Purgative Activity and Principals of the Fruits of *Rosa multiflora* and *R. wichuraiana*

Takako SETO,* Ichiro YASUDA, and Kazuyuki AKIYAMA

Department of Pharmaceutical Sciences, The Tokyo Metropolitan Research Laboratory of Public Health, Hyakunincho 3-24-1, Shinjuku-ku, Tokyo 169, Japan. *Received December 10, 1991*

Pseudocarps or seeds of *Rosa multiflora*, crude drug "Eijitsu" (営実), have been used as purgative in Japanese traditional medicine. *R. wichuraiana* was generally thought to be able to substitute for the plant. The *n*-butanol fractions of both plant seeds were tested on purgative activities with mice, and the values of the 50% effective dose (ED_{50}) were 5.6 g/kg as the seed weight for *R. multiflora* and 57 g/kg as the seed weight for *R. wichuraiana*. From pseudocarps of *R. multiflora*, a new purgative compound, multinoside A acetate, was isolated, and its ED_{50} value was tested to be 150 mg/kg (77—291 mg/kg, 95% confidence limit). The other isolated compounds were three known quercetin glycosides, quercetin 3-*O*-xyloside, isoquercitrin and hyperin. From pseudocarps of *R. wichuraiana*, three quercetin glycosides, isoquercitrin, hyperin and quercetin 3-*O*-β-D-glucuronide were isolated similarly, but no purgative components of *R. multiflora* were detected.

Keywords *Rosa multiflora*; *Rosa wichuraiana*; multinoside A acetate; quercetin 3-*O*-xyloside; isoquercitrin; hyperin; quercetin 3-*O*-β-D-glucuronide; purgative component; ^{13}C-NMR; purgative activity

Pseudocarps or seeds of *R. multiflora*, crude drug "Eijitsu" (営実), have been used as purgative in Japanese folk medicine and described in the Japanese Pharmacopoeia. *R. wichuraiana* was generally thought to be able to substitute for the plant. Seeds of *R. multiflora* have been reported[1] to have purgative activity and components as follows, multiflorin A (**1**), multiflorin B (**2**), kaemferol 3-α-L-rhamnopyranoside, multinoside A (**3**), multinoside B, quercitrin (**4**) as the flavonoid compounds, with purgative activities found in **1**, **2** and multinoside B. But components of *R. wichuraiana* are not apparent. In this paper, we compared the purgative activities and flavonoid components of the pseudocarps of both plants.

Seeds of both plants were extracted with methanol, and an *n*-butanol fraction was gained by partition with *n*-butanol and water from the ethyl ether insoluble part of the methanol extraction. The *n*-butanol fractions were tested for purgative activities with mice by the method of Takagi[1b] *et al.*, and the results are shown in Table I. No activity was found from other parts of the methanol extraction, only the *n*-butanol fraction. The ED_{50} value of *R. wichuraiana* seeds was 57 g/kg of seed weight and that of *R. multiflora* seeds was 5.6 g/kg. Therefore, the efficacy of *R. wichuraiana* seemed to be about 1/10 as weak as that of *R. multiflora*. No purgative effect was detected by the same tests in the part of pseudocarps where the seeds were removed. Next, we examined the components of both plants.

High-performance liquid chromatography (HPLC) patterns of the methanol extracts of the seeds of both plants were very different each other, as shown in Fig. 1. The pseudocarps of *R. multiflora* were extracted with methanol. The crude extract was partitioned between ethyl ether and water, and then the water layer was extracted with *n*-butanol. Compounds **5**—**7** and **9** were isolated by chromatographic separation of the *n*-butanol fraction

