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Analysis of Dye Extracted from *Phellodendron* Bark Using Liquid Chromatography

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Abstract

Berberine, palmatine, and *Phellodendron* bark dye was prepared in methanol for HPLC-DAD-MS analysis of liquid dye. Silk was dyed using berberine, palmatine, and *Phellodendron* bark dye prepared in water. The dye was extracted from the dyed silk using the HCl/methanol/water (2:1:1 v/v/v) solvent system with a slight modification. The liquid dyes and the dye extracted from the silk samples dyed with the three dye sources were examined using the HPLC-DAD-MS analysis to simultaneously detect berberine and palmatine from the plant dye and the dyeings. Colorimetric measurement was carried out using a spectrophotometer to examine the color and the intensities of berberine, palmatine, and *Phellodendron* bark dyed silk samples. From the liquid dyes, berberine eluted at 5.21 min with the molecular cation m/z=336 and the UV spectrum confirming that the product was berberine. Palmatine eluted at 5.12 min with the molecular cation m/z=352 and the UV spectrum confirming that the product was palmatine. From the silk dyed with berberine and palmatine dye, berberine and palmatine species eluted at 5.35 min and 5.24 min, respectively. From the silk dyed with *Phellodendron* bark, berberine and palmatine were detected simultaneously at 5.35 min and 5.26 min, respectively. All three dyes had yellow hue while palmatine dyed silk showed the highest hue and chroma. Palmatine dyed silk showed the highest K/S value that indicated the strongest color intensity and the highest dye uptake.

Key words: *Phellodendron* bark, Amur cork tree, Berberine, Palmatine, HPLC-DAD-MS

I. Research Background

Phellodendron, commonly called the 'cork tree,' is a deciduous tree in the family of Rutaceae. The two most well known species of the cork tree are *Phellodendron amurense* Rupr. and *Phellodendron chinense* Rupr. These two species are commonly called amur cork tree and Chinese cork tree respectively (Chan et al., 2007; Hu et al., 2010) and the plants were native to diverse regions of Asia. The bark of *Phellodendron*, often called 'huang bai,' had been widely used as the source of folk medicine in Asia from the ancient times (Drasar & Moravcova, 2004). The major chemical constituents of *Phellodendron* bark had been extensively studied for their application in modern pharmaceuticals owing to their medicinal effects such as anti-microbial, anti-inflammatory, and antiphlogistic functions (Hu et al., 2010; Lee et al., 1999). Lee et al. (1999) used narrowbore high performance liquid chromatography on *Pellodendron* bark and Coptidis Rhizoma, the biological name for huang lian, to simultaneously determine berberine and palmatine and the recovery rate of the two compounds from the plant material. They found out that in both plants berberine recovery rate was about twice as that of palmatine. Chan et al. (2007) developed a near infrared spectroscopy (NIRS) method for the rapid differentiation of the two species, amur cork tree and Chinese cork tree, and the content of berberine, palmatine, and

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jatrorrhizine. In all the extraction methods used, berberine was the most abundantly recovered compound followed by palmatine which was about three quarter of the amount of berberine, and jatrorrhizine was present in only a small amount (Chan et al., 2007). Hu et al. (2010) examined *Phellodendron amurense* Rupr. and *Phellodendron chinense* Rupr. using high performance liquid chromatography coupled with electrospray ionization mass spectrometry and identified 24 different alkaloids including berberine, palmatine, jatrorrhizine, tetrahydropalmatine. Yin et al. (2009) used high performance liquid chromatography coupled with a diode array detection method to investigate the simultaneous detection of 11 active pharmaceutical compounds including berberine from the well-known Chinese traditional medicine.

Another important characteristic of *Phellodendron* bark is that it has a deep yellow color which can be extracted to dye fibers. Having its root in Asia, *Phellodendron* bark has been one of the major sources of yellow dye used in historic Asia for fibers and paper materials not only for its color but also for its anti-bacteria and anti-insect effects (Leona & Lombardi, 2007; Nakamura et al., 2009). With the growing interest in natural dyes and dyeings, *Phellodendron* bark has been studied widely in terms of its dyeing properties on different fiber types.

Major coloring compound of *Phellodendron* bark is known as berberine ($C_{20}H_{18}NO_4^+$, molecular weight: 336.36), which belongs to the protoberberine group within the isoquinoline alkaloids (Wikipedia, 2011) (Fig. 1). Berberine is one of the few cationic dyes among the natural plant dyes. Its high affinity to protein fibers as dyeing substance and its anti-microbial, deodorizing functions as pharmaceutical substance are all due to its chemical structure containing the cationic nitrogen atom (Kim et al., 2003).

