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Antioxidant and Antimicrobial Activities of Plum Extracts

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This investigation was performed to study the antioxidant activities and the antimicrobial effect of plum (Santarosa, Oishiwase) extracts. Plums were extracted by ultrasound-assisted method and boiling method. All extracts of plums showed concentration-dependent inhibitory effects on the DPPH free radical scavenging activity. In the superoxide anion radical scavenging method, all the plum extracts showed lower activity than BHT. But in case of sonicate extract of Oishwase exhibited the highest activity in plum extracts. The antimicrobial effect of plums used for human skin- or oral cavity-presented strains; *Bacillus cereus* (KCTC 1012) and *Staphylococcus aureus* (KCTC 1927). Addition of plum extracts was used by autoclaved and filtrated. Each extract solution was added into culture media with several concentration and then the bacteria cell growth was investigated for 72 hours. The effect of antimicrobial activities showed in a higher *Staphylococcus aureus* than *Bacillus cereus*. Results indicate that the autoclaved sample showed a higher antimicrobial activity than did the filtrated sample.

Key Words: Plum, Polyphenol, Antioxidant, Antimicrobial

INTRODUCTION

Various of phenolic compounds frequently exist in foods, such as fruits and vegetables are routinely consumed in our diet. Epidemiological evidence has been provided the fact that constituents in fruits are beneficial to human health and contribute to the prevention of degenerative processes caused by oxidative stress (Kaur and Kapoor, 2001; Vinson et al., 2001). Phenolic compound in the plum have been found to play an important role in preventing cell membrane from oxidative damages induced by active oxygen radicals in living systems as dietary antioxidants and anticarcinogenic, antiallergic, antimicrobial, antimutagenic and anti-inflammatory activities as well (Sofos et al., 1998; Harborne and Williams, 2000; Kim et al., 2000; Stacewicz-Sapuntzakis et al., 2001; Chun et al., 2005). Plums contain copious amount of natural phenolic phytochemicals, such as flavonoids and phenolic acids, which may function as natural antioxidants in our daily diet (Cyril Auger et al., 2004). Yet plum, despite having very high concentrations of phenolic phytochemicals, remain underutilized and under-researched worldwide. The level of polyphenilics in plums greatly varied mainly according to the different cultivals (Kim et al., 2003c). Purpose of this study was to determine the content of total phenolic phytochemicals and flavonoids and to evaluate total antioxidant activity and antibaterial activity in various cultivars of plums.

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MATERIALS AND MATHODS

1. Preparation of plum extract

Plums were carefully cut in half and the pits removed. Freeze-dried samples were ground and then stored at -70℃ until analyzed. The freeze-dried plums were extracted by the ultrasound-assisted method (Kim et al., 2003c) and boiling for 3 hours at 80℃. The solvent was evaporated using a rotary evaporator at 40℃. The remaining phenolic concentrate was dissolved in 50 ml of distilled water.

2. Determination of total phenolics and flavonoid

The concentration of total phenolics was measured according to Kim et al. (2003c). Briefly, the absorbance

prepared blank was read at 750 nm. Total phenolic content of plums was expressed as gram per 10 g gallic acid equivalents. All samples were measured in three replications. The concentration of total flavonoid was similary to that total phenolic measured (Kim et al., 2003c) with modified method. Absorbance of the mixture was determined at 510 nm prepared water blank. Total flavonoid of plums was expressed on a fresh weight basis as gram per 10 g catechin equivalents.

3. Evaluation antioxidant activity using DPPH free radical scavenging activity

DPPH free radical scavenging activity was measured according to Lu and Foo (2001). A 2 ml methanolic solution of DPPH (0.1 mM) was added to 0.1 ml of extracted solution (0.1, 0.5, and 1 mg/ml) in methanol and 60 min after standing, the absorbance at 517 nm against methanol was measured blank the absorbance at 517 nm (against methanol as the blank). Samples were measured in three replications.

