Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Simultaneous Determination of Eight Bioactive Compounds in *Dianthus superbus* by High-performance Liquid Chromatography

Bo-Ra Yun[1], Hye Jin Yang[1], Jin Bae Weon[1], Jiwoo Lee[1], Min Rye Eom[1], Choong Je Ma[1,2*]

[1]Department of Medical Biomaterials Engineering, College of Biomedical Science, Kangwon National University, [2]Department of Biomaterials Engineering, Institute of Bioscience and Biotechnology, Kangwon National University, Chuncheon 200-701, Korea

Submitted: 06-07-2015 Revised: 04-08-2015 Published: 11-05-2016

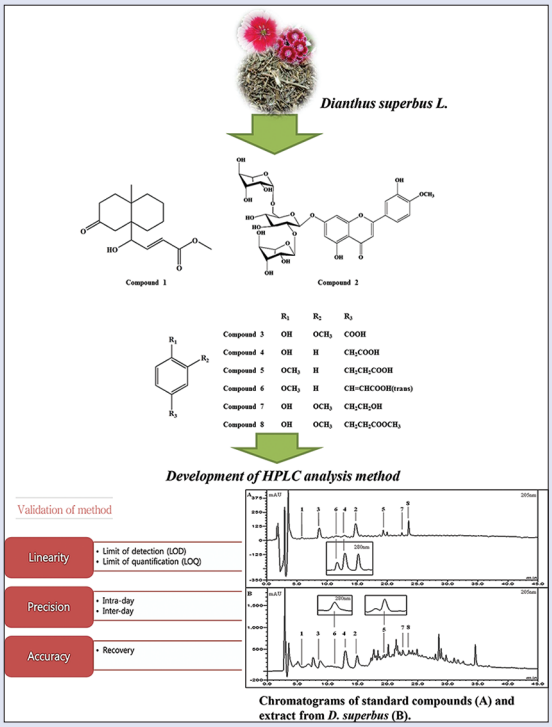
ABSTRACT

Background: *Dianthus superbus*, one of traditional herbal medicine, is widely used to treat urethritis, carbuncles and carcinoma. **Objective:** A simultaneous determination method was established for controlling the quality of *D. superbus* using the eight compounds, (E)-methyl-4-hydroxy-4-(8a-methyl-3-oxodecahydronaphthalen-4a-yl) (1), diosmetin-7-O(2'',6''-di-O-α-L-rhamnopyranosyl)-β-D-glucopyranoside (2), vanillic acid (3), 4-hydroxyphenyl acetic acid (4), 4-methoxyphenyl acetic acid (5), (E)-4-methoxycinnamic acid (6), 3-methoxy-4-hydroxyphenylethanol (7), and methyl hydroferulate (8) isolated from *D. superbus*. **Materials and Methods:** This analysis method was developed using high performance liquid chromatography coupled with diode array detector with a Shishedo C_{18} column at a column temperature of 3°C. The mobile phase was composed of 0.1% trifluoroacetic acid in water and acetonitrile. The flow rate was 1 ml/min and detection wavelength was set at 205 nm and 280 nm. Validation was performed in order to demonstrate selectivity, accuracy and precision of the method. **Results:** The calibration curves showed good linearity ($R^2 > 0.99$). The limits of detection and limits of quantification were within the ranges 0.0159–0.6205 μg/ml and 0.3210–1.8802 μg/ml, respectively. Moreover, the relative standard deviations of intra- and inter-day precision were both <2.98%. The overall recoveries were in the range of 96.23–109.87%. Quantitative analysis of eight compounds in 12 *D. superbus* samples (D-1–D-12) from various regions were analyzed and compared by developed method. **Conclusion:** As a result, this established method was accurate and sensitive for the quality evaluation of eight compounds isolated from *D. superbus* and may provide a new basis for quality control of *D. superbus*.

Key words: *Dianthus superbus*, quality control, simultaneous determination

SUMMARY

- A simultaneous determination method of eight compounds in *Dianthus superbus* was established by high performance liquid chromatography-diode array detector
- Developed analysis method is validated with linearity, precious and accuracy
- The newly established method was successfully evaluated contents of eight compounds in 12 *D. superbus* samples (D-1–D-12) from various regions and compared.



Chromatograms of standard compounds (A) and extract from *D. superbus* (B).

Abbreviations used: HPLC: High performance liquid chromatography, LOD: Limits of detection, LOQ: Limits of quantification, RSD: Relative standard deviation.

