



Supplementary Material

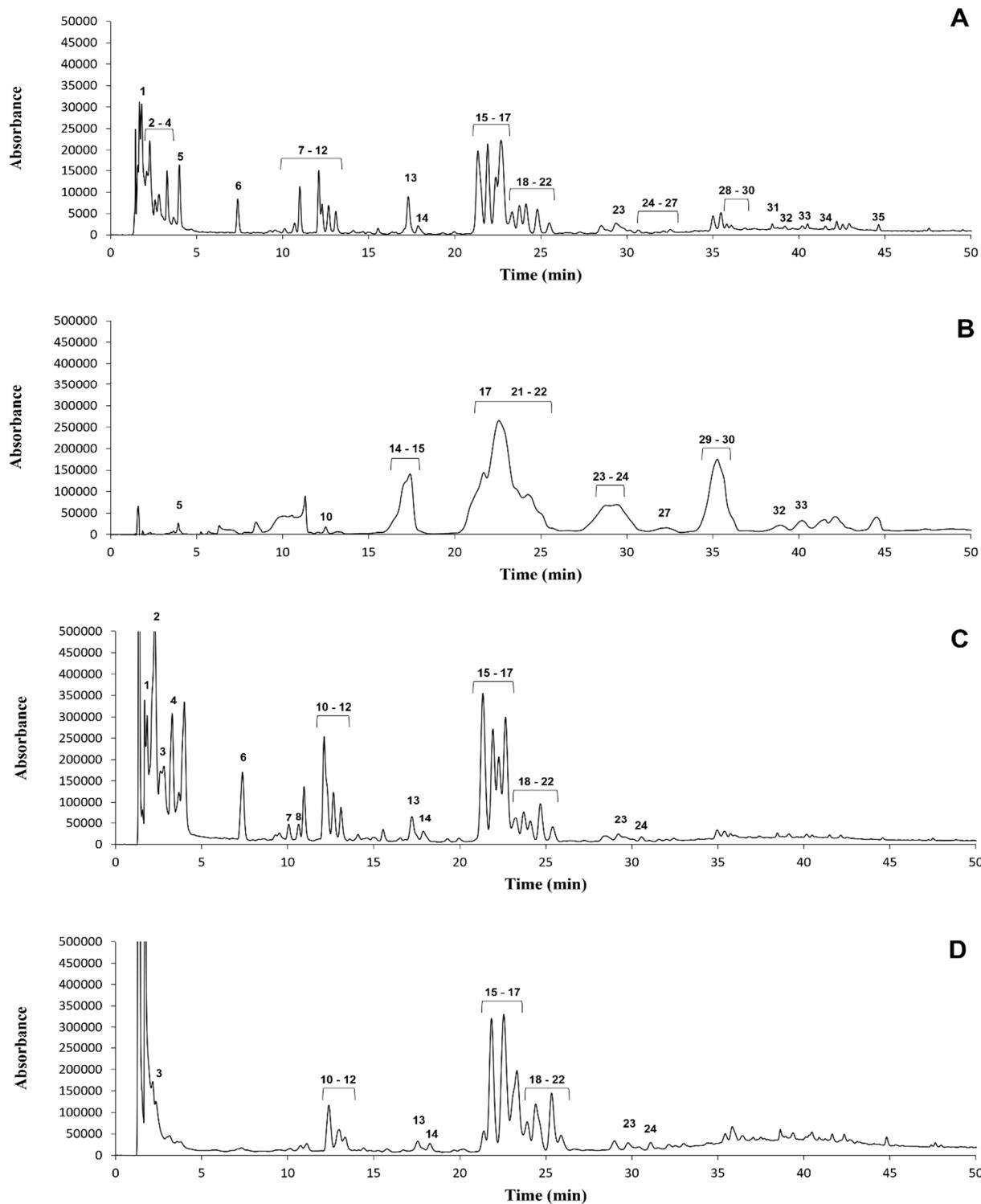


Figure S1 - Chromatographic profiles of G18.EE (A) and its fractions η -Hexane (B), EtOAc (C), and η -BuOH (D) obtained by LC-MS.

Table S1 - Chemical composition of G18.EE and its fractions η -Hexane, EtOAc, and η -BuOH according to LC-MS analysis. (“-” corresponds to a compound not present or not detected). Bold values represent the major phenolic compounds (Composition (%) > 5%).

