Determination of pectolinarin in *Cirsium* spp. using HPLC/UV analysis

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**Abstract** Pectolinarin was isolated from the ethyl acetate fraction of *Cirsium setidens* using open column chromatography and was analyzed using spectrometry. Pectolinarin content in *Cirsium* spp. was determined using HPLC/UV. Pectolinarin content in the aerial part of *Cirsium* spp. was higher than that in the root and pappus. Pectolinarin content was highest in the aerial part of *C. chlorolepis* (110.65 mg/g extract). Consequently, the aerial part of *C. chlorolepis* has potential for use in new natural medicinal products, health supplements, and beverages.

**Keywords** *Cirsium* spp. · Flavonoid · High-performance liquid chromatography · Pectolinarin

**Introduction**

*Cirsium* spp. is widely distributed in Asian and Western countries, including Korea, China, Japan, the United States, Canada, and Australia. The plants grow very well almost anywhere and breed rapidly. *Cirsium* spp. belongs to the Compositae family (Kim and Kim 2003). The roots or whole plants of *Cirsium* spp. have been widely used in the treatment of hemorrhage, liver and kidney inflammation, and various abdominal and intestinal disorders (Jung et al. 2009). *Cirsium* spp. is also bioactive and has anti-microbial, anti-diabetic, anti-oxidant, hepatoprotective, anti-inflammatory, vasorelaxant, and anti-cancer properties (Perez et al. 2001; Park et al. 2004; Nazaruk and Jakoniuk 2005; Liu et al. 2007; Jeong et al. 2008; Kim et al. 2008; Ku et al. 2008; Lim et al. 2008; Yoo et al. 2008).

*C. setidens* is distributed mainly in the mountainous regions of Kangwon Province, Korea (Lee et al. 2002). Its young leaves and shoots are edible and are also use medicinal products because it contains various nutrients such as protein, calcium, vitamin A, and pectolinarin (Park et al. 2011; Jeong et al. 2013). Among bioactive phytochemicals, pectolinarin has been isolated as a primary compound in *C. setidens* (Yoo et al. 2008). Pectolinarin has anti-tumor, hepatoprotective, and anti-inflammatory activities (Lim et al. 2008; Yoo et al. 2008). However, there have been few quantitative studies of pectolinarin in *Cirsium* spp.

In our study, we isolated pectolinarin from *C. setidens* and analyzed the content of pectolinarin in *Cirsium* spp. extracts using HPLC/UV. It can be used as a standardization data for natural food and medicinal materials.

**Materials and Methods**

**Plant materials**

*C. setidens* was collected in June 2014 from Yangyang, Republic of Korea. The pappus of *C. japonicum* var. *maackii* was obtained from Department of Herbal Crop Research, National Institute of Horticultural & Herbal Science, RDA, Eumseong, Republic of Korea.
Methanol (MeOH), and dichloromethane (CH₂Cl₂) (SamChun Pure Chemical Co., Pyeongtaek, Korea) were used in open column chromatography with silica gel (200–400 mesh ASTM; Merck Co., Darmstadt, Germany). Evaporation was performed using an Eyela rotary evaporator system (Tokyo, Japan) under reflux in vacuo. Thin-layer chromatography was performed using Kiesel gel 60 F₂₅₄ (Merck Co., Darmstadt, Germany). Evaporation was performed in vacuo, followed by heating to 100 °C. Dimethylsulfoxide (DMSO) (Cambridge Isotope Laboratories Inc., MA, USA) was used as the nuclear magnetic resonance (NMR) solution. Mass spectrometry (MS) was conducted using a JEOL JMS-600W (Tokyo, Japan) mass spectrometer. ¹H- and ¹³C-NMR spectra were recorded with a Bruker AVANCE 500 NMR spectrometer (Rheinstetten, Germany) using tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (ppm). Chemical shifts are reported in ppm (δ) and coupling constants (J) are expressed in hertz.

