# Simultaneous determination of ten bioactive compounds from the roots of *Cynanchum paniculatum* by using high performance liquid chromatography coupled-diode array detector

**Jin Bae Weon[1], Bohyoung Lee[1], Bo-Ra Yun[1], Jiwoo Lee[1], Choong Je Ma[1,2]**

*[1]Department of Biomaterials Engineering, Division of Bioscience and Biotechnology, Kangwon National University, Chuncheon 200-701, Korea*
*[2]Research Institute of Biotechnology, Kangwon National University, Chuncheon 200-701, Korea*

Submitted: 19-07-2011 Revised: 12-10-2011 Published: 02-08-12

## ABSTRACT

**Background:** *Cynanchum paniculatum* Kitagawa belongs to Asclepiadaceae and was used in traditional medicine to invigorate blood, alleviate edema, relieve pain, and relieve toxicity for a long time. **Objective:** A novel and reliable high performance liquid chromatography coupled with diode array detector method has been established for simultaneous determination of 10 bioactive compounds isolated from *Cynanchum paniculatum* Kitagawa, one of the herbal medicines. **Materials and Methods:** The chromatography analysis was performed on a SHISEIDO $C_{18}$ column (S-5 μm, 4.6 mm I.D. × 250 mm) at 35°C with a gradient elution of acetonitrile and water at a flow rate of 1ml/min and UV detection at 210, 230, and 280 nm. **Results:** The method was validated for linearity, precision, and accuracy. All calibration curves showed good linear regression ($r^2$ > 0.9996). Limits of detection (LOD) and limits of quantification (LOQ) fell in the ranges 0.01 - 0.28 μg/ml and 0.04 - 0.83 μg/ml, respectively. The relative standard deviation (RSD) of the intra- and inter- day test, precision test were within 1.92% and 2.43%, respectively. The mean recovery of all ranged from 92.82 to 107.96% with RSD values 0.12 - 2.18%. **Conclusion:** The results of validation appeared that this established method was very accurate and stabilized for the quantification of 10 bioactive compounds isolated from *C. paniculatum*.

**Key words:** *Cynanchum paniculatum* Kitagawa, high performance liquid chromatography coupled-diode array detector, simultaneous determination, validation

**Access this article online**

**Website:** www.phcog.com

**DOI:** 10.4103/0973-1296.99289

**Quick Response Code:**

## INTRODUCTION

For a long history, herbal medicine was used to treat and prevent diseases. Herbal medicines were consisted of various compounds. These compounds showed various therapeutic effects, so one herbal medicine was applied to multiple diseases.[1,2] Numerous components in herbal medicine make the quality control of herbs difficult. And the quality of these herbs has been different by many factors such as collection time, place, and cultivation environment. Thus, accurate analysis methods of herbal medicines were required for quality control of herbal medicine. Generally, analysis of herbal medicine was conducted by chromatography and relative technology such as high-performance liquid chromatography (HPLC) and gas chromatography (GC).[3-5] For effective analysis of herbal medicines, simultaneous determination was needed than individually analysis of components in herbal medicines.

*Cynanchum paniculatum* Kitagawa of the family Asclepiadaceae is one of the well-known herbal medicines. *C. paniculatum* was traditionally used as analgesic and treatment of chronic tracheitis. Recently, inhibiting activity the growth of human cancer cells, anti-inflammatory, and anti-nociceptive effects were reported.[6,7] *C. paniculatum* was known to contain paeonol, neocynaponoside A, and sarcostin. Among them, paeonol was an important active compound in *C. paniculatum*.[8] To obtain neuroprotective components from *C. paniculatum*, we isolated and identified 10 bioactive compounds which include paeonol from the roots of this

