Screening of medicinal plant extracts for estrogenic activity in combination with a glycosidase treatment

Eun-Mi AHN,[a] Teruaki AKAO,[b] Norio NAKAMURA,[a] Katsuko KOMATSU,[a] Tsutomu NISHIHARA,[c] Masao HATTORI*[a]

[a]*Institute of Natural Medicine, and* [b]*Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan.* [c]*Laboratory of Environmental Biochemistry, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamada-oka, Suita, Osaka, 565-0871, Japan.* *(Received December 19, 2003. Accepted January 26, 2004.)*

For the purpose of evaluating phytoestrogenic activity of medicinal plant extracts, a naringinase-pretreatment method was developed, monitoring with proliferation of MCF-7 human breast cancer cells and induction of β-galactosidase in a yeast two-hybrid assay system. Of various medicinal plant extracts examined, the extracts of *Alpinia katsumadai* (seeds), *Glycyrrhiza uralensis* (roots) and *Moghania philippinensis* (roots) showed higher estrogenic activity by per-treatment with naringinase than the original extract themselves. The contents of liquiritigenin and isoliquiritigenin having potent estrogenic activity, appreciably increased after the naringinase treatment of the extract of *G. uralensis*. These findings suggested that orally administered crude drugs would increase their estrogenic activity, due to the hydrolysis of some glycosylated constituents by intestinal flora.

Key words phytoestrogen, MCF-7, yeast two-hybrid assay, naringinase.

Abbreviations SD, synthetic defined; DMEM, Dulbecco's modification Eagle's medium; FBS, fetal bovine serum.

Introduction

Traditional medicines have a long history of use based on religious and cultural tradition, in which a variety of plants are viewed as sources of health remedies.[1] Recently, some of these medicinal plants and their constituents such as isoflavones, lignans and coumestans have been investigated from a view point of phytoestrogens.[2] Insufficiency of internal estrogen secretion is known to cause several physical disorders in postmenopausal women, such as osteoporosis, blood cholesterol elevation, and symptoms of menopause.[3] An estrogen replacement therapy was reported to have a curative effect on these conditions.[4] However, chronic synthetic estrogen therapy has been shown to increase the risk of certain types of cancer, such as endometrial and breast cancer.[5]

In the present paper, we screened various medicinal plants for their estrogenic activity, in which the plants were selected on the basis of their usages for treatments of menopausal syndrome, rheumatoid arthritis, bone lose, leukorrhea, menstrual irregularity, antifebrile, women's diseases *etc.* in traditional Chinese medicine.[6-7]

For evaluation of estrogenic activity, we used two estrogen assay systems, estrogen-dependent proliferation of MCF-7 (human breast cancer) cells[8] and a yeast two-hybrid assay.[9] The cell proliferation (E-screen) is a widely used assay system to determine the estrogenic potency of natural substances.[10] A disadvantage of this method is that a considerable number of substances give positive results without exerting any estrogenic activity. In addition, different MCF-7 stocks show a wide variation in regard to their sensitivity to estradiol.[11] To solve these disadvantages, we used the yeast two-hybrid assay system together with E-screen. The advantage of the former assay system is less time consuming and easy to perform. In contrast, the disadvantage is the potential for false negative results due to a difference in membrane transport of chemicals between yeast cells and animal cells. The above problems may be dissolved by the use of both estrogen assay systems.

Although, most of the studies have been performed with a single phytoestrogenic compound, we evaluated some medicinal plant extracts and those digested with naringinase, the latter being mimic to a metabolic mixture by intestinal bacteria in the gut.

Materials and Methods

Plant materials. The plant materials were purchased at crude drug markets in China, and in Japan (Tochimoto Tenkaido Co., Osaka). The botanical sources were identified by K. K. and the voucher specimens are deposited at the Herbarium of Institute of Natural Medicine, Toyama Medical and Pharmaceutical University.

