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Potent anti-obese principle from *Rosa canina*: Structural requirements and mode of action of *trans*-tiliroside

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Abstract—The 80% aqueous acetone extracts from the fruit (50 mg/kg/d) and seeds (12.5 and 25 mg/kg/d) of *Rosa canina* L., but not from the pericarps, were found to show substantial inhibitory effect on the gain of body weight and/or weight of visceral fat without affecting food intake in mice for 2 weeks after administration of the extracts. With regard to the active constituents, the principal constituent, *trans*-tiliroside (0.1–10 mg/kg/d), potently inhibited the gain of body weight, especially visceral fat weight, and significantly reduced blood glucose levels after glucose loading (1 g/kg, ip) in mice. On the other hand, kaempferol and *p*-coumaric acid lacked such effect and kaempferol 3-*O*-β-D-glucopyranoside tended to reduce the gain of body weight and visceral fat weight, but not significantly, at a dose of 10 mg/kg/d. These results indicate the importance of both kaempferol 3-*O*-β-D-glucopyranoside and *p*-coumaroyl moieties for anti-obese effects. Furthermore, a single oral administration of *trans*-tiliroside at a dose of 10 mg/kg increased the expression of PPAR-α mRNA of liver tissue in mice.

Obesity has increased at an alarming rate in recent years and is now a worldwide health problem. It is widely accepted that obesity results from disequilibrium between energy intake and expenditure, and it is known to be a strong risk factor for type 2 diabetes associated with insulin resistance.[1] Furthermore, excessive obese conditions are suggested to cause other metabolic disorders, such as hyperlipidemia, hypertension, arteriosclerosis, and so on.[2] A variety of common disorders, such as hyperglycemia, hyperlipidemia, and hypertension, are seen in individuals, and cardiovascular disease is very prevalent, and this syndrome has been called to be metabolic syndrome.[3] This syndrome is caused by the excessive accumulation of visceral fat especially. In this state, insulin resistance or hyperinsulinemic condition is observed frequently; therefore, the reduction of visceral fat weight is important to control the development of metabolic disorder in patients.

Rosa canina L. (Rosaceae), known as 'Dog Rose', is a prickly shrub (1–3 m high) with fragrant pink or white flowers followed by bright red hips, distributed in Scotland and other parts of Europe. The fruit of this plant is called 'rose hip' and has been used as a diuretic, laxative, anti-gout, anti-rheumatism, etc., in European traditional medicine.

In the course of our studies on the search for anti-diabetic and anti-obese compounds from natural medicines,[4] the 80% aqueous acetone extracts from the fruit (50 mg/kg/d) and seeds (12.5 and 25 mg/kg/d) of *R. canina* significantly suppressed the gain of body weight on the 5–14th day after administration of the extracts (Fig. 2); however, the extract from pericarps (100 and 200 mg/kg/d) did not show such an effect (data not shown). The extracts from the fruit and seeds markedly suppressed visceral fat weight (total weight of epididymal, mesenteric, and paranephric fats) without affecting food intake (Table 1) and with no obvious toxic effect (data not shown). In addition, plasma triglyceride (TG) and free fatty acid (FFA) levels were significantly reduced on the 14th day (Table 2).

Keywords: Anti-obesity; *Rosa canina*; *trans*-Tiliroside; Visceral fat; PPAR-α.
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Table 1. Effects of the extracts from the fruit and seeds of *R. canina* and *trans*-tiliroside (**1**) on food intake, visceral fat, liver weight, and liver triglyceride content in mice

