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Terpene and Phenolic Constituents of *Lactuca indica* L.

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We isolated seven terpenes and five phenolic constituents from the aerial parts of *Lactuca indica* L. using column chromatographic separation of its MeOH extract. Their structures were determined by spectroscopic methods to be *trans*-phytol (**1**), 3β-hydroxyglutin-5-ene (**2**), 5,6-epoxy-3-hydroxy-7-megastigmen-9-one (**3**), 11β-13-dihydrolactucin (**4**), 2-phenylethyl β-D-glucopyranoside (**5**), cichorioside B (**6**), 1-hydroxylinaloyl-6-*O*-β-D-glucopyranoside (**7**), (6*S*,9*S*)-roseoside (**8**), benzyl-β-D-glucopyranoside (**9**), 2-(3'-*O*-β-D-glucopyranosyl-4'-hydroxyphenyl)-ethanol (**10**), 3-(β-D-glucopyranosyloxymethyl)-2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-dihydrobenzofuran (**11**), and (+)-taraxafolin-B (**12**). Compounds **1-3**, **5**, and **7-12** were isolated for the first time from this plant source. The isolated compounds were tested for cytotoxicity against four human tumor cell lines *in vitro* using a Sulforhodamin B bioassay.

Key words: *Lactuca indica* L. Compositae, Terpene, Phenolic constituent, Cytotoxicity

INTRODUCTION

Lactuca indica L. (Compositae) is widely distributed throughout Korea. This indigenous herb is an edible wild vegetable and is used as a folk medicine for its anti-inflammatory and antibacterial activities and for treatment of intestinal disorders (Kan, 1986). Terpenoids (Hui *et al*., 1971) and flavonoids (Makoto *et al*., 1978) were isolated from this plant and the methanol extract of this species exhibits antidiabetic (Chia *et al*., 2003), antioxidant (Sheng *et al*., 2003), and anticholesterolemic activities (Park *et al*., 1995). In continuing our search for biologically active compounds from Korean Compositae medicinal plants, we investigated the constituents of the aerial parts of *L. indica* and reported hepatoprotective activity (Kim *et al*., 2007). In continuing research on this plant, we isolated seven terpenoids and five phenolic constituents from its MeOH extract and determined their structures by spectroscopic means. Compounds **1-3**, **5**, and **7-12** were isolated for the first time from this plant source. The isolated compounds were tested for cytotoxicity against four human tumor cells *in vitro* using a Sulforhodamin B bioassay. This paper describes the isolation, structural elucidation, and cytotoxic activity of these compounds.

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MATERIALS AND METHODS

General experimental procedure

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-ESIMS/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 Apollo 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).

Plant materials

The aerial parts of *Lactuca indica* L. were collected in Suwon City, Korea during May 2005. A voucher specimen (SKKU-2005-05) of the plant was deposited at the College of

Pharmacy at Sungkyunkwan University.

Extraction and isolation

The aerial parts of *L. indica* (5 kg) were extracted at room temperature with 80% MeOH and evaporated under reduced pressure to give a residue (200 g), which was dissolved in water (800 mL) and solvent-partitioned to give hexane (20 g), $CHCl_3$ (12 g), and BuOH fractions (75 g). The hexane fraction (20 g) was separated over a silica gel column with hexane: EtOAc (2:1) as the eluent to yield seven fractions (H1-H7). Fraction H2 (1.8 g) was subjected to Sephadex LH-20 column chromatography (CH_2Cl_2:MeOH = 1:1) and was purified with a silica gel prep. HPLC with hexane:EtOAc (15:1) to yield compound **1** (15 mg). Fraction H3 (1.2 g) was also subjected to Sephadex LH-20 column chromatography (CH_2Cl_2:MeOH = 1:1) and was purified with a silica gel prep. HPLC with hexane:EtOAc (5:1) to yield compound **2** (10 mg). The $CHCl_3$ fraction (12 g) was separated over a silica gel column with a solvent system of $CHCl_3$:MeOH (30:1-15:1) as the eluent to give eight fractions (C1-C8). The fraction C2 (0.6 g) was purified with a RP-C_{18} prep. HPLC (56% MeOH) to afford compound **3** (7 mg). Fraction C6 (0.6 g) was also purified with a RP-C_{18} prep. HPLC (30% MeOH) to yield compound **4** (5 mg). Similarly, the BuOH fraction (45 g) was separated over a silica gel column with a solvent system of $CHCl_3$:MeOH:H_2O (30:10:1-13:7:1) as the eluent to give six fractions (B1-B6). Fraction B1 (1.5 g) was separated over a RP-C_{18} silica gel column with 60% MeOH and was purified with a RP-C_{18} prep. HPLC (40% MeOH) to yield compound **5** (5 mg). Fraction B2 (5.0 g) was separated on a RP-C_{18} silica gel column with 55% MeOH and was purified with a silica gel prep. HPLC ($CHCl_3$: MeOH: H_2O = 20:4:0.1) to yield compounds **6** (5 mg) and **7** (5 mg), respectively. Fraction B3 (7.5 g) was chromatographed with a RP-C_{18} silica gel column chromatography with 40% MeOH as the eluent to give six subfractions (B31~B36). Subfraction B31 (3.5 g) was separated on a RP-C_{18} silica gel column with 40% MeOH and was purified with a silica Lobar A®-column ($CHCl_3$:MeOH:H_2O = 20:4:0.4) and a silica gel prep. HPLC ($CHCl_3$:MeOH:H_2O = 20:6:0.5) to give compounds **10** (9 mg) and **11** (7 mg), respectively. Subfraction B32 (1.0 g) was subjected to silica gel column ($CHCl_3$:MeOH:H_2O = 20:5:0.5) and purified with silica gel prep. HPLC ($CHCl_3$: MeOH:H_2O = 22:5:0.5) to yield compounds **8** (20 mg) and **9** (8 mg), respectively. Fraction B6 (3.0 g) was separated over a RP-C_{18} silica gel column with 30% MeOH as the eluent to give six subfractions (B61-B66). Subfraction B62 (350 mg) was purified with a RP-C_{18} prep. HPLC (28% MeOH) to afford compound **12** (10 mg).

