

Chemical Profile of Essential Oils and Antimicrobial Properties from Orange, Pummelo and Tangelo, cultivated in Greece

Eleni Anastasopoulou ¹, Konstantia Graikou ¹, Vasileios Ziogas ^{2*}, Christos Ganos ¹, Fabrizio Calapai ³ and Ioanna Chinou ^{1,*}

¹ Lab of Pharmacognosy and Chemistry of Natural Products, Department of Pharmacy, School of Health Sciences, National & Kapodistrian University of Athens, 15771, Greece; e.anastasopoulou.1@nup.ac.cy (E.A.); kgraikou@pharm.uoa.gr (K.G.); chris50ganos@hotmail.com (C.G.); ichinou@pharm.uoa.gr (I.C.)

² Institute of Olive Tree, Subtropical Plants and Viticulture, Hellenic Agricultural Organization - DIMITRA (ELGO - DIMITRA), 73134, Chania, Greece; ziogas@elgo.gr (V.Z.)

³ Department of Clinical and Experimental Medicine, University of Messina, Italy; Fabrizio.calapai@unime.it (F.C.)

* Correspondence: ichinou@pharm.uoa.gr (I.C.); ziogas@elgo.gr (V.Z)

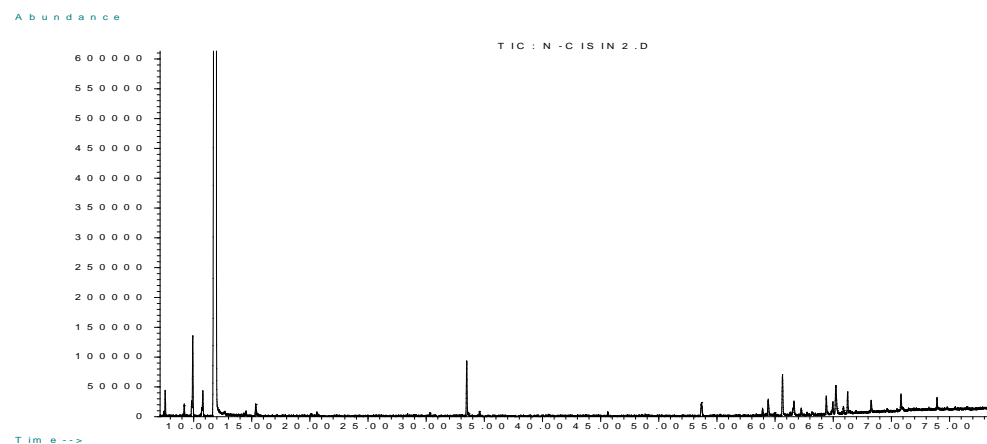


Figure S1: GC chromatogram of *Citrus sinensis* (L.) Osbeck cv. Newhall upon citrumelo rootstock peels Csp1

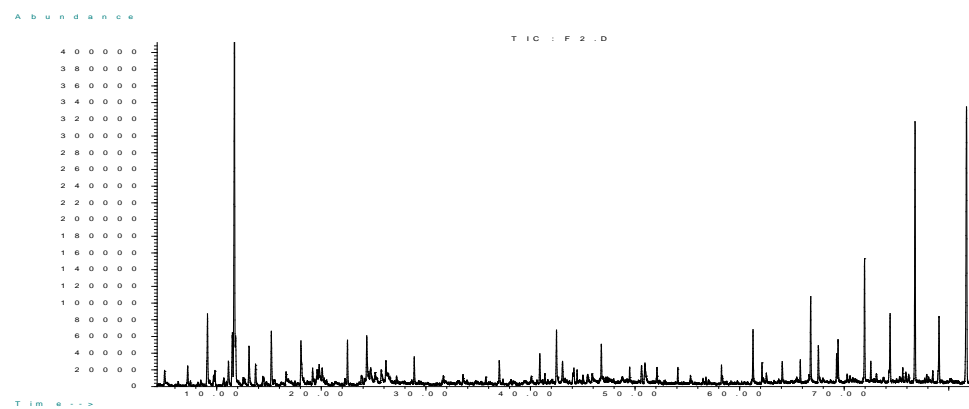


Figure S2: GC chromatogram of *Citrus sinensis* (L.) Osbeck cv. Newhall upon citrumelo rootstock leaves Csl1

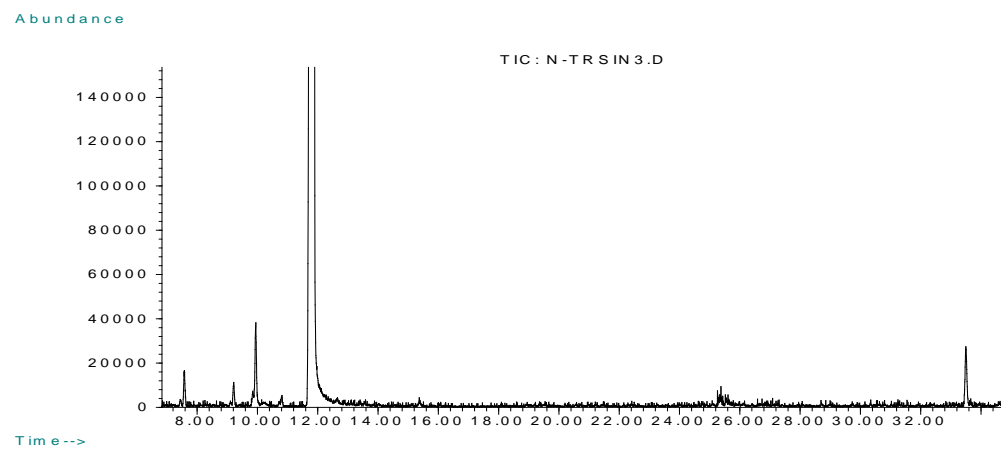


Figure S3: GC chromatogram of *Citrus sinensis* (L.) Osbeck cv. Newhall upon *Poncirus trifoliata* rootstock peels Csp2

