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Comparison of Radical Scavenging Activity of Extracts of Mulberry Juice and Cake Prepared from Mulberry (*Morus* spp.) Fruit

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Abstract

Radical scavenging activity of water and methanol extracts of mulberry juice and cake prepared from mulberry fruit (*Morus* spp.) was evaluated using three *in vitro* assay systems. Mulberry fruits were homogenized with 0.5% trifluoroacetic acid (TFA) in distilled water, filtered with cheeze-cloth and centrifuged to yield mulberry juice and cake. Mulberry juice was evaporated and solubilized in 0.5% TFA in distilled water or 0.5% TFA in 80% aqueous methanol, followed by filtration and evaporation to obtain water (WMJ) and methanol (MMJ) extracts of mulberry juice. Mulberrry cake also was extracted with the above same solvents, and thereby finally obtaining water (WMC) and methanol (MMC) extracts of mulberry cake. Among four extracts, the MMC showed the most potent radical scavenging activity against DPPH radical (IC_{50} =167.45 μg/mL), and superoxide (IC_{50}=36.18 μg/mL) and hydroxyl radicals (IC_{50}=467.08 μg/mL). The WMC also exhibited stronger radical scavenging activity than those of two other mulberry juice extract, WMJ and MMJ. Meanwhile, the MMJ exerted stronger three radical scavenging activity than the WMJ. Total phenolic content of the water and MeOH extracts from mulberry cake was higher than that of the water and MeOH extracts from mulberry juice. Thus, these results suggest that the extracts of mulberry cake with high dietary phenolics may be useful potential source of natural antioxidant as radical scavenger.

Key words: mulberry (*Morus* sp.) fruit, mulberry juice, mulberry cake, radical scavenging activity

INTRODUCTION

Recently, much attention has been focused on dietary natural antioxidants capable of inhibiting reactive oxygen radical mediated lipid peroxidation which is mainly involved in several pathological conditions such as atherosclerosis, cancer, and aging (1,2). Particularly, superoxide and hydroxyl radicals of reactive species are known to cause severe oxidative damage to susceptible biomolecules, and thereby eventually contributing to significant biological effects such as carcinogenesis, mutagenesis and cytotoxicity (3,4). For this reason, an extensive search for novel natural antioxidants acted as radical scavenger has been undertaken.

Mulberry (*Morus* spp.) fruit is rich in sugars, organic acids and anthocyanins, which are important for several palatable beverages such as juice, yoghourt and wine. Particularly, the crude drug "Sangsimja", the fruits of mulberry (*Moraceae*), has been used in folk medicine to treat diabetes, bald head, hangover, hypertension and inflammation, etc. (5,6). Recently, mulberry fruits have been reported to possess several biological effects such as antidiabetic (7,8), antioxidative (9-11), antiinflammatory (10) and antihyperlipidemic (12) activities. Therefore, demand for the mulberry fruit has increased in recent years because of its dessert and functional properties. However, few studies on antioxidant activity of mulberry juice, and mulberry cake obtained as byproduct in the process of manufacturing mulberry juices are so far available, although many studies have performed on the chemistry and biological activity of mulberry fruits.

The objective of this study was to evaluate three radical scavenging activity of the water and methanol extracts of mulberry juice and cake prepared from mulberry fruits against DPPH radical, superoxide and hydroxyl radicals.

MATERIALS AND METHODS

Materials

Mulberry fruits of Chongilppong (*M. alba* L.) tree were directly harvested in the middle of June from the farm in Yeongcheon, Gyeongbuk, Korea. The mulberry fruits

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were freeze-dried and stored at -18°C until use.

