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Content Analysis of Flavonoids in the Stems and Roots of *Acanthopanax* Species in Different Countries

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Content analysis of flavonoids in the stems and roots of *Acanthopanax* species (*A. divaricatus*, *A. koreanum*, *A. senticosus* and *A. sessiliflorus*) in different nations was conducted by high performance liquid chromatography. In the stems and roots of *Acanthopanax* species, the contents of rutin, hyperin, afzelin, quercetin and kaempferol were 0.024-0.326 and 0.059-0.531, 0.391-4.256 and 0.957-3.517, 0.935-10.572 and 1.019-1.185, 0.375-0.885 and 0.394-0.501, 0.193-0.872 and 0.179-0.500 mg/g, respectively. Total content of flavonoids in the stems and roots was highest in *A. senticosus* (Mt. Baekdu) and *A. sessiliflorus* (Gongju), respectively. Also, hyperin and afzelin were the most abundant compounds in the roots and stems of *Acanthopanax* species, respectively. This result will provide useful information in the application of these flavonoids from *Acanthopanax* species in the nutraceutical, pharmaceutical and cosmeceutical areas.

Keywords: *Acanthopanax* species, Flavonoid, Analysis, Reflux.

INTRODUCTION

Acanthopanax species, belonging to the family Araliaceae, are perennial herbaceous species. The dried roots and stem barks of *Acanthopanax* species have been used as a sedative and tonic to treat rheumatism, liver disease and diabetes, chronic bronchitis, stress, ischemic heart disease, tumor, hypertension and gastric ulcers[1-4]. The methanolic and aqueous extracts of the rhizomes of *A. senticosus* have trophic and beneficial effects, such as neuronal protection, as shown in an *in vitro* assay system for Alzheimer's disease[5]. *Acanthopanax* species have been widely used as health supplements because they have ginseng-like biological activities and are a famous tonic in Korea[6].

Flavonoids such as afzelin, antoside, isoquercitrin, hyperin, kaempferol, quercitrin and rutin have been previously isolated from *Acanthopanax* species[7-11]. Flavonoids have been used as natural antioxidants and for their health-promoting properties in humans[12]. Flavonoids having above biological activities are important compounds in *Acanthopanax* species. Until now, many studies have been reported on the analysis of triterpenoids, lignans and phenylpropanoids constituents of *Acanthopanax*[13-16], but there have been few reports on flavonoid analysis in *Acanthopanax* species.

In this study, content analysis of flavonoids (rutin, hyperin, afzelin, quercetin and kaempferol) in the stems and roots of *Acanthopanax* species in different nations (South Korea, North Korea, Russia and China) was conducted using an efficient and simple high performance liquid chromatography (HPLC) method.

EXPERIMENTAL

The stems and roots of *Acanthopanax* species (*A. divaricatus*, *A. koreanum*, *A. senticosus* and *A. sessiliflorus*) in different nations (Gongju and Jungseon, South Korea; Mt. Baekdu, North Korea; Khabarovsk, Russia; Yanbian, China) were collected and botanically identified by Prof. S. H. Cho, Gongju National University of Education, Korea.

HPLC chromatograms were recorded with a Waters Breeze system (MA, USA) equipped with a Waters 1525 binary HPLC pump and 2489 system UV/VIS detector. A Discovery® C18 (4.6 × 250 mm, 5 µm) column was purchased from Sigma-Aldrich Co. (PA, USA). Water and acetonitrile used in this research were of HPLC grade and all other reagents were of analytical grade.

Preparation of standard flavonoids: Compounds **1-5** were isolated by repeated column chromatography as reported previously. Compound **1** was isolated from the ethyl acetate fraction of *Fagopyrum tataricum*[17]. Compound **2** was isolated from the ethyl acetate fraction of *Acanthopanax chiisanensis*[18]. Compounds **3** and **5** were isolated from the ethyl acetate fraction of *Rhododendron mucronulatum* for *albiflorum*[19]. Compound **4** was isolated from the butanolic fraction of *Vaccinium koreanum*[18].

Sample preparation: For analysis of flavonoids (rutin, hyperin, afzelin, quercetin and kaempferol) in *Acanthopanax* species (*A. divaricatus*, *A. koreanum*, *A. senticosus* and *A. sessiliflorus*) in different nations, 50 g of each plant part (the stems and roots) 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.

