RESEARCH COMMUNICATION

Suppressive Properties of Extracts from Japanese Edible Plants regarding Nitric Oxide Generation

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Abstract

Acetone extracts from a total of 30 species (197 samples) of plants commonly eaten in Japan were tested for their *in vitro* inhibitory properties against nitric oxide (NO) generation in a murine macrophage cell line, RAW 264.7, that had been stimulated with lipopolysaccharide in combination with interferon-γ. Evaluation of the effects of treatment with 100 mg/mL revealed that 6 extracts (3.1%) exerted a strong inhibitory effect (inhibition rate (IR) ≥70%) with strong cell viability (CV≥70%). However, nine extracts that exhibited an IR of greater than 70% were not considered to exert a significant effect at 100 μg/mL due to their low CV (<70%). Of the 14 plant families evaluated, *Cucurbitaceae* (extracts of watermelon 1 and melon 2), *Liliaceae* (extracts of garlic 1 and 2) and *Solanaceae* (extracts of tomato 3 and eggplant 5) were shown to be promising candidates for the inhibition of NO generation at the tested concentration. When tested at 20 μg/mL, 6 extracts, one of garland-chrysamthemums (sample 5), one of lettuce (sample 2), one of tomatoes (sample 3), two of Japanese hornworts (Mitsuba 1 and 2), and one of carrots (sample 4) showed strong inhibition of NO generation (IR≥70%). Even though one of the test samples (sample 2) of Japanese hornwort had a CV of less than 70% (67.8%), Japanese hornwort was still considered to be a highly promising species for the inhibition of NO generation. Furthermore, the activity varied significantly among samples from the same species for several plants. This variation may have been due to differences between cultivars and/or growing districts, or to differences in post-harvesting treatment. Taken together, the results of the present study may provide an experimental basis for new strategies for the production of highly functional dietary plants and food items.

Key Words: Chemoprevention - dietary plants - life style-related disease - nitric oxide generation

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Introduction

Chemoprevention is regarded as an effective strategy for the reduction of cancer risk (Wattenberg, 1985; Tanaka, 1992), and several epidemiological reports have suggested that frequent consumption of fruits and vegetables resulted in a decreased cancer incidence (Shibata et al., 1992). This may be due to the presence of chemical factors such as antioxidative components (Weisburger, 1991) in fruits and vegetables. As a result of these findings, a variety of antioxidative food phytochemicals have been evaluated for their chemopreventative effects (Bertram et al,, 1987). Nitric oxide (NO) is a gaseous free radical that is produced in biological systems by inducible and constitutive NO synthases (iNOS and cNOS including eNOS and nNOS) (Vanvaskas and Schmidt, 1997). cNOS is essential to the maintenance of normal blood pressure, while iNOS contributes to the activity of phagocytes. iNOS is induced in various human cells and tissues upon stimulation with lipopolysaccharide (LPS) and/or interferon-γ (INF-γ) (Alderton et al., 2001; Bogdan, 2001). However, excess production of NO by iNOS has recently been found to be associated with life-style related diseases, including cancer (Xie et al., 1997). NO reacts rapidly with superoxide anion radical, which is concurrently generated by leukocytes such as macrophages and neutrophils to produce highly toxic peroxynitrite (ONOO-) (Ischiropoulos et al., 1992; Xia and Zweier, 1997). In the presence of oxygen, NO is also converted to the strong nitrosating trioxide (N2O3), which then forms carcinogenic N-nitrosoamines (Ohshima and Bartsch, 1999). Reactive nitrogen species (RNS) such as NO and ONOO- damage DNA, induce mutations and take part in several carcinogenic processes via activation of oncogene products or inactivation of tumor-suppressor proteins (Arroyo et al., 1992; Gal and Wogan, 1996).

To date, we have isolated and identified several cancer preventive candidates using an inhibition test against tumor promoter-induced Epstein-Barr virus activation (Ohigashi et al., 1997; Murakami et al., 1999a; 1999b). Most of these candidates have inhibited NO generation in both LPS- and IFN-γ– stimulated RAW 264.7 cells

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(Kim et al., 2000; Murakami et al., 2000a; Murakami et al., 2002; Ohata et al., 1998). To further explore potential cancer preventive food phytochemicals, screening tests of the extracts of dietary plants from Asian countries for their inhibitory properties toward NO generation have been conducted using a RAW 264.7 cell-system (Kim et al., 1998; Murakami et al., 2000b; Jiwajinda et al., 2002). However, these screening tests did not consider activity-variations due to differences in growing districts and conditions, post harvest treatments, or cultivars. Therefore, in this study, we screened 197 samples from 30 plant species that were produced using different cultivation techniques and post-harvest treatments to evaluate the importance of such variations in the ability of the plants to inhibit NO generation.

Materials and Methods

Chemicals and cells

L-Arginine and LPS were purchased from Sigma Inc. (St. Louis, MO, USA) and Difco Labs (Detroit, MI, USA), respectively. Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS) and IFN-γ were purchased from Gibco BRL (Grand Island, NY, USA). The rest of the raw chemicals used in this study were purchased from Waco Pure Chemicals Co. Ltd (Osaka, Japan). The murine machrophage cell line, RAW 264.7, was kindly donated by Ohtsuka Pharmaceutical Co. Ltd. (Ohtsu, Japan).

Sample preparation

Fruits and vegetables produced using various cultivation conditions (soil-, greenhouse-, and hydroponics-cultivation) and post-harvest treatments (fresh, boiled, cooked in a microwave oven, and nitrogen-sealing) were obtained from different districts in January, 2002 (Table). Each sample was then cut into small pieces and frozen -80°C, after which it was extracted with 10 times the volume of acetone at room temperature for 1 week. After drying in vacuo, the extract was dissolved in dimethylsulfoxide (DMSO) solution to give a final concentration of 20 or 4 mg/mL. The extracts were then evaluated in triplicate assays as described below.

LPS/IFN-γ-induced NO generation test

Murine macrophage RAW 264.7 cells were cultivated in DMEM medium containing L-glutamine supplemented with 10% heat-inactivated (55˚C, 30 min) FBS, 200 U/mL penicillin, and 250 mg/mL streptomycin at 37˚C in a humidified atmosphere of 5% CO_2 (Tayeh and Marletta, 1989). The cells were then suspended in DMEM medium at a density of $2x10^5$ cells/mL, after which they were treated with LPS (100 ng/mL), IFN-γ (100 U/mL), L-arginine (2 mM) and 100 or 20 mg/mL of each test extract. After 24 h, the level of nitrite (NO_2) in each test was measured to determine the total NO generation-inhibitory rate (IR) relative to the NO generation in a control experiment in which no test compound was used, as described below. Cells plus or minus plant-extract treated with and without LPS/IFN-γ were used for the non-treated control, stimulated control, and test sample, respectively.

Cell viability

Mitochondrial respiration, an indicator of cell viability, was determined by a mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Briefly, treated cells ($2x10^5$ cells/mL) were incubated with MTT (0.25 mg/mL) in 24-well plates for 4 h, after which they were solubilized in 0.04 N HCl in iso-propanol. The extent of the reduction of MTT within the cells was then quantified by measurement of the absorbance at 570 nm (Sladowski et al., 1992).

Measurement of NO2- production

For determination of the quantity of NO generated, the amount of NO_2 in the supernatant of the media was measured by the Griess method, as previously described (Greenwald et al., 1997). Briefly, murine macrophage cells were incubated for 24 h, after which the cell culture medium (0.5 mL) were added to 0.5 mL of an aqueous solution containing the Griess reagents (1% sulfanilamide, 0.1% naphthylethylene diamine dihydrochloride in 5% H3PO4). The NO2- production was then determined based on the absorbance at 543 nm.

