

## Antioxidant activities and determination of phenolic compounds isolated from oriental plums (Soldam, Oishiwase and Formosa)

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### Abstract

The purposes of this study were to determine phenolic compounds and to evaluate antioxidant activities of plums (Soldam, Oishiwase and Formosa). Soldam contains the highest amount of total phenolics among cultivars (Formosa: 4.0%, Oishiwase: 3.3%, Soldam: 6.4% for total phenolic) as well as the total flavonoids of which constituents were mainly myricetin and anthocyanidin. The antioxidant activities were measured by DPPH, ABTS radical scavenging, and SOD-like activities. The DPPH radical scavenging activity of Korean plum extracts (200 µg/mL) showed more than 43%, and the Soldam turned out to be the highest : ID<sub>50</sub> value: 160-177 µg/mL for Formosa and Oishiwase; 58-64 µg/mL for Soldam. The ABTS radical scavenging activity of Korean plum extracts (200 µg/mL) was found to be more than 50%. The SOD-like activity of Korean plum extracts (200 µg/mL) showed more than 70%. Among three kinds of cultivars, Soldam had the highest antioxidant activity. The nitrite scavenging activity of Soldam was 61.5%, which is the highest, compared with that of the other cultivars, about 50%. From these results, Korean plums turned out to be phytochemical rich fruit as well as to show high antioxidant activities.

**Key Words:** Antioxidant activity, phenolic compounds, oriental plums

### Introduction

With the material abundance, most modern diseases are in progress from acute to chronic metabolic disease. These are thought that involved in free radicals, reactive oxygen species and oxidative stress. Free radicals occur in everyday life, and most are removed by enzyme like superoxide dimutase (SOD) [1-5]. However, excessive reactive oxygen species is suggested to be strongly associated with cellular aging and certain metabolic diseases [6-8]. Therefore, antioxidant enzymes and antioxidant substances such as superoxide dismutase, phenolic compounds that control antioxidant have been studied and reported to protect body by food intake.

Phenolic compounds, which are widely distribute in plant, are thought to have positive effects of human health [9-10]. In the chain reaction, the phenolic compounds suppress the oxidation by donating hydrogen to alkylperoxy radical or alkyl radical for remove the radical [11]. Therefore, the phenolic compounds have been used in many antioxidant activity assays before biological system. Many methods have been used to determine the antioxidant activity, in which DPPH and ABTS radical scavenging systems [12-16] were generally used to measure the total

antioxidant activity [17].

Oriental plum in Korea, have been grown before the period of the Three States and major cultivars are Formosa, Oishiwase and soldam. It has sweet taste, sour flavor and rich juice that is the Korean favorite fruit in summer. Recently it is widely consumed because of health benefits. However, very few studies about effective against diseases of plum were carried out. According to studies reported in the food science plum contains large amounts of phenolic compounds and dietary fiber and the kind of major phenolic compounds are phenolic acid, flavonoid and antocyanin [18]. Oriental plum is known medicinal fruit, which has been extensively used in many countries to enhance immunity and treatment of constipation, mouth ulcers and irregular menstruation [19-22]. Previous studies have documented several medicinal effects as like antioxidant activity of plum [23]. It is well known that the plum may decrease blood cholesterol [24], inhibit growth on cancer cells [25], reduce food poisoning [26], inhibit nitrite scavenging [11], and inhibit growth signals of vascular smooth muscle cells [27]. Recently it has been shown to prevent bone [28].

However these studies are specific to some varieties of plum, it is not enough for the overall study on major varieties of oriental

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plums. The objectives in this study were to assess antioxidant capacity, to determine total phenolic compound content and identify the configuration of phenolic compound of various varieties of plums.

## Materials and Methods

### Preparation of plum extracts

Three varieties of plums (Formosa, Oishiwase and soldam) were purchased from Kimcheon in Gyeongbuk, Korea. They were freeze-dried and grounded into a powder from using a grinder. To determine antioxidant activity and amount of phenolic acid, 2 g of each plum powder was extract with 20 ml 80% ethanol for 12 hours by stirring extraction or ultrasonification extract. To qualitative analysis of anthocyanin, 2 g of each plum powder was extract with 20 ml methanol containing 1% HCl for 12 hours. After 12 hours, the each extract was filtered with whatman filter paper and stored at -20°C until used [29].

