Biological and chemical insight into *Gaultheria procumbens* fruits: A rich source of anti-inflammatory and antioxidant salicylate glycosides and procyanidins for food and functional application

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Supplementary Materials

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Table S1 Phenolic analytes detected in *G. procumbens* fruit dry extracts by UHPLC-PDA-ESI-MS^3

Peak	Analytes	Rt [min]	UV [nm]	$[M–H]^-$ *m/z*	MS^2 (% relative abundance)	MS^3 (% relative abundance)	Extract
1	unknown compound	3.1	285	465	447 (7); **367 (100)**	349 (29); 187 (71); 179 (90); 143 (32); 113 (21)	All
2	protocatechuic acid *	4.2	259, 293	153			All
3	vanilic acid derivative	5.3	260, 310	331	**313 (100)**; 211 (2); 167 (33); 125 (7)	297 (16), 153 (100)	ME, WE
4	3-*O*-caffeoylquinic acid (neochlorogenic acid) *	6.0	325	353	191 (100); 179 (29)		All
5	*p*-hydroxybenzoic acid *	7.0	254	137	93 (100)		All
6	unknown compound	8.8	280	293	273 (3); 195 (9); 131 (100)		All
7	procyanidin B-type dimer	10.1	280	577	**425 (100)**; 407 (64); 289 (20)	407 (100); 273 (10)	AE, ME, WE
8	procyanidin B-type dimer	10.5	280	577	**425 (100)**; 407 (63); 289 (20)	407 (100); 273 (9)	All
9	5-*O*-caffeoylquinic acid (chlorogenic acid) *	10.9	325	353	191 (100); 179 (6)		All
10	(+)-catechin *	11.1	280	289	245 (100); 205 (27)		All
11	methyl salicylate 2-*O*-(2′-*O*-β-D-glucopyranosyl)-β-D-glucopyranoside (physanguloside A, **PH**) *	11.3	285	521 [a]	475 (100); 443 (4)		All
12	sinapic acid hexoside derivative	12.1	280	431	413 (5); 385 (100); 223 (13)		All
13	caffeic acid derivative	12.4	325	327	309 (6); 179 (49); 147 (100); 119 (16)		All
14	methyl salicylate 2-*O*-(2′-*O*-β-D-glucopyranosyl-6'-*O*-β-D-xylopyranosyl)-β-D-glucopyranoside (MSTG-B, **TG**) *	13.0	285	653 [a]	607 (100); 575 (13)		All
15	procyanidin B-type trimer	13.6	280	865	847 (31); 739 (43); **713 (34)**; 695 (79); 577 (47); 451 (20); 287 (20)	695 (100); 575 (5); 561 (23); 407 (36); 243 (11)	AE, ME, WE
16	procyanidin B-type dimer	14.1	280	577	**425 (100)**; 407 (64); 289 (22)	407 (100); 273 (8)	All
17	procyanidin B-type dimer	14.6	280	577	**425 (100)**; 407 (59); 289 (18)	407 (100); 273 (10)	ME, WE
18	procyanidin B-2 (**PB2**) *	15.3	280	577	**425 (100)**; 407 (41); 289 (14)	407 (100); 273 (7)	AE, ME, WE
19	unknown compound	15.7	280	461	**415 (100)**	407 (100); 381 (4); 273 (7)	AE, ME, BE, WE
20	protocatechuic acid derivative	16.1	259, 293	481	463 (17); 345 (39); 327 (100); 165 (18); 153 (56)		All
21	(-)-epicatechin *	16.8	280	289	245 (100); 205 (16)		AE, ME, EAE, WE
22	gaultherin (**GT**) *	17.7	285	491 [a]	445 (12); 413 (4); 293 (100); 233 (2); 149 (4)		All
23	unknown compound	17.9	280	367	245 (12); 203 (61); 185 (100); 141 (26)		ME, WE

