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Antioxidative Activity of the Extracts of Japanese Apricot (*Prunus mume* Sieb. *et* Zucc.)

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ABSTRACT

In order to discern the possibility of functional food product or ingredient of a new medicine, the leaf parts and fruit parts of *Prunus mume* was partitioned with various solvents and their antioxidative activity was measured. When the antioxidative activity of MeOH extracts of leaf parts and fruit parts of Korea and China was compared, all of them showed the highest antioxidative activity in EtOAc fraction. In case of Korean *Prunus mume* leaf parts showed that quantity required for RC_{50} to be 27.04 ㎍ in EtOAc fraction and in case of China *Prunus mume* leaf parts, it was 23.31 ㎍ which is similar to that of α-tocopherol (22.14 ㎍) and showed the highest activation. In case of *Prunus mume* fruit parts MeOH extract, Korean fruit showed 29.16 ㎍, and Chinese fruit showed 31.21 ㎍ in EtOAc fraction and thus Korean fruit extract showed a higher activity of antioxidant than the Chines fruit extract. When the antioxidative activity between the fruit parts and leaf parts of *Prunus mume* was compared, the leaf parts showed a higher antioxidative activity.

Key words : Antioxidative activity, EtOAc fraction, *Prunus mume*

INTRODUCTION

Prunus mume is a drupe belonging to genus *Prunus* of *Roseaceae* and its place of origin is known to be hilly areas of Sichuan province and Hubei province in China and its wild species had distributed in Korea, Japan and Taiwan. Especially in China, it had been used as health supplement or medicine from 3,000 years ago. As for Korea, it was planted in the gardens from Three Kingdom Period to enjoy the flowers but the fruit was first used in Mid-Goryeo when Chinese medicine was imported. Plum is alkaline food and is good for recovering from fatigue and when taken as a drink, it is effective in preventing cerebral apoplexy. It is also effective for headaches, constipation, anemia, neurasthenia when taken as dried fruit or in concentrated form. Its detoxicating effect is excellent that it helps cure stomach disorder or food poisoning and its sour taste secretes gastric juice and normalize the digestive organs thus ridding indigestion and other functional gastointestinal disorder. Recently it was also discovered to have anti-cancer effects (Kim, 1998).

As the theory that the aging and geriatric diseases are caused from active oxygen, development of

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antioxidant which is known to suppress the generation of active oxygen has kept on actively. Immense research on antioxidizing enzymes such as superoxide dismutase, peroxidase, catalase, glutathione peroxidase and low molecular antioxidant originating from natural ingredients such as tocopherol, ascorbate, carotenoid, glutathione has been going on (Pratt and *Watts*, 1964; *Chang et al.*, 1977; *Hammerschmidt* and Pratt, 1977) and synthesized antioxidant such as BHT, BHA, Troxol C have also been developd and is being utilizd in pharmaceutical and food industries (Kitagara *et al.*, 1992; *Hatano.*, 1995; *Masaki et al.*, 1995).

But, due to avoiding propensity of consumers against synthesized antioxidant as well as reports on synthetic antioxidant given in large doses to animals to cause cancer (Branen, 1975), use of synthetic antioxidant is slowly being restricted. And at present development on natural antioxidants are going on actively together and various materials are being reported (Lee *et al.*, 2000, a,b *Jun et al.*, 2001; *Ha*, 2001; Bae *et al.*, 2002; Song *et al.*, 2002; *Yu et al.*, 2004; *Jeong et al.*, 2004; *Park et al.*, 2005). Even so, these natural antioxidants can't be compared to BHT and BHA, the synthetic antioxidants in efficacy as well as in cost. Therefore it is most urgent to find a safe natural antioxidant with an excellent efficacy.

