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Antioxidant and Anticancer Properties of Methanolic Extracts from Different Parts of White, Yellow, and Red Onion

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Abstract Antioxidant and anticancer effects of methanolic extracts from the flesh (WFME) and peel (WPME) of white onion, the flesh (YFME) and peel (YPME) of yellow onion, the flesh (RFME) and peel (RPME) of red onion were studied. The content of total phenolics in WFME, WPME, YPME, YFME, RPME, and RFME were 0.260±0.01, 4.480±0.23, 0.319±0.02, 719.12±37.36, 0.248±0.01, and 806.21±26.38 mg/g, respectively. The quercetin content of WFME, WPME, YFME, YPME, RFME, and RPME were 12.56±0.19, 3.57±0.14, 15.24±0.65, 755.29±22.24, 5.70±0.23, and 774.03±29.48 mg/100 g, respectively. Like total phenolics, the highest 2,2'-diphenyl-1-picrylhydrazyl (DPPH) scavenging activities were found in RPME. However, inhibitory effects on lipid oxidation of RPME were similar to those of WPME and YPME. In addition, inhibitory effect of WPME, YPME, and RPME for human breast cancer cell (MCF-7) growth were 78.43, 81.90, and 96.52% while those on human prostate cancer cell (LNcap) were 71.58, 77.93, and 98.47% at 100 μg/mL, respectively. Total phenolics, quercetin content, antioxidant, and anticancer activities exhibited significant variation among the 3 onion varieties in this experiment. Therefore, it is assumed that antioxidant and anticancer activities were affected by the total phenolics and quercetin level of onion.

Keywords: onion, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), human breast cancer cell, human prostate cancer cell

Introduction

Reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), superoxide anion radical (O_2), hydroxyl radical (OH) are generated from the autoxidation of lipids, as well as reactive nitrogen species (RNS) (1,2). Formations of these excess ROS and RNS by ultraviolet (UV) irradiation, smoking and drug metabolism are likely to damage several cellular components such as lipids, proteins, nucleic acid, and DNAs through oxidation nitration processes (3). In addition these reactive species cause inflammation or lesions on various organs and are associated with various degenerative diseases, including cancer, aging, arteriosclerosis, and rheumatism (4-8). Plants contain a wide variety of chemicals that have potential antioxidant activity. The best known phytochemical antioxidants are traditional nutrients, such as β-carotene, ascorbic acid, and α-tocopherol. However, there is a growing evidence that a significant portion of the antioxidant capacity of many food plants is due to compounds other than the traditional vitamins (9). Recently, researchers have sought to isolate powerful and nontoxic natural antioxidant from edible plants not only to prevent human from these by autoxidation and lipid peroxidation, but also to replace synthetic antioxidants (10). Therefore, the research into the determination of the natural antioxidant source is very important to promote public health.

Onion (*Allium cepa*) has been shown to contain large amounts of flavonoids, and constitute one of the major sources of flavonoids in diets (11). The onion, a vegetable member of the genus allium, has been reported to diminish atherosclerosis or thrombotic disease in populations with increased onion intake (12). These beneficial effects of onion have been attributed to its ability to inhibition platelet aggregation and thromboxane formation (13). However there is little information on antioxidant and anticancer activities of methanol extracts from onion according to varieties and parts. In line with the efforts to balance the conservation of biodiversity and encouraging controlled exploitation of plant resources for economic gains, especially in biopharming, wastage of valuable resources should be minimized. The objectives for this study were to determine the total phenolics contents, antioxidant activity, and anticancer activity of methanolic extracts from peel of white, yellow, and red onion which are normally wasted during frequent pruning, and determine correlations between antioxidant or anticancer activity and qucercetin or total phenolics.

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Materials and Methods

Sample and preparation of methanolic extracts The white onion, yellow onion, and red onion were purchased from local market (Jinju, Korea) in November 2006, and divided into flesh and peel. Each onion was skinned, chopped, and lyophilized. The lyophilized onions were ground to a fine powder. The ground peel and flesh powder were extracted with methanol at room temperature for 24 hr at 20°C (3 times with 100 mL). The methanol extracts were filtered through Adventec No. 2 filter paper (Adventec, Toyo Roshi Kaish, Ltd., Tokyo, Japan). The residues were extracted again with methanol using the same method as mentioned above. The extracts were

combined and evaporated to dryness in a rotary evaporator (N-N series; Eyela, Tokyo, Japan) at 40°C, dissolved in methanol, and used for determination of total phenolic concentration, antioxidant. Then anticancer activities of the flesh (WFME) and peel (WPME) of white onion, the flesh (YFME) and peel (YPME) of yellow onion, the flesh (RFME) and peel (RPME) of red onion were evaluated.