***trans*-Phytol (1)**

Colorless oil, $[\alpha]_D^{20}$: +4.8° (c 0.100, MeOH); UV λ_{max} (MeOH) nm (log ε): 204 (4.4); FAB-MS *m/z*: 319 $[M+Na]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 5.41 (1H, t, *J* = 6.9 Hz, H-2), 4.16 (2H, d, *J* = 6.9 Hz, H-1), 1.98 (2H, t, *J* = 7.6 Hz, H-4), 1.67 (3H, s, H-20), 1.54-1.00 (19H, m), 0.87 (9H, d, *J* = 6.6 Hz, H-16, 18, 19), 0.84 (3H, d, *J* = 6.6 Hz, H-17); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 140.5 (C-3), 123.3 (C-2), 59.6 (C-1), 40.1 (C-4), 39.6 (C-14), 37.6 (C-8), 37.6 (C-10), 37.5 (C-12), 36.9 (C-6), 33.0 (C-7), 32.9 (C-11), 28.2 (C-15), 25.3 (C-5), 25.0 (C-13), 24.7 (C-9), 22.9 (C-17), 22.8 (C-16), 19.9 (C-19), 19.9 (C-18), 16.4 (C-20).

3β-Hydroxyglutin-5-ene (2)

Colorless oil, $[\alpha]_D^{20}$: -19.9° (c 0.015, MeOH); UV λ_{max} (MeOH) nm (log ε): 204 (0.9); FAB-MS *m/z*: 449 $[M+Na]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 5.65 (1H, d, *J* = 7.0 Hz, H-10), 3.47 (1H, br s, H-3), 0.87, 0.97, 1.00, 1.02, 1.06, 1.11, 1.16, 1.18 (each 3H, s); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 141.8 (C-5), 122.3 (C-6), 76.4 (C-3), 49.9 (C-10), 47.6 (C-8), 43.3 (C-18), 41.0 (C-4), 39.5 (C-14), 39.2 (C-22), 38.0 (C-13), 36.2 (C-16), 35.3 (C-19), 35.0 (C-9), 34.8 (C-15), 34.7 (C-30), 33.3 (C-11), 32.6 (C-28), 32.3 (C-21), 32.2 (C-29), 30.5 (C-12), 30.3 (C-17), 29.1 (C-23), 28.4 (C-20), 28.0 (C-2), 25.6 (C-24), 23.8 (C-7), 19.8 (C-27), 18.6 (C-26), 18.4 (C-1), 16.4 (C-25).

5,6-Epoxy-3-hydroxy-7-megastigmen-9-one (3)

Colorless oil, $[\alpha]_D^{20}$: -54.5° (c 0.035, MeOH); IR ν_{max} cm^{-1}: 3416, 2964, 1666 and 1366 cm^{-1}; UV λ_{max} (MeOH) nm (log ε): 202 (0.9), 230 (1.5); EI-MS *m/z* (rel. int): 224 (M^+, 23), 209 (15), 191 (33), 178 (23), 165 (25), 151 (30), 137 (35), 123 (100), 109 (45) and 95 (45); ^{1}H-NMR (CD_3OD, 500 MHz): δ 7.19 (1H, d, *J* = 15.1 Hz, H-7), 6.21 (1H, d, *J* = 15.1 Hz, H-8), 3.77 (1H, m, H-3), 2.32 (1H, dd, *J* = 9.3, 5.2 Hz, H-4a), 2.30 (3H, s, H-10), 1.66 (1H, dd, *J* = 14.7, 9,3 Hz, H-4b), 1.60 (1H, dd, *J* = 12.2, 4,3 Hz, H-2a), 1.28 (1H, dd, *J* = 12.2, 10.3 Hz, H-2b), 1.20 (3H, s, H-13), 1.19 (3H, s, H-11) 0.97 (3H, s, H-12); ^{13}C-NMR (CD_3OD, 125 MHz): δ 199.0 (C-9), 144.1 (C-7), 132.6 (C-8), 69.7 (C-6), 67.5 (C-5), 63.2 (C-3), 47.0 (C-4), 40.1 (C-2), 34.9 (C-1), 29.5 (C-11), 28.5 (C-10), 23.1 (C-12), 18.8 (C-13).

