J Food Sci Nutr
Vol 15, p 36~43 (2010)
DOI: 10.3746/jfn.2010.15.1.036

J Food Science and Nutrition

Phenolic Composition and Antioxidant Activities of Different Solvent Extracts from Pine Needles in *Pinus* Species

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Abstract

The purpose of this study was to investigate the phenolic acid, proanthocyanidin (PAs), and flavonol glycoside contents, as well as the antioxidant activities of pine needle extracts from six species of young pine trees. The extracts were prepared from Section *Pinus* (Diploxylon): *P. densiflora*, *P. sylvestris*, *P. pinaster* and *P. pinea*, and Section *Strobus* (Haploxylon): *P. koraiensis* and *P. strobus*. Phenolics were extracted from pine needles with 80% acetone to obtain the soluble free fraction, and insoluble residues were digested with 4 M NaOH to obtain bound ethyl acetate and bound water fractions. Phenolics were analyzed by HPLC, and the hydrophilic antioxidant activity was measured using oxygen radical absorbance capacity (ORAC). Total phenolic and flavonoid contents of the soluble free fraction were higher than those of the bound ethyl acetate and bound water fractions. The main phenolics were monomers and polymers of PAs in the soluble free fraction, and phenolic acids and flavonol glycosides in bound ethyl acetate fraction. Flavonol glycosides found in different species of pine needles were qualitatively similar within fractions, but composition varied among *Pinus* sections. High levels of kaempferol arabinoside and an unknown compound were present in all *Strobus* species. The soluble free fraction had the highest antioxidant activity, followed by bound ethyl acetate and bound water fractions.

Key words: pine needles, solvent extract, flavonol glycosides, proanthocyanidins, antioxidant activity

INTRODUCTION

Two main classifications of Genus *Pinus* were evaluated in this research: Section *Pinus* (Diploxylon) and Section *Strobus* (Haploxylon). The former species are *P. densiflora*, *P. sylvestris*, *P. pinaster* and *P. pinea*, and the latter species are *P. koraiensis* and *P. strobus*. This classification into the two Sections is based on leaf, cone and seed characteristics (1).

Previous reports have shown that Genus *Pinus* contains bioactive substances including antioxidant phenolics from extracts of pine needles (2,3) and pine bark (4,5). However, most of the previous reports only analysed the soluble phenolics extracted using water, acetone or methanol. The main phenolic compounds previously identified included phenolic acids (6), proanthocyanidins (PAs) (7), and flavonol glycosides (8,9).

Phenol-rich plant extracts may play an important role in health promotion through numerous mechanisms, including antioxidant activity, as reported for flavanols from grape seed extracts (10) and ginkgo flavonoids (11). Kaempferol from *Ginkgo biloba* extract inhibited pancreatic cancer cell growth and induced cell apoptosis *in vitro* in a concentration dependent manner, and may have clinical applications as adjuvant therapy in the treatment of pancreatic cancer (11). Little information exists, however, regarding the characterization and content of cell wall bound phenolics from waste pine needle residue (12). Several recent studies have reported on the biological significance and activity of phenolic acids (13), flavonoids (14) and proanthocyanidins (15-17) in a variety of plants.

A previous study was conducted to determine the flavonol and tannin contents and antioxidant activity of hot water and 70% acetone extracts from pine needle (18). Rats fed a high fat diet plus pine needle extract supplement for 4 weeks demonstrated reduced weight gain and reduced serum and liver lipids (19). According to the determination of total phenolics, including both soluble free and bound forms from 80% acetone in common fruits, cranberry, had the highest soluble free phenolic content, followed by apple, red grape, strawberry, lemon, peach, orange, banana, pear, and pineapple. Pineapple had the highest bound phenolic content (20). These results suggest that in some plants, the phenolics may be highly bound to the cell wall and the concentration of phenolics may be underestimated if the bound fraction is not measured. It is well established that plant extracts

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containing high levels of phenolics exhibit strong free radical scavenging capacity, and the two variables are highly correlated (21,22).

In the present study, we investigated the contents of PAs in soluble free (Soluble-F) fraction, and phenolic acids and flavonols in bound ethyl acetate (Bound-E) fraction. Additionally, ORAC in all fractions from six pine species was determined.

MATERIALS AND METHODS

Materials

Pine needle trees were purchased from Forest Farm (Williams, OR, USA), and included four species of *Pinus* (*P. densiflora*, *P. sylvestris*, *P. pinaster*, *P. pinea*) and two species of *Strobus* (*P. koraiensis* and *P. strobus*). The growth periods of all six species were 2, 4, 2, 2, 3, and 3 years, respectively, and the plant heights were 47, 70, 142, 60, 78, and 52 cm, respectively. The lengths of pine needles evaluated were 6.5, 5.5, 12, 2.5, 10, and 8 cm, respectively. The pine needles were dried in a forced air oven at 55°C until constant weight was obtained, ground to pass through a 1 mm screen, and stored at 4°C.