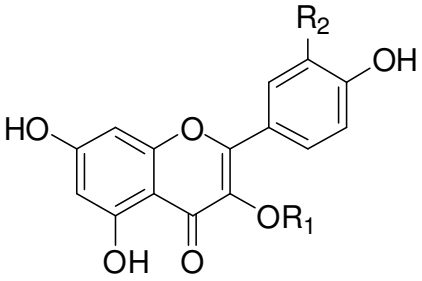
HPLC conditions: HPLC separation of flavonoids for qualitative and quantitative analysis was performed using a reverse phase system. A Discovery® C18 (4.6 × 250 mm, 5 μm) column was used with a mobile phase consisting of water (0.1 % acetic acid) and acetonitrile. The elution program was a gradient solvent system (water : 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 = 4) ± standard deviation.

Statistical analysis: Data of each sample was expressed as mean ± S.D. ANOVA using the SAS Enterprise Guide software was calculated and the significance between the mean of each group were analyzed using Duncan's multiple test.

RESULTS AND DISCUSSION

Content analysis of flavonoids in the stems and roots of *Acanthopanax* species in different nations was conducted by HPLC. Compounds **1-5** (Fig. 1) were previously isolated from *A. koreanum, A. divaricatus*, *A. chiisanensis* and *A. sciadophylloides*[8-10,18].



Compound	R_1	R_2
1	Rutinose	OH
2	Galactose	OH
3	Rhamnose	H
4	H	OH
5	H	H

Fig. 1. Chemical structures of compounds **1-5**

HPLC separation of flavonoids for qualitative and quantitative analysis was performed using a reverse phase system. HPLC condition was developed for the analysis of five standard flavonoids with good linearity ($r^2 = 0.9999$)[20]. The content of five standard flavonoids in *Acanthopanax* species was investigated in different nations. The HPLC chromatograms of standard flavonoids and 50 % methanolic extracts of *Acanthopanax* species were shown in Fig. 2. In the stems of *Acanthopanax* species, the contents of rutin (**1**), hyperin (**2**), afzelin (**3**), quercetin (**4**) and kaempferol (**5**) were 0.024-0.326, 0.391-4.256, 0.935-10.572, 0.375-0.885 and 0.193-0.872 mg/g, respectively. Also, the total content of flavonoids was 2.264-11.941 mg/g. In our results, the most abundant flavonoid in the stems was afzelin (47.994 mg/g) and the total content of flavonoids was highest in *A. senticosus* from Mt. Baekdu (11.941 mg/g) (Table-1). In the roots of *Acanthopanax* species, the contents of rutin (**1**), hyperin (**2**), afzelin (**3**), quercetin (**4**) and kaempferol (**5**) were 0.059-0.531, 0.957-3.517, 1.019-1.185, 0.394-0.501 and 0.179-0.500 mg/g, respectively. Also, the total content of flavonoids was 2.913-6.106 mg/g. In present results, the most abundant flavonoid in the roots was hyperin (18.274 mg/g) and the total content of flavonoids was highest in *A. sessiliflorus* from Gongju (6.106 mg/g) (Table-2).

