

Supplementary Materials: Development and Validation of a High-Performance Liquid Chromatography Method for Quality Assessment of Oriental Medicine, Dokhwalgisaeng-Tang

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Table S1. Information and composition of DHGST.

Herbal medicine	Scientific name	English name	Family	Used part	Origin	Amount (g)
Araliae Continentalis Radix	<i>Aralia continentalis</i> Kitag.	Aralia Contineltalis Root	Araliaceae	Root	Uiseong, Korea	411.95
Angelicae Gigantis Radix	<i>Angelica gigas</i> Nakai	Angelica Gigas Root	Apiaceae	Root	Pyeongchang, Korea	411.95
Paeoniae Radix	<i>Paeonia lactiflora</i> Pall.	Peony Root	Paeoniaceae	Root	Uiseong, Korea	411.95
Taxilli Herba	<i>Taxillus chinensis</i> (DC.) Danser	Chinese Taxillus Twig	Loranthaceae	Leaf, stem, and twig	China	411.95
Rehmanniae Radix Preparata	<i>Rehmannia glutinosa</i> (Gaertn.) DC.	Prepared Rehmannia Root	Plantaginaceae	Root	Jeongeup, Korea	294.03
Cnidii Rhizoma	<i>Cnidium officinale</i> Mak.	Cnidium Rhizome	Umbelliferae	Rhizome	Yeongyang, Korea	294.03
Ginseng Radix	<i>Panax ginseng</i> C.A.Mey.	Ginseng	Araliaceae	Root	Geumsan, Korea	294.03
Poria Sclerotium	<i>Poria cocos</i> Wolf	Poria	Polyporaceae	Sclerotium	Bonghwa, Korea	294.03
Achyranthis Radix	<i>Achyranthes bidentata</i> Blume	Achyranthes Root	Amaranthaceae	Root	Hwasun, Korea	294.03
Eucommiae Cortex	<i>Eucommia ulmoides</i> Oliv.	Eucommia Bark	Eucommiaceae	Bark	Samcheok, Korea	294.03

Gentianae Macrophyllae Radix	<i>Gentiana straminea</i> Maxim.	Gentiana Macrophylla Root	Gentianaceae	Root	China	294.03
Asiasari Radix et Rhizoma	<i>Asarum heterotropoides</i> F. Schmidt	Asiasarum Root and Rhizoma	Aristolochiaceae	Root and rhizome	China	294.03
Saposhnikoviae Radix	<i>Saposhnikovia divaricate</i> (Turcz.) Schischk.	Saposhnikovia Root	Apiaceae	Root	China	294.03
Cinnamomi Cortex	<i>Cinnamomum cassia</i> (L.) J.Presl	Cinnamom Bark	Lauraceae	Bark	Veitanam	294.03
Glycyrrhizae Radix et Rhizoma	<i>Glycyrrhiza uralensis</i> Fisch.	Licorice	Leguminosae	Root and rhizome	China	176.10
Zingiberis Rhizoma Recens	<i>Zingiber officinale</i> Roscoe	Raw Ginger	Zingiberaceae	Rhizome	Seosan, Korea	235.80
Total (g)						5000.00

Table S2. HPLC operating conditions for simultaneous quantification of the 24 marker components in DHGST.

Parameter for HPLC analysis			
HPLC system	Prominence LC-20A series (Shimadzu, Kyoto, Japan)		
Detector	Photo-diode array detector		
Detection wavelength (nm)	230, 240, 250, 270, 275, 280, 290, 300, 320, 325, 330 and 335		
Column	C18-reverse phase analytical column (SunFire C18, 4.6 × 250 mm, particle size 5 µm, Waters, Milford, MA, 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) formic acid in distilled water		
	B: 0.1% (v/v) formic acid in acetonitrile		
Gradient elution	Time (min)	A (%)	B (%)
	0	95	5
	50	40	60
	60	0	100
	70	0	100
	80	95	5
	90	95	5

Table S3. System suitability for the analysis of the 24 marker compounds with the developed HPLC method.

Compound	k'	α	N	R_s	T_f
1	1.15	1.75	16,865	8.57	1.02
2	2.02	1.13	52,309	3.12	1.03
3	2.27	1.13	89,543	3.12	0.99
4	3.26	1.27	310,553	15.12	1.08
5	4.14	1.15	289,522	10.28	1.13
6	4.77	1.06	434,085	4.84	1.07
7	5.05	1.03	562,145	2.51	0.92
8	5.20	1.03	429,662	2.51	1.06
9	5.51	1.01	695,111	1.27	1.01
10	5.58	1.01	553,186	1.27	1.20
11	6.26	1.02	455,146	1.94	1.17
12	6.40	1.02	549,249	1.94	1.08
13	6.65	1.03	522,658	2.54	1.06
14	6.82	1.03	796,159	2.54	1.07
15	7.11	1.04	968,090	5.05	1.07
16	7.91	1.11	468,111	11.02	1.03
17	8.88	1.12	443,229	10.31	1.03
18	10.38	1.11	796,587	11.47	1.03
19	11.52	1.11	582,629	11.47	1.03
20	13.88	1.13	1418,878	18.17	1.12
21	15.64	1.13	1074,775	18.17	1.02
22	17.47	1.04	1203,884	7.25	1.03
23	18.23	1.01	1894,128	1.66	1.02
24	18.38	1.01	2158,966	1.66	1.03

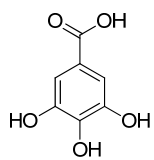
k' ; capacity factor, α ; relative retention, N ; theoretical plate number, R_s ; resolution, and T_f ; tailing factor

Table S4. Repeatability of retention time of the 24 marker analytes using HPLC ($n = 6$).

