

Supporting Information

Rec. Nat. Prod. 10:5 (2016) 617-627

Phenolic Content and Antibiofilm Activity of Propolis Against Clinical MSSA Strains

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Table S1. Tune parameters of UPLC-ESI-MS/MS.

Capillaryvoltage	: 2000 V
Conevoltage	: 25 V
Nebulizerpressure	: 7 bar
Desolvationgastemperature	: 500 °C
Gasflow	: 16.7 (L min ⁻¹)

Table S2. Method parameters for the analysis of phenolic compounds using UPLC-ESI-MS/MS.

Phenolic Compounds	Quantification > Confirmatory transition (m/z)	Cone voltage (V)	Collision Energy (V)
Pyrogallol	125.01 > 69.10, 79.04, 81.02	20	17, 17, 14
Homogentisic acid	167.03 > 123.03, 122.08, 108.00	10	20, 20, 10
Protocatechuic acid	153.06 > 108.00, 81.01, 91.01	10	20, 25, 20
Gentisic acid	153.05 > 109.04, 108.03, 81.00	10	20, 20, 12
Pyrocatechol	153.06 > 81.01, 108.00, 109.04	8	20, 25, 20
Galantamine	288.10 > 198.00, 213.09, 230.95	20	32, 23, 17
<i>p</i> -hydroxybenzoic acid	136.98 > 93.03, 65.10	10	25, 14
3,4-dihydroxybenzaldehyde	137.00 > 91.93, 107.94, 136.00	8	21, 20, 18
Catechin hydrate	288.88 > 109.15, 124.99, 245.26	30	25, 20, 15
Vanillic acid	166.98 > 151.97, 108.03, 123.03	20	18, 12, 14
Caffeic acid	179.10 > 135.14, 107.10, 133.9	32	23, 23, 24
Syringic acid	197.20 > 123.00, 167.00, 182.00	15	22, 18, 14
Vanillin	150.95 > 135.94, 91.90, 107.97	30	20, 20, 14
<i>p</i> -coumaric acid	189.18 > 151.00, 203.00, 205.00	20	20, 20, 20
Ferulic acid	163.01 > 119.04, 93.00, 117.01	5	27, 27, 15
Epicatechin	193.03 > 134.06, 178.00, 149.02	20	16, 12, 13
Catechingallate	441.00 > 168.98, 288.97	30	20, 20
Rutin	609.00 > 254.99, 270.93, 299.90	17	55, 55, 40
<i>trans</i> -2-hydroxy cinnamic acid	163.04 > 119.04, 117.01, 93.07	10	25, 22, 13
Myricetin	316.90 > 107.07, 137.01, 150.97	30	30, 25, 25
Resveratrol	227.01 > 143.01, 159.05, 185.03	30	25, 18, 18
<i>trans</i> -cinnamic acid	146.98 > 103.03, 62.18	30	10, 10
Luteolin	284.91 > 107.01, 133.05, 151.02	20	30, 33, 30
Quercetin	303.00 > 137.00, 153.00, 229.00	20	30, 32, 30
Naringenin	270.98 > 107.00, 119.04, 150.97	20	25, 25, 20
Genistein	271.00 > 153.00, 215.00, 243.00	20	27, 25, 24
Apigenin	269.10 > 107.00, 117.00, 149.00	20	30, 30, 25
Kaempferol	284.90 > 158.97, 117.10, 227.14	10	34, 40, 30
Hesperetin	301.02 > 108.01, 136.00, 163.99	20	36, 30, 24
Chrysin	252.99 > 63.05, 107.05, 142.99	20	30, 25, 25

Table S3. Analytical parameters of UPLC-MS/MS method validation.