Correspondence:

Prof. Choong Je Ma,
Department of Medical Biomaterials Engineering,
College of Biomedical Science, Kangwon
National University, Hyoja-2 Dong,
Chuncheon 200-701, Republic of Korea.
E-mail: cjma@kangwon.ac.kr
DOI: 10.4103/0973-1296.182159

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Dianthus superbus belonging to the *Caryophyllaceae* family is widely distributed in China and Korea. *D. superbus* is a traditional herbal medicine for treating urethritis, carbuncles and carcinoma. Previous studies on *D. superbus* showed various biologically activities, including antioxidant, antimicrobial, anticancer, and anti-inflammatory activity.[1-3] *D. superbus* also showed immunosuppressive effects, osteoblastic proliferative, suppress immunoglobulin E production and prevent peanut-induced

Cite this article as: Yun BR, Yang HJ, Weon JB, Lee J, Eom MR, Ma CJ. Simultaneous determination of eight bioactive compounds in *Dianthus superbus* by high-performance liquid chromatography. Phcog Mag 2016;12:S264-9.

anaphylaxis.[4-7] Especially, ethyl acetate fraction of *D. superbus* was found to possess the cytotoxic activity against cancer cells.[8] Dianthosaponins, dianthramide, flavonoid, coumarin, triterpenoid, pyran type glycoside, and cyclic peptides have been isolated from *D. superbus*.[9-15] We isolated eight compounds, (E)-methyl-4-hydroxy-4-(8a-methyl-3-oxodecahydronaphthalen-4a-yl) (1), diosmetin-7-O (2",6"-di-O-α-L-rhamnopyranosyl)-β-D- glucopyranoside (2), vanillic acid (3), 4-hydroxyphenyl acetic acid (4), 4-methoxyphenyl acetic acid (5), (E)-4-methoxycinnamic acid (6), 3-methoxy-4-hydroxyphenylethanol (7), and methyl hydroferulate (8) from *D. superbus* and these compounds were used for simultaneous determination of *D. superbus*.

Herbal medicines are composed with various chemical compounds such as flavonoids, terpenoids, saponins, and phenols. These diversity compounds exhibited therapeutic efficacy of herbal medicines, but make some difficulties at quality control of herbal medicine. The contents of bioactive compounds were depended on the location, climate, and other cultivation factor. Considering these various factors, the quality control of the raw herbal medicines such as *D. superbus* has become important. Recently, many researchers develop analytical methods for quality control due to increase use of herbal medicines. Among the analytical methods, high performance liquid chromatography (HPLC) is the most popular one, because of its easy operation, side suitability, and high accuracy.[16]

In this paper, a simple and accurate HPLC-diode array detector (DAD) method was firstly developed and validated for the simultaneous determination of the eight compounds in *D. superbus*. This method was successfully applied to simultaneous quantification of *D. superbus* samples.

MATERIALS AND METHODS

Chemicals and reagents

The herbal samples of *D. superbus* were obtained from Kyungdong traditional herbal market in Seoul, Korea and identified by Dr. Young Bae Seo, a professor of the College of Oriental Medicine, Daejeon University, Korea. A voucher specimen (CJ004M) has been deposited in the natural products laboratory, the Kangwon National University, Chuncheon, Korea. D-1–D-7 and D-8–D-12 samples were originated from Korea and China, respectively.

HPLC grade water and acetonitrile was purchased from J.T. Baker. Analytical grade trifluoroacetic acid (TFA) was obtained from Dae Jung.

Isolation of compounds

(E)-methyl-4-hydroxy-4-(8a-methyl-3-oxodecahydronaphthalen-4a-yl) (1), diosmetin-7-O (2",6"-di-O-α-L-rhamnopyranosyl)-β-D- glucopyranoside (2), vanillic acid (3), 4-hydroxyphenyl acetic acid (4), 4-methoxyphenyl acetic acid (5), (E)-4-methoxycinnamic acid (6), 3-methoxy-4- hydroxyphenylethanol (7), and methyl hydroferulate (8) were isolated in our laboratory [Figure 1]. The structures were confirmed by comparing their mass spectrometer and nuclear magnetic resonance data with literature data.[17-24] The purities of the eight standard compounds were above 98%.

Eight compound was isolated to a silica gel open column chromatography (CC) (90 cm × 10 cm, 70–230 mesh) using a gradient of n-hexane-EtOAc (100:1 → 0:1, v/v) on the EtOAc fraction.

7 fractions (A–G) were obtained from EtOAC fraction. Fraction F was performed to silica gel CC (EtOAc-MeOH from 50:1–0:1, v/v) to obtained 9 fractions (F1–F9).

Fraction F7 was subjected to medium-pressure liquid chromatography (MPLC) and four sub-fractions were collected. Compound 1 (51.3 mg) was purified with the Sephadex LH-20 and the MPLC.

