

Phytochemical composition and biological activities of extracts from early, mature, and germinated somatic embryos of *Cotyledon orbiculata* L.

Gokhan Zengin ¹, Zoltán Cziáky ², József Jekő ², Kyung Won Kang ³, José Manuel Lorenzo ^{4,5}, and Iyyakkannu Sivanesan ^{6,*}

¹ Department of Biology, Faculty of Science, Selcuk University, 42250 Konya, Turkey

² Agricultural and Molecular Research and Service Institute, University of Nyíregyháza, Nyíregyháza, Hungary

³ Babo Orchid Farm, Gyeonggi-do, 472-831 Namyangju-si, South Korea

⁴ Centro Tecnológico de la Carne de Galicia, Rúa Galicia Nº 4, Parque Tecnológico de Galicia, San Cibrao das Viñas, 32900 Ourense, Spain

⁵ Facultade de Ciencias, Universidade de Vigo, Área de Tecnoloxía dos Alimentos, 32004 Ourense, Spain

⁶ Department of Bioresources and Food Science, Institute of Natural Science and Agriculture, Konkuk University, 05029 Seoul, South Korea

* Correspondence: isivanesan@gmail.com or siva74@konkuk.ac.kr; Tel.: +82-2450-0576

Table S1. Chemical composition of early somatic embryo extract.