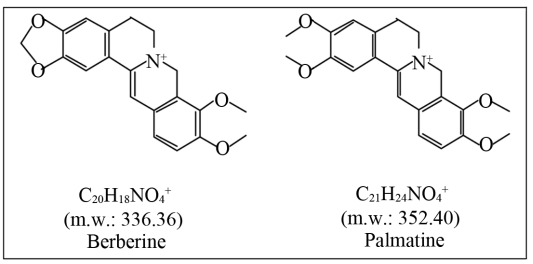

Fig. 1. Structures of berberine and palmatine (m.w. is molecular weight).

While berberine is widely known to be responsible for the color of dye extracted from *Phellodendron* bark, *Phellodendron* bark is composed of a large number of different compounds, many of them which belong to the protoberberine group with cationic nitrogen atom in their structures. Protoberberine alkaloids isolated from *Phellodendron* bark in recent studies are berberine, palmatine, jatrorrhizine, magnoflorine, phellodendrine, etc. and among these compounds more attention has been paid to berberine and palmatine ($C_{21}H_{24}NO_4^+$, molecular weight: 352.40) (Lee et al., 1999; Lee et al., 2007; Li et al., 2007; Suto et al., 1997) (Fig. 1). The isolated pure powders (or crystals) of berberine and palmatine are yellow in color (Lee et al., 2007). Another protoberberine alkaloid of *Phellodendron* bark which received interest next to palmatine in the pharmaceutical area is jatrorrhizine ($C_{20}H_{20}NO_4^+$, molecular weight: 338.38). While jatrorrhizine is known to have the same medicinal effect as berberine or palmatine, the review of previous literatures in the pharmaceuticals indicated that it has been seldomly used as the standard compound in the medicinal studies. This is probably due to the fact that the standard compound of jatrorrhizine is not sold by the reliable global chemical suppliers. While several studies reported the success in the simultaneous isolation of berberine and jatrorrhizine from the similar plant (Hu et al., 2010) their identification based on the mass spectral data is difficult since the molecular weight difference between the two compounds are extremely small, 336.36 for berberine and 338.38 for jatrorrhizine. And Rueffer et al. (1983) reported that when (S)-reticuline and (S)-protosinomenine were fed to Berberis stolonifera plant, berberine was first synthesized and then it transformed into jatrorrhizine in vivo, suggesting the transformation of berberine into jatrorrhizine during the analytical procedure. Jatrorrhizine is said to be brown red in color (Shaanxi Hongkong Biological Technology Co., Ltd., 1999-2010).

It is reported that berberine is the most abundant of all the protoberberine alkaloids in the bark of *Phellodendron*. According to Lee et al. (1999) berberine and palmatine contents in *Phellodendron* bark were 0.529%

and 0.299% respectively when the bark was extracted using 2% hydrochloric acid with cobalt thiocyanate and dichloroethane. Li et al. (2007) examined the effect of water and drought condition on the production of berberine and palmatine in 100g of amur cork tree seedlings. The seedlings were extracted using 60% ethanol and analyzed using HPLC. The results indicated that berberine content was by far higher than palmatine or any other protoberberines regardless of the growth condition. Simultaneous detection of berberine and palmatine in *Phellodendron* bark and other similar bark species had been studied extensively in pharmaceuticals using the HPLC instrument (Lee et al., 1999; Suto et al., 1997; Yin et al., 2009).

While berberine has been generally regarded as the main coloring compound of *Phellodendron* bark in the field of dyeing (Chu & Soh, 1996; Kim et al., 2001; Kim & Park, 2007; Kim & Son, 2005; Lee et al., 2010), the review of previous literatures make it clear that the extract of *Phellodendron* bark is composed of a number of different protoberberines among which palmatine needs as much attention as berberine (Cho & Kang, 2000). And considering that the pure powders of both berberine and palmatine have yellow color, it is expected that the yellow color of the fabric dyed with *Phellodendron* bark is not only due to the presence of berberine in the dye extract but it is also due to the coexistence of palmatine in the same dye. Other protoberberine alkaloids of *Phellodendron* bark such as jatrorrhizine ($C_{20}H_{20}NO_4^+$, molecular weight: 338.38), coptisine ($C_{19}H_{14}NO_4^+$, 320.31), or phellodendrine ($C_{20}H_{24}NO_4^+$, molecular weight: 342.40) would also contribute to the color of dye extracted from *Phellodendron* bark. However, the content of these compounds in Phellodendron bark is either much smaller than berberine or palmatine or it is not specified in the literatures. Jatrorrhizine, which is the most recognized amongst the three above protoberberines, is reported to be present in *Phellodendron* bark by less than one tenth of that of berberine content (Chan et al., 2007). In practical terms, the compound is not sold by the widely known global chemical suppliers. And the situation limits the usage of jatrorrhizine as the standard chemical in the experimental research.

