

*In vitro* Culture of *Rosmarinus Officinalis* L. in a Temporary Immersion System:  
Influence of two Phytohormones on Plant Growth and Carnosol Production

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## Supplementary information

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### General Experimental Procedures

All solvents and reagents used in this work were purchased from Sigma-Aldrich Co. and used without further purification. Carnosol standard was procured from Sigma-Aldrich. Analytical thin-layer chromatography (TLC) were carried out on precoated silica gel 60F254 aluminium base plates with a 230-400 mesh particle size (20 x 20 cm) (Merck), using a combination of dichloromethane ( $\text{Cl}_2\text{CH}_2$ ) and acetone ( $\text{Me}_2\text{CO}$ ) as an eluent, in proportion 95:5. In all cases, the TLC plates were visualized by exposure to ultra violet light, and then the spots were revealed by spraying with oleum (80% acetic acid, 16% water, 4% sulphuric acid) and heating at 120 °C for 15 minutes.

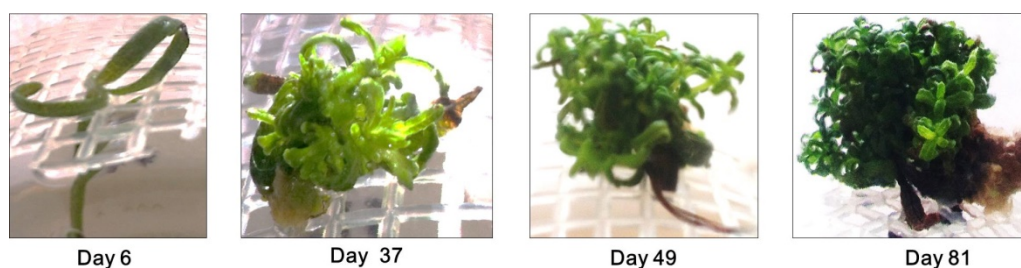
### Plant Material

Wild plants were collected in the ethnobotanical garden Francisco Peláez Roldán, located at street 2 South 1700 in the city of San Andrés Cholula, Puebla, and a specimen of *Rosmarinus officinalis* was deposited in the same ethnobotanical garden.

### Shoots regenerated from nodal explants



**Figure S1.** Effects of 5 mg L<sup>-1</sup> 6-BAP on induction of shoots from explants of *R. officinalis* L.



**Figure S2.** Effects of 5 mg L<sup>-1</sup> 6-BAP with an immersion cycle of 1 min every 12 h on induction of shoots from explants of *R. officinalis* L.

**Table S1.** Solid culture system and plant growth regulator effects on shoot number in *R. officinalis*.

Plant growth regulator		6-BAP																NAA															
Concentration (mg/L)		0.2					2.5					5					0.5					1					2.5						
Day		1A	1B	1C	PROM.	Desv Std	2A	2B	2C	PROM.	Desv Std	3A	3B	3C	PROM.	Desv Std	4A	4B	4C	PROM.	Desv Std	5A	5B	5C	PROM.	Desv Std	6A	6B	6C	PROM.	Desv Std		
% Contamination	0	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00		
	4	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00		
	7	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00		
	10	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00		
	17	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00		
28	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00			
% Necrosis	0	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00		
	4	15	1	5	7.00	7.21	40	1	15	18.67	19.76	0	40	60	33.33	30.55	5	15	15	11.67	5.77	10	0	0	3.33	5.77	40	30	5	25.00	18.03		
	7	20	1	10	10.33	9.50	45	5	15	21.67	20.82	0	45	60	35.00	31.22	30	15	15	20.00	8.66	20	0	10	10.00	10.00	40	40	15	31.67	14.43		
	10	20	1	10	10.33	9.50	55	10	35	33.33	22.55	0	75	65	46.67	40.72	50	60	20	43.33	20.82	30	0	20	16.67	15.28	60	40	40	46.67	11.55		
	17	28	9	17	18.00	9.54	70	30	45	48.33	20.21	100	100	50	83.33	28.87	85	100	30	71.67	36.86	85	0	55	46.67	43.11	100	65	90	85.00	18.03		
	28	30	24	17	23.67	6.51	98	42	48	62.67	30.75	100	100	100	100.00	0.00	100	100	100	100.00	0.00	100	100	100	100.00	0.00	100	100	100	100.00	0.00		
No. Callus	0	2	4	1	2.33	1.53	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	1	1	0.67	0.58	0	0	0	0.00	0.00		
	4	2	4	1	2.33	1.53	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	1	1	0.67	0.58	0	0	0	0.00	0.00		
	7	2	4	1	2.33	1.53	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	1	1	0.67	0.58	0	0	0	0.00	0.00		
	10	2	4	1	2.33	1.53	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	0	0	0.00	0.00	0	1	1	0.67	0.58	0	0	0	0.00	0.00		
	17	2	4	2	2.67	1.15	0	0	0	0.00	0.00	1	1	2	1.33	0.58	3	0	2	1.67	1.53	2	2	1	1.67	0.58	1	2	5	2.67	2.08		
28	2	4	2	2.67	1.15	0	0	0	0.00	0.00	1	1	4	2.00	1.73	3	0	3	2.00	1.73	3	3	3	3.00	0.00	1	4	11	5.33	5.13			
No. Shoots	0	2	5	6	4.33	2.08	4	4	2	3.33	1.15	2	2	2	2.00	0.00	2	2	2	2.00	0.00	2	0	2	1.33	1.15	6	6	2	4.67	2.31		
	4	2	5	6	4.33	2.08	4	4	2	3.33	1.15	4	2	2	2.67	1.15	2	3	2	2.33	0.58	2	0	2	1.33	1.15	6	6	2	4.67	2.31		
	7	2	5	6	4.33	2.08	4	6	4	4.67	1.15	4	2	2	2.67	1.15	2	3	2	2.33	0.58	2	0	2	1.33	1.15	6	6	2	4.67	2.31		
	10	3	5	6	4.67	1.53	4	6	4	4.67	1.15	4	2	2	2.67	1.15	2	4	2	2.67	1.15	2	0	2	1.33	1.15	6	6	2	4.67	2.31		
	17	4	5	6	5.00	1.00	4	6	4	4.67	1.15	5	2	2	3.00	1.73	2	4	2	2.67	1.15	2	0	2	1.33	1.15	6	6	2	4.67	2.31		
	28	6	5	6	5.67	0.58	5	6	4	5.00	1.00	5	2	2	3.00	1.73	2	4	4	3.33	1.15	2	0	2	1.33	1.15	6	6	2	4.67	2.31		

