

Effects of Light Quality and Chemical Elicitors on the Growth Parameters and Rosmarinic Acid Content of *in vitro* Cultures of *Hyptis pectinata* (L.) Poit.

Rita C. N. Pedroso,^{a,b} Leticia P. Pimenta,^b Marcos G. Tozatti,^{b,c} Núbia. A. A. Branquinho,^a Alessandra C. B. A. M. Hara,^a Fernando H. L. Silva,^a Alan C. Costa,^a Fabiano G. Silva,^a Carlos E. S. Miranda,^d Marcio L. A. Silva,^b Wilson R. Cunha,^b Patricia M. Pauletti^b and Ana H. Januario^b*,^b

^aInstituto Federal de Educação, Ciência e Tecnologia Goiano, Câmpus Rio Verde, 75901-970 Rio Verde-GO, Brazil

^bNúcleo de Pesquisa em Ciências Exatas e Tecnológicas, Universidade de Franca, 14404-600 Franca-SP, Brazil

^cFundação Educandário Pestalozzi, 14401-080 Franca-SP, Brazil

^dCurso de Ciências Farmacêuticas, Universidade de Ribeirão Preto, 14096-900 Ribeirão Preto-SP, Brazil

Rosmarinic acid (1)

¹H NMR (400 MHz, DMSO-*d*₆) δ 7.46 (d, *J* 15.9 Hz, H-7), 7.06 (s, H-2), 7.01 (d, *J* 8.2 Hz, H-6), 6.77 (d, *J* 8.2 Hz, H-5), 6.68 (s, H-6'), 6.64 (d, *J* 7.9 Hz, H-3'), 6.53 (d, *J* 7.9 Hz, H-2'), 6.24 (d, *J* 15.9 Hz, H-8), 5.03 (dd, *J* 4.0, 7.9 Hz, H-8'), 2.98 (dd, *J* 4.0, 14.3 Hz, H-7'a), 2.91 (dd, *J* 8.5, 14.3 Hz, H-7'b); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 170.7 (C-9'), 165.8 (C-9), 148.6 (C-4'), 145.8 (C-7), 145.5 (C-5'), 144.9 (C-4), 143.9 (C-3), 127.2 (C-1'), 125.3 (C-1), 121.5 (C-6), 120.0 (C-2'), 116.6 (C-6'), 115.7 (C-5), 115.4 (C-3'), 114.8 (C-2), 113.2 (C-8), 72.4 (C-8'), 36.1 (C-7').

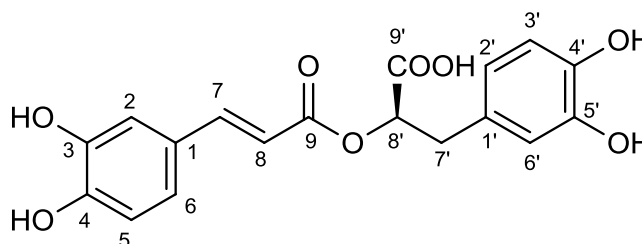


Figure S1. Chemical structure of rosmarinic acid (1).