Table S1 *(Contd.)*

Peak	Analytes	Rt [min]	UV [nm]	$[M–H]^-$ *m/z*	MS^2 (% relative abundance)	MS^3 (% relative abundance)	Extract
24	procyanidin A-type trimer	18.3	280	863	**711 (100)**; 573 (21); 559 (21); 451 (25); 411 (31); 289 (10)	693 (78); 559 (21); 541 (26); 407 (14)	AE, ME, WE
25	procyanidin B-type dimer	18.7	280	577	**425 (100)**; 407 (20); 289 (49)	407 (100); 273 (11)	AE, ME, WE
26	procyanidin A-type trimer	20.3	280	863	**711 (100)**; 693 (8); 573 (27); 559 (22); 451 (43); 411 (34); 289 (22)	693 (100); 559 (82); 541 (24); 463 (13); 407 (18); 285 (3)	AE, ME, WE
27	procyanidin B-type trimer	20.7	280	865	847 (14); 739 (18); **713 (100)**; 695 (32); 577 (29); 451 (27); 287 (17)	695 (100); 575 (22); 561 (22); 407 (20); 243 (10)	AE, ME, WE
28	procyanidin C1 *	21.3	280	865	847 (16); 739 (56); **713 (100)**; 695 (49); 577 (61); 451 (36); 287 (35)	695 (100); 575 (23); 561 (19); 407 (35); 243 (7)	AE, ME, WE
29	procyanidin B-type dimer	22.1	280	577	**425 (100)**; 407 (62); 289 (19)	407 (100); 273 (12)	AE, ME, WE
30	procyanidin A-type dimer	22.6	280	575	499 (98); 491 (54); 451 (14); **425 (100**); 407 (79); 289 (22)	407 (100); 273 (9)	All
31	procyanidin B-type trimer	23.6	280	865	847 (6); 739 (36); **713 (51)**; 695 (92); 577 (99); 451 (30); 287 (54)	695 (100); 575 (24); 561 (26); 407 (25); 243 (10)	AE, ME, WE
32	procyanidin A-type dimer	24.2	280	575	499 (27); 449 (89); **423 (41)**; 289 (79)	405 (17); 285 (100)	ME, WE
33	procyanidin A-type dimer	24.8	280	575	499 (25); 449 (49); **423 (100)**; 289 (20)	405 (11); 285 (100)	WE
34	procyanidin A-type trimer	25.7	280	863	**711 (100)**; 693 (20); 573 (24); 559 (23); 451 (32); 411 (42); 289 (12)	693 (96); 559 (100); 541 (64); 463 (5); 407 (15); 285 (2)	All
35	quercetin 3-*O*-β-D-galactopyranoside (hyperoside) *	27.6	254, 353	463	**301 (100)**	273 (38); 255 (20); 179 (100); 151 (64)	All
36	quercetin 3-*O*-β-D-glucopyranoside (isoquercitrin) *	28.6	256, 353	463	**301 (100)**	273 (54); 255 (22); 179 (100); 151 (76)	ME, WE
37	quercetin 3-*O*-β-D-glucuronopyrano-side (miquelianin) *	29.1	256, 356	477	**301 (100)**	273 (29); 257 (19); 179 (100); 151 (58)	AE, ME, WE
38	procyanidin A-type dimer	29.5	280	575	449 (56); **423 (100)**; 289 (19)	405 (8); 285 (100); 257 (5)	AE, ME, WE
39	quercetin hexoside-rhamnoside	31.8	256, 355	609	**447 (100)**; 301 (26)	301 (100)	WE
40	quercetin 3-*O*-α-L-rhamnopyranoside (quercitrin) *	32.9	276, 350	447	**301 (100)**	273 (19); 257 (23); 179 (100); 151 (59)	WE
41	kaempferol 3-*O*-β-D-glucuronopyranoside *	33.3	265, 345	461	**285 (100)**	267 (32); 257 (100); 241 (23); 229 (30); 151 (16)	ME, WE
42	unknown compound	40.3	325	663	517 (5); **487 (100)**; 469 (14)	341 (8); 307 (100); 235 (57); 205 (15); 163 (74)	ME, WE
43	unknown compound	41.5	325	693	**517 (100)**; 499 (63); 337 (12); 265 (4)	337 (90); 295 (9); 265 (36); 235 (52); 193 (100); 175 (58)	AE, ME, BE, WE
44	quercetin *	43.7	255, 364	301	273 (36); 255 (21); 179 (100); 151 (69)		All
45	kaempferol *	50.4	265, 364	285	267 (90); 257 (11); 241 (20); 229 (80); 151 (18)		All

Rt, retention times. UV λ_{max}, absorbance maxima in PDA spectra. $[M–H]^-$, pseudomolecular ions in MS spectra recorded in a negative ion mode. The bolded ions were subjected to MS^3 fragmentation. Compounds marked with an asterisk (*) were identified with authentic standards; [a] $[M+HCOO]^-$.

