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Quantification of Antidepressant Miquelianin in Mature and Immature Fruits of Korean Rubus Species

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Abstract – Antidepressant miquelianin (quercetin 3-*O*-glucuronide) was isolated from the leaves of *Rubus craetaegifolius* (Rosaceae) and identified by physical and spectroscopic data. Miquelianin was quantitatively analyzed in the leaves, mature and premature fruits of Korean wild *R. craetagifolius*, *R. pungens* var. *oldhami*, *R. coreanus*, and *R. parvifolius* by HPLC. The contents of miquelianin was highest in the mature fruit of *R. crataegifolius* (16.29 ± 0.79 mg/g); however, the content of kaempferol 3-*O*-glucuronide was 33.88 ± 7.68 mg/g. These results suggest that the mature fruit of *R. crataegifolius* would be beneficial for treating depression or stress as a functional food with its sweet taste.
Keywords – *Rubus crataegifolius*, Rosaceae, miquelianin, quantitative, HPLC

Introduction

Rubi Fructus designates the immature fruits of *Rubus coreanus* (Rosaceae) in Korean herb medicine.[1] The mature fruit of *R. coreanus* becomes black when ripening while that of *R. crataegifolius* becomes reddish. The latter fruits are widely used as a mountainous fruit with sweet taste. We previously reported the isolation and quantification of triterpenoids[2] and flavonoids[3] from *Rubus crataegifolius* (Rosaceae) and the anti-inflammatory effect of the triterpenoids, niga-ichigoside F_1 and 23-hydroxytormentic acid, isolated from *R. coreanus*.[4,5] Five flavonoids have been quantitatively analyzed in the four Rubus species growing wildly in Korea.[3]

Miquelianin is a flavonol 3-*O*-glucuronide possessing anti-stress[6] and anti-depressant activities,[7] which has been known during the study on St. John's wort (*Hypericum perforatum*, Hypericaceae). Since we isolated the two flavonol 3-*O*-glucuronides of miquelianin (quercetin 3-*O*-glucuronide) and kaempferol 3-*O*-glucuronide, their quantitative levels in the four Rubus species (the leaves and mature and immature fruits of *R. craetagifolius*, *R. pungens* var. *oldhami*, *R. coreanus*, and *R. parvifolius*) were comparatively analyzed. Therefore, this analytical study was performed because miquelianin has been reported to have anti-stress[6] and anti-depressant activities.[7]

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Experimental

Instrument and reagent – Melting point was determined on an Electrothermal 9100 melting point apparatus and was uncorrected. Optical rotation was measured on a Perkin Elmer Model 341 polarimeter at 20 ºC. The ^{1}H-NMR spectra (δ ppm, *J* in Hz) was recorded in DMSO-d_6 on a Brucker AM-500 spectrometer (500 MHz), while ^{13}C-NMR spectra was recorded in the same solvent on a Brucker AM-500 spectrometer at 125 MHz with tetramethylsilane (TMS) as an internal standard. ODS-A (12 nm S-150 um, YMC) were used for column chromatography. Thin layer chromatography was performed on TLC plate RP-18 F_{254s} Merck. All compounds were detected under UV (254 and 366 nm) or by spraying with H_2SO_4 (50%).

HPLC chromatograms were measured using an HPLC system consisting of a Varian Prostar 210 solvent delivery module, a Prostar 325 UV-Vis detector, and a 20 μL sample loop. Separation was achieved on a Shiseido Capcell Pak C18 column (5 μL, 250 mm × 4.6 mm I.D.). Solvents used for analysis were HPLC grade.

Plant material – The leaves of *R. crataegifolius*, *R. craetagifolius*, *R. pungens* var. *oldhami*, *R. coreanus*, and *R. parvifolius* were collected in the mountainous area of Wonju city, Korea, dried and crushed for extraction. The

leaves of *R. crataegifolius* and *R. coreanus* were collected on June, and the immature and mature fruits were collected on June and July, respectively. Every part of *R. pungens* var. *oldhami* was also collected in the same period as of the corresponding part of *R. crataegifolius*. The immature and mature fruits of *R. coreanus* and *R. parvifolius* were collected on August.

Extraction and fractionation – The leaves (800 g) of *R. crataegifolius* were extracted with MeOH under reflux for 5 h three times. The MeOH solution was filtered and evaporated on a rotatory evaporator under reduced pressure to give a solid MeOH extract (127.6 g). The MeOH extract (110 g) was partitioned between H_2O and diethyl ether and further H_2O layer was fractionated with BuOH. Evaporation of diethyl ether and BuOH layer produced a diethyl ether fraction (29 g) and a BuOH fraction (15.5 g).