Chemicals. Naringinase and isoliquiritigenin were purchased from Sigma Co. (St. Louis, Mo. USA). Dulbecco's modification Eagle's medium (DMEM) was obtained from Gibco BRL (Grand Island, NY, USA). Fetal bovine serum (FBS) was purchased from ICN biomedicals, Inc. (Aurora, Oh. USA). Streptomycin, 0.25 % trypsin, *o*-nitrophenyl β-D-galactoside (ONPG) were purchased from Nacalai Tesque Co. (Kyoto, Japan). 17β-Estradiol was purchased from Calbiochem Co. (Darmstadt, Germany). Human serum was obtained from Bio-whittaker (Walkersville, Md. USA). 3-(4,5-Dimethyl-2-thiazolyl)-2,3-diphenyl-2*H*-tetrazolium bromide (MTT), penicillin, norit SX-Ⅱ charcoal and liquiritin were purchased from Wako Chem. Co. (Osaka, Japan), and 20T-zymolyase from Seikagaku Kogyo Co. (Tokyo, Japan).

*To whom correspondence should be addressed. e-mail : saibo421@ms.toyama-mpu.ac.jp

Dextran 70T was obtained from Amersham Pharmacia Biotech AB. (Uppsala, Sweden). Liquiritigenin was purchased from Funakosi Co. (Tokyo, Japan). Liquiritin apioside was given from Tsumura Co. (Ibaraki, Japan).

Preparation and extraction. The dried parts of plants (10 g) were pulverized and extracted with methanol (50 ml) for 2 h under reflux. The solution was filtered and the filtrate was evaporated *in vacuo* to give a residue (MeOH extract).

Treatment of extracts with naringinase. Each MeOH extract (40 mg) was mixed with 20 mg of naringinase in 2 ml of 0.2 M acetate buffer (pH 4.7), and incubated for 3 h at 37°C. The mixture was extracted with water-saturated BuOH (3 ml×3) and the combined BuOH solutions were evaporated *in vacuo* to give a residue (naringinase-treated extract).

The MeOH extract and naringinase-treated one were tested for their estrogenic activities using both MCF-7 proliferation and yeast two-hybrid assay systems.

Cells and cell culture. Hormone-sensitive human MCF-7 breast cells were grown in DMEM supplemented with 5% fetal bovine serum (FBS), penicillin and streptomycin. The cells were harvested by trypsinization, plated in 96-well tissue-culture plates (Iwaki, Chiba, Japan) at a concentration of 5×10^3 cells/well in DMEM supplemented with 5% FBS, and allowed to stand for 24 h. Then the culture medium was replaced with phenol red-free DMEM containing 10% heat-inactivated dextran/charcoal-stripped (DC) human serum prior to the addition of test compounds.[12] Stock solutions of test compounds in DMSO were diluted with DC medium. The final DMSO concentrations in culture medium were less than 0.1%, in which this concentration affected no cell viability. After 4 days in a humidified incubator at 37°C with 5% CO_2, the growth of the cells was measured by the MTT method.[13]

Yeast two-hybrid assay. The yeast two-hybrid assay was carried out according to the method of Nishikawa *et al.*[9] Briefly, yeast cells expressing rERα were grown overnight at 30°C with shaking in synthetic defined (SD) medium lacking tryptophan and leucine. Yeast cells were treated with a test compound for 4 h at 30°C, and β-galactosidase activity was determined as follows. The growth of the yeast cells were monitored by measuring the absorbance at 600 nm. The treated yeast cells were collected by centrifugation (8000×g for 5 min) and resuspended in 200 μl of Z-buffer (0.1 M sodium phosphate, pH 7.0, 10 mM KCl and 1 mM $MgSO_4$) containing 1 mg/ml zymolyase at 37°C for 15 min. The reaction was started by addition of 40 μl of 4 mg/ml *o*-nitrophenyl β-D-galactopyranoside (ONPG). When the yellow color was developed (incubation time: *t*), 100 μl of 1 M Na_2CO_3 were added to stop the reaction. The absorbance of solution (150 μl) was measured at 420 and 550 nm. β-Galactosidase activity (*U*) was determined using the following formula:

$$U=1000\times(A_{420}-1.75\times A_{550}) / (t-\times 0.05\times A_{600})$$

Analysis by high-performance liquid chromatography (HPLC). Each extract (10 mg) was dissolved in MeOH (10 ml) and filtered through 0.45 μm Millipore filter. A 20 μl portion of each extract was analyzed by HPLC. The HPLC was performed on a CCPM-II (Tosoh co., Tokyo, Japan) equipped with a Tosoh UV-8020 spectrometer and a Shimadzu C-R 6A chromatopac (Shimadzu co., Kyoto, Japan) using a TSKgel ODS-80Ts column (Tosoh Co.; column size, 150 mm×4.6 mm i.d). The mobile phase was 2% acetic acid (v/v) (solvent A) and acetonitrile (solvent B) at a flow rate of 1 ml/min. The column was eluted with a linear gradient started from 15% B in A to 30% B at 35 min, and then 60% B at 70 min, 90% B at 100 min, and 100% B at 110 min. The elution profile was monitored at 250 nm.

