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Immunomodulatory activities of common vegetables and spices of Umbelliferae and its related coumarins and flavonoids ☆

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Abstract

Carrots, celery, coriander, fennel and parsley of the Umbelliferae family have been used as common vegetables and spices in many different cultures of the world. In this study, we evaluated the immunomodulatory activities of coumarins and flavonoids obtained from the above foods on human peripheral blood mononuclear cells (PBMC). Studies were conducted on lymphocyte transformation, ELISA assay and flow cytometry. Results provided the evidence of a health-modulating effect of these vegetables and spices which possessed a direct role in immunomodulatory function. Some of non-nutritional constituents of these foods such as coumarins and flavonoids also exhibited a similar immunomodulatory activity. At non-cytotoxic concentrations, the above phytoconstituents exhibited three types of immunomodulation including type 1 of PHA, ConA and quercetin (increased lymphocyte activation and IFN-γ secretion); type 2 of isopimpinellin (enhanced lymphocyte activation) and type 3 of rutin, bergapten and xanthotoxin (elevated IFN-γ secretion). The augmentation of lymphocyte proliferation was closely correlated to an increase in the number of lymphocyte cells including $CD8^+$ T cells and activated PBMC, whereas elevation of IFN-γ secretion was due to the activated $CD8^+$ T cells.

Keywords: Foods of Umbelliferae; Immunomodulation; Coumarins; Flavonoids

1. Introduction

Celery (*Apium graveolens* L.), coriander (*Coriandrum sativum* L.), carrots (*Daucus carota* L.), fennel (*Foeniculum vulgare* Mil.) and parsley (Petroselinum crispum Nyman Ex A.W. Hill) are popular vegetables and spices of Umbelliferae. There are many nutrients in these foods, particularly immunomodualtory nutrients, including vitamins (A, B_2, C, E) and minerals (copper, zinc, iron, selenium) (Bhaskaram, 2002; Chandra, 1991). Additionally, the above vegetables and spices also contain several bioactive phytochemicals such as flavonoids (quercetin, rutin) and coumarins (bergapten, isopimpinellin, xanthotoxin), which are reported to have curative, preventive, or nutritive value (Duke, 1992). There are several reports of the efficacy of quercetin and rutin against infections of bacteria, fungi and viruses (Bae, Han, Lee, & Kim, 2000; Chiang, Chiang, Liu, & Lin, 2003; Paulo, Gomes, Duarte, Perett, & Houghton, 1997; Wang, Hamburger, Gueho, & Hostettmann, 1989; Weidenborner, Hindorf, Jha, & Tsotsonos, 1990) and their anti-allergy (Cheong et al., 1998), anti-inflammation (Shoskes, 1999) and immunosuppression activities (Lee et al., 1995; Namgoong, Son, Chang, Kang, & Kim, 1994). The above coumarins have also been found to inhi-

Abbreviations: IFN-γ, interferon-gamma; PBMC, peripheral blood mononuclear cells; PHA, phytohemagglutinin; DMSO, dimethylsulphoxide.

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bit multiplication of bacteria, fungi and viruses (Hudson, Graham, Harris, & Ashwood-Smith, 1993; Juan, Rideout, & Ragasa, 1997; Kofinas et al., 1998; Yang & Yen, 1979), and demonstrated anti-allergy (Kimura & Okuda, 1997), anti-inflammation (Chen, Tsai, & Wu, 1995) and immunosuppression activities (Cox, Orosz, Lewis, Olsen, & Fertel, 1988; Kuzel, Roenigk, Samuelson, & Rosen, 1992).

An inappropriate immunity has been shown as a common etiology in an ever-growing array of pathological processes including infections, cancer, allergy, aging and a variety of disorders of various organs (Peakman & Vergani, 1997). To date, there is only a modest body of knowledge about the non-nutritiously active compounds in vegetables and spices and their collective roles in promoting human health by immunomodulation. Recently, we have reported that several phytochemicals in wild vegetables of Plantago species possess immunomodualtory activity, especially immunostimulating activity of phenolic compounds such as caffeic acid, chlorogenic acid, ferulic acid and *p*-coumaric acid, which have been reported to be as common phenolic acids in the above vegetables and spices of this study (Chiang, Ng, Chiang, Chang, & Lin, 2003; Duke, 1992).

