# Comparison of Berberine Content in Phellodendron amurense Rupr. Inner Bark in Relation to its Variety, Stem Position, Season and Tree Age

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# Comparison of Berberine Content in *Phellodendron amurense* Rupr. Inner Bark in Relation to its Variety, Stem Position, Season and Tree Age*

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## Abstract

A quantitative analysis for berberine was established by a high-performance liquid chromatographic (HPLC) method. The variation of berberine contents with stem position, season, and tree age were investigated in two varieties of *Phellodendron amurense* Rupr. There were significant differences in berberine content among two varieties. *P. amurense* Rupr. var. *sachalinense* Fr. Schm. (hirohano kihada, coded as HK) sampled in Miyazaki prefecture contained a higher level of berberine in its inner bark than *P. amurense* Rupr. var. *lavallei* Sprague (miyama kihada, coded as MK) sampled in Hokkaido. Higher content was observed in the stem base, then gradually decreased from the stem base to the tree top in both varieties. Berberine content showed a clear seasonal variation. During winter the content was quite rich compared with that of summer. Tree age was positively correlated with the berberine content. Older tree contained much higher contents than younger one at same stem height. The surface color of the inner bark showed no significant relationship with the berberine content.

## 1. Introduction

The Amur Cork-tree, *Phellodendron amurense* Rupr., is one of typical eastern Asian trees in the Rutaceae family having colklike bark, and it may grow a height of 20 meters. Greenish-yellow flowers bloom in summer. Female trees bear small grapelike clusters of black fruit. The leaf is dark-green, pinnate compound and changeable depend on growing conditions (Kitamura and Okamoto, 1971). The crude drug of *P.*

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* 大賀祥治・吉良今朝芳・古賀信也：キハダ内樹皮中のベルベリン含有量の変種間差異ならびに樹幹部位，伐倒時期，樹齢による変動

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*amurense* Rupr. has been used as one of important Chinese drugs (Oubaku in Japanese, Huang-po in Chinese) prescribed together with other herbs, and contains berberine as major alkaloid, and palmatine, phellodendrine and magnoflorine as minor alkaloid. It becomes evident that berberine has various pharmacological activities such as stomachic, analgesic, sedative, antidotal, hemostatic, and anti-inflammatory (Haginiwa and Harada, 1962). Therefore, a decoction or extract of crude drug can be use. Berberine is mainly produced from the inner bark of *P. amurense* Rupr.. However, there is a few reports concerning berberine contents in the bark of *P. amurense* Rupr. (Misaki et al., 1982).

This paper describes the variation of berberine contens with stem position, season, and tree age in two varieties of *P. amurense* Rupr..

## 2. Materials and methods

### 2.1. Chemicals

Chemicals and reagents were purchased from Wako Pure Chemical Industries, and were of the highest commercially available grade. All the solvents used in the chromatographic procedure were of HPLC grade.

### 2.2. Plant materials

Samples of *P. amurense* Rupr. were collected from two branches of the University Forest of Kyushu University. *P. amurense* Rupr. var. *lavallei* Sprague (miyama kihada, coded as MK) was collected from the Hokkaido branch of the University Forest of Kyushu University where is approximately 70 km northeast of Obihiro. *P. amurense* Rupr. var. *sachalinense* Fr. Schm. (hirohano kihada, coded as HK) was collected from the Miyazaki branch of the University Forest of Kyushu University where is approximately 40 km northeast of Hitoyoshi. Sampling sites and weather conditions are described in Table 1. The stands of MK grow in a dry and cold inland climate, and HK grow in a wet and cool mountainous climate.

Samples from a representative trees were taken in summer (July or August) and winter (November or December) at each site, respectively. Moreover, as for HK, old trees were felled for the purpose of examining the tree age variation (Table 2). Female trees were chosen in this experiment.

Table 1 Sites and weather conditions of two University Forest of Kyushu University.

| | Site | | | Weather | | | |
|---|---|---|---|---|---|---|---|
| | Latitude | Longitude | Altitude (m) | Mean annual temperature (℃) | January average temperature (℃) | August average temperature (℃) | Annual precipitation (mm) |
| Hokkaido branch of the University Forest | 43°17′N | 143°34′E | 300 | + 5.9 | −11.5 | +21.8 | 782 |
| Miyazaki branch of the University Forest | 32°22′N | 131°08′E | 1000 | +13.1 | + 2.3 | +23.2 | 3319 |

Table 2 Characterization of sampling trees of two *Phellodendron amurense* Rupr. varieties.