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), xanthine oxidase (EC 1.2.3.2), xanthine, nitrobluetetrazolium chloride (NBT), thiobarbituric acid (TBA), H_2O_2, 2-deoxyribose, bovine serum albumin (BSA), trifluoroacetic acid (TFA) and dimethyl sulfoxide (DMSO) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Butylated hydroxytoluene (BHT), L-ascorbic acid, and $FeCl_3 \cdot 6H_2O$ were purchased from Wako Pure Chemical Ind. (Osaka, Japan). Sodium phosphate dibasic 12 hydrate and potassium phosphate monobasic were obtained from Kanto Chemical Co., Inc. All other reagents used in this study were of analytical grade.

Preparation of mulberry juice and cake

Schematic procedure for preparation of mulberry juice and cake from mulberry fruits is shown in Fig. 1. Freeze-dried mulberry fruits (50 g) were homogenized with 0.5% trifluoroacetic acid (TFA) in distilled water (1 L) and filtered with a cheeze-cloth. The residue was washed repeatedly with the same solvent to remove anthocyanin pigments, and the filtrates were centrifuged at 5,000 rpm for 20 min to yield mulberry juice and cake. The combined mulberry juices and cakes were evaporated under reduced pressure and freeze-dried, respectively. Dried mulberry juices and cakes were further extracted twice with distilled water (1 L) and 80% aq. MeOH (1 L) under a ultrasonic cleaner (Bransonic 5210R-DTH, USA) for 2 hr, filtered and evaporated *in vacuo*, and thereby obtaining water (WMJ & WMC) and MeOH (MMJ & MMC) extracts of mulberry juice and cake.

DPPH radical scavenging activity

DPPH radical scavenging activity was determined according to the method of Tagashira and Ohtake (13). MeOH solution (200 μL) of sample at various concentrations (0.1 ~ 10 mg/mL) was added to a solution of DPPH in MeOH (1.5×10^{-4} M, 4 mL) and then vortexed vigorously. The reaction mixture was allowed to stand for 10 min at room temperature and the absorbance at 517 nm was measured. IC_{50} values were determined by regression analysis of the results obtained at three different concentrations of the sample.

