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Flavonoid Analysis of *Heloniopsis orientalis* (Thunb.) by High Performance Liquid Chromatography

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We have isolated and identified seven flavonoid compounds from the foliar extracts of *Heloniopsis orientalis*, a member of Liliaceae, which is habituated at Namhansanseong and Maranggol (Jinburyung). All are glycosylated derivatives of the flavonols isorhamnetin, kaempferol, and quercetin. Among them, quercetin 3-O-galactoside is the major compound, while isorhamnetin 3-O-arabinosylgalactoside, isorhamnetin 3-O-digalactoside, kaempferol 3,7-O-galactoside, kaempferol 3-O-arabinosylgalactoside, kaempferol 3-O-glycoside, and quercetin 3-O-arabinosylgalactoside are present in smaller amounts. Although the two populations do not differ significantly in their overall flavonol profiles, their relative amounts indicate that flavonoid levels, especially for isorhamnetin, are geographically controlled and specifically depend on the origin of the individual population.

Keywords: flavonoid, *Heloniopsis orientalis*, HPLC

Heloniopsis orientalis (Liliaceae), a perennial evergreen herb, grows under close canopies in wet soils that are rich in organic materials (Lee, 1982; Min, 2000a, b). Over the last 30 years, flavonoids have proven to be very useful markers at all levels of plant classification (Katalinic, 1997; Lim et al., 1999; Kim et al., 2000; Mendiondo et al., 2000; Oleszek et al., 2002). Moreover, they can be utilized in the identification of natural plant hybrids and recognition of plant cultivars.

Flavonols play important roles, such as in aiding the recovery of plants from damage caused by UV exposure or predators, as well as functioning as mediators of auxin signals or as phytoalexins. These compounds also affect co-pigmentation with anthocyanins. However, they are most important biologically because of their ability to act as antioxidants in animal systems (Karakaya and Nihir, 1999). Oxygen free radicals and lipid peroxidation are believed to be involved in pathological conditions, e.g., arteriosclerosis, cancer, and chronic inflammation (Briviba and Sies, 1994; Hollman et al., 1996). Food-derived flavonoids, such as quercetin, kaempferol, and myricetin, have antimutagenic and anticarcinogenic effects, both *in vitro* and *in vivo* (Hertog et al., 1992).

To explore the potential differences in flavonol composition between two plant populations and determine whether this was affected by particular habituations, we investigated the flavonol contents from leaves of *H. orientalis* growing at Namhansanseong and Jinburyung in Korea.

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MATERIALS AND METHODS

Chemicals

Standards for rutin, quercetin, and sugars were obtained from Sigma (USA), while all the solvents for extraction and HPLC analysis were from Fisher Scientific (USA) and Merck (Germany). HPLC-grade water was prepared by redistillation.

Sample Preparation

Leaves of *Heloniopsis orientalis* were harvested in April at two shaded field sites, Namhansanseong and Maranggol (Jinburyung). Their geographical and climatic properties are summarized in Table 1. The collected leaves were immediately freeze-dried and stored at −60°C.

Preparation of Crude Flavonol Extracts

Freeze-dried leaves (20 g samples) were ground in a blender and extracted with 85% MeOH at room temperature overnight. This slurry was then filtered through Whatman No. 1 filter paper with a Buchner funnel, and the residue was re-extracted with 50% MeOH at room temperature overnight. The MeOH extract was evaporated under reduced pressure, and

Table 1. Properties of two study sites.

	Site	
	Namhansanseong	Maranggol (Jinburyung)
Latitude	37°28′00″ N	37°33′00″ N
Longitude	127° 11′30″ E	128° 32′30″ E
Altitude (m)	280	650
Azimuth	0°	30°
Coverage Canopy (%)	90	60

the pellet was redissolved in H_2O. Afterward, the crude extract was separated with chloroform into flavonoid and chlorophyll portions, and the residual aqueous phase was washed with ethyl acetate. The extracts were then evaporated and stored at −4°C.

Apparatus

For the HPLC analysis, we used the Gilson Model 305-306/115 UV dual pump system (France) and a 7.8 × 30 mm Waters semi-preparative *N*-Bondapak C-18 column (USA). The detection wavelength was set at 254 nm.

