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Isolation and Cytotoxicity of Flavonoids from Daphnis Genkwae Flos

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ABSTRACT

For the purpose of quality analysis, we investigated polar constituents as marker substance for some traditional herbs. From Daphnis Genkwae Flos twelve flavonoids were isolated. They were identified as potassium apigenin 7-*O*-*β*-D-glucuronate **(1)**, apigenin 7-*O*-*β*-D-glucuronide **(2)**, apigenin 7-*O*-*β*-D- methylglucuronate **(3)**, apigenin **(4)**, genkwanin 5-*O*-*β*-D-primeveroside **(5)**, genkwanin 5-*O*-*β*-D-glucoside **(6)**, genkwanin **(7)**, tiliroside **(8)**, kaempferol **(9)**, luteolin 5-*O*-*β*-D-glucoside **(10)**, luteolin **(11)** and 7-*O*-methylluteolin **(12)**. Among them, **2**, **3**, **5**, **6**, **9** and **10** were known compounds, but were for the first time isolated from this material. Compound **1** was isolated from nature for the first time. The structures of **1–12** were established on the basis of their physical properties and spectroscopic evidence.

Treatments of human hepatoma HepG2 cells with 0.1 mM apigenin **(4)**, luteolin **(11)**, and 7-*O*-methylluteolin **(12)** for 48 hr caused 40% reduction on cell viability, whereas potassium apigenin 7-*O*-*β*-D-glucuronate **(1)**, luteolin 5-*O*-*β*-D-glucoside **(10)**, genkwanin **(7)**, genkwanin 5-*O*-*β*-D-primeveroside **(5)**, and tiliroside **(8)** caused little or no effects on the viability of HepG2 cell. These data suggest a rough structure - activity relationship of flavonoid cytotoxicity.

Key words: *Daphne genkwa*, flower, Thymelaeaceae, flavonoid, cytotoxicity

INTRODUCTION

Daphnis Genkwae Flos[(1)] is the dried flower of *Daphne genkwa* Sieb. et Zucc. (Thymelaeaceae). It was recorded in the pharmacopoeia, the *Shen Nung Pen Tsao Ching*, as a low-grade drug and reported to exhibit antifungal, purgative, expectorant and antitussive effects. It was used for the inhibition of motor activation of roundworm, treatment of cardiac asthma, edema and abdominal water retention caused by *Schistosoma cattoi*.

Previous papers reported the isolation of luteolin, 7-*O*-methylluteolin, tiliroside[(2)], apigenin, genkwanin, sitosterol, benzoic acid[(3)] and genkwadaphnin[(3, 4)] from Daphnis Genkwae Flos. Apigenin and 7-*O*-methylluteolin showed strong inhibitory activity against xanthine oxidase[(3)] Genkwadaphnin demonstrated significant antileukemic activity in low doses[(4)]. Luteolin, 7-*O*-methylluteolin and tiliroside were identified as adenosine 3', 5'-cyclic monophosphate phosphodiesterase inhibitors[(2)]. Because traditional Chinese medicine is usually prepared as a decoction, the active constituents should be contained in the polar fraction. As part of our studies on the isolation of marker constituents of traditional medicine, this paper describes the isolation, structural elucidation, and cytotoxicity of compounds **1–12**.

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MATERIALS AND METHODS

I. *Instruments and Reagents*

Melting points were determined with a Fisher-Johns melting-point apparatus and were uncorrected. Mass spectra were obtained on a JEOL JMS HX110 spectrometer. ^{1}H (300, 500 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a Bruker AM-300WB/DMX-500 SB FT-NMR spectrometer, using the solvent peak as reference standard. Column chromatography was carried out with Sephadex LH-20 (25-150 μm, Pharmacia Fine Chemical Co. Ltd.) and Diaion HP-20 (100-200 μm, Mitsubishi Chemical Industries, Ltd.).