4. Superoxide radical anion scavenging activity assay

Superoxide radical anion scavenging activity of the plum extracts was determined according to Kweon et al. (2001). Tetrazolium blue solution, the mixture of 0.1 mM xanthine and 0.1 mM NBT, was mixed in 50 mM potassium phosphate buffer (pH 7.4), which included 0.05 mM EDTA (PBE). An aliquot (0.9 ml) of the tetrazolium blue solution was added to 0.1 ml of the flavonoid sample properly diluted in 50% aqueous methanol in 9 ml of test tube. The reaction was initiated by the addition of 1 ml of xanthine oxidase solution (0.05 unit/mL) in PBE. The resulting mixture was incubated for 20 min at 37℃. By adding 2 ml of 2 N HCl to the reaction mixtures, the reaction was terminated. The coloration of NBT was measured at 560 nm against a blank that was similarly prepared. Butylated hydroxytoluene (BHT) was used as a positive control.

Table 1. Total phenolics and flavonoid contents in extract of plums

Extract (Solvent: 70% Methanol)		Total Phenol Con.[1)] (g/10 g D.W[3)])	Flavonoid Con.[2)] (g/10 g D.W)
Sonicate[4)]	Santarosa	0.014	0.004
	Oishiwase	0.020	0.008
Boiling[5)]	Santarosa	0.015	0.006
	Oishiwase	0.023	0.011

[1)] Polyphenol expressed on a dry weight basis as g/10 g gallic acid equivalents.
[2)] Flavonoid expreessed on a dry weight as g/10 g catechin equivalents.
[3)] The dry weight of plum is extracts.
[4)] Extracts were sonicated at 10℃ for 30 minute.
[5)] Extracts were boiling in water bath at 80℃ for 3 hours.

5. Antibacterial activities

1) Strains

Two kinds of bacteria that cause acne, *Staphylococcus aureus* KCTC 1927 and *Bacillus cereus* KCTC 1012. *Staphylococcus aureus* and *Bacillus cereus* were cultured at 37℃ and 30℃ respectively for 24 hours with Nutrient agar under aerobic conditions.

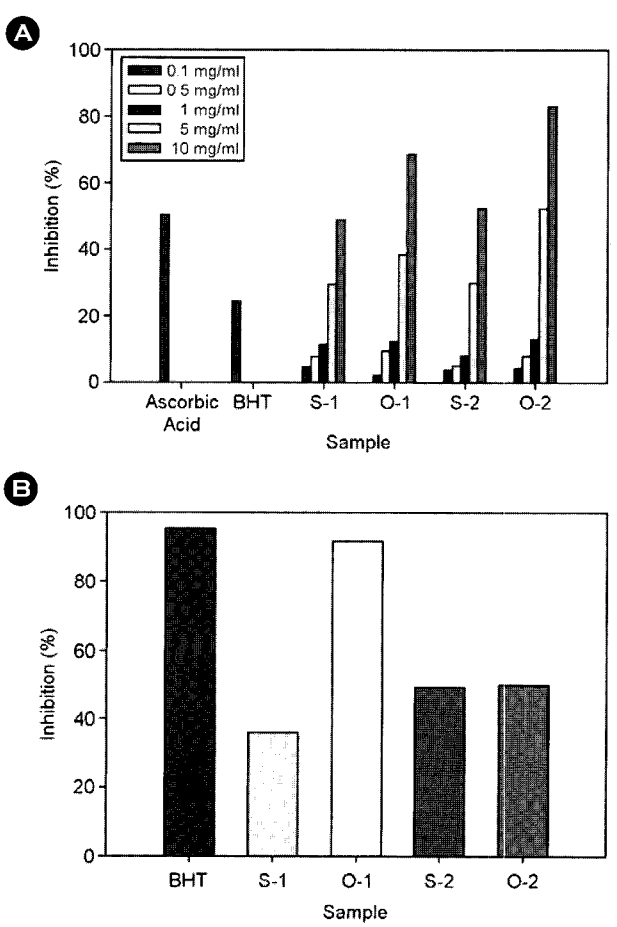

Fig. 1. Antioxidant activity of plum extracts. A): DPPH free radical scavenging activity, B): Superoxide radical anion scavenging activity assay. S: Santarosa, O: Oishiwase, 1: sonicated extract, 2: boiling extract.

2) Addition of plum extracts

Each extract solution (autoclaved and filtrated sample) was mixed into culture media with several concentration (0.1, 0.5 mg/ml) and then the bacteria cell growth was investigated for 72 hours. The cultures were grown statically for 72 hours at 30℃ (*Bacillus cereus*) or 37℃ (*Staphylococcus aureus*). The level of growth was measured as the change in absorbance of the cultures measured at 600 nm using spectrophotometer (Lee et al., 2000).

