Prev. Nutr. Food Sci. 2022;27(3):299-308
https://doi.org/10.3746/pnf.2022.27.3.299
ISSN 2287-8602

Quantitative Changes of Phenolic Compounds in Pine Twigs by Variety, Harvest Season, and Growing Region

Ju-Hee Kim, Jeong-Eun Seo, and Sang-Won Choi

Department of Food Science and Nutrition, Daegu Catholic University, Gyeongbuk 38430, Korea

ABSTRACT: Three new phenolic compounds including pinosylvin 3-methoxy-5-*O*-β-D-glucoside (PMG), taxiresinol 4'-*O*-α-L-rhamnoside (TRR), and lariciresinol 4'-*O*-α-L-rhamnoside (LRR) were first isolated and identified from red pine (*Pinus densiflora* Sieb. et Zucc.) twigs, together with four known compounds, such as (+)-catechin (CC), dihydromyricetin (DHM), dihydroquercetin 3-*O*-β-D-glucoside (DHQG), and dihydroquercetin (DHQ). Additionally, the concentrations of seven phenolic compounds in pine twigs were measured by high-performance liquid chromatography based on cultivars, harvest seasons, and growing environments. Red and black pine twigs contain 379.33 and 308.83 mg/100 g of PMG as the predominant phenolics, respectively, and their contents were significantly higher in spring than in autumn. Red pine twigs contain higher amounts of three dihydroflavonols (DHM: 87.82, DHQG: 38.47, and DHQ: 68.07 mg/100 g) and two lignans (LRR: 15.63, TRR: 30.72 mg/100 g) than black pine twigs, except for higher (+)-CC level (21.88 mg/100 g) in black pine twigs. Two pine twigs had much higher flavonoid and lignan levels in the autumn than they had in the spring. Two pine twigs harvested in several different areas do not significantly differ in their phenolic compositions and contents. These results suggest that red pine twigs possessing phytochemical phenolics may be useful as potential sources for promoting human health.

Keywords: identification, isolation, phenolic compounds, pine twigs, quantification

INTRODUCTION

A large class of naturally occurring plant secondary metabolites with a variety of biological and pharmacological effects are phenolic chemicals, including phenolic acids, cinnamic acids, flavonoids, stilbenes, lignans, and tannins (Pandey and Rizvi, 2009; Cheynier, 2012; Haminiuk et al., 2012). Recent studies have shown that plant polyphenols contributed to health benefits for the prevention of various chronic diseases, including cancer, diabetes, osteoporosis, cardiovascular, and neurodegenerative diseases (Vauzour et al., 2010; Watson et al., 2018). Thus, plant phenolic compounds are receiving much attention as promising dietary supplements for promoting human health.

Pine (*Pinus densiflora* Sieb. et Zucc., Pinaceae) trees have widely been used in traditional folk medicines in Korea for the treatment of hypertension, atherosclerosis, stroke, diabetes, cancer, balding, etc. (Im et al., 1996; Kim et al., 1997). There are numerous varieties of pine trees produced worldwide. In Korea, red pine (*P. densiflora*) and black pine (*Pinus thunbergii*) trees are the most common pine trees (Kong, 2004). In particular, the red Geumgang pine tree, which is widely grown in the Uljin province of Korea, is gaining great interest as a phytochemical source and superior wooden building material (Oh and Park, 2012; Song et al., 2016).

Stilbenes, flavonoids, proanthocyanidins, and lignans are among the numerous phenolic chemicals found in abundance in pine trees (Hovelstad et al., 2006; Li et al., 2008; Metsämuuronen and Sirén, 2019). In particular, pine needles contain large amounts of acylated flavonol glycosides and small amounts of stilbenes and lignans (Zhang et al., 2011; Metsämuuronen and Sirén, 2019; Jeon et al., 2021). These phenolics have been shown to have various biological properties such as antioxidant, antimicrobial, anti-obesity, antiaging, and hepatoprotective activities (Sharma et al., 2018; Karapandzova et al., 2019; Metsämuuronen and Sirén, 2019; Kim et al., 2021). Meanwhile, pine bark extracts (Pycnogenol) from French maritime pine (*Pinus pinaster*) possess the predominant procyanidins (condensed tannins) and several stilbenes,

Received 2 March 2022; Revised 6 June 2022; Accepted 21 June 2022; Published online 30 September 2022

Correspondence to Sang-Won Choi, E-mail: swchoi@cu.ac.kr
Author information: Ju-Hee Kim (Graduate Student), Jung-Eun Seo (Graduate Student), Sang-Won Choi (Professor)

catechins (CCs), and hydroxycinnamic acids (Rohdewald, 2002). Additionally, pine bark extracts (PineXol) from Korean red pine (*P. densiflora*) have significant amounts of procyanidin, (+)-CC, and taxifolin (Hwang et al., 2016). Pycnogenol and PineXol have been found to have potent antioxidant, antidiabetic, anticancer, antimicrobial, anti-inflammatory, antiaging, and neuroprotective activities (Rohdewald, 2002; D'Andrea, 2010; Kim et al., 2012; Ahn and Go, 2017; Kim and Choung, 2017; Kim et al., 2018). Therefore, PineXol and Pycnogenol are currently sold as health-promoting products in domestic and global markets. As a result, many studies on the phenolic analysis and biological activity of pine needles and barks have been conducted to date. However, few studies are available on the structural elucidation and quantitative analysis of phenolic compounds from pine twigs.

Every year, during the trimming and cutting of Geumgang pine trees, a substantial volume of red pine needles and twigs are obtained as industrial wastes and by-products. They represent potential sources of phytochemical polyphenols which can be dietary supplements used as nutraceutical ingredients. Therefore, it is required to conduct a phytochemical analysis of pine needles and twigs before using them as functional sources. In our previous report, several flavonol glycosides and acylated flavonol glycosides were first isolated and identified from Geumgang red pine needles (Jeon et al., 2021). Here, we discuss the separation, identification, and quantitative analysis of phenolic compounds from pine twigs as part of our ongoing search for phytochemical polyphenols from pine trees.

The objective of this study was to isolate and identify major phenolic compounds from Geumgang red pine twigs. This study performed quantitative analysis by high-performance liquid chromatography (HPLC) in relation to cultivars, harvest seasons, and growing regions.

MATERIALS AND METHODS

Materials and reagents

Geumgang pine (*P. densiflora* Sieb. At Zucc., red pine) from the lower branches of a single 15- to 18-year-old tree was harvested in the mountains in May (spring) and October (autumn) in 2020 at an altitude of 580 m, Uljin, Gyeongbuk, Korea. In addition, two other red pines in Yeongdeok and Cheongsong regions are also collected during two seasons compared to black pine. Additionally, Haesong pine (*P. thunbergii* Parlatore, black pine) was also harvested in May and October along the seashore of Sokcho, Samchuk, Uljin, Yeongdeok, and Pohang, Korea. In the Southern Regional Office of the Forest Service, National Institute of Forest Science (Uljin, Korea), two pine trees were identified by the president, and an authenticated voucher specimen was kept in the Department of Food Science and Nutrition, Daegu Catholic University. The twigs below 1-cm diameter were separated from two pine trees after removing pine needles and cut into small pieces by a mechanical cutter. The cut twigs were dried in the shade for 1 week and then finally dried in the dry oven at 45±5°C. Nuclear magnetic resonance (NMR) solvents were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All solvents for HPLC analysis were of Merck HPLC grade (Merck, Kenilworth, NJ, USA). All other reagents used in this study were of analytical grade.

