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Antioxidative Properties of Different Solvent Extracts from Persimmon (*Diospyros kaki* cv. Fuyu) Flower-Buds

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Abstract

After preparation of acetone, ethanol, methanol, and water extracts (10 g/300 mL) of dried persimmon (*Diospyros kaki* cv. Fuyu) flower-buds, total phenolic contents (TPC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (RSA), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) RSA, reducing power (RP), and tyrosinase inhibitory activity of the extracts were evaluated. The methanol extracts produced the highest TPC (113.39 mg gallic acid equivalents/g), DPPH RSA (IC_{50} = 40.25 μg/mL), ABTS RSA (IC_{50} = 58.17 μg/mL) and RP (IC_{50} = 69.43 μg/mL) activities while the water extracts generated the lowest values. The ethanol extract showed the highest tyrosinase inhibitor activity (88.90%) at a concentration of 1 mg/mL. These results indicated that persimmon flower-buds may be a useful source of natural antioxidants.

Key words: persimmon, flower-bud, antioxidant activity, tyrosinase inhibitory activity

INTRODUCTION

Plants have many phytochemicals with various bioactivities, including antioxidant, anti-inflammatory and anti-cancer activities (1). According to previous studies, extracts from natural products, such as fruits, vegetables and medicinal herbs, have positive effects against cancer, compared to chemotherapy or recent hormonal treatments (2,3). Plant phenolic compounds are secondary metabolites with salutary properties for animal and human health. The beneficial effects of those molecules are related to their antioxidant activity (4), particularly their ability to scavenge free radicals, donate hydrogen atoms or electrons, or chelate metal cations (5).

Persimmon (*Diospyros kaki*) is mainly cultivated in the countries of East Asia, such as Japan, China and Korea. Its fruits and leaves have been traditionally used for many medicinal purposes such as coughs, hypertension, dyspnea, paralysis, frostbite, burns and bleeding (6). Persimmon fruits were also reported to exercise hypercholesterolemia, antioxidant and free radical scavenging effects (7,8). Antioxidant activity of persimmon leaf tea was reported previously (9). To the best of our knowledge, however, there is no report on the study of the physiological function of persimmon flower-buds. The objective of the present study was to evaluate the antioxidant activity and tyrosinase inhibitory activities of persimmon flower-buds.

MATERIALS AND METHODS

Materials and chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) diammonium salt, dimethyl sulfoxide (DMSO), trichloroacetic acid (TCA), L-ascorbic acid (vitamin C), and butylated hydroxyltoluene (BHT) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Folin-Ciocalteu reagents were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Acetone, ethanol, and methanol were provided from Duksan Pure Chemical Co. (Ansan, Korea). Mushroom tyrosinase, L-tyrosine, hydrogen peroxide, potassium ferricyanide, and potassium phosphate were also purchased from Sigma-Aldrich Co. All the other organic solvents and chemicals used in this study were of analytical grade.

Sample preparation

Persimmon flower buds (*Diospyros kaki* cv. Fuyu) were harvested at Dagam Farm (Changwon, Korea) on May, 2010, and immediately freeze-dried (Freeze Dryer FD 5512, Ilshinlab Co., Yangju, Korea). The dried buds were crushed using a grinder (Mixer MC 811C, Novita Co., Seoul, Korea), and passed through a sieve (25 mesh). Each 10 g of powdered persimmon flower buds was extracted with 300 mL of four different solvents (acetone, ethanol, methanol, and water) overnight at room temperature, followed by filtrating with a Whatman No. 1 filter paper. The filtrate was concentrated to dryness under re-

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duced pressure on a rotary evaporator (Eyela N-1000, Tokyo Rikakikai Co., Tokyo, Japan) at 37°C. Each dried extract was dissolved in DMSO to achieve a final concentration of 50 mg/mL. All samples were placed in a glass bottle and stored at 4°C until further use.

Total phenolic contents (TPC)

TPC in the each extract were determined according to the method of Gutfinger (10). One-mL of each extract with concentration of 1 mg/mL was mixed with 1 mL of 2% Na_2CO_3 followed by standing for 3 min. Then, 0.2 mL of 50% Folin-Ciocalteau reagent was added to the mixture. After standing for 30 min, the solution was centrifuged at 13,400×*g* for 5 min. The absorbance was measured at 750 nm using a spectrophotometer (Shimadzu UV-1601, Tokyo, Japan) and TPC were expressed as gallic acid equivalents (GAE).

