RESEARCH COMMUNICATION

Screening of Indigenous Plants from Japan for Modulating Effects on Transformation of the Aryl Hydrocarbon Receptor

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Abstract

The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor with which halogenated and polycyclic aromatic hydrocarbons such as dioxins and benzo[a]pyrene interact as ligands. Since such compounds cause various toxicological effects, including cancer, through the transformation of AhR, it is important to determine influence of modulating factors. It has been reported that certain plant components such as flavonoids and indoles can affect AhR transformation. In this study, to obtain clues to novel ligands of AhR, 191 species of indigenous plants were collected in Japan, and their 50% methanolic extracts (total 368 plant parts) were tested for modulating effects on AhR transformation in a cell-free system using a rat hepatic cytosolic fraction. Among tested extracts at a concentration of 1 mg dry weight of plant/mL, 174 of 368 extracts suppressed 1 nM 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-induced AhR transformation to 50% or less, while 9 extracts *per se* induced AhR transformation equivalent to more than 20% of that induced by 1 nM TCDD. *Mallotus japonicus* (Thunb.) Muell. (leaf) and *Trichosanthes rostrata* Kitamura (fruit and fruit skin) strongly suppressed 1 nM TCDD-induced AhR transformation, while *Phellodendron amurense* Ruprecht (seed) *per se* strongly induced AhR transformation. These results suggest that a large variety of plants in Japan contain various compounds modulating, mainly suppressing, AhR transformation.

Key Words: AhR transformation - TCDD - *Mallotus japonicus* (Thunb.) Muell. - *Trichosanthes rostrata* Kitamura - *Phellodendron amurense* Ruprecht

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Introduction

The aryl hydrocarbon receptor (AhR) is a member of the basic helix-loop-helix (bHLH) protein and belongs to Per-Arnt-Sim (PAS) family. Although the AhR is at the moment an orphan receptor, it has been suggested that the AhR is involved in development of the liver and immune system from the results of study using AhR-deficient mice (Vorderstrasse et al., 2001). It is known that halogenated and polycyclic aromatic hydrocarbons (HAHs and PAHs, respectively) such as dioxins (Van den Berg et al., 1998), benzo[a]pyrene (Saeki et al., 2003), and 3-methylcholanthrene (MC) (Bhagavatula, 2000) bind to the AhR as ligands, and cause the toxicological effects including cancer (Grassman et al., 1998). These compounds have been shown to be responsible for cancer of various tissues, such as skin, ovary and liver, in experimental animals (Grassman et al., 1998), and there are also epidemiologic data that high exposure of dioxins to human being links with cancers (Steenland et al. 2004). It is known that these toxicological effects are expressed through interaction between these compounds and the AhR, that is, the transformation of AhR (Fernandez-Salguero et al., 1996). In addition, certain PAHs such as benzo[a]pyrene and MC are metabolic converted to their ultimate carcinogens by cytochrome P4501A subfamily of which expression are one of the downstream events for AhR transformation. Indeed, the skin carcinogenicity of benzo[a]pyrene was lost in AhR-/- mice (Shimizu et al., 2000). Therefore, AhR transformation is involved in carcinogenicity of HAHs and PAHs, and many researchers have attempted to find out the endogenous and/or exogenous ligands of AhR.

In many studies, it has been reported that naturally occurring compounds, such as polyphenols (Ashida et al., 2000; Amakura et al., 2003a,b), indigoids (Adachi et al., 2001; Spink et al., 2003; Guengerich et al., 2004), and indoles (Rannug et al., 1987; Bjeldanes et al., 1991), interact with the AhR. Among these compounds, flavonoids show the both agonistic and antagonistic effects on AhR transformation depending upon their concentrations. They

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act as the antagonists at the lower concentrations and as the agonists at the higher ones (Ashida et al., 2000). Lutein shows the only antagonistic effect (Fukuda et al., 2004a), whereas indigoids and indoles show agonistic effect (Rannug et al., 1987; Bjeldanes et al., 1991; Adachi et al., 2001; Spink et al., 2003; Guengerich et al., 2004). In addition, extracts of vegetables and fruits including these compounds show the agonistic or antagonistic effects on AhR transformation (Amakura et al., 2002; Jeuken et al., 2003; Park et al., 2004).

The aim of this study is a screening of plants that have modulating effects on AhR transformation for an approach to search out the novel ligands of AhR. Here, we investigated the effects of 50% methanolic extracts from 191 species of the indigenous plants (total 368 plant parts) collected in Japan on AhR transformation in a cell-free system using a southwestern chemistry-based enzyme-linked immunosorbent assay (SW-ELISA) (Fukuda et al., 2004b).

Materials and Methods

2.1. Materials

Dimethylsulfoxide (DMSO) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan) and AccuStandard (New Haven, CT), respectively. Plants used in this experiment were collected from Japan and have been registered to the National Institute of Health Sciences (NIHS) of Japan (listed in Table). Each part of the plant was pulverized with liquid nitrogen, and lyophilized. Aliquots of 100 mg of lyophilized powder were extracted with 1 mL of 50% methanol by ultrasonic waves, and centrifuged at 1,000 x g for 10 min. The obtained supernatant was dried up and used as the extract. These extracts were adjusted to a concentration at 100 mg dry weight of plant/mL with 50% methanol before use.

2.2. In vitro cell-free system

The animal treatments in this study conformed to the "Guidelines for the care and use of experimental animals, in Rokkodai Campus, Kobe University". Male Sprague-Dawley rats (6 weeks old) were purchased from Japan SLC (Shizuoka, Japan). Rat hepatic cytosolic fraction was prepared according to the previous report (Ashida et al., 2000). The cytosolic fraction (15 mg protein/mL) was incubated with 1 nM TCDD (final concentration) or DMSO (10 μL/mL) alone as a vehicle control in HEDG buffer (25 mM HEPES, pH 7.4, 1.5 mM EDTA, 1.0 mM dithiothreitol (DTT), and 10% (v/v) glycerol) at 20˚C for 2 hr in the dark. For evaluation of the antagonistic effect, each plant extract (final concentration at 1, 0.1, or 0.025 mg dry weight of plant/mL) or 50% (v/v) methanol as a vehicle control was added to the cytosolic fraction 20 min before addition of 1 nM TCDD. For evaluation of the agonistic effect, plant extract alone at 1 mg dry weight of plant/mL was added to the cytosolic fraction. After the incubation, the mixture was subjected to SW-ELISA for measurement of AhR transformation.

2.3. Measurement of AhR transformation by SW-ELISA

AhR transformation was measured by SW-ELISA according to the previous report (Fukuda et al., 2004b). Reaction mixture was consisted of 10 μL of HEDG buffer containing 750 mM KCl (final concentration at 150 mM) and 40 μL of the cytosolic fraction from the incubation as described. The reaction mixture (50 μL) containing transformed AhR was plated on a dioxin responsive element probe-bound 96-well microtiter plate, and AhR transformation was measured. Data are expressed as the mean ± SD of at least three independent determinations for each experiment.

