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Antioxidative and anti-hydrogen peroxide activities of various herbal teas

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Abstract

Herbal teas, i.e., extracts of herbs, are popular because of their fragrance and antioxidative activity. Since the antioxidative activity comes mainly from polyphenols, total polyphenol concentrations and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activities in herbal teas were measured and compared. Levels of H_2O_2 in the teas were also examined, since the production of H_2O_2 in beverages such as coffee and green tea, has been reported. Only a small amount of H_2O_2 was detected in the herbal teas just after their preparation with hot water. However, H_2O_2 was gradually produced during incubation at 25 °C after extraction with hot water, especially when the teas were incubated in phosphate buffer at pH 7.4. To examine the anti-H_2O_2 activity of herbal teas, various teas were added to a catechin-enriched green tea, which produce much H_2O_2, and they were incubated at 25 °C for one day. Addition of hibiscus and thorn apple tea decreased the production of H_2O_2 in the catechin-enriched green tea, possibly because of a lowering of the pH of the mixture.

Keywords: Antioxidant; Herbal tea; Hibiscus; Hydrogen peroxide; Polyphenol; Thorn apple

1. Introduction

Polyphenols are present in various beverages and are known to work as antioxidants (Bravo, 1998; Ina, Sakata, Tomita, & Isemura, 2002). Antioxidants are very important for human health, since the production of reactive oxygen species is thought to be a significant cause of aging and carcinogenesis (Beckman & Ames, 1997; Lambert & Yang, 2003). Actually, polyphenols can act as free radical-scavengers, quenching hydroxyl radicals (·OH) or superoxide anion radicals ($O_2^{\cdot-}$) (Hanasaki, Ogawa, & Fukui, 1994; Sichel, Corsaro, Scalia, Di Bilio, & Bonomo, 1991). Recent epidemiological studies have shown that flavonoid-rich foods and beverages, such as red wine, have beneficial effects on carcinogenesis and cardiovascular diseases (Soleas, Diamandis, & Goldberg, 1997).

Not only green and black tea but also various kinds of herbal teas are very popular in Japan because of their fragrance and anti-oxidative activity (Matsingou, Kapsokefaloi, & Salifoglou, 2001). These beverages are thought to be beneficial to both physical and mental health.

In contrast to the beneficial effects of polyphenols, the production of hydrogen peroxide (H_2O_2) from polyphenols, such as catechin derivatives, has recently been reported (Arakawa, Maeda, Okubo, & Shimamura, 2004; Cao, Sofic, & Prior, 1997; Long, Lan, Hsuan, & Halliwell, 1999; Nakayama, Ichiba, Kuwabara, Kajiya, & Kumazawa, 2002). H_2O_2 was produced from polyphenol-rich beverages under quasi-physiological conditions and increased in amount with the incubation time (Akagawa, Shigemitsu, & Suyama, 2003; Chai, Long, & Halliwell, 2003). It is known that H_2O_2 is toxic and induces cell death *in vitro* (Aoshima, Hossain, Tanaka, & Wen, 2004; Aoshima, Kadoya, Taniguchi, Satoh, & Hatanaka, 1999; Fuchs, Baier-Bitterlich, Wede, & Wachter, 1997; Whittermore, Loo, & Cotman, 1994). It has been reported that some

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polyphenols promote oxidative damage to DNA, lipids and deoxyribose under certain conditions *in vitro* (Hayakawa, Kimura, Hoshino, & Ando, 1999; Hayakawa et al., 1997; Yamanaka, Oda, & Nagao, 1997).

Various beverages in bottles are sold in vending machines in Japan. These beverages contain negligible amounts of H_2O_2 just after being opened, but their H_2O_2 levels gradually increase with time. In a previous paper, we reported methods to prevent the production of H_2O_2 by adding catalase or compounds which have reductive activity or which lower the pH of the beverages (Aoshima & Ayabe, 2007).

In this paper, total polyphenols (Ough & Amerine, 1988), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activities (Blois, 1958) and amounts of H_2O_2 in various herbal teas were measured to examine the antioxidative activity and the toxicity of the teas. Then we examined the anti-H_2O_2 activity of herbal teas by adding them to a catechin-enriched green tea which produces H_2O_2.