### Determination of total polyphenol contents

Total polyphenolic content was determined according to the method of Folin-denis [30]. The reaction was mixed to 1 mL of extracted sample, 10 mL of 10% Na<sub>2</sub>CO<sub>3</sub> and 1mL of 1N-Folin-Ciocalteau's phenol reagent. This mixture was let sit for 30 min at room temperature, and the absorbance was measured at 759 nm. The total polyphenol content was measured from the standard curve using tannic acid (Sigma, USA).

### Total flavonoid content and quantitative analysis of flavonoid

To measure the total flavonoid content, 1mL of the extract was mixed 10mL diethyleneglycol and 1mL of 1N-NaOH, reacted in the solution for 1 hr at 37°C, and measured the absorbance at 420 nm [31]. The total flavonoid content was measured from the standard curve using naringin (Sigma, USA).

For quantitative analysis of each extract, HPLC was used. Table 1 showed HPLC condition. Eriodictyol, naringenin, apigenin, hesperitin, kaempferol, myricetin, quercetin, daidzin, daidzein (EXTRASYNTHÈSE, France) were used as standard substances.

### Quantitative analysis of anthocyanin

For quantitative analysis of anthocyanin, peonidin, cyanidin, delphinidin, pelargonidin were used as standard substance (EXTRASYNTHÈSE, France) [32]. Anthocyanin is easily destroyed by light, so light is off all of the above process was performed. HPLC condition is shown in Table 1.

### Scavenging activity on DPPH (1,1-diphenyl-2-picrylhydrazyl) radical

Different concentrations (50, 100, and 200 µg/mL) of the sample prepared in 96 well plate was added to 80 µL. Each sample was added to 120 µL of DPPH 6 mg with 99% ethanol, and kept in for 30 min at 37°C. The absorbance was measured at 517 nm. BHT was used as positive control.

### ABTS radical scavenging activity

The reaction was initiated by the addition of 140 µL diluted ABTS to mixed 30 µL of sample (500 µg/mL concentration) and 30 µL of distilled water in 96 well plate. The absorbance was measured at 734 nm after for 7 min at 37°C. BHT was used as positive control.

### Superoxide dismutase (SOD)-like activity

SOD-like activity was determined using a SOD assay kit WST (Dojindo Molecular Technology, Inc., Kumamoto, Japan) [33]. This one uses the highly water-soluble tetrazolium salt WST-1 (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt), which produces a water-soluble formazan dye upon reduction with a superoxide anion.

### Nitrite scavenging ability

Nitrite scavenging ability was measured according to the method of Gray and Dugan [34]. The 1 mL of sample added 1 mL of 1 mM NaNO<sub>2</sub> was adjusted to pH 1.2 with 0.1N HCl. Reaction solution was filled up to 10 ml with distilled water and incubated at 37°C for 1 hr. 2% acetic acid and Griess reagent (1% sulfanilic acid: 1% naphthylamine in 30% acetic acid) was added to the reaction solution. After the resulting mixture was

**Table 1.** HPLC condition of flavonoid and anthocyanin analysis

	Flavonoid condition	Anthocyanin condition
Instrument	Waters UPLC system	Waters HPLC system
Column	Acquity UPLS™ HSS T3. 1.8 µm 2.1 × 10 nm	Sunfire, 5 µm, 30 × 250 mm
Detector	UV 280 nm	520 nm
Flow rate	0.45 mL/min	1.2 mL/min
Injection volume	2 µL	10 µL
Solvent A	0.1% Formic acid in H <sub>2</sub> O	Formic acid:Water = 1:10 (v/v)
Solvent B	0.1% Formic acid in Acetonitril	Formic acid:Water:MeOH = 1:9:10 (v/v)
Gradient condition (min/%A)	0/75, 10.50/65. 12.0/75.0	0/60, 5/60, 10/45, 20/0, 26/0, 27/60, 30/60

incubated at room temperature for 15 min, absorbance was measured at 520 nm by UV-VIS spectrophotometer.

### Statistical analysis

All the data are measure three times. Statistical comparisons were performed via one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. *P*-values of less than 0.05 were considered significant.

