Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 103 (2007) 181–187
Food Chemistry
www.elsevier.com/locate/foodchem

Comparison of NO-scavenging and NO-suppressing activities of different herbal teas with those of green tea

Po-Jung Tsai [a], Tzung-Hsun Tsai [b], Chun-Hsien Yu [c], Su-Chen Ho [a,*]

[a] *Department of Food Science, Yuanpei University, No. 306, Yunapei Street, Hsinchu, Taiwan 300, Taiwan, ROC*
[b] *Department of Dentistry, Keelung Chang-Gung Memorial Hospital, Keelung, Taiwan*
[c] *Department of Paediatrics, Buddist Xindian Tzu Chi General Hospital, Taipei, Taiwan*

Received 15 March 2006; received in revised form 25 May 2006; accepted 2 August 2006

Abstract

Oxidative stress caused by the production of excess nitric oxide (NO) during infection or inflammation has been implicated in the pathogenesis of several diseases, including cancer, diabetes and renal disease. Accordingly, the scavenging of NO radical or/and suppression of NO production by mitogen-activated cells may be promising indicators in screening healthy food. In this work, the NO-scavenging and NO-suppressing activities of different herbal teas were determined and compared with those of green tea. All of the tested herbal teas revealed NO-scavenging and NO-suppressing activities. The NO-scavenging activity of herbal teas can be ranked by the IC_{50}, the concentration of the tested herbal tea required to quench 50% of NO radicals released by sodium nitroprusside. The activities follow the order: green tea > rosemary, sweet osmanthus, rose and lavender > jasmine, lemongrass and daisy. The NO-suppressing activity was evaluated, based on the suppressing effect of herbal teas on the production of NO by LPS-activated RAW 264.7 macrophages. Experimental results indicated that green tea and rosemary had IC_{50} values of less than 500 μg/ml, and were proven to be good NO-suppressors, whereas lavender, sweet osmanthus, lemongrass, rose, daisy and jasmine had IC_{50} values that exceeded 500 μg/ml, and were classified as rather poor NO-suppressors. In conclusion, consumption of herbal teas promotes the NO-scavenging and NO-suppressing activities of the diet, even though their activities are weaker than that of green tea.
© 2006 Elsevier Ltd. All rights reserved.

Keywords: Nitric oxide; Antioxidant; Phenolics; Herbal tea

1. Introduction

Although nitric oxide (NO) is identified initially as a vasodilator, its multiple functions and complex physiological and pathophysiological roles are revealed. In particular, nitric oxide (NO) and reactive nitrogen species (RNS), derived from the interaction of NO with oxygen or reactive oxygen species, have both been reported to participate in the development of oxidative tissue/cellular damage, which has been established as a mechanism of tissue damage. NO has therefore attracted considerable interests in the field of human health (Burney, Caulfield, Niles, Wishnok, & Tannenbaum, 1999). NO is a gaseous free radical that can be synthesized from arginine by nitric oxide synthase (NOS) in a biological system. Among the four NOS isoforms identified, the mitochondrial NOS discovered recently is less understood (Ghafourifar & Richter, 1997). Constitutive expressions of neural NOS and endothelial NOS produce a low basal level of NO, which is an important mediator of vasodilation and neurotransmission. Inducible NOS (iNOS) is induced quantitatively by inflammatory stimuli in various cells, such as macrophages, fibroblasts, smooth muscle cells

* Corresponding author. Tel.: +886 3 5381183x8496; fax: +886 3 6102342.
E-mail address: sche@mail.yust.edu.tw (S.-C. Ho).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.08.013

and hepatocytes. It is involved in immune defence against exogenous pathogens (Mayer & Hemmens, 1997). The production of excess NO by iNOS accelerates the formation of RNS, damages cellular macromolecules, such as proteins, DNA and lipids, and triggers many detrimental cellular responses (Radi, Beckman, Bush, & Freeman, 1991a; Radi, Beckman, Bush, & Freeman, 1991b; Yermilov et al., 1995). In fact, numerous diseases, including sepsis, cancer, diabetes, renal disease and atherosclerosis, are characterized by abnormal iNOS expression and high NO production (Beckman & Koppenol, 1996; Cooke & Dzau, 1997; Yu, Gengaro, Niederberger, Burke, & Schrier, 1994). The elimination of NO by NO scavenger and suppression of its production by iNOS inactivator helps to ameliorate such disease conditions (Hooper et al., 1997; Matheis et al., 1992; Menezes et al., 1999). Therefore, the scavenging of NO or suppression of NO production by iNOS are definitely promising indices of the health effects of food.