**Extraction and isolation**

Dried samples (5 kg) of *C. setidens* were extracted with EtOH and fractionated by n-hexane, chloroform, ethyl acetate (EtOAc), and n-butanol. We obtained n-hexane (210 g), chloroform (49 g), EtOAc (21 g), and n-butanol (26 g) fractions. A portion of the EtOAc fraction (10 g) was used for open column chromatography with a stepwise gradient using a CH₂Cl₂/MeOH solvent system to yield 23 fractions. MeOH recrystallization of fraction 17 yielded compound 1 (265 mg).

**Sample preparation**

Compound 1 and Cirsium extracts (the aerial parts of *C. setidens*, *C. nipponicum*, *C. japonicum*, *C. var. maackii*, *C. chlorolepis*, *C. charoenoncium*; the root part of *C. charoenoncium*; and pappus of *C. japonicum var. maackii*) were dissolved in MeOH (20 mg/mL). The resulting solution was filtered using a 0.45-µm syringe filter and analyzes using HPLC.

**HPLC condition**

A Waters Spherisorb® INNO column C18 (4.6 × 250 mm, 5 µm) was used for analysis of compound 1. The mobile phase was dissolved in water (solvent A) and ACN (solvent B). The gradient solvent system was initially composed of solvents A/B (75:25), and then changed to solvents A/B (10:90) for 30 min, solvents A/B (0:100) for 20 min, and finally to solvents A/B (75:25) for 15 min. The injection volume was 10 µL and flow rate was 1 mL/min. The UV spectra were recorded at 254 nm for quantification of flavonoids. All injections were performed in triplicate.

**Limit of detection (LOD) and limit of quantification (LOQ)**

Validation of the HPLC method for compound 1 as a standard compound was determined by LOD and LOQ. Method linearity was established by triplicate injections in the range of 0.0001–1.0 mg/mL. Five calibration solutions were injected in triplicate. Calibration curves were constructed by linear regression of the peak height (Y), concentration (X, mg/mL), and mean value (n = 3) ± standard deviation. The relative standard deviation was used as a measure of repeatability. The percent recoveries were evaluated by calculating the ratio of amount detected versus amount added. LOD and LOQ values were determined separately at signal-to-noise ratios (S/N) of 3 and 10, respectively.

**Calibration curves**

Stock solutions (1 mg/mL) of compound 1 were prepared in MeOH, and the solution content was successively reduced to 10% in order to create different concentrations for calibration curves. The calibration curves for compound 1 were calculated using peak area (Y), concentration (X, mg/mL), and mean value (n = 3) ± standard deviation.
typical flavonoid signal was observed in the 1H-NMR spectra of compound 1. A singlet signal at δ 12.96 indicates the presence of 5-OH in the flavonoid in the 1H-NMR spectrum of compound 1. The 1H-NMR spectrum of compound 1 indicates a flavonoid with A2B2 splitting at δ 8.02 (2H, d, J = 9.0 Hz) and 7.15 (2H, d, J = 9.0 Hz) in the B-ring. The existence of H-3 and -8 was confirmed by two singlet signals at δ 6.93 and 6.90, respectively. The 1H-NMR spectrum also supported a glucoside and rhamnoside in the structure on the basis of distinguishing peaks of glucose (δ 5.4) and rhamnose (δ 1.05 and 5.2). The 13C-NMR spectrum of compound 1 indicates a carbonyl group (C-4) at δ 182.44 and A2B2 splitting at δ 128.53 (C-2',6') and δ 114.86 (C-3',5'). Anomeric carbon peaks of glucose and rhamnose appear simultaneously at δ 100.43, and the C-6 of rhamnose appears at δ 17.80. Compound 1 was identified as pectolinarin (Fig. 1) by the spectroscopic data and comparison with a previous report in the literature (Simoes et al. 2013). Pectolinarin (1) has shown significant anti-inflammatory activity in various in vivo models as well as anti-allergic and analgesic properties (Martinez-Vazquez et al. 1998; Lim et al. 2008). Therefore, large amounts of pectolinarin (1) may have some potential uses in natural medicinal products, health supplements, and beverages.

