Analysis of Flavonoid Contents in the Fruits of *Acanthopanax* Species using HPLC

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Abstract – Analysis of flavonoid contents in the fruits of *Acanthopanax* species (*A. chiisanensis*, *A. divaricatus*, *A. koreumum*, *A. senticosus*, and *A. sessiliflorus*) was conducted by high performance liquid chromatography. A Discovery® C18 (4.6 × 250 mm, 5 µm) column was used with a gradient mobile phase of water and acetonitrile (90 : 10 to 60 : 40 for 60 min) and UV detection was conducted at 350 nm. The contents of rutin, hyperin, quercetin, afzelin, and kaempferol were 0.063~0.540, 0.494~7.480, 0.584~0.704, 0.388~0.567, 0.190~0.471 mg/g, respectively, in the fruits of *Acanthopanax* species. Total content of flavonoids in the fruits of *Acanthopanax* species was highest in those of *A. chiisanensis*. Furthermore, hyperin was the most abundant compound in the fruits of *Acanthopanax* species. Consequently, our results demonstrate that the fruits of *Acanthopanax* species containing flavonoids have promising potential as a new income source of agriculture and industry in medicinal natural products, health supplements, and beverages.

Keywords – *Acanthopanax*, Flavonoid, HPLC, Hyperin.

Introduction

*Acanthopanax* species are perennial herbaceous genus of the family Araliaceae that are widely distributed in East-Asia, such as Korea, China, Russia, and Japan (Boon and Smith, 1999). Most of *Acanthopanax* species grows to 2~4 m in height, bears five leaflets, the flowering is from July to September, and the fruit ripening in October (Lee, 2003). The dried roots and stem barks of *Acanthopanax* species have been used for a long time as a sedative and tonic to treat rheumatism and hepatitis, liver disease and diabetes, chronic bronchitis, stress, ischemic heart disease, tumor, hypertension, and gastric ulcers (Fujikawa et al., 1996; Kang et al., 2005; Ni and Liu, 2006).

Until now, the study of *Acanthopanax* species has been focused on stems, roots, and leaves. There are only a few studies on the fruit of *Acanthopanax* species. Most of the fruit of *Acanthopanax* species is round and be used in health supplements, and beverages in Korea (Lee, 2003; Kim et al., 2006). Also, the biological activities of *Acanthopanax* fruits are antitumor, immunostimulating (Lee et al., 2003), antioxidant, and antimicrobial (Kim et al., 2006). The phytochemicals of fruits from *Acanthopanax* species are composed of sterols (β-sitosterol and stigmasterol), a nitrogen compound (sesilnine), lignins (sesamin, savinin, eleutherosides B and E), a coumarin (scoparone), terpenoids (ursolic acid, chiisanoside, and methyl betulin), a phenolic compound (protocatechuic acid), a flavonoid (hyperin) (Kim and Lee, 1990; Yook et al., 1992; Lee et al., 2002; Lee et al., 2002). The quantitative analysis of phytochemicals in fruits from *Acanthopanax* species are eleutherosides B and E in *A. sessiliflorus* (Kim et al., 2006), chiisanoside and hyperin in *Acanthopanax* species (Lee et al., 2007; Lee et al., 2010).

Among various phytochemical constituents in *Acanthopanax* species, flavonoids such as afzelin, aristoside, isorquercitrin, hyperin, kaempferol, quercitrin, and rutin have previously been isolated from *Acanthopanax* species (Yasue et al., 1968; Kitajima et al., 1989; Chung and Hahn, 1991; Shirasuna et al., 1997; Lee et al., 2002). Flavonoids have been used as natural antioxidants and for their health-promoting properties in humans (Bekker et al., 2006). Flavonoids having various biological activities are important compounds in *Acanthopanax* species. Until now, many studies have reported on the analysis of triterpenoids, lignans, and phenylpropanoids constituents of *Acanthopanax* species (Shin and Lee, 2002; An et al.,...
In this study, analysis of flavonoids (rutin, hyperin, quercetin, afzelin, and kaempferol) in the fruits of Acanthopanax species was conducted using high performance liquid chromatography (HPLC).

**Experimental**

**Plant materials** – The fruits of Acanthopanax species (A. chiisanensis, A. divaricatus, A. koreanum, A. senticosus, and A. sessiliflorus) were cultivated and collected at Gongju, Korea, and botanically identified by Prof. S. H. Cho, Gongju National University of Education, Korea.

**Apparatus and chemicals** – Mass spectrometry (MS) was performed using a Jeol JMS-600W (Tokyo, Japan) mass spectrometer. ¹H- and ¹³C-nuclear magnetic resonance (NMR) spectra were recorded with a Bruker Avance 300, 400, and 500 NMR (Rheinstetten, Germany) spectrometer using tetramethylsilane (TMS) as an internal standard. Evaporation was conducted using an Eyela rotary evaporator system (Tokyo, Japan) under reflux in vacuo. TLC was performed with precoated silica gel 60 F₂₅₄ plates (Art. 5715, Merck Co., Darmstädt, Germany). The compounds on the TLC plate were visualized by spraying with 10% sulfuric acid in methanol followed by heating at 100°C to detect spot color. HPLC chromatograms were recorded with a Waters Breeze system (Massachusetts, USA) equipped with a Waters 1525 binary HPLC pump and 2489 system UV/VIS detector. Water and acetonitrile used in this research were of HPLC grade, and all other reagents were of analytical grade.

**Preparation of flavonoids** – Compounds 1-5 were isolated from Fagopyrum tataricum, Acanthopanax chiisanensis, Vaccinium koreanum, and Rhododendron mucronulatum for albilflorum by repeated column chromatography as reported previously by our team. Compound 1 (rutin) was isolated from the ethyl acetate fraction of F. tataricum (Mok et al., 2011). Compound 2 (hyperin) was isolated from the ethyl acetate fraction of A. chiisanensis (Lee et al., 2008). Compound 3 (quercetin) was isolated from the butanol fraction of V. koreanum (Lee et al., 2008). Compounds 4 and 5 (afzelin and kaempferol, respectively) were isolated from the ethyl acetate fraction of R. mucronulatum for albilflorum (Mok and Lee, 2013).

**Sample preparation** – For analysis of flavonoids (rutin, hyperin, quercetin, afzelin, and kaempferol) in the fruits of Acanthopanax species (A. chiisanensis, A. divaricatus, A. koreanum, A. senticosus, and A. sessiliflorus), each 50 g of fruits from Acanthopanax species was extracted with 50% MeOH (3 × 100 mL) by reflux and evaporated in vacuo. The residue was dissolved in 1 mL of MeOH and filtered with a 0.45 µm filter. The resulting solution was used for HPLC analysis.

**HPLC conditions** – HPLC separation of flavonoids for qualitative and quantitative analysis was performed using a reverse phase system. A Discovery® C18 (4.6 × 250 mm, 5 µm) column was used with a mobile phase consisting of water (0.1% acetic acid) and acetonitrile. The elution program was a gradient solvent system of water and acetonitrile (90 : 10 to 60 : 40 for 60 min). UV detection was conducted at 350 nm. The injection volume was 10 µL and the flow rate was 1 mL/min. All injections were performed in triplicate.

**Calibration curve** – A stock solution (1 mg/mL) of each flavonoid was prepared in MeOH, successively reducing the solution content to 50% to create different concentrations. The contents of the analytes were determined from the corresponding calibration curves. The calibration functions of the flavonoids were calculated using the peak area (Y), concentration (X, µg/10 µL), and mean values (n = 5) ± standard deviation.