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# Antioxidant Activity of Herbal Teas Available on the Korean Market

**Takako Yokozawa†, Kyeoung Im Lee‡, Hiroshi Kashiwagi*, Eun Ju Cho and Hae Young Chung****

*Research Institute for Wakan-Yaku, Toyama Medical and Pharmaceutical University, Toyama 930-0194, Japan*
**Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, Toyama 930-0194, Japan*
***College of Pharmacy, Pusan National University, Pusan 609-735, Korea*

## Abstract

**The effects of aqueous extracts from Korean commercial teas on excessive free radicals were examined utilizing spin trapping, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and lipid peroxidation. A potent scavenging effect of green tea and oriental senna tea was found using spin trapping. The most effective tea against the DPPH radical was green tea, followed in order by pine leaf tea, Chinese gutta percha tea and oriental senna tea. Similar to the effects of DPPH radical, green tea, pine leaf tea, Chinese gutta percha tea and oriental senna tea had an inhibitory effect on lipid peroxidation. These findings predict that Korean tea is a promising material for scavenging free radicals, and for curing diseases related to free-radical reactions.**

Key words: Korean tea, free radical, spin trapping, 1,1-diphenyl-2-picrylhydrazyl radical, lipid peroxidation

## INTRODUCTION

In the current era of over-eating in developed countries, the importance of food now tends to be focused on health issues, particularly diseases, rather than its role as a source of nutrients and energy, and new aspects of the biological activity of food have been attracting attention. Along with recognition of the important role of active oxygen in aging, carcinogenesis, circulatory disorders, etc., antioxidants in foodstuffs are now highlighted as new edible and functional substances.

In previous studies, we investigated the antioxidant activity of various compounds chosen from Oriental medicines, paying particular attention to the correlation between molecular structure and activity. We have found that tannin and phenol compounds such as flavones have such activity (1), and that tea, which contains these compounds as its major ingredients, has high antioxidant activity (2). The tea plant is an evergreen belonging to the family Camelliaceae, and its leaves are processed to be made drinks. In recent years, infusions (decoctions) of dried leaves, flowers, fruits, seeds, bark, and roots of plants have been used as tea substitutes. Various types of such tea substitutes, which are not real tea, have been manufactured and put on the market amid recent boom in health-oriented products. This paper reports the results of our study on the antioxidant activity of herbal teas popular in Korea.

## MATERIALS AND METHODS

### Tea

Ten kinds of Korean tea, which were obtained from Korean markets, were used for screening of their antiradical effect. Korean teas that were studied are listed in Table 1. A 30 g of each tea was boiled gently in 300 ml of water for 1 h. Each extract was then evaporated under reduced pressure to leave a residue with a yield of about 25%.

### Reagent

5,5'-Dimethyl-1-pyrroline N-oxide (DMPO) was purchased from Aldrich Chemical Co., Milwaukee, USA.

**Table 1.** Korean teas used in this study

| Material | Scientific name | Maker | Used part |
|---|---|---|---|
| Barley tea | *Hordeum vulgare* var. *hexastichon* Aschers | Dongsu Foods (Chungchungbukdo Jinchun) | seed |
| Chicory tea | *Chicorium intybus* | Sulak Foods (Kyungkido Pochun) | root |
| Chinese gutta percha tea | *Eucommia ulmoides* Oliver | Taepyungyang (Kyungkido Pochun) | leaf |
| Corn tea | *Zea mays* L. | Dongsu Foods (Chungchungbukdo Jinchun) | seed |
| Green tea | *Thea sinensis* L. | Taepyungyang (Chungchungbukdo Jinchun) | leaf |
| Oriental senna tea | *Cassia tora* L. | | seed |
| Pine leaf tea | *Pinus densiflora* S. et Z. | Sanchung Foods (Kyungsangnamdo Sanchung) | leaf |
| Solomon's seal tea (1) | *Polygonatum odoratum var. pluriflorum* Ohwi | Koogje Foods (Kyungkido Hwasung) | root |
| Solomon's seal tea (2) | *Polygonatum odoratum var. pluriflorum* Ohwi | Sunnongwon (Kyungsangnamdo Yangsan) | root |
| Solomon's seal tea (3) | *Polygonatum odoratum var. pluriflorum* Ohwi | | root |

Oriental senna tea and Solomon's seal tea (3) were used as dried materials. The other teas were used as tea bag products.