Herbs are valued for their aromatic and medicinal properties. Recently, the continuous development of functional foods and herbs has attracted substantial attention (Boxer, 1999). Indeed, the sales of herbal teas in Asian countries during recent years have clearly increased (Lee, 2004). However, scant information is available on the NO-scavenging and NO-suppressing activities of herbal tea, but more is available on the widely-consumed green tea. Accordingly, in this work, numerous herbal teas that are commonly consumed in Asia were examined for their NO-scavenging and NO-suppressing activities, which were compared with those of green tea.

2. Materials and methods

2.1. Materials

RAW 264.7 cells, a murine macrophage cell line, obtained from the Food Industry Research and Development Institute (Hsinchu, Taiwan), were cultured in DMEM medium supplemented with 10% heat-inactivated fetal bovine serum (Gibco BRL Life Technologies Inc., Grand Island, NY, USA). Lipopolysaccharide (LPS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), sulphanilamide, naphthylethylenediamine, Folin–Ciocalteu reagent, gallic acid and catechin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium nitroprusside was purchased from Roche Co. (Mannheim, Germany). All other chemicals were of analytical-grade purity.

2.2. Herbal tea preparation

Eight dried herbs, including daisy, jasmine, lavender, rose, sweet osmanthus, lemongrass, rosemary and green tea, were purchased from the local supermarket (Taipei, Taiwan). Herbal extract was prepared by steeping 10 g of the dried herbs in 200 ml of distilled water at 95 °C for 5 min. The infusion was collected by filtration with two layers of gauze, and then centrifuged at 12,000×*g* for 10 min to remove marcs. The supernatants were then collected and freeze–dried. The extraction yields of solids from daisy, jasmine, lavender, rose, sweet osmanthus, lemongrass, rosemary and green tea were 36.4, 39.4, 31.7, 17.2, 33.9, 15.9, 5.0 and 18.5%, respectively. For the cell culture experiment, the weighed extract solid was dissolved in DMEM and filtered through a membrane with 0.2 μm pores, and then diluted in DMEM to the indicated concentration.

2.3. Determination of NO-scavenging activity

The NO-scavenging activity of herbal tea extract was determined by the method of Sreejayan and Rao (1997) with some modification. Briefly, 60 μl of serial diluted sample were pipetted into a 96-well flat-bottomed plate. Following this, 60 μl of 10 mM sodium nitroprusside, dissolved in phosphate buffered saline (PBS), were added to each well and the plate was then incubated under light at room temperature for 150 min. Finally, an equal volume of Griess reagent was added into each well in order to measure the nitrite content. The NO-scavenging effect of herbal tea extracts was expressed as the IC_{50} which denotes the concentration of tested herbal tea extracts required to quench 50% of the NO radicals released by sodium nitroprusside.

2.4. Determination of NO-suppressing activity

In order to determine the effect of a herbal tea extract on NO production, 1×10^5 RAW 264.7 cells were seeded into 96-well culture plates. Following incubation for 24 h, the adherent cells were washed three times with PBS. The cells were then incubated in prepared DMEM medium, containing extracts from various herbs, either with or without LPS (100 ng/ml). Following incubation for 24 h, the medium was collected for nitrite assay, at which time cell viability was evaluated using the MTT method (Mosmann, 1983). Finally, medium nitrite concentration was measured as an indicator of NO production by use of the Griess reaction (Kim et al., 1995). The NO-suppressing effect of herbal tea extracts was expressed as the IC_{50} which denotes the concentration of herbal tea extracts causing 50% inhibition of NO production by LPS-activated RAW 264.7 cells.

2.5. Evaluation of antioxidant activity

The total antioxidant capacity of herbal tea was measured using a commercial kit (Randox Laboratories Ltd., Crumlin, UK) and expressed in millimoles of trolox equivalents per gramme of solid of herbal tea extract. This assay was based on 2,2'-azinobis(3-ethylbenzothiazoline sulfonate) (ABTS) incubated with metmyoglobin and hydrogen peroxide to generate the radical cation $ABTS^+$. $ABTS^+$ has

a stable blue–green colour, and the colour can be measured at 600 nm. Antioxidants in the sample suppress the development of colour to an extent that is proportional to their concentration.

2.6. Determination of total phenolics content

The amount of total phenolics in the extract of herbal tea was determined by the Folin–Ciocalteu colorimetric method (Singleton, Orthofer, & Lamuela-Raventos, 1999). Briefly, optimal diluted sample was reacted with Folin–Ciocalteu phenol reagent in alkaline solution. The absorbance at 765 nm was measured using a spectrophotometer. The total phenolics content in each herbal tea extract was then calculated by a standard curve prepared with gallic acid and expressed as milligrammes of gallic acid equivalents per gramme of solid of herbal tea extract.