No.	Name	Formula	Rt	[M+H] ⁺	[M-H] ⁻	Fra 1	Fra 2	Fra 3	Fra 4	Fra 5
1	Trigonelline	C ₇ H ₇ NO ₂	1.27	138.05550		110.0603	96.0450	94.0656	92.0499	65.0391
2	Nicotinic acid (Niacin)	C ₆ H ₅ NO ₂	1.49	124.03986		96.0449	80.0501	78.0345		
3	Nicotinamide	C ₆ H ₆ N ₂ O	1.61	123.05584		106.0289	96.0449	80.0501		
4 ¹	Gallic acid (3,4,5-Trihydroxybenzoic acid)	C ₇ H ₆ O ₅	2.73		169.01370	125.0225	97.0281	81.0330	69.0330	
5	Phenethylamine	C ₈ H ₁₁ N	4.30	122.09698		105.0702	103.0545	79.0548		
6	Dihydroxybenzoic acid	C ₇ H ₆ O ₄	10.55		153.01879	123.0073	109.0280	108.0202	95.0123	85.0279
7	Caffeic acid	C ₉ H ₈ O ₄	15.25		179.03444	135.0438	107.0487			
8 ¹	Taxifolin (Dihydroquercetin)	C ₁₅ H ₁₂ O ₇	19.95		303.05048	285.0404	177.0181	175.0385	153.0182	125.0229
9	cis-3-[(4-hydroxy-3-methoxyphenyl)-prop-2-enoyl]oxybutanedioic acid	C ₁₄ H ₁₄ O ₈	20.64	311.07670		177.0545	149.0597	145.0284	117.0337	89.0390
10	Eriodictyol-O-hexoside	C ₂₁ H ₂₂ O ₁₁	20.85		449.10839	287.0562	151.0023	135.0438	107.0123	
11	trans-3-[(4-hydroxy-3-methoxyphenyl) prop-2-enoyl]oxybutanedioic acid	C ₁₄ H ₁₄ O ₈	21.50	311.07670		177.0546	149.0598	145.0284	117.0337	89.0390
12	Luteolin-O-hexoside isomer 1	C ₂₁ H ₂₀ O ₁₁	22.45		447.09274	327.0519	285.0405	284.0328	256.0368	151.0023
13	Luteolin-O-hexoside isomer 2	C ₂₁ H ₂₀ O ₁₁	22.92		447.09274	327.0511	285.0405	284.0327	256.0369	151.0026
14	Hyperoside (Quercetin-3-O-galactoside)	C ₂₁ H ₂₀ O ₁₂	23.26		463.08765	301.0362	300.0276	271.0248	255.0297	178.9977
15 ¹	Isoquercitrin (Quercetin-3-O-glucoside)	C ₂₁ H ₂₀ O ₁₂	23.50		463.08765	301.0356	300.0275	271.0248	255.0294	178.9975
16 ¹	Eriodictyol (3',4',5,7-Tetrahydroxyflavanone)	C ₁₅ H ₁₂ O ₆	25.46		287.05556	269.0453	151.0023	135.0438	125.0228	107.0123
17 ¹	Quercetin (3,3',4',5,7-Pentahydroxyflavone)	C ₁₅ H ₁₀ O ₇	27.57		301.03483	273.0403	178.9976	151.0024	121.0280	107.0124
