Supplementary Information

Discovery of anti-inflammatory components from Guge Fengtong Tablet based on inflammatory markers and exploration of its molecular mechanism

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Methods

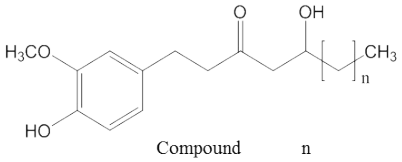
MTT assay. Assessment of cell proliferation was performed by MTT assay. RAW264.7 cells were seeded in 96-well plates at a density of 2×10^5 per well, while 293 cells were seeded at a density of 1×10^5 per well, and allowed to attach for 24 h. Then they were treated with different concentrations of compounds. After 24 h, 10 μL of MTT (5 mg/mL) was added to each well and the plates were incubated for 4 h. Then the medium was removed and 150 μL DMSO was added to dissolve the blue formazan crystals. Finally, absorbance values were measured at 570 nm with Synergy 2 Multi-Mode Microplate Reader (BioTek Instruments Inc., USA). Results were expressed as a percentage versus control.

Figure Legends

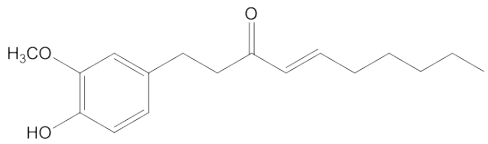
Figure S1 Chemical structures of 20 compounds in GGFTT.

Figure S2 Effect of 20 compounds in GGFTT on the viability of RAW264.7 cell. RAW264.7 cells were incubated for 24 h with various concentrations of 20 compounds. Cell viability was determined as described in Methods. Data represents the mean values of at least three experiments ± SD.

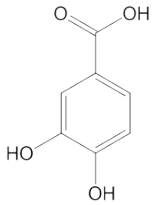
Figure S3 Effect of 20 compounds in GGFTT on the viability of 293 cells. 293 Cells were incubated for 24 h with various concentrations of 20 compounds. Cell viability was determined as described in Methods. Data represents the mean values of at least three experiments ± SD.



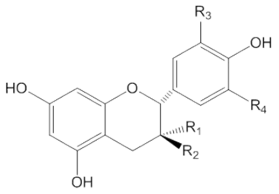
Compound	n
6-Gingerol	4
8-Gingerol	6



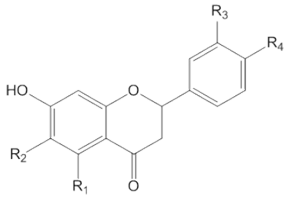
6-shogaol



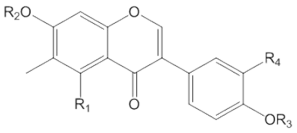
protocatechuic acid



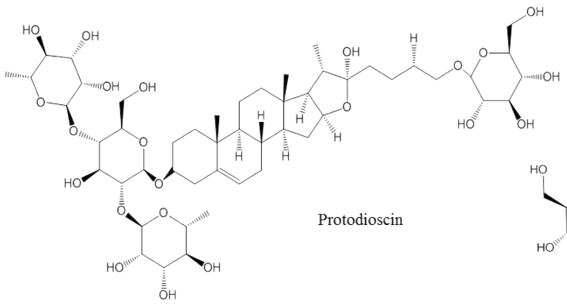
Compound	R_1	R_2	R_3	R_4
Epigallocatechin	OH	H	OH	OH
Catechin	H	OH	OH	H
Epicatechin	OH	H	OH	H



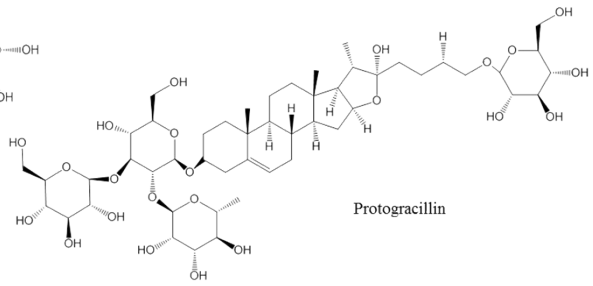
Compound	R_1	R_2	R_3	R_4
Hesperetin	OH	H	OH	OCH_3
Naringenin	OH	H	H	OH
Liquiritigenin	H	H	H	OH