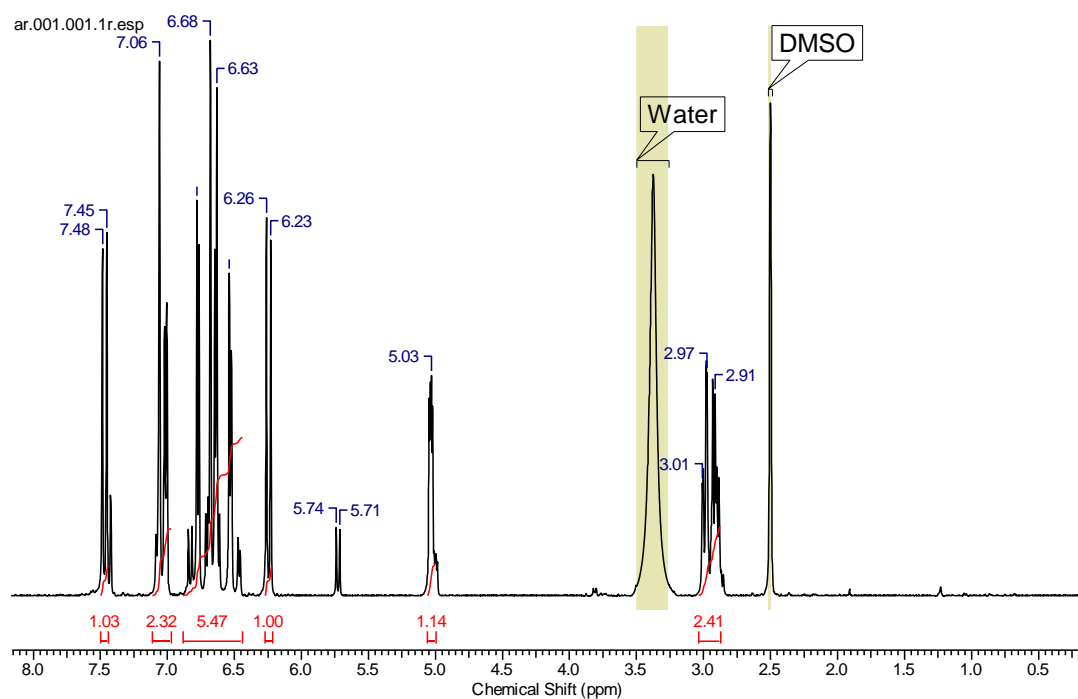


Figure S2. ^1H NMR spectrum (400 MHz, $\text{DMSO-}d_6$) of compound **1**.

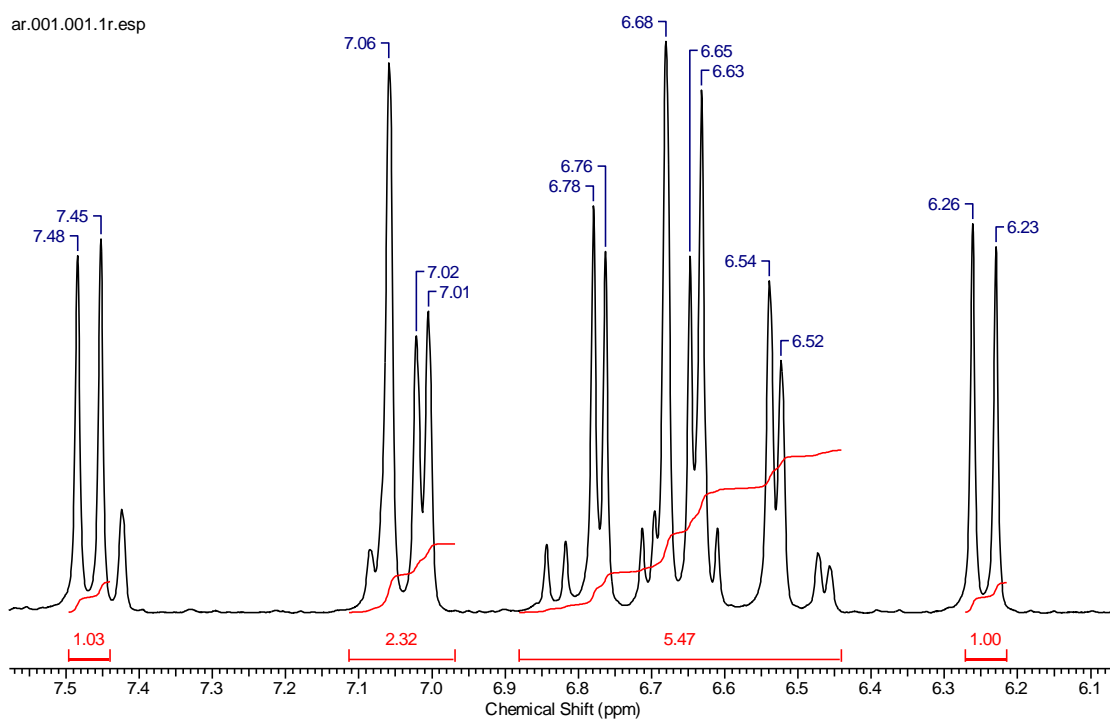


Figure S3. ^1H NMR spectrum (400 MHz, $\text{DMSO-}d_6$) of compound **1**, δ 6.0-7.5 region expansion.