Preparation of pine needle extracts

Pine needles extracts and fractions were prepared by the method of Sun et al. (20). A flow chart of the extraction process used to isolate soluble free (Soluble-F), bound ethyl acetate (Bound-E) and bound water (Bound-W) fractions from various pine needles is shown in Fig. 1. For the preparation of Soluble-F, 1 g of dried pine needles was extracted with 150 mL of chilled 80% acetone by blending with a Euro Turrax T18 Tissuemizer (Tekmar-Dohrmann Corp, Mason, OH, USA) 3 times for 5 min each time. Homogenates were filtered through filter paper (Whatman No. 1), and the filtrates were evaporated to dryness using a SpeedVac® concentrator (ThermoSavant, Holbrook, NY, USA) and reconstituted in 50 mL of distilled water. For preparation of Bound-E fraction, the residues from Soluble F were collected and hydrolyzed with 20 mL of 4 M NaOH at room temperature for 24 hr. After hydrolysis, the solutions were centrifuged for 5 min at 5000 rpm. The supernatant was neutralized with concentrated hydrochloric acid and extracted three times with ethyl acetate. Ethyl acetate extracts were evaporated to dryness using a SpeedVac® concentrator and resuspended in 10 mL of distilled water. For preparation of Bound-W, the remaining water soluble portion was evaporated to dryness using the SpeedVac®, and then reconstituted in 10 mL of water. All fractions were stored at -70°C prior to total phenolic, total flavonoid, proanthocyanidins (PAs) and antioxidant capacity analyses. One extraction was prepared from each species of pine needle, and each analysis was performed in triplicate.

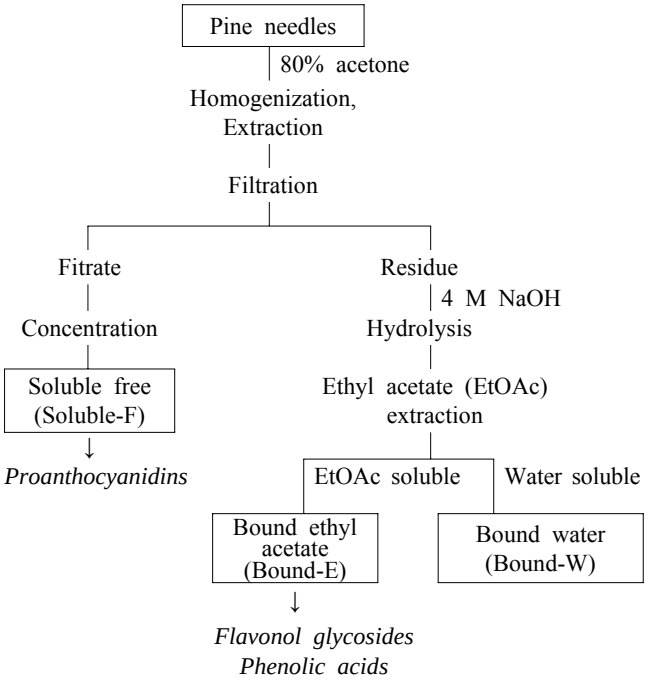

Fig. 1. Flow chart of extraction process and phenolic analysis for soluble free, bound ethyl acetate and bound water fractions from various pine needles.

Determination of total phenolics and flavonoids

Total phenolics in the fractions were determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton (23) with gallic acid as a standard. Results are expressed as mg/g dry weight. Total flavonoids in the fractions were analyzed by the method of Eum et al. (24). Naringin was used as standard with results expressed as mg/g dry weight.

HPLC analysis of flavonol glycosides and proanthocyanidins

HPLC analysis of flavonol glycosides: Bound-E fraction was passed through 0.45 μm filters (Whatman, Clifton, NJ, USA) prior to HPLC analysis. Samples (50 μL) were analysed using a Waters HPLC system (Waters Corp, Milford, MA, USA) (Table 1) (25). Separation was performed on a 250×4.6 mm Aqua C_{18} (Phenomenex, Torrance, Calif, USA) column using a binary gradient of A, 2% acetic acid and B, 0.5% acetic acid in water : acetonitrile (1:1 v/v) at a flow rate of 1.0 mL/min. The linear gradient was: 90% A to 45% A from 0～50 min; 45% A to 0% A from 50～60 min; 0% A to 90% A from 60～65 min. Peaks were monitored at wavelengths of 320 nm (phenolic acids) and

Table 1. Peak assignments, retention times (RT) and mass spectral data of *p*-coumaric acid hexoside and flavonols detected in various pine needles

Peak	HPLC RT (min)	Identification	(m/z) values	
			M^-	Fragments
1	27.8	Protocatechuic acid hexoside	315	153
2	30.9	*p*-Coumaric acid hexoside	325	163
3	32.5	Unknown		
4	34.3	Unknown		
5	37.2	Quercetin galactoside	463	301
6	38.0	Quercetin glucoside	463	301
7	41.0	Kaempferol galactoside	447	284
8	42.2	Quercetin pentoside	433	301
9	42.8	Kaempferol glucoside	447	284
10	43.8	Isorhamnetin glucoside	477	314
11	47.7	Kaempferol arabinoside	417	284
12	49.5	Unknown	461	298
13	51.3	Unknown	461	298

360 nm (flavonols) using a Waters model 996 photodiode array detector. Phenolic acids and flavonol glycosides were expressed as μg *p*-coumaric acid and μg quercetin-3-β-glucoside equivalents per g dry weight, respectively, using external calibration curves of authentic standards.