II. *Cytotoxicity Testing*

Human hepatoma HepG2 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, L-glutamine, penicillin (100 IU/mL) and streptomycin (100 μg/mL) at 37˚C in a humidified atmosphere of 5% CO_2 as described previously[(5)]. Cells were grown to confluence prior to flavonoid treatment. The flavonoids were dissolved in dimethylsulfoxide (DMSO) and added to the medium so that DMSO concentration in the medium was less than 0.1%. Cell viability was determined using a procedure modified from Carmichael's colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bro-

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mide (MTT) method [6].

III. *Plant Material*

Daphnis Genkwae Flos were purchased in Taipei, Taiwan in May 1996 and verified by Dr. Hsien-Chang Chang, National Laboratories of Foods and Drugs, Taipei, Taiwan. The voucher specimen is deposited in the National Laboratories of Foods and Drugs, Department of Health, Executive Yuan, Republic of China.

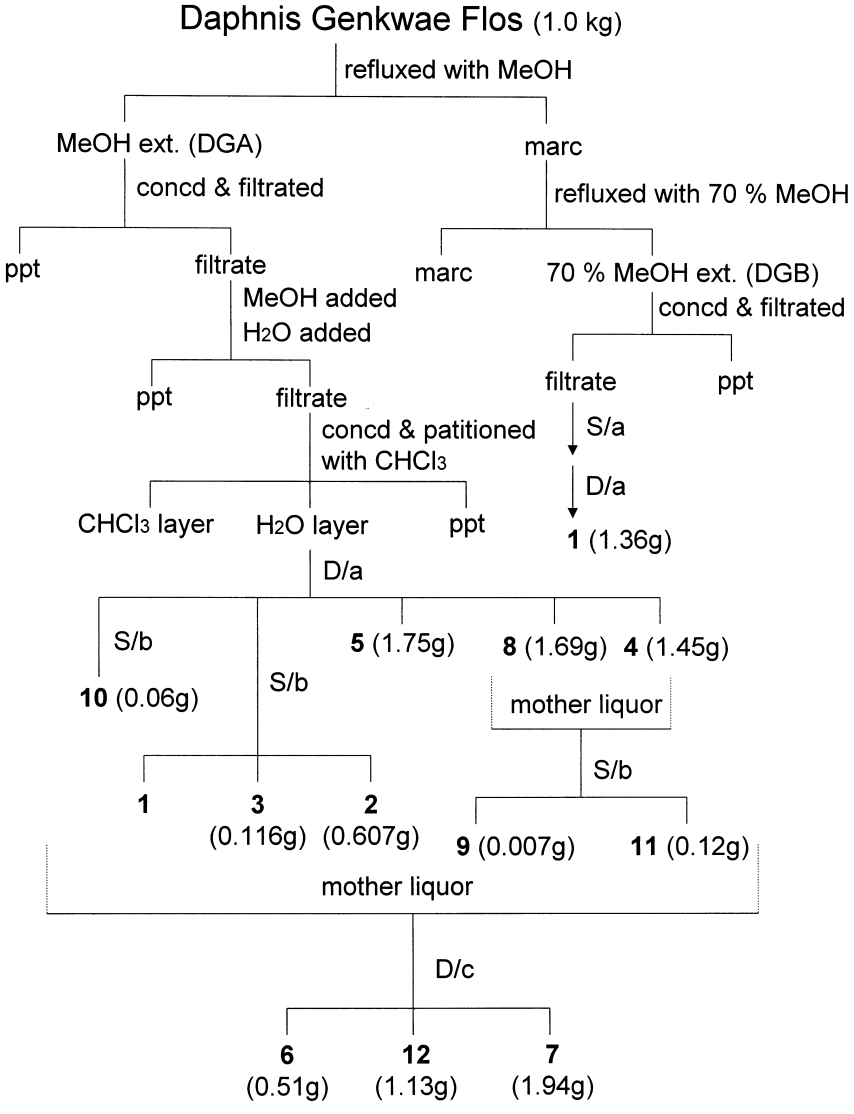
IV. *Extraction and Separation*

Daphnis Genkwae Flos (1.0 kg), was extracted twice with boiling methanol (9 L) and then twice with 70% methanol (9 L). The methanolic extract was concentrated under vacuum (ca 40˚C) and the precipitate was removed by filtration to give aqueous extract, DGA. The 70% methanolic extract was treated by the same process as for the methanolic extract to give DGB.