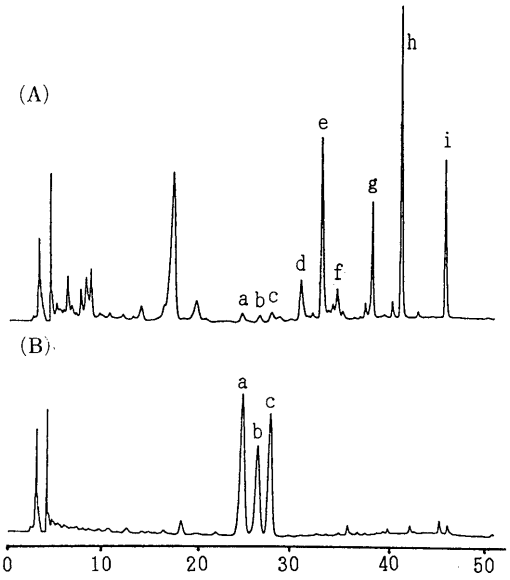


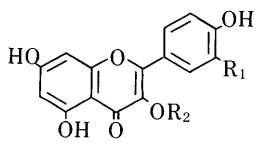
Fig. 1. HPLC Chromatograms of Methanol Extracts of *Rosa multiflora* (A) and *R. wichuraiana* (B) Pseudocarps

Conditions are described in Experimental section. a, hyperin; b, isoquercitrin; c, quercetin 3-*O*-glucuronide; d, quercetin 3-*O*-xyloside; e, multinoside A; f, quercitrin; g, multiflorin B; h, multinoside A acetate; i, multiflorin A.

TABLE I. Comparison of Purgative Activities of Rosa Seeds

	ED_{50} value (g/kg)	
	n-Butanol fraction[a] (95% confidence limit)	As seed weight[b]
R. multiflora	0.2 (0.2—0.3)	5.6
R. wichuraiana	3.3 (2.7—3.8)	57

a) Tested material. b) Converted from *n*-butanol fraction by yield.



	R1	R2	
1	H	Rha-Glc-Ac	(multiflorin A)
2	H	Rha-Glc	(multiflorin B)
3	OH	Rha-Glc	(multinoside A)
4	OH	Rha	(quercitrin)
5	OH	Xyl	(quercetin 3-*O*-xyloside)
6	OH	Glc	(isoquercitrin)
7	OH	Gal	(hyperin)
8	OH	GlcA	(quercetin 3-*O*-glucuronide)
9	OH	Rha-Glc-Ac	(multinoside A acetate)

Chart I

TABLE II. ^{13}C-NMR Spectral Data for **1**—**3** and **9** in DMSO-d_6

C	1	2	9[a]	3
2	157.2	157.2	157.3	157.3
3	134.4	134.2	134.4	134.3
4	177.6	177.6	177.7	177.7
5	161.4[b]	161.2[c]	161.3	161.3
6	98.7	98.7	98.7	98.7
7	164.2	164.2	164.2	164.2
8	93.7	93.7	93.7	93.7
9	156.5[b]	156.5[c]	156.5	156.5
10	104.1	104.1	104.1	104.1
1′	120.4	120.4	120.7	120.8
2′	130.5	130.5	115.5	115.5
3′	115.3	115.4	145.2	145.2
4′	161.4	159.9	148.4	148.4
5′	115.3	115.4	115.7	115.7
6′	130.5	130.5	121.0	121.0
Rha				
1	101.9	101.8	101.9	101.9
2	70.2	70.3	70.2	70.3
3	70.2	69.7	69.7	69.9
4	82.3	81.9	82.4	82.0
5	68.8	68.9	68.8	69.0
6	17.0	17.3	17.1	17.4
Glu				
1	104.6	104.6	104.7	104.7
2	74.2	74.4	74.3	74.5
3	76.3	76.6	76.4	76.6
4	70.2	69.7	70.2	69.8
5	73.6	76.9	73.7	76.9
6	63.5	61.0	63.6	61.0
Ac-CO	170.1		170.1	
Ac-Me	20.5		20.6	

a) Assignments were confirmed by COLOC spectrum. *b*, *c*) Reversed in the literature[5] respectively.

together with **1**—**4**. Multinoside B has been reported to be a major component of *R. multiflora*,[1] but it was not detected by HPLC[2] at this time. Pseudocarps of *R. wichuraiana* were studied with similar methods, and compounds **6**—**8** were given. These were all obtained as pale yellow substances and turned green with $FeCl_3$ on thin layer chromatography (TLC). Four compounds were identified as **5**: quercetin 3-*O*-xyloside,[3] **6**: isoquercitrin, **7**: hyperin, and **8**: quercetin 3-*O*-β-D-glucuronide,[3,4] based on spectroscopic data.