2.7. Determination of total flavonoids content

Total flavonoids content in the herbal teas was determined by the method of Zhishen, Mengcheng, and Jianming (1999). Briefly, 0.25 ml of optimally diluted sample was added into a tube containing 1 ml of double-distilled water. Then, 0.75 ml of 0.5% $NaNO_2$, 0.075 ml of 10% $AlCl_3$, and 0.5 ml of 1 M NaOH were added at 0, 5 and 6 min, sequentially. Finally, the volume of reacting solution was adjusted to 2.5 ml with double-distilled water. The absorbance at 510 nm was detected using a spectrophotometer. The flavonoid content in each herbal tea was then calculated from a standard curve prepared with catechin and expressed as milligrammes of catechin equivalents per gramme of solid of herbal tea extract.

2.8. Statistical analysis

All results are presented as means ± SEM of at least three independent tests. The significances of the differences at each group were analyzed by ANOVA and Duncan's multiple range test. The correlation between two variants was analyzed by the Pearson test. All of the statistical analyses were performed by means of SPSS software with the level of significant difference set at $p < 0.05$ (SPSS for Windows, ver. 10.0; SPSS Inc. Chicago, IL, USA).

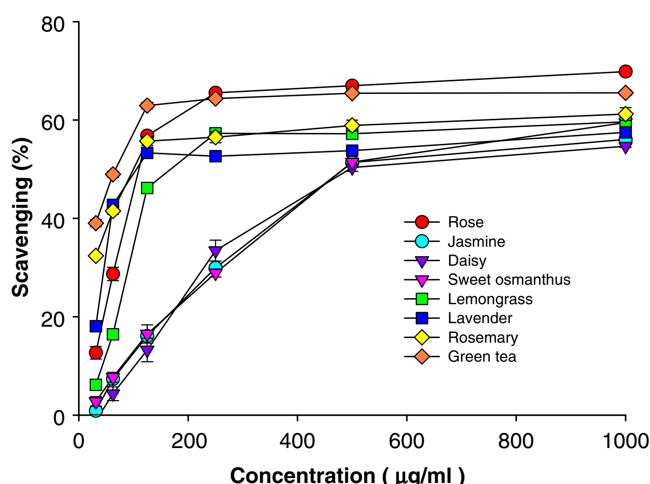

Fig. 1. NO-scavenging effect of various herbal teas.

3. Results

3.1. NO-scavenging activities of herbal teas

Fig. 1 presents the NO-scavenging effects of tested herbal teas. All of the tested herbal teas had NO-scavenging activity, with all exhibiting a two-stage NO-scavenging dose–response curve. A linear relationship between herb-concentration and NO-scavenging effect was demonstrated at concentrations below 250 µg/ml, while steady-state scavenging activity was shown at concentrations above 1000 µg/ml. The corresponding IC_{50} values for NO-scavenging ability were calculated from the linear range to concisely compare the relative NO-scavenging potencies of various herbal teas (Table 1). Green tea had an IC_{50} value below 200 µg/ml, and was a good NO-scavenger, whereas rosemary, sweet osmanthus, rose and lavender had IC_{50} values of between 200 and 400 µg/ml, indicating that they were only moderately effective NO-scavengers; jasmine, lemongrass and daisy, all of which had IC_{50} values above 600 µg/ml, were poor NO-scavengers.

3.2. NO-suppressing activities of herbal teas

The cell viability of the suspension was initially determined by a MTT method to prevent any possible cytotoxic

Table 1
IC_{50} of NO-scavenging and NO-suppressing of various herbal teas

Name	Botanical name	Used part	IC_{50} of NO-scavenging (µg/ml)	IC_{50} of NO-suppressing (µg/ml)
Daisy	*Chrysanthemum morifolium*	Flowers	730	948
Jasmine	*Jasminum officinale*	Flowers	668	ND[a]
Lavender	*Lavandula officinalis*	Flowers	270	589
Rose	*Rosa damascena*	Flowers	220	914
Sweet osmanthus	*Osmanthus fragrans Lour*	Flowers	204	740
Lemongrass	*Cymbopogon citratus*	Leaves	710	858
Rosemary	*Rosmarinus officinalis*	Leaves	200	209
Green tea	*Camellia sinensis*	Leaves	144	113