Total phenolics contents The contents of total phenol were analyzed by the method of Folin and Denis (14), reading samples on a UV/Vis-spectrophotometer (UV-1201; Shimadzu, Tokyo, Japan) at 760 nm. The each methanol extract was mixed with 5 mL of Folin-Denis phenol reagent (Sigma-Aldrich, St. Louis, MO, USA), 10 mL of 7% Na_2CO_3 and diluted by a factor 100 with distilled water. The total phenol content of each methanol extract was estimated by comparison with a standard curve generated from analysis of gallic acid (mg GAE/g extract).

Determination of quercetin One g of sample was mixed with 40 mL of 60% aqueous ethanol and 5 mL of 6 M HCl. After refluxing at 95°C for 2 hr, the hydrolysed solution was filtered into a 50-mL volumetric flask and subsequently made up to the volume with 60% aqueous ethanol. Approximately 1 mL of the final solution was allowed to cool under running water and filtered through a 0.45-μm filter (Nylon Acrodisc 13; Gelman, Ann Arbor, MI, USA) prior to injection for high performance liquid chromatography (HPLC) analysis. The analysis of quercetin in sample was carried out by the following HPLC method. Agillent 1100 series liquid chromatograph system (Agilent Technologies, Palo Alto, CA, USA), comprising vacuum degasser, quaternary pump, auto-sampler, thermostated column compartment, and diode array detector, was used. The column was a C18 reversed phase Kingsorb 5 μm C18 (150×4.6 mm) (Phenomenex, UK) with a Kingsorb 5 μm C18 (30×4.6 mm) guard column. Mobile phase consisted of 30% acetonitrile in 0.025 M KH_2PO_4 buffer solution (v/v); the pH of the mobile phase was adjusted with 6 M HCl to 2.5. The flow rate was 1.0 mL/min. The column was operated at 30°C. The sample injection volume was 20 μL. UV spectra were recorded from 200 and 400 nm, and peak areas were measured at 370 nm (15).

DPPH free radical scavenging activity This was carried according to Blois method with a slight modification (16). Briefly, a 1 mM solution of 2,2'-diphenyl-1-picrylhydrazyl (DPPH)(Sigma-Aldrich) radical solution in ethanol was prepared, and then 1 mL of this solution was mixed with 4 mL of extract solution in methanol containing 16-1,000 μg/mL of dried extract; finally, after 30 min, the absorbance was measured at 517 nm (UV-1201; Shimadzu, Tokyo, Japan). This activity is given as percent DPPH scavenging that is calculated as

$$\% \text{ DPPH scavenging} = [(Abs_{control} - Abs_{extract})/(Abs_{control})] \times 100$$

Inhibitory activity on lipid oxidation Ferric thiocyanate (FTC) method (17) using linoleic acid model system was used to measure inhibitory activity on lipid oxidation. Mixture (0.5 mL) of methanol and extract sample, 0.5 mL of 2.51% linoleic acid, 1 mL of 50 mM phosphate buffer (pH 7.0), and 0.5 mL distilled water were mixed well in a capped test tube and incubated at 40°C. Sample aliquots (0.1 mL) were taken every 24 hr, dissolved in 9.7 mL of 75% ethanol, and placed in a capped test tube. Thirty % ammonium thiocyanate solution (0.1 mL) was added to the sample suspension. After 3 min, 0.1 mL ferrous chloride solution (20 mM) dissolved in 3.5% HCl solution was added to the sample suspension as a color indicator. The absorbance was measured at 500 nm using a spectrophotometer (UV-1201; Shimadzu).