Superoxide radical scavenging activity

Superoxide radical scavenging activity was determined

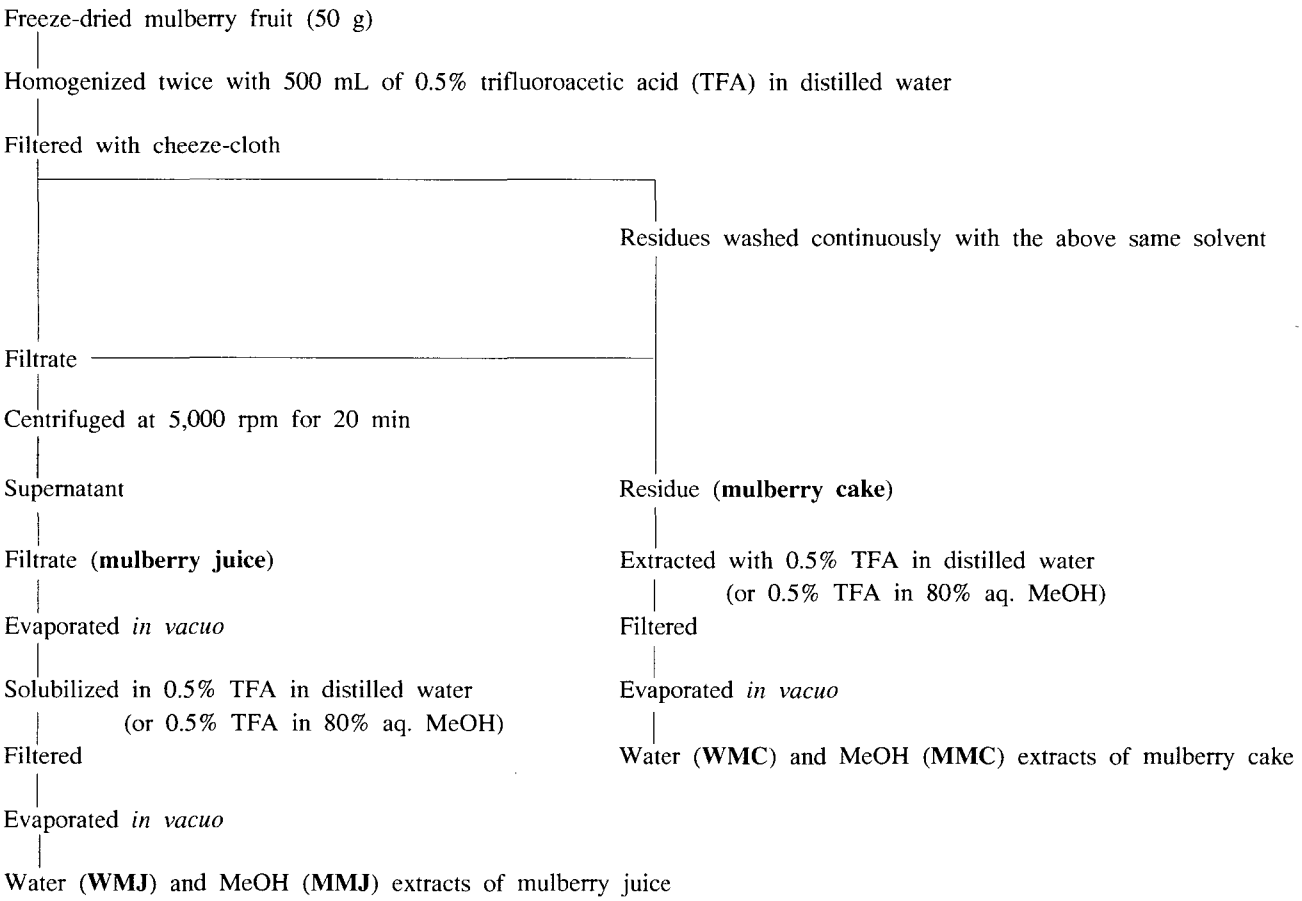

Fig. 1. Schematic procedure of water and methanol extracts of mulberry juice and cake prepared from mulberry fruits.

according to the method of Noro et al. (14), with minor modification. Sample solution [0.1 mL, 0.01~1.0% in MeOH (final concentration 5%)] was added to 0.05 M Na_2CO_3 buffer (pH 10.2, 2.4 mL) containing 3 mM xanthine (0.1 mL), 3 mM EDTA (0.1 mL), BSA (0.1 mL) and 0.75 mM NBT (0.1 mL) in a 4.5 mL glass cubic cell. The reaction mixture was allowed to stand at 25°C for 10 min and 5.0 U/mL xanthine oxidase (0.1 mL) was added to initiate the generation of superoxide anion (O^-_2). The mixture was incubated at 25°C for 20 min and 6 mM $CuCl_2$ (0.1 mL) was added and the mixture was read at 560 nm against blank samples which did not contain the enzyme. IC_{50} values were determined, as previously described.

Hydroxy radical scavenging activity

Hydroxy radical scavenging activity was determined by 2-deoxyribose assay according to the method of Halliwell et al. (15) with slight modification. The following reagents were added to the glass tubes in the order and at the final concentration stated: 0.3 mL KH_2PO_4-KOH buffer (pH 7.4, 30 mM), 0.2 mL 2-deoxyribose (2 mM), 0.2 mL $FeCl_3 \cdot 6H_2O$ (0.1 mM), EDTA (104 μM), 0.1 mL test solution [0.1~1.0% in MeOH (final concentration 5%)], 0.1 mL H_2O_2 (1.0 mM), 0.1 mL L-ascorbic acid (0.1 mM). The reaction mixtures were incubated at 37°C for 1 hr in a shaking water bath, and the peroxidation products were then determined by the thiobarbituric acid (TBA) method (16). IC_{50} values were determined, as previously described.