Isolation and purification of phenolic compounds

Spring-harvested dried pine twigs (600 g) were extracted twice with 80% aqueous solution (aq). EtOH (6 L) at 40°C under an ultrasonic cleaner (Power Sonic HT-300 L, Hwashin Tech Co., Ltd., Seoul, Korea). Under reduced pressure, the ethanol extract was evaporated to yield a dark brown residue (47.34 g). The residue was suspended in H_2O (1.0 L), successively partitioned with petroleum ether (PE, 4.0 L), ethyl acetate (EtOAc, 8.0 L), and *n*-butanol (*n*-BuOH, 8.0 L), and then evaporated *in vacuo* to obtain corresponding PE (1.86 g), EtOAc (9.95 g), and *n*-BuOH (17.35 g) fractions, respectively. The EtOAc fraction was subjected to silica gel (70∼230 mesh, Merck, Damstadt, Germany) column (6 cm×60 cm) chromatography by gradient eluting with chloroform ($CHCl_3$)-methanol (MeOH) (7:1, 5:1, 3:1, and 1:1, v/v) to obtain five fractions (Fr): Fr. 1 (1.42 g), Fr. 2 (1.12 g), Fr. 3 (0.81 g), Fr. 4 (1.82 g), and Fr. 5 (0.65 g). Fr. 2 and Fr. 3 were separately chromatographed on a Sephadex LH-20 (Pharmacia Biotech, Uppsala, Sweden) column (2 cm×80 cm) with 90% aq. MeOH and yielded three subfractions and seven subfractions, respectively. The subfraction 3 (85 mg) from Fr. 2 and subfractions 2 (93 mg), 4 (114.2 mg), and 6 (24 mg) from Fr. 3 were separately chromatographed on a octadecyl silica (ODS)-A (YMC America Inc., Devens, MA, USA) column (3 cm×30 cm) with gradient from 40% aq. MeOH to 60% aq. MeOH. As a result, four phenolic compounds, including Comp. 1 [dihydroquercetin (DHQ), 21.7 mg], Comp. 2 [dihydroquercetin 3-*O*-β-D-glucoside (DHQG), 25.5 mg], Comp. 3 [dihydromyricetin (DHM), 45.4 mg], and Comp. 4 [(+)-CC, 5.2 mg], were isolated and purified from subfraction 3 and subfractions 2, 4, and 6, respectively. Meanwhile, the *n*-BuOH fraction was subjected to silica gel CC, with elution by a gradient of increasing MeOH concentration (5:1→3:1→1:1→1:3, v/v) in $CHCl_3$ to yield four fractions, namely, Fr. 1 (3.24 g), Fr. 2 (1.28 g), Fr. 3 (2.85 g), and Fr. 4 (2.2 g). Fr. 2 and Fr. 3 were also subjected to the same purification procedure using Sephadex LH-20 and ODS-A columns, as previously described in the EtOAc fraction. Three compounds, including Comp. 5 [taxiresinol 4'-*O*-α-L-rhamnoside (TRR), 17.1 mg], Comp. 6 [la-

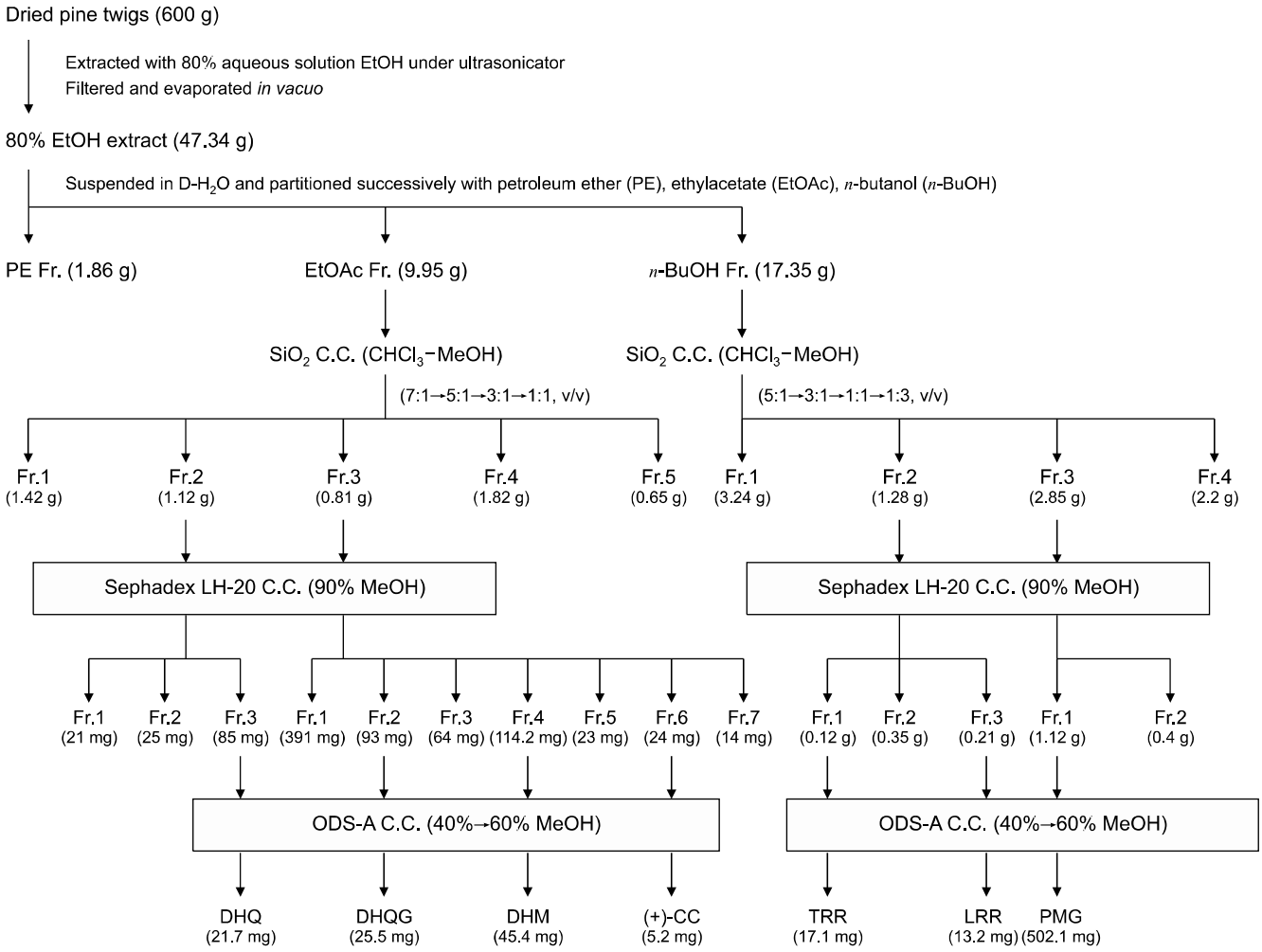

Fig. 1. Schematic procedure for isolation and purification of seven phenolic compounds from red pine twigs. PE, petroleum ether; Fr, fraction; C.C., column chromatography; ODS, octadecyl silica; DHQ, dihydroquercetin; DHQG, dihydroquercetin 3-*O*-β-D-glucoside; DHM, dihydromyricetin; CC, catechin; TRR, taxiresinol 4'-*O*-α-L-rhamnoside; LRR, lariciresinol 4-*O*-α-L-rhamnoside; PMG, pinosylvin 3-methoxy-5-*O*-β-D-glucoside.

riciresinol 4'-*O*-α-L-rhamnoside (LRR), 13.2 mg], and Comp. 7 [pinosylvin 3-methoxy-5-*O*-β-D-glucoside (PMG), 502.1 mg], were finally isolated and purified from the *n*-BuOH fraction. Fig. 1 shows a schematic procedure for isolation and purification of seven phenolic compounds from red pine twigs.

