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Evaluation of Cytotoxic Potential of Natural Products in Cultured Human Cancer Cells

Kyung Ae Nam and Sang Kook Lee*

The Research Institute of Pharmaceutical Sciences and College of Pharmacy, Ewha Womans University, Seoul 120-750, Korea

Abstract – In order to discover novel potent antitumor agents, methanolic extracts of approximately 180 herbal medicines were prepared and primarily evaluated for cytotoxic activity in cultured human lung (A549) and colon (Col 2) cancer cells. As a result, 17 natural product extracts were found to be active in the criteria of IC_{50}<20 μg/ml. Especially, the extracts of *Aristolochia debilis*, *Cynanchum ascyrifolium*, *Cynanchum paniculatum*, *Daphne genkwa*, *Euphorbia lathyris*, *Ipomoea hederacea*, *Magnolia officinalis*, *Melia azedarach* var. *japonica*, *Solanum nigrum*, *Thuja orientalis*, and *Trichosanthes kirilowii* showed a strong cytotoxic potential. The flower extract of *Daphne genkwa* was more selective cytotoxic activity against lung cancer cells (IC_{50}; 0.2 μg/ml) compared to colon cancer cells (IC_{50}>20 μg/ml). In addition, based on the cytotoxic potential of the root extract of *Cynanchum paniculatum*, the further fractionation of methylene chloride partition with silica gel column chromatography was performed. Several subfractions were considered to be active, and thus indicating that further studies for the identification of active principles from these fractions might be warranted.
Key words – Cytotoxicity, natural products, *Cynanchum paniculatum*

Introduction

It is well-known that natural products have played an important role in the discovery of useful antitumor agents (Baker *et al.*, 1995; Cragg *et al.*, 1997; Pezzuto, 1997). Indeed, clinically relevant anticancer agents such as taxol, camptothecin, vinblastine and vincristine have been uncovered from higher plants (Cragg, 1993; Wall and Wani, 1993). Nonetheless, with the increased morbidity and mortality associated with metastatic conditions, there is a still clearly need for the discovery of new agents with higher clinical efficacy.

During the course of searching for natural product-derived antitumor agents, we primarily evaluated the growth inhibitory potential of approximately 180 herbal medicine extracts with the culture of human lung and colon cancer cells. The results are presented herein.

Experimental

Chemicals – Trichloroacetic acid (TCA), and sulforhodamine B (SRB) were purchased from Sigma Chemical Co. (St. Louis, MO). Minimal essential medium with Earles' 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).

Preparation of extracts of natural products – Natural products including Korean medicinal plants were purchased from herbal markets (Han-Yang Yutong Co.) in Seoul, Korea. The botanical identification was performed by Drs. Ihnran Lee and Jungae Do (College of Pharmacy, Ewha Womans University, Seoul, Korea). The voucher specimen has been deposited in this department. Each of the dried herbs was sliced, and extracted with 100% methanol (×3). The methanol extracts were concentrated under reduced pressure below 40°C and stored at −20°C until use.

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 a CO_2 incubator.