DPPH radical scavenging activity (RSA)

The DPPH RSA of each extract was determined according to the method of Lee et al. (11). After a 0.1 mL aliquot of the extracts in DMSO was mixed with 0.9 mL of 0.041 mM DPPH in ethanol for 30 min, the optical density (OD) of the sample was measured at 517 nm. Solvent extracts were assayed at different concentrations and their relative activities were expressed as IC_{50} values, which are defined as the concentration required to scavenge 50% of DPPH radicals.

ABTS RSA

The ABTS RSA was determined by the method of Re et al. (12). Each extract (0.1 mL), potassium phosphate buffer (0.1 mL, 0.1 M, pH 5.0), and hydrogen peroxide (20 mL, 10 mM) were mixed and then pre-incubated at 37°C for 5 min. ABTS (30 mL, 1.25 mM, in 0.05 M phosphate-citrate buffer, pH 5.0) and peroxidase (30 mL, 1 unit/mL) were added to the mixture followed by incubation at 37°C for 10 min. The OD level was obtained using a multiplate reader (Sunrise RC/TS/TS Color-TC/TW/BC/6Filter, Tecan Austria GmbH, Grödig, Austria) at 405 nm, and the ABTS RSA was expressed as IC_{50} values. IC_{50} value is the concentration for scavenging 50% of ABTS radicals.

Reducing power (RP) assay

The RP assay of each extract was carried out according to the method of Oyaizu (13). One mL of the extract (concentration: 0.05, 0.1, 0.5, or 1 mg/mL), 1 mL of sodium phosphate buffer (0.2 M, pH 6.6), and 1 mL of 1% potassium ferricyanide solution were mixed and incubated at 50°C for 20 min. One mL of 10% TCA was added to the mixture, followed by centrifugation at 13,400×*g* for 5 min. One mL of supernatant was mixed with 1 mL of distilled water and 0.1 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates stronger reducing power.

Tyrosinase inhibitory activity

Tyrosinase inhibitory activity was determined with the procedure described by Vanni et al. (14). Each sample (100 μL) was diluted with 140 μL of 0.05 mM sodium phosphate buffer (pH 6.8) in a 96-well plate. Forty μL of 1.5 mM L-tyrosine solution and 20 μL of mushroom tyrosinase (1,500 units/mL) were added into appropriate wells. The test mixture (300 μL) was incubated at 37 °C for 30 min. The OD level was obtained using a multiplate reader at 492 nm and the inhibition percent of tyrosinase activity was calculated by the following formula:

$$\% \text{ inhibition} = (1 - A_{sample} / A_{control}) \times 100$$

Statistical analysis

All measurements were performed in triplicate, and analysis of variance was conducted by the General Linear Model using SAS (Statistical Analysis System) software. Student-Newman-Keul's multiple range tests were used to compare the significant differences of the mean values among treatments ($p<0.05$).

RESULTS AND DISCUSSION

Total phenolic contents (TPC)

Phenolic compounds are one of the most powerful antioxidant derivatives in plants (15), and are known to act as antioxidants not only because of their ability to donate hydrogens or electrons, but also because they are stable radical intermediates (16). The TPC in each persimmon flower bud extract, at a concentration of 1 mg/mL, were shown in Table 1. In this study, the TPC of acetone, ethanol and methanol extracts were significantly ($p<0.05$) higher than those of water extracts. The methanol extracts of persimmon flower buds showed the highest amounts of TPC (113.39 mg GAE/g), while water extracts showed the lowest TPC value (39.1 mg GAE/g).

We have determined TPC of four different parts (calyx, flesh, seed, and peel) of persimmon fruits of the same cultivar in this study (17). Acetone extracts of seeds

Table 1. Total phenolic contents of extracts from persimmon flower-buds by solvents (unit: mg GAE[1)]/g)

Solvents			
Acetone	Ethanol	Methanol	Water
103.13 ± 1.03^{b}	100.76 ± 0.77^{c}	113.39 ± 1.60^{a}	39.1 ± 0.45^{d}

All measurements were done in triplicate, and all values are means ± standard deviation. [a-d]Different letters within a row are significantly different ($p<0.05$), n=3.
[1)]GAE: gallic acid equivalents.