2. Materials and methods

2.1. Chemicals and samples

Xylenol orange, butylated hydroxytoluene (BHT), methanol and DPPH were purchased from Wako Pure Chemical Industry, Ltd., Osaka, Japan. Gallic acid was purchased from Nacalai Tesque, Kyoto, Japan. Folin and Ciocalteu's phenol reagent was obtained from Katayama Chemical Industry, Osaka, Japan. All chemicals used were of guaranteed reagent quality. Dried herbs, rose (*Rosa* species), lavender (*Lavandula* species), chamomile (*Matricaria recutita* L.), hibiscus (*Hibiscus sabdariffa* L.), lemongrass (*Cymbopogon ciratus* (DC.) Staf.), sage (*Salvia officinalis* L.), rosemary (*Rosmarinus officinalis* L.), echinacea (*Echinasea angustifolia* (DC.) Hell), thyme (*Thymus vulgaris* L.), peppermint (*Mentha piperita* L.), ginkgo (*Ginkgo biloba* L.), liquorice (*Glycyrrhiza glabra* L.), thorn apple (*Crataegus cuneata* Sieb. Et Zucc.) and green tea (*Camellia sinensis* O. Kuntze), were purchased from a local market in Japan. Catechin-enriched green tea in a polyethylene terephthalate (PET) bottle was purchased from a local market in Yamaguchi, Japan. This green tea contained 1.57 g/l of catechin derivatives, 232 mg/l of caffeine and an undefined amount of L-ascorbic acid.

The aqueous extracts of herbs, i.e., herbal teas, were prepared by addition of 10 ml of water (filtered by Yamato Millipore WQ500) at 100 °C to 0.1 g of dried herbal tea and incubation for 10 min. The teas were then filtered with filter paper (Toyo Roshi Co., Ltd., Tokyo, Japan).

2.2. Determination of total polyphenol with the Folin assay

Total polyphenol contents of herbal teas were analyzed using the Folin assay (Ough & Amerine, 1988). For the analyses, gallic acid was used as the standard and the polyphenol concentrations in beverages were expressed as mM gallic acid equivalents. One millilitre of herbal tea, diluted 20 times with deionized water, was mixed with 1 ml of Folin–Ciocalteu's reagent. After vortexing for 5 s, 1 ml of a 10% (w/w) aqueous sodium carbonate solution was added to the mixture. The mixture was incubated at room temperature for 1 h and absorbance read at 700 nm by a spectrophotometer (Hitachi U-2000A).

2.3. Measurement of DPPH radical-scavenging activity

The reaction mixture (total volume, 3 ml), consisting of 0.5 ml of a 0.5 M acetic acid buffer solution at pH 5.5, 1 ml of 0.2 mM DPPH in ethanol and 1.5 ml of herbal tea extracts diluted two hundred times by 50% (v/v) aqueous ethanol solution, was shaken vigorously for a short time to blend the mixture (Aoshima, Tsunoue, Koda, & Kiso, 2004; Blois, 1958). As a control, the reaction mixture was similarly prepared by replacing the herbal tea extracts with deionized water and shaking for a short time. After incubation at room temperature for 30 min, the DPPH remaining was determined by the absorbance at 517 nm, and the radical-scavenging activity of each sample was expressed using the ratio of the decrease in absorption of DPPH (%) relative to the control DPPH solution (100%) in the absence of the sample. That is, the radical-scavenging activity $(\%) = 100(A - B)/A$, where A and B were the 517 nm absorbance of the control and the corrected absorbance of the sample reaction mixture.

2.4. Determination of H_2O_2 by the ferrous ion oxidation–xylenol orange (FOX) assay

The concentration of H_2O_2 was measured, as previously described (Akagawa et al., 2003; Long et al., 1999). FOX reagent was prepared by adding one volume of Reagent 1 to nine volumes of Reagent 2, where Reagent 1 was 4.4 mM 2,6-di-*t*-butyl-4-methylphenol (BHT) in methanol and Reagent 2 was 1 mM xylenol orange plus 2.56 mM ammonium ferrous sulfate in 250 mM H_2SO_4. The herbal teas (100 μl), in which the H_2O_2 concentrations were to be measured, were added to the FOX reagent (3 ml) and vortexed for 5 s. After incubation for 30 min at room temperature and centrifugation, the absorbance at 560 nm was measured using a spectrophotometer (Hitachi U-2000A). The FOX assay was calibrated using a standard H_2O_2 solution whose concentration was estimated by using a molar extinction coefficient of 43 M^{-1} cm^{-1} at 240 nm.