3) Determination of inhibition zones

The dried plum extracts were dissolved in the distilled water to a final concentration of 10 mg/ml and filtrated by 0.22 μm Millipore filters. Inhibition zones of compounds were determined by the disc diffusion method. The discs (6 mm in diameter) were impregnated with 10 μl of the extracts (100 μg/disc) at the concentration of 10 mg/ml and placed on the inoculated agar. Inhibition zones formed on the medium were evaluated in millimeter.

RESULTS

1. Polyphenol and flavonoid content

Total phenolic and flavonoid content of plums (Oishiwase and Santarosa) according to extracts are shown in Table 1. The boiling extract from Oishiwase exhibited the highest total phenolics content of 0.024 g gallic acid per 10 g dry sample. Total phenolic of other extract from 10 g plum ranged from 0.014 g to 0.020 g gallic acid. It is also found that each extract from plum exhibited the similar trend of total phenolic and flavonoid content. And the boiling extract from Oishiwase exhibited the highest total flavonoid content of 0.011 g catechin per 10 g dry sample, like total phenolic content. The total phenol and flavonoid content according to the kind of plum was as follows by Oishiwase (sonicated

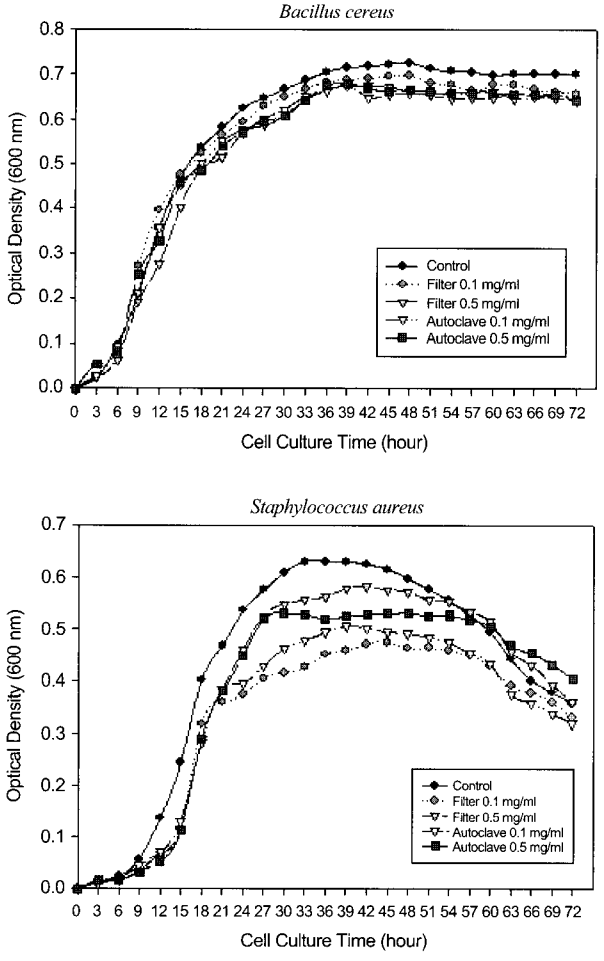

Fig. 2. Effect of boiling extract of Oishiwase on the growth of *Bacillu scereus* and *Staphylococcus aureus*.