Measurement of L-citrulline

The L-citrulline level in the medium was determined colorimetrically based on the reaction of the supernatant of the medium with diacetyl monoxime using a previously described method (Boyde and Rahmatullah, 1980). Briefly, 0.6 mL of a chromogenic reagent (5 mg of thiosemicarbazide in reagent 1 and 2, which are described below) was added to 0.4 mL of the cell culture medium, after which the reaction mixture was heated at 100˚C for 5 min. Next, the visible absorbance at 530 nm was measured. Reagent 1 was composed of 550 mL of distilled water, 250 mL of concentrated sulfuric acid, and 200 mL of concentrated phosphoric acid. After the mixture was cooled to room temperature, $FeCl_3$ (250 mg) was added. Reagent 2 was composed of 100 mL of distilled water containing 500 mg of diacetyl monoxime.

Inhibitory rate (IR) and statistical analysis

Each experiment was performed three times, and the inhibitory rates against NO generation are expressed as the mean±standard deviation (mean±SD). Samples were divided into different groups based on their cultivation method (cultivated in soil, hydroponics or greenhouse) and their post-harvesting treatments (fresh, boiled, or cooked in a microwave oven). Differences among sample groups were then assessed by a Student's t-test (two sided) that assumed unequal variance. The IR was assumed to indicate the total inhibition of NO generation, which includes the inhibition of both iNOS function (iNOS induction-inhibition and/or iNOS enzyme-inhibition) and NO scavenging. In the screening study, the total inhibitory rate (IR) was calculated using the following equation.

IR (%) = {1 - [(test sample absorbance with LPS/IFN-γ plus plant-extract) - (control absorbance without LPS/IFN-γ)]x[positive control absorbance with LPS/IFN-γ – control absorbance without LPS/IFN-γ]-1}_100.

Results

A total of 30 species (197 test samples) of dietary plants and fruits from 14 plant families commonly eaten in Japan were extracted with acetone at room temperature and then tested for their ability to inhibit the generation of NO. Thesamples included 38 extracts of tomato, 34 of spinach, 17 of onion, 11 of welsh onion, 11 of carrot and 86 of other materials. These samples varied based on their growing district, cultivation conditions (soil, hydroponic or greenhouse), post-harvesting treatment (fresh, boiled or cooked with a microwave oven) and the plant part extracted. The screening test was conducted at final concentrations of 100 and 20 μg/mL in triplicate. Throughout, we evaluated the total inhibitory rates (IRs) of extracts that also resulted in a CV of at least 70%. In addition, the IR of NO generation determined based on the amount of NO_2 includes 1) suppression of iNOS induction, 2) iNOS enzyme inhibition and 3) scavenging of NO produced (Kim et al., 1998). Inhibition of NO generation resulting from suppression of the iNOS enzyme was determined by monitoring the level of L-citrulline produced from L-arginine in response to the action of iNOS. Inhibition of iNOS function is known to occur via two pathways: (1) inhibition of signal transduction for the iNOS gene expression (protein kinase C, tyrosine kinase, mitogen-activated protein kinase, activating protein-1, nuclear factor-κB, etc.) (Lowenstein et al., 1993; Spink et al., 1995); (2) direct inhibition of iNOS enzyme activity. iNOS inhibitory activity resulting from these two pathways was measured by monitoring the level of L-citrulline selectively produced from L-arginine in response to the action of iNOS (Szabo et al., 1994). Conversely, NO scavenging activity (%) may be estimated based on the difference in the rate of total NO inhibition and inhibition of iNOS function. Subtraction of the inhibition due to the total IR from the inhibition of the iNOS function can be assumed to be the NO scavenging activity. In addition, we tentatively classified the IRs into four ranks: +++ (strongly active, IR≥70%); ++ (moderately active, 70%>IR≥50%); + , weakly active (50%>IR≥30%); and -, inactive (IR≥30%).

The results of inhibitory activity against NO generation are summarized in the Table. When tested at a

Table 1. Inhibition of NO Generation by Common Japanese Edible Plants

Sample names	N	Part tested	Growing district	Cultivation method	Cooking method	Total inhibition (IR %) 100	Total inhibition (IR %) 20	iNOS inhibition (%) 100	iNOS inhibition (%) 20	Cell viability (%) 100	Cell viability (%) 20
Araliaceae/											
Yama-Udo	1	R	NT	Soil	Fresh	77.8±0.6	69.5±2.1	77.1±0.3	56.5±2.1	20.5±1.7	60.2±7.3
Aralia chordata Thunb.											
Chenopodiaceae/											
Spinach	1	W	Chiba	Hydroponics	Fresh	30.1±1.4	34.1±3.0	20.5±1.2	29.8±1.4	36.9±1.5	100 ±0.0
Spinacia	2	W	Ibaragi	Hydroponics	Fresh	38.8±4.0	23.9±3.8	29.0±2.9	22.7±3.9	67.2± 11	100 ±0.0
oleracea L.	3	W	Ibaragi	Hydroponics	Fresh	32.2±3.0	27.9±4.1	24.8±3.5	30.6±1.4	50.7±4.1	99.6±1.6
	4	W	Gunma	Soil	Fresh	35.2±2.9	27.2±2.6	28.3±1.1	24.9±2.8	35.1±4.8	87.3 ±19
	5	W	Gunma	Soil	Fresh	45.8±2.8	14.6±5.5	39.1±3.6	19.6±4.6	56.1± 11	98.7±1.7
	6	W	Saitama	Soil	Fresh	38.6±0.9	14.9±3.0	30.8±1.5	22.7±1.5	52.3± 19	100 ±0.0
	7	W	NT	Greenhouse	Fresh	80.5±1.7	61.5±4.1	80.1±1.4	48.0±3.9	15.0±6.6	75.5±7.2
	8	W	NT	Greenhouse	Boiled	63.0±1.6	45.5±2.9	67.4±1.6	31.8±3.3	100 ±0.0	100 ±0.0
	9	W	NT	Green	MW	66.3±3.6	49.3±2.3	70.4±1.9	33.8±7.3	100 ±0.0	100 ±0.0
	10	W	NT	Greenhouse	Boiled	66.4±2.2	33.9±6.4	65.0±7.8	20.2±4.0	100 ±0.0	100 ±0.0
	11	W	NT	Greenhouse	MW	53.9±1.8	62.1±0.5	58.8±1.5	58.9±1.6	100 ±0.0	100 ±0.0
	12	W	NT	Greenhouse	Fresh	81.0±0.9	64.9±1.7	77.8±1.2	60.1±0.5	21.8±4.1	81.4±5.1
	13	W	NT	Greenhouse	Boiled	48.2±2.7	63.0±0.8	53.3±1.2	62.1±0.8	100 ±0.0	100 ±0.0
	14	W	NT	Greenhouse	MW	60.6±9.2	63.5±1.6	66.1±4.8	60.9±0.6	100 ±0.0	100 ±0.0
	15	W	NT	Greenhouse	Fresh	75.9±0.8	61.8±2.2	81.4±3.2	59.6±1.6	42.6± 15	100 ±0.0
	16	W	NT	Greenhouse	Boiled	40.7±3.4	51.5±5.4	50.0±2.0	55.8±2.9	95.4±6.8	92.415.2
	17	W	NT	Soil	Fresh	39.1±2.9	54.6±2.7	36.2±1.6	49.7±0.7	23.6±3.7	82.6±8.2
	18	W	NT	Soil	Boiled	15.5±3.3	39.6±1.4	21.5±3.9	43.7±1.9	65.7± 15	93.9±6.2
	19	W	NT	Soil	Fresh	42.7±3.6	44.5±3.4	41.5±1.9	46.3±1.2	30.2±3.3	82.0±7.3
	20	W	NT	Soil	Boiled	19.0±2.1	36.1±7.5	21.6±5.0	40.2±4.2	70.6±7.9	100 ±0.0
	21	W	NT	Soil	Fresh	31.6±3.7	52.5±2.0	31.9±1.5	50.9±1.7	55.9± 16	78.1±8.7
	22	W	NT	Soil	Boiled	16.5±3.4	41.4±2.1	20.4±7.4	43.5±0.7	76.7± 12	100 ±0.0
	23	W	NT	Soil	Fresh	50.8±0.4	48.0±0.9	41.6±1.2	49.6±3.8	29.8±1.7	100 ±0.0
	24	W	NT	Soil	Boiled	51.4±0.4	36.6±11.	42.9±1.8	45.0±6.3	34.6±3.1	100 ±0.0
	25	W	Chiba	Soil	Fresh	56.6±0.3	63.5±1.0	49.5±0.9	43.9±3.6	100 ±0.0	100 ±0.0
	26	W	Chiba	Soil	Boiled	45.7±1.0	63.1±1.1	46.5±0.6	46.8±1.2	83.9±6.5	100 ±0.0
	27	W	Iwate	Soil	Fresh	39.4±0.7	24.9±8.6	33.6±1.3	30.4±9.0	42.2±4.7	100 ±0.0
	28	W	Iwate	Soil	Fresh	24.0±2.3	26.1±3.6	18.0±2.9	30.4±3.8	47.2±8.3	100 ±0.0
	29	W	Iwate	Soil	Fresh	28.2±1.3	42.2±7.0	22.9±2.0	45.2±8.8	53.2±1.2	100 ±0.0
	30	W	Iwate	Soil	Fresh	20.5±1.3	34.5±3.8	15.4±1.2	34.1±7.0	74.5±6.3	100 ±0.0
	31	W	Fukuoka	Soil	Fresh	68.6±1.0	11.0±7.0	67.3±0.3	13.7±5.1	72.4±5.7	100 ±0.0
	32	W	Fukuoka	Soil	Boiled	45.3±1.7	4.8±10.6	48.5±0.9	5.9± 14	99.7±4.2	100 ±0.0
	33	W	Fukuoka	Greenhouse	Fresh	54.2±1.3	4.9 ±2.0	57.2±0.5	-0.1±3.4	100 ±0.0	100 ±0.0
	34	W	Fukuoka	Greenhouse	Boiled	48.1±2.3	5.3 ±4.8	52.0±2.4	6.0±4.5	90.5±4.5	100 ±0.0

