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# Simultaneous determination of methoxyflavones in selected Korean thistles

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**Abstract** Simultaneous determination of three methoxyflavones, namely, cirsimarin (**1**), hispidulin (**2**), and cirsimaritin (**3**) in selected Korean thistles was performed via reversed-phase high performance liquid chromatography system. Compound **1** was present in all the thistle species examined, whereas **2** and **3** were only detected in *Cirsium japonicum* and *C. japonicum* var. *maackii* (CJM). The concentration of compounds **1-3** in CJM varied according to the time of harvest. Plants collected in the spring (CJMS) and fall (CJMF) had the highest contents of **3** and **1**, respectively. A lower content of **2** was observed in CJMF than in CJMS. This indicates that seasonal variation affects the flavonoid content of CJM. The results of this study show that CJM is an excellent source of compounds **1-3** and it can potentially be cultivated for industrial and pharmaceutical applications involving these compounds.

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**Keywords** Cirsimarin · Cirsimaritin · High performance liquid chromatography-UV · Hispidulin · Korean thistles · Methoxyflavone

## Introduction

Thistles are perennial plants belonging to the Astraceae family that are characterized by their distinct spiny lanceolate leaves, and flowers with colors ranging from white to purple. There are approximately 250 thistle species distributed worldwide and ten of them are found in Korea [1]. They are traditionally used for herbal medicine preparations that include treatments for liver diseases, hemorrhage, edema, and inflammation [2,3]. Recent studies have revealed that thistles are rich in many bioactive compounds such as terpenoids, phytosterols, fatty acids, alkaloids, and flavonoids [4-7]. Particularly, flavonoids isolated from this genus have been shown to possess various biological activities. For example, pectolinarin and apigenin isolated from *Cirsium japonicum* exhibited anti-tumor and anti-excitotoxic activities in mice, respectively; pectolinarigenin from *C. setidens* exerted hepatoprotective and neuroprotective effects, and luteolin-5-*O*-glucoside displayed anti-inflammatory effects [8-12].

In our previous study, three methoxyflavones, namely, cirsimarin (**1**), hispidulin (**2**), and cirsimaritin (**3**) were isolated from the aerial parts of *C. japonicum* var. *maackii* (CJM) [13,14]. These compounds have been shown to exhibit bioactive properties, and thus have pharmacological importance [15-18]. Accordingly, the aim of this study was to determine the distribution of these compounds and quantify their contents in selected Korean thistles by high performance liquid chromatography (HPLC) with ultraviolet-visible detection. The results of this study will serve as a basis for the quality evaluation and selection of Korean thistles to be cultivated for industrial and pharmaceutical applications involving these compounds.

## Materials and Methods

### Plant materials and methanol extracts

Selected thistle species and varieties were analyzed in the study. *C. japonicum* var. *maackii* (CJM) collected during spring (*C. japonicum* var. *maackii* from spring: CJMS) and fall (*C. japonicum* var. *maackii* from fall: CJMF) seasons were extracted with methanol (MeOH) at 80 °C for 3 h and evaporated to dryness. The MeOH extracts of samples from several thistle species were procured from the Korea Research Institute of Bioscience and Biotechnology. These samples were from the species: *C. japonicum* (CJ), *C. chlorepsis* (CC1), *C. chanroenicum* (CC2), *Carduus crispus* (CC3), *C. nipponicum* (CN), and *C. setidens* (CS).

### Instruments and reagents

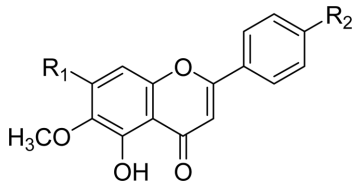
HPLC analysis was performed using a Waters HPLC system equipped with a binary pump and a UV-Vis detector (Milford, MA, USA). All solvents used were HPLC grade including, MeOH, acetonitrile (ACN), and water for the analysis.

### Preparation of standard and sample solution

A stock solution containing a mixture of compounds **1-3** was prepared by dissolving 1 mg of each standard compound together in 1 mL MeOH (Fig. 1). Serial dilution of the stock solution was performed to obtain the working solutions used for the construction of calibration curves. MeOH extracts of the selected thistles were prepared by dissolving 20 mg of each extract in 1 mL MeOH. All samples were filtered with a 0.45-μm filter prior to use.