Analysis of data. The data obtained were subjected to Students t-test and significance was assessed using 95% confidence intervals.

Results and Discussion

Estrogenic activity of medicinal plant extracts. The MeOH extracts and their naringinase-treated extracts of 29 medicinal plants were screened for estrogenic activity using two indices, MCF-7 proliferation and induction of β-galactosidase in a yeast two-hybrid assay method (Table 1).

Most of the MeOH extracts (naringinases-untreated ones) showed moderate estrogenic activity with the MCF-7 proliferation assay. However, 7 of the 29 MeOH extracts increased their estrogenic activity after treatment of naringinase. Three extracts of *Alpinia katsumadai* (seeds), *Glycyrrhiza uralensis* (roots) and *Moghania philippinensis* (roots) showed high estrogenic activity evaluated by the yeast two-hybrid assay. *A. katsumadai* (seeds) have been reported to contain a variety of diarylheptanoids, chalcones, flavonoids and stilbenes.[14-16] These stilbenes and flavonoids were reported to exhibit estrogenic activity.[17-18] From the roots of *G. uralensis*, several flavonoid glycosides (liquiritin, isoliquiritin *etc.*) and triterpene oligoglycosides have been isolated.[19-20] From *M. philippinensis*, several flavonoids, including prenylflavonoids and genistein, were isolated.[21-22]

Comparative HPLC investigations. Naringinase-treated and -untreated MeOH extracts of the roots of *G. uralensis* were analyzed by HPLC (Figure 1A-B), at a wavelength at 250 nm, which enables to detect most flavonoids.[23] The major constituents were liquiritin (**1**), liquiritin apioside (**2**), liquiritigenin (**3**) and isoliquiritigenin (**4**), which were identified by comparison of the retention times with those of authentic samples. When **1** and **2** were treated with naringinase, the former was converted to **3**, but the latter was not hydrolyzed to an aglycone. In the naringinase-treated extract, aglycones **3** and **4** were significantly increased accompanied by the decrease of a peak at 13 min, corresponding to **1** and **2**. Of these, **4** was reported to have antitumorgenic activity or estrogen-dependent growth promoting effects on MCF-7 breast cancer cells.[24]

Estrogenic activity of constituents *of Glycyrrhiza uralensis*. The estrogenic effects of constituents of *G. uralensis* are shown in Fig. 3, in which 17β-estradiol was used for positive control. In MCF-7 cell proliferation and yeast two-hybrid assays, **1** and **2** did not show any