As shown in Scheme 1, DGB was subjected to Sephadex LH-20 column chromatography using H_2O containing increasing amounts of MeOH (H_2O : MeOH = 1 : 0 → 0 : 1) and then purified on a Diaion HP-20 column (H_2O : MeOH = 1 : 0 → 0 : 1) to give compound **1**. DGA was dissolved in methanol and precipitated by the addition of water. The filtrate was concentrated and partitioned between water and chloroform. The aqueous layer was separated by repeated column chromatography over Sephadex LH-20 and Diaion HP-20 to afford compounds **1–12**.

Potassium apigenin 7-*O*-β-D-glucuronate (1)

Pale yellow micro-needles, mp 280˚C (decomp.),



Scheme 1. Separation of Flavonoids from Daphnis Genkwae Flos.
S: Sephadex LH-20 a: H_2O: MeOH (1:0 → 0:1)
D: Diaion Hp-20 b: 80% MeOH
c: H_2O: MeOH (1:1 → 0:1)

Positive FAB-MS *m/z*: 485 $[M+H]^+$. Anal. Calcd for $C_{21}H_{17}O_{11}K \cdot 3H_2O$: C, 46.75; H, 4.26. Found: C, 46.50; H, 4.31. IR (KBr) ν_{max} cm^{-1}: 3402 (OH), 1668 (flavone C=O), 1607, 1592, 1509 (aromatic C=C). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 7.87 (2H, *d*, *J*= 8.8 Hz, H-2', 6'), 6.91 (2H, *d*, *J*= 8.8 Hz, H-3', 5'), 6.78 (1H, *d*, *J*=1.9 Hz, H-8), 6.41 (1H, *d*, *J*=1.9 Hz, H-6), 6.75 (1H, s, H-3), 5.08 (1H, *d*, *J*=7.1 Hz, H-1''). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 181.8 (C-4), 171.6 (C-6"), 164.2 (C-2), 162.8 (C-7), 161.6 (C-5), 160.9 (C-4'), 156.8 (C-9), 128.2 (C-2', 6'), 120.5 (C-1'), 115.9 (C-3', 5'), 105.2 (C-10), 102.7 (C-3), 99.5 (C-6, 1"), 94.5 (C-8), 76.3 (C-5"), 74.0 (C-3"), 72.9 (C-2"), 71.8 (C-4").

Apigenin 7-*O*-*β*-D-glucuronide (2)

Pale yellow amorphous powder, mp 195˚C (decomp.), Positive ESI-MS *m/z*: 447 $[M+H]^+$. IR (KBr) ν_{max} cm^{-1}: 3411 (OH), 1737 (acidic C=O), 1665 (flavone C=O), 1608, 1595, 1498 (aromatic C=C). ^{1}H-NMR (500 MHz, DMSO-d_6): 12.97 (1H, s, OH-5), 10.44 (1H, s, OH-4'), 7.95 (2H, *d*, *J*=8.8 Hz, H-2', 6'), 6.93 (2H, *d*, *J*=8.8 Hz, H-3', 5'), 6.86 (1H, s, H-3), 6.85 (1H, *d*, *J*=2.1 Hz, H-8), 6.46 (1H, *d*, *J*=2.1 Hz, H-6), 5.26 (1H, *d*, *J*=7.3 Hz, H-1"), 4.04 (1H, *d*, *J*=9.6 Hz, H-5"). ^{13}C-NMR (125 MHz, DMSO-d_6): δ 182.2 (C-4), 170.5 (C-6"), 164.6 (C-2), 162.7 (C-7), 161.4 (C-5), 161.1 (C-4'), 157.2 (C-9), 128.9 (C-2', 6'), 121.3 (C-1'), 116.2 (C-3', 5'), 105.7 (C-10), 103.4 (C-3), 99.6 (C-6), 99.4 (C-1"), 95.0 (C-8), 75.7 (C-5"), 75.3 (C-3"), 72.9 (C-2"), 71.4 (C-4").