| | Height (m) | DBH (cm) | Age (years) |
|---|---|---|---|
| *P. amurense* Rupr. var. *lavallei* Sprague (miyama kihada, coded as MK) | 18 | 18 | 30 |
| *P. amurense* Rupr. var. *sachalinense* Fr. Schm. (hirohano kihada, coded as HK) | 20<br>21 | 19<br>30 | 25<br>60 |

Inner bark was obtained from two varieties of freshly felled *P. amurense* Rupr. trees. The samples were air-dried, and then powdered passing a 60 mesh screen by a Wiley mill. The powdered inner bark (0.1 g, dry wt) was extracted with methanol (100 ml) on a water bath at 50 °C for 30 min. The solution was centrifuged at 7,000 x *g* for 10 min, and then the yellowish supernatant was clarified by filtration through a 0.5 $\mu$m filter before injection into the HPLC column, and employed for HPLC analysis.

### 2.3. HPLC analysis

HPLC analysis of berberine was performed by using a Waters Model 208D Liquid Chromatograph. The analysis was conducted at 25 °C with a stainless steel column (4. 6 mm i.d. ; length 150 mm) packed with 5 $\mu$m ODS-silica gel (L-column, Chemical Inspection & Testing Institute) using $CH_3CN$/10 mM $NaH_2PO_4$+100 mM $NaClO_4$ (4 : 6, V/V) as eluent (flow rate : 1.0 ml/min). The eluent was monitored with a Waters Model 481 absorbance detector at 340 nm, 0.1 AUFS. The signals from the detector were processed with a Waters data module.

Identification of berberine was performed by comparsion with authentic sample by retention time, and by co-chromatography with authentic sample by HPLC analysis. The contents of berberine in the extract were calculated by the calibration curve, which was made from the peak areas of the authentic standard solutions of different concentrations ranging from 0.63 to 2.50 $\mu$g in 20 $\mu$l injected. The regression equation obtained was as follow : $Y=2.0080X-0.0695$ (correlation coefficient=0.999), where $Y$ is the peak area in integrator unit and $X$ is the amount of berberine.

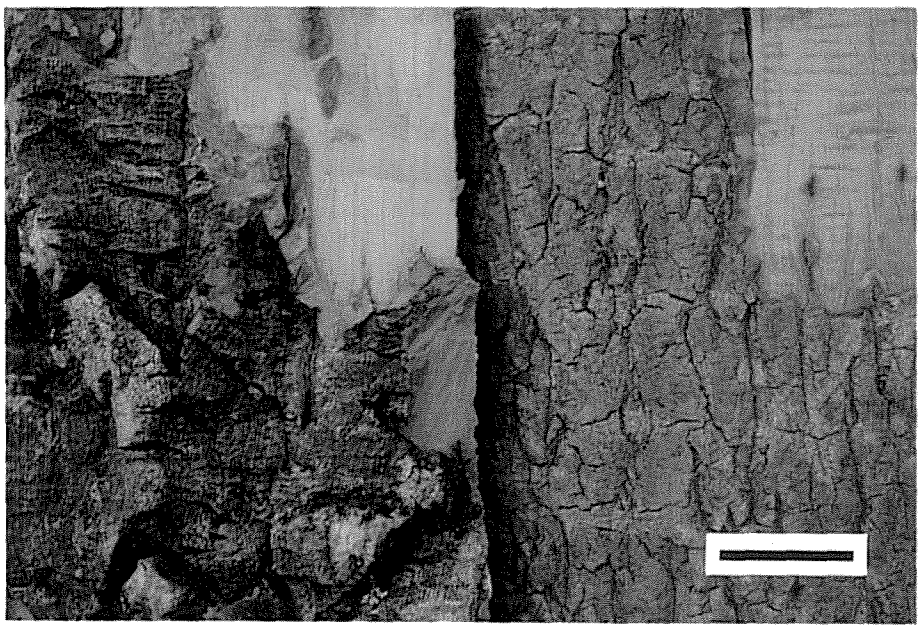
### 2.4. Surface color of inner bark

The reflection surface color of the inner bark at each position was measured using a Minolta Chromameter, Model CR-200 (Fig. 1). L* a* b* color coordinates were used for all measurements. A standard white plate (Calibration plate CR-A43 : Y=93.3, x=0.3146, y=0.3217) was employed to standardize the instrument.

## 3. Results and Discussion

### 3.1. Determination of berberine by HPLC analysis

There are distinct differences on the bark morphology in the two varieties. The



(a) (b)

Fig. 1 Outer and inner bark views of two varieties of *Phellodendron amurense* Rupr. (a), *P. amurense* Rupr. var. *lavallei* Sprague (miyama kihada, coded as MK) sampled in Hokkaido ; (b), *P. amurense* Rupr. var. *sachalinense* Fr. Schm. (hirohano kihada, coded as HK) sampled in Miyazaki prefecture. Bar=3 cm.

outer bark of MK is thick, deeply furrowed with a gray color. In contrast, HK outer bark is thin, sub-fibrous, and tessellated with a light brown color. The yellowish inner bark which contains berberine is thick in MK and thin in HK (Fig. 1).