(97.92 mg GAE/g) and calyxes (91.30 mg GAE/g) showed higher TPC. Compared to persimmon fruits, persimmon flower buds possess significantly higher phenolic compounds. Though there are many reports about the phenolics of other flowers or flower buds (18,19), little is known about phenolics of persimmon flower buds. On the other hand, Sakanaka et al. (9) reported that TPC of methanol extracts of persimmon leaves were 59.3 mg GAE/g, while that of water extracts showed 112 mg GAE/g. This result suggested that polarity of phenolics in persimmon varies according to different parts.

DPPH radical scavenging activity (RSA)

Antioxidant activity of the persimmon flower bud extracts were evaluated by measuring DPPH RSA. DPPH is a stable free radical that accepts an electron to become a stable molecule. The DPPH RSA of four different extracts from persimmon flower buds were shown in Table 2. The IC_{50} value of each extract was compared to vitamin C (IC_{50}=28.84 μg/mL) and BHT (IC_{50}=298.47 μg/mL), the positive controls in this study. The methanol extract showed the highest DPPH RSA (IC_{50}=40.25 μg/mL), while the water extract exhibited the lowest activity (IC_{50}=274.85 μg/mL). The overall trend of DPPH RSA of the extracts paralleled the TPC results.

Some flower buds showed strong antioxidant activity. For example, IC_{50} values for DPPH RSA of ethanol extracts of *Cleistocalyx operculatus* buds (20) and methanol extracts of *Mammea longifolia* buds (21) were reported as 39.27 μg/mL and 8.33 μg/mL, respectively. These data indicated that the natural buds, including persimmon flower buds, generally possessed significant antioxidant activity.

ABTS radical scavenging activity (RSA)

ABTS, another stable free radical cation, was also used to evaluate antioxidant activity of the extracts from persimmon flower buds. The ABTS RSA assay system is suitable for monitoring lipophilic antioxidants, such as carotenoids, and lipophilic extracts of nutritional components (22). The ABTS RSA of the persimmon flower bud extracts showed similar trends to that of the DPPH RSA (Table 3). The acetone, ethanol, and methanol extracts exhibited significantly ($p<0.05$) higher ABTS RSA than the water extracts. The highest ABTS RSA was detected in the methanol extracts (IC_{50}=58.17 μg/mL), which was higher than the BHT (IC_{50}=310.16 μg/mL) used as the positive control. Likewise all the other extracts also showed higher ABTS RSA than BHT. Although it is difficult to compare antioxidant activity of persimmon flower buds with pure antioxidants (vitamin C or BHT), the significant antioxidant effects of the extracts with low IC_{50} could be a result from the synergistic effects of various compounds in the extracts.

In our previous report (17), methanol extracts of calyxes and seeds of the same persimmon cultivar showed 19.45 and 29.36 μg/mL IC_{50} values, respectively, for ABTS RSA. This means calyxes and seeds possess higher ABTS RSA than flower buds. There was no correlation between TPC and ABTS RSA. In the study of the antioxidant activity of fuyu persimmon tree branches, the IC_{50} value of the acetone extract for ABTS RSA was determined as 493.76 μg/mL (23). The branch of persimmon had lower antioxidant activity, because the amount of polyphenol was lower than persimmon flower-bud (23).

Reducing power (RP) assay

The reducing capacity may serve as a significant indicator of its potential antioxidant activity and be associated with the presence of reducing substances (24,25). Duh (26) reported that the reducing properties of antioxidants are generally related to the presence of reductants. The RP of extracts also showed similar disposi-

Table 2. DPPH radical scavenging activity of extracts from persimmon flower-buds by solvents (unit: IC_{50}[1)])

Solvents				Positive Control	
Acetone	Ethanol	Methanol	Water	Vitamin C	BHT
65.13±0.71[c]	63.71±4.21[b]	40.25±1.60[a]	274.85±0.21[d]	28.84±0.24	298.47±4.82

All measurements were done in triplicate, and all values are means±standard deviation.
[a-d]Different letters within a row are significantly different ($p<0.05$), n=3.
[1)]IC_{50} (μg/mL): Concentration for scavenging 50% of DPPH radicals.