## Results

### Total polyphenol contents

In this experiment, polyphenol contents of Formosa, Oishiwase and Soldam were analyzed by using Folin-denisish and the result are shown in Fig. 1A. Total polyphenol content of Soldam 6.4% Formosa 4.3%, and Oishiwase 3.3% of the 3 different varieties that were subjected in this study. Compared with polyphenol content of domestic commercial fruit as like 2.4% peach, 2.1% pear, and 6.9% apple [35], the plums were contained relatively large amounts of polyphenols.

### Total flavonoid content and quantitative analysis of flavonoid

Distribution of flavonoid, in different plums is presented in Fig. 1B. Soldam had the highest concentration (0.98%) of flavonoid, which differed significantly from the rest of varieties (Formosa 0.55% and Oishiwase 0.62%). It is interesting to note that flavonoid concentration of apple (0.31%) and pear (0.33%) as previously reported [36]. Flavonoids of the polyphenols also had in relatively large amounts of Soldam. HPLC chromatogram by HPLC-MS analysis of flavonoid standards eriodictyol, naringenin, apigemin, hesperitin, kaempferol, myricetin, quercetin, daidzin, and daidzein is shown in Fig. 2A. The results of flavonoid composition and content on 3 kinds of Formosa, Oishiwase and Soldam are shown in Fig. 2B-2D. and Table 2. Formosa was composed of myricetin, eriodictol, quercetin, kaem-

**Table 2.** The main flavonoid contents in Korean plums (mg/100 g)

	Myricetin	Eriodictol	Quercetin
Formosa	2.10	0.11	1.02
Oishiwase	0.22	-	-
Soldam	3.23	0.19	-

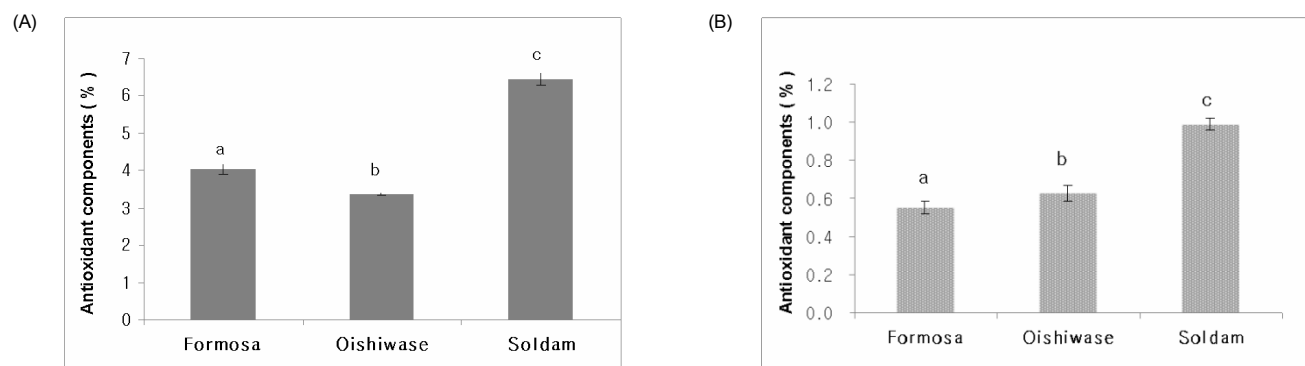
pherol, and hesperetin and especially, the contents of myricetin, eriodictol, and quercetin contained 2.1, 0.11, and 1.02 mg per 100 g edible portion, respectively. Kaempferol and contain traces of hesperetin was not possible to quantify it. Oishiwase was composed of eriodictol, hesperetin and contained eriodictol of 0.22 mg/100 g and also contain traces of hesperetin was not possible to quantify it. Soldam was composed of myricetin, eriodictol and contained myricetin of 3.2 mg, eriodictol of 0.19 mg per 100 g edible portion.

### Quantitative analysis of anthocyanin

HPLC chromatogram by HPLC-MS analysis of anthocyanin standards peonidin, cyanidin, delphinidin, and pelargonidin is shown in Fig. 3A. Peonidin, cyanidin, delphinidin, and pelargonidin were not detected in Formosa, Oishiwase, and Soldam but two unknown peaks were found. After reviewing of MS-fragment and UV-spectrum, the two peaks seem to anthocyanidin (cyanidin-3-glucose or cyanidin-3-arabinose) combined glucose (Fig. 2B-2D).