To explore the potentially health-promoting role of popular vegetables and spices of Umbelliferae, we evaluated the immunomodulatory activities of the crude extracts and two common chemical classes of five related pure compounds from Umbelliferae (Duke, 1992) on human PBMC.

2. Materials and methods

2.1. Crude extracts and chemicals

Fresh plants were collected from the southern part of Taiwan and were authenticated by Dr. Shing-Ginn Lee, Chief of Taitung Agricultural Improvement Station. Fresh plants (500 g) were boiled in 1000 ml of distilled water for 1 h and filtered by gauze. The aqueous extract was concentrated in vacua, and then lyophilized. The aqueous extract was collected and stored at 4 °C until use. Deaminated heparin, Ficoll-Hypaque, dimethylsulphoxide (DMSO), phyto-mitogens of phytohemagglutinin (PHA-L) and concanavalin A (Con A), quercetin and rutin were obtained from Sigma–Aldrich Co. (St. Louis, MO, USA). Bergapten, isopimpinellin and xanthotoxin were purchased from Carl Roth (Karlsruhe, Germany). Each of these compounds was more than 98% pure, excluding the crude extracts. 5-Bromo-2′-deoxyuridine (BrdU) labeling and detection kits were obtained from Roche Diagnostics GmbH (Mannheim, Germany). The human IFN-γ immunoassay kit was purchased from R&D Systems Inc. (Minneapolis, MN, USA).

2.2. Lymphocyte transformation

Peripheral blood from healthy volunteers (20–35 years old) was collected in a sterile syringe containing sufficient heparin to give a final concentration of 100 units/ml. Mononuclear cells were obtained by centrifuging whole blood and normal saline (1/1, v/v) mixture on Ficoll-Hypague (2.4:1, v/v) gradients at 400 g as described by manufacture's protocol (Sigma–Aldrich). After centrifugation, 0.05 ml (5.0×10^5) unfractionated PBMC was placed in a 5 ml test tube containing 0.15 ml fetal calf serum and 0.75 ml of RPMI 1640, with 0.05 ml of test samples, mitogen (positive control), medium (negative control) or additional medium with dimethylsulphoxide (DMSO: solvent control). After gentle mixing, 200 μg/well were added into the wells of a 96-well microculture plate in triplicate. The culture plate was then allowed to incubate for 3 days at 37 °C in a 5% CO_2 incubator. BrdU immunoassay was done as described by Chiang, Ng, et al. (2003). The optical densities were determined with an ELISA reader at a test wavelength of 450 nm. The stimulation index (SI) was determined by the ratio of optical density of test substance to the optical density of negative control.

2.3. Enzyme-linked immunosorbent assay (ELISA)

The solid-phase sandwich ELISA procedure was performed according to the standard protocol of IFN-γ ELISA kit. The cultivation and treatment of peripheral blood mononuclear cells was done as previous described (Chiang, Ng, et al., 2003). After 3 days, particulates were removed from the supernatants by centrifugation and the samples were stored at −70 °C until use. The sample diluent (100 μl) was added to each well and then 100 μl/well of IFN-γ standard or supernatant sample was applied. The plate was covered with an adhesive strip and incubated for 2 h at room temperature. Each well was aspirated and washed, and the process was repeated four times. The IFN-γ conjugate (200 μl/well) was added, covered with a new adhesive strip, and incubated for 2 h at room temperature. The washing process was repeated four times. The substrate solution (200 μl/well) was added and incubated for 30 min at room temperature. Finally, the stop solution (50 μl/well) was added and the optical density of each well determined within 30 min, using an ELISA reader at a wavelength of 450 nm and a reference wavelength of 540 nm.

2.4. Flow cytometry analysis

All fluoroscein isothiocyanate (FITC)- and phycoerythrin (PE)- conjugated monoclonal antibodies (mAbs) were purchased from Becton Dickinson (San Jose, CA, USA). Optimal concentrations of mAbs were determined for each mAb by titration. The isolated PBMC were cultured in triplicate with test drugs (20 μg/ml), PHA (5 μg/ml, positive control), DMSO (0.1%, solvent control) or medium (negative control) for 3 days or refed at 3 days and then harvested at 6 days in 37 °C of a 5% CO_2 incubator. Then the numbers of NK cells ($CD3^-$, $CD16^+$, $CD56^+$), activated PBMC ($CD25^+$), T cell subsets ($CD3^+$, $CD4^+$,