18 ¹	Naringenin (4',5,7-Trihydroxyflavanone)	C ₁₅ H ₁₂ O ₅	27.77		271.06065	227.0704	177.0183	151.0024	119.0487	107.0123
19 ¹	Luteolin (3',4',5,7-Tetrahydroxyflavone)	C ₁₅ H ₁₀ O ₆	28.46		285.03991	217.0500	199.0392	175.0387	151.0023	133.0281
20 ¹	Apigenin (4',5,7-Trihydroxyflavone)	C ₁₅ H ₁₀ O ₅	30.31		269.04500	225.0551	201.0556	151.0024	149.0232	117.0330
21	Chrysoeriol (3'-Methoxy-4',5,7-trihydroxyflavone)	C ₁₆ H ₁₂ O ₆	30.53		299.05556	284.0327	256.0374	227.0348	151.0023	107.0124
22	Trihydroxy-trimethoxy(iso)flavone isomer I	C ₁₈ H ₁₆ O ₈	32.95	361.09235		346.0680	345.0600	331.0444	317.0660	303.0492
23	Dihydroxy-dimethoxy(iso)flavone	C ₁₇ H ₁₄ O ₆	32.98	315.08686		300.0628	299.0549	271.0600	257.0443	
24	Chrysin (5,7-Dihydroxyflavone)	C ₁₅ H ₁₀ O ₄	33.86	255.06573		209.0600	153.0182	129.0337	103.0546	67.0185
25	Dimethoxy(iso)flavone	C ₁₇ H ₁₄ O ₄	34.16	283.09704		268.0728	267.0647	239.0700	225.0533	
26 ¹	Galangin (3,5,7-Trihydroxyflavone)	C ₁₅ H ₁₀ O ₅	34.74	271.06065		215.0697	197.0589	165.0148	153.0181	105.0338
27	Trimethoxy(iso)flavone	C ₁₈ H ₁₆ O ₅	35.01	313.10760		298.0837	297.0757	280.0729	279.0652	252.0781
28	Dihydroxy-methoxy(iso)flavone	C ₁₆ H ₁₂ O ₅	35.11	285.07630		270.0521	269.0446	242.0572	167.0339	119.0493
29	Hydroxy-trimethoxy(iso)flavone	C ₁₈ H ₁₆ O ₆	36.92	329.10251		314.0781	313.0708	299.0548	271.0599	
30	Hydroxy-methoxy(iso)flavone	C ₁₆ H ₁₂ O ₄	38.10	269.08138		254.0572	226.0622	167.0339		
31	Linoleamide	C ₁₈ H ₃₃ NO	44.47	280.26404		263.2369	245.2264	109.1015	95.0860	81.0704

32	Oleamide	C ₁₈ H ₃₅ NO	45.76	282.27969		265.2526	247.2420	135.1171	83.0861	69.0705
----	----------	------------------------------------	-------	-----------	--	----------	----------	----------	---------	---------

¹Confirmed by the standard.

Table S2. Chemical composition of mature somatic embryo extract.