HPLC analysis of proanthocyanidins: Soluble-F fraction (7 mL) was loaded onto a Sepdadex LH-20 column (6×1.5 cm), previously equilibrated in 30% (v/v) aqueous methanol (26). The column was washed with 40 mL of 30% methanol to remove contaminants, and proanthocyanidins were then eluted from the column with 70 mL of 70% acetone. The resulting extract was evaporated to dryness using a SpeedVac concentrator and reconstituted with 2 mL of solvent (acetone : water : acetic acid, 70:29.5:0.5). Samples were passed through 0.45 μm filters (Waters), and then 5 μL were analyzed using a Waters HPLC system (Milford, MA, USA). Separation was performed on a 250×4.6 mm Luna silica column (Phenomenex, Torrance, CA, USA) using a binary gradient consisting of A, dichloromethane:methanol : water : acetic acid (82:14:2:2 v/v/v/v), and B, methanol : water : acetic acid (96:2:2 v/v/v) at a flow rate of 0.8 mL/min. The linear gradient was: 100% A to 88.3% A from 0～20 min; 88.3% A to 74.4% A from 20～50 min; 74.4% A to 12.3% A from 50～55 min; 12.3% A to 12.3% A from 55～65 min; and 12.3% A to 100% A from 65～70 min. The peaks were monitored by fluorescence detection with excitation at 276 nm and emission at 316 nm using a Waters model 474 fluorescence detector. Proanthocyanidin monomers and polymers were quantified as monomer equivalents using standards previously isolated from cocoa that were provided by Mars Inc. (Hackettstown, NJ, USA). Results were expressed as mg per g dry weight.

HPLC/MS analysis of flavonols

An analytical Hewlet Packard 1100 series HPLC (Palo Alto, CA, USA) equipped with an autosampler, binary HPLC pump and UV/VIS detector was used for HPLC/MS analysis. Flavonols were separated using the HPLC conditions described above with detection at 360 nm. The HPLC was interfaced to a Bruker (Billerica, MA, USA) model Esquire-LC/MS ion trap mass spectrometer, equipped with Bruker software, which controlled the instrument and collected the signal at 360 nm. Conditions for mass spectral analysis in the negative ion electrospray mode included a capillary voltage of 4000 V, a nebulising pressure of 30.0 psi, a drying gas flow of 9.0 mL/min and a temperature of 300°C. Data were collected using the full scan mode over a mass range of m/z 500～1000 at 1.0 sec per cycle. Peak assignments based upon characteristic ions are shown in Table 1. For compounds in which chemical standards were commercially available, retention times were also used to confirm their identity.

Determination of antioxidant activity

The oxygen radical absorbance capacity (ORAC) of each fraction of pine needle extracts was measured using the method of Prior et al. (27) modified for use with a FLUOstar Optima microplate reader (BMG Labtechnologies, Durham, NC, USA). Soluble-F, Bound-E, and Bound-W pine needle extracts were diluted 50,000, 5,000, 5,000-fold, respectively, with 75 mM phosphate buffer (pH 7.0) prior to ORAC analysis. The assay was carried out at 37°C in clear 48-well Falcon plates (VWR, St. Louis, MO). A standard curve was obtained by plotting four concentrations of Trolox against the net area under the curve (AUC) of each standard. Final ORAC values were calculated using the regression equation between Trolox concentration and AUC and are expressed

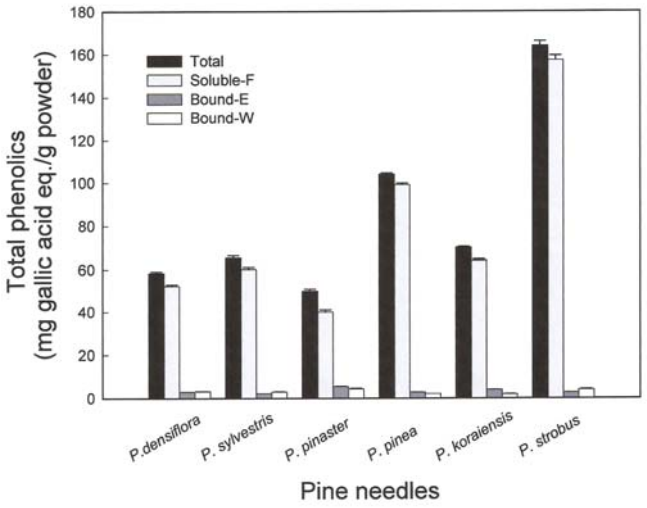

Fig. 2. Total phenolics in soluble free, bound ethyl acetate, and bound water fractions from various pine needles. Values are mean±SD of triplicate measurements.