Treatments	Dose (mg/kg/d, p.o.)	N	Food intake (g/mouse/d)	Epididymal fat (mg)	Mesenteric fat (mg)	Paranephric fat (mg)	Visceral fat (mg)	Liver weight (mg)	Liver triglyceride (mg/liver)
Control	—	7	5.6 ± 0.2	1093 ± 61	917 ± 54	442 ± 27	2451 ± 128	1626 ± 56	72.5 ± 4.5
Fruit Ext.	25	5	5.4 ± 0.2	918 ± 94	738 ± 48	394 ± 60	2049 ± 184	1588 ± 30	63.7 ± 4.0
	50	5	5.0 ± 0.2	811 ± 60	812 ± 44	330 ± 39	1953 ± 136	1557 ± 79	63.9 ± 4.0
Seeds Ext.	12.5	5	5.7 ± 0.1	1142 ± 175	745 ± 80	350 ± 63	2237 ± 306	1592 ± 79	60.4 ± 3.3
	25	5	5.0 ± 0.2	558 ± 127**	508 ± 62**	194 ± 56**	1261 ± 236**	1683 ± 185	47.4 ± 13.9*
Control	—	7	5.2 ± 0.2	1205 ± 136	846 ± 62	372 ± 48	2424 ± 235	1503 ± 20	57.3 ± 5.4
trans-Tiliroside (**1**)	0.1	7	4.7 ± 0.2	716 ± 98**	662 ± 50*	284 ± 41	1663 ± 181*	1489 ± 61	46.7 ± 9.7
	1	7	5.0 ± 0.2	407 ± 89**	509 ± 43**	141 ± 37**	1057 ± 159**	1388 ± 32	30.8 ± 5.5**
	10	5	5.3 ± 0.3	350 ± 24**	516 ± 16**	132 ± 18**	998 ± 52**	1495 ± 13	29.2 ± 4.7**

Experimental protocol is described in references and notes.[14] Each value represents the mean ± S.E.M. Significantly different from the control, $^{*}P < 0.05$, $^{**}P < 0.01$.[15]

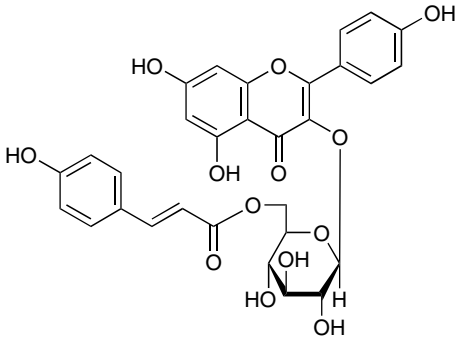
Table 2. Effects of the extracts from the fruit and seeds of *R. canina* and *trans*-tiliroside (**1**) on plasma parameters in mice

Treatments	Dose (mg/kg/d, p.o.)	N	Triglyceride (mg/dL)	Total cholesterol (mg/dL)	Free fatty acid (mEq/L)
Control	—	7	157 ± 18	143 ± 6	1.37 ± 0.09
Fruit Ext.	25	5	162 ± 8	141 ± 19	1.49 ± 0.10
	50	5	161 ± 9	144 ± 8	1.44 ± 0.07
Seeds Ext.	12.5	5	169 ± 15	156 ± 9	1.50 ± 0.09
	25	5	91 ± 11*	145 ± 19	0.88 ± 0.11**
Control	—	7	96 ± 6	95 ± 6	1.11 ± 0.06
trans-Tiliroside (**1**)	0.1	7	119 ± 8	108 ± 6	1.17 ± 0.06
	1	7	95 ± 18	102 ± 6	1.01 ± 0.16
	10	5	87 ± 5	101 ± 6	0.87 ± 0.06

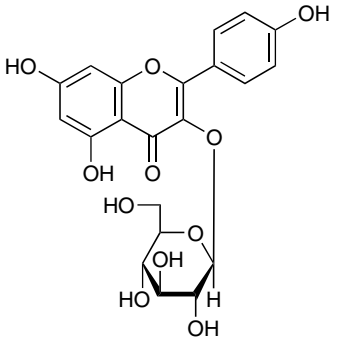
Experimental protocol is described in references and notes.[14] Each value represents the mean ± S.E.M. Significantly different from the control, $^{*}P < 0.05$, $^{**}P < 0.01$.[15]

