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Research article

Isolation of rugosin A, B and related compounds as dipeptidyl peptidase-IV inhibitors from rose bud extract powder

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Dipeptidyl peptidase-IV (DPP-IV) is a protease responsible for the degradation of the incretin hormone. A number of DPP-IV inhibitors have been approved for use in the treatment of type 2 diabetes. While these inhibitors are effective for this treatment, methods for the prevention of this disease are also required as diabetes patient numbers are currently increasing rapidly worldwide. We screened the DPP-IV inhibitory activities of edible plant extracts with the intention of using these extracts in a functional food supplement for the prevention of diabetes. Rose (*Rosa gallica*) bud extract powder was a promising material with high inhibitory activity. In this study, seven ellagitannins were isolated as active compounds through activity guided fractionations, and their DPP-IV inhibitory activities were measured. Among them, rugosin A and B showed the highest inhibitory activities and rugosin B was shown as the major contributing compound in rose bud extract powder.

Key words:

dipeptidyl peptidase IV; diabetes; ellagitannin; *Rosa gallica*

Dipeptidyl peptidase-IV (DPP-IV) is a protease responsible for the degradation of incretin hormones such as glucagon-like peptide 1 (GLP-1).[1,2] GLP-1 is a gastrointestinal hormone secreted by enteroendocrine L-cells in response to meals. It acts on pancreatic cells to inhibit the action of glucagon and stimulate insulin secretion. The actions of GLP-1 reduce the level of blood glucose which is rapidly elevated after consuming a meal.

Although the action of DPP-IV is a normal bodily response to stop the overworking of GLP-1, its inhibition is currently used as a method to treat type 2 diabetes mellitus (T2DM) patients. Inhibiting DPP-IV protects GLP-1 from degradation, which enhances the secretion of insulin to help our body decrease the blood glucose level. Currently, DPP-IV inhibiting anti-diabetes medicines such as Sitagliptin, Alogliptin, Linagliptin and more have been developed and are used to treat T2DM patients.[1]

T2DM is an enormous problem with more than 300 million patients estimated in the world. Although a variety of anti-diabetes medicine, including DPP-IV inhibitors have been developed and succeed in maintaining patients' blood glucose levels to prevent associated diseases like diabetic neuropathy, the numbers of T2DM patients are consistently increasing every year. Therefore, developing a method to prevent this disorder is still necessary.

Consuming a functional food supplement could be an effective method for preventing lifestyle-dependent diseases including diabetes. We screened edible plant extracts for DPP-IV inhibitory activity, with the aim of using these extracts in a T2DM preventing food supplement, and found rose (*Rosa gallica*) bud extract powder as a promising material. Here, we report the isolation, structure elucidation, and activities of DPP-IV inhibitors from rose bud extract powder.

Results and discussion

The isolation of DPP-IV inhibitors was accomplished by activity guided fractionations. Commercial rose bud extract powder (Maruzen Pharmaceuticals Co., Ltd.) was dissolved in water, partitioned between ethyl acetate, and then between 1-butanol. The ethyl acetate soluble part was

subjected to silica-gel column chromatography and the active fraction was then fractionated using reverse phase column chromatography. The obtained active fractions were finally purified by high performance liquid chromatography (HPLC) to obtain compounds **1–7** (see experimental section for details).

The structures of the isolated compounds were determined by nuclear magnetic resonance (NMR) and mass spectrometry (MS) analysis. The ^{1}H-NMR spectrum of all the isolated compounds indicated that they were a type of ellagitannin. Compounds **1–3**, **5**, and **6** were compared with reported data and determined as tellimagrandin I (**1**),[3] rugosin B (**2**),[4] heterophyllin A (**3**),[5] eugeniin (**5**),[6,7] and rugosin A (**6**) (Figure 1).[4] Compounds **4** and **7** did not match with reported data and were further analyzed in detail.

The ^{1}H-NMR spectrum of compound **4** was quite similar to that of rugosin B (**2**) but had an additional signal at 1.20 ppm (3H, triplet) and 4.18 ppm (2H, quartet) representing the presence of an ethoxy group (Table 1). Heteronuclear multiple bond correlation (HMBC) analysis showed a correlation between 4.18 ppm (CH_2 of ethyl) and 167.57 ppm (carbonyl carbon) (Figure 2, Table 2) indicating that this ethoxy group formed an ester bond with the carboxyl group of rugosin B (**2**). MS analysis detected a peak at *m*/*z* = 981, assisting the NMR analysis and compound **4** was determined as rugosin B ethyl ester (**4**).