Identification of phenolic compounds

A photodiode array ultraviolet (UV)-visible spectrophotometer (S-1100, Scinco, Seoul, Korea) was used to get the UV absorption spectra of seven isolated phenolic compounds (in MeOH). ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of seven phenolics were recorded on a JEOL-500 spectrometer (JEOL Ltd., Tokyo, Japan) in CD_3OD and dimethyl sulfoxide (DMSO)-d_6. Tetramethylsilane was used as an internal standard, and chemical shifts were given as δ value (ppm). The fast atom bombardment mass spectrometry (FAB-MS) was measured on a JMS-700 mass spectrometer (ion source, Xe atom beam; accelerating voltage, 10 kV, JEOL Ltd.), using nitrobenzyl alcohol as a mounting matrix.

Quantification of phenolic compounds by HPLC

Dried pine twigs (10 g) were extracted twice with 150 mL of 80% aq. EtOH by an ultrasonic cleaner for 1 h, filtered, and evaporated under reduced pressure. The EtOH extract was further redissolved in 100 mL of 80% aq. EtOH and left to stand overnight at room temperature. With the same solvent, the upper layer was collected and diluted to 100 mL. The aliquot was passed through a 0.45 μm membrane filter (polyvinylidene fluoride syringe filter, Finetech Research and Innovation Corp., Taichung, Taiwan) and finally injected into an analytical HPLC. HPLC analysis was conducted on Waters e2690/5 HPLC system equipped with 2,998 photodiode array detector (Waters Corp., Milford, MA, USA) at 280, 290, and 320 nm and autosampler. HPLC analysis was carried out using a YMC-Pack Pro C_{18} column (46 mm i.d.×250 mm, YMC America Inc.) with a Guard-Pak C_{18} precolumn (Waters Corp.) insert. The separation was conducted us-

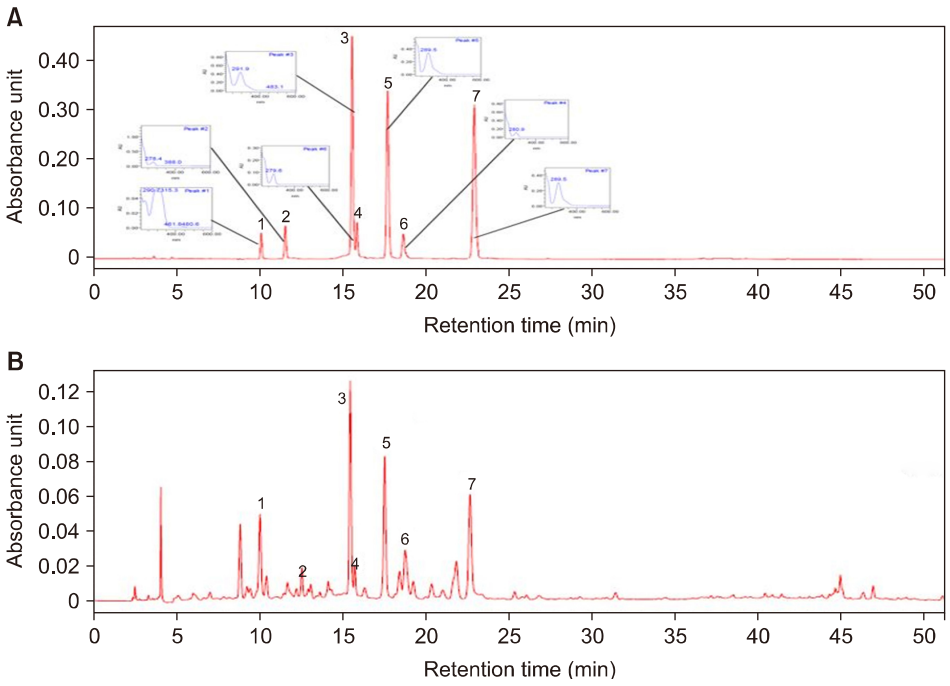

Fig. 2. High-performance liquid chromatography chromatograms of seven standard phenolic compounds (A) and the EtOH extract (B) from red pine twigs.

ing a linear gradient (0∼10 min: 10∼20% B, 10∼15 min: 20∼20% B, 15∼30 min: 20∼30% B, 30∼35 min: 30∼40% B, 35∼40 min: 40∼50% B, 40∼50 min: 50∼60% B, 50∼55 min: 60∼90% B, and 55∼60 min: 90∼10% B) of two solvent systems, namely, solvent A, 0.1% CH_3COOH in H_2O, and solvent B, CH_3CN at a flow rate of 0.8 mL/min. Each phenolic compound was identified by a comparison of its retention time (Rt) with those of the seven standard phenolics isolated previously. Linear correlation coefficients were superior to 0.996 for each phenolic. Levels of phenolic compounds were determined by calibration curves of seven standard phenolics [PMG: y=2460x+18200, (+)-CC: y=4020x+9860, DHM: y=29000x+1900, TRR: y=3740x+7020, DHQG: y=25700x+7270, LRR: y=3210x+4090, and DHQ: y=28100x–21000] and expressed as mg per 100 g of dried weight of pine twigs. Recovery rates of seven phenolics were above 95%. Fig. 2 shows the typical HPLC profiles of the seven standard phenolics and the 80% aq. EtOH extract from red pine twigs.

Statistical analysis

All data were expressed as the mean±standard deviation of three determinations, and their statistical analyses were performed using IBM SPSS Statistics 19.0 software (IBM Corp., Armonk, NY, USA). The significant difference (P<0.05) between the means was identified using one-way ANOVA followed by Duncan's multirange test.

RESULTS AND DISCUSSION

Isolation and identification of seven phenolic compounds from red pine twigs

A combination of solvent fractionation and various chromatographic methods was used to completely isolate seven phenolic components from the ethanol extract of red pine twigs. The structures of the seven phenolic compounds were identified by MS and NMR spectrometry and by comparison of published spectral data. Compounds 1, 2, and 4 were easily characterized as DHQ, DHQG, and (+)-CC, respectively, which have already been found as major abundant flavonoids in pine barks (Hwang et al., 2016; Metsämuuronen and Sirén, 2019). DHQ and (+)-CC were found in pine barks as precursors of procyanidin, which are well-known as Pycnogenol in French maritime pine and as PineXol in Korean red pine (Kim et al., 2012). In particular, DHQ, also known as taxifolin, is present in conifers, including pine, fir, yew, and spruce, and shows a wide range of pharmacological activities in the prevention of inflammation, cancer, skin hyperpigmentation, oxidative stress, and cardiovascular and liver disorders (Sunil and Xu, 2019). Four other phenolic compounds (Comp. 3 and Comp. 5∼7) firstly isolated from red pine twigs were characterized by NMR (1D-NMR and 2D-NMR) and MS spectrometry.

