

concentrations five times with one day. The inter-day test was analyzed by injecting three different concentrations five times with three different days (1, 3, and 5 days). The relative standard deviation (R.S.D.) value was taken as an evaluation of precision (R.S.D. = [standard deviation [SD] / mean measured amount] × 100).^[21] As listed in Table 2, the RSD values of

intra-day and inter-day test were investigated to be within the range of 0.07 - 1.92 % and 0.09 - 2.43 %, respectively, with accuracy ranges of 94.01 - 106.09 % for intra-day test and 95.71 - 104.80 % for inter-day test and maximum RSD was lower than 3.0 %, which showed a good reproducibility of this method. Accuracy of this developed HPLC method was

Table 1: Linear regression data, detection limit and quantification limit for the 10 compounds

Compound	Linear range (µg/ml)	Regression equation ^a	R ² (n=6)	LOD (µg/ml)	LOQ (µg/ml)
1	0.96 - 96.0	Y = 1.4856x + 0.7403	0.9998	0.06	0.17
2	0.14 - 11.0	Y = 1.8081x + 0.204	0.9999	0.03	0.09
3	0.30 - 24.0	Y = 0.6114x - 0.0839	0.9999	0.25	0.75
4	0.23 - 18.0	Y = 1.0947x + 0.0178	0.9999	0.07	0.22
5	1.45 - 145.0	Y = 0.1757x + 0.1314	0.999	0.28	0.83
6	0.33 - 26.0	Y = 1.5754x + 0.122	1.0000	0.17	0.53
7	0.22 - 17.5	Y = 0.6668x + 0.0575	0.9999	0.01	0.04
8	0.33 - 26.0	Y = 1.3433x + 0.0481	0.9998	0.02	0.08
9	0.25 - 20.0	Y = 1.9734x + 0.21	0.9999	0.15	0.45
10	0.15 - 12.0	Y = 2.6616x + 0.6503	0.9996	0.16	0.49

^aY: peak area, x: concentration injected of compounds

Table 2: Precision of 10 compounds

Components	Concentration (µg/ml)	Intra-day (n=5)			Inter-day (n=5)		
		Mean ± SD ^a (µg/ml)	RSD (%)	Accuracy (%)	Mean ± SD (µg/ml)	RSD (%)	Accuracy (%)
1	9.60	9.90 ± 0.17	1.70	103.14	9.86 ± 0.02	0.22	102.71
	4.80	4.86 ± 0.03	0.67	101.20	4.76 ± 0.06	1.24	99.20
	2.40	2.26 ± 0.01	0.19	94.01	2.36 ± 0.04	1.84	98.19
2	5.50	5.80 ± 0.01	0.07	105.50	5.74 ± 0.02	0.39	104.45
	2.75	2.92 ± 0.01	0.49	106.09	2.83 ± 0.01	0.48	102.83
	1.38	1.34 ± 0.02	1.85	97.29	1.38 ± 0.01	0.59	100.09
3	12.00	12.03 ± 0.07	0.57	100.28	12.06 ± 0.06	0.49	100.54
	6.00	6.17 ± 0.06	1.05	102.76	6.12 ± 0.05	0.79	101.94
	3.00	3.03 ± 0.06	1.92	101.22	3.12 ± 0.04	1.37	104.10
4	9.00	9.28 ± 0.02	0.16	103.12	9.28 ± 0.09	0.95	103.13
	4.50	4.53 ± 0.05	1.03	100.73	4.59 ± 0.01	0.19	101.91
	2.25	2.21 ± 0.01	0.49	98.25	2.29 ± 0.06	2.43	101.86
5	14.50	15.10 ± 0.13	0.83	104.13	14.82 ± 0.15	1.02	102.22
	7.25	7.32 ± 0.06	0.88	100.98	7.10 ± 0.05	0.65	97.88
	3.63	3.56 ± 0.02	0.64	98.28	3.47 ± 0.02	0.63	95.71
6	13.00	13.65 ± 0.22	1.63	104.99	13.54 ± 0.04	0.31	104.15
	6.50	6.80 ± 0.04	0.53	104.61	6.75 ± 0.01	0.09	103.86
	3.25	3.17 ± 0.05	1.60	97.62	3.27 ± 0.04	1.10	100.61
7	8.50	8.91 ± 0.10	1.15	104.86	8.62 ± 0.06	0.75	101.47
	4.25	4.20 ± 0.07	1.72	98.81	4.23 ± 0.02	0.56	99.56
	2.13	2.08 ± 0.03	1.49	97.92	2.18 ± 0.02	0.75	102.71
8	13.00	13.45 ± 0.03	0.25	103.46	13.46 ± 0.03	0.20	103.54
	6.25	6.83 ± 0.12	1.83	105.07	6.62 ± 0.05	0.71	101.88
	3.25	3.37 ± 0.03	0.97	103.64	3.26 ± 0.07	2.27	100.23
9	10.00	9.73 ± 0.05	0.47	97.28	10.12 ± 0.12	1.15	101.22
	5.00	5.02 ± 0.06	1.10	100.36	5.10 ± 0.04	0.80	101.94
	2.50	2.36 ± 0.02	0.94	94.21	2.61 ± 0.02	0.73	104.25
10	6.00	5.86 ± 0.09	1.52	97.67	6.11 ± 0.08	1.28	101.76
	3.00	3.14 ± 0.03	0.99	104.76	3.14 ± 0.01	0.18	104.80
	1.50	1.58 ± 0.02	1.26	105.37	1.53 ± 0.01	0.79	101.83

