated by classical column chromatography technique under optimized binary mobile phase gradients ($CHCl_3$: AcOEt(*a*), *a* = 1 to 5 %) in excellent yields.

Keywords: Optimized isolation; column chromatography; chrysophanol; physcione; emodin; *Rhamnus frangula* L.



(1) Chrysophanol, R = H
(2) Physcione, R = -OCH_3
(3) Emodine, R = -OH

Table S1. ^{1}H NMR spectral data for AQ **1-3** acquired in $CDCl_3$ and DMSO-d_6 at 300 MHz: δ (ppm), integral and J (Hz).

Position	Chemical shift δ in ppm		
	AQ-**1**	AQ-**2**	AQ-**3**
1'	12.01 (s, 1H)	12.09 (s, 1H)	12.00 (s, 1H)
2	7.65 (*dq*, 1H) $^4J_{2,4}$ = 1.1 $^4J_{2,3'}$ = 0.8	7.61 (*dq*, 1H) $^4J_{2,4}$ = 1.7 $^4J_{2,3'}$ = 0.4	7.47 (*dq*, 1H) $^4J_{2,4}$ = 1.7 $^4J_{2,3'}$ = 0.4
3'	2.47 (*dd*, 3H) $^4J_{3',2}$ = 0.8 $^4J_{3',4}$ = 0.5	2.45 (*dd*, 3H) $^4J_{3',2}$ = 0.4 $^4J_{3',4}$ = 0.7	2.40 (*dd*, 3H) $^4J_{3',2}$ = 0.4 $^4J_{3',4}$ = 0.7
4	7.10 (*dq*, 1H) $^4J_{4,2}$ = 1.1 $^4J_{4,3'}$ = 0.5	7.07 (*dq*, 1H) $^4J_{4,2}$ = 1.7 $^4J_{4,3'}$ = 0.7	7.15 (*dq*, 1H) $^4J_{4,2}$ = 1.7 $^4J_{4,3'}$ = 0.7
5	7.82 (*dd*, 1H) $^3J_{5,6}$ = 7.5 $^3J_{5,7}$ = 1.2	6.67 (*d*, 1H) $^4J_{5,7}$ = 2.5	7.10 (*d*, 1H) $^4J_{5,7}$ = 2.5
6	7.67 (*dd*, 1H) $^3J_{6,5}$ = 7.5 $^3J_{6,7}$ = 8.4		
6'		3.92 (s, 3H)	11.38 (s, 1H)
7	7.29 (*dd*, 1H) $^4J_{7,5}$ = 1.2 $^4J_{7,6}$ = 1.2	7.35 (*d*, 1H) $^4J_{7,5}$ = 2.5	6.58 (*d*, 1H) $^4J_{5,7}$ = 2.5
8'	12.12 (*d*, 1H) $^4J_{8',5}$ = 0.36	12.29 (s, 1H)	12.07 (s, 1H)

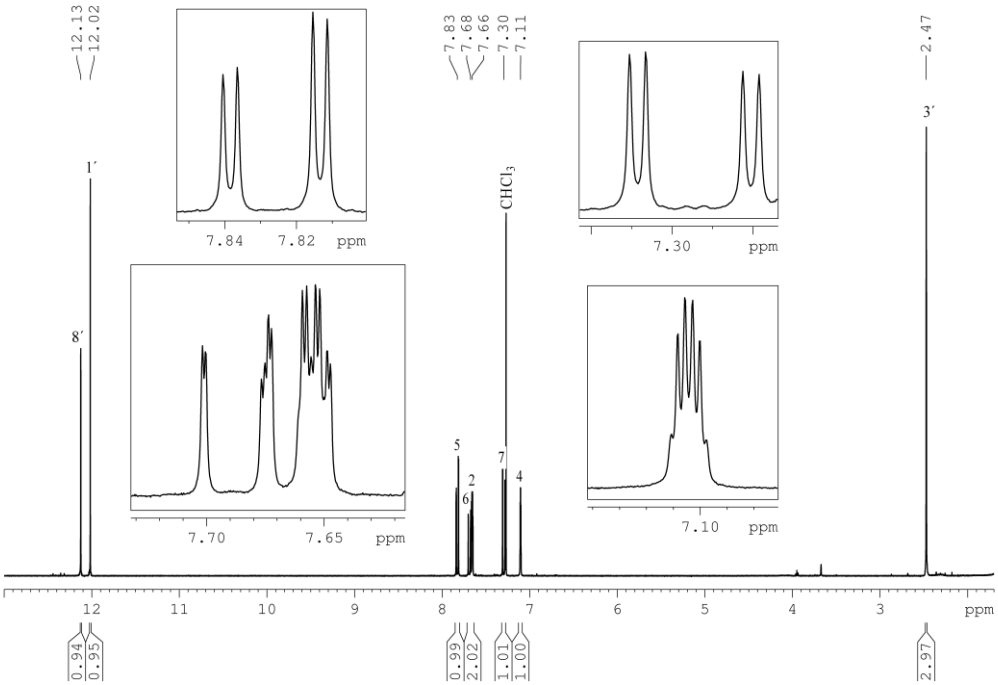


Figure S1. ^{1}H NMR spectrum of chrysophanol (AQ-**1**) recorded in $CDCl_3$ at 300 MHZ.

