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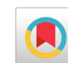

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# Phytochemical constituents of *Lactuca serriola* leaves and their content analysis by HPLC-UV

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**Abstract** This study aimed to identify the phytochemical constituents of *Lactuca serriola* leaves and perform quantitative analysis of the methanol (MeOH) extract of *L. serriola, L. indica*, *L. raddeana, L. sativa*, and *L. triangulata*. Six compounds were isolated from the MeOH extracts of *L. serriola* using open column chromatography and identified as protocatechuic acid (**1**), caffeic acid (**2**), cynaroside (**3**), apigenin 7-glucuronide (**4**), luteolin (**5**), and apigenin (**6**) using $^{1}$H-, $^{13}$C-nuclear magnetic resonance, and mass spectrometry. Quantitative analysis of the six compounds was performed on the MeOH extract of *Lactuca* species using high-performance liquid chromatography (HPLC) and an ultraviolet (UV). A reverse-phased column was used, and the UV absorbance was set to 280 nm. The contents of compounds **2** and **3** were the highest (1.58 and 2.64 mg/g ext., respectively) in *L. serriola* extracts. However, compounds **4** and **6** were higher (1.46 and 0.40 mg/g ext., respectively) in *L. triangulata*. These results provide quantitative data for the application of *Lactuca* species in the pharmaceutical and functional food industries.

**Keywords** High-performance liquid chromatography-ultraviolet · *Lactuca serriola* · Quantitative analysis

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## Introduction

*Lactuca serriola* L. (Asteraceae) is an annual or biennial plant known as prickly lettuce [1]. *Lactuca serriola* is the closest wild relative of *L. sativa*, and is native to Asia, Europe, and North Africa [2]. It is a naturalized plant and is designated as an ecosystem-disturbing species in Korea. Unlike *L. sativa*, which is the common lettuce, the leaves of *L. serriola* are oblong and lanceolate, with fine spines along the veins and leaf edges. It has traditionally been used for multiple purposes, including as a sedative, purgative, diuretic, vasorelaxant, and demulcent, and it is used to treat bronchitis, gastrointestinal injury, asthma, and pertussis. In addition, it was recently found that the aerial parts of *L. serriola* have remarkable α-glucosidase inhibitory [3] and antivenom activities [4]. *Lactuca serriola* contains lactucone, lactucin, lactucic acids [5], alkaloids, oxalic acid, lactucopicrin [1], vitamins, beta-carotene, iron [6], sesquiterpene esters [7], flavonoids [8], and phenolic acids [4].

*Lactuca serriola* has been designated an ecosystem-disturbing species by the Ministry of Environment in Korea [9]. Ecosystem-disturbing species can be artificially or naturally introduced from foreign countries and causes disturbances in the balance of the ecosystem. Genetically modified organisms can be created through genetic modification with these species. In addition, *Rana catesbeiana* [10], *Ambrosia artemisiaefolia* var. elatior [11], and *Sicyos angulatus* [12] have also been identified as ecosystem-disturbing species in Korea. Ecosystem-disturbing species have rapid reproductive power and vigorous growth, which have a serious impact on the ecosystem and cause economic damage when they enter pastures and arable land. However, in recent years, they have been studied for use as resources [13,14]. Scientific research that supports their use is still lacking. Studies on *L. serriola* have focused on its photosynthetic characteristics [15,16], genetic differences [17], genetic transformation [18], and morphological variability [19]. Studies are needed to identify the active compounds or activities of *L. serriola*.

The objectives of this study were to identify the phytochemical constituents of *L. serriola* and to quantify the methanol (MeOH) extracts of *L. serriola, L. indica*, *L. raddeana*, *L. sativa*, and *L. triangulata* using high-performance liquid chromatography-ultraviolet (HPLC-UV).

## Materials and Methods

**Plant materials**

Leaves of *L. serriola* (June, 2017) were collected from Jinwicheon, Pyeongtaek, Republic of Korea.