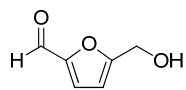
Analyte	No., Retention time (min)						Mean	SD ($\times 10^{-1}$)	RSD (%)
	1	2	3	4	5	6			
1	6.224	6.216	6.23	6.229	6.229	6.226	6.23	0.05	0.08
2	8.717	8.711	8.723	8.811	8.717	8.718	8.73	0.38	0.44
3	9.438	9.442	9.44	9.45	9.447	9.44	9.44	0.05	0.05
4	12.262	12.262	12.263	12.272	12.272	12.263	12.27	0.05	0.04
5	14.807	14.799	14.81	14.723	14.804	14.799	14.79	0.33	0.22
6	16.63	16.632	16.631	16.641	16.641	16.629	16.63	0.06	0.03
7	17.429	17.422	17.438	17.405	17.425	17.42	17.42	0.11	0.06
8	17.869	17.869	17.856	17.864	17.86	17.86	17.86	0.05	0.03
9	18.754	18.748	18.764	18.731	18.751	18.744	18.75	0.11	0.06
10	18.956	18.96	18.958	18.964	18.96	18.959	18.96	0.03	0.01
11	20.907	20.912	20.909	20.914	20.911	20.909	20.91	0.03	0.01
12	21.301	21.291	21.302	21.277	21.294	21.291	21.29	0.09	0.04
13	22.047	22.037	22.048	22.02	22.04	22.038	22.04	0.10	0.05
14	22.528	22.517	22.527	22.511	22.52	22.518	22.52	0.06	0.03
15	23.364	23.353	23.365	23.353	23.357	23.355	23.36	0.05	0.02
16	25.643	25.632	25.646	25.632	25.636	25.632	25.64	0.06	0.02
17	28.454	28.442	28.456	28.441	28.446	28.446	28.45	0.06	0.02
18	32.786	32.776	32.791	32.78	32.78	32.779	32.78	0.05	0.02
19	36.04	36.05	36.041	36.048	36.05	36.04	36.04	0.05	0.01
20	42.781	42.789	42.783	42.791	42.789	42.782	42.79	0.04	0.01
21	47.933	47.922	47.939	47.931	47.928	47.93	47.93	0.06	0.01
22	53.207	53.195	53.21	53.207	53.2	53.205	53.20	0.06	0.01
23	55.387	55.374	55.389	55.384	55.379	55.383	55.38	0.05	0.01
24	55.826	55.814	55.828	55.823	55.818	55.822	55.82	0.05	0.01

Table S5. Repeatability of peak area of the 24 marker analytes using HPLC ($n = 6$).

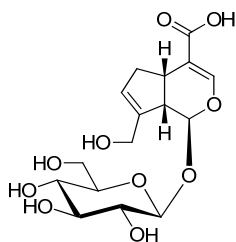
Analyte	No., Peak area						Mean	SD	RSD (%)
	1	2	3	4	5	6			
1	354,169	352,767	353,547	352,289	352,289	352,301	352893.67	793.96	0.22
2	580,179	579,153	582,550	579,154	581,123	581,578	580622.83	1370.50	0.24
3	46,461	46,104	46,327	47,142	46,994	46,813	46640.17	406.09	0.87
4	36,758	36,815	36,656	36,913	36,848	36,880	36811.67	93.15	0.25
5	329,246	329,268	322,372	329,605	330,138	324,713	327557.00	3212.75	0.98
6	139,714	140,166	139,704	142,816	141,529	140,848	140796.17	1214.95	0.86
7	59,291	58,996	59,070	58,673	58,262	58,201	58748.83	447.45	0.76
8	41,938	40,029	40,292	40,145	41,114	40,679	40699.50	725.15	1.78
9	73,910	72,959	74,385	72,788	72,952	73,104	73349.67	643.81	0.88
10	70,321	70,752	70,519	71,883	72,153	71,751	71229.83	788.68	1.11
11	19,900	20,095	19,970	20,312	20,359	20,401	20172.83	213.41	1.06
12	69,522	69,185	67,896	69,273	68,112	68,121	68684.83	716.28	1.04
13	181,259	181,380	184,363	182,171	182,270	182,476	182319.83	1116.44	0.61
14	357,121	357,250	358,619	357,336	357,971	359,389	357947.67	902.54	0.25
15	65,827	65,664	66,071	65,282	66,461	65,769	65845.67	396.58	0.60
16	346,095	344,968	348,188	344,639	346,584	345,582	346009.33	1282.89	0.37
17	260,167	259,558	262,379	261,236	261,402	259,076	260636.33	1249.04	0.48
18	68,565	68,212	68,720	68,417	68,510	68,802	68537.67	212.25	0.31
19	219,589	219,557	217,711	223,231	221,175	222,474	220622.83	2059.99	0.93
20	22,388	22,240	22,254	22,258	22,633	22,703	22412.67	206.09	0.92
21	20,090	20,041	20,158	20,157	20,160	20,441	20174.50	139.12	0.69
22	11,674	11,665	11,897	11,866	11,741	11,867	11785.00	104.39	0.89
23	12,6738	126,477	126,976	127,592	127,576	127,581	127156.67	493.01	0.39
24	81,813	81,843	82,148	82,627	82,515	82,452	82233.00	351.69	0.43