No	Compounds	LOD (mg 100 mL ⁻¹)	LOQ (mg 100 mL ⁻¹)	CV% ^a	CV% ^b	Recovery (%) ^b	StabilityCV % ^c	Accuracy(%) ^d
1	Pyrogallol	0.089	0.148	0.018	0.126	97.8-99.5	0.71	92.3-99.7
2	Homogentisicacid	0.025	0.042	0.012	0.090	92.3-98.9	1.48	94.5-99.7
3	3,4-Dihydroxybenzoic acid	0.031	0.052	0.011	0.051	98.4-100.2	0.64	92.4-100.2
4	Gentisicacid	0.048	0.080	0.014	0.134	97.9-99.9	1.29	97.9-100.1
5	Pyrocatechol	0.036	0.060	0.015	0.156	99.1-101.4	0.99	96.5-99.7
6	Galantamine	0.062	0.103	0.012	0.162	98.0-100.2	0.21	96.7-100.0
7	<i>p</i> -hydroxybenzoicacid	0.077	0.128	0.009	0.051	90.7-100.6	0.19	96.2-98.6
8	3,4-dihydroxybenzaldehyde	0.104	0.173	0.017	0.098	92.7-98.6	1.03	99.2-100.1
9	Catechinhydrate	0.053	0.088	0.021	0.218	97.5-100.4	0.88	96.7-99.9
10	Vanillicacid	0.007	0.012	0.005	0.054	93.9-100.7	0.78	91.7-98.5
11	Caffeicacid	0.021	0.035	0.011	0.155	89.9-97.5	0.45	99.0-100.5
12	Syringicacid	0.047	0.078	0.041	0.320	93.5-100.3	1.04	95.5-99.8
13	Vanillin	0.041	0.068	0.006	0.085	92.3-97.8	0.74	98.2-100.1
14	<i>p</i> -Coumaricacid	0.034	0.057	0.009	0.092	89.7-99.4	0.56	98.3-99.7
15	Ferulicacid	0.044	0.073	0.011	0.145	90.6-98.7	0.48	96.2-100.1
16	Epicatechin	0.078	0.130	0.020	0.214	91.5-99.0	0.35	98.5-99.9
17	Catechingallate	0.067	0.112	0.009	0.086	95.2-100.0	0.98	89.9-99.4
18	Rutin	0.047	0.078	0.013	0.097	96.7-98.9	1.16	92.3-97.8
19	<i>trans</i> -2-hydroxy cinnamicacid	0.059	0.098	0.016	0.198	97.2-99.7	0.72	95.0-99.8
20	Myricetin	0.018	0.030	0.004	0.080	97.6-99.5	0.48	97.6-100.0
21	Resveratrol	0.012	0.020	0.003	0.074	90.5-102.9	0.67	98.7-100.8
22	<i>trans</i> -Cinnamicacid	0.014	0.023	0.002	0.051	90.5-96.1	1.56	98.1-100.2
23	Luteolin	0.011	0.018	0.005	0.087	96.3-97.9	1.02	98.7-99.9
24	Quercetin	0.021	0.035	0.017	0.119	89.5-99.7	0.53	94.1-100.4
25	Naringenin	0.014	0.023	0.003	0.065	94.2-100.4	0.48	96.4-99.4
26	Genistein	0.010	0.017	0.023	0.142	89.9-95.2	0.72	98.6-100.7
27	Apigenin	0.024	0.040	0.017	0.051	96.1-100.9	0.32	96.5-99.8
28	Kaempferol	0.015	0.025	0.002	0.071	95.2-97.8	0.41	97.8-100.2
29	Hesperetin	0.029	0.048	0.015	0.165	92.0-98.2	1.01	97.3-100.8
30	Chrysin	0.058	0.097	0.013	0.132	96.2-100.7	0.64	96.7-100.3

^aIntra-dayrepeatability.^bInter-dayreproducibilityandrecoveryfromonce a day of threeconsecutivedays.^cStabilityweremeasuredbystandardsolutionsoverthreeconsecutivedays.^dAccuracyrepresentstheratio of themeasuredconcentrationtothetheoreticalconcentrationfrommediumconcentrationvalue of thecalibrationrange.

Table S4. Purity of standards, calibration curve and correlation coefficient for phenolic compounds.