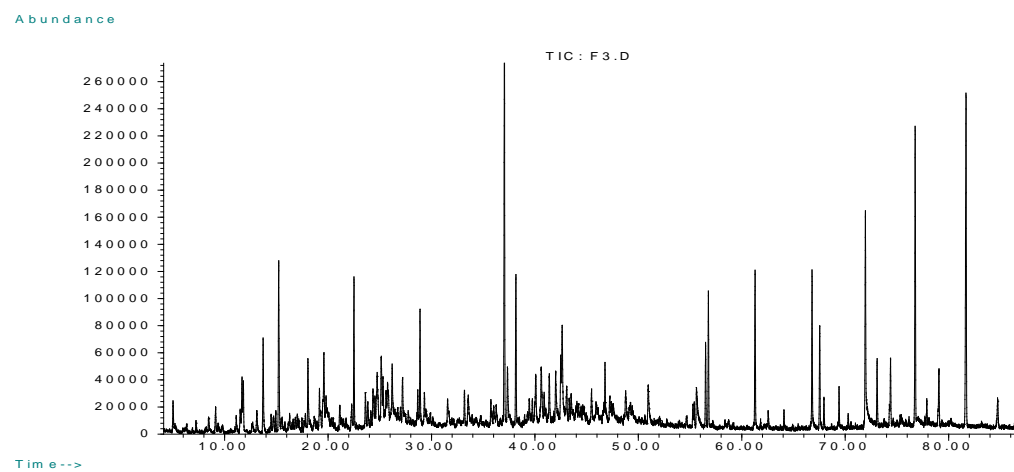


Figure S4: GC chromatogram of *Citrus sinensis* (L.) Osbeck cv. Newhall upon *Poncirus trifoliata* rootstock leaves Csl2

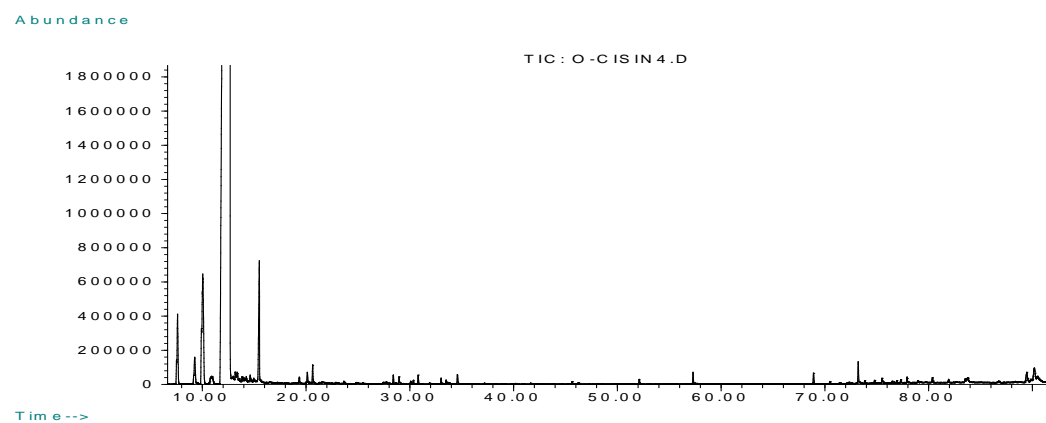


Figure S5: GC chromatogram of *Citrus sinensis* (L.) Osbeck cv. Valencia Ovale Porou upon citrumelo rootstock peels Csp3

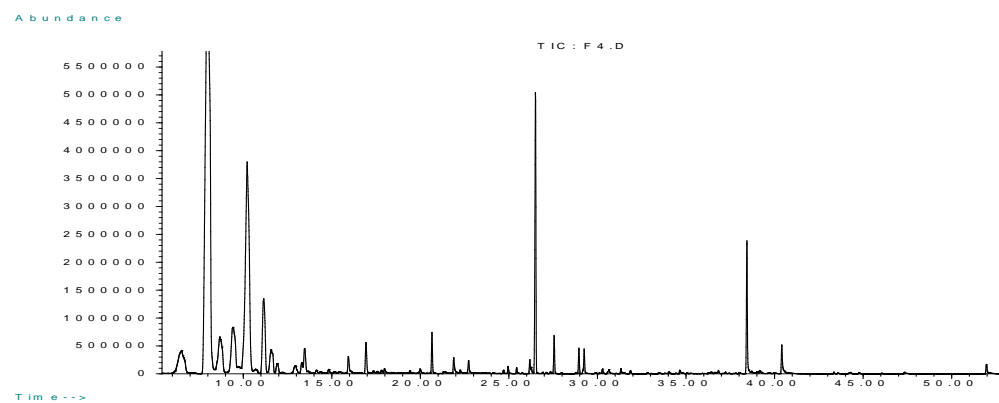


Figure S6: GC chromatogram of *Citrus sinensis*_(L.) Osbeck cv. Valencia Ovale Porou upon citrumelo rootstock leaves Csl3

