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Screening for the Active Anti-Inflammatory and Antioxidant Polyphenols of *Gaultheria procumbens* and their Application for Standardisation: From Identification, through Cellular Studies to Quantitative Determination

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Supplementary Materials

Table S1. Phenolic analytes detected in *G. procumbens* aerial part dry extract by UHPLC-PDA-ESI-MS^3.

Figure S1. Proposed fragmentation scheme of cinnamtannin B-1 (CB1) in LC-ESI-MS^3.

Table S2. NMR spectral data of cinnamtannin B-1 (CB1) in methanol-d_4 (600 MHz for ^{1}H NMR and 150.9 MHz for ^{13}C NMR).

Figure S2. Effect of the selected compounds at 25-75 μM on viability (membrane integrity) of neutrophils as indicated by propidium iodide positive [PI(+)] cells.

Table S3. Content of the marker compounds in the dry extracts of *G. procumbens*.

Table S1. Phenolic analytes detected in *G. procumbens* aerial part dry extract by UHPLC-PDA-ESI-MS^3.

Peak	Analyte	t_R (min)	UV λ_{max} (nm)	$[M–H]^-$ *m/z*	MS^2 (% relative abundance)	MS^3 (% relative abundance)
1	protocatechuic acid hexoside	4.1	259, 293	315	225 (3); 153 (100); 107 (5)	
2	protocatechuic acid *	4.7	259, 293	153		
3	protocatechuic acid hexoside	5.8	259, 293	315	225 (1); 153 (100); 107 (2)	
4	3-*O*-caffeoylquinic acid (neochlorogenic acid, NCHA) *	6.5	325	353	191 (69); 179 (46)	
5	3-*O*-*p*-coumaroylquinic acid derivative	7.0	310	371	353 (12); 325 (100); 163 (73)	
6	procyanidin A-type dimer	9.1	280	575	499 (73); 490 (100); 451 (18); 407 (2); 289 (35)	
7	procyanidin A-type dimer	9.6	280	575	499 (79); 490 (100); 451 (30); 411 (13); 289 (21)	
8	procyanidin B-type dimer	10.0	280	577	**425 (100)**; 407 (49); 289 (3)	407 (100); 381 (6); 341 (4); 273 (7)
9	procyanidin B-type dimer	10.2	280	577	**425 (100)**; 407 (56); 289 (18)	407 (100); 381 (2); 273 (5)
10	unknown compound	10.9	280	765	475 (40); 443 (24); 289 (100); 245 (8)	
11	5-*O*-caffeoylquinic acid (chlorogenic acid, CHA) *	11.4	325	353	191 (100); 179 (3)	
12	methyl salicylate 2-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside (physanguloside A) *	12.2	285	521 ***	475 (29); 443 (3); 329 (100)	
13	methyl salicylate 2-*O*-*β*-D-glucopyranosyl-(1→2)-[*O*-*β*-D-xylopyranosyl-(1→6)]-*O*-*β*-D-glucopyranoside *	12.6	285	653 ***	607 (100); 575 (11)	
14	4-*O*-caffeoylquinic acid (cryptochlorogenic acid, CCHA) *	12.8	325	353	173 (100)	
15	procyanidin B-type dimer	14.0	280	577	**425 (100)**; 407 (64); 289 (8)	407 (100); 381 (5); 273 (8)
16	procyanidin B2 (PB2) **	15.2	280	577	**425 (100)**; 407 (51); 289 (15)	407 (100); 273 (6)
17	procyanidin B-type trimer	15.5	280	865	847 (17); 739 (76); **713 (37)**; 695 (77); 577 (78); 451 (25); 287 (25)	695 (100); 575 (47); 561 (9); 425 (28); 405 (43); 287 (15)
18	gaultherin isomer	15.7	285	491 ***	445 (7); 413 (10); 293 (100); 149 (4)	
19	(–)-epicatechin (ECA) **	16.5	280	289	245 (100); 205 (23)	
20	gaultherin isomer (GT) **	17.1	285	491 ***	445 (7); 413 (4); 293 (100); 149 (2)	
21	unknown compound	17.7	280	363	345 (4); 183 (100); 179 (19); 143 (7); 121 (14)	
22	procyanidin A-type trimer	18.4	280	863	**711 (100)**; 573 (34); 559 (17); 451 (47); 411 (79); 289 (100)	693 (100); 559 (54); 541 (16); 407 (17)

