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Article

Cytotoxic Activity of Leguminous Seed Extracts against Human Tumor Cell Lines

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The cytotoxic activity of methanol extracts of 25 leguminous seeds *in vitro* was evaluated by sulforhodamine B assay, using the five human solid A549 lung, SK-OV-2 ovarian, SK-MEL-2 melanoma, XF-498 CNS and HCT-15 colon tumor cell lines. The responses varied with both cell line and leguminous seed used. Extracts of *Canavalia lineata* and *Glycine soja* revealed potent cytotoxic activity against A549 and SK-MEL-2 cell lines. Moderate activity was observed in the extracts of *Cassia obtusifolia* and *Glycine max* var. *chungtae*, and *C. lineata* and *Vigna angulasis* against SK-MEL-2 and HCT-15 cell lines, respectively. The other seed extracts were ineffective against model tumor cell lines. Because of their potent cytotoxic activities, the activity of each solvent fraction from *C. lineata* and *G. soja* was determined and the potent activity was produced from their chloroform fractions. As a naturally occurring therapeutic agent, leguminous seeds described could be useful for developing new types of anti-tumor agents.

Key words : *legume, seed extract,* Canavalia lineata, Glycine soja, *tumor cell, cytotoxic activity, anti-tumor agent.*

Carcinogenes are divided into three types; physical agent, chemical carcinogen and infectious agent such as bacteria or virus. Chemical carcinogenesis can be divided into two-defined stages, carcinogenic initiation and cancer promotion. Both initiators and promoters of carcinogenesis are both found in human environments.[1,2] Recent investigations on enviromental carcinogenesis have indicated that naturally occurring tumor promoters play a great role on the development of human cancer than initiators.[3,4] Therefore, tumor-promoting inhibitors in the two-stage carcinogenesis may be highly effective on cancer control. Since the establishment of the short-term *in vitro* colorimetric assay with reproducible results by National Cancer Institute, this assay has been used to detect tumor promoters in the environment.[5-7]

Current cancer chemotherapy is primarily dependent upon repeated administrations of synthetic anti-cancer agents. Their continued or repeated use has led to the development of resistance to the agents in the tumor cell lines[8-10] and adverse effects on human health such as alopecia, leucopenia, sterility and secondary malignancies in clinical trials.[11] Decreasing efficacy and increasing concern over possible adverse effects of chemotherapeutic agents have brought about the need for the development of new types of selective alternatives with lower toxic and more inhibitory effects.

Plants constitute a rich source of bioactive chemicals.[12,13] Since many of them are largely free from adverse effects and have excellent pharmacological actions, they could lead to the development of new classes of possibly safer anti-cancer agents. Additionally, some of plant-derived materials are found to be effective against cancer cells resistant to current chemotherapeutic agents.[8] Therefore, much efforts have been focused on the plants for potentially useful products as commercial anti-cancer agents or as lead compounds. However, relatively little work has been done on the anti-tumor activities of leguminous seed extracts compared to food[14,15] and plant origins[16,17] in spite of their excellent nutritional, pharmacological and industrial significances.[18-21]

In this study, we assessed the *in vitro* cytotoxic activity of 25 leguminous seeds to develop potentially new safer

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Abbreviation: SRB, sulforhodamine B.

types of anti-tumor agents.

Materials and Methods

Plant materials and sample preparation. The leguminous seeds were randomly and anecdotally collected (Table 1). They were dried in an oven at 60°C for 3 days and finely powdered using a blender. Each sample (50 g) was extracted two times with 500 ml methanol at room temperature and filtered (Toyo filter paper No. 2, Toyo Roshi, Japan). The combined filtrate was concentrated *in vacuo* at 35°C using a rotary vacuum evaporator.

Chemicals. SRB, benzylpenicillin potassium, and streptomycin sulfate were purchased from Sigma (St. Louis, USA) Chemical. Fetal bovine serum and RPMI 1640 were supplied by Gibco (Gaithersburg, USA). All other chemicals were of reagent grade.