Isolation – A part (15 g) of BuOH fraction was subjected to Sephadex LH-20 column (ø 45 mm × 330 mm) chromatography eluted by MeOH. Fractions collected by each 50 ml were checked on TLC and then combined into five fractions (Fr. A – Fr. E). Fr. D was concentrated to dryness and then recrystallyzed in MeOH to give compound **1** (yellowish needles, 570 mg). Fr. C was chromatographed on ODS column (ø 30 mm × 340 mm) using MeOH-H_2O (1 : 1) as a mobile phase. Fractions were collected by each 15 ml, checked on TLC and then grouped to 4 fractions (Fr.C40a – Fr.C4-D). Fr.D4-D was recrystallized in MeOH to afford compound **2** (Yellowish needles, 86 mg). Compounds **1 - 2** were identified as miquelianin and kaempferol 3-*O*-glucuronide,[8] respectively, by comparison of spectroscopic data with literature.

Compound 1 (miquelianin) – Yellow needles from MeOH-H_2O; mp 193 - 195° (190 - 191°), $[\alpha]_D$ –48.2° (c, 0.835 in pyridine-H_2O); ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 6.19 (1H, d-like, H-6), 6.39 (1H, d-like, H-8), 6.85 (1H, d-like, H-2'), 6.76 (1H, d, J = 8.0 Hz, H-5'), 7.46 (1H, dd, J = 2.0, 8.0 Hz, H-6'), 5.34 (1H, d, J = 5.0 Hz, H-1'); ^{13}C-NMR (125.5 MHz, DMSO-d_6) δ: quercetin - 156.9 (C-2), 134.3 (C-3), 177.9 (C-4), 161.4 (C-5), 99.4 (C-6), 165.5 (C-7), 94.3 (C-8), 157.5 (C-9), 104.0 (C-10), 121.2 (C-1'), 115.9 (C-2'), 145.3 (C-3'), 148.9 (C-4'), 116.4 (C-5'), 121.4 (C-6'), Glc – 102.9 (C-1"), 74.7 (C-2"), 77.1 (C-3"), 72.3 (C-4"), 74.9 (C-5"), 172.7 (C-6"); FAB-MS: m/z 477 $[M - H]^-$.

Compound 2 (kaempferol 3-*O*-glucuronide) – Yellowish needles from MeOH, mp. 189-190.5; ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 6.21 (1H, d-like, H-6), 6.44 (1H, d-like, H-8), 8.05 (1H, d, J = 8.5 Hz, C-2', 6'), 6.89 (1H, d, J = 8.0 Hz, H-3', 5'), 5.50 (1H, d, J = 7.5 Hz, anomeric proton of D-glc); ^{13}C-NMR (125.5 MHz, DMSO-d_6) δ: quercetin – 156.8 (C-2), 133.5 (C-3), 177.4 (C-4), 161.6 (C-5), 99.3 (C-6), 164.8 (C-7), 94.2 (C-8), 156.8 (C-10), 104.4 (C-10), 121.1 (C-1'), 131.4 (C-2', 6'), 115.6 (C-3',5'), 160.6 (C-4'), Glc – 101.6 (C-1"), 74.4 (C-2"), 76.3 (C-3"), 72.0 (C-4"), 76.3 (C-5"), 170.6 (C-6"); FAB-MS: m/z 461 $[M - H]^-$.

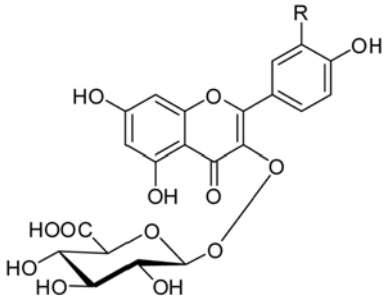

Fig. 1. Structures of miquelianin (R = OH) and kaempferol 3-*O*-glucuronide (R = H).

Standard compounds and standard calibration curves – Miquelianin and kaempferol 3-*O*-glucuronide, which were isolated from *C. crataegifolius*, were used as standard compounds. Standard compounds were dissolved with 80% aqueous methanol for injection. The concentration to plot standard curves were 100, 250, 500, and 1000 μg/g and high linearity of $R^2 > 0.999$ was obtained from each calibration curve equation.