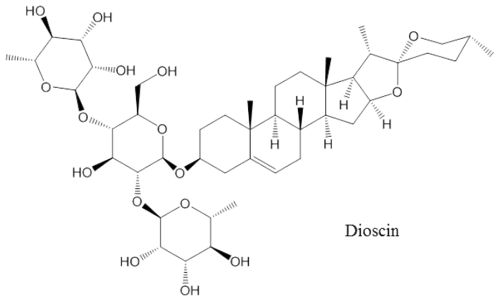
Compound	R_1	R_3	R_4	R_5
Ononin	H	Glu	CH_3	H
Daidzein	H	H	H	H
Calycosin	H	H	CH_3	OH
Genistein	OH	H	H	H
Formononetin	H	H	CH_3	H
Biochanin A	OH	H	CH_3	H



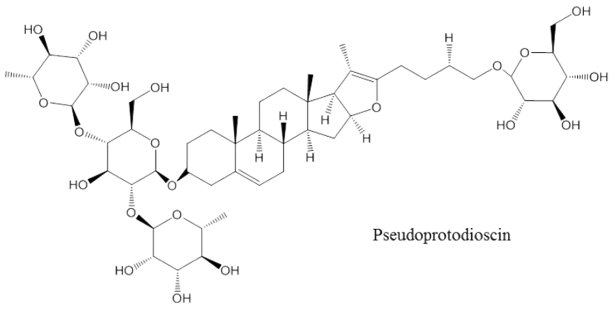
Protodioscin



Protogracillin



Dioscin



Pseudoprotodioscin

Figure S1

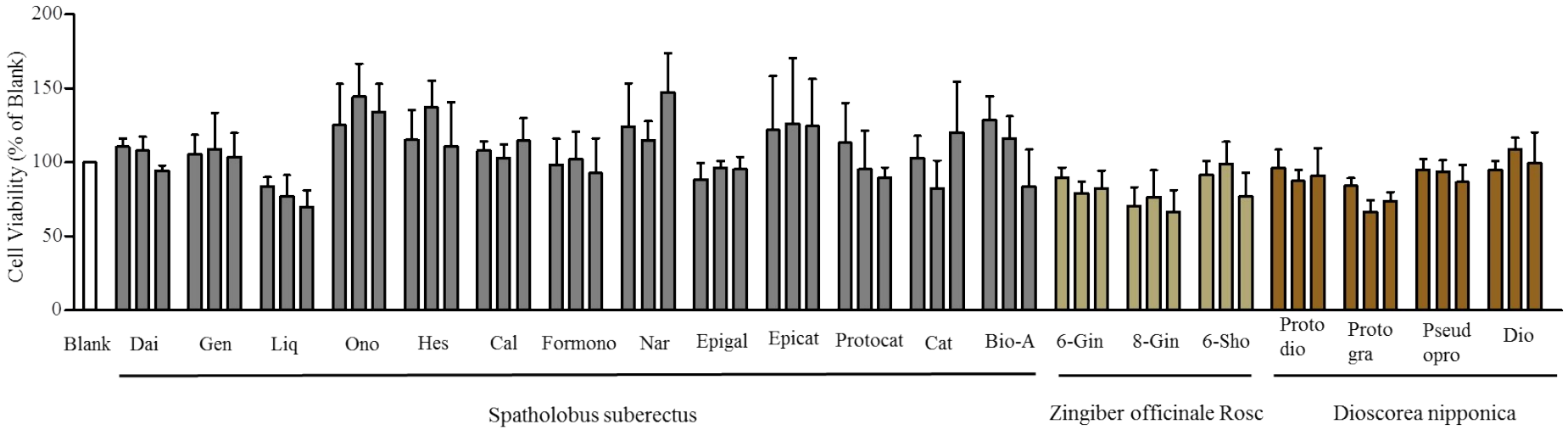

Cell Viability (% of Blank)
200
150
100
50
0
Blank
Dai
Gen
Liq
Ono
Hes
Cal
Formono
Nar
Epigal
Epicat
Protocat
Cat
Bio-A
6-Gin
8-Gin
6-Sho
Proto dio
Proto gra
Pseud opro
Dio
Spatholobus suberectus
Zingiber officinale Rosc
Dioscorea nipponica