**Chemicals and apparatus**

Ethyl acetate (EtOAc), *n*-hexane, chloroform ($CHCl_3$), *n*-butanol (*n*-BuOH), and MeOH were purchased from Samchun Pure Chemicals (Pyeongtaek, Republic of Korea). Silica gel (200-400 mesh) (Merck Co., Darmstadt, Germany) and Sephadex LH-20 (Sigma-Aldrich Co., St. Louis, MO, USA) were used as the stationary phases for open column chromatography. Silica gel 60 $F_{254}$-precoated thin-layer chromatography (TLC) (Merck KGaA, Darmstadt, Germany) was used to monitor the chromatographic separation. An Avance 500 MHz spectrometer (Bruker, Rheinstetten, Germany) was used for nuclear magnetic resonance (NMR) assignment and a JEOL-MS spectrometer (Jeol, Tokyo, Japan) was used for mass spectrometry data. Chromatographic analysis was performed using an HPLC system (Waters Alliance 2695 Separations Module, Milford, MA, USA) equipped with a pump, auto-sampler, and photodiode-array detection (Waters 996 Photodiode Array Detector). HPLC-grade solvents, such as water and acetonitrile, were purchased from J. T. Baker (Avantor, Radnor, PA, USA). Acetic acid (glacial, 100%) was purchased from Supelco® (Merck KGaA).

**Extraction, fractionation, and isolation**

Dried leaves of *L. serriola* (1.7 kg) were extracted with MeOH under reflux at 88 °C for 3 h, three times in succession. After extraction, the solvents were filtered using filter paper and evaporated using a rotary evaporator (Sunileyela Co., Ltd., Seongnam, Republic of Korea) at 55 °C to obtain MeOH extract (300 g). The extract (187 g) was suspended in distilled water ($H_2O$) and partitioned into polar fractions sequentially using *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH. Each partitioned layer of the solvent was evaporated to produce *n*-hexane (22.7 g), $CHCl_3$ (24.5 g), EtOAc (7.2 g), and *n*-BuOH (13.4 g) fractions.

Each fraction was separated using open column chromatography and silica gel was used as the stationary phase. Each fraction was eluted using a solvent mixture of a stepwise gradient system (100:0 v/v to 0:100 v/v). All small fractions on the TLC plates were visually confirmed by spraying them with 10% $H_2SO_4$ in MeOH and heating them on a hot plate. The EtOAc fraction (5.2 g) was eluted with $CHCl_3$/MeOH (100:0 v/v to 0:100 v/v) using a stepwise gradient system to obtain 146 small fractions. Fractions were grouped into four sub-fractions (LSE1-4), and LSE1-3 were re-chromatographed over a Sephadex LH-20 column with a solvent system of $H_2O$/MeOH (5:1 v/v to 1:5 v/v). LSE1 yielded 55 sub-fractions; sub-fractions (53-54) were recrystallized using MeOH to obtain compound **6**. LSE2 yielded 79 sub-fractions; sub-fractions (50-60) were identified as compound **5**. LSE3 yielded 94 sub-fractions; sub-fractions (31-33) were identified as compound **2**. The *n*-BuOH (10 g) fraction was also eluted with $CHCl_3$/MeOH to obtain 169 small fractions, which were then grouped into three sub-fractions (LSB1-3). LSB2 was re-chromatographed over a Sephadex LH-20 column with a solvent system $H_2O$/MeOH (5:1 v/v to 1:5 v/v) to obtain 86 sub-fractions. Sub-fractions (52-55) were obtained using a solvent system of $H_2O$/MeOH (1:1 v/v) and identified as compound **1**. Sub-fractions (64-66) were recrystallized using MeOH to obtain compound **4**. Sub-fractions (83-85) were identified as compound **3**.

Compound **1**: Light brown solid; $^1$H-NMR ($CD_3OD$): δ 6.76 (H-5, d, 8.0), 7.39 (H-6, dd, 2.0, 8.0), 7.42 (H-2, d, 2.0); $^{13}$C-NMR ($CD_3OD$): δ 115.8 (C-5), 117.7 (C-2), 123.6 (C-1), 125.4 (C-6), 146.1 (C-3), 150.7 (C-4), 171.9 (C-7).

Compound **2**: Yellow amorphous solid; $^1$H-NMR (DMSO-$d_6$): δ 6.15 (H-8, d, 15.5), 6.75 (H-5, d, 8.0), 6.94 (H-6, dd, 2.0, 8.0), 7.01 (H-2, d, 2.0), 7.37 (H-7, d, 15.5).