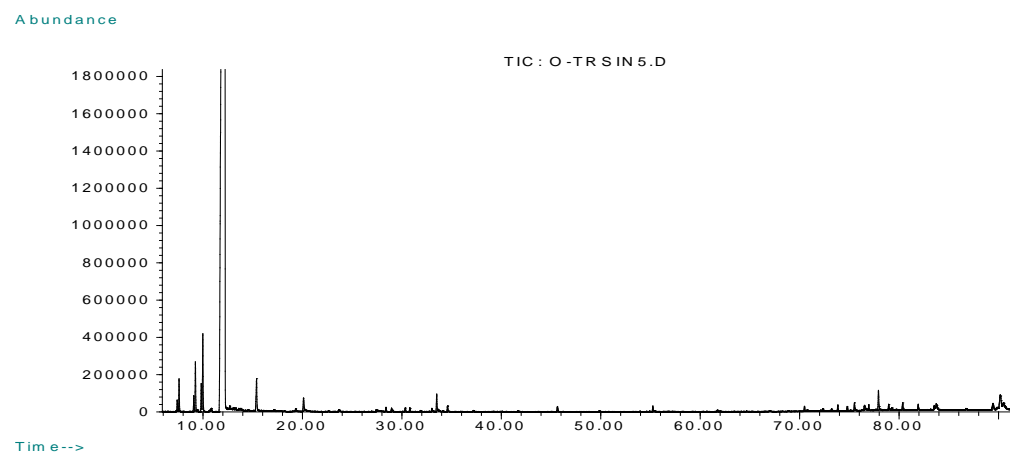


Figure S7: GC chromatogram of *Citrus sinensis*_(L.) Osbeck cv. Valencia Ovale Porou upon *Poncirus trifoliata* rootstock peels Csp4

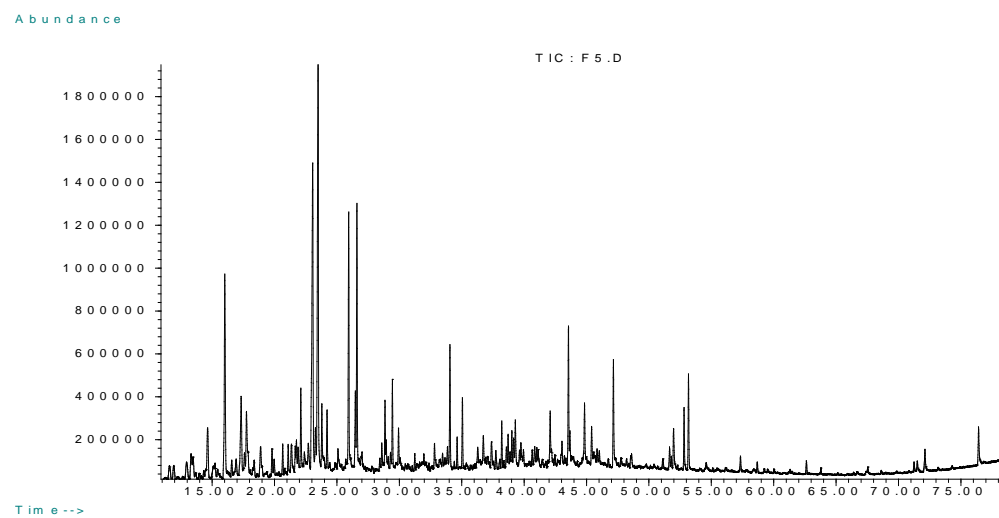


Figure S8: GC chromatogram of *Citrus sinensis* (L.) Osbeck cv. Valencia Ovale Porou on *Poncirus trifoliata* rootstock leaves Csl4

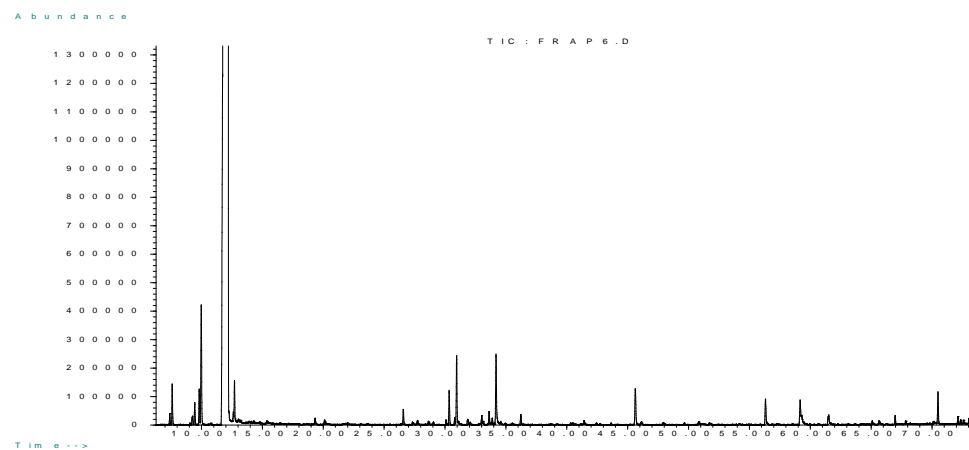


Figure S9: GC chromatogram of *Citrus maxima* (Burm.) Merr. upon *Poncirus trifoliata* rootstock peels Cgp1

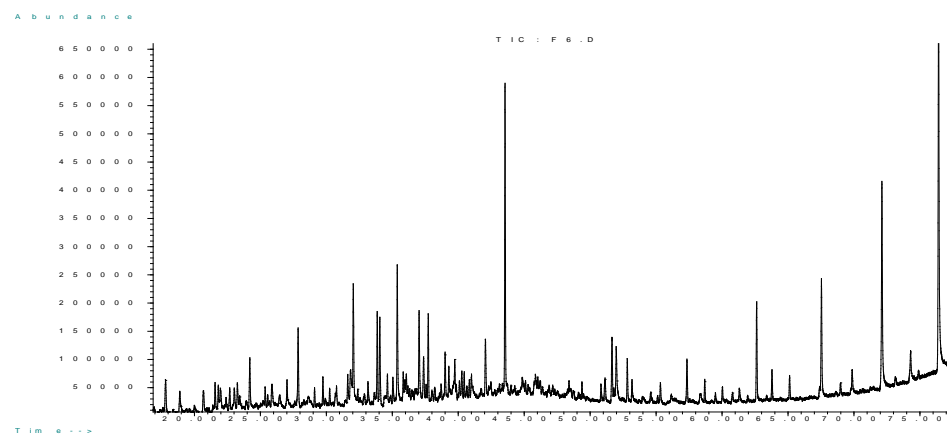


Figure S10: GC chromatogram of *Citrus maxima* (Burm.) Merr. upon *Poncirus trifoliata* rootstock leaves Cgl1