The increase of H_2O_2 in the teas was measured after extraction, 0.9 ml of tea being mixed with 0.1 ml of phosphate buffer (100 mM) at pH 7.4 or deionized water and the mixture was incubated at 25 °C for 24 h.

2.5. Prevention of H_2O_2 production in catechin-enriched green tea by addition of herbal teas

To examine the effects of herbal teas on the production of H_2O_2 in catechin-enriched green tea, 0.1 ml of herbal tea

was added to 0.9 ml of green tea and the mixtures were incubated for 24 h at 60 °C. Then the H_2O_2 concentrations in the mixtures were measured by the FOX assay.

To examine the effect of hibiscus and thorn apple tea on the production of H_2O_2 in the green tea, mixtures with various ratios of green tea and herbal tea were incubated for 24 h at 25 °C. Mixtures with various ratios of green tea and deionized water were also prepared as a control. Then the H_2O_2 concentrations in the mixtures were measured by the FOX assay.

3. Results

To examine the relationship between total polyphenol and antioxidative activity in herbal extracts, i.e., herbal teas, we measured both total polyphenol and DPPH radical-scavenging activities of herbal teas (Table 1). The correlation factor between the DPPH radical-scavenging activities and the total polyphenol concentrations was estimated to be 0.950. The pHs of the teas determined by pH test paper were: hibiscus tea, 2–3; thorn apple tea, 3–4; echinacea tea, 6–7 and other teas, 5–6.

Reportedly, H_2O_2 is produced from polyphenol-rich beverages under quasi-physiological conditions and its amount increases with incubation time (Akagawa et al., 2003; Chai et al., 2003). So we examined the production of H_2O_2 in herbal teas. Only a small amount of H_2O_2 was detected in the herbal teas, just after their preparation with hot water (Table 1). Moreover, the increase in H_2O_2 after incubation at 25 °C for 24 h was very small. However, H_2O_2 was produced in the teas after incubation at 25 °C for 24 h when they were incubated in phosphate buffer at pH 7.4 (Fig. 1), though the H_2O_2 production in herbal teas was less than that in green tea.

Since H_2O_2 is produced in polyphenol-rich beverages once the bottle has been opened, it is desirable to find easy and safe methods to prevent H_2O_2 production in beverages. So we examined the H_2O_2 production in catechin-enriched green tea sold in a PET bottle by adding various herbal teas (10%, v/v) to see whether they reduce the production of H_2O_2 at 60 °C. Fig. 2 shows that hibiscus and thorn apple teas were the only herbal teas to reduce H_2O_2 production in the green tea significantly, though some other teas caused H_2O_2 production to be significantly greater than the control. The addition of hibiscus and thorn apple teas decreased the pH of the mixture. We

Table 1
Total polyphenol, DPPH radical-scavenging activity and H_2O_2 in herbal teas

Herbal teas	Total polyphenol (mM)	DPPH radical-scavenging activity (%)	H_2O_2 (μM)
Rose	4.43 ± 0.04	82.5 ± 1.7	34 ± 2
Lavender	1.35 ± 0.02	26.6 ± 0.5	50 ± 2
Chamomile	0.60 ± 0.00	6.7 ± 0.2	0 ± 1
Hibiscus	0.43 ± 0.00	11.7 ± 0.4	57 ± 2
Lemongrass	0.57 ± 0.02	8.3 ± 0.8	14 ± 0
Sage	1.81 ± 0.01	19.8 ± 1.8	39 ± 2
Rosemary	2.29 ± 0.02	23.0 ± 1.1	24 ± 6
Echinacea	0.80 ± 0.02	6.1 ± 0.7	105 ± 3
Thyme	2.08 ± 0.01	21.5 ± 1.1	16 ± 1
Peppermint	2.63 ± 0.01	30.3 ± 1.3	23 ± 2
Ginkgo	0.53 ± 0.01	5.7 ± 1.1	7 ± 4
Liquorice	0.48 ± 0.01	0.9 ± 0.1	3 ± 1
Thorn apple	0.47 ± 0.00	9.2 ± 0.5	5 ± 2
Green tea	4.03 ± 0.02	73.1 ± 0.4	8 ± 0

Concentration of total polyphenol was expressed as mM gallic acid equivalents.
Data are means ± SD, n=3.

