Supplementary data

Identification of Chromomoric Acid C-I as Nrf2 Activator from *Chromolaena odorata*

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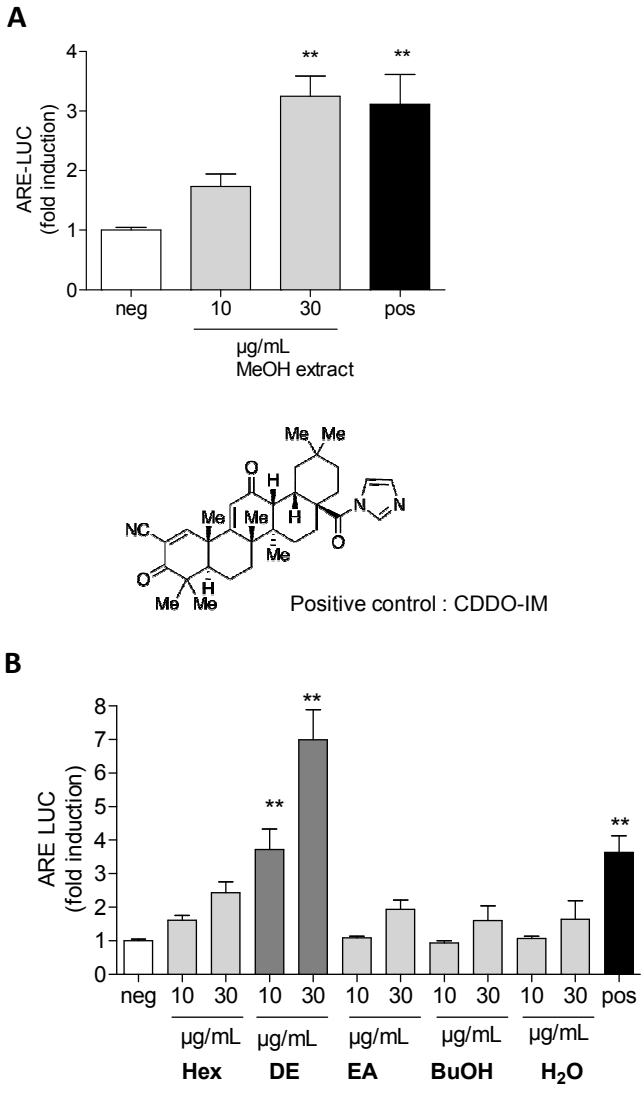
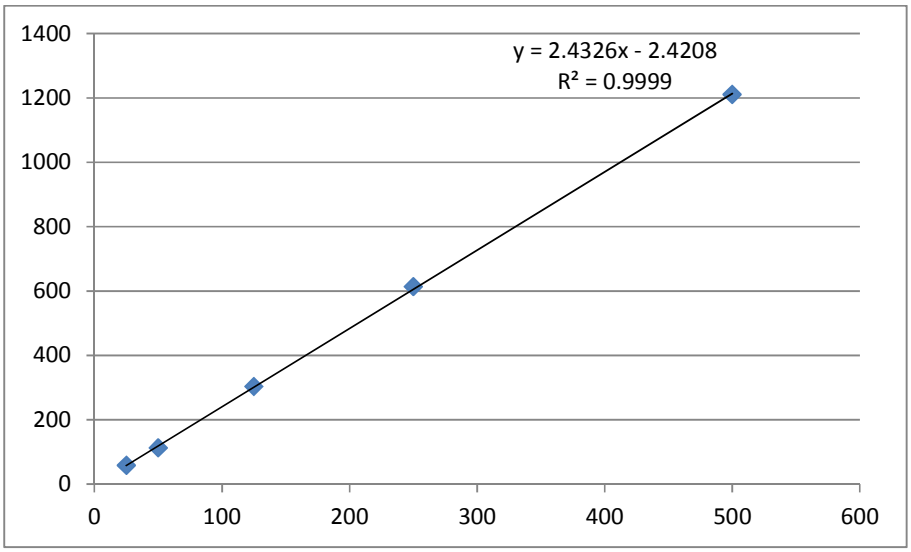
Content:

S1. Nrf2 activation by *C. odorata* (A) CHO-ARE Luc cells were treated with DMSO (0.1%, negative control, neg), 100 nM CDDO-IM (positive control, pos) and different concentrations of a crude methanol extract of *C. odorata* leaves for 18 h before the luciferase expression was determined and normalized to the cell count. The bar graph depicts data compiled of three independent experiments expressed as fold luciferase induction of the negative control cells (means + SD, ** $p < 0.01$, ANOVA). (B) CHO-ARE Luc cells were treated with DMSO (0.1%, negative control, neg), 100 nM CDDO-IM (positive control, pos) and differently polar fractions of the crude *C. odorata* leaves (*Hex*, hexane, *DE*, diethylether, *EA*, ethylacetate, *BuOH*, butanol, *H_2O*, water) methanol extract for 18 h, as indicated. Luciferase expression was assessed, normalized to the cell count and expressed as fold of the negative DMSO control. The bar graph depicts compiled data of three independent experiments (means + SD, ** $p < 0.01$, ANOVA).



S2: Quantification of Chromomoric acid C-I

Calibration curve of Chromomoric acid C-I



Preparation of samples

- Chromo-DE fraction: 5 mg of diethyl ether fraction were dissolved in 1 ml MeOH to make the solution at concentration of 5 mg/mL.
- Chromo-DE extract: The finely powdered plant material (5 g) was extracted three times with 50 mL of diethyl ether by sonication (10 min each, at ambient temperature) and then filtered. Extracts were combined, evaporated under reduced pressure and subsequently re-dissolved in methanol, quantitatively transferred to a volumetric flask and adjusted to the final volume (10 mL) with methanol.
- Prior to injection, all solutions were filtered through cotton wool. Each sample solution was assayed in triplicate.

Content (in weight %) of Chromomoric acid C-I in diethyl ether fraction and dried material.

Sample	Content (w%)
Chromo-DE fraction	0.664
Chromo-DE extract	0.014

S3: ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR data of compounds **1** and **2** ($CDCl_3$, δ in ppm, *J* in Hz).

Position	**1** δc mult	**2** δc mult	**1** δ_H mult. J (Hz)	**2** δ_H mult. J (Hz)
1	178.9 s	178.9 s	-	-
2	33.8 t	33.6 t	2.34, 2H, t (7.4)	2.34, 2H, t (7.4)
3	24.6 t	24.6 t	1.60 m	1.60 m
4	28.9 t	28.9 t	1.24 – 1.35 m	1.24 – 1.35 m
5	29.0 t	29.0 t	1.24 – 1.35 m	1.24 – 1.35 m
6	29.4 t	29.5 t	1.24 – 1.35 m	1.24 – 1.35 m
7	25.8 t	25.9 t	1.28 m	1.29 m
8	32.8 t	32.9 t	1.55 m; 1.86 m	1.56 m; 1.87 m
9	43.5 d	43.4 d	3.53 m	3.55 m
10	161.2 d	161.4 d	7.52 dd (1.8, 5.9)	7.54 dd (2.1, 5.8)
11	135.1 d	135.1 d	6.35 dd (1.7, 6.0)	6.37 dd (1.5, 5.9)
12	197.7 s	197.7 s	-	-
13	135.7 s	137.4 s	-	-
14	131.4 d	125.5 d	6.94 brd (10.0)	7.27 d (11.7)
15	124.7 d	123.1 d	6.27 dd (10.0)	6.21 dd (10.9, 11.9)
16	147.8 d	144.6 d	6.26 m	6.00 td (7.8, 10.2)
17	26.5 t	21.4 t	2.25 td (12.1; 7.4)	2.40 m
18	13.1 q	13.9 q	1.09 t (7.3)	1.05 t (7.5)

S4: HPLC chromatogram of the detection Chromomoric acid C-I in diethyl ether fraction (Chromo-DE fraction) and diethyl ether extract of dried leaves of *Chromolaena odorata* (Chromo-DE extract).