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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).

Cirsium spp. contains various phytochemicals, including flavonoids, phenolic acids, lignans, polyacetylenes, acetylenes, sterols, triterpenes, susquiterpene lactones, and alkaloids (Lee et al. 1994; Jordon-Thanden and Louda 2001; Chung et al. 2002; Yim et al. 2003; Jeong et al. 2008). The flavonoids include compounds such as pectolinarin, luteolin, linarin, hispidulin-7-neohesperidoside, pectolinarigenin, apigenin, quercetin, luteolin 5-*O*-glucoside, and 5,7-dihydroxy-6,4-dimethoxy flavones (Ganzera et al. 2005; Liu et al. 2006; Jung et al., 2012; Sun et al. 2012). *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

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Korea. Voucher specimens (*C. setidens*, No. LEE 2014-01; *C. japonicum* var. *maackii*, No. LEE 2014-02) were deposited at the Herbarium of Department of Integrative Plant Science, Chung-Ang University, Republic of Korea. *Cirsium* extracts, including the aerial parts of *C. setidens*, *C. nipponicum*, and *C. chanroenicum*, and the roots of *C. chanroenicum*, were obtained from the Plant Extract Bank of Korea Research Institute of Bioscience and Biotechnolgy in Deajeon, Korea. Extracts from the aerial parts of *C. japonicum*, *C. japonicum* var. *maackii*, and *C. chlorolepis* were obtained from the Medicinal Plant Resources Bank in Seongnam, Republic of Korea.

Instruments and reagents

Methanol (MeOH), and dichloromethane (CH_2Cl_2) (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 F_{254} plates of 0.25 mm thickness (Art. 5715, Merck Co.), and compounds were visualized by spraying with 10 % H_2SO_4 in MeOH, 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. ^{1}H- and ^{13}C-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 (δ), and coupling constants (*J*) are expressed in hertz. The HPLC system consisted of a WATERS 1525 Binary HPLC (Milford, MA, USA), a TCM column oven, and a WATERS 2489 UV/visible detector, all controlled by a computer using Empower Pro 2.0 software. The separation column was a Waters Spherisorb® INNO column C18 (4.6 × 250 mm, 5 μm). HPLC-grade solvents such as acetonitrile (ACN), MeOH, ethanol (EtOH), and distilled water (H_2O) were obtained from J.T. Baker® (PA, USA).

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 CH_2Cl_2/MeOH solvent system to yield 23 fractions. MeOH recrystallization of fraction 17 yielded compound **1** (265 mg).

Compound **1**: Yellow powder; $C_{29}H_{34}O_{15}$; FAB-MS *m/z*: 623 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 12.96 (1H, s, 5-OH), 8.04 (2H, d, *J* = 9.0 Hz, H-2',6'), 7.17 (2H, d, *J* = 9.0 Hz, H-3',5'), 6.94 (1H, s, H-8), 6.93 (1H, s, H-3), 5.20 (1H, d, *J* = 4.5 Hz, Glucose H-1), 4.57 (1H, d, *J* = 1.0 Hz, Rham H-1), 3.86 (3H, s, 6-OCH_3), 3.77 (3H, s, 4'-OCH_3), 1.06 (1H, d, *J* = 6.5 Hz, Rham H-6'''); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 182.44 (C-4), 164.22 (C-2), 162.50 (C-4'), 156.58 (C-7), 152.60 (C-5), 152.28 (C-9), 132.78 (C-6), 128.53 (C-2', 6'), 122.80 (C-1'), 114.86 (C-3', 5'), 105.99 (C-10), 103.44 (C-3), 100.43 (Glc C-1, Rham C-1), 94.44 (C-8), 60.43 (6-OCH_3), 55.64 (4'-OCH_3), 17.80 (Rham C-6).

Sample preparation

Compound **1** and *Cirsium* extracts (the aerial parts of *C. setidens*, *C. nipponicum*, *C. japonicum*, *C. japonicum* var. *maackii*, *C. chlorolepis*, *C. chanroenicum*; the root part of *C. chanroenicum*; 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) of compound **1** versus concentration (X) in mg/mL. 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.