[a] ND: not detectable.

effect of herbal teas on NO production. No herbal tea at the concentrations used herein significantly influenced cell viability (viability for all was >90% of that of the control group). Analysis of NO production, by measuring the nitrite level with the Griess reaction, indicated that placing un-stimulated RAW 264.7 cells in culture medium for

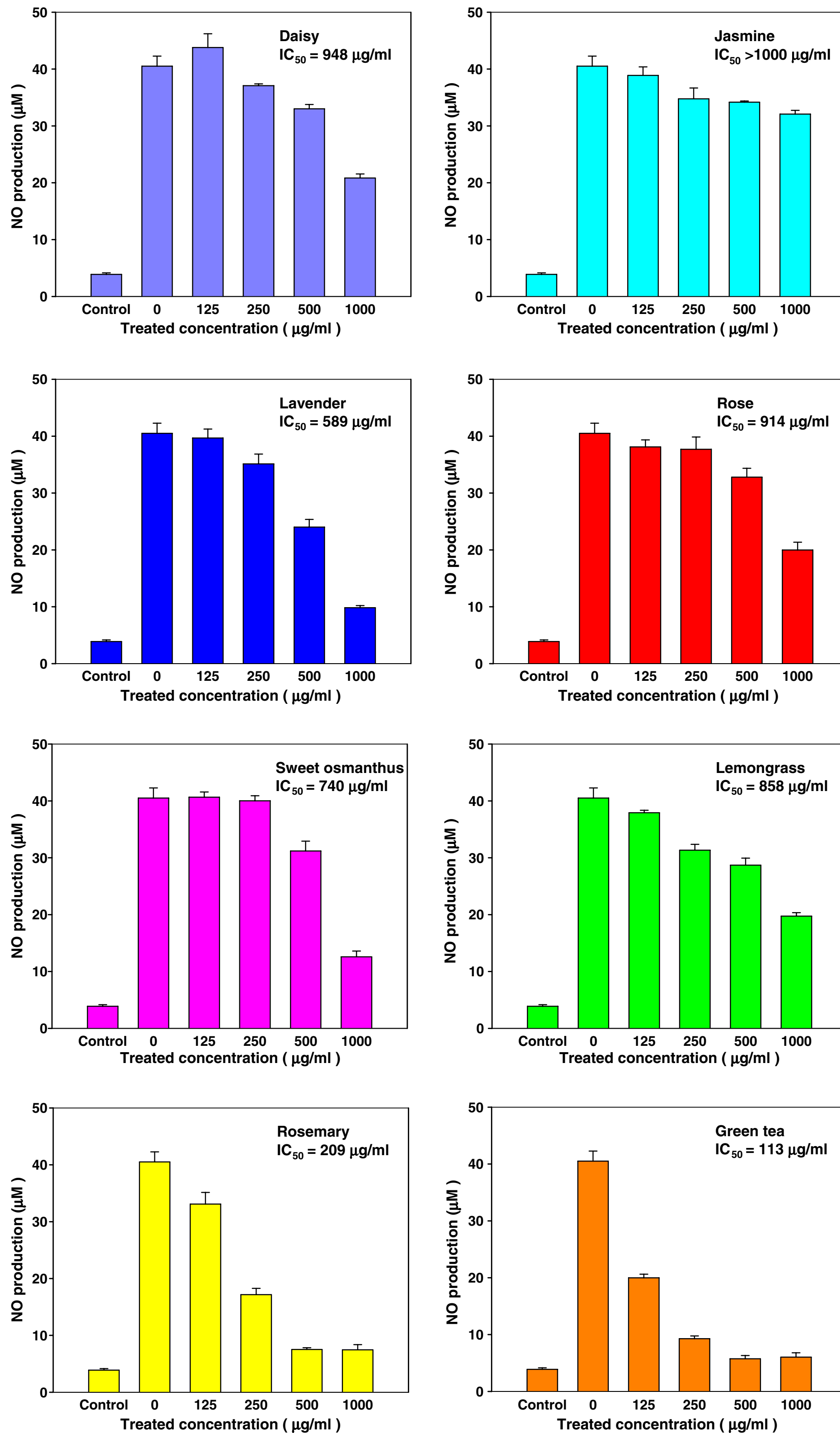

Fig. 2. Effect of various herbal teas on the NO production in LPS-activated RAW 264.7 cells. The values are expressed as means ± SEM of triplicate tests. IC_{50} denotes the concentration of herbal tea extracts causing 50% inhibition of NO production by LPS-activated RAW 264.7 cells.