Peaks	Time range (min)	λ_{max} (nm)	[M - H] m/z	Main fragments	Compound	Composition (%)			
						G18.EE	η -Hexane	EtOAc	η -BuOH
1	1.6 – 2.2	270	169	125	Gallic acid	3.39	-	1.65	-
2	2.2 – 2.5	292sh, 322	179	135, 163	Caffeic acid	7.08	-	14.97	-
3	2.3 – 3.6	253, 368	301	257, 229	Ellagic acid	2.15	0.05	0.74	2.64
4	3.1 – 3.5	309	163	119, 145, 108	p-Coumaric acid	3.33	-	7.83	-
5	3.8 – 4.2	295sh, 323	193	177, 149, 133	Ferulic acid	3.97	0.45	-	-
6	7.3 – 7.6	295sh, 322	207	163, 148, 133	3,4-Dimethyl-caffeoic acid (DMCA)	2.15	-	6.31	-
7	9.9 – 10.3	288	285	267, 239, 252	Pinobanksin-5-methyl-ether	0.49	-	1.30	-
8	10.5 – 10.8	273, 353	315	300	Quercetin-3-methyl ether	0.84	-	1.16	-
9	10.8 – 11.2	308	177	-	p-coumaric acid methyl ester	2.72	-	-	-
10	11.9 – 12.7	267, 336 291	269 271	225, 151 253, 225	Apigenin Pinobanksin	5.03	0.64	10.14	7.39
11	12.5 – 13.2	265, 363	285	285, 257, 151	Kaempferol	2.01	-	2.87	3.04
12	13.0 – 13.6	255, 368	315	300	Isorhamnetin	1.32	-	2.35	1.18
13	16.7 – 17.9	311	313	298, 269, 257, 241, 179, 153	unknown	3.30	4.16	2.47	2.66
14	17.3 – 18.5	254, 368	329	-	Quercetin-dimethyl-ether	1.01	1.11	1.12	1.52
15	20.5 – 22.4	268, 315 298, 325	253 247	209, 181, 225, 151 179, 135	Chrysin Caffeic acid isoprenyl ester	10.35	28.99	15.93	20.94
16	21.7 – 23.1	268, 332 299, 325	283 247	268 179, 135	Acacetin Caffeic acid isoprenyl ester	12.73	-	11.48	23.43
17	22.5 – 23.6	289	255	213, 211, 151	Pinocembrin	13.01	20.79	8.24	5.52

Table S1 (continued) - Chemical composition of G18.EE and its fractions η -Hexane, EtOAc, and η -BuOH according to LC-MS analysis. (“-“ corresponds to a compound not present or not detected). Bold values represent the major phenolic compounds. (Composition (%)> 5%).

Peaks	Time range (min)	λ_{max} (nm)	[M - H] m/z	Main fragments	Compound	Composition (%)			
						G18.EE	η -Hexane	EtOAc	η -BuOH
18	23.0 – 24.2	290sh, 356	269	223, 169, 249	Galangin	2.69	-	1.35	2.95
19	23.6 – 24.5	267, 362	299	284, 165	Kaempferide	2.72	-	1.93	4.38
20	24.0 – 25.1	269, 360	329	314, 297, 287, 269, 257	Kaempferol -methoxy-methyl ether	3.13	-	1.02	2.29
21	23.9 – 25.8	293	313	253, 271	Pinobanksin-3-O-acetate	2.56	4.38	3.82	9.75
22	25.2 – 29.7	298, 325	283	179, 135	Caffeic acid phenylethyl ester	1.39	0.41	1.55	2.24
23	27.6 – 30.9	311	231	163, 119	<i>p</i> -Coumaric acid isoprenyl ester (isomer)	2.00	3.49	0.50	1.64
24	30.3 – 31.4	295, 325	295	178, 134, 251, 211	Caffeic acid cinnamyl ester	0.58	0.53	0.39	1.34
25	31.5 – 31.8	268, 305b	297 403	179, 161, 151, 135 -	Caffeic acid derivative unknown	0.29	-	-	-
26	32.3 – 32.8	293	327	253, 271	Pinobanksin-3-O-propionate	0.32	-	-	-
27	31.6 – 34.4	340	269	254, 251, 236, 165	3-Hydroxy-5-methoxy flavanone	0.65	1.78	0.25	1.04
28	35.7 – 35.9	268, 309b	387 501	281, 267, 255, 293 457, 439, 247	unknown unknown	2.09	-	-	-
29	34.3 – 36.5	290	417	-	Methylated Pinobanksin-3-O-phenylpropionate	0.93	19.03	0.16	2.52
30	35.5 – 39.4	293	341	253	Pinobanksin-3-O-butyrate or isobutyrate	0.48	8.11	0.04	0.95
31	38.5 – 40.3	269, 307, 343	565	283, 269, 281, 417, 455	<i>p</i> -Coumaric acid-4-hydroxy-phenylethyl ester dimer Pinobanksin-3-O-pentenoate	0.87	-	-	1.09
32	38.1 – 41.8	292	355	253, 271 or 2-methylbutyrate		0.89	3.24	0.21	0.97
33	39.6 – 44.8	297, 320b	315	179, 135	Caffeic acid derivative	0.98	2.82	0.24	0.53

