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Determination of saikosaponins in three *Bupleurum* plants by HPLC analysis

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ABSTRACT

In this study, saikosaponins were determined from roots of three *Bupleurum* plants collected in June 2009. Bupleuri Radix (*Bupleurum* spp. root) is one of the most important crude drugs in Korea, China and Japan. High performance liquid chromatography (HPLC) was used for the determination of saikosaponin 'a', 'b' and 'c' in three *Bupleurum* plants. The highest total saikosaponin content was found in the *B. falcatum* 'Mishima' (1281.94 mg%). Total saikosaponin contents of *B. falcatum* and *B. latissimum* were 669.67 and 489.95 mg%, respectively. *B. falcatum* 'Mishima' and *B. falcatum* contained more saikosaponin 'a' than other saikosaponins. The contents of saikosaponin 'a' of *B. falcatum* 'Mishima' and *B. falcatum* were 745.74 and 484.60 mg%, respectively. In contrast, *B. latissimum*, an endemic species of Korea had higher saikosaponin 'c' (351.90 mg%) content than the other two saikosaponins and the content was higher than *B. falcatum* (181.71 mg%). *B. latissimum*, a Korean endemic species, is a good source for extraction/production of saikosaponin 'c'.

Key words : *Bupleurum falcatum*, *Bupleurum falcatum* 'Mishima', *Bupleurum latissimum*, Korean endemic species, saikosaponin

INTRODUCTION

Saikosaponins, known as the main components of *Bupleurum* spp., are oleanane saponins (Bao *et al.,* 2004). The genus of *Bupleurum* is one of the large genuses of Apiaceae and is widely distributed at Europe and Asia (Kim *et al.*, 2006). *Bupleurum* spp. is a perennial plant, a simple leaf and its venation is parallel venation, and its flower colour is yellow and it has been used in traditional Chinese herbal medicine as the major prescription for hepatitis (Yen *et al.*, 1994). It was reported that there were about 150 species at the only northern hemisphere restricted to small areas. *B. falcatum, B. longiradiatum, B. euphorbioides, B. latissimum* and *B. scorzonerifolium* are spread in Korea as five syntaxon (So *et al.*, 2008).

It was known that the roots of *B. falactum* have various pharmacological activities, such as anticomplementary, macrophages Fc receptor up-regulating and antiulcer activities have been reported (Sun *et al.*, 1991; Yamada *et al.*, 1991; Matsumoto *et al.*, 1993). *Bupleurum falcatum* 'Mishima' is one cultivar introduced in Korea Mishima island of Japan (Kim *et al.,* 2014). *B. latissimum* is an endemic species of Korea and it was observed at shoreline of Ulleungdo by 1970. But it was vanished because of the environmental variation and it has been observed since 2000 (Ahn *et al.,* 2006).

There are several kinds of saikosaponin in *Bupleurum* spp. and the content of saikosaponins has been used for the quality evaluation of Bupleuri Radix (herbal name). The content of saikosaponin has been analyzed by TLC (thin-layer chromatography) and HPLC (high perfomance liqid chromatography). Each

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analysis method has its own advantages and limitations (Xiuli *et al.*, 2005).

This study determines saikosaponin content in the three *Bupleurum* plants by HPLC analysis and is going to offer the basic information about the quality evaluation of the three *Bupleurum* plants.

MATERIALS AND METHODS

Plant Materials

The roots of the three *Bupleurum* species were collected as following : *B. falcatumm* was collected from Jeongeon, Gangwon-do (37°22' 47.16″N, 128°39″41.97″ E), *B. falcatum* 'Mishima' was collected from Goheung, Jeollanam-do (34°36′40.40″ N, 127°17′5.92″ E) and *B. latissimum* was collected from Ulleungdo island, Gyeongsangbuk-do (37°30′22.92″ N, 130°51′25.75″ E), Korea in June 2009. The materials were authenticated by one of the authors, Prof. K. W. Yun and voucher specimens were deposited in the Herbarium of Sunchon National University, Korea. The collected plant roots were air-dried in shadow for two weeks.