No.	Name	Formula	Rt	[M+H] ⁺	[M-H] ⁻	Fra1	Fra 2	Fra 3	Fra 4	Fra 5
1	Trigonelline	C ₇ H ₇ NO ₂	1.26	138.05550		110.0603	96.0450	94.0656	92.0499	65.0392
2	Nicotinic acid (Niacin)	C ₆ H ₅ NO ₂	1.54	124.03986		96.0449	80.0501	78.0345		
3	Nicotinamide	C ₆ H ₆ N ₂ O	1.63	123.05584		106.0290	96.0449	80.0500		
6	Dihydroxybenzoic acid	C ₇ H ₆ O ₄	10.41		153.01879	123.0073	109.0279	108.0201	95.0123	85.0278
7	Caffeic acid	C ₉ H ₈ O ₄	15.17		179.03444	135.0438	107.0489			
8 ¹	Taxifolin (Dihydroquercetin)	C ₁₅ H ₁₂ O ₇	19.90		303.05048	285.0403	177.0177	175.0384	153.0183	125.0228
10	Eriodictyol-O-hexoside	C ₂₁ H ₂₂ O ₁₁	20.79		449.10839	287.0563	151.0024	135.0438	107.0123	
9	cis-3-[(4-hydroxy-3-Methoxyphenyl) prop-2-enoyl]oxybutanedioic acid	C ₁₄ H ₁₄ O ₈	20.90	311.07670		177.0546	149.0597	145.0284	117.0337	89.0390
11	trans-3-[(4-hydroxy-3-Methoxyphenyl) prop-2-enoyl]oxybutanedioic acid	C ₁₄ H ₁₄ O ₈	21.45	311.07670		177.0546	149.0597	145.0284	117.0337	89.0390
33	Quercetin-O-pentosylhexoside	C ₂₆ H ₂₈ O ₁₆	21.56		595.12991	301.0354	300.0275	271.0247	255.0296	178.9975
12	Luteolin-O-hexoside isomer 1	C ₂₁ H ₂₀ O ₁₁	22.35		447.09274	327.0499	285.0404	284.0327	256.0367	151.0021
13	Luteolin-O-hexoside isomer 2	C ₂₁ H ₂₀ O ₁₁	22.83		447.09274	327.0499	285.0405	284.0328	256.0369	151.0024
14	Hyperoside (Quercetin-3-O-galactoside)	C ₂₁ H ₂₀ O ₁₂	23.23		463.08765	301.0349	300.0275	271.0248	255.0298	178.9971
15 ¹	Isoquercitrin (Quercetin-3-O-glucoside)	C ₂₁ H ₂₀ O ₁₂	23.43		463.08765	301.0360	300.0275	271.0248	255.0296	178.9975
16 ¹	Eriodictyol (3',4',5,7-Tetrahydroxyflavanone)	C ₁₅ H ₁₂ O ₆	25.42		287.05556	269.0461	151.0024	135.0438	125.0229	107.0123
17 ¹	Quercetin (3,3',4',5,7-Pentahydroxyflavone)	C ₁₅ H ₁₀ O ₇	27.55		301.03483	273.0412	178.9975	151.0024	121.0280	107.0124
18 ¹	Naringenin (4',5,7-Trihydroxyflavanone)	C ₁₅ H ₁₂ O ₅	27.75		271.06065	227.0709	177.0181	151.0023	119.0487	107.0123
19 ¹	Luteolin (3',4',5,7-Tetrahydroxyflavone)	C ₁₅ H ₁₀ O ₆	28.42		285.03991	217.0499	199.0393	175.0390	151.0024	133.0281
20 ¹	Apigenin (4',5,7-Trihydroxyflavone)	C ₁₅ H ₁₀ O ₅	30.27		269.04500	225.0554	201.0545	151.0020	149.0229	117.0330
21	Chrysoeriol (3'-Methoxy-4',5,7-trihydroxyflavone)	C ₁₆ H ₁₂ O ₆	30.49		299.05556	284.0328	256.0371	227.0366	151.0020	107.0124
34	Dihydroxy-trimethoxy(iso)flavone isomer 1	C ₁₇ H ₁₄ O ₇	31.06		329.06613	314.0436	313.0363	299.0196	271.0252	227.0345
22	Trihydroxy-trimethoxy(iso)flavone isomer I	C ₁₈ H ₁₆ O ₈	32.93	361.09235		346.0685	345.0616	331.0439	317.0653	303.0499
23	Dihydroxy-dimethoxy(iso)flavone	C ₁₇ H ₁₄ O ₆	32.96	315.08686		300.0629	299.0549	271.0602	257.0445	
35	Dihydroxy-trimethoxy(iso)flavone isomer 2	C ₁₇ H ₁₄ O ₇	33.34		329.06613	314.0435	299.0197	271.0246		
24	Chrysin (5,7-Dihydroxyflavone)	C ₁₅ H ₁₀ O ₄	33.84	255.06573		209.0597	153.0183	129.0337	103.0547	67.0186
25	Dimethoxy(iso)flavone	C ₁₇ H ₁₄ O ₄	34.13	283.09704		268.0729	267.0644	239.0701	225.0542	
26 ¹	Galangin (3,5,7-Trihydroxyflavone)	C ₁₅ H ₁₀ O ₅	34.72	271.06065		215.0700	197.0598	165.0148	153.0180	105.0338
27	Trimethoxy(iso)flavone	C ₁₈ H ₁₆ O ₅	34.97	313.10760		298.0837	297.0757	280.0730	279.0650	252.0779
28	Dihydroxy-methoxy(iso)flavone	C ₁₆ H ₁₂ O ₅	35.09	285.07630		270.0520	269.0440	242.0573	167.0341	119.0492