$CD8^+$), total T cells ($CD3^+$), total B cells ($CD3^-$, $HLA\text{-}DR^+$) and active T cells ($CD3^+$, $HLA\text{-}DR^+$) at 0, 3 or 6 days were determined by standard FACS can procedures with mAbs according to the manufacture's protocol. The stained PBMC were analyzed by a flow cytometric analyzer (FACSCalibur, Becton Dickinson, Cookeysville, MD, USA) with Cell Quest software (Becton Dickinson). The cell fractions of negative control (medium) were total T cells (0.555 ± 0.077, 0.624 ± 0.096, 0.690 ± 0.092), total B cells (0.177 ± 0.084, 0.132 ± 0.058, 0.148 ± 0.074), active T cells (0.151 ± 0.077, 0.191 ± 0.132, 0.221 ± 0.159), $CD4^+$ T cells (0.270 ± 0.067, 0.318 ± 0.067, 0.349 ± 0.093), $CD8^+$ T cells (0.439 ± 0.074, 0.453 ± 0.069, 0.461 ± 0.086), NK cells (0.280 ± 0.083, 0.270 ± 0.115, 0.243 ± 0.100) and activated PBMC (0.077 ± 0.051, 0.089 ± 0.012, 0.085 ± 0.019) at 0, 3, 6 days, respectively. There were no significant changes in the fractions of immune cells in the negative control at the three time periods.

2.5. Statistical analysis

Results were expressed as mean ± standard deviation. The one-way ANOVA and multiple comparison of Dunnett's *t*-test (>2 groups) or two-tails of Student's *t*-test (two groups) were used to evaluate the difference between the control and test samples by the SPSS Base 8.0 software for Windows. The *p*-value less than 0.05 was considered to be significant difference in all experiments.

3. Results

3.1. Immunomodulatory activity evaluated by lymphocyte transformation and secretion of IFN-γ

With the exception of aqueous extract of fennel's root, the results from crude extracts of different parts of five vegetables and spices showed that at concentrations between 50 and 200 μg/ml, all of the extracts significantly ($p < 0.05$) stimulated the proliferation of human PBMC and/or the secretion of IFN-γ (Table 1). To further explore the immuno-stimulating phytochemicals, we examined several known bioactive compounds such as flavonoids (quercetin, rutin) and coumarins (bergapten, isopimpinellin, xanthotoxin), which are very common constituents in Umbelliferae (Table 2, Fig. 1).

The IFN-γ secretion has been well-known to associate with lymphocyte activation after stimulation by antigens or mitogens, flavonoid quercetin showed this typical

Table 1
The immunomodulatory activity of aqueous extracts from common vegetables and spices of Umbelliferae

Drug	Used part	Stimulation index at concentration (μg/ml)[b]			γ-Interferon (pg/ml)[c]
		50	100	200	
Medium	(−) Control	1.0 ± 0.05			5.0 ± 2.0
ConA[a]	(+) Control	$8.5 \pm 1.34^*$			$937 \pm 55^*$
PHA[a]	(+) Control	$7.9 \pm 1.25^*$			$901 \pm 46^*$
Apium graveolens	Stem	$1.96 \pm 0.61^*$	1.82 ± 0.47	1.57 ± 0.38	$184 \pm 21.6^*$
Apium graveolens	Leaf	$3.16 \pm 0.76^*$	$2.53 \pm 0.58^*$	$2.48 \pm 0.46^*$	$21.1 \pm 2.5^*$
Apium graveolens L. var. dulce	Stem	$2.41 \pm 0.71^*$	$1.98 \pm 0.58^*$	1.88 ± 0.46	8.7 ± 1.1
Coriandrum satirum	Whole plant	1.75 ± 0.36	$2.70 \pm 0.19^*$	$2.96 \pm 0.86^*$	6.3 ± 2.1
Daucus carota	Root	$2.37 \pm 0.42^*$	$2.35 \pm 0.26^*$	$2.25 \pm 0.25^*$	6.0 ± 0.8
Foeniculum vulgare	Aerial part	$3.21 \pm 0.54^*$	$0.94 \pm 0.36^*$	$2.70 \pm 0.28^*$	$41.7 \pm 5.1^*$
Foeniculum vulgare	Root	$0.49 \pm 0.06^*$	$0.52 \pm 0.02^*$	$1.85 \pm 0.14^*$	3.0 ± 0.1
Petroselinum crispum	Whole plant	$2.89 \pm 0.87^*$	$2.71 \pm 0.66^*$	$2.53 \pm 0.57^*$	2.9 ± 0.6

