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Evaluation of chemical constituents from *Glehnia littoralis* for antiproliferative activity against HT-29 human colon cancer cells

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ABSTRACT

In order to investigate the potential of *Glehnia littoralis* as a cancer chemopreventive food, antiproliferative effects of both its crude extracts and solvent-partitioned fractions (*n*-hexane, 85% aq. MeOH, *n*-BuOH, and water) were evaluated in HT-29 human colon cancer cells. Its crude extracts and solvent-partitioned fractions exhibited dose-dependent inhibitory effects on the cell proliferation. Especially, *n*-hexane and 85% aq. MeOH fractions exhibited a high antiproliferative effect, induced apoptosis as determined by 4,6-diamidino-2-phenylindole (DAPI) staining, and reduced mRNA expression of Bcl-2, cyclooxygenase (COX-2) and inducible nitric oxide synthase (iNOS). Systematic separation of *n*-hexane and 85% aq. MeOH fractions by diverse chromatographic methods led to the isolation of furanocoumarins (**1**–**4**) and polyacetylene alcohols (**5**–**7**). All compounds exhibited dose-dependent inhibitory effects on the cell proliferation. These results indicated that potent inhibitory activity of *G. littoralis* on proliferation of cancer cells can be significantly traceable to furanocoumarines and polyacetylenic alcohols contained in *G. littoralis*.

1. Introduction

Recently, cancer chemoprevention has developed as a major field of scientic investigation, and much attention has been focused on finding cancer chemopreventive or therapeutic agents from natural resources [1–3]. Particularly, it is well-established that plants have been a useful source of clinically relevant antitumor or anticancer agent [4]. Indeed, scientists the world over have isolated antitumor or anticancer agents from plants. The cancer inhibitory potential of naturally occurring compounds derived from plants has been confirmed in various animal models. As a part of our search for bioactive compounds from natural sources, we previously reported on antioxidative and anticancer activities of halophytes [5–7]. Halophytes are salt-resistant plants that are adapted to salinity stress. Although they were recognized environmentally as one of the most important ecosystems in a tidal zone, their secondary metabolites and biological activities have been little reported to date [8–10].

Glehnia littoralis F. Schmidt ex Miquel (Apiaceae) is a species of salt-tolerant plant in the carrot family and a perennial herb native to sandy seashores of eastern Asia, particularly eastern China, Korea, Japan, and far eastern Russia, and found also in western North America from Alaska to northern California [11]. It is a long-taprooted plant and forms a basal patch of leaves, with each leaf made up of several rounded, lobular segments. It reaches height greater than half a meter and its erect stem is topped with an umbel of carrotlike white flowers. The young flower buds and leaves are edible and its root and rhizome have been traditionally used as a diaphoretic, an antipyretic, and an analgesic [12,13]. A few phytochemical studies have described the isolation of constituents of *G. littoralis* and their biological activities [14–17].

In the present study, antiproliferative activities of both crude extracts of *G. littoralis* and their solvent-partitioned fractions were evaluated in HT-29 human colon cancer cells in order to investigate its potential as a cancer chemoprevention food. Moreover, six known compounds from solvent fractions of *G. littoralis* showing antiproliferative activity against proliferation of the colon cancer cells have been isolated and their chemical structures have been determined by NMR spectral analysis and by comparison with the reported data in the literature [13, 18–22].

Potential inhibitory activity of the isolated compounds on proliferation of the colon cells was estimated by measuring MTT assay and mRNA expression of several factors such as Bcl-2, inducible NO synthase (iNOS), and cyclooxygenase-2 (COX-2).

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2. Materials and methods

2.1. Plant materials

The whole plant of *G. littoralis* was collected in September 2002, at Pohang in Korea. The collected sample was briefly dried under shade and kept at −25 °C until use.

2.2. General experimental procedures

Optical rotations were taken on a Perkin-Elmer 341 polarimeter. The UV spectra were measured on a Thermo Spectronic spectrophotometer. ^{1}H NMR and ^{13}C NMR spectral analyses were performed with a Varian Mercury NMR 300 spectrometer (300 MHz for ^{1}H and 75.5 MHz for ^{13}C, respectively) using standard pulse sequence programs. Chemical shifts were referenced to the residual solvent peaks, recorded in δ values, and expressed in ppm. The used solvents were $CDCl_3$ for compounds **1**–**7**. HMQC and HMBC spectra were recorded using pulsed field gradients. FAB-MS was measured on a Concept-1S (Kratos Co.) mass spectrometer.

