Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 106 (2008) 929–936
Food Chemistry
www.elsevier.com/locate/foodchem

Relative antioxidant and cytoprotective activities of common herbs

Kyung Mi Yoo [a,b], Choong Hwan Lee [b], Hyungjae Lee [a], BoKyung Moon [c], Chang Yong Lee [a,*]

[a] Department of Food Science and Technology, Cornell University, Geneva, NY 14456, United States
[b] Division of Bioscience and Biotechnology, BMIC, Konkuk University, Seoul, Republic of Korea
[c] Department of Food and Nutrition, Chung-Ang University, 72-6 Nae-ri, Daedeok-myeon, Anseong-si, Gyeonggi-do, Republic of Korea

Received 4 June 2007; received in revised form 29 June 2007; accepted 3 July 2007

Abstract

Many studies have been carried out on bioactivities of individual herbs, however, no collective study on their comparative antioxidant and cytoprotective activities against oxidative damage has been reported. We selected 17 common commercial herbs and studied their relative phenolic contents, antioxidant activities, and cytoprotective activities on gap–junction intercellular communication and antioxidative enzymes *in vitro* under the same conditions. Total polyphenol content ranged from 464 to 870 gallic acid equivalents (GAE) mg/100 g and total flavonoid content from 212 to 494 catechin equivalents (CE) mg/100 g. Among the samples, chamomile, rosehip, hawthorn, lemon verbena, and green tea contained relatively high total phenolics (769–844 mg GAE/100 g) and flavonoids (400–4 mg CE/100 g). Chamomile also showed the highest antioxidant activity with 960 mg/100 g of vitamin C equivalent (VCE), followed by hawthorn (929 mg VCE/100 g) and black tea (916 mg VCE/100 g). Total phenolic and total flavonoids showed a higher correlation with antioxidant activity. Most of herbs enhanced cell viability and showed protective effects against oxidative stress induced by hydrogen peroxide in Chinese hamster lung fibroblast (V79-4) cells. Furthermore, herbs used in this study showed higher protective effect on gap–junction intercellular communication (GJIC) as compared to gallic acid and catechin, and also enhanced activity of the antioxidative enzymes such as superoxide dismutase (SOD) and catalase (CAT) in a dose-dependent manner.

Keywords: Herbs; Antioxidant; V79-4 cells; Gap–junction intercellular communication; SOD; CAT

1. Introduction

Bioactive compounds commonly found in fruits, vegetables, herbs, and other plants have been shown to have possible health benefits with antioxidative, anticarcinogenic, atherosclerosis, antimutagenic, and angiogenesis inhibitory activities (Cao & Cao, 1999; Geleijnse, Launer, Hofman, Pols, & Witteman, 1999; Kahkonen et al., 1999; Yen, Duh, & Tsai, 2002). Interestingly, many herbs are known to contain large amounts of phenolic antioxidants other than well-known vitamin C, vitamin E, and carotenoids. Phenolic antioxidants in herbs are mainly composed of phenolic acids (Cao & Cao, 1999), flavonoids (Madsen & Bertelsen, 1995), and catechins (Shahidi, Janitha, & Wanasundara, 1992). Some phenolic compounds in herbs have the capacities to quench lipid peroxidation, prevent DNA oxidative damage, and scavenge reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and hydroxyl radicals (Cao & Cao, 1999; Kahkonen et al., 1999). A large number of studies have been reported using different analytical tests on individual herbs in hydrophilic and lipophilic systems (Kahkonen et al., 1999; Khokhar & Magnusdottir, 2002; Wiseman, Balentine, & Frei, 1997; Zheng & Wang, 2001). However, there is only limited information on the comparative study on phenolic contents, antioxidant activities against oxidative stress, and

* Corresponding author. Tel.: +1 315 787 2271; fax: +1 315 787 2284.
E-mail address: cyl1@cornell.edu (C.Y. Lee).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.07.006

bioactivities of herbs (Capecka, Mareczek, & Leja, 2005; Simandi et al., 2001; Weinberg, Akiri, Potoyevski, & Kanner, 1999; Zaporozhets, Krushynska, Lipkovska, & Barvinchenk, 2004).

All living organisms have endogenous defense systems against oxidative damage, such lipid peroxidation, DNA damage (Lee, Hur, Lee, & Lee, 2005), and inhibition of cell communication (Sigler & Ruch, 1993), due to reactive oxygen species (ROS). There are two main antioxidant defense mechanisms: first, antioxidant defense with enzymes such as superoxide dismutase (SOD) which catalyses dismutation of superoxide anions to hydrogen peroxide; catalase (CAT) which converts hydrogen peroxide (H_2O_2) into molecular oxygen and water: second, antioxidant defense with non-enzymatic components, such as polyphenols, ascorbic acid, and carotenoids (Rice-Evans, Miller, & Paganga, 1997; Shahidi et al., 1992).

Gap–junction intercellular communication (GJIC) is essential for maintaining the homeostatic balance by modulating cell proliferation and differentiation in multi-cellular organisms and it is related to anticancer mechanism (Trosko & Chang, 2000). The chronic inhibition of GJIC may induce a chronic dysfunctional control of homeostasis that can lead to the proliferation of initiated cells, which, in turn, can be strongly related to neoplasm conversion in carcinogenesis (Sigler & Ruch, 1993). Therefore, the maintaining normal GJIC is essential for good health.

The aim of the present study was to measure the relative content of phenolics in commonly available commercial herbs and evaluate their antioxidant capacity and cytoprotective activities under the same experimental conditions. The contents of total phenolics and flavonoids in 17 selected herbs were analyzed and their antioxidant and anticancer activities were measured by their abilities to scavenge free radical and to protect cell viability. The protective effect of herbs was measured against the inhibition of GJIC induced by H_2O_2 in V79-4 cells. In addition, the bioactivity of the herbs on the antioxidative enzymes such as SOD and CAT was investigated.

