Natural Product Sciences
8(4) : 162-164 (2002)

Evaluation of DNA Strand-Scission Activity of the Korean Indigenous Plants

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Abstract – The methanol extracts of 42 Korean indigenous plants were evaluated for the DNA strand-scission activity. As a result, the 17 extracts were found to be active in the criteria of IC_{50}<25 μg/ml. Among others, the MeOH extracts of *Caesalpinia sappan* and *Mucuna birdwoodiana* showed the most potent DNA strand-scission activity with IC_{50} values of 5.9 and 4.9 μg/ml, respectively. Therefore, the partition and fractionation for *C. sappan* were performed and tested in the DNA strand-scission assay system for further bioassay-guided fractionation.

Key words – DNA strand-scission, Plant extracts, *Caesalpinia sappan*, *Mucuna birdwoodiana*

Introduction

To find anticancer agents from higher plants, the DNA strand-scission assay has been employed as a tool of bioassay-guided fractionation. The DNA strand-scission assay was developed by Hecht *et al.* (1985), and used for bioassay-guided fractionation in the research program to find antineoplastic agents from plants by Wall and Wanis group (Chaudhuri *et al.*, 1995; Huang *et al.*, 1998; Seo *et al.*, 1999). Several natural products such as biphenyl compounds (Seo *et al.*, 1999) and benzophenone (Seo *et al.*, 2000) isolated from the Guttiferae plants, were reported previously as the DNA strand-nicking agents. In the present study, the DNA strand-scission activity of 42 methanol extracts of Korean indigenous plants were evaluated for the first time.

Experimental

Chemicals – All chemicals and reagents used were of highest purity. Bleomycin sulfate, cacodylic acid, cupric chloride, ferrous sulfate, ethylenediaminetetraacetic acid (EDTA), bromophenol blue, xylene cyanole FF, ficoll, boric acid, lauryl sulfate, glycerol, and Trizma base were all purchased from Sigma-Aldrich (St. Louis, MO, USA). Electrophoresis grade agarose and pBR322 plasmid DNA were obtained from Gibco BRL (Life Technologies, Grand Island, NY, USA). SYBR Green I Nucleic Acid Gel Stain was obtained from Roche (Indianapolis, IN, USA).

Plant material and extractions – Korean indigenous plants as test samples were purchased from an herb market (Han-Yang Yutong Co.) in Seoul, Korea, or collected. The botanical identifications were performed by Drs. Ihn-Rhan Lee and Jung-Ae Do (College of Pharmacy, Ewha Womans University, Seoul, Korea). The voucher specimens have been deposited at the herbarium of College of Pharmacy, Ewha Womans University. 50 g of each dried plant was ground and extracted with methanol by percolation. The filtered methanol extracts were evaporated under vacuum.

DNA strand-scission assay – The DNA strand-scission assay modified the procedure described by Sugiyama *et al.* and Chaudhuri *et al.*. In brief, the assay reaction mixtures (40 μl total volume) contained 25 mM cacodylate buffer pH 7.0, 0.3 mM $CuCl_2$, and 500 ng of supercoiled DNA pBR322 as a substrate, and various concentrations of the test compounds (initially dissolved in 0.5 μl of 100% DMSO, final 1.25% DMSO). The reaction mixture was incubated for 30 min at 25°C while protected from light, then stopped the reaction by addition of 5 μl of stop solution (7 mM EDTA, 0.15% bromophenol blue, 75% glycerol). The reaction mixture was analyzed by electrophoresis at 80 volts for 7 h on a 1% agarose gel in 0.5 x TBE buffer (45 mM Tris-borate, 1 mM EDTA), and then stained with SYBR Green I fluorescence, which was photographed using luminescence image analyzer, LAS-1000 plus (Fuji film, Japan). The bands of the pBR322 were measured using Image Gauge software (Fuji film, Japan). Each experiment included DMSO and bleomycin sulfate as negative and positive controls, respectively. The results were calculated as the relative percentage of the DNA scission ratios as compared to negative control group.

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DNA scission ratio =

density of DNA scission (nicked DNA)/density of total DNA (supercoiled DNA + nicked DNA)×100

Results and Discussion

The present study was conducted to evaluate the DNA strand-scission activity of Korean indigenous medicinal plants. The methanol extracts of 42 Korean indigenous plants were primarily evaluated for the DNA strand-scission activity. As judged in the criteria of DNA strand-scission activity with IC_{50}<25 μg/ml, 17 extracts were found to be active as shown in Table 1. Especially, the extracts of *Caesalpinia sappan* and *Mucuna birdwoodiana* showed the most potent DNA strand-scission activity. Although *C. sappan* and *M. birdwoodiana* have been much investigated for chemical constituents (Ding *et al.*, 1991; Fuke *et al.*, 1985; Goda *et al.*, 1987; Kwon *et al.*, 1999; Miyahara *et al.*, 1986; Nagai *et al.*, 1986; Nagai *et al.*, 1987; Nagai *et al.*, 1990; Namikoshi *et al.*, 1987; Namikoshi *et*

Table 1. DNA strand-scission activity of methanol extracts of the Korean indigenous plants.