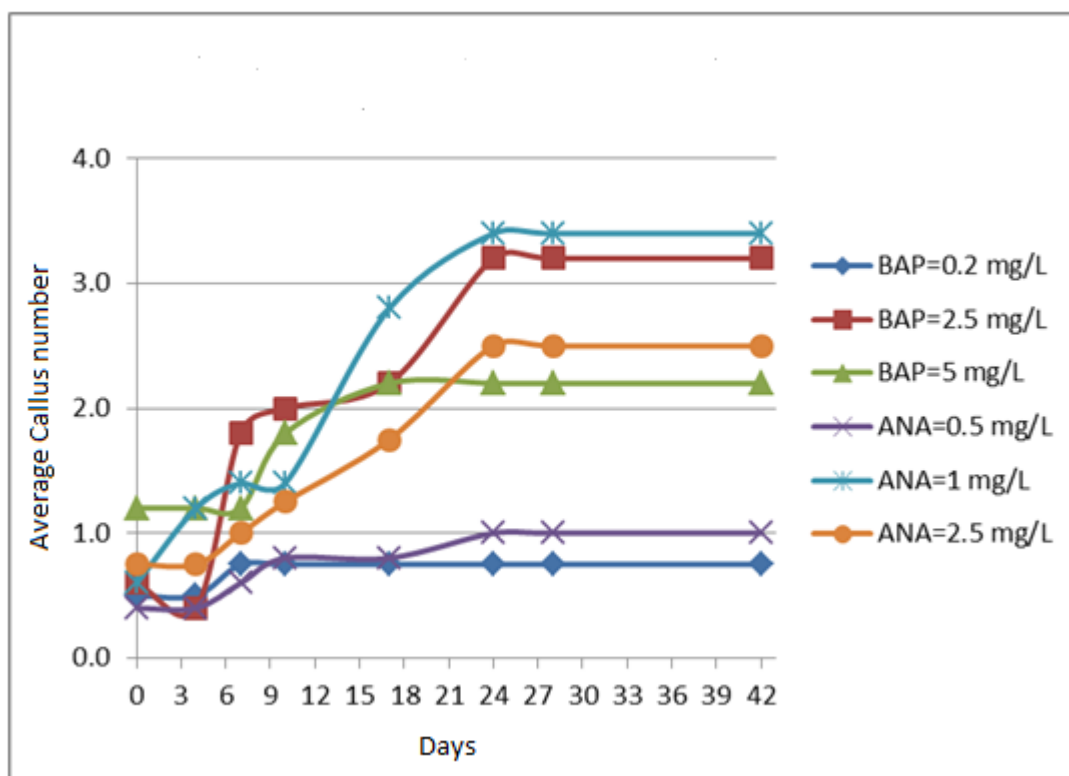
**Table S2.** Liquid culture system and plant growth regulator effects on shoot number in *R. officinalis*.

