applied sciences

MDPI

Supplementary Material

Development of a Reverse-Phase High-Performance Liquid Chromatography and Liquid Chromatography Tandem Mass Spectrometry Methods for Quality Control of Daegunjoong-Tang

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Table S1. Information on the composition of DGJT.

Herbal Medicine	Scientific Name	English name	Family	Used part	Origin	Amount (g)
Zingiberis Rhizoma	*Zingiber officinale* Rosc.	Ginger	Zingiberaceae	Rhizome	Seosan, Korea	2500.0
Ginseng Radix	*Panax ginseng* C.A.Mey.	Ginseng	Araliaceae	Root	Geumsan, Korea	1250.0
Zanthoxyli Pericarpium	*Zanthoxylum schinifolium* Sieb. et Zucc.	Zanthoxylum Peel	Rutaceae	Pericarp	Yeongyang, Korea	1250.0
					Total (g)	5000.00

Table S2. HPLC parameters for quantification of DGJT.

Parameter for HPLC Analysis			
System	Shimadzu Prominence LC-20A series (Kyoto, Japan)		
Detector	Photo diode array		
Column	Waters SunFire C18 (4.6 × 250 mm, particle size 5 µm, Milford, MI, USA)		
Column oven Temperature (°C)	40.0		
Flow rate (mL/min)	1.0		
Injection volume (µL)	10.0		
Mobile phase	A: 0.1% (v/v) Aqueous formic acid B: 0.1% (v/v) Formic acid in acetonitrile		
Gradient elution	Time (min)	A (%)	B (%)
	0	90	10
	30	40	60
	40	0	100
	45	0	100
	50	90	10
	60	90	10

Table S3. MRM parameters for LC–MS/MS analysis of 4 marker compounds in DGJT.

Compound	Mode	MW (*m*/*z*)	Transition (Q1→Q3, *m*/*z*)	Collision Energy (eV)	Cone Voltage (V)
Hyperoside	Negative	464.10	463.2→300.0	25	40
Quercitrin	Negative	448.10	447.0→301.0	25	20
Ginsenoside Rg1	Negative	800.49	799.2→637.0	20	50
6-Gingerol	Positive	294.18	295.3→177.1	10	13

Table S4. System suitability for the four marker analytes in the developed HPLC method.

Compound	*k′*	*α*	*N*	*Rs*	*Tf*
Hyperoside	3.94	1.17	501122	6.94	1.13
Quercitrin	4.60	1.14	557071	7.23	1.13
Ginsenoside Rg1	5.25	1.14	800846	7.23	1.11
6-Gingerol	9.91	1.89	1122666	40.42	1.07

Retention factor (*k′*), relative retention (*α*), theoretical plate number (*N*), resolution (*Rs*), and symmetry factor (*S*).

Table S5. Repeatability for retention time and peak areas of the four marker analytes using HPLC (*n* = 6).

No.	Retention Time (min)				Peak Area			
	Hyperoside	Quercitrin	Ginsenoside Rg1	6-Gingerol	Hyperoside	Quercitrin	Ginsenoside Rg1	6-Gingerol
1	14.152	16.043	17.880	31.244	1587117	356796	1013372	3787576
2	14.158	16.043	17.879	31.245	1594064	358826	1015568	3803418
3	14.151	16.038	17.869	31.233	1599084	359382	1021961	3818628
4	14.155	16.039	17.871	31.235	1606396	361835	1026638	3832737
5	14.158	16.044	17.878	31.241	1601466	361019	1024103	3826101
6	14.151	16.039	17.872	31.236	1592469	358462	1018940	3801593
Mean	14.15	16.04	17.87	31.24	1596766.00	359386.67	1020097.00	3811675.50
SD (× 10^{-1})	0.03	0.03	0.05	0.05	69147.41	18187.79	50869.11	170331.92
RSD (%)	0.02	0.02	0.03	0.02	0.43	0.51	0.50	0.45

Table S6. The linear range, regression equation, r^2, LODs, and LOQs of the analytes from YPS using LC–MS/MS (*n* = 3).