The ^{1}H-NMR spectrum of compound **7** was similar to that of rugosin A (**6**) but the signals corresponding to the valoneoyl group were different (Table 1). The ^{13}C-NMR spectrum showed five ester carbonyl carbon signals (Table 2), of which one is missing compared with rugosin A (**6**). HRMS analysis detected a peak with *m*/*z* 1061.1132 ($C_{47}H_{33}O_{29}$ calculated *m*/*z* 1061.1108), a molecular formula by subtracting CO_2 from that of rugosin A (**6**), indicating the decarboxylation of the gallic acid moiety. From these and using other NMR techniques, the compound was determined to be decarboxylated rugosin A (**7**). The position of the ring F was also confirmed from the heteronuclear multiple bond correlation (HMBC) and correlation in rotating frame nuclear Overhauser effect spectroscopy (ROESY). The ^{1}H-NMR signal of ring E H5’ was determined from HMBC between

3.81/5.05 (Glucose H6) and 167.33 ppm (carbonyl group of ring E), and between 6.11 ppm (ring E H5') and 167.33 ppm (see supplementary material). Correlation between ring H6'' (6.37 ppm) and ring E H5' (6.11 ppm) in ROESY spectrum confirmed the position of ring F (Figure 2 and supplementary material).

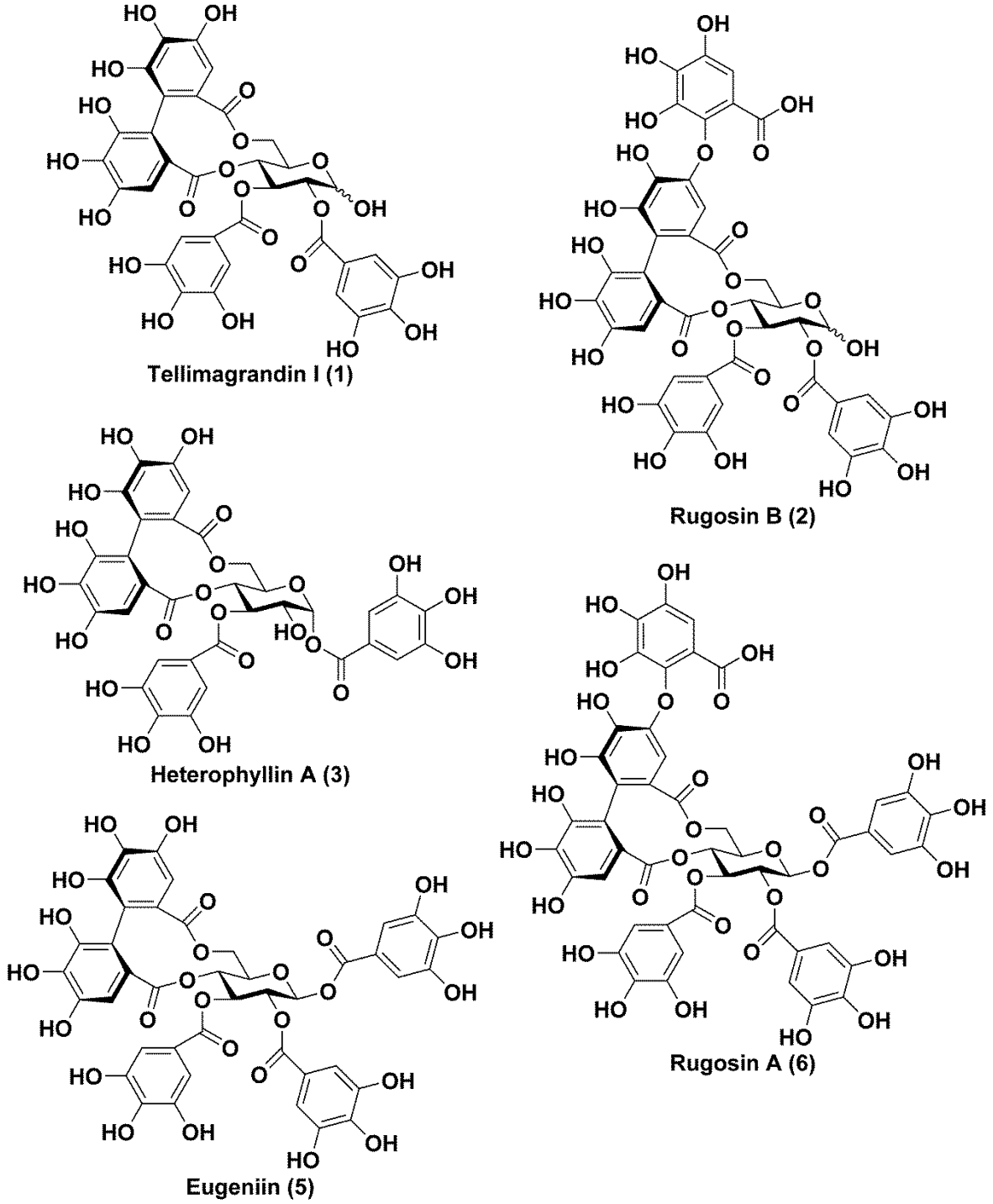

Figure 1. Structures of the isolated compounds.

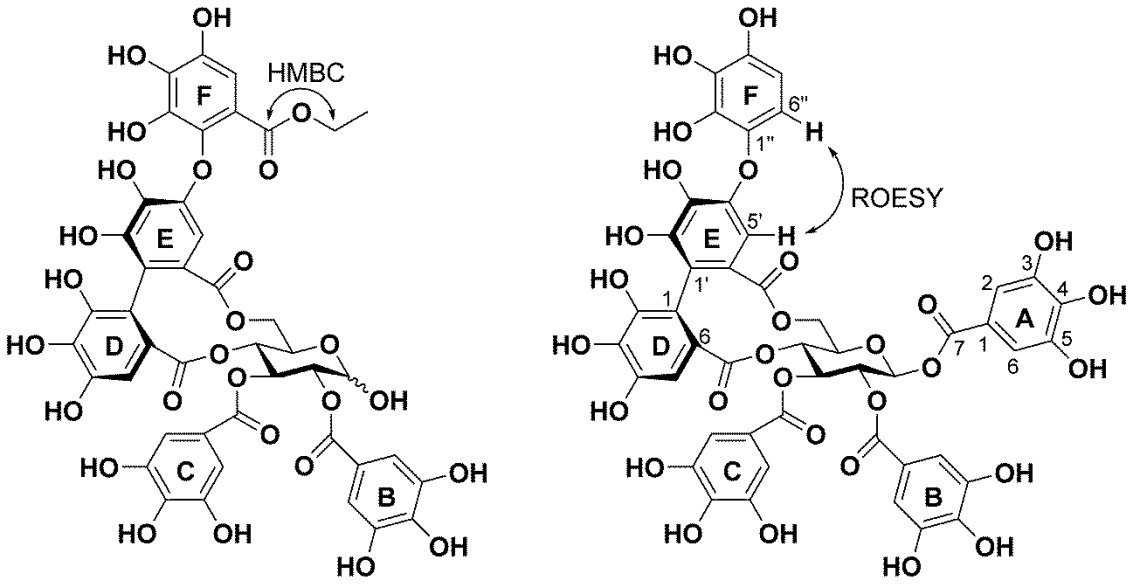

Figure 2. Structure and HMBC/ROESY correlation of compounds **4** and **7**.

Table 1. ^{1}H-NMR Spectrum data of **4** and **7** (500 MHz, rt)