†Corresponding author. E-mail: yokozawa@yi.toyama-mpu.ac.kr
Phone: 81-764-34-2281(2836), Fax: 81-764-34-4656
‡Present address: Department of Hotel Culinary Arts, Yangsan College, Yangsan 626-600, Korea

### Spin trapping assay

Electron spin resonance (ESR) spectroscopy combined with spin trapping using DMPO was employed to identify radical species. Twenty microliters of DMPO was added to 100 µl of 100 mM homoarginine solution and 200 µl of a 10 µg/ml aqueous solution of sample, followed by stirring for 10 s. The ESR spectra of this mixture were measured with a JEOL FE-3X type spectrometer (JEOL, Tokyo, Japan: X band, 100 kHz modulation) at 30°C, 5 min after the addition of DMPO. Microwave power, modulation amplitude and sweep time were set at 8 mW, 0.1 mT and 0.5 min, respectively. Two peaks of external manganese dioxide appearing at g=1.981 and g=2.034 were used to determine both the g-value and the amount of each DMPO-adduct. Radical species were assigned by comparing the observed spectra with the calculated ones. The previously reported values of the hyperfine splitting constants of the DMPO-adduct of a carbon-centered radical (DMPO-C) ($\alpha$(N)=1.58 mT and $\alpha$($\beta$H)=2.42 mT), the DMPO adduct of the hydroxyl radical (DMPO-OH) ($\alpha$(N)=1.49 mT and $\alpha$($\beta$H)=1.49 mT) and the DMPO adduct of the hydrogen radical (DMPO-H) ($\alpha$(N)=1.66 mT and $\alpha$($\beta$H)=2.25 mT) were used for the calculation. g-Factors for all the spin adducts were 2.006 (3,4).

### Determination of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

In microwells, 100 µl of an aqueous solution of the sample (control: 100 µl of distilled water) was added to ethanolic solution of DPPH (60 µM) according to the method of Hatano et al. (5). After being mixed gently and left for 30 min at room temperature, the DPPH radical was determined using a Microplate Reader, Model 3550-UV (Bio-Rad, Tokyo, Japan). The antioxidant activity of each sample was expressed in terms of $IC_{50}$ (concentration in micrograms per milliliter required to inhibit DPPH radical formation by 50%) calculated from the log-dose inhibition curve.

### Determination of antioxidant activities

Rat kidney was perfused with ice-cold 0.9% NaCl before homogenization. After being washed with 0.9% NaCl, tissue homogenates were prepared at the ratio of 1 g of wet tissue to 9 ml of 1.15% KCl using a glass homogenizer (6). The antioxidant activities were determined by quantification of thiobarbituric acid (TBA)-reactive substances using a slight modification of the method of Buege and Aust (7). The reaction mixture was composed of 0.5 ml of kidney homogenate in 0.8 ml of phosphate buffer (50 mM, pH 7.4) and 0.3 ml of a solution of $H_2O_2$ (30 mM) and $FeSO_4$ (3.3 mM) with or without 0.1 ml of an aqueous solution of tea extract at the concentration of 2~50 µg/ml. The mixture was incubated at 37°C for 20 min in a capped tube; then 4.0 ml of a stop solution, consisting of TBA/trichloroacetic acid/HCl (0.375% TBA, 15% trichloro-acetic acid, 0.25 N HCl), was added to each tube, and all of the tubes were heated at 100°C for 15 min. After a cooling period of 10 min in ice-water, centrifugation was carried out at 3000 rpm for 10 min, and then determination of the supernatant was done spectrophotometrically at 535 nm. The concentration of the TBA-reactive substance generated in the mixture was calculated using an absorption coefficient of $1.56 \times 10^5\ M^{-1}cm^{-1}l^{-1}$ (8). The antioxidant activities of tea extract were expressed in terms of $IC_{50}$ (concentration in micrograms per milliliter required to inhibit TBA-reactive substance formation by 50%) calculated from the log-dose inhibition curve.