11β-13-Dihydrolactucin (4)

Amorphous powder, mp. 91-94°C; $[\alpha]_D^{20}$: -16.6° (c 0.375, MeOH); IR ν_{max} cm^{-1}: 3402, 2904, 1771, 1682, 1636 and 1616 cm^{-1}; UV λ_{max} (MeOH) nm (log ε): 201 (2.0), 255 (2.4), 344 (2.1); FAB-MS *m/z*: 279 $[M+H]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 6.41 (1H, br s, H-3), 4.87 (1H, br d, *J* = 19.5 Hz, H-15a), 4.42 (1H, br d, *J* = 19.5 Hz, H-15b), 3.72 (1H, t, *J* = 9.8 Hz, H-6), 3.66 (1H, m, H-8), 3.64 (1H, br d, *J* = 9.8 Hz, H-5), 2.83 (1H, dd, *J* = 13.7, 1.9 Hz, H-9a), 2.67 (1H, m, H-9b), 2.41 (3H, s, H-14), 2.39 (1H, br d, *J* = 14.2 Hz, H-11), 2.17 (1H, q, *J* = 11.2 Hz, H-7), 1.38 (3H, d, *J* = 7.3 Hz, H-13); ^{13}C-NMR (CD_3OD, 125 MHz): δ 196.1 (C-2), 178.8 (C-12), 175.2 (C-4), 148.6 (C-10), 132.4 (C-3), 132.0 (C-1), 81.3 (C-6), 68.8 (C-15), 61.9 (C-8), 61.1 (C-7), 48.8 (C-5), 48.5 (C-9), 41.3 (C-11), 20.6 (C-14), 14.6 (C-13).

2-Phenylethyl β-D-glucopyranoside (5)

Colorless gum, UV λ_{max} (MeOH) nm (log ε): 205 (1.3), 267 (0.7); FAB-MS *m/z*: 279 $[M+H]^+$; ^{1}H-NMR (CD_3OD, 500

MHz): δ 7.25 (5H, m, H-2, 3, 4, 5, 6), 4.34 (1H, d, *J* = 7.6 Hz, H-1'), 4.11 (2H, m, H-β), 3.88 (1H, td, *J* = 14.1, 1.4 Hz, H-6'), 3.73 (1H, dd, *J* = 11.6, 5.6 Hz, H-3'), 3.68 (1H, td, *J* = 10.6, 7.2 Hz, H-5'), 3.36 (1H, m, H-2'), 3.25 (1H, m, H-4'), 2.97 (2H, t, *J* = 7.4 Hz, H-α); ^{13}C-NMR (CD_3OD, 125 MHz): δ 138.9 (C-1), 128.8 (C-3, 5), 128.1 (C-2, 6), 126.0 (C-4), 103.2 (C-1'), 76.9 (C-3'), 76.8 (C-5'), 73.9 (C-2'), 70.5 (C-4', C-β), 61.6 (C-6'), 36.6 (C-α).

Cichorioside B (6)

Colorless gum, $[\alpha]_D^{20}$: -35.1° (c 0.075, MeOH); IR ν_{max} cm^{-1}: 3400, 2915, 1771, 1683, 1617, 1078, and 1030 cm^{-1}; UV λ_{max} (MeOH) nm (log ε): 202 (1.5), 255 (1.8), 343 (1.0); ESI-MS *m/z*: 440 $[M]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 6.57 (1H, br s, H-3), 4.87 (1H, br d, *J* = 19.5 Hz, H-15a), 4.76 (1H, br d, *J* = 19.5 Hz, H-15b), 4.40 (1H, d, *J* = 7.8 Hz, H-1'), 3.86 (1H, dd, *J* = 14.1, 1.40 Hz, H-6'), 3.72 (1H, t, *J* = 9.8 Hz, H-6), 3.68 (1H, dd, *J* = 11.6, 5.6 Hz, H-3'), 3.66 (1H, m, H-8), 3.64 (1H, br d, *J* = 9.8 Hz, H-5), 3.56 (1H, td, *J* = 10.6, 7.2 Hz, H-5'), 3.36 (1H, m, H-2'), 3.32 (1H, m, H-4'), 2.83 (1H, dd, *J* = 13.7, 1.9 Hz, H-9a), 2.67 (1H, m, H-9b), 2.43 (3H, s, H-14), 2.39 (1H, br d, *J* = 14.2 Hz, H-11), 2.16 (1H, q, *J* = 11.2 Hz, H-7), 1.38 (3H, d, *J* = 7.3 Hz, H-13); ^{13}C-NMR (CD_3OD, 125 MHz): δ 196.1 (C-2), 178.8 (C-12), 170.6 (C-4), 148.8 (C-10), 132.5 (C-3), 132.2 (C-1), 102.9 (C-1'), 81.3 (C-6), 76.9 (C-3'), 76.8 (C-5'), 73.9 (C-2'), 70.4 (C-4'), 68.8 (C-15), 68.4 (C-8), 61.5 (C-6'), 61.0 (C-7), 48.8 (C-5), 48.5 (C-9), 41.3 (C-11), 20.6 (C-14), 14.5 (C-13).