Compound		Rugosin B ethyl ester (**4**)				Decarboxylated Rugosin A (**7**)	
Solvent		CD_3OD				DMSO-d_6	
		alpha		beta			
		ppm	multiplet, Hz	ppm	multiplet, Hz	ppm	multiplet, Hz
Ethyl	CH_3	1.2	t, 7.1	1.2	t, 7.1	-	-
	CH_2	4.14-4.20	m	4.14-4.20	m	-	-
Glucose	1	5.45	d, 3.7	4.93	m	6.17	d, 8.5
	2	5.09	dd, 3.7, 10.1	5.19	dd, 8.7, 9.3	5.38	dd, 8.5, 9.2
	3	5.80	t, 10.1	5.56	dd, 9.3, 10.0	5.77	dd, 9.2, 9.7
	4	5.06	dd, 10.0, 10.1	5.08	dd, 10.0, 10.1	4.94	t, 9.7
	5	4.59	dd, 6.9, 10.0	4.14-4.20	m	4.61	dd, 6.3, 9.7
	6a	3.75	br d, 12.0	3.82	d, 12.9	3.81	d, 13.1
	6b	5.21	dd, 6.9, 12.0	5.24	dd, 6.7, 12.9	5.05	dd, 6.3, 13.1
Galloyl A	2,6	-	-	-	-	6.90	s
Galloyl B	2,6	7.02	s	7.00	s	6.81	s
Galloyl C	2,6	6.93	s	6.89	s	6.79	s
Valoneoyl ring D	5	6.50	s	6.46	s	6.27	s
Valoneoyl ring E	5’	6.17	s	6.16	s	6.11	s
Valoneoyl ring F	5"	7.05	s	7.05	s	6.28	d, 9.0
	6"	-	-	-	-	6.37	d, 9.0

Table 2. ^{13}C-NMR Spectrum data of **4** and **7** (125 MHz, rt)

Compound		Rugosin B ethyl ester (**4**)		Rugosin A decarboxylated (**7**)
Solvent		CD_3OD		DMSO-d_6
		alpha	beta	
Ethyl	CH_3	14.40	14.44	-
	CH_2	62.21	62.21	-
Glucose	1	91.74	97.05	92.08
	2	73.47	74.74	70.62
	3	71.99[a]	74.37	71.82
	4	72.05[a]	71.70	69.71
	5	67.51	72.73	71.30
	6	64.31	64.22	62.01
Galloyl A	1	-	-	117.40
	2,6	-	-	109.06
	3,5	-	-	145.64
	4	-	-	139.60
	7	-	-	164.01
Galloyl B	1	120.61	120.94	117.98
	2,6	110.41	110.37	108.79
	3,5	146.41	146.39	145.48
	4	140.18	139.96	139.11
	7	167.42	167.12	164.43
Galloyl C	1	120.88	120.67	118.23
	2,6	110.49	110.52	108.95
	3,5	146.23	146.20	145.28
	4	139.96	139.96	138.91
	7	167.96	167.73	165.19
Valoneoyl ring D	1	116.33	116.33	115.10
	2	145.07[b]	145.07[b]	145.02
	3	137.61	137.61	135.56
	4	145.99	145.99	144.36
	5	108.11	108.11	105.33
	6	125.86[c]	125.78[c]	123.40
	7	169.15	169.07	166.93
Valoneoyl ring E	1'	118.57	118.57	117.54
	2'	145.40[b]	145.40[b]	145.42
	3'	138.21	138.21	136.84
	4'	147.66	147.71	145.76
	5'	105.89	105.89	104.67
	6'	125.91[c]	125.86[c]	123.87
	7'	169.45	169.35	167.33
Valoneoyl ring F	1"	137.28	137.28	136.20
	2"	140.76	140.76	138.86
	3"	140.83	140.83	134.53
	4"	143.91	143.91	143.24
	5"	109.91	109.91	105.79
	6"	115.73	115.73	111.55
	7"	167.49	167.49	-

a–c: Values with the same superscript may be interchanged.

The DPP-IV inhibitory activities of the isolated compounds at 10 and 100 μM are shown in Figure 3. Rugosin A (**6**) and B (**2**) exhibited the highest inhibitory activity among the tested compounds and IC_{50} values were also determined as 28.5 μM for **2**, and 25.8 μM for **6**. Plant derived inhibitors of DPP-IV are reported in several studies. [8–11] Polyphenolic compounds are most commonly explored compound and inhibitory activity ranges from few to several tens of μM. As an example of potent inhibitors among them, IC_{50} value of cyanidin 3,5-diglucoside isolated from aronia juice is reported to be 5.5 μM,[9] and (-)-vitisin B isolated from small-leaf grape (*Vitis thunbergii* var. *taiwaniana*) is reported to be 15.3 μM.[8] Thus, the activity of rugosin A (**6**) and B (**2**) are slightly milder compared to those potent inhibitors.