***Comp. 3 (DHM)*:** Comp. 3 showed a protonated molecule $[M+H]^+$ at m/z 321 in the positive FAB-MS. The ^{1}H-NMR spectrum of Comp. 3 exhibited the presence of two *meta*-coupled aromatic protons at δ 5.88 (H-6, d, J=2.0 Hz) and δ 5.91 (H-8, d, J=2.0 Hz) and an additional two *meta*-coupled aromatic protons at δ 6.54 (H-2' and H-6', s) corresponding to a pentahydroxy flavanonol. Addition-

ally, the ^{1}H-NMR spectrum showed two doublet signals with *trans*-configuration proton peak between δ 4.47 (H-3, d, *J*=11.5 Hz) and δ 4.84 (H-2, d, *J*=11.5 Hz) assignable to the 2,3-dihydroflavonol skeleton. The ^{13}C-NMR spectrum of Comp. 3 showed a typical 2,3-dihydroflavonol with A_2-type aromatic signals of benzene B ring: the presence of two *meta*-coupled aromatic carbon signals at 108.16 (C-2' and C-6') and oxymethine moiety between δ 73.75 (C-3) and δ 85.32 (C-2). From MS and NMR spectral analysis, Comp. 3 was characterized as DHM for the first time from pine trees produced in Korea, although DHM was previously isolated and identified from the bark of *Pinus contorta* (Outtrup et al., 1985). DHM was found to exert a variety of biological and pharmacological properties, including antidiabetes, antitumor, cardio-, hepato-, neuro-, and dermato-protective activities (Liu et al., 2019). Recently, DHM is receiving a renewed interest as a nutraceutical supplement to prevent alcohol hangovers (Shen et al., 2012). In Korea, the fruit stalk of *Hovenia dulcis* is known to have detoxification effects on alcohol poisoning and hepatoprotective activities and is reported to have DHM as a major principle for antihangover action (Hyun et al., 2010). Thus, red pine twigs may be used as potential sources for relieving alcohol-induced hangovers.

***Comp. 6 (LRR)*:** Comp. 6 showed a protonated molecule peak $[M+H]^+$ at *m/z* 507, together with a prominent fragment peak at *m/z* 361 [M^+-146(rhamnose)] and 137 by FAB-MS spectrometry, indicating the presence of rhamnose and 3-methoxy-4-hydroxy benzyl groups. ^{1}H-NMR spectrum of Comp. 6 showed a pair of ABX-type aromatic signals at δ 7.00 (1H, d, *J*=2.0 Hz, H-2), 6.86 (1H, d, *J*=2.0 Hz, H-6), and 6.73 (1H, s, H-5) and δ 7.05 (1H, d, *J*=8.5 Hz, H-2'), 6.87 (1H, d, *J*=2.0 Hz, H-6'), and 6.73 (1H, s, H-5'), as well as a typical furanoid skeleton connected with dibenzylbutane at δ 2.52 (H-7), δ 1.69 (H-8), and δ 3.42 (H-9) of benzene A ring and at δ 5.48 (H-7'), δ 2.50 (H-8'), and δ 3.72, 3.64 (H-9') of the benzene B ring. In addition, the ^{1}H-NMR spectrum showed one rhamnose moiety at δ 5.24 (H1"), 3.17∼3.84 (H2"∼H5"), 1.06 (H") possessing α-configuration from the coupling constant (*J*=1.5 Hz) of glycoside group, and two methoxyl moieties at δ 3.76 (3H, s) and 3.77 (3H, s). Meanwhile, ^{13}C-NMR of Comp. 6 showed dibenzylbutane-type lignan with furanoid moiety, namely, two 1,3,4-trisubstituted benzene rings at δ 110.55 (C-2), 116.45 (C-5), and 117.84 (C-6) and δ 117.96 (C-2'), 112.50 (C-5'), and 117.84 (C-6') and furanoid ring connected with two butyl groups at δ 31.29 (C-7), 34.69 (C-8), and 60.18 (C-9) and δ 86.48 (C-7'), 53.43 (C-8'), and 63.13 (C-9'). Additionally, a rhamnose moiety at 99.65 (C-1''), 70.25 (C-2"), 70.40 (C-3"), 71.73 (C-4"), 69.57 (C-5"), and 17.83 (C-6") and two methoxyl groups at δ 55.69 and 56.02 were also assigned from ^{13}C-NMR spectrometry. Thus, ^{1}H-NMR and ^{13}C-NMR of Comp. 6 showed lariciresinol rhamnoside, one of the typical furanoid lignan glycosides found in *Pinus* species (Karonen et al., 2004). Furthermore, the heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond correlation (HMBC), and correlated spectroscopy (COSY) experiments permitted the full assignment of proton and carbon signals of lariciresinol rhamnoside. In the HMQC spectrum, cross-peaks of two 1,3,4-trisubstituted benzene signals [H-2/C-2(H-2'/C-2'), H-5/C-5(H-5'/C-5'), H-6/C-6 (H-6'/C-6')], furanoid dibenzylbutane signals [H-7/C-7 (H-7'/C-7'), H-8/C-8(H-8'/C-8'), H-9/C-9(H-9'/C-9')], and rhamnose moiety signals [(H-1"/C-1"), (H-2"/C-2"), (H-3"/C-3"), (H-4"/C-4"), (H-5"/C-5"), (H-6"/C-6")] confirmed a furanoid dibenzylbutane-type lignan structure of Comp. 6. In the HMBC spectrum, correlations between H-8 and C-1 (δ 135.19), C-7 (δ 31.29), and C-9 (δ 60.18), between H-8' and C-1' (δ 128.74), C-7' (δ 86.48), and C-9' (δ 63.13), between H-7 and C-1 (δ 135.19), C-2 (δ 110.55), and C-6 (δ 117.84), and between H-7' and C-2' (δ 117.96), C-5' (δ 112.50), and C-6' (δ 117.84) established a typical furanoid skeleton connected with dibenzylbutane. Moreover, the cross-peaks between one methoxyl moiety and C-3 (δ 150.03), between another methoxyl moiety and C-3' (δ 143.35), and between anomeric H-1" and C-4' (δ 145.46) confirmed each methoxyl group at C-3 and C-3' positions and rhamnose moiety at C-4' position in the molecule. Finally, the proton and carbon signals at H-8' of Comp. 6 in CD_3OD showed an obvious downfield shift (δH 2.50→3.46; δC 53.43→55.70) at H-8' position when compared with the proton and carbon peaks in DMSO, indicating that primary alcohol (–CH_2OH) residue is located at the C-8' position. Based on the spectral data, Comp. 6 was elucidated as lariciresinol 4'-α-L-rhamnopyranoside.

***Comp. 5 (TRR)*:** Comp. 5 exhibited a sodium cationized molecule ion $[M+Na]^+$ at *m/z* 515, together with two significant ions at *m/z* 347 [M^+-146(rhamnose)] and 137 by FAB-MS spectrometry, indicating the presence of rhamnose and 3-methoxy-4-hydroxy benzyl groups. The ^{1}H-NMR spectrum of Comp. 5 is quite similar to Comp. 6, except for the absence of another methoxyl group. In the ^{1}H-NMR and ^{13}C-NMR spectra of Comp. 5, one methoxyl signal showed at δ_H 3.76 and δ_C 55.77. The HMBC correlations of H-2 to C-3 (δ 149.99), C-4 (δ 144.58), and C-5 (δ 115.76) indicated that a methoxyl group was attached at C-3 of ABX-type benzene A ring, while methoxyl proton was connected to C-3 (δ 149.99). HMBC cross-peaks of anomeric H-1" to C-4' (δ 144.70) confirmed the presence of a rhamnose moiety at the C-4' position. Judging from the respective chemical shifts of C-4 (δ 144.58) and C-3' (δ 140.68), a hydroxyl group was attached at C-4 and C-3'. From the above spectral data, Comp. 5 was determined to be taxiresinol 4'-*O*-α-L-rhamnopyranoside.