Table 1. Cytotoxic potential of natural products on human cancer cells

Plant name and Authority	Family	Part used[a]	A549[b]	Col2[c]
Acanthopanax sessiliflorum Seeman	Araliaceae	BK	>20	>20
Achyranthes japonica Nakai	Amaranthaceae	RT	>20	>20
Aconitum koreanum Raymond	Ranunculaceae	TB	>20	>20
Aconitum pseudolaeve var. *erectum* Nakai	Ranunculaceae	RT	>20	>20
Adenophora remotiflora Mig.	Campanulaceae	RT	>20	>20
Agrimonia pilosa Ledeb var. *japonica* Nakai	Rosaceae	HR	>20	>20
Ailanthus altissima (Mill) Swingle	Simarubaceae	SB	>20	>20
Akebia quinata Dence	Lardizabalaceae	ST	>20	>20
Albizzia julibrissin Durazz	Leguminosae	CR	>20	>20
Alpinia katsumadai Hayata	Zingiberaceae	SD	>20	>20
Amomum cardamomum L.	Zingiberaceae	FR	>20	>20
Amomum xanthioides Wall	Zingiberaceae	FR	19.6	>20
Ampelopsis japonica (Thunb) Makino	Vitaceae	RT	>20	>20
Angelica gigas Nakai	Umbelliferae	RT	>20	>20
Arctium lappa L.	Compositae	FR	>20	>20
Areca catechu L.	Palmae	FR	>20	>20
Aristolochia contorta Bunge	Aristolochiaceae	FR	>20	19.1
Aristolochia debilis Sieb. et Zucc.	Aristolochiaceae	RT	7.2	1.7
Artemisia apiacea Hance.	Compositae	HR	>20	>20
Artemisia capillaris Thunb	Compositae	HR	>20	>20
Asparagus cochinchinensis (Lour.) Merr.	Liliaceae	TB	>20	>20
Aster tataricus L. Fil.	Compositae	RT	>20	>20
Astragalus membranaceus (Fisch.) Bge.	Leguminosae	RT	>20	>20
Atractylodes japonica Koidz.	Compositae	ST	>20	>20
Benincasa hispida (Thunb.) Cogn.	Cucurbitaceae	SD	>20	>20
Betula platyphylla Suk. Var. *japonica* Hara.	Betulaceae	CR	>20	>20
Bletilla striata (Thunb.)Reichb. Fil.	Orchidaceae	TB	>20	>20
Broussonetia papyrifera (L.)Vent.	Moraceae	FR	>20	>20
Buddleia officinalis Maxim.	Loganiaceae	FL	>20	>20
Caesalpinia sappan L.	Leguminosae	LG	>20	>20
Caragana chamlagu Lam.	Leguminosae	LF	>20	>20
Carpesium abrotanoides L.	Compositae	FR	>20	>20
Carthamus tinctorius L.	Compositae	SD	>20	>20
Chaenomeles sinensis (Thuin) Koehne	Malaceae	FR	>20	>20
Chelidonium majus L.	Papaveraceae	HR	>20	>20
Chrysanthemum zawadskii var *latilobum* Kitamura	Compositae	HR	>20	>20
Cibotium barometz (L.) J. Sm.	Cyatheaceae	ST	>20	>20
Cichorium intybus L.	Compositae	LF	>20	>20
Cinnamomum cassia Blume	Lauraceae	TW	>20	>20
Cinnamomum loureirii Nees.	Lauraceae	BK	>20	>20
Circium japonicum DC	Compositae	RT	>20	>20
Clematis mandshurica Rupr.	Ranunculaceae	RT	>20	>20
Codonopsis pilosula (Franch) Nannf.	Campanulaceae	RT	>20	>20
Corydalis ternata Nakai	Papaveraceae	TB	>20	>20
Cremastra variabilis Nakai	Orchidaceae	TB	>20	>20
Cucumis melo L. var *makuwa* Makino	Cucurbitaceae	PF	>20	>20
Curculigo orchioides Garrtn.	Amaryllidaceae	ST	>20	>20
Curcuma longa L.	Zingiberaceae	ST	18.0	17.7
Curcuma zedoaria Roscoe	Zingiberaceae	ST	>20	>20
Cuscuta chinensis Lam.	Convolvulaceae	SD	>20	>20
Cynanchum ascyrifolium Matsumura	Asclepiadaceae	RT	3.0	2.5
Cynanchum atratum Bunge	Asclepiadaceae	RT	>20	>20
Cynanchum paniculatum Kitagawa	Asclepiadaceae	RT	15.7	14.1
Cynanchum wilfordii (Max.) Hemsl.	Asclepidaceae	TB	>20	>20
Cynomorium songaricum Rupr.	Cynomoriaceae	HR	>20	>20
Cynthus officinalis L.	Compositae	RT	>20	>20
Daphne genkwa Sieb. et Zucc.	Thymelaeaceae	FL	0.2	>20

