Short Communication

Bioactive Flavonoids from *Trapa pseudoincisa*

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Trapa pseudoincisa Nakai (Hydrocaryaceae) is an aquatic annual herb found in Korea, Japan, and China. *T. pseudoincisa* has been used for the remedy of several diseases including quadriplegia, diarrhea, and gastric ulcers [Jung and Shin, 1990]. Prior phytochemical and pharmacological studies reported some sterols as cytotoxic substances against ascites sarcoma [Irikura *et al*., 1972]. In addition, *cis*-hinokiresinol was reported as a scavenger of ABTS cation and superoxide anion radicals, and an inhibitor of LDL-oxidation, FPTase, PRL-3, and NO-production [Song *et al*., 2008]. The triterpenoids including cycloeucalenol, ursolic acid, and 2β,3α,23-trihydroxyurs-12-en-28-oic acid in *T. pseudoincisa* have also been repoted [Song *et al*., 2007].

Herein, the isolation and identification of four flavonoids, naringenin (**1**), quercetin (**2**), quercitrin (**3**), and isoquercitrin (**4**), from *T. pseudoincisa* and their biological activities including scavenging activities for ABTS [Roberta *et al*., 1999], DPPH [Han *et al*., 2004], and superoxide radical [Yoo *et al*., 2006], and their inhibitory activities against FPTase [Kwon *et al*., 1997], LDL-oxidation [Ahn *et al*., 2001], and proliferation of HUVECs [Lee *et al*., 2006], are described.

The dried whole plant of *T. pseudoincisa* (1.1 kg) was extracted with 80% aqueous MeOH (2 L×3), and evaporated *in vacuo*. The extracts were successively partitioned with water (1 L), EtOAc (1 L×2), and *n*-BuOH (1 L×2). The TIE (30 g) was subjected to the SiO_2 (500 g, 70-230 mesh, Merck, Darmstadt, Germany) cc (Φ 6×12 cm) eluted with *n*-hexane-EtOAc [10:1→5:1→3:1→1:1, v/v, 300 mL each] and $CHCl_3$-MeOH (10:1→7:1→5:1→3:1→1:1, v/v, 300 mL each) to give twenty-four fractions (TIE1~TIE24). TIE13 [2.1 g, Ve/Vt 0.56-0.62] was chromatographed by ODS (50 g, 70-230 mesh, Merck) cc (Φ 3.5×10 cm, MeOH-water=1:1→3:1, v/v, 2,000 mL each) to give compound **1** (22 mg, Ve/Vt 0.13-0.15; SiO_2 TLC R_f 0.3, $CHCl_3$-MeOH=15:1). TIE15 (3.5 g, Ve/Vt 0.65-0.69) was applied to the SiO_2 (75 g) cc (Φ 4.0×10 cm) eluted with $CHCl_3$-MeOH (15:1, v/v, 3,200 mL) to afford twelve fraction (TIE15-1~TIE15-12). TIE15-9 (0.3 g, Ve/Vt 0.61-0.73) was subjected by the SiO_2 (25 g) cc (Φ 3×7 cm) eluted with *n*-hexane-EtOAc (1:1, v/v, 2,000 mL) to give compound **2** (10 mg, Ve/Vt 0.15-0.19; ODS TLC R_f 0.7, MeOH-water=3:1). TIE21 (2.3 g, Ve/Vt 0.91-0.94) was applied to the SiO_2 (150 g) cc (Φ 4×12 cm) eluted with EtOAc-*n*-BuOH-water (15:1:0.5, v/v, 3,200 mL) to give compound **3** (1.2 g, Ve/Vt 0.24-0.45; SiO_2 TLC R_f 0.4, $CHCl_3$-MeOH-water=7:3:1). The TIB (5.1 g) was subjected to the SiO_2 (50 g, 70-230 mesh, Meck) cc (Φ 3.5×10 cm) and eluted with EtOAc-*n*-BuOH-water (5:4:1, v/v, 4,000 mL) to give four fractions (TIB1~TIB4). TIB2 (2.6 g, Ve/Vt 0.22-0.54) was applied to the SiO_2 (50 g) cc (Φ 3.5×10 cm) eluted with EtOAc-*n*-BuOH-water (60:2:1, v/v, 3,150 mL) to give seven fraction (TIB2-1~TIB2-7). TIB2-2 (1.4 g, Ve/Vt 0.12-0.31) was applied to the SiO_2 (50 g) cc (Φ 3.5×10 cm) eluted with EtOAc-*n*-BuOH-water (60:2:1, v/v, 1900