Table 1 continued. Inhibition of NO Generation by Common Japanese Edible Plants

Sample names	N	Part tested	Growing district	Cultivation method	Cooking method	Total inhibition (IR %) 100	Total inhibition (IR %) 20	iNOS inhibition (%) 100	iNOS inhibition (%) 20	Cell viability (%) 100	Cell viability (%) 20
Compositae/											
Burdock root	1	R	Aomori	Soil	Fresh	47.3±7.4	14.9±2.0	62.2±2.2	15.5±3.3	100 ±0.0	100 ±0.0
Arctium	2	R	Miyazaki	Soil	Fresh	70.1±4.8	61.5±2.6	67.3±6.6	55.9±2.6	63.9±2.8	100 ±0.0
lappa L.	3	R	Chiba	Soil	Fresh	43.9±6.5	25.8±3.9	46.7±1.5	28.3±3.8	100± 0.0	100 ±0.0
Garland-	1	W	Hyogo	Hydroponics	Fresh	56.1±2.1	45.5±8.1	57.6±3.6	46.3±8.5	88.1±8.4	100 ±0.0
chrysanth	2	W	Mie	Hydroponics	Fresh	52.5±4.3	69.3±4.8	41.2±1.1	67.5±4.1	24.6±3.1	99.6±6.0
Chrysanthe-	3	W	Miyagi	Soil	Fresh	34.7± 12	61.5±4.8	39.4±2.5	58.0±4.4	29.4±2.0	100 ±0.0
mum	4	W	Chiba	Soil	Fresh	32.6± 15	56.3±5.0	28.6±2.0	54.2±3.4	87.4± 11	100 ±0.0
coronarium L.	5	W	Chiba	Soil	Fresh	65.8± 13	75.0±0.4	70.1±2.4	67.2±0.4	31.3±0.7	56.8±5.8
Lettuce	1	W	Chiba	Soil	Fresh	33.7±8.3	69.1±0.9	31.8±7.1	71.1±0.7	26.0±1.9	66.8±1.8
Lactuca	2	W	Saitama	Soil	Fresh	59.7±1.2	40.3±0.6	55.3±0.4	42.5±0.6	26.3±1.3	100 ±0.0
sativa L.	3	W	Sizuoka	Soil	Fresh	61.5±0.6	26.6±0.9	60.9±0.6	29.2±0.8	42.1±9.6	100 ±0.0
Head lettuce	1	W	Fukuoka	Hydroponics	Fresh	63.5±0.3	46.4±1.6	66.5±2.4	73.4±1.3	100 ±0.0	100 ±0.0
L S var.	2	W	Fukuoka	Hydroponics	Fresh	65.4±0.4	71.8±0.7	64.6±0.5	12.3±7.4	100 ±0.0	70.4±0.9
capitata L.	3	W	Chiba	Hydroponics	Fresh	65.4±0.5	68.5±2.7	63.3±0.6	38.3±0.2	100 ±0.0	84.7±3.4
Convolvulaceae/											
Sweet potato	1	R	Tokushima	Soil	Fresh	17.5±2.2	6.6 ± 1.5	22.6±2.7	16.7±1.3	95.8±1.2	100 ±0.0
Ipomoea	2	R	Ibaragi	Soil	Fresh	32.0±2.4	3.0 ± 8.3	35.0±2.2	14.9±9.0	99.0±1.7	100 ±0.0
batatas L.	3	R	Chiba	Soil	Fresh	35.2±3.2	3.6±11.7	33.4±3.5	11.2±8.9	100 ±0.0	100 ±0.0
Cruciferae/											
Field mustard	1	W	Ibaragi	Soil	Fresh	65.1±0.7	54.1±0.5	64.6±0.5	59.2±0.5	63.4±4.3	93.8±1.8
Brassica	2	W	Ibaragi	Soil	Fresh	66.8±0.2	50.7±1.8	67.1±0.6	54.1±1.4	100±0.0	91.8±1.5
pekinensis L	3	W	NT	Soil	Fresh	68.5±0.5	17.3±1.5	68.0±0.8	16.0±0.9	77.6±3.1	94.6±0.5
Komatsuna	1	W	Mie	Hydroponics	Fresh	61.5±1.3	12.4±0.7	58.0±1.4	17.2±1.2	100±0.0	100 ±0.0
Brassica	2	W	Saitama	Soil	Fresh	58.6±1.9	30.2±3.9	61.6±0.7	33.0±4.4	55.2±3.1	100 ±0.0
campestris	3	W	Tokyo	Soil	Fresh	61.6±6.0	32.8±0.7	56.3±2.0	34.4±5.4	75.8±4.6	100 ±0.0
var. *perviridis*	4	W	Tokyo	Soil	Fresh	40.5±6.7	56.6±5.1	31.9±1.6	58.2±4.9	21.3±1.5	83.8±15
	5	W	Fukuoka	Soil	Fresh	59.1±2.3	36.7± 11	60.3±1.6	38.3±8.7	100±0.0	91.6±7.7
	6	W	Fukuoka	Soil	Boiled	38.0±2.4	30.6±3.7	42.4±2.0	33.1±3.4	100±0.0	89.6±3.5