Compound	Retention Time (min)	Linear Range (ng/mL)	Regression Equation ($y = ax + b$)[a]	r^2	LOD (μg/mL)[b]	LOQ (μg/mL)[c]
Hyperoside	1.63	6.25–100.00	$y = 29066.1x - 155575.0$	0.9953	0.002	0.006
Quercitrin	2.03	0.63–10.00	$y = 9346.2x + 5140.3$	0.9956	0.001	0.003
Ginsenoside Rg1	2.71	6.25–100.00	$y = 19.6x - 108.5$	0.9952	1.586	4.757
6-Gingerol	6.33	0.63–10.00	$y = 29284.3x + 10841.7$	0.9975	0.002	0.005

[a]*y* and *x* are peak area and concentration of compound, respectively [b]LOD = 3.3 × S/N [c]LOQ = 10 × S/N.

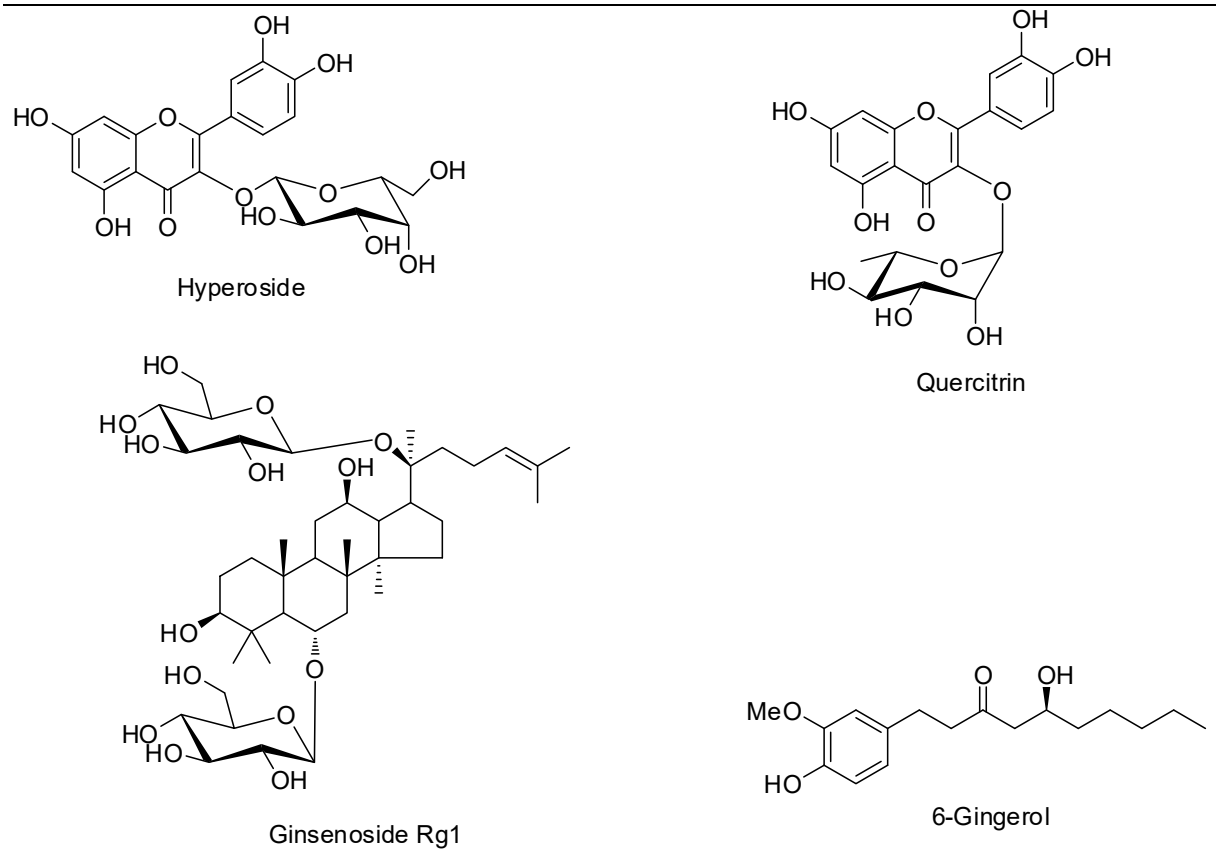
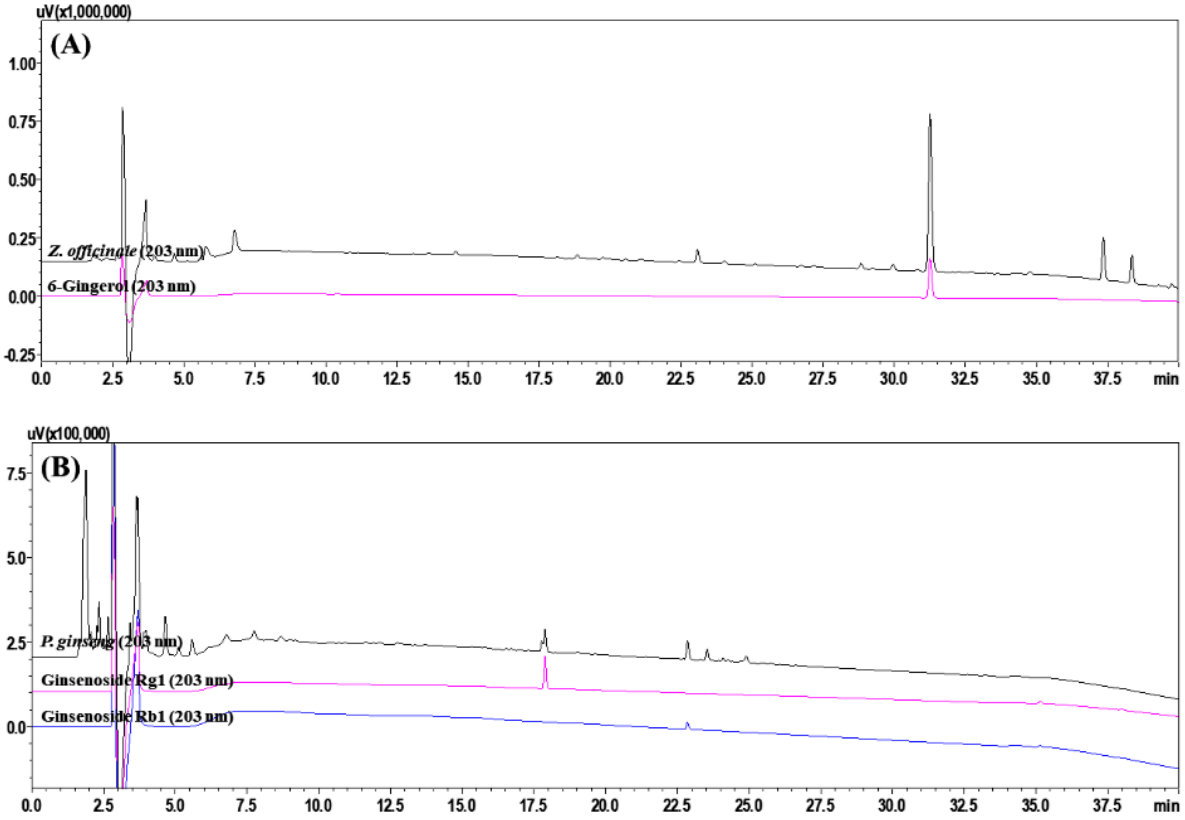
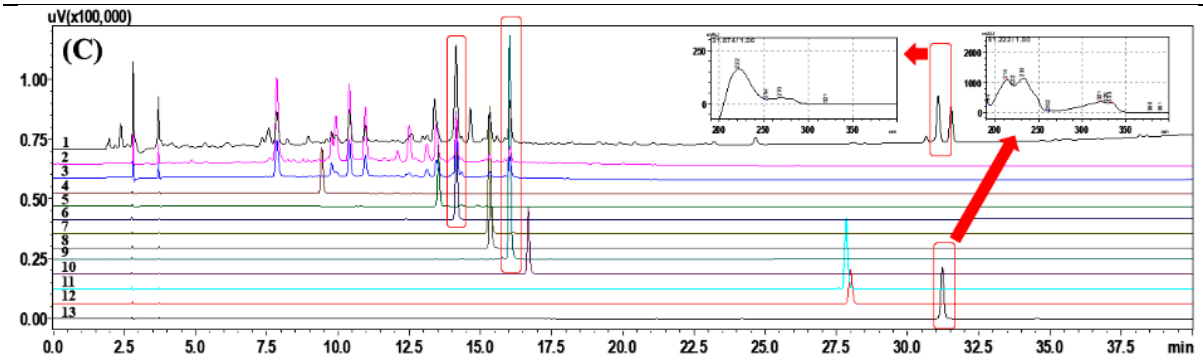


Figure S1. Chemical structures of the four marker analytes used in the quantitative analysis of DGJT.





