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Quantitative analysis of massonianoside B in *Pinus* species using HPLC/PDA

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Abstract *Pinus* species are native to the Northern Hemisphere and some parts of the tropics to temperate regions in the Southern Hemisphere. They were used as food and medicine in prehistoric times. Massonianoside B is a compound found in pine trees and possesses antioxidant activity. In order to determine the presence and content of this compound in *Pinus* species, three different parts (needles, branches, and bark) of three *Pinus* species were extracted and investigated. High-performance liquid chromatography with a gradient elution system along with a reverse-phase INNO column with photodiode array detector was employed. Results showed that the branches of the three *Pinus* species had higher massonianoside B content (5.502 to 9.751 mg/g DW) than either the needles or bark. Furthermore, among the three species, *P. rigida* × *P. taeda* had the highest concentration of total massonianoside B (11.557 mg/g DW). These findings thus provide evidence of biological activity in *Pinus* species and establish a foundation for further research.

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Keywords High-performance liquid chromatography/Photodiode Array · Massonianoside B · *Pinus* species · Quantitative analysis

Introduction

Pinus species (Pinaceae), commonly found as tall and stout trees and rarely as shrubs, have needle-shaped evergreen foliage and contain resin in their tissues. They include about 100 species widely distributed throughout the Northern Hemisphere [1,2]. Various *Pinus* preparations have been conventionally used to treat various ailments, such as ptilosis, dermatitis, toothache, etc. [3]. The bark of different *Pinus* species has been widely used in the areas of nutrition, health and medicine for more than 2000 years [4,5]. In ancient times, *Pinus* bark was used to treat inflammatory conditions and skin disorders (mainly wounds and sores) and to prevent and cure scurvy. In addition, the Sami people in Northern Scandinavia used the inner bark of *Pinus* species as food [6-8]. *Pinus* bark extracts contain abundant phenolic compounds including catechin, epicatechin, taxifolin and phenolic acids. These compounds have received much attention because of their antimutagenic, anticarcinogenic, and high antioxidant properties [9]. Many studies on the pharmacological activities of *Pinus* species have demonstrated that *Pinus* resins, their extracts, and isolated compounds exhibited antioxidant, antiviral, analgesic, anti-inflammatory, cytotoxic, and antimicrobial activities [10-14]. Moreover, *Pinus* essential oils have been shown to exhibit critical biological activities, including antifungal, acaricidal, and antiplatelet activities [15,16]. *Pinus* essential oils are also commonly used in the cosmetic industry due to their fragrance [17].

Massonianoside B is a naturally occurring phenolic compound [18]. It has been described as one of the vital active ingredients of *Pinus* polyphenol, along with catechin-3-*O*-glucose, catechin, epicatechin, cedrusin, catechin-3-*O*-rutinoside, has massonianoside C [19]. Additionally, massonianoside B have been proved to possess antioxidant activity [20,21]. Nevertheless, quantitative

analyses of this compound in *Pinus* species are still scarce.

In this study, ethanol (EtOH) extracts of needles, branches, and bark of *Pinus* species were used for assessing massonianoside B quantity by high-performance liquid chromatography (HPLC) analysis.

Materials and Methods

Plant materials

The plant materials (needles, bark, and branches) collected from *Pinus densiflora* planted in 1964, *P. rigida* planted in 1959, and *P. rigida* × *P. taeda* planted in 1959 were provided by the Department of Forest Bioresources, National Institute of Forest Science, Suwon, Korea. The samples were collected in November 2021 and confirmed by the Korea National Arboretum, Korea.

Instruments and reagents

HPLC was performed on a Waters Alliance 2695 Separations Module, USA Quat with pump, autosampler, and 996 Photodiode Array (PDA) Detector, USA. HPLC-grade solvents water, methanol (MeOH), and EtOH were purchased from J. T. Baker (Radnor, PA, USA). Massonianoside B (Fig. 1) was provided by Dr. Hee Jeong Min, Kangwondo Forest Science Institute, Korea and confirmed by the Natural Product Institute of Science and Technology (www.nist.re.kr), Anseong, Korea.

Preparation of standard and sample solutions

Massonianoside B was dissolved in MeOH (1 mg/mL) to prepare a set of standard solutions. The calibration curve was designed by diluting the standard stock solution to the desired concentrations. The needles, branches, and bark of *P. densiflora*, *P. rigida*, and *P. rigida* × *P. taeda* were extracted three times in EtOH by reflux cooling at 75 °C for 3 h. The resulting extract was filtered and evaporated to produce a concentrated EtOH extract. A portion (30 mg) of each extract was dissolved in 1 mL of MeOH, filtered through a 0.45 μm filter, and analyzed by HPLC.