Chinese	1	W	Ibaragi	Soil	Fresh	29.8±2.6	40.5±2.2	41.5±0.7	41.0±1.5	55.2±6.0	100 ±0.0
mustard	2	W	Ibaragi	Soil	Fresh	49.6±3.1	27.3±5.0	44.3±1.7	29.3±6.3	58.7±10.1	100 ±0.0
B. chinensis L	3	W	Ibaragi	Soil	Fresh	66.3±5.5	31.3±3.6	58.8±3.3	37.3±5.1	36.2±11.0	93.6±4.2
Broccoli	1	W	Gunma	Soil	Fresh	64.5±0.3	48.4±5.1	61.9±7.8	48.7±2.3	37.0±4.2	100 ±0.0
B. oleracea	2	W	Aichi	Soil	Fresh	40.3±2.2	67.0±0.7	30.8±1.4	70.0±0.8	37.0±4.5	62.1±0.6
var. *botrytis* L	3	W	Saitama	Soil	Fresh	54.5±3.4	60.2±0.7	44.8±6.9	65.1±0.2	27.0±3.2	84.7±5.8
-sprout	1	SP	NT	Hydroponics	Fresh	75.2±0.6	43.4± 10	74.2±0.2	45.4±5.7	60.9±2.7	77.8±3.7
Cabbage	1	W	Kanagawa	Soil	Fresh	68.5±0.5	-37.1±4.3	68.1±0.5	-0.1±1.4	60.1±6.8	100 ±0.0
B. oleracea	2	W	Aichi	Soil	Fresh	67.5±0.6	6.3±5.8	65.9±0.3	6.1±2.4	35.4±3.6	100 ±0.0
var.	3	W	Chiba	Soil	Fresh	65.6±0.9	8.4±12.5	60.0±1.8	7.1±8.9	32.7±7.3	100 ±0.0
capitata L.	4	W	Iwate	Soil	Fresh	50.6±0.6	10.1±1.4	56.0±0.8	8.6±4.5	100 ±0.0	97.5±6.0
	5	W	Iwate	Soil	Boiled	44.8±0.5	11.4±5.6	47.3±0.8	19.2±1.8	100 ±0.0	100 ±0.0
	6	W	Iwate	Soil	Nitrogen-sealing	48.7±1.8	9.4±1.8	50.5±1.6	8.6±3.1	100 ±0.0	100 ±0.0
	7	W	Iwate	Soil	Fresh	50.1±6.4	3.9±3.2	54.3±6.1	3.6±3.1	100 ±0.0	100 ±0.0
	8	W	Iwate	Soil	Boiled	39.6±5.6	21.1±4.7	44.4±6.0	25.7±1.5	100 ±0.0	100 ±0.0
	9	W	Iwate	Soil	Nitrogen-sealing	42.6± 14	14.6±4.9	49.5±2.4	13.8±2.1	100 ±0.0	100 ±0.0
Radish	1	R	Kanagawa	Soil	Fresh	5.0±0.2	6.6±3.2	26.0±0.6	14.1±4.1	100 ±0.0	100 ±0.0
Raphanus	2	R	Tokusima	Soil	Fresh	20.0±1.3	0.1±0.6	17.8±1.1	5.6±2.4	100 ±0.0	97.2±6.3
sativus L.	3	R	Chiba	Soil	Fresh	1.5±0.2	1.0±2.7	16.2±2.5	2.1±3.9	100 ±0.0	100 ±0.0
	4	L	NT	Soil	Fresh	39.9±0.3	34.1±0.3	43.7±0.3	42.0±8.0	100 ±0.0	100 ±0.0
	5	L	NT	Soil	Boiled	7.3±1.5	19.0±4.0	43.0±2.0	24.0±2.0	100 ±0.0	100 ±0.0
	6	R	NT	Soil	Fresh	59.3±5.0	-2.7±7.9	20.1±1.2	2.2±4.0	100 ±0.0	100 ±0.0
	7	R	NT	Soil	Boiled	29.1±0.8	-3.0±7.9	39.1±1.2	-6.5±4.0	100 ±0.0	100 ±0.0
Cucurbitaceae/											
Watermelon	1	F	Yamagata	Soil	Fresh	75.5±4.3	8.1±4.4	79.3±2.3	3.7±6.5	100 ±0.0	100 ±0.0
Citrullus	2	F	Chiba	Soil	Fresh	5.3±4.1	7.2±1.2	10.3±4.1	2.3±0.3	100 ±0.0	100 ±0.0
lanatus	3	F	NT	Soil	Fresh	15.2±0.2	7.2±1.2	21.2±0.6	0.6±3.9	100 ±0.0	100 ±0.0
Melon	1	F	Hokkaido	Soil	Fresh	28.8±3.1	2.1±3.2	38.9±2.2	-6.5±2.1	100 ±0.0	100 ±0.0
Cucumis	2	F	Chiba	Soil	Fresh	73.2±3.1	39.4±3.2	75.6±2.7	36.9±3.9	100 ±0.0	100 ±0.0
melo L.	3	F	Kumamoto	Soil	Fresh	51.1±3.9	13.8±2.5	57.6±4.3	16.3±3.3	100 ±0.0	100 ±0.0
Ebenaceae/											
Persimon	1	F	Ehime	Soil	Fresh	4.5±1.1	-4.6±2.1	9.4±5.2	-3.3±1.5	100|±0.0	100 ±0.0
Diospyros L.	2	F	Fukuoka	Soil	Fresh	4.5±0.7	-4.8±2.4	15.5±5.1	-7.2±4.8	100 ±0.0	100 ±0.0
	3	F	Wakayama	Soil	Fresh	15.1±0.1	0.9±2.0	16.0±0.4	-9.9±4.5	100 ±0.0	98.3±4.3