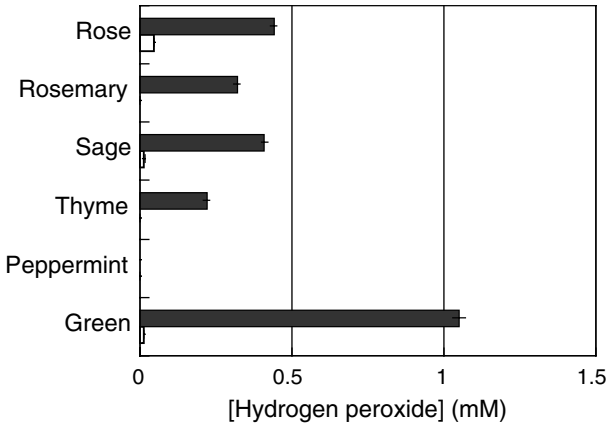

Fig. 1. The concentrations of H_2O_2 in the herbal teas in phosphate buffer at pH 7.4. The concentrations of H_2O_2 in the mixture of herbal teas (90%) with high polyphenol concentrations and 100 mM phosphate buffer (10%) were measured with the FOX assay, just after extraction of the herbs with hot water (open bars) and after incubation in a plastic tube for 24 h at 25 °C (closed bars). Data are means ± SD (bars), $n = 3$. $P < 0.01$ by Student's t test for values between before and after the incubation except peppermint.

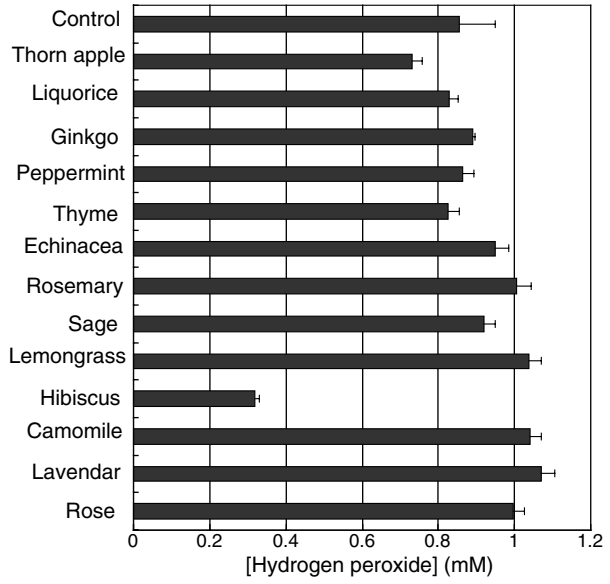

Fig. 2. Prevention of H_2O_2 production in catechin-enriched green tea by addition of various herbal teas. Mixtures of 0.9 ml of green tea and 0.1 ml of herbal tea were incubated for 24 h at 60 °C. A mixture of 0.9 ml of green tea and 0.1 ml of deionized water was prepared as a control. Then the H_2O_2 concentrations in the mixtures were measured by FOX assay. Data are means ± SD (bars), $n = 3$. $P < 0.05$ by Student's t test between the control value and the values in the presence of thorn apple, rosemary, lemongrass, hibiscus, chamomile, lavender and rose tea.