HPLC conditions

We quantitatively analyzed the *Pinus* genus in the reverse phase HPLC system using the INNO C18 column (25 cm × 4.6 mm, 5 μm). Water (A) and MeOH (B) were analyzed by gradient methods: 10 min 75% A, 30 min 53% A, 50 min 100% B, 55 min 100% B, 60 min 75% A, 70 min 75% A. The flow rate was 1 min/mL, the injection volume was 10 μL, and the wavelength was set to 280 nm.

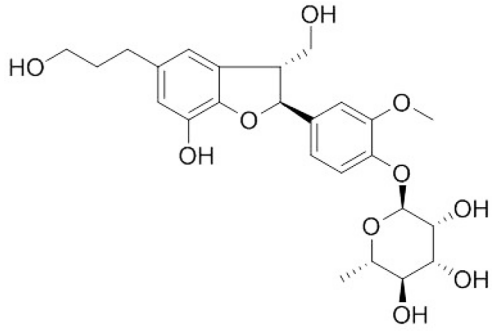

Fig. 1 Chemical structure of massonianoside B

Calibration curve

Five concentrations of standard compounds were prepared. First, the calibration curve was constructed by plotting the peak area of each solution against its corresponding concentration. Linearity was then determined based on the correlation coefficient (r^2). Next, the content of the analyte was measured on the corresponding calibration curve. Finally, the standard correction function was calculated using the peak area (Y) and concentration (X, mg/mL), and the mean ± standard deviation (n =3) (Table 1).

Results and Discussion

Phenolics are the products of secondary metabolism in plants, playing an important role in the reproduction and growth of plants [22-25]. In addition, phenolics contribute to improving human health. They are present in most fruits and vegetables such as cranberries, apples, red grapes, strawberries, broccoli, spinach, yellow onions, red peppers, carrots, and others. [26-28]. Epidemiological evidence has shown that phenolics perform crucial functions, including reducing the deposition of triglycerides, and reducing the incidence of cardiovascular disease, diabetes, cancer, stroke, and inflammation [29]. Massonianoside B (Fig. 1) is a phenolic glycoside, which has been identified as a disruptor of telomeric silencing1-like inhibitors with antileukemic activity [18]. This compound has also been isolated from two species belonging to the family Pinaceae, including the twigs and leaves of *Picea neoveitchii* and *Cedrus deodara* [21,30]. However, studies investigated on the content of massonianoside B in *Pinus* species are still limited.

This study investigated the massonianoside B content in needles, branches, and bark of three *Pinus* species using HPLC/PDA analysis. Good separations were detected in the HPLC

Table 1 The calibration curve for massonianoside B

Compound	t_R	Calibration equation [a]	Correlation factor, r^2 [b]
Massonianoside B	21.8	Y = 3479.6X – 36815	0.9992

[a]Y = peak area, X = concentration of standards (μg/mL)
[b]r^2 = correlation coefficient for five calibration data points (n =3)