Plant growth regulator		6-BAP																NAA																																	
Concentration (mg/L)		0.2								2.5								5								0.5								1								2.5									
		1A	1B	1C	1E	1D	PROM.	Desv Std	2B	2A	2C	2D	2E	PROM.	Desv Std	3A	3B	3C	3E	3D	PROM.	Desv Std	4A	4B	4C	4D	4E	PROM.	Desv Std	5A	5B	5C	5D	5E	PROM.	Desv Std	6A	6B	6C	6D	6E	PROM.	Desv Std								
% Contamination	Day	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00								
	0	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00								
	4	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00								
	7	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00								
	10	100	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	100	0.00	0.00							
	17	100	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	100	0.00	0.00							
	24	100	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	100	0.00	0.00							
% Necrosis	28	100	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0	100	0.00	0.00						
	42	100	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	100	0.00	0.00							
	0	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0.00	0.00	0	0	0	0	0	0	0.00	0.00							
	4	0	1	5	25	1	10.33	12.86	1	5	0	5	5	3.33	2.89	1	10	5	1	5	5.33	4.51	15	95	5	10	5	36.67	50.58	1	1	0	1	10	0.67	0.58	1	0	1	1	5	0.67	0.58								
	7	1	1	10	30	1	13.67	14.84	1	5	5	5	5	5.00	0.00	1	15	5	1	5	7.00	7.21	20	100	15	10	10	41.67	50.58	1	1	1	1	15	1.00	0.33	2.31	10	1	1	10	2.33	2.31								
	10	1	5	25	100	1	43.33	50.08	5	10	10	5	5	8.33	2.89	5	15	5	1	5	8.33	5.77	20	100	15	10	10	41.67	50.58	1	1	1	1	15	1.00	0.00	10	1	1	15	4.00	5.20									
	17	---	10	35	100	10	48.33	46.46	5	10	10	5	5	8.33	2.89	5	15	5	1	5	8.33	5.77	25	100	25	15	15	46.67	46.46	1	1	1	1	5	25	2.33	2.31	10	10	1	10	---	7.00	5.20							
No. Callus	24	---	75	95	100	75	90.00	13.23	45	60	25	10	5	31.67	25.66	50	40	20	5	10	36.67	15.28	50	100	70	50	60	73.33	25.17	5	65	20	40	75	41.67	22.55	30	75	20	30	---	41.67	29.30								
	28	---	95	95	100	95	96.33	2.89	45	70	50	40	15	53.33	15.28	55	45	25	5	10	36.67	15.28	65	100	75	50	50	75.00	25.00	10	65	40	45	80	50.00	13.23	35	95	90	50	---	78.33	24.66								
	42	---	100	100	100	100	100.00	0.00	65	90	70	50	15	70.00	20.00	90	65	50	5	10	68.33	20.21	100	100	100	100	100	100.00	0.00	15	95	75	80	100	81.67	7.64	70	100	100	75	---	91.67	14.43								
	0	0	0	1	0	1	0.33	0.58	0	0	2	0	1	0.67	1.15	2	0	1	1	2	1.00	1.00	0	0	1	0	1	0.33	0.58	1	0	1	1	0	0.67	0.58	1	1	1	0	1	0	1	0.67	0.58						
	4	0	0	1	0	1	0.33	0.58	0	0	1	0	1	0.33	0.58	2	0	1	1	2	1.00	1.00	0	0	1	0	1	0.33	0.58	1	1	1	3	0	1.67	1.15	1	1	1	0	1	0.67	0.58								
	7	0	1	0	1	0	0.67	0.58	3	1	2	1	2	1	1.33	0.58	2	0	1	1	2	1.00	1.00	0	0	2	0	1	0.67	1.15	1	1	1	4	0	2.00	1.73	1	1	1	1	1	1	1.00	0.00						
	10	0	1	0	1	0	1	0.67	0.58	3	1	2	2	2	1.67	0.58	3	1	1	2	2	1.67	1.15	0	0	2	1	1	1.00	1.00	1	1	1	4	0	2.00	1.73	1	1	2	1	2	1.33	0.58							
No. Shoots	17	---	1	1	0	1	0.67	0.58	3	1	2	3	2	2	2.00	1.00	3	2	2	2	2	2.33	0.58	0	0	2	1	1	1.00	1.00	1	1	4	8	0	4.33	3.51	1	2	2	2	---	2.00	0.00							
	24	---	0	1	0	1	0.67	0.58	1	5	2	3	2	3.67	2.31	3	2	2	2	2	2.33	0.58	1	1	0	2	1	1	1.00	1.00	2	2	1	5	9	0	5.00	4.00	4	2	2	2	---	2.00	0.00						
	28	---	1	1	0	1	0.67	0.58	3	1	5	2	2	2.67	2.31	3	2	2	2	2	2.33	0.58	1	0	2	1	1	1.00	1.00	2	2	1	5	9	0	5.00	4.00	4	2	2	2	---	2.00	0.00							
	42	---	1	1	0	1	0.67	0.58	3	1	5	2	2	3.67	2.31	3	2	2	2	2	2.33	0.58	1	0	2	1	1	1.00	1.00	2	1	5	9	0	5.00	4.00	4	2	2	2	---	2.00	0.00								
	0	3	2	4	2	6	2.67	1.15	6	2	7	6	6	6.67	2.31	7	2	2	2	2	3.33	2.31	2	2	2	2	2	2.00	0.00	6	6	6	6	6	6.67	0.58	7	2	4	4	12	3.33	1.15								
	4	5	4	4	4	8	4.00	0.00	7	5	6	7	11	6.00	1.00	4	6	2	5	4	4.00	2.00	3	4	4	3	8	3.67	0.58	10	9	5	10	8	5.00	2.65	9	3	6	4	12	4.33	1.53								
	7	6	4	6	6	10	5.33	1.15	8	8	6	10	15	8.00	2.00	6	6	6	6	6	6.00	0.00	5	5	5	6	9	5.33	0.58	12	10	5	14	5	9.67	4.51	11	3	6	5	14	4.67	1.53								
No. Shoots	10	6	4	6	6	10	5.33	1.15	11	8	8	14	15	10.00	3.46	6	6	6	6	6	6.00	0.00	5	5	5	6	9	5.67	0.58	13	10	8	14	5	10.67	3.06	13	4	6	5	14	5.00	1.00								
	17	---	6	6	6	13	6.00	0.00	14	9	8	14	17	10.33	3.21	8	14	8	14	15	10.00	3.46	6	5	6	6	10	5.67	0.58	15	14	10	14	5	12.67	2.31	13	4	6	5	---	5.00	1.00								
	24	---	6	6	6	13	6.00	0.00	22	13	14	17	18	14.67	2.08	14	16	14	18	19	14.67	1.15	7	5	6	6	10	5.67	0.58	17	16	10	14	6	13.33	3.06	13	6	6	8	---	6.67	1.15								
	42	---	8	6	6	13	6.67	1.15	22	14	14	17	23	15.00	1.73	15	19	15	21	29	16.33	2.31	7	5	6	6	10	5.67	0.58	19	16	10	14	6	13.33	3.06	13	6	6	8	---	6.67	1.15								