### Statistics

Data are presented as the mean±SE of 5 determinations.

## RESULTS

### Spin trapping

Fig. 1 shows the influence of the presence of 10 µg/ml Korean tea extract on the ESR spectrum of the mixture of 100 mM homoarginine aqueous solution (100 µl) and DMPO (20 µl). The ESR spectrum obtained in the absence of any tea extract (control) is shown in Fig. 1A. Although all of the peaks of DMPO adducts are not evident, DMPO-C, DMPO-OH, and DMPO-H appear in this spectrum. With regard to the spectral intensities of $Mn^{2+}$ used as a standard, the signals from the mixture containing the green tea extract decreased markedly in comparison with the control. Those of oriental senna tea also showed considerable reduction of the radical generation from homoarginine. However, the effect of pine

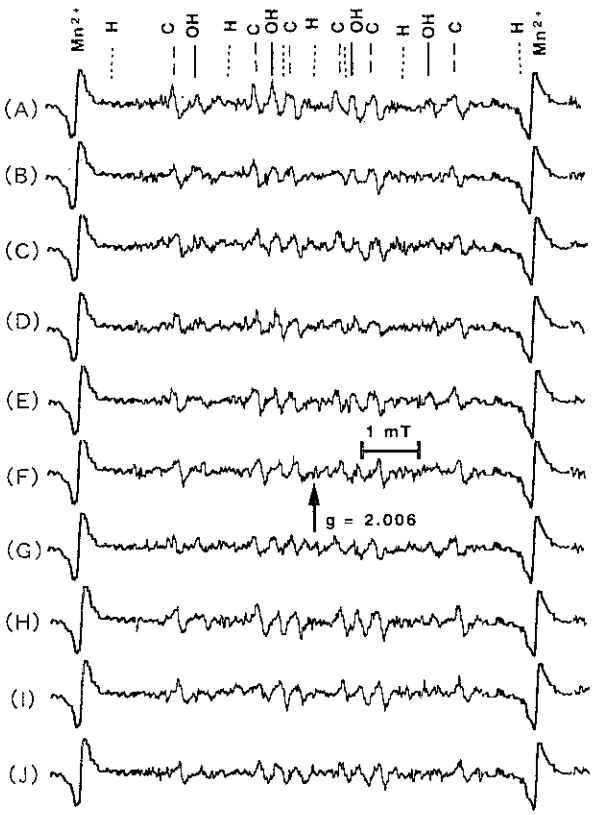


**Fig. 1.** Influence of tea extract on the ESR spectra of a mixed solution of homoarginine and DMPO: (A) none; (B) barley tea; (C) chicory tea; (D) Chinese gutta percha tea; (E) corn tea; (F) green tea; (G) oriental senna tea; (H) pine leaf tea; (I) Solomon's seal tea (1); (J) Solomon's seal tea (3). H=DMPO-H, C=DMPO-C, OH=DMPO-OH.

leaf tea and corn tea was comparatively weaker than those of the other Korean teas.

### DPPH radical

As shown in Table 2, the Korean teas tested showed significant inhibitory activity against the DPPH radical. Of the 10 kinds of Korean tea, green tea showed 50% inhibition at the concentration of 5.4 μg/ml, while the same action was shown by pine leaf tea. Chinese gutta percha tea, oriental senna tea and chicory tea also had high activity against the DPPH radical, the $IC_{50}$ values being 18.1, 19.0 and 47.6 μg/ml, respectively. Solomon's seal teas were used in the form of tea bags produced commercially by two companies, and the scavenging effect showed little difference between the two products, with $IC_{50}$ values of 125.7 and 139.1 μg/ml, respectively. In contrast, when an aqueous extract obtained from the root of Solomon's seal was used, the $IC_{50}$ value was 260.1 μg/ml, the inhibitory effect being about half that of the Solomon's seal tea in tea bag form. The aqueous extracts from corn tea and barley tea, which are very popular, commonly consumed teas in Korea, presented relatively weak activity, their $IC_{50}$ values being 153.5 and 162.9 μg/ml, respectively. These results show that green tea and pine leaf tea are excellent scavengers of the DPPH radical.

