

Supplementary material

Rosmarinic Acid and Flavonoids of the Seagrass *Zostera noltei*: New Aspects on their Quantification and their Correlation with Sunlight Exposure

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Figure S1. Map of the Bay of Cadiz (Spain) and sites of *Z. noltei* sampling.

Table S1. Statistical data.

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Figure S1. Map of the Bay of Cadiz (Spain) and sites of *Z. noltei* sampling (white circles).

Table S1. Results of the one-way ANOVA and Kruskal-Wallis test examining the differences between phenolic metabolites: rosmarinic acid (RA), apigenin 7-sulphate (APS), luteolin 7-sulphate (LS), diosmetin 7-sulphate (DS), apigenin 7-glucoside (APG), and luteolin 7-glucoside (LG). Bold numbers indicate significant differences at $p < 0.05$.

fresh <i>vs</i> air-dried <i>vs</i> freeze-dried <i>vs</i> frozen - January				
One-way ANOVA test	df	MS	F	p
Total flavonoid	3	556.7	145.4	<0.05
APG	3	0.7122	30.69	<0.05
LS	3	11.606	35.34	<0.05
APS	3	47.77	13.24	<0.05
Kruskal-Wallis test	df	X ²		p
RA	3	10.385		<0.05
LG	3	10.532		<0.05
DS	3	9.4615		<0.05
fresh <i>vs</i> air-dried <i>vs</i> freeze-dried <i>vs</i> frozen - March				
One-way ANOVA test	df	MS	F	p
Total flavonoid	3	206.40	29.58	<0.05
DS	3	84.04	28.22	<0.05
Kruskal-Wallis test	df	X ²		p
RA	3	9.2564		<0.05
LG	3	9.5595		<0.05
APG	3	10.458		<0.05
LS	3	9.0513		<0.05
APS	3	9.359		<0.05
zone-I <i>vs</i> zone-S - March				
One-way ANOVA test	df	MS	F	p
RA	1	880.5	75.91	<0.05
Total flavonoid	1	501.6	29.93	<0.05
APS	1	98.38	16.5	<0.05
DS	1	34.51	100.5	<0.05
APG	1	26.049	34.41	<0.05
LS	1	1.4145	2.335	0.157
zone-I <i>vs</i> zone-S - April				
One-way ANOVA test	df	MS	F	p
APG	1	4.477	11.35	<0.05
Kruskal-Wallis test	df	X ²		p
RA	1	8.3077		<0.05
Total flavonoid	1	7.8801		<0.05
APS	1	0.6410		0.423
DS	1	2.5641		0.109
LG	1	8.3368		<0.05
LS	1	2.1421		<0.05

control <i>vs</i> UV-Filter				
One-way ANOVA test	df	MS	F	p
RA, Day 10	1	35.15	2.144	0.193
RA, Day 30	1	246.75	10.18	<0.05
RA, Day 60	1	485.5	14.82	<0.05
One-way ANOVA test	df	MS	F	p
Total flavonoid, Day 10	1	4.867	0.637	0.455
Kruskal-Wallis test	df	X²	p	
Total flavonoid, Day 30	1	0.7590		0.383
Total flavonoid, Day 60	1	1.3333		0.248
One-way ANOVA test	df	MS	F	p
APG, Day 10	1	0.0200	0.567	0.480
APG, Day 30	1	0.2415	1.65	0.246
APG, Day 60	1	0.3486	1.894	0.218
One-way ANOVA test	df	MS	F	p
APS, Day 10	1	2.928	1.022	0.351
APS, Day 30	1	0.312	0.087	0.779
APS, Day 60	1	5.040	2.097	0.198
One-way ANOVA test	df	MS	F	p
DS, Day 10	1	0.3486	0.432	0.535
DS, Day 30	1	0.1105	0.476	0.516
DS, Day 60	1	0.2450	0.296	0.606
Kruskal-Wallis test	df	X²	p	
LG, Day 10	1	1.7943		0.180
LG, Day 30	1	0.3835		0.535
LG, Day 60	1	0.7875		0.374
One-way ANOVA test	df	MS	F	p
LS, Day 10	1	0.1081	0.045	0.839
LS, Day 30	1	0.288	0.32	0.592
LS, Day 60	1	0.042	0.023	0.885
Antioxidant activity				
Kruskal-Wallis test	df	X²	p	
Antioxidant activities (Zone-I <i>vs</i> Zone-S)	1	3.857		<0.05
One-way ANOVA test	df	MS	F	p
Antioxidant activities (Control <i>vs</i> UV-Filter)	1	357.0	8.878	<0.05