### Scavenging activity on DPPH (1,1-diphenyl-2-picrylhydrazyl) radical

These results of DPPH radical Scavenging activity experiment, there was no significant difference between as Formosa and Oishiwase well as extraction method (Table 3, Fig. 4). But Soldam showed the highest DPPH radical scavenging activity and that was concentration-dependent. In the case of the IC<sub>50</sub> value, Formosa (160-177 µg/mL), Oishiwase (16-170 µg/mL), and Soldam (58-64 µg/mL) are higher than BHT (13 µg/mL).



**Fig. 1.** Total polyphenol (A) and flavonoid (B) contents of Korean plums (% dry basis). Values are mean of three replicates. Different superscript in each column indicates the significant differences in the mean at  $P < 0.05$ .

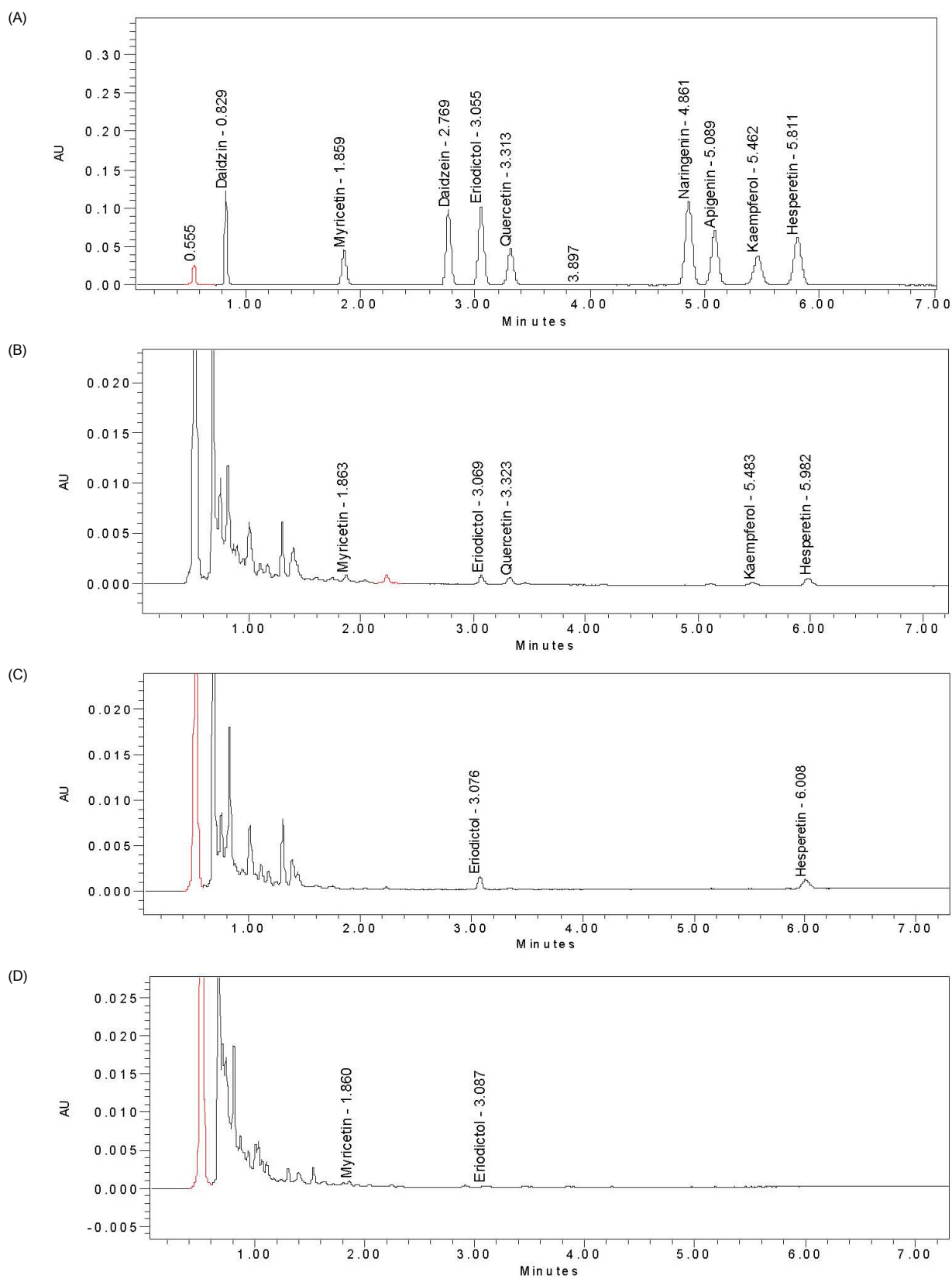


Fig. 2. Chromatogram of flavonoid composition of standard compounds (A), Formosa (B), Oishiwase (C), and Soldam (D)

