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Cytotoxic Activities of Indigenous Plant Extracts in Cultured Human Cancer Cells

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Abstract – In continuous efforts for discovery of novel potent antitumor agents from natural products, fifty-seven methanolic extracts derived from indigenous Korean plants were primarily evaluated for *in vitro* cytotoxic activity in cultured human lung (A549) and colon (Col2) cancer cells. As a result, 16 plant extracts were found to be active against A549 cells and 15 extracts were active against Col2 cells in the criteria of IC_{50} <50 μg/ml. In particular, the extracts of *Calystegia soldanella* (IC_{50} = 8.0 μg/ml in A549; IC_{50} = 27.4 μg/ml in Col2), *Heloniopsis orientalis* (IC_{50} = 4.6 μg/ml in A549; IC_{50} = 4.5 μg/ml in Col2), and *Thuja koraiensis* (IC_{50} = 1.2 μg/ml in A549; IC_{50} = 0.6 μg/ml in Col2) showed a potent cytotoxic activity. Further study for the identification of active compounds from these lead extracts might be warranted.

Keywords – Cytotoxicity, A549, Col2, *Calystegia soldanella*, *Heloniopsis orientalis*, *Thuja koraiensis*

Introduction

It is well established that natural products including plant have been a useful resource of clinically relevant antitumor agents (Cragg *et al.*, 1994; Baker *et al.*, 1995). Indeed, paclitaxel, camptothecin, podophyllotoxin, vincristine and vinblastine have been introduced as potential lead anticancer agents from higher plants (Cragg, 1993; Wani and Wall, 1993). Nonetheless, the total number of cancer deaths still continues to increase and, moreover, cancer deaths from certain solid carcinomas such as lung, colon, prostate, and rectum still remain high. Therefore, it clearly needs for the discovery of novel agents with higher efficacy.

In the course of searching for antitumor agents from natural products we primarily evaluated the in vitro cytotoxic activity of indigenous Korean plant extracts against human lung and colon cancer cells.

Experimental

Chemicals – Trichloroacetic acid (TCA), and sulforhodamine B (SRB) were purchased from Sigma Chemical Co. (St. Louis, MO). Minimal essential medium with Earle's salt (MEME), fetal bovine serum (FBS), non-essential amino acid solution (10 mM, 100X), trypsin-EDTA solution (1X) and antibiotic-antimycotic solution (PSF) were from GIBCO-BRL (Grand Island, NY).

Extracts of plant materials tested – Methanolic plant extracts used for this study was purchased from Plant Extracts Bank of Plant Diversity Research Center (Daejon, Korea).

Evaluation of cytotoxic potential with human cancer cell lines – Cytotoxic potential was determined as described previously (Lee *et al.*, 1998). Briefly, cells (in log growth phase) were counted, diluted to 5×10^4 cells/ml with fresh medium, and added to 96-well microtiter plates (190 μl/well) containing test materials (10 μl in 10% aqueous DMSO). Test plates were incubated for 3 days at 37°C in CO_2 incubator. For zero day controls, cells were incubated for 30 min at 37°C in CO_2 incubator. All treatments were performed in triplicate. After the incubation periods, cells were fixed by the addition of 50 μl of cold 50% aqueous trichloroacetic acid (4°C for 30 min), washed 4-5 times with tap water, and air-dried. The fixed cells were stained with sulforhodamin B (SRB) (0.4% w/v SRB in 1% aqueous acetic acid solution) for 30 min. Unbounded SRB solution was then removed by rinsing with 1% acetic acid. The plates were then air-dried, the bound dye was solubilized with 200 μl of 10 mM tris-base (pH 10.0), and absorbance was determined at 515 nm using an ELISA plate reader. Finally, the absorbance values obtained with each of the treatment procedures were averaged, and the averaged value obtained with the zero day control was subtracted. These results were expressed as a percentage, relative to

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Table 1. Cytotoxic potential of plant extracts against human cancer cells

