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Physiological Activities of Ginger Extracts Originated from Different Habitat

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Abstract Physiological activities of Korean-grown ginger (KG) and Chinese-grown ginger (CG) extracts were examined. Ginger was extracted with water, and 50 and 100% ethanol, and then nitrite-scavenging activity (NSA), superoxide dismutase (SOD)-like activity, and electron-donating ability (EDA) of extracts were investigated. NSA at pH 1.2 was the most effective in 50% ethanol extracts of both origins. SOD-like activities of water and 50% ethanol extracts of both samples were 8.66-35.95% lower than those of 1 and 0.1% L-ascorbate solutions. SOD-like activity of KG extracts was higher than that of CG extracts, and water extracts of samples were the highest. EDA of KG extract was higher (22.23-86.95%) than that of CG extract, while both sample extracts showed lower EDA than those of 1 and 0.1% L-ascorbate solutions.

Keywords: ginger, habitat, total phenol, electron-donating ability, antioxidant activity

Introduction

Oxidation is essential to living organisms, because it is needed for the production of energy to fuel biological processes. Oxygen-centered free radicals and other reactive oxygen species (ROS), however, are continuously produced *in vivo*, resulting in cell death and tissue damage. Oxidative damages caused by free radicals may be related to aging and diseases such as atherosclerosis, diabetes, cancer, and cirrhosis (1). Although most organisms possess antioxidant defense and repair systems that can protect them against oxidative damages, these systems are unable to prevent all damages (2). Antioxidant supplements or foods containing antioxidants, thus, can be used to help the human body reduce oxidative damages. These protective effects have been attributed partly to the various antioxidant compounds present in fruits and vegetables, for example, vitamin C and E, β-carotene, and polyphenolics (3). In vegetables, quercetin glycosides are predominant; however, the glycosides of kaempferol, luteolin, and apigenin are also present (4). Ginger extract exhibits antioxidant action by increasing the levels of cellular antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase, and scavenging ROS (5). Thus, it might be beneficial to the prevention of diseases in which the ROS plays a part.

Ginger (*Zingiber officinale*) belongs to the Zingiberaceae family. The part of the plant used is the rhizome. The major pharmacological activity of ginger appears to be due to gingerol and shogaol (6, 7). Numerous chemical investigations of this plant material have led to the isolation and identification of a large number of biologically active compounds, such as gingerols, gingerones, and shogaols (8-12). The administration of ginger has resulted in decreased symptoms of rheumatoid arthritis (13), and gingerol (a component of ginger) has been reported to have as anti-inflammatory actions, including the suppression of both cyclooxygenase and lipooxygenase metabolites of arachidonic acid (14, 15). 6-Gingerol and 6-shogaol possess varied pharmacological activities including antipyretic, analgesic, antitussive, and hypotensive effects (7).

The overall objectives of this study were to examine the potential of ginger as a functional food material by measuring its physiological activity, such as antioxidative ability. The optimum extraction conditions for the functional substances of ginger were also determined.

Materials and Methods

Preparation of ground ginger and extracts Ginger cultivated from two distinct regions, Korea and China, were harvested in January, 2005. The gingers were purchased from the Garak market in Seoul, Korea. After the ginger samples (10 g) were washed and crushed, they were extracted with 100 mL solvents (water, and 50 and 100% ethanol) for 24 hr at 37°C. The process was repeated twice. The extracts were centrifuged at 13,000×g for 10 min and filtered through Whatman filter paper No.2. Filtered extracts were evaporated under reduced pressure and redissolved in 100 mL distilled water for further experimentation.