Results

3.1. Antagonistic effect of plant extracts on AhR transformation in the cell-free system

It is predicted that plants contain the compounds modulating AhR transformation, because many phytochemicals suppress or induce AhR transformation (Amakura et al., 2003a,b). We, first, surveyed the antagonistic effect of each plant extract at 1 mg dry weight of plant/mL on AhR transformation induced by 1 nM TCDD, which is the most toxic congener among the dioxins (Van den Berg et al., 1998). Among tested extracts, 174 of 368 extracts suppressed 1 nM TCDD-induced AhR transformation to 50% or less (Table and Figure 1). A second screening was carried out using the selected 88 extracts at 0.1 mg dry weight of plant/mL, of which AhR transformation was less than 20% in the first screening (Table). As results, 13 extracts suppressed 1 nM TCDD-induced AhR transformation to 20% or less. Then, the final screening was carried out again using the selected 9 extracts at 0.025 mg dry weight of plant/mL, for which AhR transformation was less than 10% in the second screening (Table). Among these extracts, *Mallotus japonicus* (Thunb.) Muell. (leaf), *Trichosanthes rostrata* Kitamura (fruit), and *Trichosanthes rostrata* Kitamura (fruit skin) showed significant suppressive effects: They decreased 1 nM TCDD-induced AhR

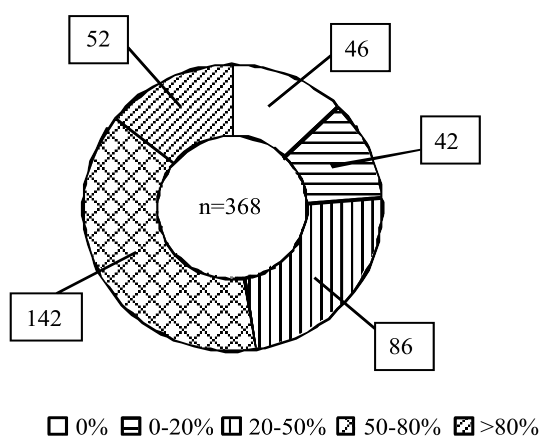

Figure 1. Antagonistic Effects of Plant Extracts on TCDD-induced AhR Transformation. Proportions of antagonistic extracts with reference to the % of 1nM TCDD-induced transformation.

Table. Antagonistic and Agonistic Effects of Plant Extracts on AhR Transformation

Scientific Name	Part	Antagonistic#			Agonistic#
		1	0.1	0.025	
Aceraceae					
Acer aidzuense Nakai (0004-02IS)*	seed	0	6.0 ± 10.5	69.0 ± 6.3	no effect
Acer mono Maxim (0702-02IS)	seed	0	0	82.0 ± 3.5	no effect
Acer rufinerve Sieb. et Zucc. (0701-02IS)	seed	0.3 ± 1.7	40.5 ±14.1		no effect
Alismataceae					
Alisma plantago-aquatica L. var. orientale Samuels. (1822-84TS)	seed	52.3 ± 5.8			10.0 ±15.6
Amaranthaceae					
Achyranthes bidentata Blume (0607-79TS)	seed	58.6 ± 3.6			no effect
Achyranthes fauriei Lev. et Van. f. rotundifolia Ohwi (1537-84TS)	seed	47.2 ± 2.3			3.5 ± 3.5
Achyranthes fauriei Leveille et Vaniot 'Hitachigoshitsu' (1247-80TS)	rhizome	81.9 ± 4.7			6.4 ± 0.8
	seed	50.6 ± 2.4			2.3 ±3.1
Anacardiaceae					
Choerospondias axillaris (Roxb.) B.L. Burtt et A.W. Hill var. japonica (Ohwi) Ohwi (0712-02IS)	seed	100 ± 13.8			no effect
Rhus succedanea L. (0713-02IS)	fruit	72.6 ± 4.0			no effect
	seed	60.4 ± 3.7			no effect
Rhus ambigua Lavalee ex Dippel (0018-02IS)	seed	28.1 ± 6.9			no effect
Apocynaceae					
Amsonia elliptica (Thunb.) Roem. et. Schult. (0478-79TS)	seed	63.1 ± 5.0			no effect
Vinca major L. (0177-97TS)	leaf	0	50.4±3.3		no effect
	stalk	66.6 ± 6.1			no effect
Araceae					
Acorus grminus Soland. (0490-79TS)	leaf	87.0 ± 8.5			8.2 ± 7.5
Berberidaceae					
Epimedium grandiflorum Morr. var. thunbergianum (Miq.) Nakai (1577-84TS)	leaf	0	8.9 ± 8.1	73.8 ± 2.8	no effect
	root	0	35.0 ± 18.8		no effect
Nandina domestica Thunb. (0014-79TS)	fruit	24.0 ± 1.0			no effect
	leaf	39.3 ± 5.1			no effect
	stalk	62.9 ± 4.9			no effect
Betulaceae					
Carpinus laxiflora (Sieb. et Zucc.) Blume (0749-02IS)	seed	51.8 ± 4.1			no effect
Boraginaceae					
Anchusa azurea Mill. (0436-79TS)	seed	45.2 ± 3.3			no effect
	flower	5.4 ±15.3	21.5 ±10.4		no effect
	leaf	0	71.1 ± 3.2		no effect
	stalk	9.8 ±14.7	67.7 ± 9.7		no effect
	root	0	79.5± 0.9		no effect
Campanulaceae					
Platycodon glandiflorum (Jacq.) A. DC. (2638-82TS)	seed	42.2 ± 5.1			0.8 ± 2.3
	leaf	41.7 ± 4.3			13.0 ± 2.2
	stalk	87.4 ± 8.9			6.7 ± 3.8
	root	99.1 ± 8.9			no effect
Caprifoliaceae					
Viburnum dilatatum Thunb. (0777-02IS)	fruit	62.2 ± 2.7			no effect
	seed	107 ± 13.0			no effect
Viburnum erosum Thunb. (0779-02IS)	fruit	77.8 ± 12.0			no effect
Caryophyllaceae					
Saponaria officinalis L. (0482-79TS)	seed	85.7 ± 1.2			3.5 ±7.4
Celastraceae					
Celastrus orbiculatus Thunb. (0080-02IS)	seed	70.7 ± 1.1			no effect
	fruit skin	65.3 ± 0.7			no effect
Chenopodiaceae					
Beta vulgaris L. var. rapa Dumort. (0120-03TS)	seed	57.0 ± 3.9			no effect
Chenopodium ambrosioides L. var. anthelminticum (L.) A. Gray (0639-79TS)	seed	57.4 ± 2.1			no effect