Table 1. ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectral data of four phenolic compounds isolated from red pine twigs

Position	PMG	TRR	LRR	DHM
^{1}H-NMR				
H-2	6.99 (1H, d, *J*=2.0 Hz)	7.02 (1H, d, *J*=2.0 Hz)	7.00 (1H, d, *J*=2.0 Hz)	4.84 (1H, d, *J*=11.5 Hz)
H-3				4.47 (1H, d, *J*=11.5 Hz)
H-4	6.98 (1H, t, *J*=2.0 Hz)			
H-5		6.49 (1H, d, *J*=2.0 Hz)	6.73 (1H, brs)	
H-6	6.99 (1H, d, *J*=2.0 Hz)	6.88 (1H, d, *J*=2.0 Hz)	6.86 (1H, d, *J*=2.0 Hz)	5.88 (1H, d, *J*=2.0 Hz)
H-7		2.45 (1H, t, *J*=7.8 Hz)	2.52 (1H, m)	
H-8		1.64 (1H, m)	1.69 (1H, m)	5.91 (1H, d, *J*=2.0 Hz)
H-9		3.40 (1H, m)	3.42 (1H, m)	
H-2′	7.38 (1H, d, *J*=8.5 Hz)	7.05 (H, d, *J*=2.0 Hz)	7.05 (1H, d, *J*=8.5 Hz)	6.54 (1H, s)
H-3′	7.05 (1H, d, *J*=8.5 Hz)			
H-4′	7.09 (1H, d, *J*=1.5 Hz)			
H-5′	7.05 (1H, d, *J*=8.5 Hz)	6.53 (1H, s)	6.73 (1H, brs)	
H-6′	7.38 (1H, d, *J*=8.5 Hz)	6.90 (1H, d, *J*=2.0 Hz)	6.87 (1H, d, *J*=2.0 Hz)	6.54 (1H, s)
H-7′		5.45 (1H, d, *J*=6.5 Hz)	5.48 (1H, d, *J*=6.5 Hz)	
H-8′		2.50 (1H, m)→3.96 m	2.50 (1H, brs)→3.46 m	
H-9′		3.70 (1H, m), 3.65 (1H, m)	3.72 (1H, m), 3.64 (1H, m)	
H-α	6.31 (1H, d, *J*=16.0 Hz)			
H-β	7.28 (1H, d, *J*=16.0 Hz)			
Hexose	Glucose	Rhamnose	Rhamnose	
H-1	4.84 (1H, d, *J*=7.5 Hz)	5.24 (1H, d, *J*=1.5 Hz)	5.24 (1H, d, *J*=1.5 Hz)	
H-2～H5	3.20～3.67	3.17～3.84	3.17～3.84	
H6	3.79	1.10 (3H, d, *J*=6.5 Hz)	1.06 (3H, d, *J*=6.5 Hz)	
OCH_3	3.81 (3H, s)	3.76 (3H, s)	3.76 (3H, s)	
OCH_3			3.77 (3H, s)	
^{13}C-NMR				
1	141.55	134.92	135.19	
2	101.21	110.59	110.55	85.32
3	151.09	149.99	150.03	73.75
4	105.32	144.58	144.78	198.33
5	149.13	115.76	116.45	165.35
6	109.21	117.82	117.84	97.43
7		31.27	31.29	168.82
8		34.66	34.69	96.40
9		63.24	60.18	164.50
10				101.87
1′	132.28	128.71	128.74	129.19
2′	112.27	118.04	117.96	108.16
3′	122.60	140.68	143.35	146.93
4′	117.91	144.70	145.46	134.98
5′	122.60	114.85	112.50	146.93
6′	112.27	117.82	117.84	108.16
7′		86.11	86.48	
8′		53.69→56.06	53.43→55.70	
9′		60.18	63.13	
Cα	128.10			
Cβ	129.75			
Hex-1	Glu-1 (102.61)	Rha-1 (99.66)	Rha-1 (99.65)	
2	75.02	70.24	70.25	
3	78.39	70.39	70.40	
4	71.46	71.73	71.73	
5	78.00	69.56	69.57	
6	62.60	17.82	17.83	
OCH_3	56.86	55.77	55.69	
OCH_3			56.02	
FAB-MS	389 [M+H]	515 [M+Na]	507 [M+H]	321 [M+H]

Chemical shift in δ ppm, coupling constant (*J*) expressed in Hz in parenthesis and measured in the solvent CD_3OD (PMG and DHM) and dimethyl sulfoxide (TRR and LRR), taking tetramethylsilane as an internal standard. Fast atom bombardment mass spectrometry (FAB-MS) spectra were determined by nitrobenzyl alcohol as a matrix.
PMG, pinosylvin 3-methoxy-5-*O*-β-D-glucoside; TRR, taxiresinol 4′-*O*-α-L-rhamnoside; LRR, lariciresinol 4′-*O*-α-L-rhamnoside; DHM, dihydromyricetin.

Two lignan glycosides, namely, Comp. 5 and Comp. 6, are being reported for the first time from *Pinus* species, although its derivatives have already been isolated from several conifers, including spruce, pine, and *Taxus* (Willför et al., 2003; Erdemoglu et al., 2004; Karonen et al., 2004). Recently, two lignan derivatives have been found to have antioxidant, antimicrobial, antiulcerogenic, anti-influenza, and hepato- and neuroprotective activities (Nguyen et al., 2004; Välimaa et al., 2007; Metsämuuronen and Sirén, 2019). However, information on the screening of lignan from Korean pine trees and its biological activity is still unknown.

***Comp. 7 (PMG)*:** Comp. 7 showed a protonated molecule $[M+H]^+$ at *m/z* 389 in the positive FAB-MS spectrum, together with two fragment ion peaks at *m/z* 359 $[M^+-30]$ and at *m/z* 227 $[M^+-162]$, indicating the presence of pinosylvin skeleton substituted with methoxyl and hexosyl moieties. The ^{1}H-NMR of Comp. 7 showed a pinosylvin unit attached with methoxyl and hexosyl groups: A_3-type aromatic protons (A ring) at δ 6.98 ppm (1H, t, *J*=2.0 Hz, H-4) and 6.99 ppm (1H, d, *J*=2.0 Hz, H-2 and H-6) and A_5-type aromatic protons (B ring) at δ 7.38 ppm (1H, d, *J*=8.5 Hz, H-2' and H-6'), 7.09 ppm (1H, d, *J*=1.5 Hz, H-4'), and 7.05 ppm (1H, dd, *J*=8.5 Hz, H-3' and H-5') and methoxyl proton at δ 3.81 ppm (3H, s) and glucosyl proton at δ 4.84 ppm (H_1") and 3.20∼3.79 ppm (H_2"∼H_6"). The coupling constant (*J*=7.5) of anomeric proton (H_1") of glucose moiety showed β-configuration of glucose. The ^{13}C-NMR spectrum of Comp. 7 was very similar to that of pinosylvin (Ngo and Brown, 1998) attached to a methoxyl group at the C-3 position and a glucosyl group at the C-5 position, i.e., a downfield signal at the C-3 position (δ 151.09 ppm) and an upfield signal at C-5 (δ 149.13 ppm). Therefore, Comp. 7 was identified as pinosylvin 3-methoxy-5-*O*-β-D-glucoside for the first time from pine twigs, even though several pinosylvin derivatives, including pinosylvin monomethyl ether and resveratrol methylglucoside, have been identified from pine trees and other plants (Nyemba et al., 1995; Ngo and Brown, 1998; Simard et al., 2008). Pinosylvin is a natural phytoalexin stilbenoid that is synthesized in plants in response to UV light and fungal attacks (Hovelstad et al., 2006). Pinosylvin and its derivatives in *Pinus* species showed strong antioxidant, anticarcinogenic, antimicrobial, anti-inflammatory, and antiaging activities (Ioannidis et al., 2017). As a result, red pine twigs included phenolic compounds of the dihydroflavonol, pinosylvin, and lignan types, such as DHM, PMG, TRR, and LRR, whose derivatives have already been discovered in other plants to have hepatoprotective and hangover-preventing properties (Nguyen et al., 2004; Hyun et al., 2010; Dergachova et al., 2019). Thus, red pine twigs may be taken much attention as potential functional ingredients for relieving alcohol-induced hangover and hepatoxicity. Table 1 presents the detailed ^{1}H-NMR and ^{13}C-NMR spectral data of the four phenolic compounds. Fig. 3 shows the chemical structures of the seven phenolic compounds from red pine twigs.