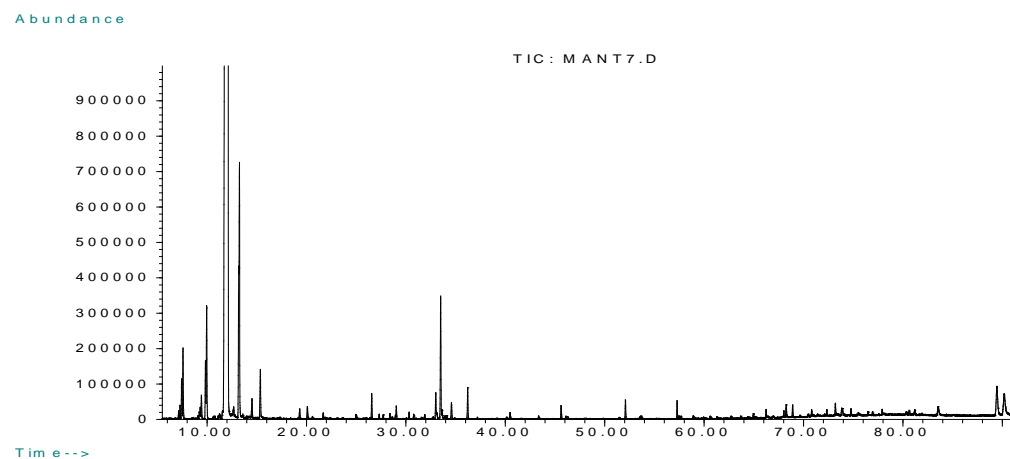


Figure S11: GC chromatogram of *Citrus paradisi* x *Citrus tangerina* cv. Minneola upon *Poncirus trifoliata* rootstock peels Crp1

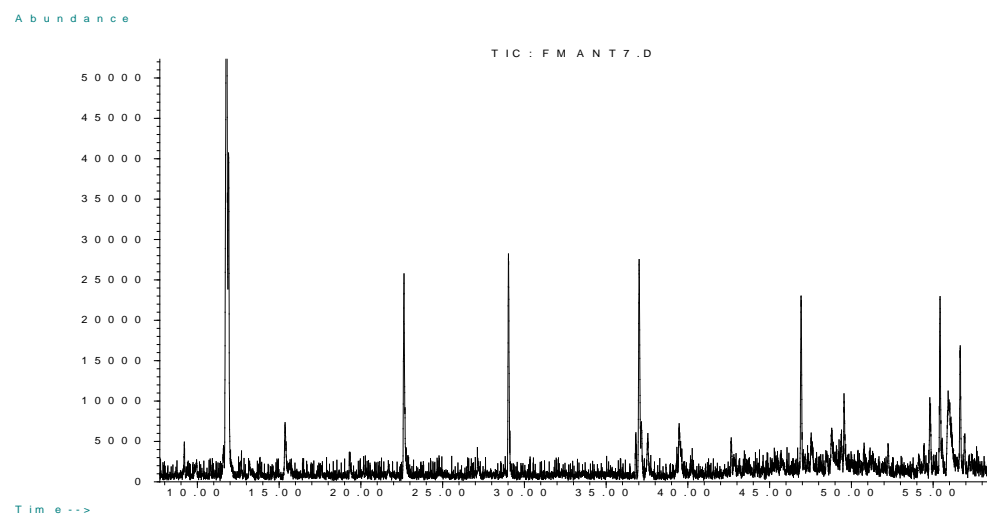
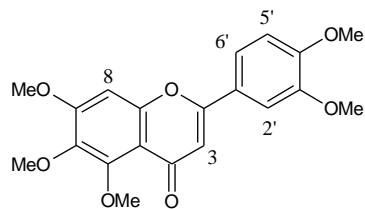


Figure S12: GC chromatogram of *Citrus paradisi* x *Citrus tangerina* cv. Minneola upon *Poncirus trifoliata* rootstock leaves Crl1

NMR data of the isolated PMF's

- 5,6,7,3',4'-pentamethoxyflavone (sinensetin)



δ (ppm)		multiplicity/ J	
3.93	3H	s	-OCH ₃
3.97	3H	s	-OCH ₃
3.98	3H	s	-OCH ₃
3.99	6H	s	-OCH ₃
6.60	1H	s	H3
6.80	1H	s	H8
6.97	1H	d ($J=9\text{Hz}$)	H5'
7.33	1H	d ($J=2\text{Hz}$)	H2'
7.52	1H	dd ($J=9\text{ Hz}, 2\text{Hz}$)	H6'