Table 1 continued. Inhibition of NO Generation by Common Japanese Edible Plants

Sample names	N	Part tested	Growing district	Cultivation method	Cooking method	Total inhibition (IR %) 100	Total inhibition (IR %) 20	iNOS inhibition (%) 100	iNOS inhibition (%) 20	Cell viability (%) 100	Cell viability (%) 20
Chestnut	1	F	Chiba	Soil	Fresh	28.2±2.2	3.2±10.3	34.7±0.6	-2.1±6.6	100±0.0	84.6±3.4
Castanea crenata											
Leguminosae/											
Mungbean	1	Sp	Yamanashi	Soil	Fresh	45.6±0.7	5.3±4.5	49.3±2.7	1.9±2.5	100±0.0	100±1.5
Vigna mungo	2	Sp	Tochigi	Soil	Fresh	49.8±0.7	7.8±2.2	49.7±1.8	7.1±0.8	100±0.0	99.3±0.7
	3	Sp	Fukushima	Soil	Fresh	38.3±2.9	8.2±2.7	42.9±2.31	6.3±3.4	100±0.0	98.0±3.0
Liliaceae/											
Onion	1	R	Hyogo	Soil	Fresh	63.5±6.7	37.2±5.3	70.0±2.4	40.9±5.1	100±0.0	100±0.0
Allium cepa L.	2	R	Hokkaido	Soil	Fresh	63.4±6.7	48.0±2.5	69.2±1.1	50.0±2.8	100±0.0	100±0.0
	3	R	Hiroshima	Soil	Fresh	38.7±4.6	21.7±2.6	30.3±2.2	16.2±1.4	100±0.0	100±0.0
	4	R	Hiroshima	Soil	Fresh	51.1±4.1	22.9±1.1	33.0±3.8	18.5±3.1	100±0.0	96.8±12.4
	5	R	Hiroshima	Soil	Fresh	59.8±4.1	7.8±0.3	35.1±1.9	3.4±0.6	100±0.0	100±0.0
	6	R	Hiroshima	Soil	Boiled	65.0±3.5	20.1±1.8	27.2±1.5	19.8±3.1	96.7±7.1	87.0±3.6
	7	R	Hiroshima	Soil	Boiled	62.2±2.4	36.2±0.9	27.0±3.4	38.5±1.3	100±0.0	100±0.0
	8	R	Hiroshima	Soil	Boiled	67.4±0.2	30.1±1.9	25.6±1.7	35.1±4.3	100±0.0	92.3±11.7
	9	R	Hyogo	Soil	Fresh	18.1±6.1	22.3±10.3	22.5±6.8	24.2±10.7	100±0.0	100±0.0
	10	R	Hyogo	Soil	Boiled	2.2±1.8	10.6±3.5	7.6±3.1	11.1±3.4	100±0.0	100±0.0
	11	R	Hokkaido	Soil	Fresh	14.3±9.4	5.8±6.4	20.3±6.7	7.8±6.9	100±0.0	100±0.0
	12	R	Hokkaido	Soil	Boiled	32.8± 15	19.9±3.3	31.2±3.5	15.5±3.3	100±0.0	100±0.0
	13	R	Saga	Soil	Fresh	36.2±7.2	9.1±2.2	46.0±2.8	6.0±1.8	90.4±1.8	100±0.0
	14	R	Saga	Soil	Boiled	23.5± 10	13.8±8.5	25.3±4.2	10.5±6.9	100±0.0	98.8±6.5
	15	R	USA	Soil	Fresh	43.6±2.1	34.8±1.5	68.5±1.0	23.9±19.9	100±0.0	81.9±24.5
	16	R	USA	Soil	Fresh	25.9± 13	15.5±5.7	40.9±8.1	12.5±5.1	100±0.0	100±0.0
	17	R	USA	Soil	Boiled	29.1± 11	12.5±6.5	35.2±10.2	9.7±5.4	100±0.0	99.1±3.0
Welsh onion	1	W	Chiba	Soil	Fresh	55.5±6.6	26.7±8.7	67.1±0.8	27.7±8.3	100±0.0	100±0.0
Allium	2	W	Chiba	Soil	Fresh	68.5±1.5	54.0±1.3	55.6±4.7	506±3.1	31.8±0.1	89.3±5.3
fistulosum	3	W	Tochigi	Soil	Fresh	54.7±9.1	65.8±2.2	52.7±0.1	62.4±2.0	35.3±1.0	96.1±7.5
	4	W	NT	Soil	Fresh	10.2±3.4	-1.8±2.7	14.7±3.8	-2.5±2.7	100±0.0	100±0.0
	5	W	NT	Soil	Fresh	59.9±1.3	43.2±4.5	65.1±1.6	42.4±2.0	100±0.0	85.8±8.4
	6	W	NT	Soil	Fresh	53.0±4.4	16.2±2.8	61.6±1.7	12.8±2.1	100±0.0	100±0.0
	7	W	NT	Soil	Boiled	66.1±3.7	40.7±3.3	70.6±2.1	36.7±3.5	100±0.0	96.7±1.5
	8	W	Fukuoka	Soil	Fresh	38.2±1.0	35.5±9.4	29.6±2.5	28.2±8.3	73.0±8.5	90.1±3.4
	9	W	Fukuoka	Soil	Boiled	35.6±1.6	31.0±9.1	25.9±1.1	25.2±12.2	71.9±13.2	96.7±2.4
	10	W	Fukuoka	Hydroponics	Fresh	45.0±2.9	31.6±3.1	35.6±2.9	27.9±3.5	86.9±5.3	88.9±4.4
	11	W	Fukuoka	Hydroponics	Boiled	22.9±0.7	22.4±3.5	17.3±2.6	18.0±3.5	78.4±2.2	99.3±0.6
Leek	1	W	Tochigi	Soil	Fresh	30.7±1.6	52.2±0.7	19.6±5.6	50.2±1.7	31.8±3.2	81.9±3.4
A. fistulosum	2	W	Tochigi	Soil	Fresh	32.3±2.7	49.0±2.3	22.0±2.2	47.7±2.5	30.6±3.0	95.8±2.0
Rottl.	3	W	Tochigi	Soil	Fresh	23.6±0.8	44.9±3.3	14.7±2.1	45.0±1.2	39.0±2.8	100±0.0
Garlic	1	R	Aomori	Soil	Fresh	72.5±0.2	29.2±4.3	44.7±1.1	28.3±4.2	70.6±9.0	100±0.0
Allium	2	R	Aomori	Soil	Fresh	70.8±0.2	24.5±4.5	48.8±7.6	26.0±3.1	70.8±8.4	100±0.0
sativum L.	3	R	China	Soil	Fresh	70.9±0.4	45.7±3.6	36.9±7.5	42.6±4.3	32.2±2.1	100±0.0
Rosaceae/											
Apple	1	F	Aomori	Soil	Fresh	5.7±0.2	-2.9±2.5	1.5±0.2	0.5±4.4	100±0.0	100±0.0
Malus	2	F	Yamagata	Soil	Fresh	5.2±2.7	6.1±6.4	0.8±2.7	3.1±2.0	100±0.0	92.6±0.5
domestica	3	F	Nagano	Soil	Fresh	8.2±2.0	5.2±3.6	7.4±1.3	5.9±4.0	100±0.0	100±0.0
Rutaceae/											
Orange	1	P	Miyazaki	Soil	Fresh	62.6±3.6	-2.2±3.9	59.6±4.3	45.2±4.2	93.4±2.1	100±0.0
Citrus sinensis	2	F	Miyazaki	Soil	Fresh	26.8±2.7	2.0±2.8	26.9±2.4	0.4±5.4	100±0.0	100±0.0
Solanaceae/											
Tomato	1	F	Mie	Hydroponics	Fresh	20.0±8.9	0.2±6.7	18.8±5.9	58.2±7.0	100±0.0	100±0.0
Lycopersicon	2	F	Aichi	Hydroponics	Fresh	29.4±5.7	7.9±3.9	28.0±13.1	60.0±5.0	99.0±10.3	100±0.0
esculentum	3	F	Mie	Hydroponics	Fresh	75.4±0.1	75.0±0.5	72.2±5.6	-28.0±8.5	79.6±4.4	86.5±8.8
Mill.	4	F	Kumamoto	Soil	Fresh	30.7±1.7	3.4±2.7	27.6±4.2	4.6±3.0	100±0.0	98.5±7.1
	5	F	Sizuoka	Soil	Fresh	33.0±5.6	15.1±3.5	30.4±6.8	14.9±3.7	98.3±7.4	96.9±1.1
	6	F	Aichi	Soil	Fresh	29.4±3.4	17.8±2.8	29.7±0.2	18.2±0.7	98.7±1.3	96.8±1.2
	7	F	NT	Soil	Fre	16.7±2.5	14.7±2.9	26.5±2.1	4.4±3.1	100±0.0	100±0.0
	8	F	Fukushima	Soil	Fresh	43.4±3.0	12.8±2.3	51.5±2.0	15.9±2.9	92.9±2.9	100±0.0
	9	F	Fukushima	Soi	Nitrogen-sealing	50.0±8.2	16.9±4.4	55.3±6.4	14.9±5.8	96.1±2.9	100±0.0
	10	F	Fukushima	Soil	Fresh	52.8±3.4	16.2±5.1	55.8±2.8	14.0±4.3	95.1±0.7	100±0.0
	11	F	Fukushima	Soil	Nitrogen-sealing	53.0±2.4	38.4±3.7	54.8±3.7	38.1±2.3	95.8±3.5	100±0.0
	12	F	Fukushima	Soil	Fresh	47.3±11.6	22.6±7.0	53.9±10.8	25.2±5.8	94.4±7.6	100±0.0
	13	F	Fukushima	Soil	Nitrogen-sealing	45.0±2.8	10.5±3.9	49.5±3.9	12.9±1.1	98.4±2.1	100±0.0
	14	F	Fukushima	Soil	Fresh	45.2±4.9	17.2±5.3	51.8±4.4	21.1±0.1	100±0.0	100±0.0