Apigenin 7-*O*-*β*-D-methylglucuronate (3)

Pale yellow amorphous powder, mp 257-259˚C, Positive ESI-MS *m/z*: 461 $[M+H]^+$. IR (KBr) ν_{max} cm^{-1}: 3422 (OH), 1742 (ester C=O), 1666 (flavone C=O), 1610, 1595, 1500 (aromatic C=C). ^{1}H-NMR (500 MHz, DMSO-d_6): δ 12.96 (1H, s, OH-5), 10.44 (1H, s, OH-4'), 7.94 (2H, *d*, *J*=8.8 Hz, H-2', 6'), 6.93 (2H, *d*, *J*=8.8 Hz, H-3', 5'), 6.85 (1H, s, H-3), 6.85 (1H, *d*, *J*=2.0 Hz, H-8), 6.46 (1H, *d*, *J*=2.0 Hz, H-6), 5.30 (1H, *d*, *J*=7.3 Hz, H-1"), 4.19 (1H, *d*, *J*=9.5 Hz, H-5"), 3.65 (3H, s, -OCH_3). ^{13}C-NMR (125 MHz, DMSO-d_6): δ 182.2 (C-4), 169.6 (C-6"), 165.0 (C-2), 162.7 (C-7, 5), 161.3 (C-4'), 157.3 (C-9), 129.0 (C-2', 6'), 120.6 (C-1'), 116.7 (C-3', 5'), 105.9 (C-10), 103.2 (C-3), 99.6 (C-6, 1"), 95.1 (C-8), 75.5 (C-3", 5"), 73.0 (C-2"), 71.6 (C-4"), 52.5 (OCH_3).

Enzymatic Hydrolysis of 1, 2 and 3: Each of **1** (1.0 mg), **2** (0.5 mg) and **3** (0.5 mg) in 50% MeOH was incubated with *β*-glucuronidase at 37˚C. The reaction mixture was monitored by SiO_2 TLC/$CHCl_3$: MeOH : H_2O = 6: 4: 0.5/5% $FeCl_3$. **1** and **2** were hydrolyzed after 48 and 4 hours, respectively, and **3** was not hydrolyzed at all.

Genkwanin 5-*O*-*β*-D-primeveroside (5)

Pale yellow needles, mp 200-202˚C, positive FAB MS *m/z* : 579 $[M+H]^+$, 285 [M-glucose-xylose+H]$^+$, ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.23 (1H, s, OH-4'), 7.91 (2H, *d*, *J*=8.7 Hz, H-2', 6'), 6.92 (2H, *d*, *J*=8.7 Hz, H-3', 5'), 7.01 (1H, *d*, *J*=2.2 Hz, H-8), 6.87 (1H, *d*, *J*=2.2 Hz, H-6), 6.67 (1H, s, H-3), 4.77, 4.19 (each 1H, *d*, *J*=7.3 Hz, anom. H), 3.89 (3H, s, -OCH_3). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 177.1 (C-4), 163.7 (C-7), 161.5 (C-2), 160.8 (C-4'), 158.6 (C-9), 158.1 (C-5), 128.2 (C-2', 6'), 121.2 (C-1'), 116.0 (C-3', 5'), 109.2 (C-10), 105.9 (C-3), 104.1 (C-1'"), 103.5 (C-1"), 102.8 (C-6"), 96.6 (C-8"), 76.4 (C-3'"), 76.0 (C-3"), 75.5 (C-5"), 73.3 (C-2", C-2'"), 69.8 (C-4"), 69.5 (C-4'"), 68.7 (C-6"), 65.6 (C-5'"), 56.2 (OCH_3).