Results and Discussion

Confirmation of the chemical structure of compound **1** was carried out by a combination of ^{1}H-, ^{13}C-NMR, and FAB-MS. A

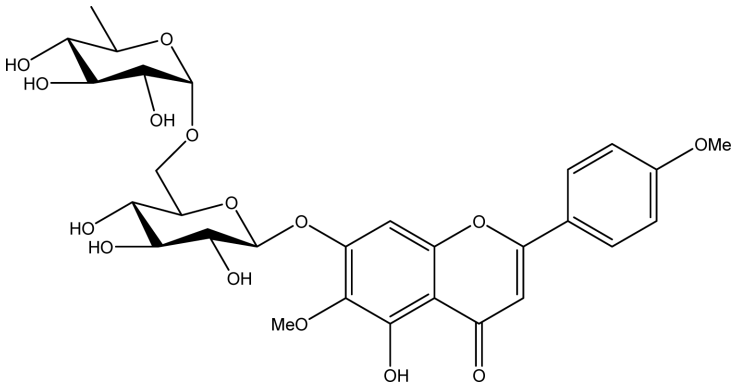

Fig. 1 Structure of pectolinarin (**1**)

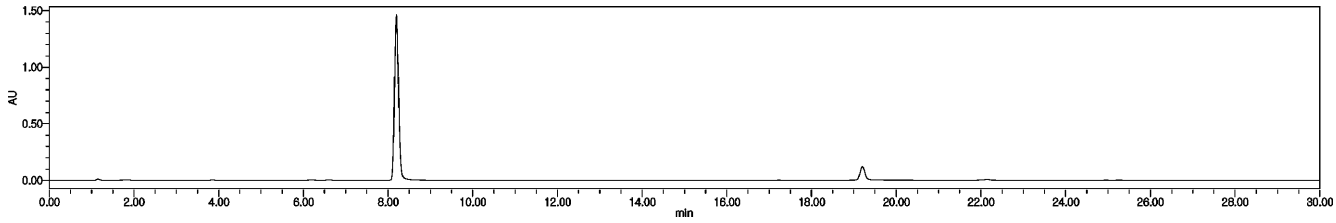

Fig. 2 HPLC chromatogram of pectolinarin (**1**)

Table 1 Calibration curve of pectolinarin (**1**)

Compound	Linear range (mg/mL)	Linear regression equation Y = aX – b		Correlation coefficient (r^2)
		Slope (a)	Intercept (b)	
1	0.0001-1.0	958906	51982	0.9996

Y = Peak area, X = Concentration of standard (mg/mL)
r^2 = Correlation coefficient for three data points from calibration curve

typical flavonoid signal was observed in the ^{1}H-NMR spectra of compound **1**. A singlet signal at δ 12.96 indicates the presence of 5-OH in the flavonoid in the ^{1}H-NMR spectrum of compound **1**. The ^{1}H-NMR spectrum of compound **1** indicates a flavonoid with A_2B_2 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 ^{1}H-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 ^{13}C-NMR spectrum of compound **1** indicates a carbonyl group (C-4) at δ 182.44 and A_2B_2 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. charnroenicum* (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. nipponcium*	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.