In light of such premise, the purpose of present research was to examine the dye extracted from *Phellodendron* bark to simultaneously detect berberine and palmatine using the HPLC-DAD-MS instrument. And to verify the method of extracting dye from the dyed silk samples so that both berberine and palmatine can be detected simultaneously from the silk samples dyed with *Phellodendron* bark. This research also examined the color difference between the *Phellodendron* bark dyed silk and the silk dyed with reference standards to investigate the effect of berberine and palmatine on the yellow color of the *Phellodendron* bark dyed silk. This research utilized the high performance liquid chromatography (HPLC) instrument tripled with a diode array detector (DAD) and a mass selective detector (MSD) for examining the dye extracted from *Phellodendron* bark and the dyed silk samples.

II. Experimental

1. Materials

The *Phellodendron* bark was purchased from the Kyungdong traditional pharmaceutical market. Berberine chloride and palmatine chloride hydrate were purchased from Sigma-Aldrich (USA) and used as reference standards. Aluminum potassium sulfate [$AlK(SO_4)_2 \cdot 12\ H_2O$)] was purchased from Shinyo Pure Chemicals, Co (Osaka, Japan) and used as alum mordant. Methanol (Acros Organics) and acetonitrile (EMD Chemicals) were HPLC grades. Formic acid and HPLC water were purchased from Mallinckrodt Baker. Hydrochloric acid (36-38wt%) was purchased from VWR Inc. Silk fabric (KS K0905 Standard Adjacent Fabrics for Colorfastness Test) was purchased from the Korea Apparel Testing & Research Institute. Glass fiber enhanced 0.45μm syringe filters were purchased from Alltech (Deerfield, IL). Water used for dyeing and sample preparation was deionized using a Corning Megapure MP6 with Barnstead Nanopure System.

2. Preparation of Liquid Dyes of *Phellodendron* Bark, Berberine, and Palmatine for HPLC-DAD-MS Analyses

Weight of 13g of powdered *Phellodendron* bark was

refluxed for 1 hour with 130mL methanol and centrifuged for 5 min at 5,000rpm, 21°C. About 2mL of the supernatant was filtered into an HPLC vial using a glass fiber enhanced 0.45μm syringe filter and used as the extract of *Phellodendron* bark for HPLC analysis. Berberine chloride and palmatine chloride hydrate were separately weighed 0.01g and were dissolved in 10mL methanol and were used as the reference standards. The standard dyes were filtered into the HPLC vials using the glass fiber enhanced 0.45μm syringe filters.

3. Dyeing of Silk Samples with *Phellodendron* Bark, Berberine, and Palmatine

Berberine and palmatine dye liquors were prepared by dissolving 0.3g of each of the standard compounds in 50mL water. For the first extraction of *Phellodendron* bark, 5g of powdered *Phellodendron* bark and 50mL water were heated on a hotplate at 80°C for 1 hour. The second extraction was carried out by adding 50mL water onto the residue. The two extracts were mixed and used as the *Phellodendron* dye liquor.

Silk was dyed using the pre-mordanting procedure and repeating the dyeing process twice. Silk specimens each weighing approximately 2g were mordanted with 0.3g of aluminum potassium sulfate in 50mL of water for 30 min at 60°C. Then the silk was dyed with dye liquors of *Phellodendron* bark, berberine, and palmatine for 1 hour at 60°C. The dye liquor was saved. Mordanting and dyeing procedure was repeated using the fresh mordant solution and the dye liquor saved from the first dyeing. After each mordanting and dyeing, the silk was rinsed first with running tap water, then soaked in the beaker with fresh deionized water for final rinsing.

4. Extraction of Dye from Dyed Silk Samples for HPLC-DAD-MS Analyses

Dye was extracted from the silk samples dyed with *Phellodendron* bark, berberine, and palmatine by modifying the HCl/methanol/water (2:1:1 v/v/v) solvent system used in the previous literatures (Berghe et al., 2009; Wouters, 1985; Wouters et al., 1990). An aliquot of 400μL of HCl/methanol/water (2:1:1 v/v/v) was placed in a 20mL beaker with approximately 0.5-0.8mg of silk specimen. The beaker was placed in an oven at 105°C for 15 min followed by rapid cooling in cold water. Then, the beaker was placed in a vacuum desiccator above NaOH pellets until the liquid completely evaporated. After this treatment the silk sample showed tan color. An aliquot of 1.1mL methanol was added to the beaker while gently rotating the beaker so that the silk sample was completely soaked. By this treatment methanol became yellow and the silk sample became white. The methanol extract was filtered using the glass fiber enhanced 0.45μm syringe filters and analyzed using HPLC-DAD-MS.

