Activity-Guided Screening of the Antioxidants from *Paulownia tomentosa* var. *tomentosa* Bark

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Tree barks, as a type of forestry residues, are a rich and renewable bioresource that can produce high value-added products. *Paulownia tomentosa* var. *tomentosa (PTT)* has been extensively used in traditional Chinese medicine to cure various diseases. However, the antioxidative activity of the chemical constituents of the tree has not yet been investigated. In this study, the bark of *PTT* were extracted and fractioned. Then the resulting ethyl acetate (EtOAc) soluble fraction, which exhibited the strongest antioxidative effect, was subjected to repeated open column chromatography for purification. The screening process was carried out under the guidance antioxidative activity via diphenylpicrylhydrazyl (DPPH) radical scavenging assay. Eight phenolic compounds, glucodistylin (**I**), luteolin (**II**), ellagic acid (**III**), cistanoside F (**IV**), campneoside II (**V**), isocampneoside II (**VI**), verbascoside (**VII**), and isoverbascoside (**VIII**), were isolated and their structures were elucidated by various spectroscopic analyses. Among the phenolics, **II**~**VIII** showed significant antioxidative activity.

Keywords: Isolation and purification; Structure elucidation; Total phenolic contents; Paulownia tomentosa var. tomentosa; Antioxidative activity; Activity-guided screening

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INTRODUCTION

Some reactive oxygen species (ROS) and free radicals have been widely accepted as being harmful to human health by triggering many diseases, including cancer, inflammatory disorders, coronary heart diseases (*e.g.*, arteriosclerosis), and aging (Hosoya *et al.* 2008; Lane 2008; Si *et al.* 2011a). Synthetic antioxidants, such as tertiary butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT), and butylated hydroxyl-anisole (BHA), are effective, but may possess mutagenic activity (Tepe *et al.* 2006). Fortunately, natural antioxidants from plants, either as crude solvent extracts or as individually isolated compounds, can dramatically decrease the risk of the above-mentioned diseases and show no or negligible side effects (Si *et al.* 2009a). Accordingly, considerable effort has been directed towards finding safe, effective, and naturally occurring antioxidants from plants.

Paulownia tomentosa var. *tomentosa* (*PTT*) (Scrophulariaceae), a variety of *P. tomentosa*, is a hardwood native to China and distributed throughout eastern and southern Asian countries (Hong *et al.* 1998; Si *et al.* 2008). The leaves of this variety are densely hairy, which distinguishes it from the sparse-haired leaves of other *P. tomentosa* varieties

(Hong *et al.* 1998; Smejkal *et al.* 2007). In traditional medicine, the bark, fruit, xylem, and leaves of *PTT* are used to cure or prevent various disorders, including hemorrhoid, carbuncle, inflammatory bronchitis, gonorrhea, upper respiratory tract infection, parotitis, asthma, traumatic bleeding, erysipelas, bacteriologic diarrhea, swelling, bronchopneumonia, enteritis, conjunctivitis, high blood pressure, and tonsillitis (Jiang 2003; Jiangsu New Medical College 1977). Notably, it has been proposed that the antioxidant constituents of *PTT* account for its beneficial therapeutic effects (Jiangsu New Medical College 1977). Our previous phytochemical investigation of the acetone extracts of *PTT* led to the isolation of one new apigenin and three previously known apigenin derivatives, elucidated as 7-caffeoyl-4′-methoxylapigenin, apigenin-7-*O*-*β*-D-glucopyranoside, apigenin-7-*O*-*β*-D-glucuronopyranoside, and apigenin-7-*O*-*β*-D-glucuronopyranosyl(1→2)-*O*-*β*-D-glucuronopyranoside (Si *et al.* 2009b). From the *n*-BuOH-soluble fraction of the extracts from *PTT* barks, nine low molecular weight extractives with chemotaxonomic significance were obtained (Si *et al.* 2011a). However, the antioxidative potency evaluation of *PTT* and its individual antioxidative compounds purification have not been carried out to date.