Table S1 (conclusion) - Chemical composition of G18.EE and its fractions η-Hexane, EtOAc, and η-BuOH according to LC-MS analysis. (“-“ corresponds to a compound not present or not detected). Bold values represent the major phenolic compounds. (Composition (%)) > 5%).

Peaks	Time range (min)	λ_{max} (nm)	[M - H] ⁻ m/z	Main fragments	Compound	Composition (%)			
						G18.EE	η -Hexane	EtOAc	η -BuOH
34	41.4 – 41.7	280	293	185, 197, 275, 249	<i>p</i> -Methoxy-cinnamic acid cinnamyl ester	0.85	-	-	-
35	44.5 – 44.8	310	473	-	<i>p</i> -Coumaric acid derivative	0.85	-	-	-

Table S2 - Analysis of the effect of the selected propolis fractions' treatments on cell biomass along the time. Statistical analyses were performed with Two-way ANOVA for both cell lines (A375 and WM9). Bold p-values show statistically significant effects.

A375 Cell Line						
Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F ratio	p-value	Partial Eta Squared
Time	31319	3	10440	F(2.096,20.96) = 72.40	<0.0001	0.879
Treatment	3404	4	850.9	F(4,10) = 4.774	0.0205	0.440
Time vs Treatment	1494	12	124.5	F(12,30) = 0.8633	0.5901	0.257
Residual	4326	30	144.2	-----	-----	-----

WM9 Cell Line						
Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F ratio	p-value	Partial Eta Squared
Time	4413	3	1471	F(1.192,11.92) = 15.83	0.0013	0.613
Treatment	7092	4	1773	F(4,10) = 2.344	0.1252	0.718
Time vs Treatment	3330	12	277.5	F(12,30) = 2.986	0.0074	0.544
Residual	2788	30	92.94	-----	-----	-----

vs = versus

Table S3 - Analysis of the effect of the selected propolis fractions' treatments on melanoma cells' ROS levels. Statistical analyses were performed with One-way ANOVA for both cell lines (A375 and WM9). Bold p-values show statistically significant effects.

A375 Cell Line						
Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F ratio	p-value	Partial Eta Squared
Treatment	52679542	6	8779924	F(6,14) = 59.85	<0.0001	0.9625
Residual	2053646	14	146689	-----	-----	-----
WM9 Cell Line						
Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F ratio	p-value	Partial Eta Squared
Treatment	41822810	6	6970468	F(6,14) = 3.972	0.0157	0.6299
Residual	24570069	14	1755005	-----	-----	-----

Table S4 - Analysis of the effect of the selected propolis fractions' treatments on mitochondrial activity. Statistical analyses were performed with One-way ANOVA for both cell lines (A375 and WM9).

A375 Cell Line						
Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F ratio	p-value	Partial Eta Squared
Treatment	51.67	6	8.612	F(6,14) = 2.467	0.0769	0.5139
Residual	48.88	14	3.491	-----	-----	-----
WM9 Cell Line						
Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F ratio	p-value	Partial Eta Squared
Treatment	8.320	6	1.387	F (6, 14) = 2.176	0.1082	0.4826
Residual	8.921	14	0.6372	-----	-----	-----

Table S5 - Analysis of the effect of the selected propolis fractions' treatments on mitochondrial biomass. Statistical analyses were performed with One-way ANOVA for both cell lines (A375 and WM9). Bold p-values show statistically significant effects.