Table 1. Estrogenic activity of medical plants

Medicinal plants			MCF-7 cell proliferation (% control)[a] 10 μg/mL		β-Galactosidase activity[b] (U) 100 μg/mL	
Scientific name	Family name	Part used	MeOH ext.	Naringinase treat.	MeOH ext.	Naringinase treat.
Adenophora lilifolioides PAX.et HOFFIM	Campanulaceae	root	101.8 ± 2.90	117.2 ± 2.47**[d]	15.4 ± 0.92	21.6 ± 1.54
Alpinia katsumadai HAYATA	Zingiberaceae	mature seed	108.2 ± 2.30	108.3 ± 3.39	251.5 ± 13.24***	262.2 ± 8.19***
Angelica acutiloba KITAGAWA	Umbelliferae	root	110.2 ± 5.33	112.2 ± 3.65	61.8 ± 1.39	22.3 ± 2.22
Arisaema flavum (FORSK.) SCHOTT	Araceae	tuber	100.8 ± 3.89	95.6 ± 3.91	74.8 ± 3.14	73.1 ± 2.59
Astragalus membranaceus BUNGE	Fabaceae	root	123.0 ± 5.51*	132.2 ± 11.7*	93.8 ± 4.60	63.5 ± 4.96
Butea monosperma (LAN.) KUNTZE	Fabaceae	mature seed	112.2 ± 6.81*	136.6 ± 2.54**	67.3 ± 9.67	87.3 ± 3.22
Carthamus tinctorius L.	Compositae	flower	107.4 ± 4.39	128.7 ± 3.79**[d]	29.0 ± 5.52	27.6 ± 5.84
Carthamus tinctorius L.	Compositae	seed	113.9 ± 3.38**	97.7 ± 3.14	56.5 ± 5.77	5.6 ± 4.31
Cnidium monnieri CUSSON	Umbelliferae	mature seed	94.2 ± 4.87	57.1 ± 1.96**	35.9 ± 1.69	55.8 ± 1.40
Cnidium officinale MAKINO	Umbelliferae	root	160.0 ± 2.35**	124.1 ± 3.66**	60.9 ± 8.38	21.9 ± 2.92
Codonopsis pilosula (FRANCH.) NANNF	Campanulaceae	root	135.7 ± 4.59**	111.9 ± 9.09	33.8 ± 2.95	39.4 ± 3.62
Codonopsis subscaposa KOM	Campanulaceae	root	98.2 ± 3.61	80.1 ± 2.51**	33.3 ± 4.35	45.5 ± 3.84
Cuminum cyminum L.	Apiaceae	mature fruit	97.9 ± 2.21	97.7 ± 3.05	48.1 ± 0.97	70.2 ± 5.38
Cyperus rotundus L.	Cyperaceae	root	140.3 ± 5.98**	115.1 ± 2.39 **	29.0 ± 7.46	15.9 ± 1.67
Gentiana algida PALL	Gentianaceae	flower	128.1 ± 4.79**	140.2 ± 4.00**[e]	88.5 ± 2.99	70.2 ± 1.81
Glycyrrhiza uralensis FISCH	Fabaceae	root	165.7 ± 2.07**	181.2 ± 2.17**[e]	159.4 ± 5.00	515.8 ± 17.07***[e]
Hedvotis diffusa WILLD.	Rubiaceae	root	58.0 ± 2.58**	51.9 ± 1.62**	34.2 ± 1.22	17.2 ± 1.04
Malus yunnanensis (FRANCH.) SCHNEID	Rosaceae	fruit, whole plant	154.9 ± 12.85**	130.2 ± 7.76**	63.1 ± 1.56	39.0 ± 4.46
Moghania philippinensis MERR. et HEYNE	Fabaceae	root	152.6 ± 7.95**	164.0 ± 2.40**	364.1 ± 16.81***	501.7 ± 19.35***[d]
Myristica fragrants HOUTT	Myristicaceae	aril	96.3 ± 3.21	89.6 ± 2.19*	16.9 ± 6.29	37.5 ± 3.42
Nigella glandulifera FREYN	Ranunculaceae	mature seed	103.9 ± 3.71	120.1 ± 5.09**[c]	85.5 ± 0.93	59.7 ± 4.69
Onosma hookeri C.B.CLARKE	Boraginaceae	root	131.1 ± 2.96**	122.5 ± 5.89**	-27.9 ± 5.38	8.8 ± 3.02
Paeonia suffruticosa ANDREWS	Paeoniaceae	root bark	116.5 ± 5.24*	96.9 ± 5.25	70.6 ± 5.86	70.7 ± 1.87
Panax japonicum C.A.MEYER var. MAJOR	Araliaceae	root	89.2 ± 2.17**	124.1 ± 4.47**[e]	-4.05 ± 2.50	30.7 ± 14.52
Panax notginseng (BURKILL) F.H.CHEN	Araliaceae	flower	100.8 ± 4.69	88.1 ± 3.42	58.7 ± 3.97	23.7 ± 4.07
Polygonatum cirrhifolium (WALL.) ROYLE	Liliaceae	rhizome	117.1 ± 5.46*	96.0 ± 1.89	52.3 ± 1.27	26.3 ± 2.87
Salviae yunnanensis C.H.WRIGHT	Labiatae	root	83.9 ± 3.55	106.5 ± 2.83[d]	33.2 ± 3.39	21.1 ± 3.23
Saussurea laniceps HAND. MAZZ	Asteraceae	whole plant	101.1 ± 3.95	95.2 ± 4.56	22.7 ± 2.37	37.8 ± 5.97
Saussurea namikawae KITAM	Asteraceae	root	119.6 ± 9.17	122.3 ± 2.57**	55.3 ± 3.36	60.5 ± 2.42