**Address for correspondence:**
Dr. Choong Je Ma, Department of Biomaterials Engineering, Division of Biotechnology and Bioengineering, Kangwon National University, Hyoja-2 Dong, Chuncheon 200-701, Republic of Korea. E-mail: cjma@kangwon.ac.kr

plant. Compounds 1-10 were identified with paeonol (1), 4-acetylphenol (2), 2,5-dihydroxy-4-methoxyacetophenone (3), 4-O-methyl ether gallacetophenone (4), acetoveratrone (5), 2,5-dimethoxyhydroquinone (6), vanillic acid (7), resacetophenone (8), m-acetylphenol (9), and 3,5-dimethoxyhydroquinone (10). Paeonol (1) has shown to protect rat neurons from oxygen-glucose deprivation-induced injury and had anti-inflammatory and analgesic effects.[9,10] 2,5-dihydroxy-4-methoxyacetophenone (3) was found to selectively inhibit the aggregation of rabbit platelets induced by arachidonic acid.[11] Vanillic acid (7) has shown the protective effect on ROS induced cytotoxicity and melanogenesis in cultured human skin melanoma cells.[12] Resacetophenone (8) has been reported to protective effect against lipid peroxidative injury induced by n-BuOH on mice.[13] Bioactivities of the other compounds were not reported. The quality of *C. paniculatum* such as amount of compounds was affected by culture environment. Now, no quantitative analytical method has been developed for identification of amount of compounds in *C. paniculatum* include major compound, paeonol. Thus, quality control method of *C.paniculatum* was needed.

In this study, simultaneous determination by high performance liquid chromatography with diode-array detection (HPLC-DAD) was developed for efficient qualitative and quantitative analysis of 10 bioactive compounds, paeonol (1), 4-acetylphenol (2), 2,5-dihydroxy-4-methoxyacetophenone (3), 4-O-methyl ether gallacetophenone (4), acetoveratrone (5), 2,5-dimethoxyhydroquinone (6), vanillic acid (7), resacetophenone (8), m-acetylphenol (9), and 3,5-dimethoxyhydroquinone (10) based on reported papers.[14-17]

## MATERIALS AND METHODS

### Materials

*C. paniculatum* was purchased from Kyungdong traditional herbal market (Seoul, Korea). HPLC grade water and acetonitrile used in HPLC analysis were purchased from the J.T. Baker (USA). And analytical grade trifluoroacetic acid (TFA) was obtained from DAE JUNG (Korea). Reference compounds 1-10 were isolated from *C. paniculatum* by repeated silica gel, Sephadex LH-20 column chromatography, and preparative HPLC. The chemical structures of 10 compounds were identified by spectroscopic data of $^{1}H$ NMR and $^{13}C$ NMR and comparison with published references of these compounds. The purities of 10 reference compounds were above 98% by normalization of the peak areas detected by HPLC. Their chemical structures are shown in Figure 1.

