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Phytochemical Constituents of *Nelumbo nucifera*

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Abstract – Phytochemical investigation of the MeOH extract of the leaves of *Nelumbo nucifera* resulted in the isolation of five norsesquiterpenes, four flavonoids, two triterpenes and one alkaloid. Their chemical structures were characterized by spectroscopic methods to be (*E*)-3-hydroxymegastigm-7-en-9-one (**1**), (3*S*,5*R*,6*S*,7*E*)-megastigma-7-ene-3,5,6,9-tetrol (**2**), dendranthemoside B (**3**), icariside B_2 (**4**), sedumoside F_1 (**5**), luteolin (**6**), quercetin 3-*O*-β-D-glucuronide (**7**), quercetin 3-*O*-β-D-glucoside (**8**), isorhamnetin 3-*O*-rutinoside (**9**), alphitolic acid (**10**), maslinic acid (**11**), and *N*-methylasimilobine (**12**). Norsesquiterpenoids (**1-5**) and triterpenes (**10-11**) were isolated for the first time from this plant. Compounds **6** and **10-12** exhibited considerable cytotoxicity against four human cancer cell lines *in vitro* using a SRB bioassay.
Keywords – *Nelumbo nucifera*, Nymphaeaceae, Norsesquiterpenes, Cytotoxicity

Introduction

Nelumbo nucifera GAERTN (Nymphaeaceae) is a perennial aquatic plant, which is distributed throughout Asia (Van Bergen *et al*., 1997; Kim, 1996). The seeds of *N. nucifera* have been used in traditional medicine for the alleviation of fever and treatment of bleeding, dizziness and hematuria (Bensky and Gamble, 1993). Thus far, much works have been done on the phytochemical constituents of *N. nucifera*. Mainly, various alkaloids such as nuciferine, *N*-nornuciferine, roemerine, liensinine, neferine and (−)-1(*S*)-norcoclaurine, have been reported from this herb (Furukawa, 1996; Luo *et al.,* 2005; Kashiwada *et al*., 2005; Wu *et al*., 2004; Agnihotri *et al*., 2008). Flavonoids and their glycosides including (+)-catechin, quercetin, kaempferol and nelumboroside A have been also isolated (Kim *et al*., 2001; Jung *et al*., 2003; Hyun *et al*., 2006). The extract of this herb exhibited anti-diabetic (Lee *et al*., 2001), anti-oxidant (Hyun *et al*., 2006) and anti-obesity activities (Ono *et al*., 2006). As parts of our continuing search for bioactive constituents from Korean natural sources, we investigated the constituents of the leaves of *N. nucifera.* As a consequence, we isolated five norsesquiterpenes (**1-5**), four flavonoids (**6** - **9**), two triterpenes (**10** and **11**) and one alkaloid (**12**) from its MeOH extract. This paper is the first report of isolation of norsesquiterpenoids from this plant source. The isolated triterpenes (**10** - **11**) were also isolated for the first time from this plant. All the isolated compounds were tested for their cytotoxic activities against four human cancer cell lines *in vitro* using a SRB bioassay.

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Experimental

General – Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1020 Polarimeter. IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. UV spectra were recorded with a Schimadzu UV-1601 UV-Visible spectrophotometer. NMR spectra were recorded on a Varian UNITY INOVA 500 NMR spectrometer. EIMS and FABMS data were obtained on a JEOL JMS700 mass spectrometer, and LC-ESI/MS data on an Agilent 1100LC/ MSD trap SL LC/MS. Preparative HPLC was performed using a Gilson 306 pump with a Shodex refractive index detector and Alltech Silica 5 μ column (250 × 22 mm) or Econosil® RP-18 10 μ column (250 × 22 mm). Silica gel 60 (Merck, 70~230 mesh and 230~400 mesh) was used for column chromatography. TLC was performed using Merck precoated Silica gel F_{254} plates and RP-18 F_{254s} plates. The packing material for molecular sieve column chromatography was Sephadex LH-20 (Pharmacia Co.). Low-pressure liquid chromatography was performed over Merck LiChroprep Lobar®-A Si 60 (240 × 10 mm) or LiChroprep Lobar®-A RP-18 (240 × 10 mm)

columns with a FMI QSY-0 pump (ISCO). Sep-Pak® (Waters, Vac 6cc) and RediSep® (ISCO, C-18 Reverse Phase 4.3 g) were also used for column chromatography.