Compounds **2**, **4**, **6**, and **7** had higher activities than **1**, **3**, and **5**. The difference between these two groups of ellagitannins was the structure of condensed gallic acid esterified to the 4-OH and 6-OH of glucose. The former group had a valoneoyl group or modified valoneoyl group where three gallic acids were connected to each other, and the latter group had a hexahydroxydiphenoyl (HHDP) group formed from two gallic acids. Thus, the additional gallic acid moiety in the valoneoyl group could be an important part contributing towards the DPP-IV inhibitory activity.[11] The importance of the additional gallic acid in the valoneoyl group was also confirmed from the change in activities between compounds **2** and **4**, or **6** and **7**. Compound **4** is an ester derivative of **2** and compound **7** is a decarboxylated derivative of **6**. Both **4** and **7** had lower activities compared with their original structures, indicating the importance of the acid formed carboxyl moiety of the valoneoyl group.

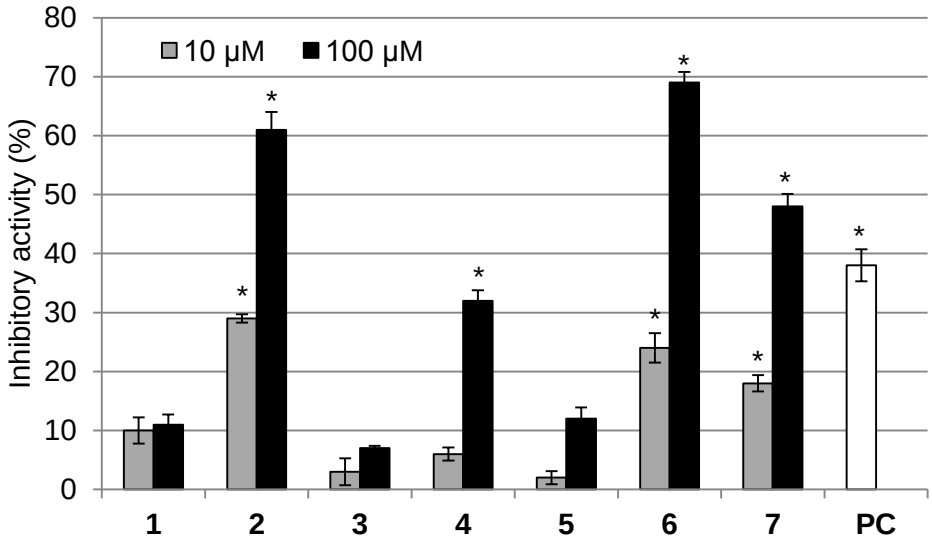

Figure 3. DPP-IV inhibitory activities of the isolated compounds.

PC: positive control, 25 μM diprotin. * $p < 0.05$ vs. control

Ellagitannins are the major polyphenol contained in roses.[12–15] Few studies are reporting about the constituents of *R. gallica* and among the ellagitannins, tellimagrandin I (**1**) and rugosin A (**6**) has been previously isolated from *R. gallica* as inhibitors of digestive enzymes.[16,17] Rugosin B ethyl ester (**4**) and decarboxylated rugosin A (**7**) are new derivatives that have not been reported to date. We assumed that rugosin B ethyl ester (**4**) was a product formed during the processing of rose bud extract powder from *R. gallica* flower buds. According to the manufacturer's information, the extraction of *R. gallica* buds uses aqueous ethanol and therefore, ethyl ester could be formed during this step. Decarboxylated rugosin A (**7**) may also be an artificial product formed during the processing, because the decarboxylation of the valoneoyl group can be accomplished by relatively mild conditions in the case of mallotinic acid; an ellagitannin with valoneoyl moiety esterified through the 3-OH and 6-OH groups of glucose.[18] However, we tried replicating the same conditions against rugosin A (**6**) but failed to identify **7** in the reaction mixture (Data not shown).