Next, to clarify the active constituents of the extract from the seeds, the *n*-butanol (*n*-BuOH)-soluble fraction (2.1%) of the extract was subjected to ODS and SiO_2 column chromatographies and finally HPLC to give *trans*-tiliroside (**1**,[5,6] 0.013% from the dried seeds) (Fig. 1), *cis*-tiliroside[6] (0.00089%), buddlenoids A[7] (0.00034%) and B[7] (0.00038%), dihydrodehydrodiconiferyl alcohol[8] (0.014%), and urolignoside[9] (0.0077%).[10]

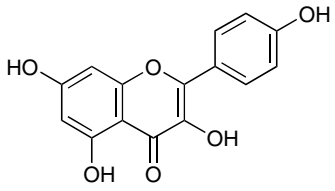
Recently, many bioactivities of the principal constituent, *trans*-tiliroside (**1**), were reported, such as hepatoprotective,[11] inhibition of the production of nitric oxide and



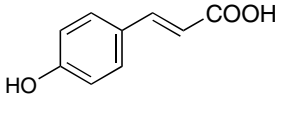
trans-tiliroside (**1**)



kaempferol 3-*O*-β-D-glucopyranoside (**2**)



kaempferol (**3**)



p-coumaric acid (**4**)

Figure 1. Chemical structures of *trans*-tiliroside (**1**) and related compounds **2**–**4**.

tumor necrosis factor-α in macrophages,[11,12] antiinflammatory and anticomplement activities,[13] etc.; however, an anti-obese effect of **1** has not been reported so far. We therefore examined the anti-obese effect of **1** in the first step. As shown in Figure 2 and Table 1, compound **1** (0.1–10 mg/kg/d) markedly suppressed the gain of body weight, visceral fat weight, and liver TG levels. Plasma FFA levels tended to be reduced, but not significantly (Table 2). No suitable positive control (reference compound) was found in this experiment, but a well-known lipase inhibitor, orlistat, was examined; however, orlistat at 1 and 10 mg/kg/d could not show such an effect in mice fed standard laboratory chow (data not shown).

To the best of our knowledge, *trans*-tiliroside (**1**) is the first example of potent anti-obese effects at low doses without toxic effects from natural products. In addition, after administration of **1** (0.1–10 mg/kg/d) for 15 d, D-glucose (1 g/kg, ip) was administered to the mice. As shown in Figure 3, compound **1** significantly inhibited the increase in plasma glucose levels especially 1 h after the loading of D-glucose. These results suggested that compound **1** could be effective for the improvement of abnormal glucose tolerance.

With regard to the structural requirements of *trans*-tiliroside (**1**) for the anti-obese effect, the effects of keampferol 3-*O*-β-D-glucopyranoside (**2**), kaempferol (**3**), and