Plant materials – The leaves of *Nelumbo nucifera* (4.5 kg) were purchased from Kyungdong herbal market, Seoul, Korea, in July 2005. A voucher specimen (SKKU-2005-7) of the plant was deposited in the herbarium of the College of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and isolation – The leaves of *N. nucifera* (4.5 kg) were extracted at room temperature with 80% MeOH and evaporated under reduced pressure to give a residue (656 g), which was dissolved in water (800 mL) and partitioned with solvent to give hexane (49 g), CH_2Cl_2 (16 g), EtOAc (10 g), and BuOH (30 g) soluble portions. The hexane fraction (49 g) was separated over a silica gel column (hexane : EtOAc = 7 : 1 – 1 : 1) to yield six fractions (H1 - H6). Fraction H5 (1.1 g) was further separated over an RP-C_{18} silica gel column (60% MeOH) to yield ten fractions (H51 - H510). Fraction H53 (25 mg) was purified over a silica gel prep. HPLC (CH_2Cl_2 : MeOH = 90 : 1) to yield compound **1** (11 mg, R_t = 15.5 min). In turn, fraction H58 (25 mg) and H59 (42 mg) were purified over a silica gel prep. HPLC (hexane : EtOAc = 1 : 1) to give compounds **10** (10 mg, R_t = 17.0 min) and **11** (9 mg, R_t = 14.5 min), respectively. The EtOAc fraction (10 g) was separated over a silica gel column with a solvent system of $CHCl_3$: MeOH (10 : 1 – 1 : 1) as the eluant to give sixteen fractions (E1 - E16). Fraction E10 (519 mg) was further separated over an RP-C_{18} silica gel column (50% MeOH) and purified by repeating recrystallization using MeOH to give compound **6** (9 mg). In turn, fraction E16 (473 mg) was further separated over an RP-C_{18} silica gel column (50% MeOH) and purified with RediSep® (50% MeOH) to afford compound **7** (6 mg). Similarly, the BuOH fraction (30 g) was separated over a silica gel column ($CHCl_3$: MeOH : H_2O = 7 : 3 : 0.5) to give seven fractions (B1 - B7). Fraction B1 (1.0 g) was separated over a Sephadex LH-20 column (CH_2Cl_2 : MeOH = 1 : 1) and silica gel column ($CHCl_3$: MeOH = 10 : 1), and purified with RediSep® (60% MeOH) to give compound **12** (7 mg). Fraction B3 was further separated over an RP-C_{18} silica gel column (20% MeOH) to yield four fractions (B31 - B34). In turn, fraction B31 (178 mg) was purified with a silica Lobar A®-column (CH_2Cl_2 : MeOH = 8 : 1) and silica gel prep. HPLC (hexane : $CHCl_3$: MeOH = 3 : 5 : 1) to give compound **2** (11 mg, R_t = 13.5 min). Fraction B33 (100 mg) was separated over an RP-C_{18} silica Lobar A®-column (40% MeOH) and purified with a silica gel prep. HPLC (CH_2Cl_2 : MeOH = 8 : 1) to give compound **4** (6 mg, R_t = 13.0 min). Fraction B32 (230 mg) was separated over a silica Lobar A®-column (hexane : $CHCl_3$: MeOH = 1 : 5 : 1) and purified with a silica gel prep. HPLC (EtOAc : MeOH : H_2O = 11 : 1 : 0.5) to give compound **3** (38 mg, R_t = 16.5 min). Fraction B34 (57 mg) was separated over RediSep® (80% MeOH) and purified with a silica gel prep. HPLC ($CHCl_3$: MeOH = 11 : 1) to give compound **5** (5 mg, R_t = 12.5 min). Fraction B5 (1.8 g) was further separated over a silica gel column (EtOAc : MeOH : H_2O = 10 : 1 : 0.5) to give two fractions (B51 - B52). In turn, fraction B51 (20 mg) was purified with Sep-Pak® ($CHCl_3$: MeOH = 5 : 1) to give compound **8** (5 mg). Fraction B52 (171 mg) was purified with an RP-C_{18} prep. HPLC (45% MeOH) to give compound **9** (5 mg, R_t = 20.0 min).

(*E*)-3-Hydroxymegastigm-7-en-9-one (1) – Coloress gum; $[\alpha]_D^{25}$: −7.7° (*c* 0.3, $CHCl_3$); FAB-MS *m/z* : 211 $[M+H]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.83 (3H, d, *J* = 6.5 Hz, H-13), 0.89 (3H, s, H-11), 0.92 (3H, s, H-12), 0.95 (1H, m, H-4a), 1.17 (1H, m, H-2a), 1.54 (1H, dd, *J* = 10, 11 Hz, H-6a), 1.67 (1H, m, H-5a), 1.79 (1H, m, H-2b), 2.08 (1H, m, H-4b), 2.27 (3H, s, H-10), 3.84 (1H, dddd, *J* = 4.5, 4.5, 11.5, 12.0 Hz, H-3), 6.07 (1H, d, *J* = 16.0 Hz, H-8), 6.53 (1H, dd, *J* = 10.0, 16.0 Hz, H-7); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 21.3 (C-13), 21.8 (C-11), 27.4 (C-10), 31.1 (C-5), 31.5 (C-12), 35.8 (C-1), 44.5 (C-4), 50.3 (C-2), 57.8 (C-6), 66.8 (C-3), 133.9 (C-8), 149.2 (C-7), 199.5 (C-9).