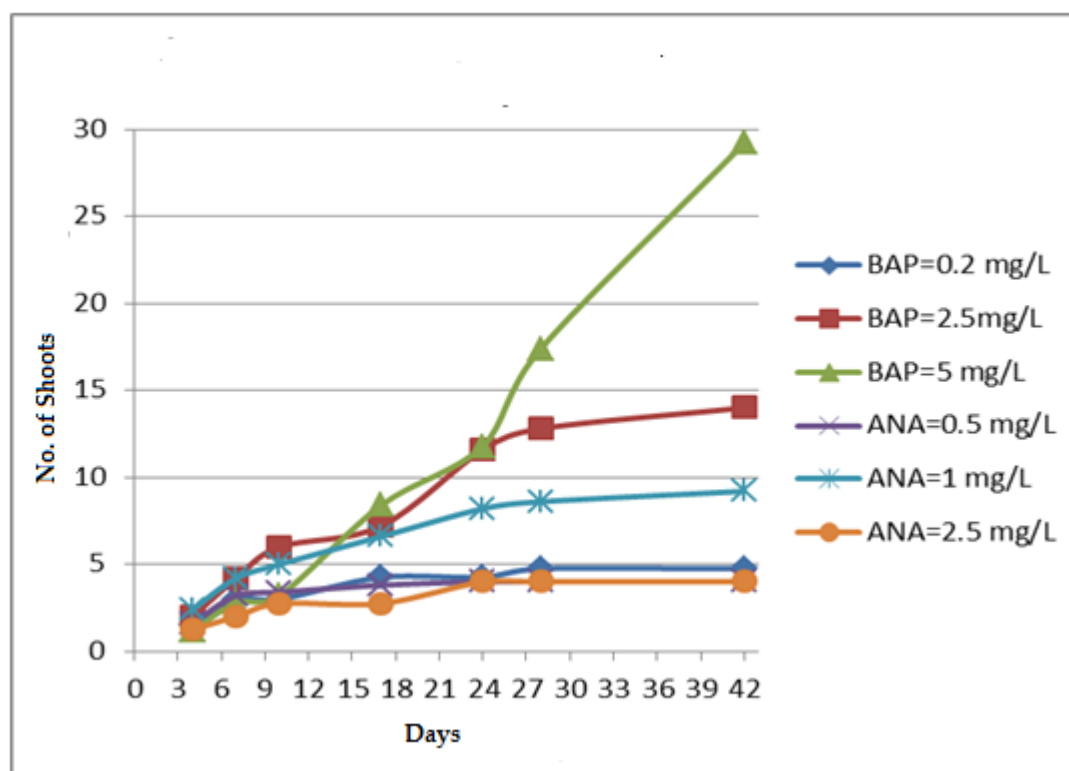
**Table S3.** Effects of immersion time and frequency on the contamination, necrosis, number of Callus and shoots.