5. HPLC-MS Analyses

An Agilent 1200 series binary HPLC-DAD-MS system (Foster City, CA) with diode-array detector (DAD), and mass selective detector (MSD) consisting of a single quadrupole mass analyzer with the multi-mode source atmospheric pressure chemical ionization (APCI) in the positive mode was used to detect the target compounds. LC separation was achieved by 150mm length, 4.6mm i.d. stainless steel C18 column, 5μm particle size (Restek Corporation, Bellefonte, PA). The gradient elution applied in the analysis with solvent A (acetonitrile) and solvent B (0.5% formic acid in water) was: 0-5.7 min, 90-20% B; 5.7-10 min, 20-61% B, 10-15 min, 61% B. The flow rate was 1.0mL/min, and the injection volume was 20μL. Detection wavelength for DAD was set at 275nm following Petroviciu et al. (2010). The column temperature of the MSD was 25°C and the drying gas (N_2) temperature 350°C, vaporizer 230°C, capillary voltage of 3kV in positive ion mode, fragmentor voltage 160V following Hua et al. (2007). After the HPLC-MS analyses of the liquid dyes, the instrument was reset to check the possibility of nitrogen gas leak, and this resulted in the shift of retention times in the analyses of the dye extracted from the silk dyeings.

6. Color Measurement of the Dyed Silk Samples

A spectrophotometer (JS-555, Color Techno System, Co. Ltd., Japan) was used to measure the color and the reflectance value (R) of the silk samples dyed with berberine, palmatine, and *Phellodendron* bark. Color

measurement was carried out under D_{65} illuminant and 10° standard observer. L*a*b* and H, V/C values were obtained for describing the color. Three measurements were taken for each sample and the average data were reported. The K/S value was calculated using the Kubelka-Munk formula to compare the color intensities and the degree of dye uptake of the dyed silk samples (Jung et al., 1997).

III. Results and Discussion

1. HPLC-DAD-MS Analyses of Berberine and Palmatine Reference Standards

The results of HPLC-DAD-MS analyses of berberine and palmatine standards are shown in <Fig. 2> to <Fig. 5>. The chromatogram generated by the HPLC-DAD-MS instrument on the berberine reference standard showed a major peak eluted at 5.2 min (Fig. 2) (Ahn et al., in print). The mass spectra indicated that the mass-to-charge-ratio (m/z) of the peak was 336 <Fig. 3>, which is the fingerprint molecular cation of berberine (Chen et al., 2000; Deevanhxay et al., 2009; Hua et al., 2007). A small peak eluted at 8.30 min appears to be an impurity in the berberine reference standard. The chromatogram generated by the HPLC-DAD-MS instrument on the palmatine reference standard showed a major peak eluted at 5.1 min (Fig. 4) (Ahn et al., in print). The mass spectra indicated that the mass-to-charge-ratio (m/z) of the peak was 352 <Fig. 5> which is the fingerprint molecular cation of palmatine (Chen et al., 2000; Deevanhxay et al., 2009). Berberine and palmatine showed maximum absorption at three wavelengths within the UV range, 232nm, 264nm, and 344-348nm <Fig. 2> and <Fig. 4> (Ahn et al., in print) and this was consistent with the UV spectra of quaternary alkaloids, such as berberine and palmatine, reported in the literatures (Deevanhxay et al., 2009; Zhang et al., 2009). By the HPLC-DAD-MS analyses of the reference standards, the retention times, the target mass-to-charge-ratios, and the UV spectra of berberine and palmatine were confirmed. And these data were used to identify the two compounds in the dye extracted from *Phellodendron* bark and the dye extracted from the dyed silk samples.

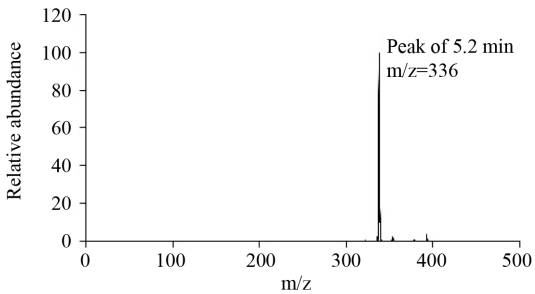

Fig. 3. Mass spectrum of the peak of 5.21 min showing the molecular cation of berberine.