ar.001.001.1r.esp

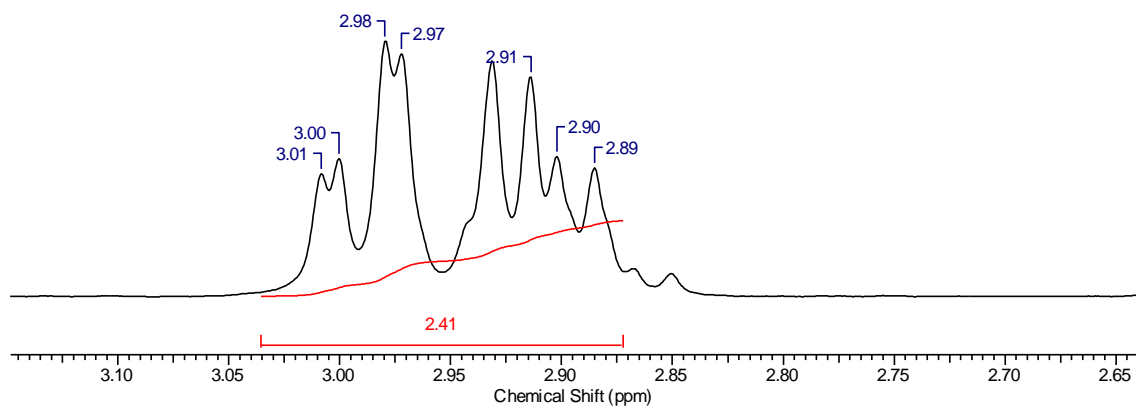


Figure S4. ¹H NMR spectrum (400 MHz, DMSO-*d*₆) of compound **1**, δ 2.65-3.10 region expansion.

ar.015.001.1r.esp

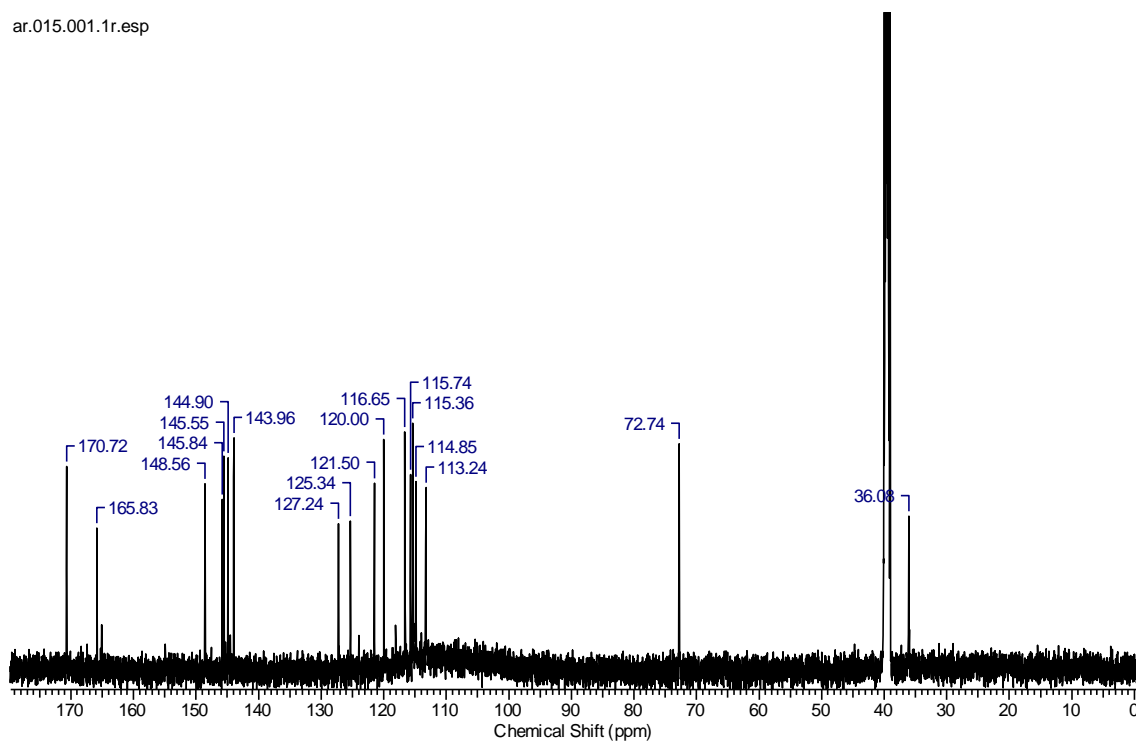


Figure S5. ¹³C NMR spectrum (100 MHz, DMSO-*d*₆) of compound **1**.