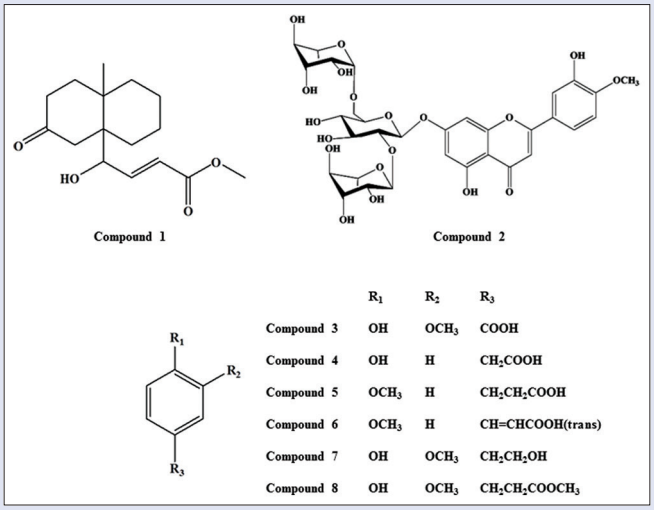


Figure 1: Chemical structures of compounds 1–8. 1: (E)-methyl-4-hydroxy-4-(8a-methyl-3-oxodecahydronaphthalen-4a-yl); 2: Diosmetin-7-O (2",6"-di-O-α-L-rhamnopyranosyl)-β-D-glucopyranoside; 3: Vanillic acid; 4: 4-hydroxyphenyl acetic acid; 5: 4-methoxyphenyl acetic acid; 6: (E)-4-methoxycinnamic acid, 7: 3-methoxy-4-hydroxyphenylethanol 8: Methyl hydroferulate

Among the compounds, compound 1, (E)-methyl-4-hydroxy-4-(8a-methyl-3-oxodecahydronaphthalen- 4a-yl) was first isolated.

(E)-methyl-4-hydroxy-4-(8a-methyl-3-oxodecahydronaphthalen-4a-yl)

Yellow oil; ^{1}H NMR (CD_3OD 400 MHz): δ 0.94 (3H, *s*, H-11), δ 1.17–2.31 (10H, *m*, H-4, 5, 6, 7, 9), δ 2.40–2.77 (4H, *m*, H-2, 10), δ 3.73 (3H, *s*, H-16), δ 3.89 (1H, *dd*, *J* = 7.58, 2.85 Hz, H-12), δ 6.01 (1H, *dd*, *J* = 15.26, 5.43 Hz, H-13), δ 6.16 (1H, *dd*, *J* = 15.26, 1.06 Hz, H-14). ^{13}C NMR (CD_3OD 100 MHz) δ 18.20 (C-11), δ 23.01 (C-5), δ 23.77 (C-6), δ 29.00 (C-4), δ 29.14 (C-7), δ 33.77 (C-8), δ 36.85 (C-9), δ 42.28 (C-10), δ 50.99 (C-16), δ 52.27 (C-3), δ 52.95 (C-2), δ 77.40 (C-12), δ 139.67 (C-13), δ 124.67 (C-14), δ 174.99 (C-15), δ 210.25 (C-1).

Standard solution and Sample preparation

Eight Standard compounds, (E)-methyl-4-hydroxy-4-(8a-methyl-3-oxodecahydronaphthalen-4a-yl) (200 μg/ml), diosmetin- 7-O (2",6"-di-O-α-L-rhamnopyranosyl)-β-D-glucopyranoside (200 μg/ml), vanillic acid (200 μg/ml), 4-hydroxyphenyl acetic acid (200 μg/ml), 4-methoxyphenyl acetic acid (200 μg/ml), (E)-4-methoxycinnamic acid (200 μg/ml), and 3-methoxy-4-hydroxyphenylethanol (200 μg/ml) were accuracy weighed and prepared in methanol. These were diluted to prepare solutions with six different concentrations for the establishment of calibration curves. Solutions were filtered through 0.45 μm membrane filter before HPLC analysis. Then, all solutions were stored in the refrigerator at 4°C.

Dried *D. superbus* extract powders were correctly weighed (10 mg) and dissolved in methanol (1 ml). These extract solutions also were filtered through a 0.45 μm membrane filter before sample injection.

High performance liquid chromatography-diode array detector analysis

The HPLC system is consisted of Dionex system with LPG 3X00 pump, ACC-3000 auto sampler, column oven, and DAD-3000(RS) DAD ultraviolet (UV)–visible. Separation was accomplished using a Shiseido C_{18} column (4.6 mm ID × 250 mm, 5 μm pore size). Column temperature was maintained at 30°C. The mobile phase was composed of 0.1% TFA aqueous

solution (A) and acetonitrile (B) at a flow rate of 1.0 ml/min. The gradient flows were as follows: 15% B at 0–10 min, 15–25% B at 10–15 min, 25–70% B at 15–40 min, 70% B at 40–45 min. The injection volume was 20 µL. The wavelength of UV detection was set at 205 and 280 nm, respectively.

Calibration curves and limits of detection and quantification

The stock standard mixture was diluted with methanol to appropriate concentration ranges for the construction of calibration curves. Calibration curves were established by plotting the peak area (y) versus concentration (x) of each analyte. The linearity was measured by correlation coefficient (R^2) values. Limit of detection (LOD) and quantification (LOQ) were determined using signal-to-noise ratios (S/N) of 3 and 10, respectively.