### HPLC analytical conditions

HPLC analysis of compounds **1-3** was performed using a reversed-phase HPLC system utilizing an INNO $C_{18}$ (25 cm×4.6 mm, 5 μm) column with a mobile phase of 0.5% acetic acid-water (solvent A) and ACN (solvent B). The total running time of the analysis was 55 min and the gradient elution system was performed as follows: 83% A at 0 min, decreased to 70% A between 0-10 min and maintained until 25 min, 20% A at 30 min, increased to 100% B between 30-35 min and maintained until 40 min, increased to 83% A between 40-50 min and maintained until 55 min. The flow rate, injection volume, and UV absorbance were 1 mL/min, 10 μL, and 270 nm, respectively. The temperature of the column was held constant at 30 °C.




| Compound | $R_1$ | $R_2$ |
|---|---|---|
| Cirsimarin (**1**) | $OCH_3$ | O-Glc |
| Hispidulin (**2**) | OH | OH |
| Cirsimaritin (**3**) | $OCH_3$ | OH |

**Fig. 1** Structures of compounds **1-3**

### Calibration curve

Calibration curves were constructed by plotting the concentrations of each standard solution with their respective peak areas. The linearity of each calibration curve was determined based on the correlation coefficient ($r^2$). The concentrations of compounds **1-3** in the samples were calculated from the calibration curve of each compound. The calibration functions were determined based on the peak area (*Y*), concentration (*X*, μg/mL), and mean values (n =5) ± standard deviation.

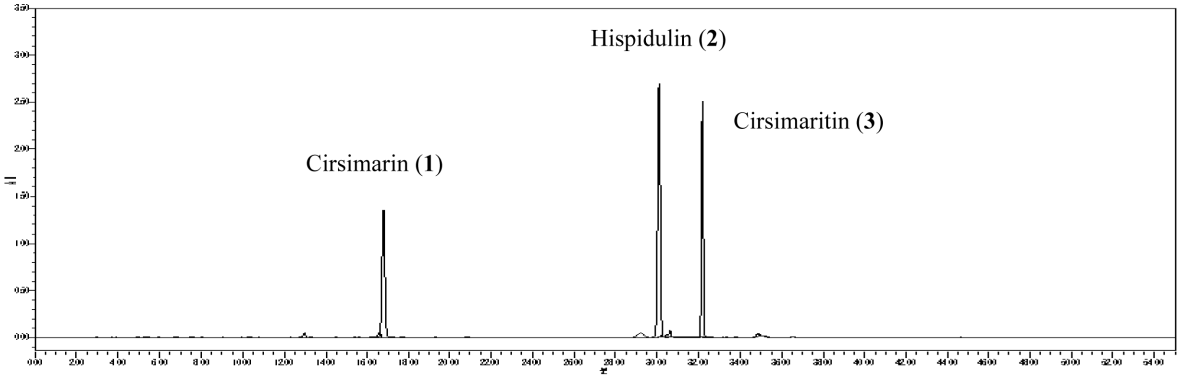
## Results and Discussion

The simultaneous determination of compounds **1-3** in selected Korean thistles was performed using a reverse-phase HPLC system. The analytical method showed good linearity as displayed in the calibration curves for each standard compound (Table 1). The chromatographic separation of compounds **1-3** showed a high resolution in all thistles examined (Fig. 2). The peaks of all chromatograms were confirmed by spiking the HPLC samples with the reference compounds and by UV comparison for qualitative analysis. The concentrations and distributions of the methoxyflavones in the samples analyzed are summarized in Table 2. Compound **1** was present in all the thistles examined, and the samples CJMS, CS, CN, and CC2 contained especially high concentrations of the compound. Compounds **2** and **3** were only detected in CJ, CJMS, and CJMF. Among the thistle species examined, the presence of all three methoxyflavones in CJM is

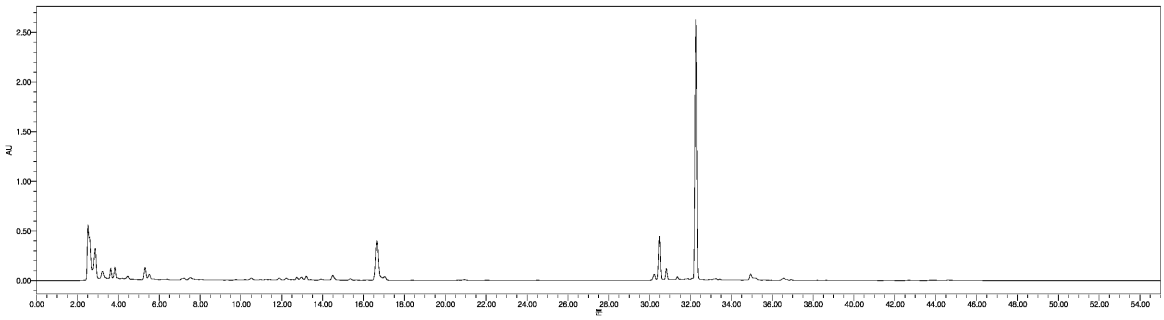
**Table 1** Calibration curves for compounds **1-3**

