Determination of Hyperin in the Fruits of Acanthopanax Species by High Performance Liquid Chromatography

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Abstract – The content of hyperin in Acanthopanax species was determined by high performance liquid chromatography (HPLC). Hyperin was quantified by a reverse-phase column with elution program [initially gradient solvent (acetonitrile : water = 85 : 15 to 80 : 20 for 20 min), then isocratic solvent (acetonitrile : water = 80 : 20 for 20 min), and finally gradient solvent (acetonitrile : water = 80 : 20 to 65 : 35 for 20 min)]. UV detection was conducted at 210 nm. The content of hyperin in the fruits of Acanthopanax was measured in the species A. chiisanensis (2.04 mg/g), A. sessiliflorus (1.13 mg/g), A. divaricatus (0.98 mg/g), A. koreanum (0.75 mg/g) and A. senticosus (0.05 mg/g). The content of hyperin in A. chiisanensis was higher than that of other Acanthopanax species.

Keywords – Acanthopanax, Araliaceae, quantitative analysis, flavonoid, hyperin

Introduction

Acanthopanax species, belonging to the family Araliaceae, are perennial herbaceous species distributed in Korea, China, and Japan. They have traditionally been used as a tonic and a sedative, as well as in the treatment of rheumatism and diabetes (Perry and Metzger, 1980; Yook, 1990).

Many studies have shown that Acanthopanax exhibits a variety of pharmacological activities such as antibacterial, anti-cancer, anti-inflammatory, anti-gout, anti-hepatitis, anti-hyperglycemic, anti-leshinomic acid, anti-oxidant, anti-pyretic, choleric, hemostatic anti-xanthine oxidase, immunostimulatory, hypo-cholesterolemic, and radio-protectant (Davydov and Krikorian, 2000). These plants have been widely used as health supplements in Korea. Triterpenoid saponins such as sliphioside F, copteroside B, hederagenin 3-O-β-D-glucuronopyranoside 6'-O-methyl ester, and gypsogenin 3-O-β-D-glucuronic acid from A. senticosus fruits are reported to be pancreatic lipase inhibitors (Fang et al., 2007). Aqueous extracts from the fruits of A. senticosus are reported to have antioxidant and antimicrobial effects (Kim et al., 2006).

Isolation and characterization of several lignans, coumarins, flavonoids, and terpenes from Acanthopanax species have previously been reported (Shin and Lee, 2002). Among them, hyperin, quantified by HPLC from the stem of A. senticosus and A. sessiliflorus (Lee et al., 2004a), has been reported to show antioxidant (Han et al., 2000), anti-inflammatory (Lee et al., 2004b) and aldose reductase inhibitory activities (Lee et al., 2003).

However, there has been no report on the determination of hyperin in the fruits of Acanthopanax species. Therefore, the determination of hyperin in fruits of Acanthopanax species was conducted by efficient and simple analytical methods.

Experimental

Plant materials – Acanthopanax species (Acanthopanax chiisanensis, A. divaricatus, A. koreanum, A. senticosus, and A. sessiliflorus) were cultivated and collected by Wolga Agro-Products Co. Ltd., Gongju, Korea, and botanically identified by Prof. S. H. Cho, Gongju National University of Education, Korea.

Instruments and reagents –\textsuperscript{1}H- and \textsuperscript{13}C-nuclear magnetic resonance (NMR) spectra were recorded with a Bruker AVANCE 400 NMR spectrometer (Rheinstetten, Germany) in DMSO using TMS as an internal standard. The mass spectrometry (MS) was measured with a Jeol JMS-AX505WA mass spectrometer (Tokyo, Japan) with a direct inlet. HPLC chromatograms were recorded with a GILSON 305 system pump with a GILSON 188 system UV/VIS detector (Villiers le Bel, France). Water and

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Preparation of hyperin – Air-dried grounded powder of A. chiisanensis fruits (1.5 kg) was extracted with MeOH under reflux for 3 h. The MeOH extract (524.5 g) was suspended in water, and then fractionated successively with equal volumes of n-hexane, CHCl₃, EtOAc, and n-BuOH (yield: 28.5, 23.7, 19.9, and 82.5 g, respectively). Among them, a portion of the EtOAc fraction (5.0 g) was chromatographed on a silica gel, and eluted with a stepwise gradient of CHCl₃-MeOH (100% CHCl₃ up to 100% MeOH). Hyperin was isolated from the 15% MeOH fraction after recrystallization with MeOH.

