

## SUPPLEMENTARY MATERIAL

# Extraction optimization and Qualitative/Quantitative determination of bioactive abietane-type diterpenes from three *Salvia* Species (common sage, Greek sage and rosemary) by $^1\text{H}$ -qNMR

Panagiotis Kallimanis <sup>1</sup>, Prokopios Magiatis <sup>1,\*</sup>, Angeliki Panagiotopoulou <sup>2</sup>, Ioanna Chinou <sup>1,\*</sup>

<sup>1</sup> Laboratory of Pharmacognosy & Chemistry of Natural Products, Department of Pharmacy, National & Kapodistrian University of Athens, University Campus 157 71 Zografou, Greece

<sup>2</sup> Institute of Biosciences & Applications, National Centre for Scientific Research "Demokritos", 15310 Agia Paraskevi Attikis, Greece

\* Correspondence: P.M. Tel.: +30 2107274052; magiatis@pharm.uoa.gr. I.C.: ichinou@pharm.uoa.gr

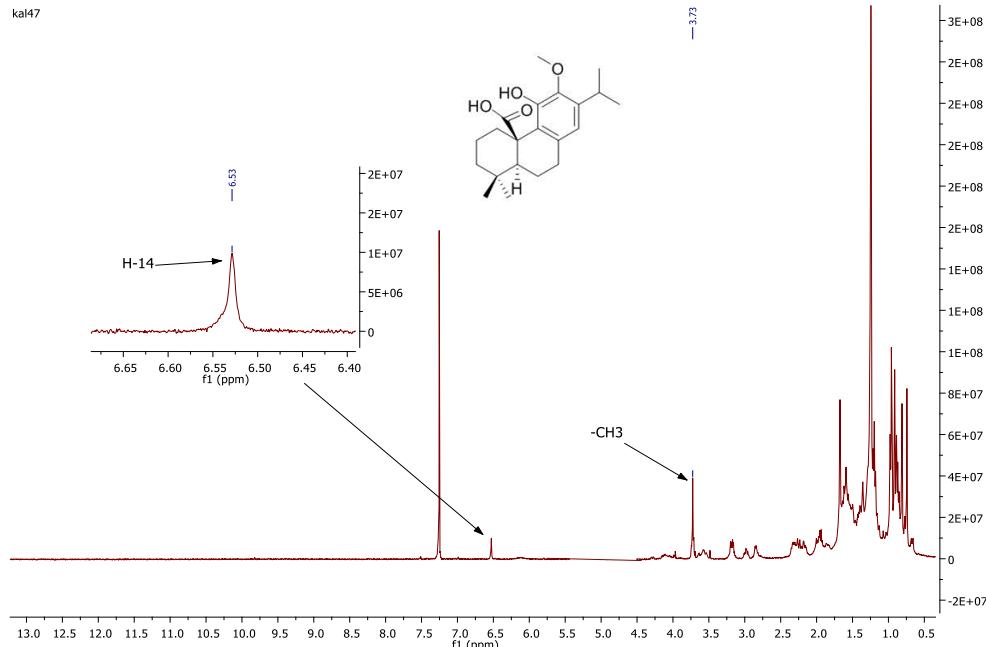
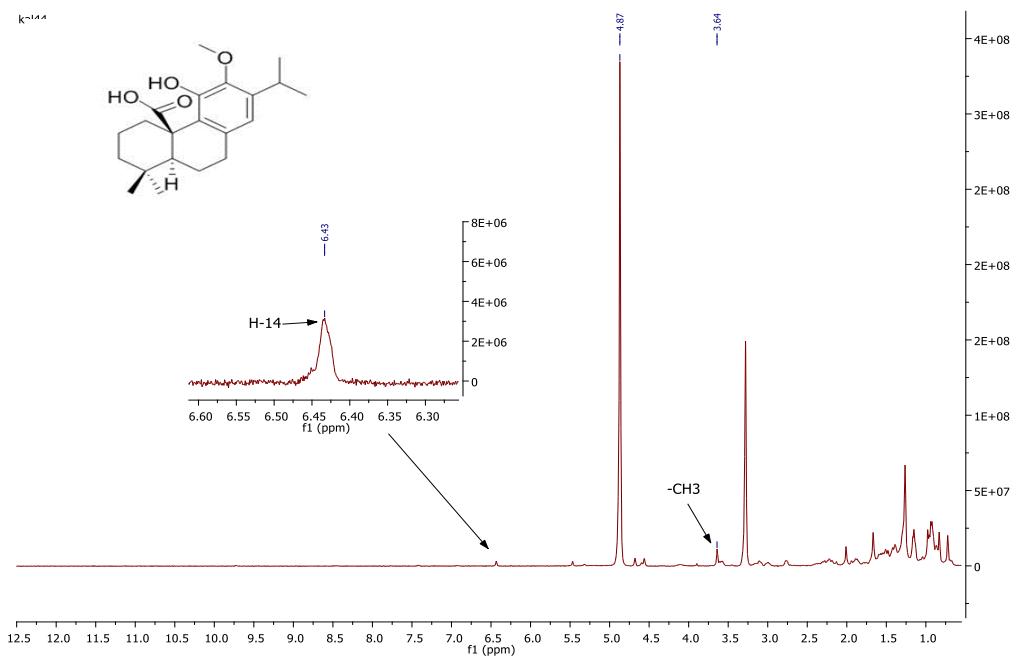