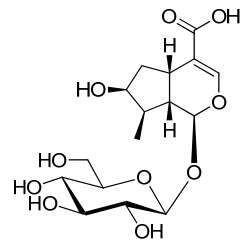
Gallic acid (1)



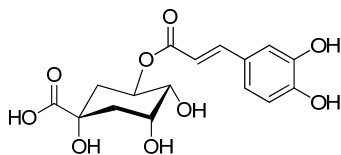
5-Hydroxymethylfurfural (2)



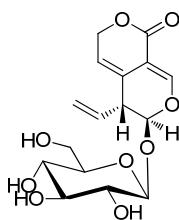
Geniposidic acid (3)



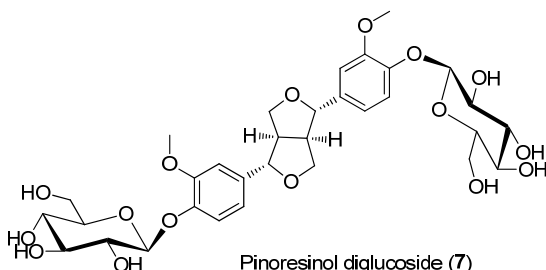
Loganic acid (4)



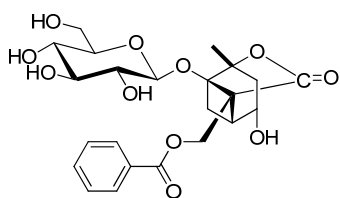
Chlorogenic acid (5)



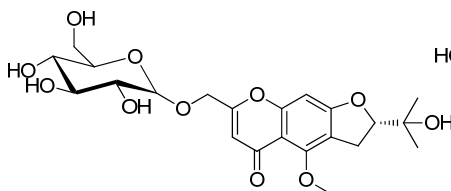
Gentiopicroside (6)



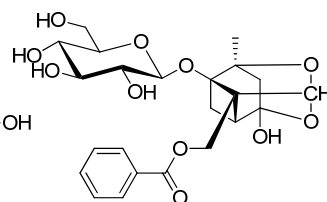
Pinoresinol diglucoside (7)



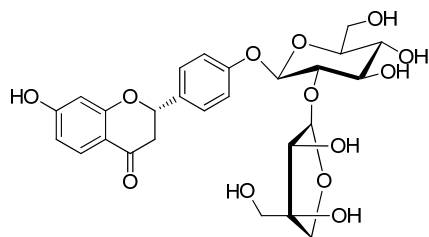
Albiflorin (8)



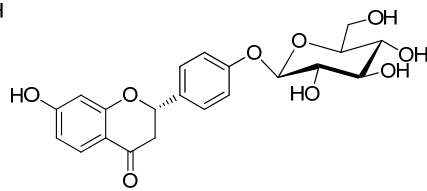
prim-O-Glucosylcimifugin (9)



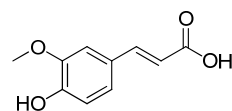
Paeoniflorin (10)



Liquiritin apioside (11)



Liquiritin (12)



Ferulic acid (13)