^aThe values were means ± SD of five injection

determined by recovery test. Recovery test was executed by the standard addition method. Three different concentrations of mixed 10 standards were spiked in sample solution. The solutions were injected three times. Recoveries varied from 92.82 to 107.96% at all concentration of 10 compounds. And RSD values were all less than 2.18% [Table 3].

Table 3: Recovery of 10 compounds

Components	Spiked amount (µg/ml)	Measured amount (µg/ml)	Recovery ^a (n=3, %)	RSD (%)
1	4.80	5.14 ± 0.04	107.11	0.75
	2.40	2.58 ± 0.01	105.87	0.18
	1.20	1.18 ± 0.02	98.74	1.95
2	2.75	2.93 ± 0.03	106.55	0.86
	1.38	1.47 ± 0.02	106.73	1.53
	0.69	0.65 ± 0.01	94.45	2.18
3	6.00	6.35 ± 0.09	105.82	1.48
	3.00	3.02 ± 0.01	100.73	0.45
	1.50	1.48 ± 0.02	98.90	1.59
4	4.50	4.65 ± 0.04	103.37	0.95
	2.25	2.25 ± 0.03	99.82	1.12
	1.13	1.12 ± 0.01	99.47	0.46
5	7.25	7.76 ± 0.06	107.05	0.81
	3.63	3.50 ± 0.06	96.47	1.71
	1.81	1.79 ± 0.01	98.56	0.22
6	6.50	6.46 ± 0.10	99.36	1.49
	3.25	3.25 ± 0.05	100.03	1.44
	1.63	1.59 ± 0.01	97.99	0.48
7	4.25	4.45 ± 0.01	104.62	0.30
	2.13	2.27 ± 0.02	106.85	0.89
	1.06	1.00 ± 0.02	94.57	1.54
8	6.50	7.02 ± 0.02	107.96	0.29
	3.25	3.41 ± 0.01	104.79	0.26
	1.63	1.70 ± 0.01	104.67	0.80
9	5.00	5.11 ± 0.08	102.22	1.48
	2.50	2.20 ± 0.03	99.39	1.43
	1.25	1.24 ± 0.01	93.71	0.87
10	3.00	3.24 ± 0.01	107.88	0.12
	1.50	1.39 ± 0.01	92.82	0.64
	0.75	0.75 ± 0.01	99.58	1.77

^aThe recovery (%) was measured from the equation of (the amount from sample and added standard - amount from sample) / the amount from added standard × 100

Analysis of *C. paniculatum* samples

The developed HPLC-DAD method was applied to a simultaneous determination of the 10 bioactive compounds in *C. paniculatum* sample and well separated [Figure 2b]. Peaks were identified by comparison of retention time and UV spectra with standards. The amounts of compounds 1-10 in seven *C. paniculatum*