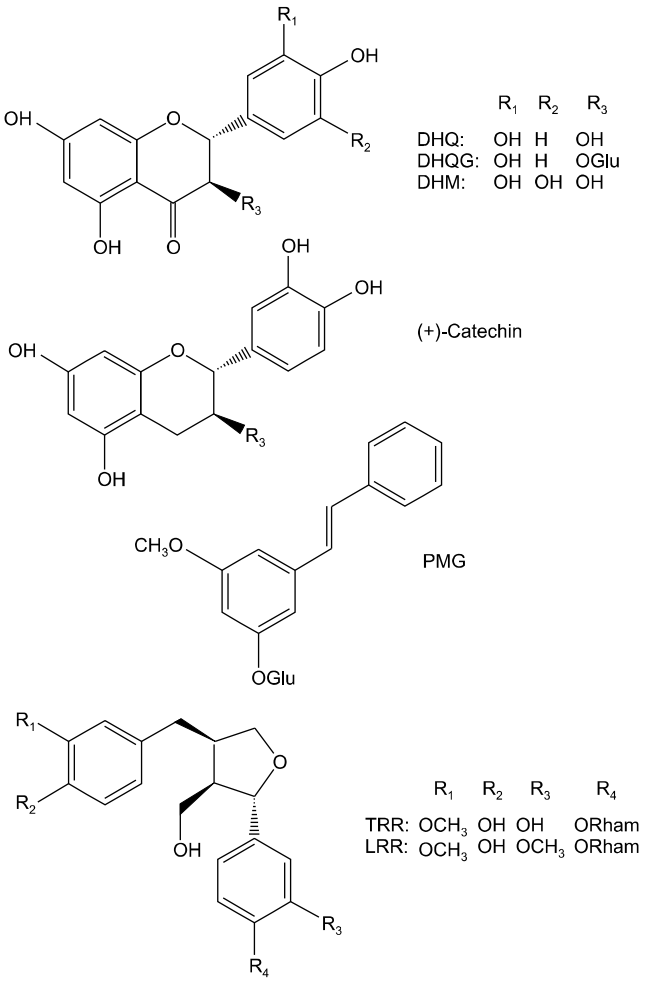

Fig. 3. Chemical structures of seven phenolic compounds isolated from red pine twigs. DHQ, dihydroquercetin; DHQG, dihydroquercetin 3-*O*-β-D-glucoside; DHM, dihydromyricetin; PMG, pinosylvin 3-methoxy-5-*O*-β-D-glucoside; TRR, taxiresinol 4'-*O*-α-L-rhamnoside; LRR, lariciresinol 4-*O*-α-L-rhamnoside.

Quantitative change of seven phenolic compounds by variety, harvest seasons, and growing regions

According to cultivars, harvest times, and growing areas, HPLC analysis was used to quantify the seven phenolic chemicals in red pine twigs. As shown in Fig. 2, the seven phenolic compounds were easily distinguished from the 80% aq. EtOH extract of red pine twigs by comparing the Rts of each standard phenolic compound that had been previously extracted. These Rts were as follows PMG (Rt: 10.2 min), CC (Rt: 11.6 min), DHM (Rt: 15.6 min), LRR (Rt: 15.9 min), DHQG (Rt: 17.7 min), TRR (Rt: 18.7 min), and DHQ (Rt: 22.9 min). The HPLC profiles of the Geumgang red pine twigs were similar to that of the Haesong black pine twigs (data not shown), but phenolic content varied considerably by the two pine cul-

Table 2. Quantitative changes of seven phenolic compounds of red pine and black pine twigs produced in Uljin according to harvest season (unit: mg/100 g, dry weight)

Harvest season (mon)	Phenolic compound						
	PMG	CC	LRR	TRR	DHM	DHQG	DHQ
Red pine							
Spring (May)	379.33±5.55^{a}	3.14±0.29^{d}	15.63±0.31^{b}	30.72±0.55^{b}	87.82±1.90^{b}	38.47±0.35^{c}	68.07±0.25^{b}
Autumn (October)	231.08±2.55^{d}	9.72±0.41^{c}	36.45±0.98^{a}	39.80±0.99^{a}	109.41±1.97^{a}	96.93±0.94^{a}	143.89±1.88^{a}
Black pine							
Spring (May)	308.83±3.22^{b}	21.88±0.78^{b}	6.20±0.76^{d}	12.05±0.26^{d}	4.46±0.15^{d}	25.98±0.29^{d}	15.47±0.30^{d}
Autumn (October)	257.21±2.32^{c}	46.13±1.98^{a}	9.63±0.32^{c}	23.35±0.21^{c}	16.27±0.42^{c}	76.13±1.20^{b}	22.21±0.25^{c}

Data are presented as mean±SD of triplicate determinations.
Values with different superscript letters (a-d) within a column indicate significant differences (P<0.05) by Duncan's multiple range test.
PMG, pinosylvin 3-methoxy-5-*O*-β-D-glucoside; CC, (+)-catechin; LRR, lariciresinol 4′-*O*-α-L-rhamnoside; TRR, taxiresinol-4′-*O*-α-L-rhamnoside; DHM, dihydromyricetin; DHQG, dihydroquercetin 3-*O*-β-D-glucoside DHQ, dihydroquercetin.

tivars. As presented in Table 2, the seven phenolic compounds of two pine twigs were determined at an early (spring) and late (autumn) vegetative growth period of the pine tree (Metsämuuronen and Sirén, 2019). The red pine twigs harvested in spring possessed higher amounts of PMG (379.33 mg/100 g), three dihydroflavonols (DHM: 87.82, DHQG: 38.47, DHQ: 68.07 mg/100 g), and two lignans (LRR: 15.63, TRR: 30.72 mg/100 g) than the black pine twigs. Meanwhile, the black pine twigs had higher amounts of (+)-CC (21.88 mg/100 g) than the red pine twigs. Additionally, the level of PMG, the main phenolic in both pine twigs, was higher in spring than in autumn, while levels of other phenolic compounds were higher in autumn than in spring. On the other hand, as presented in Table 3, phenolic levels of two pine twigs harvested in autumn varied considerably by growing regions. The red pine twigs, which were sampled in the Geumgang pine forest regions including Uljin, Cheongsong, and Yeongdeok of Gyeongbuk province in Korea, contained large amounts of PMG (209.09～268.18 mg/100 g) and dihydroflavonols (DHM: 104.23～109.41, DHQG: 84.50～109.02, DHQ: 120.02～143.89 mg/100 g) as the main phenolics but contained moderate and small amounts of two lignans (LRR: 14.81～36.45, TRR: 18.93～39.80 mg/100 g) and CC (9.72～15.97 mg/100 g), respectively. Meanwhile, the black pine twigs harvested from five different regions, including Sokcho, Samchuk, Uljin, Yeongdeok, and Pohang native to Haesong pine trees, contained considerable amounts of PMG (257.21～315.67 mg/100 g) and CC (27.56～46.13 mg/100 g) as the main phenolics except for DHQG but contained small amounts of two lignans (LRR: 4.92～9.63, TRR: 18.28～24.02 mg/100 g) and three dihydroflavonols (DHM: 2.75～25.16, DHQG: 25.54～103.81, DHQ: 20.75～38.29 mg/100 g), although their levels varied by growing districts. There are no big differences in phenolic composition and content among red and black pine twigs grown in different areas, respectively. Thus, pine twigs possessed