*(Collection number of plant at NIHS) # (% of 1 nM TCDD) mg of dry weight/mL

Table. Antagonistic and Agonistic Effects of Plant Extracts on AhR Transformation (continued)

Scientific Name	Part	Antagonistic 1	Antagonistic 0.1	Antagonistic 0.025	Agonistic
Clethraceae					
Clethra barbinervis Sieb. et Zucc. (0097-02IS)	seed	0	73.6 ± 9.8		no effect
Compositae					
Arctium lappa L. (0116-03TS)	seed	61.1 ± 8.4			no effect
Artemisia annua L.(0435-84TS)	root	0	62.3 ± 3.9		no effect
	leaf	28.5 ± 1.4			no effect
	stalk	46.8 ± 0.4			no effect
	seed	75.6 ± 18.3			no effect
Atractylodes ovata DC. (0018-94TS)	leaf	95.3 ± 4.2			no effect
	root	111 ± 4.3			0.2 ± 1.9
	seed	13.5 ± 1.1	50.6± 5.0		7.1 ± 1.2
Bidens frondosa L. (0120-02IS)	seed	52.7 ± 2.4			no effect
Carthamus tinctorius L. (0439-79TS)	flower	89.7 ± 5.3			2.7 ± 0.4
	leaf	54.8 ± 1.5			8.1 ± 0.2
	root	90.6 ± 3.1			10.0 ± 1.2
	seed	30.8 ± 1.5			5.4 ± 4.4
Chrysanthemum morifolium Ramat. 'Daizyo' (0131-93TS)	flower	0	24.6 ± 12.5		no effect
	leaf	0	34.6 ± 1.1		no effect
	stalk	35.3 ± 2.4			no effect
	root	28.1 ± 3.3			no effect
Cichorium intybus L. (0467-79TS)	seed	56.7 ± 10.5			no effect
Cynara scolymus L. (0551-79TS)	leaf	39.5 ± 1.9			no effect
Matricaria chamomilla L. (0498-79TS)	flower	50.1 ± 3.5			5.9 ± 1.7
	stalk+leaf	77.2 ± 1.3			2.3 ± 2.7
Pyrethrum cinerariifolium Trevir. (0500-79TS)	flower	29.4 ± 11.2			no effect
	stalk	47.1 ± 1.7			no effect
	root	50.5 ± 2.4			no effect
Spilanthes fusca Hort. (0393-90TS)	seed	34.8 ± 1.2			no effect
Spilanthes oleracea Jacq. (1819-84TS)	seed	52.4 ± 0.4			no effect
Xanthium strumarium L. (0163-02IS)	seed	14.3 ± 3.6	65.1 ± 4.6		no effect
Convolvulaceae					
*Pharbitis ni*l Choisy (0636-79TS)	seed	32.9 ± 10.7			no effect
Coriariaceae					
Cicuta virosa L. (0062-79TS)	leaf	0	65.2 ± 11.6		no effect
	stalk	53.7 ± 2.0			no effect
Cruciferae					
Isatis tinctoria L. (0324-80TS)	seed	0	30.1 ± 5.0		no effect
	flower	63.1 ± 7.2			no effect
	leaf	43.4 ± 14.8			no effect
	stalk	54.7 ± 8.8			no effect
Cucurbitaceae					
Trichosanthes cucumeroides Maxim. (0181-02IS)	fruit	69.4 ± 7.0			no effect
	fruit skin	122 ± 6.7			no effect
	seed	55.6 ± 8.5			no effect
Trichosanthes rostrata Kitamura (0414-79TS)	fruit	0	0	0	no effect
	fruit skin	0	0	33.0 ± 1.7	no effect
	leaf	0	1.8 ± 1.9	73.6 ± 2.9	no effect
	seed	23.9 ± 4.9			no effect
Cupressaceae					
Thuja orientalis L. (1512-84TS)	fruit	67.5 ± 7.5			0.3 ± 0.5
	leaf	69.2 ± 7.0			1.7 ± 1.4
Chamaecyparis obtusa (Sieb. et Zucc.) Sieb.et Zucc. ex Endl. (0186-02IS)	pine corn	42.3 ± 1.1			no effect
	seed	35.4 ± 14.4			no effect
Chamaecyparis pisifera (Sieb. et Zucc.) Sieb.et Zucc ex Endl. (0157-79TS)	seed	65.4 ± 3.4			no effect
Cyperaceae					
Carex kobomugi Ohwi (0188-02IS)	seed	83.5 ± 13.0			38.4 ± 25.0

Table. Antagonistic and Agonistic Effects of Plant Extracts on AhR Transformation (continued)