Determination of nitrite-scavenging activity (NSA) A procedure described by Kausar *et al.* (16) was used to measure the NSA. One milliliter of a 1 mM $NaNO_2$ solution was added to 1 mL of each ginger extract, and the pH values of the resulting mixtures were adjusted to 1.2, 3.0, and 4.2 using 8 mL buffer solutions: 0.1 N HCl for pH 1.2, and 0.2 N citric acid for pH 3.0, and 4.2. The final volume of each sample was adjusted to 10 mL. The samples were allowed to react at 37°C for 1 hr, and 1 mL of each sample was taken from the solutions, mixed thoroughly with 5 mL of 2% acetic acid and 0.4 mL

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Griess reagent, and kept at room temperature for 15 min. Prior to usage, the Griess reagent was prepared by mixing equal amounts of 1% sulfanilic acid and 1% naphthylamine, which were made with 30% acetic acid. The residual nitrite content was determined by measuring the absorbance at 520 nm (Jasco, SSE-343, Hachioji, Japan). NSA was also expressed in a percentage using the following equation:

$$\text{NSA}(\%)=\frac{1-(A-C)}{B}\times 100$$

where, A is the absorbance of the sample mixture and 1 mM of $NaNO_2$ after 1 hr reaction; B is the absorbance of the mixture of distilled water and 1 mM of $NaNO_2$ after 1 hr reaction; and C is the absorbance of ginger extracts.

Determination of superoxide dismutase (SOD)-like activity SOD-like activity was measured using a modified method of Marklund (17). Briefly, the pH of each sample was adjusted to 8.5 using a Tris-HCl buffer (50 mM tris[hydroxymethyl]amino-methane+10 mM EDTA, pH 8.5). Three milliliters of the Tris-HCl buffer and 0.2 mL of 7.2 mM pyrogallol were added to 0.2 mL each sample. The mixtures were held at 25°C for 10 min before stopping the reaction by adding 1 mL of 1 N HCl. Absorbances were determined at 420 nm using a UV/VIS spectrometer. SOD-like activity was expressed in a percentage using the following equation:

$$\text{SOD-like activity}(\%)=\frac{1-A}{B}\times 100$$

where, A is the absorbance difference between the treated sample and control, and B is the absorbance difference between the untreated sample and control.

Determination of electron donating ability (EDA) The EDA was determined in terms of reducing power of α,α-diphenyl-picrylhydrazyl (DPPH) in each extract according to a modified method of Kim *et al.* (18). One milliliter of each extract was mixed with 1 mL of 4×10^{-4} M DPPH dissolved in 99.9% ethanol to make a total volume of 2 mL. After vortexing the mixtures for 10 sec and holding them at room temperature for 30 min, the absorbances were measured at 525 nm using a UV/VIS spectrophotometer. The EDA was expressed in a percentage using the following equation:

$$\text{EDA}(\%)=1-\frac{A}{B}\times 100$$

where, A is the absorbance of the sample treated with the extract and B is that of an untreated sample. All data represent means of three values measured separately.

Determination of the inhibitory effect on tyrosinase The inhibitory effect on tyrosinase was measured by a method reported by Wong *et al.* (19). A crude tyrosinase solution was prepared by dissolving mushroom tyrosinase (Sigma Chemical Co., St Louis, MO, USA; t7755) in a 50 mM sodium phosphate buffer (pH 7.0). Subsequently, 0.2 mL crude tyrosinase solution and 0.1 mL ginger extract were added to 2.8 mL of 10 mM catechol solution. The absorbance of the resulting mixture was determined at 420 nm by a UV/VIS spectrometer to measure the tyrosinase activity. The inhibitory effect on tyrosinase was calculated by measur-ing changes in the absorbance per unit of time as follows:

$$\text{Inhibitory effect}(\%)=\frac{1-(A-B)}{C}\times 100$$

where, A is the difference in the absorbance of samples treated by the enzyme solution; B is the difference in the absorbance of samples treated by a buffer solution in lieu of an enzyme solution; and C is the difference in the absorbance of samples treated by distilled water in lieu of extracts.

Determination of total polyphenol content Total polyphenol content was measured by the Folin-Denis method (20). Ginger extract (0.1 mL), 8.4 mL distilled water, and 0.5 mL of 2 N Folin reagent were set for 3 min before adding 1 mL of 20% Na_2CO_3 solution. After holding the mixed solution for 1 hr, absorbances were measured at 765 nm using a UV/VIS spectrometer. Total polyphenol content was determined from the standard curve obtained using (+)-catechin.