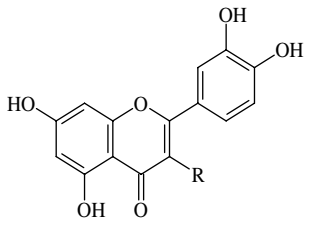
[a] ConA (10 μg/ml), PHA (5 μg/ml).
[b] The various concentrations of aqueous extracts were evaluated on their ability to direct stimulation of PBMC without mitogen in three independent experiments. One-way ANOVA and multiple comparison of Dunnett's *t*-test were used to evaluate the difference between test drug and medium control ($^*p < 0.05$).
[c] The data were the response of the maximum stimulation index group of each drug.

Table 2
The common phytochemical constituents of Umbelliferae[a]

Vegetable plant	Coumarins			Flavonoids	
	Bergapten	Isopimpinellin	Xanthotoxin	Quercetin	Rutin
Apium graveolens L.	+	+	+		+
Coriandrum sativum L.				+	+
Daucus carota L.	+	+	+	+	
Foeniculum vulgare Mil.	+	+	+	+	+
Petroselinum crispum Nyman Ex A.W. Hill	+	+	+	+	+
Ratio	4/5	4/5	4/5	4/5	4/5

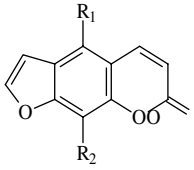
[a] Cited from Duke (1992).

A. Flavonoids



	R	Type
Quercetin	OH	1
Rutin	O-rutinoside	3

B. Coumarins



	R_1	R_2	Type
Bergapten	OCH_3	H	3
Isopimpinellin	OCH_3	OCH_3	2
Xanthotoxin	H	OCH_3	3

Fig. 1. Structure of bioactive constituents of the two chemical classes (A: flavonoids; B: coumarins) obtained from the common vegetables and spices. There are three types of immunomodulation including type 1 (enhanced lymphocyte activation and secretion of IFN-γ), type 2 (enhanced lymphocyte activation) and type 3 (enhanced secretion of IFN-γ).

response, which significantly ($p < 0.05$) stimulated the proliferation of human PBMC and the secretion of IFN-γ. However, flavonoid rutin, coumarins bergapten and xanthotoxin exhibited a potent stimulator for the secretion of IFN-γ ($p < 0.05$) but they did not enhance the proliferation of human PBMC. In addition, coumarin isopimpinellin expressed another response, which significantly ($p < 0.05$) promoted the proliferation of human PBMC but did not modulate the secretion of IFN-γ (Table 3).

Table 3
The immunomodulatory activity of common constituents from popular vegetables and spices of Umbelliferae

Drug	Dose (μg/ml)	Stimulation index[a]	γ-Interferon (pg/ml)
DMSO	0.10%	1.0 ± 0.05	5.1 ± 1.9
ConA	10	8.5 ± 1.34*	937 ± 55*
PHA	5	7.9 ± 1.25*	901 ± 46*
Bergaten	2	1.18 ± 0.26	139 ± 10.4*
	20	1.02 ± 0.11	91.8 ± 6.5*
Isopimpinellin	2	0.92 ± 0.16	1.0 ± 0.2
	20	1.71 ± 0.35*	4.6 ± 0.5
Xanthotoxin	2	1.25 ± 0.14	20.7 ± 1.2*
	20	1.05 ± 0.21	1.0 ± 0.1
Quercetin	2	0.76 ± 0.10	14.1 ± 0.8
	20	1.74 ± 0.26*	161 ± 12.6*
Rutin	2	1.07 ± 0.12	49.6 ± 2.8*
	20	0.82 ± 0.10	123 ± 9.2*

[a] The various concentrations of pure compounds were evaluated on their ability to direct stimulation of PBMC without mitogen in three independent experiments. One-way ANOVA and multiple comparison of Dunnett's *t*-test were used to evaluate the difference between test drug and DMSO control (*$p < 0.05$).