1-Hydroxylinaloyl-6-*O*-β-D-glucopyranoside (7)

Colorless gum, $[\alpha]_D^{20}$: -19.5° (c 0.065, MeOH); IR ν_{max} cm^{-1}: 3380, 2928, 1563, 1413, 1078, and 1041 cm^{-1}; UV λ_{max} (MeOH) nm (log ε): 203 (1.8), 224 (1.1), 275 (0.8); ESI-MS *m/z*: 355 $[M+Na]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 5.93 (1H, dd, *J* = 17.1, 11.1 Hz, H-7), 5.49 (1H, dt, *J* = 7.4, 1.4 Hz, H-3), 5.21 (1H, dd, *J* = 17.1, 1.6 Hz, H-8a), 5.04 (1H, dd, *J* = 11.1, 1.6 Hz, H-8b), 4.25 (1H, d, *J* = 7.9 Hz, H-1'), 4.21 (1H, d, *J* = 11.2 Hz, H-1a), 4.05 (1H, d, *J* = 11.2 Hz, H-1b), 3.87 (1H, dd, *J* = 14.1, 1.4 Hz, H-6'), 3.67 (1H, dd, *J* = 11.6, 5.6 Hz, H-3'), 3.55 (1H, td, *J* = 10.6, 7.2 Hz, H-5'), 3.36 (1H, m, H-2'), 3.32 (1H, m, H-4'), 2.11 (2H, m, H-4), 1.69 (3H, s, H-9), 1.55 (2H, m, H-5), 1.26 (3H, s, H-10); ^{13}C-NMR (CD_3OD, 125 MHz): δ 145.0 (C-7), 131.7 (C-8), 128.9 (C-2), 110.9 (C-3), 101.4 (C-1'), 77.0 (C-3'), 76.7 (C-5'), 74.7 (C-1), 73.9 (C-2'), 72.5 (C-6), 70.5 (C-4'), 61.6 (C-6'), 41.7 (C-5), 26.5 (C-10), 22.2 (C-4), 12.8 (C-9).

(6*S*,9*S*)-Roseoside (8)

Colorless gum, $[\alpha]_D^{20}$: +57.7° (c 0.065, MeOH); IR ν_{max} cm^{-1}: 3419, 2974, 2932, 1654, 1374, 1078, and 1040 cm^{-1}; UV λ_{max} (MeOH) nm (log ε): 205 (1.5), 237 (1.8); ESI-MS *m/z*: 387 $[M+H]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 5.97 (1H, d, *J* = 15.6 Hz, H-7), 5.76 (1H, s, H-4), 5.73 (1H, dd, *J* = 15.6, 7.2 Hz, H-8), 4.53 (1H, m, H-9), 4.28 (1H, d, *J* = 7.9 Hz, H-1'), 3.85 (1H, dd, *J* = 14.1, 1.4 Hz, H-6'), 3.63 (1H, dd, *J* = 11.6, 5.6 Hz, H-3'), 3.59 (1H, td, *J* = 10.6, 7.2 Hz, H-5'), 3.36 (1H, m, H-2'), 3.32 (1H, m, H-4'), 2.61 (1H, d, *J* = 17.1 Hz, H-2a), 2.18 (1H, d, *J* = 17.1 Hz, H-2b), 1.94 (3H, s, H-13), 1.29 (3H, d, *J* = 6.6 Hz, H-10), 1.08 (3H, s, H-11), 1.05 (3H, s, H-12); ^{13}C-NMR (CD_3OD, 125 MHz): δ 200.0 (C-3), 165.9 (C-5), 132.6 (C-7), 132.5 (C-8), 125.9 (C-4), 100.1 (C-1'), 78.8 (C-6), 77.2 (C-3'), 77.0 (C-5'), 73.8 (C-9), 73.4 (C-2'), 70.5 (C-4'), 61.6 (C-6'), 49.6 (C-2), 41.2 (C-1), 23.5 (C-12), 22.3 (C-11), 21.0 (C-10), 18.3 (C-13).