2. Materials and methods

2.1. Chemicals

H_2O_2, vitamin C, gallic acid, catechin, catalase, 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as diammonium salt, hesperidin, naringin, 2-deoxyribose, sodium monobasic phosphate, sodium dibasic phosphate, 2′,7′-dichlorodihydrofluorescein diacetate (DCF-DA), and Folin–Ciocalteu phenol reagent were obtained from Sigma (St. Louis, MO, USA). 2,2′-Azobis-(2-amidinopropane)-dihydrochloride (AAPH), DPPH (1,1-diphenyl-2-picrylhydrazyl), and ethylenediaminetetraacetic acid (EDTA) were obtained from Wako Chemicals (Richmond, VA, USA). All other chemicals used were analytical grade (Fisher, Springfield, NJ, USA). All solvents were of HPLC grade.

2.2. Herbs

With the exception of chamomile (*Chamaemelum nobilis* L.), hawthorn (*Crataegus monogyna* Jacq.), dandelion (*Taraxacum officinalis*), fennel (*Foeniculum vulgare*), green tea (*Camellia sinensis* L.), jasmine (*Jasminum*), lavender (*Lavendula angustifolia* Mill), lemon grass (*Cymbopogon citratus*), lemon verbena (*Aloysia triphylla*), peppermint (*Mentha piperita* L.), rooibos (*Aspalathus iinearis* R dahlgr.), rosehip (*Rosa rubiginosa*), rosemary (*Rosemarinus officinalis* L.), and thyme (*Thymus vulgaris* L.) leaves that were harvested from their natural habitat in the Jangwon environ, the rest of samples, bergamot (*Citrus bergamia*), black tea (*C. sinensis* L.), and blue gum tree (*Eucalyptus globules LABILL*) leaves, were bought from the local markets in two cities (Seoul and Yongpang) in Korea. Each sample was cleaned with tap water and freeze dried. The freeze-dried samples were ground to powder (FM-700W food mixer, Han Il, Korea) and then stored at −20 °C until analyzed.

2.3. Preparation of extraction

Herbs leaves were extracted from 10 g of ground freeze-dried herbs using 100 mL of 70% aqueous methanol in a 250 mL round-bottomed flask. The mixture of herbs powder and solvent was sonicated for 30 min with continual nitrogen gas purging. The mixture was filtered through Whatman #2 filter paper (Whatman International Limited, Kent, UK) using a chilled Bűchner funnel and rinsed with 50 mL of 70% aqueous methanol. The filter cake was re-extracted by repeating the above steps under the same conditions. The two filtrates were obtained and transferred into a 1 L round-bottomed flask with an additional 50 mL of distilled water. The solvent was evaporated under reduced pressure at 40 °C. The phenolic concentrate was dissolved in 50 mL of methanol and made to a final volume of 100 mL with methanol. The solution was then centrifuged in a Sorvall RC-5B refrigerated superspeed centrifuge (Du Pont Company, Biomedical Products Department, Wilmington, DE, USA) at 8000*g* for 15 min at 4 °C. The final extract solution was stored at −4 °C until analyzed.

2.4. Evaluation of antioxidant activity

2.4.1. Total phenolics contents

The total contents of phenolic phytochemicals were measured using the Folin–Ciocalteu method (Rakitzis, 1975). Briefly, 1 mL of appropriately diluted samples or a standard solution of gallic acid was added to a 25 mL volumetric flask containing 9 mL of distilled water. A reagent blank was prepared using distilled water. One milliliter of Folin–Ciocalteu phenol reagent was added to the mixture and mixed by shaking. After 5 min, 10 mL of 7% Na_2CO_3 solution were added with mixing. The solution was then immediately diluted to a volume of 25 mL with ddH_2O and mixed thoroughly. After incubation for 90 min at

23 °C, the absorbance relative to that of a prepared blank was read at 750 nm using a spectrophotometer (Model DU 530, Beckman, MO, USA). The total phenolic contents are expressed here in milligrams of gallic acid equivalents (GAE) per 100 g of fresh herb.

2.4.2. Total flavonoids contents

The flavonoid content was measured using a colorimetric assay developed previously (Zhishen, Mengcheng, & Jianming, 1999). One milliliter of the extracts or standard solutions of catechin was added to a 10 mL volumetric flask. Distilled water was added to make a volume of 5 mL. At zero time, 0.3 mL of 5% (w/v) sodium nitrite was added to the flask. After 5 min, 0.6 ml of 10% (w/v) $AlCl_3$ was added and, then 6 min, 2 mL of 1 M NaOH were also added to the mixture, followed by the addition of 2.1 mL distilled water. Absorbance was read at 510 nm against the blank (water) and flavonoid content was expressed as mg catechin equivalents per 100 g of fresh herb. Samples were analyzed in triplicate.

2.4.3. Total antioxidant capacity

The total antioxidant capacity was expressed by VCEAC (vitamin C equivalent antioxidant capacity) (Kim, Lee, Lee, & Lee, 2002). The AAPH (1 mM) was mixed with 2.5 mM ABTS as diammonium salt in phosphate buffered saline (PBS) solution (100 mM potassium phosphate buffer (pH 7.4) containing 150 mM NaCl). After the mixture was heated in a water bath at 68 °C for 13 min, the blue–green ABTS solution was adjusted with fresh PBS solution to an absorbance of 0.650 at 734 nm. Twenty microliters of the sample solution added to 980 μL of the ABTS radical solution were incubated in a water bath at 37 °C for 10 min. The decrease of absorbance at 734 nm was measured at 10 min. The control consisted of 20 μL of 50% methanol and 980 μL of ABTS solution. The ABTS radical scavenging capacities of herb extracts were expressed on the fresh weight basis as mg of vitamin C equivalent antioxidant capacity per 100 g (VCEAC). Samples of each extraction were analyzed in triplicate. Vitamin C standard curves at, 2.5, 5, 10, 20, 40, 60, 80, and 100 mg/L concentrations of L-ascorbic acid, were obtained using the ABTS. The absorbance reduction at 734 nm of ABTS solution by selected pure polyphenol was measured at concentrations of 2.5, 5, 10, and 20 mg/L.