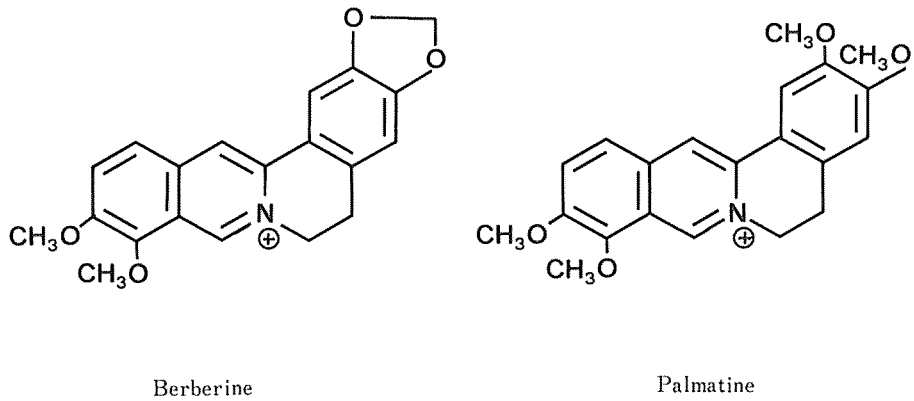
Figure 2 shows the structures of berberine and palmatine. Figure 3 shows the HPLC profiles of authentic berberine and methanol extracts of two varieties of *P. amurense* Rupr. inner bark. The retention time of berberine was apporoximately 9.9 min under the conditions in this experiment. It was clear that berberine was the major alkaloid contained in both varieties. On the other hand, palmatine was detected only in the extract of MK as minor alkaloid.

### 3.2. Variety variation

The data given in Fig. 4 show that berberine content in the inner bark was quite different depending on variety of *P. amurense* Rupr.. The berberine content in HK was higher about three to four-fold than that of MK.

### 3.3. Stem position variation

Figure 4 shows the changes of berberine content at different stem heights in each



Berberine Palmatine

Fig. 2 Structures of berberine and palmatine as the major components of the hot methanol extract of *Phellodendron amurense* Rupr. inner bark.

tree. The higher content was observed in the stem base, then gradually decreased from the stem base to the tree top in two varieties. The highest content was 8.3 % in the stem base of HK. The reasons why these phenomena occurrence are not clear. It may be suggested that the biosynthesizing and/or accumulating part of berberine or metabolism of berberine are different from stem position.

### 3.4. Seasonal variation

Figure 4 shows the seasonal variation of berberine content in two varieties. Higher content was detected in winter samples at all stem positions of two varieties. This phenomenon may be due to higher level accumulation of berberine in winter. It is known that the extractives-content in spruce increases to be a maximum in winter, and decreases during spring to be a minimum in early summer (Dahm, 1970). From these results it is suggested that winter is the most suitable season for berberine production in high yields.

### 3.5. Tree age variation

Figure 5 shows the differences in berberine content in two varieties with respect to different age. It shows clearly that the older tree contains a larger amount of berberine than the younger tree. In other word, accumulation of berberine is promoted in the old tree rather than younger tree.

