Comparative Analyses of Bioactive Constituents from *Forsythia suspensa* and *Forsythia viridissima* by HPLC-DAD

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Abstract – A high-performance liquid chromatography (HPLC) with diode array detector (DAD) method was established for the discrimination of a folk medicine *Forsythia suspensa* and *Forsythia viridissima*. Five and three representative metabolites of the lignan and phenolic glycoside classes were selected for the analysis from *F. suspensa* and *F. viridissima*, respectively. The optimal chromatographic conditions were obtained on an ODS column (5 µm, 4.6 × 250 mm) with the column temperature at 40°C. The mobile phase was composed of methanol and 0.3% acetic acid using an isocratic elution with the flow rate 1 mL/min. Detection wavelength was set at 280 nm. All calibration curves showed good linear regression ($r^2 > 0.996$) within test ranges. Limits of detection (LOD) and limits of quantitation (LOQ) values were lower than 0.096 and 0.291 µg/mL, respectively. The developed method provided satisfactory precision and accuracy with overall intra-day and inter-day variations of 0.07-0.63% and 0.14-0.62%, respectively, and the overall recoveries of 97.79-102.46% for all of the compounds analyzed. In addition, effectiveness of diverse extraction methods was compared to each other for the development of standard analytical method. The verified method was successfully applied to the quantitative determination of representative metabolites in fifty-three commercial *F. suspensa* samples and fifteen commercial *F. viridissima* samples from diverse sources. The overall analytical results showed the unequivocal differences in bioactive constituents between *F. suspensa* and *F. viridissima*.

Keywords – HPLC-DAD, *Forsythia suspensa*, *Forsythia viridissima*, lignan, phenylethanoide glycoside

Introduction

Herbal medicines have been practiced to maintain good health and treat diseases in the Asia communities and recently in worldwide. Increment of worldwide attention and concomitant pharmaceutical research has made it essential to carry out the quality control measurement for the herbal medicines. However, serious hindrance has been attributed to the lack of recognition and regulation of profession, qualified practitioners, quality-controlled herbal medicines, and evidence-based clinical studies (Normile, 2003; Chan, 2005). Thus it is urgently needed to establish a comprehensive qualified evaluation method based on analysis of the whole bioactive compounds in order to accurately reflect the quality of herbal medicines.

The dried fruits of *Forsythia suspensa* and *Forsythia viridissima*, named Forsythia fructus (Oleaceae), are commonly used as herbal medicines. *F. suspensa* and *F. viridissima* is listed in Korea and Japan Pharmacopoeia but in China Pharmacopoeia only *F. suspensa* is listed. Although there are officially differences, these two species are widely distributed in Korea, China and Japan, East Asia. Traditionally *F. suspensa* has been used as an antipyretic, detoxicant and anti-inflammatory agent. (Piao *et al.*, 2008; Chang *et al.*, 2008). Also *F. suspensa* extract suppresses vomiting, resist hepatic injury, inhibit elastase activity and exhibit diuretic, analgesic, antioxidant, antiendotoxic and antiviral effects (Zhang, 2000; Liu, 2007). Flavonoids, lignans, terpenes, phenylethanoloid glycosides, and volatile oils, have been isolated from *F. suspensa* (Li and Feng, 2005).

On the other hand, *F. viridissima* has been used as an anti-inflammatory agent and it has diuretic, antidote, extrusion of pus and antibacterial effects (Lee *et al.*, 2010). Flavonoids, lignans, triterpenoids, and their glycoside derivatives analogous to *F. suspensa* have been isolated from *F. viridissima* (Nishibe *et al.*, 1977; Chiba *et al.*, 1978). However, there are significant differences in the amount and distribution of individual constituent between

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F. suspensa and F. viridissima, such as the lack of as phylligenin, pinoresinol, phyllirin and pinoresinol-β-D-glucoside in F. viridissima and arctigenin, matairesinol, arctiin and matairesinoside in F. suspensa (Kitagawa et al., 1988; Mariko et al., 1979). Despite these differences, both plants have been commonly used as herbal medicines without discrimination. Therefore the quality control for the discrimination between the two species is essential.

In this study, we initially aimed at developing an HPLC-DAD method for the simultaneous identification and quantitation of bioactive constituents in F. suspensa and F. viridissima. Based upon the prior researches, among the components selected for analysis were five representative compounds from F. suspensa and three representative compounds from F. viridissima with significant bioactivities and large contents: forsythiaside (1) of phenylethanoide glycoside and lariciresinol (2), phyllirin (3), pinoresinol (4), and phylligenin (5) of lignan (Fig. 1.) in F. suspensa and arctiin (6), matairesinol (7) and arctigenin (8) of lignan in F. viridissima (Fig. 2.). The developed methods have been verified for their effectiveness against diverse validation parameters. In addition, the contents of bioactive compounds in fifty-three commercial

F. suspensa from China and fifteen commercial F. viridissima from Korea were analyzed and compared to each other.

**Experimental**

**Plant materials** – Fifty-three samples of Forsythia suspensa and fifteen samples Forsythia viridissima grown in different regions were provided by the National Center for Standardization of Herbal Medicine, such as C-1 (Pan’an, China), C-2–C-5 (Hanam, China), C-6–C-19 (Shanxi, China), C-20–C-53 (unidentified, China), K-1–K-2 (Buyeo, Korea), K-3 (Jeonbuk, Korea), K-4 (Jeonju, Korea), K-5–K-12 (Kyungbuk, Korea), K-13–K-15 (unidentified, Korea).

**Reagents, chemicals and samples** – forsythiaside (1), lariciresinol (2), phyllirin (3), pinoresinol (4), phillygenin (5), arctiin (6), matairesinol (7), and arctigenin (8), isolated and purified from Forsythia suspensa and Forsythia viridissima by a series of chromatographic procedures were provided from National Center for Standardization of Herbal Medicine, Korea and the structures were elucidated by comparison of spectral data (UV, IR, MS, 1H-NMR, 13C-NMR) with the literature.

![Fig. 1. Chemical structures of the standard compounds from Forsythia suspensa.](image-url)