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NOTE

Three new aromatic glycosides from the ripe fruit of cherry tomato

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Abstract Three new aromatic glycosides were isolated from the ripe fruit of cherry tomato [*Lycopersicon esculentum* var. *cerasiforme* (Dunal) Alef. (Solanaceae)] along with six known aromatic glycosides and one known steroidal alkaloid glycoside. Their chemical structures were determined on the basis of spectroscopic data as well as chemical evidence.

Keywords *Lycopersicon esculentum* · Cherry tomato · Solanaceae · Aromatic glycoside · Methyl salicylate

Introduction

Tomato, the fruit of *Lycopersicon esculentum* (syn. *Solanum lycopersicum* L., Solanaceae), is most widely used as a fresh vegetable and for cooking. The species of tomato on the market are roughly classified into two groups, red color-type and pink color-type; the former is mainly used for pasta sauce and in cooking, and the latter as a fresh vegetable. We earlier reported the isolation and structural elucidation of a steroidal alkaloid glycoside, esculeoside A, from the ripe pink color-type tomato (*L. esculentum*, 'Momotaro'); a solanocapsine-type glycoside, esculeoside B, from the ripe red color-type tomato (*L. esculentum*, 'Italian San Marzano'); six steroidal glycosides, esculeosides A, B, C, and D, lycoperoside G, and 3-*O*-*β*-lycotetraosyl 3*β*,26-dihydroxy-cholestan-16, 22-dione 26-*O*-α-L-arabinopyranosyl-(1 → 6)-*β*-D-glucopyranoside, and six aromatic compounds, zizybeoside I, benzoyl alcohol *β*-gentiobioside, rutin, methyl caffeate, phenylalanine, and 4-hydroxyphenyl *β*-D-glucopyranosyl-(1 → 2)-*β*-D-glucopyranoside, from the ripe cherry tomato [*L. esculentum* var. *cerasiforme* (Dunal) Alef.]; and two pregnane glycosides, tomato pregnane and 3-*O*-*β*-lycotetraosyl 5α-pregna-3*β*,16*β*-diol-20-one, from the overripe cherry tomato [1–6]. As for the constituents of the tomato, differences were observed among cultivars as was mentioned above. Therefore, as part of our continuing study on the chemical constituents of the tomato, we examined the constituents of the ripe fruit of cherry tomato ('Komomo'). The present paper deals with the isolation and structural characterization of three new aromatic glycosides along with seven known compounds from the ripe fruit.

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Results and discussion

The ripe fruit of cherry tomato was smashed with H_2O in a mixer then filtered through filter paper to give a filtrate, which was subjected to Diaion HP20, Sephadex LH-20, Chromatorex NH_2, Chromatorex ODS, and silica gel column chromatography, as well as HPLC on ODS to afford nine aromatic glycosides (**1**–**9**) and one steroidal alkaloid glycoside (**10**).

Compound **1** was obtained as an amorphous powder and exhibited an $[M + Na]^+$ ion peak at *m/z* 631 in the positive FAB–MS; the high-resolution (HR) positive FAB–MS indicated the molecular formula of **1** to be $C_{25}H_{36}O_5$. The ^{1}H-NMR spectrum of **1** revealed the presence of four aromatic protons [δ 7.84 (1H, d, $J = 1.5$, 7.5 Hz), 7.67 (1H, d, $J = 8.0$ Hz), 7.46 (1H, ddd, $J = 1.5$, 7.5, 8.0 Hz), 6.88 (1H, dd, $J = 7.5$, 7.5 Hz)] assigned to an *ortho*-substituted phenyl group, three anomeric protons [δ 5.62 (1H, d, $J = 7.5$ Hz), 5.56 (1H, d, $J = 8.0$ Hz), 4.90 (1H, d, $J = 7.5$ Hz)], and one methoxy group [δ 3.90 (3H, s)]. The ^{13}C-NMR spectrum of **1** gave 25 carbon signals, including those corresponding to one carboxyl carbon (δ 166.3), six aromatic carbons (δ 157.4, 134.1, 131.6, 121.5, 121.0, 116.4), three anomeric carbons (δ 105.9, 105.7, 100.0), and one methoxy carbon (δ 52.0). These ^{1}H- and ^{13}C-NMR signals were assigned with the aid of ^{1}H–^{1}H COSY, HMQC, and HMBC spectra (Tables 1, 2), and **1** was determined to be a methyl salicylate 2-*O*-triglycoside, which was composed of 1 mol of pentose and 2 mol of hexose.