Total phenolic content

The total phenolic contents of the mulberry extracts were determined with the Folin-Ciocalteu reagent (17). Each mulberry extracts (0.5 mL) was diluted in a test tube with distilled water to 5.0 mL. Folin-Ciocalteu reagent (5.0 mL) was added, and then mixed thoroughly. After an interval of 3.0 min, 10% Na_2CO_3 solution (5.0 mL) was added, and then mixture was allowed to stand for 1 hr with intermittent mixing. The optical density (O.D.) was measured on an UV-vis JASCO spectrophotometer (Tokyo, Japan). The concentration of the total phenolic content of the mulberry extracts was determined by comparison with the O.D. values of different concentrations of a standard phenolic compound, gallic acid. The experiment was carried out in triplicate, and the total phenolic contents were expressed as gallic acid equivalent per 100 g of dried extracts.

Statistical analysis

All experiments were performed in three replicates. A curve of the concentration against plotted the percentage inhibition was used to calculate the half-maximal inhibition concentration (IC_{50}). Statistical analysis was performed using Duncan's multiple range test (18).

RESULTS AND DISCUSSION

Yield of mulberry juice and cake prepared from mulberry fruits

The yields (%) of water (H_2O) and methanol (MeOH) extracts of mulberry juice and cake prepared from mulberry fruits are shown in Table 1. The yields of the H_2O and MeOH extracts from the mulberry juice were 70.01 and 65.38%, respectively, while those of H_2O and MeOH extracts from mulberry cake were 1.68 and 1.82%, respectively. In the mulberry juice, the yield of the H_2O extract was slightly higher than that of the MeOH extract, while, in the mulberry cake, the yield of the MeOH extract was slightly higher than that of the H_2O extract. Thus, the yields of the extracts from mulberry juice were significantly higher than those of the extracts from mulberry cake. It is considered that the higher yield of H_2O and MeOH extracts from mulberry juice could largely be ascribed to the presence of high amounts of anthocyanin pigments in mulberry juice (19).

DPPH, superoxide and hydroxyl radical scavenging activity

DPPH, superoxide and hydroxyl radical scavenging activities (RSA) of the H_2O and MeOH extracts from mulberry juice and cake are shown in Fig. 2~Fig. 4. Four extracts from mulberry juice and cake showed significant RSA in a dose-dependent manners, and the 50% inhibitory concentration (IC_{50}) of each extracts was calculated from the results. Among four extracts, the MeOH extract

Table 1. Yield of acidified water and methanol extracts from mulberry juice and cake

Mulberry	Extraction solvent	Yield (%)[1)]
Mulberry juice	0.5% TFA in distilled water (WMJ)	70.01±1.30[2)a3)]
	0.5% TFA in 80% aq. MeOH (MMJ)	65.38±1.07[b]
Mulberry cake	0.5% TFA in distilled water (WMC)	1.68±0.21[c]
	0.5% TFA in 80% aq. MeOH (MMC)	1.82±0.19[c]

[1)]Dry base of mulberry fruit.
[2)]Values are mean±SD of triplicate analyses.
[3)]Values with the different superscript letters are significantly different at $p<0.05$.

from mulberry cake exhibited the strongest DPPH RSA (IC_{50}=167.45 μg/mL), followed by the H_2O extract (IC_{50} =278.02 μg/mL) from mulberry cake, the MeOH extract (IC_{50}=317.16 μg/mL) and the H_2O extract (IC_{50}=407.52 μg/mL) from mulberry juice, in descending order (Fig. 2). Thus, DPPH RSA of the extracts from mulberry cake was higher than those of mulberry juice, and the MeOH extracts were higher than the water extracts.

