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

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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 (ED₅₀) 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 ED₅₀ 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; ¹³C-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 reported¹⁾ 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 ED₅₀ 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)	
	<i>n</i> -Butanol fraction ^{a)} (95% confidence limit)	As seed weight ^{b)}
<i>R. multiflora</i>	0.2 (0.2—0.3)	5.6
<i>R. wichuraiana</i>	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- <i>O</i> -xyloside)
6	OH	Glc (isoquercitrin)
7	OH	Gal (hyperin)
8	OH	GlcA (quercetin 3- <i>O</i> -glucuronide)
9	OH	Rha-Glc-Ac (multinoside A acetate)

Chart 1

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

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 literature³⁾ 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 HPLC²⁾ 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 [$\text{M}^+ + 1$] 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 $\rightarrow$ 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 $\rightarrow$ 4)- α -L-rhamnoside. The assignments of carbon signals of 9 were confirmed by correlation spectroscopy via ^1H - ^{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. wichuraiana* 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 ($\text{DMSO}-d_6$) at room temperature. ^1H (500 MHz)-NMR spectra and ^1H - ^{13}C long-range correlation spectroscopy was recorded on a JEOL α -500 using $\text{DMSO}-d_6$ 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. $\times$ 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. $\times$ 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 pre-coated 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. $\times$ 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. $\times$ 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. $\times$ 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: *R_f* 0.50, brown. HPLC (*t_R*): 46 min. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 266, 296 (sh), 344. FAB-MS *m/z*: 637 ($M^+ + 1$), 287 (aglycon + 1 ion), $^{13}\text{C-NMR}^5$: Table II, C-5 and C-9 were reversed in the literature.

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

Multinoside A Acetate (9) TLC: *R_f* 0.47 green. HPLC (*t_R*): 41 min. $[\alpha]_D^{22} - 133.5^\circ$ (*c* = 1.34, MeOH), mp 169–173 °C. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 259, 308 (sh), 353. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400 (br, OH), 1724 (acetyl CO), 1656 (conjugated CO), 1608, 1507, 1446, 1363, 1272. High resolution (HR)-MS (FAB): Calcd for $\text{C}_{29}\text{H}_{33}\text{O}_{17}$ ($M^+ + 1$): 653.1718. Found: 653.1725. FAB-MS *m/z*: 653 ($M^+ + 1$), 303 (aglycon + 1 ion). $^1\text{H-NMR}$ δ : 0.86 (3H, d, *J* = 5.5 Hz, rha-CH₃), 1.95 (3H, s, -OCOCH₃), 2.98–3.04 (2H, m, glu-2,4H), 3.16 (1H, brdd, *J* = 8.9 Hz, glu-3H), 3.30–3.44 (m, glu-5H, rha-5,4H, overlapped with H₂O), 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). $^{13}\text{C-NMR}$: Table II.

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

Quercitrin (4) TLC: *R_f* 0.55 green. HPLC (*t_R*): 35 min. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 259, 308 (sh), 354. FAB-MS *m/z*: 449 ($M^+ + 1$), 303 (aglycon + 1 ion). $^{13}\text{C-NMR}$ 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: *R_f* 0.51 green. HPLC (*t_R*): 31 min. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 259, 308 (sh), 359. HR-MS (FAB): Calcd for $\text{C}_{20}\text{H}_{19}\text{O}_{11}$ ($M^+ + 1$): 435.0927. Found: 435.0950. FAB-MS *m/z*: 453 ($M^+ + 1$), 303 (aglycon + 1 ion). $^{13}\text{C-NMR}$ 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: *R_f* 0.49 green. HPLC (*t_R*): 27 min. $[\alpha]_D^{22} - 25.9^\circ$ (*c* = 1.75, MeOH). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 258, 359. FAB-MS *m/z*: 465 ($M^+ + 1$), 303 (aglycon + 1 ion). $^{13}\text{C-NMR}$ 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: *R_f* 0.50 green. HPLC (*t_R*): 25 min. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 257, 359. FAB-MS *m/z*: 465 ($M^+ + 1$), 303 (aglycon + 1 ion). $^{13}\text{C-NMR}$ 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: *R_f* 0.25 green. HPLC (*t_R*): 28 min. $[\alpha]_D^{22} - 27.5^\circ$ (*c* = 1.5, MeOH). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 258, 359. HR-MS (FAB): Calcd for $\text{C}_{21}\text{H}_{19}\text{O}_{13}$ ($M^+ + 1$): 479.0846. Found: 479.0826. FAB-MS *m/z*: 479 ($M^+ + 1$), 303 (aglycon + 1 ion). $^{13}\text{C-NMR}$ 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, $^{13}\text{C-NMR}$ 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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