Benzyl-β-D-glucopyranoside (9)

Colorless gum, UV λ_{max} (MeOH) nm (log ε): 206 (2.3), 236 (1.3); FAB-MS *m/z*: 271 $[M+H]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 7.43 (2H, m, H-3, 5), 7.35 (2H, m, H-2, 6), 7.27 (1H, m, H-4), 4.36 (1H, d, *J* = 7.6 Hz, H-1'), 3.89 (1H, td, *J* = 14.1, 1.4 Hz, H-6'), 3.72 (1H, dd, *J* = 11.6, 5.6 Hz, H-3'), 3.68 (1H, td, *J* = 10.6, 7.2 Hz, H-5'), 3.36 (1H, m, H-2'), 3.29 (1H, m, H-4'), 3.24 (2H, t, *J* = 7.4 Hz, H-α); ^{13}C-NMR (CD_3OD, 125 MHz): δ 138.8 (C-1), 128.0 (C-3, 5), 128.0 (C-2, 6), 127.5 (C-4), 102.1 (C-1'), 76.9 (C-3'), 76.8 (C-5'), 73.9 (C-2'), 70.5 (C-4', C-α), 61.6 (C-6').

2-(3'-*O*-β-D-Glucopyranosyl-4'-hydroxyphenyl)-ethanol (10)

Colorless gum, UV λ_{max} (MeOH) nm (log ε): 204 (2.5), 221 (1.6), 278 (1.1); FAB-MS *m/z*: 339 $[M+Na]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 7.09 (1H, d, *J* = 1.9 Hz, H-5), 6.80 (1H, dd, *J* = 8.3, 1.9 Hz, H-2), 6.78 (1H, d, *J* = 8.3, 1.9 Hz, H-6), 4.76 (1H, d, *J* = 7.8 Hz, H-1'), 3.91 (1H, dd, *J* = 14.1, 1.4 Hz, H-6'), 3.73 (1H, dd, *J* = 11.6, 5.6 Hz, H-3'), 3.71 (2H, t, *J* = 7.1 Hz, H-β), 3.49 (1H, td, *J* = 10.6, 7.2 Hz, H-5'), 3.41 (1H, m, H-2'), 3.40 (1H, m, H-4'), 2.73 (2H, t, *J* = 7.1 Hz, H-α); ^{13}C-NMR (CD_3OD, 125 MHz): δ 145.7 (C-4), 145.3 (C-3), 130.9 (C-1), 124.0 (C-2), 118.5 (C-5), 115.7 (C-6), 103.3 (C-1'), 77.1 (C-3'), 76.5 (C-5'), 73.7 (C-2'), 70.2 (C-4') 63.1 (C-β), 61.3 (C-6'), 38.3 (C-α).

3-(β-D-Glucopyranosyloxymethyl)-2-(4-hydroxy-3-methoxy-phenyl)-5-(3-hydroxypropyl)-7-methoxy-dihydrobenzofuran (11)

Colorless gum, $[\alpha]_D^{20}$: -30.7° (c 0.420, MeOH); IR ν_{max} cm^{-1}: 3391, 2937, 2882, 1608, 1518, 1500, and 1077 cm^{-1}; UV λ_{max} (MeOH) nm (log ε): 232 (2.7), 284 (2.3); FAB-MS *m/z*: 545 $[M+Na]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 7.00 (1H, d, *J* = 2.0 Hz, H-2"), 6.87 (1H, dd, *J* = 8.1, 2.0 Hz, H-6"), 6.78 (1H, br t, *J* = 1.1 Hz, H-4), 6.74 (1H, d, *J* = 7.9 Hz, H-5"), 6.73 (1H, br d, *J* = 1.1 Hz, H-6), 5.60 (1H, d, *J* = 6.5 Hz, H-2), 4.36 (1H, d, *J* = 7.9 Hz, H-1'"), 4.21 (1H, dd, *J* = 9.5, 6.1 Hz, 3-CH_{2a}), 3.84 (3H, s, 7-OCH_3), 3.81 (3H, s, 3"-OCH_3), 3.77 (1H, m, 3-CH_{2b}), 3.69 (1H, dd, *J* = 12.2, 5.1 Hz, H-6'"), 3.62 (1H, m, H-3), 3.58 (2H, t, *J* = 6.5 Hz, H-3'), 3.36-3.26 (3H, m, H-3'", 4'", 5'"), 3.24 (1H, dd, *J* = 9.1, 8.0 Hz, H-2'"), 2.63 (2H, t, *J* = 7.6 Hz, H-1'), 1.83 (2H, m, H-2'); ^{13}C-NMR (CD_3OD, 125 MHz): δ 147.8 (C-3"), 146.3 (C-4"), 146.2 (C-1a), 144.0 (C-7), 135.7 (C-5), 133.6 (C-1"), 128.5 (C-3a), 118.5 (C-6"), 117.0 (C-4), 114.9 (C-5"), 113.1 (C-6), 109.5 (C-2"), 103.4 (C-1'"), 87.8 (C-2), 77.1 (C-3'"), 76.9 (C-5'"), 74.0 (C-2'"), 71.2 (C-3-CH_2), 70.5 (C-4'"),