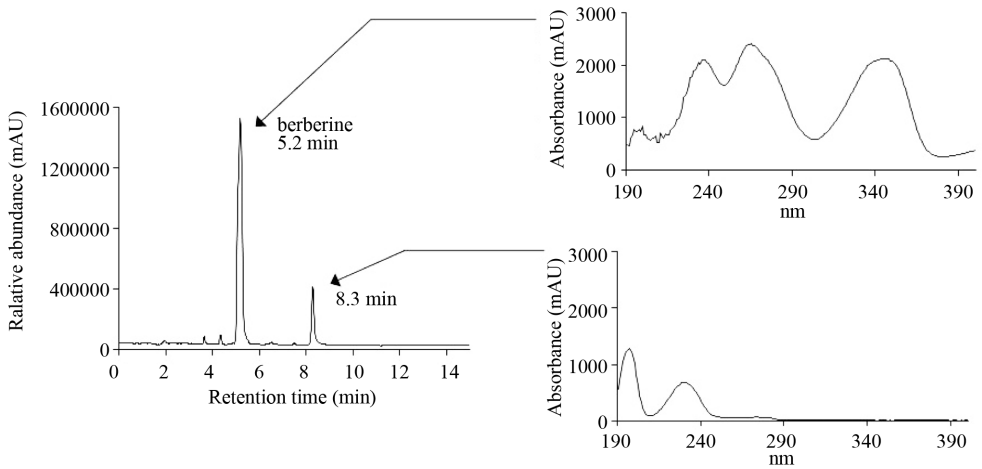

Fig. 2. HPLC-DAD-MS chromatogram of berberine standard, UV spectra of the peaks on the right.

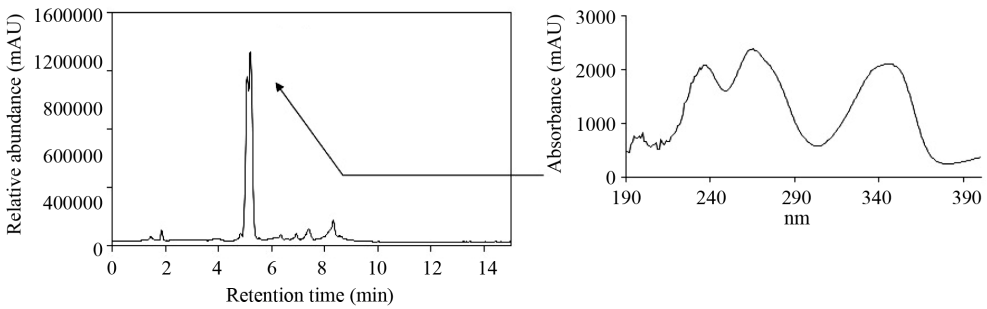

Fig. 4. HPLC-DAD-MS chromatogram of palmatine standard, UV spectrum of the peak on the right.

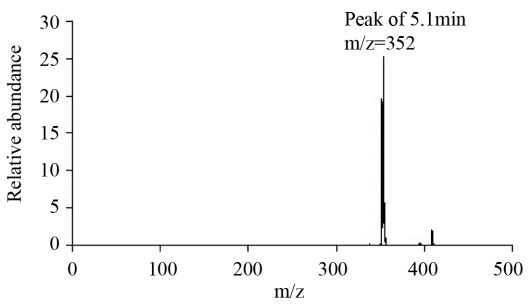

Fig. 5. Mass spectrum of the peak of 5.12 min showing the molecular cation of palmatine.

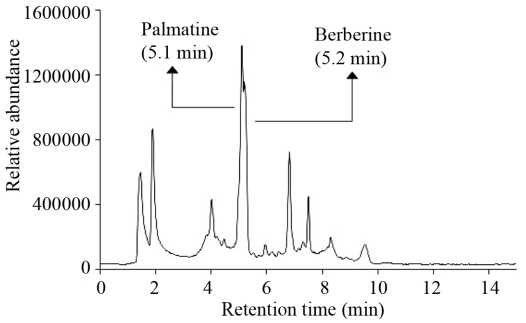

Fig. 6. HPLC-DAD-MS chromatogram of the dye extracted from *Phellodendron* bark.

2. HPLC-DAD-MS Analyses of Dye Extracted from *Phellodendron* Bark

HPLC-DAD-MS analysis of the dye extracted from *Phellodendron* bark exhibited that the extract contained both berberine and palmatine. The peaks generated by HPLC-DAD-MS instrument exhibited two major peaks which slightly overlap at retention time range of 5.1-5.2 min. The mass spectra of the two peaks indicated that the two peaks represent the molecular cations of berberine (m/z=336) and palmatine (m/z=352), each eluted at retention times 5.2 min and 5.1 min respectively (Fig. 6) (Ahn et al., in print). The UV spectra of the two compounds were consistent with those of the quaternary alkaloids (Deevanhxay et al., 2009; Zhang et al., 2009). The retention times, mass-to-charge-ratios (m/z), and the UV spectra confirmed that the two major products of the liquid dye extracted from *Phellodendron* bark were berberine and palmatine. Therefore, it was concluded that the present HPLC-DAD-MS method is successful in the simultaneous detection of berberine and palmatine in the dye extracted from *Phellodendron* bark.