2.4.4. DPPH radical scavenging activity

Effect of the herb extracts on DPPH free radical was measured, based on previous study (Brand-Williams, Cuvelier, & Berset, 1995). Positive control was prepared by mixing 4 mL of ascorbic acid (0.05 mg/mL) and 1 mL of DPPH (0.4 mg/mL), whereas negative control was prepared by mixing distilled water with 1 mL of DPPH. Four milliliter of the extract was added to 1 mL DPPH. The mixture was gently homogenized and left to stand at room temperature for 30 min. Absorbance was read at 520 nm using a spectrophotometer. The ability of extract to scavenge DPPH free radical was calculated using the following equation:

$$\text{Radical scavenging activity } (\%) = (A - A_1)/A \times 100$$

where A was the absorbance of the control, and A_1 was the absorbance of the test sample.

2.5. Evaluation of various herbs on the intracellular antioxidant systems

2.5.1. Cell culture

Chinese hamster lung fibroblast (V79-4) cells from the American Type Culture Collection (ATCC) were maintained at 37 °C in an incubator with a humidified atmosphere of 5% CO_2 and cultured in Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal bovine serum, streptomycin (100 μg/mL), and penicillin (100 units/mL).

2.5.2. Cell viability

The effect of various herbs on the viability of the V79-4 cells was determined using the MTT (3-4,5-dimethylthiazol-*Z*-yl-2,5-diphenyltetrazolium bromide) assay, which is based on the reduction of a tetrazolium salt by mitochondrial dehydrogenases in viable cells (Kang et al., 2005). V79-4 cells were seeded in a 96 well plate at a concentration of 1.5×10^5 cells/mL. Sixteen hours after plating, cells were treated with two concentrations of herbs. Cells were incubated for 24 h at 37 °C. During the last 4 h, cells were incubated with 20 μL medium of MTT stock solution in 200 μL medium at 37 °C. Each cell was then extracted with acidic isopropanol and the absorbance was measured with an ELISA reader (Bio-Rad, Hercules, CA, USA) at 570 nm. The relative cell viability was determined by the amount of MTT converted to the insoluble formazan salt. Absorbance reductions of the samples viability was determined by absorbance reductions of the samples at various concentrations when compared with untreated control. All data are presented as mean ± standard deviation (SD) for at least six replications for each prepared sample.

2.5.3. Bioassay of GJIC

The GJIC was measured by the scrape-loading/dye-transfer technique as shown by Lee et al. (2005). Briefly, V79-4 cells were treated with various concentrations of herb extract with 300 μM H_2O_2 for 1 h. The GJIC assay was conducted at non-cytotoxic doses of the samples, as determined by MTT assay. Following incubation, the cells were washed twice with 2 mL of phosphate buffer solution (PBS). Lucifer yellow was added to the washed cells, and three scrapes were made with a surgical steel-bladed scalpel at low light intensities. Each scrape was performed so as to ensure that it traversed a large group of confluent cells. After an incubation period of 3 min, the cells were washed four times with 2 mL of PBS and then fixed with 2 mL of a 4% formalin solution. The number of communicating cells as indicated by the dye was counted under an inverted fluorescence microscope (Olympus I × 70, Okaya, Japan).

2.5.4. Superoxide dismutase (SOD) activity

SOD activity was measured, based on Kang et al. (2005). NBT was reduced to blue formazan by O_2^-, which has a strong absorbance at 560 nm; however, the presence of SOD inhibits this reaction. The cells were homogenized in 0.05 M sodium carbonate buffer (pH 10.2). The assay mixture consisted of 0.05 M sodium carbonate buffer (pH 10.2) containing 3 mM xanthine, 0.75 mM NBT, 3 mM EDTA, 1.5 mg/mL BSA and 50 μL of homogenate. The reaction was initiated by adding 50 μL of xanthine oxidase (0.1 mg/mL) and incubated for 30 min at room temperature. The reaction was stopped by adding 6 mM of copper (II) chloride and centrifuged at 1500*g* for 10 min. The absorbance of formazan at 560 nm was then measured in the supernatant. Each unit was expressed as activity per milligram protein.

2.5.5. Catalase (CAT) activity

The reaction mixture contained 12 μL of 3% (v/v) H_2O_2 and 100 μL of cell lysates in 50 mM phosphate buffer (pH 7.0) to a final volume of 1.0 mL. Samples were incubated for 2 min at 37 °C and the absorbance of the sample was monitored for 5 min at 240 nm. Changes in absorbance were taken to be proportional to the breakdown of H_2O_2. Each unit was expressed as activity per mg protein.

2.6. Statistical analysis

Significance was evaluated by analysis of variance (ANOVA) followed by Duncan's protected least significant difference test. Probability value of $p < 0.05$ was used as the criteria for significant differences.