(3*S*,5*R*,6*S*,7*E*)-Megastigma-7-ene-3,5,6,9-tetrol (2) – Coloress gum; $[\alpha]_D^{25}$: −22.7° (*c* 0.23, MeOH); FAB-MS *m/z* : 245 $[M+H]^+$; ^{1}H-NMR (500 MHz, CD_3OD): δ 0.88 (3H, s, H-11), 1.11 (3H, s, H-13), 1.23 (3H, s, H-12), 1.27 (3H, d, *J* = 6.0 Hz, H-10), 1.47 (1H, m, H-2a), 1.68 (1H, t, *J* = 12.0 Hz, H-2b), 1.72 (1H, t, *J* = 12.0 Hz, H-4a), 1.75 (1H, m, H-4b), 4.05 (1H, m, H-3), 4.34 (1H, m, H-9), 5.79 (1H, dd, *J* = 6.0, 16.0 Hz, H-8), 6.09 (1H, dd, *J* = 1.0, 16.0 Hz, H-7); ^{13}C-NMR (125 MHz, CD_3OD): δ 22.9 (C-10), 25.0 (C-12), 25.8 (C-13), 26.3 (C-11), 39.5 (C-1), 44.5 (C-4), 45.3 (C-2), 64.1 (C-3), 68.3 (C-9), 76.5 (C-5), 77.7 (C-6), 129.9 (C-7), 134.9 (C-8).

Dendranthemoside B (3) – Coloress gum; $[\alpha]_D^{25}$: −42.1° (*c* 1.0, MeOH); FAB-MS *m/z* : 411 $[M+Na]^+$; ^{1}H-NMR (500 MHz, CD_3OD): δ 0.83 (3H, d, *J* = 6.5 Hz, H-13), 0.89 (3H, s, H-11), 1.06 (3H, s, H-12), 1.51 (1H, m, H-4a), 1.60 (1H, m, H-2a), 1.69 (1H, m, H-2b), 1.87 (1H, m, H-4b), 2.12 (1H, m, H-5), 2.29 (3H, s, H-10), 3.14-3.88 (5H, m, sugar-H), 3.99 (1H, m, H-3), 4.38 (1H, d, *J* = 8.0 Hz, H-1'), 6.35 (1H, d, *J* = 16.0 Hz, H-8), 6.89 (1H, d, *J* = 16.0 Hz, H-7); ^{13}C-NMR (125 MHz, CD_3OD): δ 15.3 (C-13), 23.9 (C-12), 24.7 (C-11), 26.2 (C-10), 34.1 (C-5), 36.6 (C-4), 39.7 (C-1), 41.2 (C-2), 61.6 (C-6'), 70.5 (C-

4'), 73.9 (C-2'), 74.2 (C-3), 76.6 (C-5'), 76.8 (C-3'), 77.8 (C-6), 101.5 (C-1'), 130.4 (C-8), 153.0 (C-7), 199.6 (C-9).

Icariside B_2 (4) – Coloress gum; $[\alpha]_D^{25}$: -69.8° (*c* 0.07, MeOH); FAB-MS *m/z* : 386 $[M]^+$; ^{1}H-NMR (500 MHz, CD_3OD): δ 0.97 (3H, s, H-11), 1.17 (3H, s, H-12), 1.22 (3H, s, H-13), 1.43 (1H, m, H-2a), 1.74 (1H, m, H-4a), 1.80 (1H, m, H-2b), 2.30 (3H, s, H-10), 3.14-3.85 (5H, m, sugar-H), 3.87 (1H, m, H-3), 4.35 (1H, d, *J* = 8.0 Hz, H-1'), 6.20 (1H, d, *J* = 16.0 Hz, H-8), 7.17 (1H, d, *J* = 16.0 Hz, H-7); ^{13}C-NMR (125 MHz, CD_3OD): δ 19.0 (C-13), 24.3 (C-12), 26.2 (C-10), 28.2 (C-11), 34.7 (C-1), 36.9 (C-4), 44.0 (C-2), 61.5 (C-6'), 67.1 (C-5), 69.9 (C-6), 70.4 (C-4'), 71.5 (C-3), 73.9 (C-2'), 76.7 (C-5'), 76.9 (C-3'), 101.7 (C-1'), 132.6 (C-8), 144.0 (C-7), 199.0 (C-9).