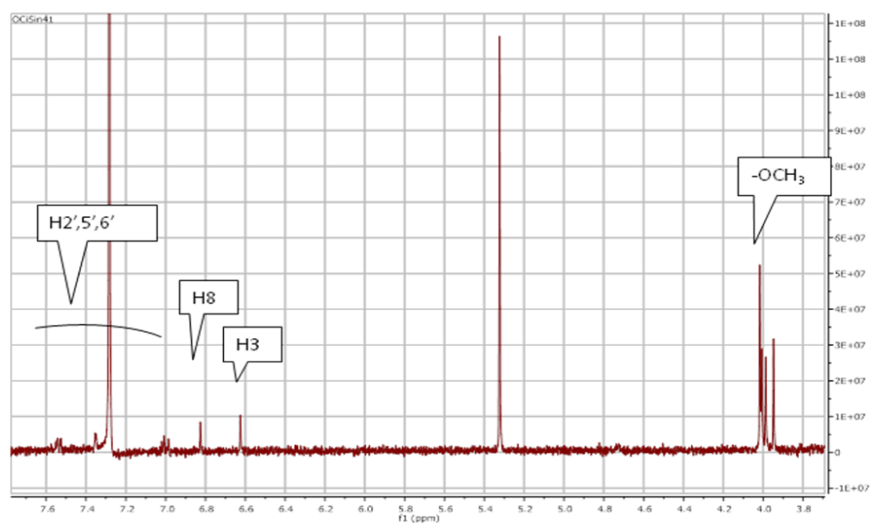
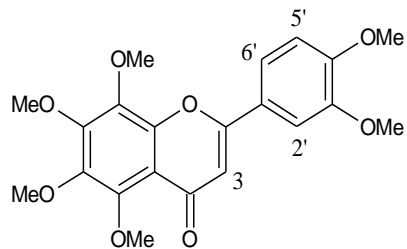


Figure S13: ^1H -NMR of the compound 5,6,7,3',4'-pentamethoxyflavone

- 5,6,7,8,3',4',-hexamethoxyflavone (nobiletin)



δ (ppm)		multiplicity/ J	
3.95	6H	s	$-\text{OCH}_3$
3.97	3H	s	$-\text{OCH}_3$
3.98	3H	s	$-\text{OCH}_3$

4.03	3H	s	-OCH ₃
4.11	3H	s	-OCH ₃
6.63	1H	s	H3
7.00	1H	d(<i>J</i> =9Hz)	H5'
7.42	1H	d(<i>J</i> =2Hz)	H2'
7.57	1H	dd(<i>J</i> =9Hz, 2Hz)	H6'

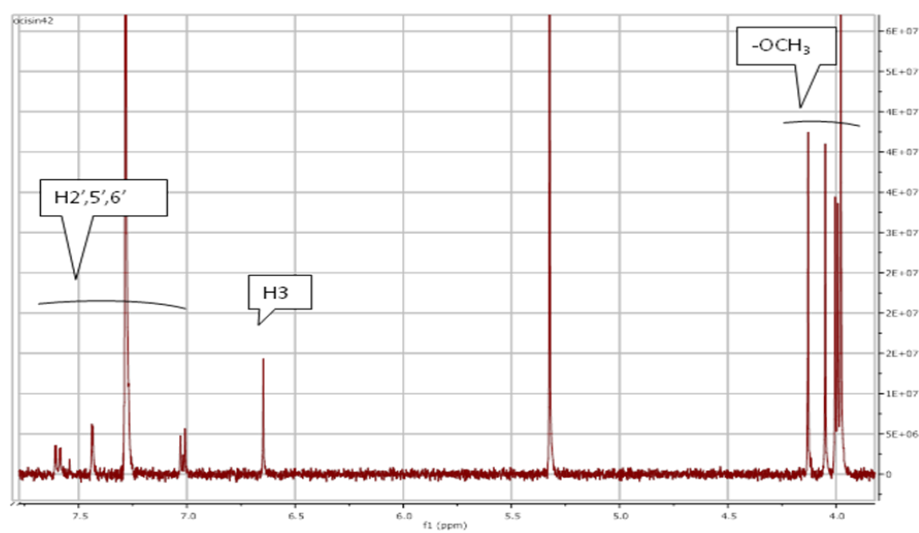
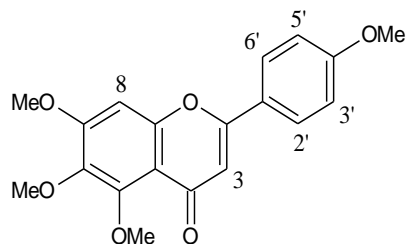


Figure S14: ¹H-NMR of the compound 5,6,7,8,3',4',-hexamethoxyflavone

Mixture of 5,6,7,4'-tetramethoxyflavone (tetra-*O*-methylscutellarein) & 3,5,6,7,3',4'-hexamethoxyflavone (3-methoxy-sinensetin)

- 5,6,7,4'-tetramethoxyflavone (tetra-*O*-methylscutellarein)



δ (ppm)		multiplicity/ <i>J</i>	
3.89	3H	s	-OCH ₃
3.92	3H	s	-OCH ₃
3.98	3H	s	-OCH ₃
3.99	3H	s	-OCH ₃
6.59	1H	s	H3
6.80	1H	s	H8
7.01	2H	d (<i>J</i> =9Hz, 2Hz)	H3', 5'
7.83	2H	d (<i>J</i> =9Hz, 2Hz)	H2', 6'