29	Hydroxy-trimethoxy(iso)flavone	C ₁₈ H ₁₆ O ₆	36.89	329.10251		314.0781	313.0704	299.0548	271.0599	
30	Hydroxy-methoxy(iso)flavone	C ₁₆ H ₁₂ O ₄	38.07	269.08138		254.0574	226.0624	167.0341		
31	Linoleamide	C ₁₈ H ₃₃ NO	44.45	280.26404		263.2369	245.2265	109.1016	95.0860	81.0705
32	Oleamide	C ₁₈ H ₃₅ NO	45.71	282.27969		265.2528	247.2419	135.1169	83.0861	69.0705

¹Confirmed by the standard.

Table S3. Chemical composition of germinated somatic embryo extract.

No.	Name	Formula	Rt	[M+H] ⁺	[M-H] ⁻	Fra 1	Fra 2	Fra 3	Fra 4	Fra 5
1	Trigonelline	C ₇ H ₇ NO ₂	1.26	138.05550		110.0604	96.0449	94.0656	92.0499	65.0392
2	Nicotinic acid (Niacin)	C ₆ H ₅ NO ₂	1.48	124.03986		96.0449	80.0501	78.0345		
3	Nicotinamide	C ₆ H ₆ N ₂ O	1.61	123.05584		106.0291	96.0449	80.0501		
4 ¹	Gallic acid (3,4,5-Trihydroxybenzoic acid)	C ₇ H ₆ O ₅	2.68		169.01370	125.0230	97.0278	81.0330	69.0329	
6	Dihydroxybenzoic acid	C ₇ H ₆ O ₄	10.43		153.01879	123.0073	109.0280	108.0202	95.0123	85.0279
7	Caffeic acid	C ₉ H ₈ O ₄	15.18		179.03444	135.0438	107.0488			
8 ¹	Taxifolin (Dihydroquercetin)	C ₁₅ H ₁₂ O ₇	19.92		303.05048	285.0404	177.0183	175.0391	153.0179	125.0229
9	cis-3-[(4-hydroxy-3-Methoxyphenyl)-prop-2-enoyl]oxybutanedioic acid	C ₁₄ H ₁₄ O ₈	20.78	311.07670		177.0546	149.0597	145.0284	117.0337	89.0391
10	Eriodictyol-O-hexoside	C ₂₁ H ₂₂ O ₁₁	20.85		449.10839	287.0562	151.0024	135.0438	107.0123	
11	trans-3-[(4-hydroxy-3-methoxyphenyl) prop-2-enoyl]oxybutanedioic acid	C ₁₄ H ₁₄ O ₈	21.45	311.07670		177.0546	149.0598	145.0284	117.0337	89.0390
12	Luteolin-O-hexoside isomer 1	C ₂₁ H ₂₀ O ₁₁	22.39		447.09274	327.0509	285.0405	284.0331	256.0380	151.0024
14	Hyperoside (Quercetin-3-O-galactoside)	C ₂₁ H ₂₀ O ₁₂	23.22		463.08765	301.0361	300.0277	271.0248	255.0298	178.9975
15 ¹	Isoquercitrin (Quercetin-3-O-glucoside)	C ₂₁ H ₂₀ O ₁₂	23.44		463.08765	301.0359	300.0277	271.0248	255.0297	178.9975
16 ¹	Eriodictyol (3',4',5,7-Tetrahydroxyflavanone)	C ₁₅ H ₁₂ O ₆	25.43		287.05556	269.0454	151.0024	135.0439	125.0230	107.0124
17 ¹	Quercetin (3,3',4',5,7-Pentahydroxyflavone)	C ₁₅ H ₁₀ O ₇	27.55		301.03483	273.0410	178.9976	151.0024	121.0280	107.0124
18 ¹	Naringenin (4',5,7-Trihydroxyflavanone)	C ₁₅ H ₁₂ O ₅	27.76		271.06065	227.0712	177.0183	151.0024	119.0488	107.0124
19 ¹	Luteolin (3',4',5,7-Tetrahydroxyflavone)	C ₁₅ H ₁₀ O ₆	28.42		285.03991	217.0497	199.0391	175.0388	151.0024	133.0282
20 ¹	Apigenin (4',5,7-Trihydroxyflavone)	C ₁₅ H ₁₀ O ₅	30.26		269.04500	225.0552	201.0544	151.0025	149.0232	117.0331
36 ¹	Isorhamnetin (3'-Methoxy-3,4',5,7-tetrahydroxyflavone)	C ₁₆ H ₁₂ O ₇	30.41		315.05048	300.0276	283.0247	271.0249	164.0105	151.0026
22	Trihydroxy-trimethoxy(iso)flavone isomer I	C ₁₈ H ₁₆ O ₈	31.35	361.09235		346.0681	345.0606	331.0447	317.0661	303.0498
37	Rhamnetin (7-Methoxy-3,3',4',5-tetrahydroxyflavone)	C ₁₆ H ₁₂ O ₇	32.41		315.05048	300.0273	193.0135	165.0181	121.0281	97.0280
38	Trihydroxy-trimethoxy(iso)flavone isomer II	C ₁₈ H ₁₆ O ₈	32.94	361.09235		346.0680	345.0609	331.0452	317.0654	303.0490
23	Dihydroxy-dimethoxy(iso)flavone	C ₁₇ H ₁₄ O ₆	32.96	315.08686		300.0628	299.0549	271.0601	257.0446	
24	Chrysin (5,7-Dihydroxyflavone)	C ₁₅ H ₁₀ O ₄	33.84	255.06573		209.0594	153.0183	129.0335	103.0546	67.0184
25	Dimethoxy(iso)flavone	C ₁₇ H ₁₄ O ₄	34.13	283.09704		268.0729	267.0653	239.0701	225.0549	
26 ¹	Galangin (3,5,7-Trihydroxyflavone)	C ₁₅ H ₁₀ O ₅	34.72	271.06065		215.0700	197.0596	165.0148	153.0181	105.0338