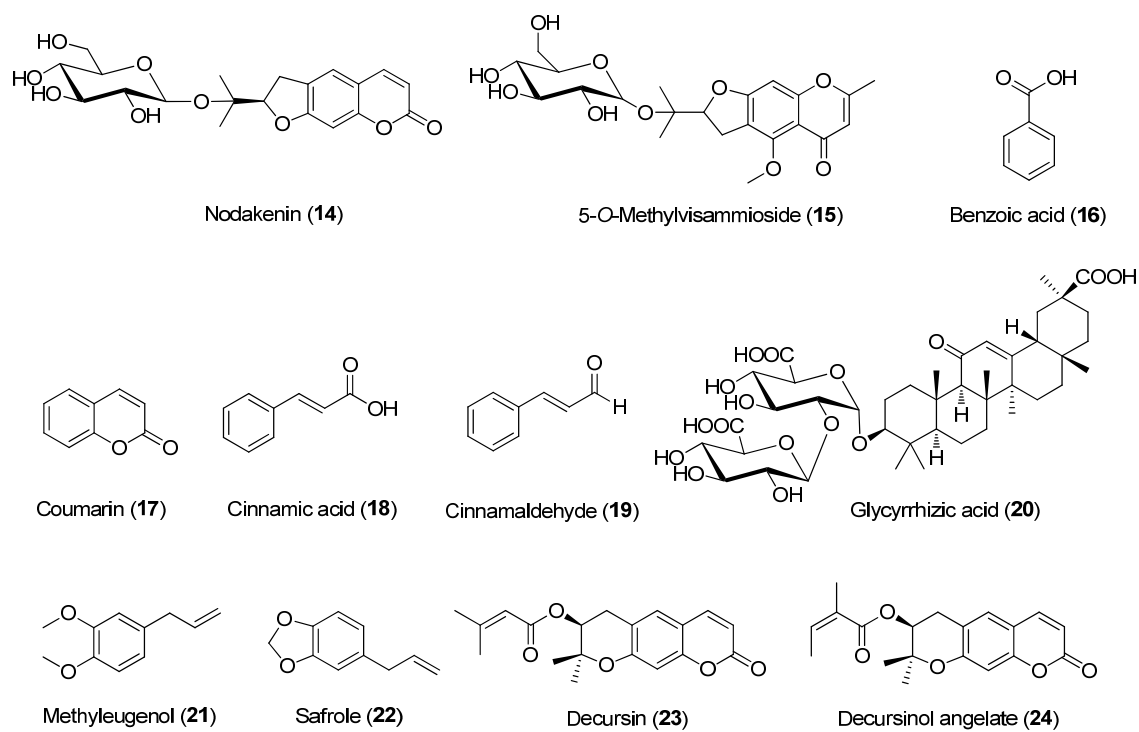
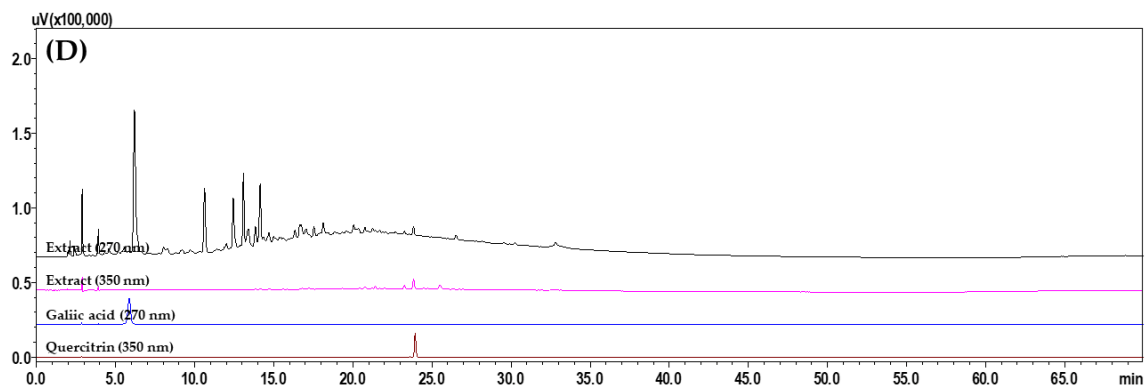
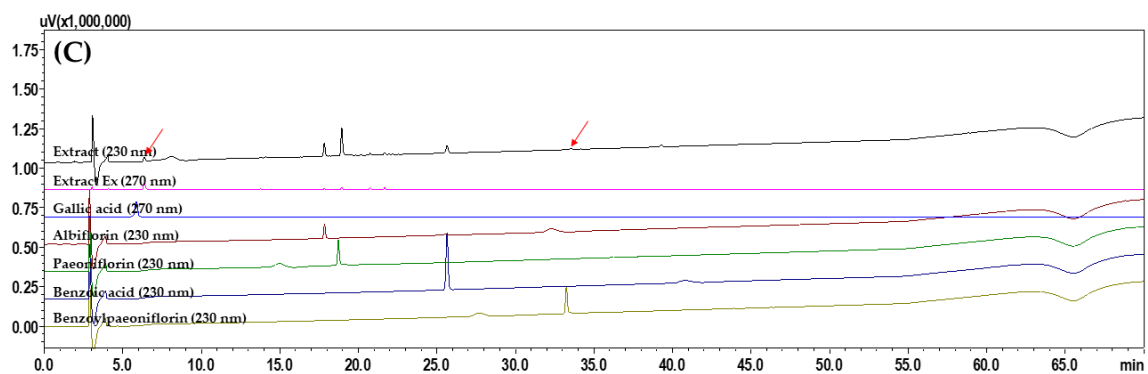