Table 2
Total antioxidant activity, total phenolics and flavonoid contents of various herbal teas[a]

Name	Total antioxidant (mmol TE/g extract)	Total phenolics (mg GAE/g extract)	Total flavonoids (mg CE/g extract)
Daisy	0.43 ± 0.08^{e}	38.0 ± 1.2^{e}	26.3 ± 0.2^{e}
Jasmine	0.43 ± 0.09^{e}	45.3 ± 2.1^{e}	15.2 ± 0.3^{f}
Lavender	0.88 ± 0.01^{d}	74.0 ± 2.1^{d}	68.6 ± 2.0^{c}
Rose	1.35 ± 0.17^{c}	141 ± 5.2^{b}	22.0 ± 1.2^{e}
Sweet osmanthus	1.76 ± 0.05^{b}	149 ± 3.8^{b}	183 ± 2.4^{a}
Lemongrass	0.45 ± 0.09^{e}	39.2 ± 1.0^{e}	17.2 ± 0.8^{f}
Rosemary	1.66 ± 0.01^{b}	123 ± 2.8^{c}	124 ± 2.4^{b}
Green tea	3.04 ± 0.21^{a}	237 ± 14.5^{a}	44.9 ± 1.3^{d}

[a] The values are expressed as means ± SEM of triplicate tests. Means not sharing a common letter were significantly different ($p < 0.05$) when analyzed by ANOVA and Duncan's multiple range test.

24 h yielded a basal quantity of nitrite, whereas stimulating them with LPS for 24 h rapidly increased the concentration of nitrite (Fig. 2). Moreover, significant concentration-dependent inhibition of NO production was observed when cells were co-treated with LPS and various concentrations of the various herbal teas (Fig. 2). The NO-suppressing activity of herbal teas was ranked based upon IC_{50}, as follows: green tea (113 μg/ml) > rosemary (209 μg/ml) > lavender (589 μg/ml) > sweet osmanthus (740 μg/ml) > lemongrass (858 μg/ml) > rose (914 μg/ml) > daisy (948 μg/ml) > jasmine (>1000 μg/ml).

3.3. Total antioxidant capacity of herbal teas

This study adopted an ABTS assay, which has been demonstrated to be better than the DPPH assay for evaluating the total antioxidant capacity of foods (Lee, Kim, Lee, & Lee, 2003). Table 2 presents total antioxidant capacities of various herbal teas. Green tea had the strongest antioxidant capacity of all of the tested herbal teas, with 3.04 mmol of trolox equivalents per gramme of tea extract. The total antioxidant capacities of the other tested herbal teas were ranked in decreasing order as follows: sweet osmanthus and rosemary > rose > lavender > lemongrass, daisy and jasmine.

3.4. Total phenolic and flavonoid content of herbal teas

Table 2 lists the total phenolic and flavonoid contents of various herbal teas. Green tea contained more phenolics than did any of the other tested herbal teas, with 237 mg gallic acid equivalent per gramme of tea extract. Daisy, lemongrass and jasmine were low in phenolics, containing only 38.0, 39.2 and 45.3 mg of gallic acid equivalents per gramme of tea extract (Table 2). Clearly, the tested herbal teas had widely ranging phenolic contents. Additionally, the phenolic content of a particular herbal tea was highly correlated with its total antioxidant capacity ($r = 0.984$, $p < 0.001$) and the IC_{50} for NO-scavenging ($r = -0.843$, $p < 0.01$). However, no significant correlation existed between phenolic content and NO-suppressing activity. The relative concentration pattern of flavonoids in various herbal teas clearly differed from that of phenolics. Sweet osmanthus contained the most flavonoids, with 183 mg of catechin equivalents per gramme of extract. Unlike the phenolic content, the flavonoid content of green tea was rather poor, with only 44.9 mg of catechin equivalents per gramme of tea extract. Furthermore, the flavonoid content of herbal tea was correlated with neither the total antioxidant capacity nor NO-scavenging activity.