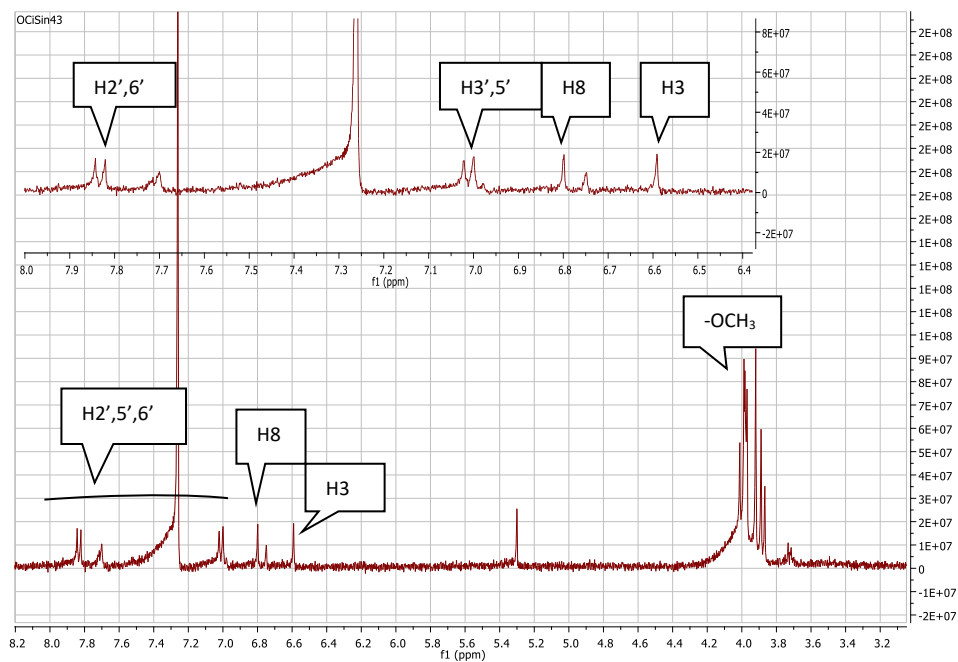
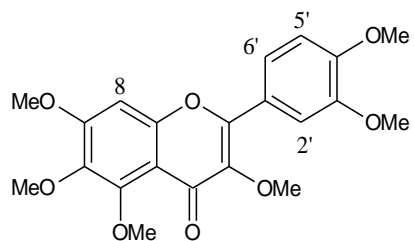


Figure S15: ¹H-NMR of the compound 5,6,7,4'-tetramethoxyflavone

- 3,5,6,7,3',4'-hexamethoxyflavone (3-methoxy-sinensetin)



δ (ppm)		multiplicity/ J	
3.87	3H	s	-OCH ₃
3.92	3H	s	-OCH ₃
3.97	3H	s	-OCH ₃
3.98	3H	s	-OCH ₃
4.01	3H	s	-OCH ₃
6.75	1H	s	H8
6.99	1H	d ($J=9\text{Hz}$)	H5'
7.70	1H	d ($J=2\text{Hz}$)	H2'
7.71	1H	dd ($J=9\text{Hz}, 2\text{Hz}$)	H6'

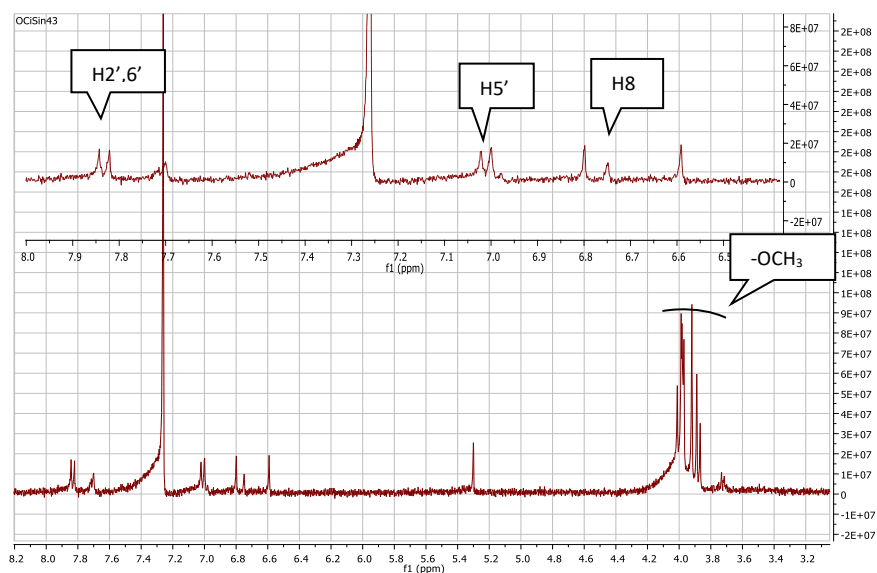
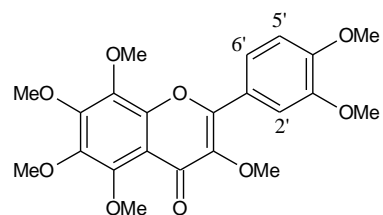


Figure S16: ¹H-NMR of the compound 3,5,6,7,3',4'-hexamethoxyflavone

Mixture of 3,5,6,7,8,3',4'-heptamethoxyflavone (3-methoxy-nobiletin) & 5,6,7,8,4'-pentamethoxyflavone (tangeretin)

- 3,5,6,7,8,3',4'-heptamethoxyflavone (3-methoxy-nobiletin)



δ (ppm)		multiplicity/ J	
3.89	3H	s	-OCH ₃
3.95	3H	s	-OCH ₃
3.97	9H	s	-OCH ₃
4.00	3H	s	-OCH ₃
4.10	3H	s	-OCH ₃
7.01	1H	d ($J=9\text{Hz}$)	H5'
7.80	1H	d ($J=9\text{Hz}$)	H2'
7.84	1H	dd ($J=9\text{Hz}, 2\text{Hz}$)	H6'