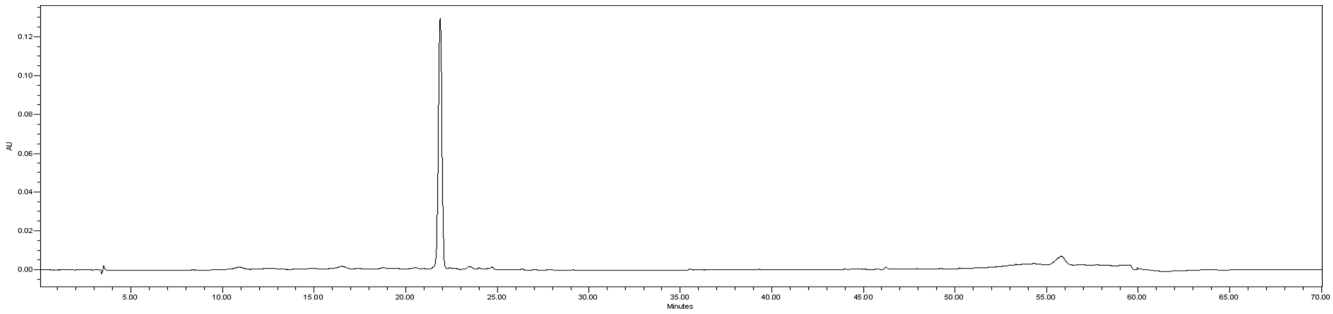

Fig. 2 HPLC chromatogram of massonianoside B

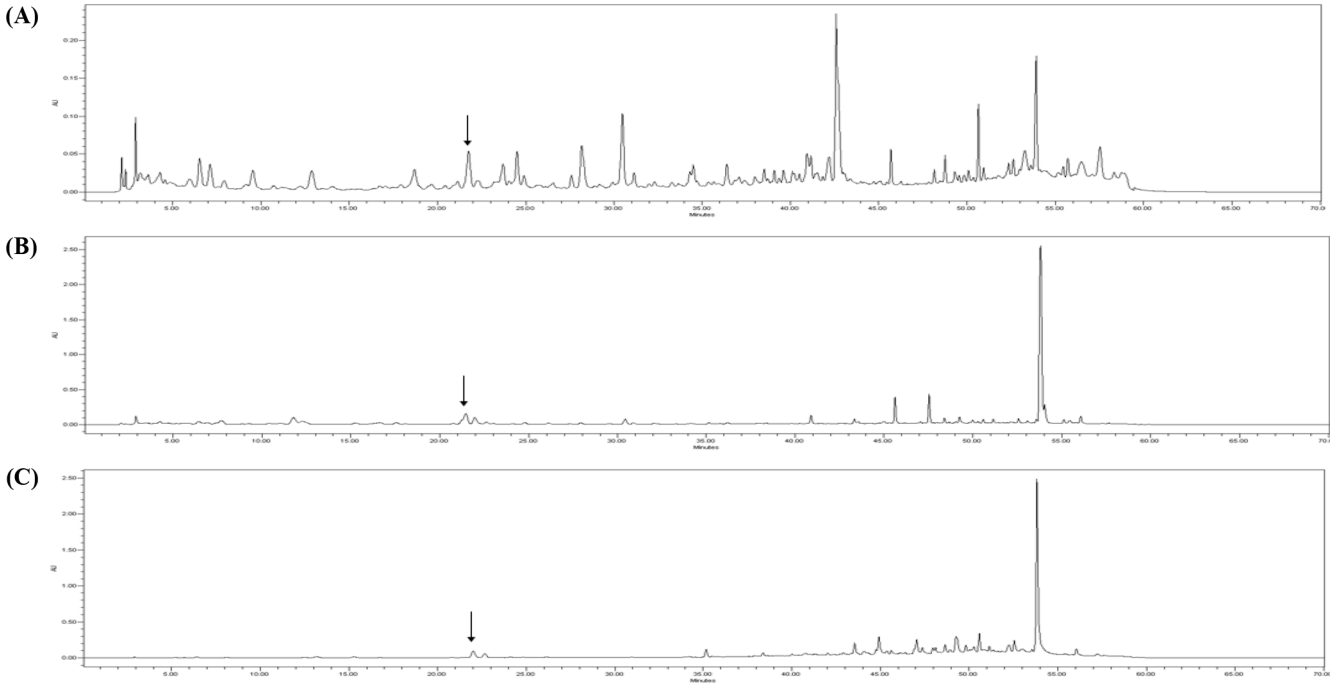

Fig. 3 HPLC chromatograms of needles (A), branches (B), and bark (C) of *P. densiflora*

chromatogram at the retention time of 21.80 min and experimented with the matrix spike samples. The HPLC conditions and results of massonianoside B quantification are shown in Fig. 2. The linear calibration curve equation was Y =3479.6X – 36815, where Y and X stand for a given peak area and the corresponding massonianoside B concentration, respectively. The correlation coefficient (r^2) was greater than 0.9992, illustrating good linearity of the analytical method (Table 1). The amount of massonianoside B in each sample was calculated using the calibration curve. The chromatographic separation of massonianoside B and the EtOH extract of needles, branches, and bark of three different *Pinus* species are shown in Figs. 2-5. The results of the quantitative analyses are summarized in Table 2.