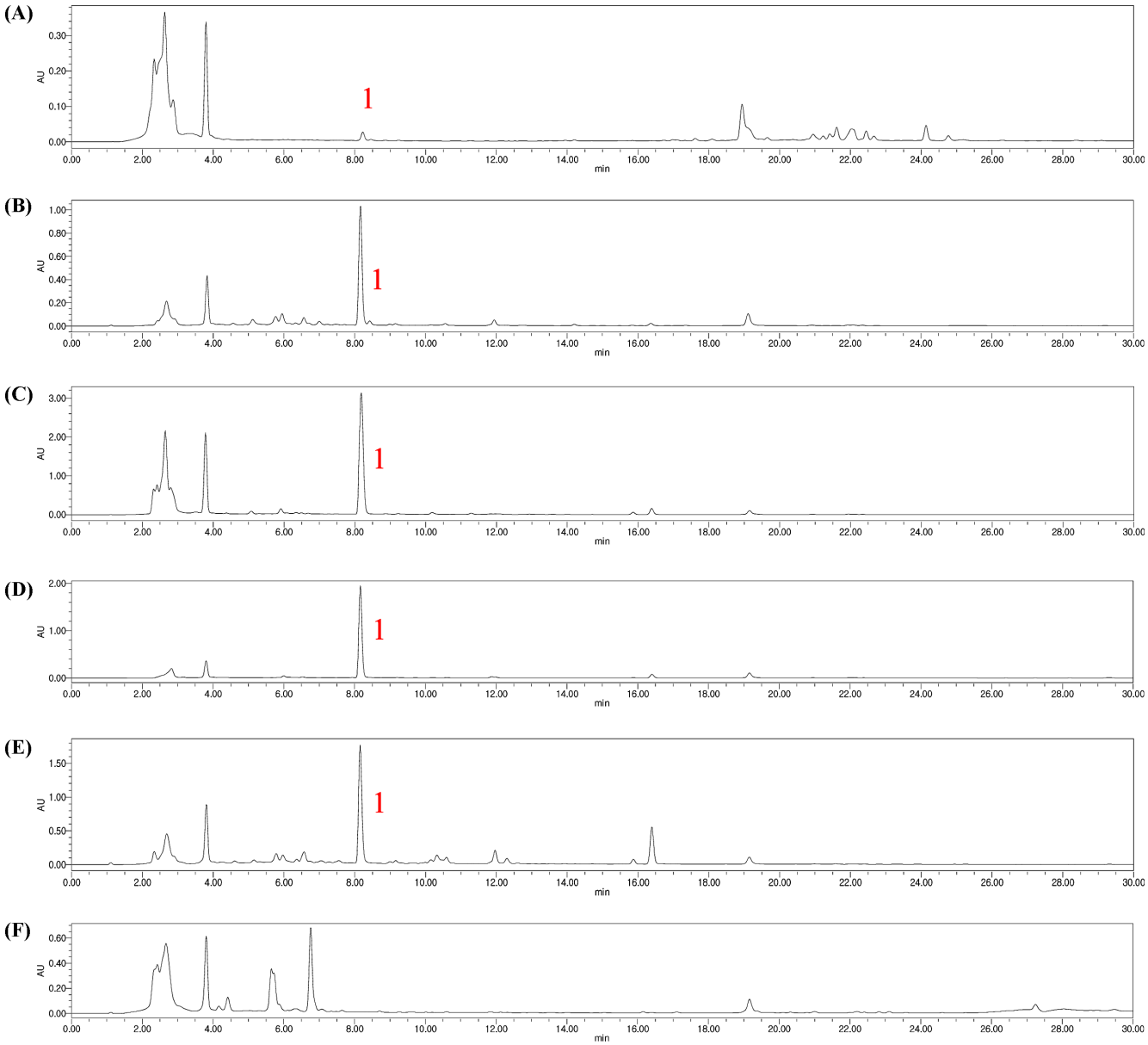

Fig. 3 HPLC chromatograms of the MeOH extracts from the aerial part of *C. japonicum* (A), *C. setidens* (B), *C. chlorolepis* (C), *C. nipponcium* (D), *C. chanroenicum* (E), and *C. japonicum* var. *maackii* (F)

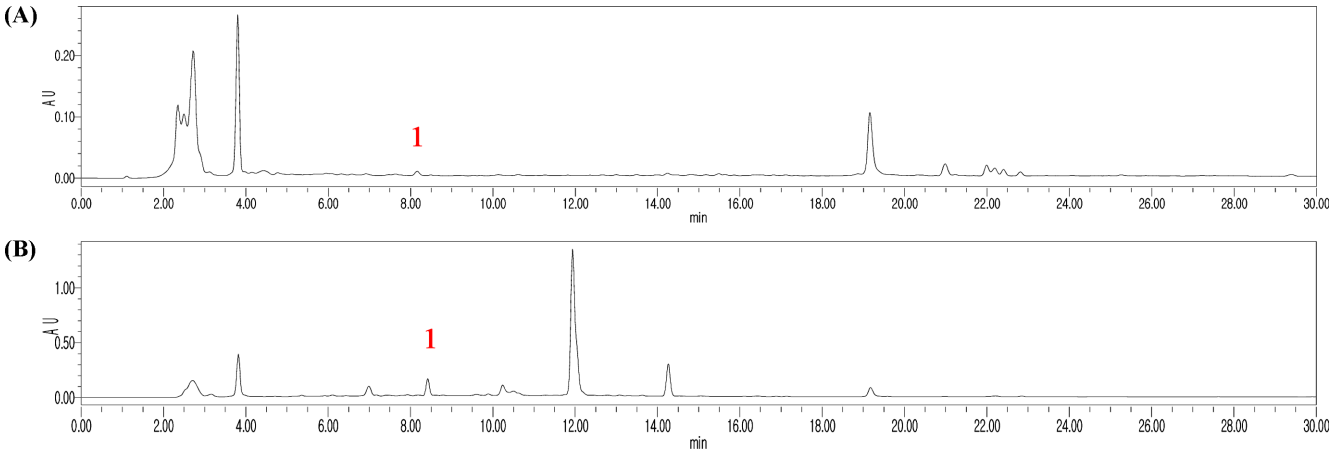

Fig. 4 HPLC chromatograms of the MeOH extracts from the root of *C. chanroenicum* (A) and the pappus of *C. japonicum* var. *maackii* (B)

Table 3 LOD and LOQ values of pectolinarin (**1**)

Compound	Regression equation	r^2	Linear range (mg/mL)	LOD (mg/mL)	LOQ (mg/mL)
1	Y = 145055X – 6665.2	0.9996	0.0001-1.0	0.048	0.052

Y = Peak height, X = Concentration of standard (mg/mL)
r^2 = Correlation coefficient for three data points from calibration curve

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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