Precision and recovery

Intra- and inter-day test were conducted to determine the precision of the developed method. Validation of HPLC method with precision and recovery was performed according to the International Conference on Harmonization guidelines.[25] The intraday test was analyzed by standard solutions at three different concentrations on the single day and the inter-day test was performed on the three different days (1, 3, 5 days). In order to confirm the repeatability, each sample was analyzed 5 times as described above. The relative standard deviation (RSD) was taken as a measure of repeatability. RSD value determined repeatability of method.

Recovery test was used to investigate the accuracy of this analysis method. The standard solutions with known three different concentrations (5.56, 2.78, and 1.39 µg/mL) were added to *D. superbus* sample and then analyzed in 3 times. Recovery was calculated by the equation.

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

RESULTS AND DISCUSSION

Method development

To obtain optimal analytic conditions, various HPLC parameters were tested, including column, mobile phase, and gradient elution system and flow rate. Based on the resolution, baseline and retention time, the analytical conditions were optimized. To improve the peak shape, TFA was added to the water. The detection wavelength was optimized at 205 nm for (E)-methyl-4-hydroxy-4-(8a-methyl-3-oxodecahydronaphthalen-4a-yl), diosmetin-7-O (2”,6”-di-O-α-L-rhamnopyranosyl)-β-D- glucopyranoside, vanillic acid, 3-methoxy-4-hydroxyphenylethanol and methyl hydroferulate and 280 nm for 4-hydroxy-phenylacetic acid, 4-methoxyphenylacetic acid, (E)-4-methoxycinnamic acid. About 20 µl was found to be the suitable injection volume. All peak of each compound were separated successful within 45 min. HPLC chromatogram of the eight compounds is shown in Figure 2. The peaks of each compound were confirmed by comparing their retention time in the HPLC chromatogram and the UV spectrum.

Method validation

Calibration curves with six concentrations of standard solution were conducted in the concentration range of 0.69–22.22 µg/ml. The calibration data of the eight compounds showed a good linearity ($R^2 > 0.98$). LOD and LOQ, which were measured by calibration curve, were in the range 0.12–1.91 µg/ml and 0.37–5.80 µg/ml, respectively [Table 1].

In inter- and intra-day test, the RSD values were taken as a measure of precision. As a result, the RSD values of the intra- and inter-day tests were found to be within the ranges 0.32–2.81% and 0.25–2.92%, respectively. And accuracy of intra- and inter-day was ranged from 91.14–109.70% and 93.61–108.49%, respectively [Table 2]. The recovery of the selected maker compounds ranged from 96.23 to 109.87%, and their RSD values were <2.73% [Table 3].

Dianthus superbus samples analysis and cluster analysis

The newly established analytical method was applied to the analysis of eight compounds in 12 *D. superbus* samples (D-1–D-12) from various regions. The samples were analyzed using the optimized HPLC

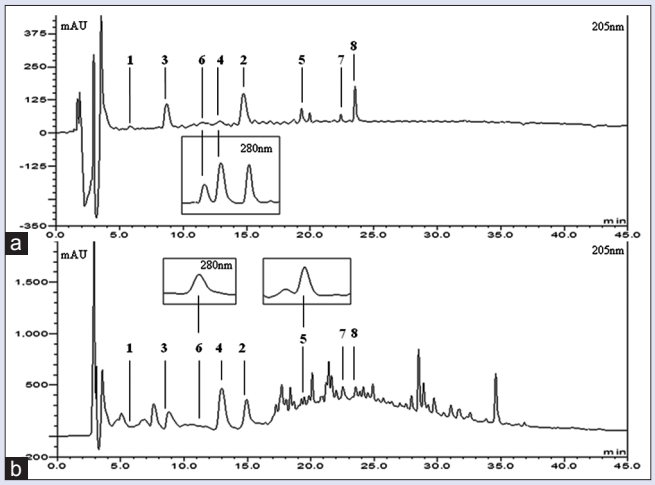


Figure 2: Chromatograms of standard compounds (a) and extract from *Dianthus superbus* (b). 1: (E)-methyl-4-hydroxy-4-(8a-methyl-3-oxodecahydronaphthalen-4a-yl); 2: Diosmetin-7-O (2”,6”-di-O-α-L-rhamnopyranosyl)-β-D-glucopyranoside; 3: Vanillic acid; 4: 4-hydroxyphenyl acetic acid; 5: 4-methoxyphenyl acetic acid; 6: (E)-4-methoxycinnamic acid; 7: 3-methoxy-4-hydroxyphenylethanol; 8: Methyl hydroferulate

Table 1: The regression data, limits of detection and limits of quantifications for eight compounds analyzed by HPLC-DAD