Radical scavenging activities (RSA) of the H_2O and MeOH extracts from mulberry juice and cake against superoxide anion radical induced by xanthine-xanthine oxidase, and hydroxy radical generated via the Fenton reaction are present in Fig. 3 and Fig. 4, respectively. All extracts exhibited concentration-dependent radical scavenging activity against two above radicals, and the IC_{50} values are listed in Fig. 3 and Fig. 4. The superoxide radical scavenging activity of four extracts follows the order: the MeOH extract (IC_{50}=36.18 μg/mL) > the H_2O extract (IC_{50}=102.21 μg/mL) from mulberry cake > the MeOH extract (IC_{50}=165.49 μg/mL) > the H_2O extract (IC_{50}=183.28 μg/mL) from mulberry juice. In particular, the MeOH extract from mulberry cake exhibited the most potent superoxide radical scavenging activity.

Meanwhile, the hydroxyl radical scavenging activity of four extracts in decreasing order was the MeOH extract (IC_{50}=467.08 μg/mL) > the H_2O extract (IC_{50}= 714.86 μg/mL) from mulberry cake > the MeOH extract (IC_{50}=920.20 μg/mL) > the H_2O extract (IC_{50}=987.60 μg/mL) from mulberry juice. Thus, the order of hydroxyl radical scavenging activity of four extracts was very similar to that of their DPPH and superoxide radical scavenging activity, although the extent of their radical scavenging activity was considerably different among four extracts from mulberry juice and cake. From the three above results, the MeOH extract of mulberry cake was found to have antioxidative potential as radical