3.2. Immunomodulatory activity evaluated by flow cytometry analysis

There were three types of immunomodulation after treatment with phytochemicals including type 1 (enhanced lymphocyte activation and secretion of IFN-γ), type 2 (augmented lymphocyte activation) and type 3 (elevated secretion of IFN-γ). The immunomodulatory effects of type 1 response by adding positive control (PHA) at 3 days after treatment exhibited significant elevation of active T, $CD8^+$ T and activated PBMC, whereas the $CD4^+$ T cells were significantly decreased ($p < 0.05$) (Table 4). Similar responses were found at 6 days post-treatment with PHA, but it showed different T cell patterns such as significant ($p < 0.05$) increment of total T and $CD4^+$ T and decrement of $CD8^+$ T cells (Table 4). The significant changes of immune cell populations at 3 days after culture with quercetin showed that total T, active T and $CD8^+$ T cells increased, but NK and $CD4^+$ T cells decreased. Similar responses at 6 days of quercetin treatment were found after 6 days of PHA treatment, except that there were no significant changes of total T and activated PBMC (Table 4).

The immunomodulation of type 2 response at 3 days after culture with isopimpinellin was found to increase total T and active T cells, whereas at 6 days it elevated the populations of active T and $CD4^+$ T cells (Table 5). The type 3 immunomodulatory response at 3 days after treatment with bergapten showed that it significantly increased $CD8^+$ T cells and decreased $CD4^+$ T cells, whereas it did not significantly change the immune cells populations at 6 days post-treatment (Table 6).

Table 4
The cell fractions in type 1 immunomodulatory activity of control drugs and quercetin

	DMSO (0.1%)[a]	PHA (5 μg/ml)[b]	Quercetin (20 μg/ml)[b]
Cells at 3 days			
Total T	0.614 ± 0.039	0.627 ± 0.014	0.640 ± 0.015*
Total B	0.143 ± 0.014	0.160 ± 0.032	0.14 ± 0.036
Active T	0.175 ± 0.003	0.241 ± 0.043*	0.210 ± 0.034*
CD4+	0.333 ± 0.010	0.30 ± 0.025*	0.324 ± 0.004*
CD8+	0.450 ± 0.012	0.479 ± 0.036*	0.465 ± 0.015*
NK cell	0.275 ± 0.033	0.292 ± 0.051	0.25 ± 0.010*
Activated PBMC	0.090 ± 0.011	0.104 ± 0.017*	0.091 ± 0.007
Cells at 6 days			
Total T	0.689 ± 0.010	0.733 ± 0.069*	0.689 ± 0.044
Total B	0.144 ± 0.022	0.153 ± 0.006	0.150 ± 0.039
Active T	0.208 ± 0.019	0.383 ± 0.143*	0.282 ± 0.097*
CD4+	0.336 ± 0.004	0.385 ± 0.066*	0.360 ± 0.022*
CD8+	0.459 ± 0.016	0.436 ± 0.046	0.448 ± 0.015
NK cell	0.236 ± 0.025	0.228 ± 0.052	0.250 ± 0.025
Activated PBMC	0.091 ± 0.017	0.248 ± 0.089*	0.104 ± 0.027

[a] The solvent controls (DMSO) were not changed the fractions of immune cells by comparison with the results of negative control (medium) at the same time period.

[b] The positive controls (PHA-L) or quercetins were evaluated on their ability to stimulate PBMC in triplicate of four healthy volunteers and were also used to determine the difference between test and solvent control (*$p < 0.05$).

Table 5
The cell fractions of type 2 immunomodulatory activity of isopimpinellin

	DMSO (0.1%)	Isopimpinellin (20 μg/ml)[a]
Cells at 3 days		
Total T	0.614 ± 0.039	0.671 ± 0.048*
Total B	0.143 ± 0.014	0.135 ± 0.020
Active T	0.175 ± 0.003	0.190 ± 0.020*
CD4+	0.333 ± 0.010	0.333 ± 0.009
CD8+	0.450 ± 0.012	0.452 ± 0.006
NK cell	0.275 ± 0.033	0.261 ± 0.026
Activated PBMC	0.090 ± 0.011	0.092 ± 0.009
Cells at 6 days		
Total T	0.689 ± 0.010	0.696 ± 0.068
Total B	0.144 ± 0.022	0.135 ± 0.029
Active T	0.208 ± 0.019	0.268 ± 0.035*
CD4+	0.336 ± 0.004	0.358 ± 0.014*
CD8+	0.459 ± 0.016	0.450 ± 0.016
NK cell	0.236 ± 0.025	0.233 ± 0.047
Activated PBMC	0.091 ± 0.017	0.101 ± 0.025

[a] The isopimpinellin was evaluated on their direct stimulation of PBMC without mitogen in triplicate of four healthy volunteers and was used to determine the difference between test and solvent controls (*$p < 0.05$).