Acetylation of genkwanin 5-*O*-*β*-D-primeveroside: Compound **5** (30 mg) was acetylated overnight with acetic anhydride and pyridine at room temperature. Usual work-up afforded the genkwanin 5-*O*-*β*-D-primeveroside octaacetate (**5a**, 32 mg) as white amorphous powder. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.86, 7.22 (each 2H, *d*, *J*=8.5 Hz, H-2', 3', 5', 6'), 6.75, 6.74 (each 1H, *d*, *J*=2.0 Hz, H-6, 8), 6.58 (1H, *s*, H-3), 4.52 (1H, *d*, *J*=6.5 Hz, H-1'"), 4.86 (1H, *dd*, *J*=6.5, 7.8 Hz, H-2'"), 5.04 (1H, *t*, *J*= 7.8 Hz, H-3'"), 4.92 (1H, *m*, H-4'"), 4.10 (1H, *dd*, *J*=4.7, 11.8 Hz, H-5'"), 3.34 (1H, *dd*, *J*= 8.6, 11.8 Hz, H-5'"), 5.13 (1H, *d*, *J*=7.8 Hz, H-1"), 5.39 (1H, *dd*, *J*=7.8, 9.3 Hz, H-2"), 5.30 (1H, *t*, *J*=9.3 Hz, H-3"), 5.01 (1H, *t*, *J*=8.3 Hz, H-4"), 3.83 (1H, *m*, H-5"), 3.77 (1H, *dd*, *J*= 4.2, 11.5 Hz, H-6"), 3.71 (1H, *dd*, *J*=7.3, 11.5 Hz, H-6"), 3.93 (3H, *s*, -OCH_3), 2.32, 2.11, 1.89 (each 3 H, 3 × OAc), 2.03, 2.02, 2.01 (15 H in total, 5 × OAc).

Genkwanin 5-*O*-*β*-D-glucoside (6)

Pale brown amorphous powder, mp 200˚C (decomp.), ^{1}H-NMR (500 MHz, DMSO-d_6): δ 10.21 (1H, *s*, OH-4'), 7.79 (2H, *d*, *J*=8.6 Hz, H-2', 6'), 6.87 (2H, *d*, *J*=8.6 Hz, H-3', 5'), 6.86 (1H, *d*, *J*=2.2 Hz, H-8), 6.69 (1H, *d*, *J*=2.2 Hz, H-6), 6.53 (1H, s, H-3), 4.79 (1H, *d*, *J*=7.5 Hz, H-1"), 3.80 (3H, *s*, -OCH_3). ^{13}C-NMR (125 MHz, DMSO-d_6): δ 178.5 (C-4), 164.6 (C-7), 162.2 (C-2), 161.4 (C-5), 159.5 (C-4'), 158.5 (C-9), 129.1 (C-2', 6'), 121.8 (C-1'), 116.8 (C-3', 5'), 109.4 (C-10), 106.3 (C-3), 103.2 (C-1"), 102.8 (C-6), 96.8 (C-8), 77.8 (C-5"), 76.1 (C-3"), 73.8 (C-2"), 70.4 (C-4"), 61.5 (C-6"), 56.9 (OCH_3).

Genkwanin (7)

Pale Yellow amorphous powder, mp 294-296˚C, ^{1}H-NMR (300 MHz, DMSO-d_6): δ 12.92 (1H, *s*, OH-5), 10.31 (1H, *s*, OH-4'), 7.94 (2H, *d*, *J*=8.7 Hz, H-2', 6'), 6.93 (2H, *d*, *J*=8.7 Hz, H-3', 5'), 6.82 (1H, *d*, *J*=1.8 Hz, H-8), 6.36 (1H, *d*, *J*=1.8 Hz, H-6), 6.75 (1H, s, H-3), 3.87 (3H, *s*, -OCH_3). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 180.2 (C-4), 165.2 (C-7), 164.2 (C-2), 161.4 (C-5), 161.2 (C-4'), 157.3 (C-9), 128.7 (C-2', 6'), 121.1 (C-1'), 116.1 (C-3', 5'), 104.7 (C-10), 103.1 (C-3), 98.1 (C-6), 92.8 (C-8), 56.1 (OCH_3).