HPLC condition for flavonol 3-*O*-glucuronides – One gram of the pulverized plant material was sonicated in pure methanol (MeOH) (40 ml) at 40 °C for 2 h, filtered and concentrated to dryness on a rotatory evaporator at 55 °C, and finally on a freeze dryer. The concentrated extracts were dissolved in 80% aqueous MeOH (2 ml) and filtered through 0.50 μm syringe filter, and the filtrate (20 μl) was injected into HPLC system. The mobile phase was 0.5% aqueous phosphoric acid (solvent A) and methanol (solvent B). The gradient was: 0 min, 70% A : 30% B; 0 - 3 min, 50% A : 50% B; 3 - 10 min, 47% A : 53% B; 10 - 15 min, 44% A : 56% B; 15 - 18 min, 41% A : 59% B; 18 - 20 min, 30% A : 70% B; 20 - 22 min, 20% A : 80% B; 22 - 25 min, 0% A : 100% B; 25 - 28 min, 0% A : 100% B; 28 - 29 min, 70% A : 30% B; 29 - 35 min, 70% A : 30% B. Chromatography was performed at the flow rate of 1.00 ml/min in 40 min, and the eluted compounds were detected at 254 nm.

Results and Discussion

The present research was performed to develop the fruits of Korean Rubus species as a healthy food or functional food with sweet taste. The fruits of *R. coreanus*

which are used for Rubi Fructus are quite different from those of other Rubus species with respect to the fruit color when ripening. Premature fruits of *R. coreanus* are used for Rubi Fructus, but no mature or premature fruits of *R. crataegifolius*, *R. pungens* var. *oldhami* and *R. parvifolius* are used for it.[1] The mature fruit color of *R. coreanus* is black while that of other three Rubus species is reddish.

Compounds miquelianin and kaempferol 3-*O*-glucuronide isolated from the leaves of *R. crataegifolius* were identified by comparison of spectroscopic data with literature data, as described in the experimental section. Since miquelianin possessing the structure of quercetin 3-*O*-glucuronide has been reported to have anti-depressant[7] and anti-stress[6] activities, the two flavonol 3-*O*-glucuronides were quantitatively analyzed in the leaves and the mature and premature fruits of *R. coreanus*, *R. crataegifolius*, *R. pungens* var. *oldhami* and *R. parvifolius* by HPLC. Although quantitative levels of flavonoids and triterpenoids have been reported from the Rubus species, the contents of flavonol 3-*O*-glucuronides have not been measured. In addition, anti-inflammatory[9] and anti-hyperlipidemic[10] effects of 19α-hydroxyursane-type triterpenoids of Rubus species were also previously reported.

Calculation of calibration curve of miquelianin and kaempferol 3-*O*-glucuronide led to the equation of $y = 262.31x - 3096.1$ and $y = 247.39x + 3801.7$, respectively, where y is the peak area (μV) and x is the concentration (μg/ml). The R^2 values of both equations were more than 0.999, demonstrating the linearity of calibration curves. HPLC chromatograms of the leaves and the mature and premature fruits were shown in Fig. 2. The contents of miquelianin and kaempferol 3-*O*-glucuronide in the plant parts of the four species were shown in Table 1. Each part of *R. crataegifolius* exhibited higher concentration than the corresponding parts of other three species. Mean values of miquelianin in the leaves, mature and premature fruits of *R. crataegifolius* were 8.33, 16.3 and 5.25 mg/g dry weight, while those of kaempferol 3-*O*-glucuronide were 3.61, 33.9 and 14.7 mg/g dry weight. In particular, the concentration of miquelianin was highest in the mature fruits. However, the content of flavonol 3-*O*-glucuronide in the mature fruits of *R. coreanus* was lower than in the premature ones. The levels of flavonol 3-*O*-glucuronides were relatively low in *R. pungens var oldhami* and *R. parvifolius*, although the qunatitative differences were observed among the plant parts.

Gudej *et al*.[11] reported the isolation of the glycosides of kaempferol and quercetin, but not of their glucuronides from *Rubus idaeus*. Miquelianin, which was isolated in the present study, is quercetin 3-*O*-glucuronide[12], whereas