3. Results

3.1. Total phenolics and flavonoids

Table 1 shows common and scientific names, total phenolics and total flavonoids of 17 different herbs. The total phenolic content ranged in a wide range from 464.2 to 844.4 mg of gallic acid equivalents (GAE)/100 g of fresh herb. Based on the total phenolics, they could be divided into three groups: high, middle, and low phenolic groups. Chamomile, rosehip, hawthorn, lemon verbena, green tea, and rosemary belonged to the high phenolic group with total phenolics ranged between 769.8 and 844.4 mg GAE/100 g of fresh herb. Chamomile had the highest total phenolics among the 17 herbs, whereas bergamot had the lowest. The level of total phenolics in chamomile was about 2 fold higher than bergamot. Black tea, dandelion, jasmine, and fennel are in the middle phenolic group with total phenolics ranged between 700.7 and 745.5 mg GAE/100 g of fresh herb; Thyme, lemon grass, booibos, lavender, peppermint, blue gum three, and bergamot belonged to the low phenolic group (464.2–678.6 mg GAE/100 g of fresh herb).

The total flavonoid contents followed a similar pattern as the total phenolics, but they were different in order. Rosemary was ranked sixth in total phenolics, but it contained the second highest flavonoids, while rosehip contained the second highest total phenolics, but it ranked seventh in flavonoids among 17 herb samples. Among the three major groups, the high flavonoid group includes chamomile, rosehip, hawthorn, lemon vervena, green tea, rosemary and black tea. Chamomile (494.5 mg catechin equivalent (CE)/100 g of fresh herb) was the highest, followed by rosemary (448.4 mg of fresh herb), black tea (431.8 mg), lemon verbena (431.6 mg), hawthorn (407.8 mg), rosehip (400.5 mg), and green tea (412.6 mg). Dandelion, jasmine, fennel, lemon grass, rooibos, lavender, peppermint, and blue gum tree (300.5–399.7 mg) were among of the middle flavonoid group. The thyme (299.9 mg) and bergamot (211.0 mg) were included the low flavonoids group.

3.2. Total antioxidant activity

The total antioxidant activity expressed by VCEAC is shown in Table 1. The total antioxidant activities also showed a wide range from 227.4 to 960.4 mg VCE/100 g of fresh herb. Chamomile, hawthorn, black tea, rosehip, dandelion, and rosemary showed a very high level of total antioxidant capacity (between 852.1 and 960.4 mg VCE/100 g of fresh herb). Lemon verbena, green tea, and lemon grass were in the middle group (between 730.1 and 847.4 mg VCE/100 g of fresh herb). Jasmine, fennel, booibos, lavender, peppermint, and blue gum tree were in the low group (between 631.0 and 697.4 mg VCE/100 g of fresh herb).

3.3. Radical scavenging activity

The results of DPPH radical scavenging activities showed (Table 1) that chamomile exhibited the greatest free radical scavenging activity (91%), followed by rosehip with 88.7% and lemon verbena with 86.9%, which were greater than that of vitamin C at the level of 10 μg/mL (74%) (data not shown). Fennel, lemon grass, lavender, peppermint, and bergamot showed relative lower radical scavenging activity (78.5–60.1%).

3.4. Effect of various herbs on cells viability of V79-4 cells

The effect of various herb extract on cell viability in V79-4 cells is shown in Fig. 1. The cell viability of V79-4 cells was increased with the treatment of various herbs. The treatment with H_2O_2 on V79-4 cells for 24 h significantly showed cell toxicity. Gallic acid exhibited 36% and 40% of cell viability at the concentration of 100 and 200 μg/mL, whereas catechin showed 32% and 34% under the same condition. Treatments with various herbs for 1 h prior to the addition of 300 μM of H_2O_2 induced a dose-dependent increase on cell viability. At the 200 μg/mL level, chamomile generally induced cell viability two times more than gallic acid. In particular, the cell viabilities by

Table 1
The total phenolics, total flavonoids, total antioxidant activity, and DPPH radical quenching activity of various herb extracts[A]

Samples	Species (family)	Total phenolics[B] (mg GAE/100 g)	Total flavonoids[C] (mg CE/100 g)	TAA[D] (mg VCE/100 g)	DPPH (%)[E]
Chamomile	*Chamaemelum nobilis* L.	844.4 ± 0.9a	494.5 ± 2.2a	960.4 ± 2.1a	91.0 ± 2.4a
Rosehip	*Rosa rubiginosa*	818.5 ± 1.0a	400.5 ± 2.1a	900.2 ± 0.9a	88.7 ± 1.9a
Hawthorn	*Crataegus pinnatifida*	817.0 ± 2.8a	407.8 ± 2.4a	929.4 ± 0.9a	84.5 ± 2.3a
Lemon verbena	*Aloysia triphylla*	770.7 ± 2.2a	431.6 ± 1.4a	839.4 ± 1.2b	86.9 ± 2.2a
Green tea	*Camellia sinensis* L.	769.8 ± 0.7a	412.6 ± 1.5a	847.4 ± 11.0b	84.0 ± 1.3a
Rosemary	*Rosemarynus officinalis* L.	766.4 ± 0.6a	448.4 ± 1.1a	852.1 ± 1.3a	85.4 ± 2.1a
Black tea	*Camellia sinensis* L.	745.5 ± 0.9b	431.8 ± 0.8a	916.2 ± 1.8a	84.2 ± 2.2a
Dandelion	*Taraxacum officinalis*	745.1 ± 2.6b	399.7 ± 1.3b	865.8 ± 0.5a	76.4 ± 1.0b
Jasmine	*Jasminum*	734.0 ± 4.9b	305.5 ± 2.3c	665.8 ± 1.2c	72.5 ± 2.8b
Fennel	*Foeniculum vulgare*	700.7 ± 1.0b	328.7 ± 0.7c	638.6 ± 0.9c	70.1 ± 1.2c
Thyme	*Thymus vulgaris* L.	678.6 ± 2.9c	299.9 ± 0.5d	625.8 ± 0.5c	74.5 ± 2.6b
Lemon grass	*Cymbopogon citratus*	662.0 ± 3.2c	300.5 ± 0.4c	730.1 ± 1.5b	70.4 ± 1.4c
Rooibos	*Aspalathus iinearis* R	659.2 ± 2.1c	381.0 ± 1.6b	631.0 ± 0.8c	78.5 ± 1.5b
Lavender	*Lavendula angustifolia* Mill	651.6 ± 2.4c	390.4 ± 2.0b	653.5 ± 2.4c	64.2 ± 0.7c
Peppermint	*Mentha piperita* L.	638.1 ± 0.5c	316.4 ± 0.1c	654.4 ± 1.1c	63.7 ± 1.4c
Blue gum tree	*Eucalyptus globules* LABILL	621.6 ± 0.9d	395.4 ± 1.3b	697.4 ± 1.9c	60.1 ± 0.2d
Bergamot	*Citrus bergamia*	464.2 ± 0.6d	211.0 ± 0.2d	227.4 ± 2.7d	65.4 ± 1.5c

