Simultaneous determination of saikosaponin derivatives in *Bupleurum falcatum* by HPLC-ELSD analysis using different extraction methods

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**Abstract** Saikosaponin derivatives such as saikosaponins A, B1, B2, B3, B4, C, and D present in *Bupleurum falcatum* were analyzed by a high performance liquid chromatograph equipped with an evaporative light scattering detector, using different extraction solvents (water and 70% ethanol). The samples were injected into a YMC Pack Pro C18 column and separated using a gradient elution system with a mobile phase composed of acetonitrile and water at a flow rate of 1.1 mL/min. The content of saikosaponin derivatives was higher in 70% ethanol extract than in water extract. This study provides an efficient analytical method for determining the optimal conditions for extraction of saikosaponin derivatives, which can be used as a basis for development of functional foods and pharmaceutical products from *B. falcatum*.

**Keywords** *Bupleurum falcatum* · Extraction method · High performance liquid chromatograph · Quantitative analysis · Saikosaponin

**Introduction**

Recent pharmacological studies have shown that the medicinal herb, *Bupleurum falcatum* (BF), is effective in improving cognitive function [1,2]. BF has been used as a traditional herbal medicine for centuries. Ancient doctors in Korea used a variety of herbs to treat mental stress, which produced similar effects as described in BF [3]. In East Asia, it is being used to treat women's health problems, depression, digestive problems, hepatitis and infections [4-8]. Many studies have reported the presence of various bioactive components in its roots, such as polysaccharides, triterpenoid glycosides, phenethyl alcohol glycosides, and flavonoid glycosides [9-13].

Phytochemical studies have demonstrated that saikosaponins are the major components present in BF. Saikosaponins are oleanane-type triterpenoid saponins and divided into seven types according to their characteristic aglycone moiety [14]. Saikosaponins can be broadly divided depending on the number of rings present, but generally five-ring structures tend to dominate [15]. Saikosaponins isolated from BF are known for their numerous pharmacological activities, including anti-inflammatory, anti-bacterial, anti-tumor, anti-cancer, anti-hepatitis, and anti-allergic [16-20].

In this study, we aimed to and quantify saikosaponins A, B1, B2, B3, B4, C, and D in BF extract using a high performance liquid chromatograph (HPLC) equipped with an evaporative light scattering detector (ELSD).

**Materials and Methods**

**Plant materials**
The 70% ethanol (EtOH) (3-19-0097) and water (3-19-0054) extracts of BF were provided by Korea Institute of Oriental Medicine, Daejeon 34054, Republic of Korea.

**Instruments and reagents**
HPLC analysis was performed using an instrument (Gilson 72, Rue Gambetta-BP45 95400, Villers Le Bel France) equipped with a pump, auto-sampler, and ELSD. The HPLC grade solvents (water and acetonitrile) were used for analysis and purchased from J. T. Baker (Philipsburg, PA, USA). Analytical grade methanol...
(MeOH) reagent was purchased from Samchun Pure Chemical Co., Ltd. (Pyeongtaek, Korea). Saikosaponins A, B1, B2, B3, B4, C, and D were obtained from Natural Product Institute of Science and Technology (www.nist.re.kr), Anseong, Korea.

**Extraction of samples**

Dried and crushed BF (1 kg) was extracted with 70% EtOH (4 L) under sonication for 1 h and the process was repeated 2 times. After extraction, the sample was filtered, evaporated at 37 °C, freeze-dried, and homogenized using 600 μm sieve to obtain 70% EtOH extract of BF (EEB). Dried and crushed BF (1 kg) was extracted with water (4 L) under reflux for 3 h. The resulting extract was filtered using 53 μm sieve, evaporated at 37 °C, and homogenized using 600 μm sieve to obtain water extract of BF (WEB). The homogenized powders were stored in tight-sealed bottles and kept in a refrigerator with light protection until used for analysis.

**Preparation of standards and HPLC conditions**

The EEB and WEB (1 mg each) were dissolved in MeOH and filtered using a 0.45 μm polyvinylidene fluoride (PVDF) membrane filter. Quantitative analysis was performed using a reverse phase HPLC with a YMC Pack Pro C18 column (5 μm, 25 cm × 4.6 mm). The injection volume was 10 μL, and the ELS detector was used. The temperature of the column was maintained at 25 °C, and the flow rate was set at 1.1 mL/min. The eluent used in mobile phase consisted of 90% acetonitrile (A) and 10% acetonitrile (B). The lamp discharge system of A started at 20% until 5 min, increased to 50% at 35 min, and 100% at 40 min which was maintained until 50 min. It was then decreased to 20% at 51 min and maintained until 60 min.

**Calibration curves**

The standard stock solution was prepared by dissolving the compound in MeOH (1 mg/mL). The calibration curve was constructed by continuously diluting the selected stock solutions to the desired concentration. The standard calibration curve values were calculated as peak area (Y), concentration (X, mg/mL), and mean value ± standard deviation (n = 3).