Table S2 NMR spectral data of **PH**, **GT** and **TG** in methanol-d_4 (600 MHz for ^{1}H and 150.9 MHz for ^{13}C) [a]

Pos. [b]	PH		GT		TG	
	δ_H	**δ_C**	**δ_H**	**δ_C**	**δ_H**	**δ_C**
1		120.1		121.0		120.0
2		156.2		157.2		156.1
3	7.28 (1H, *dd*, J_1=0.8, J_2=8.7)	114.9	7.47 (1H, *dd*, J_1=1.1, J_2=7.9)	117.8	7.34 (1H, *dd*, J_1=1.5, J_2=8.7)	115.3
4	7.54 (1H, *ddd*, J_1=1.5, J_2=7.2, J_3=8.7)	133.4	7.59 (1H, *ddd*, J_1=1.9, J_2=7.9, J_3=7.9)	133.9	7.57 (1H, *ddd*, J_1=1.9, J_2=7.5, J_3=8.7)	133.7
5	7.09 (1H, *ddd*, J_1=0.8, J_2=7.2, J_3=7.9)	121.1	7.15 (1H, *ddd*, J_1=1.1, J_2=7.9, J_3=7.9)	122.3	7.09 (1H, *ddd*, J_1=1.5, J_2=7.5, J_3=8.7)	121.2
6	7.79 (1H, *dd*, J_1=1.5, J_2=7.9)	130.9	7.79 (1H, *dd*, J_1=1.9, J_2=7.9)	130.6	7.79 (1H, *dd*, J_1=1.9, J_2=7.5)	130.9
7		166.6		167.2		166.5
8	3.90 (3H, *s*)	51.2	3.92 (3H, *s*)	51.4	3.90 (3H, *s*)	51.2
	2-β-D-glucopyranosyl:		2-β-D-glucopyranosyl:		2-β-D-glucopyranosyl:	
1'	5.36 (1H, *d*, *J*=7.5)	98.3	4.90 (1H, *d*, *J*=7.5)	102.5	5.33 (1H, *d*, *J*=7.5)	98.3
2'	3.82 (1H, *dd*, J_1=7.5, J_2=9.0)	81.6	3.56 (1H, *dd*, J_1=7.5, J_2=9.0)	73.6	3.83 (1H, *dd*, J_1=7.5, J_2=9.0)	81.6
3'	3.71 (1H, *dd*, J_1=9.0, J_2=9.8)	76.3	3.50 (1H, *dd*, J_1=9.0, J_2=9.8)	76.2	3.70 (1H, *dd*, J_1=9.0, J_2=9.8)	76.2
4'	3.54 (1H, *dd*, J_1=9.0, J_2=9.8)	69.5	3.43 (1H, *dd*, J_1=9.8, J_2=9.8)	69.9	3.86 (1H, *dd*, J_1=9.0, J_2=9.8)	69.6
5'	3.52 (1H, *ddd*, J_1=1.5, J_2=6.4, J_3=9.0)	76.5	3.69 (1H, *ddd*, J_1=1.9, J_2=6.4, J_3=9.8)	76.1	3.49 (1H, *ddd*, J_1=1.5, J_2=6.4, J_3=9.0)	75.8
6'	3.88 (1H, *dd*, J_1=1.5, J_2=11.7, H-6'$_a$) 3.73 (1H, *dd*, J_1=6.4, J_2=11.7, H-6'$_b$)	61.0	4.16 (1H, *dd*, J_1=1.9, J_2=11.7, H-6'$_a$) 3.81 (1H, *dd*, J_1=6.4, J_2=11.7, H-6'$_b$)	68.5	4.12 (1H, *dd*, J_1=1.5, J_2=11.7, H-6'$_a$) 3.81 (1H, *dd*, J_1=6.4, J_2=11.7, H-6'$_b$)	68.3
	2′-β-D-glucopyranosyl:		6′-β-D-xylopyranosyl:		6′-β-D-xylopyranosyl:	
1"	4.84 (1H, *d*, *J*=7.5)	103.0	4.36 (1H, *d*, *J*=7.5)	104.1	4.31 (1H, *d*, *J*=7.5)	103.9
2"	3.25 (1H, *dd*, J_1=7.5, J_2=9.4)	74.7	3.24 (1H, *dd*, J_1=7.5, J_2=9.0)	73.6	3.21 (1H, *dd*, J_1=7.5, J_2=9.0)	73.6
3"	3.41 (1H, *dd*, J_1=9.0, J_2=9.4)	76.3	3.31 (1H, *dd*, J_1=9.0, J_2=9.8)	76.3	3.28 (1H, *dd*, J_1=8.7, J_2=9.0)	76.2
4"	3.40 (1H, *dd*, J_1=8.3, J_2=9.0)	69.4	3.52 (1H, *ddd*, J_1=5.3, J_2=9.8, J_3=10.2)	69.8	3.48 (1H, *ddd*, J_1=5.3, J_2=9.0, J_3=10.5)	69.8
5"	3.17 (1H, *ddd*, J_1=2.3, J_2=6.4, J_3=8.3)	76.0	3.18 (1H, *dd*, J_1=10.2, J_2=11.7, H-5"$_a$)	65.5	3.11 (1H, *dd*, J_1=10.5, J_2=11.3, H-5"$_a$)	65.5
6"	3.57 (1H, *dd*, J_1=2.3, J_2=11.7, H-6″$_a$) 3.22 (1H, *dd*, J_1=6.4, J_2=11.7, H-6″$_b$)	60.3	3.87 (1H, *dd*, J_1=5.3, J_2=11.7, H-5"$_e$)		3.82 (1H, *dd*, J_1=5.3, J_2=11.3, H-5"$_e$)	
					2′-β-D-glucopyranosyl:	
1‴					4.85 (1H, *d*, *J*=7.5)	103.0
2‴					3.25 (1H, *dd*, J_1=7.5, J_2=7.9)	74.7
3‴					3.42 (1H, *dd*, J_1=7.9, J_2=9.0)	76.0
4‴					3.40 (1H, *dd*, J_1=9.0, J_2=9.0)	69.4
5‴					3.17 (1H, *ddd*, J_1=1.1, J_2=6.4, J_3=9.0)	76.1
6‴					3.52 (1H, *dd*, J_1=1.1, J_2=12.1, H-6‴$_a$) 3.23 (1H, *dd*, J_1=6.4, J_2=12.1, H-6‴$_b$)	60.2