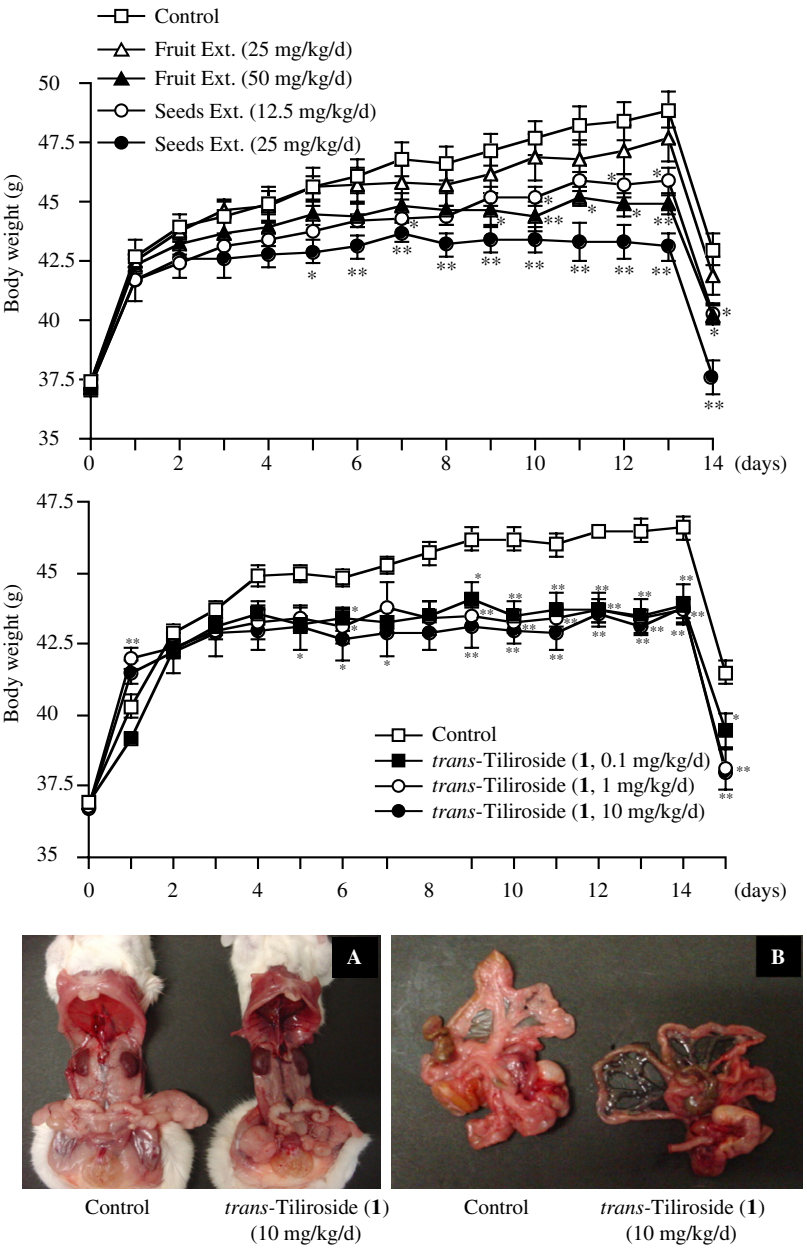


Figure 2. Effects of the extracts from the fruit and seeds of *R. canina* and *trans*-tiliroside (**1**) on body weight in mice for 14 or 15d and photographs of epididymal and paranephric fats (A) and mesentric fat (B) of mice on 15th day. Experimental protocol is described in references and notes.[14] Each point represents the mean with S.E.M. of 5–7 animals. Significantly different from the control, $^{*}P < 0.05$, $^{**}P < 0.01$.[15]

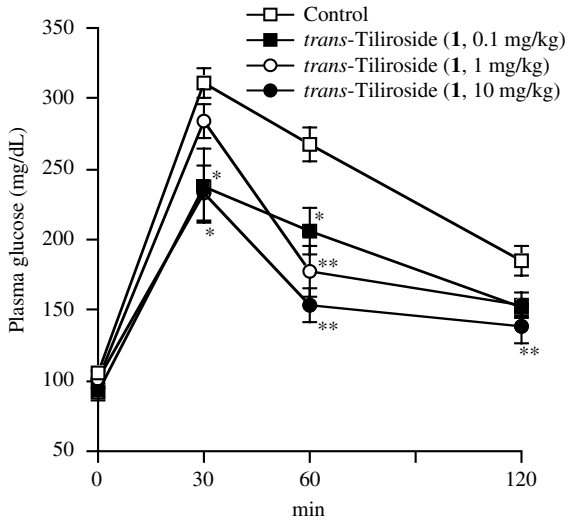


Figure 3. Effects of *trans*-tiliroside (**1**) on glucose tolerance test in mice. Test sample was administered to ddY mice for 15 d. After fasting for 20 h, 10% (w/v) glucose solution was intraperitoneally (ip) administered to mice at 10 mL/kg. Blood samples (ca. 0.2 mL) were collected from the infraorbital venous plexus before (0 h) and 0.5, 1, and 2 h after loading of glucose. Each point represents the mean with S.E.M. of 5–7 animals. Significantly different from the control, $^{*}P < 0.05$, $^{**}P < 0.01$.[15]