Sedumoside F_1 (5) – Coloress gum; $[\alpha]_D^{25}$: −3.3° (*c* 0.03, MeOH); FAB-MS *m/z* : 397 $[M+Na]^+$; ^{1}H-NMR (500 MHz, CD_3OD): δ 0.82 (3H, d, *J* = 6.5 Hz, H-13), 0.88 (3H, s, H-11), 0.91 (3H, s, H-12), 0.92 (1H, m, H-4a), 1.11 (1H, m, H-2a), 1.28 (3H, d, *J* = 6.5 Hz, H-10), 1.32 (1H, m, H-6), 1.53 (1H, m, H-5), 1.69 (1H, m, H-2b), 1.96 (1H, H-4b), 3.14-3.80 (5H, m, sugar-H), 3.81 (1H, m, H-3), 4.35 (1H, d, *J* = 8.0 Hz, H-1'), 4.36 (1H, m, H-9), 5.35 (1H, dd, *J* = 10.0, 16.0 Hz, H-7), 5.53 (1H, dd, *J* = 7.0, 16.0 Hz, H-8); ^{13}C-NMR (125 MHz, CD_3OD): δ 21.6 (C-10), 26.8 (C-11), 26.9 (C-13), 32.1 (C-5), 32.2 (C-12), 35.8 (C-1), 45.5 (C-4), 51.1 (C-2), 58.5 (C-6), 62.4 (C-6'), 67.3 (C-3), 71.2 (C-4'), 75.3 (C-2'), 77.9 (C-5'), 78.0 (C-3'), 78.0 (C-9), 102.2 (C-1'), 133.1 (C-7), 136.4 (C-8).

Luteolin (6) – Yellow amorphous powder; ESI-MS (negative) *m/z* : 285 $[M-H]^-$; UV λ_{max} (MeOH) nm: 253, 267, 291 (sh), 349; IR ν_{max} (KBr): 3400, 1600, 1605, 1498 cm^{-1}; ^{1}H-NMR (500 MHz, CD_3OD): δ 6.22 (1H, s, H-6), 6.45 (1H, s, H-8), 6.54, (1H, s, H-3), 6.91 (1H, d, *J* = 8.5 Hz, H-5'), 7.37 (1H, br. s, H-2'), 7.38 (1H, br. s, H-6'); ^{13}C-NMR (125 MHz, CD_3OD): δ 93.8 (C-8), 98.9 (C-6), 102.7 (C-3), 104.1 (C-10), 113.0 (C-6'), 115.6 (C-5'), 119.1 (C-2'), 122.5 (C-1'), 145.9 (C-3'), 149.8 (C-4'), 158.2 (C-9), 162.0 (C-5), 164.8 (C-7), 165.2 (C-2), 182.7 (C-4).

Quercetin 3-*O*-β-D-glucuronide (7) – Yellow amorphous powder; ESI-MS (negative) *m/z* : 477 $[M-H]^-$; UV λ_{max} (MeOH) nm: 253, 267, 291; ^{1}H-NMR (500 MHz, CD_3OD): δ 3.31-3.95 (5H, m, sugar-H), 5.24 (1H, d, *J* = 8.0 Hz, H-1"), 6.22 (1H, s, H-6), 6.41, (1H, s, H-8), 6.85 (1H, d, *J* = 8.5 Hz, H-5'), 7.58 (1H, dd, *J* = 2.0, 8.5 Hz, H-6'), 7.60 (1H, d, *J* = 2.0 Hz, H-2'); ^{13}C-NMR (125 MHz, CD_3OD): δ 71.4 (C-2"), 73.8 (C-3"), 75.6 (C-4"), 75.6 (C-5"), 93.6 (C-8), 98.8 (C-6), 101.4 (C-1"), 103.9 (C-10), 115.2 (C-2'), 116.2 (C-5'), 120.6 (C-1'), 120.9 (C-6'), 133.2 (C-3), 144.9 (C-3'), 148.6 (C-4'), 156.3 (C-2), 156.3 (C-9), 161.3 (C-5), 164.3 (C-7), 168.7 (C-6"), 177.2 (C-4).