2.3. Extraction, fractionation, and isolation

The air-dried material of *G. littoralis* was chopped into small pieces and extracted with CH_2Cl_2 (3 L 2×) for 24 h at room temperature. After removal of the solvent, the residue was extracted with MeOH (3 L 2×) for 24 h at room temperature. The combined crude extracts (340 g) were suspended between CH_2Cl_2 and water. The organic layer was further partitioned between 85% aq. MeOH and *n*-hexane and then the aqueous layer was fractionated with *n*-BuOH and H_2O successively, to afford the *n*-hexane (22.09 g), 85% aqueous MeOH (8.95 g), *n*-BuOH (16.92 g), and water (125.0 g) fractions.

A portion of the 85% aq. MeOH fraction (4 g) was fractionated into 7 subfractions by C_{18} (YMC-GEL ODS-A, 12 nm, S-75 μm) reversed-phase vacuum flash chromatography eluting with stepwise gradient mixtures of MeOH–H_2O (50, 60, 70, 80, 90% aq. MeOH and 100% MeOH). The 60% aqueous MeOH fraction (0.12 g) was further separated into 10 subfractions by a silica preparative TLC with the solvent system 100% $CHCl_3$. Pure compound **1** (bergapten, 22.8 mg) was obtained from the first subfraction. The fourth fraction (0.021 g) was further separated by HPLC (2 mL/min, YMC ODS-A column, 65% aq. MeOH) to afford compounds **2** (isopimpinellin, 2.5 mg) and **3** (xanthotoxin, 9.2 mg). The 80% aq. MeOH fraction (0.250 g) was separated into 14 subfractions by a silica preparative TLC with 5% MeOH in $CHCl_3$. Compound **4** (imperatorin, 1.6 mg) was separated from the second subfraction (0.007 g) by HPLC (2 mL/min, YMC ODS-A column, 80% aq. MeOH). Purification of the fifth subfraction was carried out using C_{18} (YMC-GEL ODS-A, 12 nm, S-75 μm) reversed-phase vacuum flash chromatography eluting with 65% aq. MeCN, to afford pure compound **5** (panaxydiol, 0.5 mg). The sixth and seventh subfractions (0.018 g) were combined on the basis of their behavior and subjected to reversed-phased HPLC (2 mL/min, YMC ODS-A, 65% aq. MeCN) to give pure compound **6** (falcaindiol, 4.0 mg).

A portion of the *n*-hexane fraction (6.1 g) was separated into 11 subfractions by a silica column chromatography (silica gel 60, 0.063–0.200 mm, Merck) eluting with stepwise gradient mixtures of *n*-hexane-EtOAc (100% hexane, 10, 20, 30, 40, 50, 60, and 70% EtOAc in hexane, and 100% EtOAc), 100% acetone and 100% MeOH. Further separation of the third subfraction (0.300 g) by preparative TLC on a silica gel column with the solvent system *n*-hexane/ethyl ether (2:1) EtOAc as eluent afforded pure compound **7** (falcarinol, 8.2 mg). The chemical structures of the purified compounds are illustrated in Fig. 1.

Compound **1** (bergapten): yellow crystal; mp: 185–187°; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.25 (1H, d, J = 9.6 Hz, H-3), 8.13 (1H, d, J = 9.6 Hz, H-4), 7.11 (1H, s, H-8), 7.57 (1H, d, J = 2.0 Hz, H-2′), 7.00 (1H, d, J = 2.0 Hz, H-3′), 4.26 (3H, s, 5-OMe); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 161.1 (C, C-2), 112.5 (CH, C-3), 139.1 (CH, C-4), 149.4 (C, C-5), 112.6 (C, C-6), 158.2 (C, C-7), 93.8 (CH, C-8), 152.6 (C, C-9), 106.3 (C, C-10), 144.7 (CH, C-2′), 105.0 (CH, C-3′), 60.1 (CH_3, 5-OMe); EI-MS m/z 216 $[M]^+$.

Compound **2** (isopimpinellin): pale yellow crystal; $[\alpha]_D^{25}$: 9.52° (c 0.21, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.27 (1H, d, J = 9.6 Hz, H-3), 8.10 (1H, d, J = 9.6 Hz, H-4), 7.60 (1H, d, J = 2.3 Hz, H-2′), 7.00 (1H, d, J = 2.3 Hz, H-3′), 4.26 (3H, s, 5-OMe), 4.15 (3H, s, 8-OMe); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 160.3 (C, C-2), 112.8 (CH, C-3), 139.3 (CH, C-4), 144.2 (CH, C-5), 114.7 (C, C-6), 149.9 (C, C-7), 128.1 (C, C-8), 143.6(C, C-9), 107.6 (C, C-10), 145.0 (CH, C-2′), 105.0 (CH, C-3′), 60.9 (CH_3, 5-OMe), 61.7 (CH_3, 8-OMe); EI-MS m/z 246 $[M]^+$.