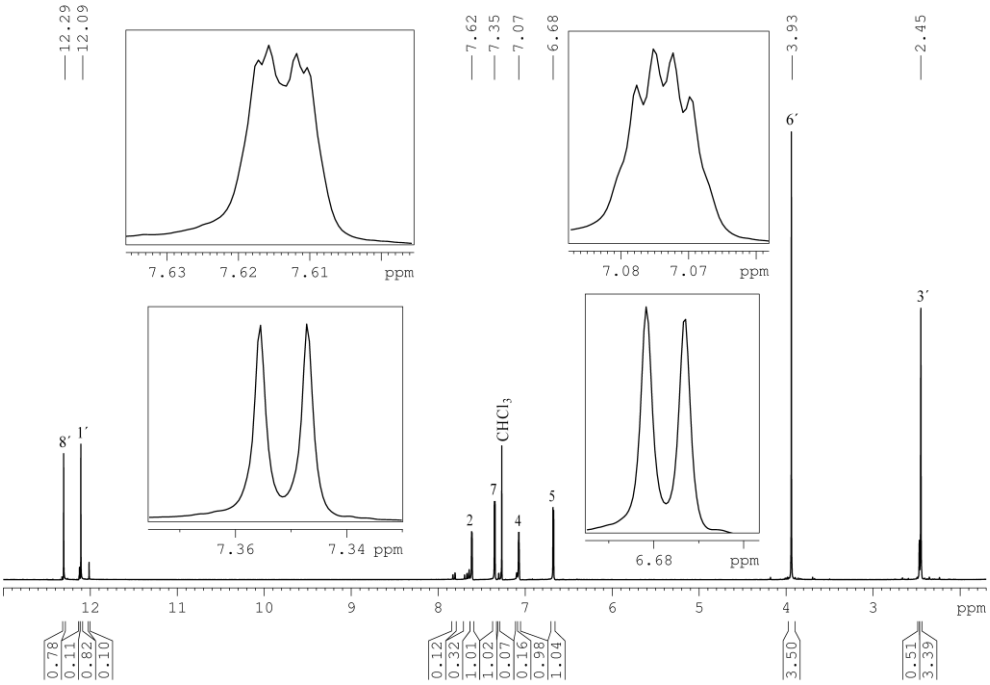


Figure S2. ^{1}H NMR spectrum of physcione (AQ-**2**) recorded in $CDCl_3$ at 300 MHZ.

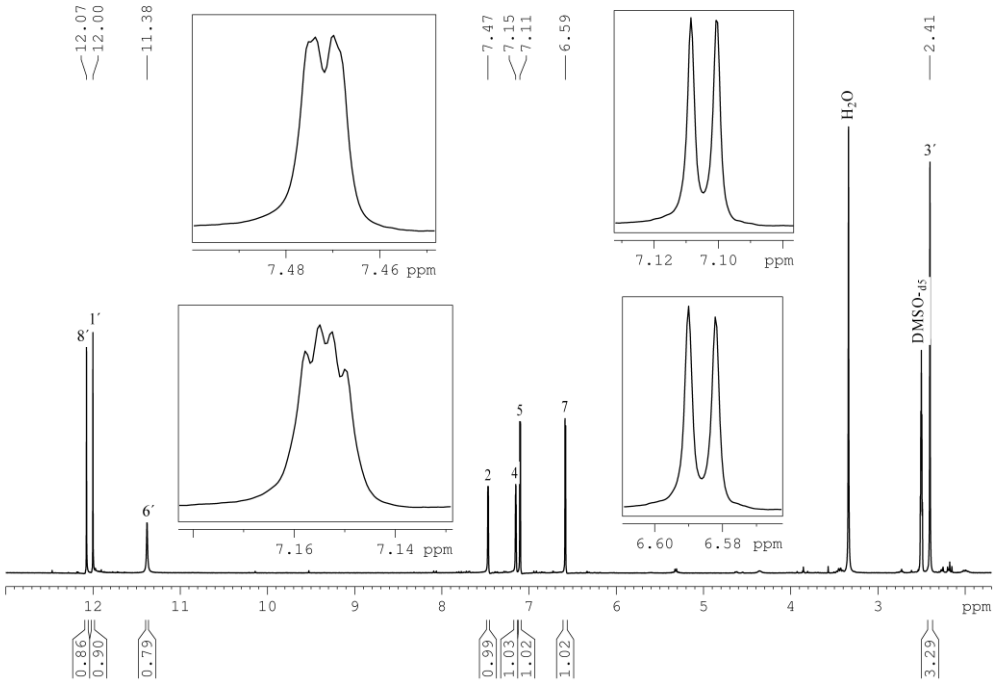


Figure S3. ^{1}H NMR spectrum of emodine (AQ-**3**) recorded in DMSO-d_6 at 300 MHZ.