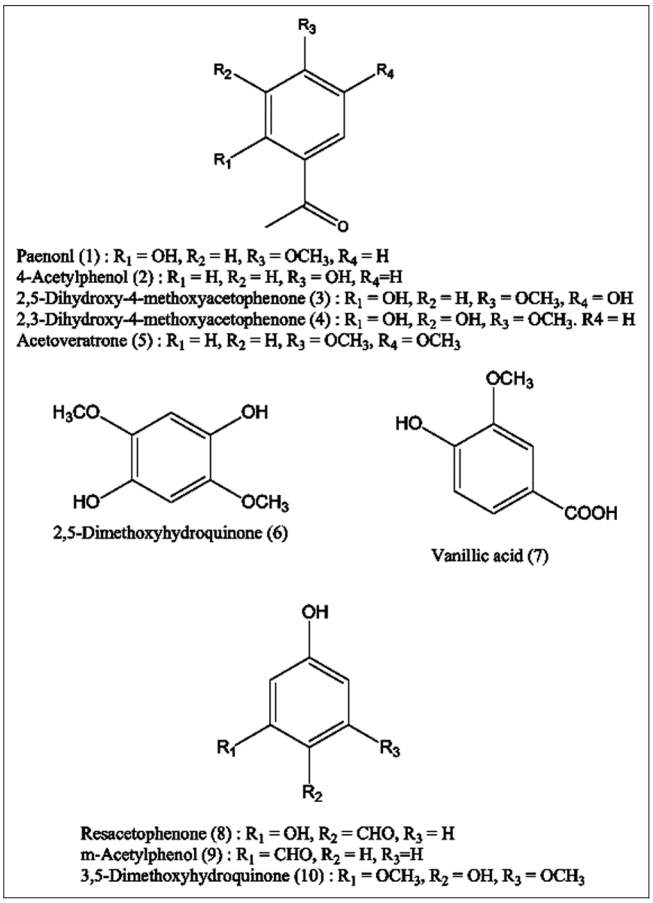


**Figure 1:** Chemical structure of compounds 1-10 isolated from the roots of *Cynanchum paniculatum* Kitagawa

### Extraction and isolation

Roots of *Cynanchum paniculatum* Kitag. (5.2 kg) were extracted using 80% methanol (3 × 2L) by ultrasonication-assisted extraction. The MeOH solution was evaporated to residue. The residue suspended in water and partitioned with hexane, $CHCl_3$, EtOAc, and n-BuOH to afford a hexane (4.84 g), $CHCl_3$ (36.86 g), EtOAc (12.60 g), and n-BuOH (41.70 g) fractions, respectively. The $CHCl_3$ fraction was chromatographed in a silica gel column (90 × 10 cm, 70 - 230 mesh) and eluted with a gradient of $CHCl_3$ / MeOH (100:1 → 0:1, v/v) to obtain eight fractions, denoted as A - H. Compound **1** (2.16 g) and **3** (2.23 mg) were obtained from fraction A and B, respectively, by Sephadex LH-20 using 100% MeOH. Fraction C was subjected to Sephadex LH-20 column (eluent; 100% MeOH) to give five fraction (C1 - C5). C3 was separated by Sephadex LH-20 (eluent; 100% MeOH) to give three fraction (C3a - C3c). Compound **2** (5.39 mg), **4** (12.15 mg) and **5** (11.19 mg) were obtained from fraction C3b by preparative HPLC on a $C_{18}$ YMC hydrosphere (250 mm × 20 mm I.D. S-5 μm) with acetonitrile / water (20: 80, v/v). Compound **8** (14.05 mg) was obtained from C4 by preparative HPLC on a $C_{18}$ YMC hydrosphere (250 mm × 20 mm I.D. S-5 μm) with acetonitrile / water (20: 80

→ 24: 76, v/v). Using a Sephadex LH-20 column eluted with 100% MeOH, fraction D was further purified to four fractions (D1 - D4). D3 was subjected to preparative HPLC on $C_{18}$ YMC hydrosphere (250 mm × 20 mm I.D. S-5 μm) eluted with acetonitrile /water (20: 80 → 60: 40, v/v) to yield compounds **9** (4.3 mg) and **10** (9.54 mg). Fraction F was separated by passage over a silica gel column (90 × 5 cm, 70 - 230 mesh), eluted with $CHCl_3$ / MeOH (30:1 → 0:1, v/v) to give four fractions (F1 - F4). Fraction F was continuously chromatographed on a silica gel column (90 × 5 cm, 70 - 230 mesh, $CHCl_3$ / MeOH, 40:1 → 0:1), sephadex LH-20 column (eluent; 100% MeOH), and preparative HPLC on $C_{18}$ YMC hydrosphere (250 mm × 20 mm I.D. S-5 μm, acetonitre / 0.1% TFA water, 5: 95 → 35: 65, v/v) to give compounds **6** (5.44 mg) and **7** (7.72 mg).