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Abbreviations: ABTS, 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid); BHA, butylated hydroxyanisole; bFGF, basic fibroblast growth factor; cc, column chromatography; DPPH, diphenylpicrylhydrazyl; FPTase, farnesyl protein transferase; HUVECs, hunan umbilical vein endothelial cells; LDL, low-density lipoprotein; ODS, octadecyl silica gel; PRL-3, phosphatase of regenerating liver-3; TIB, *n*-butanol fraction; TIE, ethyl acetate fraction; Ve/Vt, elution volume/total volume

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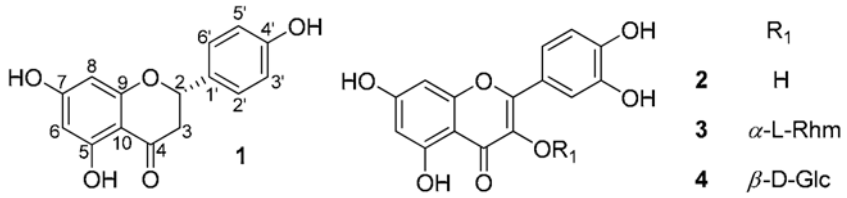

Fig. 1. Chemical structures of flavonoids from *T. pseudoincisa*.

mL) to give six fraction (TIB2-1~TIB2-6). TIB2-2-3 (160 mg, Ve/Vt 0.22-0.45) was applied to the SiO_2 (50 g) cc (Φ 3.5×10 cm) eluted with $CHCl_3$-MeOH-water (10:3:1, lower layer of 1,400 mL) to produce compound **4** (49 mg, Ve/Vt 0.63-0.72; SiO_2 TLC R_f 0.5, $CHCl_3$-MeOH-water=65:35:10) (Fig. 1).

Naringenin (1) Yellow powder (MeOH-H_2O); $[\alpha]_D$ +15.8 (*c* 0.3, EtOH); EIMS *m/z* 272[M]$^+$, 179, 166, 153, 124, 119, 107, 107, 91, 69; IR (KBr, ν) 3,250, 1,630, 1,605, 1,520, 1,500 cm^{-1}; ^{1}H-NMR (400 MHz, CD_3OD, δ) 7.30 (1H, d, *J*=8.0 Hz, H-2', 6'), 6.81 (1H, d, *J*=8.0 Hz, H-3', 5'), 5.89 (1H, s, H-6), 5.88 (1H, s, H-8), 5.32 (1H, dd, *J*=10.0, 2.8 Hz, H-2), 3.09 (1H, dd, *J*=16.8, 10.0 Hz, H-3a), 2.68 (1H, dd, *J*=16.8, 10.0 Hz, H-3b); ^{13}C-NMR (100 MHz, CD_3OD, δ) Refer to Table 1.

Quercetin (2) Yellow powder (MeOH-H_2O); EIMS *m/z* 302[M]$^+$, 301, 274, 273, 245, 153, 137, 109; IR (KBr, ν) 3380, 1669, 1614, 1512 cm^{-1}; ^{1}H-NMR (400 MHz, CD_3OD, δ) 7.73 (1H, d, *J*=2.0 Hz, H-2'), 7.63 (1H, dd, *J*=8.6, 2.0 Hz, H-6'), 6.79 (1H, d, *J*=8.6 Hz, H-5'), 6.38 (1H, d, *J*=1.8 Hz, H-8), 6.18 (1H, d, *J*=1.8 Hz, H-6); ^{13}C-NMR (100 MHz, CD_3OD, δ) Refer to Table 1.