Table 6
The cell fractions of type 3 immunomodulatory activity of bergapten

	DMSO (0.1%)	Bergapten (20 μg/ml)[a]
Cells at 3 days		
Total T	0.614 ± 0.039	0.637 ± 0.014
Total B	0.143 ± 0.014	0.152 ± 0.030
Active T	0.175 ± 0.003	0.192 ± 0.030
CD4+	0.333 ± 0.010	0.318 ± 0.013*
CD8+	0.450 ± 0.012	0.463 ± 0.009*
NK cell	0.275 ± 0.033	0.268 ± 0.027
Activated PBMC	0.090 ± 0.011	0.093 ± 0.007
Cells at 6 days		
Total T	0.689 ± 0.010	0.710 ± 0.057
Total B	0.144 ± 0.022	0.139 ± 0.014
Active T	0.208 ± 0.019	0.199 ± 0.041
CD4+	0.336 ± 0.004	0.333 ± 0.027
CD8+	0.459 ± 0.016	0.467 ± 0.016
NK cell	0.236 ± 0.025	0.219 ± 0.010
Activated PBMC	0.091 ± 0.017	0.081 ± 0.017

[a] The bergapten was evaluated on their direct stimulation of PBMC without mitogen in triplicate of four healthy volunteers and was used to determine the difference between test and solvent controls (*$p < 0.05$).

4. Discussion

Since ancient times several species of Umbelliferae such as carrots, celery, coriander, fennel and parsley have been used as common vegetables and spices in many different cultures of the world. They provide many dietary phytonutrient including immunomodulatory nutrients of vitamins, and they also contain a variety of phytochemicals of unknown function (Bhaskaram, 2002; Chandra, 1991; Duke, 1992). Recently, our studies have documented that wild vegetables of Plantaginaceae possess antiviral, antitumor and immunomodulatory activities and many constituents, namely chlorogenic acid, ferulic acid, *p*-coumaric acid and caffeic acid, enhanced the activity of human lymphocyte proliferation and secretion of IFN-γ (Chiang, Chiang, Chang, & Lin, 2003; Chiang, Ng, et al., 2003). The above bioactive organic acids have been reported to be present in the celery, coriander, carrots, fennel and parsley (Duke, 1992). In addition, the above vegetables and spices also contain several bioactive phytochemicals including flavonoids (quercetin, rutin) and coumarins (bergapten, isopimpinellin, xanthotoxin), which are reported to have the efficacy against infections (Bae et al., 2000; Chiang, Chiang, Liu, et al., 2003; Hudson et al., 1993; Juan et al., 1997; Kofinas et al., 1998; Paulo et al., 1997; Wang et al., 1989; Weidenborner et al., 1990; Yang & Yen, 1979), anti-allergy (Cheong et al., 1998; Kimura & Okuda, 1997), anti-inflammation (Chen et al., 1995; Shoskes, 1999) and immunosuppression (Cox et al., 1988; Kuzel et al., 1992; Lee et al., 1995; Namgoong et al., 1994).

An unsuitable immunity has been well known to play a central role in a variety of ailments including infections, cancer, allergy, aging and many disorders of various organs (Peakman & Vergani, 1997). Although the above common foods contribute phytonutrients as human nutrition, the roles in promoting human health from non-nutritional constituents by immunomodulation have not yet been studied. Therefore, we evaluated the immunomodulatory activities of the crude extracts and two common chemical classes of five related pure compounds from Umbelliferae (Duke, 1992) on human PBMC. Lymphocyte transformation or activation is an in vitro technique commonly used to assess cellular immunity in human and other animals and this response refers to an in vitro correlate of an in vivo immune response (Horsmanheimo, 1974). IFN-γ is produced by T lymphocytes, natural killer cells, macrophages and neutrophils and has receptors on virtually all cell types of the body. It is a hallmark of Th1-type response and exerts a multitude of cellular biological effects. Thus, high-level production of IFN-γ is typically associated with effective host defense against intracellular pathogens and cancer (Billiau & Vandenbroeck, 2001).