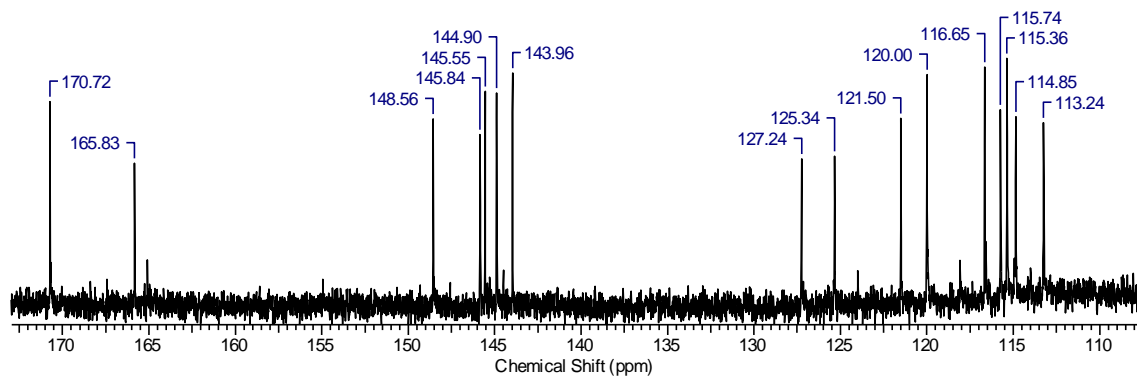


Figure S6. ^{13}C NMR spectrum (100 MHz, $\text{DMSO-}d_6$) of compound **1**, δ 110-172 region expansion.

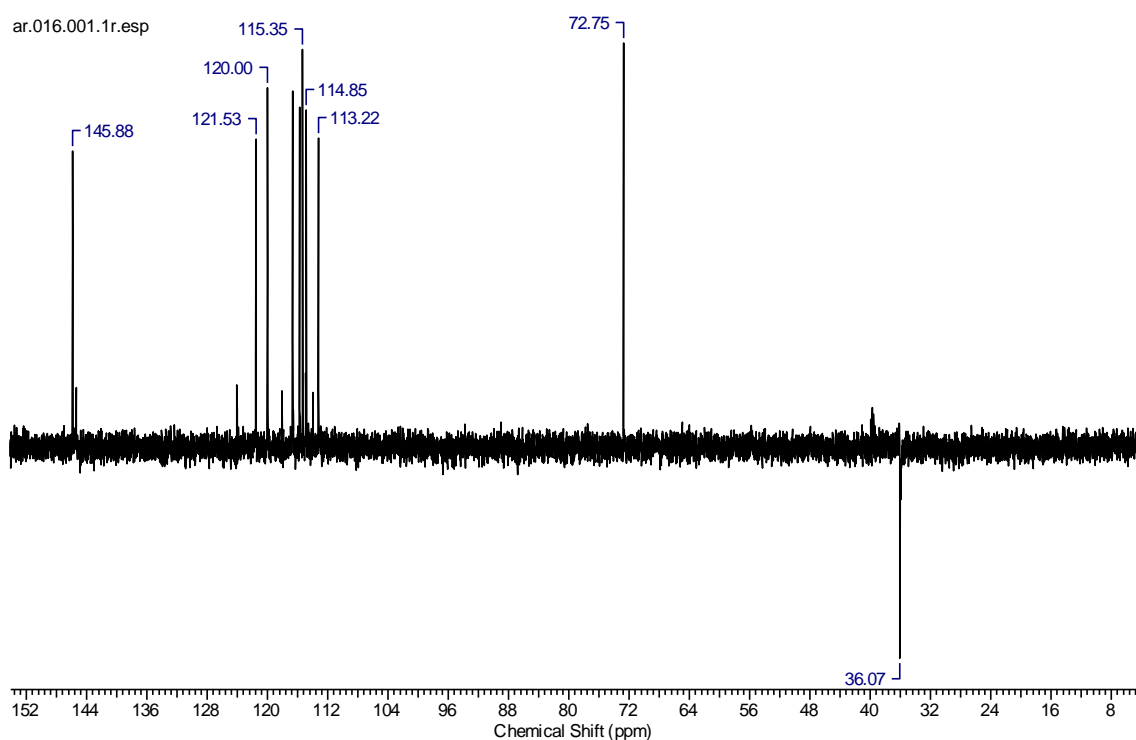


Figure S7. ^{13}C DEPT spectrum (100 MHz, $\text{DMSO-}d_6$) of compound **1**.