Quercitrin (3) Yellow powder (MeOH-H_2O); $[\alpha]_D$ -178 (*c* 0.1, MeOH); FABMS *m/z* 449.2[M+H]$^+$, 303.1[M-Rhm+1]$^+$; IR (KBr, ν) 3228, 1655, 1614, 1549, 1449, 1176, 1086 cm^{-1}; ^{1}H-NMR (400 MHz, CD_3OD, δ) 7.25 (1H, d, *J*=2.0 Hz, H-2'), 7.21 (1H, dd, *J*=8.4, 2.0 Hz, H-6'), 6.86 (1H, d, *J*=8.4 Hz, H-5'), 6.28 (1H, d, *J*=2.0 Hz, H-8), 6.22 (1H, d, *J*=2.0 Hz, H-6), 5.26 (1H, d, *J*=2.0 Hz, H-1''), 0.80 (3H, d, *J*=6.0 Hz, H-6''); ^{13}C-NMR (100 MHz, CD_3OD, δ) Refer to Table 1.

Isoquercitrin (4) Yellow powder (MeOH-H_2O); $[\alpha]_D$ -12.5 (*c* 0.9, MeOH); FABMS *m/z* 465[M+H]$^+$; IR (KBr, ν) 3300, 1650 cm^{-1}; ^{1}H-NMR (400 MHz, CD_3OD, δ) 7.56 (1H, dd, *J*=2.4, 8.4 Hz, H-6'), 7.70 (1H, d, *J*=1.6 Hz, H-2'), 6.89 (1H, d, *J*=8.4 Hz, H-5'), 6.33 (1H, d, *J*=1.6 Hz, H-8), 6.14 (1H, d, *J*=1.6 Hz, H-6), 5.24 (1H, d, *J*=7.2 Hz, H-1''); ^{13}C-NMR (100 MHz, CD_3OD, δ) Refer to Table 1.

Structural identifications of these compounds were carried out by interpretation of several spectroscopic data and comparison with the data described in the literature. Compounds **1-4** were readily identified as naringenin (**1**) [Exarchou *et al*., 2003], quercetin, (**2**) [Jung *et al*., 2007], quercetin 3-*O*-α-L-rhamnopyranoside (quercitrin, **3**) [Jung *et al*., 2007], and quercetin 3-*O*-β-D-glucopyranoside (isoquercitrin, **4**) [Han *et al*., 2004].

The inhibitory activities of compounds **2** and **3** on LDL-oxidation (IC_{50}: 2, 30.1 mM; 3, 111.6 mM) was almost ten times higher than that of probucol (IC_{50}: 5.4 mM), a well known inhibitor that is much more potent than the other active components obtained from natural sources [Kim *et al*., 2005]. LDL-oxidation has been proposed as an important step in the formation of atherosclerotic lesions. Evidence to support this hypothesis is based in part on the observational studies that show associations between oxidized LDL cholesterol, as well as the presence of atherosclerotic lesions and the progression of carotid artery atherosclerosis.

Compounds **1-4** exhibited scavenging activity against

Table 1. ^{13}C-NMR data (100 MHz, δ_C) of compounds 1-4 from *T. pseudoincisa* in CD_3OD

No. of Carbon	Compound **1**	Compound **2**	Compound **3**	Compound **4**
2	80.41	148.59	157.90	158.71
3	46.13	139.61	135.87	135.41
4	197.55	177.13	179.02	179.12
5	165.27	162.27	162.49	162.68
6	96.98	99.15	99.58	99.73
7	168.21	165.37	167.12	165.69
8	96.10	94.33	94.58	94.61
9	165.27	158.03	158.78	158.11
10	114.13	103.00	105.60	105.47
1'	130.94	124.02	122.77	123.06
2'	128.91	115.86	116.08	115.82
3'	116.21	146.34	145.78	145.63
4'	158.83	147.84	149.20	149.61
5'	116.21	116.11	116.77	117.41
6'	128.91	121.54	122.69	122.82
1''			103.13	104.19
2''			71.73	75.61
3''			71.89	78.22
4''			73.09	71.02
5''			71.68	77.96
6''			17.56	62.40