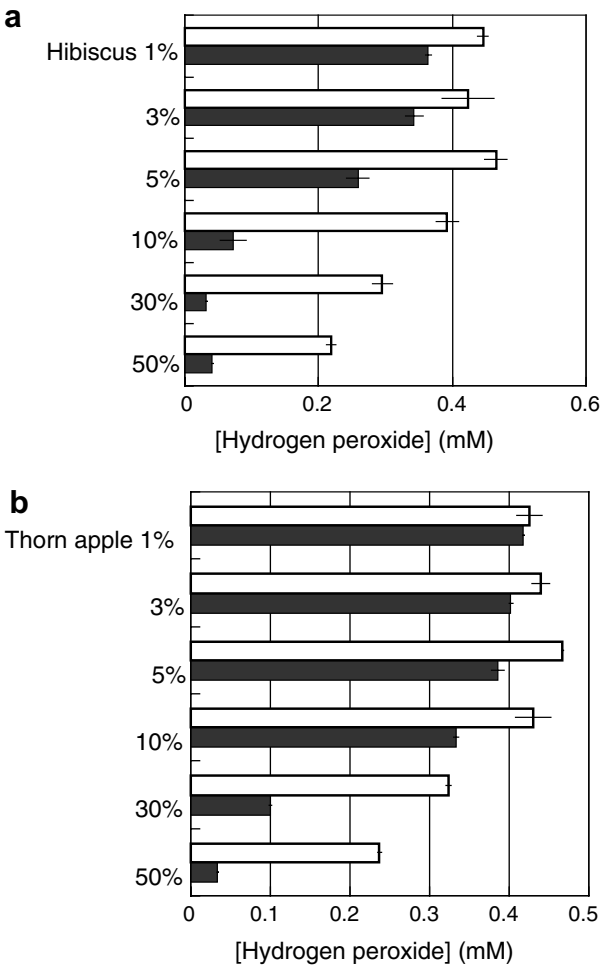

Fig. 3. Dose-dependent effects of: (a) hibiscus tea and (b) thorn apple tea on the production of H_2O_2 in catechin-enriched green tea. The green tea was mixed with hibiscus and thorn apple tea in various ratios. The green tea was also mixed with deionized water in the same ratios as a control. These mixtures were incubated for 24 h at 25 °C and their H_2O_2 concentrations were measured by FOX assay. Data are means ± SD (bars), $n = 3$. $P < 0.01$ by Student's t test between the control values and the values in the presence of the herbal teas except that in the presence of 1% thorn apple.

examined the effect of dose on the production of H_2O_2 in the mixture of green tea and hibiscus tea or thorn apple tea (Fig. 3). However, addition of these herbal teas to green tea did not prevent H_2O_2 production when the pH of the mixture was kept at 7.4 by addition of the phosphate buffer (data not shown). We examined the time-dependence of the production of H_2O_2 and found that the herbal teas suppressed the production for at least 24 h.

4. Discussion

Polyphenols in beverages are popular because of their beneficial physiological effects on health (Bravo, 1998; Ina et al., 2002). So total polyphenol concentrations in herbal teas were measured by the Folin method and compared with their DPPH radical-scavenging activities. As expected, they showed a close relationship (correlation factor 0.950). The total polyphenol and DPPH radical-scavenging activity of rose tea were greater than those of green tea, possibly because of the presence of anthocyanin. However, other herbal teas had less activity than had green tea, possibly because green tea contained much greater amounts of catechin derivatives, which possess beneficial physiological effects, including antioxidative, dietary and bactericidal actions, as well as antitumour activity (Hara, 2001; Ina et al., 2002).

Green tea and black tea, together with coffee, are the most popular beverages in the world. They contain xanthine derivatives, such as caffeine, which have various physiological activities, indicating the inhibition of cellular phosphodiesterase activity, resulting in an increased concentration of cAMP, increased free Ca^{2+} concentration inside nerve cells through ryanodine receptor opening, antagonism of adenosine actions on adenosine receptors (Cardinali, 1980) and inhibition of $GABA_A$ receptors (Hossain, Hamamoto, Aoshima, & Hara, 2002a) and they work as central nervous system stimulants. On the other hand, most herbal teas contain no caffeine, though they include polyphenols, usually at levels lower than those in green tea. Herbal teas contain various fragrances, which may have a tranquillizing effect on the mind (Aoshima & Hamamoto, 1999). So as people need mental relaxation after working hard, herbal teas have recently become very popular in Japan, because they are milder to the human nervous system than are tea or coffee, containing caffeine.