Though microbes other than plants have been found to contain natural antioxidant, still constituents extracted from plants are by far dominant. Because plants photosynthesize, density of oxygen within its body is high and is likely to produce antioxidant materials and also because plants can move to a safer place even in a bad environment that they tend to have a high adaptability to environment. Because of these reasons, plants are likly to contain polyphenol type antioxidant within its cells to protect itself and so development of natural antioxidant with plants as its ingredients are being in process actively. Especially, the natural ingredients of flavonoid type and acidic phenol compounds known to exist in large quantity in various fruits and vegetables have been found out to have diverse physiologically active functions such as antioxidant, anti-allergy and anti-cancer and so on and research on them has grown quite large (*Ames* and Saul, 1987; *Kim*, 1998).

This thesis focused on activation of antioxidant in the extracts of *Prunus mume* with leaf parts and fruit parts extracted from Japanese apricot trees a in order to discern th possibility of *Prunus mume* in making it into functional food and ingredient of new medicines.

MATERIALS AND METHODS

Leaf parts and immature fruit parts without the seed of *Prunus mume* from China and Korea were used after having cleanly washed and dried in shade.

400 g of each dried ingredients are prepared by being cut into small pieces then the leaves were deposited in CH_2Cl_2 (5 L) for 2 days twice to extract chlorophyll. Lead acetate 10% (w/v) solution was mixed in the ratio of 1:1 with this CH_2Cl_2 extract to filter chlorophyll then it was concentrated and dried in a vacuum evaporator and CH_2Cl_2 fraction(China : 2.3 g, Korea : 2.7 g) was used separately for antioxidative activity. Fruit parts without the stone and leaf parts without chlorophyll were deposited in MeOH (5 L) for two days twice and the extract was concentrated and dried in a vacuum evaporator at 40℃ 115 g of MeOH extract was put out from fruit part of Chinese and 122 g from Korean fruit part, 99 g from leaf parts of Chinese and 95 g from Korean leaf parts were extracted.

In order to each MeOH extract partitioned by polar solvent, they were suspended in a distilled water, using n-Hexane, Ethylacetate (EtOAc) and n-Butanol (BuOH) in separatory funnel (3 L) then was successively partitioned to n-Hexane fraction 4.21 g (Chines fruit parts), 4.18 g (Korean fruit parts), 3.69 g (Chinese leaf parts), 3.42 g (Korean leaf parts), EtOAc fraction 18.34

g, 19.75 g, 16.84 g, 17.47 g, BuOH fraction 40.99 g, 42.78 g, 34.28 g, 31.05 g, and finally H2O fraction 51.46 g, 55.29 g, 44.19 g, 43.06 g. Each fraction was filtered, then concentrated at 40℃ using a rotary vacuum evaporator then was stored in the refrigerator at 4℃ A certain amount of each fraction was dissolved in MeOH then antioxidative activity was measured using DPPH free radical scavenging activity.