Scientific Name	Part	Antagonistic 1	0.1	0.025	Agonistic
Ebenaceae					
Diospyros kaki Thunb. (1725-84TS)	leaf	18.4 ± 13.3	71.3 ± 16.1		no effect
	stalk	4.4 ± 8.8	68.9 ± 12.2		no effect
Elaeagnaceae					
Elaeagnus ubbellata var. rotundifolia Makino (0226-02TS)	fruit	45.2 ± 4.0			no effect
Ephedraceae					
Ephedra distachya L. (0381-79TS)	ground region	74.6 ± 4.7			7.4 ± 1.1
Ephedra distachya L. (EP-13)(0381-79TS)	ground region	32.0 ± 6.2			no effect
	root	16.6 ± 0.9	65.6 ± 0.5		no effect
Ericaceae					
Pieris jponica D. Don (0081-79TS)	fruit	74.7 ± 3.6			7.6 ± 3.0
	leaf	84.9 ± 3.9			no effect
Rhododendron oomurasaki Makino (1719-84TS)	flower (white)	19.2 ± 7.6	49.2 ± 8.9		no effect
	leaf	24.9 ± 16.3			no effect
	stalk	92.0 ± 13.5			no effect
Eucommiaceae					
Eucommia ulmoides Oliv. (0057-79TS)	leaf	65.9 ± 3.6			no effect
Euphorbiaceae					
Aleurites cordata (Thunb.) R. Br. ex Steud. (0234-02IS)	seed	38.9 ± 4.5			no effect
Euphorbia cyparissias L. (0574-79TS)	flower	0	41.6 ± 10.6		9.6 ± 7.7
	leaf	46.8 ± 6.7			no effect
	stalk	70.8 ± 5.3			no effect
	root	80.8 ± 12.4			no effect
Mallotus japonicus (Thunb.) Muell. (0095-79TS)	leaf	0	6.9 ± 9.7	42.4 ± 2.3	no effect
	stalk	92.9 ± 1.7			no effect
Mallotus japonicus (Thunb.) Muell. Arg. (0095-79TS)	seed	19.4 ± 0.5	38.7 ± 2.4		no effect
Ricinus communis L. (0610-79TS)	leaf	84.4 ± 4.6			no effect
	stalk	62.2 ± 1.8			no effect
	seed	94.1 ± 20.5			no effect
Sapium sebiferum (L.) Roxb. (0887-02IS)	seed	17.1 ± 6.8	84.0 ± 18.8		no effect
Fagaceae					
Castanea crenata Siebold et Zucc. (0891-02IS)	seed	111 ± 6.3			no effect
Castanopsis cuspidata (Thunb.) Schottky var. sieboldii (Makino) Nakai (0892-02IS)	seed	56.4 ± 4.6			1.9 ± 1.1
Castanopsis cuspidata Schott. var. sieboldii Nakai (0892-02IS)	seed	57.7 ± 3.4			no effect
Pasania edulis Makino (0001-93TN)	seed	22.4 ± 6.9			no effect
Quercus myrsinaefolia Blume (0894-02IS)	seed	59.8 ± 6.1			no effect
Quercus serrata Thunb. (0256-02IS)	seed	53.6 ± 14.7			no effect
Geraniaceae					
Geranium thunbergii Sieb. et Zucc. (0444-79TS)	leaf	0	40.4 ± 13.0		no effect
	root	84.2 ± 10.6			no effect
	stalk	6.3 ± 1.0	43.0 ± 9.4		no effect
Ginkgoaceae					
Ginkgo biloba L. (0900-02IS)	seed	63.8 ± 3.5			no effect
Gramineae					
Coix lacryma-jobi L. (0903-02IS)	seed	73.8 ± 7.5			2.2 ± 3.9
Coix lacryma-jobi L. var. ma-yuen (Roman.) Stapf (0905-02IS)	seed	65.1 ± 13.3			2.4 ± 8.7
Pennisetum alopecuroides (L.) Spreng. (0299-02IS)	seed	56.0 ± 7.6			no effect
Phragmites communis Trin. (0304-02IS)	seed	74.7 ± 2.6			4.3 ± 5.1
Guttiferae					
Hypericum erectum Thunb. (0429-79TS)	seed	62.9 ± 5.2			no effect
Labiatae					
Melissa officinalis L. (0819-83TS)	ground region	0	25.3 ± 11.3		26.5 ± 10.2
	rhizome	14.0 ± 6.9	50.5 ± 12.9		no effect
	root	0	76.3 ± 16.8		no effect
Mentha arvensis L. var. piperascens Malinvaud 'Ayanami' (0396-79TS)	leaf	67.7 ± 0.8			no effect
	stalk	0	52.2 ± 2.3		no effect
	root	23.5 ± 1.1			no effect

Table. Antagonistic and Agonistic Effects of Plant Extracts on AhR Transformation (continued)

Scientific Name	Part	Antagonistic			Agonistic
		1	0.1	0.025	
Labiatae (continued)					
Mentha piperita L. 'Eikoku Kuro' (0391-79TS)	leaf	73.5 ± 2.8			no effect
	stalk	71.7 ± 2.2			no effect
	root	77.8 ± 1.7			no effect
Mentha pulegium L. (0184-95TS)	stalk	55.9 ± 7.0			no effect
	root	72.7 ± 9.4			no effect
Perilla frutescens Britton var. acuta Kudo f. crispa	seed	64.6 ± 4.6			1.9 ± 2.5
Makino (0931-02IS)	leaf	55.3 ± 2.8			no effect
	root	72.9 ± 3.5			no effect
	flower	48.8 ± 0.4			no effect
Makino (0932-02IS)	stalk	49.5 ± 2.9			no effect
Perilla frutescens Britton var. acuta Kudo f. viridi-crispa	seed	68.8 ± 1.7			4.0 ± 4.2
Plectranthus japonicus (Burm. fil.) Koidz. (0548-79TS)	leaf	13.8 ± 1.0	32.4 ± 12.6		no effect
	stalk	60.7 ± 5.3			no effect
	root	60.8 ± 1.6			5.6 ± 5.1
	seed	22.0 ± 9.9			no effect
Salvia miltiorrhiza Bunge (1851-81TS)	ground region	8.4 ± 5.9	47.2 ± 6.9		no effect
	root	0	60.0 ± 1.0		no effect
Schizonepeta tenuifolia Briquet (0166-95TS)	leaf	8.2 ± 2.1	32.1 ± 13.5		no effect
	stalk	39.6 ± 1.6			no effect
	root	33.6 ± 2.4			no effect
	seed	52.4 ± 5.1			no effect
Stachys officinalis (L.) Trevis. (0124-80TS)	whole grass	0	31.2 ± 6.9		no effect
Lardizabalaceae					
Akebia trifoliata (Thunb.) Koidz. (0349-02IS)	fruit	73.3 ± 2.5			no effect
	fruit skin	49.1 ± 1.6			no effect
	leaf	17.5 ± 6.6	69.1 ± 6.2		no effect
	stalk	75.3 ± 1.6			no effect
Stauntonia hexaphylla (Thunb.) Decaisne (1587-84TS)	flower	0	68.1 ± 18.8		no effect
	leaf	0	54.1 ± 15.9		no effect
	stalk	8.3 ± 16.1	43.2 ± 19.7		no effect
Lauraceae					
Lindera strychnifolia (Sieb. et Zucc.) f. Vill. (0107-79TS)	leaf	48.8 ± 5.1			no effect
	stalk	68.9 ± 5.0			no effect
Lindera umbellata Thunb. (1544-84TS)	leaf	5.1 ± 2.9	24.2 ± 6.2		no effect
Neolitsea sericea (Blume) Koidz. (0355-02IS)	seed	84.9 ± 6.4			no effect
	leaf	47.3 ± 1.6			28.4 ± 14.4
	branch	71.1 ± 3.1			2.0 ± 10.8
Parabenzoin praecox Nakai (0158-02IS)	fruit	55.8 ± 3.1			no effect
	seed	32.9 ± 1.7			no effect
Leguminosae					
Cajanus cajan (L.) Millsp. (0959-02TS)	seed	70.0 ± 5.9			19.1 ± 22.2
Cassia nomame (Sieb.) Honda (0597-79TS)	seed	81.3 ± 9.4			no effect
Cassia obtusifolia L. (0599-79TS)	leaf	72.2 ± 2.8			no effect
	stalk	70.1 ± 5.3			no effect
	root	48.7 ± 2.4			no effect
	seed	20.8 ± 4.0			no effect
Cassia torosa Cav. (0604-79TS)	leaf	52.2 ± 3.7			2.9 ± 3.5
	stalk	69.4 ± 3.9			2.6 ± 2.0
	root	79.2 ± 2.0			13.4 ± 2.4
	seed	46.0 ± 2.8			no effect
Glycyrrhiza uralensis Fisch. (0125-93TS)	leaf	9.6 ± 16.6	26.9 ± 2.6		5.2 ± 5.4
	rhizome	10.4 ± 6.1	52.0 ± 2.5		no effect
Pueraria lobata (Willd.) Ohwi (0415-79TS)	leaf	101 ± 7.7			2.5 ± 2.3
	stalk	85.0 ± 7.1			no effect
Wisteria floribunda (Willd.) DC. (1663-84TS)	seed	10.3 ± 8.4	34.9 ± 5.9		no effect
	flower	28.7 ± 17.7			no effect
	leaf	7.3 ± 2.4	23.8 ± 19.5		13.8 ± 21.8
	stalk	70.7 ± 1.4			no effect