Phenolics are secondary plant metabolites that are involved in a wide range of specialized physiological functions. They appear to be very important for the normal growth, development, and defense mechanisms of plants (Rusak *et al.* 2008). These compounds are capable of modulating the activity of many enzymes, suggesting their involvement in biochemical and physiological processes, not only in plants, but also in animals and humans (Di Carlo *et al.* 1999). The medicinal use of extracts prepared from *PTT* barks dated back to ancient times, and we assumed that its antioxidative phenolic components may account for its medicinal value.

For antioxidative activity determination, there are many methods of assessment, including *in vitro* and *in vivo* assays. The antioxidative activity of the chemical constituents may vary depending upon the evaluation protocol. However, DPPH is a radical scavenging, which is an easy to handle and accurate assay and one of the most used and effective probes for studying antioxidative effects (Blois 1958). As one chain of our systematically searching potential antioxidants from woody plants, the 95% EtOH extracts of *PTT* bark were investigated in this work. Under the guidance of DPPH radical scavenging assay, fractionation and purification of the 95% ethanol extracts resulted in the isolation of eight compounds. Structure elucidation of the isolated compounds were primarily analyzed by NMR and mass spectroscopy and compared with reported data.

EXPERIMENTAL

General Experiment Procedures

^{1}H- and ^{13}C-NMR, DEPT (distortionless enhancement by polarization transfer), and 2D NMR spectra including (heteronuclear multiple-bond correlation), HMQC (Heteronuclear single-quantum correlation spectroscopy), and TOCOSY (Total correlation spectroscopy) were recorded in MeOH-d_4, DMSO-d_6, or acetone-d_6 (Sigma) with tetramethylsaline (Sigma) as an internal standard. NMR spectra were obtained on a Bruker Avance DPX 400 spectrometer at an operating frequency of 400 MHz (^{1}H) and 100 MHz (^{13}C) at the Central Laboratory of Kangwon National University, Korea, and State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, P.R. China. EI-MS (electron ionization mass) and positive FAB-MS (fast

atom bombardment mass) spectroscopy were carried out using a Micromass Autospec M363 spectrometer, and MALDI-TOF-MS (matrix assisted laser desorption ionization/time of flight mass spectroscopy) was performed with a Model Voyager-DE STR spectrometer at the Central Laboratory of Kangwon National University, Korea.

Open column chromatography was carried out with Sephadex LH-20 and silica gel (Merck). An SBS-160 fraction collector was used to collect the eluents. Solvent evaporations were done with a RE-52AA rotary evaporator (Shanghai Yarong Biochemical Equipment Company). Thin layer chromatography (TLC) analyses, 1D and 2D, were conducted on DC-Plastikfolien Cellulose F (Merck) plates and developed with *t*-BuOH-HOAc-H_2O (3:1:1, v/v/v) and HOAc-H_2O (3:47, v/v). TLC visualization and detection were carried out by exposing to UV light at 254 and 365 nm wavelengths, and then by spraying with 1% $FeCl_3$ in EtOH or vanillin-HCl-EtOH (60:0.15:6, w/v/v) solutions followed by heating. Samples were freeze-dried with a LGJ-12 lyophilizer (Beijing Songyuan Huaxing Technology Development Co., Ltd).

Plant Materials

PTT bark (5.28 kg in total after air-dried) were collected from Laiwu, Shandong Province, P.R. China in June 20, 2010. The plant materials were authenticated by Dr. Dan Wang (Institute of Chemical Industry of Forest Products, Chinese Academy of Forestry, P.R. China). A voucher specimen (No. CMSCE–100618) was deposited at the herbarium of Tianjin Key Laboratory of Pulp and Paper, College of Materials Science and Chemical Engineering, Tianjin University of Science and Technology, Tianjin, P.R. China.