Determination of angiotensin I-converting enzyme (ACE) inhibitory effect The angiotensin I-converting enzyme inhibitory effect was measured using the method of Cushman and Cheung (21). A 50 μL ginger extract was added to 100 μL of 100 mM sodium borate buffer (pH 8.3), which contained 450 mM NaCl and 50 μL of 50 mM hippuryl-histidyl-leucine solution. The mixture was then dissolved in a 100 mM sodium borate buffer (pH 8.3) containing 300 mM NaCl. Preincubation of the mixture was carried out at 37°C for 30 min. The resulting reaction solution was added to 50 mL coenzyme ACE solution, which was extracted from rabbit lung acetone powder (Sigma; L-0756) using a 100 mM sodium borate buffer. The mixture was allowed to react at 37°C for 30 min before the reaction was terminated by adding 100 μL of 1.75 N HCl. One milliliter of ethyl acetate was added to the mixture and stirred for 15 sec. Subsequently, 500 μL supernatant was dried and dissolved with 1 mL distilled water to measure the absorbance at 228 nm using a spectrophotometer. A blank test was performed using 50 μL distilled water in place of the sample. To serve as a control, 100 μL of 1.75 N HCl and 50 μL ACE coenzyme solution were sequentially added. The percentage of the ACE inhibition effect was calculated as follows:

$$\text{ACE}(\%)=\left[1-\frac{(A-B)}{(B-C)}\right]\times 100$$

where, A and B are the absorbances of samples treated with ginger extract and distilled water, respectively, and C is the absorbance of the control.

Statistical analysis One way analysis of variance (ANOVA) test and factorial analysis were carried out for all experiments using the statistical analysis system (SAS, Windows, v8) program (22). Duncan's multiple range tests were also used to examine the significance of the averages of values among the experimental groups.

Results and Discussion

Effects of nitrite-scavenging activity (NSA) The ingestion of large portions of nitrite-containing food leads to the development of toxic symptoms such as methemoglobin symptoms. Due to the ready formation of a nitrosyl reaction between nitrite and second or third class amine to form a carcinogen-like nitrosamine under low acidic conditions in the stomach, many attempts have been made to search for a natural substance that can remove these nitrites (17, 18, 23).

The nitrite removal abilities of different types of ginger were compared at pH 1.2 using various extraction solvents (Fig. 1). As a whole, nitrite removal abilities of water and the 50% ethanol extracts of ginger (47-84%) were lower than those of 0.1 and 1% L-ascorbic acid, 87.03 and 99.99% (Table 1). Because nitrosamine is readily formed at low acidic pH in the stomach, high nitrite removal ability at low pH (e.g. pH 1.2) is considered to effectively suppress the formation of nitrosamine (16). All ginger extracts showed higher levels of ability to remove nitrites, as compared to 1% L-ascorbic acid. This result was in good agreement with several reports that, in some plant extracts, phenolic compounds or ascorbate can remove nitrites, thereby reducing hazards associated with them (17, 18, 23, 24).

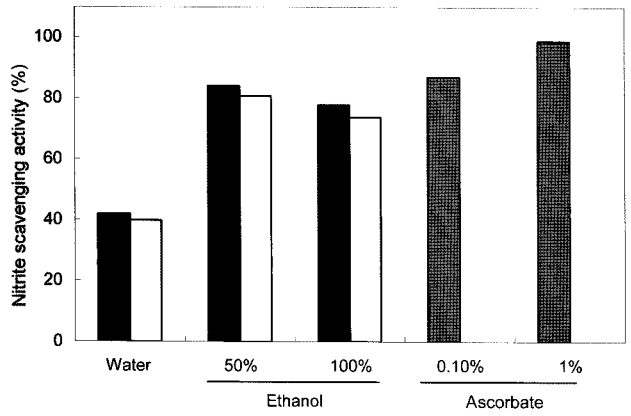

Fig. 1. Nitrite scavenging activities of ginger extracts (pH 1.2, ■ KG, □ CG). Data are expressed as mean values.