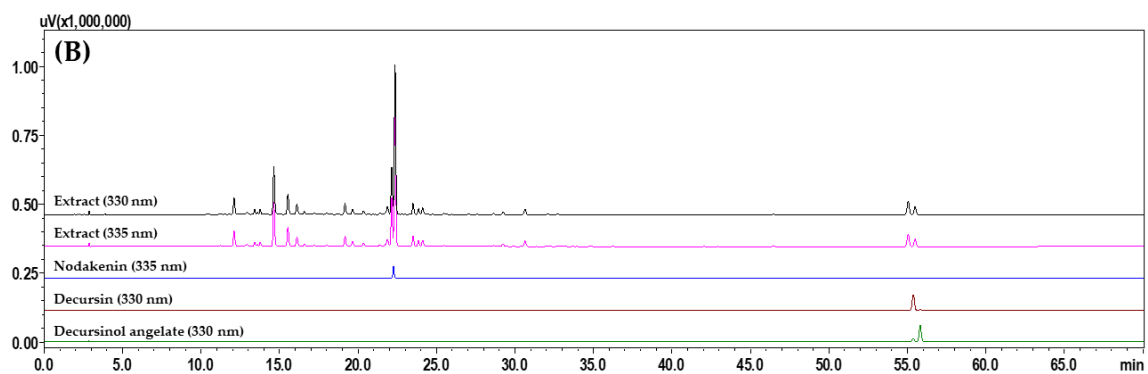
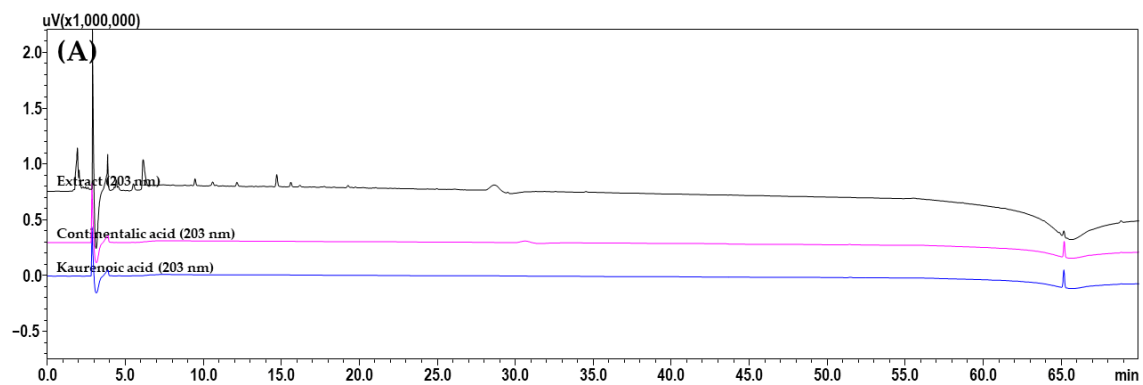
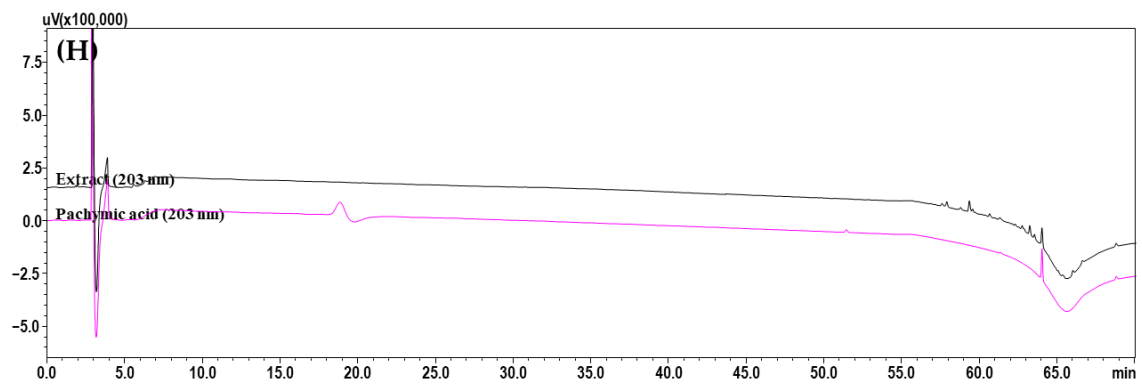
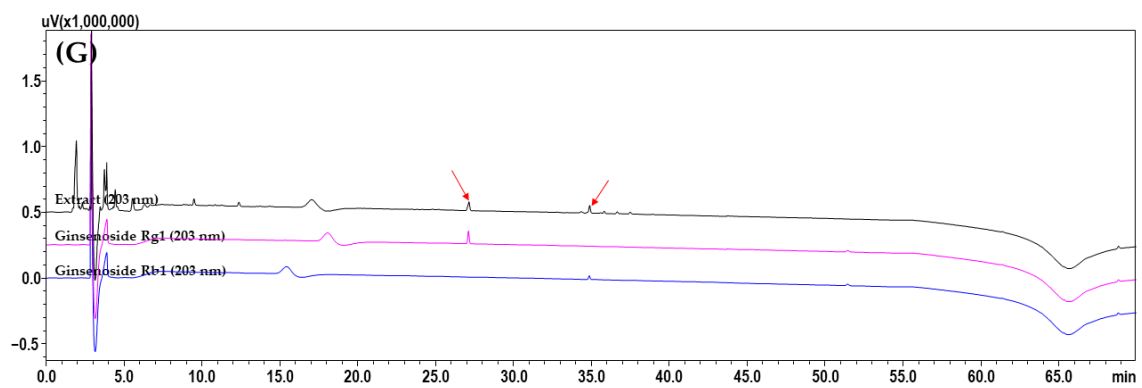