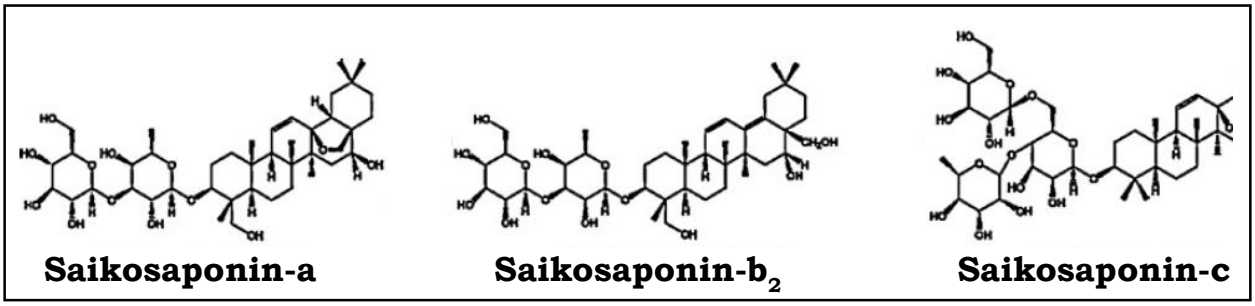

Fig. 1. Structure of saikosaponins.

Chemicals and Reagents

We purchased HPLC grade acetonitrile from J. T. Baker SOLUSORB® (J. T. Baker, USA). Saikosaponin 'a', 'b_2' and 'c' (Fig. 1) were purchased from the Sigma-Aldrich (USA). Ultrapure water was generated with a Millipore II (Nihon Millipore, Tokyo, Japan).

Apparatus and Chromatographic Conditions

Waters associates M 411 (Waters Co., USA) equipped with a 515 binary pump, manual sample injector, and a UV 486 detector were used to perform HPLC analysis. The HPLC fingerprint was carried out on a μ-Bondapak C_{18} column (4.6 mm I. D. × 150 mm, Waters Co., USA) at 30°C with a sample injection volume of 20 μl. Detection wavelength was 206 nm and the flow rate was 1.0 ml/min.

HPLC for Analysis of Saikosaponin

Eighty per cent ethanol was mixed with each 1 g powdered sample and it was pulverized using a homogenizer. The extract was centrifuged at 3,000 rpm for 30 min. Its supernatant was filtered through Whatman No. 2 filter paper. The filtrate was filtered through 0.45 μm membrane filter and it was used as the sample for HPLC analysis. The content was calculated on external standard method (Wang *et al.*, 2004).

Statistical Analysis

To verify the statistical significance, mean±SD of three independent measurements were calculated. Statistical analysis was performed with the software program SPSS (Version 16.0). The level of significance was set at $P<0.05$.

RESULTS AND DISCUSSION

Saikosaponin Contents of Three *Bupleurum* Plants

Fig. 2 shows the HPLC chromatogram of saikosaponin standards and the results for saikosaponins content of the three *Bupleurum* species are presented in Table 1.

The saikosaponin content is one of the most important criteria for determining the quality of Bupleuri Radix (Pan, 2006; Zhu *et al.*, 2007). Nishiura *et al.* (1994) suggested that saikosaponins were known to have various pharmacological effects including stabilization

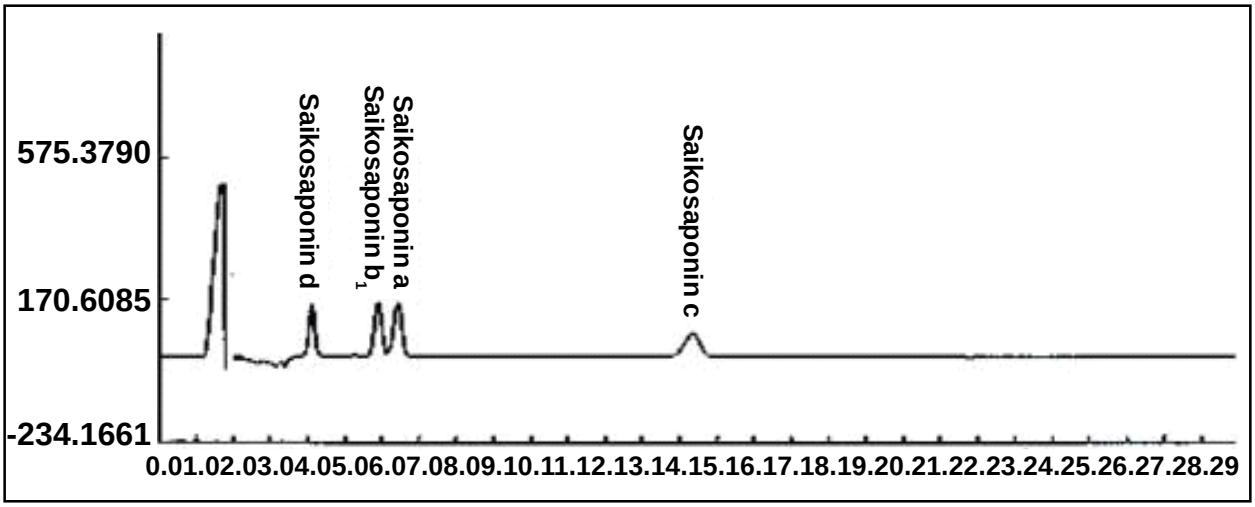

Fig. 2. HPLC chromatogram of saikosaponin standards.