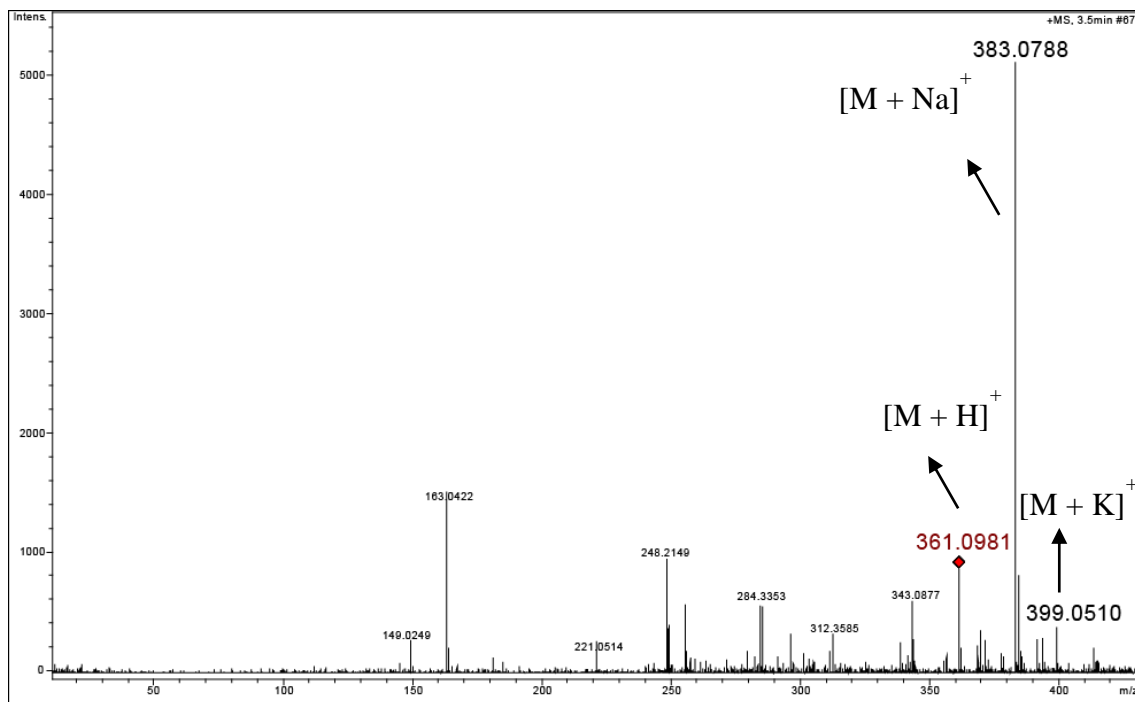


Figure S8. HR-ESIMS spectrum of compound 1 (positive mode).