Measurement and Analysis

Our HPLC method was one proposed by Mun and Park (1995). Conditions for the mobile phase included the following: pump A, acetonitrile; pump B, water-acetic acid-acetonitrile; with a gradient elution for 27 min per cycle; 15 to 30% acetonitrile in 2% HOAc; and a flow rate of 3 ml min^{-1}. All solvents were HPLC-grade, which were filtered and degassed before use. The preliminary analysis of the flavonoid extracts was done by two-dimensional TLC (cellulose plate; TBA, 15% HOAc). Bulk isolation and purification of the flavonoids was conducted via one-dimensional PC, using Whatman 3MM paper, followed by HPLC. The purified flavonoids were identified through a combination of UV spectral-analysis HPLC and TLC, as described by Harbone (1980) and Markham (1982).

RESULTS AND DISCUSSION

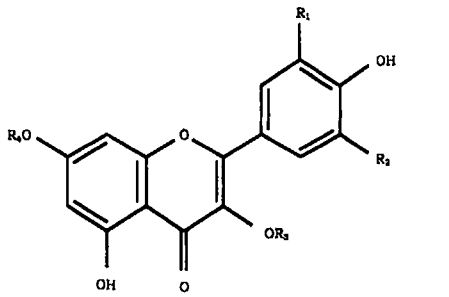
We have isolated seven flavonoid compounds from the foliar extracts of *Heloniopsis orientalis*, a member

Table 2. UV spectral data for flavonoids in *Heloniopsis orientalis*. sh: shoulder

Compound	Absorption maxima (nm)					
	MeOH	NaOMe	$AlCl_3$	AlCl/HCl	NaOAc	NaOAc/H_3
Isorhamnetin	360.6	415.2	406.2	399.6	387.2	381.2
3-O-arabinosylgalactoside	302sh	332.6	371.6	367.0	267.8	301.0sh
	258.0	274.4	301.8sh	300.8sh		263.2
			270.8	267.6		
Isorhamnetin	360.6	421.8	415.0	397.0	403.4	380.6
3-O-diaglactoside	301.8sh	321.6sh	368.2	361.0	267.4	305.0sh
	257.6	276.0	307.6sh	298.8sh		262.8
			273.6	267.4		
Kaempferol	350.2	400.8	384.0sh	393.4sh	358.6	354.0
3,7-O-galactoside	296sh	328.0	356.6	348.8	306.6	298.4
	267.0	275.6	305.8sh	301.0sh	268.2	267.2
			272.4	274.4		
Kaempferol	356.0	407.0	406.8sh	395.8	411.0	394.8sh
3-O-arabinosylgalactoside	266.0	326.2	360.4	350.8	355.2	359.8
		275.4	306.2sh	302.0sh	274.2	305.2sh
			273.8	274.2		269.0
Kaempferol	356.6	403.6	390.0sh	393.0sh	367.2	354.0
3-O-glycoside	292.6sh	327.2	354.0	349.4	305.6sh	302.4sh
	267.0	277.4	306.2sh	301.4sh	272.8	267.4
			274.8	275.2		
Quercetin	361.6	409.0	425.6	405.2	379.8	371.0
3-O-galactoside	298.4sh	328.8sh	274.4	368.2sh	296.2sh	268.0
	257.2	272.4		298.2sh	262.4	
				268.8		
Quercetin	353.8	414.0	365.2	400.0	372.4	360.6
3-O-arabinosylgalactoside	255.8	332.6sh	305.6sh	354.2	320.2	255.2
		274.8	267.6	309.0sh	274.8	
				267.0		

of Liliaceae (Tables 2 and 3). All were identified as glycosylated derivatives of three flavonols -- isorhamnetin, kaempferol, and quercetin. Among them, quercetin 3-O-galactoside was the major compound, while isorhamnetin 3-O-arabinosylgalactoside, isorhamnetin 3-O-digalactoside, kaempferol 3,7-O-galactoside, kaempferol 3-O-arabinosylgalactoside, kaempferol 3-O-glycoside, and quercetin 3-O-arabinosylgalactoside were present in smaller amounts. Few reports have been made about flavone profiles in members of Liliaceae. Williams (1975) found that the flavonol aglycone quercetin and kaempferol are ubiquitous in the acid hydrolysates of *Tricyrtis* (Liliaceae). In addition, Hong et al. (1999) have recovered various flavonoids, such as myricetin, quercetin, kaempferol, and isorhamnetin, from the leaves of *Tricyrtis* species. Carotenuto et al. (1996, 1997) have also analyzed the flavonoid glycosides derived from quercetin, kaempferol, and isorhamnetin in *Allium ursinum* and *A. neapolitanum*. However, in the present study, we found only quercetin, kaempferol, and isorhamnetin, but not myricetin, in the leaves of *H. orientalis*. Although glucose and rhamnose have been detected as conjugated forms in Liliaceae (Carotenuto et al., 1996, 1997; Williams et al., 1993), the sugars we found in the flavonol-O-glycoside from *H. orientalis* were galactose, arabinose, arabinosylgalactose and galactosylgalactose (Tables 2 and 3, Fig. 1).