Plant name and Authority	Family	Part used[a]	A549[b]	Col2[c]
Abelia tyaihyoni Nakai	Caprifoliaceae	ST	> 50	> 50
Actaea asiatica Hara	Ranunculaceae	WP	> 50	> 50
Actinodaphne lancifolia (S. et Z.) Meissn	Lauraceae	TW	47.6	> 50
Asperula odorata L.	Rubiaceae	WP	> 50	> 50
Caesalpinia japonica S. et Z.	Leguminosae	LS	> 50	> 50
Calystegia soldanella Roem. et Schult.	Convolvulaceae	WP	8.0	27.4
Capsella bursa-pastoris (L.) Medicus	Cruciferae	HR	> 50	> 50
Cardamine amaraeformis Nakai	Cruciferae	WP	> 50	> 50
Cardamine flexuosa With	Cruciferae	HR	> 50	> 50
Carpinus laxiflora Bl.	Betulaceae	SB	20.6	36.2
Cayratia japonica (Thunb.) Gagnep.	Vitaceae	FR	> 50	> 50
Celtis choseniana Nakai	Ulmaceae	FR	> 50	> 50
Cinnamomum japonicum Sieb.	Lauraceae	SB	> 50	42.4
Citrus tachibana (Makino) C. Tanaka	Rutaceae	SB	> 50	> 50
Cleyera japonica Thunb.	Theaceae	SB	> 50	> 50
Cornus walteri Wagner.	Cornaceae	ST	46.3	39.6
Crataegus pinnatifida Bunge	Rosaceae	ST	36.2	38.9
Daphniphyllum glaucescens Blume	Euphorbiaceae	ST	> 50	> 50
Dystaenia takeshimana (Nak.) Kitagawa	Umbelliferae	HR	> 50	> 50
Dystaenia takeshimana (Nak.) Kitagawa	Umbelliferae	RT	> 50	> 50
Erysimum aurantiacum Max.	Cruciferae	LS	> 50	> 50
Heloniopsis orientalis (Thunb.) C. Tanaka	Liliaceae	WP	4.6	4.5
Hovenia dulcis Thunb.	Rhamnaceae	ST	28.3	> 50
Ilex macropoda Miq.	Aquifoliaceae	ST	> 50	> 50
Ixeris dentate var. albiflora Nak.	Compositae	RT	> 50	> 50
Kirengeshoma koreana Nakai	Saxifragraceae	RT	> 50	> 50
Kirengeshoma koreana Nakai	Saxifragraceae	WP	16.9	33.9
Lamium album var. barbatum (S. et Z.) Fr. et Sav.	Labiatae	WP	> 50	> 50
Lepidium ruderale L.	Cruciferae	WP	> 50	> 50
Ligularia fischeri (Ledeb.) Turcz.	Compositae	WP	> 50	> 50
Ligustrum japonicum Thunb.	Oleacae	TW	> 50	> 50
Lindera obtusiloba Bl.	Lauraceae	LS	> 50	> 50
Lonicera maackii Max.	Caprifoliaceae	ST	> 50	> 50
Lonicera vidalii Fr. et Sav.	Caprifoliaceae	LF	> 50	> 50
Maackia fauriei (Lev.) Takeda	Leguminosae	SB	44.3	41.4
Meliosma oldhamii Max.	Sabiaceae	SB	11.3	30.6
Mitchella undulata S. et Z.	Rubiaceae	WP	> 50	> 50
Myrica rubra S. et Z.	Myricaceae	SB	18.6	27.8
Neolitsea aciculate (Bl.) Koidz	Lauraceae	LF	> 50	> 50
Osmanthus insularis Koidz.	Oleacae	SB	> 50	> 50
Phlomis umbrosa Turcz	Labiatae	WP	> 50	> 50
Sambucus sieboldiana Bl.	Caprifoliaceae	SB	> 50	> 50
Sorbus alnifolia (S. et Z.) K. Koch	Rosaceae	ST	> 50	> 50
Staphylea bumalda Dc.	Staphyleaceae	FR	> 50	> 50
Stewartia koreana Nakai	Theaceae	ST	41.6	40.7
Symplocos paniculata Miq.	Symplocaceae	LF	17.9	13.1
Syringa velutina var. kamibayashi T. Lee.	Oleacae	LS	> 50	> 50
Tetragonia tetragonoides O. Kuntze	Aizoaceae	WP	> 50	> 50
Thuja koraiensis Nakai	Cupressaceae	LF	1.2	0.6
Tiarella polyphylla D. Don.	Saxifragraceae	RT	18.2	35.3
Vaccinium bracteatum Thunb.	Ericaceae	SB	> 50	> 50
Vaccinium oldhami Miq.	Ericaceae	ST	> 50	> 50
Viburnum awabuki K. Koch	Caprifoliaceae	LF	45.5	49.9
Viburnum erosum Thunb.	Caprifoliaceae	FR	> 50	> 50
Vicia angustifolia var. segetilis K. Koch	Leguminosae	WP	> 50	> 50
Wasabia koreana Nakai	Cruciferae	RT	> 50	> 50
Wasabia koreana Nakai	Cruciferae	WP	> 50	> 50
Ellipticine			0.2	0.8

[a]Part used: FT (Fruit), HR (Herb), LF (Leaf), LS (Leaf+stem), RT (Root), SB (Stem bark), ST (Stem), TW (Twig), WP (Whole plant).
[b]A549: IC_{50} (μg/ml) in cultured human lung carcinoma.
[c]Col2: IC_{50} (μg/ml) in cultured human colon carcinoma.

solvent-treated control incubations, and IC_{50} values were calculated using non-liner regression analyses (percent survival versus concentration).

Results and Discussion

During the course of our continuous efforts for discovery of antitumor agents from natural products, the present study was undertaken to evaluate the cytotoxic potential of indigenous Korean plants that have been used for alleviating diverse diseases or dietary foods. Based on the highest mortality, five-year survival rate and rapid increase of cancer patients recently in Korea, we selected two solid carcinoma cell lines lung (A549) and colon (Col2) for evaluating cytotoxicity of natural products. Among tested fifty-seven methanolic extracts of plant materials, 16 extracts were found to be active against A549 cells and 15 extracts were active against Col2 cells as shown in Table 1 as judged in the criteria of IC_{50} < 50 μg/ml. In particular, the extracts of *Calystegia soldanella*, *Heloniopsis orientalis*, and *Thuja koraiensis* showed a potent cytotoxic activity against both cell lines. Further, the whole plant extract of *C. soldanella* showed more susceptible against lung cells (IC_{50} = 8.0 μg/ml) than colon cancer cells (IC_{50} = 27.4 μg/ml). In addition, since the indigenous Korean plant *T. koraiensis* showed a strong cytotoxicity (IC_{50} = 1.2 μg/ml in A549; IC_{50} = 0.6 μg/ml in Col2) and has not been thoroughly studied for phytochemical investigation, the monitoring of cytotoxic principles for this extract might be valuable.

In conclusion, for the discovery of novel antitumor agents from natural products, we primarily approached and evaluated the cytotoxicity for indigenous Korean plant extracts. Several plant extracts exhibited potential cytotoxicity and selectivity in cultured human lung or colon cancer cells. Therefore, the information from this study will be useful to the isolation of active compounds against human cancer cells.

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