| Compound | $t_R$[a] | Calibration equation[b] | Correlation factor, $r^2$ [c] |
|---|---|---|---|
| Cirsimarin (**1**) | 17.41 | Y=2,000,000X+77,179 | 0.999 |
| Hispidulin (**2**) | 30.19 | Y=2,000,000X+14,006 | 1.000 |
| Cirsimaritin (**3**) | 32.21 | Y=1,000,000X+366,477 | 0.989 |

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




(A)



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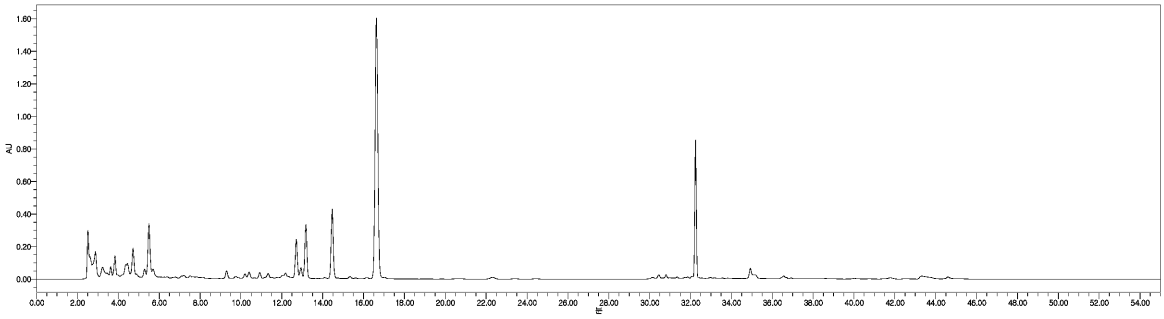

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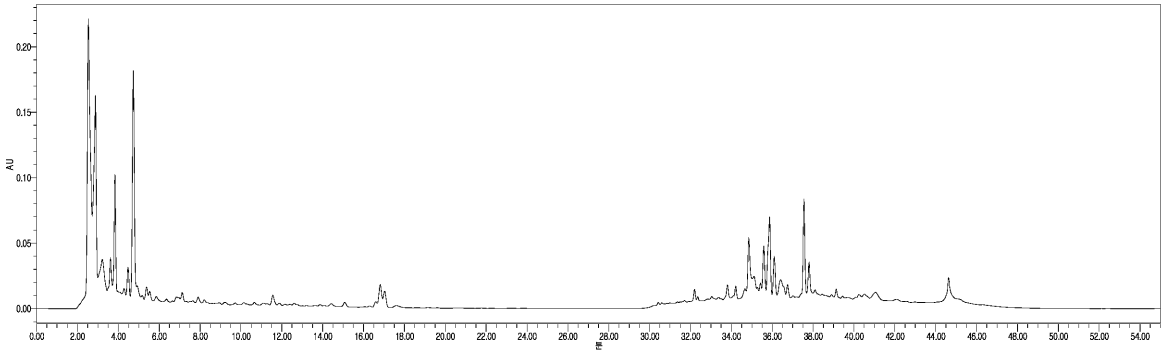
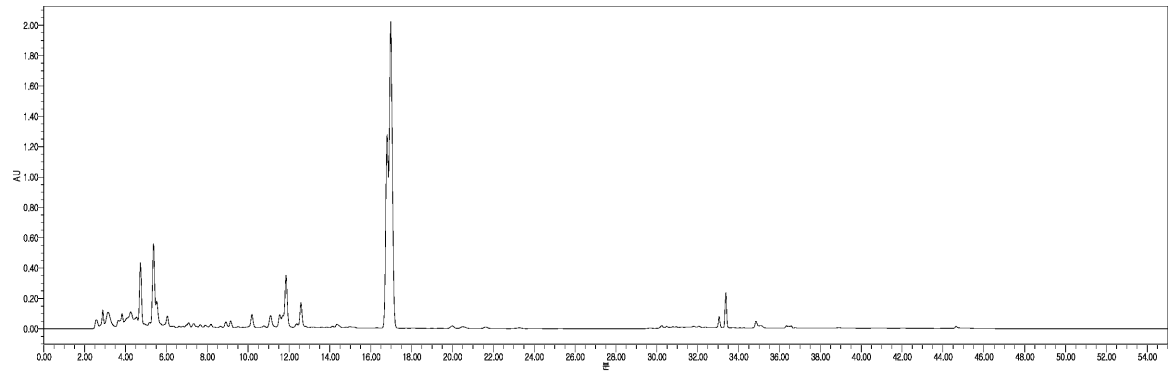
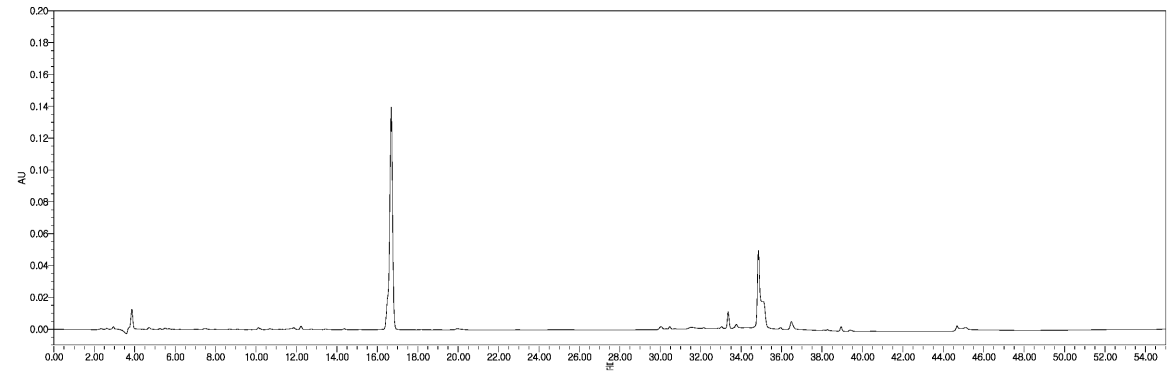