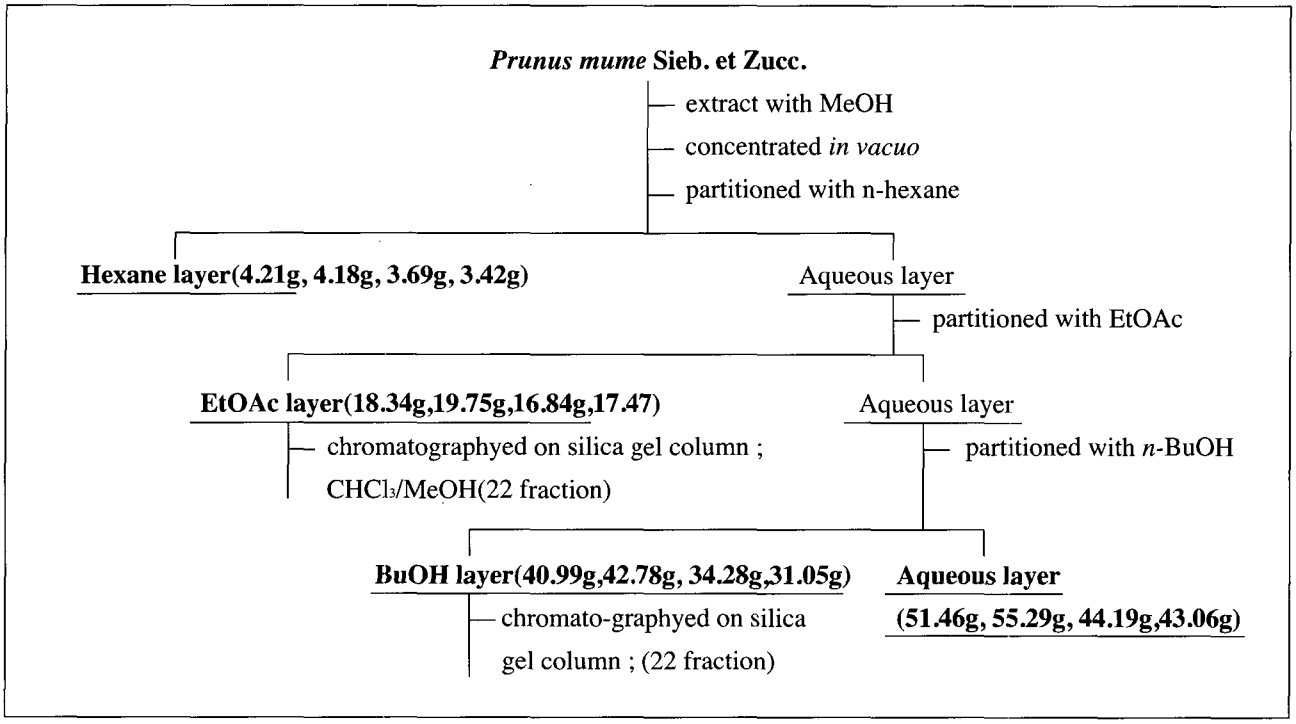

Fig. 1. Isolation scheme of each fractions.

To measure antioxidative activity, DPPH (1,1-diphenyl-2-2-picrylhydrazyl) and *α-tocopherol* (vitamine E) of Sigma and BHA (3-tert-butyl-4-hydroxyanisole), a synthetic antioxidant, of Kanto were used. As for solvents, except 1st grade CH_2C_{12} and methanol (MeOH) of Daejung used for initial extraction from the ingredients, ACS grade or HPLC of TEDIA and CARLO ERBA were used. To measure absorbance, MILTON ROY company spectronic 20D made in U.S.A spectrophotometer was used.

Each fraction of refinement used DPPH free radical scavenging activity (Choi *et al.*, 1993) to measure the antioxidative activity. Various samples were dissolved in 4 mL of MEOH, 1 mL of DPPH solution (1.5×10^{-4}M DPPH in MeOH) was added then after placed for 30 minutes at room temperature, absorbance was measured at 517 nm. Control group without the samples showed the quantity required to half the absorbance(μg) in RC_{50}, and these were compared with *α-tocopherol* (natural antioxidant) and BHA (synthetic antioxidant).

RESULTS AND DISCUSSION

MeOH extracts from *Prunus mume* fruit parts and leaf parts were measured for antioxidative activity using DPPH free radical scavenging activity in order to use Chinese and Korean *Prunus mume* as natural antioxidant by analyzing their antioxidative activity.

The antioxidative activity of MeOH extract and fraction from MeOH extract are shown in Table 1 and 2 together with that of standard antioxidant (*α-tocopherol* and BHA).

When the antioxidative activity of leaf parts extract of Chinese and Korean *Prunus mume* was compared, the MeOH extract of Chinse leaf parts showed a slightly higher antioxidative activity and according to the difference of polar solvent, the MeOH extract showing high activity were made into solvent fraction with *n*-Haxane, EtOAc, BuOH, H_2O to look into activity according to each fraction and as a result of measuring the antioxidative activity using DPPH free radical scavenging activity, it didn't show antioxidative activity in n-Haxane fraction but showed a high activity at EtOAc. Such results revealed that the antioxidants contained in *Prunus mume* can be extracted by combining with an matter with strong polarity and when compared α-tocopherol used in human life or BHA, one of the most excellent synthetic antioxidant, through these fractions had not been refined 100%, the leaf parts of Chinese *Prunus mume* showed 60~95% of antioxidative activity of BHA or α-tocopherol in EtOAc fraction, 53~84% in BuOH fraction, 26~40% in H_2O fraction. As for Korean leaf parts extract, it showed 52~81% in EtOAc fraction, 47~73% in BuOH fraction and 24~38% in H2O fraction.

