Studies on Lymphangiogenesis Inhibitors from Korean and Japanese Crude Drugs

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Metastasis occurs when cancer cells detach from a tumor, travel to distant sites in the body and develop into tumors in these new locations. Most cancer patients die from metastases. Among the various forms of cancer metastasis, lymphatic metastasis is an important determinant in cancer treatment and staging. In this study, we investigated lymphangiogenesis inhibitors from crude drugs used in Japan and Korea. The three crude drugs Saussureae Radix, Psoraleae Semen and Aurantii Fructus Immaturus significantly inhibited the proliferation of temperature-sensitive rat lymphatic endothelial (TR-LE) cells in vitro. By a chromatographic method using bioassay-guided fractionation methods, costunolide (1) and dehydrocostus lactone (2) from S. Radix, p-hydroxybenzaldehyde (3), psoralen (=ficusin) (4), angelicin (=isopsoralen) (5), psoracorylifol D (6), isobavachalcone (7), bavachinin (8) Δ3,2-hydroxybakuchiol (9) and bakuchiol (10) from P. Semen and cis-octadeccyl ferulate (11), (2R)-3β,7,4′-trihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (12), (25′)-7,4′-dihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (13) and umbelliferone (14) from A. F. Immaturus were obtained. Three compounds (compounds 11–13) from A. F. Immaturus were isolated for the first time from this medicinal plant. Among isolated compounds, ten compounds (compounds 1, 2, 6–12, 13) showed an inhibitory effect on the proliferation and the capillary-like tube formation of TR-LE cells. In addition, all compounds except compound 12 showed selective inhibition of the proliferation of TR-LE cells compared to Hela and Lewis lung carcinoma (LLC) cells. These compounds might offer clinical benefits as lymphangiogenesis inhibitors and may be good candidates for novel anti-cancer and anti-metastatic agents.

Key words lymphangiogenesis; Saussureae Radix; Psoraleae Semen; Aurantii Fructus Immaturus; costunolide; bakuchiol

Lymphatic vessels comprise a system of thin-walled, low pressure vessels that collect fluid, proteins and cells released by the blood vessels into the interstitial spaces of tissues. Lymphangiogenesis is the formation of new lymphatic vessels from pre-existing lymphatic vessels.

Lymphatic vasculature plays an important role in the pathogenesis of human diseases such as cancer. Lymphatics are an important determinant of metastasis and usually serve as the primary pathway for the metastatic spread of tumor cells to regional lymph nodes, and possibly, also to distant organs. Metastasis of tumor cells is the primary reason for cancer deaths, and with few exceptions all cancers can metastasize.

Therapeutics targeting tumor-associated lymphangiogenesis has not established yet, and natural products that inhibit lymphangiogenesis have not been identified. We investigated the methanol (MeOH) extracts of 126 crude drugs used in Japan and Korea to assess their effects on the proliferation of temperature-sensitive rat lymphatic endothelial (TR-LE) cells in vitro. Among them, three crude drugs, namely Saussureae Radix (the root of Saussuea lappa), Psoraleae Semen (the seed of Psoralea corylifolia) and Aurantii Fructus Immaturus (the immature fruits of Poncirus trifoliata) showed significant inhibitory effects on the proliferation of TR-LE cells. When the MeOH extracts of these crude drugs were analyzed by a chromatographic method using bioassay-guided fractionation, costunolide (1) and dehydrocostus lactone (2) from S. Radix, p-hydroxybenzaldehyde (3), psoralen (=ficusin) (4), angelicin (=isopsoralen) (5), psoracorylifol D (6), isobavachalcone (7), bavachinin (8) Δ3,2-hydroxybakuchiol (9) and bakuchiol (10) from P. Semen and cis-octadeccyl ferulate (11), (2R)-3β,7,4′-trihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (12), (25′)-7,4′-dihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (13) and umbelliferone (14) from A. F. Immaturus were identified and isolated as major extract constituents. The isolated compounds, except for compounds 3, 4, 5 and 14, showed an inhibitory effect on the proliferation of TR-LE cells in vitro, suggesting they might have an anti-lymphangiogenic effect.