[a] MCF-7 cell proliferation (% control), 17β-estradiol (10^{-10} M): 214.2 ± 7.56
[b] β-galactosidase activity ; control (DMSO): 47.3 ± 2.48 (*U*), 17β-estradiol (10^{-7} M): 1191 ± 50.49 (*U*)
Each value represents the mean ± S.E of six experiments. Asterisks denote significant differences from the control at $p< 0.05$, $**p< 0.01$.
Significant difference from the MeOH extract at a [c] $p< 0.05$, [d] $p< 0.01$, [e] $p< 0.001$.

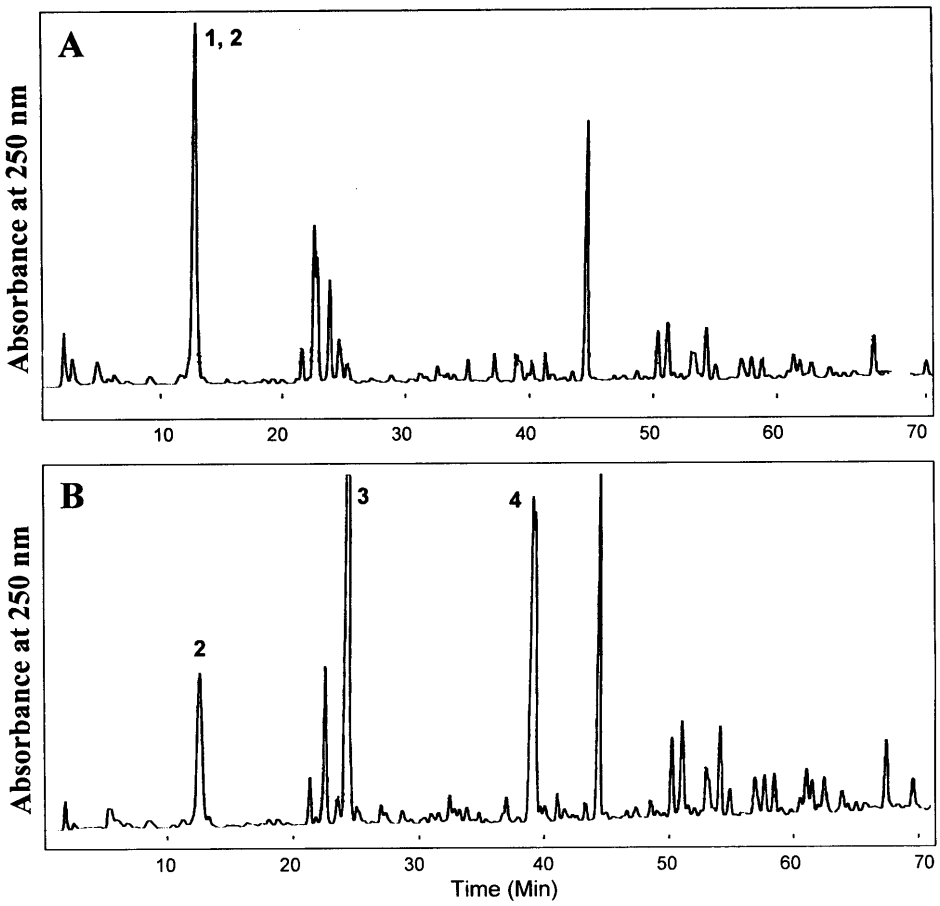
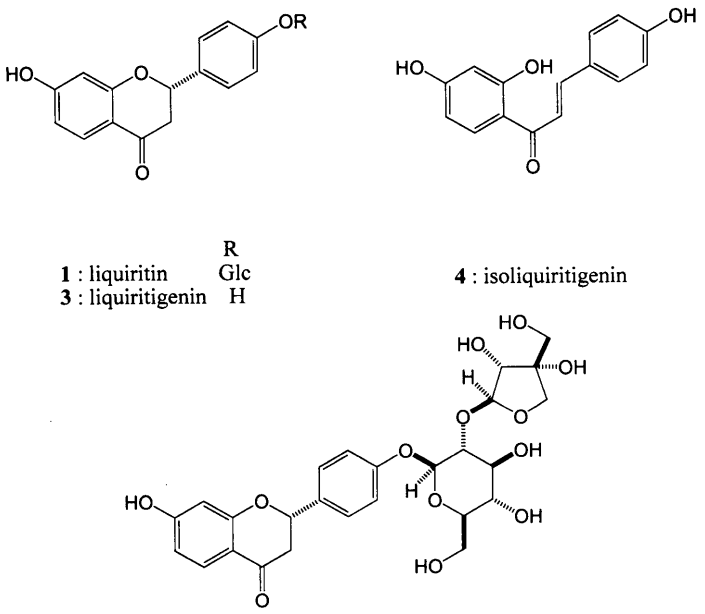