Luteolin 5-*O*-*β*-D-glucoside (10)

Pale yellow amorphous powder, mp 200˚C (decomp.), ^{1}H-NMR (300 MHz, DMSO-d_6): δ 7.37~7.34 (2H, *m*, H-2', 6'), 6.87 (1H, *d*, *J*=8.9 Hz, H-5'), 6.78 (1H, *d*, *J*=2.1 Hz, H-

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8), 6.69 (1H, *d*, *J*=2.1 Hz, H-6), 6.52 (1H, *s*, H-3), 4.70 (1H, *d*, *J*=7.1 Hz, H-1"). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 176.8 (C-4), 162.5 (C-2), 161.3 (C-7), 158.6 (C-5), 158.3 (C-9), 149.2 (C-4'), 145.6 (C-3'), 121.5 (C-1'), 118.5 (C-6'), 116.0 (C-5'), 113.2 (C-2'), 108.5 (C-10), 105.7 (C-3), 104.4 (C-1"), 104.2 (C-6), 98.1 (C-8), 77.5 (C-5"), 75.6 (C-3"), 73.6 (C-2"), 69.7 (C-4"), 60.9 (C-6").

RESULTS AND DISCUSSION

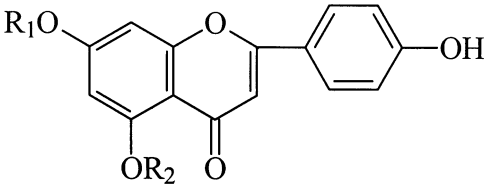
From the methanolic and 70% methanolic extract of Daphnis Genkwae Flos, twelve flavonoids **(1-12)** were isolated and their structures are shown in Figure 1. The ^{1}H-NMR spectra of compounds **1-7** showed signals due to four A_2B_2 type protons near δ 7.9 and 6.9 (each 2H, *d*, *J* = 8.6~8.8 Hz), two meta-coupling protons near δ 6.8 and 6.4 (each 1H, *d*, *J* = 1.8~2.2 Hz) and a proton as a singlet near δ 6.7 (1H, s). These data implied that **1-7** possessing an apigenin moiety. Compounds **4** and **7** were identical with apigenin[7-8] and genkwanin[8], respectively, by directly comparison of their ^{1}H-NMR data with the values in literatures.

Compound **2** was obtained as a pale yellow amorphous powder, soluble in methanol and slightly soluble in water. The ^{1}H-NMR spectrum showed the signal due to an anomeric proton at δ 5.26 (1H, *d*, *J*= 7.3 Hz) and the ^{13}C-NMR spectrum showed six carbon signals [δ 170.5 (C-6"), 99.4 (C-1"), 75.7 (C-5"), 75.3 (C-3"), 72.9 (C-2"), 71.4 (C-4")] in addition to the signals of apigenin, suggested the presence of a glucuronic acid. This assumption was supported by the evidence that **2** was hydrolyzable by glucuronidase. The electrospray ionization mass spectrum (ESI MS) showed a prominent peak at *m/z* 447 $[M+H]^+$. The HMBC and HMQC spectra confirmed the structure of **2** as apigenin 7-*O*-*β*-D-glucuronide and was further confirmed by direct comparison of its physical data with literature values[9].

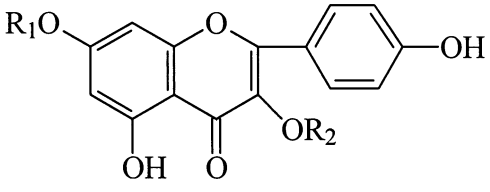
Compound **1**, a pale yellow amorphous powder, showed similar ^{1}H- and ^{13}C-NMR spectra data as **2**. It can also be hydrolyzed by glucuronidase but with quite slower rate than **2**. The positive FAB mass spectrum showed M+1 at 485, which was 38 mass units (+K–H) higher than **2**. The existence of potassium ion was also confirmed by the atomic absorption detection. This was supported by the result of elementary analysis, which showed the data consistent to the formula $C_{21}H_{17}O_{11}K \cdot 3H_2O$. Compound **1** was very different from **2** because **1** was easily soluble in water and slightly soluble in methanol. Additionally, the IR spectrum of compound **2** showed the absorption band at 1738 cm^{-1} due to the carboxylic acid group of glucuronic acid, which was not present in **1**. Therefore **1** was confirmed as a potassium salt of **2**.