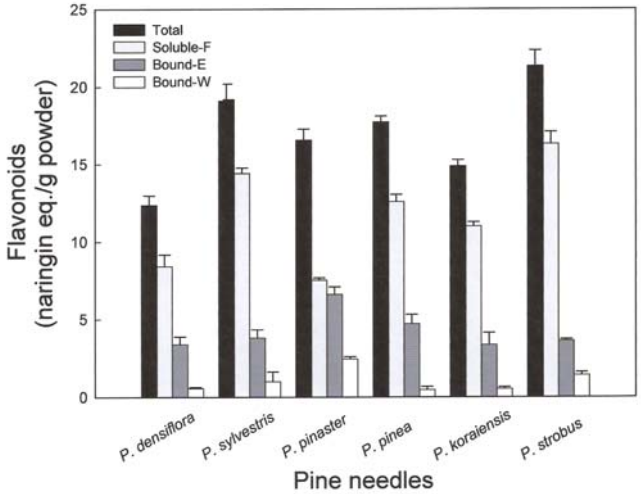

Fig. 3. Total flavonoids in soluble free, bound ethyl acetate, and bound water fractions from various pine needles. Values are mean±SD of triplicate measurements.

as μM of Trolox equivalents per gram dry weight.

Statistical analysis

The experimental data were analyzed with JMP software (Version 8, SAS Institute, NC, USA). Results are expressed as mean values±standard deviation.

RESULTS AND DISCUSSION

Total polyphenol and flavonoids

The total phenolic contents of the three fractions, including their sum, from the six pine needle species are shown in Fig. 2. Among all pine needles analyzed, *P. strobus* had the highest Soluble-F phenolic content (157.1±2.0 mg/g), followed by *P. pinea* (99.1±0.7 mg/g), *P. koraiensis* (64.3±0.4 mg/g), *P. sylvestris* (60.1±1.0 mg/g), *P. densiflora* (52.2±0.7 mg/g), and *P. pinaster* (40.0±1.0 mg/g). *P. pinaster* had the highest Bound-E phenolic content (5.4±0.1 mg/g), followed by *P. koraiensis* (3.9±0.1 mg/g), *P. densiflora* (2.9±0.1 mg/g), *P. pinea* (2.8±0.1 mg/g), *P. strobus* (2.7±0 mg/g), and *P. sylvestris* (2.3±0 mg/g). *P. pinaster* had the highest Bound-W phenolic content (4.4±0.3 mg/g), followed by, *P. strobus* (4.0±0.2 mg/g), *P. densiflora* (3.2±0.1 mg/g), *P. syvestris* (3.0±0.1 mg/g), *P. pinea* (2.1±0 mg/g), and *P. koraiensis* (1.8±0.1 mg/g). The phenolic content of the Soluble-F fraction was higher than that of Bound-E and Bound-W fractions. *P. strobus* had the highest total phenolic (Soluble-F+Bound-E+Bound-W) content (163.8±2.1 mg/g), followed by *P. pinea* (103.9±0.4 mg/g), *P. koraiensis* (70.0±0.4 mg/g), *P. sylvestris* (65.3±1.1 mg/g), *P. densiflora* (58.3±0.7 mg/g), and *P. pinaster* (49.8±1.0 mg/g).

The flavonoid contents of the three fractions from the six pine needle species are shown in Fig. 3. Among all the pine needles analyzed, *P. strobus* had the highest Soluble-F flavonoid content (16.3±0.8 mg/g), followed by *P. sylvestris* (14.4±0.4 mg/g), *P. pinea* (12.6±0.5 mg/g), *P. koraiensis* (11.0±0.3 mg/g), *P. densiflora* (8.4±0.8 mg/g), and *P. pinaster* (7.5±0.2 mg/g). *P. pinaster* had the highest Bound-E flavonoid content (6.6±0.5 mg/g), followed by *P. pinea* (4.7±0.6 mg/g), *P. sylvestris* (3.8±0.5 mg/g), *P. strobus* (3.6±0.1 m/g), *P. densiflora* (3.4±0.5 mg/g), and *P. koraiensis* (3.3±0.8 mg/g). Among all the species, the pine needles of *P. pinaster* had the highest ratio of total flavonoids to total phenolics. *P. pinaster* had the highest Bound-W flavonoid content (2.4±0.2 mg/g), followed by *P. strobus* (1.4±0.2 mg/g), *P. sylvestris* (1.0±0.6 mg/g), *P. densiflora* (0.6±0.1 mg/g), *P. koraiensis* (0.5±0.1 mg/g), and *P. pinea* (0.5±0.2 mg/g).