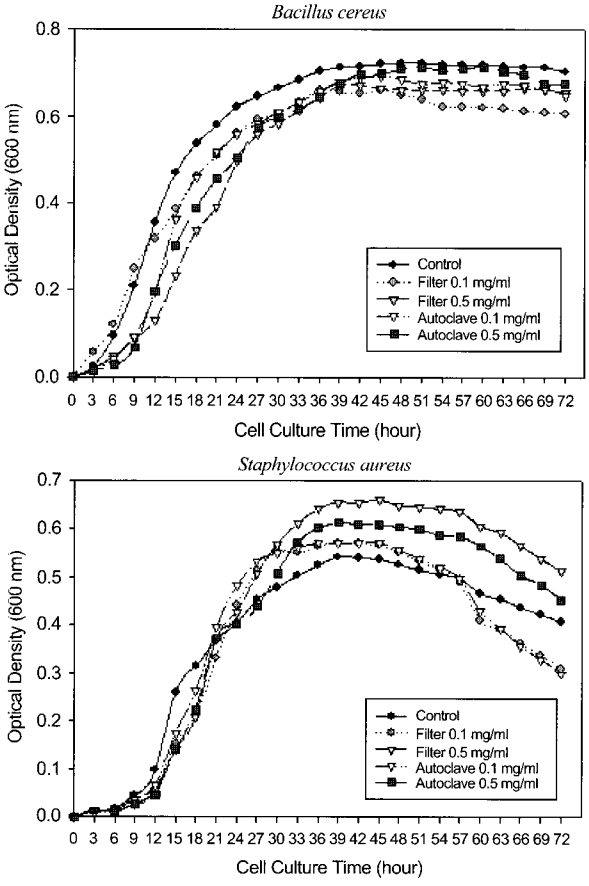

Fig. 3. Effect of sonicated extract of Oishiwase on the growth of *Bacillus cereus* and *Staphylococcus aureus*.

extract) > Santarosa (boiling extract) > Santarosa (sonicated extract).

2. DPPH free radical scavenging activity and superoxide radical anion scavenging activity assay

DPPH free radical scavenging activity showed strongly in Oishiwase (sonicated extract) compare with BHT (Fig. 1a). All extracts of plums showed concentration-dependent inhibitory effects on the free radical scavenging activity. In the superoxide anion radical scavenging method. All the plum extracts showed lower activity than BHT. But in case of sonicate extract of Oishwase exhibited the highest activity among the plum extracts (Fig. 1b).

3. Antimicrobial activity

The antimicrobial effect of plums on *Bacillus cereus* (KCTC 1012) and *Staphylococcus aureus* (KCTC 1927) is shown in Fig. 2~5. The inhibition zone measured in millimeters, including the diameter of the paper disk (6 mm), was used as the criterion for measuring the antimicrobial activity of plum extracts. Growth inhibition zones of plum extraxts against human skin- or oral cavity-presented microorganisms are shown in Table 2, 3. Results indicate that the autoclaved sample showed a higher antimicrobial activity than did the filtrated sample.

DISCUSSION

Flavonoid is generally known as a important antioxidant (Cao et al., 1997; Duthie et al., 1997; Rice-Evans et al., 1997; Arora et al., 1998; Ohshima et al., 1998; Nuutila et al., 2003). Superoxide anion radical scavenging method has been used to evaluate the antioxidant activity of various chemicals (Re et al., 1999; Kweon et al., 2001). Plums

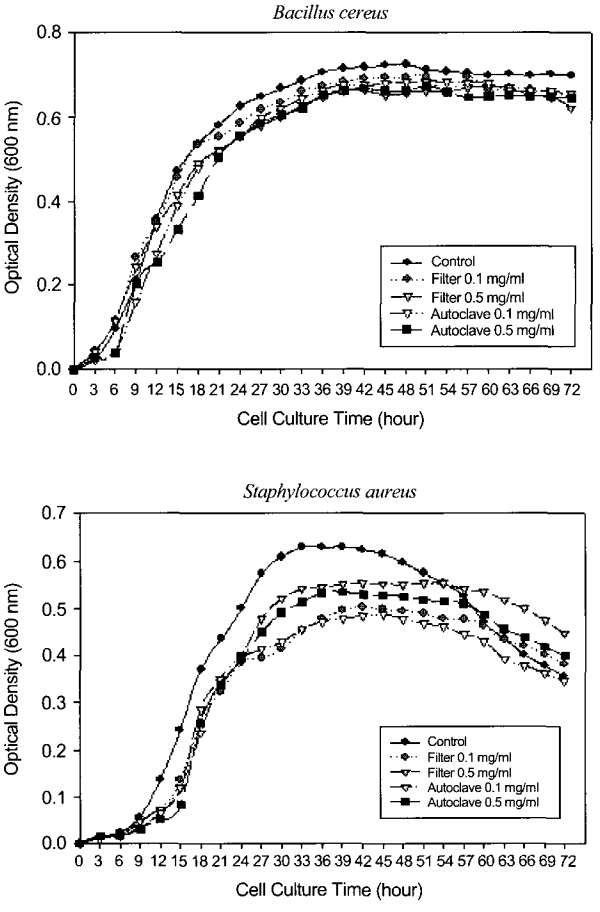

Fig. 4. Effect of boiling extract of Santarosa on the growth of *Bacillus cereus* and *Staphylococcus aureus*.

