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 : ID50 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 dismutase (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...
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, 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-denish [30]. The reaction was mixed to 1 mL of extracted sample, 10 mL of 10% Na₂CO₃ and 1 mL 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, 1 mL of the extract was mixed 10 mL diethylenglycol and 1 mL 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. Erioodictyol, naringenin, apigemin, hesperitin, kaempferol, myricetin, quercetin, daidzin, daidzein (EXTRASYNTHESE, France) were used as standard substances.

Table 1. HPLC condition of flavonoid and anthocyanin analysis

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Waters UPLC system</th>
<th>Waters HPLC system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Acquity UPLS™ HSS T3. 1.8 um 2.1 × 10 nm</td>
<td>Sunfire, 5 um, 30 × 250 mm</td>
</tr>
<tr>
<td>Detector</td>
<td>UV 280 nm</td>
<td>520 nm</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.45 mL/min</td>
<td>1.2 mL/min</td>
</tr>
<tr>
<td>Injection volume</td>
<td>2 µL</td>
<td>10 µL</td>
</tr>
<tr>
<td>Solvent A</td>
<td>0.1% Formic acid in H₂O</td>
<td>Formic acid:Water = 1:10 (v/v)</td>
</tr>
<tr>
<td>Solvent B</td>
<td>0.1% Formic acid in Acetonitril</td>
<td>Formic acid:Water:MeOH = 1:9:10 (v/v)</td>
</tr>
<tr>
<td>Gradient condition (min/%A)</td>
<td>0/75, 10,50/65, 12.0/75.0</td>
<td>0/60, 5/60, 10/45, 20/0, 260, 27/60, 30/60</td>
</tr>
</tbody>
</table>

Quantitative analysis of anthocyanin

For quantitative analysis of anthocyanin, peonidin, cyanidin, delphinidin, pelargonidin were used as standard substance (EXTRASYNTHESE, 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 NaNO₂ 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% naphthylammine in 30% acetic acid) was added to the reaction solution. After the resulting mixture was