Korean J. Crop Sci. 45(2): 83~87(2000)

Isolation and Determination of Phenolic Compounds in Peony (*Paeonia lactiflora* Pall.)Root

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ABSTRACT : The five phenolic compounds of peony root were isolated by Sephadex LH-20 column chromatography. Their chemical structures were identified by spectroscopic methods (UV, FT-IR, FAB-MS and $^{1}H \cdot ^{13}C$-NMR). The complete structures of these compounds were elucidated to be (+)-taxifolin-3-O-β-D-glucopyranoside, benzoic acid, gallic acid, (−)-epicatechin and (+)-catechin. The concentrations of five phenolic compounds in the peony root of three Korean cultivars (Youngchonjakyak, Euisungjakyak and Jomjakyak) were determined by reverse-phase HPLC. The constituents concentration in Youngchonjakyak were generally higher than in Euisungjakyak and Jomjakyak. The concentrations of (+)-taxifolin-3-O-β-D-glucopyranoside, benzoic acid, gallic acid, (−)-epicatechin and (+)-catechin in three different cultivars were ranged 0.23~0.52%, 0.20~0.30%, 0.26~0.28%, 0.09~0.12% and 0.34~0.63%, respectively.

Keywords : peony root, *Paeonia lactiflora* Pall., (+)-taxifolin-3-O-β-D-glucopyranoside, benzoic acid, gallic acid, (−)-epicatechin, (+)-catechin, HPLC.

Peony root (roots of *Paeonia lactiflora* Pall.) is well-known as one of the most important crude drugs used in several Korean medicinal prescriptions (Choung, 1996; Choung & Kang, 1997). The crude drug has been used extensively in oriental medicine as an analgesic, an anti-spasmodic, an astringent and a sedative for the treatment of a variety of painful afflictions (Hatakeyama *et al.*, 1994).

Recently, the extract of herbal medicine prepared from peony root diminishes cognitive disruption caused by central cholinergic dysfunction, thus showing therapeutic potential in Alzheimer's disease (Kobayashi *et al.*, 1990; Hatakeyama *et al.*, 1994).

Paeoniflorin (Shibata & Nakahara, 1963; Choung & Kang, 1997), albiflorin (Kaneda *et al.*, 1972; Choung & Kang, 1997), benzoylpaeoniflorin (Kaneda *et al.*, 1972) and gallotannins (Nishizawa *et al.*, 1980) were isolated from peony root as physiological active principles. Among these, paeoniflorin has several pharmacological effects including anti-allergic, anti-convulsive, analgesic, muscle relaxant and anti-inflammatory actions, and the therapeutic effects of peony root are explained by the pharmacological actions of paeoniflorin (Takagi & Harada., 1969; Takeda *et al.*, 1995). However, Sugaya and co-workers have reported that paeoniflorin showed less potent activity of anti-convulsant effects and suggested that in the evaluation of the quality of peony roots, albiflorin and phenolic compounds concentration were more important than paeoniflorin (Sugaya *et al.*, 1991).

In the peony root, paeoniflorin, albiflorin and phenolic compounds are present along with other glycoside and other possibly effective constituents. Therefore, the effects of physiological activity of peony root is presumed due to combination and synergic effect of these compounds (Choung, 1996; Choung *et al.*, 1999)

In this study, in order to furnish basic information of quality evaluation in Korean cultivated peony root, we report the isolation of some phenolic compounds from peony root and the concentrations of some phenolic compounds in three Korean cultivated peony root.

MATERIAL AND METHOD

Materials

The four-year-old peony root (*Paeonia lactiflora* Pall.) of three cultivars (Youngchon jakyak, Euisungjakyak and Jomjakyak) which used in this study were grown under controlled conditions in the experimental field of Yeungnam University, Korea.

Methanol, acetonitrile and water were obtained from MERCK chemical Co. (Germany). Acetic acid, acetone, tetramethylsilane (TMS), D_2O, CD_3OD and Sephadex LH-20 resin were purchased from Sigma Chemical Co. (USA). All laboratory chemicals were used of reagent grade.

Extraction, isolation and determination of phenolic compounds

The dried four-year-old peony root of Euisungjakyak was ground and then the powder was extracted three times with

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70% acetone at room temperature for 24h. The combined extract were filtered and concentrated at 40°C *in vacuo*. The crude acetone extract was loaded onto the Sephadex LH-20 column (30 mm × 700 mm). The column was eluted stepwise with H_2O, 10% MeOH, 20% MeOH, 30% MeOH and 40% MeOH. Each fraction were collected and concentrated as before.