1: *Z. schinifolium* (255 nm), 2: *Z. schinifolium* (320 nm), 3: *Z. schinifolium* (350 nm),

4: Xeroboside (340 nm), 5: Rutin (355 nm), 6: Hyperoside (355 nm), 7: Feulic acid (320 nm), 8: Foeniculin (355 nm),

9: Quercitrin (350 nm), 10: Isorhamnetin-7-glucoside (370 nm), 11: 3,4,5-Trihydroxy-7,4′-dimethoxyflavone (285 nm),

12: Dictamnin (310 nm), 13: Schinifolin (320 nm)

Figure S2. HPLC chromatogram of constituent herbal medicine and its main components. A: *Z. officinale*, B: *P. ginseng*, and C: *Z. schinifolium*.

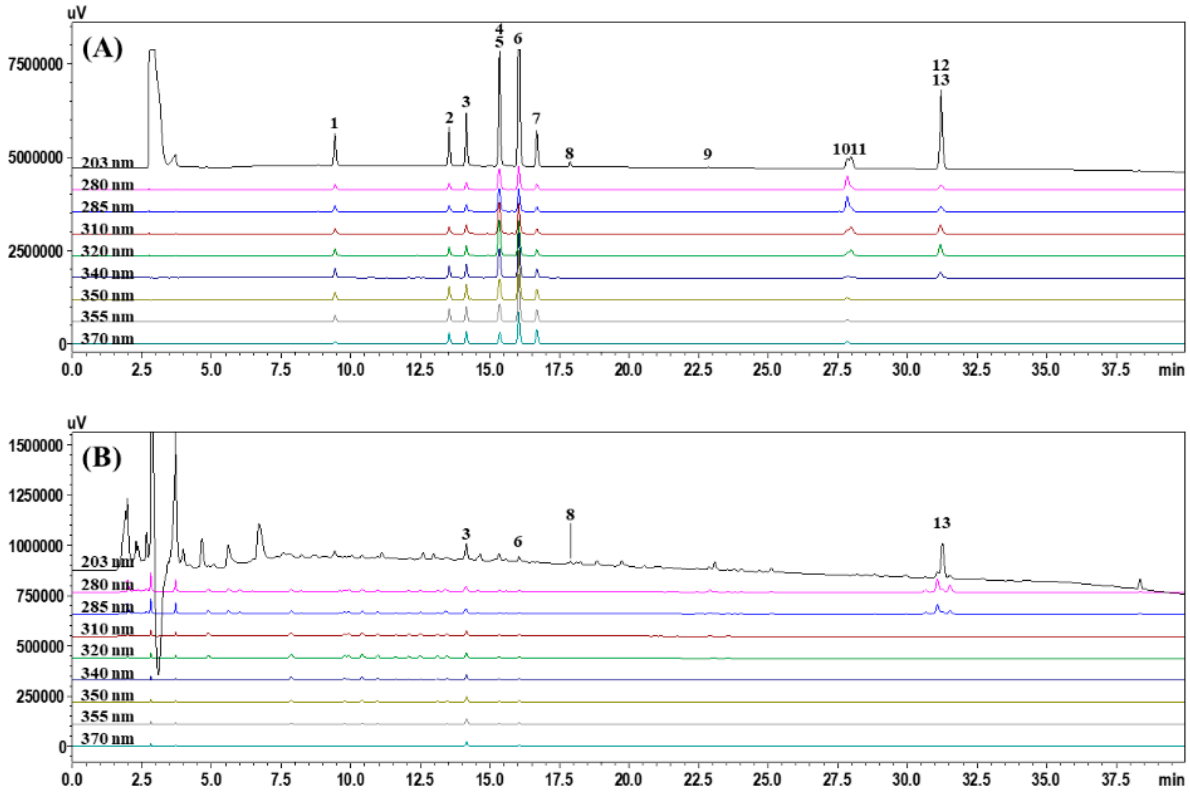
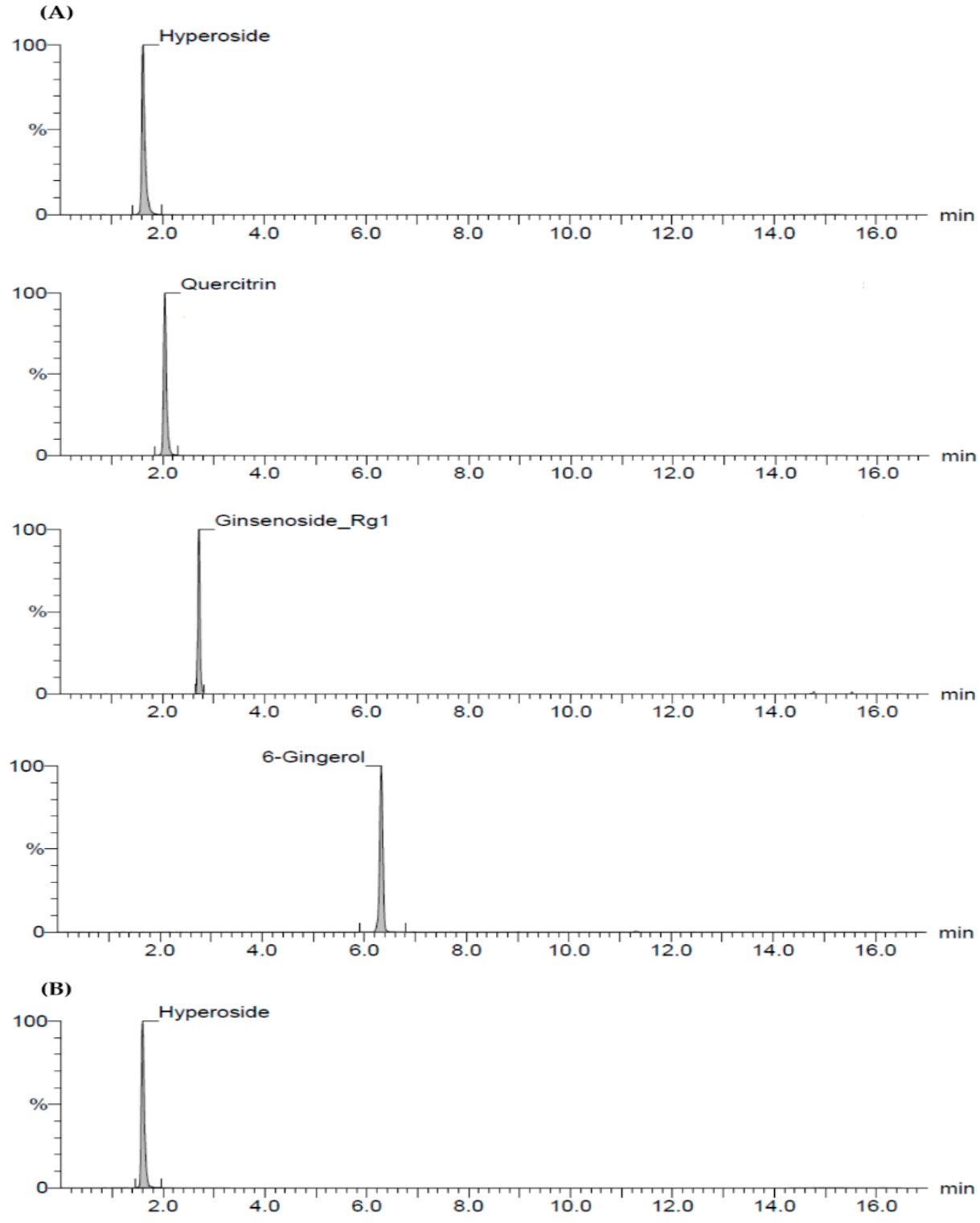


Figure S3. HPLC chromatograms of the standard solution (A) and 70% methanol extract of DGJT sample (B). Xeroboside (**1**), rutin (**2**), hyperoside (**3**), ferulic acid (**4**), foeniculin (**5**), quercitrin (**6**), isorhametin-7-glucoside (**7**), ginsenoside Rg1 (**8**), ginsenoside Rb1 (**9**), 3,4,5-trihydroxy-7,4′-dimethoxyflavone (**10**), dictamnin (**11**), schinifolin (**12**), 6-gingerol (**13**).