Tumor cell lines and culture conditions. Five human tumor cell lines used in this study were SK-MEL-2 human melanoma, A549 lung, SK-OV-3 ovarian, HCT-15 colon and XF-498 central nerve system (CNS) tumor cell lines. They have been maintained in the laboratory as stocks in RPMI 1640 supplemented with 10% fetal bovine serum. Cell cultures were passaged once or twice weekly using trypsin-EDTA to detach the cells from their culture flasks.

Bioassy for cytotoxicity. SRB assay was applied for the measurement of the cytotoxicity of the test materials against model tumor cell lines.[5-7] The rapidly growing cells were harvested, counted, and inoculated at the appropriate concentrations ($1\text{-}2\times10^4$ cells/well) into 96 well microtiter plates. After incubation for 24 hr, the materials dissolved in culture medium were applied to the culture wells in triplicate followed by incubating for 48 hr at 37°C under 5% CO_2 atmosphere. The cultures fixed with cold TCA were stained by 0.4% SRB dissolved in 1% acetic acid. After solubilizing the bound dye with 10 mM unbuffered Tris base by gyratory shaker, the absorbance at 520 nm was measured with a microplate reader (Dynatech Model MR 700). All tests were replicated three times. Fifty percent inhibitory dosage (ED_{50}) was defined as the dosage which reduced absorbance by 50% of untreated wells as of the control in the SRB assay.

It has been generally acknowledged that plant extracts having cytotoxic effect at <40 μg/ml may be useful for developing anti-tumor agents. Therefore, cytotoxic activity was classified as follows: very strong activity ++++, <ED_{50} 10 μg/ml; strong +++, ED_{50} 11~40 μg/ml; moderate ++, ED_{50} 40~100 μg/ml; weak +, ED_{50} 100~200 μg/ml; and little or no activity -, >ED_{50} 200 μg/ml.

Results and Discussion

The *in vitro* cytotoxic activity of methanol extracts of 25 leguminous seeds was determined by sulforhodamine B assay, using the five human solid tumor cell lines (Table

Table 1. List of leguminous plants tested.

Scientific name	Chracteristics				
	Seed colour	Flower colour	Size (cm)	Shape	Yield[a] (%)
Amphicarpaea edgeworthii	Purple	Light-purple	0.5	Ellipse	10.7
Arachis hypogaea	Dark-brown	Yellow	1.3	Ellipse	5.3
Canavalia lineata	Brown	Purple	0.9	Rod	12.0
Cassia obtusifolia	Dark-brown	Yellow	0.4	Rod	13.3
Dunbaria villosa	Light-brown	Yellow	0.9	Ellipse	5.6
Glycine max var. *solitae*	Black	White	1.1	Ellipse	10.0
Glycine max var. *yagkong*	Black	White	0.5	Spherical	5.5
Glycine max var. *hooktae*	Black	Purple	0.8	Spherical	6.6
Glycine max var. *bangkong*	Dark-brown	Purple	1.1	Ellipse	5.4
Glycine max var. *geumdu*	Dark-purple	Purple	0.6	Spherical	4.8
Glycine max var. *chungtae*	Light-green	White	0.8	Spherical	11.1
Glycine max var. *wooltalikong*	Purple	Purple	1.1	Ellipse	1.9
Glycine max var. *mejukong*	Yellow	White	0.8	Spherical	7.1
Glycine soja	Brown	Light-purple	2.0	Rod	10.7
Lathyrus japonica	Black	Red	1.5	Ellipse	12.0
Phaseolus multiflorus	Dark-purple	Red	1.2	Rod	5.3
Phaseolus nipponensis	Dark-green	Yellow	2.1	Ellipse	5.7
Phaseolus radiatus var. *geodu*	Black	White	0.5	Spherical	7.8
Phaseolus radiatus var. *aurea*	Green	Yellow	0.5	Rod	5.2
Pisum sativum	Light-green	White-blue	0.7	Spherical	3.6
Rhynchosia volubilis	Brown	Yellow	1.1	Ellipse	5.3
Vicia hirsuta	Black	Light-purple	1.2	Ellipse	11.8
Vicia tetrasperma	Light-purple	Light-purple	1.1	Ellipse	12.3
Vigna angulasis	Red	Yellow	0.6	Spherical	4.8
Vigna sinensis	Light-yellow	Yellow	0.7	Ellipse	6.2

[a](Dried weight of methanol extract/dried weight of sample)×100.