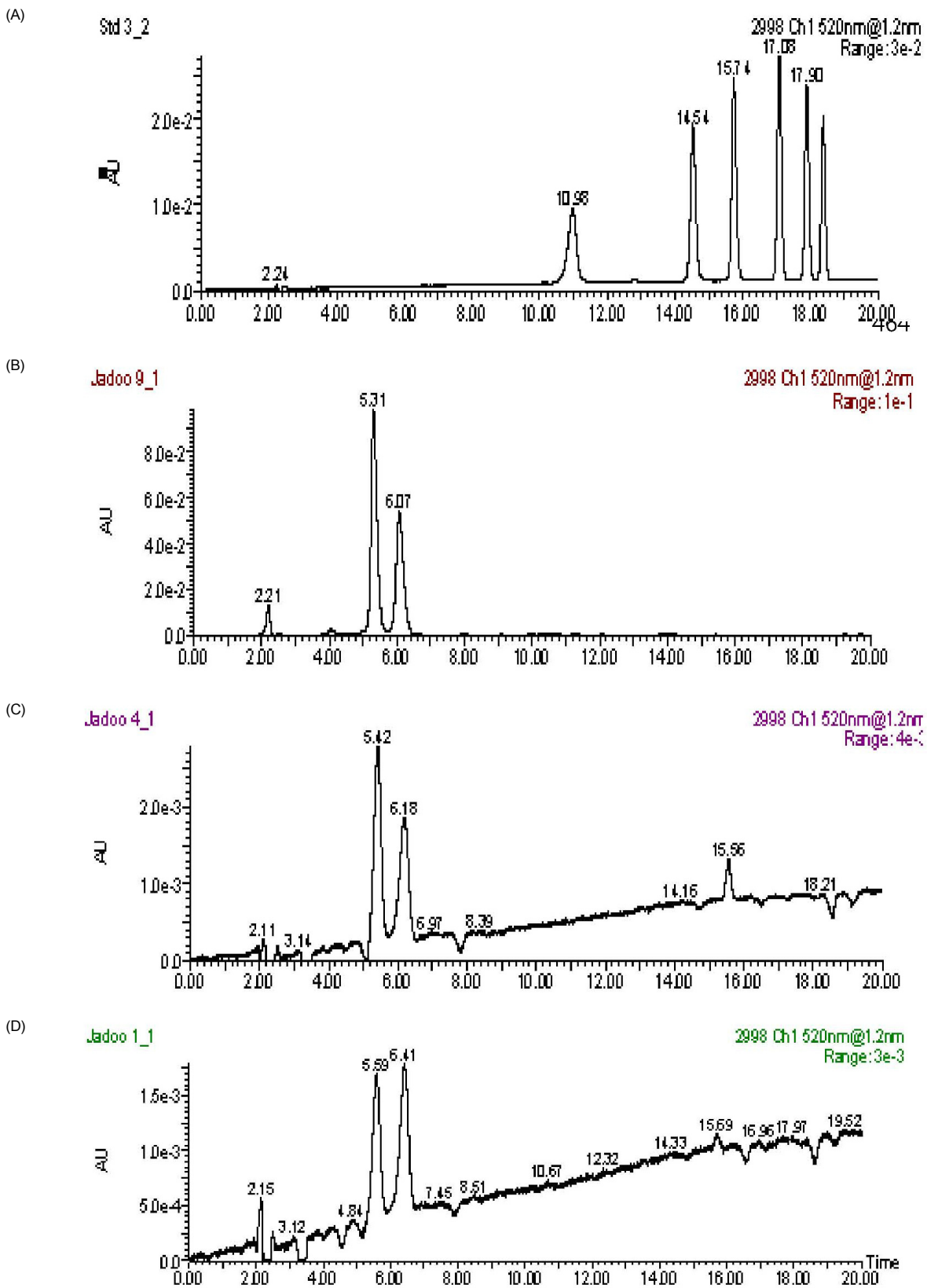


Fig. 3. Chromatogram of anthocyanin standard compounds (A), Formosa (B), Oishiwase (C), and Soldam (D)