Anticancer effect Anticancer activity was measured using the sulforhodamine B (SRB) assay (18). All samples were sterilized by filtration through a 0.22-μm filter (Nylon Acrodisc 13; Gelman) prior to analysis in the anticancer activity test. The test-cells used in this assay were MCF-7 (human breast cancer cell) and LNcap (human prostate cancer cell). Cells were maintained as adherent cell cultures in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 10 units of penicillin and 10 μg/mL streptomycin (Gibco, Grand Island, NY, USA) at 37°C in a humidified incubator (Sanyo Electric Co., Ltd., Gunma-ken, Japan) containing 5% CO_2. Cells were transferred into 96-well plates and incubated for 24 hr prior to the addition of test samples. Samples were added and incubated for 48 hr. The effects of the samples on the growth of human cancer cell lines were evaluated for their cytotoxicity activity using a SRB.

Statistical analysis Results were expressed as mean values. Comparison of means was performed by Duncan's multiple-range test using Statistical analysis system (SAS). Significance was determined at $p<0.05$. All data were reported as the mean±standard deviation (SD) of 3 replications.

Results and Discussion

Total phenolics contents Methanol was the most suitable solvent in the extraction in polyphenolic compounds from plant tissue, due to its ability to inhibit the action of polyphenol oxidase that causes the oxidation of polyphenol and its ease of evaporation compared to water (19). The total phenolic compound contents of WFME, WPME,

Table 1. Contents of phenolics and quercetin in different parts of white, yellow, and red onion

Sample[1)]	Total phenolic compounds (mg/g)	Quercetin level (mg/100 g)
WFME	0.260±0.015[2)d]	12.56±0.19[c]
WPME	4.480±0.23[c]	3.57±0.14[d]
YFME	0.319±0.017[d]	15.24±0.65[c]
YPME	719.12±37.36[b]	755.29±22.24[a]
RFME	0.248±0.013[d]	5.70±0.23[d]
RPME	806.21±26.38[a]	774.03±29.48[a]

[1)]WFME, white onion flesh methanol extract; YFME, yellow onion flesh methanol extract; RFME, red onion flesh methanol extract; WPME, white onion peel methanol extract; YFME, yellow onion peel methanol extract; RPME, red onion peel methanol extract.
[2)]Values are mean±SD of triplicates.

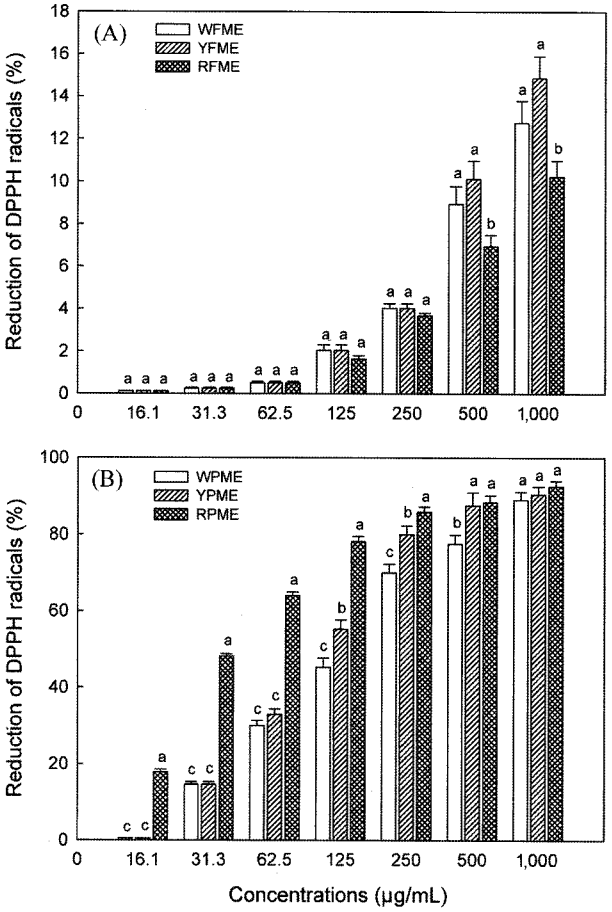

Fig. 1. DPPH radical scavenging activities of methanol extracts from flesh (A) and peel (B) of white, yellow, and red onion. WFME, white onion flesh methanol extract; YFME, yellow onion flesh methanol extract; RFME, red onion flesh methanol extract; WPME, white onion peel methanol extract; YFME, yellow onion peel methanol extract; RPME, red onion peel methanol extract.