3. HPLC-DAD-MS Analysis of Dye Extracted from Silk Samples Dyed with Berberine, Palmatine, and *Phellodendron* Bark

Silk dyed with berberine and palmatine, and *Phellodendron* bark dye were extracted using the slightly modified HCl/methanol/water (2:1:1 v/v/v) solvent system (Berghe et al., 2009; Wouters, 1985; Wouters et al., 1990). The extractions from dyed silk samples were examined using the same HPLC-DAD-MS method as the liquid dyes. In addition to the dyed silk samples, the silk sample undyed was also extracted using the above solvent system and the extract was examined using the same HPLC-DAD-MS method.

<Fig. 7> is the HPLC chromatogram of the undyed silk. From the undyed silk sample, three peaks eluted near 5.0-5.5 min, which is the retention time range of interest in this study. However, the mass-to-charge-

ratios (m/z) of the two peaks did not match those of berberine or palmatine. Using the HPLC chromatogram of the undyed silk sample as the reference, the HPLC-DAD-MS chromatograms of the silk dyed with berberine, palmatine, and *Phellodendron* bark were examined.

<Fig. 8> is the HPLC chromatogram of the silk sample dyed with berberine reference standard. <Fig. 8> exhibits an apparent similarity with the HPLC chromatogram of the undyed silk in <Fig. 7>. The pattern of the peak distribution and the retention times of the peaks in <Fig. 7>–<Fig. 8> appear to be almost identical. One difference between the two chromatograms is the intensity of the peaks at 5.0-5.5 retention time range. The retention time of the major peak at 5.0-5.5 min range was 5.3 min (Fig. 8). After the HPLC-MS analyses of the liquid dyes, the instrument was reset to check the possibility of nitrogen gas leak, and this resulted in the shift of retention times in the analyses of the dye extracted from the silk dyeings. Same condition holds for the anayses of all the silk dyeing. The mass spectrum of the peak at 5.3 min indicated that the mass-to-charge-ratio (m/z) of thepeak was 336 (Fig. 9) (Ahn et al., in print). The UV spectrum also matched those of the quaternary alkaloids. It was concluded that product represented by the peak eluted at 5.3 min was berberine.

<Fig. 10> is the HPLC chromatogram of the silk sample dyed with palmatine reference standard. Similar to berberine dyed silk, the HPLC chromatogram of palmatine dyed silk in <Fig. 10> showed an apparent similarity with the HPLC chromatogram of the undyed silk in <Fig. 7>. The retention time of the major peak at 5.0-5.5 min range was 5.2 min (Fig. 10) (Ahn et al., in print). The mass spectrum of the peak at 5.2 min indicated that the mass-to-charge-ratio (m/z) of the peak was 352 (Fig. 11). The UV spectrum also matched those of the quaternary alkaloids. It was concluded that product represented by the peak eluted at 5.2 min was palmatine.

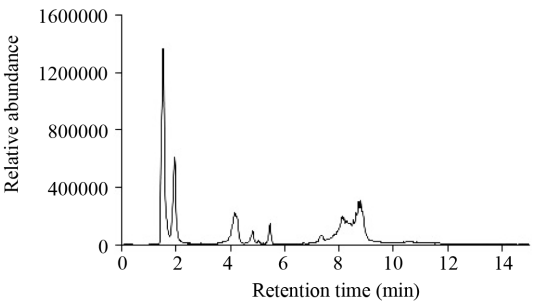

Fig. 7. HPLC-DAD-MS chromatogram of undyed silk.

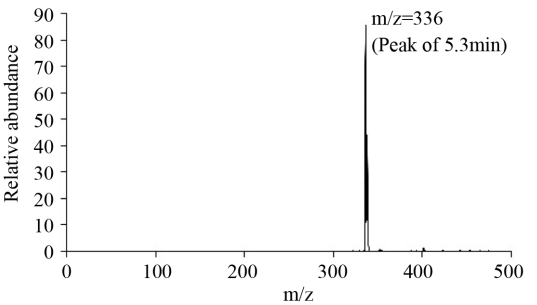

Fig. 9. Mass spectrum of the peak of 5.33 min showing the molecular cation of berberine.

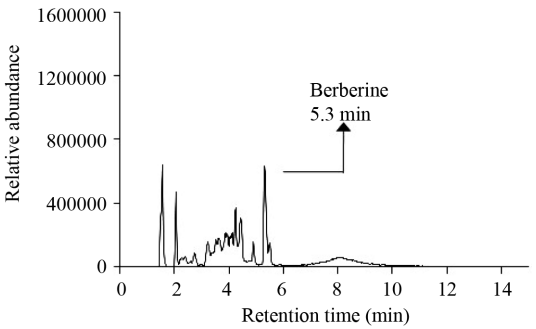

Fig. 8. HPLC-DAD-MS chromatogram of the dye extracted from silk dyed with berberine standard.