Quercetin 3-*O*-β-D-glucoside (8) – Yellow amorphous powder; FAB-MS *m/z* : 465 $[M+H]^+$; UV λ_{max} (MeOH) nm : 257, 264 (sh), 295, 355; IR ν_{max} (KBr): 3415, 1655, 1605, 1205 cm^{-1}; ^{1}H-NMR (500 MHz, CD_3OD): δ 5.25 (1H, d, *J* = 7.5 Hz, H-1"), 6.21 (1H, *J* = 2.0 Hz, H-6), 6.40 (1H, *J* = 2.0 Hz, H-8), 6.88 (1H, d, *J* = 8.0 Hz, H-5'), 7.59 (1H, dd, *J* = 8.0, 2.0 Hz, H-6'), 7.73 (1H, d, *J* = 2.0 Hz, H-2'); ^{13}C-NMR (125 MHz, CD_3OD) : δ 61.3 (C-6"), 70.0 (C-4"), 74.6 (C-2"), 76.9 (C-3"), 77.2 (C-5"), 93.6 (C-8), 98.7 (C-6), 103.2 (C-1"), 104.5 (C-10), 114.9 (C-2'), 116.4 (C-5'), 121.7 (C-6'), 121.9 (C-1'), 134.5 (C-3), 144.6 (C-3'), 148.7 (C-4'), 157.3 (C-2), 157.8 (C-9), 161.8 (C-5), 164.9 (C-7), 178.3 (C-4).

Isorhamnetin 3-*O*-rutinoside (9) – Yellow amorphous powder; FAB-MS *m/z* : 625 $[M+H]^+$; ^{1}H-NMR (500 MHz, CD_3OD): δ 1.10 (1H, d, *J* = 6.0 Hz, H-6'''), 3.97 (1H, s, OCH_3), 4.53 (1H, s, H-1'''), 5.24 (1H, d, *J* = 7.5 Hz, H-1"), 6.23 (1H, s, H-6), 6.43 (1H, s, H-8), 6.93 (1H, d, *J* = 8.0 Hz, H-5'), 7.65 (1H, d, *J* = 8.0 Hz, H-6'), 7.96 (1H, s, H-2'); ^{13}C-NMR (125 MHz, CD_3OD): δ 16.7 (C-6'''), 55.6 (C-3'-OCH_3), 67.3 (C-6"), 68.6 (C-5'''), 70.4 (C-4"), 70.8 (C-2'''), 70.9 (C-3'''), 72.7 (C-4'''), 74.7 (C-2"), 76.2 (C-5"), 77.0 (C-3"), 93.8 (C-8), 98.9 (C-6), 101.3 (C-1'''), 103.0 (C-1"), 104.5 (C-10), 113.4 (C-2'), 114.9 (C-5'), 121.8 (C-6'), 122.7 (C-1'), 134.3 (C-3), 147.2 (C-3'), 149.6 (C-4'), 157.4 (C-2), 157.7 (C-9), 161.9 (C-5), 165.1 (C-7).

Alphitolic acid (10) – White powder, mp 275 - 278 °C; $[\alpha]_D^{25}$: +176.8° (*c* 0.065, $CHCl_3$); ESIMS *m/z* : 471 $[M-H]^-$; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.82 (3H, s, H-24), 0.92 (3H, s, H-25), 0.96 (3H, s, H-26), 0.99 (3H, s, H-23), 1.03 (3H, s, H-27), 1.71 (3H, s, H-30), 3.01 (1H, m, H-19), 3.70 (1H, m, H-2), 4.63 (1H, d, *J* = 1.5 Hz, H-29β), 4.75 (1H, d, *J* = 1.5 Hz, H-29α); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 14.9 (C-27), 16.3 (C-26), 16.7 (C-24), 17.6 (C-25), 18.5 (C-6), 19.6 (C-30), 21.2 (C-11), 25.6 (C-12), 28.7 (C-23), 29.9 (C-21), 30.8 (C-15), 32.4 (C-16), 34.4 (C-7), 37.2 (C-22), 38.5 (C-13), 38.8 (C-10), 39.4 (C-4), 41.0 (C-8), 42.7 (C-14), 47.0 (C-19), 47.1 (C-1), 49.5 (C-18), 50.7 (C-9), 55.7 (C-5), 56.5 (C-17), 69.8 (C-2), 84.1 (C-3), 110.3 (C-29), 150.5 (C-20), 177.0 (C-28).