Table 3. ABTS radical scavenging activity of extracts from persimmon flower-buds by solvents (unit: IC_{50}[1)])

Solvents				Positive Control	
Acetone	Ethanol	Methanol	Water	Vitamin C	BHT
61.01±3.42[b]	68.53±2.16[c]	58.17±0.97[a]	212.77±4.81[d]	28.05±0.17	310.16±3.98

All measurements were done in triplicate, and all values are means±standard deviation.
[a-d]Different letters within a row are significantly different ($p<0.05$), n=3.
[1)]IC_{50} (μg/mL): Concentration for scavenging 50% of ABTS radicals.

Table 4. Reducing power of extracts from persimmon flower-buds by solvents (unit: IC_{50}[1)])

Solvents				Positive Control	
Acetone	Ethanol	Methanol	Water	Vitamin C	BHT
88.75±1.64[c]	80.34±0.48[b]	69.43±1.57[a]	327.50±0.57[d]	24.01±0.15	52.32±0.22

All measurements were done in triplicate, and all values are means±standard deviation.
[a-d]Different letters within a row are significantly different ($p<0.05$), n=3.
[1)]IC_{50} (μg/mL): Concentration for increasing 0.500 value in optical density.

Table 5. Tyrosinase inhibitory activity of extracts from persimmon flower-buds by solvents (unit: %)

Solvents				Positive Control
Acetone	Ethanol	Methanol	Water	Vitamin C
51.85±0.62[b]	88.90±1.48[a]	45.47±1.74[c]	18.49±0.45[d]	44.27±1.16

All measurements were done in triplicate, and all values are means±standard deviation.
[a-d]Different letters within a row are significantly different ($p<0.05$), n=3.
Concentration: all samples (1 mg/mL), vitamin C (0.1 mg/mL).

tions to that of DPPH and ABTS RSA (Table 4). Persimmon flower-bud methanol extracts were similar to the positive control BHT. The highest RP of extracts was shown in the acetone extract (IC_{50}=69.43 μg/mL). These results coincide with the values of TPC, DPPH RSA, and ABTS RSA.

Tyrosinase inhibitory activity

Tyrosinase inhibitors are chemical agents capable of reducing enzymatic reactions, such as food browning and melanisation of human skin. Therefore, these agents have good commercial potential in both food processing and cosmetic industries. The tyrosinase inhibitory activity of persimmon flower-bud extracts is expressed in Table 5. All samples were first dissolved in DMSO at 50 mg/mL and then diluted to 1 mg/mL using DMSO. The ethanol extracts (88.90%) showed the highest tyrosinase inhibitory activity, while water extracts (18.49%) showed the lowest tyrosinase inhibitory activity. The ethanol extracts of guava leaves at 1 mg/mL (27) and 70% ethanol extracts of waxy type whole barely at 250 ppm (28) showed 69.56% and 26.76% of tyrosinase inhibitory activity, respectively. The results suggested that ethanol extracts of persimmon flower-buds possesses significant tyrosinase inhibitory activity. Because ethanol is a permitted solvent in food and cosmetic industries, the outlook for applications using persimmon buds is positive.