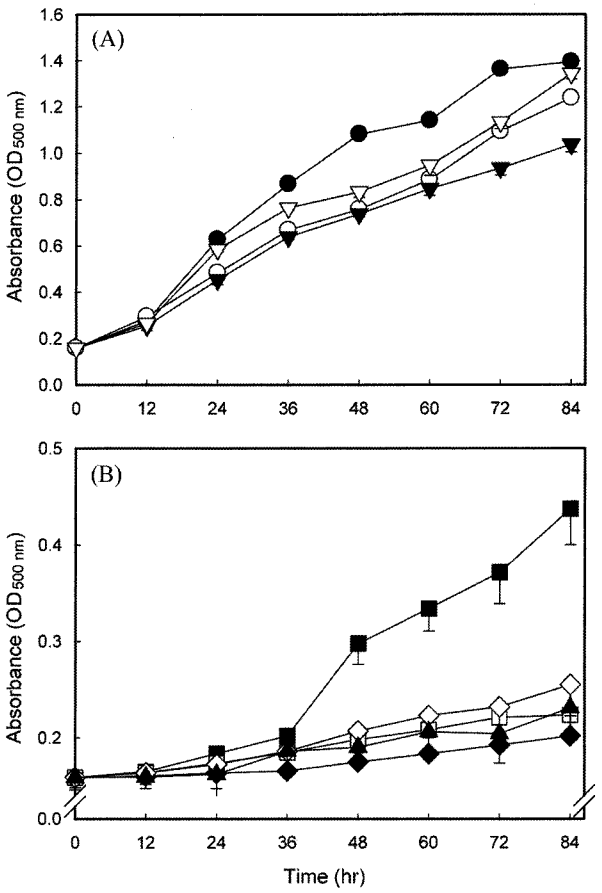

Fig. 2. Antioxidant activities of methanol extracts from flesh (A) and peel (B) of white, yellow, and red onion at the level of 1 mg/mL. ●, Control; ○, WFME; ▼, YFME; ▽, RPME; ■, WPME; □, YFME; ◆, RPME; ◇, α-tocopherol; ▲, BHA. WFME, white onion flesh methanol extract; YFME, yellow onion flesh methanol extract; RFME, red onion flesh methanol extract; WPME, white onion peel methanol extract; YFME, yellow onion peel methanol extract; RPME, red onion peel methanol extract.

YFME, YPME, RFME, and RPME were 0.260±0.015, 4.48±0.23, 0.319±0.017, 719.12±37.36, 0.248±0.013, and 806.21±26.38 mg gallic acid equivalent/g, respectively (Table 1). It was noted that WPME, YPME, and RPME, had significant higher total phenol contents than those of WFME, YFME, and RFME ($p<0.05$). Phenolics are widely found in food products derived from plant sources and they have been shown to possess significant antioxidant activities. Studies have shown that increasing levels of flavonoids in the diet could decrease certain human diseases (20).

Quercetin contents As shown in Table 1, the amounts of quercetin in the WFME, WPME, YFME, YPME, RFME, and RPME were 12.56±0.19, 3.57±0.14, 15.24±0.65, 755.29±22.24, 5.70±0.23, and 774.03±29.48 mg/100 g, respectively (Table 1). Kim and Kim (21) reported that the level of quercetin of onion was increased with greater distance from the core. Skin, the nonedible part, showed outstanding levels of quercetin comparing to the other edible parts.

Scavenging effect on DPPH radical The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. Figure 1 shows the dose-response curves of DPPH radical scavenging activities of the 4 methanol extracts from onion. It was found that the radical scavenging activities of all the extracts increased with increasing concentration. The scavenging effect was in the order: RPME (92.53%)>YPME (90.39%)>WPME (89.02%) >YFME (14.86%)>WFME (12.75%)>RFME (10.24%) at a concentration of 1.0 mg/mL ($p<0.05$). Kim and Kim (21) reported that DPPH radical scavenging activity was affected by the quercetin level of onion. Thus, the DPPH radical scavenging activity of onion methanol extracts may be mostly related to their phenolic hydroxyl group.