A375 Cell Line						
Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F ratio	p-value	Partial Eta Squared
Treatment	10580118	6	1763353	F(6,14) = 5.993	0.0028	0.7198
Residual	4119184	14	294227	-----	-----	-----
WM9 Cell Line						
Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F ratio	p-value	Partial Eta Squared
Treatment	22875260	6	3812543	F (6, 14) = 10.33	0.0002	0.8158
Residual	5165729	14	368981	-----	-----	-----

Table S6 - Analysis of the effect of selected propolis fractions' treatments on mitochondrial polarization. Statistical analyses were performed with One-way ANOVA for both cell lines (A375 and WM9).

A375 Cell Line						
Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F ratio	p-value	Partial Eta Squared
Treatment	9159783	6	1526631	F(6,14) = 2.662	0.0616	0.5329
Residual	8030032	14	573574	-----	-----	-----
WM9 Cell Line						
Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F ratio	p-value	Partial Eta Squared
Treatment	3083553	6	513926	F (6, 14) = 1.248	0.3407	0.3485
Residual	5763906	14	411708	-----	-----	-----

Table S7 - Analysis of the effect of propolis fractions' treatments on the levels of apoptotic markers. Statistical analyses were performed with One-way ANOVA for both cell lines (A375 and WM9). Bold p-values show statistically significant effects.

A375 Cell Line – Bax protein						
Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F ratio	p-value	Partial Eta Squared
Treatment	0.4133	4	0.1033	F(4,10) = 2.431	0.1161	0.4930
Residual	0.4250	10	0.04250	-----	-----	-----
WM9 Cell Line – Bax protein						
Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F ratio	p-value	Partial Eta Squared
Treatment	0.4526	4	0.1131	F(4,10) = 0.6521	0.6385	0.2069
Residual	1.735	10	0.1735	-----	-----	-----
A375 Cell Line – Bcl-2 protein						
Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F ratio	p-value	Partial Eta Squared
Treatment	0.3866	4	0.09666	F(4,10) = 0.6709	0.6269	0.2115
Residual	1.441	10	0.1441	-----	-----	-----
WM9 Cell Line – Bcl-2 protein						
Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F ratio	p-value	Partial Eta Squared
Treatment	0.1520	4	0.03800	F(4,10) = 0.4382	0.7785	0.1491
Residual	0.8673	10	0.08673	-----	-----	-----
A375 Cell Line – Caspase 3 protein						
Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F ratio	p-value	Partial Eta Squared
Treatment	0.3043	4	0.07606	F(4,10) = 2.131	0.1514	0.4644
Residual	0.3510	10	0.03570	-----	-----	-----
WM9 Cell Line – Caspase 3 protein						
Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F ratio	p-value	Partial Eta Squared
Treatment	0.3399	4	0.08498	F(4,10) = 0.9050	0.4969	0.2658
Residual	0.9390	10	0.09390	-----	-----	-----
A375 Cell Line – Caspase 9 protein						
Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F ratio	p-value	Partial Eta Squared
Treatment	0.5295	4	0.1324	F(4,10) = 4.811	0.0201	0.6581
Residual	0.2751	10	0.02751	-----	-----	-----
WM9 Cell Line – Caspase 9 protein						
Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F ratio	p-value	Partial Eta Squared
Treatment	2.920	4	0.7299	F(4,10) = 12.98	0.0006	0.8385
Residual	0.5623	10	0.05623	-----	-----	-----

Table S7 (continued) - Analysis of the effect of propolis fractions' treatments on the levels of apoptotic markers. Statistical analyses were performed with One-way ANOVA for both cell lines (A375 and WM9). Bold p-values show statistically significant effects.

A375 Cell Line – Bcl-XL protein						
Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F ratio	p-value	Partial Eta Squared
Treatment	1.937	4	0.4843	F(4,10) = 10.31	0.0014	0.8048
Residual	0.4697	10	0.04697	-----	-----	-----
WM9 Cell Line – Bcl-XL protein						
Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F ratio	p-value	Partial Eta Squared
Treatment	0.02661	4	0.006653	F(4,10) = 0.3726	0.8231	0.1298
Residual	0.1786	10	0.01786	-----	-----	-----
A375 Cell Line – p53 protein						
Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F ratio	p-value	Partial Eta Squared
Treatment	0.5521	4	0.1380	F(4,10) = 1.667	0.2331	0.4000
Residual	0.8281	10	0.08281	-----	-----	-----
WM9 Cell Line – p53 protein						
Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F ratio	p-value	Partial Eta Squared
Treatment	0.8925	4	0.2231	F(4,10) = 1.426	0.2949	0.3632
Residual	1.565	10	0.1565	-----	-----	-----