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Quantitative Determination of Kurarinone and Trifolirhizin from the Roots of *Sophora flavescens*

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Abstract – A simple reversed phase HPLC method was developed for extracting pharmacologically active compounds kurarinone and trifolirhizin from the roots of *Sophora flavescens* using a binary gradient of acetonitrile : H_2O with UV detection at 254 nm. The kurarinone and trifolirhizin contents of the roots of *S. flavescens* collected from ten district markets in Korea and China were 77.24~686.89 μg/g and 46.89~288.58 μ/g, respectively.
Keywords – *Sophora flavescens*, HPLC, kurarinone, trifolirhizin

Introduction

The dried root of *Sophora flavescens* Aiton (Leguminosae) is a traditional Chinese medicine with antibacterial, anti-inflammatory, antipyretic, antiarrhythmic, antiasthmatic, antiulcerative and antineoplastic activities, and has been used to treat jaundice, leucorrhea, carbuncles, pyrogenic infections of the skin, scabies, and dysentery (Shen *et al*., 2006). In the previous studies, alkaloids, flavonoids, alkylxanthones, quinones, triterpene glycosides, fatty acids, and essential oil has been isolated from the roots of this plant (Sun *et al*., 2007). The alkaloids isolated from *S. flavescens* have been reported to have antitumor, antiviral, and anti-inflammatory bioactivities (Cheng *et al*., 2006; Zhang and Huang, 2004). The biological effects of some of the flavonoids have been demonstrated *in vitro* antibacterial, antiviral, cytotoxic, antioxidant, glycosidase inhibitory, anti-inflammatory, anti-advanced glycation end products (AGEs), and estrogenic activities (Kim *et al*., 2006; Naeyer *et al*., 2004; Piao *et al*., 2006; Ryu *et al*., 1997; Woo *et al*., 1998;). It was previously reported that the CH_2Cl_2- and BuOH-soluble fractions of this plant roots have a scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. From the above fractions, *trans*-hexadecyl ferulic acid, *cis*-octadecyl ferulic acid, *trans*-hexadecyl sinapic acid, (–)-4-hydroxy-3-methoxy-8,9-methylenedioxypterocarpan, desmethylanhydroicaritin, and 8-lavandulykaempferol were isolated and found to have scavenging activities against DPPH and $ONOO^-$ (Jung *et al*., 2005a; Jung *et al*., 2005b). *S. flavescens* contains numberous flavonoids such as kurarinone, trifolirhizin, kuraridinol, formononetin, kushenols, kuraridin, maackiain, *nor*-kurarinone, and sophoraflavone G (Piao *et al*., 2006). This study quantified the levels of kurarinone and trifolirhizin from the roots of *S. flavescens* collected from the district markets in Korea and China.

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Experimental

General – The chromatographic system for quantitative analysis consisted of a 306 pump (Gilson, USA), 811C dynamic mixer (Gilson, USA), UV/VIS-156 detector (Gilson, USA), 231 XL sample injector (Gilson, USA), and GILSON UniPoint data processor (Gilson, USA). Separations were performed using an Agilent Eclipse XD8-C18 (Agilent Technologies, USA; 5 μm, 4.6 × 150 mm). Methanol (Burdick & Jackson, USA) and acetonitrile (Burdick & Jackson, USA) used in this work were of HPLC grade and other reagents were of analytical grade. Milli-Q (Millipore, MA, USA) treated water (with resistivity more than 17.5 MΩ cm) was used throughout the experiments.

Plant material – The roots of *S. flavescens* were purchased from oriental medicinal markets, such as, SF-K-1 (Jeongseon-mart, Gangwon, Korea, cultured in Korea), SF-K-2 (Jecheon-mart, Chungbuk, Korea, cultured in Korea), SF-K-3 (Kyungdong-mart, Seoul, Korea, cultured

in China), SF-K-4 (Pocheon-mart, Gyeonggi, Korea, cultured in Korea), SF-C-1 (Bojoo-mart, China, cultured in China), SF-C-2 (Ankook-mart, China, cultured in China), SF-C-3 (Nanjoo-mart, China, cultured in China), SF-C-7 (Kimpo-mart, Gyeonggi, Korea, cultured in China), SF-C-9 (Kyungdong-mart, Seoul, Korea, cultured in China), and SF-C-10 (Bojoo-mart, China, cultured in China). All of plant materials identified by Prof. Je-Hyun Lee, Kyung-Hee University, Korea and voucher specimens have been deposited at the Herbarium of College of Pharmacy, Catholic University of Daegu, Korea.