The ^{1}H- and ^{13}C-NMR spectrum of **3** were also closely similar to **2** except an extra methoxy signal at δ 3.65 (3H, *s*). The positive ESI mass spectrum of **3** exhibited the $[M+H]^+$ ion peak at *m/z* 461 which was 14 mass units (CH_3 — H) higher than **2**, corresponding to a methylene group. The IR spectrum showed the absorption band at 1741 cm^{-1}, which arises from ester linkage. Therefore **3** was suggested to be apigenin 7-*O*-*β*-D-methylglucuronate[9].

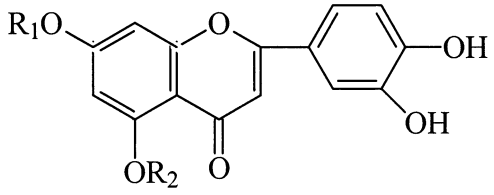
Compound **5** was obtained as a pale yellow amorphous powder. The ^{1}H-NMR spectrum showed signals indicating the existence of genkwanin and disaccharide [δ 6.68, 4.75 (each 1H, *d*, *J* = 7.5 Hz, anomeric H). The ^{13}C-NMR spectrum of **5** exhibited eleven more carbon signals than that of genkwanin, suggesting that the disaccharide was composed of a pentose and a hexose. Further support was obtained by a positive FAB mass spectrum, which showed the $[M+H]^+$ ion peak at *m/z* 579, and a prominent peak at *m/z* 285 arising



	R_1	R_2
1	K glucuronate	H
2	glucuronic acid	H
3	methyl glucuronate	H
4	H	H
5	CH_3	primeverose
6	CH_3	glucose
7	CH_3	H



	R_1	R_2
8	H	coumaroyl glucose
9	H	H



	R_1	R_2
10	H	glucose
11	H	H
12	CH_3	H

Figure 1. Structures of compounds **1-12** isolated from Daphnis Genkwae Flos.

from genkwanin. By inspecting the ^{1}H-^{1}H COSY spectrum of its acetate, the structure of **5** was established as genkwanin 5-*O*-primeveroside and was confirmed by direct comparison of the ^{13}C-NMR data with literature values[10-11]. Compound **5** was isolated from the Thymeleaceae plant for the first time.

Compounds **6** and **8-13** were identified as genkwanin 5-*O*-*β*-D-glucoside **(6)**[12], tiliroside **(8)**[13], kaempferol **(9)**[8, 14-15], luteolin 5-*O*-*β*-D-glucoside **(10)**[8, 12], luteolin **(11)**[8, 15, 16-17] and 7-*O*-methylluteolin **(12)**[17-18], respectively.

Among these compounds, apigenin 7-*O*-*β*-D-glucuronide **(2)**, apigenin 7-*O*-*β*-D-methylglucuronate **(3)**, genkwanin 5-*O*-*β*-D-primeveroside **(5)**, genkwanin 5-*O*-*β*-D-glucoside **(6)**, kaempferol **(9)** and luteolin 5-*O*-*β*-D-glucoside **(10)** were isolated from Daphnis Genkwae Flos for the first time. Potassium apigenin 7-*O*-*β*-D-glucuronate was the major component and isolated from nature for the first time.