MATERIALS AND METHODS

Materials S. Radix and A. F. Immaturus were purchased from Uchida Wakanyaku Ltd. (Tokyo, Japan). The MeOH extract of P. Semen was donated from the library of Prof. Y.-C. Kim (University of Wonkang, Korea). The Anti-cancer drugs, paclitaxel and vincristine sulfate were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Extraction and Isolation S. Radix (ca. 500 g) was extracted with MeOH. The MeOH extract was evaporated in vacuo, and partitioned between n-hexane and MeOH to afford an n-hexane soluble part (11.7 g). The active n-hexane fraction was subjected to silica gel column chromatography with n-hexane-acetone (6:1) and then purified by recrystallization (JAIHEL-1H×2, Japan Analytical Industry Co., Ltd., Tokyo, Japan) with chloroform (CHCl3) to give compound 1 (0.46%...
from n-hexane extract) and 2 (1.57% from n-hexane extract). P. Semen (ca. 500 g) was extracted with MeOH to give MeOH extract (85.1 g). Part of the MeOH extract (552.0 mg) was partitioned between n-hexane and MeOH to afford an n-hexane soluble part (286 mg). The active n-hexane fraction was subjected to silica gel column chromatography with CHCl₃/MeOH, n-hexane–ethylacetate (EtOAc), and purified by reversed phase HPLC (YMC-Pack Pro C18, 65–100% MeOH/H₂O) to give compounds 3 (0.9 mg), 4 (1.2 mg), 5 (1.4 mg), 6 (2.5 mg), 7 (3.1 mg), 8 (2.0 mg), 9 (4.8 mg) and 10 (10.2 mg). The MeOH extract (179.6 g) of A. F. Immaturus was partitioned between n-hexane and MeOH to afford an n-hexane soluble part (7.3 g). The active n-hexane fraction was subjected to silica gel column chromatography with n-hexane/EtOAc, Sephadex LH-20 with MeOH, and finally purified with reversed phase HPLC (YMC-Pack Pro C18, 50–100% MeOH/H₂O) to give compounds 11 (0.7 mg), 12 (1.5 mg), 13 (0.6 mg) and 14 (1.2 mg), respectively.

The structures of all compounds were identified by NMR and electron ionization (EI)-MS spectral data together with published data.

**Cell Culture**  
TR-LE cells were donated from the library of Prof. I. Saiki and maintained on Type I collagen coated cell culture dishes (Iwaki, Tokyo, Japan) in HuMedia-EB2 and HuMedia-EG (Kurabo Industries, Ltd., Osaka, Japan) supplemented with 10% fetal bovine serum (FBS) at a permissive temperature (33°C). Lewis lung carcinoma (LLC) was donated from the library of Prof. M. Ono and cultured in Roswell Park Memorial Institute medium (RPMI 1640) supplemented with 10% FBS at 37°C in a humidified air containing 5% CO₂. Human malignant epithelial cells (Hela) were cultured in Eagle’s Minimum Essential medium (EMEM) supplemented with 10% FBS kept in an incubator at 37°C in a humidified air containing 5% CO₂. FBS was purchased from Nichirei Bioscience Inc. (Tokyo, Japan).

**Cell Proliferation Assay**  
TR-LE cells (5×10³ cells/well) were seeded in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% FBS in Type I collagen coated 96 well cell culture plates (Nippi Inc., Tokyo, Japan). Cells were allowed to adhere for 2h, and then grown with compounds at the final concentration of 1–50 µM for additional 48h. Cell viability was determined by a Cell-Titer 96 AQueous Non-Radioactive Cell Proliferation (MTS) Assay (Promega, WI, U.S.A.) according to the manufacturer’s protocol. LLC cells (5×10³ cells/well) were seeded in RPMI 1640 containing 10% FBS in 96 well cell culture plates (Nunc, Roskilde, Denmark). Cells were allowed to adhere for 2h, and then grown with or without compounds at the same concentration for additional 48h and cell proliferation assay was performed. Hela cells (3×10³ cells/well) were seeded in EMEM containing 10% FBS in 96 well plates and incubated for 24h, then grown with compounds for additional 48h and cell proliferation assay was performed. In
all experiments, the final concentration of vehicle (DMSO) concentration did not exceed 0.5% (v/v).

Tube Formation Assay Sub-confluent TR-LE cells were harvested with trypsin-ethylenediaminetetraacetic acid (EDTA) and centrifuged at 1000 rpm for 5 min. A cell suspension (2 x 10⁴ cells/well) was prepared in 40 µL of DMEM supplemented with 10% FBS in matrigel pre-coated 96 well plates (Becton Dickinson Labware, Bedford, MA, U.S.A.). After 4 h incubation at 37°C, cells were fixed by using glutaraldehyde and stained with hematoxylin. Morphological changes were photographed at x10 magnification with a phase-contrast microscope. The length of the capillary-like tube structures was measured by hand, and we used three photographs per well, and measured the length about ten tube structures.