Table. Antagonistic and Agonistic Effects of Plant Extracts on AhR Transformation (continued)

Scientific Name	Part	Antagonistic			Agonistic
		1	0.1	0.025	
Liliaceae					
Fritillaria verticillata Willd. var. thunbergii Bak. (0518-02TS)	root	0	52.0 ± 14.5		no effect
Hosta montana F. Maekawa (0409-02IS)	seed	82.2 ± 8.4			no effect
Lilium cordatum Koidz.	seed	60.0 ± 1.7			no effect
Liriope platyphylla Wang et Tang (0225-97TS)	seed	68.6 ± 2.7			no effect
Smilax china L. (0419-02IS)	fruit	40.8 ± 2.7			no effect
Magnoliaceae					
Liriodendron tulipifera L. (1022-02IS)	seed	57.9 ± 18.3			no effect
Magnolia kobus DC. (0028-79TS)	seed	73.9 ± 1.4			no effect
	fruit	83.7 ± 16.9			4.3 ± 11.4
Magnolia obovata Thunb. (0052-79TS)	leaf	99.0 ± 1.0			13.0 ± 1.6
Magnolia officinalis Rehd. et. Wils. (0045-95TS)	leaf	90.1 ± 5.6			4.3 ± 4.3
Michelia figo Spreng. (0140-79TS)	leaf	53.4 ± 16.4			no effect
	stalk	59.1 ± 0.1			no effect
Malvaceae					
Hibiscus manihot L. (0646-79TS)	leaf	78.6 ± 10.0			no effect
	stalk	117 ± 8.2			1.5 ± 1.8
	rhizome	69.7 ± 8.6			no effect
	seed	28.5 ± 17.6			no effect
Hibiscus manihot L. cv. Nagatoro 2 gou (0646-79TS)	seed	39.9 ± 14.6			no effect
Hibiscus manihot L. 'Nagatoro 2 gou' (0646-79TS)	seed	55.1 ± 9.1			no effect
Martyniaceae					
Proboscidea louisinica Thell. (0577-85TS)	seed	42.9 ± 7.5			no effect
Meliaceae					
Melia azedarach L. (1044-02IS)	branch	78.8 ± 18.2			no effect
	fruit	83.8 ± 7.4			no effect
	leaf	0	56.4 ± 12.2		no effect
	seed	55.1 ± 9.5			no effect
Nymphaeaceae					
Nelumbo nucifera Gaertn. (1059-02IS)	seed	29.6 ± 12.9			no effect
Oleaceae					
Ligustrum lucidum Ait. (1733-84TS)	seed	73.5 ± 10.4			no effect
Paeoniaceae					
Chaenomeles speciosa (Sweet) Nakai (0221-97TS)	seed	27.2 ± 14.8			no effect
	fruit skin	0	46.7 ± 1.1		no effect
Paeonia lactiflora Pall. (1071-02IS)	seed	28.9 ± 1.8			49.8 ± 7.8
	fruit	50.4 ± 13.0			no effect
	leaf	34.6 ± 8.0			no effect
	stalk	51.9 ± 5.6			no effect
Paeonia lactiflora Pall. (White, Single) (0130-93TS)	leaf	4.9 ± 0.9	38.9 ± 12.2		no effect
	stalk	83.2 ± 6.8			no effect
	root	59.8 ± 4.2			no effect
Paeonia suffruticosa Andr. (0226-97TS)	leaf	0	56.0 ± 5.4		no effect
	flower	23.8 ± 15.3			no effect
	fruit	39.2 ± 3.0			no effect
	stalk	31.6 ± 15.5			no effect
Papaveraceae					
Eschscholzia californica Cham. (1674-81TS)	ground region	0	84.2 ± 12.9		no effect
	root	0	38.3 ± 13.3		no effect
Papaver pseudo-orientale Medw. (0013-95TS)	root	77.4 ± 1.2			no effect
Phytolaccaceae					
Phytolacca americana L. (0479-02IS)	seed	73.5 ± 5.0			no effect
Pinaceae					
Pinus densiflora Sieb. et Zucc. (0480-02IS)	pine corn	18.8 ± 0.2	31.7 ± 13.1		no effect
Pinus thunbergii Parlat. (0481-02IS)	pine corn	0	57.7 ± 14.3		no effect
	leaf	31.1 ± 8.7			no effect
	branch	80.3 ± 9.4			21.5 ± 17.2

Table. Antagonistic and Agonistic Effects of Plant Extracts on AhR Transformation (continued)