Isolation of standard compounds – The roots (10 kg) of *S. flavescens* were refluxed with MeOH for three hours (3 × 20 L). The total filtrate was concentrated to dryness in vacuum at 40 °C in order to render the MeOH extract (2.3 kg) and this extract was suspended in distilled water and sequentially partitioned with CH_2Cl_2 (280 g), EtOAc (228 g), BuOH (710 g), and H_2O (1,048 g) in sequence. The CH_2Cl_2-soluble fraction (290 g) was initially chromatographed over a Si gel column using CH_2Cl_2-MeOH under gradient conditions in order to yield 25 subfractions (Fr. 1 - Fr. 25). A portion of fraction 19 (4.4 g) was chromatographed on a RP C-18 column eluted with H_2O-MeOH (3 : 7) and then Sephadex LH-20 column using MeOH to yield trifolirhizin (9 g) and kurarinone (3.8 g) (Jung *et al*., 2005a).

Trifolirhizin (Maackiain *O*-*β*-D-glucopyranoside) – colorless powder; mp. 142 - 144 °C; UV λ_{max} (CH_3CN): 286, 310; EI-MS *m/z*: 446 $[M]^+$; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.36 (1H, d, J = 8.6 Hz, H-1), 6.99 (1H, s, H-7), 6.70 (1H, dd, J = 8.3, 2.4 Hz, H-2), 6.55 (1H, d, J = 2.4 Hz, H-4), 6.53 (1H, s, H-10), 5.93 (2H, d, J = 15.6 Hz, OCH_2), 5.57 (1H, d, J = 7.2 Hz, H-11a), 4.84 (1H, d, J = 7.5 Hz, H-1'), 4.27 (1H, dd, J = 10.4, 4.0 Hz, H-6), 3.62 (1H, dd, J = 7.1, 4.2 Hz, H-6), 3.32~3.43 (2H, m, H-5',6'), 3.15~3.28 (3H, m, H-2',3',4'); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 158.4 (C-3), 156.1 (C-4a), 153.6 (C-10a), 147.4 (C-9), 141.1 (C-8), 131.8 (C-1), 118.2 (C-6b), 114.1 (C-11b), 110.3 (C-2), 105.3 (C-7), 104.0 (C-4), 101.0 (OCH_2), 100.2 (C-1c), 93.2 (C-10), 77.6 (C-11a), 69.1 (C-2',3',4'), 65.8 (C-6), 60.6 (C-6'), 39.7 (C-6a).

Kurarinone – colorless powder; mp. 115 - 117 °C; UV λ_{max} (MeOH): 288; EI-MS *m/z*: 438 $[M]^+$; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 10.30 (1H, s, OH-7), 9.55 (1H, s, OH-2'), 9.31 (1H, s, OH-4'), 7.21 (1H, d, J = 8.6 Hz, H-6'), 6.40 (1H, dd, J = 8.6, 2.1 Hz, H-5'), 6.33 (1H, d, J = 2.1 Hz, H-3'), 6.11 (1H, s, H-6), 5.42 (1H, dd, J = 13.4, 2.4 Hz, H-2), 4.91 (1H, m, H-4''), 4.55 (1H, br s, H-9''a), 4.48 (1H, br s, H-9''b), 3.70 (3H, s, OCH_3), 2.80 (1H, dd, J = 16.4, 13.4 Hz, H-3a), 2.50 (2H, m, H-1''), 2.48 (1H, m, H-2''), 2.43 (1H, dd, J = 16.4, 2.4 Hz, H-3b), 1.94 (2H, m, H-3''), 1.57 (3H, s, H-10''), 1.52 (3H, s, H-6''), 1.42 (3H, s, H-7''); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 188.8 (C-4), 162.4 (C-5), 162.0 (C-7), 159.5 (C-9), 158.0 (C-4'), 155.2 (C-2'), 147.8 (C-8''), 130.5 (C-5''), 127.2 (C-6'), 123.3 (C-4''), 116.3 (C-1'), 110.6 (C-9''), 106.9 (C-8), 106.2 (C-5'), 104.3 (C-10), 102.2 (C-3'), 92.4 (C-6), 73.4 (C-2), 55.3 (OCH_3), 46.7 (C-7), 44.5 (C-3), 30.7 (C-3''), 26.8 (C-1''), 25.4 (C-6''), 18.5 (C-10''), 21.1 (C-3), 20.7 (C-9), 18.9 (C-13) (Fig. 1).