Recently, the column chromatography using several synthetic resins were developed for isolation of polyphenols from plants and foods (Nishizawa *et al.*, 1980; Cho, 1992). In particular, the Sephadex LH-20 resin could be more generally used because this resin offered a better resolution and high recovery efficiency of polyphenols by means of stepwise elution of polyphenols using a mixture solvent system of water-alcohol. In this advantage, we applied Sephadex LH-20 open column chromatography to isolate phenolic compounds in peony root using stepwise system.

As a result of Sephadex LH-20 open column chromatography, the five compounds were isolated in crude acetone extract. To determine the purity of isolated compounds, reverse-phase HPLC was used.

In order to determine the concentration of phenolic compounds in three different cultivars (Youngchonjakyak, Euisungjakyak and Jomjakyak), each peony roots were divided into two groups according to existence of a cortex; that is, cortex-removed and cortex-unremoved peony root and then dried for 30days at room temperature. The dried peony roots were ground and the powder (1 g) was extracted with distilled water (100 m*l*) by using ultrasonic cleaner for 30 min. The all extract samples were analyzed by reverse-phase HPLC. Prior to analysis, all samples were filtered through a 0.45 μm Millipore membrane filter.

Instrumentation and conditions

UV-spectrophotometer : UV absorption spectra of purified phenolic compounds were recorded on a spectrophotometer with SHIMADZU Model 5840 in H_2O.

$^1H \cdot ^{13}C$-NMR : ^1H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) were measured on a BRUKER ARX-300 in D_2O or CD_3OD containing tetramethylsilane (TMS) as the internal standard, and the chemical shift were given in δ value.

FT-IR and FAB-MS : IR spectra were recorded on a JASCO FT/IR-5300 with KBr. The Fast Atom Bombardment mass spectra (FAB-MS) were recorded on a JEOL JMS-AX 505WA with glycerol as the mounting matrix.

HPLC : The HPLC system was composed of L-6200 intelligent pump, a L-4250 UV-VIS variable wavelength detector and a D-2500 integrator (HITACHI). Injections were carried out with a Rheodyne 7725i injector equipped with a 20 μ*l* loop. The column was a TOSOH ODS-120T (250 × 4.6 mm I.D), the flow rate was set at 0.7 m*l* min^{-1} by isocratic elution, using a solvent (H_2O : CH_3CN : CH_3OH : CH_3COOH, 80: 15:5:0.2) with monitoring 254 nm, and the column temperature was set at 30°C. For protection of the analytical column, Nova-pak C_{18} guard insert column (Waters) was used. The five phenolic compounds standard calibration curve for quantitative analysis were shown in Fig. 1., and all simple correlation coefficients were above 0.999, respectively.

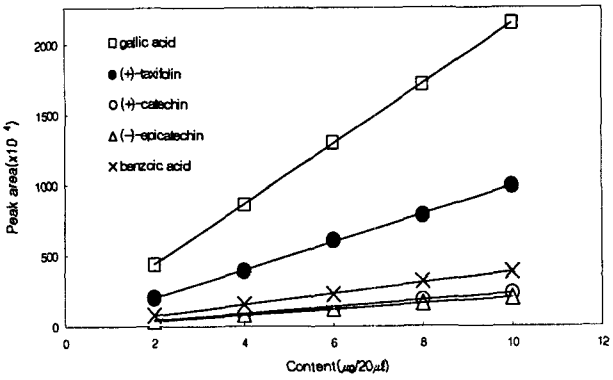


Fig. 1. Calibration curves of five phenolic compound standards by HPLC.

RESULTS AND DISCUSSION

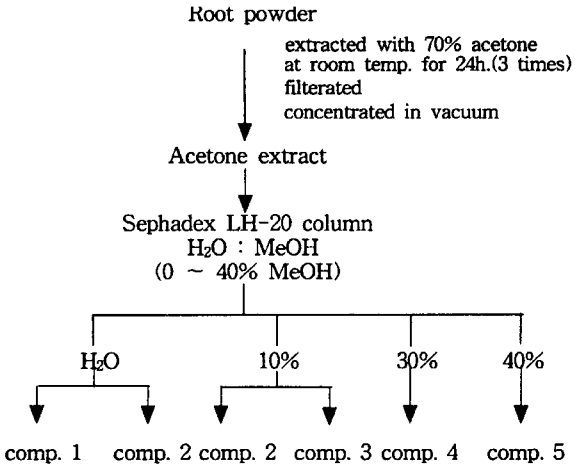
Extraction and isolation of phenolic compounds

The scheme for extraction and isolation of phenolic compounds from peony root is shown in Scheme 1. On Sephadex LH-20 open column chromatography, the compound 1 and 2 were separated by H_2O as eluant, and then compound 2 and 3 were separated by 10% MeOH. In 30% and 40% MeOH elution, compound 4 and 5 were separated respectively. As a result of column chromatography, the five compounds (compound 1~5) were isolated from acetone extract of peony root.