Scientific Name	Part	Antagonistic			Agonistic
		1	0.1	0.025	
Pittosporaceae					
Pittosporum tobira (Thunb.) Ait. (0488-02IS)	seed	106 ± 2.2			no effect
	fruit skin	97.3 ± 10.7			no effect
	leaf	93.6 ± 5.9			27.7 ± 5.2
Plantaginaceae					
Plantago asiatica L. (0490-02IS)	seed	114 ± 15.8			13.3 ± 5.7
Polygonaceae					
Polygonum bistorta L. (0470-79TS)	flower	18.7 ± 15.6	44.0 ± 1.3		no effect
	leaf	27.8 ± 16.5			no effect
	stalk	48.7 ± 3.1			no effect
	root	0	18.4 ± 8.3		no effect
	seed	38.1 ± 9.6			no effect
Rheum undulatum L. (0147-80TS)	leaf	0	35.7 ± 11.8		no effect
	root	0	16.6 ± 16.1		15.7 ± 19.1
Punicaceae					
Punica granatum L. (0108-79TS)	fruit skin	0	18.3 ± 3.0		no effect
	seed	76.3 ± 3.8			8.4 ± 6.5
Ranunculaceae					
Aconitum carmichaeli Debx. (0512-79TS)	flower	32.3 ± 5.2			no effect
	leaf	96.5 ± 3.5			no effect
	stalk	71.4 ± 4.1			no effect
	root	54.7 ± 2.3			no effect
Clematis terniflora DC. (0521-02IS)	seed	43.5 ± 3.5			no effect
Coptis japonica (Thunb.) Makino var. dissecta (Yatabe)	leaf	58.1 ± 5.5			no effect
Nakai (3205-81TS)	root	0	42.3 ± 5.6		no effect
Rosaceae					
Agrimonia japonica (Miq.) Koidz. (0529-02IS)	seed	45.1 ± 1.4			no effect
Chaenomeles sinensis (Thouin.) Koehn. (0051-79TS)	seed	52.9 ± 5.2			no effect
	fruit	0	66.0 ± 17.9		no effect
	leaf	0	68.0 ± 16.3		no effect
	stalk	40.4 ± 7.1			no effect
Chaenomeles japonica (Thunb.) Spach (0529-79TS)	seed	71.2 ± 2.3			no effect
	fruit skin	4.2 ± 0.4	61.4 ± 3.2		no effect
	leaf	0	39.2 ± 3.3		no effect
	stalk	41.7 ± 0.8			no effect
Prunus pauciflora Bunge (0065-79TS)	seed	61.7 ± 2.9			no effect
	fruit	30.9 ± 1.4			no effect
	stalk	10.4 ± 7.9	63.3 ± 11.4		no effect
	leaf	0.3 ± 2.1	40.8 ± 17.8		no effect
Rhodotypos scandens (Thunb.) Makino (0141-79TS)	seed	65.4 ± 5.8			no effect
Rosa multiflora Thunb. (0033-85TS)	fruit	70.9 ± 5.5			no effect
	leaf	52.2 ± 2.1			no effect
	stalk	81.3 ± 6.5			no effect
Rosa rugosa Thunb. (0150-79TS)	fruit	78.3 ± 2.2			6.1 ± 3.0
Rosa wichuraiana Crep. (0549-02IS)	fruit	98.8 ± 6.1			no effect
Sanguisorba officinalis L. (0124-03TS)	seed	72.2 ± 11.9			no effect
Sanguisorba tenuifolia Fisch. var. purpurea Trautv. et. Mey. (1853-81TS)	seed	60.8 ± 1.8			no effect
Spiraea cantoniensis Lour. (0131-79TS)	flower	27.4 ± 2.2			no effect
	leaf	38.1 ± 3.4			no effect
	stalk	64.6 ± 6.3			no effect
Rubiaceae					
Rubia tinctorum L. (0161-87TS)	leaf	61.5 ± 12.0			no effect
	stalk	1.5 ± 5.0	95.5 ± 3.5		no effect
	root	17.6 ± 3.4	25.6 ± 9.9		no effect
Rutaceae					
Citrus leiocarpa hort. ex T. Tanaka "Hukuremikan" (1673-84TS)	fruit	173 ± 1.2			33.1 ± 0.8
	fruit skin	13.8 ± 0.3	28.8 ± 4.4		no effect
Phellodendron amurense Ruprecht (0097-79TS)	seed	75.7 ± 0.5			71.4 ± 2.4

Table. Antagonistic and Agonistic Effects of Plant Extracts on AhR Transformation (continued)

Scientific Name	Part	Antagonistic			Agonistic
		1	0.1	0.025	
Rutaceae (continued)					
Ruta graveolens L. (0462-79TS)	ground region	0.2 ± 2.6	6.2 ± 8.9	65.1 ± 3.6	no effect
	stalk	18.2 ± 5.0	25.1 ± 7.6		no effect
Zanthoxylum piperitum (L.) DC. (0100-79TS)	fruit	79.6 ± 1.9			6.7 ± 1.2
	leaf	79.1 ± 1.3			27.9 ± 1.7
Zanthoxylum piperitum (L.) DC. ((f. inerme (Makino) Makino)) (1677-84TS)	fruit skin +seed	48.8 ± 3.6			no effect
Zanthoxylum schinifolium Sieb. et Zucc. (0577-02IS)	seed	58.4 ± 5.5			no effect
Saxifragaceae					
Hydrangea macrophylla (Thunb.) Ser. var. thunbergii (Siebold) Makino (2639-82TS)	leaf	0	10.4 ± 13.4		no effect
	stalk	30.5 ± 4.7			no effect
Schisandraceae					
Kadsura japonica Dunal (0526-79TS)	fruit	33.4 ± 4.4			no effect
	leaf	20.6 ± 6.8			no effect
	stalk	53.8 ± 4.0			no effect
Scrophulariaceae					
Rehmannia glutinosa Liboschitz forma hueichingensis (Chao et Schih) Hsiao (1765-84TS)	rhizome	74.1 ± 15.3			no effect
Rehmannia glutinosa Liboschitz var. purpurea Makino (0521-84TS)	rhizome	92.2 ± 3.7			no effect
Veronicastrum sibiricum (L.) Pennell (0487-79TS)	leaf	0	8.4 ± 7.8	76.4 ± 0.4	no effect
	stalk	42.7 ± 12.9			no effect
	root	53.6 ± 12.0			no effect
Solanaceae					
Atropa belladonna L. (0590-79TS)	leaf	75.6 ± 2.6			15.1 ± 8.2
	stalk	63.8 ± 4.5			no effect
Capsicum annuum L. (1174-02IS)	fruit	104 ± 7.0			no effect
	seed	15.3 ± 18.8	67.9 ± 18.5		no effect
Lycium chinense Mill. (0541-82TS)	fruit	58.5 ± 8.4			no effect
	leaf	35.9 ± 0.5			0.4 ± 0.5
	stalk	52.0 ± 1.2			3.6 ± 2.0
	seed	0	45.4 ± 11.8		no effect
Physalis alkekengi L. var. franchetii (Masters) hort. (0635-79TS)	fruit	66.6 ± 5.0			no effect
	leaf	28.1 ± 0.5			no effect
	stalk	74.0 ± 1.3			no effect
	root	63.8 ± 18.2			no effect
	seed	15.3 ± 11.8	36.2 ± 15.6		no effect
Scopolia lurida Dunal (0113-03TS)	leaf	58.6 ± 2.8			no effect
	stalk	67.4 ± 1.4			no effect
	root	48.8 ± 0.8			no effect
Solanum lyratum Thunb.(0117-03TS)	seed	2.4 ± 9.4	69.0 ± 4.1		no effect
Withania somnifera Dunal (0177-80TS)	seed	74.7 ± 13.4			no effect
Stachyuraceae					
Euscaphis japonica (Thunb.) Kanitz (0612-02IS)	seed	66.6 ± 1.6			0.9 ± 6.3
Stachyurus praecox Sieb. et Zucc. (0610-02IS)	fruit	73.7 ± 1.5			no effect
	seed	82.9 ± 19.0			no effect
Sterculiaceae					
Firmiana simplex W. F. Wight (0338-01TS)	seed	64.2 ± 6.5			no effect
Styracaceae					
Styrax japonica Sieb. et Zucc. (0618-02IS)	seed	7.6 ± 1.1	31.6 ± 5.1		no effect
Taxaceae					
Taxus cuspidata Sieb. et Zucc. (2874-81TS)	leaf	74.0 ± 4.8			no effect
Torreya nucifera (L.) Sieb. et Zucc. (0063-79TS)	seed	106 ± 9.0			no effect
Taxodiaceae					
Cryptomeria japonica D. Don (1202-02IS)	pine corn	60.2 ± 2.5			no effect
	leaf	19.4 ± 1.1	58.8 ± 6.4		no effect
	seed	50.7 ± 15.6			no effect
Theaceae					
Camellia japonica L. var. hortensis Makino (1203-02IS)	seed	67.1 ± 8.9			no effect
Ternstroemia gymnanthera (Wight er Arn.) Beddome (1210-02IS)	fruit	60.7 ± 2.0			no effect