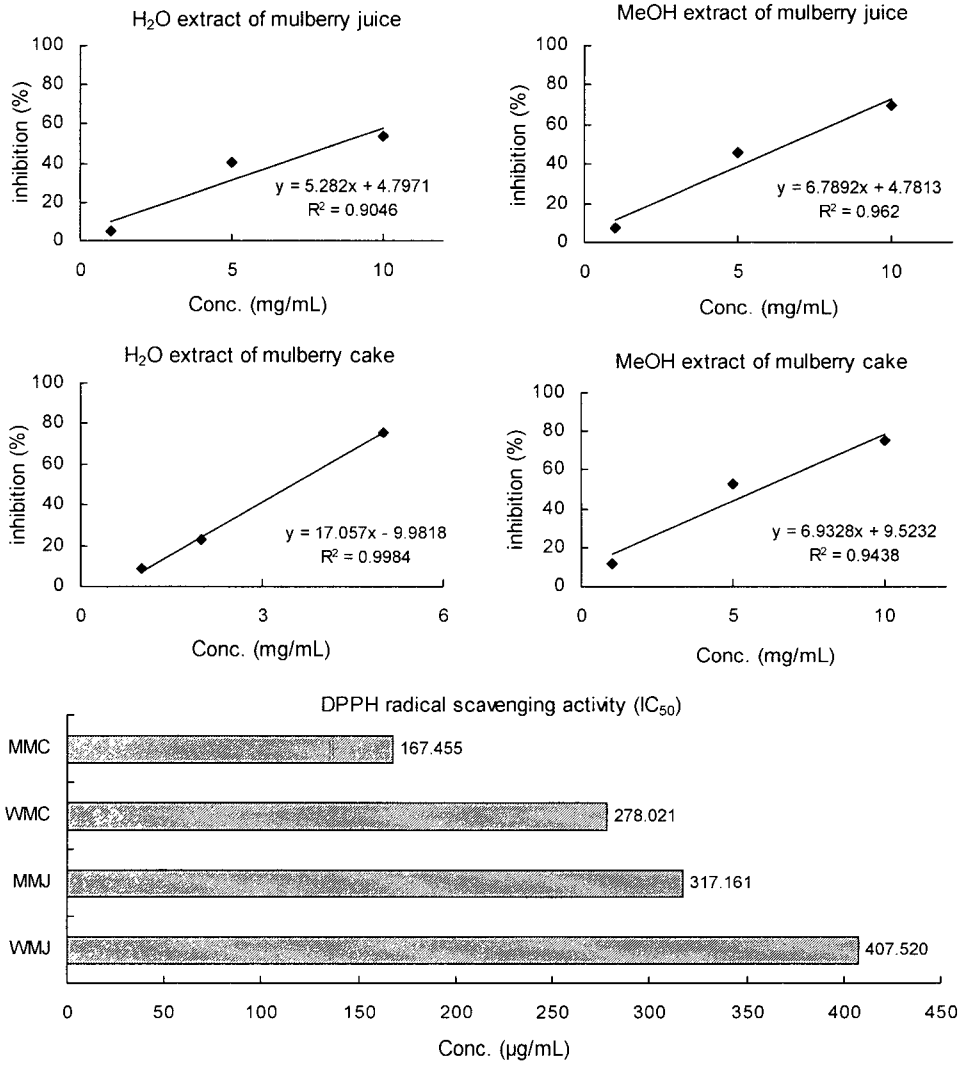

Fig. 2. DPPH radical scavenging activity of water and methanol extracts of mulberry juice and cake prepared from mulberry fruits.

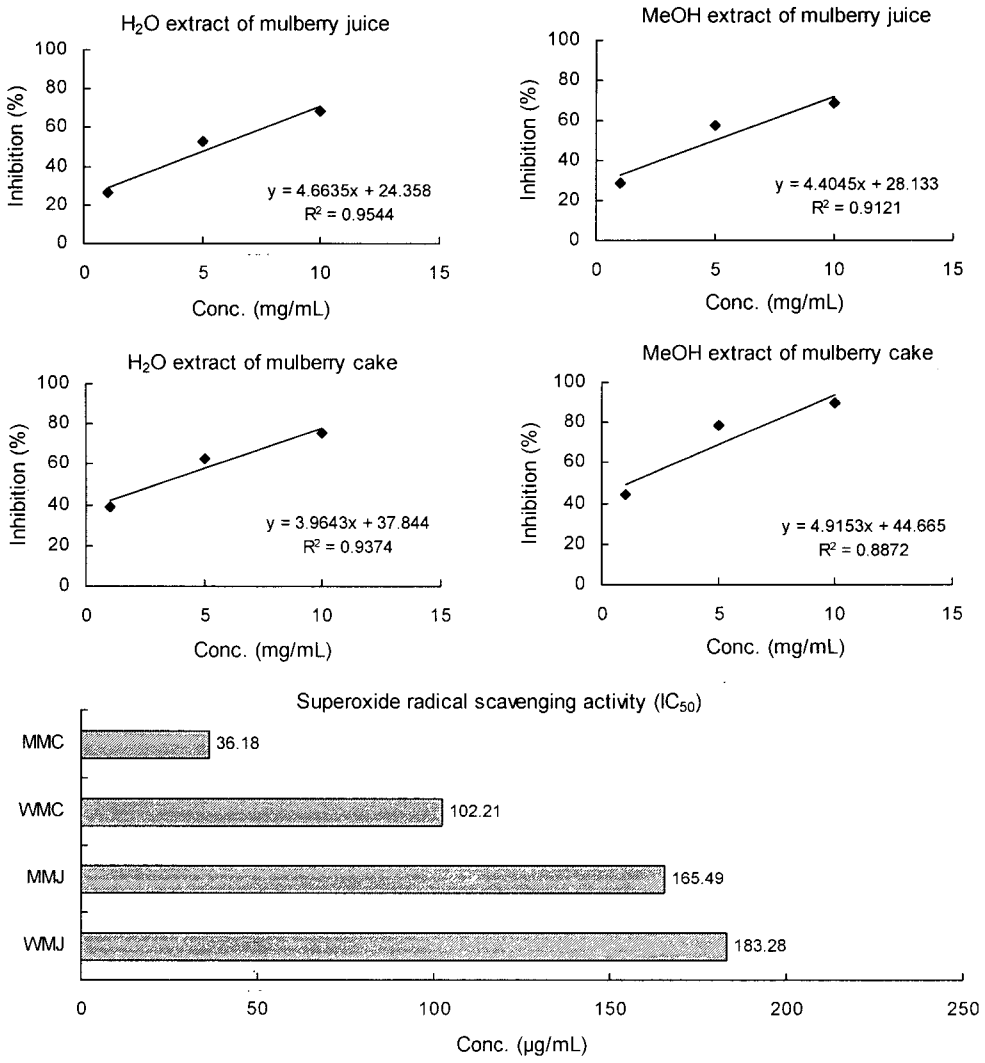

Fig. 3. Superoxide radical scavenging activity of water and methanol extracts of mulberry juice and cake prepared from mulberry fruits.