Table S2. Content (mg/g dw) of rosmarinic acid (RA), sulfated flavonoids APS, LS, DS, flavonoid glycosides LG, APG, and total flavonoids in fresh, air-dried, freeze-dried, and frozen leaves of *Z. noltei* collected in (A) January and (B) March. Values are mean \pm SE ($n=3$). For each compound different letters indicate significant differences ($p<0.05$, Table S1).

(A)

Compound	fresh	air-dried	freeze-dried	frozen
RA	29.99 \pm 1.59 ^a	2.48 \pm 0.24 ^b	17.67 \pm 1.58 ^c	1.36 \pm 0.17 ^b
APS	12.98 \pm 1.46 ^a	4.28 \pm 0.31 ^b	7.56 \pm 1.37 ^{bc}	11.76 \pm 0.83 ^{ac}
LS	5.51 \pm 0.36 ^a	0.86 \pm 0.05 ^b	2.35 \pm 0.46 ^b	2.21 \pm 0.31 ^b
DS	3.93 \pm 0.20 ^a	0.96 \pm 0.05 ^b	2.79 \pm 0.61 ^a	4.04 \pm 0.25 ^a
APG	1.26 \pm 0.04 ^a	0.35 \pm 0.02 ^b	0.65 \pm 0.08 ^b	1.37 \pm 0.15 ^a
LG	0.53 \pm 0.03 ^a	nq	0.30 \pm 0.02 ^b	0.42 \pm 0.01 ^c
Total flavonoids	24.22 \pm 1.96 ^a	6.45 \pm 0.39 ^b	13.66 \pm 2.52 ^{bc}	19.81 \pm 1.39 ^{ac}

nq=not quantified, below limit of quantification.

(B)

Compound	fresh	air-dried	freeze-dried	frozen
RA	30.12 \pm 0.40 ^a	4.56 \pm 0.71 ^b	6.00 \pm 1.01 ^b	31.98 \pm 1.00 ^a
APS	11.74 \pm 1.42 ^a	3.12 \pm 0.19 ^b	4.24 \pm 0.56 ^b	12.50 \pm 1.48 ^a
LS	3.88 \pm 0.62 ^a	0.65 \pm 0.07 ^b	0.97 \pm 0.13 ^b	3.52 \pm 0.26 ^a
DS	2.92 \pm 0.20 ^a	0.44 \pm 0.14 ^b	1.06 \pm 0.19 ^b	2.09 \pm 0.32 ^a
APG	1.33 \pm 0.20 ^a	0.16 \pm 0.01 ^b	0.34 \pm 0.04 ^{bc}	0.79 \pm 0.02 ^c
LG	0.45 \pm 0.03 ^a	nq	0.18 \pm 0.01 ^b	0.43 \pm 0.02 ^a
Total flavonoids	20.31 \pm 2.11 ^a	4.36 \pm 0.31 ^b	6.79 \pm 0.87 ^b	19.34 \pm 2.00 ^a

nq=not quantified, below limit of quantification.