No	Compounds	M.W. (g.mol ⁻¹)	Purity of Standard and Supplier	Calibration equations	r ²
1	Pyrogallol	126.11	≥99 % (Sigma-Aldrich)	y = 3952.61x + 189.447	0.992438
2	Homogentisic acid	168.15	≥98% (Sigma-Aldrich)	y = 4625.53x - 79.189	0.998501
3	3,4-Dihydroxybenzoic acid	154.12	≥99 % (Sigma-Aldrich)	y = 163.811x + 269.959	0.999716
4	Gentisic acid	154.12	≥99.5 % (Sigma-Aldrich)	y = 15802.05x + 2143.53	0.999339
5	Pyrocatechol	110.11	≥99 % (Sigma-Aldrich)	y = 8177.83x - 26.315	0.999817
6	Galantamine	368.30	≥94 % (Sigma-Aldrich)	y = 4050.44x + 2264.44	0.999190
7	<i>p</i> -hydroxybenzoic acid	138.12	≥99 % (Sigma-Aldrich)	y = 569.645x + 3815.13	0.999187
8	3,4-dihydroxybenzaldehyde	138.12	≥99 % (Sigma-Aldrich)	y = 876.228x - 168.443	0.997171
9	Catechin hydrate	290.27	≥98% (Sigma-Aldrich)	y = 31975x + 3434.29	0.995962
10	Vanillic acid	168.15	≥97 % (Sigma-Aldrich)	y = 9201.29x - 393.299	0.998502
11	Caffeic acid	180.16	≥98% (Sigma-Aldrich)	y = 194.377x + 585.655	0.998902
12	Syringic acid	198.17	≥95 % (Sigma-Aldrich)	y = 413.173x + 235.852	0.999168
13	Vanillin	152.15	≥99 % (Sigma-Aldrich)	y = 1542.35x + 1626.98	0.994994
14	<i>p</i> -Coumaric acid	164.16	≥98 % (Sigma-Aldrich)	y = 499.659x - 1449.58	0.998894
15	Ferulic acid	194.19	≥99 % (Sigma-Aldrich)	y = 580.716x + 168.08	0.998408
16	Epicatechin	290.27	≥98 % (Sigma-Aldrich)	y = 7646.83x + 6034.60	0.989468
17	Catechingallate	442.40	≥98% (Sigma-Aldrich)	y = 54507.70x + 120.14	0.991150
18	Rutin	610.52	≥94 % (Sigma-Aldrich)	y = 517.736x + 715.49	0.999923
19	<i>trans</i> -2-hydroxy cinnamic acid	164.16	≥98% (Sigma-Aldrich)	y = 367.979x - 1097.20	0.999166
20	Myricetin	318.24	≥96 % (Sigma-Aldrich)	y = 39242.70x + 134.89	0.997791
21	Resveratrol	228.24	≥99 % (Sigma-Aldrich)	y = 24706.70x + 853.14	0.998549
22	<i>trans</i> -Cinnamic acid	148.16	≥99 % (Sigma-Aldrich)	y = 635.896x + 744.66	0.995543
23	Luteolin	286.20	≥98% (Sigma-Aldrich)	y = 28712x + 162.10	0.999877
24	Quercetin	338.26	≥95 % (Sigma-Aldrich)	y = 33013.60x + 214.63	0.999763
25	Naringenin	272.26	≥95 % (Sigma-Aldrich)	y = 43739x + 6163.03	0.996296
26	Genistein	270.24	≥98 % (Sigma-Aldrich)	y = 21417.80x + 204.87	0.999464
27	Apigenin	270.24	≥95 % (Sigma-Aldrich)	y = 1485.35x - 649.90	0.994972
28	Kaempferol	286.20	≥97 % (Sigma-Aldrich)	y = 708.133x - 417.65	0.993051
29	Hesperetin	302.30	≥95 % (Sigma-Aldrich)	y = 23581.30x + 1836.25	0.999437
30	Chrysin	254.24	≥97 % (Sigma-Aldrich)	y = 66179.10x + 114.99	0.998183

Table S5. Principal component analysis and Eigen values of propolis, extraction solvents and phenolic compounds.

Compounds	Factor 1	Factor 2		
Homogentisic acid	0.329518	0.920763		
3,4-Dihydroxybenzoic acid	0.939507	0.193701		
Gentisic acid	0.968054	0.089375		
Pyrocatechol	0.855893	-0.44141		
<i>p</i> -hydroxy benzoic acid	0.902741	-0.34058		
3,4-dihydroxybenzaldehyde	0.520726	-0.39483		
Vanillic acid	0.948275	-0.20604		
Caffeic acid	0.788065	0.55367		
Syringic acid	0.654466	0.688517		
Vanillin	0.837251	-0.49474		
<i>p</i> -coumaric acid	0.958227	0.065961		
Ferulic acid	0.820816	0.435669		
Rutin	0.904623	-0.27374		
<i>trans</i> -2-hydroxy cinnamic acid	0.517173	0.777617		
Myricetin	0.871151	-0.43654		
<i>trans</i> -cinnamic acid	0.932029	-0.22655		
Luteolin	0.217611	0.928061		
Quercetin	0.895773	-0.38669		
Naringenin	0.661765	0.715219		
Genistein	0.927942	-0.0358		
Apigenin	0.883656	-0.37354		
Kaempferol	0.957617	0.13782		
Hesperetin	0.76117	-0.60043		
Chrysin	0.765018	0.545316		
	% total	Cumulative	Cumulative	
	Eigenvalues	Variance	Eigenvalue	%
1	15.71323	65.4718	15.71323	65.4718
2	5.883592	24.51497	21.59682	89.98677

Table S6. Effect of extracts ($\mu\text{g/g}$) on phenolic compounds.