Cell Cycle Analysis TR-LE cells were treated with 10 µM of bakuchiol, and after 6–48 h incubation, cells were harvested and stained with propidium iodide (PI) using the Cycle Test Plus DNA Reagent kit (BD Biosciences, San Jose, CA, U.S.A.) according to the manufacturer’s recommendations. Cell distribution according to cell cycle phase was determined by measuring the DNA content using a BD FACSCalibur flow cytometer employing the Cell Quest Software. The percentage of cells in the G0/G1, S and G2/M phases was determined using Modfit LT software (Verity Software House Inc., Topsham, ME, U.S.A.). Cells with hypodiploid DNA (content less than that of G0/G1-phase cells) were considered to be apoptotic (sub-G1).

Statistical Analysis Results are given as means±S.D.

RESULTS

Bioassay-Guided Fractionation MeOH extracts prepared from 126 crude drugs were screened for anti-lymphangiogenic activity. Among them, nine crude drugs showed a selective inhibitory effect on TR-LE proliferation. Based on these results, three of them, S. Radix, P. Semen and A. F. Immaturus, were selected to identify lymphangiogenesis inhibiting compounds (Table 1). From the n-hexane soluble part of the MeOH extract of S. Radix, compound 1 (costunolide) and 2 (dehydropseudo lactone) were identified as active ingredients. Five active compounds 6 (psoracorylifol D), 7 (isobavachalcone), 8 (bavachinin), 9 (Δ3, 2-hydroxybakuchiol), and 10 (bakuchiol) were isolated from the MeOH extract of P. Semen together with three inactive compounds 3 (p-hydroxybenzaldehyde), 4 (psoralen), 5 (angelicin). From the MeOH extract of A. F. Immaturus, two active prenylflavones 12, 13 and 11 (cis-octadecyl ferulate) were identified together with one inactive compound 14 (umbelliferone).

Inhibitory Effect on the Proliferation of TR-LE, LLC and Hela Cells Inhibitory effects of compounds 1–14 on the proliferation of TR-LE, Hela, and LLC cells were determined by MTT assay. Paclitaxel and Vincristine were used as positive controls. The results are shown in Table 2.

Table 1. The Selected Nine Crude Drugs and Their Inhibitory Effects on the Proliferation of TR-LE and Hela Cells

| Crude drugs          | Cell viability TR-LE (%) | Cell viability Hela (%) |
|----------------------|--------------------------|-------------------------|
|                      | 10 µg/mL | 1 µg/mL | 10 µg/mL | 1 µg/mL |
| Fritillariae Bulbus  | 22.5     | 46.3    | 58.8     | 67.0    |
| Hoelen               | 20.2     | 39.6    | 56.2     | 74.5    |
| Piperis Longi Fructus| 14.2     | 34.2    | 35.1     | 54.9    |
| Saussureae Radix     | 0.50     | 30.3    | 16.0     | 39.7    |
| Equiseti Herba       | 28.5     | 58.6    | 35.0     | 66.5    |
| Ledebouriellae Radix | 26.8     | 47.0    | 50.4     | 64.4    |
| Psoraleae Semen      | 7.3      | 40.0    | 35.1     | 53.6    |
| Curcumae Radix       | 14.7     | 49.6    | 40.3     | 63.6    |
| Auranti Fructus Immaturus | 9.8 | 36.7 | 54.3 | 69.7 |

Table 2. IC₅₀ Values of Compounds 1–14 against TR-LE, Hela, and LLC Proliferation