Table 1 continued. Inhibition of NO Generation by Common Japanese Edible Plants

Sample names	N	Part tested	Growing district	Cultivation method	Cooking method	Total inhibition (IR %) 100	Total inhibition (IR %) 20	iNOS inhibition (%) 100	iNOS inhibition (%) 20	Cell viability (%) 100	Cell viability (%) 20
Tomato	15	F	Fukushima	Soil	Nitrogen-sealing	33.5±2.7	17.5±3.6	41.0±0.7	16.4±4.3	72.1±4.2	100 ±0.0
continued	16	F	Fukushima	Soil	Fresh	35.0±1.0	21.6± 11	40.6±3.3	20.8±0.8	77.8±7.0	100 ±0.0
	17	F	Fukushima	Soil	Nitrogen-sealing	50.4±1.9	24.2±7.1	52.8±1.8	28.8±10.8	78.6±5.1	100 ±0.0
	18	F	Fukushima	Soil	Fresh	46.9±3.3	21.5±0.9	51.3±2.7	29.9±3.7	70.3±3.6	100 ±0.0
	19	F	Fukushima	Soil	Nitrogen-sealing	37.9±3.6	15.9±1.4	41.0±3.5	22.5±1.4	89.1±2.1	100 ±0.0
	20	F	Fukushima	Soil	Fresh	45.4±2.5	21.6±4.8	46.6±0.8	18.7±4.2	88.5±3.9	100 ±0.0
	21	F	Fukushima	Soil	Nitrogen-sealing	25.9±3.9	16.8±2.9	21.3±2.0	18.4±3.9	74.8±5.8	100 ±0.0
	22	F	Fukushima	Soil	Fresh	41.0±0.8	25.5±2.0	47.9±3.3	37.6±8.2	100 ±0.0	100 ±0.0
	23	F	Fukushima	Soil	Nitrogen-sealing	28.6±1.9	9.0±5.1	36.0±2.1	17.5±4.9	100 ±0.0	100 ±0.0
	24	F	Fukushima	Soil	Fresh	57.7±2.2	23.9±5.2	59.3±2.9	31.2±4.1	100 ±0.0	100 ±0.0
	25	F	Fukushima	Soil	Nitrogen-sealing	34.6±1.6	14.4±9.8	38.2±3.0	22.8±9.1	100 ±0.0	100 ±0.0
	26	F	Chiba	Soil	Fresh	38.5|±2.3	9.8±1.8	47.2±3.9	16.1±1.7	100 ±0.0	100 ±0.0
	27	F	Chiba	Soil	Fresh	30.5±1.2	3.8±1.0	40.1±0.7	7.9±0.7	100 ±0.0	100 ±0.0
	28	F	Fukushima	Soil	Fresh	40.1±9.9	15.3±6.4	49.5±5.2	8.8±8.3	100 ±0.0	100 ±0.0
	29	F	Fukushima	Soil	Fresh	27.7±2.1	18.0±3.3	35.8±1.9	10.6±1.4	100 ±0.0	96.3±2.9
	30	F	Chiba	Soil	Fresh	12.6±4.2	22.8±1.5	21.9±4.1	5.4±12.4	100 ±0.0	99.6±0.5
	31	F	NT	Soil	Fresh	39.2±6.6	18.2±3.8	50.1±3.8	9.0±2.8	100 ±0.0	100 ±0.0
	32	F	Fukushima	Soil	Nitrogen-sealing	25.6±2.7	26.1± 12	42.2±2.1	25.2±0.9	100 ±0.0	100 ±0.0
	33	F	Fukushima	Soil	Nitrogen-sealing	17.7±3.9	20.5±5.9	34.7±4.0	9.2±7.7	100 ±0.0	100 ±0.0
	34	F	Fukushima	Soil	Nitrogen-sealing	18.5±0.4	25.6±4.5	35.7±1.2	18.3±2.8	100 ±0.0	100 ±0.0
	35	F	Kumamoto	Soil	Fresh	19.6±5.3	2.7±5.4	21.0±3.3	4.6±2.21	100 ±0.0	100 ±0.0
	36	F	Fukuoka	Soil	Fresh	8.2±3.0	18.6±1.3	13.1±4.3	15.9±2.0	100 ±0.0	86.2±9.0
	37	F	Fukuoka	Soil	Fresh	16.6±0.8	21.6±7.6	22.3±0.8	22.9±5.3	100 ±0.0	92.8±1.5
	38	F	Fukuoka	Soil	Fresh	15.9±2.8	5.9±7.3	18.4±3.0	8.7±2.9	100 ±0.0	95.4±3.6
Egg plant	1	F	Kumamoto	Soil	Fresh	57.9±1.9	7.9±3.5	56.0±1.0	10.0±6.4	100 ±0.0	100 ±0.0
Solanum	2	F	Kumamoto	Soil	Boiled	49.8±1.0	10.7±1.6	48.3±0.5	11.8±2.7	98.3±1.4	100 ±0.0
melongena L.	3	F	Fukuoka	Soil	Fresh	69.6±1.2	15.0±1.9	70.4± 11	18.6±3.9	89.3±4.4	100 ±0.0
	4	F	Fukuoka	Soil	Boiled	60.2±1.3	5.4±2.4	62.5±1.6	13.0±7.6	100 ±0.0	100 ±0.0
	5	F	Fukuoka	Hydroponics	Fresh	72.7±1.0	18.6±1.5	70.1±3.0	38.4± 15	72.7±3.5	100 ±0.0
	6	F	Fukuoka	Hydroponics	Boiled	51.7±3.5	6.8±1.3	51.2±3.3	12.2±1.3	100 ±0.0	100 ±0.0
Potato	1	R	Hokkaido	Soil	Fresh	34.1±3.3	-0.1±3.3	41.4±3.3	3.7±3.1	100 ±0.0	100 ±0.0
Solanum	2	R	Nagasaki	Soil	Fresh	59.9±3.9	12.8±5.3	63.5±3.4	19.8±3.9	100 ±0.0	100 ±0.0
tuberosum L.	3	R	Hokkaido	Soil	Fresh	56.9±3.4	13.7±4.1	62.0±2.1	24.1±7.2	96.0±3.7	100 ±0.0
Umbelliferae/											
Mitsuba	1	W	Shizuoka	Hydroponics	Fresh	65.1±4.4	84.1±0.6	65.0±0.5	81.5±0.9	34.0±2.7	100 ±0.0
Cryptotaenia	2	W	Ibaragi	Hydroponics	Fresh	72.7±1.5	77.0±3.2	67.8±1.5	76.4±2.3	39.3±5.1	63.0±7.8
japonica Hassk.Japanese hornwort											
Carrot	1	R	Chiba	Soil	Fresh	71.4±0.6	50.6±3.7	68.8±1.2	40.5±4.2	38.7±0.6	98.6±8.0
Daucus	2	R	Saitama	Soil	Fresh	55.0±2.0	36.6±4.3	65.5±0.0	31.4±6.0	100±0.0	100 ±0.0
carota L.	3	R	Ibaragi	Soil	Fresh	47.7±1.8	39.7±2.7	65.8±0.6	32.9±1.7	70.6± 21	100 ±0.0
var. *sativa*	4	L	Iwate	Soil	Fresh	58.7±0.8	70.9±2.0	50.9±1.1	70.4±2.2	34.5±2.9	95.8±3.6
	5	L	Iwate	Soil	Boiled	43.8±0.2	61.2±7.2	43.6±2.0	61.7±6.7	29.8±5.0	100 ±0.0
	6	R	Iwate	Soil	Fresh	63.2±3.5	30.7±3.3	65.3±1.2	28.6±4.7	100 ±0.0	100 ±0.0
	7	R	Iwate	Soil	Fresh	66.9±2.0	46.2±4.3	65.9±1.5	46.2±3.1	100 ±0.0	100 ±0.0
	8	R	Iwate	Soil	Boiled	60.5±3.7	61.4±0.4	64.2±0.5	58.3±0.3	37.4±4.4	100 ±0.0
	9	R	Iwate	Soil	Boiled	62.4±1.2	57.4±5.9	56.0±2.2	56.3±4.7	47.6± 23	100 ±0.0
	10	R	Iwate	Soil	Microwave oven	66.2±1.3	48.7±9.6	62.4±1.4	48.8±7.5	91.7± 13	100 ±0.0
	11	R	Iwate	Soil	Microwave oven	61.2±2.3	10.4±4.7	61.2±1.0	0.2±7.1	100 ±0.0	100 ±0.0
Parsley	1	W	Miyazaki	Soil	Fresh	50.0±2.1	68.6±0.6	45.5±3.1	66.9±0.7	39.6±5.1	91.9±1.5
Petroselinum sativum Hoffm.											