**Table 3.** IC<sub>50</sub> of BHT and Korean plum extracts on DPPH radicals

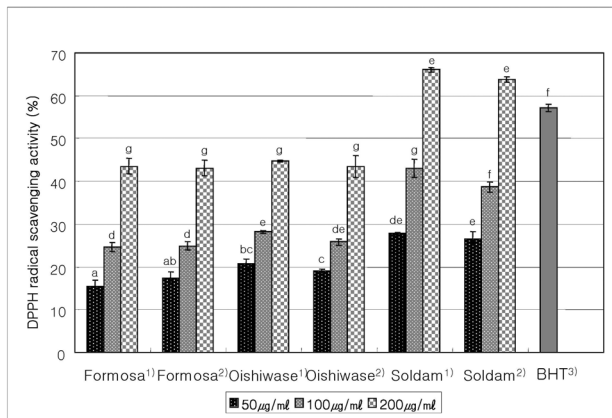
Cultivar	IC <sub>50</sub> (µg/mL)	
	A <sup>1)</sup>	B <sup>2)</sup>
Formosa	160 <sup>a</sup>	177 <sup>a</sup>
Oishiwase	170 <sup>a</sup>	166 <sup>a</sup>
Soldam	58 <sup>b</sup>	64 <sup>b</sup>
BHT <sup>3)</sup>	13 <sup>c</sup>	

<sup>1)</sup> Ultrasonification extracts at 60°C

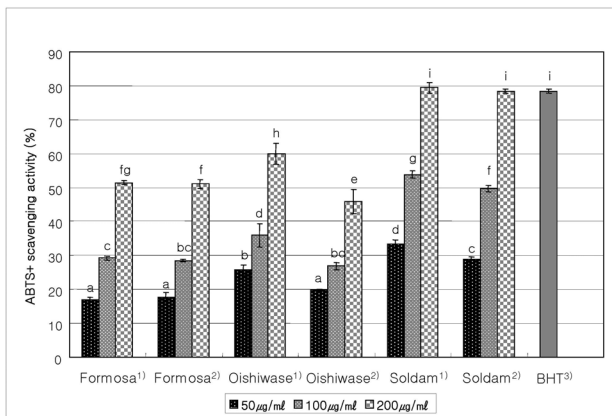
<sup>2)</sup> Shaking extracts at 60°C

<sup>3)</sup> The concentration was 25 µg/mL values are means (n=3).

Different superscript in each column indicates the significant differences in the mean at P<0,05.



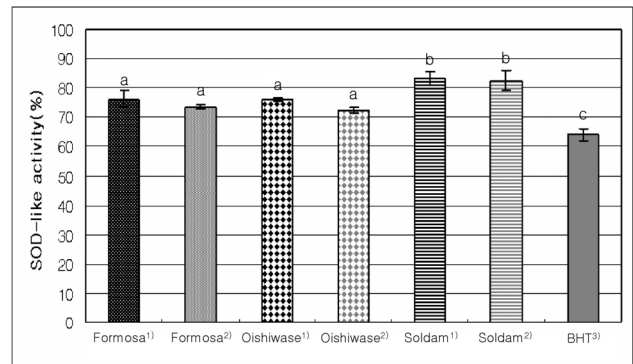
**Fig. 4.** DPPH radical scavenging activity of BHT and Korean plum extracts. <sup>1)</sup> Ultrasonification extracts at 60°C, <sup>2)</sup> Shaking extracts at 60°C, <sup>3)</sup> The BHT is used at the concentration of 25 µg/mL (n=3). Different superscript in each column indicates the significant differences in the mean at P<0,05.