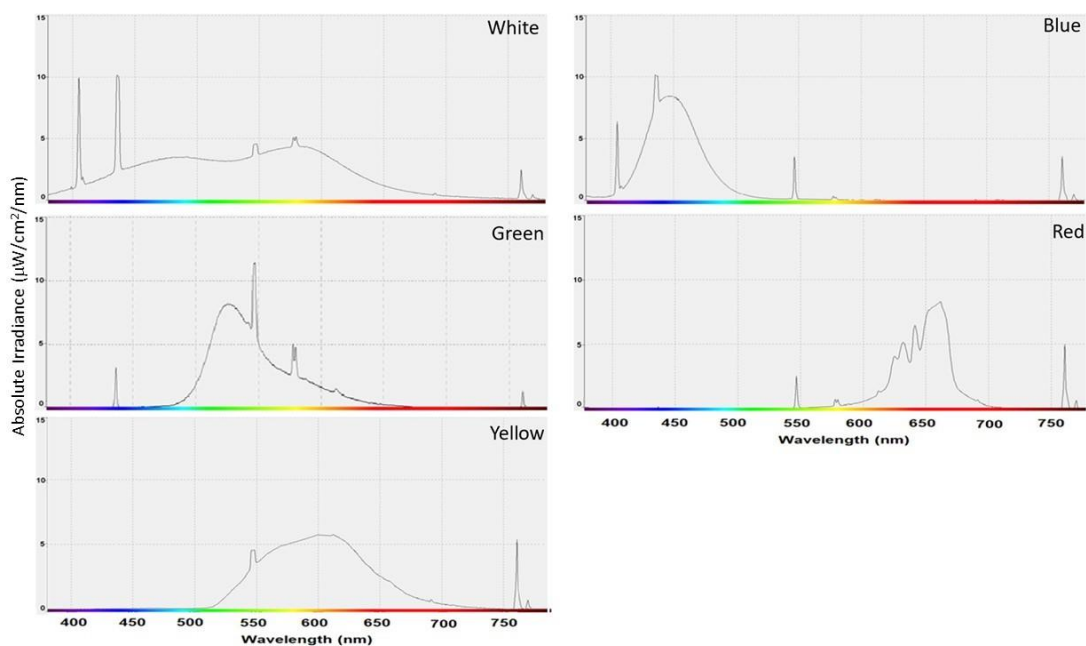


Figure S9. Spectral characteristics of the fluorescent lights used in the treatments of *H. pectinata* seedlings at 20 and 30 days of exposure to light spectra: white (300-750 nm), blue (400-490 nm), green (490-560 nm), red (600-700 nm), and yellow light (560-590 nm).