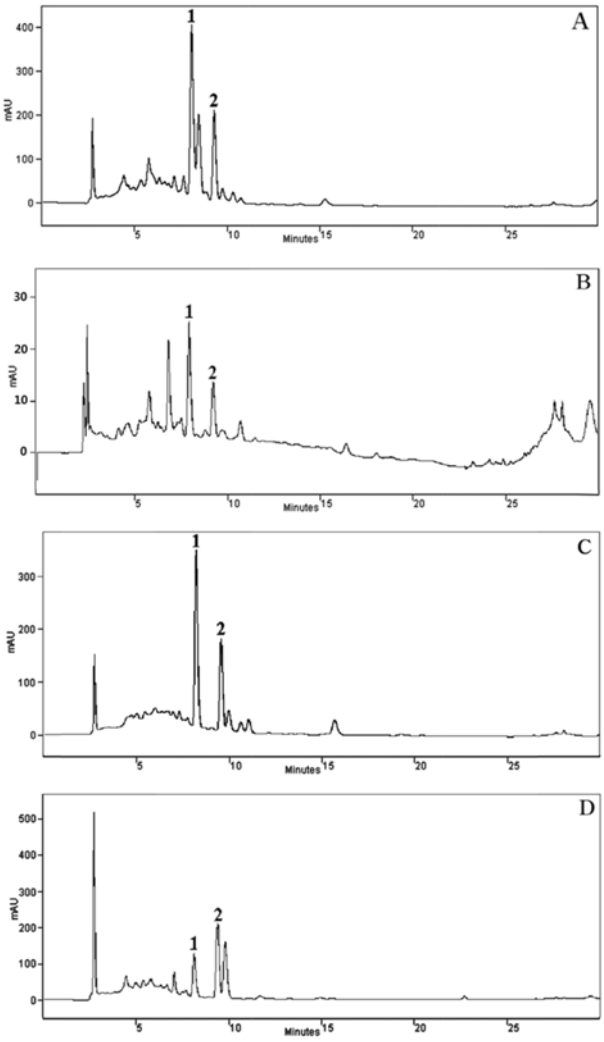

Fig. 2. HPLC chromatograms of the flavonoids analysis from the leaves of four *Rubus* species. **A** : *R. crataegifolius,* **B** : *R. pungens var. oldhami,* **C** : *R. parvifolius,* D; *R. coreanus*; **1** (miquelinain), **2** (kaempferol 3-*O*-glucuronide).

kaempferol 3-*O*-glucuronide has no special common name. Butterweck *et al*.[7] reported that miquelianin of St. John's wort (the herb of *Hypericum perforatum*) has anti-depressant activity similar to imipramine, an anti-depressant agent and that quercetin and its 3-*O*-glucoside do not. In particular, the anti-depressant activity of miquelianin has been demonstrated at the forced swimming test in the rat (0.6 mg/kg dose for 12 d, *p.o.*).[7] Juergenliemk *et al*.[12] demonstrated that miquelianin could reach central nervous system (CNS), after absorbed from the intestine without hydrolysis. This suggests that glycoside bonds in flavonol 3-*O*-glucuronides pass through intestinal mucosa without hydrolysis, although many of glycoside bonds can be hydrolyzed in the intestine.[13,14]

Based on these backgrounds, the present experiment

Table 1. Contents of miquelianin and kaempferol 3-*O*-glucuronide in the leaves and mature and immature of four Rubus species

(Unit: mg/g)

Plant name	Part	Miquelianin	Kaempferol 3-*O*-glucuronide	Sum
R. crataegifolius	Mature fruit	16.29 ± 0.79[a]	33.88 ± 7.68	50.18 ± 8.47
	Immature fruit	5.25 ± 0.95	14.69 ± 1.47	19.93 ± 1.76
	Leaf	8.33 ± 0.34	3.61 ± 0.34	11.94 ± 0.36
R. pungens var. oldhami	Mature fruit	1.18 ± 0.10	tr[b]	1.18 ± 0.10
	Immature fruit	0.90 ± 0.00	tr	0.90 ± 0.00
	Leaf	0.55 ± 0.02	tr	0.55 ± 0.02
R. parvifolius	Mature fruit	0.80 ± 0.07	0.18 ± 0.04	0.98 ± 0.10
	Immature fruit	2.97 ± 0.15	5.98 ± 0.61	8.95 ± 0.75
	Leaf	7.21 ± 0.41	2.96 ± 0.41	10.08 ± 0.81
R. coreanus	Mature fruit	0.79 ± 0.04	0.82 ± 0.04	1.60 ± 0.02
	Immature fruit	1.05 ± 0.04	0.95 ± 0.08	2.00 ± 0.05
	Leaf	3.30 ± 0.31	3.79 ± 0.63	7.09 ± 0.38

[a]Values represent mean ± S.D. based on three experiments. [b]trace (peak with integral value at 206 nm).

was aimed to demonstrate a high content of miquelianin in some Rubus species. As shown in Table 1, the highest level of miquelianin was observed in the mature fruits of *R. crataegifolius* by the HPLC analysis. In the mature fruit of *R. crataegifolius*, the sum of miquelianin and kaempferol 3-*O*-glucuronide reached approximately 5.0% of dry weight. The taste of mature fruits is commonly sweeter than of the premature ones. Therefore, the mature fruits may be developed as a functional food possessing anti-depressant activity and sweet taste. However, in *R. coreanus*, the flavonol 3-*O*-glucuronide decreased with ripening, although those of *R. crataegifolius* increased. In conclusion, the mature fruits of *R. crataegifolius* could be developed as a functional food with taste and antide-pressant activity, based on the high content of antide-pressant miquelianin.

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