Compound	Regression equation	R^2	Linear range (µg/ml)	LOD (µg/ml)	LOQ (µg/ml)
1	Y[a]=0.202x−0.14	0.9869	0.69-22.22	0.81	2.45
2	Y=3.864x+3.122	0.9934	0.69-22.22	0.55	1.67
3	Y=1.744x−0.578	0.9974	0.69-22.22	1.91	5.80
4	Y=0.421x−0.081	0.9999	0.69-22.22	0.18	0.55
5	Y=0.678x+0.089	0.9999	0.69-22.22	0.12	0.37
6	Y=0.174x−0.025	0.9998	0.69-22.22	0.44	1.35
7	Y=0.170x+0.305	0.9883	0.69-22.22	1.36	4.12
8	Y=1.002x+0.692	0.9990	0.69-22.22	0.20	0.61

[a]Y: Peak area; x: Amount (µg); 1: (E)-methyl-4-hydroxy-4-(8a-methyl-3-oxodecahydronaphthalen-4a-yl); 2: Diosmetin-7-O (2”,6”-di-O-α-L-rhamnopyranosyl)-β-D-glucopyranoside; 3: Vanillic acid; 4: 4-hydroxyphenyl acetic acid; 5: 4-methoxyphenyl acetic acid; 6: (E)-4-methoxycinnamic acid; 7: 3-methoxy-4-hydroxyphenylethanol; 8: Methyl hydroferulate; LOD: Limits of detection; LOQ: Limits of quantification; HPLC-DAD: High performance liquid chromatography-diode array detector

Table 2: Intra- and inter-day precision of eight compounds. (E)-methyl-4-hydroxy-4-(8a-methyl-3-oxodecahydronaphthalen-4a-yl) (1), diosmetin-7-O (2″,6″-di-O-α-L-rhamnopyranosyl)-β-D-glucopyranoside (2), vanillic acid (3), 4-hydroxyphenyl acetic acid (4), 4-methoxyphenyl acetic acid (5), (*E*)-4-methoxycinnamic acid (6), 3-methoxy-4-hydroxyphenylethanol (7), and methyl hydroferulate (8)

Compound	Concentration (μg/ml)	Intra-day			Inter-day		
		Mean (μg/ml)	RSD[a] (%)	Accuracy (%)	Mean (μg/ml)	RSD[a] (%)	Accuracy (%)
1	11.11	11.34±0.16	1.40	102.07	10.91±0.13	1.20	98.21
	5.56	5.85±0.16	2.81	105.22	6.03±0.11	1.84	108.49
	2.78	2.70±0.05	1.76	97.04	2.87±0.03	1.10	103.18
2	11.11	10.55±0.08	0.73	94.91	11.48±0.03	0.25	103.36
	5.56	5.39±0.11	2.10	97.02	5.59±0.03	0.49	100.52
	2.78	2.61±0.06	2.19	93.97	2.72±0.04	1.37	97.70
3	11.11	11.85±0.25	2.08	106.44	11.49±0.17	1.47	103.43
	5.56	5.75±0.16	2.77	103.44	5.86±0.12	2.03	105.46
	2.78	3.05±0.04	1.28	109.70	2.96±0.03	1.15	106.60
4	11.11	11.49±0.27	2.38	103.45	11.39±0.11	0.94	102.56
	5.56	5.87±0.11	1.89	105.64	5.60±0.14	2.42	100.80
	2.78	2.53±0.06	2.17	91.14	2.69±0.07	2.61	96.76
5	11.11	11.48±0.15	1.31	103.37	11.57±0.11	0.98	104.18
	5.56	5.77±0.08	1.31	103.86	5.79±0.08	1.36	104.20
	2.78	2.58±0.01	0.46	92.97	2.72±0.08	2.90	97.71
6	11.11	11.72±0.32	2.73	105.49	11.73±0.27	2.28	105.58
	5.56	5.90±0.13	2.17	106.20	5.78±0.14	2.37	103.90
	2.78	2.63±0.01	0.39	94.55	2.79±0.07	2.53	100.26
7	11.11	10.26±0.13	1.23	92.34	10.40±0.30	2.92	93.61
	5.56	5.44±0.12	2.26	97.82	5.50±0.07	1.32	98.93
	2.78	2.93±0.01	0.47	105.51	3.00±0.07	2.43	107.87
8	11.11	11.96±0.13	1.07	107.66	11.98±0.28	2.37	107.82
	5.56	6.07±0.16	2.65	109.25	5.87±0.16	2.71	105.58
	2.78	2.65±0.01	0.32	95.42	2.68±0.06	2.13	96.24

RSD[a] = [a]Relative standard deviation

Table 3: Recovery of the eight compounds. (E)-methyl-4-hydroxy-4-(8a-methyl-3-oxodecahydronaphthalen-4a-yl) (1), diosmetin-7-O (2″,6″-di-O-α-L-rhamnopyranosyl)-β-D-glucopyranoside (2), vanillic acid (3), 4-hydroxyphenyl acetic acid (4), 4-methoxyphenyl acetic acid (5), (*E*)-4-methoxycinnamic acid (6), 3-methoxy-4-hydroxyphenylethanol (7), and methyl hydroferulate (8)