In case of CH_2C_{12} extract that has gone through chlorophyll elimination process, comparatively low activity was shown and when the leaf parts of Korean and Chinese *Prunus mume* were compared, the leaf parts of Chinese *Prunus meme* showed a higher activation on the whole.

When the antioxidative activity of the fruit parts of Chinese and Korean *Prunus meme* was compared, MeOH extract of Korean fruit parts showed a slightly higher activity compared to Chinese fruit parts which was a different result from the leaf parts' comparison. According to the difference of polarity, the activation was measured using DPPH free radical scavenging activity and though it didn't show activity in *n*-Haxane fraction, it did show a high activity at EtOAc fraction. Also in the measurement of antioxidative activity using the root parts of Rhodiolacea (*Song et al.*, 2002; *Park et al.*, 2005) also showed a high activity at EtOAc fraction.

Also the result of measuring the antioxidative activity of a young stem of *Morus alba* also showed a

Table 1. DPPH free radical scavenging activities of methanol extracts from the leaf parts of Korean and Chinese *Prunus mume* and their solvent fraction.

Fractions	RC_{50}[z] (μg)	
	Chinese *P. mume*	Korean *P. mume*
CH_2C_{12} extract	98.58±0.04[y]	109.73±0.05
MeOH extract	62.26±0.01	69.55±0.02
Hexane fraction	-	-
EtOAc fraction	23.31±0.01	27.04±0.03
BuOH fraction	26.86±0.03	30.82±0.05
H_2O fraction	54.19±0.02	58.32±0.03
BHA	14.03±0.02	14.03±0.02
α-tocopherol	22.14±0.03	22.14±0.03

[z] Amount required for 50% reduction of DPPH after 30min.

[y] Data values are expressed as mean±SD of triplicate determinations.

Table 2. DPPH free radical scavenging activities of methanol extracts from the fruit parts of Korean and Chinese *Prunus mume* and their solvent fraction.

Fractions	RC_{50}[z](μg)	
	Chinese *P. mume*	Korean *P. mume*
MeOH extract	87.64±0.04	79.39±0.03
Hexane fraction	-	-
EtOAc fraction	31.21±0.02	29.16±0.01
BuOH fraction	35.37±0.02	32.98±0.02
H2O fraction	62.96±0.03	60.08±0.04
BHA	14.03±0.02	14.03±0.02
α-tocopherol	22.14±0.03	22.14±0.03

[z] Amount required for 50% reduction of DPPH after 30min.

[y] Data values are expressed as mean±D of triplicate determinations.

similar degree of antioxidative activity when compared to that of BHA and BHT in EtOAc fraction. In case of antioxidative activity in the leaf parts of *Castanea creanata* (*Jeong et al.*, 2002) was reported to have shown a higher measure than BHA and BHT. These results are giving us a similar results on that antioxidative activity in EtOAc fraction is high in both the leaf parts and fruit parts of *Prunus mume*.

When the antioxidative activity between the fruit parts and leaf parts of *Prunus mume* was compared, the leaf parts showed a higher activity. Such result shows a similar tendency to the report saying the leaf part showed a higher activity when compared to the fruit part or its peel part of *Eriobotrya japonica* (*Bae et al.*, 2002) and also the fact the antioxidative activity was highest in EtOAc fraction is also quite similar. In the research of antioxidative activity using extracts of leaves, bark and the stem part of Ficus carica also showed considerably a good reaction in leaves rather than other parts (*Moon et al.*, 1997).

The research on antioxidative activity in immature and mature fruit of *Prunus salicina* Lindl. cv. Soldam (Yu *et al.*, 2004) with the fruits collected at a different time showed that the fruits collected between June to the beginning of July measured a higher activity than BHA. Therefore, *Prunus mume* should in the future also needs to have the antioxidative activity between the immature and mature fruit measured and investigated.

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