Hyperin: EI-MS m/z 302 (100) [M-Gal]+, 273 (7.3), 245 (4.2), 207 (11.1), 153 (6.1), 137 (7.4), 128 (7.1); FAB-MS m/z 465 [M + H]⁺; 1H-NMR (400 MHz, DMSO-d₆) δH 12.64 (1H, s, 5-OH), 7.67 (1H, d, δ = 7.8 Hz, galactosyl H-145), 6.41 (1H, d, δ = 2.0 Hz, H-2'), 6.82 (1H, d, δ = 8.5 Hz, H-6'), 7.53 (1H, d, δ = 2.0 Hz, H-2), 6.82 (1H, d, δ = 8.5 Hz, H-5'), 6.41 (1H, d, δ = 1.9 Hz, H-8), 6.21 (1H, d, δ = 1.9 Hz, H-6), 5.38 (1H, d, δ = 7.8 Hz, galactosyl H-1); 13C-NMR (100 MHz, DMSO-d₆) δC 177.9 (C=O), 146.45 (C-7), 161.6 (C-5), 156.6 (C-2, C-9), 148.9 (C-4'), 145.2 (C-3'), 133.9 (C-3), 122.4 (C-6'), 121.5 (C-1'), 116.3 (C-5'), 115.6 (C-2'), 104.3 (C-10), 102.2 (C-1''), 99.1 (C-6), 93.9 (C-8), 76.2 (C-5''), 73.6 (C-3''), 71.6 (C-2''), 68.3 (C-4''), 60.5 (C-6'').

Sample preparation – For the analysis of hyperin in Acanthopanax species, 300 mg of each of the fruits from Acanthopanax species (A. chiisanensis, A. diiviricatus, A. koreanum, A. senticosus, and A. sessiliflorus) were extracted with 50% MeOH (20 ml × 3 times) by reflux and evaporated in vacuo. The residue was dissolved in 6 ml of 50% MeOH and filtered with a 0.45 µm filter. The resulting solution was used for HPLC analysis.

HPLC condition – The HPLC separation of hyperin for qualitative and quantitative analysis was performed using a reverse phase system. A Nucleodur 100-5 C18 ec (4.6 × 250 mm, 5 µm) column was used, with a mobile phase consisting of acetonitrile and water. The elution program was initially gradient solvent (acetonitrile : water = 85 : 15 to 80 : 20 for 20 min), then isocratic solvent (acetonitrile : water = 80 : 20 for 20 min), and finally gradient solvent (acetonitrile : water = 80 : 20 to 65 : 35 for 20 min) UV detection was conducted at 210 nm. The injection volume was 10 µl and the flow rate was 1 ml/min. All injections were performed in triplicate.

Calibration – A stock solution (3 mg/6 ml) of hyperin isolated from A. chiisanensis was prepared in 50% MeOH, and blended with 50% MeOH, repeatedly, diminishing the solution content to 50% percent to different concentrations. The contents of the analytics were determined from the corresponding calibration curves. The calibration functions of hyperin are calculated with peak area (Y), concentration (X, µg/10 µl), and mean values (n = 5) ± standard deviation.

Results and Discussion

A chromatographic separation of the MeOH extract from the fruits of A. chiisanensis led to the isolation of hyperin (Fig. 1). The presence of hyperin in the fruits of A. chiisanensis was identified by spectroscopy. The typical flavonoid signals were identified in the ¹H-NMR spectrum. The singlet at δ 12.64 showed the typical aromatic 5-OH of the flavonoid A ring. The proton signals at δ 7.67 (dd, J = 2.0, 8.5 Hz), 7.53 (d, J = 2.0 Hz) and 6.82 (d, J = 8.5 Hz) showed the ABX splitting type of the B ring. The proton signals at δ 6.30–5.00 represented glycoside. The ¹³C-NMR spectrum showed C=O at δ 177.9. The signals at δ 10.2 represented an anomic carbon of sugar moiety. The FAB-MS showed an [M + H]⁺ ion at m/z 465. The molecular formula was determined to be C₂₃H₂₀O₁₂. Accordingly, the structure was elucidated as hyperin by comparing its spectral data in the literature (Lee et al., 2002).

Hyperin was detected in Acanthopanax senticosus (Chen et al., 2002), Epimedium koreanum (Sha et al., 1995), Hypericum perforatum (Wu et al., 2002), Sanguisorba officinalis (Sha et al., 1998) and apple (Lommen et al., 2000; Chinnici et al., 2004). Hyperin contents of 0.47 and 0.14 mg/g were identified in the stems of A. senticosus and A. sessiliflorus, respectively. No hyperin was detected in the root of A. senticosus, but 0.30 mg/g in that of A. sessiliflorus (Lee et al., 2004a). However, there have been no reports on the determination of hyperin in the fruits of Acanthopanax species.

Hyperin content in the various Acanthopanax fruits was determined by HPLC. The standard curve for hyperin is...
Acknowledgment

This research was supported in part by a grant from Wolga Agro-Products Co. Ltd., Korea.

References