HPLC separation of pectolinarin (1) for qualitative and quantitative analyses was performed using a reverse phase system with a mobile phase consisting of water and ACN. Pectolinarin (1) was detected in 8.20 min (Fig. 2). The standard calibration curve for pectolinarin (1) is shown in Table 1. Pectolinarin content in Cirsium extracts were determined using optimized analytical methods (Table 2). Pectolinarin (1) was detected in the aerial part of C. japonicum (1.04 mg/g extract), C. setidens (33.90 mg/g extract), C. nipponicum (61.44 mg/g extract), C. charoenicum (49.43 mg/g extract), C. chlorolepis (110.65 mg/g extract), the roots of C. charoenicum (0.48 mg/g extract), and the pappus of C. japonicum var. maackii (4.97 mg/g extract) (Table 2). Pectolinarin (1) was not detected in the aerial part of C. japonicum var. maackii. Pectolinarin content in the aerial parts of Cirsium spp. were greater than that in the roots and the pappus (Table 2; Figs. 3, 4). Pectolinarin content was highest in the aerial parts of C. chlorolepis (110.65 mg/g extract) (Table 2; Fig. 3).

A previous study has shown the pectolinarin content in MeOH, EtOH, and water extracts from C. japonicum as 18.7, 16.5, and 12.7 mg/g extract, respectively (Liu et al. 2007). Pectolinarin
Table 2 Content of pectolinarin (1) in the MeOH extracts of *Cirsium* spp.

| Sample                          | 1 (mg/g extract) |
|---------------------------------|------------------|
| Aerial part of *C. japonicum*   | 1.036±0.009      |
| Aerial part of *C. setidens*    | 33.899±0.460     |
| Aerial part of *C. chlorolepis* | 110.652±13.440   |
| Aerial part of *C. nipponicum*  | 61.441±0.545     |
| Aerial part of *C. chanroenicum*| 49.434±7.133     |
| Aerial part of *C. japonicum* var. maackii | -          |
| Root part of *C. chanroenicum*  | 0.479±0.082      |
| Pappus of *C. japonicum* var. maackii | 4.967±0.133    |

Content in MeOH extracts from the aerial parts of *C. setidens*, *C. nipponicum*, *C. chanroenicum*, and *C. chlorolepis* in the present study was higher than that in MeOH, EtOH, and water extracts from *C. japonicum* in previous studies. Among MeOH extracts from the aerial part of *C. setidens*, *C. nipponicum*, *C. chanroenicum*, and *C. chlorolepis*, pectolinarin content was highest in the aerial part of *C. chlorolepis*. In our study, pectolinarin content in *C. setidens*, *C. nipponicum*, *C. chanroenicum*, and *C. chlorolepis* was higher than that in *C. japonicum*. However, we obtained data showing that *C. japonicum* from China have higher pectolinarin content than *C. japonicum* from Korea.

Fig. 3 HPLC chromatograms of the MeOH extracts from the aerial part of *C. japonicum* (A), *C. setidens* (B), *C. chlorolepis* (C), *C. nipponicum* (D), *C. chanroenicum* (E), and *C. japonicum* var. maackii (F)
The linear calibration equation was \( y = 958906x - 51982 \), where \( y \) is the peak area and \( x \) is amount of pectolinarin (1). The correlation coefficient \( (r^2) \) was 0.9996 (Table 1). The LOD and LOQ under the present chromatographic conditions were determined at signal to noise ratios (S/N) of 3 and 10, respectively. The LOD and LOQ of pectolinarin (1) were 0.048 and 0.052 mg/mL, respectively (Table 3).

In our study, the aerial parts of *Cirsium* spp. were found to be particularly valuable because of its pectolinarin content. Because of its relatively higher pectolinarin content, the aerial part of *C. chlorolepis*, in particular, has potential for use in new natural medicinal products, health supplements, and beverages. We also determined optimal conditions for analyzing pectolinarin (1), which can be used for additional analysis.

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