Based on the calibration equation of the standard curve, the content of massonianoside B was determined (Table 2). Among the three parts (needles, branches, and bark), branches contained the highest amount of massonianoside B in all species (*P. densiflora*: 6.967 mg/g DW, *P. rigida*: 5.502 mg/g DW, and *P. rigida* × *P. taeda*: 9.751 mg/g DW). A large amount of massonianoside B was found in the needles of *P. rigida* (3.477 mg/g DW) than in the needles of either *P. densiflora* (1.317 mg/g DW) or *P. rigida* × *P. taeda* (0.927 mg/g DW). No massonianoside B was found in the bark of *P. rigida*, whereas concentrations of massonianoside B in the bark of *P. densiflora* (1.483 mg/g DW) and the bark of *P. rigida* × *P. taeda* (0.879 mg/g DW) were similar to concentrations of massonianoside B in the needles of these two species. Additionally, *P. rigida* × *P. taeda* showed the highest total massonianoside B content (11.557 mg/g DW), followed by *P. densiflora* (9.767 mg/g DW), and *P. rigida* (8.979 mg/g DW).

Few studies have been conducted on the massonianoside B content in *Pinus* species; however, one such study found a massonianoside B concentration of 129.7 mg/100 g DW in

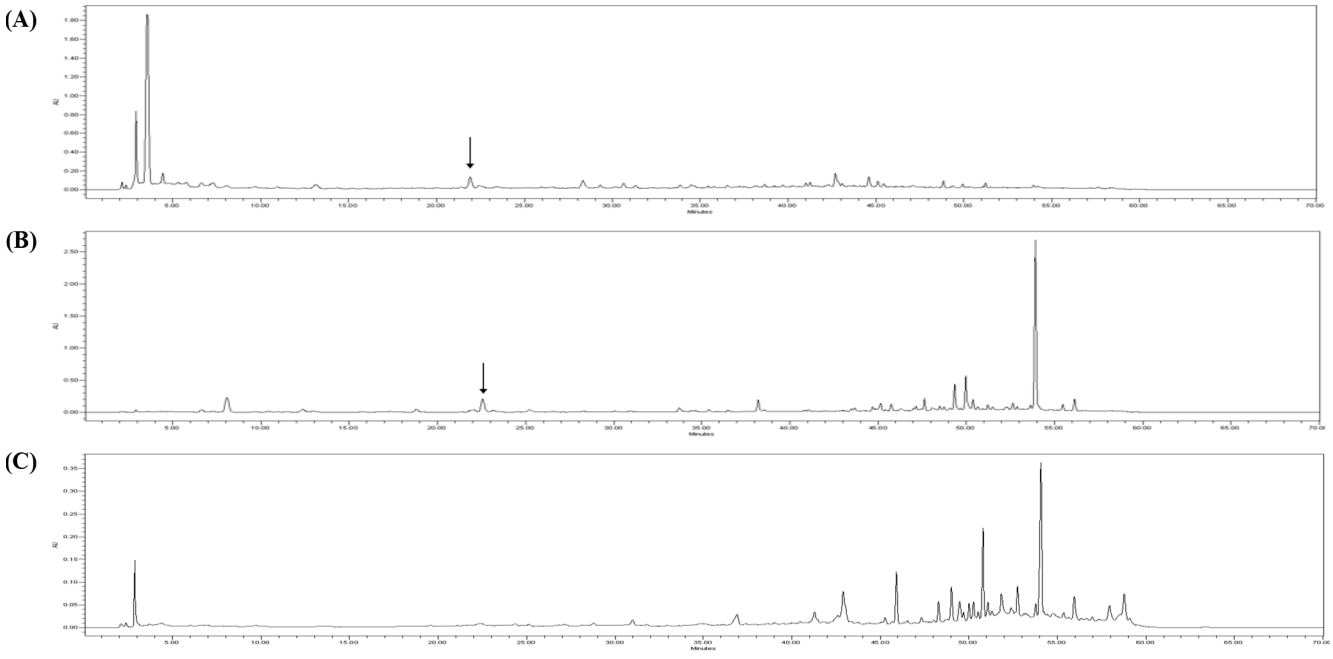

Fig. 4 HPLC chromatograms of needles (A), branches (B), and bark (C) of *P. rigida*