Figure S1  $^1\text{H}$ -NMR spectrum of 12-O-methylcarnosic acid (12MCA) in  $\text{CDCl}_3$



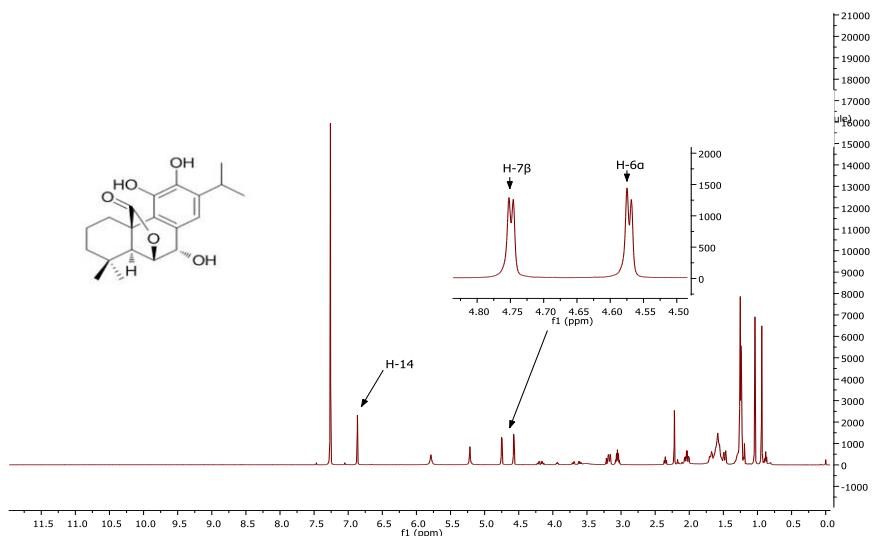
**Figure S2**  $^1\text{H}$ -NMR spectrum of 12-O-methylcarnosic acid (12MCA) in  $\text{CD}_3\text{OD}$