Compound **3**: Yellow powder; $^1$H-NMR (DMSO-$d_6$): δ 5.08 (H-1″, d, 7.5), 6.43 (H-6, d, 2.0), 6.73 (H-3, s), 6.78 (H-8, d, 2.0), 6.86 (H-5′, d, 8.5), 7.40 (H-2′, d, 2.0), 7.44 (H-6′, dd, 2.5, 8.5), 13.04 (5-OH, s); $^{13}$C-NMR (DMSO-$d_6$): δ 60.6 (C-6″), 69.5 (C-4″), 73.1 (C-2″), 76.4 (C-3″), 77.1 (C-5″), 94.7 (C-8), 99.5 (C-6), 99.8 (C-1″), 102.8 (C-3), 105.3 (C-10), 113.2 (C-2′), 115.9 (C-5′), 119.3 (C-6′), 121.5 (C-1′), 145.9 (C-3′), 150.1 (C-4′), 156.9 (C-9), 161.1 (C-5), 162.9 (C-7), 164.6 (C-2), 181.8 (C-4).

Compound **4**: Yellow powder; $^1$H-NMR (DMSO-$d_6$): δ 5.02 (H-1′, d, 7.5), 6.41 (H-6, d, 1.5), 6.81 (H-8, d, 1.5), 6.83 (H-3, s), 6.89 (H-3′,5′, d, 8.5), 7.92 (H-2′,6′, d, 8.5), 13.00 (5-OH, s); $^{13}$C-NMR (DMSO-$d_6$): δ 72.0 (C-4″), 72.9 (C-2″), 73.6 (C-3″), 76.6 (C-5″), 94.6 (C-8), 99.6 (C-6), 99.6 (C-1″), 102.8 (C-3), 105.2 (C-10), 116.1 (C-3′,5′), 121.0 (C-1′), 128.5 (C-2′,6′), 156.9 (C-9), 161.0 (C-4′), 162.0 (C-5), 163.1 (C-7), 164.3 (C-2), 171.7 (C-6″), 181.9 (C-4).

Compound **5**: Yellow powder; $^1$H-NMR (DMSO-$d_6$): δ 6.17 (H-6, d, 2.0), 6.42 (H-8, d, 2.5), 6.66 (H-3, s), 6.87 (H-5′, d, 8.5), 7.38 (H-2′, d, 2.5), 7.41 (H-6′, dd, 2.5, 8.5), 12.99 (5-OH, s).

Compound **6**: Yellow powder; $^1$H-NMR (DMSO-$d_6$): δ 6.01 (H-6, s), 6.28 (H-8, s), 6.66 (H-3, s), 6.90 (H-3′,5′, d, 9.0), 7.87 (H-2′,6′, d, 9.0), 12.93 (5-OH, s).

**Preparation of samples and stock solutions for HPLC**

The MeOH extracts of *L. indica*, *L. raddeana*, *L. sativa*, and *L. triangulate* were purchased from Korea Research Institute of Bioscience and Biotechnology, Daejeon, Republic of Korea.

Sample stock solutions were prepared by dissolving each extract (30 mg/mL) in MeOH, sonicating for 20 min, and filtering through a 0.45 μm polyvinylidene difluoride membrane. Stock solutions of the six standards (1 mg/mL) were prepared using the same procedure as above.

**HPLC conditions**

The six compounds were quantified using HPLC-UV using a reverse-phase YMC-Pack Pro C18 column (4.6×250 mm, 5 μm) (YMC Korea Co. Ltd., Seongnam, Republic of Korea) maintained at 30 °C. All the samples were detected at a wavelength of 280 nm. The flow rate was 1 mL/min and the injection volume was 10 μL. The mobile phases were 0.5% acetic acid in water (A) and acetonitrile (B). Samples and standards were eluted using a gradient program as follows: 0 min, 95% A; 22 min, 30% B; 29 min, 30% B; 32 min, 40% B; 42 min, 100% B; 45 min, 100% B; 47 min, 95% A; and 55 min, 95% A. All samples were injected in triplicate.

**Calibration curves**

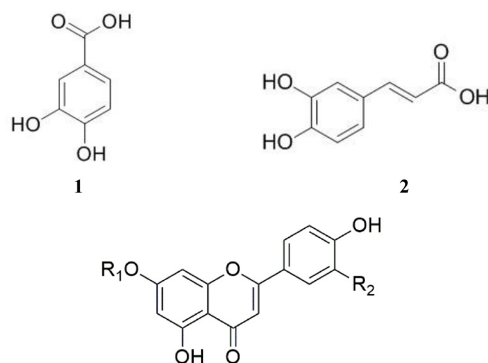
The working solutions were prepared by serially diluting the standard stock solution of each standard to the specified concentrations and were used to construct the calibration curve. The calibration functions of the standards were calculated from the peak area (Y) and concentration (X, μg/mL), and the values are presented as means ± standard deviation (n =3).