Fig. 1. HPLC Chromatograms of constituents in the naringinase-treated (B) and nariginase-untreated (A) MeOH extracts from roots of *Glycyrrhiza uralensis*.
1 : liquiritin, **2** : liquiritin apioside, **3** : liquiritigenin, **4** : isoliquiritigenin



2 : liquiritin apioside

Fig. 2. Chemical structures of constituents of *G. uralensis*.

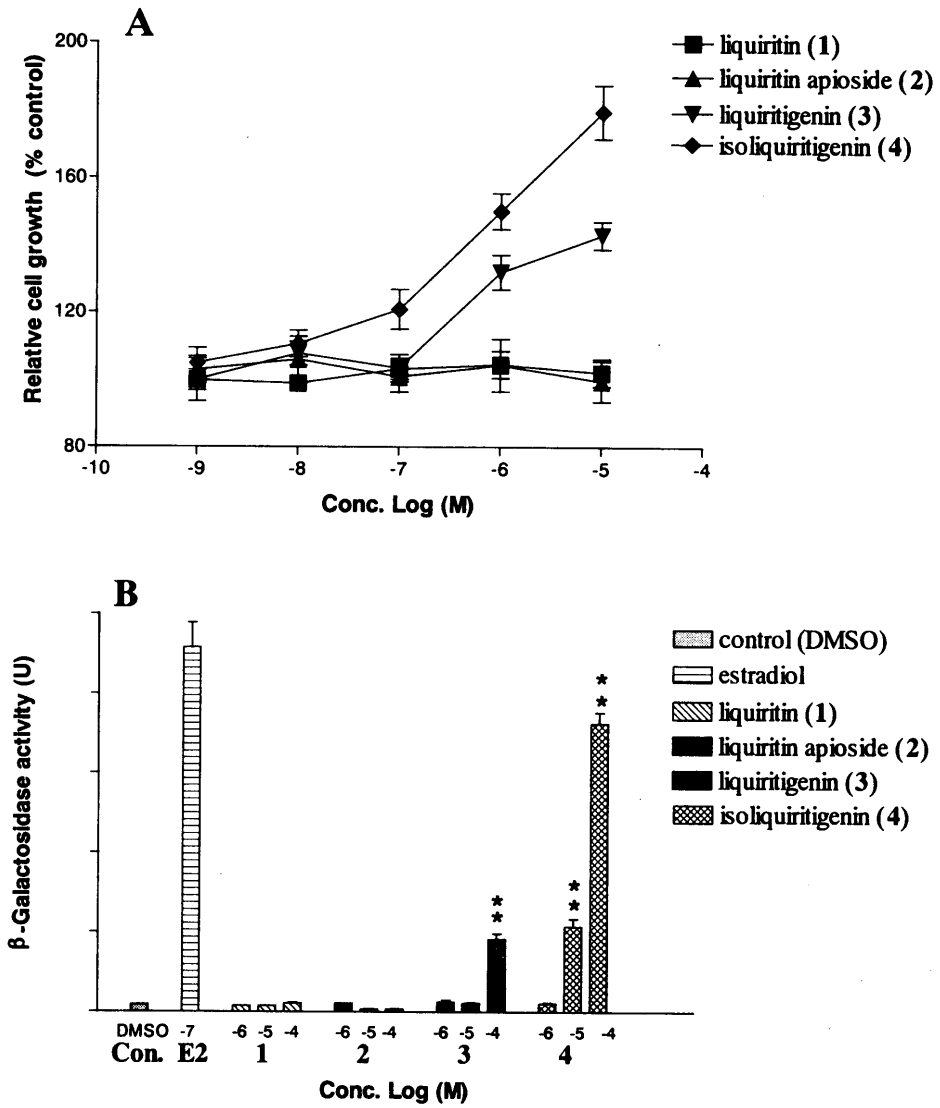


Fig. 3. A: Dose-response effect of some constituents on the proliferation of MCF-7 cells. The bar at each point is the standard error of at least three independent experiments (n=6).
B: Induction of β-galactosidase dependent on an estrogen receptor (ERα) binding. The bar at each point is the standard error of three independent experiments (n=3). Significantly different from the control at $p<0.01$ (**).

appreciable estrogenic activities. On the other hand, **3** and **4**, which were increased in contents after treatment with naringinase, showed significant MCF-7 cell proliferation and high β-galactosidase activities. When compared to the corresponding glycosides, the higher estrogenic activities of these aglycones are attributable to the remove of sugar moieties by naringinase.

In conclusion, we developed a glycosidase-pretreatment method to evaluate estrogenic activity of various medicinal plant extracts, which may mimic the bacterial hydrolysis of the glycosidic precursors of phytoestrogens. Therefore, the glycosidase-pretreatment method is more reliable to evaluate phytoestrogenic activity of crude drugs, especially in the case of oral administration of these drugs.

Acknowledgment

This paper forms part of 21st Century COE program sponsored by the Ministry of Education, Culture, Sports, Science and Technology.

References

1) Huxtable, R.J.: The myth of beneficent nature: The risks of herbal preparations. *Ann. Internal. Med.* **117**, 165-166, 1992.
2) Mousavi, Y., Adlercreutz, H.: Enterolactone and estradiol inhibit each other's proliferation effect on MCF-7 breast cancer cells in culture. *J. Steriod Biochem. Mol. Biol.* **41**, 615-619, 1992.
3) Baird, D., Umbach, D.: Dietary intervention study to assess estrogenicity of dietary soy, among postmenopausal women. *Clin. Endocr.* **80**, 1685-1690, 1995.
4) Knight, D.C., Eden, J.A.: A review of the clinical effects of