Figure S2

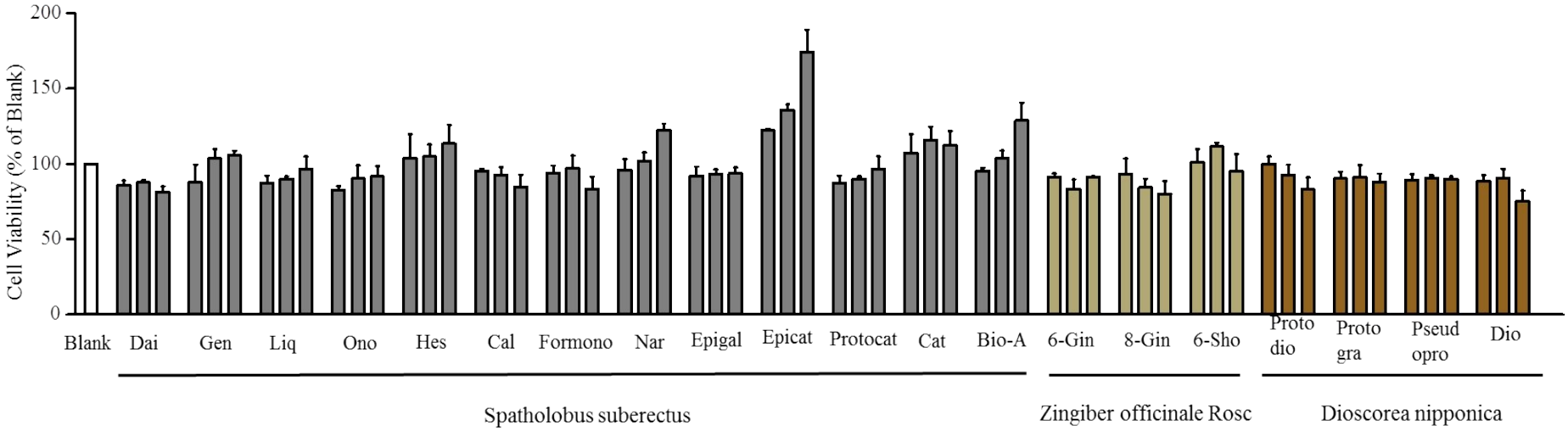

Cell Viability (% of Blank)
200
150
100
50
0
Blank
Dai
Gen
Liq
Ono
Hes
Cal
Formono
Nar
Epigal
Epicat
Protocat
Cat
Bio-A
6-Gin
8-Gin
6-Sho
Proto dio
Proto gra
Pseud opro
Dio
Spatholobus suberectus
Zingiber officinale Rosc
Dioscorea nipponica

Figure S3

Table S1 Linear regression data, LOD, LOQ, recoveries of 10 active components in GGFTT

Peak	compounds	Regression equations	R^2	LOD（ng）	LOQ（ng）	Linear range（ng）	Recovery			
							Original (μg)	Spiked (μg)	Found (μg)	Recovery (%)
1	genistein	y = 1.8564x + 0.0375	0.999	0.04	0.125	0.125-25	0.155	0.156	0.336	115.83±6.08
2	hesperetin	y = 1.1307x + 0.0107	0.9995	0.04495	0.1495	0.15-20	0.080	0.080	0.174	119.22±6.5
3	biochanin A	y = 2.0768x + 0.246	0.9961	0.03	0.1	0.1-50	0.414	0.414	0.813	96.34±7.89
4	protogarcillin	y = 0.0067x + 0.0086	0.9985	3.5	12.5	0.4-20	74.485	74.550	168.133	125.62±1.95
5	pseudoprotodioscin	y = 0.0066x + 0.0062	0.999	2.25	7	12.5-1000	360.108	360.034	653.315	81.44±2.38
6	naringenin	y = 0.7029x + 0.0465	0.9961	0.0745	0.249	7-700	0.761	1.617	2.488	106.85±6.09
7	6-gingerol	y = 0.363x + 0.2152	0.9925	0.2	0.75	4-200	87.353	87.420	179.233	105.1±2.95
8	dioscin	y = 0.0052x + 0.0257	0.9979	15	32.5	32.5-2000	1342.984	1200.000	2670.564	107.02±3.73
9	8-gingerol	y = 0.3324x - 0.0234	0.9993	0.15	0.7	2-100	22.390	22.356	43.466	94.27±1.59
10	6-shogaol	y = 0.4655x + 0.2473	0.9965	0.325	1	0.7-350	231.334	231.384	450.265	94.62±3.06