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REFERENCES

1. Lee JY, Hwang WI, Lim ST. 2004. Antioxidant and anticancer activities of organic extracts from *Platycodon grandiflorum* A. De Candolle roots. *J Ethnopharmacol* 93: 409-415.
2. Pezzuto JM. 1997. Plant-derived anticancer agents. *Biochem Pharmacol* 53: 121-133.
3. Wu J, Wu Y, Yang BB. 2002. Anticancer activity of *Hemsleya amabilis* extract. *Life Sci* 71: 2161-2170.
4. Heim KE, Tagliaferro AR, Bobilya DJ. 2002. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *J Nutr Biochem* 13: 572-584.
5. Balasundram N, Sundram K, Samman S. 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chem* 99: 191-203.
6. George AP, Redpath S. 2008. Health and medicinal benefits of persimmon fruit: a review. *Adv Hort Sci* 22: 244-249.
7. Matsuo T, Ito S. 1978. The chemical structure of kakitannin from immature fruit of the persimmon (*Diospyros kaki* L.). *J Agric Biol Chem* 42: 1637-1640.
8. Shela G, Elzbieta B, Gustaw K, Marina Z, Simon T. 1998. Dietary persimmon improves lipid metabolism in rats fed diets containing cholesterol. *J Nutr* 128: 2023-2027.
9. Sakanaka S, Tachibana Y, Okada Y. 2005. Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). *Food Chem* 89: 569-575.
10. Gutfinger T. 1981. Polyphenols in olive oil. *J Am Oil Chem Soc* 58: 966-968.
11. Lee SC, Kim JH, Jeong SM, Kim DR, Ha JU, Nam KC, Ahn DU. 2003. Effect of far-infrared radiation on the antioxidant activity of rice hulls. *J Agric Food Chem* 51: 4400- 4403.
12. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evan C. 1999. Antioxidant activity applying improved ABTS radical cation decolorization assay. *Free Radical Biol Med* 26: 1231-1237.
13. Oyaizu M. 1986. Studies on product of browning reaction prepared from glucose amine. *Jpn J Nutr* 44: 307-315.
14. Vanni A, Gastaldi D, Giunata G. 1990. Kinetic investigations on the double enzymatic activity of the tyrosinase mushroom. *Ann Chim-Rome* 80: 35-60.
15. Maillard MN, Soum MH, Boivia P, Berset C. 1996. Antioxidant activity of barley and malt: relationship with phenolic content. *Lebensm-Wiss Technol* 29: 238-244.
16. Rice-Evans CA, Miller NJ, Paganga G. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol Med* 20: 933-956.

17. Jang IC, Jo EK, Bae MS, Lee HJ, Jeon GI, Park E, Yuk HG, Ahn GH, Lee SC. 2010. Antioxidant and antigenotoxic activities of different parts of persimmon (*Diospyros kaki* cv. Fuyu) fruit. *J Med Plant Res* 4: 155-160.
18. Lee EJ, Kim JS, Kim HP, Lee JH, Kang SS. 2010. Phenolic constituents from the flower buds of *Lonicera japonica* and their 5-lipoxygenase inhibitory activities. *Food Chem* 120: 134-139.
19. Dan C, Liang J, Bai BR, Peng SL, Ding LS. 2010. A new phenolic glucoside gallate from the flower buds of *Eugenia caryophyllate*. *Chin J Nat Med* 8: 180-182.
20. Dung NT, Kim JM, Kang SC. 2008. Chemical composition, antimicrobial and antioxidant activities of the essential oil and the ethanol extract of *Cleistocalyx operculatus* (Roxb.) Merr and Perry buds. *Food Chem Toxicol* 46: 3632-3639.
21. Rathee JS, Hassarajani SA, Chattopadhyay S. 2006. Antioxidant activity of *Mammea longifolia* bud extracts. *Food Chem* 99: 436-443.
22. Nicoletta P, Roberta R, Min Y, Catherine RE. 1999. Screening of dietary carotenoids and carotenoid-rich fruit extracts for antioxidant activities applying 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation decolorization assay. *Method Enzymol* 299: 379-389.
23. You DH, Jang IC, Seo GU, Kim SJ, Yang EK, Ahn GH, Matsushita Y, Lee SC. 2010. Antioxidant activity of branches of fuyu and nishimurawase persimmon trees. *J Korean Soc Appl Biol Chem* 53: 82-86.
24. Mier S, Kaner J, Akiri B, Hadas SP. 1995. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. *J Agric Food Chem* 43: 1813-1817.
25. Shimada K, Fujikawa K, Yahara K, Nakamura T. 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *J Agric Food Chem* 40: 945-948.
26. Duh PD. 1998. Antioxidant activity of budrock (*Arctium lappa* L.): its scavenging effect on free radical and active oxygen. *J Am Oil Chem Soc* 75: 455-461.
27. You DH, Park JW, Yuk HG, Lee SC. 2011. Antioxidant and tyrosinase inhibitory activities of different parts of guava (*Psidium guajava* L.). *Food Sci Biotechnol* 20: 1095-1100.
28. Lee NY, Kim YK, Choi I, Cho SK, Hyun JN, Choi JS, Park KH, Kim KJ, Lee MJ. 2010. Biological activity of barely (*Hordeum vulgare* L.) and barely by-product extracts. *Food Sci Biotechnol* 19: 785-791.

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