Compound **3** (xanthotoxin): pale yellow crystal; $[\alpha]_D^{25}$: 9.52° (c 0.21, MeOH); mp: 146–148°; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.36 (1H, d, J = 9.5 Hz, H-3), 7.75 (1H, d, J = 9.5 Hz, H-4), 7.36 (1H, s, H-5), 7.67 (1H, d, J = 2.0 Hz, H-2′), 6.80 (1H, d, J = 2.0 Hz, H-3′), 4.29 (3H, s, 8-OMe); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 160.3 (C, C-2), 114.7 (CH, C-3), 144.2 (CH, C-4), 112.8 (CH, C-5), 126.0 (C, C-6), 147.5 (C, C-7), 132.7 (CH, C-8), 142.8 (C, C-9), 116.4 (C, C-10), 146.5 (CH, C-2′), 106.7 (CH, C-3′), 61.3 (CH_3, 8-OMe); EI-MS m/z 216 $[M]^+$.

Compound **4** (imperatorin): pale yellow crystal; mp: 97.5–99°; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.36 (1H, d, J = 9.6 Hz, H-3), 7.75 (1H, d, J = 9.6 Hz, H-4), 7.33 (1H, s, H-5), 7.67 (1H, d, J = 2.0 Hz, H-2′), 6.79 (1H, d, J = 2.0 Hz, H-3′), 4.99 (2H, d, J = 7.2 Hz, H-1″), 5.62 (1H, t, J = 7.2 Hz, H-2″), 1.71 (3H, s, H-4″), 1.73 (3H, s, H-5″); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 160.4 (C, C-2), 114.6 (CH, C-3), 144.2 (CH, C-4), 113.1 (CH, C-5), 125.7 (C, C-6), 148.4 (C, C-7), 131.5 (CH, C-8), 143.6 (C, C-9), 116.4 (C, C-10), 146.5 (CH, C-2′), 106.7 (CH, C-3′), 70.1 (CH_2, C-1″), 119.6 (CH, C-2″), 139.7 (CH, C-3″), 25.9 (CH_3, C-4″), 18.2 (CH_3, C-5″); EI-MS m/z 270 $[M]^+$.