4. Discussion

Of the herbal teas tested herein, green tea is the strongest NO-scavenger and NO-suppressor. Indeed, the potent NO-scavenging and NO-suppressing abilities of *Camellia sinensis* have been demonstrated previously. Catechins, particularly EGCG, are assumed to be the main constituents of green tea that are responsible for NO-scavenging and NO-suppressing abilities (Nakagawa & Yokozawa, 2002; Paquay et al., 2000). Although tea produced from *C. sinensis* is the most popular healthy beverage throughout the world and so, would contribute a large proportion of the NO-suppressing capacity of the typical diet, the consumption of tea prepared from *C. sinensis* is still limited for people who suffer from the side effects of caffeine. Additionally, around 80% of the dried tea manufactured annually is consumed as black tea. Our earlier study indicated that black tea exhibited only 50% of the NO-suppressing capacity of green tea, possibly because of the reduction in polyphenolic content caused by fermentation (Lin, Lu, Chen, & Ho, 2006). Therefore, alternative beverages with good NO-suppressing characteristics must be evaluated and developed. In this work, all of the tested herbal teas revealed some NO-scavenging and NO-suppressing activities, and rosemary was the second most powerful, after green tea. Numerous polyphenolic compounds with antioxidant activity have been identified in rosemary (Uhl, 2000). The main constituents, carnosol and carnosic acid, which contribute 90% of the antioxidant activity, have been shown to suppress the LPS-induced production of NO by mouse peritoneal cells (Chan, Ho, & Huang, 1995). In addition to being used as a culinary spice, rosemary traditionally acts as an analgesic, a sedative, a diuretic and an antibacterial agent (Tainter & Grenis, 2001). Furthermore, this work provided experimental results to demonstrate that rosemary is appropriate to develop as a health beverage.

The phenolic content of foodstuffs correlated strongly with $ABTS^{+}$, DPPH and O_2^- radical-scavenging activities, so phenolics are recognized as main contributors to free radical- scavenging activity (Katsube et al., 2004; Parejo et al., 2002; Tsai, Tasi, & Ho, 2005). This study also reveals a significant correlation between total phenolic content and $ABTS^{+}$-scavenging activity. Additionally, NO-scavenging activity is herein correlated with total phenolic content, indicating that the NO-scavenging capacity of herbs was also governed by the phenolic content. Various components

of foods or herbs, such as polyphenols that are present in wine, catechins in tea, and tannins and alkaloids in herbs, have been reported to be responsible for NO-scavenging activity (Paquay et al., 2000; Verhagen, Haenen, & Bast, 1996). Furthermore, several flavonoids (Van Acker, Tromp, Haenen, Van der Vijgh, & Bast, 1995), curcuminoids (Sreejayan & Rao, 1997) and some phenolics in olive oil (De la Puerta, Domingue, Rui-Gutierre, Flavill, & Hoult, 2001) have also been established to be powerful NO-scavengers. The structural feature of flavonoids that is responsible for scavenging NO has been examined. For instance, the catechol group is a basic requirement for excellent NO-scavenging and gallic acid linked to flavan-3-ol is more important in the scavenging of NO by catechins (De la Puerta et al., 2001). In fact, the structural features of flavonoids needed for high NO-scavenging activity are also applicable to the scavenging of other reactive oxygen/nitrogen species, such as superoxide anions and peroxynitrite (Chung et al., 1998; Haenen, Paquay, Korthouwer, & Bast, 1997; Heijnen, Haenen, Van Acker, Van der Vijgh, & Bast, 2001; Taubert et al., 2003).

Unlike NO-scavenging activity, the NO-suppressing activity of herbs is correlated with neither phenolic nor flavonoid contents. This finding is consistent with the authors' earlier study (Tsai et al., 2005). The suppressing effect of herbs on the production of NO by LPS-activated cells is the combined effect of three actions: (1) the blocking of iNOS expression, (2) the inactivation of iNOS catalytic function and (3) the scavenging of NO radical (Sheu & Yen, 2001). Various compounds in herbs are differently involved in NO-suppression and have additive or synergic effects. Consequentially, the NO-suppressing activity of herbs is not attributable simply to the phenolic content. Flavones and saponins have also been suggested to be the main active constituents of herbs that are responsible for anti-inflammatory activity (Sasikumar, 2004). The structure–activity relationships of flavonoids, in inhibiting NO production, and their mechanisms of action, have been clarified. Flavones exhibit stronger activity than do the corresponding flavonols, flavanones and isoflavones. The glycoside moiety reduces activity. The inhibition of iNOS gene expression is documented as the main NO-suppressing action of flavonoids. (Kim, Cheon, Kim, Kim, & Kim, 1999; Matsuda, Morikawa, Ando, Toguchida, & Yoshikawa, 2003). However, the molecules in the tested herbs that participate in the regulation of iNOS have not been clearly identified and further research is required in this regard.

Owing to abundant phytochemicals and their aroma, herbs have become a vital source of chemopreventive agents. This work provides comparative information about several herbs with respect to their NO-scavenging and NO-suppressing activities. According to those results, rosemary, next to green tea, exhibits potent NO-scavenging and NO-suppressing activities, and is highly promising for development as an antioxidant healthy beverage, protective against NO-mediated damage.