Maslinic acid (11) – White powder, mp 241 - 245 °C; $[\alpha]_D^{25}$: +45.1° (*c* 0.17, MeOH); IR λ_{max} ($CHCl_3$) : 3400, 2910, 1675 cm^{-1}; EIMS *m/z* : 472 (M^+), 426, 408, 248, 223, 203; ^{1}H-NMR (500 MHz, CD_3OD): δ 0.81 (3H, s, H-29), 0.82 (3H, s, H-26), 0.91 (3H, s, H-25), 0.95 (3H, s, H-30), 1.01 (3H, s, H-24), 1.02(3H, s, H-27), 1.17 (3H, s, H-23), 2.91 (1H, d, *J* = 10.0 Hz, H-3), 3.62 (1H, m, H-2), 5.27 (1H, t, *J* = 3.5 Hz, H-12); ^{13}C-NMR (125 MHz, CD_3OD):

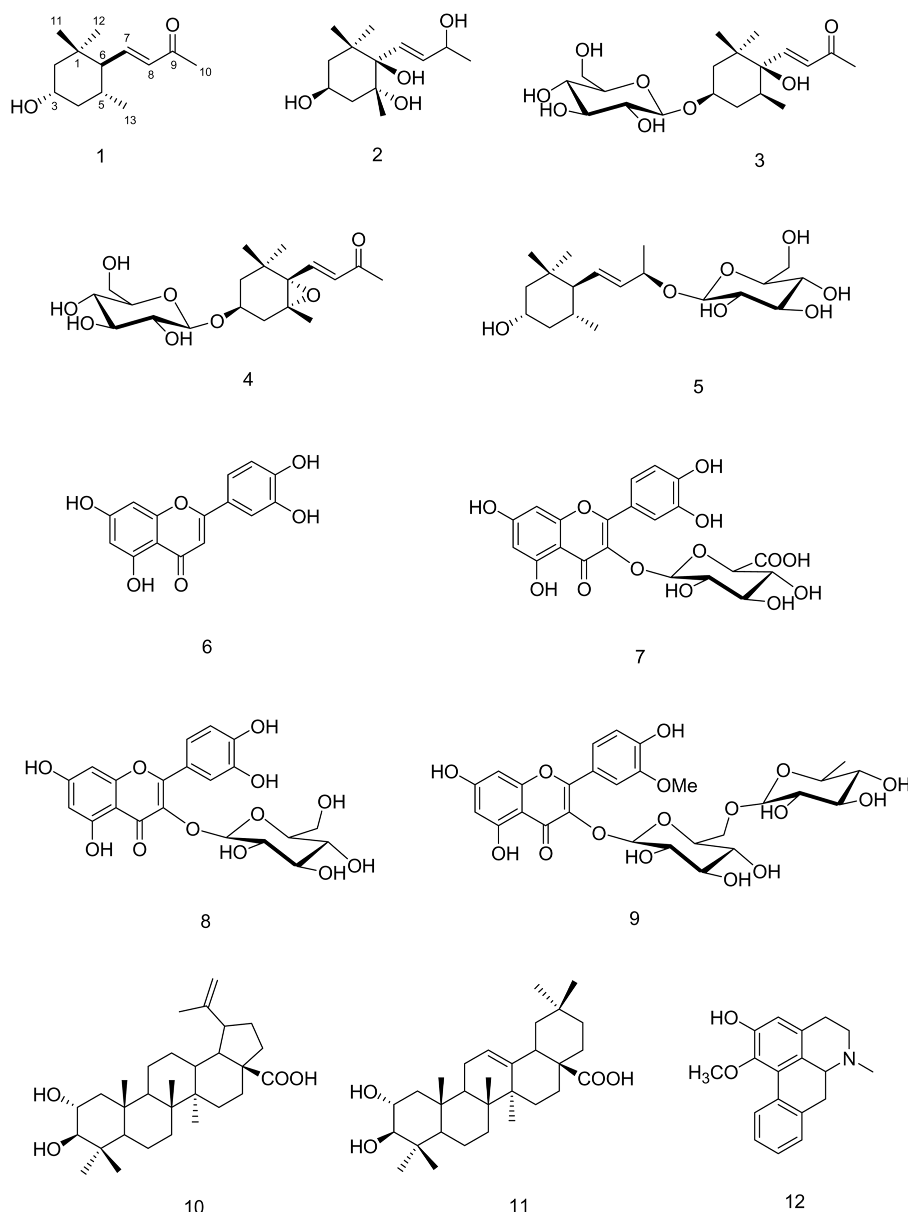

Fig. 1. The structures of **1-12** from *N. nucifera*

δ 16.4 (C-25), 17.1 (C-24), 17.2 (C-26), 18.4 (C-6), 23.3 (C-30), 23.3 (C-16), 23.5 (C-11), 25.7 (C-27), 27.8 (C-15), 28.9 (C-23), 30.5 (C-20), 32.7 (C-22), 32.8 (C-29), 32.8 (C-7), 33.8 (C-21), 38.0 (C-10), 39.3 (C-8), 39.4 (C-4), 41.5 (C-18), 41.7 (C-14), 46.0 (C-19), 46.2 (C-17), 47.3 (C-1), 47.7 (C-9), 55.4 (C-5), 68.0 (C-2), 83.2 (C-27), 121.7 (C-12), 144.4 (C-13), 179.0 (C-30).