| Compounds | IC₅₀ (µM) |
|-----------|----------|
|           | TR-LE    | Hela    | LLC     | Tube formation |
| 1         | 1.37     | 15.59   | 3.49    | 0.09            |
| 2         | 3.27     | 22.31   | 5.43    | 0.08            |
| 3         | >50      | >50     | nt      | nt              |
| 4         | >50      | >50     | nt      | nt              |
| 5         | >50      | >50     | nt      | nt              |
| 6         | 8.52     | >50     | 18.20   | 1.08            |
| 7         | 9.24     | >50     | 21.10   | 1.41            |
| 8         | 15.93    | >50     | 23.26   | 1.48            |
| 9         | 9.33     | >50     | 20.96   | 1.18            |
| 10        | 7.43     | 27.9    | 12.71   | 0.49            |
| 11        | 4.38     | 7.02    | 12.92   | 0.44            |
| 12        | 6.73     | 6.09    | 19.95   | 0.93            |
| 13        | 7.66     | >50     | 21.07   | 0.74            |
| 14        | >50      | >50     | nt      | nt              |
| Paclitaxel| 0.018    | 0.0017  | nt      | nt              |
| Vincristine| 0.080    | 0.0043  | nt      | nt              |
proliferation of TR-LE, Hela and LLC cells were examined. As shown in Table 2, compounds 1 and 2 showed an efficient inhibitory effect on TR-LE proliferation with IC\textsubscript{50} values of 1.37 and 3.27 µM. Furthermore, compounds 6, 7, 8, 9, and 13 showed selective inhibition of TR-LE proliferation compared with Hela proliferation. Prenyl flavones, i.e. compounds 12 and 13, showed an inhibitory effect on TR-LE proliferation with IC\textsubscript{50} values of 6.73 and 7.66 µM, however, the IC\textsubscript{50} value of compound 13 inhibiting Hela proliferation was more than 50 µM. The mitotic inhibitors, paclitaxel and vincristine showed strong inhibitory effects on the proliferation of both TR-LE and Hela cells; however, the IC\textsubscript{50} values for TR-LE cells have been estimated to be about 10–20 times lower than those for Hela cell proliferation.

**Effect on Capillary-Like Tube Formation of TR-LE Cells**

To further investigate the effect of compounds on lymphangiogenesis, we measured the lengths of the capillary-like tubes formed by TR-LE cells grown on matrigel after addition of non-toxic doses of compounds. Ten compounds 1, 2, 6, 7, 8, 9, 10, 11, 12 and 13, which showed selective inhibition of TR-LE proliferation, were examined. As shown in Fig. 2A, compounds 1, 10 and 13 inhibited tube formation resulting in shortened capillary-like tubes on TR-LE cells. This effect was dose-dependently (Fig. 2B). The IC\textsubscript{50} values of ten compounds are listed in Table 2. Compounds 1 and 2 showed a significant inhibitory effect on the formation of the capillary-like tube network.

**Effect of Compound 10 on Cell Cycle**

The effect of compound 10 (bakuchiol), which is the major ingredient of P. Semen, on cell cycle parameters was examined using flow cytometric analysis. Ten micromole of compound 10 were added to the medium of TR-LE cells for 6, 12, 24 and 48 h. Then the cells were harvested and analyzed with a FACScalibur. As shown in Fig. 3, no effect was seen within 24 h, however, the percentage of sub-G1-phase cells increased rapidly between 24–48 h.
DISCUSSION

Clinically, tumor progression through the blood or lymphatic vessels represents the most critical problem of human cancer, with regional lymph node metastasis often being the most important prognostic factor for the cancer patient. From the sentinel lymph node, which is the primary regional lymph node to which tumor cells metastasize, further dissemination may occur to other lymph nodes and to distant organs. For this reason, it is important for the advancement of cancer treatment to search for, identify, and develop lymphangiogenesis inhibitors from natural resources.

The three crude drugs, S. Radix, P. Semen and A. F. Immaturus, significantly inhibited TR-LE cell proliferation in vitro. Subsequently, fourteen compounds were identified and isolated from these crude drugs. Ten of these compounds (compounds 1, 2, 6–12 and 13) showed a selective inhibitory effect on the proliferation and the capillary-like tube formation of TR-LE cells. Several biological activities of compound 1 (costunolide), a major ingredient of S. Radix, have been reported, such as inhibition of nuclear factor (NF)-κB activation, nitric oxide (NO) production, and interleukin (IL)-1β expression. These biological effects are closely related to the process of inflammation. Anti-inflammatory activity of compound 10 (bakuchiol) has been also reported, and the close association of inflammation, angiogenesis, lymphangiogenesis, and cancer progression has been recognized. The mechanisms of action of the identified compounds or their involvement in signaling pathways have not been elucidated in this study. However, in the case of compound 1, it was explained in our previous study that this compound inhibited the vascular endothelial growth factor (VEGF)-induced autophosphorylation of VEGFR-2 (KDR/Flik-1), and thus, in the present study, might inhibit cell proliferation by a similar mechanism due to TR-LE cells both expressing VEGFR2 and VEGFR3 (Flt-4).

It is well known that prenyl flavonoids show several biological activities. For example, prenyl chalcone 7 (isobavachalcone) has been reported to have activities such as abrogation of Akt signaling, inhibition of NO synthase, and induction of apoptosis. Prenyl flavanone 8 (bavachinin) has been reported as an anti-oxidant, and as an inhibitor of the human 20S proteasome, and compound 13 has been also reported as an anti-oxidant and as an inhibitor of aldose reductase. However, this is the first report showing that a prenyl flavone has an anti-lymphangiogenic activity.