Table 2. *In vitro* cytotoxic activity of leguminous seed extracts against the five human tumor cell lines.

Legume	Cytotoxic Activity,[a] ED_{50} (μg/ml)				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
A. edgeworthii	-	-	-	-	-
A. hypogaea	-	-	-	-	-
C. lineata	+++	-	+++	-	++
C. obtusifolia	-	-	++	-	-
D. villosa	-	-	-	-	-
G. max var. *seolitae*	-	-	+	-	-
G. max var. *yagkong*	-	-	-	-	-
G. max var. *hooktae*	-	-	-	-	-
G. max var. *bangkong*	-	-	-	-	-
G. max var. *geumdu*	-	-	-	-	-
G. max var. *chungtae*	-	-	++	-	-
G. max var. *wooltalikong*	-	-	-	-	-
G. max var. *mejukong*	-	-	-	-	-
G. soja	+++	-	++++	+	+
L. japonica	-	-	-	-	-
P. multiflorus	-	-	-	-	-
P. nipponensis	-	-	-	-	-
P. radiatus var. *geodu*	-	-	-	-	-
P. radiatus var. *aurea*	+	-	+	-	-
P. sativum	-	-	-	-	-
R. volubilis	-	-	-	-	-
V. hirsuta	-	-	-	-	-
V. tetrasperma	-	-	-	-	-
V. angulasis	+	-	-	-	++
V. sinensis	-	-	-	-	-

[a]++++, <ED_{50} 10 μg/ml; +++, ED_{50} 11~40 μg/ml; ++, ED_{50} 40~100 μg/ml; +, ED_{50} 100~200 μg/ml; -, >ED_{50} 200 μg/ml.

2). The responses varied with both leguminous see and cell line used. In tests with A549 lung tumor cell line, the strong cytotoxicity was produced by extracts of *Canavalia lineata* (ED_{50}, 39 μg/ml) and Glycine soja (ED_{50}, 31 μg/ml). However, the other leguminous seeds exhibited little or no cytotoxic activity against the cell line (ED_{50}, >300 μg/ml).

For tests with SK-MEL-2 melanoma tumor cell line, extracts from *G. soja* and *C. lineata* revealed highly effective (ED_{50}, 9 μg/ml) and strong (ED_{50}, 37 μg/ml) cytotoxic activity, respectively, whereas moderate activity (ED_{50}, 40~100 μg/ml) was obtained in extracts from *Glycine max* var. *chungtae* and *Cassia obtusifolia* (Table 2).

In tests with HCT-15 colon tumor cell line, extracts from *C. lineata* and *Vigna angulasis* exhibited moderate cytotoxicity, whereas the other test leguminous seeds exhibited very weak or no cytotoxic activity (Table 2).

The results from SK-OV-3 ovarian and XF-498 CNS tumor cell lines showed that extracts from all the leguminous seeds used exhibited little or no cytotoxic activity (Table 2).

Because of their potent cytotoxic activity against A549 and SK-MEL-2 cell lines, the activity of each solvent fraction from the extracts of *C. lineata* and *G. soja* was evaluated (Table 3). Chloroform fraction from the extract of *C. lineata* showed potent cytotoxic activity. In the fractionation of the methanol extract from *G. soja,* strong cytotoxic activity was not observed in any fraction, whereas potent cytotoxic activity was detected by application of chloroform+ethyl acetate fractions (1 : 1, v/v). These results suggest that various compounds including alkaloids, phenolics and terpenoids exist in leguminous seeds and jointly contribute to anti-tumor activities.