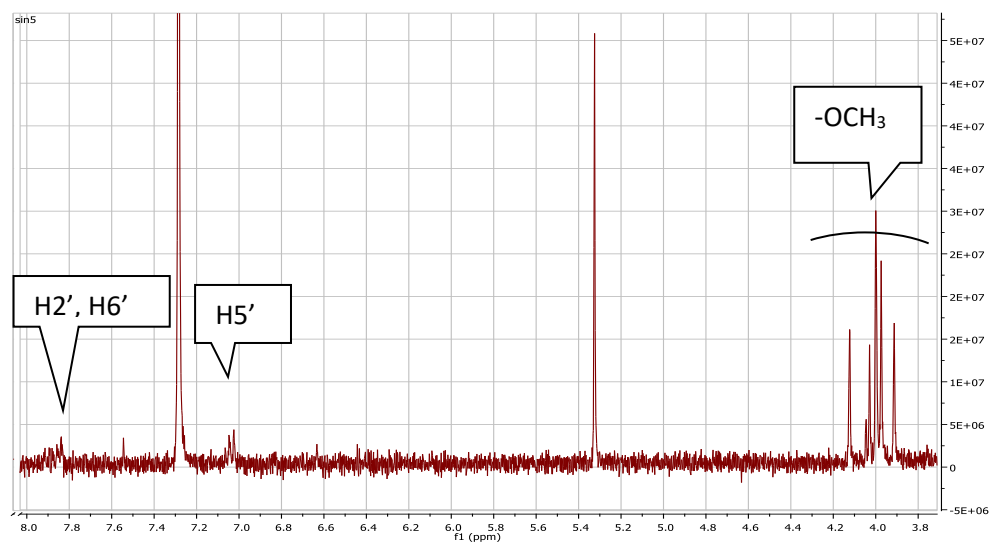
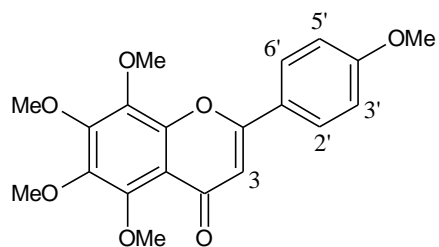


Figure S17: ^1H -NMR of the compound 3,5,6,7,8,3',4'-heptamethoxyflavone

- 5,6,7,8,4'-pentamethoxyflavone (tangeretin)



δ (ppm)		multiplicity / J	
3.89	3H	s	-OCH ₃
3.95	6H	s	-OCH ₃
4.02	3H	s	-OCH ₃

4.10	3H	s	-OCH ₃
6.61	1H	s	H3
7.02	2H	d (<i>J</i> =9Hz)	H3' H5'
7.87	2H	d (<i>J</i> =9Hz)	H2' H6'

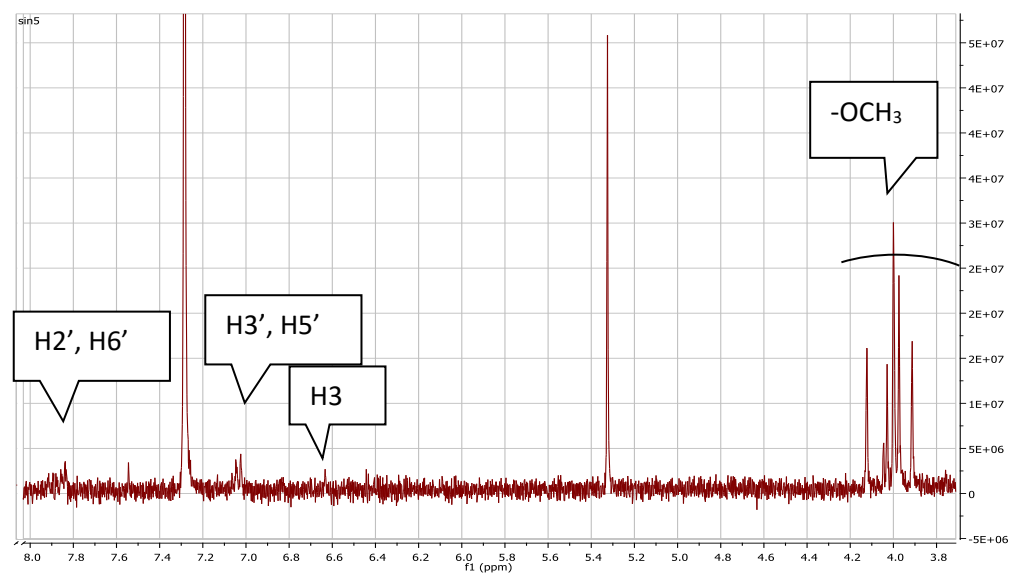


Figure S18: ¹H-NMR of the compound 5,6,7,8,4'-pentamethoxyflavone

Table S1: Chemical composition of the essential oils from peels and leaves of six Citrus cultivar/rootstock combinations

Compounds	KI	Relative concentration (area %)											
		Csp1	Csp2	Csp3	Csp4	Cgp1	Crp1	Csl1	Csl2	Csl3	Csl4	Cgl1	Cr11

[illegible]