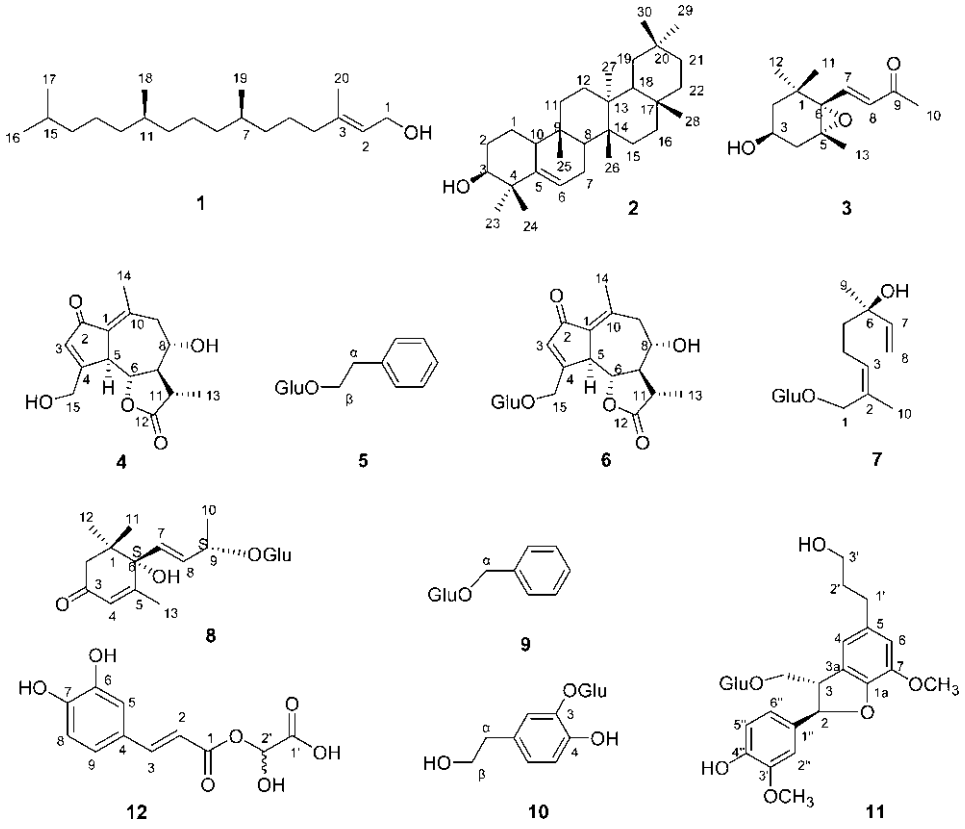

Fig. 1. The structures of compounds **1-12** from the aerial parts of *L. indica*

61.6 (C-6'"), 61.0 (C-3'), 55.6 (C-7-OCH_3), 55.2 (C-3"-OCH_3), 52.1 (C-3), 34.6 (C-2'), 31.7 (C-1').

(+)-Taraxafolin-B (12)

Brown powder, mp. 120-125°C; $[\alpha]_D^{20}$: +50.6° (c 0.510, MeOH); IR ν_{max} cm^{-1}: 3392, 2713, 1701, 1601, 1525, 1385, and 1157 cm^{-1}; UV λ_{max} (MeOH) nm (log ε): 217 (2.8), 235 (2.3), 243 (2.3), 299 (2.8), and 325 (3.0); EI-MS *m/z* (rel. int): 253 ([M-H]$^+$, 20), 237 (17), 191 (65), 167 (30), 149 (100), 147 (50), and 111 (43); ^{1}H-NMR (CD_3OD, 500 MHz): δ 7.62 (1H, d, *J* = 16.0 Hz, H-2), 7.08 (1H, d, *J* = 1.7 Hz, H-5), 6.92 (1H, dd, *J* = 8.1, 1.7 Hz, H-9), 6.79 (1H, d, *J* = 8.1 Hz, H-8), 6.43 (1H, d, *J* = 16.0 Hz, H-3), 5.74 (1H, s, H-2'); ^{13}C-NMR (CD_3OD, 125 MHz): δ 173.3 (C-1'), 167.9 (C-1), 148.1 (C-7), 145.7 (C-3), 145.5 (C-6), 126.9 (C-4), 121.7 (C-9), 115.3 (C-8), 114.6 (C-5), 114.0 (C-2), 75.3(C-2').

Test for cytotoxicity *in vitro*

Sulforhodamin B bioassays (SRB) were used as cytotoxicity screening methods (Skehan *et al*., 1990). Cytotoxicity assays for each compound were performed *in vitro* against four cultured human tumor cell lines at the Korea Research Institute of Chemical Technology: A549 (non-small cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), SK-MEL-2 (skin melanoma), and HCT15 (colon cancer cells).

RESULTS AND DISCUSSION

Compounds **1-2**, **4-6**, and **10** were identified by comparing the ^{1}H-, ^{13}C-NMR, and MS spectra with the literature to be *trans*-phytol (**1**) (Brown, 1994), 3β-hydroxyglutin-5-ene (**2**) (Antinio *et al*., 1987), 11β-13-dihydrolactucin (**4**) (Sag *et al*., 1982; Mamoru, *et al*., 1988), 2-phenylethyl β-D-glucopyranoside (**5**) (Chi *et al*., 1994; Kaoru *et al*., 1988), cichorioside B (**6**) (Sag *et al*., 1982; Mamoru, *et al*., 1988), and 2-(3'-O-β-D-glucopyranosyl-4'-hydroxyphenyl)-ethanol (**10**) (Armandodoriano *et al*., 1998). Compounds **1-3**, **5**, and **7-12** were isolated for the first time from this plant. The following describes the structural elucidation of compounds **3**, **7-9**, and **11-12**, which were not so often isolated from natural sources.