One Immersion every 24 HORAS																																						
Plant growth regulators		6-BAP																																				
Concentration (mg/L)		2.5 mg/L														5 mg/L																						
Immersion time (min)		1					5					10					1					5					10											
	Day	1A	1B	1D	1C	PROM.	Desv std	2B	2A	2C	2D	PROM.	Desv std	3A	3B	3C	3D	PROM.	Desv std	4B	4A	4C	4D	PROM.	Desv std	5B	5A	5C	5D	PROM.	Desv std	6A	6B	6D	PROM.	Desv std		
% Contamination	0	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	
	5	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	100	0	0	0	0.00	0.00	100	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	
	34	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	100	0	0	0	0.00	0.00	100	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	
	48	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00		0	0	0	0.00	0.00	100	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	
% Necrosis	70	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00		0	0	0	0.00	0.00	100	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	
	0	0	0	1	2	1	1.00	1.00	0	0	0	8	2.50	4.33	0	0	0	0	0.00	0.00		28	3	28	19.67	14.43	0	0	27	6	11.00	14.18	0	20	10	0	10.00	10.00
	5	1	2	2	2	1.53	0.50	0	2	0	60	20.50	34.22	60	0	1	75	25.33	43.02		45	5	63	37.67	29.69	1	25	77	6	36.00	36.76	1	35	100	0	45.33	50.30	
	34	2	3	5	100	3.33	1.53	4	15	100	90	68.33	46.46		3	100	100	67.67	56.00		88	100	100	96.00	6.93	11	100	95	66	87.00	18.36	15	100	100	2	71.67	49.07	
No. of Callus	48	2	4	6	100	4.00	2.00	6	22	100	90	70.67	42.44		4	100	100	68.00	55.43		100	100	100	100.00	0.00	12	100	100	100	100.00	0.00	15	100	100	3	71.67	49.07	
	70	7	6	6	100	6.33	0.58	6	54	100	100	84.67	26.56		17	100	100	72.33	47.92		100	100	100	100.00	0.00	88	100	100	100	100.00	0.00	100	100	100	6	100.00	0.00	
	0	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00		0	0	0	0.00	0.00		0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	
	5	1	0	0	0	0.33	0.58	1	0	0	0	0.00	0.00		0	0	0	0.00	0.00		0	0	0	0.00	0.00	1	0	0	0	0.00	0.00	1	0	0	0	0.33	0.58	
No. of Shoots	34	4	1	3	0	2.67	1.53	2	1	0	0	0.33	0.58		2	0	0	0.67	1.15		1	2	0	1.00	1.00	1	2	0	1	1.00	1.00	1	0	0	1	0.33	0.58	
	48	4	2	3	0	3.00	1.00	4	2	0	0	0.67	1.15		2	0	0	0.67	1.15		1	2	0	1.00	1.00	1	2	0	1	1.00	1.00	1	0	0	1	0.33	0.58	
	70	4	3	3	0	3.33	0.58	5	2	0	0	0.67	1.15		2	0	0	0.67	1.15		1	2	0	1.00	1.00	1	2	0	2	1.33	1.15	1	0	0	1	0.33	0.58	
	0	7	9	8	6	8.00	1.00	6	10	2	4	5.33	4.16	2	4	6	2	4.00	2.00	3	4	2	4	3.33	1.15	6	2	6	6	4.67	2.31	6	4	1	5	3.67	2.52	
No. of Shoots	5	7	12	8	6	9.00	2.65	6	10	4	8	7.33	3.06	2	4	12	2	6.00	5.29	3	4	2	4	3.33	1.15	12	2	6	6	4.67	2.31	6	4	1	6	3.67	2.52	
	34	13	47	23	12	27.67	17.47	26	23	12	15	16.67	5.69		28	18	12	19.33	8.08		4	2	4	3.33	1.15	51	2	6	8	5.33	3.06	16	4	1	28	7.00	7.94	
	48	26	73	86	12	61.67	31.56	67	33	19	26	26.00	7.00		43	23	25	30.33	11.02		4	2	4	3.33	1.15	60	2	6	8	5.33	3.06	16	4	1	52	7.00	7.94	
	70	94	99	95	12	96.00	2.65	76	33	28	35	32.00	3.61		50	35	38	41.00	7.94		4	2	4	3.33	1.15	60	2	6	8	5.33	3.06	16	4	1	101	7.00	7.94	