In our study, significant differences between cytotoxic activities of the test materials were observed. Cytotoxic activity of extracts from *C. lineata, C. obtusifolia, G. max* var. *chungtae* and *V. angulasis* which have the determinate nodule with ureide-exporting structure[22] indicated that the ureide-exporting plants might posses higher cytotoxic activity against human tumor cell lines than amide-exporting plants such as *Pisum sativum, Rhynchosia volubilis* and *Vicia tetrasperma.* Furthermore, although the cytotoxic activity was observed in four ureide-exporting plants tested in this study, these results suggest that certain metabolites and enzymes induced by ureide-exporting metabolism might be considered for cytotoxic activity.

It has been well acknowledged that plant-derived extracts and phytochemicals are potential alternatives to synthetic anti-cancer agents.[15-18] Barclay and Perdue[15] suggested that the most promising botanicals as sources of novel plant-based anti-tumor agents to use at present (1976) and in the future are species of the families Cephalo-

Table 3. Cytotoxic activity of solvent fractions of methanol extracts from *Canavalia lineata* and *Glycine soja*.

Legume Fraction	Cytotoxic Activity, ED_{50} (μg/ml)				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
C. lineata					
Hexane	-	-	-	-	+
Chloroform	+++	-	+++	-	-
Ethyl acetate (EtOAc)	-	-	-	-	-
Butanol	-	-	-	-	-
Water	-	-	-	-	-
G. soja					
Hexane	-	-	-	-	-
Chloroform	+	-	++	-	+
Ethyl acetate	-	-	-	+	-
Butanol	-	-	-	-	-
Water	-	-	-	-	-
Chloroform+EtOAc (1 : 1)	++	-	+++	-	-

taxaceae, Podocarpaceae, Taxaceae, Aunonaceae, Menispermaceae, Thymelaeaceae, Celestraceae, Celestraceae, Euphorbiaceae, Rutaceae, Simanubaceae, Apocynaceae, and Liliaece. In our study, *C. lineata* and *G. soja* (Family Apiaceae) seeds showed potent cytotoxic activity against A549 and SK-MEL-2 cell lines, suggesting an indication of at least one of their pharmacological actions. Although the active principles of these seeds remain unknown at present, soybean seed-derived isoflavone β-glycoside conjugates and aglucones inhibit mammary tumorigenesis in animal models.[23]

In conclusion, the strong cytotoxic activity of leguminous seeds described confirms their superiority and usefulness on an anti-tumor agent. Additionally, natural product-derived materials are found to be effective against cancer cells resistant to current chemotherapeutic agents.[8] The isolation and characterization of their antitumorigenic components are in progress.