Values in the same column that are followed by a different letter are significantly different $p < 0.05$ by Duncan's multiple range tests.
[A] Mean values for all determinations based on $n = 3$.
[B] Total phenol content, expressed in milligrams of gallic acid equivalents per 100 g of fresh herb.
[C] Total flavonoids content, expressed in milligrams of catechin equivalents per 100 g of fresh herb.
[D] Total antioxidant activity, expressed in milligrams of vitamin C equivalents per 100 g of fresh herb.
[E] Means of DPPH radical scavenging activity of 100 μg/mL of each extract.

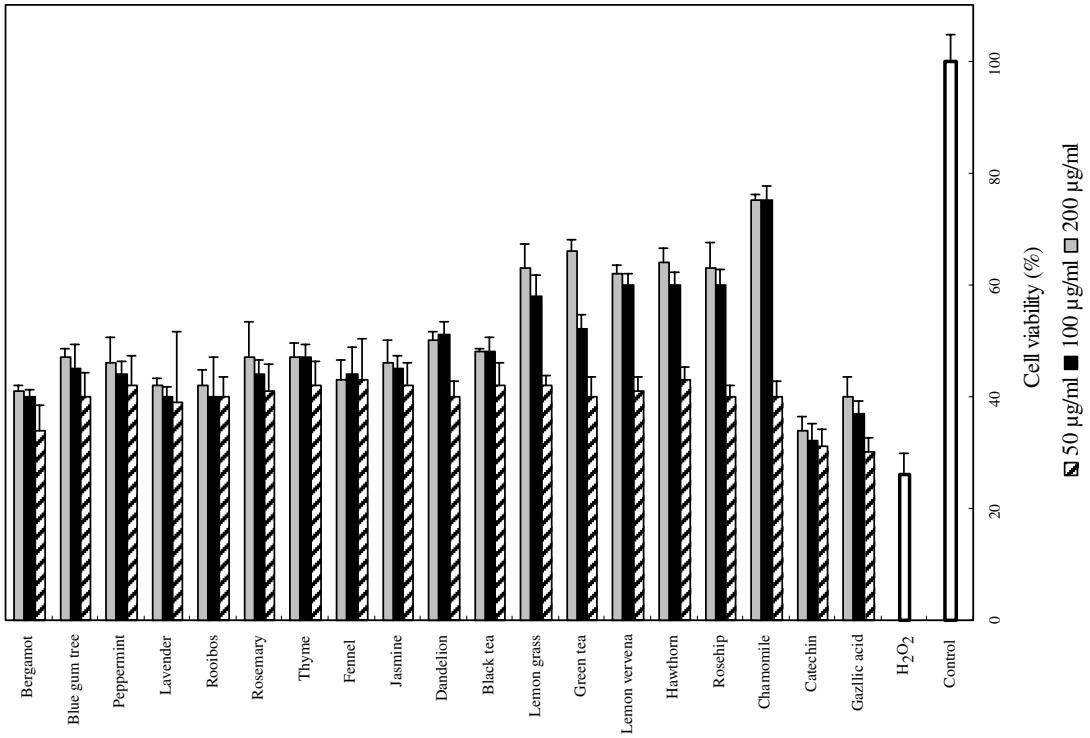

Fig. 1. Effect of various herbs on V79-4 cells viability. V79-4 cells were treated with different concentrate of various herbs for 24 h. Cell viability was determined by MTT and expressed as a percentage of viable cells in the total number of cells counted. The figure shows means ± SD ($n = 5$) for each herb of 0.01 mg/mL: (1) control, (2) H_2O_2, (3) gallic acid, (4) catechin, (5) chamomile, (6) rosehip, (7) hawthorn, (8) lemon verbena, (9) green tea, (10) rosemary, (11) black tea, (12) dandelion, (13) jasmine, (14) fennel, (15) thyme, (16) lemon grass, (17) rooibos, (18) lavender, (19) peppermint, (20) blue gum tree, (21) bergamot.

chamomile, rosehip, hawthorn, lemon verbena, and green tea were significantly greater than the other herbs at the same concentration. On the other hand, fennel, lavender, peppermint, blue gum tree, and bergamot showed relatively low degrees of cell viability, but they exhibited higher rates of cell viability than gallic acid and catechin.

3.5. Effect of various herbs on H_2O_2-induced inhibition of GJIC in V79-4 cells

The protective effects of various herbs on H_2O_2-induced oxidative stress in the inhibition of GJIC in V79-4 cells are shown in Fig. 2. The number of communicating cells observed with microscope also revealed clearly that the H_2O_2 had a cytotoxicity effect on V79-4 cells, but the cells treated with the high antioxidant herbs were protected better. The treatment with chamomile, rosehip, hawthorn, lemon verbena, and green tea extracts caused higher numbers of communicating cells compared with gallic acid, catechin, and other herbs.