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

Table S3 Correlation (*r*) coefficients and probability (*p*) values of linear relationships between antioxidant and anti-inflammatory activity parameters and phenolic contents of *G. procumbens* fruit dry extracts

r (*p*) for:	Antioxidant activity						Anti-inflammatory activity		
	DPPH	FRAP	TBARS	$O_2^{\bullet-}$	$^{\bullet}OH$	H_2O_2	COX-2	HYAL	LOX
TPC	–0.8696 (0.130)	0.7930 (0.207)	–0.8114 (0.189)	–0.7378 (0.262)	0.1976 (0.802)	–0.5858 (0.414)	–0.1391 (0.861)	–0.8119 (0.188)	–0.5837 (0.416)
TPH	–0.3849 (0.615)	0.8807 (0.119)	–0.3807 (0.619)	–0.4155 (0.585)	–0.4449 (0.555)	–0.8594 (0.141)	–0.8253 (0.175)	–0.6946 (0.305)	–0.9331 (0.067)
TPA	–0.9042 (0.096)	0.8115 (0.188)	–0.8532 (0.147)	–0.7878 (0.212)	0.2405 (0.759)	–0.6345 (0.365)	–0.1221 (0.878)	–0.8516 (0.148)	–0.6229 (0.377)
TLPA	–0.7617 (0.238)	0.9939 (0.006) **	–0.7469 (0.253)	–0.7506 (0.249)	–0.0756 (0.924)	–0.9469 (0.053)	–0.5443 (0.456)	–0.9376 (0.062)	–0.9693 (0.031) *
TPHA	0.1530 (0.847)	0.2445 (0.756)	0.0826 (0.917)	–0.0342 (0.917)	–0.3246 (0.675)	–0.5059 (0.494)	–0.5123 (0.488)	–0.1682 (0.832)	–0.5275 (0.473)
TSAL	–0.3367 (0.663)	0.8553 (0.145)	–0.3338 (0.666)	–0.3717 (0.628)	–0.4801 (0.520)	–0.8374 (0.163)	–0.8452 (0.155)	–0.6572 (0.343)	–0.9158 (0.084)
TFL	–0.2752 (0.725)	0.6931 (0.307)	–0.1843 (0.816)	–0.1050 (0.895)	–0.5773 (0.423)	–0.3318 (0.668)	–0.7156 (0.284)	–0.3981 (0.602)	–0.4606 (0.539)

TPC: total phenolic content (Folin-Ciocalteau assay) in gallic acid equivalents, TPH: total phenolic content (HPLC), TSAL: total salicylates (HPLC), TPA: total proanthocyanidins (*n*-butanol/HCl assay) in cyanidin chloride equivalents, TLPA: total proanthocyanidins (HPLC), TPHA: total phenolic acids (HPLC), TFL: total flavonoids (HPLC). Asterisks mean significance of the estimated linear relationship (* $p < 0.05$, ** $p < 0.01$) for four extracts ($n = 4$).