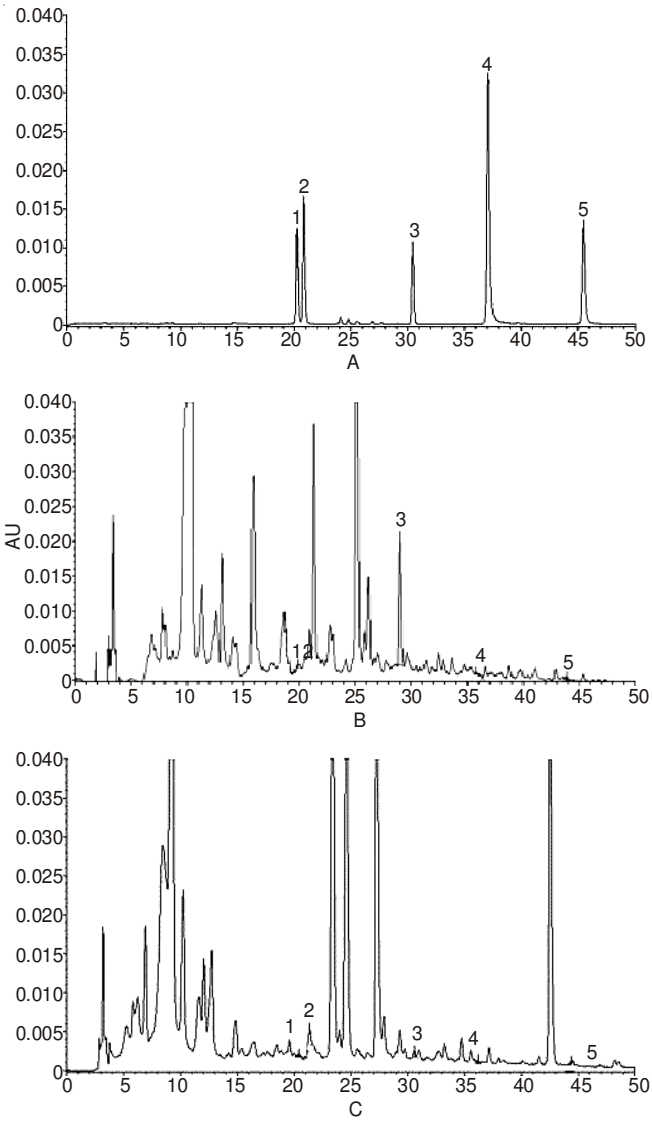


Fig. 2. HPLC chromatograms of standard flavonoids (**A**) and the 50 % MeOH extracts of *A. senticosus* stems (**B**) from Mt. Baekdu and *A. divaricatus* roots (**C**) from Gongju (**1**, rutin; **2**, hyperin; **3**, afzelin; **4**, quercetin; **5**, kaempferol

TABLE-1
CONTENTS OF COMPOUNDS **1-5** IN THE 50 % METHANOLIC EXTRACTS OF THE STEMS OF *Acanthopanax* SPECIES

Sample	Content (mg/g)					
	1	2	3	4	5	Total
A. koreanum from Gongju	0.062 ± 0.008 [d]	0.450 ± 0.010 [e]	0.935 ± 0.028 [f]	0.412 ± 0.001 [b]	0.405 ± 0.007 [c]	2.264 ± 0.054
A. divaricatus from Gongju	Trace	4.256 ± 0.137 [a]	1.105 ± 0.059 [f]	0.885 ± 0.009 [a]	0.628 ± 0.020 [b]	6.874 ± 0.225
A. sessiliflorus from Gongju	0.029 ± 0.005 [e]	0.807 ± 0.011 [d]	3.030 ± 0.053 [e]	0.382 ± 0.001 [d]	0.193 ± 0.001 [f]	4.441 ± 0.071
A. senticosus from Gongju	0.255 ± 0.007 [b]	1.140 ± 0.033 [c]	5.830 ± 0.013 [d]	0.388 ± 0.001 [d]	0.402 ± 0.004 [c]	8.015 ± 0.058
A. senticosus from Jungseon	0.326 ± 0.014 [a]	0.391 ± 0.003 [e]	9.798 ± 0.329 [a]	0.393 ± 0.002 [c]	0.872 ± 0.016 [a]	11.780 ± 0.364
A. senticosus from Mt. Baekdu	0.024 ± 0.003 [e]	0.664 ± 0.008 [d]	10.572 ± 0.323 [a]	0.386 ± 0.001 [d]	0.295 ± 0.005 [e]	11.941 ± 0.340
A. senticosus from Khabarovsk	0.095 ± 0.009 [c]	1.715 ± 0.052 [b]	8.647 ± 0.255 [b]	0.380 ± 0.001 [d,e]	0.342 ± 0.010 [d]	11.179 ± 0.327
A. senticosus from Yanbian	0.065 ± 0.003 [d]	0.468 ± 0.006 [e]	8.077 ± 0.331 [c]	0.375 ± 0.001 [e]	0.398 ± 0.003 [c]	9.383 ± 0.344
Total	0.856 ± 0.049	9.891 ± 0.260	47.994 ± 1.391	3.601 ± 0.017	3.535 ± 0.066	–

Data are represented as the mean ± S.D. (n = 4) in mg/g of the methanolic extracts of samples.