p-coumaric acid (**4**) were compared to that of **1** at a dose of 10 mg/kg/d. As a result, compounds **3** and **4** did not show such an effect, and compound **2** tended to reduce the gain of body weight and visceral fat weight, although its effect was not significant. Only compound **1** showed a significant anti-obese effect (Fig. 4 and Tables 3 and 4). In the previous study, we reported that *trans*-tiliroside (**1**) isolated from linden, the flower of *Tilia argentea*, inhibited liver damage induced by D-galactosamine/lipopolysaccharide in mice, and keampferol 3-*O*-β-D-glucopyranoside moiety is essential and *p*-coumaroyl moiety enhanced the activity.[11] Similarly, our results in the present study indicated that both kaempferol 3-*O*-β-D-glucopyranoside and *p*-coumaroyl moieties are necessary for the anti-obese effect of **1**.

Finally, the effect of **1** on the expression of peroxisome proliferator-activated receptor α (PPAR-α) mRNA levels in liver tissue 24 h after a single oral administration of **1** in mice was examined using a RT-PCR method. As shown in Figure 5, the expression of the PPAR-α mRNA level was apparently increased by **1** in liver tissue. These findings suggested that lipid metabolism was promoted by the oral administration of **1** in mice.

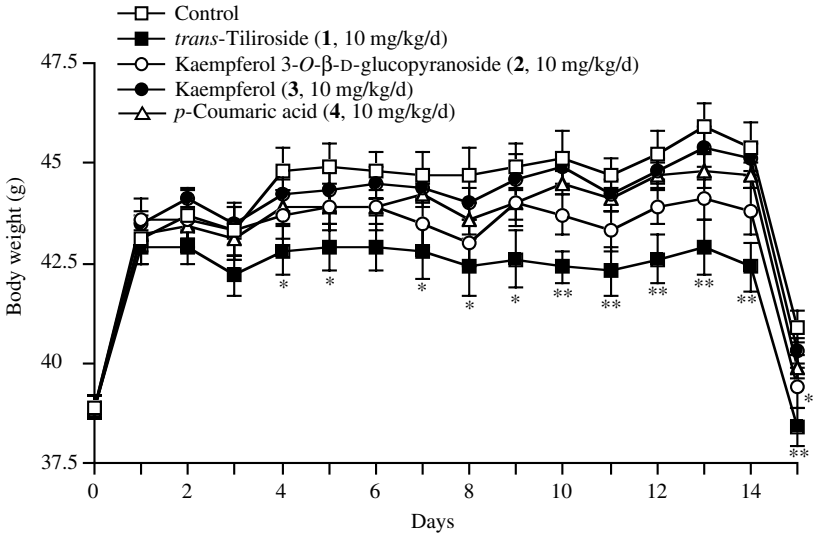


Figure 4. Effects of *trans*-tiliroside (**1**) and related compounds **2**–**4** on body weight in mice for 15 d. Experimental protocol is described in references and notes.[14] Each point represents the mean with S.E.M. of 6 animals. Significantly different from the control, $^{*}P < 0.05$, $^{**}P < 0.01$.[15]

Table 3. Effects of *trans*-tiliroside (**1**) and related compounds **2**–**4** on food intake, visceral fat, liver weight, and liver triglyceride content in mice

Treatments	Dose (mg/kg/d, p.o.)	N	Food intake (g/mouse/d)	Epididymal fat (mg)	Mesenteric fat (mg)	Paranephric fat (mg)	Visceral fat (mg)	Liver weight (mg)	Liver triglyceride (mg/liver)
Control	—	6	4.28 ± 0.16	940 ± 115	773 ± 32	378 ± 35	2091 ± 169	1525 ± 7	71.7 ± 5.5
trans-Tiliroside (**1**)	10	6	4.22 ± 0.14	626 ± 51*	561 ± 43*	266 ± 32	1453 ± 123*	1497 ± 66	51.9 ± 5.7
Kaempferol 3-*O*-glucopyranoside (**2**)	10	6	4.34 ± 0.16	797 ± 59	680 ± 32	293 ± 33	1770 ± 108	1638 ± 51	64.8 ± 7.3
Kaempferol (**3**)	10	6	4.20 ± 0.14	896 ± 78	804 ± 65	418 ± 48	2119 ± 183	1522 ± 68	60.3 ± 6.5
p-Coumaric acid (**4**)	10	6	4.30 ± 0.10	847 ± 87	761 ± 88	307 ± 77	1916 ± 236	1637 ± 43	58.2 ± 5.0