Extraction and Fractionation

PPT bark was air-dried and ground to a fine powder with a Wiley mill (40-mesh sieve). Then, a precisely weighed amount (3.61 kg) was extracted in a jar (20 L × 5 times) with 95% EtOH (v/v) for 5 days at 20 °C. The combined extracts were filtered and concentrated with a rotary evaporator *in vacuo* to remove the EtOH solvent. The resulting residue mixture was then suspended in H_2O and successively fractionated by liquid-liquid extraction (LLE) in fractionators with a serious of solvents, including *n*-hexane, methylene chloride (CH_2Cl_2), ethyl acetate (EtOAc), and *n*-butanol (*n*-BuOH), followed by freeze-drying to give fractions soluble in *n*-hexane (4.24 g, yield 0.12%), CH_2Cl_2 (3.63 g, yield 0.10%), EtOAc (28.43 g, yield 0.79%), *n*-BuOH (36.15 g, yield 1.01%), and H_2O (226.84 g, yield 6.28%).

DPPH Radical Scavenging Assay

Diphenylpicrylhydrazyl (DPPH) radical scavenging assay was conducted according to the procedure described by Blois (1958) with a minor modification. Methanol solutions (4 mL) of the samples at different concentrations were added to a solution of DPPH (1.5×10^{-4} M, 1 mL) in MeOH in tubes, then the tubes were shaken on a shaker (IKA MS3 basic, Germany) for 10 s. After standing at 20 °C for 30 min, the optical density was measured at 517 nm with a UV-visible spectrophotometer. IC_{50} (50% inhibitory concentration) values were obtained through extrapolation from concentration of sample necessary to scavenge 50% of the DPPH radicals. Butylated hydroxytoluene (BHT) and α-tocopherol were used as positive controls.

Statistical Analyses

All tests were carried out independently in triplicate (n = 3). Data are expressed as the mean ± the standard derivation (SD). Excel 2007 (Microsoft, Redmond, WA USA) was used to process the statistical results of the experiments.

RESULTS AND DISCUSSION

Antioxidant Activity of EtOH Extracts and Its Soluble Fractions

The DPPH radical has been widely accepted as a model compound to assess the antioxidative potency of various antioxidants (Blois 1958; Juma and Majinda 2004). Thus, in this work, a DPPH-free radical scavenging assay was used to evaluate the 95% EtOH extracts and the resulted *n*-hexane, CH_2Cl_2, EtOAc, *n*-BuOH, and H_2O soluble fractions for general antioxidative effect.

Table 1. DPPH Radical Scavenging Activity of 95% EtOH Extracts and Its Soluble Fractions from *PTT* Bark

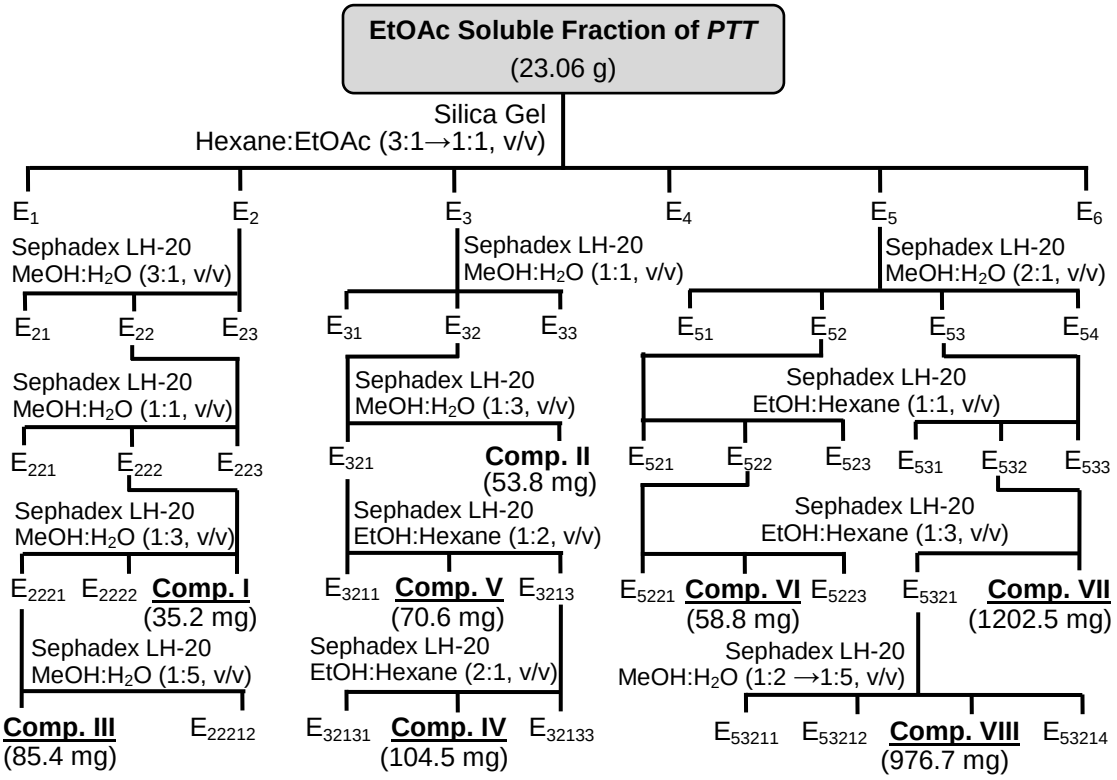
Samples	IC_{50} (μg/mL)[†]
95% EtOH extracts	3.53 ± 0.02
n-Hexane soluble fraction	16.65 ± 0.01
CH_2Cl_2 soluble fraction	5.27 ± 0.02
EtOAc soluble fraction	2.18 ± 0.01
n-BuOH soluble fraction	2.84 ± 0.02
H_2O soluble fraction	3.41 ± 0.02
BHT[‡]	1.99 ± 0.01
α-Tocopherol[‡]	1.94 ± 0.01

[†] Values are mean±SD of three independent trials; [‡] Positive controls.

As demonstrated in Table 1, overall, the antioxidant activity of the extracts and soluble fractions evaluating against DPPH free radical decreased in the following order: EtOAc > *n*-BuOH > H_2O > EtOH > CH_2Cl_2 > *n*-hexane fraction. These results indicated that the EtOAc soluble fraction, which could scavenge 50% DPPH radicals with the lowest inhibitory concentration, exhibited the most significant antioxidative ability comparable with positive controls of BHT and α-tocopherol, while the other soluble fractions showed weaker or negligible activities. It was evident that the EtOAc soluble fraction could be a promising source for antioxidants, which might serve as excellent radical inhibitors or scavengers. Therefore, the EtOAc soluble fraction was further investigated to isolate and purify individual antioxidative compounds.

Purification and Isolation of Individual Components

As shown in Scheme 1, a portion of the above freeze-dried EtOAc soluble fraction powder (23.06 g), which exhibited the strongest antioxidative potency (2.18 ± 0.01 μg/mL) among all the soluble fractions, was applied to a silica gel column with hexane-EtOAc used as the gradient eluent (3:1→1:1, v/v) to give six main fractions labeled E_1 (1.33 g, IC_{50} 6.73 ± 0.01 μg/mL), E_2 (4.12 g, IC_{50} 2.56 ± 0.01 μg/mL), E_3 (5.28 g, IC_{50} 2.27 ± 0.02 μg/mL), E_4 (2.03 g, IC_{50} 4.68 ± 0.02 μg/mL), E_5 (8.15 g, IC_{50} 2.04 ± 0.01 μg/mL), and E_6 (1.46 g, IC_{50} 5.15 ± 0.01 μg/mL).



Scheme 1. Purification and Isolation procedure of individual components from bark of *PTT*

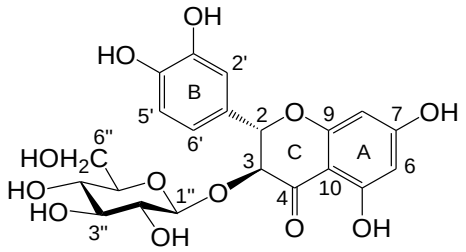
Fraction E_2, which exhibited significant DPPH radical scavenging, was loaded to a Sephadex LH-20 column chromatography using MeOH-H_2O (3:1, v/v) for further purification to yield three subfractions E_{21}, E_{22}, and E_{23}, among which, E_{22} (3.06 g) revealed the strongest antioxidative activity (IC_{50} 2.30 ± 0.01 μg/mL). The E_{22} fraction was re-subjected to a Sephadex LH-20 column with MeOH-H_2O gradient eluent (1:1→1:3→1:5, v/v) to separate compounds **I** (35.2 mg) and **III** (85.4 mg) (refer to Fig. 2). The E_3 fraction was likewise eluted with MeOH-H_2O (1:1 and 1:3, v/v) and EtOH-hexane (1:2 and 2:1, v/v) over a Sephadex LH-20 column, and compounds **II** (53.8 mg), **IV** (104.5 mg), and **V** (70.6 mg) were obtained. Fraction E_5, which exhibited the most significant DPPH radical scavenging, was also purified over a Sephadex LH-20 column using MeOH-H_2O (2:1, v/v) as the mobile phase to obtain four sub-fractions E_{51}–E_{54}. Sub-fractions E_{52} and E_{53}, which showed strongest radical scavenging potential, were reloaded to a Sephadex LH-20 column for purification with EtOH-hexane (1:1→1:3, v/v) and MeOH-H_2O (1:2→1:5, v/v) used as the eluting solvent gradients to yield 58.8 mg of compound **VI**, 1202.5 mg of compound **VII**, and 976.7 mg of compound **VIII**.

Identification and Structure Elucidation of the Isolated Compounds

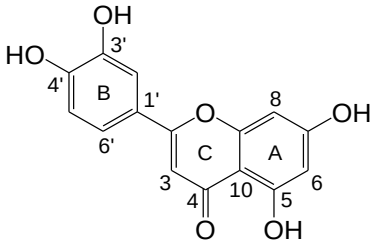
Through repeated open column chromatography and further DPPH radical assays of the active EtOAc soluble fraction, 8 compounds (**I**~**VIII**, as presented in Fig. 1) were isolated and purified.

Compound **I**, obtained as amorphous powder, revealed a gray-green color spot on a cellulose plate when spraying 1% $FeCl_3$, which indicated the presence of a phenolic

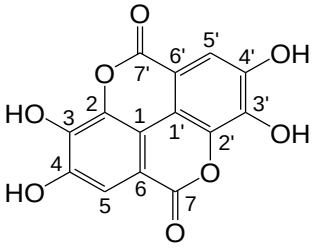
hydroxyl group in the molecule (Imakura *et al*. 1985; Si *et al*. 2008). In an FAB-MS spectrum, **I** exhibited an $[M+H]^+$ ion peak at *m/z* 467 as the base peak, suggesting the molecular formula $C_{21}H_{22}O_{12}$. In ^{1}H-NMR spectrum of **I**, the presence of two *meta* coupled doublets (J = 1.9 Hz) in aromatic region at δ 5.89 and 5.94 were assigned to H-6 and H-8. Also, one *ortho* coupled doublet at δ 6.77 (1H, *d*, J = 8.1 Hz, H-5'), a double doublet at δ 6.83 (1H, *dd*, J = 8.1 & 1.9 Hz, H-6'), and a *meta* coupled doublet at δ 6.96 (1H, *d*, J = 1.9 Hz, H-2') indicated the typical ABX proton system B-ring. The presence of a β-configuration glucose was evidenced by a doublet (J = 7.5 Hz) at δ 3.87, together with 6 protons between δ 3.12~3.78 (Andary *et al*. 1982). In support of these assignments, long range HMBC spectra of **I** were observed between anomeric proton H-1" (δ 3.87) and C-3 (δ 76.14). The above elucidation and ^{13}C-NMR data of **I** were in correspondence with glucodistylin (Ishimaru *et al*. 1988).



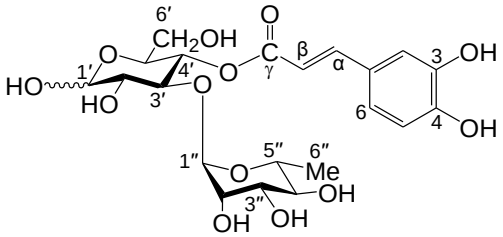
Glucodistylin (**I**)



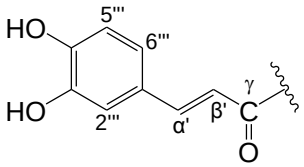
Luteolin (**II**)



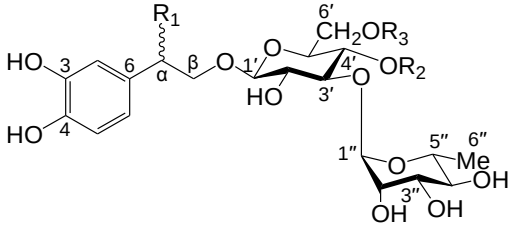
Ellagic acid (**III**)



Cistanoside F (**IV**)



(*E*)-caffeoyl



Campneoside II (**V**): R_1 = OH, R_2 = (*E*)-caffeoyl, R_3 = H
Isocampneoside II (**VI**): R_1 = OH, R_2 = H, R_3 = (*E*)-caffeoyl
Verbascoside (**VII**): R_1 = H, R_2 = (*E*)-caffeoyl, R_3 = H
Isoverbascoside (**VIII**): R_1 = R_2 = H, R_3 = (*E*)-caffeoyl

Fig. 1. Phenolic extractives (**I~VIII**) isolated from bark of *PTT*

Compound **II**, an amorphous powder, gave a gray-green color on a cellulose plate when 1% $FeCl_3$ sprayed or yellow with vanillin-HCl-EtOH. R_f values were 0.57 (solvent A) and 0.08 (solvent B) on TLC plate. The molecular formula of **II** was determined as $C_{15}H_{10}O_6$ based on EI-MS (M^+ *m/z*, 286). In ^{1}H-NMR spectrum, the 5,7-disubstitution pattern of A-ring of compound **II** was suggested by the two doublets at δ 6.53 (H-8) and 6.26 (H-6) and with a *meta* coupling constant value (J = 1.9 Hz). The aromatic ABX coupled system at δ 7.02, 7.48 and 7.50 was assigned to H-5', 6' and 2', respectively, on B-ring. In ^{13}C-NMR spectrum, peaks at δ 146.48 and 150.10 were originated from hydroxy-bearing C-3' and 4', while signals at δ 114.12, 116.63, 120.14 and 123.75 were derived from 2', 5', 6', and 1', indicating the catechol B-ring. Based on the above evidences and a comparison with literature (Agrawal 1989), **II** was identified as luteolin.

Compound **III**, isolated as an amorphous powder with R_f values 0.14 and 0.02 in solvents A and B, respectively, presented gray-green on TLC when reacting with $FeCl_3$. The positive FAB-MS spectrum of **III** showed its molecular ion peak $[M+H]^+$ at *m/z* 303, well coincided with $C_{14}H_6O_8$. ^{1}H-NMR spectrum of compound **III** displayed only one singlet at δ 7.48 integrated to two protons due to a pair of symmetric protons H-5 and 5'. ^{13}C-NMR spectrum of **III** forwarded instinctive carboxylic acid carbons at δ 159.06 attributed to C-7 and 7'. C-5 and 5', the protonated aromatic carbons, and C-3,3'/C-4,4', the hydroxy-bearing carbons, gave three strong peaks at δ 110.12, 139.49 and 148.01, respectively, because of symmetrical skeleton. The rest six quaternary carbons at δ 107.53, 136.27, and 112.23 were assigned to C-1,1', C-2,2', and C-6,6', respectively. On the basis of foregoing spectral clues and a direct comparison of authentic samples (Ishimaru *et al.* 1988; Nawwar *et al.* 1994), **III** was elucidated as ellagic acid.

Compounds **IV**~**VIII** were all isolated as yellow amorphous powders. The presence of the phenolic hydroxyl groups in the molecules were recognized from the gray-green color arose with ethanolic $FeCl_3$ solution on TLC (Imakura *et al*. 1985; Si *et al*. 2008). We have previously reported the isolation of **IV**~**VIII** from *Paulownia corena* and structure elucidation from their physiochemical data (Kim *et al*. 2007 and 2008).

Compound **I**: Yellowish amorphous powder; $FeCl_3$ test: gray-green; R_f: 0.64 (solvent A) & 0.53 (solvent B); FAB-MS: $[M+H]^+$ *m/z* 467; ^{1}H-NMR (400 MHz, δ, MeOH-d_4): 3.12~3.30 (4H, *m*, H-2",3",4",5"), 3.60 (1H, *dd*, J = 12.0 & 5.8 Hz, H_a-6"), 3.78 (1H, *dd*, J = 12.0 & 2.2 Hz, H_b-6"), 3.87 (1H, *d*, J = 7.5 Hz, H-1"), 4.93 (1H, *d*, J = 9.7 Hz, H-3), 5.24 (1H, *d*, J = 9.7 Hz, H-2), 5.90 (1H, *d*, J = 2.0 Hz, H-6), 5.94 (1H, *d*, J = 2.0 Hz, H-8), 6.77 (1H, *d*, J = 8.1 Hz, H-5'), 6.83 (1H, *dd*, J = 8.1 & 1.9 Hz, H-6'), 6.96 (1H, *d*, J = 1.9 Hz, H-2'). ^{13}C-NMR (100 MHz, δ, MeOH-d_4): 61.58 (C-6"), 70.20 (C-4"), 73.53 (C-2"), 76.14 (C-3), 76.51 (C-3"), 77.07 (C-5"), 82.54 (C-2), 96.54 (C-8), 96.55 (C-6), 101.59 (C-1"), 103.82 (C-10), 115.14 (C-2'), 115.39 (C-5'), 120.36 (C-6'), 128.07 (C-1'), 145.26 (C-3'), 146.28 (C-4'), 163.11 (C-9), 164.31 (C-5), 167.90 (C-7), 195.05 (C-4).

Compound **II**: Yellowish amorphous powder; $FeCl_3$ test: gray-green; Vanillin-HCl-EtOH test: yellow; R_f: 0.57 (solvent A) & 0.08 (solvent B); EI-MS: $[M]^+$ *m/z* 286; ^{1}H-NMR (400 MHz, δ, acetone-d_6): 6.25 (1H, *d*, J = 1.9 Hz, H-6), 6.52 (1H, *d*, J = 1.9 Hz, H-8), 6.57 (1H, *s*, H-3), 7.02 (1H, *d*, J = 8.3 Hz, H-5'), 7.48 (1H, *dd*, J = 1.9 Hz and J = 8.3 Hz, H-6'), 7.50 (1H, *d*, J = 1.9 Hz, H-2'); ^{13}C-NMR (100 MHz, δ, acetone-d_6) : 94.70 (C-8), 99.71 (C-6), 104.20 (C-3), 105.33 (C-10), 114.12 (C-2'), 116.63 (C-5'), 120.14 (C-6'), 123.75 (C-1'), 146.48 (C-3'), 150.10 (C-4'), 158.78 (C-9), 163.36 (C-5), 164.90 (C-2), 165.14 (C-7), 183.05 (C-4).