### 3.6. Relationship between berberine content and surface color (b* value : yellowish degree) of inner bark

As mentioned above (paragraph 3.2), the berberine content of MK was lower

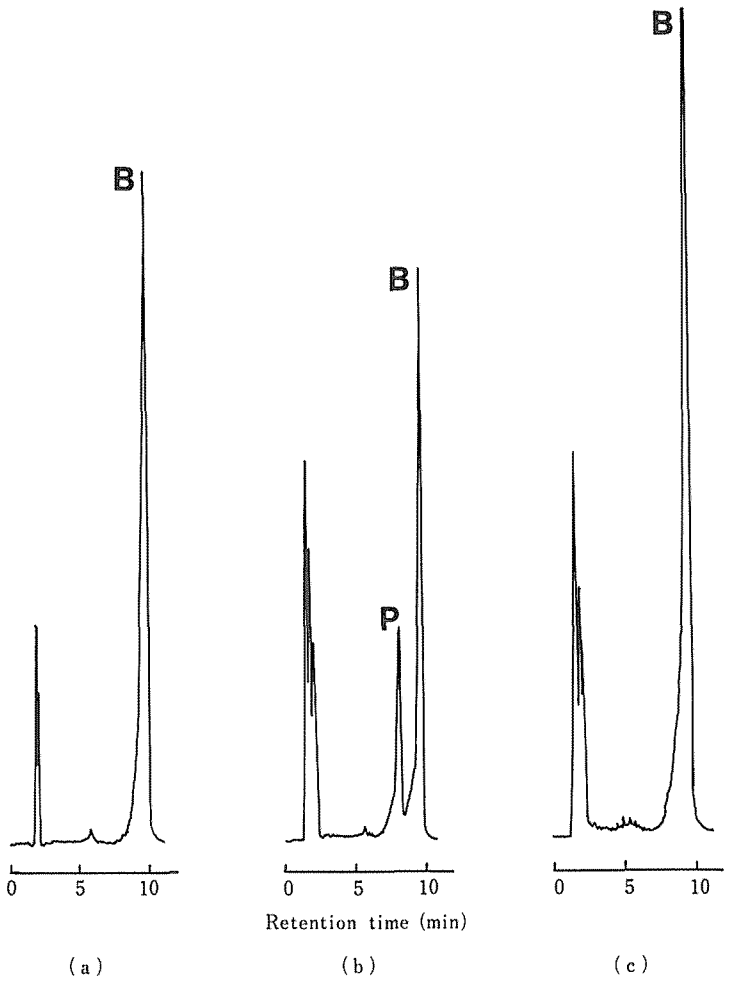



Fig. 3 High-performance liquid chromatography profiles of authentic berberine and hot methanol extracts of two *Phellodendron amurense* Rupr. varieties. (a), authentic berberine ; (b), *P. amurense* Rupr. var. *lavallei* Sprague (miyama kihada, coded as MK) ; (c), *P. amurense* Rupr. var. *sachalinense* Fr. Schm. (hirohano kihada, coded as HK). B, berberine ; P, palmatine. Apparatus : Waters high-performance liquid chromatography Model 208D ; Conditions, L-column ODS (4.6 mm i.d., 150 mm) 25°C ; eluent, acetonitrile : 10 mM sodium dihydrogenphosphate+100 mM sodium perchlorate=4 : 6 ; flow rate, 1.0 ml/min ; detector, 340 nm, 0.1 AUFS ; sample vol., 20 $\mu$l.

compared with HK. However, the b* value (yellowish degree) of the inner bark surface in MK was higher than that of HK (Fig. 6). This indicates that the surface yellowish degree of inner bark is not directly attributable to the berberine content. Some other color compound like flavonoid may affect for the depth of inner bark color.

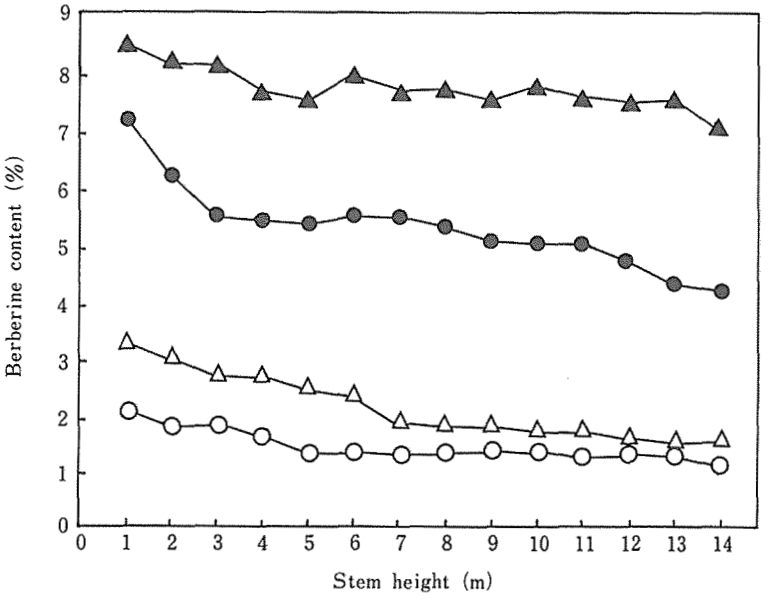



Fig. 4 Berberine content of individual stem heights of two varieties felled in summer and winter. Open circle, July ; open triangle, December (*P. amurense* Rupr. var. *lavallei* Sprague, miyama kihada, coded as MK) ; filled circle, August ; and filled triangle, November ( *P. amurense* Rupr. var. *sachalinense* Fr. Schm., hirohano kihada, coded as HK).