Experimental protocol is described in references and notes.[14] Each value represents the mean ± S.E.M. Significantly different from the control, $^{*}P < 0.05$, $^{**}P < 0.01$.[15]

Table 4. Effects of *trans*-tiliroside (**1**) and related compounds **2**–**4** on plasma parameters in mice

Treatments	Dose (mg/kg/d, p.o.)	N	Triglyceride (mg/dL)	Total cholesterol (mg/dL)	Free fatty acid (mEq/L)	Glucose (mg/dL)
Control	—	6	122 ± 20	126 ± 9	1.88 ± 0.09	117 ± 4
trans-Tiliroside (**1**)	10	6	128 ± 6	125 ± 11	1.84 ± 0.08	93 ± 4**
Kaempferol 3-*O*-glucopyranoside (**2**)	10	6	113 ± 5	119 ± 11	1.70 ± 0.11	99 ± 4
Kaempferol (**3**)	10	6	139 ± 15	149 ± 10	1.72 ± 0.10	110 ± 6
p - Coumaric acid (**4**)	10	6	162 ± 18	143 ± 9	1.67 ± 0.16	106 ± 3

Experimental protocol is described in references and notes.[14] Each value represents the mean ± S.E.M. Significantly different from the control, $^{**}P < 0.01$.[15]

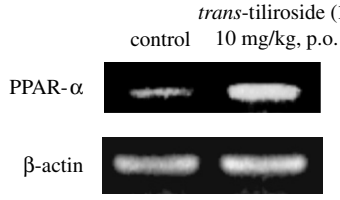


Figure 5. Effects of *trans*-tiliroside (**1**) on expression of PPAR-α mRNA levels of liver tissue in mice. Liver tissues of mice (11 weeks old) were removed 24 h after administration of **1**, and mRNA levels were analyzed by RT-PCR method.[16]

In conclusion, the 80% aqueous acetone extracts from the seeds of *R. canina* (12.5 and 25 mg/kg/d) were found to show an inhibitory effect on the gain of body weight and visceral fat weight without affecting food intake in mice for 2 weeks. With regard to active constituents, the principal constituent, *trans*-tiliroside (**1**), potently inhibited the gain of body weight, especially visceral fat weight, and increase in plasma glucose levels after glucose loading in mice. With regard to the structural requirements of **1**, both kaempferol 3-*O*-β-D-glucopyranoside and *p*-coumaroyl moieties are important for the anti-obese effects. The anti-obese effect of **1** was apparently stronger than that of orlistat, and compound **1** could be useful for the development of a new class of anti-obese agents. The mechanism of action of compound **1** should be studied further.

Acknowledgments

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10. The dried fruit (21 g), seeds (619 g), and pericarps (5.5 kg) of *R. canina* were crushed and extracted two times with 80% aq acetone at room temperature for 20 h. Evaporation of the solvent under reduced pressure provided a 80% aq acetone extract (4.7 g, 22.4% from the dried fruit; 27.1 g, 4.4% from the dried seeds; 1211 g, 22.0% from the dried pericarps), and a part of the extract (26.8 g) of seeds was partitioned into an *n*-butanol (*n*-BuOH) and H_2O (1:1, v/v) mixture to furnish an *n*-BuOH-soluble fraction (12.8 g, 2.1%) and H_2O-soluble fraction (13.5 g, 2.2%). The *n*-BuOH-soluble fraction (12.8 g) was subjected to reversed-phase silica gel column chromatography [400 g, MeOH–H_2O (50:50 → 70:30, v/v) → MeOH] to give 10