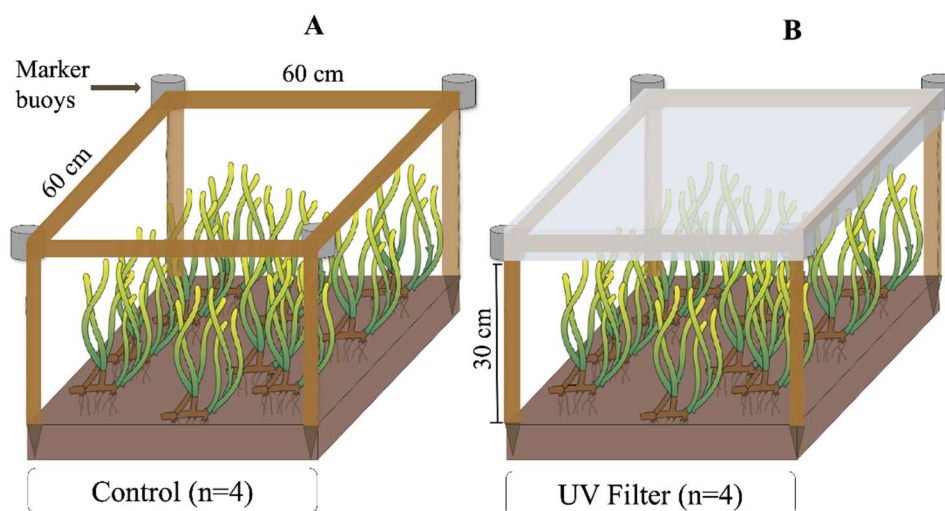


Figure S2. Scheme of the *in situ* experiment with *Z. noltei*: (A) control plants; (B) plants under an UV-filter.

Table S3. Content (mg/g dw) of rosmarinic acid (RA), sulfated flavonoids APS, LS, DS, flavonoid glycosides LG, APG, and total flavonoids in leaves of *Z. noltei* under a UV-filter for 10, 30, and 60 days and the corresponding controls. Values are mean \pm SE ($n=4$). For each day and compound different letters indicate significant differences ($p<0.05$, Table S1).

Compound	Day 0	Day 10		Day 30		Day 60	
		control	UV-filter	control	UV-filter	control	UV-filter
RA	30.35 \pm 1.34	24.25 \pm 2.31	20.06 \pm 1.70	38.16 \pm 3.37 ^a	27.06 \pm 0.88 ^b	40.02 \pm 2.05 ^a	24.43 \pm 3.49 ^b
APS	12.58 \pm 2.28	12.48 \pm 0.23	13.69 \pm 1.17	11.68 \pm 1.31	11.29 \pm 0.29	16.44 \pm 0.96	14.86 \pm 0.53
LS	3.25 \pm 0.52	4.20 \pm 0.89	4.43 \pm 0.63	2.50 \pm 0.41	2.88 \pm 0.53	4.20 \pm 0.80	4.06 \pm 0.53
DS	0.86 \pm 0.16	1.45 \pm 0.23	1.72 \pm 0.44	0.63 \pm 0.24	0.40 \pm 0.25	1.17 \pm 0.43	0.82 \pm 0.48
APG	0.59 \pm 0.06	0.81 \pm 0.10	0.71 \pm 0.09	0.82 \pm 0.26	0.47 \pm 0.08	1.15 \pm 0.27	0.73 \pm 0.14
LG	0.18 \pm 0.10	0.25 \pm 0.08	0.20 \pm 0.07	0.14 \pm 0.08	0.12 \pm 0.07	0.26 \pm 0.09	0.27 \pm 0.02
Total Flav	17.47 \pm 2.33	19.20 \pm 1.45	20.76 \pm 1.31	15.77 \pm 1.90	15.16 \pm 0.60	23.22 \pm 1.17	20.73 \pm 1.38

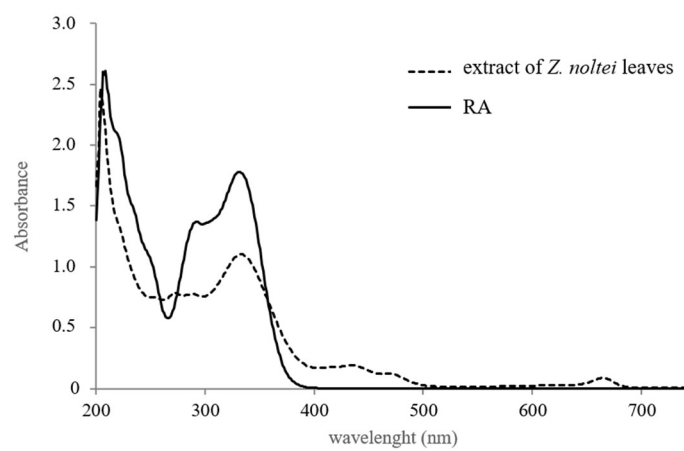


Figure S3. UV-Vis spectra of a methanolic extract of fresh leaves of *Z. noltei* and of rosmarinic acid (RA).