**Table S1**  $^1\text{H}$ -NMR,  $^{13}\text{C}$ -NMR data of 12MCA in  $\text{CDCl}_3$ .

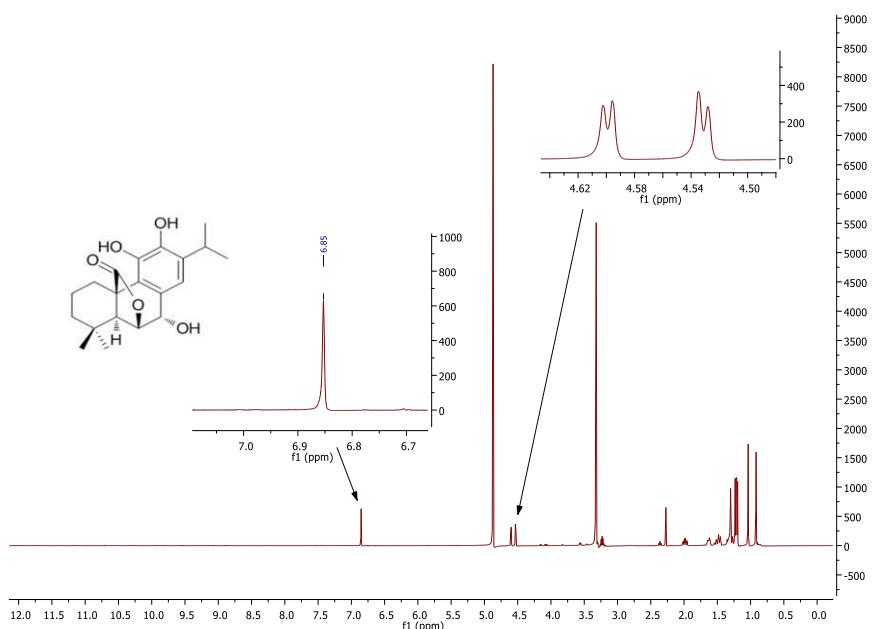
No	$\delta$ $^1\text{H}$ (ppm)	Integration	Multiplicity	J (Hz)	$\delta$ $^{13}\text{C}$ (ppm)
1	$\alpha$ 1.16 $\beta$ 3.57	1 1	m m	- -	33.8
2	$\alpha$ 2.16 $\beta$ 1.56	1 1	m m	- -	19.6
3	$\alpha$ 1.53 $\beta$ 1.30	1 1	m m	- -	41.7
4	-	-	-	-	34.1
5	1.59	1	m	-	59.8
6	$\alpha$ 1.85 $\beta$ 2.25	1 1	m m	- -	19.7
7	$\alpha$ 2.84 $\beta$ 0.96	1 1	m d	- 2.5	34.3
8	-	-	-	-	134.6
9	-	-	-	-	125.3
10	-	-	-	-	47.8
11, -OH	6.13	1	br s		145.9
12	-	-	-	-	147.1
13	-	-	-	-	139.7
14	6.53	1	s	-	117.5
15	3.18	1	sept	6.7	26.7
16	1.22	3	m	-	23.5
17	1.19	3	m	-	23.9
18	0.98	3	s		33.3
19	0.87	3	s		18.9
20	-	-	-	-	178.9
-CH <sub>3</sub>	3.73	3	s		54.8

**Table S2**  $^1\text{H}$ -NMR of 12MCA in  $\text{CD}_3\text{OD}$ .

No	$\delta$ $^1\text{H}$ (ppm)	Integration	Multiplicity	J (Hz)
1	$\alpha$ 1.07 $\beta$ 3.59	1 1	ddd m	12.9/ 12.2/ 4.4
2	$\alpha$ 2.22 $\beta$ 1.53	1 1	m m	
3	$\alpha$ 1.53 $\beta$ 1.32	1 1	m m	
4	-	-	-	-
5	1.51	1	m	
6	$\alpha$ 1.77 $\beta$ 2.22	1 1	brd m	12
7	$\alpha$ 2.76 $\beta$ 2.76	1 1	m m	
8	-	-	-	-
9	-	-	-	-
10	-	-	-	-
11	-	-	-	-
12	-	-	-	-
13	-	-	-	-
14	6.43	1	s	
15	3.13	1	m	
16	1.13	3	d	7.1
17	1.15	3	d	7.3
18	0.98	3	s	
19	0.83	3	s	
20	-	-	-	-
-CH <sub>3</sub>	3.64	3	s	