Table S1. *Cont.*

Peak	Analyte	t_R (min)	UV λ_{max} (nm)	$[M–H]^-$ *m/z*	MS^2 (% relative abundance)	MS^3 (% relative abundance)
23	procyanidin A-type trimer (cinnamtannin B-1, CB1) **	20.0	280	863	**711 (100)**; 693 (24); 573 (23); 559 (21); 451 (33); 411 (42); 289 (11)	693 (88); 559 (100); 541 (8); 463 (3); 407 (6); 285 (1)
24	procyanidin B-type trimer (procyanidin C1) *	21.2	280	865	847 (6); 739 (42); **713 (43)**; 695 (100); 577 (80); 451 (31); 287 (59)	695 (100); 575 (39); 561 (89); 407 (23); 243 (9)
25	procyanidin A-type trimer	21.6	280	863	**711 (100)**; 573 (43); 559 (16); 451 (45); 411 (70); 289 (20)	693 (100); 559 (69); 541 (22); 463 (24); 407 (58)
26	caffeoylquinic acid derivative	22.0	325	391	225 (26); 179 (100); 161 (12); 143 (7); 119 (8); 113 (5)	
27	procyanidin A-type dimer	22.5	280	575	499 (100); 491 (28); 451 (8); 411 (9); 289 (37)	
28	procyanidin A-type dimer	23.4	280	575	499 (57); 491 (38); 451 (11); 411 (9); 289 (95)	
29	procyanidin A-type trimer	24.2	280	863	**711 (100)**; 573 (49); 559 (16); 451 (35); 411 (64); 289 (21)	693 (100); 559 (65); 541 (20); 463 (21); 407 (55)
30	procyanidin A-type dimer	24.7	280	575	499 (18); 491 (23); 451 (43); 411 (14); 289 (26)	
31	procyanidin A-type trimer	25.1	280	863	**711 (100)**; 573 (68); 559 (19); 451 (6); 411 (11); 289 (11)	693 (100); 559 (45); 541 (28); 463 (27); 407 (50)
32	quercetin 3-*O*-*β*-D-xylopyranosyl-(1→2)-*β*-D-glucuronopyranoside (wintergreenoside A, DGQ) **	25.5	257, 356	609	477 (2); **301 (100)**	273 (51); 255 (22); 179 (100); 151 (69)
33	procyanidin A-type dimer	26.4	280	575	499 (30); 491 (19); 451 (12); 411 (39); 289 (100)	
34	procyanidin A-type trimer	26.8	280	863	**711 (100)**; 573 (60); 559 (11); 451 (35); 411 (92); 289 (39)	693 (29); 559 (11); 541 (12); 463 (38); 407 (21)
35	quercetin 3-*O*-*β*-D-galactopyranoside (hyperoside, HY) **	27.6	254, 353	463	**301 (100)**	273 (49); 255 (19); 179 (95); 151 (75)
36	quercetin 3-*O*-*β*-D-glucopyranoside (isoquercitrin, IQ) **	28.5	256, 353	463	**301 (100)**	273 (54); 255 (22); 179 (100); 151 (76)
37	quercetin 3-*O*-*β*-D-glucuronopyranoside (miquelianin, MQ) **	29.1	256, 356	477	**301 (100)**	273 (29); 257 (19); 179 (100); 151 (58)
38	kaempferol 3-*O*-*β*-D-xylopyranosyl-(1→2)-*β*-D-glucuronopyranoside (wintergreenoside B, DGK) **	29.6	275, 345	593	461 (3); **285 (100)**	267 (32); 257 (100); 241 (23); 229 (30); 151 (16)
39	quercetin 3-*O*-*α*-L-arabinopyranoside (guaijaverin, GV) **	30.8	258, 356	433	**301 (100)**	273 (44); 255 (21); 179 (100); 151 (68)
40	kaempferol 3-*O*-*β*-D-glucuronopyranoside (KG) **	33.5	265, 345	461	**285 (100)**	267 (28); 257 (100); 241 (15); 229 (45); 151 (22)
41	quercetin (QU) *	43.7	255, 364	301	273 (41); 255 (18); 179 (100); 151 (74)	

t_R, retention times; UV λ_{max}, absorbance maxima in PDA spectra; $[M–H]^-$, pseudomolecular ions in MS spectra recorded in a negative ion mode; in bold – ions subjected to MS^3 fragmentation; * compounds identified with authentic standards; ** compounds isolated during the present study; *** $[M+HCOO]^-$. The nomenclature of caffeoylquinic acids isomers is according to IUPAC.