Table 1. Nitrite scavenging abilities of ginger extracts by habitat and solvents

Habitat & Solvents	Nitrite scavenging ability (%)		
	pH 3.0	pH 4.5	pH 6.0
Korea ginger			
Water	30.52	21.62	18.83
50% ethanol	79.88	66.31	24.75
100% ethanol	75.53	54.19	17.11
China ginger			
Water	29.95	18.47	13.44
50% ethanol	70.79	64.04	22.78
100% ethanol	68.62	54.86	25.55

All values are expressed as mean of triplicate determinations.

Superoxide dismutase (SOD)-like activity SOD-like activity is derived not from enzymes but from low molecular weight materials that play a role similar to the SOD. They are phytochemicals for the most part and can protect oxidative hindrance by suppressing the reactivity of superoxide. Nice *et al.* (25) purified SOD along with substances showing high thermal stability as well as SOD-like activity. They reported that these materials are phenolic compounds bound with SOD. Kim *et al.* (26) suggested that vitamin C has high SOD-like activity. With respect to the 100% ethanol extract of ginger, the SOD-like activity was high; 51.87% activity was retained in the KG extracts, whereas 8.66-35.53% and 29.59-35.95% activities were observed in water and 50% ethanol extract samples, respectively (Fig. 2). On the other hard, the SOD-like activities of 0.1 and 1% L-ascorbic acids were 43.12 and 98.71%, respectively, which were similar to the results reported by Kim *et al.* (26). On the whole, the SOD-like activity of the KG extract was higher than that of the CG extract, and the water extract was more effective than ethanol extract. These results were in agreement with those of Kim *et al.* (26). Therefore, the antioxidative activity of substances associated with the inhibition of pyrogallol oxidation varies according to plant material and its habitat. Kim *et al.* (17) demonstrated that a specific substance could repress the reactivity of a superoxide throughout the oxidation or radical reaction in a living body. Because the ginger extracts had high inhibitory and eliminative activities against superoxides, the study of antioxidative materials capable of repressing the reactivity of superoxides according to the type of active oxygens or reaction mechanism is necessary.

Effects of electron donating ability (EDA) The EDA measures hydrogen atom (or one electron) donating ability and hence provides a measure of free radical-scavenging antioxidant ability. The test used DPPH (Fig. 3), a purple-colored stable free radical, which became a yellow-colored diphenylpicrylhydrazine (27). A higher EDA leads to a greater ability to eliminate active oxygen, which causes problems in the body (27). Kang *et al.* (18) determined the EDAs of

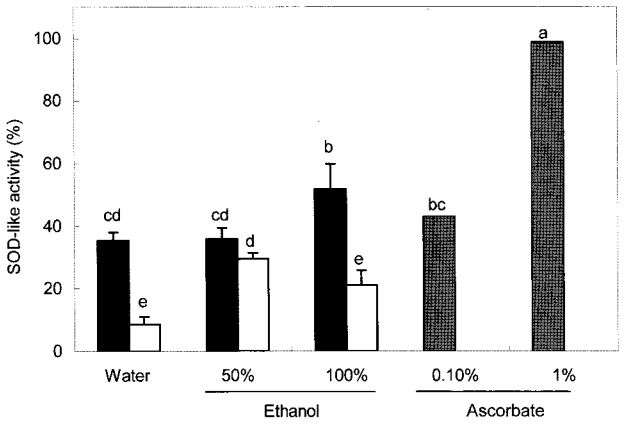

Fig. 2. Superoxide dismutase (SOD)-like activity of ginger extracts (■ KG, □ CG). Data are expressed as mean ± SD. Significant differences within a set of experiment were analyzed by ANOVA test ($p< 0.05$).