Inhibitory effect of lipid oxidation The inhibitory effect of the 6 onion methanol extracts (WFME, WPME, YFME, YPME, RFME, and RPME), α-tocopherol and ascorbic acid on lipid peroxidation in the linoleic acid system are shown in Fig. 2. It was also found that the inhibitory effects on the lipid peroxidation of all the tested extracts were

Table 2. Anticancer activity of methanol extracts from fresh and peel of white, yellow, and red onion on human breast cancer cell line (MCF-7) and prostate cancer cell line (LNcap)

Sample[1)]	Conc. (μg/mL)	Anticancer activity (%)	
		MCF-7	LNcap
WFME	1	1.21±0.22[2)d]	1.01±0.15[d]
	10	13.92±1.35[c]	10.64±1.06[cd]
	100	33.86±2.32[bc]	17.47±2.35[c]
WPME	1	3.26±0.54[d]	2.52±0.61[d]
	10	17.89±1.64[c]	11.64±1.26[cd]
	100	78.43±3.69[b]	71.58±2.97[b]
YFME	1	1.28±0.32[d]	1.31±0.21[d]
	10	18.32±1.93[c]	19.32±1.25[cd]
	100	37.29±2.47[bc]	27.29±2.72[c]
YPME	1	4.26±0.73[d]	3.79±0.73[d]
	10	19.24±1.73[c]	12.63±1.37[cd]
	100	81.90±3.93[b]	77.93±3.19[b]
RFME	1	1.26±0.12[d]	1.09±0.12[d]
	10	15.39±1.74[c]	12.39±1.02[cd]
	100	32.65±3.10[bc]	22.65±3.21[c]
RPME	1	9.05±1.38[d]	10.62±1.02[cd]
	10	25.68±4.42[c]	19.31±3.19[c]
	100	96.52±5.05[a]	98.47±6.96[a]

[1)]WFME, white onion flesh methanol extract; YFME, yellow onion flesh methanol extract; RFME, red onion flesh methanol extract; WPME, white onion peel methanol extract; YFME, yellow onion peel methanol extract; RPME, red onion peel methanol extract.
[2)]Values are mean±SD of triplicates.

concentration-dependent. At the concentration of 1 mg/mL, the inhibitory effects of the 6 extracts followed the order RPME, YPME, WPME, YFME, RFME, and WFME ($p<0.05$). The WPME, YPME, and RPME had a better inhibitory effect than the WFME, YFME, and RFME at 1 mg/mL. Ramarathnam *et al.* (22) discovered that phenolic compounds play an important role in blocking autoxidation of the oils. Additionally, Nuutila *et al.* (23) observed that the edible part of onion was clearly less effective than the skin and the edible part of onion was a concentration of 1,000 mg/mL resulted in only 40% lipid peroxidation inhibition.

Anticancer effect Recently, with increased breast cancer and prostatic carcinoma due to westernization and changes in food habits, attention has been focused on cancer preventive effects of some foods and in particular *Allium*. Growth inhibitory activities of the WFME, WPME, YFME, YPME, RFME, and RPME against human breast cancer cells (MCF-7) are shown in Table 2. RPME exhibited the highest growth inhibitory activity of 96.52% and YFME exhibited the next highest growth inhibition at 81.90%. The YFME exhibited low inhibitory activity of below 10% and the WFME and RFME exhibited no significantly inhibitory activity against MCF-7. Growth inhibitions of the WFME, WPME, YFME, YPME, RFME, and RPME against human prostate cancer cells (LNcap) are shown in Table 2. RPME exhibited the strongest inhibitory activity of 98.47% at a concentration of 100 μg/mL. The RPME had the highest total phenolic content exhibiting strong growth inhibitory activity. Yang *et al.* (24) reported that several onion varieties including shallots, Northern Red, Western Yellow, and New York Bold showed relatively high antiproliferative activities toward both HepG2 and Caco-2 cell in a dose-dependent manner. It seems to have a strong relation among 3 factors including HepG2 cell proliferation, phenolics, and total antioxidant activity. This activity may be due to the presence of phenolics compounds especially quercetin. Total phenolic and quercetin contents and antioxidant and anticancer effect exhibited a significant variation among the 6 methanol extracts from onion flesh and peel in this experiment. RPME exhibited the greatest total phenolic content and quercetin content, thus also exhibiting the greatest antioxidant and anticancer activities. Strong correlations were observed between total phenolic content, quercetin content, antioxidant and anticancer activities. In our results, quercetin and total phenolics may have important roles in the antioxidant and anticancer activities of onion flesh and peel. Finally, our results verified that the methanol extract of onion flesh and peel has a very strong antioxidant and anticancer activities and can be utilized as an effective and safe antioxidant source.

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