Compound **9** was a new naturally occurring flavonoid. Its ultraviolet (UV) spectrum had the same absorption maxima as **3** and **4** at 259 and 308 nm, and its fast-atom bombardment mass spectrum (FAB-MS) ion peak at *m/z* 653 [$M^+ + 1$] was only 16 mass units larger than the peak given by **1**. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) data of **1**—**3** and **9** were very similar to each other, as shown in Table II. The carbon signals of the aglycon part of **9** completely agreed with those of **3**, and the glycosyl part signals were similar to those of **1**. Compound **9** was thought to have a quercetin group at the aglycon part and 6-*O*-acetyl-β-D-glucosyl-(1→4)-α-L-rhamnose group as its glycosyl part. Thus, **9** was confirmed to be multinoside A acetate, quercetin 3-(6-*O*-acetyl)-β-D-glucosyl-(1→4)-α-L-rhamnoside. The assignments of carbon signals of **9** were confirmed by correlation spectroscopy *via* ^{1}H–^{13}C long-range couplings (COLOC).

Purgative activity was also examined on **9** and **6**, and as the result, the ED_{50} value of **9** was 150 mg/kg (77—291 mg/kg, 95% confidence limit). The activity was weaker than ED_{50} 30 mg/kg (22—39 mg/kg) of **1**[1b] but stronger than 222 mg/kg (161—304 mg/kg) of **2**.[1b] No effectivity on purgative action was shown by **6** at a dose of 200 mg/kg.

The lower purgative activity of *R. wichuraiana* seeds was corresponding to the lack of purgative components of *R. multiflora* seeds, **1**, **2** and **9**. In conclusion, the pseudocarps of *R. wichuraiana* were not valuable as the substitute for "Eijitsu" (営実), the pseudocarps of *R. multiflora*, because *R. wichurajana* seeds had only poor activity and lacked the purgative components of the *R. multiflora* seeds.

Experimental

Apparatus Melting points are not corrected. UV spectra were measured in MeOH with a Hitachi 320 spectrophotometer. Infrared (IR) spectra were recorded on a Bio-Rad FTS-60. ^{13}C-NMR (68 MHz) spectra were obtained on a JEOL FX-270 spectrometer with tetramethylsilane (TMS) as an internal standard in dimethyl sulfoxide-d_6 (DMSO-d_6) at room temperature. ^{1}H (500 MHz)-NMR spectra and ^{1}H–^{13}C long-range correlation spectroscopy was recorded on a JEOL α-500 using DMSO-d_6 as solvent at room temperature, and COLOC was measured with an average J_{CH} value of 8 Hz for two- and three-bond coupling. FAB-MS (thioglycerol matrix) were recorded on a VG-70S mass spectrometer. Analytical HPLC was carried out with a JASCO LC-800, on a TSK gel ODS-80TM (Tosoh, 4.6 i.d. × 250 mm, 5 μm) column by a gradient mode in a mobile phase (CH_3CN–H_2O–H_3PO_4 mixture, initial concentration, CH_3CN 18%, H_3PO_4 0.05%; 20—40 min linear gradient; 40—60 min, CH_3CN 30%, H_3PO_4 0.05%), flow rate, 1 ml/min, detection, 280 nm, and column temperature, 25 °C. Preparative HPLC was carried out with a Tosoh HLC-837 on a TSKgel ODS-80TM (Tosoh, 25.4 i.d. × 250 mm, 5 μm) column by CH_3CN– H_2O–H_3PO_4 (200 : 800 : 0.5) solution (A) or CH_3CN–H_2O–H_3PO_4 (300 : 700 : 0.5) solution (B). Each eluate, after concentration *in vacuo* to 1/3 volume, was loaded on a Sephadex LH-20 column (10 cm length), washed with H_2O to remove H_3PO_4, and then the substances were eluated with MeOH and concentrated. TLC was carried out with precoated Kieselgel 60 F_{254} plates (Merck) and solution C ($CHCl_3$: MeOH : H_2O = 65 : 35 : 10, under layer), and detection was achieved by spraying them with a 1% $FeCl_3$ MeOH solution.