Table 3. Contents of seven phenolic compounds of red pine and black pine twigs produced in several different areas in autumn season (unit: mg/100 g, dry weight)

Growing area	Phenolic compound						
	PMG	CC	LRR	TRR	DHM	DHQG	DHQ
Red pine							
Uljin	231.08±2.55^{e}	9.72±0.41^{f}	36.45±0.98^{a}	39.80±0.99^{a}	109.41±1.97^{a}	96.93±0.94^{c}	143.89±1.88^{a}
Yeongduk	268.18±3.93^{c}	15.97±0.27^{e}	25.05±0.49^{b}	30.09±0.39^{b}	104.23±2.31^{b}	109.02±2.18^{a}	130.73±2.48^{b}
Cheongsong	209.09±2.78^{f}	10.87±0.25^{f}	34.81±0.32^{a}	18.93±0.28^{e}	106.15±2.21^{b}	84.50±1.12^{d}	120.02±1.51^{c}
Black pine							
Uljin	257.21±2.32^{d}	46.13±1.98^{b}	9.63±0.32^{c}	23.35±0.21^{c}	16.27±0.42^{d}	76.13±1.20^{e}	22.21±0.25^{g}
Sokcho	315.67±4.11^{a}	27.56±0.27^{d}	5.74±0.13^{d}	21.12±0.39^{d}	25.16±0.34^{c}	25.54±0.62^{g}	20.75±0.21^{g}
Samchuk	312.19±3.97^{a}	32.92±0.19^{c}	4.92±0.11^{d}	18.28±0.27^{e}	14.02±0.29^{e}	53.29±0.71^{f}	31.19±0.19^{f}
Yeongduk	289.05±3.58^{b}	54.93±1.82^{a}	8.02±0.21^{c}	21.72±0.26^{d}	24.29±0.17^{c}	97.49±2.02^{c}	33.02±0.28^{e}
Pohang	270.19±4.32^{c}	45.93±1.93^{b}	9.50±0.45^{c}	24.02±0.28^{c}	2.75±0.11^{f}	103.81±2.83^{b}	38.29±0.39^{d}

Data are presented as mean±SD of triplicate determinations.
Values with different superscript letters (a-g) within a column indicate significant differences (P<0.05) by Duncan's multiple range test.
PMG, pinosylvin 3-methoxy-5-*O*-β-D-glucoside; CC, (+)-catechin; LRR, lariciresinol 4′-*O*-α-L-rhamnoside; TRR, taxiresinol-4′-*O*-α-L-rhamnoside; DHM, dihydromyricetin; DHQG, dihydroquercetin 3-*O*-β-D-glucoside DHQ, dihydroquercetin.

stilbenes, dihydroflavonols, and lignans as major phenolics, and their compositions and contents varied greatly by cultivars, harvest seasons, and growing regions. These results were consistent with an earlier report that levels of phenolic compounds in pine species are affected by variety, harvesting time, and growing district (Metsämuuronen and Sirén, 2019). The concentrations of pine phenolics are also influenced by the wood parts, such as needles, branches, stem barks, and roots. Recent studies reported that the flavonol glycosides, as well as acetylated and acylated flavonol glycosides, were presented as major abundant flavonoids in red and black pine needles, respectively. On the other hand, stilbenes, dihydroflavonols (e.g., DHM), and lignans were scarcely not nearly detectable in two pine needles (Jeon et al., 2021) and pine barks (Hwang et al., 2016). In particular, two flavonol-type glycosides in pine needles, such as kaempferol and quercetin, showed the greatest content in spring and then decreased gradually during the vegetative growth period of pine trees, whereas the acetylated and acylated flavonol glycosides increased considerably by autumn and then decreased (Jeon et al., 2021). Moreover, it was found that pine barks contained large amounts of lignans like nortrachelogenin but small amounts of lariciresinol, matairesinol, and isoliovil (Willför et al., 2003; Pietarinen et al., 2006). In addition, some lignan glucosides, xylosides, and rhamnosides have been found in the bark of Scots pine, but other lignans, including lariciresinol and taxiresinol glycosides, have not been found in pine barks (Karonen et al., 2004). As a result, the primary phenolic profiles of pine twigs and needles harvested from the two well-known red and black pines growing in Korea varied significantly depending on the variety and harvest season. The presence of significant levels of pinosylvin-type stilbenes, dihydroflavonol-type flavonoids, and dibenzylbutane-type lignans with alcohol-decomposing and hepatic detoxifying activities was found in red pine twigs in addition to red pine needles and barks, which is important to note (Nguyen et al., 2004; Hyun et al., 2010; Dergachova et al., 2019). Thus, pine twig extract is receiving renewed interest as a potential source for the prevention of alcohol-induced hepatoxicity and hangover.

In conclusion, the seven phenolic compounds from Geumgang red pine twigs were first isolated and identified. The structure of seven phenolic compounds has been determined by using extensive two-dimensional NMR and MS methods, and their quantitative analysis has been undertaken by HPLC. Their composition and content were considerably influenced by cultivars, harvest times, and growing regions. In particular, the red pine twigs were found to contain three major phenolic compounds, including pinosylvin, dihydroflavonol, and lignan, whose derivatives are known to have alcohol-decomposing and hepatic detoxicating activities. As a result, pine twigs collected as industrial wastes or as a by-product of the trimming and cutting of pine trees are a sustainable and renewable source of valuable phenolic compounds. The phytochemical phenolic analysis of red pine twigs contributes significantly to establish standardization and evaluate the biological properties of Geumgang pine twig extracts. Further study on the effect of alleviating ethanol-induced hepatotoxicity and hangover of red pine twig extract and phenolic compounds will be presented soon.

ACKNOWLEDGEMENTS

The authors sincerely thank staff members in Southern Regional Office of Forest Service in Uljin for providing Geumgang pine trees.

FUNDING

This study was supported by a research fund from Daegu Catholic University.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

JHK and JES prepared tables and figures; all authors drafted manuscript; JHK and SWC approved final version of manuscript.

REFERENCES

Ahn H, Go GW. *Pinus densiflora* bark extract (PineXol) decreases adiposity in mice by down-regulation of hepatic *de novo* lipogenesis and adipogenesis in white adipose tissue. J Microbiol Biotechnol. 2017. 27:660-667.

Cheynier V. Phenolic compounds: from plants to foods. Phytochem Rev. 2012. 11:153-177.

D'Andrea G. Pycnogenol: A blend of procyanidins with multifaceted therapeutic applications?. Fitoterapia. 2010. 81:724-736.

Dergachova DI, Klein OI, Marinichev AA, Gessler NN, Bogdanova ES, Smirnova MS, et al. Hepatoprotective effect of polyphenols in rats with experimental thioacetamide-induced toxic liver pathology. Bull RSMU. 2019. 6:70-76.

Erdemoglu N, Sahin E, Sener B, Ide S. Structural and spectroscopic characteristics of two lignans from *Taxus baccata* L.. J Mol Struct. 2004. 692:57-62.

Haminiuk CWI, Maciel GM, Plata-Oviedo MSV, Peralta RM. Phenolic compounds in fruits—an overview. Int J Food Sci Technol. 2012. 47:2023-2044.