### HPLC apparatus and conditions

The HPLC analyses were performed with a Dionex Ultimate 3000 system (Germany) equipped with a pump (LPG 3X00), an auto sampler (ACC-3000), a column oven (TCC-3000SD) and diode array UV/VIS detector (DAD-3000(RS)). System control and data analyses were executed by Dionex Chromelon™ Chromatography Data System. We tested various columns such as XTerra™ RP18 (5 μm, 4.60 mm I.D. × 250 mm), SHISEIDO $C_{18}$ column (5 μm, 4.60 mm I.D. × 250 mm), and a LUNA $C_{18}$ column (5 μm, 4.60 mm I.D. × 250 mm). As a result of the test, chromatography was conducted on a SHISEIDO $C_{18}$ column (5 μm, 4.60 mm I.D. × 250 mm). The mobile phase was made up from acetonitrile (A) and 0.1% TFA (trifluoroacetic acid) water (B). The mobile phase was eluted by suitable gradient program at flow rate of 1.0 ml/ min: 5% A (v/v) at 0-10 min, 5-10% A at 10-15 min, 10% A at 15-30 min, 10-12% A at 30-40 min, 12-20% A at 40-50 min, 20-50% A at 50-60 min, and 50% A at 60-70 min. UV detector was set in 190 ~ 400 nm. The injection volume was 20 μl.

### Preparation of standard solutions and sample solution

The accurately weighed standard stock solutions of isolated 10 compounds, paeonol (1), 4-acetylphenol (2), 2,5-dihydroxy-4-methoxyacetophenone (3), 4-O-methyl ether Gallacetophenone (4), acetoveratrone (5), 2,5-dimethoxyhydroquinone (6), vanillic acid (7), resacetophenone (8), m-acetylphenol (9), and 3,5 dimethoxyhydroquinone (10) were prepared in methanol. Working solutions were prepared by diluting the stock solutions with methanol to obtain the calibration curve and conduct method validation. Working solutions were analyzed with mixture. Stock solutions and working solutions was stored 4─ before HPLC analysis.

To obtain the powder, a part of *C. paniculatum* extract was conducted by freeze drying. The powder accurately weighed (110.5 mg) was dissolved in 5 ml MeOH. All standard solution and sample solution was filtered through 0.45 μm membrane filters before injection into HPLC.

## RESULTS AND DISCUSSION

### Optimization of HPLC chromatographic conditions

To develop a better simultaneous determination of 10 compounds of *C. paniculatum*, the chromatographic condition was optimized. Various gradient elution systems including compositions of water and acetonitrile were tested for suitable mobile phase. For the high resolution of the peaks and depressing the peak tailing by lowering pH, trifluoroacetic acid (0.1 %) was added into the water.[18] Column temperature also tested from 2,5─ to 35─. We separated in 35─. Peak area value of each 10 compounds was measured at the maximal UV absorption of these compounds through investigated UV spectra. The UV wavelengths of compounds (6, 7, 9 and 10) were set at 210 nm, compounds (3 and 5) were set at 230 nm and compounds (1, 2, 4 and 8) were set at 280 nm. The HPLC-DAD chromatogram of the 10 standard compounds under the optimization conditions was shown in Figure 2a. The peaks of compounds were identified by comparing retention time and UV spectra.

### Validation of HPLC method

#### *Calibration curves, LOD, and LOQ*

Standard solutions of compounds 1-10 were diluted by MeOH to serials of concentrations to establish the calibration curve. Six different concentrations were analyzed by three replicate injections. Calibration curves were constructed by plotting the peak area versus concentration of 10 compounds. The regression equations of 10 compounds were obtained from calibration curve to investigate linearity. In regression equation, $Y = ax + b$, $x$ is referred to the concentration of marker compounds, $Y$ is peak area, a is slope of calibration curve, and b is intercept of calibration curve. Correlation coefficient ($r^2$) value indicated linearity. The limits of detection (LOD) and limits of quantification (LOQ) in chromatographic condition were determined at signal-to-noise ratios (S/N) of 3 and 10, respectively.[19] The regression equation and correlation coefficient ($r^2$) of 10 compounds were given in Table 1. All calibration curves of compounds 1-10 exhibited good linearity ($r^2 > 0.9996$). The LOD and LOQ were measured by calibration curve. The LOD was less than 0.28 % and LOQ was less than 0.83% of 10 compounds showed high sensitivity.