***N*-Methylasimilobine** (**12**) – Brown gum; FAB-MS *m/z* : 282 $[M+H]^+$; $[\alpha]_D^{25}$: –211.2° (*c* 0.66, $CHCl_3$); UV λ_{max} (MeOH) nm : 253, 267, 291 (sh), 349; IR λ_{max} (KBr): 3400, 1600, 1589, 1570, 1496, 1471, 1452, 1423, 1373, 1298, 1273, 1242, 1173, 1142 cm^{-1}; ^{1}H-NMR (500 MHz, CD_3OD): δ 2.53 (3H, s, N-CH_3), 3.01 (2H, m, H-4), 3.12 (2H, m, H-5), 3.18 (2H, m, H-7), 3.56 (3H, s, 1-OCH_3), 6.64 (1H, s, H-3), 7.21 (1H, m, H-10), 7.24 (1H, m, H-9), 7.31 (1H, m, H-8), 8.30 (1H, d, J = 7.5 Hz, H-11); ^{13}C-NMR (125 MHz, CD_3OD): δ 28.0 (C-4), 34.4 (C-7), 42.6 (C-6, N-CH_3), 53.1 (C-5), 59.2 (C-1, OCH_3), 62.7 (C-6a), 115.0 (C-3), 126.0 (C-1a), 126.5 (C-1b), 127.0 (C-11), 127.4 (C-9), 127.6 (C-10), 127.9 (C-8), 128.9 (C-3a), 132.1 (C-11a), 135.9 (C-7a), 144.1 (C-1), 150.0 (C-2).

Test for cytotoxicity *in vitro* – Sulforhodamin B bioassay (SRB) was used as for cytotoxicity screening (Skehan *et al.,* 1990). The *in vitro* cytotoxicity of each

compound against four cultured human tumor cells was assessed at the Korean Research Institute of Chemical Technology. The cell lines used were A549 (non small cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), SK-MEL-2 (skin melanoma), and HCT15 (colon cancer cells). Doxorubicin was used as a positive control. The cytotoxicities of doxorubicin against A549, SK-OV-3, SK-MEL-2, and HCT cell lines were IC_{50} 0.007, 0.056, 0.117, and 0.164 μM, respectively.

Results and Discussion

Compounds **2 - 4**, and **6 - 12** were identified by comparing the ^{1}H-, ^{13}C-NMR, and MS spectral data with the literature values to be (3*S*,5*R*,6*S*,7*E*)-megastigma-7-ene-3,5,6,9-tetrol (**2**) (Kijima *et al*., 1996), dendranthemoside B (**3**) (Pauli *et al*., 1990), icariside B_2 (**4**) (Otsuka *et al*., 1995), luteolin (**6**) (Kwak *et al*., 2009), quercetin 3-*O*-β-D-glucuronide (**7**) (Ishimatsu *et al*., 1989), quercetin 3-*O*-β-D-glucoside (**8**) (Kim, 2001), isorhamnetin 3-*O*-rutinoside (**9**) (Xu *et al*., 2006), alphitolic acid (**10**) (Bae *et al*, 1996), maslinic acid (**11**) (Kim *et al*., 2005), and *N*-methylasimilobine (**12**) (Han *et al*., 1989). The terpenoids **1-5**, and **10-11** were isolated for the first time from this plant. The following describes the structural elucidation of compounds **1** and **5**, which were for the second time isolated from natural sources.

Compound **1** was obtained as a colorless gum with a negative optical rotation ($[\alpha]_D^{25}$: −7.7° in $CHCl_3$). From the FAB-MS (*m/z* 211 $[M+H]^+$) and ^{1}H- and ^{13}C-NMR spectral data, the molecular formula of **1** was deduced to be $C_{13}H_{22}O_2$. The ^{1}H-NMR spectrum showed four methyls at δ 0.83 (3H, d, J = 6.5 Hz, H-13), 0.89 (3H, s, H-11), 0.92 (3H, s, H-12), and 2.27 (3H, s, H-10), one methine bearing an oxygen function at δ 3.84 (1H, dddd, J = 4.5, 4.5, 11.5, 12.0 Hz, H-3), and an *trans*-olefinic protons at δ 6.07 (1H, d, J = 16.0 Hz, H-8), and 6.53 (1H, dd, J = 10.0, 16.0 Hz, H-7). The ^{13}C-NMR spectrum demonstrated the presence of 13 carbon signals, consisting of one carbonyl carbon signal at δ 199.5, and two olefinic carbon signals at δ 133.9 and 149.2, which indicated the presence of an α,β-unsaturated ketone moiety. These spectral data suggested that **1** was an ionane-type norsesquiterpene (Weyerstahl *et al*., 1994). The coupling pattern (dddd, J = 4.5, 4.5, 11.5, 12.0 Hz) of H-3 implied that the configuration of OH group at C-3 was an equatorial form based on comparison with published data (Weyerstahl *et al*., 1994). This was also confirmed by the NOESY experiment, in which correlations of H-3 with H-5 and H-11, and of H-12 with H-13 were observed. Consequently, the structure of **1** was determined to be (*E*)-3-hydroxymegastigm-7-en-9-one.