Acidic hydrolysis of **1** afforded D-xylose and D-glucose, which were confirmed by optical rotation using chiral detection in HPLC analyses. The coupling constants of signals due to anomeric and methine protons of xylosyl and glucosyl units in the ^{1}H-NMR spectrum indicated that all the monosaccharide units were of the pyranose type, and all glycosidic linkages were β in $^4C_1$ conformations. The ^{13}C-NMR data for the monosaccharide units were compared with those of corresponding methyl pyranosides in the literature [7]. Glycosylation shifts [7, 8] were observed at C-2 (+7.9 ppm) and C-6 (+7.0 ppm) of the inner glucosyl unit (Glc). Moreover, key correlations were observed between H-1 of the terminal glucosyl unit (Glc′) and C-2 of Glc; H-1 of xylosyl unit (Xyl) and C-6 of Glc; and H-1 of Glc and C-2 of aglycone moiety (Ag) in the HMBC spectrum of **1** (Fig. 1). The structure of **1** was therefore defined as methyl salicylate 2-*O*-β-D-glucopyranosyl-(1 → 2)-[*O*-β-D-xylopyranosyl-(1 → 6)]-*O*-β-D-glucopyranoside (Fig. 2).

Compound **2** was obtained as an amorphous powder, and gave D-glucose and L-arabinose on acidic hydrolysis. The positive FAB–MS of **2** showed an $[M + Na]^+$ ion peak at *m/z* 601. The molecular formula of **2** was determined to be $C_{25}H_{38}O_{15}$ using HR-positive FAB–MS. The ^{1}H-NMR spectrum of **2** indicated signals due to five aromatic protons [δ 7.32 (2H, d, $J = 8.5$ Hz), 7.26 (2H, dd, $J = 8.5$, 8.5 Hz), 7.15 (1H, t, $J = 8.5$ Hz)], one oxygenated methylene group [δ ca. 4.27 (1H), 3.78 (1H, ddd, $J = 7.5$, 8.5, 8.5 Hz)], one methylene group [δ 3.09 (2H, m)], and three anomeric protons [δ 5.29 (1H, d, $J = 8.0$ Hz), 4.86 (1H, d, $J = 7.0$ Hz), 4.82 (1H, d, $J = 8.0$ Hz)]. The ^{13}C-NMR spectrum of **2** showed signals due to one phenyl group [δ 139.5, 129.6 (×2), 128.6 (×2), 126.4], three anomeric carbons (δ 106.4, 105.5, 102.9), and one methylene carbon (δ 36.6). In the same manner as for **1**, these ^{1}H- and ^{13}C-NMR signals were examined in detail and the data suggested that **2** was composed of 1 mol of phenethyl alcohol, 2 mol of glucose, and 1 mol of arabinose. The data also indicated that the glycosidic linkages of the glucose units were β in $^4C_1$ conformations and that of the arabinose unit was α in a $^4C_1$ conformation. Glycosylation shifts [7, 8] in the ^{13}C-NMR data of **2** were observed at C-2 (+9.0 ppm) and C-6 (+6.9 ppm) of Glc. In addition, the HMBC spectrum of **2** showed the key correlations between H-1 of Glc′ and C-2 of Glc; H-1 of arabinosyl unit (Ara) and C-6 of Glc; and H-1 of Glc and C-8 of Ag. Consequently, **2** was elucidated as phenethyl alcohol 8-*O*-β-D-glucopyranosyl-(1 → 2)-[*O*-α-L-arabinopyranosyl-(1 → 6)]-*O*-β-D-glucopyranoside.

Compound **3** was obtained as an amorphous powder. The positive FAB–MS of **3** showed an $[M + Na]^+$ ion peak at *m/z* 587, which was 14 mass units (CH_2) smaller than that of **2**, and the molecular formula of **3** was determined to be $C_{24}H_{36}O_{15}$ using HR-positive FAB–MS. The ^{1}H- and ^{13}C-NMR spectra of **3** were superimposable on those of **2**, except for the appearance of the signals due to a benzyl alcohol unit and the lack of the signals due to a phenethyl alcohol unit. On the basis of these data, **3** was concluded to be benzyl alcohol 7-*O*-β-D-glucopyranosyl-(1 → 2)-[*O*-α-L-arabinopyranosyl-(1 → 6)]-β-D-glucopyranoside.