Acknowledgment

The authors would like to thank the Keelung Chang-Gung Memorial Hospital, Keelung, Taiwan, for financially supporting this research under Contract No. CMRPG-240241.

References

Beckman, J. S., & Koppenol, W. H. (1996). Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *American Journal of Physiology, 271*, C1424–C1437.

Boxer, A. (1999). *Herb and spice handbook*. San Francisco, Calif: Soma.

Burney, S., Caulfield, J. L., Niles, J. C., Wishnok, J. S., & Tannenbaum, S. R. (1999). The chemistry of DNA damage from nitric oxide and peroxynitrite. *Mutation Research, 424*, 37–49.

Chan, M. M-Y., Ho, C-T., & Huang, H-I. (1995). Effects of three dietary phytochemicals form tea, rosemary and turmeric on inflammation-induced nitrite production. *Cancer Letters, 96*, 23–29.

Chung, H. Y., Yokozawa, T., Soung, D. Y., Kye, I. S., No, J. K., & Baek, B. S. (1998). Peroxynitrite-scavenging activity of green tea tannin. *Journal of Agricultural and Food Chemistry, 46*, 4484–4486.

Cooke, J. P., & Dzau, V. J. (1997). Nitric oxide synthase: role in the genesis of vascular disease. *Annual Review of Medicine, 48*, 489–509.

De la Puerta, R., Domingue, M. E. M., Rui-Gutierre, V., Flavill, J. A., & Hoult, J. R. S. (2001). Effects of virgin olive oil phenolics on scavenging of reactive nitrogen species and upon nitrergic neurotransmission. *Life Science, 69*, 1213–1222.

Ghafourifar, P., & Richter, C. (1997). Nitric oxide synthase activity in mitochondria. *FEBS Letters, 418*, 291–296.

Haenen, G. R. M. M., Paquay, J. B. G., Korthouwer, R. E. M., & Bast, A. (1997). Peroxynitrite scavenging by flavonoids. *Biochemical and Biophysical Research Communications, 236*, 591–593.

Heijnen, C. G. M., Haenen, G. R. M. M., Van Acker, F. A. A., Van der Vijgh, W. J. F., & Bast, A. (2001). Flavonoids as peroxynitrite scavengers: the role of the hydroxyl groups. *Toxicology in Vitro, 15*, 3–6.

Hooper, D. C., Bagasra, O., Marini, J. C., Zborek, A., Ohnishi, S. T., Kean, R., et al. (1997). Prevention of experimental allergic encephalomyelitis by targeting nitric oxide and peroxynitrite: implications for the treatment of multiple sclerosis. *Proceedings of the National Academy of Science of the USA, 94*, 2528–2533.

Katsube, T., Tabata, H., Ohta, Y., Yamasaki, Y., Anuurad, E., Shiwaku, K., et al. (2004). Screening for the antioxidant activity in edible plant products: comparison of low-density lipoprotein oxidation assay, DPPH radical scavenging assay, and Folin–Ciocalteu assay. *Journal of Agricultural and Food Chemistry, 52*, 2391–2396.

Kim, H. K., Cheon, B. S., Kim, Y. H., Kim, S. Y., & Kim, H. P. (1999). Effects of naturally occurring flavonoids on nitric oxide production in the macrophage cell line RAW 264.7 and their structure–activity relationships. *Biochemical Pharmacology, 58*, 759–765.

Kim, H., Lee, H. S., Chang, K. T., Ko, T. H., Baek, K. J., & Kwon, N. S. (1995). Chloromethyl ketones block induction of nitric oxide synthase in murine macrophages by preventing activation of nuclear factor-κB. *Journal of Immunology, 154*, 4741–4748.

Lee, H. (2004). Have a cuppa, with fruits & herbs. *Asia-Pacific-Food-Industry, 16*(9), 56–59.

Lee, K. W., Kim, Y. J., Lee, H. J., & Lee, C. Y. (2003). Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. *Journal of Agricultural and Food Chemistry, 51*, 7292–7295.

Lin, C.-C., Lu, M.-J., Chen, S.-J., & Ho, S.-C. (2006). Heavy fermentation impacts NO-supressing activity of tea in LPS-activated RAW 264.7 macrophages. *Food Chemistry, 98*, 483–489.

Matheis, G., Sherman, M. P., Buckberg, G. D., Haybron, D. M., Young, H. H., & Ignarro, L. J. (1992). Role of L-arginine–nitric oxide pathway in myocardial reoxygenation injury. *American Journal of Physiology, 262*, H616–H620.