The results from crude extracts of five vegetables and spices from Umbelliferae exhibited that most of the aqueous extracts significantly stimulated the proliferation of human PBMC and/or the secretion of IFN-γ. The immunostimulating activity may probably due partly to the phenolic compounds such as chlorogenic acid, caffeic acid, ferulic acid and p-coumaric acid, which have been shown to possess this activity and to be present in the above-mentioned foods from Umbelliferae (Chiang, Chiang, Chang, et al., 2003; Chiang, Ng, et al., 2003; Duke, 1992). However, this study showed that flavonoid quercetin and coumarin isopimpinellin might also contribute to the immuno-enhancing activity of lymphocyte activation, whereas flavonoids (quercetin and rutin) and coumarins (bergapten, xanthotoxin) might also serve as candidates for the immuno-stimulating activity of IFN-γ secretion. Therefore, dietary phytonutrient of some vitamins and minerals is presumed to be important in modulation of human immunity. In addition, other food constituents such

as flavonoids and coumarins of non-nutrients most likely provide immunomodulation as well.

IFN-γ was first identified in mitogen-activated lymphocyte supernatants as a distinctive antiviral activity, and this pleotropic cytokine plays an important role in modulating nearly all phases of immune and inflammatory responses (Billiau & Vandenbroeck, 2001; Wheelock, 1965). This concomitance of activated lymphocytes and secretion of IFN-γ was only in agreement with the results of type 1 immunomodulation in this study such as phytomitogen (PHA, ConA) and flavonoid (quercetin). However, in the type 2 immunomodulation, the similar flavonoid rutin (quercetin-3-rutinoside) significantly stimulated the secretion of IFN-γ, but did not elevate the proliferation of human PBMC, indicating the sugar moiety as the key point for different responses between type 1 (quercetin) and type 2 (rutin) immunomodulations (Fig. 1, Table 3). Among three coumarins tested, two types of immunomodulation were exhibited which correlated to the number of methoxy group including type 2 response of isopimpinellin (two methoxy group and lymphocyte activation) and type 3 immunomodulation of bergapten and xanthotoxin (one methoxy group and elevation of IFN-γ secretion), whereas the position of methoxy group affected the immunomodulating potency such as bergapten (5-methoxypsoralen) and xanthotoxin (8-methoxypsoralen) (Fig. 1, Table 3).

To determine which types of lymphocytes direct the patterns of immunomodulation, we used the flow cytometric analysis. As in type 1 immunomodulation (classical pattern) at 3 days post-treatment, lymphocyte activation was correlated to increase the number of lymphocyte cells including $CD8^+$ T cells and activated PBMC, while the enhancement of the secretion of IFN-γ might be due to the $CD8^+$ T cells because cell numbers also elevated in type 3 but not in type 2 response. The results showing $CD8^+$ T cells as the main cell types to secrete IFN-γ were in accordance with the observation made by Sad et al., despite the different species tested (Sad, Marcotte, & Mosmann, 1995). There were significant changes the $CD4^+/CD8^+$ ratio in type 1 immunomodulation because the ratio declined at 3 days and significantly increased at 6 days after treatment (Table 4).

The five popular vegetables and spices of Umbelliferae and its several bioactive constituents including flavonoids and coumarins were previously known to have nutritional, antimicrobial, anti-allergy and anti-inflammation activities. However, the immunosuppressive effects of the above-mentioned ingredients were at cytotoxic concentrations such as 140 μM of xanthotoxin (30 μg/ml) (Cox et al., 1988) on mitogen-activated lymphocytes (Lee et al., 1995; Namgoong et al., 1994) or UV-irradiated modification of xanthotoxin (Kuzel et al., 1992). In this study, we propose another way that non-toxic doses ($\leqslant$20 μg/ml) of the above-mentioned foods and their related ingredients might act to affect health as immuno-stimulating agents, i.e. directly enhancing of lymphocyte activation and/or secretion multipotent cytokine IFN-γ.

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