Identification of isolated five phenolic compounds

The chemical structures of five isolated compounds were identified by means of spectroscopic methods (UV, FT-IR, FAB-MS and $^1H \cdot ^{13}C$-NMR).

Compound 1 : UV λmax nm=211, 260 nm, ^1H-NMR(D_2O) δ=0.90~1.65(1H, ddd, J=9, 6, 3 Hz, H-5″), 1.88~2.00(2H, H-2″, 3″), 2.29(1H, d, J=13 Hz, H-4″), 3.73(2H, dd, J=13,3 Hz, H-6″), 4.57(1H, d, J=8 Hz, H-1″), 4.62(1H, d, J=4 Hz, H-2), 5.36(1H, d, J=9 Hz, H-3), 5.45~5.56(2H, H-6,8), 6.89 ~7.10(3H, H-2′, 5′, 6′), ^{13}C-NMR(D_2O) δ=63.30(C-6″), 72.28(C-4″), 75.69(C-2″), 78.55(C-3), 84.13(C-2), 101.14 (C-1″), 103,36(C-10), 118.23(C-2′), 123.62(C-6′), 147.38



Scheme 1. Extraction and isolation of five phenolic compounds in peony root.

(C-3'), 163.71 (C-5), 170.92(C-7)

Compound 2 : UV λmax nm=228, 270 nm, IR νmax cm^{-1} (KBr)=3069~2561, 1687, 1454, 1423, 1327, 1292, 1182, 933, 806, 707, 665, 551 cm-1, FAB-MS=122 (M^+), 105, 77, 51 m/z, ^{1}H-NMR(CD_3OD)=7.44(2H, m, H-3,5), 7.53(1H, m, H-4), 8.04(2H, m, H-2,6), ^{13}C-NMR(CD_3OD)=129.34(C-3,5), 130.64(C-2,6), 131.66(C-1), 133.95(C-4), 169.89(C-7)

Compound 3 : UV λmax nm=213, 264 nm, IR νmax cm^{-1} (KBr)=3288(OH), 1703, 1618, 1541(aromatic C=C), 1448, 1338, 1246, 1026, 868, 790, 765, 731, 702 cm^{-1}, ^{1}H-NMR (D_2O) δ=7.12(2H, s, H-2,6), ^{13}C-NMR(D_2O) δ=112.75(C-2,6), 124.02(C-1), 140.65(C-4), 147.32(C-3,5), 173.23(C-7)

Compound 4 : UV λmax nm=203, 278 nm, IR νmax cm^{-1} (KBr)=3456(OH), 1624, 1521, 1469, 1440, 1184, 1143, 1095, 1070, 1045, 1016, 808, 794 cm^{-1}, ^{1}H-NMR(D_2O) δ=2.77 (1H, d, J=16 Hz, H-4), 2.92(1H, dd, J=17,4 Hz, H-10), 4.28(1H, br s, H-3), 4.92(1H, s, H-2), 5.91(1H, d, J=1 Hz, H-8), 5.99(1H, d, J=1 Hz, H-6), 6.91~7.01(3H, m, H-2', 5', 6'), ^{13}C-NMR(D_2O) δ=30.12(C-4), 68.37(C-3), 80.84(C-2), 97.61(C-8), 98.53(C-6), 102.44(C-10), 117.08(C-2'), 118.77 (C-5'), 121.69(C-6'), 133.72(C-1'), 146.43(C-4'), 146.58(C-3'), 157.77(C-9), 158.05(C-5), 158.32(C-7)

Compound 5 : UV λmax nm=203, 279 nm, IR νmax cm^{-1} (KBr)=3358 (OH), 1626, 1521, 1464, 1373, 1286, 1257, 1182, 1147, 1030, 825 cm^{-1}, ^{1}H-NMR(D_2O) δ=2.46(1H, dd, J=16,8 Hz, H-4), 2.82(1H, dd, J=16,5 Hz, H-10), 4.09(1H, m, H-3), 4.60 (1H, d, J=8 Hz, H-2), 5.91(1H, d, J=1 Hz, H-8), 6.00(1H, s, H-6), 6.78(1H, d, J=8 Hz, H-6'), 6.86 (2H, d, J=8 Hz, H-2', 5'), ^{13}C-NMR(D_2O) δ=29.42(C-4), 69.37(C-3), 83.65(C-2), 97.58(C-8), 98.50(C-6), 103.31(C-10), 117.64 (C-2'), 118.94(C-5'), 122.74(C-6'), 132.98(C-1'), 146.79(C-4'), 147.04(C-3'), 157.57(C-9), 157,91(C-5), 157.97(C-7)