Table 1. Content (mg%) of saikosaponins in three *Bupleurum* plants

Components	*Bupleurum falcatum*	*Bupleurum latissimum*	*Bupleurum falcatum* 'Mishima'
Saikosaponin 'a'	484.60±9.28b	129.82±13.04c	745.74±10.18a
Saikosaponin 'b_2'	3.36±0.27b	8.23±0.28b	37.96±1.41a
Saikosaponin 'c'	181.71±8.52c	351.90±7.92b	498.24±24.88a
Total saikosaponin	669.67	489.95	1281.94

Values with different letters in the same line were significantly (P<0.05) different.

of cell membranes and the protective action of saikosaponin against halothane-induced hepatitis may be partly due to stabilization of the cell membrane of hepatocytes. Zhu *et al.* (2006) stated that the concentration of saikosaponins depended on the growth location, the time of harvest and the part of the root.

B. falcatum 'Mishima' showed more total saikosaponin content (1281.94 mg%) than the two other plants. Total saikosapon contents of *B. falcatum* and *B. latissimum* were 669.67 and 489.95 mg%, respectively. The content of saikosaponin 'a' in *B. falcatum* 'Mishima' and *B. falcatum* was higher than the other saikosaponins and the average contents were 745.74 and 484.60 mg%, respectively. The content of saikosaponin 'b_2' in *B. falcatum* 'Mishima' was higher than those of the other two species. Also, the content of saikosaponin 'c' of *B. falcatum* 'Mishima' was higher than that of *B. falcatum* and *B. latissimum*. The results showed that, in particular, the saikosaponin 'c' content of *B. latissimum* (351.90 mg%) was higher than the other two saikosaponins.

There are many studies to demonstrate that saikosaponin 'a' content of *B. falcatum* and *B. falcatum* 'Mishima' was more than the content of saikosapinin 'c' of the other two *Bupleurum* plants (Kim *et al.*, 2000; Kim *et al.*, 2008). Also, a research showed that the contents of saikosaponin 'a' and 'c' of *B. falcatum* and *B. falcatum* 'Mishima' were higher than the saikosaponin 'b' content of the two plants (Park, 2004).

Saikosaponin 'a' is a compound which has been widly used in treating liver diseases such as liver fibrosis (Wang *et al.,* 2013). Saikosaponin 'c' has the same effects as saponin without glucose (Yamamoto *et al.*, 1975). We can anticipate that the three *Bupleurum* plants have pharmacological activity, especially, *B. falcatum* 'Mishima' has stronger pharmacological activity than the other *Bupleurum* plants.

Saikosaponins, including saikosaponin 'a', 'b_2' and 'd' were reported to have properties of cell growth inhibition, inducing cancer cells differentiation and apoptosis (Hsu *et al.*, 2004). Shyu *et al.* (2004) reported that saikosaponin 'c' had the potential for therapeutic angiogenesis but was not suitable for cancer

theraphy. In this study, *B. falcatum* 'Mishima' and *B. latissimum* had more saikosaponin 'c' than that of *B. falcatum*. It can also suggest the potential of *B. falcatum* 'Mishima' and *B. latissimum* for use in the therapeutic angiogenesis. Saikosaponin 'c' was transformed to saikosaponin 'h' and 'i' by acid hydrolysis and to saikogenin 'c' by mouse intestinal bacteria, respectively. Saikosaponin 'c' was easily transformed to their aglycones and saikosaponin 'c' was transformed to four metabolites, prosaikogenin E_1, prosaikogenin E_2, prosaikogenin E_3 and saikogenin E_4 by human intestinal bacteria. Sapogenin showed a different activity on cells from saponin. The saponin showed anti-inflammatory action and antiedematous effect, but sapogenin did not show anti-inflammatory action and antiedematous effect (Kim *et al.*, 2009). However, the saikosapogenin showed radioprotective and cytotoxic activities, antioxidant, immunomodulatory activity and antiproliferative activity (Papiya *et al.*, 2009). It can be insisted that these metabolic processes are important in the pharmacological effect of traditional medicines.

The contents of saikosaponins from *B. latissimum* were high in the orders of saikosaponin 'c', saikosaponin 'a' and saikosaponin 'b_2'. It is possible to develop functional ingredient consisting saikosaponin 'c'. Therefore, we insist that *B. latissimum*, a Korean endemic species, is a good source for extraction/production of saikosaponin 'c' with important application in pharmaceutical industries.

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