The flavonoid contents of the Soluble-F fractions were higher than those of Bound-E and Bound-W fractions. *P. strobus* had the highest total flavonoid (Soluble-F+Bound-E+Bound-W) content (21.3±1.0 mg/g), followed by *P. sylvestris* (19.2±1.0 mg/g), *P. pinea* (17.7±0.4 mg/g), *P. pinaster* (16.6±0.70 mg/g), *P. koraiensis* (14.9±0.4 mg/g), and *P. densiflora* (12.4±0.6 mg/g).

Flavonol glycosides

The HPLC profiles of phenolic acids and flavonol glycosides detected at 320 nm and 360 nm, respectively, in the bound ethyl acetate fraction of pine needles are shown in Fig. 4. Peak 1 was tentatively identified as protocatechuic acid hexoside, and peak 2 was tentatively identified as *p*-coumaric acid hexoside, while peaks 3 and 4 represent unknown phenolic acids. The flavonol

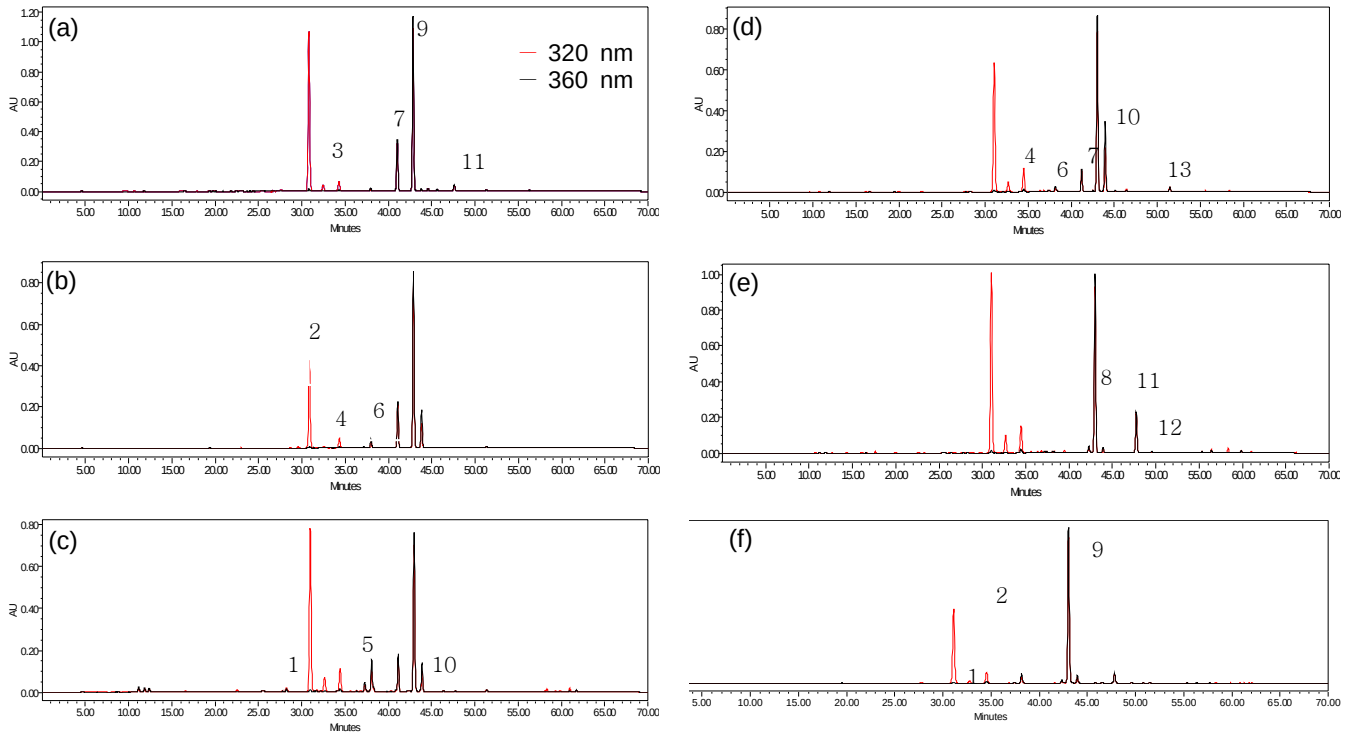

Fig. 4. Detection of flavonol glycosides (360 nm) and phenolic acids (320 nm) in bound ethyl acetate fraction of various pine needles. (a) *P. densiflora*, (b) *P. sylvestris*, (c) *P. pinaster*, (d) *P. pinea*, (e) *P. koraiensis*, and (f) *P. strobus*. See Table 1 for peak identification.