## Results and Discussion

The chemical structures of the six isolated compounds were identified using NMR and MS and compared with previous literature. Two phenolic acids and four flavonoids were isolated and identified from the MeOH extracts of *L. serriola* (Fig. 1).

The NMR spectra of compounds **1** and **2** indicated phenolic acids [protocatechuic acid (**1**) and caffeic acid (**2**)], and their spectral data were compared with those of previous studies [20-23]. Signals of the ABX splitting type in the aromatic ring were observed in both compounds. The spectrum of compound **1** showed aromatic proton signals at δ 6.76 (d, 8.0), 7.39 (dd, 2.0, 8.0), and 7.42 (d, 2.0) and carbonyl carbon signals at δ 171.9. Furthermore, the $^1$H-NMR spectrum of compound **2** showed trans-ene double bond signals at δ 6.15 (d, 15.5) and 7.37 (d, 15.5).

Compounds **3-6** were identified as flavonoids and their derivatives [cynaroside (**3**), apigenin 7-glucuronide (**4**), luteolin (**5**), and apigenin (**6**)] by comparing their spectral data with those of previous studies [24-29]. “Flavonoids” belong to a class of secondary metabolites of polyphenols found in plants, and originate from the Latin word “*flavus*”, meaning yellow. Flavonoids have a basic skeleton of 15-carbons and are composed of two phenyl rings and one heterocyclic ring containing an embedded oxygen [30]. The spectra of compounds **3-6** indicated a 5-OH signal of the flavonoid A-ring and a carbonyl (C=O) signal. Compound **3** had a glucose attached to compound **5**, and




| Compound | $R_1$ | $R_2$ |
|---|---|---|
| **3** | Glc | OH |
| **4** | Gln | H |
| **5** | OH | OH |
| **6** | OH | H |

**Fig. 1** Chemical structures of compounds **1-6**: protocatechuic acid (**1**), caffeic acid (**2**), cynaroside (**3**), apigenin 7-glucuronide (**4**), luteolin (**5**), and apigenin (**6**)

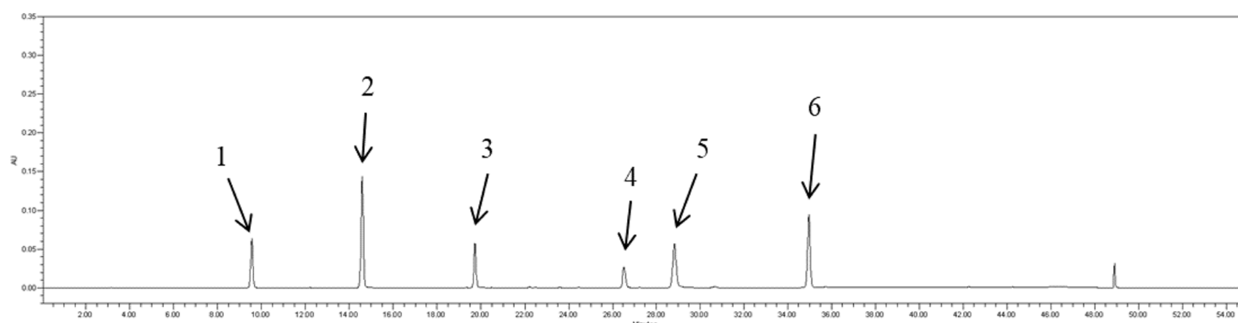



**Fig. 2** HPLC chromatograms of compounds **1**–**6**: protocatechuic acid (**1**), caffeic acid (**2**), cynaroside (**3**), apigenin 7-glucuronide (**4**), luteolin (**5**), and apigenin (**6**)