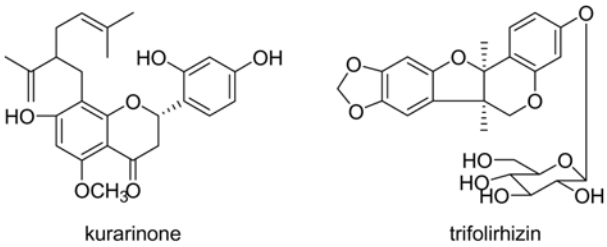

Fig. 1. Structure of kurarinone and trifolirhizin.

Preparation of test sample – Air-dried roots (2 g) was finely powdered and refluxed with 100 mL MeOH for 2 hours, and filtered using filter paper. The methanol extracts were evaporated to dryness in vacuum. The residue was dissolved in 10 mL methanol and the crude extract then filtered with a 0.45 μm pore size and a 5 μL sample subjected to HPLC analysis.

HPLC analysis – Method for flavonoid analysis was modified from those previously described (Ha *et al*., 2006) by using a reverse phase system (Agilent Eclipse XD8-C18, 5 μm, 4.6 × 150 mm i.d.). Elution was initially with acetonitrile-water (10 : 90), which was changed according to linear gradient over 40 min to acetonitrile-water (70 : 30). The flow rate was 1 mL/min, and 5 μL aliquots of samples were injected for analysis and UV detection was carried out at 254 nm.

Calibration – Stock solutions (2 mg/mL) of kurarinone and trifolirhizin isolated from *S. flavescens* were prepared individually in methanol, and different concentrations (trifolirhizin: 5, 10, 25, 50, 100 μg/mL; kurarinone: 0.5, 1, 2.5, 5, 10 μg/mL) of these were loaded onto an HPLC for the preparation of the calibration functions. The calibration function of kurarinone and trifolirhizin calculated with peak area (y), concentration (x, mg/mL), and mean values (n = 5) ± standard deviation.

Results and Discussion

The optimal mobile phase composition for the analysis of kurarinone and trifolirhizin from the MeOH extracts of

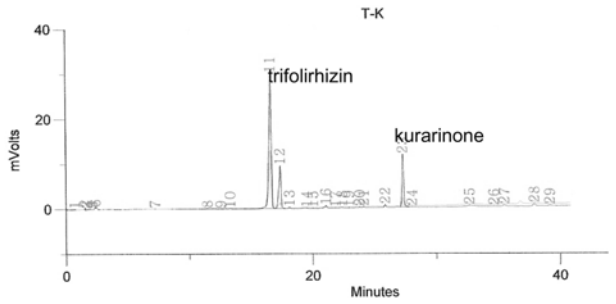

Fig. 2. HPLC chromatogram of kurarinone and trifolirhizin.

the roots of *S. flavescens* was selected by performing several HPLC runs with various concentrations of acetonitrile in water as the mobile phase. A solution of the initial 10% acetonitrile in water, which was changed gradually over 40 min to 70% acetonitrile, was selected as the mobile phase. The HPLC peaks of the kurarinone and trifolirhizin contained in each sample were verified using the standard reference material. The chromatographic system used produced symmetrical peaks with a baseline resolution for kurarinone and trifolirhizin using a simple gradient profile (Lee *et al*., 2004). The retention time of kurarinone and trifolirhizin were 16.92 and 27.15 min, respectively (Fig. 2). The correlation coefficients of each calibration curve of kurarinone and trifolirhizin were 0.9998 and 0.9994, respectively (Fig. 3, Table 1). Under the above HPLC conditions the detection limits of kurarinone and trifolirhizin were 0.05 μg/mL (UV_{254} nm) and 0.1 μg/mL (UV_{254} nm), respectively.

The performance of the expressed method was tested by applying it to a simultaneous assay of kurarinone and trifolirhizin in the roots of *S. flavescens* obtained from the ten of oriental medicinal markets in Korea and China. The test samples were prepared as described previously and injected in duplicate (Li and Wang, 2004). The results are summarized in Table 2. It was found that the flavonoid contents of the herbal samples were quite different. Of these roots of *S. flavescens*, the sample from Bojoo-mart in China had the highest kurarinone content (686.89 ± 10.79 μg/g). On the other hand, the sample from kyungdong-mart cultured in China had the highest trifolirhizin content (288.58 ± 1.05 μg/g). These results suggest that this method might be used more conveniently for the monitoring the quality of flavonoids from the roots of *S. flavescens*.

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