TABLE-2
CONTENTS OF COMPOUNDS **1-5** IN THE 50 % METHANOLIC EXTRACTS OF THE ROOTS OF *Acanthopanax* SPECIES

Sample	Content (mg/g)					
	1	2	3	4	5	Total
A. koreanum from Gongju	0.142 ± 0.016 [d]	0.957 ± 0.011 [g]	1.077 ± 0.003 [b,c,d]	0.419 ± 0.001 [d]	0.318 ± 0.007 [c]	2.913 ± 0.038
A. divaricatus from Gongju	0.521 ± 0.005 [a]	1.920 ± 0.019 [f]	1.063 ± 0.001 [c,d]	0.394 ± 0.001 [e]	0.500 ± 0.003 [a]	4.398 ± 0.029
A. sessiliflorus from Gongju	0.531 ± 0.004 [a]	3.517 ± 0.079 [a]	1.185 ± 0.041 [a]	0.501 ± 0.010 [a]	0.372 ± 0.013 [b]	6.106 ± 0.147
A. senticosus from Gongju	0.059 ± 0.014 [f]	2.723 ± 0.033 [b]	1.069 ± 0.011 [c,d]	0.421 ± 0.002 [c,d]	0.264 ± 0.003 [d]	4.536 ± 0.063
A. senticosus from Jungseon	0.107 ± 0.011 [d,e]	2.187 ± 0.020 [d]	1.160 ± 0.019 [a,b]	0.440 ± 0.003 [b,c]	0.187 ± 0.001 [f]	4.081 ± 0.054
A. senticosus from Mt. Baekdu	0.291 ± 0.023 [c]	2.335 ± 0.028 [c]	1.090 ± 0.058 [a,b,c]	0.406 ± 0.005 [d,e]	0.179 ± 0.003 [f]	4.301 ± 0.117
A. senticosus from Khabarovsk	0.373 ± 0.020 [b]	1.982 ± 0.076 [e]	1.052 ± 0.013 [c,d]	0.444 ± 0.002 [b]	0.241 ± 0.004 [e]	4.092 ± 0.115
A. senticosus from Yanbian	0.071 ± 0.005 [e,f]	2.653 ± 0.036 [b]	1.019 ± 0.032 [d]	0.411 ± 0.002 [d,e]	0.184 ± 0.003 [f]	4.338 ± 0.078
Total	2.095 ± 0.098	18.274 ± 0.302	8.715 ± 0.178	3.436 ± 0.026	2.245 ± 0.037	–

Data are represented as the mean ± S.D. (n = 4) in mg/g of the methanolic extracts of samples.

The contents of rutin (**1**), hyperin (**2**), afzelin (**3**), quercetin (**4**) and kaempferol (**5**) detected in the stems and roots of *Acanthopanax* species were 0.856 and 2.095, 9.891 and 18.274, 47.994 and 8.715, 3.601 and 3.436, 3.535 and 2.245 mg/g, respectively. Consequently, the total content of flavonoids in the stems and roots was highest in *A. senticosus* from Mt. Baekdu and *A. sessiliflorus* from Gongju, respectively. Also, hyperin (**2**) and afzelin (**3**) were the most abundant compounds in the roots and stems of *Acanthopanax* species, respectively. In a previous paper, total content of flavonoids in the fruits of *Acanthopanax* species was highest in those of A. chiisanensis and the most abundant flavonoid in the fruits of *Acanthopanax* species was hyperin[20]. As results, among flavonoids, hyperin (**2**) was the most abundant compound in the roots, stems and fruits of *Acanthopanax* species.

Rutin (**1**) has been reported to prevent or delay the onset of diabetes, lower the risk of heart disease[21,22] and to have antioxidative and antihypertensive activities[23,24]. In addition, there are many reports on anti-inflammatory, anticancer and beneficial cardiovascular effects[25,26] of hyperin (**2**); antimicrobial and antioxidant activities[27] of afzelin (**3**); antiviral, anticancer and antioxidant activities[28-30] of quercetin (**4**); and cardiovascular, neuroprotective, antidiabetic, antiosteoporotic and antiallergic activities[31] of kaempferol (**5**).

On the basis of these results, it may be concluded that HPLC remains the method of choice for analyzing the most relevant flavonoids in *Acanthopanax* species. This result will be useful information in the application of these flavonoids from *Acanthopanax* species in the nutraceutical, pharmaceutical and cosmeceutical areas.

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