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Spectrophotometric Comparison of the Content of Chlorophylls in Weld (*Reseda luteola*)

The absorption spectrum of an absolute ethanol extract of weld sample **B**[20] is presented in figure S1. The absorbance at the main red absorption band at ~665 nm was used to compare different samples.

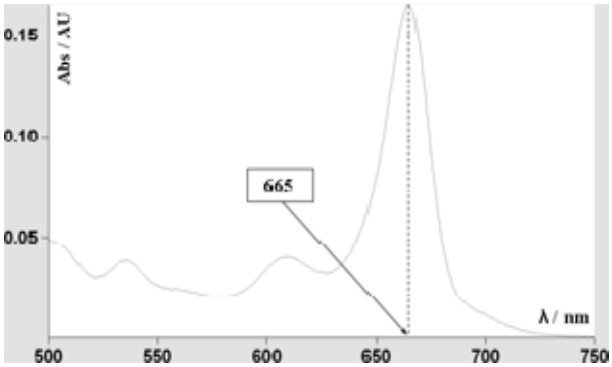

Figure S1. Absolute ethanol extract of weld sample **B**[20] in 10 mm-pathlength quartz cuvette. The wavelength of the main red absorption band is indicated.

Precision of the analytical method using absolute ethanol and 10 mm-pathlength plastic cuvettes[19] was evaluated. The dependence of the extraction efficiency on the particle size of the samples and the correlation between the absorbance of ethanolic and DMF extracts of different *R. luteola* samples using 10 mm-pathlength cuvettes were verified. Results are presented below.

Table S1. Absorbance of extracts of weld samples due to chlorophylls and their structurally similar breakdown products obtained with ethanol and *N,N*-dimethylformamide, using two types of cuvettes.[a,b]

Entry	Plant material	n	Extraction procedure[c] and series of measurements	Solvent[d]	Absorbance (AU) and s_r (%)[e]

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			10 mm-pathlength plastic cuvette		
1	**A**	3	3-min vortexing	Abs EtOH	0.096 (4.4)
2	**B**	3	3-min vortexing; day *a*	Abs EtOH	0.159 (0.3)
3	**B**	3	3-min vortexing; day *a*	Abs EtOH	0.164 (2.5)
4	**B**	3	3-min vortexing; day *a*	Abs EtOH	0.160 (3.8)
5[f]	**B**	9	3-min vortexing; day *a*	Abs EtOH	0.161 (2.7)
6	**B**	3	3-min vortexing; day *(a + 3)*	Abs EtOH	0.158 (3.1)
7	**B**	3	3-min vortexing; day *(a + 3)*	Abs EtOH	0.157 (1.1)
8	**B**	3	3-min vortexing; day *(a + 3)*	Abs EtOH	0.164 (1.7)
9[f]	**B**	9	3-min vortexing; day *(a + 3)*	Abs EtOH	0.159 (2.9)
10	**C 0.75**	3	3-min vortexing	Abs EtOH	0.149 (6.1)
11	**C 0.50**	3	3-min vortexing	Abs EtOH	0.180 (4.6)
12	**C 0.25**	3	3-min vortexing	Abs EtOH	0.216 (1.5)
13	**D**	3	3-min vortexing; day *a* (n = 2) and day *(a + 3)* (n = 1)	Abs EtOH	0.271 (2.7)
14	**E**	3	3-min vortexing	Abs EtOH	0.643 (0.6)
			10 mm-pathlength quartz cuvette		
15	**B**	3	3-min vortexing	DMF	0.215 (1.8)
16	**C 0.25**	3	3-min vortexing	DMF	0.287 (1.3)
17	**D**	3	3-min vortexing	DMF	0.390 (3.2)
18	**E**	3	3-min vortexing	DMF	1.026 (1.1)

[a] λ of detection: entries 1 through 14, 665 nm − 750 nm (*i.e.* absorbance at 750 nm subtracted from that at 665 nm); entries 15 through 18, 664 nm − 750 nm. *NB* Subtraction of absorbance at 750 nm was only done for consistency with data in the Technical Note. In all cases the absorbance at 750 nm varied between −6 and 10 mAU;

[b] **A** through **E** are the codes of (dried and ground-sieved) weld samples in order of increasing concentration of **chl**s and breakdown products. Note: 1) differences among samples include cultivar and plant parts used.[20] 2) Numbers behind **C**: pore size of sieves used during the grinding-sieving process (in mm);

[c] Always at room temperature and under reduced light;

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[d] Abs EtOH = absolute ethanol and DMF = *N,N*-dimethylformamide;

[e] Average absorbance (s_r = relative standard deviation);

[f] Combining results of previous 3 entries.

Precision: repeatability was assessed by analysis of sample **B** (n = 9; entry 5 of table S1): s_r < 3.0%. Sample **B** was analysed again on another day (n = 9; entry 9). The difference between both results was 1%. Based on the results seen in entries 15 and 18 of the table in the Technical Note itself, and 1 and 14 of table S1 above, samples with absorbance of 190 mAU using 2 mm-pathlength plastic cuvettes should display absorbance ≤ 1.0 AU using 10 mm-pathlength plastic cuvettes. Thus, users having many samples with absorbance ≤ 190 mAU (with 2 mm-pathlength cuvettes) may use the 10 mm-pathlength cuvettes for increased precision.