Considering the isolation yield, rugosin A (**6**) and rugosin B (**2**) are the major contributors of DPP-IV inhibitory activities in rose bud extract powder. To confirm this, we quantitated the amount of these two compounds in the rose bud extract powder by the UPLC-MS method. The quantitated

amount of each compounds contained in 1 g of powder was rugosin A (**6**) at 13.7±0.3 μmol and rugosin B (**2**) at 39.7±1.2 μmol, Compound **2** was the highest compound contributing inhibitory activity in the powder. As the rose bud extract powder showed a 44% inhibition at 1 g/L, the combined amounts of these two compounds can explain the inhibitory activity of the extract powder.

In conclusion, rose bud extract powder is a promising candidate material for the development of a functional food for the prevention of type 2 diabetes. The isolation of active compounds from this material yielded seven ellagitannins (**1–7**). Among them, a compound with a valoneoyl moiety demonstrated higher activity than the others, and rugosin B (**2**) was the major contributor of DPP-IV inhibitory activity of this material.

Materials and methods

General

Commercially available chemicals were purchased from Wako Pure Chemical Industries, Ltd. unless otherwise noted. Fluorescence was measured using a Synergy™ MX microplate reader (BioTek Instruments, Inc.). A Bruker AMX 500 (Bruker BioSpin K.K.) or Jeol EX270 (Jeol Ltd.) were used to obtain NMR spectra and residual solvents were used as an internal standard (methanol-d_4: ^{1}H 3.31 ppm, ^{13}C 49.0 ppm; acetone-d_6: ^{1}H 2.04 ppm, ^{13}C 29.8 ppm; DMSO-d_6: ^{1}H 2.49 ppm, ^{13}C 39.5 ppm). A Waters LCT Premier Spectrometer (Waters Co.) was used to obtain mass spectra and was combined with the Waters Acquity UPLC system (Waters Co.) for UPLC-MS analysis.

Rose bud extract powder

A commercial product of Maruzen Pharmaceuticals Co., Ltd. was purchased and used (Lot No. 50717012 and 60808013). The powder was produced from the extract of *Rosa gallica* flower buds.

Isolation of DPP-IV inhibitory compounds from rose bud extract powder

Rose bud extract powder (50 g) was dissolved in water and an aqueous solution was extracted with

ethyl acetate and then with 1-butanol to give water soluble (38.88 g), 1-butanol soluble (7.98 g) and ethyl acetate soluble (4.39 g) fractions. The ethyl acetate soluble fraction (2.10 g) was separated using silica gel column chromatography by stepwise elution with chloroform/methanol (10/1 → 5/1 → 2/1 →0/1). The fraction eluted by chloroform/methanol = 2/1 was then separated using Cosmosil® 75C18-OPN column chromatography by stepwise elution with 10% aq. methanol (Fr. 1–2), 20% aq. methanol (Fr. 3–4), 30% aq. methanol (Fr. 5–6), and 50% aq. methanol (Fr. 7–8). The active fraction (Fr. 3 and 5) was further purified by HPLC. Fr. 3 was separated using Inertsil ODS-3 (GL Science Co., φ20×250 mm) with 0.1% formic acid, and 30% aq. methanol as an eluent to obtain tellimagrandin I (**1**, 40.8 mg), rugosin B (**2**, 38.1 mg), and heterophyllin A (**3**, 17.4 mg). Fr. 5 was separated using InertSustain® C18 (GL Science Co., φ20×250 mm) with 0.1% formic acid and 35% aq. methanol as an eluent to obtain rugosin B ethyl ester (**4**, 19.0 mg), eugeniin (**5**, 13.4 mg), rugosin A (**6**, 57.5 mg) and decarboxylated rugosin A (**7**, 15.6 mg). Using the ^{1}H NMR spectrum obtained, the data of each compound were matched with reported data for structure determination. The ^{1}H NMR spectrum data in CD_3OD is also provided as supplementary data.