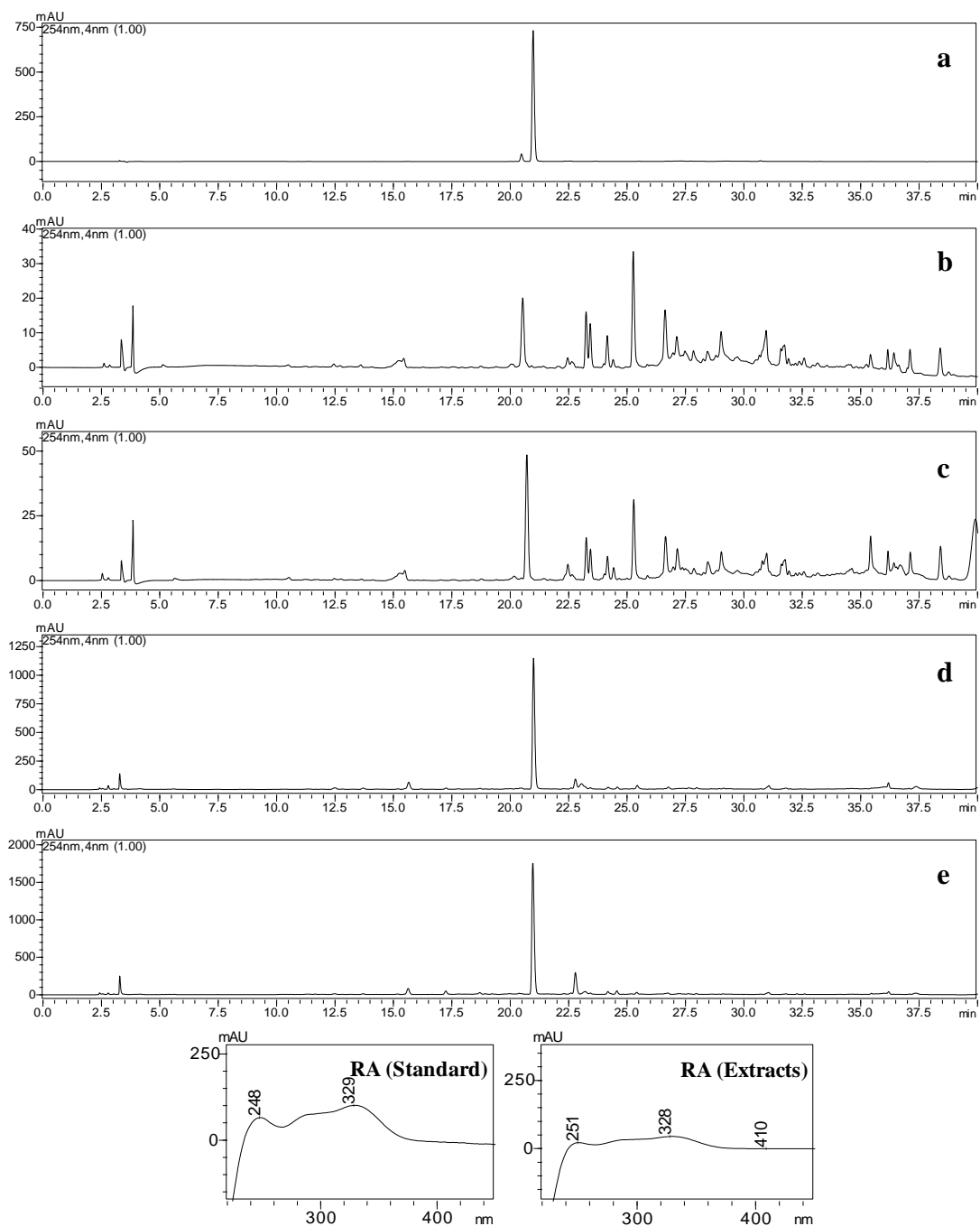


Figure S10. (a) HPLC-PDA chromatogram of rosmarinic acid (**1**, RT = 20.981 min) and HPLC-PDA chromatogram of methanolic extract of *H. pectinata* seedling cultures under (b) white color; (c) red color; (d) effect of elicitor silver nitrate (SN, 30 μ M); (e) effect of elicitor salicylic acid (SA, 60 μ M) and UV spectra of standard RA and UV spectra of RA in the extracts. Chromatographic conditions: CH₃OH/H₂O/HCOOH (5:94.8:0.2, v/v/v) in a linear gradient until 100% CH₃OH for 30 min, followed by 10-min elution with 100% CH₃OH. The volume of injection was 20 μ L, and the UV detector was set at 254 nm and 40 °C.

Table S1. Shoot length and average number of expanded leaves of *Hyptis pectinata* seedlings submitted to 20 and 30 days of light treatment

Light treatment	Evaluation time / days		Evaluation time / days	
	20	30	20	30
	Average shoot length ^a / cm		Average leaf number ^a	
White	7.16 ± 1.57	8.68 ± 2.11	8.23 ± 2.35	19.48 ± 7.30
Blue	11.56 ± 3.53	15.09 ± 3.87	7.68 ± 2.34	11.60 ± 3.76
Green	11.33 ± 2.79	11.19 ± 3.25	6.80 ± 2.51	11.30 ± 6.46
Red	10.65 ± 2.99	12.91 ± 2.49	5.60 ± 2.34	12.30 ± 4.63
Yellow	10.98 ± 2.16	12.71 ± 2.14	7.70 ± 3.39	12.35 ± 4.03
CV / %	25.23	22.87	35.90	39.05

^aMean ± standard error. CV: coefficient of variation.

Table S2. Effect of the type and concentration of abiotic elicitor on the shoot length and leaf number of *H. pectinata* seedlings

Treatment	Evaluation time / days			Evaluation time / days		
	3	6	20	3	6	20
	Average shoot length ^a / cm			Average leaf number		
Salicylic acid 30 µM	4.87 ± 0.90	6.12 ± 2.86	4.05 ± 0.32	12.81 ± 4.75	17.30 ± 5.31	10.90 ± 2.10
Salicylic acid 60 µM	4.88 ± 0.77	7.3 ± 0.83	4.65 ± 0.88	10.70 ± 1.4	16.95 ± 4.4	12.10 ± 1.06
Silver nitrate 30 µM	5.57 ± 0.30	5.34 ± 1.22	6.97 ± 1.06	12.5 ± 3.93	16.55 ± 1.34	17.20 ± 4.65
Silver nitrate 60 µM	5.26 ± 0.59	6.82 ± 0.85	4.9 ± 2.13	12.95 ± 1.1	16.30 ± 6.30	14.50 ± 4.93
Control	4.65 ± 0.52	6.19 ± 0.63	6.70 ± 0.71	15.35 ± 1.34	16.75 ± 1.31	18.10 ± 2.31
CV / %	12.20	20.11	18.70	19.46	22.25	20.67

^aMean ± standard error. CV: coefficient of variation.