fractions [Fr. 1 (2.68 g), Fr. 2 (1.34 g), Fr. 3 (0.85 g), Fr. 4 (0.43 g), Fr. 5 (0.43 g), Fr. 6 (1.04 g), Fr. 7 (0.37 g), Fr. 8 (0.12 g), Fr. 9 (0.98 g), and Fr. 10 (3.78 g)]. Fraction 2 (1.34 g) was further purified by ordinary-phase silica gel column chromatography [40 g, $CHCl_3$–MeOH–H_2O (10:3:1, v/v/v, lower layer) → MeOH] and HPLC [YMC-Pack ODS-A, CH_3CN–H_2O (15:85, v/v)] to give uroligno-side (13.0 mg, 0.0077%). Fraction 4 (0.43 g) was also purified by ordinary-phase silica gel column chromatography [13 g, $CHCl_3$–MeOH–H_2O (20:3:1, v/v/v, lower layer) → MeOH] to give dihydrodehydrodiconiferyl alcohol (82.0 mg, 0.014%). Fraction 7 (0.37 g) was separated by ordinary-phase silica gel column chromatography [12 g, $CHCl_3$–MeOH–H_2O (15:3:1 → 10:3:1 → 7:3:1, v/v/v, lower layer) → MeOH] and HPLC [YMC-Pack ODS-A, CH_3CN–H_2O 30:70 or 25:75, v/v] to give *trans*-tiliroside (**1**, 62.7 mg, 0.013%), *cis*-tiliroside (4.4 mg, 0.00089%), buddlenoids A (2.1 mg, 0.00034%) and B (2.4 mg, 0.00038%). The known compounds were identified by comparison of their physical data ($[\alpha]_D$, UV, IR, ^{1}H NMR, ^{13}C NMR, MS) with reported values. [5–9] Compounds **1** and **2** isolated from the flower of *T. argentea* previously [11] were also used for the pharmacological evaluation.

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14. Test sample was administered to ddY male mice (11 w old) once a day (10:00–12:00) for 14 or 15 d fed a standard laboratory chow (MF, Oriental Yeast Co., Ltd., Japan). Body weight was measured every day before administration of the test sample. After fasting for 20 h, blood samples (ca. 0.2 mL) were collected from the infraorbital venous plexus and put into tubes containing 10 unit heparin Na, mice were killed by cervical dislocation, and then epididymal, mesenteric, and paranephric fats were removed and weighed. Plasma glucose, triglyceride (TG), total cholesterol, and free fatty acid (FFA) levels were determined using commercial kits (Glucose CII-test Wako, Triglyceride E-test Wako, Cholesterol E-test Wako, and NEFA C-test Wako, respectively). After removing the liver, ca. 100 mg of liver tissue was cut and homogenized with 5 mL H_2O, and the TG concentration in the suspension was determined using Triglyceride E-test Wako.
15. For statistical analysis, one-way analysis of variance followed by Dunnett's test was used. Probability (*P*) values of less than 0.05 were considered significant.
16. Total RNA was extracted from the liver (25–30 mg) using RNeasy™ Mini (Qiagen) according to the manufacturer's instructions. RT-PCR was performed using inllustra™ Ready-to-Go™ RT-PCR Beads (GE Healthcare). Equal amounts of total RNA (2 μg) corresponding to each priming dose were reversed transcribed using oligo(dT)$_{12\text{-}18}$ (0.5 μg/μL) as a first-strand primer. The following specific primers (Invitrogen) were used: PPAR-α mRNA, sense 5′ATGCCAGTACTGCCGTTTTC3′, and antisense 5′GGCCTTGACCTTGTTCATGT3′; β-actin 5′GGGAAATCGTGCGTGACAT3′ and antisense 5′CA GGAGGAGCAATGATCTC3′. Reverse transcription was performed at 42 °C for 30 min. Thermocycling parameters were as follows: denaturation at 95 °C for 1 min and 19 cycles for PPAR-α and 20 cycles for β-actin, consisting of incubations at 95 °C for 1 min, 58 °C for 1 min, and 72 °C for 2 min. After PCR, 15 μL of the reaction mixture was subjected to electrophoresis on a 2% agarose gel and visualized by ethidium bromide staining.