### Precision and accuracy

Precision was evaluated by repeatability test, intra- and inter-day. Repeatability test was conducted with the three different concentrations of mixture standard solutions.[20] The intra-day test was analyzed by injecting three different

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 |

[a]*Y*: 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 |

[a]The 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 |

[a] The 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*

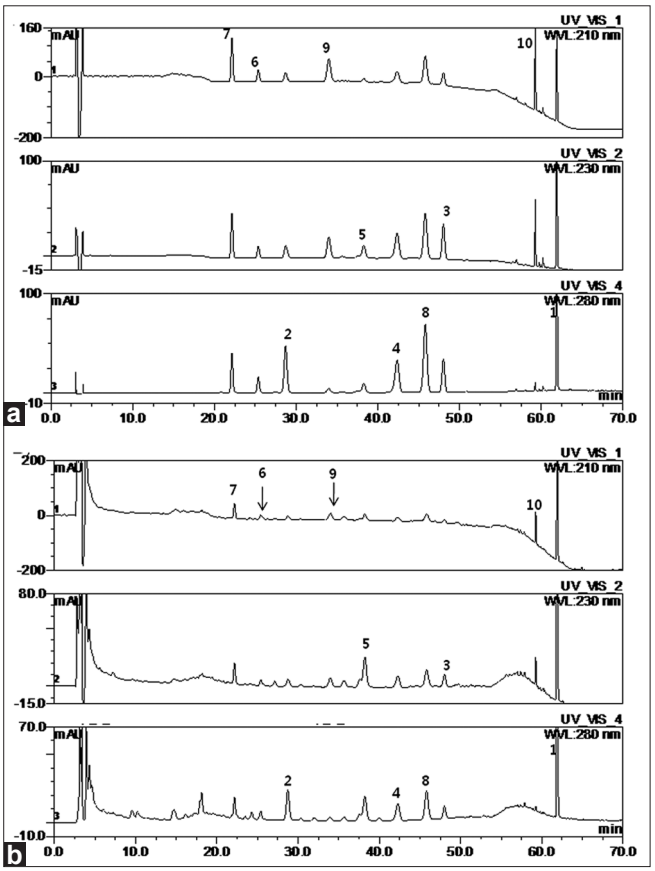


**Figure 2:** The HPLC chromatogram of standard mixture (a) and *Cynanchum paniculatum* Kitagawa (b). (1) paeonol, (2) 4-acetylphenol, (3) 2,5-dihydroxy-4-methoxyacetophenone, (4) 4-O-methyl ether Gallacetophenone, (5) acetoveratrone, (6) 2,5-dimethoxyhydroquinone, (7) vanillic acid, (8) resacetophenone,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