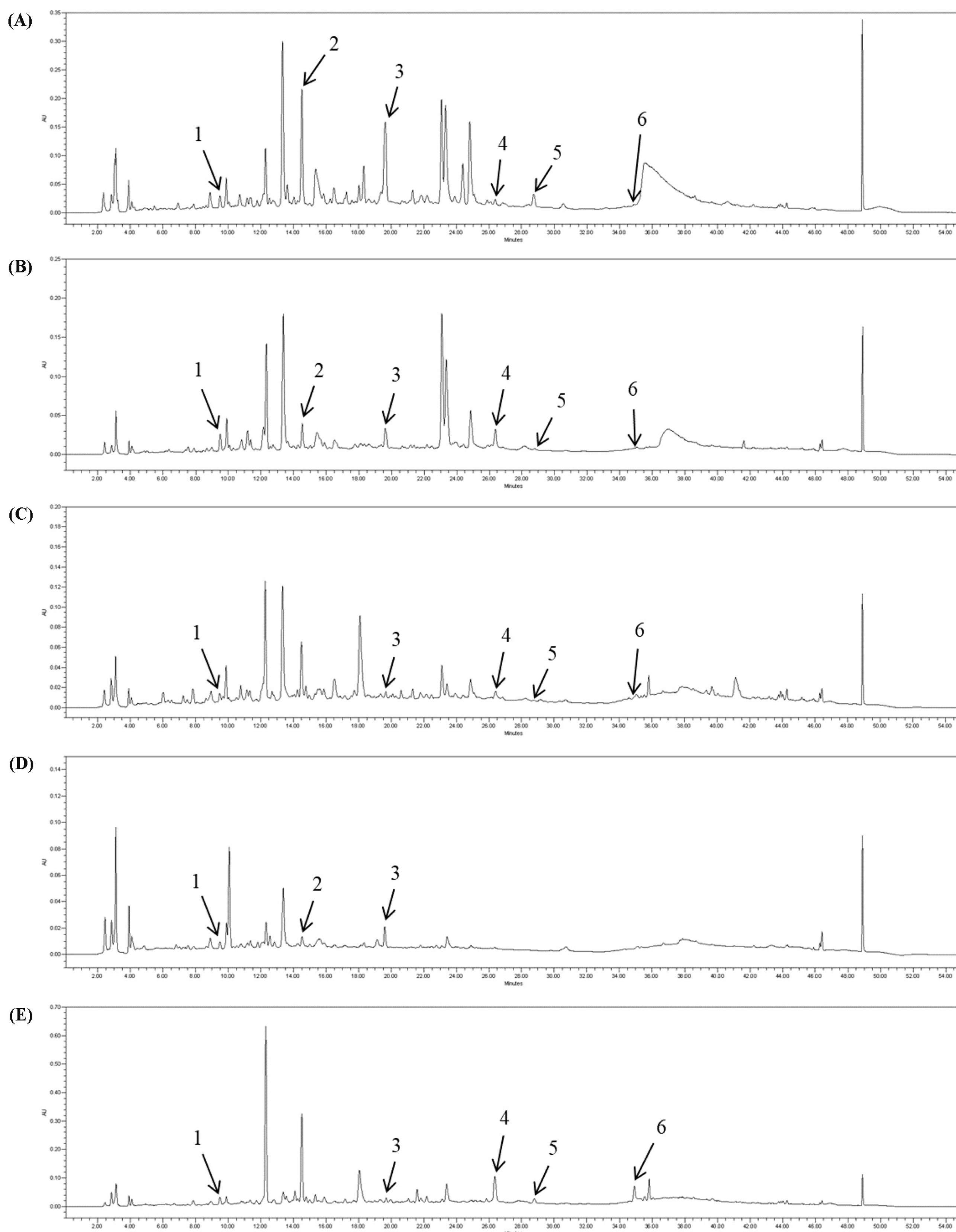



Fig. 3 HPLC chromatograms of the methanol extracts [protocatechuic acid (**1**), caffeic acid (**2**), cynaroside (**3**), apigenin 7-glucuronide (**4**), luteolin (**5**), and apigenin (**6**)] of (A) *Lactuca serriola*, (B) *L. indica*, (C) *L. raddeana*, (D) *L. sativa*, and (E) *L. triangulata*

**Table 1** Calibration curves of methanol extracts of *Lactuca serriola*: protocatechuic acid (**1**), caffeic acid (**2**), cynaroside (**3**), apigenin 7-glucuronide (**4**), luteolin (**5**), and apigenin (**6**)

| Compound | $t_R$ [a] | Calibration equation[b] | Correlation factor, $r^2$ [c] |
|---|---|---|---|
| **1** | 9.5 | Y = 14486X - 2266.9 | 0.9999 |
| **2** | 14.5 | Y = 26598X - 10400 | 0.9994 |
| **3** | 19.7 | Y = 15814X + 16652 | 0.9992 |
| **4** | 26.5 | Y = 16062X - 29206 | 0.9991 |
| **5** | 28.8 | Y = 20652X + 3588.9 | 0.9992 |
| **6** | 34.9 | Y = 25529X + 3059.4 | 0.9994 |