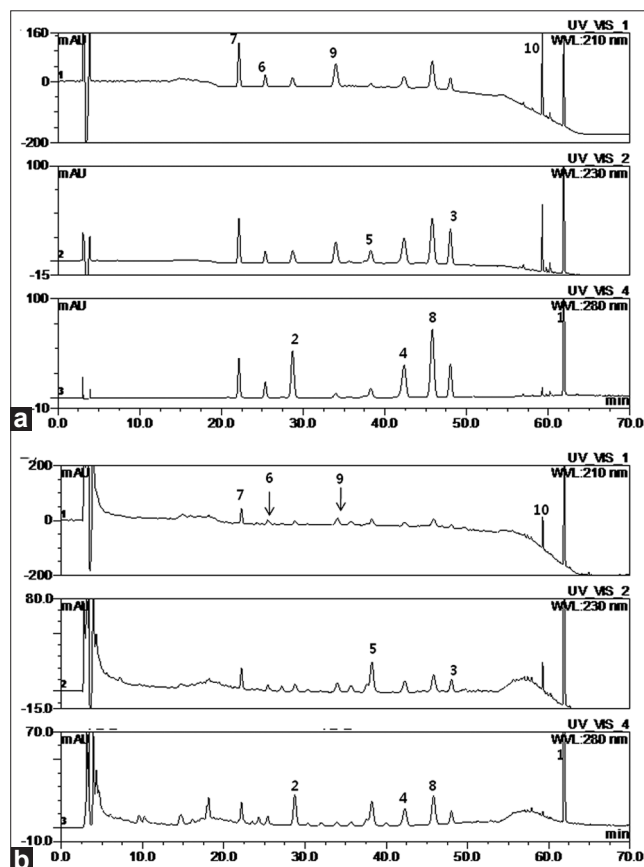


Figure 2: The HPLC chromatogram of standard mixture (a) and *Cynanchum paniculatum* Kitagawa (b). (1) paeonol, (2) 4-acetylphenol, (3) 2,5-dihydroxy-4-methoxyacetophenone, (4) 4-O-methyl ether Gallacetophenone, (5) acetoveratrone, (6) 2,5-dimethoxyhydroquinone, (7) vanillic acid, (8) resacetophenone, (9) m-acetylphenol, and (10) 3,5-dimethoxyhydroquinone

Table 4: Contents of 10 compounds in seven *C. paniculatum* samples

Compound	Content (µg/mg)						
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
1	27.60 ± 0.001	26.00 ± 0.003	25.96 ± 0.002	26.02 ± 0.010	25.99 ± 0.005	26.17 ± 0.001	26.01 ± 0.001
2	0.17 ± 0.002	0.19 ± 0.005	0.16 ± 0.004	0.15 ± 0.002	0.15 ± 0.002	0.15 ± 0.003	0.15 ± 0.004
3	0.02 ± 0.003	0.03 ± 0.004	0.03 ± 0.005	0.02 ± 0.004	0.04 ± 0.006	0.02 ± 0.004	0.03 ± 0.005
4	0.14 ± 0.001	0.15 ± 0.001	0.15 ± 0.002	0.13 ± 0.003	0.12 ± 0.004	0.16 ± 0.005	0.12 ± 0.004
5	3.97 ± 0.089	3.87 ± 0.022	4.21 ± 0.045	4.42 ± 0.054	4.36 ± 0.032	3.88 ± 0.041	3.97 ± 0.032
6	0.10 ± 0.006	0.11 ± 0.007	0.17 ± 0.005	0.19 ± 0.004	0.15 ± 0.005	0.13 ± 0.007	0.12 ± 0.005
7	0.21 ± 0.006	0.24 ± 0.004	0.30 ± 0.006	0.29 ± 0.005	0.25 ± 0.004	0.24 ± 0.005	0.28 ± 0.06
8	0.08 ± 0.001	0.08 ± 0.002	0.09 ± 0.002	0.08 ± 0.005	0.09 ± 0.004	0.07 ± 0.004	0.09 ± 0.003
9	0.07 ± 0.008	0.07 ± 0.004	0.08 ± 0.005	0.07 ± 0.007	0.07 ± 0.006	0.07 ± 0.005	0.07 ± 0.004
10	0.08 ± 0.001	0.09 ± 0.002	0.08 ± 0.003	0.07 ± 0.004	0.08 ± 0.012	0.07 ± 0.004	0.08 ± 0.005

samples obtained from markets of different regions were calculated from calibration curve. The results shown in Table 4 indicated that the contents of 10 compounds in seven *C. paniculatum* samples were slightly different. As results, we identified that quality of *C. paniculatum* was affected by the growth environment.

CONCLUSION

A HPLC-DAD method for simultaneous determination of 10 bioactive compounds isolated from *C. paniculatum* has been newly developed and successfully separated. Validation of this method was evaluated by linearity, precision, and accuracy test. The results of validation identified that developed method was rapid, accurate, and reliable. This method was useful for qualitative and quantitative analysis of bioactive compounds in *C. paniculatum*.

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