Table 2. Scavenging activities of compounds 1-4 from *T. pseudoincisa* and BHA

Compounds	IC_{50} (μM)[a]		
	ABTS radical scavenging activity	DPPH radical scavenging activity	Superoxide radical scavenging activity
Compound **1**	15.6	49.2	142.2
Compound **2**	28.1	91.7	30.1
Compound **3**	34.6	87.7	13.4
Compound **4**	67.5	73.1	87.3
BHA[b]	70.6	45.6	141.6

[a]IC_{50} values indicate 50% inhibition concentration (mM), and were determined based on the regression lines at five different concentrations.
[b]BHA was used as the positive control inhibitor of ABTS, DPPH, and superoxide radical-scavenging activities.

ABTS cation (IC_{50} values: **1**, 15.6 μM; **2**, 28.1 μM; **3**, 34.6 μM; **4**, 67.5 μM), DPPH (IC_{50} values: **1**, 49.2 μM; **2**, 91.7 μM; **3**, 87.7 μM; **4**, 73.1 μM), and superoxide anion radicals (IC_{50} values: **1**, 142.2 μM; **2**, 30.1 μM; **3**, 13.4 μM; **4**, 87.3 μM). BHA, a well known antioxidant, was also evaluated as the positive control; IC_{50} values on the ABTS cation, DPPH, and superoxide anion radicals were 70.6, 45.6, and 141.6 μM, respectively. Compounds **1-3** showed higher scavenging activity on ABTS than BHA. In DPPH radical-scavenging activity, compound **1** was revealed to have almost the same level of activity as the positive control, and the compounds **2** and **3** exhibited significantly higher superoxide radical-scavenging activity than BHA. *T. pseudoincisa* has also been reported to show high scavenging activity against the DPPH free radical-generating system [Kim *et al*., 1997]. Thus, the four flavonoids could be principal contributors to the radical-scavenging activity of *T. pseudoincisa*.

The methanol extract of *T. pseudoincisa* strongly inhibited the activity of FPTase by 90% at 100 μg/mL. Compounds **1** and **3** had IC_{50} of 70.2 and 117.2 μM, respectively. On the other hand, 2-hydoxycinnamaldehyde, a well known FPTase inhibitor, showed IC_{50} value of 172.3 μM [Kwon *et al*., 1997], lower than those of compounds **1** and **3**. Compounds **1** and **3** also exhibited PRL-3 inhibitory activity with IC_{50} values of 133.1 and 130.6 μM, respectively, whereas some biflavonoids such as ginkgetin and sciadopitysin were reported to show inhibitory activity on PRL-3 with IC_{50} values of 25.8 and 46.2 μM [Choi *et al*., 2006] (Table 2). In addition, compounds **1** and **3** showed more effective inhibitory activity against PRL-3 than ginkgetin and sciadopitysin. Therefore, compounds **1** and **3** may be useful lead compounds for the development of antitumor drugs through the control of FPTase and PRL-3-mediated signal pathways.

Though compound **3** did not show any significant cytotoxic effect on the non-stimulated HUVECs at 100 μg/mL, it inhibited the bFGF-induced endothelial proliferation by 80% at 25 μg/mL. Compound 3 showing inhibitory activity on the proliferation of HUVECs without cytotoxicity could be a useful source in the development of drugs for the prevention and remedy of cancer.

In conclusion, four flavonoids, naringenin, quercetin, quecitrin, and isoquercitrin, were isolated from *T. pseudoincisa*, and their inhibitory activities, including scavenging activities on ABTS, DPPH, and superoxide anion radicals inhibitory activities against LDL-oxidation, FPTase, PRL-3, and proliferation of HUVECs, were evaluated for use as antioxidant, antiatherogenic, and anticancer materials.

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