(D)

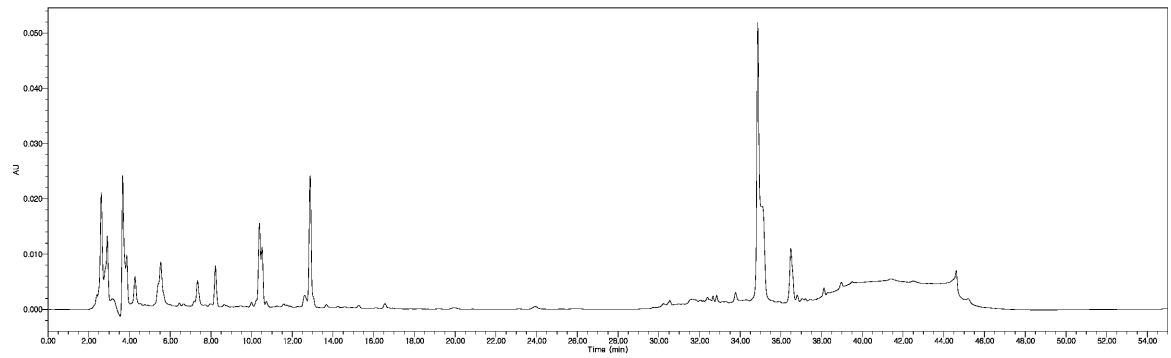
**Fig. 2** HPLC chromatograms of compounds **1-3** (**A**) and the MeOH extracts of CJMS (**B**), CJMF (**C**), CJ (**D**), CC1 (**E**), CC2 (**F**), CC3 (**G**), CN (**H**), and CS (**I**)



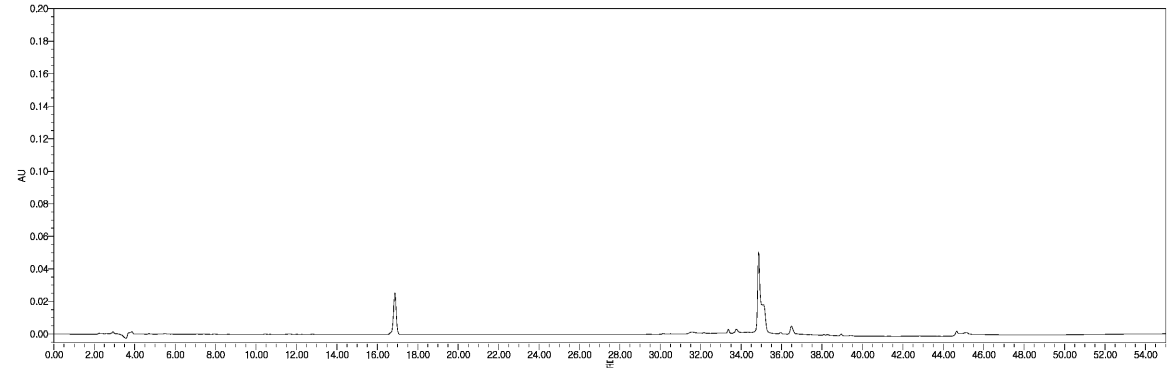

(E)