The production of hydrogen peroxide (H_2O_2) from polyphenols in beverages was reported and its mechanism was recently proposed (Akagawa et al., 2003; Chai et al., 2003). Production of hydrogen peroxide in herbal teas was very minor after the extraction of herbs with hot water, except for echinacea tea. Hydrogen peroxide in echinacea tea may be produced during the extraction of echinacea in hot water because the pH of echinacea tea is higher than that of other teas. Polyphenols are known to autoxidize at a higher rate as the pH value is increased, because the dissociated phenolic group has a stronger electron-donating capacity than has the undissociated form (Mochizuki, Yamazaki, Kano, & Ikeda, 2002). However, production of hydrogen peroxide in herbal teas incubated under quasi-physiological conditions at pH 7.4 increased the concentrations causing cell toxicity. The toxicity of H_2O_2 was measured *in vitro*. It causes apoptosis in cells through oxidative stress (Aoshima et al., 2004; Aoshima et al., 1999; Fuchs et al., 1997, 1994). Green tea or polyphenols in green tea also cause cell death in PC12 cells or bacteria (Arakawa et al., 2004; Chai et al., 2003). However, the toxic side effects of beverages with large amounts of polyphenols have not been reported *in vivo* until now, possibly for the following reasons. Only 5–8% of all the polyphenols in foods and beverages are taken into the body through the intestines. Since reactive oxygen species, including $O_2^{\cdot-}$ and H_2O_2, are constantly produced during oxidative phosphorylation, cells have an ubiquitous protective system against oxidative stress, e.g. superoxide dismutase or catalase (Richter & Schweizer, 1997). The epithelial cells in the

stomach and intestines are always being reproduced, even when the superficial ones die.

Though some polyphenols produce toxic H_2O_2, they also have beneficial activity, being antioxidative. However, since some polyphenols also cause cell death by perturbing the membrane structure (Aoshima et al., 2005; Hossain et al., 2002b), much attention should be paid to their cell toxicity when they are consumed in large quantities. It is necessary to clarify the beneficial (Duthie et al., 1998; Ina et al., 2002) and toxic (Fujita, Wakabayashi, Nagao, & Sugimura, 1985; Yang et al., 2000) effects of each polyphenol *in vivo* and to evaluate the uptake of each polyphenol for human health. It may be better not to take supplements with extraordinary large amounts of polyphenols, even though they have strong antioxidative activity.

In a previous paper (Aoshima & Ayabe, 2007), we found that H_2O_2 is produced in green tea stored in a bottle after opening the cap and exposing it to air and reported that addition of catalase, a reducing agent, such as L-cysteine, or acidic compounds, such as L-aspartic acid or citric acid, had a suppressive effect. In this paper, we have found that the addition of hibiscus and thorn apple teas reduced the production of H_2O_2 in green tea. Addition of some herbal teas, such as lavender or lemongrass tea produced more H_2O_2 than a control to which had been added 10% deionized water instead of herbal teas, possibly because the teas themselves contained polyphenols. The addition of herbal teas is simple and is possibly accepted by consumers, since both green tea and herbal teas have been drunk safely by many people for a long time. The production of H_2O_2 was prevented because the pH of the mixture was lowered to 2–3. However, addition of hibiscus or thorn apple tea to green tea changes the taste and colour of green tea. So it is necessary to develop a new method without affecting the taste and colour of green tea. We have been searching for natural products which prevent H_2O_2 production at high temperature and pH 7.4, but have had no real success so far. Ascorbic acid (vitamin C) is usually added to green teas sold in a PET bottle. However, ascorbic acid itself reportedly produces H_2O_2 (Clement, Ramalingam, Long, & Halliwell, 2001; Wee, Long, Whiteman, & Halliwell, 2003).

5. Conclusions

Both the total polyphenol concentration and DPPH radical-scavenging activities of various herbal teas were measured and found to have a close correlation. Production of H_2O_2 in herbal teas was very low but became high at pH 7.4. Addition of hibiscus and thorn apple teas to catechin-enriched green tea prevented the production of H_2O_2 dose-dependently, possibly because of a decrease in the pH of the mixture.

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