glycosides detected at 360 nm in pine needles were identified as quercetin galactoside (peak 5), quercetin glucoside (peak 6), kaempferol galactoside (peak 7), quercetin pentoside (peak 8), kaempferol glucoside (peak 9), isorhamnetin glucoside (peak 10), and kaempferol arabinoside (peak 11), while peaks 12 and 13 represent unknown flavonols. Flavonol glycosides found in different species of pine needles were qualitatively similar within fractions, but composition varied among *Pinus* sections. Previous reports mainly focused on soluble fractions, but in our study various kaempferol glycosides were identified that have not been identified previously. The total phenolic acid contents of bound ethyl acetate fractions in the six pine needles ranged from 2483.6 μg/g in *P. sylvestris* to 6631.2 μg/g in *P. koraiensis* (Table 2). The total flavonol glycoside content of the six pine needles ranged from 1924.2 μg/g in *P. sylvestris* to 2983.1 μg/g in *P. strobus*.

In terms of composition, *p*-coumaric acid hexoside was the predominant phenolic acid, and kaempferol glu-

Table 2. Flavonol glycoside and *p*-coumaric acid contents of bound ethyl acetate fraction in various pine needles

Compound	Pine needles					
	P. densiflora	*P. sylvestris*	*P. pinaster*	*P. pinea*	*P. koraiensis*	*P. strobus*
1[1)] Protocatechuic acid hexoside	63.2±0.4	0	97.7±5.1	10.2±2.0	24.2±1.0	36.6±0.5
2[1)] *p*-Coumaric acid hexoside	5644.5±32.9[3)]	2209.0±15.5	4118.4±25.7	3311.0±28.6	5267.0±38.5	4115.0±17.4
3[1)]	215.2±1.2	44.6±0.3	391.5±6.1	258.7±3.8	543.0±2.9	130.4±0.7
4[1)]	340.1±2.6	230.0±0.9	578.3±2.8	602.9±6.6	797.0±3.1	571.0±3.1
5[2)] Quercetin galactoside	8.6±0.2	6.2±0.9	76.4±2.9	18.2±1.3	19.9±5.2	4.0±0.8
6[2)] Quercetin glucoside	25.0±5.9	48.2±12.1	261.5±14.3	41.4±14.6	13.2±0.4	153.4±8.8
7[2)] Kaempferol galactoside	512.7±38.2	331.4±13.9	281.0±13.0	151.7±20.7	0	0
8[2)] Quercetin pentoside	7.0±3.2	0	5.7±0.3	5.4±0.4	61.1±0.5	52.8±1.8
9[2)] Kaempferol glucoside	1752.8±110.2	1278.0±73.6	1185.6±33.0	1305.1±71.0	1546.7±21.5	2481.6±66.9
10[2)] Isorhamnetin glucoside	18.3±1.5	246.7±60.0	221.7±6.9	467.7±106.2	48.4±0.2	117.3±3.3
11[2)] Kaempferol arabinoside	63.2±3.7	3.9±0.4	4.7±0.1	2.1±0.5	362.4±7.2	165.2±6.2
12[2)]	4.7±1.0	0.6±0.4	1.4±0.3	0	8.0±0.6	5.6±0.7
13[2)]	15.1±0.4	9.2±0.1	13.5±1.0	33.8±3.1	2.4±0.1	3.2±0.6

[1)]Data expressed as *p*-coumaric acid. [2)]Quercetin glucoside equivalents. [3)]μg/g dry weight pine needle.

coside was the predominant flavonol glycoside in the six types of pine needles. Typically, the flavonols that were present in large amounts in all pine needles were in glycoside forms: however, the levels of flavonol glycosides in the four *Pinus* (Section) species (*P. densiflora*, *P. sylvestris*, *P. pinaster*, *P. pinea*) were different from the two *Strobus* species (*P. koraiensis*, *P. strobus*). Kaempferol galactoside was present only in *Pinus* species, whereas quercetin pentoside was present only in *Strobus* species. High levels of kaempferol arabinoside and unknown compound 12 were present in all the *Strobus* species.

P. koraiensis and *P. strobus* also contained low levels of unknown compound 13 compared to the four *Pinus* species. The only flavonols that were previously identified in *Pinus* were the 3-*O*-glucosides of kaempferol and isorhamnetin, taxifolin 7-glucoside, and mono- and di-coumaroyl-glucosides of kaempferol (28). We did not detect taxifolin 7-glucoside or the mono- and di-coumaroyl-glucosides of kaempferol in our samples. The content of *p*-coumaric acid hexoside ranged from 2209 μg/g in *P. sylvestris* to 5644.5 μg/g in *P. densiflora*. The content of kaempferol glucoside ranged from 1185.6 μg/g in *P. pinaster* to 2481.6 μg/g in *P. strobus*. The content of isorhamnetin glucoside ranged from 18.3 μg/g in *P. densiflora* to 467.7 μg/g in *P. pinea*. In a recent study, kaempferol was reported to be an effective inhibitor of human cytochrome *P*450 enzymes, an important step in cancer chemoprevention (29). Due to an abundance of kaempferol glycosides, pine needle extracts appear to be an excellent candidate for further study involving chemopreventive properties.