(F)

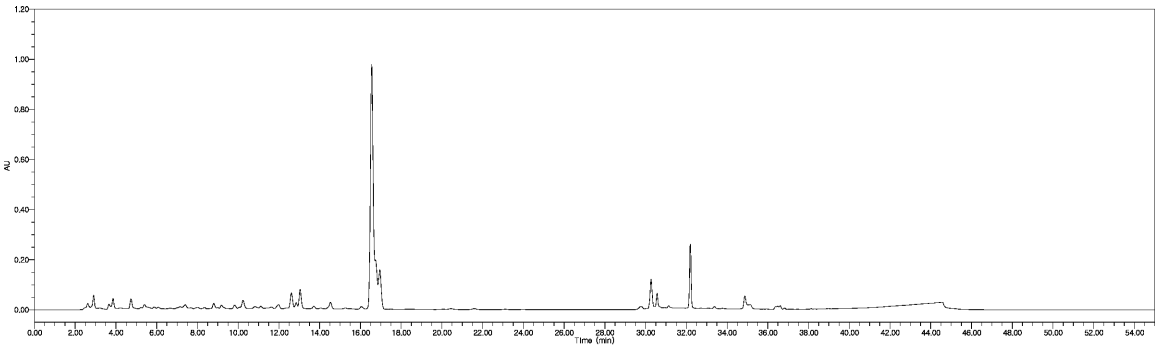



(G)




(H)

**Fig. 2** Contined



(I)

**Fig. 2** Contined

**Table 2** Contents of compounds **1-3** in the MeOH extracts of selected Korean thistles

| Sample | Content (mg/g extract) | | |
|---|---|---|---|
| | Cirsimarin (**1**) | Hispidulin (**2**) | Cirsimaritin (**3**) |
| CJMS | 17.65±0.60 | 3.31±0.01 | 79.73±0.10 |
| CJMF | 74.99±0.04 | 0.16±0.00 | 21.07±0.01 |
| CJ | 0.04±0.00 | ND | 1.62±0.00 |
| CC1 | 9.28±0.20 | ND | ND |
| CC2 | 44.77±0.23 | ND | ND |
| CC3 | 0.37±0.00 | ND | ND |
| CN | 46.75±0.11 | ND | ND |
| CS | 73.59±0.46 | ND | ND |

ND=not detected

consistent with previous studies that investigated on its phytochemistry [14]. The concentration of compounds **1-3** in CJM varied according to the time of harvest as shown by the differences in flavonoid content between the samples collected in the spring (CJMS) and in the fall (CJMF). Particularly, the concentration of **1** was highest in the samples harvested in the spring while that of **3** was highest in the fall. This indicates that seasonal variation affects the flavonoid content of CJM, which should be taken into consideration to optimize the yield of these compounds upon harvest. Especially because the reported biological activities of CJM is attributed to the presence of compounds **1-3** in its extracts, and that variations in their yield can have profound effects on the bioactivity of CJM. In our previous research works, we have shown that compounds **1** and **2** from CJM confer potential therapeutic effects against diabetes, as indicated by its strong inhibitory effects on the polyol pathway which is a major mechanism linked to the pathogenesis of diabetic complications [13,19]. Moreover, compounds **1-3** in CJM has also been reported to exhibit beneficial against menopausal symptoms in animal models [18]. Moreover, the results showed that although CJ and CJM belong to the same species, the two varieties showed distinct flavonoid contents in that all three methoxyflavones were present in CJM whereas **2** was absent in CJ. The contents of **1** and **3** were also higher in CJM than in CJ. This indicates that the flavonoid contents of CJ and CJM can be used to distinguish the two varieties similar to a previous study in which *C. setosum* and *C. japonicum* were differentiated from each other based on their flavonoid contents [3].

There have been few studies on the chemical composition of Korean thistles and this study provides new information regarding the distribution and concentration of methoxyflavones in several Korean thistle species. It was observed that compounds **1-3** are the major constituents of CJM and therefore could be cultivated as a source of these compounds for industrial and pharmaceutical applications.

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