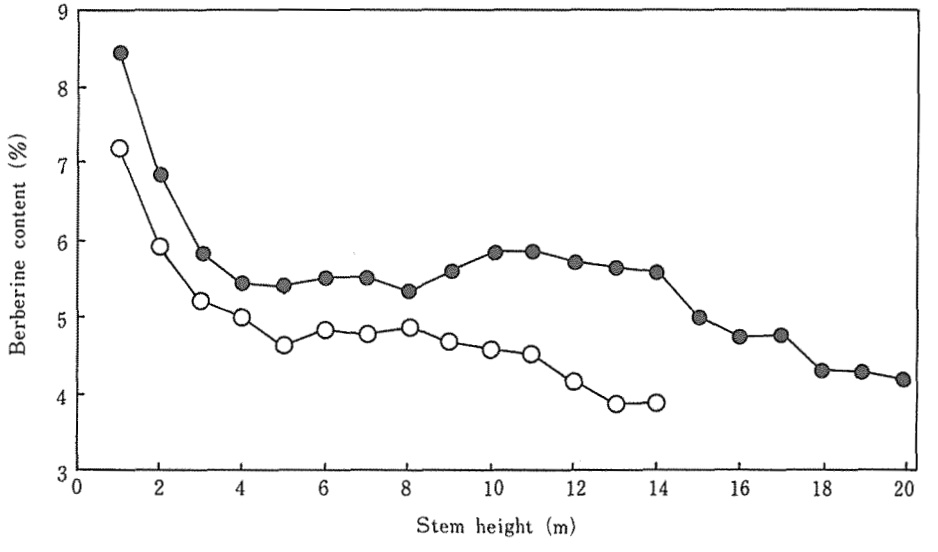



Fig. 5 Berberine content of individual stem heights of *P. amurense* Rupr. var. *sachalinense* Fr. Schm. (hirohano kihada, coded as HK) with different tree age. Open circle, 19 cm in DBH ; and filled circle, 30 cm in DBH.

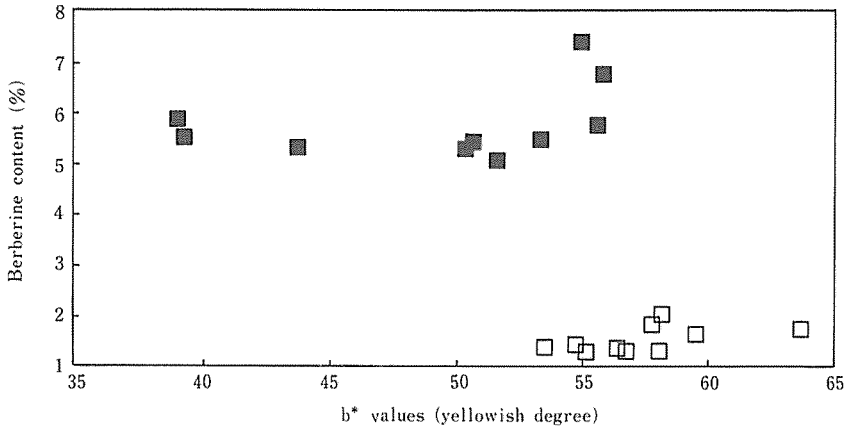



Fig. 6 Relationship between b* values and berberine content of *P. amurense* Rupr. inner bark. Open square, *P. amurense* Rupr. var. *lavallei* Sprague (miyama kihada coded as MK) ; and filled square, *P. amurense* Rupr. var. *sachalinense* Fr. Schm. (hirohano kihada coded as HK).

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# キハダ内樹皮中のベルベリン含有量の変種間差異ならびに樹幹部位，伐倒時期，樹齢による変動

大賀祥治・吉良今朝芳・古賀信也

## 要　約

キハダ（*Phellodendron amurense* Rupr.）の2変種について内樹皮部に含有されるベルベリンを部位，伐倒時期，樹齢を主因子として定量的に検討した．分析には高速液体クロマトグラフィー法を用いた．2変種間でベルベリン含有率に大きな差異があることが明らかになり，宮崎産のヒロハノキハダの方が北海道産のミヤマキハダに比べ3-4倍ものベルベリンを含有することが示された．さらに，樹高毎の採取部位によりその含有率は大きく異なり，基部の方が特に多く2-5 mまでで急激に低下し，その後ほぼ一定値を示し梢端付近では含有率が低いことが分かった．また，冬期の方が夏期に比べベルベリン含有率が2変種とも多いことが示された．そして，樹齢が大きな因子で高齢木の方がベルベリン含有率が高い結果が得られた．また，内樹皮部表面の黄色味の度合とベルベリン含有率とは直接相関がみられないことが分かった．