## ACKNOWLEDGEMENT

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

## REFERENCES

1. Jiang WY. Therapeutic wisdom in traditional Chinese medicine: A perspective from modern science. Trends Pharmacol Sci 2005;26:558-63.
2. Lu AP, Jia HW, Xiao C, Lu QP. Theory of traditional Chinese medicine and therapeutic method of diseases. World J Gastroenterol 2004;10:1854-6.
3. Drasar P, Moravcova J. Recent advances in analysis of Chinese medical plants and traditional medicines. J Chromatogr B Analyt Technol Biomed Life Sci 2004;812:3-21.
4. Merlin NJ, Parthasarathy V, Manavalan R, Kumaravel S. Chemical investigation of aerial parts of *Gmelina asiatica* Linn by GC-MS. Phcog Res 2009;3:152-6.
5. Djemgou PC, Ngandeu F, Hegazy ME, Nkanwen ER, Neguim G, Chosson E, *et al*. GC-MS Analysis of Terpenes from *Ficus mucuso*. Phcog Res 2009;4:197-201.
6. Lee SK, Nam KA, Heo YH. Cytotoxic activity and G2/M cell cycle arrest mediated by antofine, a phenanthroindolizidine alkaloid isolated from *Cynanchum paniculatum*. Planta Med 2003;69:21- 5.
7. Choi JH, Jung BH, Kang OH, Choi HJ, Park PS, Cho SH, *et al*. The anti-inflammatory and anti-nociceptive effects of ethyl acetate fraction of *Cynanchi paniculati* radix. Biol Pharm Bull 2006;29:971-5.
8. Mitsuhashi H, Hayashi K, Nomura T. Studies on the constituents of asclepiadaceae plants. 8. Components of *Cynanchum paniculatum* Kitagawa. Chem Pharm Bull 1966;14:779-83.
9. Wu JB, Song NN, Wei XB, Guan HS, Zhang XM. Protective effects of paeonol on cultured rat hippocampal neurons against oxygen-glucose deprivation-induced injury. J Neurol Sci 2008;264:50-5.
10. Chou TC. Anti-inflammatory and analgesic effects of paeonol in carrageenan-evoked thermal hyperalgesia. Br J Pharmacol 2003;139:1146-52.
11. Lin HC, Ding HY, Ko FN, Teng CM, Wu YC. Aggregation inhibitory activity of minor acetophenones from *Paeonia* species. Planta Med 1999;65:595-9.
12. Ha DH, Choi YJ, Yoo SM. Effects of vanillic acid on the cell viability and melanogenesis in cultured human skin melanoma cells damaged by ROS-induced cytotoxicity. J Exp Biomed Sci 2007;13:251-379.
13. Sheng XF, Zhou Y, Liu YZ, Feng X, Huang TF. The effects of resacetophenone against lipid peroxidative injury induced by n-butane on mice. J Hunan Normal Univ 2007;2:6304-66.
14. Sinha AK, Verma SC, Sharma UK. Development and validation of an RP-HPLC method for quantitative determination of vanillin and related phenolic compounds in *Vanilla planifolia*. J Sep Sci 2007;30:15-20.
15. Qi LW, Yu QT, Li P, Li SL, Wang YX, Sheng LH, *et al*. Quality evaluation of Radix Astragali through a simultaneous determination of six major active isoflavonoids and four main saponins by high-performance liquid chromatography coupled with diode array and evaporative light scattering detectors. J Chromatogr A 2006;1134:162-9.
16. Xu YJ, Zhao DX, Fu CX, Cheng LQ, Wang NF, Han LJ, *et al*. Determination of flavonoid compounds from *Saussurea involucrata* by liquid chromatography electrospray ionisation mass spectrometry. Nat Prod Res 2009;23:1689-98.
17. Tang D, Yang D, Tang A, Gao Y, Jiang X, Mou J, *et al*. Simultaneous chemical fingerprint and quantitative analysis of *Ginkgo biloba* extract by HPLC-DAD. Anal Bioanal Chem 2010;396:3087-95.
18. Heyrman AN, Henry RA. Importance of controlling mobile phase pH in reversed phase HPLC. Keystone Technical Bulletin; 1999.
19. Won JB, Ma JY, Um YR, Ma CJ. Simultaneous determination of five marker constituents in Ssanghwa tang by HPLC/DAD. Pharmacogn Mag 2010;22:111-5.
20. Shekarchi M, Hajimehdipoor H, Khanavi M, Adib N, Bozorgi M, Akbari-Adergani B. A validated method for analysis of Swerchirin in *Swertia longifolia* Boiss. by high performance liquid chromatography. Pharmacogn Mag 2010;21:13-8.
21. Rai P, Patil P, Rajput SJ. Simultaneous determination of phyllanthin and hypophyllanthin in herbal formulation by derivative spectrophotometry and liquid chromatography. Pharmacogn Mag 2009;18:151-8.

**Cite this article as:** Weon JB, Lee B, Yun B, Lee J, Ma CJ. Simultaneous determination of ten bioactive compaounds from the roots of *Cynanchum paniculatum* by using high performance liquid chromatography coupled-diode array detector. Phcog Mag 2012;8:231-6.

**Source of Support:** Supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0005149), **Conflict of Interest:** None declared.