Chemical analysis of flavonoids has been extensively used to document the hybridization frequency of many species (King, 1977; Wyatt and Hunt, 1991; Lim et al., 1999). In general, the profile of a hybrid is the summation of its two parental profiles, although occasionally having some novel or missing compounds (Lim et al., 1999; Kim et al., 2000). Therefore, we measured



Kaempfer : R1 = H, R2 = H, R3 = H, R4 = H
Quercetin : R1 = OH, R2 = H, R3 = H, R4 = H
Isorhamnetin : R1 = OCH3, R2 = H, R3 = H, R4 = H
R3 = galactose, arabinose, arabinosylgalactose, galactosylgalactose
R4 = galactose

Figure 1. Chemical structure of flavonols from leaves of *H. orientalis*.

flavonoid contents to determine the effect of geographical location. Here, the relative amount of isorhamnetin 3-O-digalactoside seemed to vary the most -- from Namhansanseong, its content was 3.4% whereas from Jinburyung, it ranged from 5.0 to 6.0% (Table 4). This difference suggests that flavanol composition is affected mainly by growing conditions, e.g., the amount of canopy coverage. Oleszek et al. (2002) have also reported some variation in the concentrations of particular compounds among samples of Scots pine (*Pinus sylvestris*) collected from different origins.

We also examined the relationship among habitat, petal color, and flavonoid profile (Table 4). For example, in plants from the Namhansanseong population that had purple petals, the relative contents of quercetin 3-O-galactoside, isorhamnetin 3-O-arabinosylgalactoside, and isorhamnetin 3-O-digalactoside were 22.9%, 12.9%,

Table 3. Chromatographic properties of flavonoids identified from leaves of *H. orientalis*. RT = absolute retention time; = RT of sample/RT of internal standard. Solvents: TBA = tert-butanol:acetic acid:water (3:1:1); HOAc = acetic acid:water (15:85)

Compound	RT		R_f value	
	(min)	α	TBA	HOAc
Isorhamnetin 3-O-arabinosylgalactoside	9.20	0.92	0.30	0.46
Isorhamnetin 3-O-digalactoside	9.91	0.99	0.50	0.49
Kaempferol 3,7-O-galactoside	11.70	1.17	0.26	0.39
Kaempferol 3-O-arabinosylgalactoside	9.91	0.99	0.46	0.56
Kaempferol 3-O-glycoside	10.84	1.09	0.62	0.57
Quercetin 3-O-galactoside	10.61	1.06	0.56	0.31
Quercetin 3-O-arabinosylgalactoside	10.38	1.04	0.39	0.51

Table 4. Distribution of flavonoid compounds identified from the leaves of H. orientalis, based on sampling site and petal color. Relative contents are presented as percentages.

Compound	Sampling site (petal colar)			
	Namhansanseong (purple)	Jinburyung (purple)	Jinburyung (white)	Jinburyung (whitish purple)
Quercetin 3-O-galactoside	22.9	21	21.3	17
Isorhamnetin 3-O-arabinosylgalactoside	12.9	8.8	13.4	8.6
Isorhamnetin 3-O-digalactoside	3.4	5.1	6.4	5.9

and 3.4% of the flavonoid leaf extract, respectively. In contrast, those from the Jinburyung population, also with purple petals, showed relative amounts of quercetinu-3-O-galactoside, isorhamnetin-3-O-arabinosylgalactoside, and isorhamnetin-3-O-digalactoside that were 21.0%, 8.8%, and 5.1%, respectively, of the total. For plants with white petals from the same population, the relative contents were 21.3%, 13.4% and 6.4% compared with 17.0%, 8.6%, and 5.9%, respectively, for those having whitish-purple petals. Within the Jinburyung population itself, flavonol profiles did not differ significantly among the three petal colors.

Although several secondary metabolites, such as 3,26-dihydroxycholest-5-ene-16,22-dione, spirost-5-ene-3,12-diol, spirost-5-ene-3,17-diol, and spirost-3,5-diene, have been identified in *H. orientalis* (Buckingham, 1994), our report is the first concerning flavonol composition in this species. Our data further support the idea that the levels of flavonoids in plants are geographically controlled, and are specific to the origin of the individual population.

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