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Cytotoxic Activity of Vietnamese Herbal Medicines against A549 Cells

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A549세포에 대한 베트남 약용식물의 세포독성

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Abstract – Eighty methanol extracts prepared from Vietnamese plants were tested for their cytotoxic activity against A549 cells, a human lung carcinoma cell line. Nine extracts showed survival rates of tumor cells of less than 30% at 100 μg/mL of the methanol extracts. Among them, three were less than 15%; *Meliosma pinnata* (VK03, 4%), *Goniothalamus vietnamensis* (VK05, 06, 0%), *Garcinia* sp. (VK50, 51, 0%) and *Aglaia aphanamixis* (VK63, 11%). Both leaf and root extracts of *Goniothalamus vietnamensis* and the leaf extract of a *Garcinia* species completely inhibited the growth of A549 cells at the concentration of 100 μg/mL methanol extract.

Key words – Cytotoxicity, Vietnamese plant, *Goniothalamus vietnamensis.*

Various efforts have been taken to find antitumor agents from the plant kingdom, and thus resulted in the discovery of some clinically useful anticancer drugs. As described by Ho, Vietnam has an abundant and diverse flora which accommodates about 12,000 vascular species.[1)] Of these, more than 11,000 species have been mentioned in his books. More than 4000 species are currently used in traditional medicine.[2, 3)] Recently, we have reported a number of Vietnamese medicinal plants showing inhibitory effects on the tube formation of human umbelical veinous endothelial (HUVE) cells as well as some cancer cell lines.[4)] As a continuity of our ongoing studies, we have implemented a preliminary screening for another part of our voucher samples from Vietnam on A549 cells. Here we report our results from this screening.

MATERIALS AND METHODS

Plant Material – Plant materials were collected from some provinces in the North of Vietnam. The identification of plant was determined by Dr. Vu Xuan Phuong, Institute of Ecology and Biological Resources (NCST) and Dr. Ngo Van Trai, National Institute of Materia Medica. The voucher specimen of studied plants have been deposited at the above institute.

Extraction – Plant materials (including leaves, stems, root, and fruit if available) were collected with dried amounts of 30-50 g for bioassay and 500-1000 g or more for isolation process of active compounds. The plant parts (leaves, roots, flower or stems) were dried in shade and grinded. The materials were extracted with 95% methanol in water for 3 times (8 hrs each) and filtered. The methanol filtrates were combined and concentrated *in vacuo*. The extract was dissolved in DMSO (dimethyl sulfoxide) at the stock concentration of 100 mg/mL and subjected to cytotoxic assay.

Cytotoxicity assay – Human lung carcinoma cell line (A549), obtained from Korea Research Institute of Bioscience and Biotechnology (KRIBB), were maintained as a monolayer in RPMI1640 media supplemented with 10% fetal bovine serum (GIBCO, Grand Island, NY), sodium bicarbonate, penicillin G, and streptomycin at 37°C under a humidified atmosphere of 5% CO_2. Cytotoxicity was measured by the sulforhodamine B (SRB) method.[5)] Viable cells were

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Table 1. Cytotoxic activity of plant extracts against A549 cells