Compound	Spiked (μg/ml)	Found (μg/ml)	RSD[a] (%)	Recovery[b] (%)
1	5.56	5.47±0.02	0.30	98.39
	2.78	3.03±0.06	2.03	109.03
	1.39	1.44±0.02	1.50	103.58
2	5.56	6.11±0.01	0.17	109.87
	2.78	2.77±0.07	2.38	99.49
	1.39	1.46±0.01	0.90	104.89
3	5.56	5.58±0.04	0.63	100.28
	2.78	3.04±0.04	1.17	109.46
	1.39	1.68±0.04	2.43	101.19
4	5.56	5.95±0.07	1.21	107.05
	2.78	3.02±0.03	1.05	108.58
	1.39	1.35±0.01	0.51	97.37
5	5.56	5.48±0.08	1.40	98.73
	2.78	2.92±0.02	0.60	105.24
	1.39	1.46±0.04	2.73	104.81
6	5.56	6.10±0.03	0.51	109.78
	2.78	2.98±0.07	2.39	107.17
	1.39	1.34±0.02	1.24	96.23
7	5.56	5.65±0.06	0.98	101.53
	2.78	2.82±0.02	0.58	101.42
	1.39	1.46±0.03	2.33	105.34
8	5.56	5.60±0.01	0.27	100.74
	2.78	2.91±0.01	0.36	104.80
	1.39	1.44±0.03	1.81	103.58

RSD[a] = [a]Relative standard deviation. [b]Recovery (%) = (amount found-original amount)/amount spiked × 100%.

condition in triplicate to determine the mean content. The contents of eight compounds in *D. superbus* were calculated from the calibration curves of standard. Table 4 exhibited the results of sample analysis. The results show that 4-hydroxy-phenylacetic acid was the highest content (83.21–85.58 μg/mg) among the isolated compounds in *D. superbus* sample. Contents of 4-methoxyphenylacetic acid (0.87–1.27 μg/mg) and methyl hydroferulate (1.35–1.68 μg/mg) were lower than other compounds in 12 *D. superbus* samples.

We performed hierarchical cluster analysis (HCA) using IBM SPSS Statistics 21 (SPSS Inc., an IBM Company). Samples were investigated based on contents of eight compounds. HCA analysis has shown difference among different *D. superbus* sample origins. Difference from samples was represented by Euclidean distance [Figure 3]. Samples were divided into three clusters (I, II, III). Cluster I consisted of samples originated from Korea exclude D-10 and D-11 samples. Cluster II and III consisted of samples originated from China exclude D-3 sample. The samples clustered one group was associated with similar content of compounds. The result showed contents of compounds in *D. superbus* samples is different by cultivation environment and provided reference for the quality control of *D. superbus*.

Simultaneous determination of eight compounds, (E)-methyl-4-hydroxy-4-(8a-methyl-3- oxodecahydronaphthalen-4a-yl) (1), diosmetin-7-O (2”,6”-di-O-α-L-rhamnopyranosyl)-β-D-gluc opyranoside (2), vanillic acid (3), 4-hydroxyphenyl acetic acid (4), 4-methoxyphenyl acetic acid (5), (E)-4-methoxycinnamic acid (6), 3-methoxy-4- hydroxyphenylethanol (7), and methyl hydroferulate (8) in *D. superbus* was established for quantitative analysis of *D. superbus* and method validation including linearity, precision and accuracy. The results of linearity indicate that this established method has high

Table 4: Contents of eight compounds in 12 *Dianthus superbus* samples

Sample	Content (μg/mg)							
	1	2	3	4	5	6	7	8
D-1	13.63±0.59	2.54±0.02	5.28±0.08	84.12±0.08	1.27±0.02	3.12±0.40	10.25±0.70	1.58±0.09
D-2	14.71±0.28	2.55±0.01	5.34±0.04	84.27±0.02	1.22±0.11	3.89±0.27	11.02±0.16	1.50±0.02
D-3	13.76±0.55	2.57±0.01	5.20±0.15	84.16±0.82	0.87±0.06	3.21±0.59	11.65±0.10	1.68±0.01
D-4	14.05±0.87	2.54±0.04	5.00±0.07	84.13±0.54	1.03±0.02	3.11±0.26	10.40±0.90	1.53±0.15
D-5	14.26±0.44	2.55±0.01	5.03±0.07	84.15±0.06	1.11±0.18	3.61±0.35	10.09±0.38	1.51±0.10
D-6	13.71±0.48	2.76±0.02	5.09±0.03	84.14±0.01	0.89±0.11	3.16±0.23	10.21±0.08	1.54±0.09
D-7	14.73±0.42	2.54±0.01	4.92±0.18	84.16±0.07	1.12±0.02	2.95±0.38	9.24±0.09	1.46±0.01
D-8	13.66±0.44	2.56±0.05	4.86±0.14	85.58±0.72	1.02±0.02	3.46±0.25	9.08±0.91	1.43±0.01
D-9	12.99±0.48	2.51±0.03	5.15±0.11	84.13±0.54	1.04±0.01	4.12±0.25	9.28±0.58	1.44±0.09
D-10	13.71±0.80	2.59±0.04	5.21±0.06	84.13±0.48	1.12±0.02	3.27±0.15	8.66±0.77	1.43±0.04
D-11	14.16±0.77	2.57±0.01	5.09±0.08	84.16±0.39	1.15±0.02	3.53±0.88	8.19±0.48	1.35±0.01
D-12	13.27±0.54	2.51±0.01	5.20±0.05	83.21±1.25	1.19±0.01	3.02±0.14	9.95±0.98	1.65±0.16