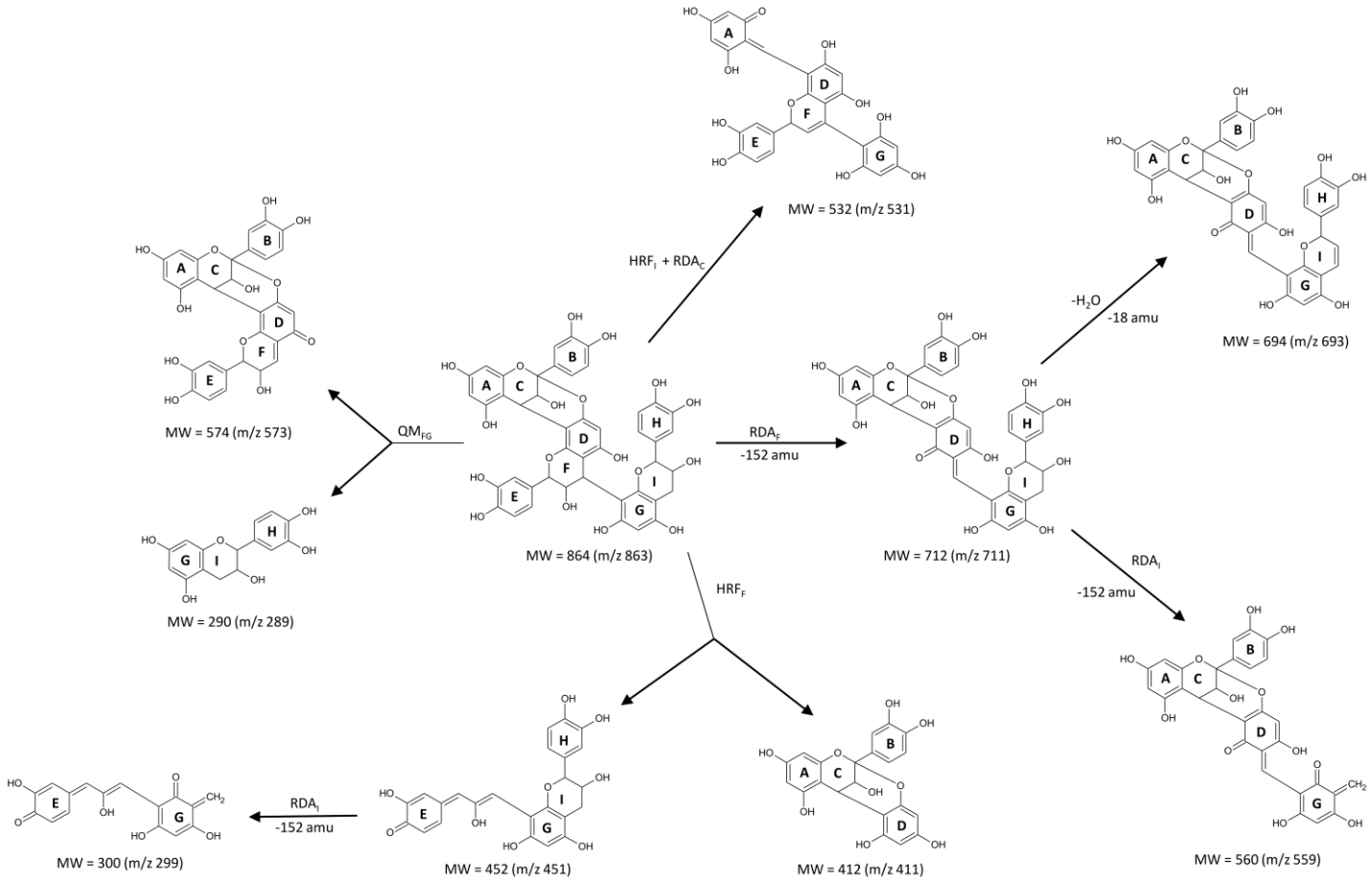

Figure S1. Proposed fragmentation scheme of cinnamtannin B-1 (CB1) in LC-ESI-MS^3. MW, molecular weight of a ion; HRF, heterocyclic ring fission; QM, quinone methide cleavage; RDA, retro-Diels-Alder fragmentation.

Table S2. NMR spectral data of cinnamtannin B-1 (CB1) in methanol-d_4 (600 MHz for ^{1}H NMR and 150.9 MHz for ^{13}C NMR) [a].