**Figure S3**  $^1\text{H}$ -NMR spectrum of Rosmanol (RO) in  $\text{CDCl}_3$



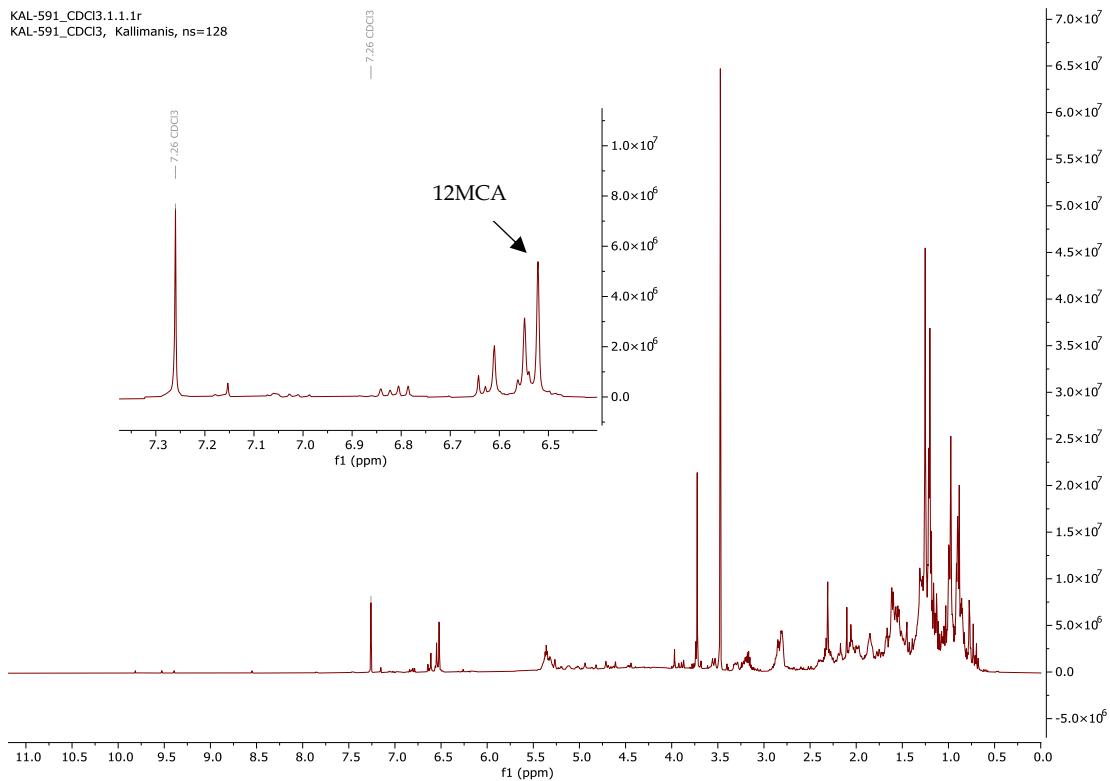
**Figure S4**  $^1\text{H}$ -NMR spectrum of Rosmanol (RO) in  $\text{CD}_3\text{OD}$