Plant name and Authority	Family	Part used[a)]	DNA strand-scission (IC_{50} μg/ml)
Albizzia julibrissin Durazz.	Leguminosae	CR	>25
Angelica gigas Nakai	Umbelliferae	RT	>25
Areca catechu L.	Palmae	FR	>25
Astragalus membranaceus (Fisch.) Bge.	Leguminosae	RT	>25
Benincasa hispida (Thunb.) Cogn.	Cucurbitaceae	SD	15.9
Broussonetia papyrifera (L.) Vent.	Moraceae	FR	>25
Caesalpinia sappan L.	Leguminosae	LG	5.9
Caragana chamlagu Lam.	Leguminosae	LF	>25
Chrysanthemum zawadskii var. *latilobum* Kitamura	Compositae	HR	>25
Cibotium barometz (L.) J. Sm.	Cyatheaceae	ST	>25
Cinnamomum cassia Blume	Lauraceae	TW	>25
Circium japonicum DC.	Compositae	RT	22.2
Curcuma longa L.	Zingiberaceae	ST	>25
Davallia maruesii Moore	Davalliaceae	ST	>25
Eriocaulon sieboldianum Sieb. et Zucc.	Eriocaulaceae	FT	>25
Gallus domesticus Blume	Phasianidae	ST	>25
Glechoma longituba (Nakai) Kupr.	Labiatae	HR	>25
Gleditsia japonica var. *koraiensis* Nakai	Leguminosae	TN	24.5
Glycine max (L.) Merr.	Leguminosae	SD	24.1
Glycyrrhiza uralensis Fischer	Leguminosae	RT	>25
Humulus japonicus Sieb. et Zucc.	Moraceae	TW, LF	23.9
Lespedeza cuneata G. Don	Leguminosae	TW,LF	23.7
Melia azedarach L. var. *japonica* Makino	Meliaceae	FR	>25
Morus alba L.	Moraceae	FR	23.6
Mucuna birdwoodiana Tutcher	Leguminosae	ST	4.9
Phragmites communis Trin.	Graminae	RT	18.9
Polygonum cuspidatum Sieb. et Zucc.	Polygonaceae	RT	17.6
Pueraria thunbergiana Bentham	Leguminosae	RT	>25
Pueraria thunbergiana Bentham	Leguminosae	FL	>25
Rehmannia glutinosa Liboschitz var. *purpurea* Makino	Scrophulariaceae	RT	>25
Rheum coreanum Nakai	Polygonaceae	ST	13.7
Rhus verniciflua Stokes	Anacardiaceae	FD	21.3
Rosa laevigata Michx	Rosaceae	FR	>25
Salvia militiorrhiza Bunge	Labiatae	RT	17.3
Selaginella tamariscina Spring	Selaginellaceae	HR	23.7
Sophora japonica L.	Leguminosae	RT	>25
Thuja orientalis L.	Cupressaceae	FT	>25
Trigonella foenum-graecum L.	Leguminosae	SD	>25
Vicia venosa Max.	Leguminosae	TW, LF	24.8
Zelkova serrata (Thunberg) Makino	Ulmaceae	LF	22.4
Zingiber officinale Roscoe	Zingiberaceae	ST	>25
Zizyphus jujuba Mill. var.	Rhamnaceae	FR	>25
Positive control	bleomycin		3.1

[a)]Part used: BK (bark), CR (cortex), FD (fluid), FL (flower), FR (fruit), FS (seed in fruit), FT (flower + twig), HR (herb), LF (leaf), LG (lignum) PF (peduncle of fruit), PL (pollen), RT (root), SB (stem bark), SC (sclerotium), SD (seed), TB (tuber), TN (thorn), TW (twig), WP (whole plant)

Table 2. DNA strand-scission activity of partitions of *C. sappan* and *M. birdwoodiana*.

Plant name	Fraction	DNA strand-scission activity (IC_{50} μg/ml)
C. sappan	Hexane ext.	6.2
	EtOAc ext.	4.5
	Aqueous ext.	4.9
M. birdwoodiana	Hexane ext.	8.4
	EtOAc ext.	4.6
	Aqueous ext.	13.7

al., 1987; Shimokawa *et al.*, 1985), any evaluation for the DNA strand-scission activity has never been reported before. Each of the methanol extract of *C. sappan* and *M. birdwoodiana* was suspended in water and partitioned with *n*-hexane and ethyl acetate, successively, and all fractions were tested as shown in Table 2. The ethyl acetate soluble fractions of *C. sappan* and *M. birdwoodiana* showed significant activity with IC_{50} values of 5.9 μg/ml and 4.6 μg/ml, respectively. Therefore, further bioassay-guided fractionations with these active fractions are encouraged for the discovery of new anticancer potentials. Novel anticancer agents are expected to be isolated from these medicinal plants in future study.

Acknowledgements

This research was supported by a grant (PF002201-02) from Plant Diversity Research Center of 21st Century Frontier Research Program funded by Ministry of Science and Technology of Korean government.

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(Accepted November 1, 2002)