Tellimagrandin I (**1**, mixture of α,β-anomer)

^{1}H-NMR (500 MHz, acetone-d_6, rt):

α-anomer: 3.75 (1H, d, *J* = 12.5 Hz), 4.67 (1H, dd, *J* = 6.5, 10.0 Hz), 5.10 (1H, dd, *J* = 3.8, 10.1 Hz), 5.15 (1H, dd, *J* = 9.8, 10.0 Hz), 5.32 (1H, dd, *J* = 6.5, 12.5 Hz), 5.56 (1H, d, *J* = 3.8 Hz), 5.89 (1H, dd, *J* = 9.8, 10.1 Hz), 6.41 (1H, s), 6.59 (1H, s), 6.97 (2H, s), 7.06 (2H, s) ppm

β-anomer: 3.83 (1H, d, *J* = 12.9 Hz), 4.27 (1H, dd, *J* = 6.6, 10.2 Hz), 5.09 (1H, dd, *J* = 10.0, 10.2 Hz), 5.15 (1H, dd, *J* = 8.7, 9.9 Hz), 5.24 (1H, d, *J* = 8.7 Hz), 5.34 (1H, dd, *J* = 6.6, 12.9 Hz), 5.62 (1H, dd, *J* = 9.9, 10.0 Hz), 6.38 (1H, s), 6.60 (1H, s), 6.92 (2H, s), 7.05 (2H, s) ppm

ESI-MS (negative) *m*/*z* 785 $[M-H]^-$

Rugosin B (**2**, mixture of α,β-anomer)

^{1}H-NMR (270 MHz, acetone-d_6, rt):

α-anomer: 3.67 (1H, d, *J* = 12.9 Hz), 4.62 (1H, dd, *J* = 6.6, 9.8 Hz), 5.05 (1H, dd, *J* = 9.8, 10.1 Hz), 5.10 (1H, dd, *J* = 3.8, 10.0 Hz), 5.19-5.24 (1H, m), 5.50 (1H, d, *J* = 3.8 Hz), 5.85 (1H, dd, *J* = 10.0, 10.1 Hz), 6.35 (1H, s), 6.47 (1H, s), 6.98 (2H, s), 7.05 (2H, s), 7.16 (1H, s) ppm

β-anomer: 3.75 (1H, d, *J* = 13.2 Hz), 4.24 (1H, dd, *J* = 6.6, 10.1 Hz), 5.03-5.11 (2H, m), 5.19-5.24 (2H, m), 5.59 (1H, t, *J* = 9.9 Hz), 6.35 (1H, s), 6.44 (1H, s), 6.94 (2H, s), 7.05 (2H, s), 7.16 (1H, s) ppm

ESI-MS (negative) *m*/*z* 953 $[M-H]^-$

Heterophyllin A (**3**)

^{1}H-NMR (270 MHz, acetone-d_6 + D_2O, rt): 3.76 (1H, d, *J* = 13.4 Hz), 4.22 (1H, dd, *J* = 4.0, 9.9 Hz), 4.56 (1H, dd, *J* = 6.2, 10.1 Hz), 5.06 (1H, dd, *J* = 9.9, 10.1 Hz), 5.24 (1H, dd, *J* = 6.2, 13.4 Hz), 5.65 (1H, t, *J* = 9.9 Hz Hz), 6.41 (1H, d, *J* = 4.0 Hz), 6.49 (1H, s), 6.62 (1H, s), 7.04 (2H, s), 7.24 (2H, s);

ESI-MS (negative): *m*/*z* = 785 $[M-H]^-$

Rugosin B ethyl ester (**4**, 2:1 mixture of α,β-anomer)

See Table 1 and Table 2 for NMR data.

IR (neat) ν: 1038, 1096, 1221, 1338, 1457, 1507, 1540, 1616, 1646, 1653, 1716, 3397 cm^{-1}; $[\alpha]_D^{25}$ +1306.8° (*c* 0.100, CH_3OH); HR-ESI-MS (negative): $[M-H]^-$, found *m*/*z* 981.1222, $C_{43}H_{34}O_{27}^-$ requires *m*/*z* 981.1215.

Eugeniin (**5**)

^{1}H-NMR (500 MHz, acetone-d_6, rt): 3.87 (1H, d, *J* = 13.2 Hz), 4.54 (1H, dd, *J* = 6.6, 9.9 Hz), 5.23 (1H, t, *J* = 9.9 Hz Hz), 5.39 (1H, dd, *J* = 6.6, 13.2 Hz), 5.58 (1H, dd, *J* = 8.3, 9.9 Hz), 5.84 (1H, t, *J* = 9.9 Hz), 6.20 (1H, d, *J* = 8.3 Hz), 6.44 (1H, s), 6.63 (1H,s), 6.96 (2H, s), 7.00 (2H, s), 7.11 (2H, s);