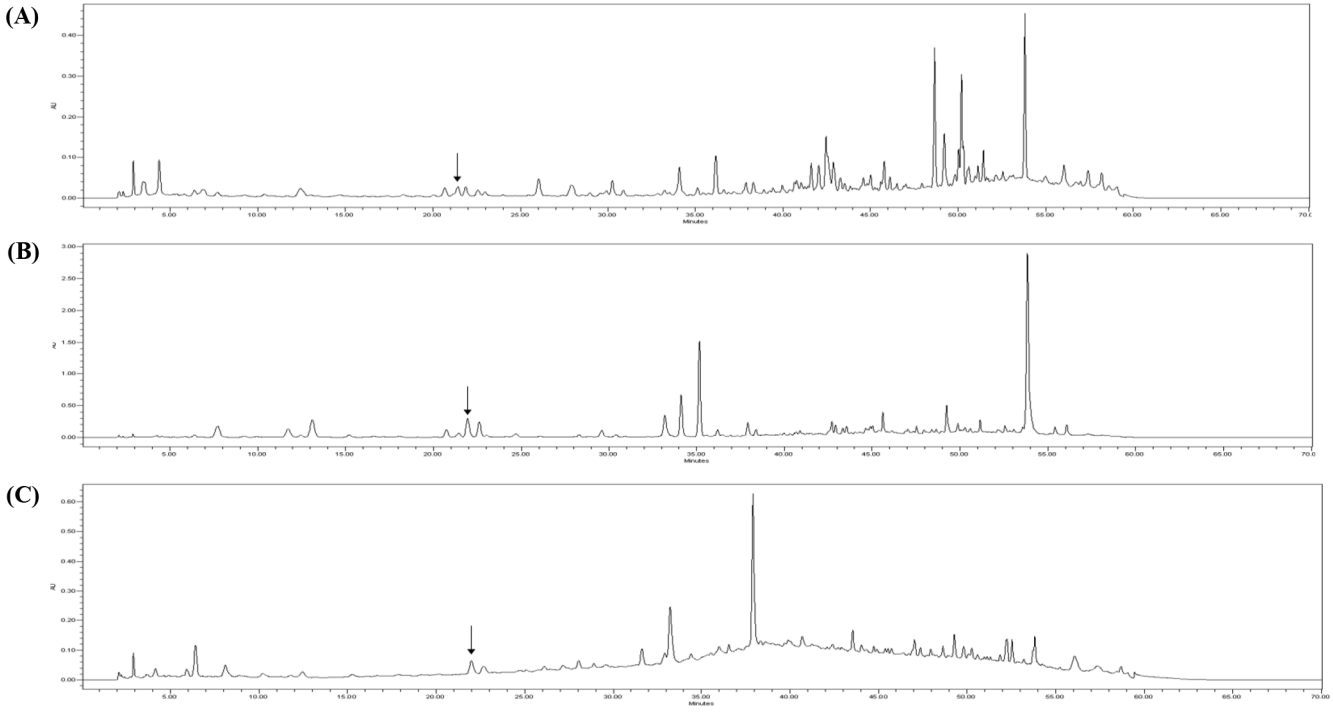

Fig. 5 HPLC chromatograms of needles (A), branches (B), and bark (C) of *P. rigida* × *P. taeda*

needles of *Cedrus deodara* [30]. This concentration is lower than that found in *P. densiflora* and *P. rigida* but higher than that found in *P. rigida* × *P. taeda* in the present study. Massonianoside B has been reported to have antioxidant properties [20], hence their presence in *Pinus* trees may affect the antioxidant capacity in this species. This is demonstrated by studies in which massonianoside B was separated and purified from the ethyl acetate fraction of *P. densiflora* extract and was shown to have the strongest antioxidant capacity among all constituents analyzed [31,32]. Therefore, it can be concluded that massonianoside B is a naturally occurring compound in *Pinus* species and contributes to the biological activity of those species, particularly antioxidant activity.

Table 2 Content of massonianoside B in EtOH extracts in different parts of three *Pinus* species

Sample	Content (mg/g DW)		
	P. densiflora	*P. rigida*	*P. rigida* × *P. taeda*
Needle	1.317±0.000	3.477±0.001	0.927±0.001
Branch	6.967±0.002	5.502±0.000	9.751±0.019
Bark	1.483±0.000	-	0.879±0.000
Total amount	9.767	8.979	11.557

In conclusion, this study investigated the massonianoside B content in three different parts (needles, branches, and bark) of three *Pinus* species using HPLC/PDA analysis. Results of this study demonstrated the presence of massonianoside B in all three *Pinus* species, with the highest concentration found in the branches of all samples examined. Additionally, the highest total massonianoside B content was found in *P. rigida* × *P. taeda.* These results provide the basis for further research on *Pinus* species and the potential use of their needles, branches, and bark in the preparation of herbal medicines.

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