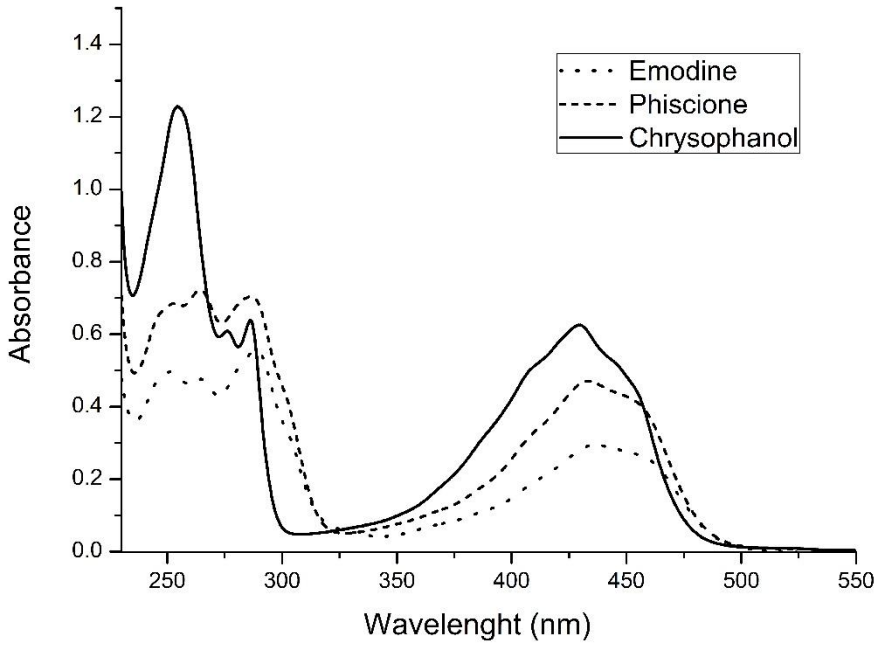


Figure S4. UV-Vis spectra of AQ **1**-**3** recorded in EtOH.

Experimental

General

Dry samples were purchased in packs of 60 g from *Cha & Cia Produtos Naturais* (SP, Brazil). Chrysophanol (≥ 98), physcione (≥ 98), emodine (≥ 90), hydrogen chloride (HCl), and solvents of HPLC-grade: chloroform ($CHCl_3$), dichloromethane (DCM), ethyl acetate (AcOEt), hexane (hex), methanol (MeOH), were purchased from Sigma-Aldrich and used as received. Thin-layer chromatography (TLC) plates and silica gel 60 Å**,** 230-400 mesh were purchased from Sigma-Aldrich.

Extraction process

Dry *Cortex Frangulae* (180 g) were washed, tritured, and exhaustively macerated in MeOH (500 mL) at room temperature for 24 h. All the MeOH extracts were combined, filtered off, and concentred on a rotary evaporation to give 115 g a mixture of AQs glycosides. The glycosides were hydrolysed with a solution of HCl 6 M (250 mL) at 100 ^{o}C for 1 h. The solution was cooled, poured in water (1 L), and neutralized with Na_2CO_3. The precipitate formed was filtered off and washed with water to produce (after drying under vacuum), 46 g of dark solid. Next, the material was exhaustively extracted with DCM in a Soxhlet apparatus yielding of (after solvent evaporation) 34 g (19 %) of brown solid crude AQs aglycones.

Purification process

Purification process was performed by silica gel CC (glass columns, 60 x 600 mm) using two binary mobile phase combinations: hex : AcOEt and $CHCl_3$: AcOEt with a gradient of 0-15 %, in both cases. The eluted fractions were analysed by TLC using DCM : hex (9:1, v/v) as a mobile phase and combined according to reference of the retention time (Rf) values.

Spectroscopic Measurement

^{1}H NMR spectra were recorded on a Bruker AVANCE III 300 MHz spectrometer operating at 300.133 MHz, equipped with direct field gradient and probe of 5 mm, at a constant temperature of 25° C. All samples were prepared with an addition of 0.7 mL of deuterated solvents in 5-10 mg of AQs. ^{1}H NMR data was recorded in $CDCl_3$ and DMSO-d_6 and listed as residual internal: $CHCl_3$ (δ 7.27) and DMSO-d_5 (δ 2.50) and the spectra were referenced to TMS peak. Data are presented as follows: chemical shift (ppm), multiplicity (s = singlet, d = doublet, dd = doublet of doublet, dq = doublet of quartet), integration and coupling constant (*J*). All spectra were

processed using the Bruker TopSpin 3.1 software. UV-vis absorption spectra were recorded on a Beckman Coulter DU800 UV-Vis Spectrophotometer at room temperature.