Table 1. Continued

Plant name and Authority	Family	Part used[a]	A549[b]	Col2[c]
Davallia mariesii Moore	Davalliaceae	ST	>20	>20
Dendrobium nobile Lindl.	Orchidaceae	HR	>20	>20
Dictamnus albus L.	Rutaceae	SB	>20	>20
Dioscorea japonica Thunb.	Dioscoreaceae	TB	>20	>20
Dioscorea tokoro Makino	Dioscoreaceae	TB	>20	>20
Diospyros kaki Thunb.	Ebenaceae	LF	>20	>20
Dipsacus japonicus Mig.	Dipsacaceae	RT	>20	>20
Draba nemorosa L.	Cruciferae	SD	>20	>20
Eclipta prostrata L.	Compositae	HR	>20	>20
Ephedra sinica Stapf.	Ephedraceae	RT	>20	>20
Equisetum hiemale L.	Equisetaceae	HR	>20	>20
Eriobotrya japonica (Thunb) Lindl.	Malaceae	LF	>20	>20
Eriocaulon sietoldianun Sieb. et Zucc.	Eriocaulaceae	FT	>20	>20
Erycibe obtusifolia B.	Convolvulaceae	ST	>20	>20
Eucommia ulmoides Oliv.	Eucommiaceae	TW	>20	>20
Eucommia ulmoides Oliv.	Eucommiaceae	SB	>20	>20
Eugenia caryophyllata Thunb.	Myrtaceae	SB	>20	>20
Euphorbia lathyris L.	Euphorbiaceae	SD	7.3	>20
Euphoria longana Steud.	Sapindaceae	FR	>20	>20
Gallus domesticus Blume	Phasianidae	ST	>20	>20
Ganoderma lucidum Karst.	Polyporaceae	WP	>20	>20
Gardenia jasminoides Ellis.	Rubiaceae	FR	>20	>20
Gastrodia elata Blume	Orchidaceae	ST	>20	>20
Ginkgo biloba L.	Ginkgoaceae	FR	>20	>20
Glechoma longituba (Nakai) Kupr.	Labiatae	HR	>20	>20
Gleditsia japonica var. *koraiensis* Nakai	Leguminosae	TN	>20	>20
Glycine max (L.) Merr.	Leguminosae	SD	>20	>20
Glycyrrhiza uralensis Fischer	Leguminosae	RT	>20	>20
Hemerocallis fluva L.	Liliaceae	LF	>20	>20
Hordeum vulgale L.	Graminae	FR	>20	>20
Houttuynia cordata Thunb.	Saururaceae	HR	>20	>20
Ipomoea hederacea Jacq.	Convolvulaceae	SD	9.1	>20
Juncus effusus L. var. *decipiens* Buchen.	Juncaceae	HR	>20	>20
Kalopanax pictus (Thunb.) Nakai	Araliaceae	CR	>20	>20
Kochia scoparia (L.) Schrad.	Chenopodiaceae	SD	>20	>20
Leonurus sibiricus L.	Labiatae	SD	>20	>20
Ligustrum lucidum Ait.	Oleaceae	FR	17.4	>20
Litchi chinensis Sonn.	Sapindaceae	FR	>20	>20
Lycium chinensis Mill.	Solanaceae	SB	>20	>20
Lycopus lucidus Turcz.	Labiatae	HR	>20	>20
Magnolia denudata Desr.	Magnoliaceae	FL	>20	>20
Magnolia officinalis Rehd. et Wils.	Magnoliaceae	CR	>20	13.1
Malva verticillata L.	Malvaceae	SD	>20	>20
Melandrium firmum Rohrb.	Caryophyllaceae	SD	>20	>20
Melia azedarach L. var. *japonica* Makino	Meliaceae	FR	15.1	4.6
Momordica cochinchinensis Spr.	Cucurbitaceae	SD	>20	>20
Morinda officinalis How.	Rubiaceae	RT	>20	>20
Morus alba L.	Moraceae	FR	>20	>20
Morus alba L.	Moraceae	LF	>20	>20
Morus alba L.	Moraceae	TW	>20	>20
Mucuna birdwoodiana Tutcher.	Leguminosae	ST	>20	>20
Nepeta japonica Max.	Labiatae	HR	>20	>20
Oldenlandia diffusa (Willd.) Roxb.	Rubiaceae	HR	>20	>20
Orostachys japonicus A. Berger.	Crassulaceae	HR	>20	>20
Paeonia moutan Sims.	Paeoniaceae	SB	>20	>20
Panax notoginseng F. H. Chen.	Araliaceae	RT	>20	>20
Panax quinquefolium L.	Araliaceae	RT	>20	>20