27	Trimethoxy(iso)flavone	C ₁₈ H ₁₆ O ₅	34.99	313.10760		298.0837	297.0757	280.0729	279.0647	252.0779
28	Dihydroxy-methoxy(iso)flavone	C ₁₆ H ₁₂ O ₅	35.05	285.07630		270.0523	269.0445	242.0573	167.0340	119.0492
29	Hydroxy-trimethoxy(iso)flavone	C ₁₈ H ₁₆ O ₆	36.90	329.10251		314.0785	313.0693	299.0549	271.0601	
30	Hydroxy-methoxy(iso)flavone	C ₁₆ H ₁₂ O ₄	38.07	269.08138		254.0573	226.0623	167.0338		
31	Linoleamide	C ₁₈ H ₃₃ NO	44.45	280.26404		263.2369	245.2262	109.1015	95.0860	81.0705
32	Oleamide	C ₁₈ H ₃₅ NO	45.74	282.27969		265.2528	247.2417	135.1168	83.0861	69.0705

¹Confirmed by the standard.

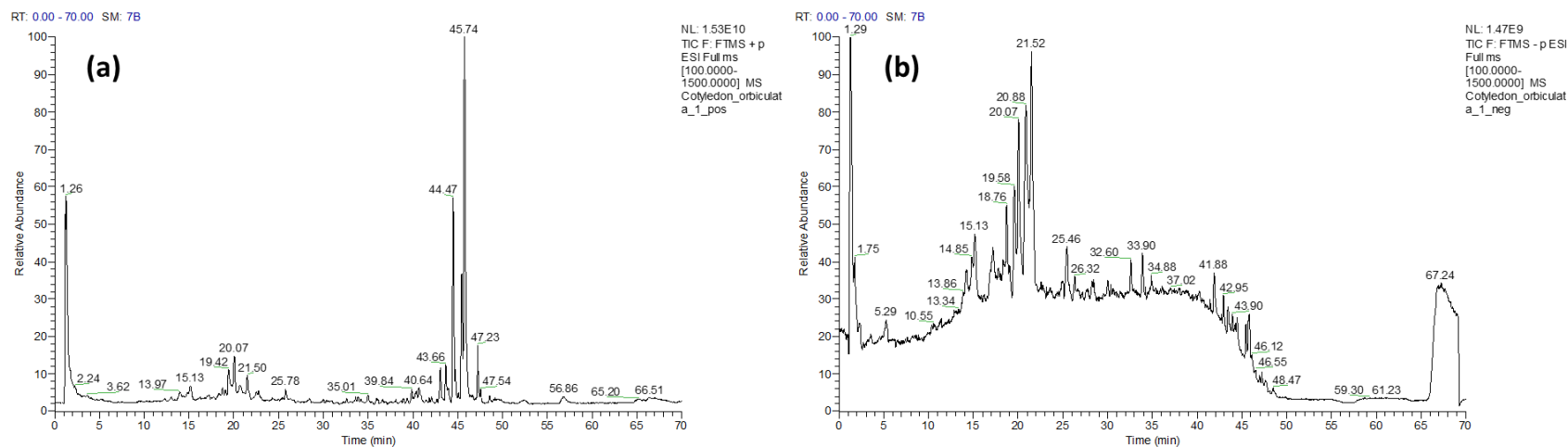


Figure S1. Total ion chromatogram of early somatic embryos extract of *C. orbiculata* in positive (a) and negative (b) mode.