Compound 1~5 were identified as (+)-taxifolin-3-O-β-D-glucopyranoside, benzoic acid, gallic acid, (-)-epicatechin and (+)-catechin, respectively (Fig. 2). Among these, (+)-taxifolin-3-O-β-D-glucopyranoside is a novel compound, however benzoic acid, gallic acid, (−)-epicatechin and (+)-catechin were well-known as anti-oxidant and/or anti-hypertension agent.

Comparison of phenolic compounds concentration in three different cultivars

The aqueous extracts of Youngchonjakyak, Euisungjakyak and Jomjakyak were analyzed by reverse-phase HPLC. The typical HPLC chromatogram of peony root is shown in Fig. 3.

In the case of cortex-unremoved peony root, the concentrations of (+)-taxifolin-3-O-β-D-glucopyranoside and (+)-catechin in Youngchonjakyak (0.518% and 0.629%) were higher than in Euisungjakyak (0.288% and 0.337%) and Jomjakyak (0.233% and 0.421%). The concentrations of (+)-taxifolin-3-O-β-D-glucopyranoside and (+)-catechin in cortex-removed peony root were showed the same tendency as those in cortex-unremoved peony root. Comparing cortex-removed and cortex-unremoved peony root, the concentrations of (+)-taxifolin-3-O-β-D-glucopyranoside and (+)-catechin in cortex-

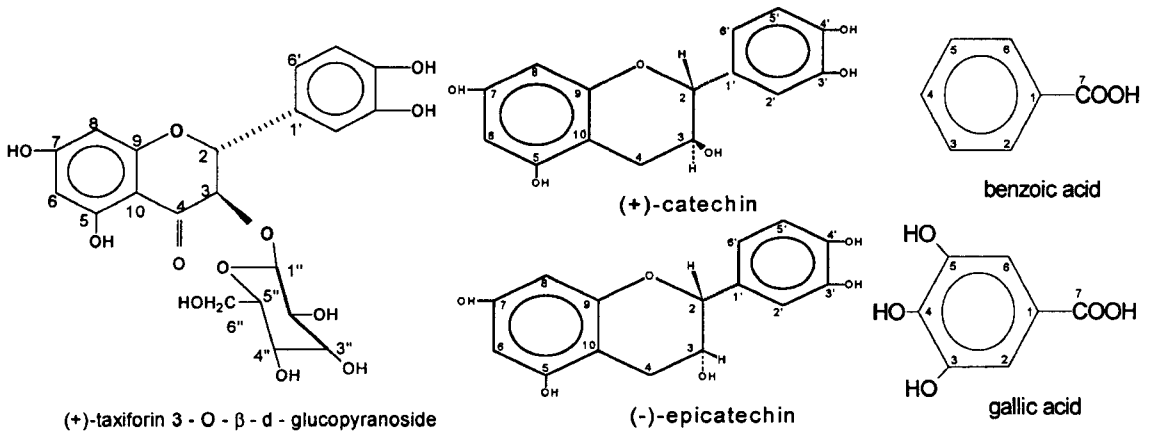


Fig. 2. Chemical structures of five phenolic compounds in peony root.

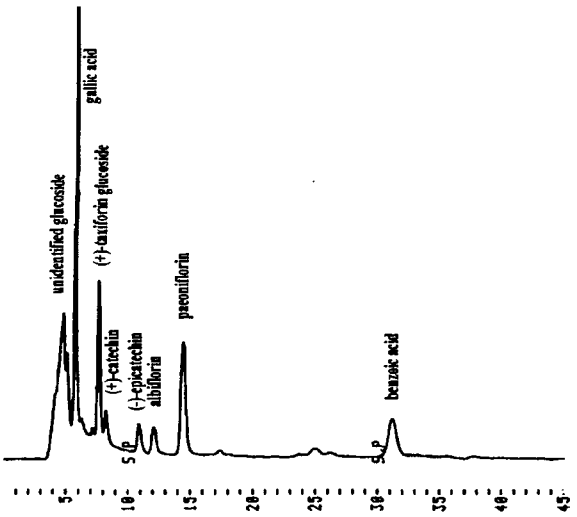


Fig. 3. HPLC chromatogram of aquous extract in peony root.