Ring	Pos. [b]	δ_H	δ_C
C	2		101.2
	3	3.34 (1H, *d*, *J*=3.4)	68.2
	4	4.19 (1H, *d*, *J*=3.4)	29.9
A	5		157.8
	6	6.01 (1H, *d*, *J*=2.3)	99.4
	7		158.9
	8	6.06 (1H, *d*, *J*=2.3)	97.6
	9		155.2
	10		105.9
B	1'		133.5
	2'	7.08 (1H, *d*, *J*=1.9)	117.2
	3'		146.8
	4'		146.9
	5'	6.88 (1H, *d*, *J*=8.3)	117.1
	6'	6.90 (1H, *dd*, J_1=2.3, J_2=8.3)	120.9
F	2	5.74 (1H, *s*)	79.9
	3	4.17 (1H, *br s*)	73.6
	4	4.6 (1H, *br s*)	39.3
D	5		156.8
	6	5.85 (1H, *s*)	97.2
	7		152.1
	8		107.5
	9		152.8
	10		107.8
E	1'		132.8
	2'	7.35 (1H, *d*, *J*=1.5)	117.8
	3'		147.3
	4'		147.6
	5'	6.83 (1H, *d*, *J*=8.3)	116.8
	6'	7.22 (1H, *dd*, J_1=1.5, J_2=8.3)	122.4
I	2	4.43 (1H, *s*)	81.4
	3	3.91 (1H, *br s*)	68.6
	4	2.88 (2H, *m*)	30.9
G	5		157.1
	6	6.15 (1H, *s*)	97.6
	7		156.6
	8		109.9
	9		156.8
	10		100.9
H	1'		134.2
	2'	6.86 (1H, *d*, *J*=1.5)	116.4
	3'		146.4
	4'		146.5
	5'	6.79 (1H, *d*, *J*=8.3)	116.5
	6'	6.76 (1H, *dd*, J_1=1.5, J_2=8.3)	120.6

[a] Data acquired with TMS as the internal standard, δ in ppm. Multiplicities and coupling constants (*J*, in Hz) are given in parentheses. Assignments confirmed by ^{1}H-^{1}H COSY, HMQC, HMBC and ROESY experiments. [b] For trivial atom numbering see chemical formula of CB1 (Fig. 2).

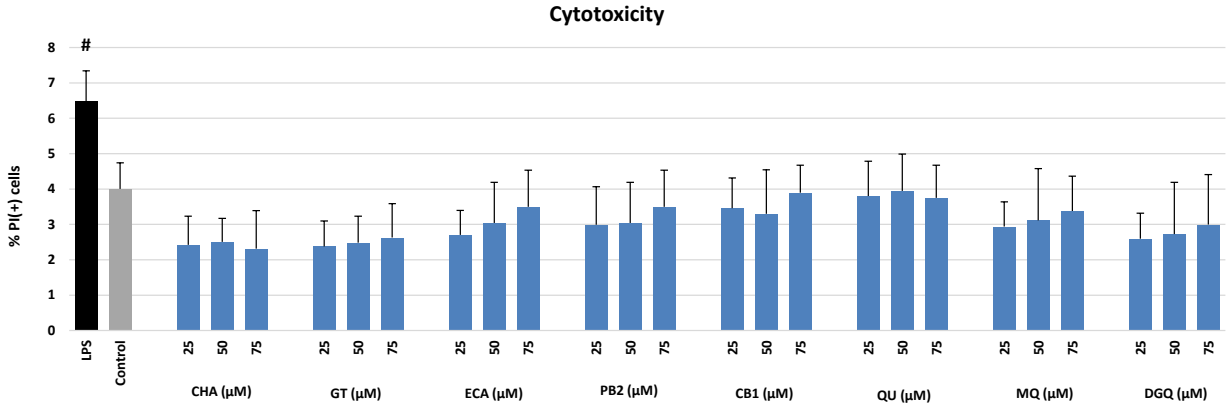

Figure S2. Effect of the selected compounds at 25-75 μM on viability (membrane integrity) of neutrophils as indicated by propidium iodide positive [PI(+)] cells. Data expressed as means ± SD of three independent experiments performed with cells isolated from five independent donors. Statistical significance: #$p < 0.05$ compared to the non-stimulated control. Analytes: QU, quercetin; MQ, miquelianin; DGQ, wintergreenoside A; ECA, (–)-epicatechin; PB2, procyanidin B2; CB1, cinnamtannin B-1; CHA, chlorogenic acid; GT, gaultherin.

Table S3. Content of the marker compounds in the dry extracts of *G. procumbens*.