Compound **3** was obtained as a colorless oil. The IR spectrum showed the bands of a hydroxyl group at 3416 cm^{-1} and a carbonyl group at 1666 cm^{-1}. From the EI-MS (*m/z* 224 [M]$^+$) and ^{1}H- and ^{13}C-NMR spectral data, the molecular formula was deduced to be $C_{13}H_{20}O_3$. The ^{1}H-NMR spectrum showed olefinic proton signals at δ 7.19 (1H, d, *J* = 15.1 Hz) and 6.21 (1H, d, *J* = 15.1 Hz), an oxygenated proton signal at δ 3.77 (1H, m), and four methyl groups at δ 2.30 (3H, s), 1.20 (3H, s), 1.19 (3H, s), and 0.97 (3H, s). The ^{13}C-NMR spectrum exhibited 13 carbon signals, consisting of a carbonyl carbon signal at δ 199.0, two olefinic carbon signals at δ 144.1 and 132.6, and three oxygenated carbon signals at δ 69.7, 67.5, and 63.2. In

the ^{13}C-NMR spectrum, the two olefinic carbon signals at δ 144.1 (C-7) and 132.6 (C-8) and the carbonyl carbon at δ 199.0 (C-9) indicated the presence of an α,β-unsaturated ketone moiety. These spectral data suggested that **3** was an ionane-type norsesquiterpene (Brigida *et al*., 2004). Based on further comparison with published data (Brigida *et al*., 2004), the structure of **3** was identified as 5,6-epoxy-3-hydroxy-7-megastigmen-9-one.

Compound **7** was obtained as a colorless gum. From the ESI-MS (*m*/*z* 355 $[M+Na]^+$) and ^{1}H- and ^{13}C-NMR spectral data, the molecular formula of **7** was deduced to be $C_{16}H_{28}O_7$. The ^{1}H-NMR spectrum showed four olefinic protons at δ 5.93 (1H, dd, *J* = 17.1, 11.1 Hz), 5.49 (1H, dt, *J* = 7.4, 1.6 Hz), 5.21 (1H, dd, *J* = 17.1, 1.6 Hz), and 5.04 (1H, dd, *J* = 11.1, 1.6 Hz), including two terminal methylene protons at δ 5.21 and 5.04, two oxygenated proton signals at δ 4.21 (1H, d, *J* = 11.2 Hz), and 4.05 (1H, d, *J* = 11.2 Hz), and two methyl groups at δ 1.69 (3H, s) and 1.26 (3H, s). The ^{13}C-NMR spectrum demonstrated the presence of 16 carbon signals, consisting of four olefinic carbon signals at δ 145.0, 131.7, 128.9, and 110.9 and two oxygenated carbon signals at δ 74.7 and 72.5. An anomeric carbon signal at δ 101.4 and five oxygenated carbon signals (δ 77.0, 76.7, 73.9, 70.5, and 61.6) suggested the presence of D-glucose (Stephen *et al*., 1977). The coupling constant (*J* = 7.9 Hz) of the anomeric proton of D-glucose indicated that it was the β-form (Stephen *et al*., 1977). These spectral data suggested that **7** was a monoterpene glycoside. The structure of compound **7** was identified as 1-hydroxylinaloyl-6-*O*-β-D-glucopyranoside based on further comparison with literature data (Funda *et al*., 1998).

Compound **8** was obtained as a colorless gum. The ^{1}H-NMR spectrum showed three olefinic protons at δ 5.97 (1H, d, *J* = 15.6 Hz), 5.76 (1H, s), and 5.73 (1H, dd, *J* = 15.6, 7.2 Hz), an oxygenated proton at δ 4.53 (1H, m), and four methyl groups at δ 1.94 (3H, s), 1.29 (3H, d, *J* = 6.6 Hz), 1.08 (3H, s), and 1.05 (3H, s). An anomeric proton signal of sugar at δ 4.28 (1H, d, *J* = 7.9 Hz) was also observed. The ^{13}C-NMR spectrum demonstrated the presence of 19 carbon signals, consisting of a carbonyl carbon signal at δ 200.0, four olefinic carbon signals at δ 165.9, 132.6, 132.5, and 125.9, and two oxygenated carbon signals at δ 78.8 and 73.8. An anomeric carbon signal at δ 100.1 and five oxygenated carbon signals (δ 77.2, 77.0, 73.4, 70.5, and 61.6) suggested the presence of D-glucose (Stephen *et al*., 1977). These spectral data suggested that **8** was an iononol-type norsesquiterpene glycoside (Yves *et al*., 1999). Based on the above consideration and the comparison of the data with those previously published (Yves *et al*., 1999; Yumiko *et al*., 1985), the structure of **8** was identified as (6*S*,9*S*)-roseoside.