Table 1. Continued

Plant name and Authority	Family	Part used[a]	A549[b]	Col2[c]
Paonia lactiflora Pall.	Paeoniaceae	RT	>20	>20
Persicaria tinctoria H.Gross.	Polygonaceae	HR	>20	>20
Phaenosperma globosa M.	Graminae	HR	>20	>20
Pharbitis nil Choisy.	Convolvulaceae	SD	16.4	>20
Phragmites communis Trin.	Graminae	RT	>20	>20
Phyllostachys nigra M. *var. henonis* S.	Bambusaceae	CR	>20	>20
Phytolacca esculentum Var Houtt.	Phytolaccaceae	RT	>20	>20
Pinus densiflora Sieb et. Zucc.	Pinaceae	TW	>20	>20
Piper longum L.	Piperaceae	SD	>20	>20
Polygala japonica Houtt.	Polygalaceae	HR	>20	>20
Polygala tenuifolia Willd.	Polygalaceae	RT	>20	>20
Polygonum aviculare L.	Polygonaceae	HR	>20	>20
Polygonum multiflorum Thunb.	Polygonaceae	TB	>20	>20
Poncirus trifloliata Ratin.	Rutaceae	FR	>20	>20
Poria cocos (S.) Wolf	Polyporaceae	ST	>20	>20
Poria cocos (S.) Wolf	Polyporaceae	SB	>20	>20
Poria cocos (S.) Wolf	Polyporaceae	SC	>20	>20
Portulaca oleracea L.	Portulacaceae	HR	>20	>20
Prunella vulgaris L. var. *lilacina* Nakai	Labiatae	HR	>20	>20
Prunus japonica var. nakaii Rhed.	Amygdalaceae	SD	>20	>20
Pterocarpus santalinus Lf.	Leguminosae	LG	>20	>20
Pueraria thunbergiana Bentham	Leguminosae	RT	>20	>20
Pueraria thunbergiana Bentham	Leguminosae	FL	>20	>20
Pyrrosia lingua (Thunb) Far W.	Polypodiaceae	LF	>20	>20
Rehmania glutinosa Liboschitz	Scrophulariaceae	RT	>20	>20
Rehmannia glutinosa Liboschitz var. *purpurea* Making	Scrophulariaceae	RT	>20	>20
Rheum coreanum Nakai	Polygonaceae	ST	>20	>20
Rhus verniciflua Stokes	Anacardiaceae	FD	>20	>20
Rosa laevigata Michx	Rosaceae	FR	>20	>20
Rosa rugosa Thunb.	Rosaceae	RT	>20	>20
Rubia akane Nakai	Rubiaceae	RT	>20	>20
Rubus coreanus Mig.	Rosaceae	FR	>20	>20
Salvia miltiorrhiza Bunge	Labiatae	RT	>20	>20
Sargassum fusiforme (Harv.) Setch.	Sargassaceae	HR	>20	>20
Schizandra chinensis Baill.	Schizandraceae	FR	>20	>20
Scirpus yagara Ohwi	Cyperaceae	ST	>20	>20
Scutellaria baicalensis Georgi.	Labiatae	RT	>20	>20
Sedum albroseum Bak.	Crassulaceae	HR	>20	>20
Selaginella tamariscina Spring	Selaginellaceae	HR	>20	>20
Sesamum indicum DC.	Pedaliaceae	SD	>20	>20
Siegesbeckia orientalis L. var. *pubescens* Mak.	Compositae	HR	13.2	>20
Sinomenium acutum Rehder et Wilson	Menispermaceae	ST	>20	>20
Solanum nigrum L.	Solanaceae	HR	16.9	15.8
Sophora japonica L.	Leguminosae	RT	>20	>20
Sophora subprostrata Chun. Et. T. Chen.	Leguminosae	RT	>20	>20
Spirodela polyrrhiza (L.) Schleid	Lemnaceae	HR	>20	>20
Strychnos ignatii Berg.	Loganiaceae	FR	>20	>20
Thuja orientalis L.	Cupressaceae	FT	0.6	0.3
Tribulus terrestris L.	Zygophyllaceae	FR	>20	>20
Trichosanthes kirilowii Max	Cucurbitaceae	RT	1.8	0.8
Trigonella foenum-graecum L.	Leguminosae	SD	>20	>20
Triticum aestivum L.	Graminae	SD	>20	>20
Typha orientalis Schum. et Thonn.	Typhaceae	PL	>20	>20
Ulmus macrocarpa Hance	Ulmacea	FR	>20	>20
Ulmus parvifolia Jacq.	Ulmaceae	SB	>20	>20
Ulmus pumila L.	Ulmaceae	SB	>20	>20
Uncaria sinensis Havil.	Rubiaceae	TW	>20	>20

Table 1. Continued

Plant name and Authority	Family	Part used[a]	A549[b]	Col2[c]
Undaria pinnatifida Sur.	Lamirariaceae	HR	>20	>20
Xanthium strumarium L.	Compositae	FR	>20	>20
Zea mays L.	Graminae	CS	>20	>20
Zingiber officinale Roscoe	Zingiberaceae	ST	>20	>20
Zizyphus jujuba Mill.	Rhamnaceae	FS	>20	>20
Zizyphus jujuba Mill. var. *inermis* Rehol.	Rhamnaceae	FR	>20	>20
Taxol			0.007	0.003
Ellipticine			0.5	0.3
Streptonigrin			0.2	0.2