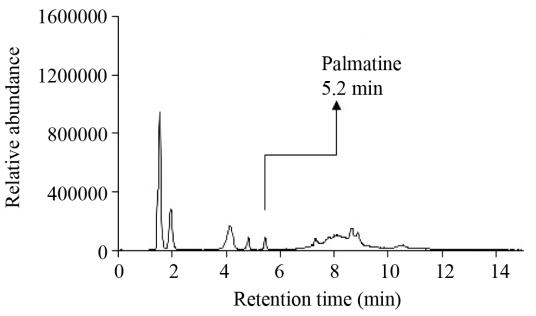

Fig. 10. HPLC-DAD-MS chromatogram of the dye extracted from silk dyed with palmatine standard.

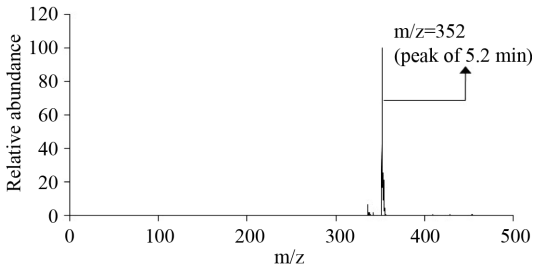

Fig. 11. Mass spectrum of the peak of 5.24 min showing the molecular cation of palmatine.

From the HPLC-DAD-MS analyses of the silk dyed with berberine and palmatine reference standards, it was concluded that the present method of extracting the dye from dyed silk was successful in extracting both berberine and palmatine from the silk. And the same HPLC-DAD-MS method which was used to examine the dye liquor can be used to detect the two compounds in the dyed silk samples.

<Fig. 12> is the HPLC-DAD-MS chromatogram of the dye extracted from the silk dyed with *Phellodendron* bark. Two peaks appeared in 5.0-5.5 retention time range. Although the intensity of the peaks were lower than those of berberine dyed and palmatine dyed silk samples, the retention times of the peaks (berberine=5.3 min, palmatine=5.2 min) <Fig. 12> (Ahn et al., in print), the mass-to-charge-ratio (m/z) of the peaks (berberine=336m/z, palmatine=352m/z) <Fig. 13>, and the UV spectra were the same as those of berberine and palmatine species in the berberine dyed and palmatine dyed silk samples. From the HPLC-DAD-MS analysis of the *Phellodendron* bark dyed silk samples, it is

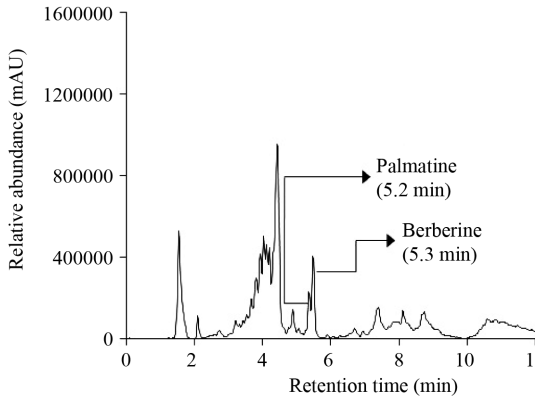

Fig. 12. HPLC-DAD-MS chromatogram of the dye extracted from silk dyed with *Phellodendron* bark.

concluded that both berberine and palmatine co-exist in the dyed silk, and that the extraction method and the instrumental method used in this study can separate and detect both species from the dyed textiles.

4. Color Measurement of the Dyed Silk Samples

<Table 1> illustrates the result of color measurement of the silk samples dyed with berberine, palmatine, and *Phellodendron* bark. While the silk samples dyed with all three dye types exhibited a yellow hue in the Munsell color system, the silk dyed with *Phellodendron* bark showed the weakest hue (H=6.14Y) with the lowest value (V=6.81) and chroma (C=8.54) compared to the two other samples. Among the three silk samples, the silk dyed with palmatine exhibited the strongest yellow hue (H=6.83Y) with the highest chroma (C=11.80). Similar result was observed in the measurements of the

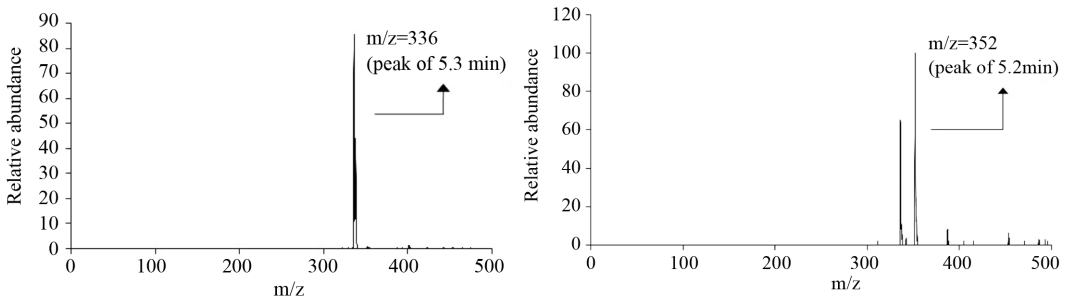

Fig. 13. Mass spectra of the peaks of 5.35 min and 5.26 min showing the molecular cations of berberine and palmatine, respectively.