Matsuda, H., Morikawa, T., Ando, S., Toguchida, I., & Yoshikawa, M. (2003). Structural requirements of flavonoids for nitric oxide production inhibitory activity and mechanism of action. *Bioorganic and Medicinal Chemistry, 11*, 1995–2000.

Mayer, B., & Hemmens, B. (1997). Biosynthesis and action of nitric oxide in mammalian cells. *Trends in Biochemical Science., 22*, 477–481.

Menezes, J., Hierholzer, C., Watkins, S. S., Lyons, V., Peitzman, A. B., Billiar, T. R., et al. (1999). A novel nitric oxide scavenger decreases liver injury and improves survival after hemorrhagic shock. *American Journal of Physiology, 277*, G144–G151.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assay. *Journal of Immunological Methods, 65*, 55–63.

Nakagawa, T., & Yokozawa, T. (2002). Direct scavenging of nitric oxide by green tea. *Food and Chemical Toxicology, 40*, 1745–1750.

Paquay, J. B. G., Haenen, G. R. M. M., Stender, G., Wiseman, S. A., Tijburg, L. B. M., & Bast, A. (2000). Protection against nitric oxide toxicity by tea. *Journal of Agricultural and Food Chemistry, 48*, 5768–5772.

Parejo, I., Viladomat, F., Bastida, J., Rosas-Romero, A., Flerlage, N., Burillo, J., et al. (2002). Comparison between the radical scavenging activity and antioxidant activity of six distilled and nondistilled Mediterranean herbs and aromatic plants. *Journal of Agricultural and Food Chemistry, 50*, 6882–6890.

Radi, R., Beckman, J. S., Bush, K. M., & Freeman, B. A. (1991a). Peroxynitrite oxidation of sulhydryls. *Journal of Biological Chemistry, 266*, 4244–4250.

Radi, R., Beckman, J. S., Bush, K. M., & Freeman, B. A. (1991b). Peroxynitrite induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. *Archive of Biochemistry and Biophysiology, 288*, 481–487.

Sasikumar, B. (2004). Rosemary. In K. V. Peter (Ed.). *Hand book of herbs and spices* (2, pp. 243–255). Boca Raton: CRC.

Sheu, F., & Yen, G-C. (2001). Modulation of nitric oxide production by foodstuffs. *Food Science and Agricultural Chemistry, 3*(2), 42–58.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocateu reagent. *Methods in Enzymology, 299*, 152–178.

Sreejayan, N., & Rao, M. N. A. (1997). Nitric oxide scavenging by curcuminoids. *Journal of Pharmacology and Pharmacology, 49*, 105–107.

Tainter, D. R., & Grenis, A. T. (2001). *Spices and seasonings*. New York: Wiley.

Taubert, D., Breitenbach, T., Lazar, A., Censarek, P., Harlfinger, S., Berkels, R., et al. (2003). Reaction rate constants of superoxide scavenging by plant antioxidants. *Free Radical Biology and Medicine, 35*(12), 1599–1607.

Tsai, T-H., Tasi, P-J., & Ho, S-C. (2005). Antioxidant and anti-inflammatory activities of several commonly used spices. *Journal of food Science, 70*(1), C93–C97.

Uhl, S. R. (2000). *Handbook of spices, seasonings, and flavorings*. Lancaster, Pa: Technomic Pub.

Van Acker, S. A. B. E., Tromp, M. N. J. L., Haenen, G. R. M. M., Van der Vijgh, W. J. F., & Bast, A. (1995). Flavonoids as scavengers of nitric oxide radical. *Biochemical and Biophysical Research Communications, 214*(3), 755–759.

Verhagen, J. V., Haenen, G. R. M. M., & Bast, A. (1996). Nitric oxide radical scavenging by wines. *Journal of Agricultural and Food Chemistry, 44*, 3733–3734.

Yermilov, V., Rubio, J., Becchi, M., Friesen, M. D., Pignatelli, B., & Ohshima, H. (1995). Formation of 8-nitroguanine by the reaction of guanine with peroxynitrite in vitro. *Carcinogenesis, 16*, 2045–2050.

Yu, L., Gengaro, P. E., Niederberger, M., Burke, T. J., & Schrier, R. W. (1994). Nitric oxide: a mediator in rat tubular hypoxia/reoxygenation injury. *Proceedings of the National Academy of Science of the USA, 91*, 1691–1695.

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry, 64*, 555–559.