31.	myrtenol	1192						1.22	-	-	2.92	1.69	-
32.	estragole	1194						2.17	-	-	-	-	-
33.	<i>cis</i> -piperitol	1194						-	1.67	-	-	-	-
34.	decanal	1196	-	-	0.16	-	-	-					
35.	<i>trans</i> -carveol	1216						-	-	-	1.71	-	-
36.	<i>cis</i> -carveol	1226						-	-	-	-	1.41	-
37.	thymol methyl ether	1232	-	-	-	-	-	0.08					
38.	citral B (neral)	1235						-	-	-	2.05	-	-
39.	cuminal	1238						-	-	-	0.67	-	-
40.	car-3-en-2-one	1250						-	1.21	-	-	-	-
41.	linalyl acetate	1255						1.77	2.77	0.95	0.83	-	7.70
42.	<i>trans</i> -anethole	1281						-	1.21	0.49	-	-	-
43.	thymol	1288	-	-	-	-	-	0.08					
44.	cuminol	1289						-	-	-	2.10	-	-
45.	carvacrol	1294						-	2.05	-	-	-	-
46.	<i>p</i> -mentha-1,4 dien-7-ol	1295						-	-	-	1.55	-	-
47.	δ -elemene	1336	-	-	-	-	0.22	0.33					
48.	citronellyl acetate	1350	-	-	-	-	-	0.06					
49.	neryl acetate	1358	-	-	-	-	0.07	-	-	0.15	-	-	-
50.	isocyclocitral	1363						-	-	-	0.50	-	-
51.	α -copaene	1373	-	-	0.08	0.09	-	0.08					
52.	β -elemene	1387	-	-	-	-	-	0.17	1.23	2.39	7.23	1.87	7.48
53.	β -cubebene	1410	-	-	0.08	0.03	-	0.40					
54.	<i>trans</i> -caryophyllene	1415	-	-	-	-	-	0.09					
55.	β -caryophyllene	1417	-	-	0.04	-	0.55	-	-	0.86	-	-	-
56.	γ -elemene	1431	-	-	-	-	-	0.51					
57.	calarene	1432	-	-	-	0.07	-	-					
58.	α -bergamotene	1433	-	-	-	-	1.02	-					
59.	citronellyl propionate	1443						-	-	0.18	-	-	-
60.	aromadendrene	1438						-	-	-	0.72	-	-

61.	α -humulene	1450							-	-	0.57	-	-	-
62.	<i>trans</i> - β -farnesene	1453							-	-	0.61	-	-	-
63.	germacrene D	1483	-	-	0.13	0.08	0.34	-						
64.	β -selinene	1487							-	1.73	-	-	-	-
65.	valencene	1493	1.36	1.26	0.03	0.32	-	1.73						
66.	pentadecane	1498							-	3.23	-	-	-	-
67.	β -bisabolene	1504												
68.	δ -cadinene	1520	-	-	0.10	0.14	-	0.25						
69.	elemol	1550							-	0.25	-	-	-	-
70.	germacrene B	1559							-	-	-	0.94	-	-
71.	spathulenol	1575							-	-	-	0.95	3.27	8.20
72.	globulol	1581							-	1.45	-	-	-	-
73.	caryophyllene oxide	1582							1.10	7.88	-	3.93	3.74	-
74.	β -oplopenone	1606							-	-	-	-	1.94	-
75.	isospathulenol	1619							-	3.54	-	0.93	-	-
76.	aromadendrenepoxide	1630							-	-	-	2.66	-	-
77.	longifolenaldehyde	1631							-	-	-	1.89	-	-
78.	alloaromadendrene	1638							-	0.83	-	-	-	-
79.	caryophylla-3,8(13)-dien-5- β -ol	1639							-	-	-	-	2.43	-
80.	caryophylla-4(12),8(13)-dien-5- β -ol	1641							-	-	-	-	-	1.95
81.	valerenol	1697							-	-	-	0.81	-	-
82.	β -sinensal	1698							-	-	3.10	-	-	-
83.	2-carene-10-al	1707							-	-	-	0.62	-	-
84.	pentadecanal	1708	-	-	-	-	0.51	-						
85.	isolongifolol	1727							-	-	-	-	1.88	-
86.	α -sinensal	1753							-	-	0.88	-	-	-
87.	nootkatone	1805	-	-	0.03	-	0.74	0.20	-	1.59	-	-	-	-
88.	hexahydrofarnesyl acetone	1830							-	1.43	-	3.53	12.00	5.80
89.	neophytadiene	1836							-	2.44	-	2.29	-	-
90.	phytol	1939							-	1.45	0.27	1.45	2.73	5.36

91.	palmitic acid	1968	0.58	-	0.03	-	-	0.25			-	14.52	4.80	5.63
92.	eicosane	1997							-	0.61	-	1.15	-	-
93.	ledenoxide	2059							-	1.27	-	-	-	-
94.	linoleic acid	2126	-	-	0.10	0.25	-	-			99.63	95.28	91.55	90.93
95.	oleic acid	2133	-	-	-	-	-	0.15						
96.	O-methylated coumarin (osthole)	2139	-	-	-	-	0.49	-						
97.	docosane	2198							-	-	-	-	10.54	-
98.	7-methoxy-8-(2-oxo-3-methylbutyl)coumarin	2259	-	-	-	-	0.06	-						
99.	tricosane	2298							2.26	-	-	-	1.95	-
100.	tetracosane	2398							0.82	-	-	-	-	-
101.	n-pentacosane	2489	-	-	-	-	0.14	-						
102.	pentacosane	2500							3.41	3.09	-	-	4.20	-
103.	hexacosane	2600							1.83	0.80	-	-	-	-
104.	heptacosane	2700							5.04	5.39	-	-	5.86	-
105.	(z)14-tricosenyl formate	2730	-	-	-	-	0.36	-						
106.	octacosane	2800							3.10	1.33	-	-	-	-
107.	nonacosane	2900							10.49	6.02	-	-	-	-
108.	triacontane	3000							3.19	-	-	-	-	-
109.	phytosterol	>3000							-	-	-	-	-	3.90
110.	hentriacontane	>3000							15.25	8.27	-	-	18.87	-
111.	pentamethoxyflavone	>3000	-	-	0.13	0.16	-	-						
112.	tetramethoxyflavone	>3000	1.24	-	0.12	-	-	-						
113.	heptamethoxyflavone	>3000	-	-	0.14	0.13	-	0.20						
114.	hexamethoxyflavone	>3000	-	-	0.12	0.28	-	0.18						