**Table S3**  $^1\text{H}$ -NMR,  $^{13}\text{C}$ -NMR data of RO in  $\text{CDCl}_3$ 

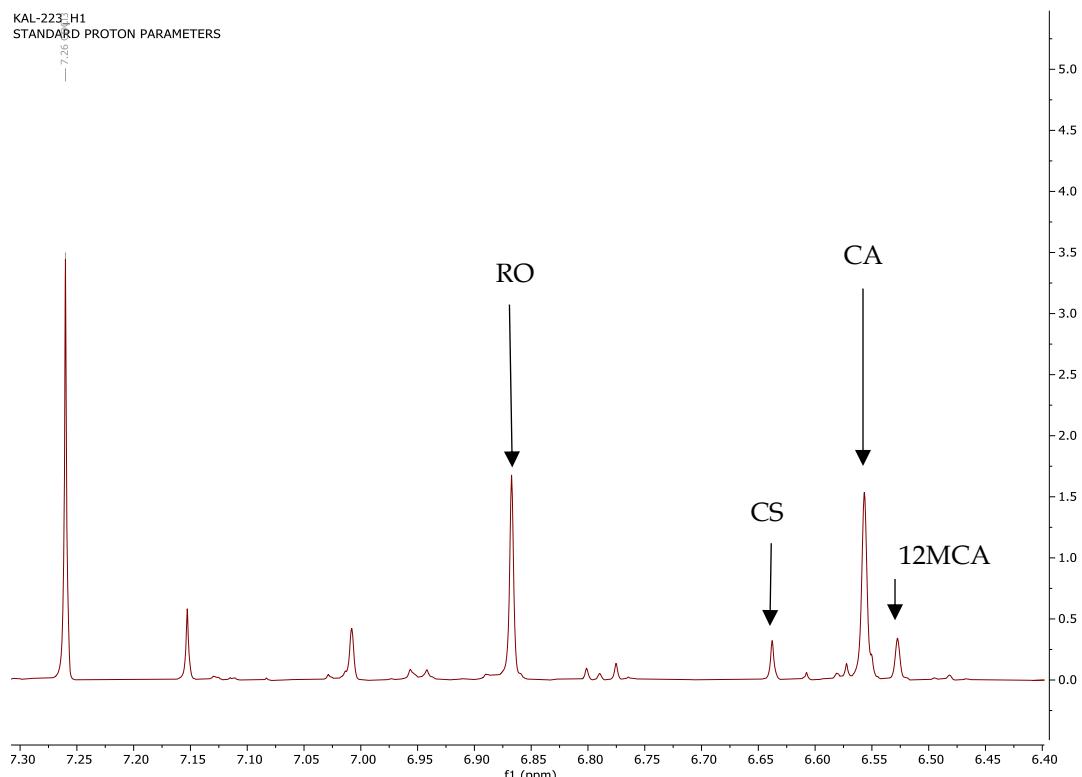
Position	$\delta$ $^1\text{H}$ (ppm)	Integration	Multiplicity	J (Hz)	$\delta$ $^{13}\text{C}$ (ppm)
1	$\alpha$ 2.03	1	td brd	13.8	28.5
	$\beta$ 3.17	1			
2	$\alpha$ 1.52	1	m		19.7
	$\beta$ 1.45	1	m		
3	$\alpha$ 1.17	1	m		38.4
	$\beta$ 1.43	1	m		
4	-	-	-	-	31.9
5	2.2	1	s		55.6
6	4.57	1	d	3.3	70.9
7	4.75	1	d	3.3	80.3
8	-	-	-	-	131.2
9	-	-	-	-	136.8
10	-	-	-	-	48.8
11	-	-	-	-	144.3
12	-	-	-	-	145.3
13	-	-	-	-	125.4
14	6.86	1	s		118.9
15	3.05	1	sept	7	27.7
16	1.23	3	d	4.5	22.2
17	1.25	3	d	4	22.9
18	1.04	3	s		31.6
19	0.94	3	s		23.3
20	-	-	-	-	178.9