One Immersion every 12 HORAS																																					
Plant growth regulators		6-BAP																																			
Concentration (mg/L)		2.5 mg/L														5 mg/L																					
Immersion time (min)		1				5				10				1				5				10															
	Day	7A	7B	7C	7D	PROM.	Desv std	8A	8B	8C	8D	PROM.	Desv std	9A	9C	9B	9D	PROM.	Desv std	10C	10B	10D	10A	PROM.	Desv std	11B	11C	11A	11D	PROM.	Desv std	12C	12A	12B	12D	PROM.	Desv std
% Contamination	0	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00
	5	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	100	0	0	0	0.00	0.00	100	0	0	0	0.00	0.00
	34	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	0	100	0.00	0.00	0	0	0	0	0.00	0.00	100	0	0	0	0.00	0.00		0	0	0	0.00	0.00
	48	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	0		0.00	0.00	0	0	0	0	0.00	0.00		0	0	0	0.00	0.00		0	0	0	0.00	0.00
% Necrosis	70	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	0		0.00	0.00	0	0	0	0	0.00	0.00		0	0	0	0.00	0.00		0	0	0	0.00	0.00
	0	2	0	4	0	2.00	2.00	50	20	4	8	24.67	23.35	13	0	20	0	11.00	10.15	8	0	0	0	0.00	0.00	10	20	0	0	6.67	11.55	0	56	7	0	21.00	30.51
	5	3	1	11	0	5.00	5.29	83	30	12	23	41.67	36.91	13	0	50	82	21.00	25.94	40	1	0	0	0.17	0.29	30	20	1	0	7.00	11.27	0	100	7	0	35.67	55.82
	34	6	40	77	5	41.00	35.51	100	100	60	98	86.67	23.09	36	5	50	82	30.33	23.03	100	7	1	1	3.00	3.46		93	4	43	46.67	44.61	9	100	83	100	94.33	9.81
No. of Callus	48	11	78	85	7	58.00	40.85	100	100	80	100	93.33	11.55	40	5	50		31.67	23.63	100	9	1	2	4.00	4.36		100	4	43	49.00	48.28	33	100	100	100	100.00	0.00
	70	12	100	85	7	65.67	47.08	100	100	80	100	93.33	11.55	100	5	50		51.67	47.52	100	10	2	2	4.67	4.62		100	4	43	49.00	48.28	100	100	100	100	100.00	0.00
	0	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00
	5	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	1	1	0	1	0.67	0.58	1	0	0	1	0.33	0.58
No. of Shoots	34	2	2	2	1	2.00	0.00	2	0	2	1	1.33	1.15	3	8	0	0	3.67	4.04	1	2	1	2	1.67	0.58		2	1	2	1.67	0.58	2	0	2	2	1.33	1.15
	48	2	2	2	1	2.00	0.00	2	0	2	1	1.33	1.15	3	8	0		3.67	4.04	2	5	2	5	4.00	1.73		3	1	3	2.33	1.15	2	0	2	2	1.33	1.15
	70	2	4	2	1	2.67	1.15	2	0	2	1	1.33	1.15	3	8	0		3.67	4.04	2	5	2	5	4.00	1.73		3	1	3	2.33	1.15	2	0	2	2	1.33	1.15
	0	5	4	7	10	5.33	1.53	4	2	5	6	3.67	1.53	4	6	4	6	4.67	1.15	2	6	4	4	4.67	1.15	2	2	4	4	3.33	1.15	7	5	6	6	5.67	0.58
No. of Shoots	5	5	4	7	10	5.33	1.53	4	2	5	6	3.67	1.53	4	6	4	6	4.67	1.15	2	6	4	4	4.67	1.15	2	2	4	4	3.33	1.15	7	5	6	6	5.67	0.58
	34	33	5	7	64	15.00	15.62	4	2	5	6	3.67	1.53	10	24	10		14.67	8.08	2	50	62	91	67.67	21.08	2	4	7	4.33	2.52	51	5	6	6	5.67	0.58	
	48	33	5	8	139	15.33	15.37	4	2	5	6	3.67	1.53	12	54	12		26.00	24.25	2	97	148	143	129.33	28.11	2	12	7	7.00	5.00	71	5	6	6	5.67	0.58	
	70	33	7	8	139	16.00	14.73	4	2	5	6	3.67	1.53	14	118	16		49.33	59.48	2	146	203	162	170.33	29.40	2	57	7	22.00	30.41	91	5	6	6	5.67	0.58	