D-1–D-12: 12 *Dianthus superbus* samples. 1: (E)-methyl-4-hydroxy-4-(8a-methyl-3-oxodecahydronaphthalen-4a-yl); 2: Diosmetin-7-O (2”,6”-di-O-α-L-rh amnopyranosyl)-β-D-glucopyranoside; 3: Vanillic acid; 4: 4-hydroxyphenyl acetic acid; 5: 4-methoxyphenyl acetic acid; 6: (E)-4-methoxycinnamic acid; 7: 3-methoxy-4-hydroxyphenylethanol; 8: Methyl hydroferulate

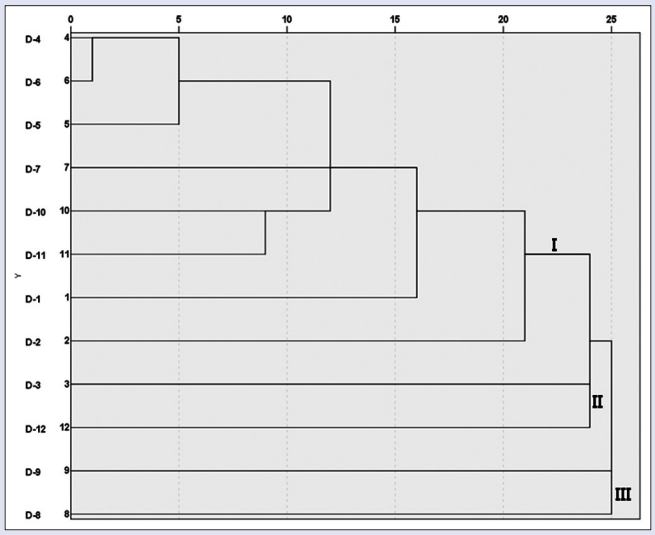


Figure 3: Dendrogram of cluster analysis for 12 *Dianthus superbus* samples

sensitivity. Intra- and inter-day variability assays were used to determine the precision of the developed method and this result demonstrated good reproducibility of this analytical method. Recovery test was performed to evaluate the accuracy of this method. The results showed that the established method was reliable and accurate.

This developed method was successfully analyzed contents of eight compounds in 12 *D. superbus* samples. In addition, we confirmed that content of *D. superbus* is different between cultural environment, such as area and climate.

CONCLUSION

In this study, we developed the simultaneous determination of eight compounds, (E)-methyl-4-hydroxy-4- (8a-methyl-3-oxodecahydronaphthalen-4a-yl) (1), diosmetin-7-O (2”,6”-di-O-α-L-rham nopyranosyl)-β-D-glucopyranoside (2), vanillic acid (3), 4-hydroxyphenyl acetic acid (4), 4-methoxyphenyl acetic acid (5), (E)-4-methoxycinnamic acid (6), 3-methoxy-4- hydroxyphenylethanol (7), and methyl hydroferulate (8) in *D. superbus* for quality control using HPLC-DAD.

This HPLC-DAD method was applied for simultaneous determination of *D. superbus* sample under optimized HPLC conditions. The results of method validation demonstrated that this developed method was sensitive, reliable and reproducible for simultaneous determination of *D. superbus*. Thus, this study may be provided analysis method for quality evaluation and identification of medicinal effect of *D. superbus* by using a HPLC-DAD.

Acknowledgments

This research was supported by a Basic Science Research Program grant from the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (2010-0005149).