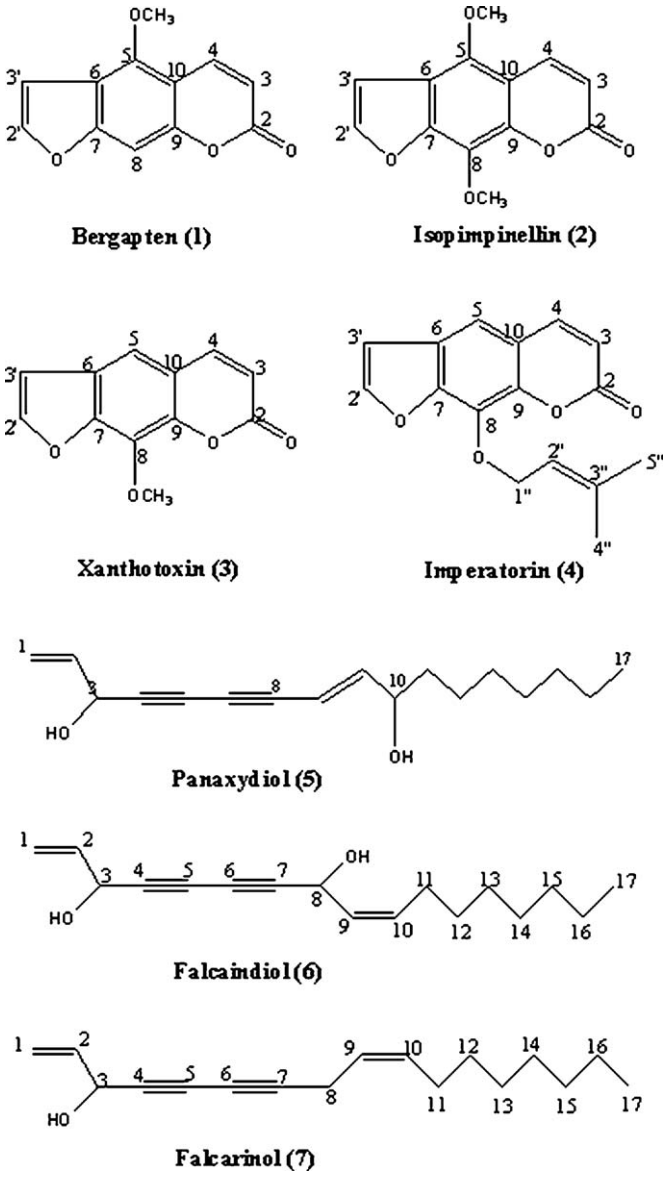

Fig. 1. Chemical structure of compounds isolated from 85% aq. MeOH and *n*-hexane fractions of *Glehnia littoralis*.

Compound **5** (panaxydiol): yellow oil; $[\alpha]_D^{20}$: −20° (c 0.25, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.47 (1H, ddd, J = 17.0, 1.4, 1.0 Hz, H-1), 5.25 (1H, ddd, 10.8, 1.4, 1.0 Hz, H-1), 5.94 (1H, ddd, J = 17.1, 10.8, 5.5 Hz, H-2), 4.97 (1H, brs, H-3), 5.75 (1H, dqui, 16.0, 8.0 Hz, H-8), 6.32 (1H, dd, 16.0, 5.8 Hz, H-9), 4.19 (1H, br q, 5.8 Hz, H-10), 1.53 (2H, m, H-11), 1.21–1.42 (2H, m, H-12/-13/-14/-15/-16), 0.88 (3H, br t, J = 0.02 Hz, H-17); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 117.3 (CH_2, C-1), 135.8 (CH, C-2), 63.7 (CH, C-3), 80.4 (C, C-4), 70.9 (C, C-5), 73.5 (C, C-6), 77.1 (C, C-7), 107.9 (CH, C-8), 149.8 (CH, C-9), 72.0 (CH, C-10), 37.0 (CH_2, C-11), 29.5 (CH_2, C-12), 29.5 (CH_2, C-13), 29.3 (CH_2, C-14), 31.8 (CH_2, C-15), 22.7 (CH_2, C-16), 14.2 (CH_3, C-17); EI-MS m/z 260 $[M]^+$.

Compound **6** (falcaindiol): yellow oil; $[\alpha]_D^{20}$: +280° (c 0.15, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.47 (1H, ddd, J = 17.1, 1.2, 1.0 Hz, H-1), 5.25 (1H, ddd, J = 10.2, 1.2, 1.0 Hz, H-1), 5.93 (1H, ddd, J = 17.1, 10.2, 5.2 Hz, H-2), 4.93 (1H, brs, H-3), 5.19 (1H, brd, J = 8.0 Hz, H-8), 5.51 (1H, m, H-9), 5.61 (1H, m, H-10), 2.10 (2H, m, H-11), 1.38 (2H, m, H-12), 1.27 (2H, m, H-13/-14/-15/-16), 0.88 (3H, t, J = 6.9 Hz, H-17); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 117.3 (CH_2, C-1), 135.7 (CH, C-2), 63.5 (CH, C-3), 78.2 (C, C-4), 70.3 (C, C-5), 68.7 (C, C-6), 79.8 (C, C-7), 58.6 (CH, C-8), 127.6 (CH, C-9), 134.6 (CH, C-10), 27.8 (CH_2, C-11), 29.4 (CH_2, C-12), 29.2 (CH_2, C-13), 29.3 (CH_2, C-14), 31.9 (CH_2, C-15), 22.7 (CH_2, C-16), 14.2 (CH_3, C-17); EI-MS m/z 260 $[M]^+$.

Compound **7** (falcarinol): yellow oil; $[\alpha]_D^{20}$: −34.29° (c 0.23, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.44 (1H, dt, J = 17.1, 1.2, 1.0 Hz, H-1), 5.21 (1H, dt, J = 10.2, 1.2, 1.0 Hz, H-1), 5.91 (1H, ddd, J = 17.1, 10.2, 5.5 Hz, H-2), 4.90 (1H, brs, H-3), 3.01 (2H, d, J = 6.6 Hz, H-8), 5.35 (1H, m, H-9), 5.50 (1H, m, H-10), 2.01 (2H, m, H-11), 1.27 (2H, m, H-12/-13/-14/-15/-16), 0.88 (3H, t, J = 6.9 Hz, H-17); ^{13}C NMR (75 MHz, $CDCl_3$) δ: