PHENOLIC EXTRACTIVES WITH CHEMOTAXONOMIC SIGNIFICANCE FROM THE BARK OF *PAULOWNIA TOMENTOSA* VAR. *TOMENTOSA*

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Chemotaxonomy, also known as chemosystematics, can be regarded as a hybrid science which can classify plants based on their unique extractives (secondary metabolites). In this work, we investigated the chemotaxonomic marks of *Paulownia* species by studying the extractives of *Paulownia tomentosa* (Thunb.) Steud. var. *tomentosa,* a hardwood species widely used in Chinese medicine and pulping industries. Nine phenolic extractives, including two flavonoids (naringenin (**1**), and quercetin (**2**)), two phenolic acids (cinnamic acid (**3**), and gallic acid (**4**)), and five phenylpropanoid glycosides (cistanoside F (**5**), acteoside (**6**), isoacteoside (**7**), campneoside II (**8**), and isocampneoside II (**9**)), were isolated from the *n*-BuOH soluble fraction of *P. tomentosa* var. *tomentosa* bark, by repeated sephadex LH-20 open column chromatography coupled by Thin Layer Chromatography detection. The structures of the phenolic extractives were elucidated and characterized on the basis of their spectroscopical data and physiochemical properties. This was the first time to report the nine extractives from *P. tomentosa* var. *tomentosa* bark. Our chemotaxonomic analysis demonstrated that phenylpropanoid glycosides in *P. tomentosa* var. *tomentosa* were interesting and phenylpropanoid glycosides may possibly be considered as a useful chemotaxonomic marker within the species of *Paulownia*.

Keywords: Paulownia tomentosa *var.* tomentosa*; Bark; Phenolic extractives; Spectroscopic analysis; Chemotaxonomic significance*

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INTRODUCTION

Chemotaxonomy, also called chemosystematics, is the attempt to classify and identify plants, according to demonstrable differences and similarities in their biochemical compositions. Thus, chemotaxonomic markers are powerful tools for the identification of a wide variety of plants (Bohm 1987). For example, previously, we reported that galloyl glucoses may possibly be considered as a useful chemotaxonomic marker within the species of *Juglans* (Si et al. 2011), and glucosides of 1,2-dihydroxy-cyclohexane acylated by *ρ*-coumaric acid (or *ρ*-coumaric acid derivatives) could be treated as chemotaxonomic clues within the Salicaceae family (Si et al. 2009a). We also concluded that the phenolic constituents of *Conyza sumatrensis* may indicate it has a

closer relationship to the genus *Erigeron* than the other species of *Conyza* (Chai et al. 2008). *Paulownia tomentosa* (Thunb.) Steud. var. *tomentosa* (Scrophulariaceae), a medicinal hardwood that is native to China and extensively cultured in east and south Asian countries (Si et al. 2009b; Hong et al. 1998), has long been widely used in folk remedies to treat various diseases, including upper respiratory tract infection, cough, inflammatory bronchitis, phlegm, carbuncle, traumatic bleeding, erysipelas, hemorrhoid, asthma, gonorrhea, high blood pressure, bronchopneumonia, bacteriologic diarrhea, parotitis, tonsillitis, conjunctivitis, enteritis, and swelling (Jiang 2003; Jiangsu New Medical College 1977).

Phytochemical investigation on other species of *Paulownia* led to the isolation of different classes of extractives, including phenylethanoid glycosides (Si et al. 2008a, 2008b; Kim et al. 2008, 2007; Du 2003; Damtoft and Jensen 1993; Ota et al. 1993; Schilling 1982), lapachol type naphthoquinones (Huang et al. 2004), iridoids (Damtoft and Jensen 1993), and lignans (Okazaki et al. 1997; Ayres and Loike 1990; Takahash and Nakagawa 1963). Our previous phytochemical investigation of EtOAc soluble fraction of *P. tomentosa* var. *tomentosa* bark led to the isolation of four apigenin derivatives, elucidated as apigenin-7-O-β-D-glucopyranoside, apigenin-7-O-β-D-glucuronopyranoside, apigenin-7-O-β-D-glucuronopyranosyl(1→2)-O-β-D-glucuronopyranoside, and 7-caffeoyl-4′-methoxylapigenin (Si et al. 2009b). However, to the best of our knowledge, no other researcher has accomplished the same job, and systematically screening the extractives from *P. tomentosa* var. *tomentosa* with chemotaxonomic significance within *Paulownia* species has never been carried out to date.

EXPERIMENTAL

Plant Materials

Bark of *P. tomentosa* var. *tomentosa* was collected in June of 2010, from Laiwu, Shandong Province, China. The tree was authenticated by Prof. Dr. Dan Wang (Institute of Chemical Industry of Forest Products, Chinese Academy of Forestry, China). A voucher specimen (CMSCE–100618) has been deposited at herbarium of Tianjin Key Laboratory of Pulp and Paper, College of Materials Science and Chemical Engineering, Tianjin University of Science and Technology.

Equipment and Reagents

Melting points were determined on an Electrothermal IA 9200 apparatus (uncorrected). Optical rotations were measured on a JASCO DIP 1000 polarimeter in MeOH. 1D and 2D Thin Layer Chromatography (TLC) analyses were carried out on DC-Plastikfolien Cellulose F (Merck) plates and developed with *t*-BuOH-HOAc-H_2O (3:1:1, v/v, solvent A) and HOAc-H_2O (3:47, v/v, solvent B). Column chromatography was performed with Sephadex LH-20 (Merck). Eluents were collected with an SBS-160 fraction collector. Visualization and detection was conducted by UV light at 365 and 254 nm wavelength and then by spraying with 1% ethanolic $FeCl_3$ or vanillin-HCl-EtOH (60:0.15:6, w/v/v) solutions followed by heating.

^1H and ^{13}C-NMR, DEPT and correlation NMR spectra including HMQC, HMBC, and TOCOSY were recorded in $(CD_3)_2CO$ or CD_3OD (Sigma) with TMS (Sigma) as an internal standard using a Bruker Avance DPX 400 spectrometer at the operating frequency of 400 MHz (^1H) and 100 MHz (^{13}C) at State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, China, and at Central Laboratory of Kangwon National University, Korea. Positive FAB and EI-MS spectroscopy were done with a Micromass Autospec M363 spectrometer at Limerick Pulp and Paper Center, University of New Brunswick, Canada; MALDI-TOF-MS were also performed on a Model Voyager-DE STR spectrometer at Central Laboratory of Kangwon National University, Korea.

Solvents concentration was done with a RE-52AA rotary evaporator (Shanghai Yarong Biochemical Equipment Company). Samples were freeze dried with a LGJ-12 lyophilizer (Beijing Songyuan Huaxing Technology Development Co., Ltd).

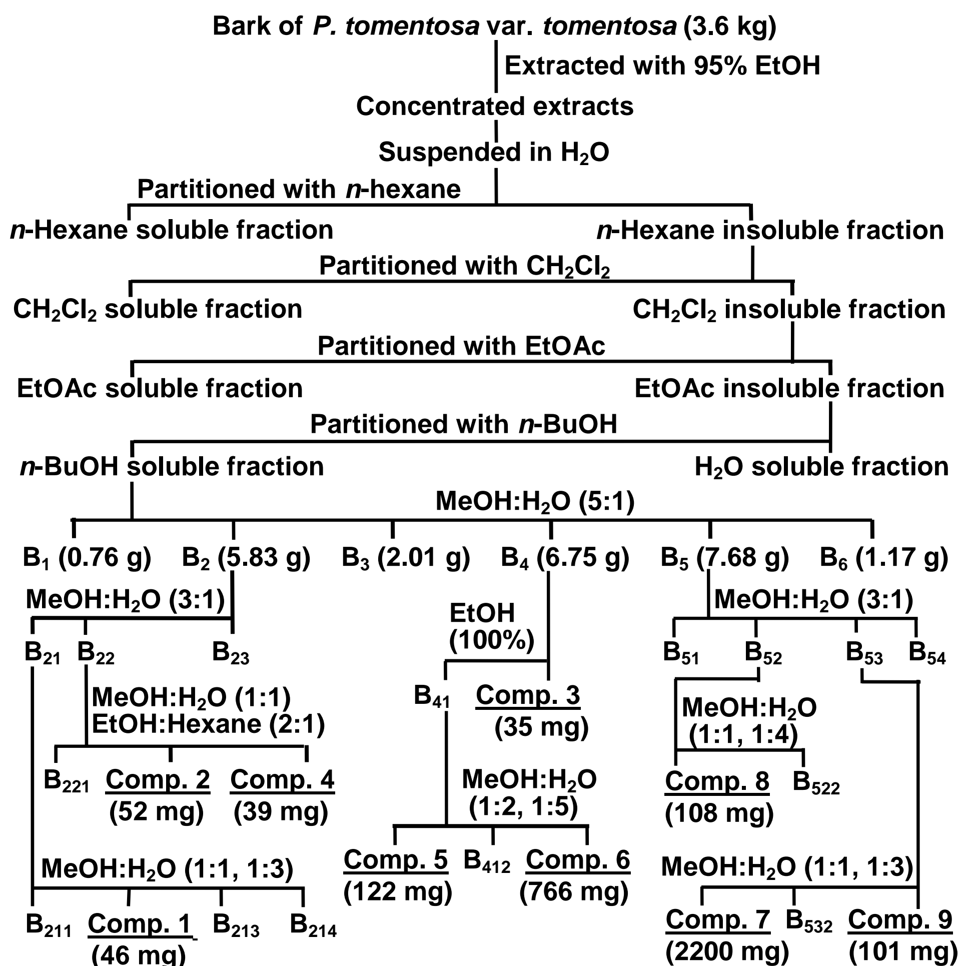

Fig. 1. Extraction, fractionation and isolation procedures of phenolic extractives from *P. tomentosa var. tomentosa* bark (The method was adopted from the literature (Si et al. 2009a)).

Extraction and Fractionation (Si et al. 2009a)

Bark samples of *P. tomentosa* var. *tomentosa* (3.6 kg) were dried and finely ground, then extracted with 95% aqueous EtOH (volume ratio) at room temperature (each 20 l for 96 h $\times$ 5 times). As shown in Fig. 1, after filtration and evaporation of the EtOH solvent under reduced pressure, residue of the aqueous EtOH extract was suspended in H_2O, and then followed by partition sequentially with different polar solvents, then freeze-dried to give soluble fractions of *n*-hexane (4.2 g, yield 0.12%), CH_2Cl_2 (3.6 g, yield 0.10%), EtOAc (28.4 g, yield 0.79%), *n*-BuOH (36.2 g, yield 1.01%) and H_2O (226.8 g, yield 6.30%). Soluble fractions were kept in dark, cool, and sealed vials.

Isolation of Phenolic Extractives

As presented in Fig. 1, a portion of the above freeze-dried *n*-BuOH soluble fraction powders (24.5 g) was subjected to a Sephadex LH-20 column with MeOH-H_2O (5:1, v/v) solution used as mobile phase to produce six main fractions: B_1 (0.76 g), B_2 (5.83 g), B_3 (2.01 g), B_4 (6.75 g), B_5 (7.68 g), and B_6 (1.17 g), which were guided and monitored by TLC performance. Fraction B_2 was applied to a Sephadex LH-20 column with MeOH-H_2O (3:1, v/v) as eluting mixtures for further separation to yield three subfractions referred to as B_{21} (2.43 g), B_{22} (2.74 g), and B_{23} (522 mg). The first subfraction (B_{21}) was further eluted on a Sephadex LH-20 column with MeOH-H_2O (1:1 and 1:3, v/v) stepwisely, and compound **1** (46 mg) was obtained as a yellow amorphous powder after lyophilization. Subfraction B_{22} was also submitted to Sephadex LH-20 column chromatography using MeOH-H_2O (1:1, v/v) and EtOH-Hexane (2:1, v/v) to get compounds **2** (52 mg) and **4** (39 mg). When treated in 100% EtOH, 35 mg of compound **3** were obtained as white crystalline precipitation in B_4. And the supernatant B_{41} was also purified with a Sephadex LH-20 column with MeOH-H_2O (1:2 and 1:5, v/v) as washing solvents to give 122 mg of compound **5** (122 mg) and 766 mg of compound **6**.

Fraction B_5 was also subjected to passage over a Sephadex LH-20 open column eluting with MeOH-H_2O (3:1, v/v) to provide four subfractions labeled as B_{51}–B_{54} according to TLC. And repeated purification of the second (B_{52}, 1.2 g) and the third (H_{53}, 4.18 g) subfractions on Sephadex LH-20 column chromatography with MeOH-H_2O (1:1 and 1:4, v/v) and MeOH-H_2O (1:1 and 1:3, v/v) as eluting systems, yellow amorphous powders **8** (108 mg), **7** (2200 mg) and **9** (101 mg) were obtained, respectively.

RESULTS AND DISCUSSION

Phenolic Extractives Isolation and Elucidation

Eight yellow amorphous powders (**1**, **2**, and **4**–**9**), and one white crystalline extractives **3** were isolated and purified through repeated chromatographic and TLC screening of an *n*-butanol soluble fraction of *P. tomentosa* var. *tomentosa* bark. The structures of the nine isolated phenolic extractives, as shown in Fig. 2, including two flavonoids (one flavanone naringenin (**1**, Harborne and Mabry 1982), and one flavonol quercetin (**2**, Markham and Chari 1982), two phenolic acids (cinnamic acid (**3**, Kashiwada et al. 1988), and gallic acid (**4**, Si et al. 2011)), and five phenylpropanoid glycosides (cistanoside F (**5**, Kobayashi et al. 1985), acteoside (**6**, Si et al. 2008a),

isoacteoside (**7**, Ota et al. 1993), campneoside II (**8**, Wu et al. 2004), and isocampneoside II (**9**, Kim et al. 2007)), were elucidated and determined by comparing their MS, ^{1}H and ^{13}C-NMR spectral data with those of reported in literature and a verification of other physiochemical evidence (such as 2D TLC in Fig. 3, melting points, and optical rotations). To the best of our knowledge, this was the first time of reporting phenolic extractives **1–9** from *P. tomentosa* var. *tomentosa* bark.

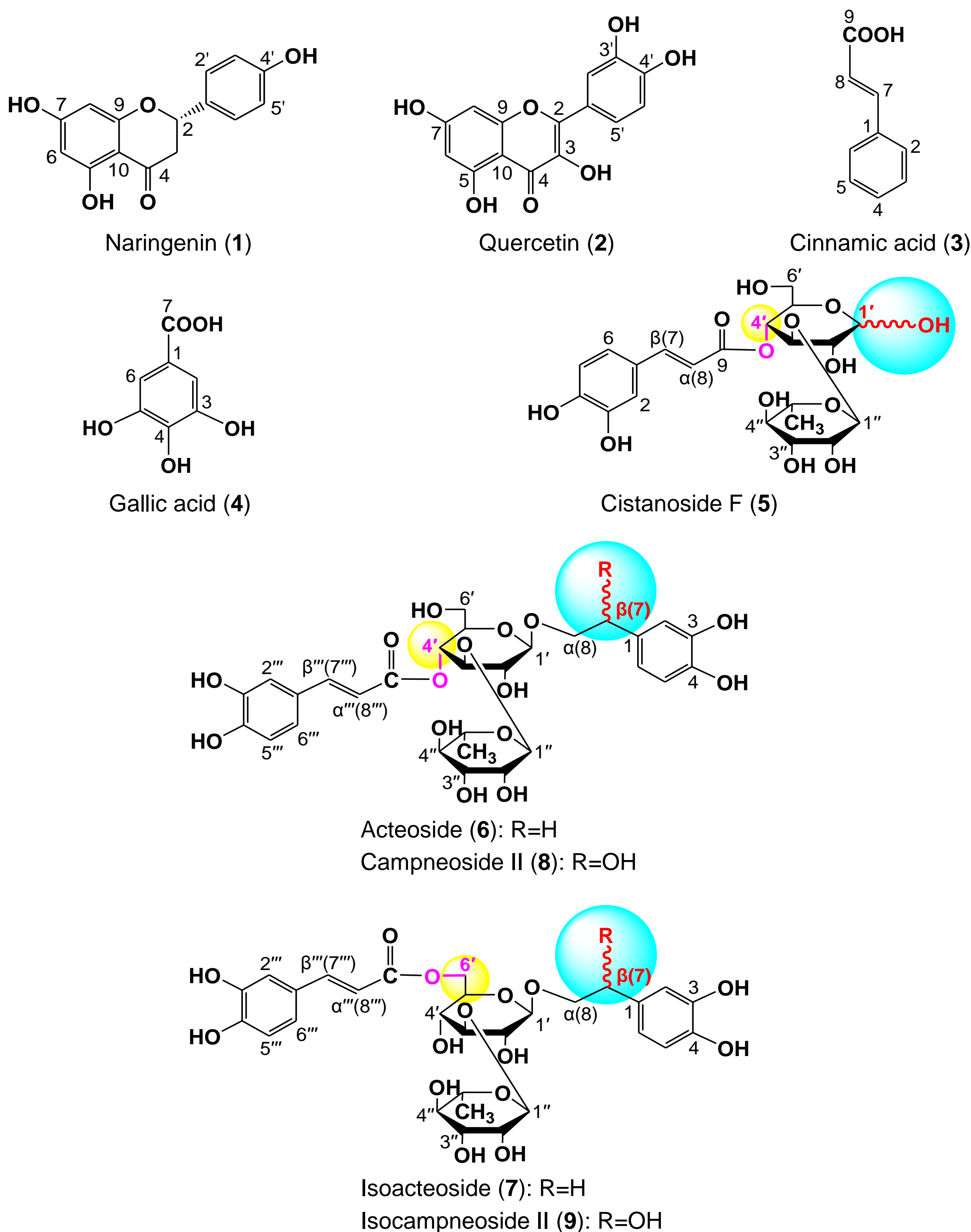

Fig. 2. Structures of the elucidated phenolic extractives from *P. tomentosa* var. *tomentosa* bark

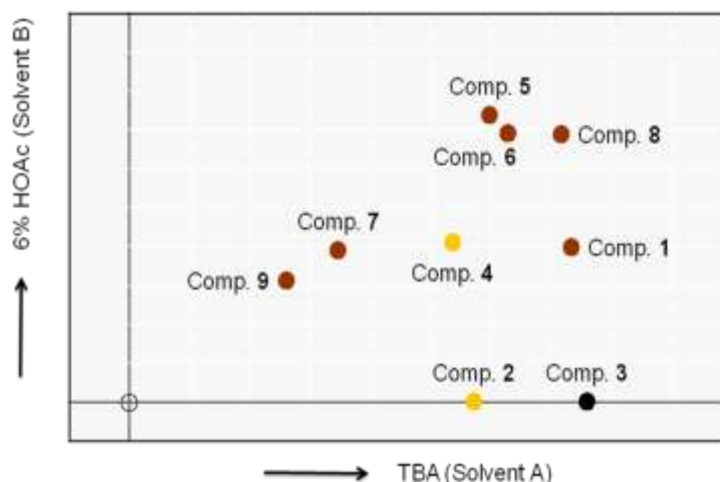

Fig. 3. Distribution and chromogenic reaction of the phenolic extractives **1**–**9** on 2D TLC plate developed with TBA and 6% HOAc (Comp. **3** was detected under UV at wavelength 254 nm)

*Compound **1** (Naringenin)*

Yellowish amorphous powder; $FeCl_3$ test: Positive (dark brown); R_f: 0.74 (solvent A) and 0.40 (solvent B); EI-MS: Calculated for $C_{15}H_{12}O_5$ 272; Found $[M]^+$ *m/z* 272, $[A_1+H]^+$ *m/z* 153, $[A_1]^+$ *m/z* 152 and $[B_1+2H]^+$ *m/z* 120; ^{1}H-NMR (400 MHz, δ, $(CD_3)_2CO$): 2.73 (1H, *dd*, *J*=3.0 Hz and *J*=17.1 Hz, H-3eq), 3.19 (1H, *dd*, *J*=12.9 Hz and *J*=17.1 Hz, H-3ax), 5.46 (1H, *dd*, *J*=3.0 Hz and *J*=12.9 Hz, H-2), 5.96 (1H, *d*, *J*=2.1Hz, H-6), 5.97 (1H, *d*, *J*=2.1 Hz, H-8), 6.90 (2H, *d*, *J*=8.6 Hz, H-3',5'), 7.40 (2H, *d*, *J*=8.6 Hz, H-2',6'); ^{13}C-NMR (100 MHz, δ, $(CD_3)_2CO$): 43.49 (C-3), 79.95 (C-2), 95.85 (C-8), 96.82 (C-6), 103.25 (C-10), 116.19 (C-3',5'), 129.05 (C-2',6'), 130.81 (C-1'), 158.69 (C-4'), 164.39 (C-9), 165.31 (C-5), 167.30 (C-7), 197.27 (C-4).

*Compound **2** (Quercetin)*

Yellowish amorphous powder; Vanillin-HCl-EtOH test: Positive (yellow); R_f: 0.58 (solvent A) and 0.00 (solvent B); EI-MS: Calculated for $C_{15}H_{10}O_7$ 302; Found *m/z* 302 $[M]^+$, 153 $[A_1+H]^+$ and 137 $[B_2]^+$; ^{1}H-NMR (400 MHz, δ, $(CD_3)_2CO$) : 6.30 (1H, *d*, *J*=1.9 Hz, H-6), 6.55 (1H, *d*, *J*=1.9 Hz, H-8), 7.02 (1H, *d*, *J*=8.3 Hz, H-5'), 7.70 (1H, *dd*, *J*=8.3 Hz and *J*=2.1 Hz, H-6'), 7.80 (1H, *d*, *J*=2.1 Hz, H-2'); ^{13}C-NMR (100 MHz, δ, $(CD_3)_2CO$): 94.94 (C-8), 99.55 (C-6), 104.36 (C-10), 116.11 (C-2'), 116.61 (C-5'), 121.89 (C-1'), 123.84 (C-6'), 137.04 (C-3), 146.26 (C-3'), 147.89 (C-2), 148.78 (C-4'), 158.03 (C-9), 162.04 (C-5), 165.49 (C-7), 177.03 (C-4).

*Compound **3** (Cinnamic acid)*

White crystal; $FeCl_3$ test: Negative; Vanillin-HCl-EtOH test: Negative; UV light: Dark spot; R_f: 0.76 (solvent A) and 0.00 (solvent B); EI-MS: Calculated for $C_9H_8O_2$ 148; Found $[M]^+$ *m/z* 148, $[M\text{-}OH]^+$ *m/z* 131 and $[M\text{-}COOH]^+$ *m/z* 103; ^{1}H-NMR (400 MHz, δ, CD_3OD): 6.47 (1H, *d*, *J*=16.0 Hz, H-8), 7.38 (2H, *m*, H-3,5), 7.38 (1H, *m*, H-4), 7.57 (2H, *m*, H-2,6), 7.66 (1H, *d*, *J*=16.0 Hz, H-7); ^{13}C-NMR (100 MHz, δ, CD_3OD): 118.39 (C-8), 128.18 (C-3,5), 129.01 (C-2,6), 130.40 (C-4), 134.83 (C-1), 145.30 (C-7), 169.38 (C-9).

*Compound **4** (Gallic acid)*

Yellowish amorphous powder; Vanillin-HCl-EtOH test: Positive (yellow); R_f: 0.54 (solvent A) and 0.41 (solvent B); EI-MS: Calculated for $C_7H_6O_5$ 170; Found $[M]^+$ *m/z* 170, $[M\text{-}OH]^+$ *m/z* 153 and $[M\text{-}COOH]^+$ *m/z* 125; ^{1}H-NMR (400 MHz, δ, CD_3OD): 7.13 (2H, *s*, H-2,6); ^{13}C-NMR (100 MHz, δ, CD_3OD): 109.94 (C-2,6), 122.43 (C-1), 138.60 (C-4), 145.83 (C-3,5), 170.78 (C-7).

*Compound **5** (Cistanoside F)*

Yellowish amorphous powder; Vanillin-HCl-EtOH test: Positive (yellow); R_f: 0.60 (solvent A) and 0.72 (solvent B); $FeCl_3$ test: Positive (dark brown); Melting point: 129-130 ℃; Optical rotation: $[\alpha]_D^{20}$ -79.6 °(*c,* 0.0025 in MeOH); MALDI-TOF-MS: Calculated for $C_{21}H_{28}O_{13}$ 488; Found $[M+Na]^+$ *m/z* 511, $[M+K]^+$ *m/z* 527 and $[2M+Na]^+$ *m/z* 999; ^{1}H (400 MHz, δ, CD_3OD) and ^{13}C-NMR (100 MHz, δ, CD_3OD): *α*-form and *β*-form, see Table 1.

Table 1. ^{1}H and ^{13}C-NMR spectral data of compound **5** in CD_3OD

Position	δ_C *α*/*β*-form	δ_H (multi. and J_{HH} (Hz)) *α*/*β*-form
Caffeoyl		
1	127.65/127.69	–
2	115.21*	7.07 (1H, *d*, 2.0)/7.06 (1H, *d*, 2.0)
3	146.82*	–
4	149.74/149.78	–
5	116.51*	6.79 (1H each, *d*, 8.1)*
6	123.24*	6.97 (1H, *dd*, 8.1&2.0)/6.96 (1H, *dd*, 2.0&8.1)
7	147.90/148.01	7.60 (1H each, *d*, 15.9)*
8	114.70/114.85	6.29 (1H, *d*, 15.9)/6.28 (1H, *d*, 15.9)
9	168.42/168.33	–
Glucose		
1'	94.04/98.16	5.13 (1H, *d*, 3.6)/4.56 (1H, *d*, 7.9)
2'	76.22/77.38	3.56 (1H, *m*)/3.38 (1H, *m*)
3'	79.21/81.73	4.06 (1H, *m*)/3.81 (1H, *m*)
4'	70.70/70.80	4.94 (1H, *m*)/4.93 (1H, *m*)
5'	71.23/74.71	4.02 (1H, *m*)/3.58 (1H, *m*)
6'a,b	62.37/62.50	3.18 (1H, *m*), 3.49 (1H, *m*)
Rhamnose		
1''	103.03/103.12	5.20 (1H, *d*, 1.6)/5.15 (1H, *d*, 1.6)
2''	72.32*	3.94 (1H each, *m*)*
3''	72.06*	3.61 (1H each, *m*)*
4''	73.78/73.82	3.32 (1H, *m*)/3.31 (1H, *m*)
5''	70.38/70.70	3.58 (1H, *m*)/3.57 (1H, *m*)
6''	18.48*	1.10 (3H each, *d*, 6.1)*

*: Overlapping signals

*Compound **6** (Acteoside)*

Yellowish amorphous powder; $FeCl_3$ test: Positive (dark brown); Melting point: 135-136 ℃; Optical rotation: $[\alpha]_D^{20}$ -79.8 °(*c,* 0.0025 in MeOH); R_f: 0.63 (solvent A) and 0.68 (solvent B); FAB-MS: Calculated for $C_{29}H_{36}O_{15}$ 624; Found $[M+H]^+$ *m/z* 625 and $[M+Na]^+$ *m/z* 647; ^1H (400 MHz, δ, CD_3OD) and ^{13}C-NMR (100 MHz, δ, CD_3OD): See Table 2.

Table 2. ^1H and ^{13}C-NMR spectral data of compounds **6** and **7** in CD_3OD

	δ_C		δ_H (multi. and J_{HH} (Hz))	
Position	**6**	**7**	**6**	**7**
Aglycone				
1	131.50	131.43	–	–
2	117.15	117.12	6.70 (1H, *d*, 1.8)	6.68 (1H, *d*, 1.9)
3	146.15	146.15	–	–
4	144.69	144.68	–	–
5	116.35	116.40	6.68 (1H, *d*, 8.2)	6.64 (1H, *d*, 8.1)
6	121.31	121.31	6.56 (1H, *dd*, 8.2, 1.8)	6.53 (1H, *dd*, 8.1, 1.9)
7a,b	36.59	36.71	2.79 (1H, *m*)	2.78 (1H, *m*)
8a,b	72.29	72.38	3.72 (1H, *m*), 4.04 (1H, *m*)	3.72 (1H, *m*), 3.96 (1H, *m*)
Caffeoyl				
1''	127.68	127.72	–	–
2''	115.26	115.13	7.06 (1H, *d*, 1.9)	7.04 (1H, *d*, 2.0)
3''	146.85	146.80	–	–
4''	149.82	149.65	–	–
5''	116.56	116.58	6.78 (1H, *d*, 8.2)	6.77 (1H, *d*, 8.2)
6''	123.27	123.20	6.95 (1H, *dd*, 8.2, 1.9)	6.68 (1H, *dd*, 2.0, 8.2)
7''	148.06	147.29	7.59 (1H, *d*, 15.8)	7.56 (1H, *d*, 15.9)
8''	114.72	114.87	6.28 (1H, *d*, 15.8)	6.29 (1H, *d*, 15.9)
9''	168.34	169.18	–	–
Glucose				
1'	104.22	104.41	4.38 (1H, *d*, 7.8)	4.33 (1H, *d*, 7.9)
2'	76.22	75.72	3.39 (1H, *m*)	3.35 (1H, *m*)
3'	81.70	83.99	3.81 (1H, *m*)	3.53 (1H, *m*)
4'	70.60	70.42	4.93 (1H, *m*)	3.41 (1H, *m*)
5'	76.04	75.43	3.54 (1H, *m*)	3.55 (1H, *m*)
6'a,b	62.38	64.66	3.52 (1H, *m*), 3.62 (1H, *m*)	4.35 (1H, *m*), 4.50 (1H, *m*)
Rhamnose				
1'''	103.07	102.74	5.19 (1H, *d*, 1.4)	5.18 (1H, *d*, 1.3)
2'''	72.37	72.45	3.92 (1H, *m*)	3.94 (1H, *m*)
3'''	72.07	72.29	3.59 (1H, *m*)	3.69 (1H, *m*)
4'''	73.81	74.03	3.29 (1H, *m*)	3.39 (1H, *m*)
5'''	70.45	70.07	3.57 (1H, *m*)	4.00 (1H, *m*)
6'''	18.49	17.91	1.09 (3H, *d*, 6.0)	1.25 (3H, *d*, 6.0)

*Compound **7** (Isoacteoside)*

Yellowish amorphous powder; Vanillin-HCl-EtOH test: $FeCl_3$ test: Positive (dark brown); Melting point: 134-135 ℃; Optical rotation: $[\alpha]_D^{20}$ -47.5 °(*c,* 0.0025 in MeOH); R_f: 0.43 (solvent A) and 0.39 (solvent B); FAB-MS: Calculated for $C_{29}H_{36}O_{15}$ 624; Found

$[M+Na]^+$ *m/z* 647; ^1H (400 MHz, δ, CD_3OD) and ^{13}C-NMR (100 MHz, δ, CD_3OD): See Table 2.

*Compound **8** (Campneoside II)*

Yellowish amorphous powder; $FeCl_3$ test: Positive (dark brown); Melting Point: 157-159 ℃; Optical rotation: $[\alpha]_D^{20}$ -61.6 °(*c*, 0.0025 in MeOH); R_f: 0.72 (solvent A) and 0.69 (solvent B); MALDI-TOF-MS: Calculated for $C_{29}H_{36}O_{16}$ 640; Found $[M+Na]^+$ *m/z* 663, $[M+K]^+$ *m/z* 679, $[2M+Na]^+$ *m/z* 1303 and $[2M+K]^+$ *m/z* 1319; ^1H (400 MHz, δ, CD_3OD) and ^{13}C-NMR (100 MHz, δ, CD_3OD): See Table 3.

Table 3. ^1H and ^{13}C-NMR spectral data of compound **8** in CD_3OD

Position	δ_C	δ_H (multi. and J_{HH} (Hz))
Aglycone		
1	133.61/133.87	–
2	114.65/114.67	6.85 (1H each, *d*, 2.0)*
3	146.12/146.03	–
4	146.31/146.26	–
5	116.21/116.17	6.77 (1H each, *d*, 8.1)*
6	119.00/119.11	6.56 (1H each, *dd*, 8.1&2.0)*
7	73.57/74.21	4.75 (1H each, *m*)*
8	76.18/76.69	3.95 (1H, *m*), 3.60 (1H, *m*)/3.83 (1H, *m*), 3.71 (1H, *m*)
Caffeoyl		
1''	127.66*	–
2''	115.24*	7.06 (1H each, *d*, 1.2)*
3''	146.85*	–
4''	149.82*	–
5''	116.54*	6.80 (1H each, *d*, 8.3)*
6''	123.28*	6.96 (1H each, *dd*, 8.3&1.2)*
7''	148.08*	7.60 (1H each, *d*, 15.8)*
8''	114.79*	6.28 (1H each, *d*, 15.8)*
9''	168.30/168.29	–
Glucose		
1'	104.60/104.06	4.43 (1H, *d*, 7.8)/4.41 (1H, *d*, 7.8)
2'	76.12/76.43	3.42 (1H each, *m*)*
3'	81.31/81.43	3.82 (1H each, *m*)*
4'	70.45*	4.92 (1H each, *m*)*
5'	76.07/76.09	3.54 (1H each, *m*)*
6'a,b	62.32*	3.52 (1H each, *m*)*, 3.61 (1H each, *m*)*
Rhamnose		
1'''	102.94/103.00	5.22 (1H, *s*)/5.20 (1H, *s*)
2'''	72.37*	3.96 (1H each, *m*)*
3'''	72.06*	3.60 (1H each, *m*)*
4'''	73.79*	3.31 (1H each, *m*)*
5'''	70.38*	3.56 (1H each, *m*)*
6'''	18.48*	1.07 (3H each, *d*, 6.0)*

*: Overlapping signals

*Compound **9** (Isocampneoside II)*

Yellowish amorphous powder; $FeCl_3$ test: Positive (dark brown); Melting Point: 153-154 ℃; Optical rotation: $[\alpha]_D^{20}$ -39.4 °(*c*, 0.0025 in MeOH); R_f: 0.26 (solvent A) and 0.31 (solvent B); MALDI-TOF-MS: Calculated for $C_{29}H_{36}O_{16}$ 640; Found $[M+Na]^+$ *m/z* 663, $[M+K]^+$ *m/z* 679, $[2M+Na]^+$ *m/z* 1303 and $[2M+K]^+$ *m/z* 1319; ^1H (400 MHz, δ, CD_3OD) and ^{13}C-NMR (100 MHz, δ, CD_3OD): See Table 4.

Table 4. ^1H and ^{13}C-NMR spectral data of compound **9** in CD_3OD

Position	δ_C	δ_H (multi. and J_{HH} (Hz))
Aglycone		
1	133.78/133.58	–
2	114.56*	6.82 (1H, *d*, 1.5)/6.83 (1H, *d*, 1.5)
3	146.01/146.08	–
4	146.24/146.30	–
5	116.21/116.25	6.66 (1H each, *d*, 8.2)*
6	119.13/119.01	6.69 (1H each, *dd*, 8.2&1.5)*
7	73.55/74.23	4.72 (1H, *t*)/4.74 (1H, *t*)
8	76.44/76.84	3.87 (1H, *m*), 3.57 (1H, *m*)/3.82 (1H, *m*), 3.70 (1H, *m*)
Caffeoyl		
1''	127.70/127.67	–
2''	115.06/115.04	7.03 (1H, *d*, 1.9)/7.05 (1H, *d*, 1.9)
3''	146.78*	–
4''	149.65*	–
5''	116.57*	6.78 (1H each, *d*, 8.1)*
6''	123.26*	6.86 (1H, *dd*, 8.1&1.9)/
7''	147.31*	7.54 (1H each, *d*, 15.8)*
8''	114.79*	6.27 (1H each, *d*, 15.8)*
9''	169.15*	–
Glucose		
1'	104.30/104.78	4.40 (1H, *d*, 7.9)/4.37 (1H, *d*, 6.0)
2'	75.51/75.65	3.40 (1H each, *m*)*
3'	83.54/83.45	3.58 (1H each, *m*)*
4'	70.26/70.22	3.41 (1H each, *m*)*
5'	75.94*	3.59 (1H each, *m*)*
6'a,b	64.58*	4.33 (1H each, *m*)*, 4.50 (1H each, *m*)*
Rhamnose		
1'''	102.66/102.61	5.22 (1H, *d*, 1.3)/5.20 (1H, *d*, 1.4)
2'''	72.25*	3.94 (1H each, *m*)*
3'''	72.37*	3.73 (1H each, *m*)*
4'''	74.00*	3.41 (1H each, *m*)*
5'''	70.04*	4.01 (1H each, *m*)*
6'''	17.92*	1.25 (3H each, *d*, 6.0)*

*: Overlapping signals

Chemotaxonomic Significance of the Phenolic Extractives

The genus *Paulownia*, comprising 6-17 species in the Scrophulariaceae family, is characterized by producing several types of secondary metabolites, including iridoids

(Damtoft and Jensen 1993), lapachol type naphthoquinones (Huang et al. 2004), and lignans (Okazaki et al. 1997; Ayres and Loike 1990; Takahash and Nakagawa 1963). Previously, we elucidated new epimeric phenylethanoid glycosides from *Paulownia coreana* (Kim et al., 2008, 2007; Si et al. 2008a, 2008b).

In this work, nine phenolic extractives (naringenin (**1**), quercetin (**2**), cinnamic acid (**3**), gallic acid (**4**), cistanoside F (**5**), acteoside (**6**), isoacteoside (**7**), campneoside II (**8**), and isocampneoside II (**9**)) were isolated from the bark of *P. tomentosa* var. *tomentosa* for the first time. What is noteworthy is that naringenin (**1**), quercetin (**2**), cinnamic acid (**3**), gallic acid (**4**), and have never been obtained from *Paulownia* genus previously. Also, this was the first time of report cistanoside F (**5**) from the title tree species.

Phenylpropanoid glycosides in *Paulownia* species have widely been reported, such as cistanoside F (**5**) has been purified from inner bark of *P. coreana* (Kim et al. 2007); acteoside (**6**) and isoacteoside (**7**) have been isolated from sapwood (Ota et al. 1993), leaf (Schilling et al. 1982), stem (Jang 1992), young plant (Damtoft and Jensen 1993), and flower (Du 2003) of *P. tomentosa*; campneoside I from wood of *P. tomentosa* var. *tomentosa* (Si et al. 2008a), flower (Du 2003), and stem (Jang 1992) of *P. tomentosa*; isocampneoside I from wood of *P. tomentosa* var. *tomentosa* (Si et al. 2008a); campneoside II (**8**) from flower (Du 2003) and stem (Jang 1992) of *P. tomentosa*; isocampneoside II (**9**) and campneoside II (**8**) from wood of *P. tomentosa* var. *tomentosa* (Si et al. 2008b), inner bark, and leaf of *P. coreana* (Kim et al. 2008, 2007); ilicifolioside A and isoilicifoliodise A from wood of *P. tomentosa* var. *tomentosa* (Si et al. 2008b). Thus, the isolation of the five phenylpropanoid glycosides (**5**–**9**) in such a significant amount here in bark of *P. tomentosa* var. *tomentosa* was interesting and phenylpropanoid glycosides may possibly be considered as a useful chemotaxonomic marker within the species of *Paulownia*.

CONCLUSIONS

1. Based on the successive Sephadex LH-20 column chromatographic separation of a portion of the *n*-BuOH soluble fraction, which was extracted with 95% EtOH from bark of *P. tomentosa* var. *tomentosa* aided by TLC, nine phenolic extractives, including two flavonoids (naringenin (**1**), and quercetin (**2**)), two phenolic acids (cinnamic acid (**3**), and gallic acid (**4**)), and five phenylpropanoid glycosides (cistanoside F (**5**), acteoside (**6**), isoacteoside (**7**), campneoside II (**8**), and isocampneoside II (**9**)), were isolated.
2. To the best of knowledge, this was the first time that the nine phenolic extractives from bark of *P. tomentosa* var. *tomentosa* have been reported. In this work, compounds **1**–**4** found their first occurrence in *Paulownia*. Particularly, the isolation of the five phenylpropanoid glycosides (**5**–**9**) in such a significant amount here in bark of *P. tomentosa* var. *tomentosa* was interesting, and phenylpropanoid glycosides may possibly be considered as a useful chemotaxonomic marker within the species of *Paulownia*.
3. Phenylpropanoid glycosides, which are significant anti-cytotoxic reagents (Inoue et al.

1998), have been obtained from the title plant. Hence, this investigation will provide ample opportunities for further investigation to develop high value added products from *P. tomentosa* var. *tomentosa*. Also, the phenylpropanoid glycosides obtained in this work shall give useful clues in chemotaxonomic study of *Paulownia* species.

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