3.6. Effect of various herbs on the intracellular antioxidative enzymes

We measured bioactivity of various herbs on antioxidative enzymes, SOD, and CAT, in V79-4 cells and compared the activities with those of gallic acid and catechin as references (Fig. 3). Chamomile, rosehip, hawthorn, lemon verbena, and green tea induced higher SOD and CAT activities than the other herbs; for the SOD activity at a concentration of 100 μg/mL, chamomile, rosehip, hawthorn, lemon verbena, and green tea increased the activity by 70.1, 64.4, 61.8, 61.1, 60.9, and 60.0 unit/mg protein, respectively, as compared with 49.6 and 36.8 unit/mg protein from gallic acid and catechin; on CAT activity, chamomile, rosehip, hawthorn, lemon verbena, and green tea enhanced the enzyme activity by 77.8, 76.6, 76.9, 76.2, and 67.7 unit/mg protein, respectively. Gallic acid and catechin increased the CAT activity of 57.0 and 40.1 unit/mg protein, respectively. However, peppermint, blue gum tree, and bergamot were found the lower effect on SOD and CAT activity.

4. Discussion

Several studies have been carried out on antioxidant and anticancer activities of individual herbs. Cao and Cao (1999) reported angiogenesis of green tea; Geleijnse et al. (1999) studied the relationship between tea and atheosclerosis; Zheng and Wang (2001) reported oxygen radical absorbance capacity of 12 medicinal herbs; however, there is no information on antioxidant and anticancer activities of herbs based reactive oxygen species (ROS) damage on tissues. ROS or high level of free radicals leads to oxidative stress that induces degenerative disorders, such as cancer, cardiovascular disease and neurodegenerative diseases (Yen et al., 2002). The present study was aimed to elucidate effects of selected hers on antioxidant and anticancer activities on gap–junction intercellular communication and antioxidative enzymes *in vitro* study.

We selected 17 common herbs obtained from local region and studied their relative phenolic contents, antioxidant and anticancer activities under the same conditions. Total polyphenol content in the herbs ranged from 464 to 870 GAE mg/100 g and total flavonoids from 212 to 494 CE mg/100 g. Among the samples, chamomile, rosehip, hawthorn, lemon verbena, and green tea exhibited relatively high total phenol and total flavonoids. The total phenol content of green tea was 769.8 mg GAE/100 g, while that of black tea was 745.5 mg GAE/100 g. The

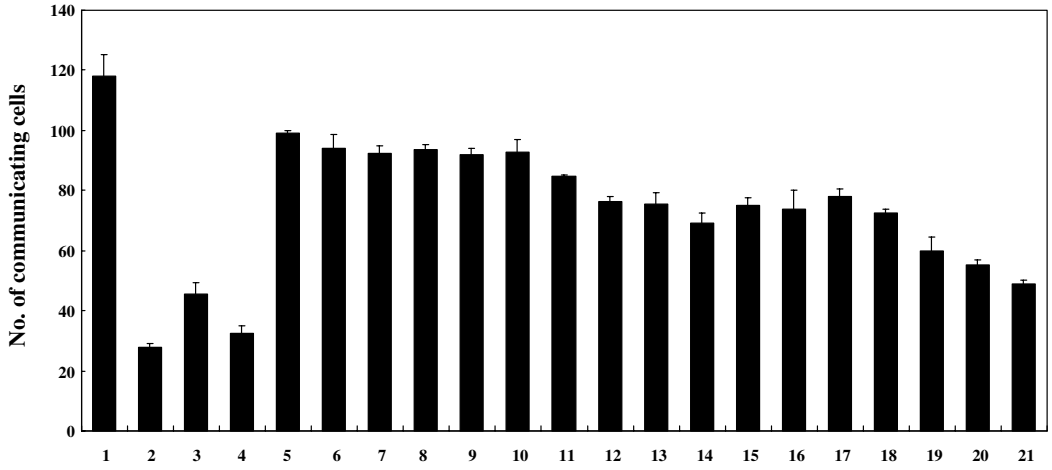

Fig. 2. Protective effects of various herbs on H_2O_2-induced oxidative stress in the inhibition of GJIC in V79-4 cells. Cells (1) were exposed to 300 μM H_2O_2 (2), 100 μg/mL gallic acid (3), 100 μg/mL catechin (4), or 100 μg/mL chamomile (5), rosehip (6), hawthorn (7), lemon verbena (8), green tea (9), rosemary (10), black tea (11), dandelion (12), jasmine (13), fennel (14), thyme (15), lemon grass (16), rooibos (17), lavender (18), peppermint (19), blue gum tree (20), and bergamot (21) for 1 h, respectively. GJIC was assessed using the scrape-loading/dye-transfer method, and the number of communication cells visualized with the dye was counted under an inverted fluorescence microscope. Error bar = SD, data are representative of six separate experiments.