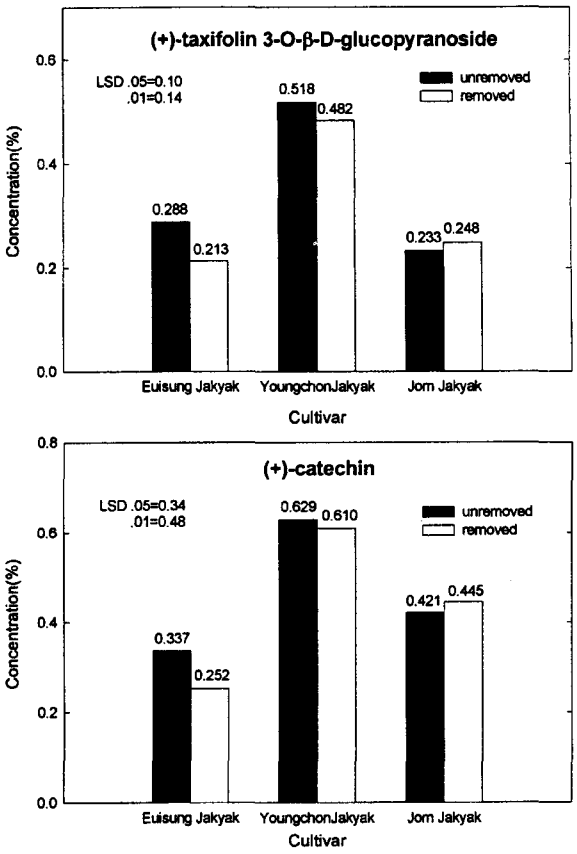


Fig. 4. Comparison of (+)-taxifolin(-3-O-β-D-)glucopyranoside and (+)-catechin concentrations in cortex-removed and -unremoved four-year-old penoy root among three peony cultivars.

unremoved Youngchonjakyak and Euisungjakyak were higher than those in cortex-removed one, while (+)-taxifolin-3-O-β-D-glucopyranoside and (+)-catechin concentration of cortex-unremoved Jomjakyak were lower than those of cortex-removed one on the contrary (Fig. 4).

The concentrations of gallic acid were ranged from 0.19% to 0.28%, which were not significantly different in all samples. In cortex-unremoved peony root, (−)-epicatechin concentrations of three different cultivars were ranged from 0.09% to 0.12%, which were approximately similar, but in case of cortex-removed one, the concentrations of (−)-epicatechin were significantly different. The concentration of benzoic acid was not significantly different among three cultivars, and ranged from 0.19% to 0.30% (Table 1).

As results of this experiment, the concentrations of gallic acid, (−)-epicatechin and benzoic acid were not significantly different in three cultivars, however the concentrations of (+)-taxifolin-3-O-β-D-glucopyranoside and (+)-catechin were significantly different in three cultivars. Especially, the concentrations of (+)-taxifolin-3-O-β-D-glucopyranoside and (+)-catechin in Youngchonjakyak were about twofold higher than those in Euisungjakyak and Jomjakyak.

Table 1. Comparison of gallic acid, (−)-epicatechin and benzoic acid concentrations in cortex-removed and -unremoved four-year-old peony root among three peony cultivars.

Compound	Cortex	Concentration (%)		
		Euisungjakyak	Youngchonjakyak	Jomjakyak
Gallic acid	Unremoved	0.254 a†	0.271 a	0.278 a
	Removed	0.189 a	0.257 a	0.235 a
	Difference	0.075 ns	0.014 ns	0.043 ns
(-)-Epicatechin	Unremoved	0.096 a	0.121 a	0.094 a
	Removed	0.193 a	0.147 b	0.101 c
	Difference	0.097 **	0.026 ns	0.007 ns
Benzoic acid	Unremoved	0.202 a	0.300 a	0.201 a
	Removed	0.259 a	0.285 a	0.185 a
	Difference	0.057 ns	0.015 ns	0.016 ns

†Means within a row followed by the same letters are not significantly different at the 5% level by DMRT.
*,**Singnificant at 5% and 1% level. nsNot significant at 5% level.

In conclusion, up to now, the pharmacological effects of peony root are only explained by the paeoniflorin. However, these are many physiological effective constituents such as albiflorin, (+)-taxifolin-3-O-β-D-glucopyranoside, benzoic acid, gallic acid, (−)-epicatechin and (+)-catechin in peony roots. The diverse physiological activities of peony root are presumed due to combined and synergic effect of these constituents. Therefore, the real quality evaluation of peony root should be judged by the whole constituents contents, but not by the specific one such as paeoniflorin.

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