Because Snapka and colleagues reported that bakuchiol (10) inhibited replicative DNA polymerase using the SV40 bioassay, we examined the effect of bakuchiol on cell cycle parameters. As a result, bakuchiol induced apoptosis after treatment for 24–48 h; however, the percentage of S-phase cells did not change within 24 h. Generally, the structures of DNA polymerase are highly conserved in species, and this has not been due to species deference. The programs of controlling cell proliferation are cell cycle arrest or inhibition of proliferation and apoptosis. Bakuchiol did not arrest the cell cycle, but apoptotic cell death was detected after treatment for 48 h.

Snapka and colleagues also reported that the 4-hydroxystyryl moiety is a pharmacophore associated with DNA polymerase inhibitory activity. Interestingly, the “4-hydroxy-, 4-methoxy-, or 4-oxystyryl” moieties have been found in the structures of compounds 7, 8, 9, 10, 12, and 13, which showed selective inhibition of proliferation of TR-LE cells.

In conclusion, this is the first pharmacological evidence that S. Radix, P. Semen and A. F. Immaturus and ten compounds, isolated from these crude drugs might have anti-lymphangiogenic activities as they inhibited cell proliferation and capillary-like tube formation of TR-LE cells. These compounds might offer clinical benefits as lymphangiogenesis inhibitors and might be good candidates or novel anti-cancer and anti-metastatic agents.
REFERENCES

1) Stacker SA, Achen MG, Jussila L, Baldwin ME, Alitalo K. Lymphangiogenesis and cancer metastasis. Nat. Rev. Cancer, 2, 573–583 (2002).

2) Achen MG, McColl BK, Stacker SA. Focus on lymphangiogenesis in tumor metastasis. Cancer Cell, 7, 121–127 (2005).

3) Pepper MS. Lymphangiogenesis and tumor metastasis: myth or reality? Clin. Cancer Res., 7, 462–468 (2001).

4) Matsuo M, Koizumi K, Yamada S, Takeda Y, Ueda M, Terasaki T, Obinata M, Ohtani O, Iwatsuki K. Establishment and characterization of conditionally immortalized endothelial cell lines from the thoracic duct and inferior vena cava of the ap58/EGFP double-transgenic rats. Cell Tissue Res., 326, 749–758 (2006).

5) Li A, Sun A, Liu R. Preparative isolation and purification of costunolide and dehydrocostuslactone from Aucklandia lappa Decne by high-speed counter-current chromatography. J. Chromatogr. A, 1076, 193–197 (2005).

6) Yin S, Fan CQ, Dong L, Yue JM. Psoracorylifols A–E, five novel compounds with activity against Helicobacter pylori from the stem bark of Magnolia grandiflora. Tetrahedron, 62, 2569–2575 (2006).

7) Pistelli L, Spera K, Flamini G, Mele S, Morelli I. Isoflavonoids from Sophora flavescens: Isolation and characterization of a new prenylated chalcone. Phytochemistry, 42, 1455–1458 (1996).

8) Kim YJ, Lee HN, Park EH, Shim SH. Inhibition of human 20S proteasome by compounds from seeds of Psoralea corylifolia. J. Korean Chem. Soc., 30, 1867–1869 (2009).

9) Zhao G, Zheng XW, Qin GW, Gai Y, Jiang ZH, Guo LH. In vitro dopaminergic neuroprotective and in vivo antiparkinsonian-like effects of Δ3,2-hydroxybakuchiol isolated from Psoralea corylifolia roots. Cell. Mol. Life Sci., 66, 1617–1629 (2009).

10) Hsu PI, Miller JS, Berger JM. Bakuchiol, an antibacterial component of Psoralidium tenuiflorum. Nat. Prod. Res., 23, 781–788 (2009).

11) Speth E, Okahara K, Kuffner F. Identity of fisconin and psoralene. Berichte der Deutschen Chemischen Gesellschaft, 70B, 73 (1937).

12) Jakupovic J, Paredes L, Bohlmann F, Watson L. Prenyl flavanes and their chemopreventive effects. Phytochemistry, 27, 3273–3275 (1988).

13) Kang SS, Kim JS, Son KH, Chang HW, Kim HP. A new prenylated flavanone from the roots of Sophora flavescens. Fitoterapia, 71, 511–515 (2000).