Compounds **4**–**10** were identified as kaempferol 3-*O*-rutinoside (**4**) [9], rutin (**5**) [9], quercetin 3-*O*-β-apiofuranosyl-(1 → 2)-*O*-[α-rhamnopyranosyl-(1 → 6)]-β-glucopyranoside (**6**) [9], phenethyl alcohol β-gentiobioside (**7**) [10], zizybeoside I (**8**) [11], dihydroconiferyl 4-*O*-β-D-glucopyranoside (**9**) [12], and esculeoside B (**10**) [2], respectively, based on comparison of their physical and spectral data with authentic samples or those already reported.

To gain insight into the biological functions of tomato constituents, the antioxidative properties of MeOH-eluted fractions (using a Diaion HP20 column) obtained from H_2O extracts of 'Komomo' and 'Momotarofight' (*L. esculentum*) cultivars were analyzed. The 'Komomo' fraction revealed ca. threefold lower 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (EC_{50} value of 48.5 μg/ml) than that of the 'Momotarofight' fraction (EC_{50} value of 16.5 μg/ml) (Fig. 3a). In a superoxide anion radical scavenging assay, the activity of the 'Komomo' fraction was ca. 1.4-fold higher (EC_{50} value of 174 μg/ml) than that of the 'Momotarofight' fraction (EC_{50} value of 246 μg/ml) (Fig. 3b). It is conceivable that the antioxidative activities of these tomato fractions may be because of the different compositions and/or amount of active compounds present in

Table 1 ^{1}H-NMR spectral data for **1–3** (in pyridine-d_5, 500 MHz)

	1	**2**	**3**
Ag-2		7.32 d (8.5)	7.72 d (7.5)
Ag-3	7.67 d (8.0)	7.26 dd (8.5, 8.5)	7.38 dd (7.5, 7.5)
Ag-4	7.46 ddd (1.5, 7.5, 8.0)	7.15 t (8.5)	7.22 t (7.5)
Ag-5	6.88 dd (7.5, 7.5)	7.26 dd (8.5, 8.5)	7.38 dd (7.5, 7.5)
Ag-6	7.84 dd (1.5, 7.5)	7.32 d (8.5)	7.72 d (7.5)
Ag-7		3.09 m	5.18 d (12.0)
Ag-7		3.09 m	4.81 d (12.0)
Ag-8		ca. 4.27	
Ag-8		3.78 ddd (7.0, 8.5, 8.5)	
OCH_3	3.90 s		
Glc-1	5.62 d (7.5)	4.82 d (8.0)	4.94 d (7.5)
Glc-2	4.47 dd (7.5, 9.0)	4.06 dd (8.0, 9.5)	4.15 dd (7.5, 9.0)
Glc-3	4.35 dd (9.0, 9.0)	ca. 4.25	4.21 dd (9.0, 9.0)
Glc-4	4.14 dd (9.0, 9.0)	ca. 4.10	4.12 dd (9.0, 9.0)
Glc-5	ca. 4.21	3.96 ddd (2.0, 5.5, 9.5)	4.00 ddd (2.5, 6.0, 9.0)
Glc-6	4.75 dd (1.5, 11.5)	4.77 dd (2.0, 11.0)	4.80 dd (2.5, 11.0)
Glc-6	ca. 4.25	ca. 4.23	4.26 dd (6.0, 11.0)
Glc′-1	5.56 d (8.0)	5.29 d (8.0)	5.31 d (7.5)
Glc′-2	4.08 dd (8.0, 9.0)	ca. 4.10	4.10 dd (7.5, 9.0)
Glc′-3	ca. 4.26	ca. 4.25	4.26 dd (9.0, 9.0)
Glc′-4	4.30 dd (8.0, 8.0)	ca. 4.29	ca. 4.30
Glc′-5	3.99 m	ca. 3.95	3.91 ddd (3.0, 4.5, 9.0)
Glc′-6	4.34 dd (4.5, 11.5)	4.53 dd (2.5, 11.5)	4.48 dd (3.0, 11.5)
Glc′-6	ca. 4.24	4.39 dd (5.0, 11.5)	4.41 dd (4.5, 11.5)
Xyl-1	4.90 d (7.5)		
Xyl-2	4.00 dd (7.5, 9.0)		
Xyl-3	4.08 dd (9.0, 9.0)		
Xyl-4	ca. 4.19		
Xyl-5	ca. 4.25		
Xyl-5	3.56 dd (11.0, 11.0)		
Ara-1		4.86 d (7.0)	4.91 d (7.0)
Ara-2		4.44 dd (7.0, 8.5)	4.48 dd (7.0, 8.5)
Ara-3		4.12 dd (3.0, 8.5)	4.15 dd (3.5, 8.5)
Ara-4		ca. 4.29	ca. 4.31
Ara-5		ca. 4.28	ca. 4.30
Ara-5		3.72 dd (3.0, 13.0)	3.74 dd (2.5, 13.0)

δ in ppm from TMS (coupling constants (J) in Hz are given in parentheses)

Glc glucopyranosyl, *Xyl* xylopyranosyl, *Ara* arabinopyranosyl, *Ag* aglycone moiety

the fruits of both cultivars. Investigations into the biological functions of the isolated compounds from the fruit of 'Komomo' and constituents from the fruit of 'Momotarofight' are now in progress.

Experimental

General

Optical rotations were performed with a JASCO DIP-1000 KYU digital polarimeter (JASCO, Tokyo). MS were recorded on a JEOL JMS-700T (JEOL, Tokyo). ^{1}H- and ^{13}C-NMR spectra were recorded with JEOL ECA 500 spectrometer (JEOL), and chemical shifts are given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. Silica gel 60 (Merck, Art. 9385; Merck, Darmstadt, Germany), Sephadex LH-20 (Pharmacia Fine Chemicals, Uppsala, Sweden), Chromatorex ODS (Fuji Silysia Chemical, Ltd., Aichi), Chromatorex NH_2 (Fuji Silysia Chemical, Ltd.), and Diaion HP20 (Mitsubishi Chemical Industries Co., Ltd., Tokyo) were used for column chromatography. HPLC separation was run on a Shimadzu LC-10AS micro pump (Shimadzu, Kyoto) with Shimadzu RID-10A RI-detector (Shimadzu). For HPLC column chromatography, COSMOSIL 5C18-AR-II

Table 2 ^{13}C-NMR spectral data for **1–3** (in pyridine-d_5, 125 MHz)

	1	2	3		1	2	3
Ag-1	121.0	139.5	138.9	Glc′-1	105.7	106.4	106.6
Ag-2	157.4	129.6	128.1	Glc′-2	76.8	76.7	76.8
Ag-3	116.4	128.6	128.6	Glc′-3	78.1	78.7	78.6
Ag-4	134.1	126.4	127.6	Glc′-4	71.2	71.4	71.4
Ag-5	121.5	128.6	128.6	Glc′-5	78.3	78.2	78.1
Ag-6	131.6	129.6	128.1	Glc′-6	62.3	62.8	62.6
Ag-7	166.3	36.6	70.9	Xyl-1	105.9		
Ag-8		70.8		Xyl-2	75.0		
OCH_3	52.0			Xyl-3	78.1		
Glc-1	100.0	102.9	102.2	Xyl-4	70.8		
Glc-2	82.7	83.8	84.1	Xyl-5	67.1		
Glc-3	77.8	77.9	78.0	Ara-1		105.5	105.7
Glc-4	71.1	71.6	71.5	Ara-2		72.3	72.4
Glc-5	77.5	76.8	76.9	Ara-3		74.4	74.4
Glc-6	69.5	69.4	69.5	Ara-4		69.2	69.3
				Ara-5		66.6	66.7

δ in ppm from TMS

Glc glucopyranosyl, *Xyl* xylopyranosyl, *Ara* arabinopyranosyl, *Ag* aglycone moiety

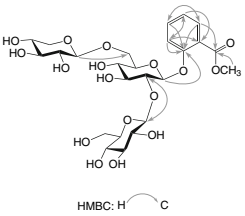

Fig. 1 ^{1}H–^{13}C long-range correlations observed for **1** in the HMBC spectrum (in pyridine-d_5, 500 MHz)

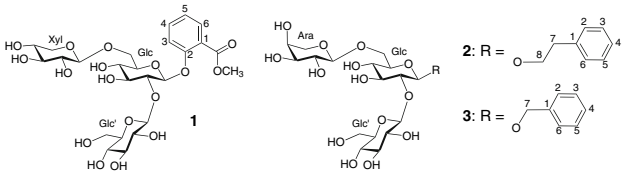

Fig. 2 Structures of **1–3**

(Nacalai Tesque Inc., Kyoto, 20-mm i.d. × 250 mm) was used.

Plant material

The ripe fruits of cherry tomato ('Komomo') were collected in October 2008 at the farm of Tokai University, Kumamoto prefecture, Japan.

Extraction and isolation

The ripe fruits (17836 g) of cherry tomato were smashed with H_2O (26650 ml) in a mixer then filtered through filter

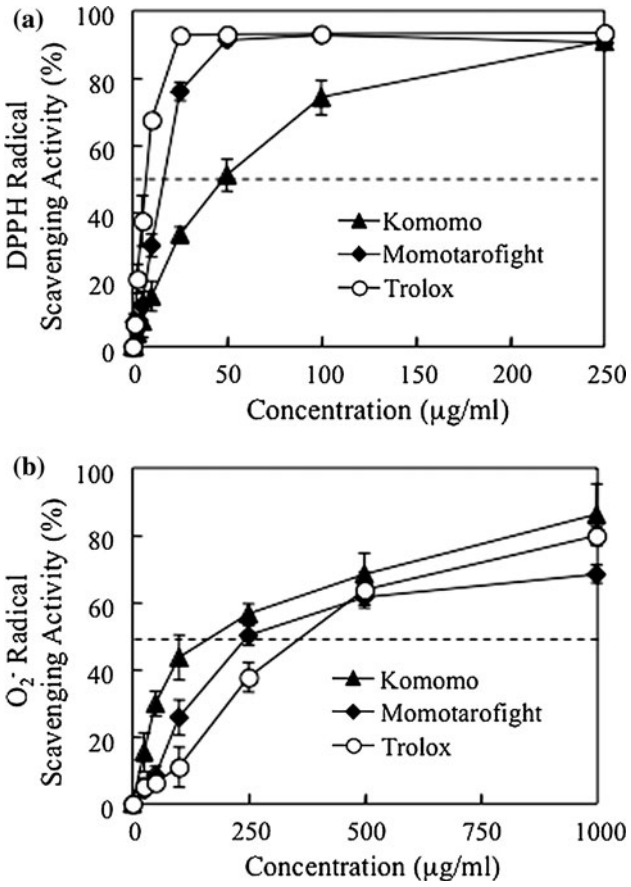

Fig. 3 Antioxidative activities of two different tomato fractions from 'Komomo' and 'Momotarofight' cultivars on the DPPH radical (**a**) and superoxide anion radical scavenging analyses (**b**)

paper to give a filtrate, which was subjected to Diaion HP20 column chromatography (H_2O, MeOH, acetone) to afford the MeOH-eluted fraction (fr.) and acetone-eluted fr. The MeOH-eluted fr. (17.9 g) was chromatographed over a Sephadex LH-20 column (90% MeOH, MeOH) to give frs. 1–4. Chromatography of fr. 1 (16.8 g) over a Sephadex LH-20 column (30% MeOH, MeOH) furnished frs. 1.1–1.4. Fraction 1.3 (1.1 g) was chromatographed over a Chromatorex NH_2 column [$CHCl_3$–MeOH–H_2O (8:2:0.1, 7:3:0.5, 6:4:1, 5:5:1, 3:6:1), 100% MeOH, 90% MeOH, 80% MeOH, 70% MeOH, 60% MeOH, 50% MeOH, 40% MeOH, 30% MeOH, 20% MeOH, H_2O] to give frs. 1.3.1–1.3.19. Chromatography of fr. 1.4 (9.1 g) over a Chromatorex NH_2 column under the same conditions to those of fr. 1.3 furnished frs. 1.4.1–1.4.20. Fractions 1.3.5 (130 mg), 1.3.8 (419 mg), 1.4.5 (268 mg), 1.4.7 (356 mg), and 1.4.11 (258 mg) were each subjected to HPLC (30% MeOH) to afford **7** (7 mg) and **8** (3 mg) from fr. 1.3.5, **10** (32 mg) from fr. 1.3.8, **9** (7 mg) from fr. 1.4.5, **7** (17 mg) from fr. 1.4.7, and **1** (6 mg), **2** (8 mg), and **3** (3 mg) from fr. 1.4.11. Fraction 2 (0.8 g) was chromatographed over a Chromatorex ODS column (40% MeOH, 50% MeOH, 60%

MeOH, 70% MeOH, 80% MeOH, 90% MeOH, 100% MeOH) to furnish frs. 2.1–2.11 and **5** (11 mg). Chromatography of fr. 2.6 (89 mg) over a Diaion HP20 column (H_2O, MeOH, acetone) furnished **6** (9 mg), fr. 2.6.1 (58 mg), and fr. 2.6.2 (11 mg). Fraction 2.10 (49 mg) was subjected to silica gel column chromatography [$CHCl_3$–MeOH–H_2O (10:2:0.1, 8:2:0.2, 7:3:0.5, 6:4:1, 0:1:0)] to afford **4** (9 mg), fr. 2.10.1 (20 mg), fr. 2.10.2 (7 mg), fr. 2.10.3 (6 mg), and fr. 2.10.4 (13 mg).

1: Amorphous powder. $[\alpha]_D^{11}$ −64.1° (*c* 0.7, MeOH). Positive FAB–MS *m/z*: 631 $[M + Na]^+$. HR-positive FAB–MS *m/z*: 631.1855 (calcd for $C_{25}H_{36}O_{17}Na$: 631.1850). ^{1}H-NMR spectral data: see Table 1. ^{13}C-NMR spectral data: see Table 2.

2: Amorphous powder. $[\alpha]_D^{11}$ −23.3° (*c* 0.9, MeOH). Positive FAB–MS *m/z*: 601 $[M + Na]^+$. HR-positive FAB–MS *m/z*: 601.2133 (calcd for $C_{25}H_{38}O_{15}Na$: 601.2108). ^{1}H-NMR spectral data: see Table 1. ^{13}C-NMR spectral data: see Table 2.

3: Amorphous powder. $[\alpha]_D^{11}$ −22.6° (*c* 0.3, MeOH). Positive FAB–MS *m/z*: 587 $[M + Na]^+$. HR-positive FAB–MS *m/z*: 587.1962 (calcd for $C_{24}H_{36}O_{15}Na$: 587.1952). ^{1}H-NMR spectral data: see Table 1. ^{13}C-NMR spectral data: see Table 2.

Sugar analysis

Compounds **1** (1 mg) and **2** (1 mg) were each heated in 2 M HCl–dioxane (2:1, 1.5 ml) at 95°C for 1 h. The reaction mixture was extracted with AcOEt (2 ml). The aqueous layer was neutralized with Amberlite MB-3 (Organo Co., Tokyo) and then evaporated under reduced pressure to give a monosaccharide fr. This fr. was analyzed by HPLC under the following conditions: column, Shodex RS-Pac DC-613 (Showa Denko, Tokyo, 150 mm × 6.0 mm); solvent, CH_3CN–H_2O (3:1); flow rate, 1.0 ml/min; column temperature, 70°C; detector, JASCO OR-2090 plus (JASCO); pump, JASCO PU-2080 plus (JASCO); and column oven, JASCO CO-2060 plus (JASCO). The retention time (t_R) and optical activity of each of the monosaccharides were as follows. D-Xylose [t_R (min) 5.5; optical activity, positive] and D-glucose [t_R (min) 7.2; optical activity, positive] for **1**; L-arabinose [t_R (min) 5.8; optical activity, positive] and D-glucose [t_R (min) 7.1; optical activity, positive] for **2**.

Assay of antioxidative effects

The ripe fruits of pink color-type tomato ('Momotarofight') were also collected in October 2008 at the farm of Tokai University, Kumamoto prefecture, Japan. The fraction of 'Momotarofight' eluted from the Diaion HP20 column was prepared by a similar method to that used for 'Komomo'. The DPPH radical scavenging effect was measured based on the following method [13]. Briefly, the reaction was started by the addition of DPPH into the assay mixture containing varying concentrations of test samples, then allowed to proceed for 30 min at room temperature. The absorbance of the resulting solution was measured at 517 nm. The superoxide anion (O_2^-) radical scavenging effect was measured by using the phenazine methosulfate (PMS)–NADH system according to previously described methods [14, 15]. Briefly, the reaction was started by the addition of NADH into the assay mixture containing nitro blue tetrazolium (NBT) plus varying concentrations of test samples, then allowed to proceed for 10 min at room temperature. The absorbance of the resulting solution was measured at 570 nm. In both cases, Trolox was used as a standard sample. Data shown represent mean ± SD derived from four determinations.

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