Compound **III**: Yellowish amorphous powder; $FeCl_3$ test: gray-green; R_f: 0.14 (solvent A) & 0.02 (solvent B); FAB-MS: $[M+H]^+$ *m/z* 303; ^{1}H-NMR (400 MHz, δ, DMSO-d_6): 7.48 (2H, *s*, H-5,5'); ^{13}C-NMR (100 MHz, δ, DMSO-d_6): 107.53 (C-1,1'), 110.12 (C-5,5'), 112.23 (C-6,6'), 136.27 (C-2,2'), 139.49 (C-3,3'), 148.01 (C-4,4'), 159.06 (C-7,7').

Compounds **IV**~**VIII**: Yellow amorphous powders; $FeCl_3$ test: gray-green; R_f values, MALDI-TOF-MS, FAB-MS and NMR data: see (Kim *et al*. 2007 and 2008; Si *et al*. 2011b).

Antioxidative Activity of the Isolated Compounds

DPPH radical scavenging assay was thereby conducted to evaluate the antioxidative properties of the isolated individual compounds.

Table 2. DPPH Radical Scavenging Activity of the Phenolics from *PTT* Bark

Samples	IC_{50} (μM)[†]
I	37.58 ± 0.02
II	6.50 ± 0.01
III	5.96 ± 0.01
IV	6.45 ± 0.02
V	5.95 ± 0.01
VI	6.14 ± 0.02
VII	5.99 ± 0.01
VIII	6.22 ± 0.01
BHT[‡]	6.88 ± 0.01
α-Tocopherol[‡]	6.83 ± 0.02

[†] Values are mean ± SD of three independent trials.
[‡] Positive controls.

From the investigation results depicted in Table 2, luteolin, ellagic acid, cistanoside F, campneoside II, isocampneoside II, verbascoside, and isoverbascoside were obviously more efficient than positive controls. Notably, campneoside II, exhibiting the lowest 50% of inhibitory concentration value, indicated it had the most remarkable radical scavenging potency. In contrast, glucodistylin, a flavonoid glycoside in structure, revealed almost no inhibition against the DPPH radical when compared to the standard antioxidants of BHT and α-tocopherol. Our previous studies revealed that free hydroxyl moieties, especially 3,4′-*O*-dihydroxyl units act as free radical scavengers. For flavonoid, the 2,3-double bond combined with a 4-keto group in the C-ring are good radical donators. However, glycosidation will dramatically decrease the flavonoid's antioxidant activity (Si *et al*. 2011a). The results in this investigation were in good agreement with respect to their implications, helping to enrich knowledge of the structure-activity relationships. These observations suggested that the isolated extractives **II**–**VIII** possess significant antioxidative potency, and are possibly responsible for the antioxidative activity of the EtOAc extracted fraction of the *PTT* bark.

CONCLUSIONS

1. Eight phenolics, including two flavonoids (glucodistylin (**I**) and luteolin (**II**)), one phenolic acid (ellagic acid (**III**)), and five phenylpropanoid glycosides (cistanoside F (**IV**), campneoside II (**V**), isocampneoside II (**VI**), verbascoside (**VII**), and

isoverbascoside (**VIII**)), were identified from EtOAc soluble fraction of *PTT* bark, on the basis of their physiochemical data as well as NMR and MS spectroscopic evidence.

2. This was the first report, to our knowledge, of the antioxidants from *PTT*. Results clearly demonstrated that phenolic compounds II~VIII exhibited significant antioxidative activity when compared with the positive controls.

ACKNOWLEDGMENTS

The authors thank the National Natural Science Foundation of China (NSFC, 31170541 and 31000279), Program for New Century Excellent Talents in University (NCET-10-0951), and the Foundation (No. 2012IM002) of Key Laboratory of Industrial Fermentation Microbiology of Ministry of Education, and Tianjin Key Lab of Industrial Microbiology (Tianjin University of Science & Technology) for their financial support.

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Article submitted: August 29, 2012; Peer review completed: November 27, 2012; Revised version received and accepted: December 9, 2012; Published: December 12, 2012.