14) Balde AM, Claeys M, Pieters LA, Wray V, Vliegenck AJ. Ferulic acid esters from stem bark of Pavetta owariensis. Phytochemistry, 30, 1024–1026 (1991).

15) Kong LY, Li Y, Min ZD, Li X, Zhu TR. Coumaraines from Peucedanum praetortorum. Phytochemistry, 41, 1423–1426 (1996).

16) Alitalo K, Tammela T, Petrova TV. Lymphangiogenesis in development and human disease. Nature, 438, 946–953 (2005).

17) Koo TH, Lee JH, Park YJ, Hong YS, Kim HS, Kim KW, Lee JJ. A sesquiterpene lactone, costunolide, from Magnolia grandiflora inhibits NF-κB by targeting IκB phosphorylation. Planta Med., 67, 103–107 (2001).

18) Park HJ, Jung WT, Basnet P, Kadota S, Namba T, Syringin O-β-glucoside, a new phenylpropanoid glycoside, and costunolide, a nitrile oxide synthase inhibitor, from the stem bark of Magnolia sieboldii. J. Nat. Prod., 59, 1128–1130 (1996).

19) Kang JS, Yoon YD, Lee KH, Park SK, Kim HM. Costunolide inhibits interleukin-1β expression by down-regulation of AP-1 and MAPK activity in LPS-stimulated RAW 264.7 cells. Biochem. Biophys. Res. Commun., 313, 171–177 (2004).

20) Backhouse CN, Delporte CL, Negrete RE, Erazo S, Zuñiga A, Pinto A, Cassels BK. Active constituents isolated from Psoralea glandulosa L. with antiinflammatory and antipyretic activities. J. Ethnopharmacol., 78, 27–31 (2001).

21) Watari K, Nakao S, Fotovati A, Basaki Y, Hosoi F, Berczky B, Higuchi R, Miyamoto T, Kawanu Ono M, Ono M. Role of macrophages in inflammatory lymphangiogenesis: Enhanced production of vascular endothelial growth factor C and D through NF-kappaB activation. Biochem. Biophys. Res. Commun., 377, 826–831 (2008).

22) Jeong SJ, Itokawa T, Shibuya M, Kawanu M, Ono M, Higuchi R, Miyamoto T. Costunolide, a sesquiterpene lactone from Saussurea lappa, inhibits the VEGFR KDR/Flik-1 signaling pathway. Cancer Lett., 187, 129–133 (2002).

23) Jin H, Zhou X, Dong X, Cao J, Zhu H, Lou J, Hu Y, He Q, Yang B. Abrogation of Akt signaling by Isobavachalcone contributes to its anti-proliferative effects towards human cancer cells. Cancer Lett., 294, 167–177 (2010).

24) Akihisa T, Tokuda H, Ukiya M, Iwatsuki K, Ogasaawara K, Mukainaka T, Iwatsuki K, Suzuki T, Nishino H. Chalcones, coumarins, and flavanones from the exudate of Angelica keiskei and Scrophularia nodosa. Planta Med., 67, 61–65 (2001).

25) Nishimura R, Tabata K, Arakawa M, Ito Y, Kimura Y, Akihisa T, Nagai H, Sakuma A, Kohno H, Suzuki T. Isoavachalcone, a chalcone constituent of Angelica keiskei, induces apoptosis in neuroblastoma. Biol. Pharm. Bull., 30, 1878–1883 (2007).

26) Xiao G, Li G, Chen L, Zhang Z, Yin JJ, Wu T, Cheng Z, Wei X, Wang Z. Isolation of antioxidants from Psoralea corylifolia fruits using high-speed counter-current chromatography guided by thin layer chromatography-antioxidant autographic assay. J. Chromatogr. A, 1217, 5470–5476 (2010).

27) Jung HJ, Kang SS, Hyun SK, Choi JS. In vitro free radical and ONOO− scavengers from Sophora flavescens. Arch. Pharm. Res., 28, 534–540 (2005).

28) Jung HA, Yoon NY, Kang SS, Kim YS, Choi JS. Inhibitory activities of prenylated flavonoids from Sophora flavescens against aldose reductase and generation of advanced glycation endproducts. J. Pharm. Pharmacol., 60, 1227–1236 (2008).

29) Sun NJ, Woo SH, Cassidy JM, Snapka RM. DNA polymerase and topoisomerase II inhibitors from Psoralea corylifolia. J. Nat. Prod., 61, 362–366 (1998).