Table S4 Extraction yield and phenolic contents including the levels of individual methyl salicylate glycosides (**GT**, **PH** and **TG**) in *G. procumbens* fruits extracted with different solvents

	Extraction solvent	
	methanol-water (7:3, *v/v*)	water
Yield [a]	43.69 ± 2.47	48.09 ± 2.60
TPC [a]	7.66 ± 0.12 *	4.93 ± 0.04 *
TPC [b]	36.40 ± 0.53 *	23.43 ± 0.71 *
TPA [b]	28.49 ± 0.47 *	15.09 ± 0.16 *
TSAL [b]	38.21 ± 0.40 *	15.63 ± 0.30 *
TSA [b]	9.87 ± 0.10 *	3.57 ± 0.07 *
GT [b]	22.33 ± 0.69 *	1.37 ± 0.02 *
PH [b]	5.69 ± 0.19 *	3.91 ± 0.06 *
TG [b]	10.19 ± 0.32	10.35 ± 0.37

Results are presented as means ± SD (n = 3) and calculated per: [a] fresh fruit weight (mg/g fw); [b] dry fruit weight (mg/g dw). Means in the same row marked with asterisks differ significantly (p < 0.05). TPC: total phenolic content (Folin-Ciocalteau assay) in gallic acid equivalents, TSAL: total salicylates (HPLC), TSA: total salicylates in equivalents of salicylic acid, TPA: total proanthocyanidins (*n*-butanol/HCl assay) in cyanidin chloride equivalents.

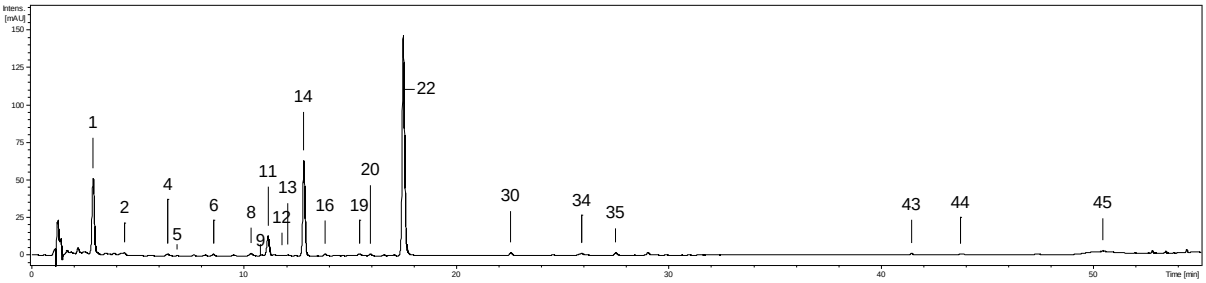

Fig. S1 UHPLC-PDA chromatogram of *n*-butanol extract of *G. procumbens* fruits (**BE**) at 280 nm. The peak numbers refer to those implemented in Table S1. The following compounds were isolated: **PH** (peak 11), **TG** (peak 14) and **GT** (peak 22).

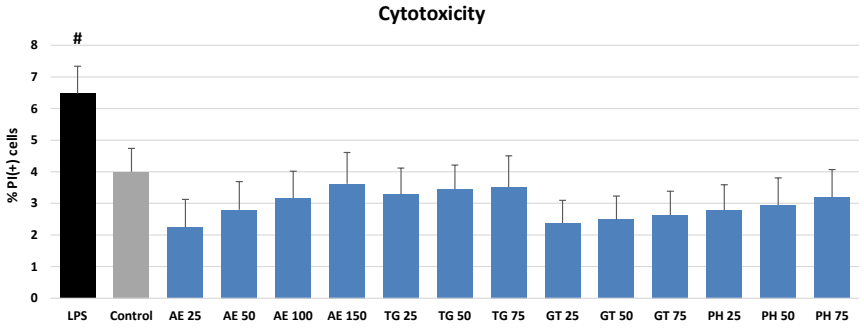

Fig. S2 Effect of acetone fruit extract (**AE**) at 25, 50, 100, 150 µg/mL and the isolated salicylates (**PH**, **GT** and **TG**) at 25, 50, and 75 µM on viability (membrane integrity) of neutrophils as indicated by propidium iodide positive PI(+) cells. Data expressed as means ± SD of three independent experiments performed with cells isolated from five independent donors. Statistical significance: #p < 0.05 compared to the non-stimulated control.