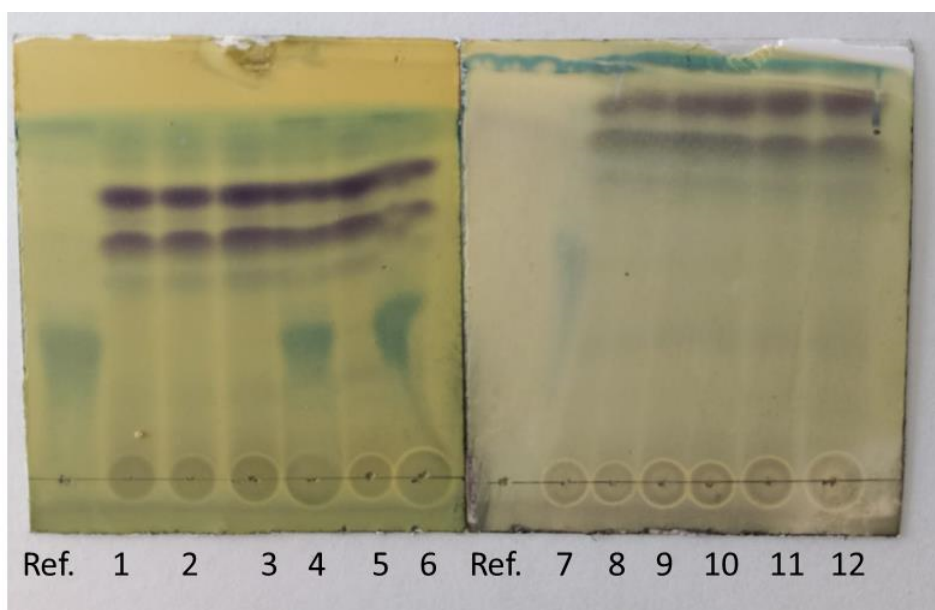


**Figure S3.** Effect of 6-BAP and NAA on callus induction in *R. officinalis* explants versus days of cultivation.



**Figure S4.** Effect of 6-BAP and NAA on number of shoots in *R. officinalis* explants versus days of cultivation.

### Thin-layer chromatography



**Figure S5.** Thin-layer chromatography (TLC, SiO<sub>2</sub>) of acetonic extracts of callus samples from fractions 1-12 (Table 1) developed with dichloromethane: acetone (95:5) and revealed with oleum (80% acetic acid, 16% water, 4% sulphuric acid).

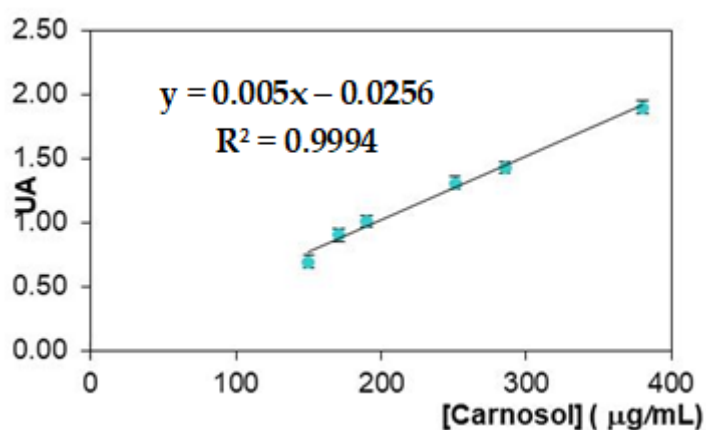
### HPLC chromatogram

**Table S4.** Validation parameters for the determination of carnosol by HPLC-DAD.

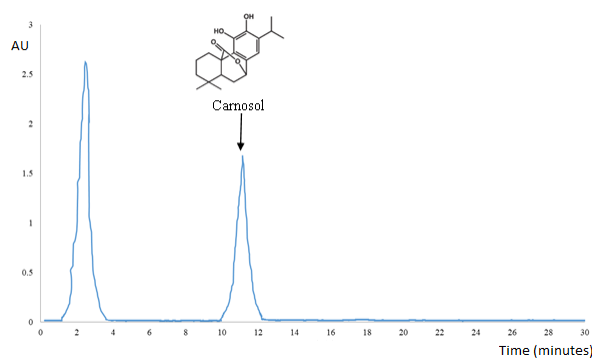
Validation parameters	Results
Wavelength (nm)	293
Linearity range (µg / mL)	150-385
Retention time (min)	10.7
Calibration equation	y= 0.005x + 0.0256
Correlation coefficient (R <sup>2</sup> )	0.9894
Standard deviation of the intercept	3.0 x 10 <sup>-4</sup>
Standard deviation of the slope	0.0256
Limit of detection (µg/mL)	3.68
Limit of quantification (µg/mL)	12.62

**Table S5.** Influence of the flow of the mobile on retention time ( $t_R$ ), dead time ( $t_0$ ) and retention factors ( $k'$ ).