Recovery = (found− original amount) / spiked × 100

Table S2 Repeatability of 10 active components in GGFTT (n=6)

Peak	compounds		1	2	3	4	5	6	content (mg/g)	
									Mean ± SD	RSD（%）
1	genistein	PI	0.05065	0.05168	0.05162	0.05113	0.05212	0.05076	0.00062±0.00002	2.49
		content	0.00060	0.00063	0.00063	0.00061	0.00064	0.00060		
2	hesperetin	PI	0.04560	0.04541	0.04568	0.04828	0.04731	0.04796	0.00008±0.000001	3.6
		content	0.03087	0.03070	0.03094	0.03324	0.03237	0.03296		
3	biochanin A	PI	0.08789	0.09065	0.08899	0.08769	0.08768	0.08545	0.00165±0.00004	2.14
		content	0.00165	0.00171	0.00167	0.00165	0.00165	0.00160		
4	protogarcillin	PI	0.04835	0.04930	0.04860	0.04897	0.04774	0.04818	0.29794±0.0042	1.41
		content	0.29665	0.30377	0.29847	0.30130	0.29210	0.29535		
5	pseudoprotodioscin	PI	0.19828	0.19641	0.19489	0.19161	0.20626	0.19057	1.44043±0.04284	2.97
		content	1.45515	1.44101	1.42948	1.40462	1.51560	1.39675		
6	naringenin	PI	0.06816	0.06736	0.06647	0.06863	0.06887	0.06785	0.00076±0.000001	4.12
		content	0.77033	0.74194	0.71017	0.78723	0.79556	0.75928		
7	6-gingerol	PI	2.72031	2.70840	2.73004	2.72681	2.80689	2.83096	0.34941±0.01429	1.99
		content	0.34963	0.34800	0.35097	0.35052	0.36150	0.36480		
8	dioscin	PI	0.60983	0.60241	0.60956	0.57579	0.55583	0.55287	5.37194±0.254	4.73
		content	5.61666	5.54534	5.61401	5.28932	5.09736	5.06893		
9	8-gingerol	PI	0.56077	0.56394	0.57105	0.57230	0.59064	0.52886	0.08956±0.00306	3.41
		content	0.08899	0.08946	0.09053	0.09072	0.09347	0.08419		
10	6-shogaol	PI	8.93019	9.04039	9.15658	9.11938	9.24808	9.38060	0.92534±0.01651	1.78
		content	0.90272	0.91428	0.92646	0.92256	0.93605	0.94995		

Note：Six replicates of the same samples were extracted and analyzed to confirm the repeatability. PI represented the relative area of the peak, and RSD was analyzed by the three contents.

Table S3 Intra-day precision of 10 active components in GGFTT (n=6)

Peak	compounds	Intra-day precision (standards solutions on PI, n=6)							
		1	2	3	4	5	6	Mean ± SD.	RSD (%)
1	genistein	2.0695	2.0774	2.0482	2.0188	2.0042	1.9917	2.03496±0.03537	1.74
2	hesperetin	0.4967	0.4819	0.4687	0.4720	0.4648	0.4519	0.47268±0.0153	3.24
3	biochanin A	5.7194	5.9660	5.9371	5.8748	5.7372	5.7079	5.82375±0.11621	2.00
4	protogarcillin	0.1551	0.1572	0.1568	0.1550	0.1575	0.1577	0.15656±0.00118	0.75
5	pseudoprotodioscin	0.4152	0.4310	0.4366	0.4312	0.4353	0.4081	0.42622±0.01173	2.75
6	naringenin	0.0687	0.0694	0.0698	0.0701	0.0681	0.0684	0.06749±0.00239	1.17
7	6-gingerol	3.2116	3.2153	3.1873	3.1465	3.1162	3.1124	3.16489±0.04624	1.46
8	dioscin	0.0288	0.0288	0.0286	0.0286	0.0285	0.0285	0.02843±0.00068	0.50
9	8-gingerol	1.2390	1.2753	1.2514	1.2350	1.2241	1.2021	1.23782±0.02475	2.00
10	6-shogaol	7.4034	7.7033	7.6994	7.6136	7.5090	7.4056	7.55572±0.13688	1.81

Note：The samples were analyzed for six times within the same day for intra-day test. PI represented the relative area of the peak.