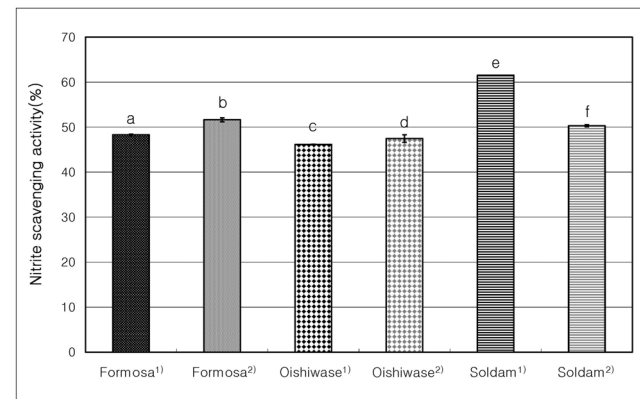
**Fig. 5.** ABTS<sup>+</sup> Scavenging activity of BHT and Korean plum extracts. <sup>1)</sup> Ultrasonification extracts at 60°C, <sup>2)</sup> Shaking extracts at 60°C, <sup>3)</sup> The BHT is used at the concentration of 25 µg/mL (n=3). Different superscript in each column indicates the significant differences in the mean at P<0,05.

*ABTS radical scavenging activity*

The results of ABTS radical scavenging activity measured are indicated in Fig. 5. The highest scavenging effect was shown in the Soldam, followed by the Oishiwase and Formosa in order. Soldam at the 50-200 µg/mL concentration exhibited the most powerful scavenging activity against ABTS radical in concentra-



**Fig. 6.** SOD-like activity of BHT and Korean plum extracts. <sup>1)</sup> Ultrasonification extracts at 60°C, <sup>2)</sup> Shaking extracts at 60°C, <sup>3)</sup> The BHT is used at the concentration of 25 µg/mL (n=3). Different superscript in each column indicates the significant differences in the mean at P<0,05.



**Fig. 7.** Nitrite scavenging of Korean plum extracts. <sup>1)</sup> Ultrasonification extracts at 60°C, <sup>2)</sup> Shaking extracts at 60°C. Values are mean of three replicates. Different superscript in each column indicates the significant differences in the mean at P<0,05.

tion dependant manner, and it exhibit about 80% higher electron donating ability. The Oishiwase extracted by ultrasonification had electron donating ability below 50%, which was the lowest activity among the extracts. Moreover, the antioxidant activity of the Soldam was higher than well-known antioxidants, such as BHT.

*Superoxide dismutase (SOD)-like activity*

The results of SOD-like activity measured are shown in Fig. 6. There was no significant difference between as Formosa and Oishiwase depending on extraction method and the activity value at the 200 µg/mL concentration of Formosa ranged from low level of 73 to 76%, Oishiwase ranged from low level of 72 to 76%. The highest SOD-like activity was in the Soldam extract ranged from low level of 82 to 83% that was significantly different compared with others. On the other hand, SOD-like activity of BHT (25 µg/mL) was 64% that is relatively lower than Formosa, Oishiwase and Soldam.

Soldam, which contain the highest phenolic compound, was

shown the strong antioxidant activities against ABTS radical, DPPH radical scavenging, and SOD-like activity. This result is same with the report that high contents of phenolic compound have stronger antioxidant capacity [37].

#### *Nitrite scavenging ability*

Nitrite scavenging ability measured results are shown in Fig. 7. Most of the plums exhibited nitrite scavenging ability ranged from low level of 46.1% to 61.5%. The highest scavenging abilities of Soldam obtained by stirring and ultrasonification extraction were in 61.5 and 50.3%, respectively.

There was no significant difference between as stirring extraction method well as ultrasonification extraction method in Formosa and Oishiwise at the 200 µg/mL concentration. However, in case of Soldam, ultrasonification extract was higher than stirring extract in nitrite scavenging.

#### **Discussion**

Free radicals occur in everyday life, and most are removed by enzyme like superoxide dismutase (SOD) [1-5]. The free radical, reactive oxygen species and peroxides that cause aging, cancer, various diseases are fatal to humans. Currently synthetic antioxidants were developed to inhibit that. But due to the toxicity usage limitation and low activity of synthetic antioxidants, the research on safer and stronger natural antioxidants is needed. Therefore, antioxidant enzymes and substances such as superoxide dismutase, phenolic compounds that control antioxidant have been studied and reported to protect body by food intake. Polyphenol, flavonoid, and anthocyanin are strong antioxidants and are associated with many useful biological effects of plant product. Therefore, phenolic compounds have been used in many antioxidant activity assays before biological system.