phytoestrogens. *Obstet. Gynecol.* **87**, 897-904, 1996.
5) Schafer, J.M., Lee, E.S., O'Regan, R.M., Yao, K., Jordan, V.C.: Rapid development of tamoxifen-stimulated mutant p53 breast tumor (T47D) in athymic mice. *Clin. Cancer Res.* **6**, 4373-4380, 2000.
6) Jiang Su New Medical College: Encyclopedia of Chinese Materia Medica, Shanghai Science and Technology Publisher, Shanghai, p. 212, 1977.
7) Sashida, Y., Yamasaki, K.: Integrated essential pharmacognosy (6th edition), Nankodo, Japan, 2002.
8) Horwitz, K.B., Costlow, M.E., McGuire, W.L.: MCF-7: a human breast cancer cell line with estrogen, androgen, progesterone, and glucocorticoid receptor. *Steroids* **26**, 785-795, 1975.
9) Nishikawa, J., Saito, K., Goto, J., Dakeyama, F., Matsuo, M., Nishihara, T.: New screening methods for chemicals with hormonal activities using interaction of nuclear hormone receptor with coactivator. *Toxicol. Appl. Pharmacol.* **154**, 76-83, 1999.
10) Yoshikawa, M., Uemura, T., Shimoda, H., Kishi, A., Kawahara, Y., Matsuda, H.: Medicinal foodstuffs. XVIII. Phytoestrogens from the aerial part of *Petroselinum crispum* Mill. (parsely) and structures of 6"-acetylapiin and a new monoterpene glycoside, petroside. *Chem. Pharm. Bull.* **48**, 1039-1044, 2000.
11) Villalobos, M., Olea, N., Brotons, J.A., Olea-Serrano, M.F., Ruiz de Almodovar, J.M., Pedraza, V.: The E-screen assay: a comparison of different MCF7 cell stocks. *Environ. Health. Perspect.* **103**, 844-850, 1995.
12) Soto, A.M., Sonnenschein, C.: The role of estrogens on the proliferation of human breast tumor cells (MCF-7). *J. Steroid Biochem.* **23**, 87-94, 1985.
13) Carmichael, J., DeGraph, W.G., Gazer, A.F., Minna, J.D., Mitchell, J.B.: Evaluation of a tetrazolium-based semiautomated colorimetric assay: Assessment of chemosensitivity testing. *Cancer Res.* **47**, 936-942, 1987.
14) Yang, Y., Kinoshita, K., Koyama, K., Takahashi, K., Tal, T., Nunoura, Y., Watanabe, K.: Two novel anti-emetic principles of *Alpinia katsumadai*. *J. Nat. Prod.* **62**, 1672-1674, 1999.
15) Kimura, Y., Takahashi, S., Yoshida, I.: Studies on the constituents of Alpinia. XII. On the constituents of the seeds of *Alpinia katsumadai* Hayata. The structure of cardamomin. *Yakugaku Zasshi* **88**, 239-241, 1968.