VSN[a]	Plant Name and Authority	Family	Part used[b]	SR (%)[c]
VK01	*Murraya glabra* (L.) Thw.	Rutaceae	ST	36
VK02	*Embelia parviflora* Wall. ex DC.	Myrsinaceae	SB	13
VK03	*Meliosma pinnata* (Roxb.) Walpers.	Sabiaceae	LF	4
VK04	*Bumelia harmandii* (L.) Thw.	Sapotaceae	SB	43
VK05	*Goniothalamus vietnamensis* Ban	Annonaceae	RT	0
VK06	*Goniothalamus vietnamensis* Ban	Annonaceae	LF	0
VK07	*Micromelum falcatum* Tanaka	Rutaceae	LF	88
VK08	*Desmos cochinchinensis* Lour.	Annonaceae	LF	24
VK09	*Balanophora fungosa* J.R. et Forster	Balanophoraceae	WP	53
VK10	*Glycosmis stenocarpa* (Drake) Tanaka	Rutaceae	LF	25
VK11	*Buxus myrica* Levl.	Buxaceae	LF	59
VK12	*Micromelum falcatum* Tanaka	Rutaceae	LF	83
VK13	*Polyanthia oligogyna* Merr.	Annonaceae	LF	85
VK14	*Entada phaceoleudes* (L.) Merr.	Fabaceae	LF	49
VK15	Tabernaemontana pallida	Apocynaceae	LF	85
VK16	*Millettia setigera* Dunn	Fabaceae	LF	30
VK17	*Gomphandra mollis* Merr.	Icacinaceae	ST	33
VK18	*Elaeagnus loureiri* Champ.	Elaeagnaceae	LF	49
VK19	*Canarium tonkinense* Engl.	Burseraceae	SB	19
VK20	*Garcinia* sp.	Cluciaceae	LF	50
VK21	*Loxogramma* sp.	Grammitidaceae	LF	59
VK22	*Acronychia pedunculata* (L.) Miq.	Rutaceae	LF	42
VK23	*Dischidia tonkinensis* Cost.	Asclepiadaceae	LF	77
VK24	*Gymnema reticulata* (Moon) Alst.	Asclepiadaceae	LF	>100
VK25	*Myxopyrum nervosum* Blume	Oleaceae	LT	74
VK26	*Tapiscia affinis* Merr. et Chun.	Staphyleaceae	LF	47
VK27	*Diospyros susarticulata* Lec.	Ebenaceae	FR	51
VK28	*Myxopyrum nervosum* Blume	Oleaceae	RH	>100
VK29	*Glycosmis stenocarpa* (Drake) Tanaka	Rutaceae	RT	51
VK30	*Fissistigma polyanthoides* (DC.) Merr.	Annonaceae	LF	75
VK31	*Fissistigma polyanthoides* (DC.) Merr.	Annonaceae	SB	84
VK32	*Abroma angusta* (L.) Willd.	Sterculiaceae	ST	93
VK33	*Abroma angusta* (L.) Willd.	Sterculiaceae	LF	59
VK34	*Heteropanax fragrans* (Roxb.) Seem.	Araliaceae	LF	57
VK35	*Disporopsis longifolia* Craib.	Convallariaceae	LF	>100
VK36	*Disporopsis longifolia* Craib.	Convallariaceae	RH	>100
VK37	*Heteropanax fragrans* (Roxb.) Seem.	Araliaceae	SB	>100
VK38	*Elaeocarpus angustifolius* Blume	Elaeocarpaceae	FL	>100
VK39	*Aristolochia roxburghliana* Klotsch.	Aristolochiaceae	WP	15
VK40	*Ardicia thorelli* Pit.	Myrsinaceae	LF	>100
VK41	*Phoebe lanceolata* Nees.	Lauraceae	LF	89
VK42	*Ficus glaberrima* Blume	Moraceae	FR	98
VK43	*Ficus glaberrima* Blume	Moraceae	LF	26
VK44	*Lasianthus anamicus* Pit.	Rubiaceae	ST	>100
VK45	*Lasianthus anamicus* Pit.	Rubiaceae	LF	>100
VK46	*Capparis radula* Gagnep.	Capparaceae	LF	>100