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Ding C, Zhang W, Li J, Lei J, Yu J. Cytotoxic constituents of ethyl acetate fraction from *Dianthus superbus*. Nat Prod Res 2013;27:1691-4.
2. Yu JO, Liao ZX, Lei JC, Hu XM. Antioxidant and cytotoxic activities of various fractions of ethanol extract of *Dianthus superbus*. Food Chem 2007;104:1215-9.
3. Gou J, Zou Y, Ahn J. Enhancement of antioxidant and antimicrobial activities of *Dianthus superbus*, *Polygonum aviculare*, *Sophora flavescens*, and *Lygodium japonicum* by pressure-assisted water extraction. Food Sci Biotechnol 2011;20:83-7.
4. López-Expósito I, Castillo A, Yang N, Liang B, Li XM. Chinese herbal extracts of *Rubia cordifolia* and *Dianthus superbus* suppress IgE production and prevent peanut-induced anaphylaxis. Chin Med 2011;6:35.
5. Shin IS, Lee MY, Ha H, Jeon WY, Seo CS, Shin HK. *Dianthus superbus* fructus suppresses airway inflammation by downregulating of inducible nitric oxide synthase in an ovalbumin-induced murine model of asthma. J Inflamm (Lond) 2012;9:41.
6. Tong Y, Luo JG, Wang R, Wang XB, Kong LY. New cyclic peptides with osteoblastic proliferative activity from *Dianthus superbus*. Bioorg Med Chem Lett 2012;22:1908-11.
7. Reid-Adam J, Yang N, Song Y, Cravedi P, Li XM, Heeger P. Immunosuppressive effects of the traditional Chinese herb Qu Mai on human alloreactive T cells. Am J Transplant 2013;13:1159-67.
8. Yu JQ, Yin Y, Lei JC, Zhang XQ, Chen W, Ding CL, *et al.* Activation of apoptosis by ethyl acetate fraction of ethanol extract of *Dianthus superbus* in HepG2 cell line. Cancer Epidemiol 2012;36:e40-5.
9. Shimizu M, Hayashi T, Shimizu K, Morita N. A pyran-type glycoside from *Dianthus superbus* var. longicalycinus. Phytochemistry 1982;21:245-7.
10. Wang YC, Tan NH, Zhou J, Wu H. Cyclopeptides from *Dianthus superbus*. Phytochemistry 1998;49:1453-6.

11. Hsieh PW, Chang FR, Wu CC, Wu KY, Li CM, Chen SL, *et al.* New cytotoxic cyclic peptides and dianthramide from *Dianthus superbus*. J Nat Prod 2004;67:1522-7.
12. Hsieh PW, Chang FR, Wu CC, Li CM, Wu KY, Chen SL, *et al.* Longicalycinin A, a new cytotoxic cyclic peptide from *Dianthus superbus* var. longicalycinus (MAXIM.) WILL. Chem Pharm Bull (Tokyo) 2005;53:336-8.
13. Dahiya R. Synthesis and biological activity of a cyclic hexapeptide from *Dianthus superbus*. Chem Pap 2008;62:527-35.
14. Chen X, Luo JG, Kong LY. Two new triterpenoid saponins from *Dianthus superbus* L. J Asian Nat Prod Res 2010;12:458-63.
15. Luo JG, Chen X, Kong LY. Three new triterpenoid saponins from *Dianthus superbus*. Chem Pharm Bull (Tokyo) 2011;59:518-21.
16. Jiang Y, David B, Tu P, Barbin Y. Recent analytical approaches in quality control of traditional Chinese medicines – A review. Anal Chim Acta 2010;657:9-18.
17. Hon YS, Lu L, Chang RC, Linb SW, Sunb PP, Leea CF. Syntheses of α, β-unsaturated carbonyl compounds from the reactions of monosubstituted ozonides with stable phosphonium ylides. Tetrahedron 2000;56:9269-79.
18. Emam AM, Elias R, Moussa AM, Faure R, Debrauwer L, Balansard G. Two flavonoid triglycosides from *Buddleja* madagascariensis. Phytochemistry 1998;48:739-42.
19. Huang Z, Dostal L, Rosazza JP. Mechanisms of ferulic acid conversions to vanillic acid and guaiacol by *Rhodotorula* rubra. J Biol Chem 1993;268:23954-8.
20. Lehnert W, Hunkler D. Possibilities of selective screening for inborn errors of metabolism using high-resolution 1H-FT-NMR spectrometry. Eur J Pediatr 1986;145:260-6.
21. Rosecke J, Konig WA. Odorous compounds from the fungus *Gloeophyllum odoratum*. Flavour Fragr J 2000;15:315-9.
22. Yu J, Whitney PS, Spencer JB. Direct comparison between the mechanism of hydrometalation and β-elimination in heterogeneous and homogeneous hydrogenation. J Mol Catal A Chem 1999;146:199-210.
23. Shimoji Y, Tamura Y, Nakamura Y, Nanda K, Nishidai S, Nishikawa Y, *et al.* Isolation and identification of DPPH radical scavenging compounds in Kurosu (Japanese unpolished rice vinegar). J Agric Food Chem 2002;50:6501-3.
24. Doyle MP, Hu W, Valenzuela MV. Total synthesis of (S)-(+)-imperanene. Effective use of regio- and enantioselective intramolecular carbon-hydrogen insertion reactions catalyzed by chiral dirhodium (II) carboxamidates. J Org Chem 2002;67:2954-9.
25. Anonymous. ICH Guidelines: Validation of Analytical Procedures. Methodology, 2, 2003.


Choong Je Ma

ABOUT AUTHOR

Choong Je Ma has completed his PhD at the age of 32 years from Seoul National University and postdoctoral studies from University of Michigan. He is the professor of Department of Medical Biomaterials Engineering, College of Biomedical science, Kangwon National University, Korea. He has published more than 20 papers in reputed journals.