Table 1. Cytotoxic activities of compounds (**1 - 12**) isolated from *N. nucifera*

Compound	IC_{50} (μM)[a]			
	A549	SK-OV-3	SK-MEL-2	HCT-15
1	>30.0	>30.0	>30.0	>30.0
2	>30.0	>30.0	>30.0	>30.0
3	>30.0	>30.0	>30.0	>30.0
4	>30.0	>30.0	>30.0	>30.0
5	>30.0	>30.0	>30.0	>30.0
6	12.1	9.8	7.0	9.7
7	>30.0	>30.0	>30.0	>30.0
8	>30.0	>30.0	>30.0	>30.0
9	>30.0	>30.0	>30.0	>30.0
10	10.0	13.5	12.1	8.1
11	13.7	16.5	15.8	11.7
12	13.1	14.6	6.0	2.8
Doxorubicin	0.007	0.056	0.117	0.164

[a]IC_{50} value of compounds against each cancer cell line, which was defined as the concentration (μM) that caused 50% inhibition of cell growth *in vitro*.

Compound **5** was obtained as a colorless gum with a negative optical rotation ($[\alpha]_D^{25}$: −3.3° in MeOH). From the FAB-MS (*m/z* 397 $[M+Na]^+$) and ^{1}H- and ^{13}C-NMR spectral data, the molecular formula of **5** was deduced to be $C_{19}H_{34}O_7$. The ^{1}H-NMR spectrum showed four methyls at δ 0.82 (3H, d, J = 6.5 Hz, H-13), 0.88 (3H, s, H-11), 0.91 (3H, s, H-12), and 1.28 (3H, d, J = 6.5 Hz, H-10), two methines bearing an oxygen function at δ 3.81 (1H, m, H-3), and 4.36 (1H, m, H-9), an *trans*-olefinic protons at δ 5.35 (1H, dd, J = 10.0, 16.0 Hz, H-7), and 5.53 (1H, dd, J = 7.0, 16.0 Hz, H-8), together with β-D-glucopyranosyl moiety at 3.14-3.80 (5H, m, sugar-H), and 4.35 (1H, d, J = 8.0 Hz, H-1'). The ^{13}C-NMR spectrum demonstrated the presence of 19 carbon signals, consisting of two olefinic carbon signals at δ 133.1 and 136.4, and two oxygenated carbon signals at δ 67.3 and 78.0. An anomeric carbon signal at δ 102.2 and five oxygenated carbon signals (δ 78.0, 77.9, 75.3, 71.2, and 62.4) suggested the presence of glucopyranoside (Stephen *et al*., 1977). The coupling constant (J = 8.0 Hz) of the anomeric proton of D-glucose indicated that it was β-form (Stephen *et al*., 1977). The HMBC correlation between H-1' (δ 4.35) and C-9 (δ 78.0) indicated that sugar moiety was located at C-9. In the NOESY experiment, NOESY correlations between H-3 and H-5, H-11 and between H-11 and H-5, H-7 and between H-12 and H-13 were observed. On the basis of above information and optical rotation ($[\alpha]_D^{25}$: −3.3°) of **5** in comparison with published data ($[\alpha]_D$: −11.2°) (Morikawa

et al., 2007), the stereochemistry of carbon at C-3, 5, 6, and 9 was assigned to be *S*, *R*, *S*, and *R* form, respectively. Based on further comparison with published data (Morikawa *et al*., 2007), the structure of **5** was identified as sedumoside F_1.

The isolated compounds (**1** - **12**) were tested *in vitro* for cytotoxicity against the A549, SK-OV-3, SK-MEL-2, and HCT15 human tumor cells using the SRB assay. Compounds **6** and **10-12** exhibited considerable cytotoxicity against the A549, SK-OV-3, SK-MEL-2, and HCT15 cell lines as shown in Table 1.

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