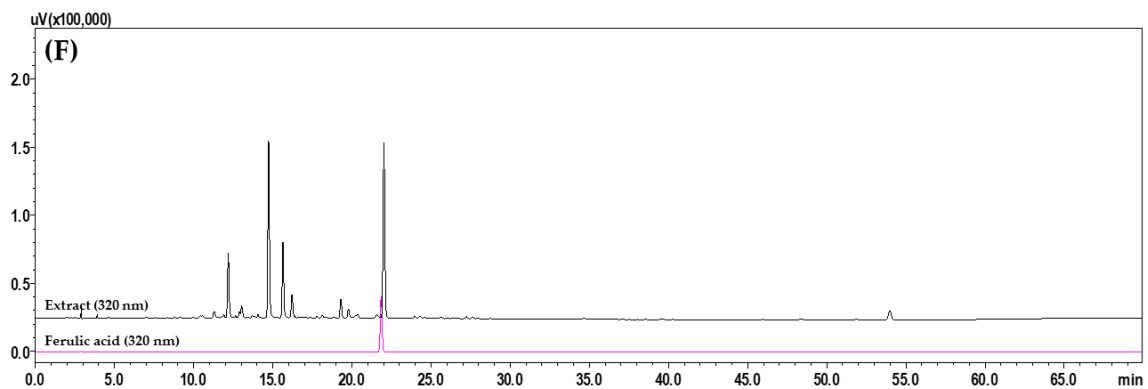
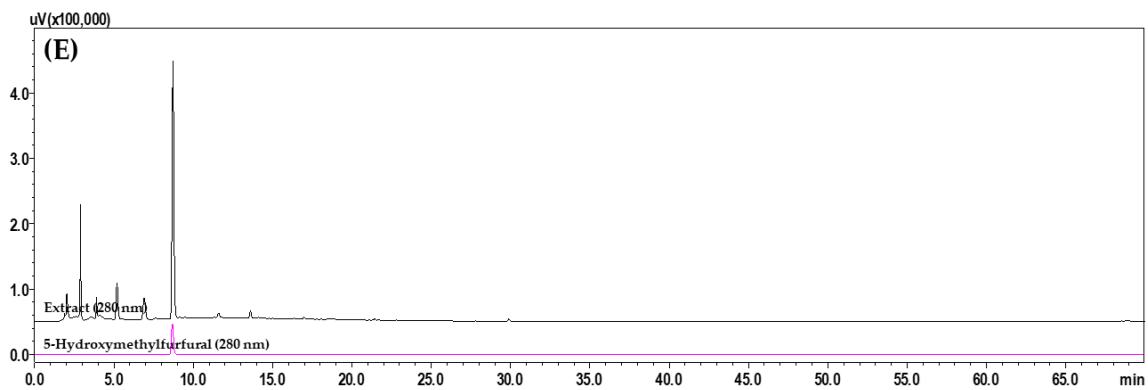
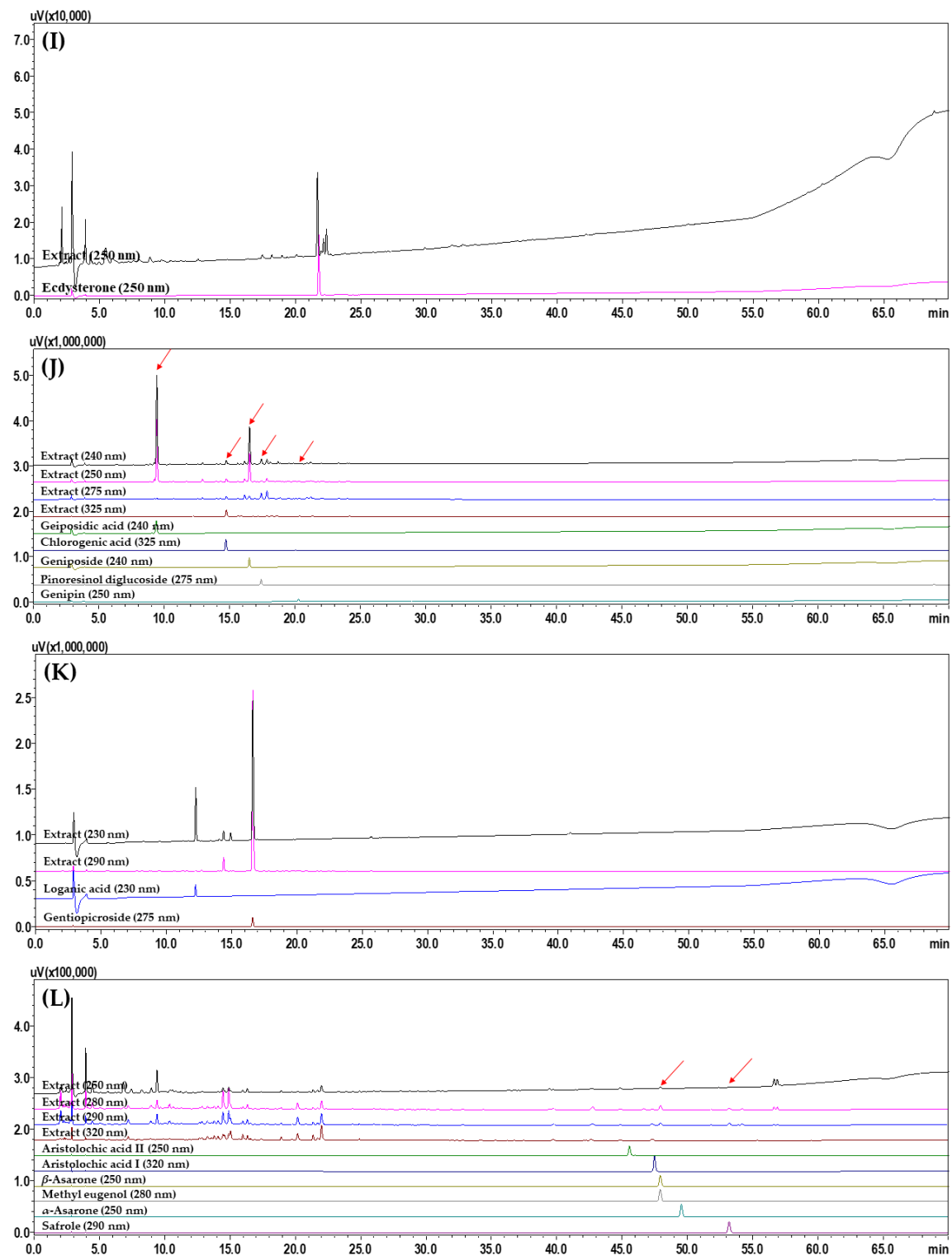