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References

1. MaCann, J., Choi, E., Yamasaki, E. and Ames, B. N. (1975) Detection of carcinogens as mutagens in the *Salmonella*/microsome test: Assay of 300 chemicals. *Proc. Natl. Acad. Sci.* **72**, 5135-5139.
2. Fujiki, H., Hecker, E., Moore, R. E., Sugimura, T. and Weinstein, I. B. (1984) In *Cellular interaction by environmental tumor promoters,* Japan Scientific Society Press, Tokyo/VNU Science Press, Utrecht, Netherlands.
3. Pitot, H. C. and Campbell, H. A. (1988) In *Tumor promoters: Biological approaches for mechanistic studies and assay systems*, Langenbach, R., Elmore, E. and Barett, J. C. (eds.) pp. 79-95, Raven Press, New York, USA.
4. Boyd, M. R. (1989) In *Cancer: Principles and practice of oncology updates*, DeVita, V. T. Jr., Hellman, S. and Rosenberg, S. A. (eds.) pp. 1-12, Lippincott, Philadelphia, USA.
5. Monks, A., Scudiero, D. A., Skehan, P., Shoemaker, R. H., Paull, K. D., Vistica, D. T., Hose, C., Langley, J., Cronise, P., Vaigro-Woiff, A., Gray-Goodrich, M., Campbell, H., Mayo, J. and Boyd, M. R. (1991) Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. *J. Natl. Cancer Inst.* **83**, 757-766.
6. Rubinstein, L. V., Shoemaker, R. H., Paull, K. D., Simon, R. M., Tosini, S. Skehan, P., Scudiero, D. A., Monks, A. and Boyd, M. R (1990) Comparison of *in vitro* anticancer-drug screening data generated with a tetrazolium assay versus a protein assay against a diverse panel of human tumor cell lines. *J. Natl. Cancer Inst.* **82**, 1113-1118.
7. Skehan, P., Storeng, R., Scudiero, D. A., Monks, A., McMahon, J. B., Visca, D. T., Warren, J. T., Bokesch, H., Kenney, S. and Boyd, M. R. (1990) New colorimetric cytotoxicity assay for anticancer drug screening. *J. Natl. Cancer Inst.* **82**, 1107-1112.
8. Colegate, S. M. and Molyneux, J. J. (1993) In *Bioactive natural products: Detection, isolation and structure determination*, pp. 9-58, CRC Press, Boca Raton, USA.
9. Stark, G. R. and Calvert, H. (1986) Drug resistance. *Cancer Surv.* **5**(2).
10. van der Bliek, A. M. and Borst, P. (1989) Multidrug resistance. *Advance in Cancer Res.* **52**, 165-204.
11. Malpas, J. S. (1991) In *Introduction to the cellular and molecular biology of cancer,* Franks, L. M. and Teich, N. M. (2nd ed.) pp. 451-469, Oxford University Press, Oxford, UK.
12. Swain, T. (1977) Secondary compounds as protective agents. *Ann. Rev. Plant Physiol.* **28**, 479-501.
13. Wink, M. (1993) In *Phytochemistry and agriculture*, van Beek, T. A. and Breteler, H. (eds.) *Proc. Phytochem. Soc. Europe* vol. **34**, pp. 171-213, Clarendon Press, Oxford, UK.
14. Waldron, K. W., Johnson, I. T. and Fenwick, G. R. (1993)

In *Food and cancer prevention: Chemical and biological aspects,* The Royal Society of Chemistry, Thomas Graham House, Cambridge, UK.

15. Perchellet, J. P., Gali, H. U., Perchellet, E. M., Laks, P. E., Botari, V., Hemingway, K. W. and Scalbert, A. (1994) In *Food phytochemicals for cancer prevention: Fruits and vegetables,* Huang, M. T., Osawa, T., Ho, C. T. and Rosen, R. T. (eds.) *ACS Symp. Ser.* No. 546, pp. 303-327, Am. Chem. Soc., Washington, D.C., USA.
16. Barclay, A. S. and Perdue, R. E. Jr. (1976) Distribution of anticancer activity activity in higher plants. *Cancer Treat. Rep.* **50**, 1081-1113.
17. Cassady, J. M. and Douros, J. D. (1980) In *Anticancer agents based on natural product models,* Academic Press, New York, USA.
18. Sharpe, D. B. (1984) In *Proc. World Soybean Research Conference III*, Richard, S. (ed.) pp. 25-31.
19. Namba, T. (1986) Coloured illustrations of Wakan-Yaku: The crude drugs in Japan, China and the neighbouring countries (4th ed.) Hoikusha Publishing, Osaka, Japan.
20. Smith, K. J. and Huyser, W. (1987) Soybeans: Improvement, production and uses. *Agronomy* **16**, 1-21.
21. Lee, H. S. and Ahn, Y. J. (1997) Growth response of lactic acid bacteria to leguminous seed ectracts. *Agric. Chem. Biotechnol.* **40**, 167-171.
22. Hirsch, A. M. (1992) Developmental biology of legume nodulation. *New Phytol.* **122**, 211-237.
23. Coward, L., Barnes, N. C., Setchell, K. D. R. and Barnes, S. (1993) Genistein, daidzein and their beta-glycoside conjugates: antitumor isoflavones in soybean food from american and asian diets. *J. Agric. Food Chem.* **41**, 1961-1967.