Extraction and Isolation Air-dried *R. multiflora* pseudocarps (800 g) collected at Kawasaki City in Kanagawa prefecture were crushed and extracted three times with MeOH at 50 °C. The extract, after evaporation of MeOH, was added to H_2O and partitioned with ether and then *n*-BuOH. The *n*-BuOH fraction (54 g) was passed through an ODS short column with H_2O, 50% MeOH and MeOH as eluents. 50% MeOH eluate was applied to Sephadex LH-20 column (4.3 i.d. × 20 cm), and eluted with H_2O, an increasing amount of MeOH in H_2O and MeOH. 30%—50% MeOH fractions, which had many $FeCl_3$ positive spots on TLC, were gathered and divided into 5 fractions, fractions 1—5, by preparative HPLC with solution B. Fractions 2, 3 and 4 were repeatedly subjected to preparative HPLC with solution B to afford multiflorin B (**2**, 60 mg), multinoside A acetate (**9**, 390 mg) and multiflorin A (**1**, 75 mg) respectively. Fraction 1 was separated by preparative HPLC with solution A into 4 further fractions, fractions 1a, 1b, 1c and 1d. Fraction 1a was found to be a mixture of two substances by analytical HPLC, and afforded hyperin (**7**, 25 mg) and isoquercitrin (**6**, 28 mg) after separation by preparative HPLC in a recycle mode with solution A. Fraction 1b was rechromatographed on a silica gel column (2.5 i.d. × 15 cm, Merck) with $CHCl_3$–MeOH (2 : 1) to give quercitrin (**4**, 27 mg). Fraction 1c was purified in the same manner as fraction 1; it yielded multinoside A (**3**, 530 mg). Without removal of H_3PO_4, quercetin 3-*O*-xyloside (**5**, 30 mg) separated from fraction 1d as yellow needles.

Extraction and preliminary isolation by preparative HPLC were performed in a way similar to the case of *R. multiflora* from 350 g of *R. wichuraiana* pseudocarps which were gathered at Miura of Kanagawa prefecture. The eluate from Sephadex LH-20 column similarly to *R. multiflora* was applied to preparative HPLC with solution A. The fraction of main peaks was chromatographed on a silica gel column (3 i.d. × 25 cm, Merck) with $CHCl_3$–MeOH (2 : 1), $CHCl_3$–MeOH (1 : 1), solution C and solution D (solution C : MeOH = 9 : 1) one after another, to divide it into 6 fractions, fractions 1—6. Fractions 2, 4 and 6 were eluates of $CHCl_3$–MeOH (1 : 1), solution C and solution D respectively, and each was purified with a Sephadex LH-20 column to afford hyperin (**7**, 18 mg),

isoquercitrin (**6**, 20 mg) and quercetin 3-*O*-*β*-D-glucuronide (**8**, 60 mg).

Multiflorin A (1) TLC: *Rf* 0.50, brown. HPLC (t_R): 46 min. UV λ_{max}^{MeOH} nm: 266, 296 (sh), 344. FAB-MS *m/z*: 637 ($M^+ + 1$), 287 (aglycon + 1 ion), ^{13}C-NMR[5]: Table II, C-5 and C-9 were reversed in the literature.

Multiflorin B (2) TLC: *Rf* 0.39 brown. HPLC (t_R): 38 min. UV λ_{max}^{MeOH} nm: 266, 296 (sh), 344. FAB-MS *m/z*: 595 ($M^+ + 1$), 303 (aglycon + 1 ion). ^{13}C-NMR[5]: Table II, C-5 and C-9 were reversed in the literature.