16) Ngo, K.S., Brown, G.D.: Stilbenes, monoterpense, diarylheptanoids, labdanes and chalcones from *Alpinia katsumadai*. *Phytochemistry* **47**, 1117-1123, 1998.
17) Inano, H., Suzuki, K., Onoda, M., Kobayashi, H., Wakabayashi, K.: Comparative effect of chlormadinone acetate and diethylstilbestrol as promoters in mammary tumorigenesis of rats irradiated with gamma-rays during lactation. *Breast Cancer Res. Treat.* **53**, 153-160, 1999.
18) Miksicek, R.J.: Commonly occurring plant flavonoids have estrogenic activity. *Mol. Pharmacol.* **44**, 37-43, 1993.
19) Saitoh, T., Noguchi, H., Shibata, S.: A new isoflavone and corresponding isoflavanone of licorice root. *Chem. Pharm. Bull.* **26**, 144-147, 1978.
20) Kitagawa, I., Hori, K., Uchida, E., Chen, W.Z., Yoshikawa, M., Ren, J.: Saponin and sapogenol. L. On the constituents of the roots of *Glycyrrhiza uralensis* Fischer from Xinjiang, China. Chemical structures of licorice-saponin L3 and isoliquiritin apioside. *Chem. Pharm. Bull.* **41**, 1567-1572, 1993.
21) Chen, M., Lou, S.Q., Chen, J.H.: Studies on the chemical constituents of *Flemingia philippinensis*. *Acta Pharmaceutica Sinica* **26**, 42-48, 1990.
22) Chen, M., Lou, S.Q., Chen, J.H.: Two isoflavones from *Flemingia philippinensis*. *Phytochemistry* **30**, 3842-3844, 1991.
23) Marken, H.M., Beecher, G.R.: Measurement of food flavonoids by high-performance liquid chromatography: a review. *J. Agric. Food. Chem.* **48**, 577-599, 2000.
24) Maggiolini, M., Statti, G., Vivacqua, A., Gabriele, S., Rago, V., Loizzo, M., Menichini, F., Amdo, S.: Estrogenic and antiproliferative activities of isoliquiritigenin in MCF-7 breast cancer cells. *J. Steriod Biochem. Mol. Biol.* **82**, 315-322, 2002.

Japanese abstract

薬用植物エキスの植物エストロゲン活性を評価する目的で，naringinase で前処理し，MCF-7 ヒト乳癌細胞の増植及び yeast two-hybrid assay 法による β-galactosidase 活性の誘導を指標とする方法を開発した。検討したエキスの中で *Alpinia katsumadai, Glycyrrhiza uralensis, Moghania philippinensis* は naringinase 処理することにより，元のエキスよりエストロゲン活性が強くなった。*G. uralensis* のエキスを naringinase 処理すると，エストロゲン活性のある liquiritigenin と isoliquiritigenin の含有量は顕著に増加した。この結果は，生薬を経口投与すると腸内細菌による配糖体の加水分解が起り，エストロゲン活性が増大することを示唆している。

*〒930-0194 富山市杉谷 2630
富山医科薬科大学和漢薬研究所 服部征雄