ESI-MS (negative): *m*/*z* = 937 $[M-H]^-$

Rugosin A (**6**)

^{1}H-NMR (500 MHz, acetone-d_6, rt): 3.80 (1H, d, J = 13.8 Hz), 4.48 (1H, dd, J = 6.1, 10.2 Hz), 5.17 (1H, dd, J = 10.1, 10.2 Hz), 5.31 (1H, dd, J = 6.1, 13.8 Hz), 5.58 (1H, dd, J = 8.2, 10.2 Hz), 5.85 (1H, d, J = 10.1, 10.2 Hz), 6.18 (1H, d, J = 8.2 Hz), 6.37 (1H, s), 6.50 (1H, s), 6.99 (2H, s), 7.01 (2H, s), 7.11 (2H, s), 7.16 (1H, s); ESI-MS (negative): m/z = 1105 $[M-H]^-$

Decarboxylated rugosin A (**7**).

See Table 1 and Table 2 for NMR data.

IR (neat) ν: 1032, 1065, 1202, 1349, 1448, 1493, 1508, 1613, 1719, 3388 cm^{-1}; $[\alpha]_D^{25}$ +474.3° (c 0.100, CH_3OH); HR-ESI-MS (negative): $[M-H]^-$, found m/z 1061.1132, $C_{47}H_{33}O_{29}^-$ requires m/z 1061.1108.

DPP-IV inhibitory activity assay

DPP-IV inhibitory activity was measured using the DPP (IV) Inhibitor Screening Assay Kit (Cayman Chemical) following the manufacturer's instruction. Briefly, the sample solution was dissolved in dimethyl sulfoxide (DMSO, 10 μL) and DPP-IV enzyme solution (10 μL) was added to half the area of a 96-well white microplate and incubated for 5 min at 37°C. The substrate solution (50 μL) was added to the plate and incubated for 30 min at 37°C. Fluorescence (ex 360 nm, em 460 nm) was measured every 5 min and the reaction rate was measured. For the control, DMSO was added instead of the sample solution. Diprotin (25 μM) was used as the positive control.

In the case of plant extracts or in some of the samples, the inhibition of fluorescence was seen. To exclude the effect of fluorescence inhibition, the fluorescent inhibition ratio (FI) was also measured. Sample solution (10 μL), 7-amino-4-methylcoumarin (50 μM, 10 μL), and 20 mM Tris-HCl buffer (pH 8.0, contains 100 mM sodium chloride and 1 mM EDTA) were mixed and the fluorescence was measured [F(AMC+Sample)]. The fluorescence of 5 μM 7-amino-4-methylcoumarin [F(AMC)] and

the sample [F(Sample)] were measured individually and the fluorescent inhibition ratio was calculated according to the following equation.

$$FI = \frac{F(AMC + Sample)}{F(AMC) + F(Sample)}$$

Inhibitory activity was calculated using the following equation.

$$Inhibitory\ activity\ (\%) = \left\{1 - \frac{reaction\ rate\ (sample) \times FI}{reaction\ rate\ (control)}\right\} \times 100$$

Experiments were carried out in duplicate, repeated more than twice and the average value is shown as the result.

Quantitation by LC-MS

Rose bud extract powder was dissolved in water at a concentration of 1.0 g/L and analyzed by UPLC-MS. The analysis was achieved with ESI as an ion source in negative detection mode. An InertSustain® C18 column (2 μm, φ2.1 mm×100 mm, GL Science Co.) was employed for the separation, and gradient elution from 5% aq. acetonitrile with 0.1% formic acid to 95% acetonitrile with 0.1% formic acid for 20 min was used as a mobile phase with flow rate of 0.2 mL/min. Mass spectrum was measured between *m/z* 100–1500. A standard line of rugosin A (**6**) was made between 5–25 μM and rugosin B (**2**) was made between 10–50 μM. The experiment was repeated three times and the average value was calculated.

Supplementary material

GC-MS data and NMR spectrum of isolated compounds. The supplemental material for this paper is available at XXX.

Author contributions

The study was design by EK, YI, MK, and JK. The methods were constructed and performed by: EK and YU. The results were discussed by all of the authors. The manuscript was written by EK,

commented and revised by all of the authors.

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