Dependence of extraction efficiency on particle size: an increase of absorbance with decreasing particle size is seen (entries 10 through 12). Additionally, as expected, s_r decreases with the decrease of the particle size. This indicates that it is harder to obtain representative samples from coarse plant materials than from fine ones.

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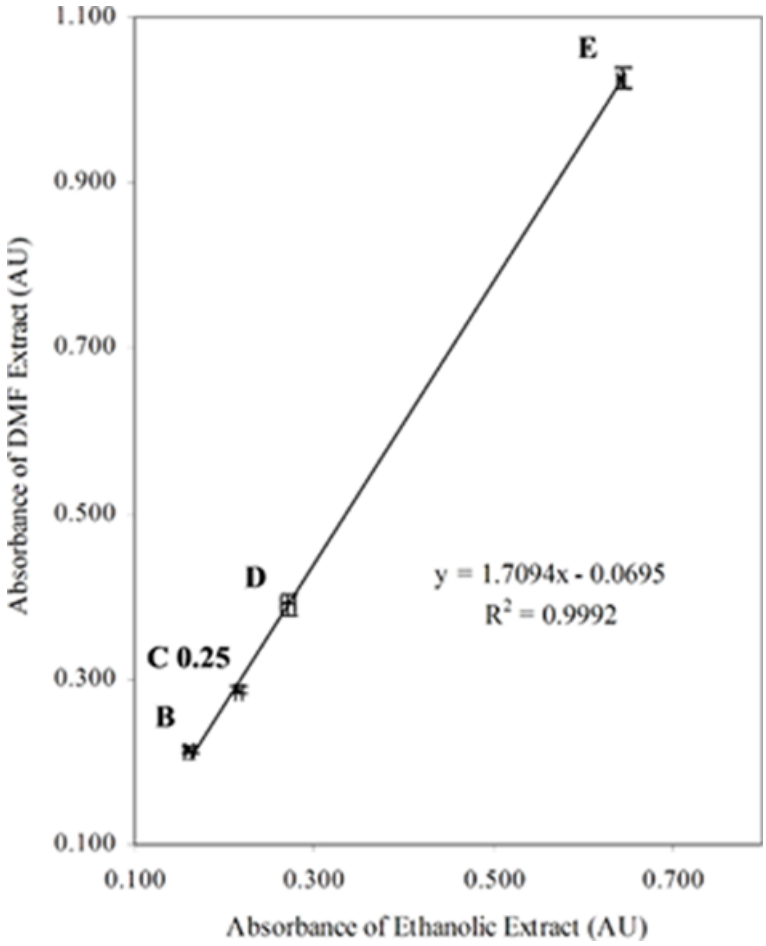

Figure S2. Correlation between absorbance of ethanolic and DMF extracts of *R. luteola*. Average absorbances are plotted; error bars = 1 standard deviation. Ethanolic extracts: 10 mm-pathlength plastic cuvette; n = 3 (**C 0.25**, **D** and **E**) and n = 9 (**B**). DMF extracts: 10 mm-pathlength quartz cuvette; n = 3 (all).

As also seen previously, the absorbance of ethanolic and DMF extracts linearly correlate. Note: the slopes of the curves seen in the figure in the Technical Note and in figure S2 above are different due to different pathlengths of the cuvettes used.

A gradual increase in absorbance from sample 1 to 20 after simultaneous extraction of 20 samples (Vortex Genie-2; set to maximum speed) followed by

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sequential absorbance measurements was observed. The results (entries 1, 3 and 5 of table S2) indicate that the extraction of **chl**s and their breakdown products continues statically if the plant material is left in contact with the extraction solvent. Simultaneously, there is evidence that the five-minute centrifugation step at 13,000 rpm enhanced the extraction efficiency. In spite of the observed gradual increase in absorbance, the absorbance of the extracts seen in entry 2 is lower than those seen in entries 1 and 3 and the absorbance of the extracts seen in entry 4 is lower than those seen in entries 3 and 5.

Table S2. Absorbance of acetone extracts of weld sample **B** obtained with two methods of extraction, after simultaneous extraction of 20 samples followed by sequential absorbance measurements.[a]

Entry	*n*	*Extraction procedure and sample treatment*[b]	*Position of the samples within a series*[c]	*Absorbance (AU) and* s_r *(%)*[d]
		10 mm-pathlength quartz cuvette		
1	3	3-min vortex; 5-min centrifuge	1-3	0.158 (3.5)
2	4	3-min vortex	4-7	0.149 (5.3)
3	3	3-min vortex; 5-min centrifuge	8-10	0.170 (2.6)
4	4	3-min vortex	14-17	0.160 (4.4)
5	3	3-min vortex; 5-min centrifuge	18-20	0.184 (4.6)

[a] λ of detection: 664 − 740 nm. Note: range of readings at 740 nm: 1 - 4 mAU;

[b] Room temperature;

[c] Position in the analysed series of samples;

[d] Average absorbance (s_r = relative standard deviation).