Table. Antagonistic and Agonistic Effects of Plant Extracts on AhR Transformation (continued)

Scientific Name	Part	Antagonistic			Agonistic
		1	0.1	0.025	
Theaceae (continued)					
Thea sinensis L. (0630-20IS)	fruit skin	21.6 ± 1.3			no effect
	seed	90.0 ± 1.4			no effect
	leaf	0	21.9 ± 10.8		no effect
	stalk	20.3 ± 4.4			no effect
Typha latifolia L. (0635-02IS)	fruit	19.4 ± 14.4	48.7 ± 11.4		no effect
Umbelliferae					
Angelica acutiloba Kitagawa (0050-92TS)	leaf	73.1 ± 4.0			no effect
	rhizome	78.6 ± 9.0			no effect
Angelica acutiloba Kitagawa subsp. Iwatensis Kitagawa (0050-92TS)	seed	2.5 ± 2.3	37.8 ± 18.8		11.0 ± 3.7
Angelica acutiloba Kitagawa var. sugiyamae Hikino (0112-00TS)	rhizome	64.7 ± 2.1			no effect
Bupleurum fruticosum L. (1108-82TS)	leaf	60.9 ± 5.2			no effect
	branch	49.1 ± 2.8			no effect
Cnidium officinale Makino (0121-03TS)	rhizome	49.9 ± 2.9			no effect
Foeniculum vulgare Mill. (0430-79TS)	stalk	49.5 ± 1.3			no effect
	seed	80.0 ± 14.4			no effect
Valeriana fauriei Briquet (0496-90TS)	leaf	0	43.4 ± 4.9		no effect
	root	4.6 ± 7.9	54.5 ± 2.8		no effect
Valerianaceae					
Patrinia scabiosaefolia Fisch.(0770-98TS)	flower	104 ± 1.5			no effect
	leaf	75.9 ± 5.0			no effect
	stalk	97.3 ± 3.8			no effect
	root	102 ± 4.8			no effect
	seed	42.8 ± 1.6			no effect
Patrinia villosa (Thunb.) Juss. (1776-84TS)	flower	37.6 ± 1.0			no effect
	leaf	44.5 ± 1.2			no effect
	stalk	44.9 ± 1.8			no effect
	root	27.9 ± 1.2			no effect
	seed	61.7 ± 4.0			no effect
Verbenaceae					
Verbena officinalis L. (0495-79TS)	fruit	57.0 ± 3.2			no effect
Zingiberaceae					
Curcuma aromtica Salisb (0541-02TS)	leaf	69.7 ± 2.6			no effect
	stalk	60.0 ± 1.7			4.1 ± 28.6
	root	74.1 ± 11.7			no effect
Curcuma longa L. (0534-02TS)	rootstock	120 ± 7.0			4.5 ± 1.7
	leaf	87.6 ± 7.0			no effect
	stalk	53.8 ± 12.5			no effect
	root	48.0 ± 2.1			no effect
Curcuma xanthorrhiza Roxb. (0543-02TS)	leaf	95.0 ± 6.5			no effect
	stalk	54.6 ± 3.6			no effect
	root	38.2 ± 6.1			no effect
Curcuma zedoaria Rosc. (0530-02TS)	leaf	97.4 ± 0.7			6.7 ± 0.7
	stalk	35.7 ± 2.1			no effect
	root	49.9 ± 13.0			no effect

transformation to 42, 0, and 33%, respectively. They also showed a dose-dependent suppressive effect against 1 nM TCDD (Figure 2). The value for a concentration causing 50% of inhibition (IC_{50}) was determined by plotting concentrations of each extract against percent (%) of 1 nM TCDD-induced AhR transformation: The IC_{50} values for *Mallotus japonicus* (leaf), *Trichosanthes rostrata* (fruit), and *Trichosanthes rostrata* (fruit skin) were 19, 0.96, and 11 µg dry weight of plant/mL, respectively. These results indicate that these plants contain compounds that have a strong potency to suppress AhR transformation.

3.2 Induction of AhR transformation by plant extracts in the cell-free system

Previously, it has been reported that plant components, such as indigo, indirubin, tryptophan, and indole-3-carbitol, induce AhR transformation (Rannug et al., 1987; Bjeldanes et al., 1991; Adachi et al., 2001; Spink et al., 2003; Guengerich et al., 2004), indicating that certain plants also contain the agonist(s) of AhR. Thus, we investigated whether plant extract itself induces AhR transformation. Among tested extracts, the extract from *Phellodendron amurense* Ruprecht (seed) had the strongest agonistic effect