(A)
Hyperoside
Quercitrin
Ginsenoside_Rg1
6-Gingerol
(B)
Hyperoside
100
%
0
min
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0

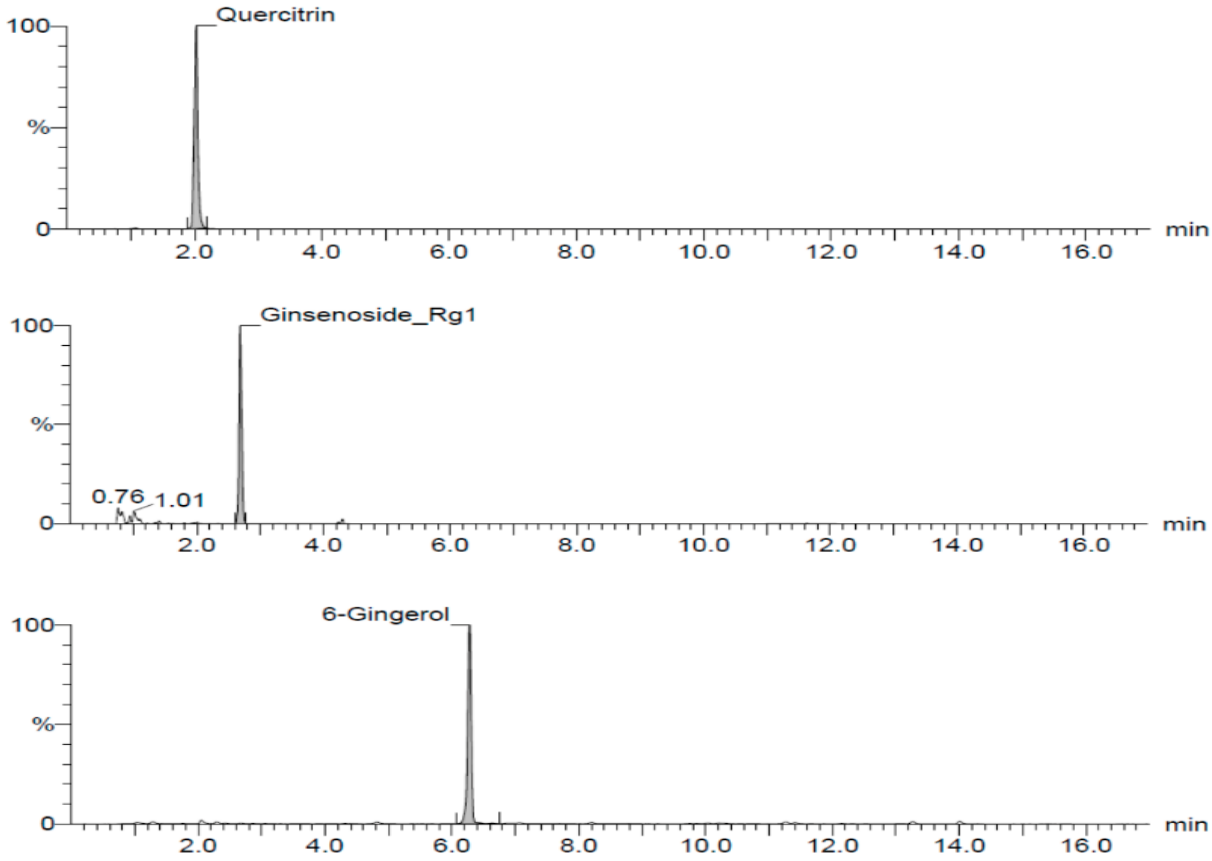


Figure S4. Extracted ion chromatograms of the reference standard (A) and DGJT sample (B) by LC–MS/MS MRM mode.