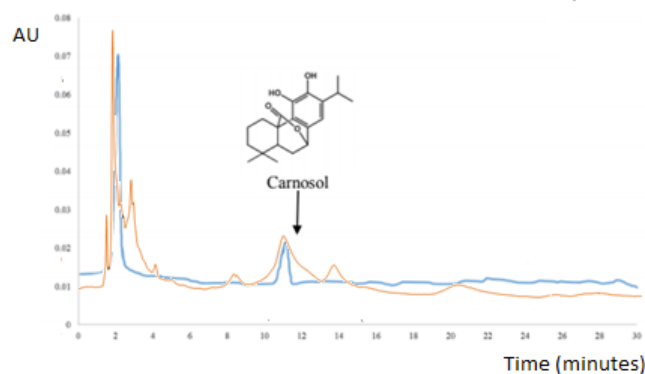
Flow (mL/min)	Retention time (min)	Dead time (min)	Retention factors
1.0	14.81	2.513	4.89
1.1	13.44	2.293	4.86
1.2	10.74	2.680	3.01
1.5	11.32	2.147	4.27



**Figure S6.** Calibration Curve for carnosol. Six different concentrations of carnosol ( $n= 3$ ) were prepared from the standard solution. 20  $\mu$ L of each concentration were injected and response was measured at 293 nm to plot calibration curve.

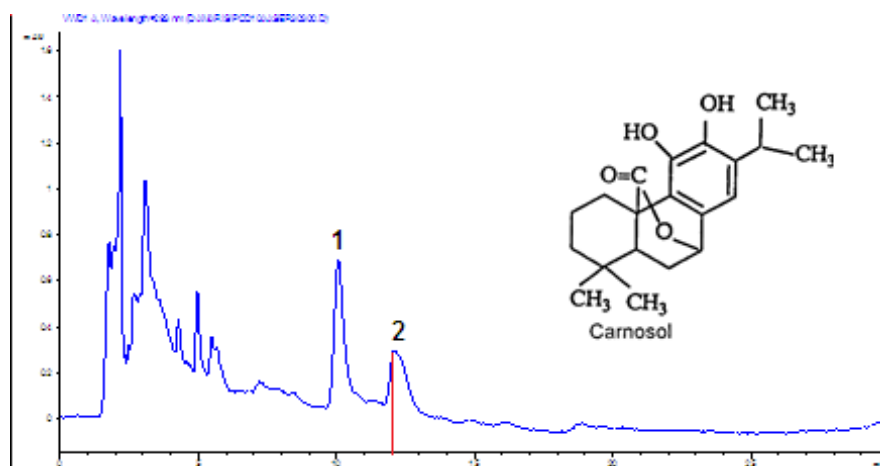


**Figure S7.** Chromatogram of carnosol at 370  $\mu$ g mL<sup>-1</sup>. Chromatography conditions: flow at 1.2 mL min<sup>-1</sup>; isocratic elution, 60-40% 10 mM formic (pH 3) - CH<sub>3</sub>CN (v/v); the injection volume was 20  $\mu$ L, and the wavelength of the detector was set at 293 nm.



**Figure S8.** Chromatogram of the Carnosol standard at 220 mg mL<sup>-1</sup> (blue line) vs chromatogram of a sample of *R. officinalis* acetonetic extract (orange line). Chromatography conditions: flow at 1.2 mL min<sup>-1</sup>; isocratic elution, 60-40% 10 mM formic (pH 3) - CH<sub>3</sub>CN (v/v); the injection volume was 20 µL, and the wavelength of the detector was set at 293 nm.

Chromatogram of carnosol at 370 µg mL<sup>-1</sup>. Chromatography conditions: flow at 1.2 mL min<sup>-1</sup>; isocratic elution, 60-40% 10 mM formic (pH 3) - CH<sub>3</sub>CN (v/v); the injection volume was 20 µL, and the wavelength of the detector was set at 293 nm.

**Figure S9.** Characteristic HPLC profile of the acetonetic extract of a callus sample of *R. officinalis* (media supplemented with 6- BAP; Table 3, entries 1 and 4). Peak 1, carnosol; Peak 2, undefined.

Chromatography conducted using Hypersil ODS C18 (250mm x 4.6 mm, 5  $\mu$ m particle size) column; flow at 1.2 mL/min; isocratic elution, 60-40% 10 mM formic (pH 3) - CH<sub>3</sub>CN (v/v) as mobile phase which was filtered and degassed before analysis; Retention times (r.t) in min correspond to the analysis at 25°C; the injection volume was 20  $\mu$ L, and the wavelength of the detector was set at 254 and 293 nm.