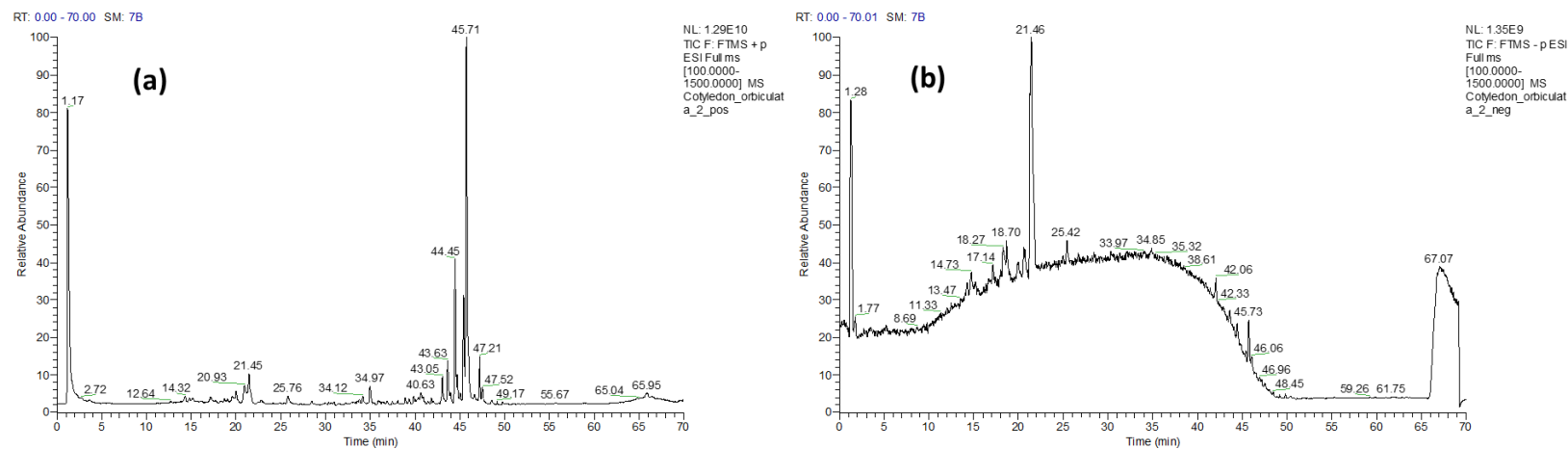


Figure S2. Total ion chromatogram of mature somatic embryos extract of *C. orbiculata* in positive (a) and negative (b) mode.

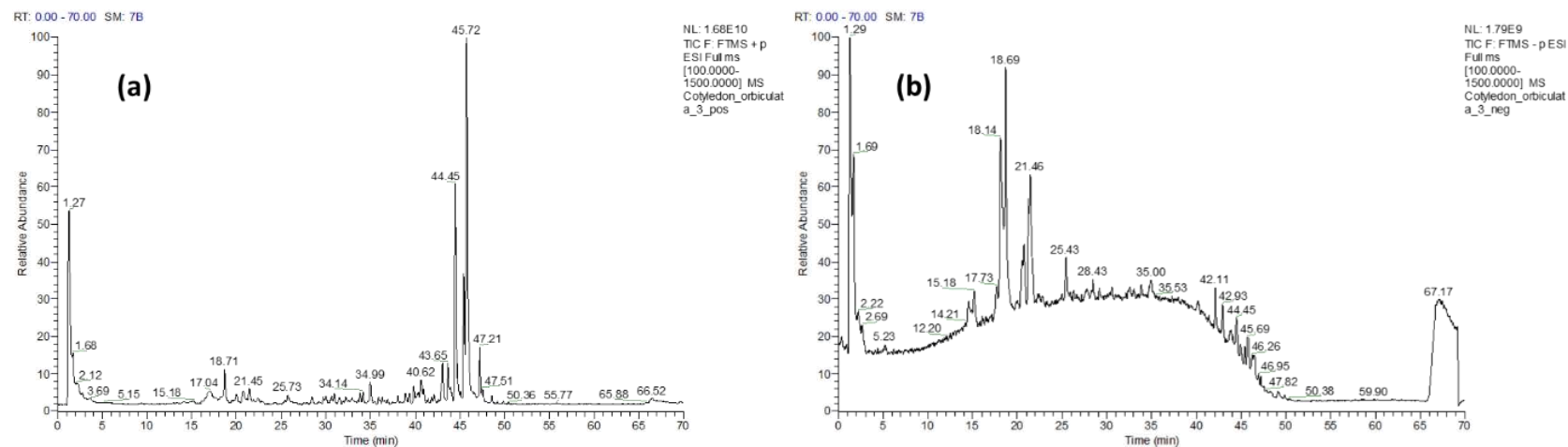


Figure S3. Total ion chromatogram of germinated somatic embryos extract of *C. orbiculata* in positive (a) and negative (b) mode.