Table S4 Inter-day precision of 10 active components in GGFTT (n=9)

Peak	compound	Inter-day precision (standards solutions on PI, n=9)									Mean ± SD	RSD (%)
		Day 1			Day 2			Day 3				
		1	2	3	1	2	3	1	2	3		
1	genistein	2.24316	2.07226	2.05910	2.06954	2.07739	2.04819	2.05954	2.23816	2.12117	2.10983±0.07694	3.65
2	hesperetin	0.51112	0.50800	0.49924	0.50793	0.50269	0.49669	0.51720	0.50189	0.49898	0.50486±0.00668	1.32
3	biochanin A	5.83192	5.82684	5.81406	5.96605	5.93714	5.87481	5.89473	5.96932	5.93858	5.89483±0.06106	1.04
4	protogarcillin	0.15763	0.15634	0.15776	0.15718	0.15683	0.15751	0.15682	0.15698	0.15504	0.1569±0.00083	0.53
5	pseudoprotodioscin	0.43737	0.42399	0.42789	0.41971	0.43655	0.42350	0.43101	0.43656	0.43122	0.42976±0.00643	1.50
6	naringenin	0.07360	0.07648	0.07646	0.07613	0.07901	0.07540	0.07643	0.07493	0.07362	0.07488±0.003	2.20
7	6-gingerol	3.17152	3.22377	3.22594	3.21159	3.21531	3.18734	3.12636	3.31668	3.23699	3.21283±0.05184	1.61
8	dioscin	0.02737	0.02736	0.02724	0.02719	0.02729	0.02727	0.02723	0.02740	0.02749	0.02722±0.00072	0.35
9	8-gingerol	1.25377	1.25271	1.25140	1.29106	1.27534	1.25136	1.26587	1.28549	1.26759	1.26606±0.01522	1.20
10	6-shogaol	7.86718	7.63064	7.77541	7.70332	7.69941	7.61357	7.79864	8.17279	7.82725	7.78758±0.16821	2.16

Note：The samples were examined in duplicates for consecutive three days for inter-day test. PI represented the relative area of the peak.

Table S5 The concentrations of the 20 compounds in RAW264.7 cells

Compounds	Concentrations (μM)		
daidzein	2.5	5	10
genistein	12.5	25	50
liquiritigenin	25	50	100
ononin	25	50	100
hesperetin	25	50	100
calycosin	12.5	25	50
formononetin	25	50	100
naringenin	12.5	25	50
epigallocatechin	25	50	100
epicatechin	25	50	100
protocatechuic acid	20	25	50
catechin	25	50	100
biochanin A	12.5	25	50
6-gingerol	25	50	100
8-gingerol	0.625	1.25	2.5
6-shogaol	1.5625	3.125	6.25
protodioscin	0.625	1.25	2.5
protogarcillin	5	10	20
pseudoprotodioscin	3.125	6.25	12.5
dioscin	0.1875	0.375	1.25

Table S6 The concentrations of the 20 compounds in 293 cells

Compounds	Concentrations (μM)		
daidzein	2.5	5	10
genistein	5	10	20
liquiritigenin	25	50	100
ononin	25	50	100
hesperetin	25	50	100
calycosin	12.5	25	50
formononetin	25	50	100
naringenin	12.5	25	50
epigallocatechin	25	50	100
epicatechin	25	50	100
protocatechuic acid	10	20	25
catechin	10	20	40
biochanin A	6.25	12.5	25
6-gingerol	25	50	100
8-gingerol	0.25	0.5	1
6-shogaol	1.25	2.5	5
protodioscin	0.3125	0.625	1.25
protogarcillin	1.25	2.5	5
pseudoprotodioscin	4	8	16
dioscin	0.25	0.5	1