Compounds	Hexane Extract	Ethanol Extract	Ethylacetate Extract
Homogentisic acid	482.15b	1804.2ab	3508.2a
3,4-Dihydroxybenzoic acid	152.59c	552.99b	1162.2a
Gentisic acid	368.40c	2628.0b	6064.9a
Pyrocatechol	33.784b	129.57b	573.85a
<i>p</i> -hydroxy benzoic acid	100.53c	1004.9b	2295.2a
3,4-dihydroxybenzaldehyde	179.8	1428.5	1516.2
Vanillic acid	681.95c	2550.7b	4898.9a
Caffeic acid	2927.6c	13649.0b	21311.0a
Syringic acid	385.08c	1251.5b	1660.8a
Vanillin	56.146b	125.06b	338.42a
<i>p</i> -coumaric acid	1644.9	3841.1	6772.7
Ferulic acid	1125300c	105600b	146700a
Rutin	181.59b	614.84b	2305.9a
<i>trans</i> -2-hydroxy cinnamic acid	320.06b	561.05b	1211.6a
Myricetin	128.29b	316.16b	785.58a
<i>trans</i> -cinnamic acid	89.074c	290.94b	652.45a
Luteolin	7.97b	43.38ab	123.27a
Quercetin	101.68b	283.33b	731.39a
Naringenin	180.02c	695.05b	1567.3a
Genistein	245.49c	877.59b	1507.9a
Apigenin	36.65b	86.82b	315.41a
Kaempferol	111.19c	534.46b	1240.9a
Hesperetin	6.126b	19.734b	62.708a
Chrysin	9.638c	23.714b	74.821a

Means followed by the same letter in the same column are not significantly different ($p : 0.05$)

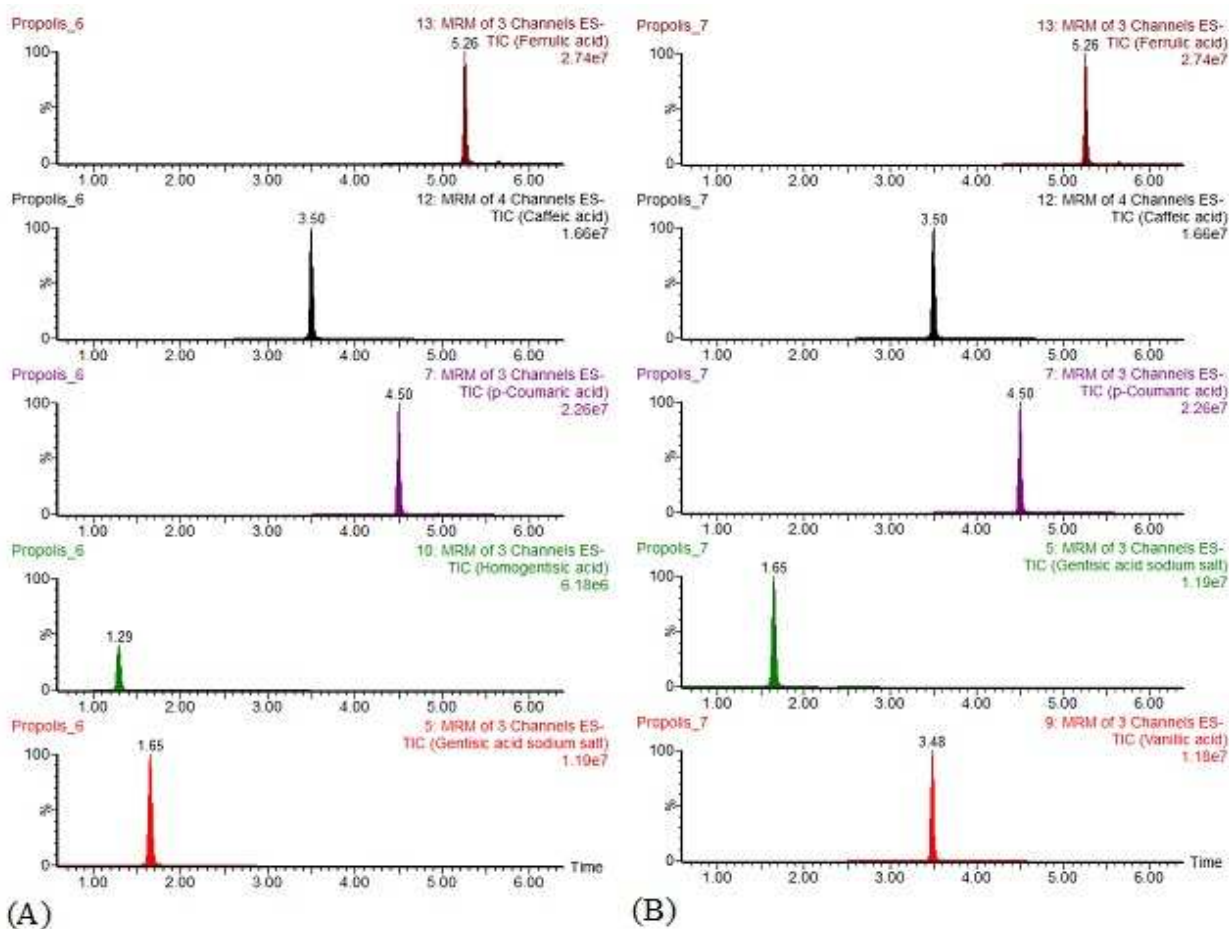


Figure S1. The total ion chromatograms (TIC) of major phenolic compounds found in BP-1 (Propolis 6) and BP-2 (Propolis-7).