The cytotoxicity of flavonoids **1–12** was evaluated against the human hepatoma HepG2 cell. The major reason for choosing this cell line is that HepG2 cells contain the essential drug-metabolizing enzymes and are frequently used for xenobiotic metabolism and toxicity studies[5]. Treatments with apigenin **(4)** and genkwanin **(7)** caused a 42% and a 6% decrease of cell viability, respectively (Table 1), whereas, treatments with potassium apigenin 7-*O*-*β*-D-glucuronate **(1)** and genkwanin 5-*O*-*β*-D-primeveroside **(5)** had no effects on cell viability. Treatments with luteolin **(11)** and 7-O-methylluteolin **(12)** resulted in a 41% decrease of cell viability. However, treatments with luteolin 5-*O*-*β*-D-glucoside **(10)** and tiliroside **(8)** caused a 10% decrease and a 9% increase of cell viability, respectively.

Comparison of the structures and cytotoxicity data suggests a possible structure-activity relationship on the cytotoxicity of flavonoids in human liver cells. The cytotoxicity of apigenin **(4)** markedly decreased with the presence of a methoxy (in **7**) or a glucuronyl (in **1**) group at C-7, or a coumaroyl glucosyl group at C-3 (in **8**) (Table1; Figure 1). However, the presence of a hydroxyl group at C-3' position (in **11**) has no apparent effect on the cytotoxicity of apigenin. The cytotoxicity of luteolin **(11)** was slightly affected with the presence of a methoxy group at C-7 position (in **12**), but was decreased by a glucose at C-5 position (in **10**). These data appear to suggest that substitution at the C-2, C-5, or C-7 position may be an important factor for the cytotoxicity of apigenin or luteolin. Further studies are needed to investigate the action mechanism of flavonoid cytotoxicity.

Table 1. Effects of flavonoids isolated from Daphnis Genkwae Flos on the viability of human hepatoma HepG2 cells

Compound	Cell viability (%)
Control	100
Apigenin **(4)**	58 ± 1*
Potassium apigenin 7-*O*-*β*-D-glucuronate **(1)**	106 ± 4
Genkwanin **(7)**	94 ± 1*
Genkwanin 5-*O*-*β*-D-primeveroside **(5)**	103 ± 2
Tiliroside **(8)**	109 ± 2*
Luteolin **(11)**	59 ± 1*
7-*O*-Methylluteolin **(12)**	59 ± 1*
Luteolin 5-*O*-*β*-glucoside **(10)**	90 ± 1*

Each value represents the mean ± S.E. of three determinations significantly different from control ($p < 0.05$).

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芫花之類黃酮類成分及細胞毒性研究

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摘 要

我們自芫花藥材分離得到12種黃酮類化合物，其結構經確認為：potassium apigenin 7-*O*-*β*-D-glucuronate **(1)**，apigenin 7-*O*-*β*-D-glucuronide **(2)**，apigenin 7-*O*-*β*-D-methylglucuronate **(3)**，apigenin **(4)**，genkwanin 5-*O*-*β*-D-primeveroside **(5)**，genkwanin 5-*O*-*β*-D-glucoside **(6)**，genkwanin **(7)**，tiliroside **(8)**，kaempferol **(9)**，luteolin 5-*O*-*β*-D-glucoside **(10)**，luteolin **(11)** 及7-*O*-methylluteolin **(12)**。其中化合物**2**、**3**、**5**、**6**、**9**和**10**是已知化合物而首次由此植物中分離得到。化合物**1**是首次由天然物中分離而得。各成分之結構係依其物理性質及光譜數據確認。

使用0.1mM apigenin **(4)**, luteolin **(11)**及7-*O*-methylluteolin **(12)**處理人類肝癌HepG2細胞48小時造成細胞存活率下降約40%，但是potassium apigenin 7-*O*-*β*-D-glucuronate **(1)**, genkwanin 5-*O*-*β*-D-primeveroside **(5)**, genkwanin **(7)**, tiliroside **(8)**及luteolin 5-*O*-*β*-D-glucoside **(10)**處理對細胞存活率則較無顯著影響。本實驗結果顯示類黃酮細胞毒性可能有複雜的構造效應關係。

關鍵詞：芫花，瑞香科，類黃酮類成分，細胞毒性