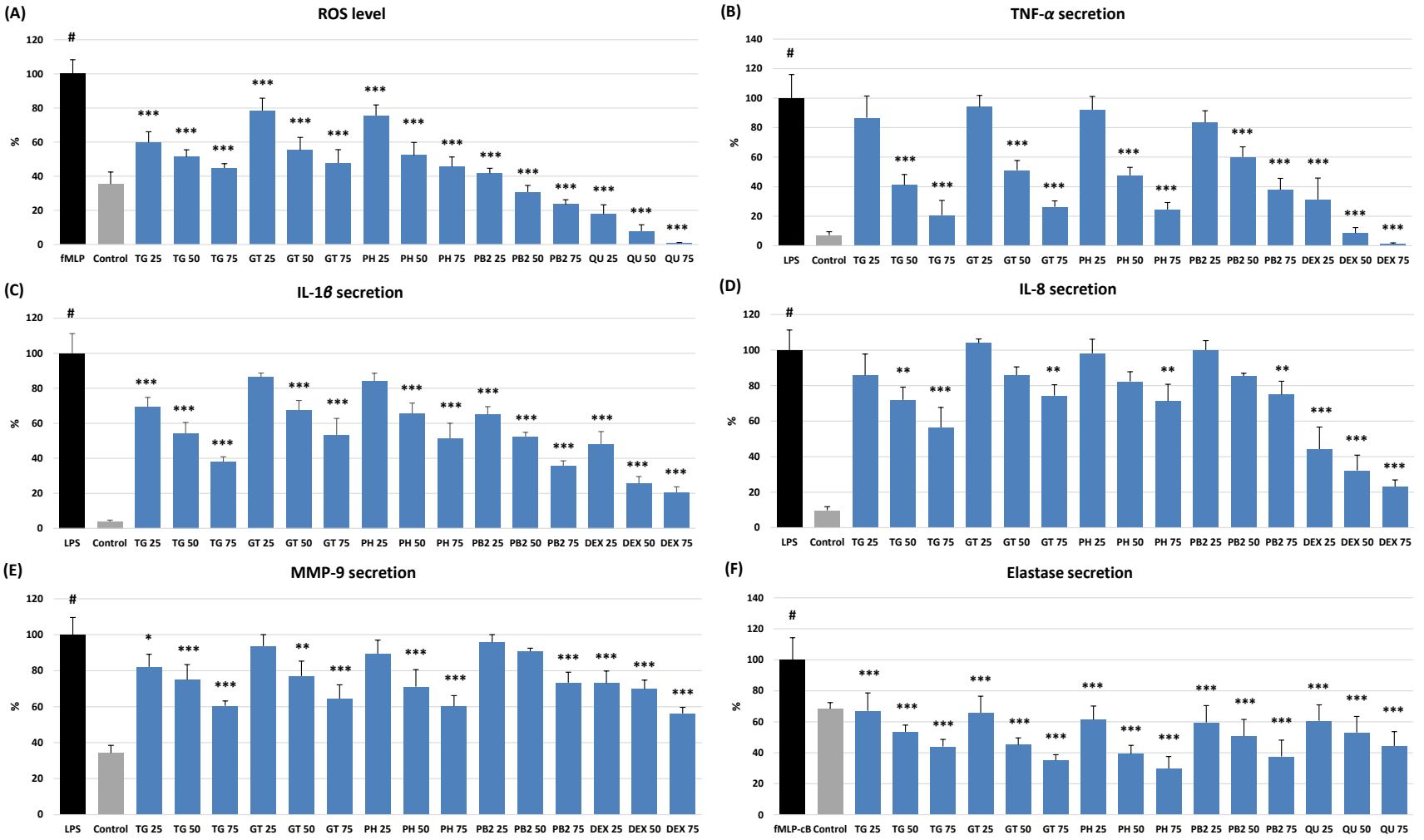

Fig. S3 Effect of the isolated salicylates (**TG**, **GT** and **PH**) and positive controls (**QU** and **DEX**) at 25, 50, and 75 µM on: (A) ROS production, and secretion of (B) TNF-α, (C) IL-1β, (D) IL-8, (E) MMP-9, and (F) ELA-2 by stimulated human neutrophils. Data expressed as means ± SD of three independent experiments performed with cells isolated from five independent.