Hovelstad H, Leirset I, Oyaas K, Fiksdahl A. Screening analyses of pinosylvin stilbenes, resin acids and lignans in Norwegian

conifers. Molecules. 2006. 11:103-114.
Hwang YJ, Yin J, Le TT, Youn SH, Ahn HS, Kwon SH, et al. Quantitative analysis of taxifolin, (+)-catechin and procyanidin B1 from the preparation of *Pinus densiflora* (PineXol®). Korean J Pharmacogn. 2016. 47:246-250.
Hyun TK, Eom SH, Yu CY, Roitsch T. *Hovenia dulcis*—An Asian traditional herb. Planta Med. 2010. 76:943-949.
Im WK, Park SK, Ryu JW, Sa DM, Lee MS, Im KO. Plant resource. Seoil Publishing, Seoul, Korea. 1996. p 59-63.
Ioannidis K, Melliou E, Alizoti P, Magiatis P. Identification of black pine (*Pinus nigra* Arn.) heartwood as a rich source of bioactive stilbenes by qNMR. J Sci Food Agric. 2017. 97:1708-1716.
Jeon YH, Seo JE, Kim JH, Lee YJ, Choi SW. Quantitative changes of flavonol glycosides from pine needles by cultivar, harvest season, and thermal process. Prev Nutr Food Sci. 2021. 26:100-108.
Karapandzova M, Stefkov G, Karanfilova IC, Panovska TK, Stanoeva JP, Stefova M, et al. Chemical characterization and antioxidant activity of mountain pine (*Pinus mugo* Turra, Pinaceae) from Republic of Macedonia. Rec Nat Prod. 2019. 13:50-63.
Karonen M, Hämäläinen M, Nieminen R, Klika KD, Loponen J, Ovcharenko VV, et al. Phenolic extractives from the bark of *Pinus sylvestris* L. and their effects on inflammatory mediators nitric oxide and prostaglandin E_2. J Agric Food Chem. 2004. 52:7532-7540.
Kim CM, Shin MG, Ahn DG, Lee GS. The encyclopedia of oriental herbal medicine. Jungdam, Seoul, Korea. 1997. p 2488-2489.
Kim J, Choung SY. *Pinus densiflora* bark extract prevents selenite-induced cataract formation in the lens of Sprague Dawley rat pups. Mol Vis. 2017. 23:638-648.
Kim JW, Im S, Jeong HR, Jung YS, Lee I, Kim KJ, et al. Neuroprotective effects of Korean red pine (*Pinus densiflora*) bark extract and its phenolics. J Microbiol Biotechnol. 2018. 28:679-687.
Kim NH, Yoon S, Um BH, Kim JW. Bioactive compounds isolated from *Pinus densiflora* and *koraiensis* and their applications. Appl Chem Eng. 2021. 32:132-138.
Kim SM, Kang SW, Jeon JS, Um BH. A comparison of Pycnogenol® and bark extracts from *Pinus thunbergii* and *Pinus densiflora*: Extractability, antioxidant activity and proanthocyanidin composition. J Med Plants Res. 2012. 6:2839-2849.
Kong WS. Species composition and distribution of native Korean conifers. J Korean Geogr Soc. 2004. 39:528-543.
Li YL, Yang XW, Zhang WD. Stilbenes, lignans, and phenols from *Abies chensiensis*. Biochem Syst Ecol. 2008. 36:932-934.
Liu D, Mao Y, Ding L, Zeng XA. Dihydromyricetin: A review on identification and quantification methods, biological activities, chemical stability, metabolism and approaches to enhance its bioavailability. Trends Food Sci Technol. 2019. 91:586-597.
Metsämuuronen S, Sirén H. Bioactive phenolic compounds, metabolism and properties: a review on valuable chemical compounds in Scots pine and Norway spruce. Phytochem Rev. 2019. 18:623-664.
Ngo KS, Brown GD. Stilbenes, monoterpenes, diarylheptanoids, labdanes and chalcones from *Alpinia katsumadai*. Phytochemistry. 1998. 47:1117-1123.
Nguyen NT, Banskota AH, Tezuka Y, Le Tran Q, Nobukawa T, Kurashige Y, et al. Hepatoprotective effect of taxiresinol and (7'R)-7'-hydroxylariciresinol on D-galactosamine and lipopolysaccharide-induced liver injury in mice. Planta Med. 2004. 70:29-33.
Nyemba AM, Mpondo TN, Kimbu SF, Connolly JD. Stilbene glycosides from *Guibourtia tessmannii*. Phytochemistry. 1995. 39:895-898.
Oh N, Park JA. Users' characteristic in Uljin Kumgang pine (*Pinus densiflora* for. erecta) eco-forest (UKPEF). J KIFR. 2012. 16:141-152.
Outtrup H, Schaumburg K, Madsen JØ. Isolation of dihydromyricetin and dihydroquercetin from bark of *Pinus contorta*. Carlsberg Res Commun. 1985. 50:369. https://doi.org/10.1007/BF02907158
Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev. 2009. 2:270-278.
Pietarinen SP, Willför SM, Ahotupa MO, Hemming JE, Holmbom BR. Knotwood and bark extracts: strong antioxidants from waste materials. J Wood Sci. 2006. 52:436-444.
Rohdewald P. A review of the French maritime pine bark extract (Pycnogenol), a herbal medication with a diverse clinical pharmacology. Int J Clin Pharmacol Ther. 2002. 40:158-168.
Sharma A, Sharma L, Goyal R. A review on Himalayan pine species: ethnopharmacological, phytochemical and pharmacological aspects. Pharmacogn J. 2018. 10:611-619.
Shen Y, Lindemeyer AK, Gonzalez C, Shao XM, Spigelman I, Olsen RW, et al. Dihydromyricetin as a novel anti-alcohol intoxication medication. J Neurosci. 2012. 32:390-401.
Simard F, Legault J, Lavoie S, Mshvildadze V, Pichette A. Isolation and identification of cytotoxic compounds from the wood of *Pinus resinosa*. Phytother Res. 2008. 22:919-922.
Song WY, Kwon HA, Kim BR, Shin SJ. Hemicelluloses comparison between Korean red pine and Geumgang red pine. J Korea TAPPI. 2016. 48:86-91.
Sunil C, Xu B. An insight into the health-promoting effects of taxifolin (dihydroquercetin). Phytochemistry. 2019. 166:112066. https://doi.org/10.1016/j.phytochem.2019.112066
Välimaa AL, Honkalampi-Hämäläinen U, Pietarinen S, Willför S, Holmbom B, von Wright A. Antimicrobial and cytotoxic knotwood extracts and related pure compounds and their effects on food-associated microorganisms. Int J Food Microbiol. 2007. 115:235-243.
Vauzour D, Rodriguez-Mateos A, Corona G, Oruna-Concha MJ, Spencer JP. Polyphenols and human health: prevention of disease and mechanisms of action. Nutrients. 2010. 2:1106-1131.
Watson RR, Preedy VR, Zibadi S. Polyphenols: prevention and treatment of human disease. 2nd ed. Academic Press, London, UK. 2018. p 125-241.
Willför SM, Ahotupa MO, Hemming JE, Reunanen MH, Eklund PC, Sjöholm RE, et al. Antioxidant activity of knotwood extractives and phenolic compounds of selected tree species. J Agric Food Chem. 2003. 51:7600-7606.
Zhang JM, Shi XF, Ma QH, He FJ, Fan B, Wang DD, et al. Chemical constituents from pine needles of *Cedrus deodara*. Chem Nat Compd. 2011. 47:272. https://doi.org/10.1007/s10600-011-9901-9