Analyte	Content (mg/g dw) [a]							
	ME-AP	ME-L	ME-S	MED-AP	DEF-AP	EAF-AP	BF-AP	WF-AP
NCHA	3.68 ± 0.13 ^{D}	4.24 ± 0.08 ^{E}	0.96 ± 0.04 ^{A}	2.48 ± 0.15 ^{C}	1.67 ± 0.06 ^{B}	5.68 ± 0.22 ^{F}	6.47 ± 0.06 ^{G}	2.34 ± 0.15 ^{C}
CHA	1.25 ± 0.09 ^{C}	1.68 ± 0.11 ^{D}	0.96 ± 0.05 ^{B}	1.67 ± 0.10 ^{D}	2.46 ± 0.13 ^{F}	2.72 ± 0.13 ^{G}	2.04 ± 0.08 ^{E}	0.41 ± 0.01 ^{A}
CCHA	0.65 ± 0.03 ^{A}	0.88 ± 0.10 ^{B}	*nd*	1.95 ± 0.11 ^{D}	0.81 ± 0.05 ^{B}	3.78 ± 0.22 ^{E}	3.56 ± 0.20 ^{E}	1.24 ± 0.03 ^{C}
PB2	14.25 ± 0.45 ^{E}	13.15 ± 0.37 ^{D}	17.22 ± 0.19 ^{D}	13.46 ± 0.25 ^{D}	6.12 ± 0.55 ^{B}	35.05 ± 0.65 ^{F}	8.36 ± 0.21 ^{C}	2.10 ± 0.07 ^{A}
ECA	12.47 ± 0.67 ^{C}	9.07 ± 0.29 ^{B}	24.35 ± 0.93 ^{D}	12.31 ± 0.16 ^{C}	0.31 ± 0.07 ^{A}	79.73 ± 1.58 ^{E}	*nd*	*nd*
GT	96.51 ± 0.80 ^{F}	98.41 ± 0.31 ^{E}	93.76 ± 0.81 ^{D}	75.68 ± 0.80 ^{B}	21.37 ± 1.11 ^{A}	30.25 ± 1.47 ^{C}	127.69 ± 4.06 ^{G}	27.81 ± 1.99 ^{B}
CB1	19.84 ± 0.22 ^{C}	18.61 ± 0.28 ^{B}	25.11 ± 0.52 ^{D}	14.42 ± 0.22 ^{A}	*nd*	154.57 ± 4.52 ^{E}	15.51 ± 0.78 ^{A}	*nd*
DGQ	5.13 ± 0.38 ^{E}	7.26 ± 0.24 ^{F}	2.16 ± 0.14 ^{C}	3.90 ± 0.38 ^{D}	1.02 ± 0.04 ^{B}	0.40 ± 0.01 ^{A}	5.05 ± 0.09 ^{E}	10.65 ± 0.52 ^{G}
HY	3.24 ± 0.25 ^{B}	5.01 ± 0.16 ^{D}	1.04 ± 0.03 ^{A}	5.34 ± 0.25 ^{D}	4.30 ± 0.17 ^{C}	30.04 ± 1.69 ^{F}	9.88 ± 0.08 ^{E}	*nd*
MQ	27.75 ± 0.78 ^{D}	32.14 ± 0.69 ^{E}	17.54 ± 0.57 ^{B}	30.38 ± 0.78 ^{C}	9.72 ± 0.55 ^{A}	36.90 ± 1.34 ^{F}	42.74 ± 0.24 ^{G}	22.84 ± 0.88 ^{C}
QU	0.19 ± 0.01 ^{C}	0.22 ± 0.01 ^{C}	0.1 ± 0.01 ^{B}	0.48 ± 0.01 ^{D}	8.68 ± 0.46 ^{F}	5.36 ± 0.18 ^{E}	7.74 ± 0.29 ^{G}	0.07 ± 0.01 ^{A}
Total	184.96	190.67	181.04	162.07	56.46	384.48	229.04	67.46

Analytes: QU, quercetin; MQ, miquelianin; HY, hyperoside; DGQ, wintergreenoside A; ECA, (–)-epicatechin; PB2, procyanidin B2; CB1, cinnamtannin B-1; NCHA, neochlorogenic acid; CHA, chlorogenic acid; CCHA, cryptochlorogenic acid; GT, gaultherin. Extracts: methanol-water (75:25, *v*/*v*) extracts (ME) of the aerial parts (ME-AP), leaves (ME-L), and stems (ME-S); deffated methanol-water (75:25, *v*/*v*) extract of aerial parts (MED-AP), and its fractions: DEF-AP (diethyl ether fraction), EAF-AP (ethyl acetate fraction), BF-AP (*n*-butanol fraction), and WF-AP (water fraction). All data expressed as mean values ± SD ($n = 3 \times 3 \times 1$); [a] dry weight of the plant extract; different superscripts (A-G) indicate significant differences in the mean value at $\alpha = 0.05$; *nd* – not detected.