Figure S1. Chemical structures of the 24 marker components in DHGST.







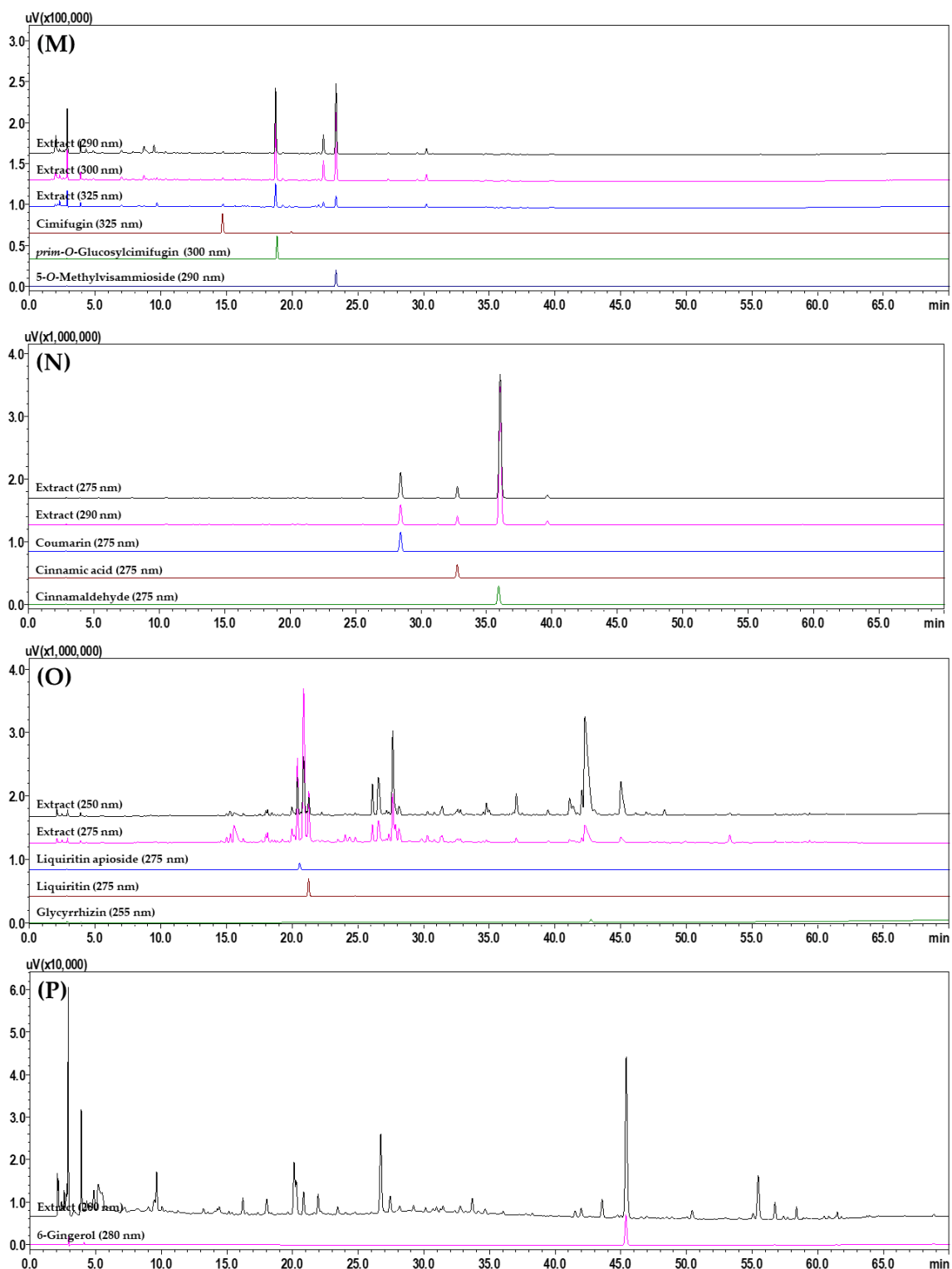


Figure S2. HPLC chromatogram of herbal medicine extract and its main components. A: *A. continentalis*; B: *A. gigas*; C: *P. lactiflora*; D: *T. chinensis*; E: *R. glutinosa*; F: *C. officinale*; G: *P. ginseng*; H: *P. cocos*; I: *A.*

bidentata; J, *E. ulmoides*; K, *G. straminea*; L, *A. heterotropoides*; M, *S. divaricate*; N, *C. cassia*; O, *G. uralensis*; and P, *Z. officinale*.

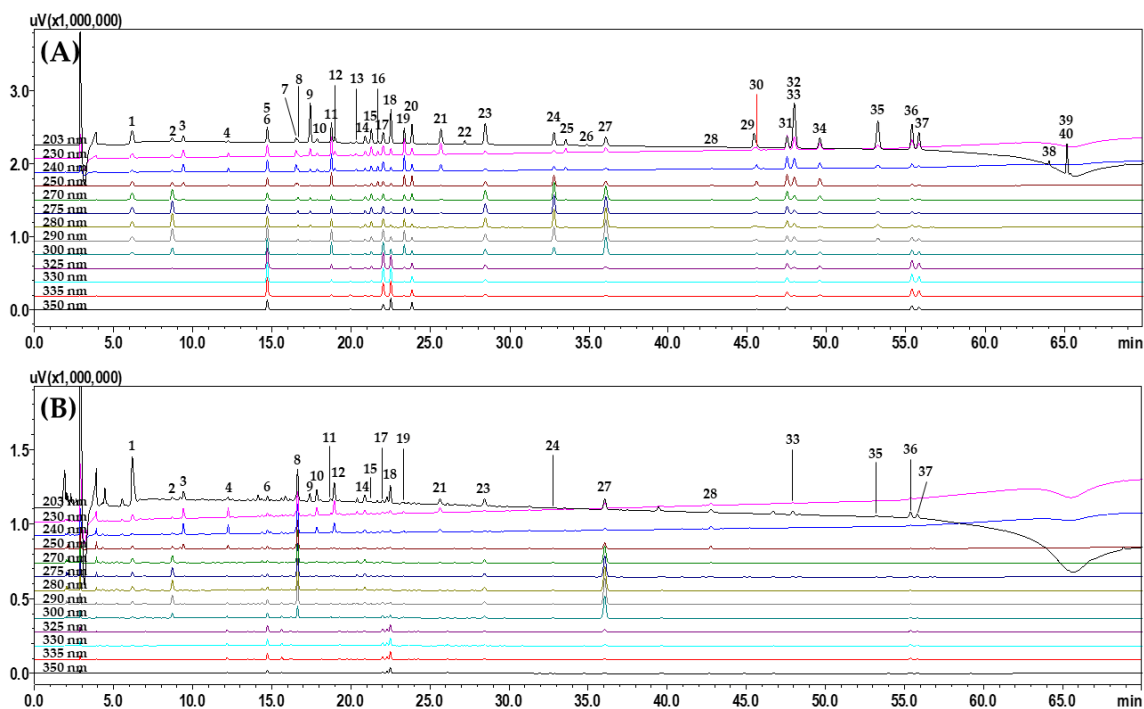


Figure S3. HPLC chromatograms of the standard solution (A) and 70% MeOH extract of DHGST sample (B). Gallic acid (1), 5-hydroxymethylfurfural (2), geniposidic acid (3), loganic acid (4), cimufugin (5), chlorogenic acid (6), geniposide (7), gentiopicroside (8), pinorelinol diglucoside (9), albiflorin (10), *prim-O*-glucosylcimufugin (11), paeoniflorin (12), genipin (13), liquiritin apioside (14), liquiritin (15), ecdysterone (16), ferulic acid (17), nodakenin (18), 5-*O*-methylvisammioside (19), quercitrin (20), benzoic acid (21), ginsenoside Rg1 (22), coumarin (23), cinnamic acid (24), benzoylpaeoniflorin (25), ginsenoside Rb1 (26), cinnamaldehyde (27), glycyrrhizin (28), 6-gingerol (29), aristolochic acid II (30), aristolochic acid I (31), β -asarone (32), methyleugenol (33), α -asarone (34), safrole (35), decursin (36), decursinol angelate (37), pachymic acid (38), continentalic acid (39), and kaurenoic acid (40).