[a]Part used: HR (herb), SB (stem bark), RT (root), ST (stem), LF (leaf), TW (twig), FL (flower), FT (flower + twig), FR (fruit), SD (seed), SC (sclerotium), TB (tuber), PF (penduncle of fruit), PL (pollen), CR (cortex), FD (fluid), CS (corn silk), FS (seed in fruit), WP (whole plant), BK (bark), TN (thorn).
[b]A549 : IC_{50}(μg/ml) in cultured human lung cancer cells
[c]Col2 : IC_{50}(μg/ml) in cultured human colon cancer cells

For zero day controls, cells were incubated for 30 min at 37°C in a 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 sulforhodamine B (SRB) (0.4% w/v SRB in 1% aqueous acetic acid) for 30 min. Free 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 solvent-treated control incubations, and IC_{50} values were calculated using non-linear regression analyses (percent survival versus concentration).

Results and Discussion

The present study was undertaken to evaluate the cytotoxic potential of traditional herbal medicines that have been used for several diseases including cancer and cancer related illness. The methanol extracts of approximately 180 natural products were primarily evaluated for the cytotoxic activity in cultured human non-small lung (A549) and colon (Col2) cancer cells. As judged in the criteria of cytotoxic activity with IC_{50}<20 μg/ml, 17 extracts were found to be active as shown in Table 1. Especially, the extracts of *Aristolochia debilis*, *Cynanchum ascyrifolium*, *Cynanchum paniculatum*, *Daphne genkwa*, *Euphorbia lathyris*, *Ipomoea hederacea*, *Magnolia officinalis*, *Melia azedarach* var. *japonica*, *Solanum nigrum*, *Thuja orientalis*, and *Trichosanthes kirilowii* showed a strong cytotoxic potential against either lung or colon cancer cell. As can be seen in Table 1, some natural product extracts also showed a selective cytotoxicity against cell types. In the case of the flower extract of *Daphne genkwa*, lung cells (IC_{50}; 0.2 μg/ml) were more susceptible than colon (IC_{50}; >20 μg/ml) cancer cells. Cytotoxic selectivity is likely to be due to the presence of different classes of compounds in the extract, as it has been documented previously with known classes of compounds (Cragg *et al.*, 1994). In addition, based on the cytotoxic potential of the root extract of *Cynanchum ascyrifolium*, other species such as *C. atratum*, *C. paniculatum* were also tested for the activity. Compared to the less active of *C. atratum*, the extract of *C. paniculatum* was considered to be active. Since *C. paniculatum* is the indigenous species in Korea and not much investigated for chemical constituents (Lee *et al*, 1980; Sugama *et al*, 1985; Lee, 1996), the cytotoxic principles of the extract were monitored by bioassay-guided fractionation. As a result, several subfractions from the methylene chloride partition through silica gel column chromatography were found to be active as shown in Table 2. Subfraction F9-2 showed a very strong activity both lung and colon cells with the IC_{50} value of 0.007 μg/ml and 0.004 μg/ml, respectively. Therefore, further fractionation for elucidation of active principle from

Table 2. Cytotoxic potential of subfractions of methylene chloride partition from *Cynanchum paniculatum*

Fraction	A549 (IC_{50}: μg/ml)	Col2 (IC_{50}: μg/ml)
F1	>20	>20
F2	>20	>20
F3	>20	>20
F4	>20	>20
F5	>20	>20
F6	>20	>20
F7	>20	>20
F8	12.5	9.0
F9	1.0	1.3
F 9-1	2.0	1.0
F 9-2	0.007	0.004
F 9-3	3.5	2.1
F 9-4	19.2	18.3
F 9-5	1.5	1.1
F10	>20	>20

this active fraction is highly encouraged and warranted for the discovery of potential antitumor agents from natural products.

In conclusion, approximately 10% of the natural product extracts tested showed the cytotoxic potential against lung or colon cells in culture. In addition, some natural product extracts exhibited selectivity. Therefore, the information will be helpful to the isolation of active principle with selectivity against certain cancer cells. With the potent cytotoxic activity of subfraction of *C. paniculatum*, the study to isolate the active principles from the extract will be strongly recommended.

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