Each sample was tested at a concentration of 100 μg/mL and 20 μg/mL in triplicate experiments. RAW 264.7 cells were stimulated with both LPS (100 ng/mL) and IFN-γ (100 U/mL) to induce the generation of NO. R, root; Sp, sprout; W, whole part; F, fruit; L, leaf; Rh, rhizome; P, peel; NT, not traceable. The total activity was evaluated using the Griess method. iNOS inhibitory activity was measured based on the citrulline level

concentration of 100 mg/mL, the inhibitory activities of 53 samples including some of spinach, garland-chrysanthemum, lettuce, and some species belonging to the families of Cruciferae (genus *Brassica*) and Liliaceae (genus *Allium*) were determined to be insignificant due to the low cell viability observed after treatment (CVs<70%). An activity profile of the 144 extracts with significant cell viabilities (CVs≥70%) following treatment with a concentration of 100 mg/mL is shown in Figure 1a. Six samples (watermelon 1, melon 2 (Andes), garlic 1 and 2, tomato 3 and egg plant 5) were found to be strongly active (+++), while 49 samples were found to be moderately active (++), 47 samples were found to be weakly active (+), and 42 samples were found inactive (-). Next, screening of a lower concentration (20 μg/mL) was conducted. The results of this analysis revealed that 192 samples had an acceptable CV (Figure 1b). Of these samples, 4 (tomato 3, Japanese hornwort 1, head lettuce

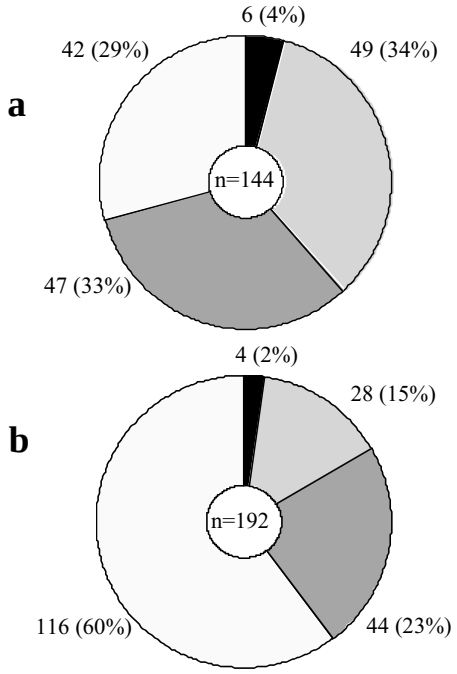

Figure 1. Activity Profile of the Total NO Inhibitory Activities of the Tested Samples of Edible Plants Commonly Eaten in Japan. +++black, highly active (IR≥70%); ++ dark grey, moderately active (70%>IR≥50%); + medium grey, weakly active (50%>IR≥30%); - light grey, inactive (IR<30%). A, data tested at 100 μg/mL, CV≥70%; B, data tested at 20 μg/mL, CV≥70%

2 and carrot 4) that were not selected for further analysis following treatment with 100 μg/mL were selected. Garland-chrysantimum 5 and Japanese hornwort (mitsuba) 2, which exhibited IR of more than 70%, had still nonpreferable CVs.

Based on the results of this screening test, watermelon 1 and melon 2 (cultivar. Andes) in Cucurbitaceae, tomato 3 and egg plant 5 in Solanaceae, garlic 1 and 2 in Liliaceae, head lettuce 2 in Compositae, and Japanese hornwort and carrot 4 in Umbelliferae were found to possess NO generation inhibitory activity. Of the strongly active species, Japanese hornwort was found to be the most promising species among the common dietary plants.

In most cases, the activities were a result of the

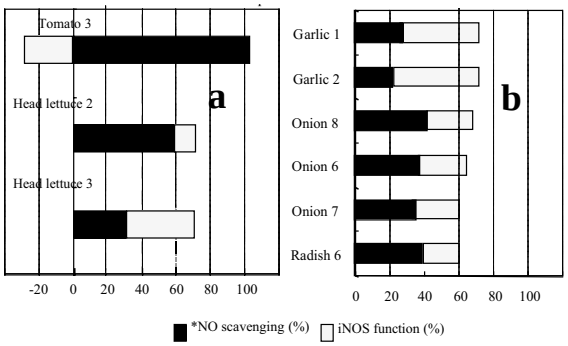

Figure 2. Plant Extracts Showing a Significant Difference in the Rates of Total NO and iNOS Inhibition Activities. a, treatment with 20 μg /mL; b, treatment with 100 μg /ml. *NO scavenging =Total NO suppressive activity obtained by adding the NO scavenging activity and iNOS inhibition. Error bars are deleted

inhibition of iNOS function. However, there were several samples whose inhibitory activities (more than 70% inhibition) were believed to be a result of both the inhibition of iNOS function and NO scavenging effect. For example, most of the inhibition of NO generation that was observed in response to treatment with tomato 3 at a concentration of 20 μg/mL was believed to arise from NO scavenging (Figure 2a). Furthermore, treatment with garlic samples (sample 2 and 3) at a concentration of 100 mg/mL was found to prevent NO generation via NO scavenging and the inhibition of iNOS (Figure 2b). This tendency was also observed in head lettuce samples (sample 2 and 3 at 20 μg/mL) and onion samples (boiled sample 6, 7 and 8 at 100 mg/mL) as shown in Figure 2a and 2b, although the total inhibitory activities of these samples was moderate.

It is interesting to note that different NO inhibition-levels were observed within the same species in some cases. For example, of the 38 tomato samples tested, only sample 3 showed strong inhibitory activity at 100 and 20 mg/mL, while the inhibitory activity of the other tomato extracts ranged from ++ to -. Although sample 3 was produced by hydroponic cultivation, two other tomato samples that did not have strong inhibitory activity were also produced by hydroponic cultivation; therefore, the reason for the differences in activity is unclear. Large variations in activity were also found among spinach (4.9-63.6%), burdock root (14.9-61.5%), garland-chrysanthemum (45.5-75.0%), lettuce (26.6-69.1%), welsh onions (-1.8-65.8%) and carrots (10.4-70.9%) at 20 μg/mL (CV≥70%), and onions (2.2-67.4%) at 100 μg/mL (CV≥70%).