Table 1. Results of the color measurement of the dyed silk samples

	L*	a*	b*	ΔE	H	V	C	K/S	λ_{max} (nm)
Undyed silk	96.68	−0.33	3.56	-	-	-	-	-	-
Berberine dyed silk	81.33	−3.26	74.65	72.78	6.45Y	7.99	10.75	10.28	440
Palmatine dyed silk	80.37	−4.32	83.31	81.50	6.83Y	7.89	11.80	19.13	440
Phellodendron bark dyed silk	69.82	−1.25	58.84	61.46	6.14Y	6.81	8.54	9.71	420

CIELAB color system. Although the difference was small, among the three samples the *Phellodendron* bark dyed silk sample showed the largest redness (a*= −1.25) while the yellowness was the smallest (b*= 58.84). Greenness (a*) and yellowness (b*) were the largest in palmatine dyed silk (a*=−4.32, b*=83.31). As expected from the color measurements, the color difference (⊿E) between the undyed silk and the dyed silk samples was the greatest in palmatine dyed silk sample (⊿E=81.50).

<Table 1> also shows the K/S values of the dyed silk samples calculated using the reflectance (R) at the wavelength of maximum absorbance (λ_{max}). The maximum absorbance wavelength of *Phellodendron* bark dyed silk was slightly lower (420nm) than those of berberine and palmatine dyed silk (440nm). A relatively larger color difference in *Phellodendron* bark dyed silk compared to berberine and palmatine dyed silk can be explained by its different maximum absorbance wavelength compared to berberine or palmatine dyed silk samples. Surprisingly, it was found that the K/S value of palmatine dyed silk was close to twice that of berberine dyed silk. This suggests that the dye uptake of palmatine dyed silk was greater than that of berberine dyed silk.

Considering that berberine and palmatine dye liquors prepared in this experiment were of the same concentration (0.3g/50mL), the present results suggest some significant implications regarding the behavior of berberine and palmatine compounds in *Phellodendron* bark dye. It is possible that palmatine allows for a higher dye exhaustion possibly due to the higher adsorption rate. Or it may be that palmatine has higher solubility in water which consecutively affected the availability of dye molecules in the dye liquor. Whether the structure of palmatine allows for a higher dye absorption than berberine also needs to be investigated.

The solubility of the salts of berberine- such as the berberine chloride used as the reference standard in this study- in water is said to be low except at boiling temperature. While palmatine is said to be soluble in water even below the boiling temperature (ChemYQ, 2005; LKT Laboratories, Inc., 2010). However, specific solubilities of berberine and palmatine in particulate amount are not reported in previous literatures. Dyeing characteristics of berberine on the exhaustion rate or adsorption and diffusion behaviors on different fiber types had been studied previously (Kim & Park, 2002; Kim & Son, 2005; Kim et al., 2003; Kim et al., 2004). Dyeing characteristics of *Phellodendron* bark extract had been extensively dealt with (Cho & Kang, 2000; Chu & Soh, 1996; Kang et al., 2003; Kim et al., 2001; Kim & Park, 2007; Kim et al., 2003; Lee et al., 2010; Yong et al., 1999). However, there had been no attempt to study the characteristics of palmatine as the source of dye. The result of present investigation calls for an in depth research effort on the comparison and characterization of berberine and palmatine as dye sources, and their effects on the dye extracted from *Phellodendron* bark. An analysis which quantitatively examines berberine and palmatine concentration in the *Phellodendron* bark dyes and dyeings is also in need (Ahn et al., in print).

IV. Conclusions

The purpose of present research was to examine the dye extracted from *Phellodendron* bark and the dyed silk samples to simultaneously detect berberine and palmatine using the HPLC-DAD-MS instrument. It was found that the HPLC-DAD-MS method utilized in this research was successful in separating and detecting berberine and palmatine from the *Phellodendron* bark dye and the dyeings. It was also found that the method used to extract the dye from the dyed silk samples successfully extracted both berberine and palmatine from the dyed silk. The color measurement indicated that the silk samples dyed with berberine, palmatine,

and *Phellodendron* bark all had yellow hue but the color intensity of palmatine dyed silk was the greatest in hue and chroma. The concentration of dye in the dyed silk represented by the K/S values indicated that the dye concentration in the palmatine dyed silk was the greatest. The present result suggests a significant implication that palmatine needs future investigation in terms of its dyeing properties as a new natural dye source. The present investigation implies that equal attention should be given to palmatine as the dye component affecting the color and dyeability of the textiles dyed with *Phellodendron* bark dye.

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