**Table S4**  $^1\text{H}$ -NMR,  $^{13}\text{C}$ -NMR data of RO in  $\text{CD}_3\text{OD}$ .

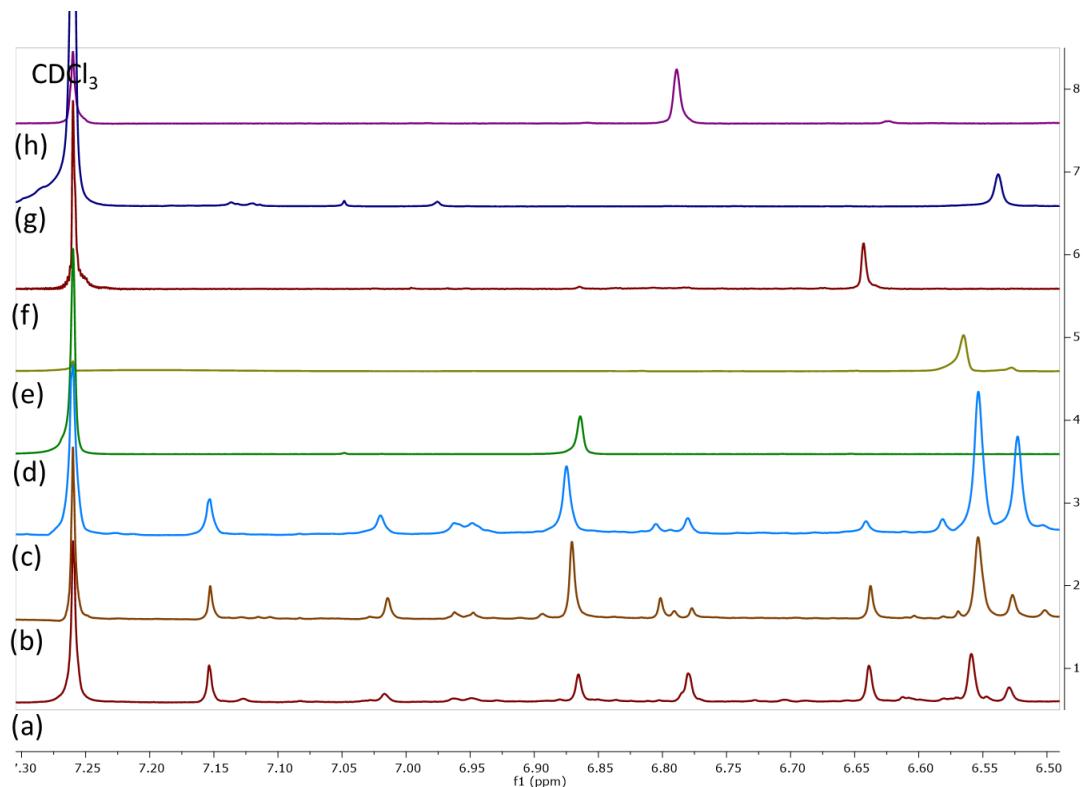
Position	$\delta$ $^1\text{H}$ (ppm)	Integration	Multiplicity	J (Hz)	$\delta$ $^{13}\text{C}$ (ppm)
1	$\alpha$ 1.98	1	td	14.4/ 5.2	28.7
	$\beta$ 1.48	1	m		
2	$\alpha$ 1.45	1	m		20.5
	$\beta$ 1.61	1	m		
3	$\alpha$ 1.25	1	td	13.2/ 3	39.9
	$\beta$ 3.32	1	m		
4	-	-	-	-	32.3
5	2.27	1	s		52.1
6	4.53	1	d	3.2	80.6
7	4.60	1	d	3.3	69.5
8	-	-	-	-	129.8
9	-	-	-	-	125.6
10	-	-	-	-	48.9
11	-	-	-	-	145.6
12	-	-	-	-	143.9
13	-	-	-	-	138.2
14	6.85	1	s		120.7
15	3.23	1	sept	6.9	27.9
16	1.22	3	d	6.9	22.8
17	1.20	3	d	6.9	23.3
18	1.03	3	s		32.1
19	0.92	3	s		22.6
20	-	-	-	-	181.2