When the activities of 2 sets of 2 cultivation types, soil and hydroponics, were compared no large variations were observed within spinach and tomato samples (Figure 3a). However, a significant difference in activity was observed between spinach produced by greenhouse-cultivation and that produced by soil-cultivation at 100 mg/mL (Figure 3-B). In addition, a significant difference in the activity of fresh spinach samples and boiled spinach samples was observed (Figure 3-C, $p<0.01$ based on a Student-t test). Specifically, the activity of the fresh samples was greater than that of the boiled samples.

Discussion

Umbelliferae plants as a promising plant family

The present study demonstrated that the acetone extracts from a variety of plants commonly eaten in Japan had strong inhibitory activities toward LPS/IFN-γ stimulated NO generation in RAW 264.7 murine macrophages. Macrophage-generated NO has been reported to cause mutagenesis (Arroyo et al., 1992) and deamination of DNA bases (Wink et al., 1991). However, Chan et al. (1995) reported that well-known cancer preventive phytochemicals, such as (-)-epigallocatechin-3-gallate, carnosol and curcumin, inhibit the production of peroxynitrite and nitrite in animal cells in culture. Therefore, reduction of the excess NO level by phytochemicals may be an effective and reasonable strategy for cancer prevention.

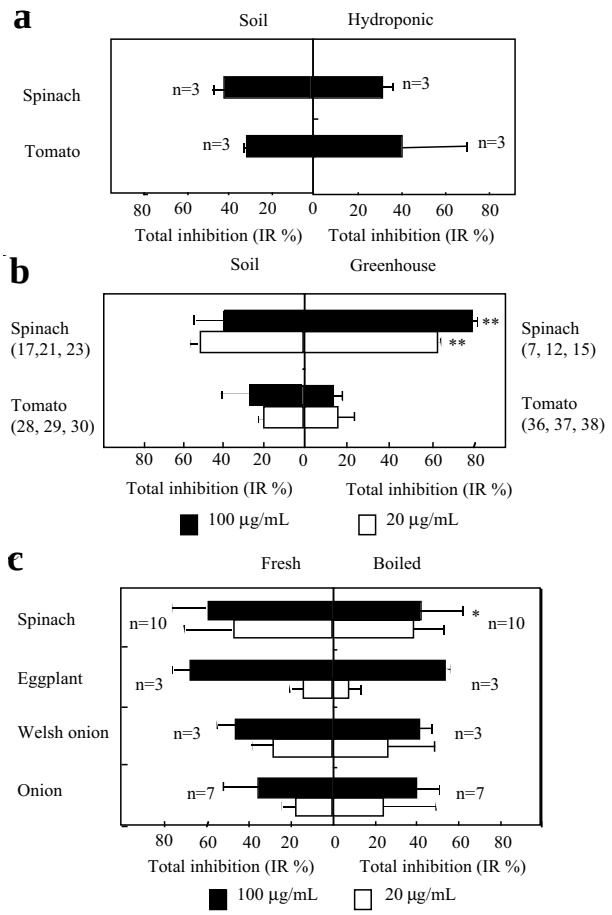

Figure 3. Suppressive Effects of Edible Japanese Plant Extracts against NO Generation Based on Different Cooking Conditions and Cultivation Methods. a, samples produced by hydroponics and soil cultivation, tested at 100 μg /mL; b, samples produced by soil and vinylhouse cultivation, tested at 100 μg /mL and 20 μg /mL, **, <0.01 vs. soil cultivation; c, fresh and boiled samples, tested at 100 μg / mL and 20 μg /mL, *, <0.05 vs. conventional

It has been reported that naturally occurring components present in edible plants possess substantial anticarcinogenic and antimutagenic activities. Because oxidative and inflammatory tissue damage is closely related to tumor-promotion, substances with pronounced anti-oxidative or anti-inflammatory effects are expected to exert suppressive effects on carcinogenesis, particularly during the promotion stage (Surh et al., 2001; Surh, 2002). In this study, the leaves of Japanese hornwort (mitsuba in Japanese: Umbelliferae) were found to be the most promising product for the treatment of cancer. Moreover, the number of active species with IRs greater than 50% belonging to Umbelliferae suggests that this family of plants is a promising source of effective cancer-preventive agents. Liliaceae was also found to be a promising plant family for the inhibition of NO generation. Plants belonging to Umbelliferae and Liliacae have long been used as common foodstuffs and spices as well as traditional folk medicine in Asian countries. Accordingly, vegetables belonging to both families have been investigated as functional foods, and some have exhibited anti-carcinogenic effects (Baba et al., 2000; 2002; Mizuno et al.,1994). Plants in Cruciferae may also be useful as anti-carcinogens, as indicated in our previous study conducted to evaluate edible Thai and Indonesian plants (Murakami et al., 1995; Murakami et al., 1998).

Modes of action for NO suppression

In the present study, we found remarkable differences in the inhibition rates of the total NO and iNOS function in tomato sample 3 (Solanaceae) at 20 μg/mL, head lettuce samples 2 and 3 (Ebenaceae) at 100 μg/mL, onion samples 9, 7 and 8 (Liliaceae) at 100 μg/mL, and radish sample 6 (Cruciferae) at 20 μg/mL. These differences in activity may have resulted from the NO scavenging effect of the samples rather than inhibition of the generation of NO; however, more detailed analyses would be required to confirm this. Most activities of the samples, with the exception of the above-mentioned samples, occur due to the inhibition of NO production via inhibition of iNOS function. However, this was not the case for tomato 3. This may have occurred due to the occurrence of compounds that promote NO generation due to iNOS, and/ or citrulline, or NO scavenging factors. Additional study should be conducted to evaluate these findings.

Different activities within species

In the present study, we observed significantly different activities within some plant species. For example, among the 38 tomato samples, one (tomato 3) showed strong activity (+++), five showed moderate activity (++), seventeen showed weak activity (+), and fifteen showed inactivity (-) at 100 μg/mL. Furthermore, an extract from melon sample 3 (Andes) from Chiba prefecture (IR=73.2%, at 100 μg/mL) more strongly inhibited NO generation than that of melon 1 (Yubari) from Hokkaido prefecture (IR=28.8% at 100 μg/mL) (Table). One of the reasons for such variation in activity may be variations in the cultivars and/or growing district. Other causes may include differences in temperature, humidity, or light during cultivation of the plants. Additionally, cultivation on soil, hydroponics and in a vinylhouse must also influence the inhibition of NO generation, as indicated by the data observed for spinach in this study (Table); however, other factors should also be considered. Furthermore, post-harvest treatment may have important effects on the activity of the extracts, as indicated by the results observed when extract from spinach plants (sample 6~24), radishes (samples 6 and 7), onions and egg plants were evaluated.

In conclusion, when the inhibitory activity of total NO production was evaluated 101 of 144 samples (70%) showed inhibitory activity (IR≥30%) at 100 μg/mL, while 76 of the 144 samples (39%) showed inhibitory activity at 20 μg/mL. In this study, extracts of watermelon 1 and melon 2 (Cucurbitaceae), garlic 1 and 2 (Liliaceae), and tomato 3 and eggplant 5 (Solanaceae) showed promise for the inhibition of NO generation at a concentration of 100 μg/mL. In addition, when extracts were evaluated at a concentration of 20 μg/mL, the extract of garland-chrysamthemums 5, head lettuce 2 (Compositae), tomato 3 (Solanaceae), Japanese hornworts and carrot 4 (Umbelliferae) showed strong inhibition of NO production. Furthermore, the results of this study indicated that the activity of different cultivars differed within

species, as did the activities of the same species when they were obtained from different growing districts, cultivated using different conditions, or subjected to different post-harvest treatments. Taken together, the results of this study provide an experimental basis for the development of new strategies to produce highly functional plants and food items.

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