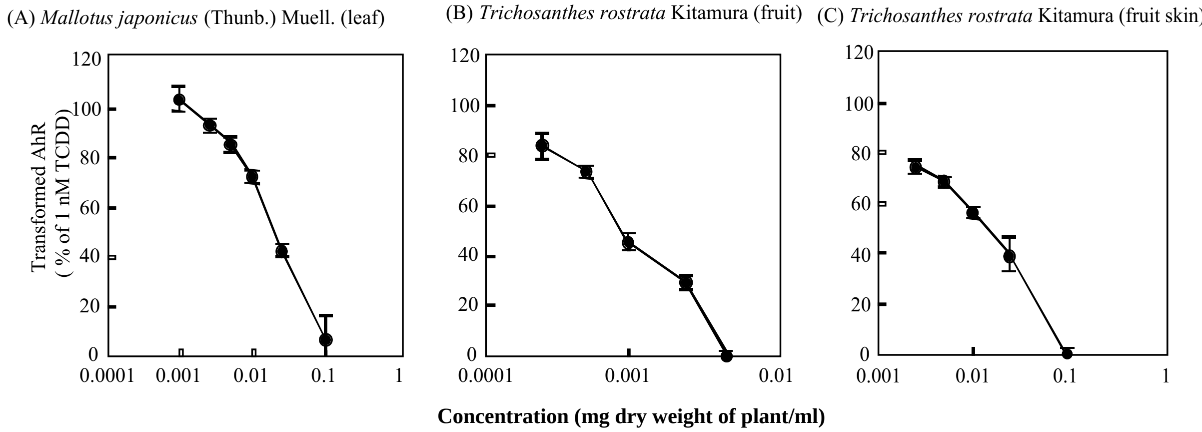

Figure 2. Dose-dependent Antagonistic Effects of Plant Extracts on TCDD-induced AhR Transformation
The rat hepatic cytosolic fraction containing AhR was pre-incubated with indicated concentrations of each extract dissolved in 50% methanol (10 µL/mL): (A) *Mallotus japonicus* (Thunb.) Muell. (leaf), (B) *Trichosanthes rostrata* Kitamura (fruit), and (C) *Trichosanthes rostrata* Kitamura (fruit skin). After 20 min, the cytosolic fraction was treated with 1 nM TCDD or DMSO (10 µL/mL) as a vehicle control and incubated for further 2 hr at 20˚C. AhR transformation was measured by SW-ELISA as described in the Materials and Methods. Data are mean ± SD values shown as the percentages of AhR transformation induced by 1 nM TCDD from independent triplicate experiments

and other 8 of 368 extracts also had a weak effect that is equivalent to more than 20% of 1 nM TCDD-induced transformation (Table). Transformation induced by *Phellodendron amurense* (seed) at 1 mg dry weight of plant/mL was equal to 71% of that by 1 nM TCDD (Table), and a value for a 50% effective concentration (EC_{50}) was 75 µg dry weight of plant/mL (Figure 3). These results indicate that *Phellodendron amurense* (seed) has a strong compound(s) that is able to induce AhR transformation.

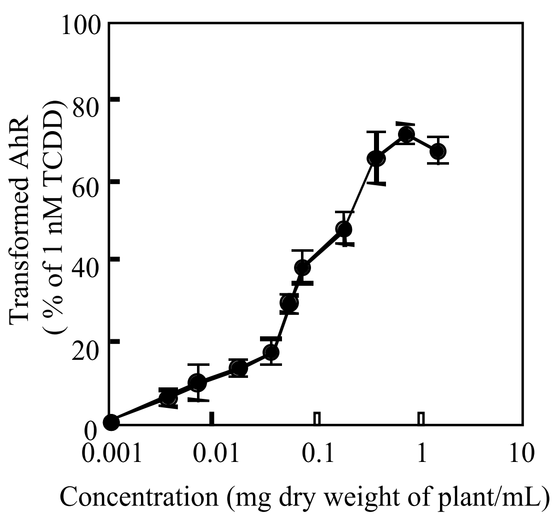

Figure3. Dose-dependent Induction of AhR Transformation by *Phellodendron amurense* Ruprecht Seeds
The rat hepatic cytosolic fraction containing AhR was incubated with indicated concentrations of *Phellodendron amurense* Ruprecht (seed) extract dissolved in 50% methanol (10 µL/mL) for 2 hr at 20˚C. AhR transformation was measured by SW-ELISA as described in Materials and Methods. Data are shown as the percent of 1 nM TCDD-induced transformation, and the values represent as the mean ± SD from the independent triplicate experiments.

Discussion

Since the AhR is at the moment an orphan receptor and its transformation is an initial step to express HAHs and PAHs-induced toxicity including cancer (Fernandez-Salguero et al., 1996; Vorderstrasse et al., 2001), many researchers attempted to find the compounds modulating AhR transformation. Certain polyphenols, indigoids, and indoles were reported to be the agonists and/or antagonists of AhR (Rannug et al., 1987; Bjeldanes et al., 1991; Ashida et al., 2000; Adachi et al., 2001; Amakura et al., 2003a,b; Spink et al., 2003; Guengerich et al., 2004). It was also reported that chlorophylls and dietary fibers were able to interact with dioxins (Morita et al., 1995; 2001). Although these compounds are widely distributed in plant kingdom, plants may also contain the novel compound(s) modulating AhR transformation. This study was carried out to obtain a clue for finding out a novel ligand(s) of AhR from plants. Among tested extracts, about half of extracts decreased 1 nM TCDD-induced AhR transformation to 50% or less at 1 mg dry weight of plant/mL, while some extracts induced AhR transformation *per se*. This indicates that many extracts mainly contain an antagonist(s) but not agonist(s), and certain polyphenols and/or chlorophylls contribute, at least in part, to the antagonistic effect of these extracts.

In this study, *Mallotus japonicus* (Thunb.) Muell. (leaf) and *Trichosanthes rostrata* Kitamura (fruit and fruit skin) showed the strong antagonistic effect. Although it was reported that *Mallotus japonicus* (leaf) contained rutin, unsaturated aliphatic compounds, and tannins (Arisawa, 2003), tannins did not have the antagonistic effect (Amakura et al., 2003b) and rutin had the weak effect (Ashida et al., 2000). Regarding *Trichosanthes rostrata* (fruit or fruit skin), there are no reports identifying its constituents yet. Therefore, isolation and identification of the novel active compound(s) from these plants will be important issues in the future.

In the case of *Phellodendron amurense* Ruprecht (seed), which strongly induced AhR transformation, it was reported that this plant contains phenolcarboxylic acids and limonoids such as limonin, obakunone, limonin 17-β-D-glucopyranoside, and obakunone 17-β-D-glucopyranoside (Miyake et al., 1992). However, phenolcarboxylic acids and limonin did not induce AhR transformation (Amakura et al., 2003a). Therefore, *trans*-stilbenes are the candidate for compounds inducing AhR transformation, because they exist widely in many seeds, and certain *trans*-stilbenes are reported to induce AhR transformation (Kato et al., 2002). For example, *trans*-3,4',5-trihydroxystilbene (resveratrol), which is abundantly contained in the seed and the fruit skin of grapes, induces AhR transformation *per se* (Casper et al., 1999; Amakura et al., 2003a), although it suppresses TCDD-induced AhR transformation (Lee and safe, 2001). *Phellodendron amurense* (seed) will also contain a strong agonist(s) such as a derivative of *trans*-stilbene, but it will not show the dioxin-like actions because this plant is used as an herbal medicine (Li et al., 2003). Indigo and indirubin in *Polygonum tinctorium* induce AhR transformation (Adachi et al., 2001; Spink et al., 2003; Guengerich et al., 2004), but they are also ingredients of the herbal medicine, 'Dang gui Long hui wang'. Thus, the identification of active compound(s) in *Phellodendron amurense* Ruprecht (seed) and investigation of its molecular mechanism is also needed in the future.

In conclusion, we showed basic data on search for the novel ligands of AhR. Many plants contain the compounds modulating, especially suppressing, AhR transformation, and identification of the active compounds from *Mallotus japonicus* (leaf), *Trichosanthes rostrata* (fruit and fruit skin), and *Phellodendron amurense* (seed) is an important issue in the future to give novel information on the ligands of AhR.

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