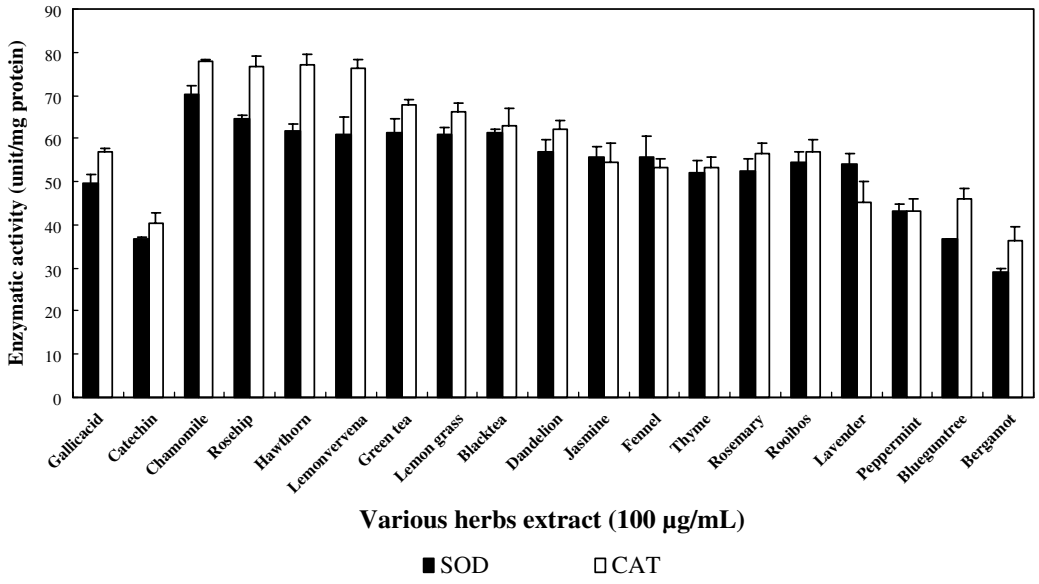

Fig. 3. SOD and CAT enzymes activities of various herbs in V79-4 cells. The figure shows means ± SD ($n = 6$) for each herb of 100 μg/mL.

reported value of total phenolics in teas varied in a wide range between 86.3 and 8.050 mg of GAE/100 g (Kahkonen et al., 1999; Richelle, Tavazzi, & Offord, 2001; Zaporozhets et al., 2004; Zheng & Wang, 2001). These differences may be mainly attributed to diversities of the analytical methods, variety, maturity, extraction method of samples and geographic origin where tea plants were grown (Lee, Park, & Choi, 1996; Shiraki et al., 1994; Zaporozhets et al., 2004). Kahkonen et al. (1999) found a similar level of total phenolics in chamomile (900 mg GAE/100 g) and thyme (Simandi et al., 2001). However, there has been no report on total flavonoid contents in herbs.

The total antioxidant activities also showed a wide range from 227.4 mg to 960.4 of VCE/100 g. Chamomile, hawthorn, black tea, rosehip, dandelion, and rosemary showed a very high level of total antioxidant capacity (between 852.1 and 960.4 mg VCE/100 g). Chamomile consistently showed the highest total phenolics, total flavonoids, and total antioxidant capacity, however, black tea that belonged to the intermediate group in total phenolics showed the third highest antioxidant capacity among the herb samples studied. Bergamot contained the lowest total phenolics, flavonoids, and the lowest antioxidant capacity among samples. An interesting note is that the relative levels of total phenolics and total antioxidant capacity showed relatively the same pattern. The relationships between particular antioxidants and antioxidant activity are difficult to be explained on the basis of quantitative analysis only. Not only the level of antioxidants but also an interactions occurring among them and other constituents might influence on the antioxidant activity of herbs (Zaporozhets et al., 2004).

Previous studies (Lee, Kim, Lee, & Lee, 2003) have reported on the relationship between total phenolics and antioxidant activity in tea, fruits, and red wine. Several reports (Kahkonen et al., 1999; Yoo, Lee, Park, Lee, & Hwang, 2004) showed a strong relationship between the total phenolic content and antioxidant capacity in selected fruit, vegetable, and grain. The antioxidant mechanisms in biological systems include direct quenching free radicals to terminate the radical chain reaction, chelating transition metals, acting as reducing agents, or stimulating the antioxidative enzyme activities (Khokhar & Magnusdottir, 2002; Trojakova, Reblova, Nguyen, & Pokorny, 2001; Weinberg et al., 1999). The variation of relative radical scavenging capacity of individual herb against different radicals should be explained by the different mechanisms involved. The extraction methods or solvent selection in different testing systems may also affect the capacity of scavenge on different radicals. We found a higher correlation between total antioxidant activity and total phenolics ($r^2 = 0.822$) and between antioxidant activity and total flavonoids ($r^2 = 0.717$) (data not shown).

The V79-4 cells exposed to H_2O_2 were distinctively low in the cell viability and prevented the gap–junction intercellular communication. However, the cells pretreated with herbs extract increased cell viability and protected GJIC more than gallic acid and catechin. Previous studies (Lee et al., 2005; Lee, Lee, Kang, & Lee, 2002) reported that gallic acid and catechin protected GJIC in the WB cells. In this study, the morphological observation and the number of communicating cells treated with herbs were very similar to those of the control cells (under no oxidative stress). Some of the herbs, such as chamomile, hawthorn, rosehip, lemon verbena, and green tea protected the V79-4 cells from the H_2O_2-induced oxidative stress and maintained high level of cellular communication. In addition, most of the herbs tested here also enhanced the activities of antioxidative enzymes, SOD and CAT.

Therefore, the antioxidant effects of herbs on cell viability can be explained by two mechanisms: direct antioxidant action by scavenging ROS and indirect antioxidant action through induction of antioxidative enzymes and protecting intercellular communications.

5. Conclusions

Among 17 selected commercial herbs, chamomile, rosehip, hawthorn, lemon verbena, green tea, and rosemary were higher in total phenolics and flavonoids, while peppermint, blue gum tree, and bergamot were relatively low. The extracts of chamomile, rosehip, hawthorn, lemon verbena, green tea had high levels of DPPH radical scavenging activity, increased the cell viability, inhibited H_2O_2-induced oxidative stress, protected GJIC and enhanced SOD and CAT activities. The total phenolics and total flavonoids had a higher correlation with total antioxidant capacity.