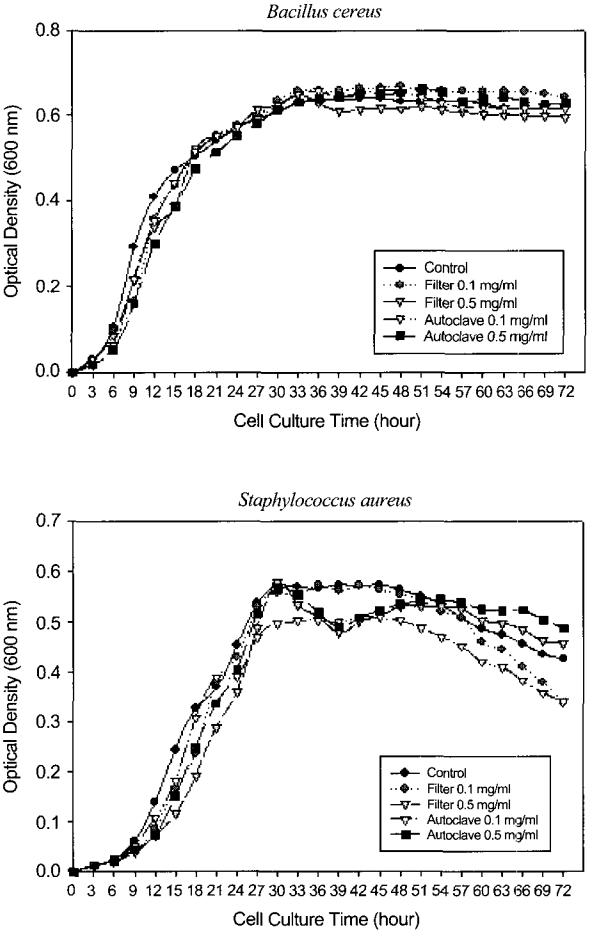

Fig. 5. Effect of sonicated extract of Santarosa on the growth of *Bacillus cereus* and *Staphylococcus aureus*.

Table 2. Antimicrobial activity of different extracts (0.1, 0.5 mg/disc) of Oishiwase

Strain	Extract	Oishiwase (mg/disc)			
		F0.1[a]	A0.1[b]	F0.5	A0.5
B. cereus	boiling	–[c]	–	–	–
	sonicate	–	–	–	–
S. aureus	boiling	7.0[d]	9.0	10.0	15.0
	sonicate	10.0	9.0	–	10.0

[a] Filtrated sample
[b] Autclaved sample with medium
[c] No inhibition
[d] Diameter of inhibition zone (mm) including the diameter of the paper disk (6 mm)

Table 3. Antimicrobial activity of different extracts (0.1, 0.5 mg/disc) of Santarosa

Strain	Extract	Santarosa (mg/disc)			
		F0.1[a]	A0.1[b]	F0.5	A0.5
B. cereus	boiling	–[c]	–	–	–
	sonicate	–	–	–	–
S. aureus	boiling	7.0[d]	8.0	10.0	10.0
	sonicate	7.0	8.0	9.0	10.0

[a] Filtrated sample
[b] Autclaved sample with medium
[c] No inhibition
[d] Diameter of inhibition zone (mm) including the diameter of the paper disk (6 mm)

have the potential to contribute greatly to human nutrition because of their richness in fiber and antioxidants (Kim et al., 2003b; Stacewicz-Sapuntzakis et al., 2001).

Similar to our results, a strong correlation between total phenolic and antioxidant capacity of plums has been reported (Gil et al., 2002; Kim et al., 2003b,c; Chun and Kim, 2004). In this study, the general antioxidant effects through the potential on DPPH free radical scavenging activity and superoxide anion radicals were investigated. The antimicrobial activity of different extracts against strains of bacteria performed by the addition of sample and disc paper method showed that plums have antimicrobial activity which would differ according to the kind of extracts and the strain of bacteria tested; the technique of extraction of active compounds would interfere, leading to more or less effective activities. The most active constituents of the extracted parts of plums with a wide spectrum of antimicrobial effectiveness are the boiling extract. Their antimicrobial activity would be changed to many factors such as phenological stage of plants, concentration and type of target microorganisms, extraction methods and extract types as well. From the above results, it appears important to develop natural antioxidants and microbial inhibitors from plums, and this may be a good way for extensively utilizing the antioxidant resource or functional food.

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