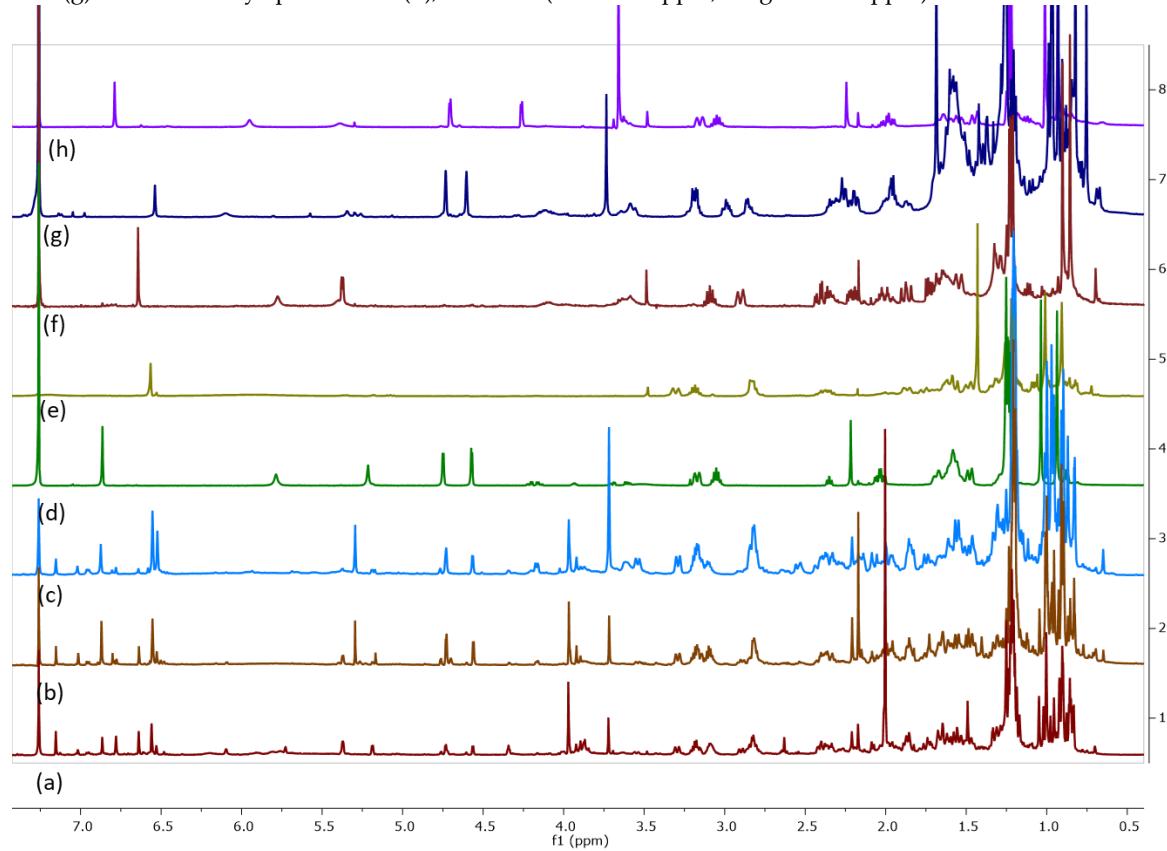
**Figure S5**  $^1\text{H}$ -NMR spectrum in CDCl<sub>3</sub> of methanolic extract of *Salvia microphylla* Kunth.



**Figure S6**  $^1\text{H}$ -NMR spectrum in CDCl<sub>3</sub> of *Salvia fruticosa* Mill. decoction in 5 min (CA: carnosic acid, CS: carnosol, 12MCA: 12-O-methylcarnosic acid, RO: rosmarinol).



**Figure S7**  $^1\text{H}$ -NMR spectra of aqueous extracts (decoction in 5 min) of *Salvia rosmarinus* Spenn. (a), *Salvia fruticosa* Mill. (b) and *Salvia officinalis* L. (c), Rosmanol (d), Carnosic acid (e), Carnosol (f), 12-O-methylcarnosic acid (g) and 7-methoxy epirosmanol (h), in  $\text{CDCl}_3$  (set at 7.26 ppm, range 7.3 -6.5 ppm).



**Figure S8**  $^1\text{H}$ -NMR spectra of aqueous extracts (decoction in 5 min) of *Salvia rosmarinus* Spenn. (a), *Salvia fruticosa* Mill. (b) and *Salvia officinalis* L. (c), Rosmanol (d), Carnosic acid (e), Carnosol (f), 12-O-methylcarnosic acid (g) and 7-methoxy epirosmanol (h), in  $\text{CDCl}_3$  (set at 7.26 ppm, range 7.4 -0.5 ppm).