Acknowledgement

This research was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (KRF-2006-F00025).

References

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel Wissenschaft Technologie, 28*, 25–30.

Cao, Y. H., & Cao, R. H. (1999). Angiogenesis inhibited by drinking tea. *Nature, 398*(6726), 381.

Capecka, E., Mareczek, A., & Leja, M. (2005). Antioxidant activity of fresh and dry herbs of some Lamiaceae species. *Food Chemistry, 93*, 223–226.

Geleijnse, J. M., Launer, L. J., Hofman, A., Pols, H. A. P., & Witteman, J. C. M. (1999). Tea flavonoids may protect against atherosclerosis – The Rotterdam study. *Archives of Internal Medicine, 159*, 2170–2174.

Kahkonen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S., et al. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry, 47*, 3954–3962.

Kang, K. A., Lee, K. H., Chae, S., Koh, Y. S., Yoo, B. S., Kim, J. H., et al. (2005). Triphlorethol-A from *Ecklonia cava* protects V79-4 lung fibroblast against hydrogen peroxide induced cell damage. *Free Radical Research, 39*, 883–892.

Khokhar, S., & Magnusdottir, S. G. M. (2002). Total phenol, catechin, and caffeine contents of teas commonly consumed in the United Kingdom. *Journal of Agricultural and Food Chemistry, 50*, 565–570.

Kim, D. O., Lee, K. W., Lee, H. J., & Lee, C. Y. (2002). Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. *Journal of Agricultural and Food Chemistry, 50*, 3713–3717.

Lee, J. H., Park, J. C., & Choi, J. S. (1996). The antioxidant activity of *Ecklonia stolonifera*. *Archives of Pharmacal Research, 19*, 223–227.

Lee, K. W., Hur, H. J., Lee, H. J., & Lee, C. Y. (2005). Antiproliferative effects of dietary phenolic substances and hydrogen peroxide. *Journal of Agricultural and Food Chemistry, 53*, 1990–1995.

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.

Lee, K. W., Lee, H. J., Kang, K. S., & Lee, C. Y. (2002). Preventive effects of vitamin C on carcinogenesis. *Lancet, 359*(9301), 172.

Madsen, H. L., & Bertelsen, G. (1995). Spices as antioxidants. *Trends in Food Science and Technology, 6*, 271–277.

Rakitzis, E. T. (1975). Reaction of thioureas with Folin–Ciocalteu reagent. *Analytica Chimica Acta, 78*, 495–497.

Rice-Evans, C. A., Miller, J., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science, 2*, 152–159.

Richelle, M., Tavazzi, I., & Offord, E. (2001). Comparison of the antioxidant activity of commonly consumed polyphenolic beverages (coffee, cocoa, and tea) prepared per cup serving. *Journal of Agricultural and Food Chemistry, 49*, 3438–3442.

Shahidi, F., Janitha, P. K., & Wanasundara, P. D. (1992). Phenolic antioxidants. *Critical Reviews in Food Science and Nutrition, 32*, 67–103.

Shiraki, M., Hara, Y., Osawa, T., Kumon, H., Nakayama, T., & Kawakishi, S. (1994). Antioxidative and antimutagenic effects of theaflavins from black tea. *Mutation Research, 323*(1–2), 29–34.

Sigler, K., & Ruch, R. J. (1993). Enhancement of gap junctional intercellular communication in tumor promoter-treated cells by components of green tea. *Cancer Letters, 69*(1), 15–19.

Simandi, B., Hajdu, V., Peredi, K., Czukor, B., Nobik-Kovacs, A., & Kery, A. (2001). Antioxidant activity of pilot-plant alcoholic and supercritical carbon dioxide extracts of thyme. *European Journal of Lipid Science and Technology, 103*, 355–358.

Trojakova, L., Reblova, Z., Nguyen, H. T. T., & Pokorny, J. (2001). Antioxidant activity of rosemary and sage extracts in rapeseed oil. *Journal of Food Lipids, 8*, 1–13.

Trosko, J. E., & Chang, C. C. (2000). Modulation of cell–cell communication in the cause and chemoprevention/chemotherapy of cancer. *Biofactors, 12*, 259–263.

Weinberg, Z. G., Akiri, B., Potoyevski, E., & Kanner, J. (1999). Enhancement of polyphenol recovery from rosemary (*Rosmarinus officinalis*) and sage (*Salvia officinalis*) by enzyme-assisted ensiling (ENLAC). *Journal of Agricultural and Food Chemistry, 47*, 2959–2962.

Wiseman, S. A., Balentine, D. A., & Frei, B. (1997). Antioxidants in tea. *Critical Reviews in Food Science and Nutrition, 37*, 705–718.

Yen, G. C., Duh, P. D., & Tsai, H. L. (2002). Antioxidant and pro-oxidant properties of ascorbic acid and gallic acid. *Food Chemistry, 79*, 307–313.

Yoo, K. M., Lee, K. W., Park, J. B., Lee, H. J., & Hwang, I. K. (2004). Variation in major antioxidants and total antioxidant activity of yuzu (*Citrus junos* Sieb ex Tanaka) during maturation and between cultivars. *Journal of Agricultural and Food Chemistry, 52*, 5907–5913.

Zaporozhets, O. A., Krushynska, O. A., Lipkovska, N. A., & Barvinchenk, V. N. (2004). A new test method for the evaluation of total antioxidant activity of herbal products. *Journal of Agricultural and Food Chemistry, 52*, 21–25.

Zheng, W., & Wang, S. Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural and Food Chemistry, 49*, 5165–5170.

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.