### Lipid peroxidation

Table 3 shows the inhibitory effect on TBA-reactive substance formation in a reaction mixture treated with $H_2O_2$ and $Fe^{2+}$. Green tea showed the most potent protection against TBA-reactive substance formation, with an $IC_{50}$ value of 28.8 μg/ml. Pine leaf tea and Chinese gutta percha tea also showed considerably strong suppression of lipid peroxidation, with $IC_{50}$ values of 156.8 and 246.6 μg/ml, respectively. On the other hand, oriental senna tea appeared to have comparatively weak antioxidant activity, which was about 29 times lower than that of green tea. However, barley tea, chicory tea, corn tea and Solomon's seal tea appeared to have no activity, since their $IC_{50}$ values exceeded 1,000 μg/ml.

**Table 2.** $IC_{50}$ Values of tea against the DPPH radical

| Material | $IC_{50}$ (μg/ml) |
|---|---|
| Barley tea | 162.9±5.7 |
| Chicory tea | 47.6±1.7 |
| Chinese gutta percha tea | 18.1±0.4 |
| Corn tea | 153.5±7.4 |
| Green tea | 5.4±0.3 |
| Oriental senna tea | 19.0±2.6 |
| Pine leaf tea | 7.3±0.2 |
| Solomon's seal tea (1) | 139.1±16.4 |
| Solomon's seal tea (2) | 125.7±13.0 |
| Solomon's seal tea (3) | 260.1±17.6 |

**Table 3.** $IC_{50}$ Values of tea on lipid peroxidation induced by $H_2O_2$ and $Fe^{2+}$ in renal homogenate

| Material | $IC_{50}$ (mg/ml) |
|---|---|
| Barley tea | >1000 |
| Chicory tea | >1000 |
| Chinese gutta percha tea | 246.6± 3.4 |
| Corn tea | >1000 |
| Green tea | 28.8± 0.6 |
| Oriental senna tea | 840.8±60.8 |
| Pine leaf tea | 156.8± 4.1 |
| Solomon's seal tea (1) | >1000 |
| Solomon's seal tea (2) | >1000 |
| Solomon's seal tea (3) | >1000 |

## DISCUSSION

Koreans have developed various traditional teas with medicinal functions and unique flavors. With the use of available source materials, teas have become an essential part of the Korean diet, and some of them are now appreciated as healthy drinks. However, we could hardly find any scientific studies about Korean teas except for green tea and ginseng tea (9).

Various substances in the body possess a guanidine group in their molecular structures. Mori et al. (10) and Yokoi et al. (11) have reported that intraventricular injection of α-guanidinoglutaric acid or homoarginine induces generalized seizures in rats, suggesting the involvement of the formation of hydroperoxyl radical ($\cdot O_2H$) and hydroxyl radical ($\cdot OH$). On the other hand, as shown in Fig. 1, the ESR spectrum of homoarginine solution shows the 1:2:2:1 quartet pattern peculiar to DMPO-OH. There are many possible mechanisms by which ·OH radicals are generated from an aqueous solution of a guanidine compound. In general, there is equilibrium between guanidine ions and electrons in a guanidine solution. These electrons are considered to react with oxygen molecules in the solution to generate superoxide ($\cdot O_2^-$), resulting in formation of ·OH through the Haber-Weiss reaction (12). However, in the presence of green tea extract at the concentration of 2 μg/ml, the production of ·OH was markedly low. A similar ·OH radical-eliminating action was noted for all tea extracts, although it was less potent than that of green tea.