Multinoside A Acetate (9) TLC: *Rf* 0.47 green. HPLC (t_R): 41 min. $[\alpha]_D^{22}$ −133.5° (*c* = 1.34, MeOH), mp 169—173 °C. UV λ_{max}^{MeOH} nm: 259, 308 (sh), 353. IR ν_{max}^{KBr} cm^{-1}: 3400 (br, OH), 1724 (acetyl CO), 1656 (conjugated CO), 1608, 1507, 1446, 1363, 1272. High resolution (HR)-MS (FAB): Calcd for $C_{29}H_{33}O_{17}$ ($M^+ + 1$): 653.1718. Found: 653.1725. FAB-MS *m/z*: 653 ($M^+ + 1$), 303 (aglycon + 1 ion). ^{1}H-NMR *δ*: 0.86 (3H, d, *J* = 5.5 Hz, rha-C$\underline{H}_3$), 1.95 (3H, s, –OCOC$\underline{H}_3$), 2.98—3.04 (2H, m, glu-2,4H), 3.16 (1H, br dd, *J* = 8.9 Hz, glu-3H), 3.30—3.44 (m, glu-5H, rha-5,4H, overlapped with H_2O), 3.76 (1H, br d, *J* = 8.5 Hz, rha-3H), 4.03—4.09 (2H, m, glu-6H_a, rha-2H), 4.20 (1H, br d, *J* = 9.8 Hz, glu-6H_b), 4.34 (1H, d, *J* = 7.3 Hz, glu-1H), 5.01—5.2 (5H, br m and d, *J* = 2.3 Hz, rha-1H), 5.45 (1H, br s), 6.20 (1H, d, *J* = 1.8 Hz, 6H), 6.38 (1H, d, *J* = 2.4 Hz, 8H), 6.87 (1H, d, *J* = 8.5 Hz, 5′H), 7.23 (1H, dd, *J* = 1.8, 8.5 Hz, 6′H), 7.30 (1H, d, *J* = 2.5 Hz, 2′H), 9.35 (1H, br s), 9.73 (1H, br s), 11.85 (1H, br s), 12.61 (1H, s, 5-OH). ^{13}C-NMR: Table II.

Multinoside A (3) TLC: *Rf* 0.31 green. HPLC (t_R): 33 min. mp 187—190 °C. UV λ_{max}^{MeOH} nm: 259, 308 (sh), 353. FAB-MS *m/z*: 611 ($M^+ + 1$), 303 (aglycon + 1 ion). ^{13}C-NMR[6]: Table II.

Quercitrin (4) TLC: *Rf* 0.55 green. HPLC (t_R): 35 min. UV λ_{max}^{MeOH} nm: 259, 308 (sh), 354. FAB-MS *m/z*: 449 ($M^+ + 1$), 303 (aglycon + 1 ion). ^{13}C-NMR *δ*: 17.4 (q, C-6″), 70.1, 70.4, 70.6 (each d, C-1″, 2″, 5″), 71.2 (d, C-4″), 93.6 (d, C-8), 98.7 (d, C-6), 101.8 (d, C-1″), 104.9 (s, C-10), 115.4 (d, C-2′), 115.7 (d, C-5′), 120.8 (s, C-1′), 121.1 (d, C-6′), 134.2 (s, C-3), 145.2 (s, C-3′), 148.4 (s, C-4′), 156.4 (s, C-2), 157.3 (s, C-6), 161.3 (s, C-5), 164.1 (s, C-7), 177.7 (s, C-4).

Quercetin 3-*O*-Xyloside (5) Yellow needles, mp 218—223 °C. TLC: *Rf* 0.51 green. HPLC (t_R): 31 min. UV λ_{max}^{MeOH} nm: 259, 308 (sh), 359. HR-MS (FAB): Calcd for $C_{20}H_{19}O_{11}$ ($M^+ + 1$): 435.0927. Found: 435.0950. FAB-MS *m/z*: 453 ($M^+ + 1$), 303 (aglycon + 1 ion). ^{13}C-NMR *δ*: 66.0 (t, C-5″), 69.4 (d, C-4″), 73.6 (d, C-2″), 76.0 (d, C-3″), 93.6 (d, C-8), 98.7 (d, C-6), 101.8 (d, C-1″), 103.9 (s, C-10), 115.4 (d, C-2′), 116.2 (d, C-5′), 121.0 (s, C-1′), 121.5 (d, C-6′), 133.2 (s, C-3), 144.9 (s, C-3′), 148.6 (s, C-4′), 156.3 (s, C-2,9), 161.2 (s, C-5), 164.2 (s, C-7), 177.4 (s, C-4).