Table 1. Continued

VSN[a]	Plant Name and Authority	Family	Part used[b]	SR (%)[c]
VK47	*Diospyros susarticulata* Lec.	Ebenaceae	LF	73
VK48	*Elaeocarpus angustifolius* Blume	Elaeocarpaceae	LF	28
VK49	*Hydnocarpus macrocarpa* (Bedd.) Warb. subsp. burmanica Sleum.	Elacourtiaceae	LF	>100
VK50	*Garcinia* sp.	Cluciaceae	LF	0
VK51	*Garcinia* sp.	Cluciaceae	ST	0
VK52	*Gouania javanica* Miq.	Rhamnaceaee	ST	86
VK53	*Glycosmis* sp.	Rutaceae	LF	18
VK54	*Brassaiopsis variabilis* Shang.	Araliaceae	AP	97
VK55	*Phyllanthus reticulatus* Champ. ex Benth.	Euphorbiaceae	ST	81
VK56	*Phyllanthus reticulatus* Champ. ex Benth.	Euphorbiaceae	LF	71
VK57	*Glycosmis stenocarpa* (Drake) Tanaka	Rutaceae	ST	84
VK58	*Naravelia zeylanica* (L.) DC.	Ranunculaceae	LT	>100
VK59	*Viburnum lutescens* Blume	Verbenaceae	LF	57
VK60	*Rubus cochichinensis* Tratt.	Rubiaceae	LF	63
VK61	*Strophioblachia fimbricalyx* Boerl.	Euphorbiaceae	LF	51
VK62	*Gymnema reticulata* (Moon) Alst.	Asclepiadaceae	LF	76
VK63	*Aglaia aphanamixis* Pellegr.	Meliaceae	LF	11
VK64	*Vitex pubescens* Vahl.	Verbenaceae	LT	30
VK65	*Ampelopsis javanica* (Thunb.) Makino.	Vitaceae	LF	94
VK66	*Glochidion daltoni* Muell.-Arg. Kurz	Euphorbiaceae	WP	56
VK67	*Chloranthus glabra* (Thunb.) Nakai	Chloranthaceae	LF	57
VK68	*Machilus cochinchinensis* Lec.	Lauraceae	LF	32
VK69	*Eurya ciliata* Merr.	Theaceae	LF	23
VK70	*Lasia spinosa* (L.) Thw.	Araceae	LF	77
VK71	*Conarus semidecandrus* Jack.	Connaraceae	LT	68
VK72	*Ardicia conspersa* Walk.	Myrsinaceae	LF	96
VK73	*Mussaenda erosa* Champ. ex Benth.	Rubiaceae	LT	76
VK74	*Bousigonia mekongense* Pierre in Planch.	Apocynaceae	LT	63
VK75	*Croton cascarilloides* Raeusch.	Euphorbiaceae	RT	22
VK76	*Croton cascarilloides* Raeusch.	Euphorbiaceae	LF	56
VK77	*Uvaria microcarpa* Champ. ex Benth. & Hook.f.	Annonaceae	SB	54
VK78	*Ceiba pentandra* (L.) Gaertn.	Bombacaceae	FL	65
VK79	*Elaeagnus loureiri* Champ.	Elaeagnaceae	LF	82
VK80	*Callicarpa longifolia* Lam.	Verbenaceae	WP	52

VSN: voucher specimen number. [b]Part used: LF (Leaf), WP (Whole plants), SB (Stem Bark), LT (Leaves and twigs), ST (Stem), (AP) aerial part, RT (Root), (RH) Rhizome. [c]SR: survival rate of A549 cells (human lung carcinoma).

seeded in the growth medium (180 μL) into 96 well microtiter plates (4×10^4 cells per each well) and allowed to attach overnight. The test samples were dissolved in DMSO and adjusted for the final sample concentrations 100 μg/ml by diluting with the growth medium. Each sample was prepared in triplicate. The final DMSO concentration was adjusted to < 0.1%. After 72 hr incubation, the medium was removed and the remaining cells were fixed using 10% trichloroacetic acid (TCA) for 1 hr at 4°C. The TCA-treated cells were washed extensively with water and then dried in air. Subsequently, 50 μL of SRB solution (0.4% in acetic acid) was added to each well at room temperature. After standing for 1 hr, the wells were washed 3-4 times with 1% acetic acid and dried in air. The bound dye was dissolved in Tris base (100 μL of 10 mM). The absorbance

was measured using a micro-plate reader at 520 nm. The OD of treated well was substracted by OD at time-zero (TZ) plate and divided by calculated value of untreated control. The survival rate of cells was calculated by the following formula:

$SR = [(T - Tz) / (To - Tz)] \times 100$

In which: SR: Survival rate of cell

T: OD value at day 3

Tz: OD value at time-zero

To: OD value of the untreated control

RESULTS AND DISCUSSION

The results of the screening are summarized in Table 1. Since the plants materials were extracted with methanol, the amount of extracts should be severely dependent on the used part of a plant. Methanol extraction of a leaf material containing chlorophylls and other polar small molecules normally offers much more sample amount than that of a root or a bark. Since the same concentration of the methanol extracts was used for the cytotoxic measurement, the cytotoxic activity of a methanol extract might be underestimated compared to extract of root or bark. For this reason, it was arbitrarily taken as the survival rate of 30% or less being active for a leaf sample and 15% or less for a root sample. Forty seven among the 85 samples are prepared from leaves (LF), 7 from leaves and twigs (LT) and others from the barks and roots.

The LF extracts showing SR of less than 30% are as follows; *Meliosma pinnata* (VK03, 4%), *Goniothalamus vietnamensis* (VK06, 0%), *Desmos cochinchinensis* (VK08, 24%), *Glycosmis stenocarpa* Tanaka (VK10, 25%), *Millettia setigera* (VK16, 30%), *Ficus glaberrima* (VK42, 26%), *Elaeocarpus angustifolius* (VK48, 28%), *Garcinia* sp. (leaves and stems) (VK50, 51, 0%), *Glycosmis* sp. (VK53, 18%) and *Aglaia aphanamixis* (VK63, 11%). Three of the above leaf extracts were the interesting samples showing the SR values of <15%, categorized as being strongly cytotoxic. The whole plant extract from *Aristolochia roxburghliana* (VK39) displayed a good activity with SR of 15%. Among LT extracts, only *Vitex pubescens* extract (VK64) showed SR of 30%. Among the stem bark (SB), stem (ST) and root (RT), *Embelia parviflora* (VK02, 13%) and *Garcinia* sp. (VK50, 0%) were found to be active.

The cytotoxic activity of *Goniothalamus vietnamensis* may be attributed to the presence of small molecules. Some acetogenins from *G. giganteus* such as gigantetronenin, gigantrionen and annomontacin[6] and 4-deoxyannomonatcin, annomontacinon[7] as well as goniotriocin, xylomaticinone[8] showed cytotoxic activity. It is worthy to examine whether the similar metabolites are present in *G. vietnamensis* and responsible for its cytotoxic activity.

The cytotoxicity of the *Aristolochia* sp. was reported[9] and is expected to be due to aristolochic acid and its derivatives.[10]

Some *Garcinia* species like *G. kola*[11] and *G. macgregorii*[12] showed antimalarial activity. It would be interesting to investigate whether the cytotoxic and antimalarial activity comes from the same compound(s) from the *Garcinia* species.

Aglaia species were screened for their cytotoxicity. Bohnenstengel and coworkers confirmed that the cytotoxic activity ensued from the presence of cyclopentabenzofuran lignans of Aglaia species.[13] Horgen and coworkers[12] screened some rain forest trees against the cytotoxicity of some cancer cell lines and found that *Croton argyratus* and *C. leophyllus* showed a relatively strong cytotoxic activity. *Croton cascarilloides* having a moderate cytotoxic activity could contain the same cytotoxic principle(s) as both of the above mentioned species. Cytotoxic activity on *Meliosma* and *Embelia* species has not been described in the literature. We selected the methanol extracts showing less than 15% survival for further study.

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초 록 – 71종의 베트남 약용식물을 메탄올로 추출하여 얻은 80개의 추출물을 100 μg/mL의 농도로 조제하여 인체 폐암세포인 A549세포에 대한 세포독성을 측정하였다. 9개의 추출물이 30% 이하의 암세포 생존율을 나타내었으며, 이들 중 *Meliosma pinnata* (VK03, 4%), *Goniothalamus vietnamensis* (VK05, 06, 0%), *Garcinia* sp. (VK50, 51, 0%) 그리고 *Aglaia aphanamixis* (VK63, 11%)는 15% 이하의 암세포 생존율을 나타내었다. *Goniothalamus vietnamensis*의 잎과 뿌리 그리고 *Garcinia species*의 잎의 메탄올 추출물은 100 μg/mL의 농도에서 암세포의 성장을 100% 억제하였다.