scavenger. A systematic research in search of major active principles for strong radical scavenging activity of the MeOH extract from mulberry cake is now in progress.

Total phenolic content

The total phenolic contents of the H_2O and MeOH extracts from mulberry juice and cake are given in Table 2. Among four extracts, total phenolic content was highest in the MeOH extract (4.48%) from mulberry cake, followed by the H_2O extract (2.30%) from mulberry cake, the MeOH extract (2.17%) and the H_2O extract (1.61%) from mulberry juice, in descending order. Thus, total phenolic contents of the extracts from mulberry cake was higher than those of mulberry juice, and the MeOH extracts were higher than the H_2O extracts. Furthermore, it was found that there are a positive correlation between the total phenolic content of mulberry extract and the DPPH, superoxide and hydroxyl radical scavenging potentials (data not shown). Thus, these findings indicate that phytochemical phenolics in mulberry cake may be mainly responsible for the strong radical scavenging activities of the MeOH extract from mulberry cake. In the previous report (19), we found that the mulberry cake prepared from mulberry fruits contained several phenolic constituents such as anthocyanins and flavonoids which were well-known as potent oxygen radical scavengers (20,21). Further study on the isolation and identification of some phenolic compounds from mulberry cake, and on evaluation of their scavenging activities against DPPH, superoxide and hydroxyl radicals is now in progress.

The superoxide and hydroxyl radicals of reactive oxygen species are known to have a high and indiscriminate activity and cause severe oxidative damage to susceptible biomolecules (2,4). However, this damage can be modulated by dietary antioxidant phenolics acted as radical