**Results and Discussion**

BF has been used since ancient times to improve cognitive impairment in people, and compounds isolated from its roots have now been shown to be effective in improving cognitive impairment [3]. Several types of saikosaponins are the main components found in BF [21]. These substances act as therapeutic agents for various diseases and play an important role in improving cognitive ability. Quantitative analyses of saikosaponins A, B1, B2, B3, B4, C, and D in EEB and WEB were performed using reverse phase HPLC with gradient elution system. The results of our HPLC analyses showed good separation of the components, and the use of ELSD was found to be effective in the detection of saikosaponins. As saikosaponins A, B1, B2, B3, B4, C, and D have no UV chromophores, the optimized analysis was performed at short wavelength of 203 nm using ELSD owing to the fact that the analysis is susceptible to interference from other substances. The calibration curve was created by linearly plotting peak area vs. prepared concentration and analyzed using linear regression. The linear regression coefficients (r²) for the standard were 0.9983 to 1. The calibration curves of saikosaponins A, B1, B2, B3, B4, C, and D are shown in Table 1.

The structures of saikosaponins A, B1, B2, B3, B4, C, and D are shown in Fig. 1. Peak retention times of 30.7, 33.9, 31.7, 26.8, 27.7, 23.6 and 39.8 min of saikosaponins A, B1, B2, B3, B4, C, and D are shown in Table 1. HPLC chromatograms of saikosaponins A, B1, B2, B3, B4, C, and D are shown in Fig. 2. HPLC chromatograms of EEB and WEB are shown in Fig. 3 and 4, respectively. Table 2 shows the content of saikosaponins A, B1, B2, B3, B4, C, and D in EEB and WEB. The overall contents of total saikosaponins was 6.085 and 1.93 mg/mL in EEB and WEB, respectively. The results revealed that 70% EtOH was more efficient in extraction of saikosaponins from BF. The parameters affecting extraction efficiency of saikosaponins A, B1, B2, B3, B4, C, and D were extraction method and solvent used. In the

### Table 1 Calibration curves of saikosaponins A (1), B1 (2), B2 (3), B3 (4), B4 (5), C (6), and D (7)

| Compound | t<sub>a</sub> | Calibration equation<sup>a</sup> | Correlation factor, r<sup>b</sup>² |
|----------|--------------|---------------------------------|-------------------------------|
| 1        | 30.7         | Y = 4.3627X-50.57                | 1                             |
| 2        | 33.9         | Y = 21.302X-1380.9               | 0.9995                        |
| 3        | 31.7         | Y = 5.315X-230.35                | 0.9983                        |
| 4        | 26.8         | Y = 10.937X-1209.1               | 1                             |
| 5        | 27.7         | Y = 4.5337X-633                  | 0.9999                        |
| 6        | 23.6         | Y = 16.298X-1246                 | 0.9998                        |
| 7        | 39.8         | Y = 13.166X-1738.5               | 1                             |

<sup>a</sup>Y = peak area, X = concentration of standards (mg/mL)

<sup>b</sup>r² = correlation coefficient based on three data points in the calibration curves
extraction method performed using an ultrasonic shaker and 70% EtOH, the extraction yield was 9.46%. The extraction yield of reflux extraction using water was 20.26%. As shown in Table 2, the content of all standards except saikosaponin B2 were higher in the 70% EtOH extract than in the water extract.

Park et al. (2015) previously reported that extraction with 70% EtOH could increase the content of saikosaponins, consistent with our results [22]. Lee (2008) reported the importance of the extraction solvent during plant extraction. Previous studies on saikosaponin extraction suggested that extracting saikosaponins using 70% MeOH and ultrasonic shaker for 40 min could result in the highest extraction yield. Our results revealed that the saikosaponin content was significantly higher when extracted with 70% EtOH than with water. However, we performed water extraction under reflux, and 70% EtOH extraction using ultrasonic shaker. Consequently, the use of ultrasonic extraction method with water may result in a higher yield of saikosaponins [23].

The overall content of saikosaponins A, B1, B2, B3, B4, C, and D was higher in the 70% EtOH extract than in the water extract. Our study provides an optimized analytical method for determining the best extraction conditions for saikosaponins, which can be used as a basis for development of functional food and pharmaceutical products derived from BF.

### Table 2 Contents of saikosaponins A (1), B1 (2), B2 (3), B3 (4), B4 (5), C (6), and D (7) in EEB and WEB

| Compound | Content (mg/g) | EEB | WEB |
|----------|---------------|-----|-----|
| 1        | 31.35 ± 2.19  | 1.87 ± 0.04 |
| 2        | 1.78 ± 0.00   | 1.70 ± 0.00 |
| 3        | 1.10 ± 0.00   | 2.61 ± 0.04 |
| 4        | 2.81 ± 0.00   | 2.78 ± 0.01 |
| 5        | 6.31 ± 0.00   | 3.64 ± 0.00 |
| 6        | 5.63 ± 0.12   | 2.12 ± 0.00 |
| 7        | 12.10 ± 0.29  | 3.31 ± 0.01 |

Fig. 1 Chemical structures of saikosaponins A (1), B1 (2), B2 (3), B3 (4), B4 (5), C (6), and D (7)

Fig. 2 HPLC chromatogram of saikosaponins A (1), B1 (2), B2 (3), B3 (4), B4 (5), C (6), and D (7)
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