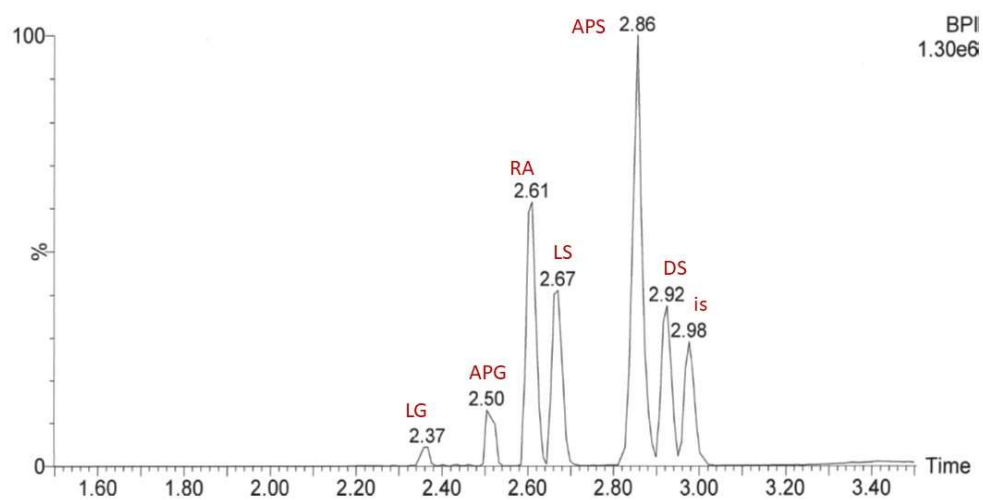


Figure S4. UPLC-MS chromatogram of a methanolic extract of fresh leaves of *Z. noltei*.
LG: luteolin 7-glucoside; APG: apigenin 7-glucoside; RA: rosmarinic acid; LS: luteolin 7-sulfate;
APS: apigenin 7-sulfate; DS: diosmetin 7-sulfate; is: internal reference.

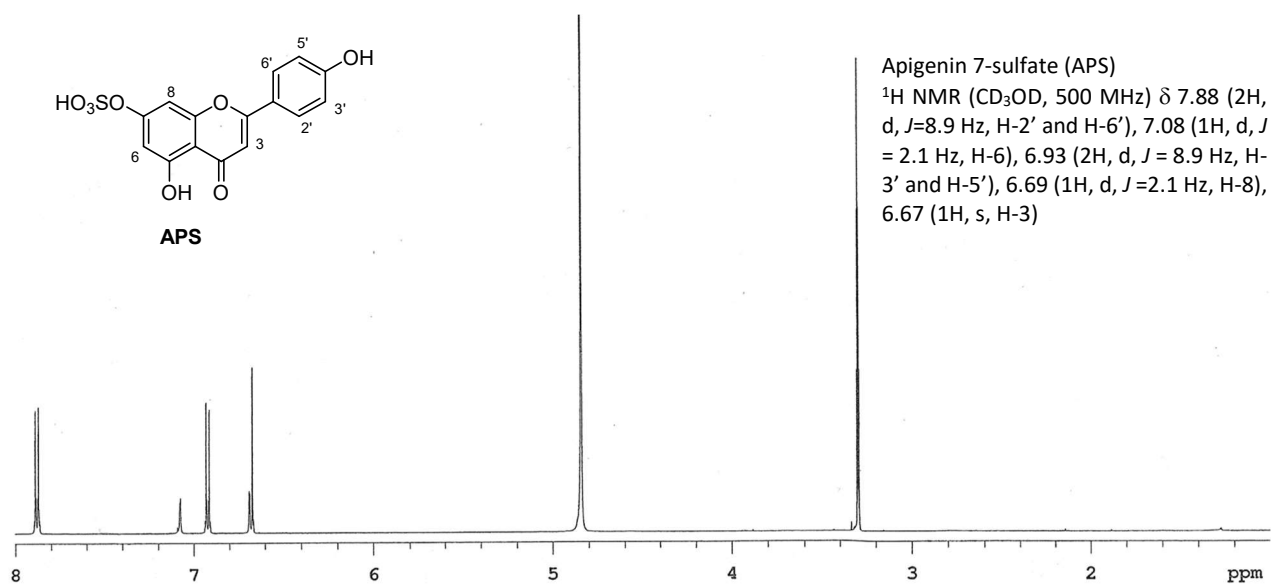


Figure S5. ^1H NMR spectrum (CD_3OD , 500 MHz) of apigenin 7-sulfate (APS) isolated from *Z. noltei* leaves.