[a] $t_R$ = retention time
[b] Y = peak area, X = concentration of the standard (μg/mL)
[c] $r^2$ = correlation coefficient for five data points in the calibration curve

**Table 2** Content of protocatechuic acid (**1**), caffeic acid (**2**), cynaroside (**3**), apigenin 7-glucuronide (**4**), luteolin (**5**), and apigenin (**6**) in methanol extracts of *Lactuca* species

| Sample | Content (mg/g ext.) | | | | | |
|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** |
| *L. serriola* | 0.29±0.05 | 1.58±0.36 | 2.64±0.45 | 0.21±0.02 | 0.30±0.02 | 0.01±0.00 |
| *L. indica* | 0.35±0.00 | 0.29±0.01 | 0.46±0.02 | 0.51±0.01 | 0.01±0.01 | tr |
| *L. raddeana* | 0.10±0.01 | ND | 0.04±0.01 | 0.23±0.04 | 0.01±0.00 | tr |
| *L. sativa* | 0.09±0.00 | 0.09±0.00 | 0.22±0.01 | ND | ND | ND |
| *L. triangulata* | 0.32±0.01 | ND | 0.13±0.01 | 1.46±0.08 | 0.22±0.03 | 0.40±0.02 |

ND, not detected; tr, trace

an anomeric proton signal was observed at δ 5.08 (d, 7.5). The $^{13}$C-NMR signals of glucose and anomeric carbon were indicated at δ 60.6, 69.5, 73.1, 76.4, 77.1, and 99.8. Compound **4** had glucuronic acid combined with compound **6**, and the carbon signal at δ 171.7 (C-6″) indicated a glucuronic acid moiety. Compound **5** showed proton signals at δ 6.87 (d, 8.5), 7.38 (d, 2.5), and 7.41 (dd, 2.5, 8.5), indicating the ABX splitting type of the flavonoid B-ring. Compound **6** showed proton signals at δ 6.90 (d, 9.0) and 7.87 (d, 9.0), indicating A2B2 splitting of the B-ring.

Six compounds were quantified in the extract of *L. serriola*, and extracts of four plants of the same genus were also quantitatively analyzed and their contents were compared. The six compounds were well separated by HPLC, and the chromatograms of the standards and extracts are shown in Fig. 2 and Fig. 3, respectively. The correlation coefficients ($r^2$) of the standards ranged from 0.9991 to 0.9999 (Table 1). The contents of each compound in the extracts of *Lactuca* species are shown in Table 2, and all six compounds were detected in the extract of *L. serriola*. There was a high content of compound **3** (2.64 mg/g ext.) in the *L. serriola* extract, which was also higher than the other extracts; compound **2** was high. The other compounds were present in relatively small amounts in *L. serriola* extracts. Unlike *L. serriola*, the content of compound **4** was the highest in *L. triangulata* compared to other extracts (1.46 mg/g ext.). Compound **1** was detected in all extracts, and the contents were similar. In contrast to the other extracts, only compounds **1-3** were detected in *L. sativa*.

In other studies, several flavonoids and phenolic acids, including chlorogenic acid, *p*-coumaric acid, luteolin, caffeic acid, and cynaroside, have been isolated from *L. serriola* and their antioxidant activity has been reported [8,14,31]. Wang et al. isolated and identified compounds **1-3** from *L. indica* and evaluated the free radical-scavenging activity in the extract [32]. Nugroho et al. quantified phenolic compounds, including luteolin and cynaroside, from the extract of *L. raddeana,* suggesting its anti-obesity activity [33]. *Lactuca sativa* contains vitamins, carotenoids, and phenolic compounds [34], and polyphenols are mainly composed of caffeic acid derivatives and flavonoids [35]. Kim et al. conducted an HPLC analysis from the seventeen extracts of the Compositae plants; among them, *L. triangulata* showed the highest total phenolic content and peroxynitrite-scavenging activity [36]. Although there are several studies on *L. serriola*, their photosynthetic characteristics and genetic and morphological variability have been studied, and studies on the phytochemical constituents of *L. serriola* are still lacking. In addition, *L. serriola* is an ecosystem-disturbing species with high research value and potential as a usable resource.

In conclusion, we isolated six phytochemical constituents from *L. serriola* and performed quantitative analysis of the MeOH extracts of *L. serriola, L. indica*, *L. raddeana*, *L. sativa*, and *L. triangulata* using HPLC-UV. These results can be used as key data for economic and industrial utilization of *L. serriola* and *Lactuca* species.

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