Proanthocyanidins

Fig. 5 shows chromatograms representing proanthocyanidins in the soluble-F fraction following a Sephadex LH-20 column clean-up step. A peak with retention time of 13.5 min was consistent with the retention time of a proanthocyanidin monomer standard, while the peak with a retention time of 57 min represented proanthocyanidin polymers with degree of polymerization greater than 10.

(+) Catechin and proanthocyanidins in common foods are well known for health beneficial effects (30,31). Proanthcyanidins, also known as condensed tannins, are oligomeric and polymeric flavan-3-ols. The compounds that consist exclusively of (epi)catechin are called procyanidins (PCs).

The content of (+) catechin ranged from 0.97 mg/g in *P. pinaster* to 2.84 mg/g in *P. densiflora*, while the content of polymers ranged from 0.74 mg/g in *P. koraiensis* to 13.5 mg/g in *P. pinea* (Table 3). We suspect that the actual concentration of polymers in the extracts may actually be much higher because an authentic standard of polymers was unavailable, and the peaks were

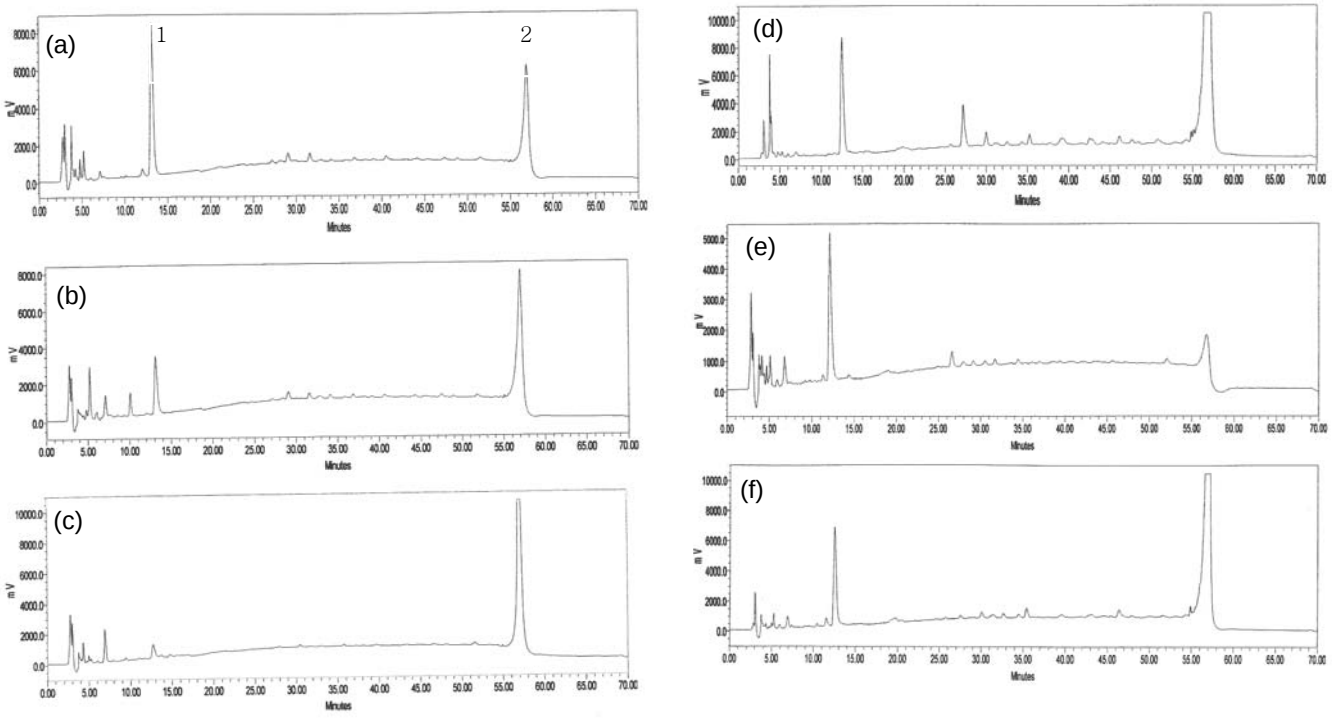

Fig. 5. Detection of proanthocyanidins in soluble free fraction of various pine needles. (a) *P. densiflora*, (b) *P. sylvestris*, (c) *P. pinaster*, (d) *P. pinea*, (e) *P. koraiensis*, and (f) *P. strobus*. Peak 1, monomer; catechin, 2; polymer.