Compound **9** was obtained as a colorless gum. The ^{1}H- and ^{13}C- NMR data were almost the same as those of **5**, except for the presence of a CH_2 group (δ 2.97) in **5**. Based on further comparison with published data (Kaoru *et al*., 1988), the structure of **9** was identified as benzyl-β-D-glucopyranoside.

Compound **11** was obtained as a colorless gum. The ^{1}H-NMR spectrum showed five aromatic proton signals at δ 7.00 (1H, d, *J* = 2.0 Hz), 6.87 (1H, dd, *J* = 8.1, 2.0 Hz), 6.78 (1H, br t, *J* = 1.1 Hz), 6.74 (1H, d, *J* = 7.9 Hz), and 6.73 (1H, br d, *J* = 1.1 Hz) and two methoxyl proton signals at δ 3.84 (3H, s) and 3.81 (3H, s). In addition, the ^{1}H-^{1}H COSY spectrum showed correlations among signals at δ 3.58 (H-3'), 2.63 (H-1'), and 1.83 (H-2') and signals at δ 5.60 (H-2), 3.77 (3-CH_{2b}), and 3.62 (H-3). The ^{13}C-NMR spectrum demonstrated the presence of 26 carbon signals, consisting of twelve aromatic carbon signals at δ 147.8, 146.3, 146.2, 144.0, 135.7, 133.6, 128.5, 118.5, 117.0, 114.9, 113.08, and 109.5 and three oxygenated carbon signals at δ 87.81, 71.2, and 61.0. The ^{1}H-NMR spectrum [δ 4.36 (1H, d, *J* = 7.9 Hz, H-1'''), 3.69 (1H, dd, *J* = 12.2, 5.1 Hz, H-6'''), 3.36-3.26 (3H, m, H-3''', 4''', 5'''), 3.24 (1H, dd, *J* = 9.1, 8.0 Hz, H-2''')] and ^{13}C-NMR spectrum [δ 103.4 (C-1'''), 77.1 (C-3'''), 76.9 (C-5'''), 74.0 (C-2'''), 70.5 (C-4'''), 61.6 (C-6''')] suggested the presence of D-glucopyranose (Stephen *et al*., 1977). The coupling constant (*J* = 7.9 Hz) of the anomeric proton of D-glucose indicated it was the β-form (Stephen *et al*., 1977). The ^{1}H-NMR and ^{13}C-NMR spectra showed the dihydrobezofuran type of neolignan glycoside (Veronique *et al*., 2000). Based on further comparison with published data (Veronique *et al*., 2000, Tsutomu *et al*., 2004), the structure of **11** was identified as 3-(β-D-glucopyranosyloxymethyl)-2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-dihydrobenzofuran.

Compound **12** was obtained as a brown powder. The EI-MS (*m*/*z* 253 $[M-H]^+$) and ^{1}H- and ^{13}C-NMR spectral data of **12** gave a molecular formula of $C_{11}H_{10}O_7$. The ^{1}H-NMR spectrum showed three aromatic proton signals at δ 7.08 (1H, d, *J* = 1.7 Hz), 6.92 (1H, dd, *J* = 8.1, 1.7 Hz), and 6.79 (1H, d, *J* = 8.1 Hz), two olefinic proton signals at δ 7.62 (1H, d, *J* = 16.0 Hz) and 6.43 (1H, d, *J* = 16.0 Hz), and an oxygenated proton signal at δ 5.74 (1H, s). The signals at δ 7.62 (1H, d, *J* = 16.0 Hz) and 6.43 (1H, d, *J* = 16.0 Hz) also indicated the presence of *trans*-olefinic protons. The ^{13}C-NMR spectrum demonstrated the presence of 11 carbon signals, composed of six aromatic carbon signals at δ 148.1, 145.5, 126.9, 121.7, 115.3, and 114.6 and two olefinic carbon signals at δ 145.7 and 114.0, which implied the presence of a *trans*-caffeoyl moiety. In addition, a carboxyl carbon signal at δ 173.3 and an oxygenated carbon signal at δ 75.3 were observed in the ^{13}C-NMR spectrum. These data implied that **12** was a phenyl propanoid with an oxalic acid moiety. The structure of **12** was identified as (+)-taraxafolin-B based on additional HMQC and HMBC experiments and comparison with published data (Yann *et al*., 2005). However, the configuration of the OH group at C-2' could not be determined due to the paucity and instability of the compound.

The isolated compounds were tested for cytotoxicity against four human tumor cells *in vitro* using the SRB assay. Compound **1** exhibited moderate cytotoxic activity against the A549, SK-OV-3, SK-MEL-2, and HCT15 cell lines (ED_{50}: 12.15, 13.77, 14.29, and 12.23 μM, respectively), but the other compounds showed little cytotoxicity (ED_{50} > 30 μM).

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