Active oxygen species currently known to be produced in the body include $\cdot O_2^-$, $H_2O_2$, ·OH, singlet oxygen ($^1O_2$) and lipid hydroperoxide (LOOH). The reactivity of ·OH is particularly high among various different radicals. On the other hand, the DPPH radical is stable in ethanolic solution for more than 60 min (5). Antioxidants react with the DPPH radical directly and restore it by transfer of electrons or hydrogen. Therefore, we used this system for assessing the radical-scavenger activity of Korean tea extracts. We found that the descending order of radical scavenging activity was green tea > pine leaf tea > Chinese gutta percha tea > oriental senna tea > chicory tea > Solomon's seal tea > corn tea > barley tea. Of the 10 tea extracts tested, green tea and pine leaf tea extracts showed the most active antiradical property, having 50% inhibitory activity at a low concentration of 5.4 and 7.3 μg/ml, respectively. Chinese gutta percha tea and oriental senna tea also revealed significant scavenging activities on the DPPH radical. These results suggest that Korean tea is a promising agent for scavenging of free radicals. On the other hand, the $IC_{50}$ values of Solomon's seal tea extracted from tea bags on the DPPH radical were 125.7 and 139.1 μg/ml, respectively, the values being roughly the same as

the two products. In an aqueous extract from the unprocessed root of Solomon's seal, the scavenging activity on DPPH radical was lower than that of the tea bag extract. It was found that the scavenging activity on the DPPH radical was excellent for tea extracts prepared with leaf parts, such as green tea, pine leaf tea and Chinese gutta percha tea.

Tea (*Camellia sinensis* L.) can be divided into three types, i.e., unfermented, semifermented and fermented, in terms of the degree of leaf fermentation. Each type of tea has a distinct aroma, color and flavor, which appeal to our senses of taste, smell and sight. The scavenging activities of these teas on the DPPH radical have been compared. As reported previously (2), it was found that the green tea extract had a very significant scavenging activity. Other tea extracts seemed to have relatively weak effect. On the other hand, the inhibitory effect of 28 crude drugs on the DPPH radical was examined. Gallae Rhois, Rhei Rhizoma, Cinnamomi Cortex and Ephedrae Herba appear to be promising crude drugs for scavenging DPPH radical, and for curing diseases related to free-radical reactions (13). A similar DPPH radical-scavenging action was noted for Korean teas such as green tea and pine leaf tea, although it was less potent than Japanese green tea and Gallae Rhois.

Although green tea extract is known to have various physiological effects such as anti-mutagenic, antitumoral, antioxidative and antiradical activities (2,14-17), there have been only a few studies on the components and physiological action of pine leaf. Iwata et al. (18) recently reported the triglyceride-decreasing action of gallic acid and galloyl gallic acid extracted from Japanese red pine leaves. However, the components of the pine leaf have not been clarified, though there have been a few studies on essential oil and resin, and others have shown the presence of a large amount of various amino acids and carbohydrates in the ethanol extract (19-21). We have been interested in its physiologically active natural components and have tried to detect the active components of pine leaves and their anti-radical effects.

Lipid peroxides produced from unsaturated fatty acid by radicals have histotoxicity by themselves, and also increase the production of free radicals in chain-reaction-like way (22, 23). On the basis of these findings, we added various concentrations of tea extracts to the incubation medium of an experimental system in which the Fenton reaction was induced in a kidney homogenate in the presence of $H_2O_2$ and $Fe^{2+}$, and obtained their $IC_{50}$ values. Of the 10 teas tested, green tea showed the most active antiradical property, having 50% inhibitory activity at a low concentration of 28.8 μg/ml. Following it in order were pine leaf tea, Chinese gutta percha tea and oriental senna tea. However, chicory tea, which has been confirmed to suppress the DPPH radical, appeared to have no action on the formation of TBA-reactive substance, because this $IC_{50}$ value exceeded 1,000 μg/ml. This suggested that the radical scavenging effects of teas depend on not only their components but also the species of radical scavenged, as well as the path of action.

As the involvement of active oxygen and lipid peroxidation in various diseases including atherosclerosis, ischemic disorders and inflammation has been clarified, prevention and treatment have been attempted from the aspect of antioxidative defense. Edible natural antioxidants are now viewed as new functional substances which inhibit the peroxidation in the body associated with aging and carcinogenesis. In the present study, a tea substitute processed from pine needles proved to have high antioxidant activity, like green tea. We intend to further investigate the effects of this pine needle tea on the biological antioxidative system in comparison with green tea, and search for its active ingredient.

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