In this study, we determined the phenolic compounds and evaluated antioxidant activities of oriental plums (Soldam, Oishiwise and Formosa). Total polyphenols widely distributed in the plant as one of the secondary metabolites that has various structures and molecular weight. Because of phenolic hydroxyl (OH) groups of these, it is easily to combine with proteins and other macromolecules and they have antioxidant, anticancer and variety of biological activities [38]. We determinate total polyphenol content according to the method of Folin-denish. Total polyphenol ranged from low level of 3.3 (Oishiwise) to 6.4% (Soldam) of the 3 different varieties that were subjected in this study. Flavonoid, which is the largest class of phenolic compound, is synthesized by plants. Flavonoids has a antioxidant as effective free radical capture. Flavonoid content was analyzed by using diethylenglycol and NaOH. Soldam had the highest flavonoid content of 0.98%, which differed significantly from the rest of varieties (Formosa 0.55% and Oishiwise 0.62%). It is interesting to note that flavonoid concentration of apple and pear were 0.31%

and 0.33% [36]. Flavonoid quantitative results analyzed by HPLC were as follows. Formosa was composed of myricetin, eriodictol, quercetin, kaempferol, and hesperetin and Oishiwise was composed of eriodictol, hesperetin and contain eriodictol. Soldam was composed of myricetin and eriodictol. In the above results, we obtained that Oishiwise and Soldam comprising an antioxidant compound and especially Soldam contained far more than others. These results were not proved directly the physiological activity effects, but refer to already reported physiological activity effects; antioxidant, anti-aging, antitumor, inhibit cholesterol, etc. [26-28] of phenolic compounds, it is thought to be useful in measurement of physiological activity. We compared the antioxidant activity effects of Soldam, Oishiwise and Formosa, using the DPPH, ABTS, nitrite scavenging method and SOD-like activity. These DPPH and ABTS radical scavenging systems [12-16] were generally used to measure the total antioxidant activity. There is a number of ways for certain materials to measure the antioxidant activity, but among them DPPH radical scavenging method is relatively simple to measure multiple samples simultaneously is commonly used [17]. DPPH is a stable free radical. When antioxidants react with this stable radical, the electrons become paired off [39]. According to the result of DPPH radical scavenging activity, the Soldam variety showed the highest DPPH radical scavenging activity and that was concentration-dependent. The antioxidants activity of Soldam depending on growth times [38], the IC<sub>50</sub> value of the time of immature to mature ranged from low level of 2.23 to 105.50 µg/mL. Compared with that IC<sub>50</sub> value of this experiment was lower. It seems climate and maturation. ABTS assay is a decolorization assay, which concern the direct generation of ABTS radical mono cation, which has a long wavelength absorption spectrum without the involvement of any intermediary radical. The antioxidant activity of plums by this assay implies that action may be inhibited or scavenged either the ABTS radicals since both inhibition and scavenging properties of antioxidants towards this radical have been reported in earlier studies [40]. This result of ABTS radical scavenging activity experiment, the highest scavenging effect was in the Soldam, followed by the Oishiwise and Formosa in order. Soldam at the 50-200 µg/mL concentration exhibited the most powerful scavenging activity against ABTS radical in concentration dependant manner, and it exhibit about 80% higher electron donating ability. ABTS radical scavenging activity is higher than DPPH radical scavenging activity. It seems that although radicals, ABTS is cation radical but DPPH is free-radical. Because of it, they have different ability to scavenge radicals [34]. SOD-like activity materials are not enzymes but phytochemicals in plant that return superoxide (O<sup>2-</sup>) generated by breathing to hydrogen peroxide or oxygen. These materials have SOD-like activity and protect the body. SOD-like activity experiment, the highest SOD-like activity was in the Soldam extract ranged from low of 82 to 83% that was significantly different compared with others. Case a large amount of nitrite intake, nitrite reacts with the amine to produce

cancer-causing substance nitrosamine in body. Most of the plums exhibited nitrite scavenging ability ranged from low level of 46.1% to 61.5%. The highest scavenging ability was in Soldam of 61.5% (stirring extract) and 50.3% (ultrasonification extract).

Soldam had the highest phenolic compound, showing strong antioxidant activities against ABTS radical, DPPH radical scavenging, SOD-like activity and nitrite scavenging. This result is same with the report that high contents of phenolic compound have stronger antioxidant capacity [37]. From these results, Korean plums turned out to be phytochemical rich fruit as well as to show high antioxidant activities. Also, it is suggested that Soldam is a potent candidate for development of the functional food material. This is a natural antioxidant and has endless possibilities.

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