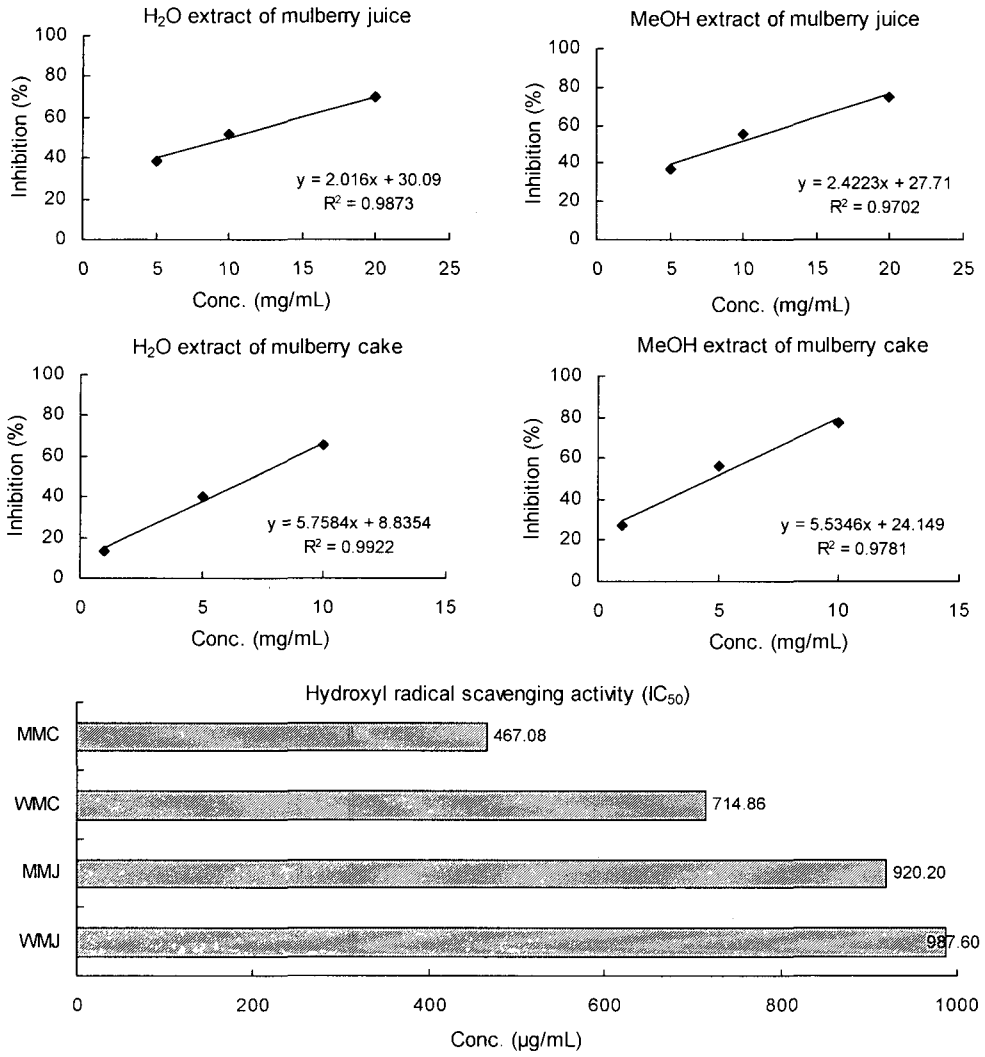

Fig. 4. Hydroxyl radical scavenging activity of water and methanol extracts of mulberry juice and cake prepared from mulberry fruits.

Table 2. Contents of total phenolics in the water and methanol extracts from mulberry juice and cake

Sample	Extraction solvent	Phenolics (g/100 g, dried extract)[1)]
Mulberry juice	0.5% TFA in distilled water (WMJ)	1.61±0.14[2)c3)]
	0.5% TFA in 80% aq. MeOH (MMJ)	2.17±0.27[b]
Mulberry cake	0.5% TFA in distilled water (WMC)	2.30±0.31[b]
	0.5% TFA in 80% aq. MeOH (MMC)	4.48±0.47[a]

[1)]Gallic acid equivalent.
[2)]Values are mean±SD of triplicate analyses.
[3)]Values with the different superscript letters are significantly different at $p<0.05$.

scavengers. Previously, antioxidant phenolic compounds such as flavonoids and anthocyanins present in mulberry fruits have been reported to play a beneficial role against oxidative damage (19,22). Additionally, this study speculated that phenolics in mulberry cake may be acted as free DPPH radical, superoxide, and hydroxyl radical scavengers. Thus, these results indicate that mulberry cake obtained as byproduct in the process of manufacturing mulberry juice might be a useful source of phenolic antioxidants as radical scavenger. Therefore, development of new functional foods using whole mulberry fruits is essential in place of mulberry juice. This is the first report on radical scavenging activity of the extracts of mulberry juice and cake prepared from mulberry fruits.

In conclusion, the active oxygen radical-mediated lipid peroxidation in cell membranes has been implicated as

the primary cause of many heart-related diseases, atherosclerosis, cancer, and aging (1-4). In the previous, mulberry fruits have been reported to have strong antioxidant activity (9-11). In the present study, particularly, the extracts of mulberry cake were found to have radical scavenging activity stronger than those of mulberry juice. These results suggest that consumption of whole mulberry fruits with high levels of phenolics may be useful potential therapeutic agents capable of regulating radical-mediated pathological disorders, such as coronary heart disease, atherogenesis, cancer, and aging. Further study on the DPPH, superoxide and hydroxyl radical scavenging activity of several phenolic compounds isolated from mulberry fruits is being undertaken to report soon.

ACKNOWLEDGEMENTS

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