Table S3. Fresh and dry weight of *Hyptis pectinata* seedlings submitted to 20 and 30 days of light treatment

Light treatment	Evaluation time / days		Evaluation time / days	
	20	30	20	30
	Fresh weight ^a / g		Dry weight ^a / g	
White	0.28 ± 0.11	0.43 ± 0.18	0.03 ± 0.01	0.04 ± 0.01
Blue	0.26 ± 0.08	0.40 ± 0.14	0.03 ± 0.01	0.040 ± 0.02
Green	0.29 ± 0.09	0.42 ± 0.17	0.03 ± 0.01	0.06 ± 0.05
Red	0.29 ± 0.09	0.56 ± 0.17	0.03 ± 0.01	0.07 ± 0.02
Yellow	0.27 ± 0.10	0.45 ± 0.15	0.02 ± 0.01	0.03 ± 0.01
CV / %	33.81	35.84	35.71	45.83

^aMean ± standard error. CV: coefficient of variation.

Table S4. Effect of the type and concentration of abiotic elicitors on the fresh and dry weight of *H. pectinata* seedlings

Treatment	Evaluation time / days			Evaluation time / days		
	3	6	20	3	6	20
	Fresh weight ^a / g			Dry weight ^a / g		
Salicylic acid 30 μ M	0.27 \pm 0.07	0.48 \pm 0.16	0.24 \pm 0.08	0.03 \pm 0.00	0.05 \pm 0.01	0.03 \pm 0.00
Salicylic acid 60 μ M	0.29 \pm 0.09	0.50 \pm 0.04	0.29 \pm 0.03	0.03 \pm 0.00	0.05 \pm 0.00	0.03 \pm 0.00
Silver nitrate 30 μ M	0.47 \pm 0.09	0.38 \pm 0.08	0.21 \pm 0.04	0.04 \pm 0.00	0.038 \pm 0.00	0.02 \pm 0.00
Silver nitrate 60 μ M	0.43 \pm 0.05	0.33 \pm 0.12	0.18 \pm 0.10	0.04 \pm 0.00	0.04 \pm 0.01	0.02 \pm 0.00
Control	0.37 \pm 0.12	0.52 \pm 0.20	0.29 \pm 0.06	0.04 \pm 0.01	0.05 \pm 0.00	0.03 \pm 0.01
CV / %	22.95	27.14	25.61	5.55	8.77	7.69

^aMean \pm standard error. CV: coefficient of variation.

Table S5. Rosmarinic acid content in *Hyptis pectinata* seedlings submitted to 20 and 30 days of light treatment

Light treatment	Evaluation time	
	Rosmarinic acid content (20 days) ^a /	Rosmarinic acid content (30 days) ^a /
	(μ g g ⁻¹ dry weight)	(μ g g ⁻¹ dry weight)
White	37.92 \pm 0.11	n.q.
Blue	n.q.	n.q.
Green	41.40 \pm 0.85	27.01 \pm 0.15
Red	161.64 \pm 8.64	21.70 \pm 1.65
Yellow	82.82 \pm 3.28	61.18 \pm 9.21
CV / %	3.98	17.00

^aMean \pm standard error. n.q.: not quantified; CV: coefficient of variation.

Table S6. Effect of abiotic elicitor type and concentration on rosmarinic acid content in *Hyptis pectinata* seedlings

Treatment	Evaluation time / days		
	3	6	20
	Rosmarinic acid content / (μ g g ⁻¹ of dry weight)		
Salicylic acid 30 μ M	2475.045 \pm 125.81	1586.49 \pm 45.90	2575.48 \pm 46.54
Salicylic acid 60 μ M	3203.60 \pm 75.07	1917.01 \pm 64.47	7402.95 \pm 109.18
Silver nitrate 30 μ M	4213.38 \pm 21.89	5156.95 \pm 92.53	4647.95 \pm 103.46
Silver nitrate 60 μ M	6456.94 \pm 216.95	2729.71 \pm 58.17	5964.13 \pm 115.18
Control	3863.10 \pm 251.59	2892.00 \pm 133.55	2146.31 \pm 50.11
CV / %	3.42	2.76	1.86

^aMean \pm standard error. CV: coefficient of variation.