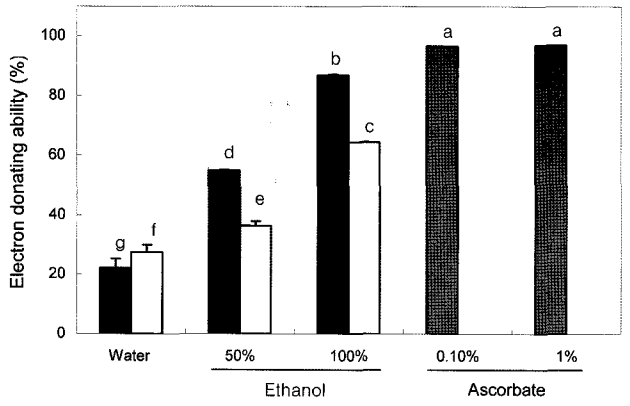

Fig. 3. Electron donating ability (EDA) of ginger extracts (■ KG, □ CG). Data are expressed as mean ± SD. Significant differences within a set of experiment were analyzed by ANOVA test ($p<0.05$).

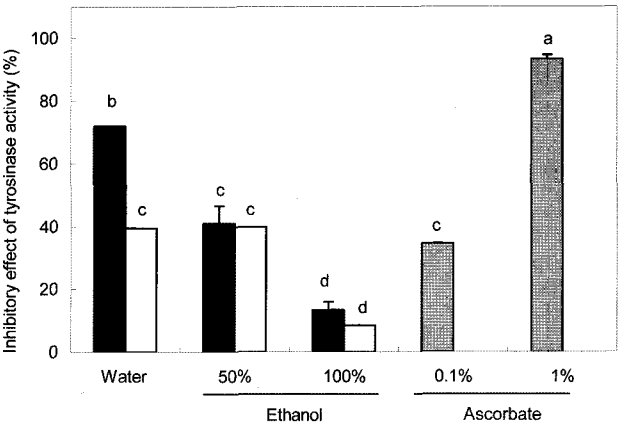

Fig. 4. Tyrosinase inhibition effects of ginger extracts (■ KG, □ CG). Data are expressed as mean ± SD. Significant differences within a set of experiment were analyzed by ANOVA test ($p<0.05$).

phenolic acid, flavonoids, and other phenolic compounds as indices of antioxidative ability. They noted that compounds with a higher reducing ability have higher EDA.

In our experiment, the EDAs of 0.1 and 1% L-ascorbic acids, widely used as antioxidants, were 96.75 and 97.02%, respectively. The EDAs of all ginger extracts were lower than those of the L-ascorbic acid solutions. The EDAs of all sample extracts, except for the 100% ethanol extract of CG, were between 62-87%. Although the EDAs of ginger were not very high, the highest amount not higher than 30%, they were presumed to have a certain degree of free radical binding ability. Thus, the ability to form a stable radical varies depending upon the antioxidative substances in the extract samples.

Effects of tyrosinase inhibition Tyrosinase (dihydroxy-L-phenylalanine oxygen oxidoreductase, EC 1.14.18.1) is known to be responsible for the browning reaction of phenolic substances during processing and storage, because it utilizes a wide range of phenolic compounds, which results in enzymatic coloration (28). The inhibitory activity of the two ginger extracts ranged between 39.53 and 71.94% ($p<0.05$) with KG having generally higher activity than CG (Fig. 4). Tyrosinase was inhibited 34.65 and 93.41% by 0.1 and 1% L-ascorbic acids, respectively, which were used as reference materials. Although the inhibition effects of the ginger extracts were found to be somewhat lower than that of the 1% L-ascorbic acid, they were considerably higher than that of the 0.1% L-ascorbic acid. Due to some safety and efficiency problems associated with various compounds in functioning as tyrosinase inhibitors, Jung *et al.* (28) searched for plant materials capable of inhibiting tyrosinase activity. They suggested the high tyrosinase activity of green and black teas were linked with the phenolic components in tea. They also reported that radishes, radish sprouts, and red peppers showed relatively high tyrosinase inhibition activity.