Table 3. Proanthocyanidins content of soluble free fraction in various pine needles

	Monomer[1)]	Polymer	Total	Percent of total phenolic content
P. densiflora	2.84±0.33[2)]	3.81±0.09	6.65	12.7
P. sylvestris	1.30±0.58	5.65±0.63	6.96	11.6
P. pinaster	0.97±1.61	5.93±1.61	6.90	17.3
P. pinea	2.59±0.17	13.49±0.44	16.09	16.2
P. koraiensis	1.23±0.09	0.74±0.23	1.96	3.0
P. strobus	2.05±0.08	10.52±0.30	12.57	8.0

[1)]Data expressed as cocoa proanthocyanidin equivalents.
[2)]mg/g dry weight pine needle.

quantified as monomer equivalents. Consistent with our findings, a hot water extract from *Pinus radiate* bark contained ample quantities of polymeric proanthocyanidins (DP>13), comprising 94% of total procyanidins (5). However, the total proanthocyanidin content (4.5 mg/g extract) determined by the HCl-butanol method of a hot water extract obtained from *Pinus densiflora* (7) was much lower than the values obtained in this study.

Antioxidant activity

Antioxidant capacities of the six pine needle species are shown in Fig. 6. *P. strobus* had the highest Soluble-F antioxidant activity (1223.3±12.6 μmole Trolox/g), followed by *P. pinea* (901.5±35.2 μmole Trolox/g), *P. sylvestris* (560.0±36.3 μmole Trolox/g), *P. pinaster* (478.8 ±32.8 μmole Trolox/g), *P. densiflora* (466.1±27.3 μmole Trolox/g), and *P. koraiensis* (402.0±7.5 μmole Trolox/g).

P. pinaster had the highest Bound-E antioxidant activity (128.0±9.6 μmole Trolox/g), followed by *P. koraiensis* (111.6±6.2 μmole Trolox/g), *P. sylvestris* (91.7±3.2 μmole Trolox/g), *P. strobus* (82.3±3.1 μmole Trolox/g), *P. pinea* (70.9±0.9 μmole Trolox/g), and *P. densiflora* (61.4±3.7 μmole Trolox/g).

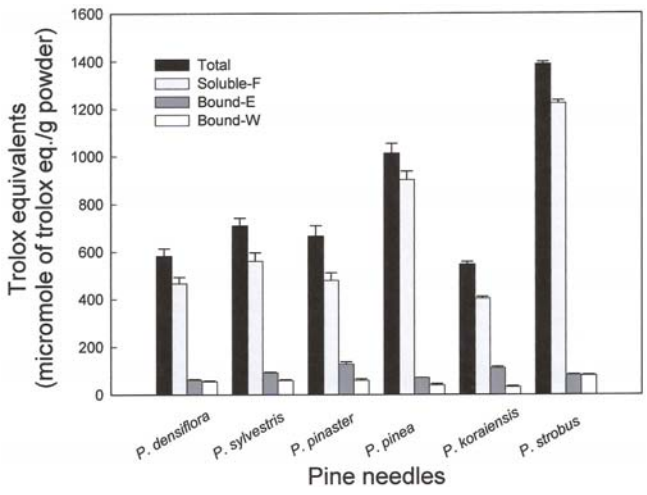

Fig. 6. Oxygen radical absorbing capacity (ORAC) in the soluble free, bound ethyl acetate, and bound water fraction of various pine needles. Values are mean±SD of triplicate measurements.

P. strobus had the highest Bound-W antioxidant activity (81.3±2.4 μmole Trolox/g), followed by *P. pinaster* (60.2±7.1 μmole Trolox/g), *P. sylvestris* (59.3±4.0 μmole Trolox/g), *P. densiflora* (55.3±2.8 μmole Trolox/g), *P. pinea* (39.7±5.5 μmole Trolox/g), and *P. koraiensis* (32.0±4.5 μmole Trolox/g).

The antioxidant capacities of the Soluble-F fractions of the six pine needles were higher than those of Bound-E and Bound-W fractions. *P. strobus* had the highest total antioxidant (Soluble-F+Bound-E+Bound-W) capacity (1386.8±10.5 μmole Trolox/g), followed by *P. pinea* (1012.1±40.3 μmole Trolox/g), *P. sylvestris* (710.7±31.7 μmole Trolox/g), *P. pinaster* (667.0±43.4 μmole Trolox/g), *P. densiflora* (582.7 ±31.1 μmole Trolox/g), and *P. koraiensis* (545.6±11.4 μmole Trolox/g). This work confirms that free and cell wall bound phenolics in pine needle extracts exhibit total antioxidant activity.

ACKNOWLEDGEMENTS

Kang Yoon-han wishes to express gratitude to Cindi R. Brownmiller for her help in instrumental analysis, and to Dr. Mi Jin Cho for her advice on the data processing. This study was supported by Gangneung-Wonju National University Research Fund in 2008.

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(Received January 14, 2010; Accepted March 9, 2010)