Isoquercitrin (6) TLC: *Rf* 0.49 green. HPLC (t_R): 27 min. $[\alpha]_D^{22}$ −25.9° (*c* = 1.75, MeOH). UV λ_{max}^{MeOH} nm: 258, 359. FAB-MS *m/z*: 465 ($M^+ + 1$), 303 (aglycon + 1 ion). ^{13}C-NMR *δ*: 60.9 (t, C-6″), 69.8 (d, C-4″), 74.0 (d, C-2″), 76.4 (d, C-5″), 77.4 (d, C-3″), 93.6 (d, C-8), 98.6 (d, C-6), 101.0 (d, C-1″), 103.7 (s, C-10), 115.2 (d, C-2′), 116.2 (d, C-5′), 121.1 (s, C-1′), 121.5 (d, C-6′), 133.3 (s, C-3), 144.8 (s, C-3′), 148.5 (s, C-4′), 156.2 (s, C-2, 9), 161.1 (s, C-5), 164.0 (s, C-7), 177.3 (s, C-4).

Hyperin (7) TLC: *Rf* 0.50 green. HPLC (t_R): 25 min. UV λ_{max}^{MeOH} nm: 257, 359. FAB-MS *m/z*: 465 ($M^+ + 1$), 303 (aglycon + 1 ion). ^{13}C-NMR *δ*: 60.1 (t, C-6″), 67.8 (d, C-4″), 71.2 (d, C-2″), 73.2 (d, C-3″), 75.7 (d, C-5″), 93.6 (d, C-8), 98.8 (d, C-6), 101.9 (d, C-1″), 103.9 (s, C-10), 115.2 (d, C-2′), 116.0 (d, C-5′), 121.0 (s, C-1′), 121.9 (d, C-6′), 133.5 (s, C-3), 144.8 (s, C-3′), 148.5 (s, C-4′), 156.3 (s, C-2, 9), 161.1 (s, C-5), 164.4 (s, C-7), 177.3 (s, C-4).

Quercetin 3-*O*-*β*-D-Glucuronide (8) Yellow needles, mp 195—196 °C. TLC: *Rf* 0.25 green. HPLC (t_R): 28 min. $[\alpha]_D^{22}$ −27.5° (*c* = 1.5, MeOH). UV λ_{max}^{MeOH} nm: 258, 359. HR-MS (FAB): Calcd for $C_{21}H_{19}O_{13}$ ($M^+ + 1$): 479.0846. Found: 479.0826. FAB-MS *m/z*: 479 ($M^+ + 1$), 303 (aglycon + 1 ion). ^{13}C-NMR *δ*: 71.3 (d, C-4″), 73.7 (d, C-2″), 75.8, 75.9, (each d, C-3″, 5″), 93.5 (d, C-8), 98.7 (d, C-6), 101.0 (d, C-1″), 103.8 (s, C-10), 115.1 (d, C-2′), 116.0 (d, C-5′), 120.8 (s, C-1′), 121.6 (d, C-6′), 133.0 (s, C-3), 144.8 (s, C-3′), 148.5 (s, C-4′), 156.1 (s, C-2,9), 161.1 (s, C-5), 164.1 (s, C-7), 169.6 (s, C-6″), 177.1 (s, C-4).

Compounds **1**, **2** were identified by direct comparison with authentic samples on TLC and HPLC, and compound **4**, **7** and **8** on FAB-MS, ^{13}C-NMR in addition to TLC and HPLC.

Preparation for Measurements of Purgative Activities From 100 g of pseudocarps, BuOH fractions were made in a way similar to the items of extraction and isolation and measured by the method in the literature.[1)]

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References and Notes

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