Total polyphenol content Many researchers have reported that the plant polyphenols contained in fruits and vegetables play important roles in preventing degenerative diseases when consumed as part of a daily diet (29, 30). The total polyphenol content was determined using the standard curve (R^2=0.9919) for catechins (Fig. 5). The total polyphenol content varied depending upon the habitat and solvents, with the water extracts showing between 37.69 and 41.35 mg%, while those of the 50 and 100% ethanol extracts showed 51.53-149.08 and 44.16-93.04 mg%, respectively. The results showed that the total polyphenol content of the KG extracts were relatively higher than that of the CG extracts. Polyphenol compounds such as caffeic, chlorogenic, ferulic, and *p*-coumaric acids showed antioxidant activity (30, 31). Similar results were also reported by Kahkonen *et al.* (32), who found that total phenolic contents of fruits, vegetables, cereals, and medicinal plants were 1100.0-1000.8, 60-740, 20-130, and 80-4200.1 mg%, respectively.

Angiotensin I-converting enzyme inhibitory effect The angiotensin I-converting enzyme (ACE) is known to cut the C-terminal dipetide (His-Leu) of inactive angiotensin-I, not only to form angiotensin-II, which is linked with the increase in blood pressure by the contraction of the blood vessels walls, but to decompose and inactivate bradykinin, which is responsible for the lowering of blood pressure. This phenomenon eventually causes high blood pressure in

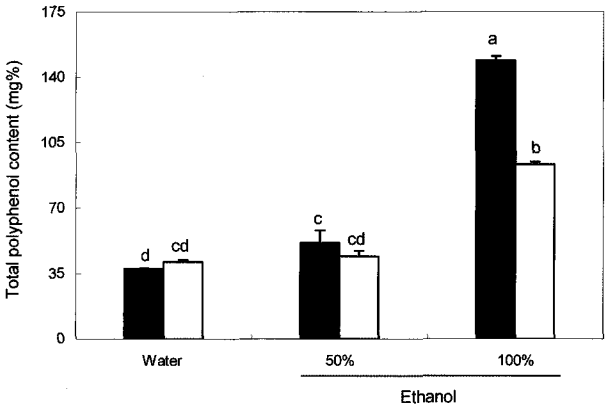

Fig. 5. Total polyphenol content of ginger extracts (■ KG,□CG). Data are expressed as mean ± SD. Significant differences within a set of experiment were analyzed by ANOVA test ($p<0.05$).

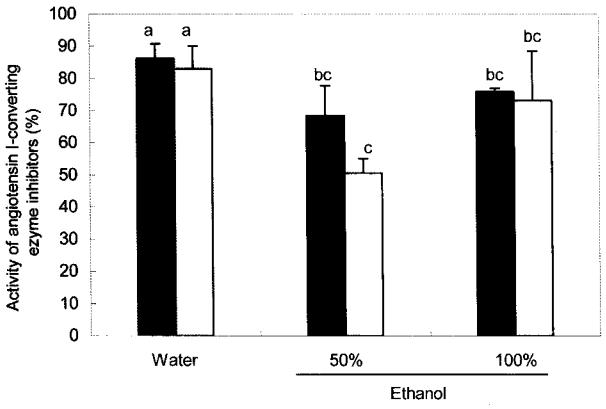

Fig. 6. Angiotesin I-converting enzyme inhibition effect of ginger extracts (■ KG, □ CG). Data are expressed as mean ± SD. Significant differences within a set of experiment were analyzed by ANOVA test ($p<0.05$).

the human body (33-35). The ACE inhibitory effect of 1 mg ginger extract was 86.3%, which increased with increasing concentration level of the extract. Similar results were also reported for pine needles (36), *Zizyphus jujaba* leaves (37), and green tea (38), with over 50% ACE inhibitory effect.

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