DOI:10.1021/jf902103b

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Flavonoid Composition and Antioxidant Activity of Tree Peony (*Paeonia* Section *Moutan*) Yellow Flowers

Chonghui Li,[†,§] Hui Du,[†,§] Liangsheng Wang,*[,†] Qingyan Shu,[†] Yuanrun Zheng,[‡] Yanjun Xu,[#] Jingjing Zhang,[†,§] Jie Zhang,[†,⊥] Ruizhen Yang,[†,§] and Yuxuan Ge[†,§]

[†]Beijing Botanical Garden and [‡]Research Center for Plant Ecology and Biodiversity Conservation, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China, [§]Graduate University, Chinese Academy of Sciences, Beijing 100049, China, [#]Department of Applied Chemistry, China Agricultural University, Beijing 100094, China, and [⊥]College of Science, Northwest A&F University, Shaanxi 712100, China

Tree peony flowers are edible and traditional Chinese medicine materials. In the present study, 26 flavonoids were identified and quantified in yellow flowers of tree peony by high-performance liquid chromatography with diode array detector (HPLC-DAD) and by HPLC−electrospray ionization−mass spectrometry (HPLC-ESI-MS). Seventeen of them were first reported in flowers of tree peony, and glycosides of kaempferol, luteolin, and apigenin as well as isosalipurposide were the main flavonoids investigated. Furthermore, the petal extracts showed high antioxidant activity according to DPPH$^{\bullet}$, ABTS$^{\bullet+}$, and OH$^{\bullet}$ scavenging assays and ferric reducing antioxidant power assay. There were significant correlations between antioxidant activity and both the total polyphenol content (determined by Folin−Ciocalteu method) and the total content of quercetin, kaempferol, and luteolin glycosides. This work is valuable for elucidation of phenolic composition in tree peony flowers and for further utilization of them as functional food and medicine materials.

KEYWORDS: Tree peony; yellow flower; flavonoids; polyphenol; antioxidant activity; HPLC-MS

INTRODUCTION

Tree peony (a woody shrub of the section *Moutan*, genus *Paeonia*, family Paeoniaceae) originates in China, including eight species and more than a thousand cultivars. Considered to be a traditional ornamental and medicinal plant with large and beautiful flowers, tree peony has been widely cultivated in Shandong, Henan, Gansu, and Anhui provinces of China and has been introduced to Japan, America, and Europe (*1*). The flowers of tree peony are rich in nutrient proteins, microelements, and vitamins, and they have been used to make various delicious folk foods, such as casseroles, cakes, teas, and drinks since the Song dynasty (A.D. 960−1279) (*2*). Moreover, the flowers and roots of tree peony are used as herbal medicine. The flowers have been prescribed for treatment of diseases related to irregular menstruation and dysmenorrheal for women (*3*). In addition, extracts of flowers are employed to make skin care products, which help to enhance skin flexibility, reduce pigment accumulation, and inhibit fleck formation. It is well-known that degenerative or pathological processes such as atherosclerosis, aging, and cancer in the human body are caused by oxidative damage (*4*), and tree peony flowers are used in traditional functional food and medicine due to their antioxidant activity. The previous study suggests the flower extract efficiently removes superoxide anion ($O_2^{\bullet-}$), hydroxyl radical (OH$^{\bullet}$), and hydrogen peroxide (H_2O_2) and also protects DNA from being damaged by OH$^{\bullet}$ (*5*).

Plants are considered to be natural antioxidant resources because they are rich in phenolic components, which have antimutagenic, anticarcinogenic, anti-inflammation, and antioxidant activities (*4*). The flavonoids especially have been highlighted in recent research because of their potential role as contributing factors to the antioxidant activity of the diet (*6*). Flavonoids existing as glycosides contribute to the bioactivity of tree peony flowers. The anthocyanin fractions in purple, pink, and red flowers had been well investigated (*7*). However, there is limited information of other flavonoids' composition. So far, six flavonoid aglycones, quercetin (Querc), kaempferol (Kaempf), isorhamnetin (Isorhmnt), luteolin (Lutln), apigenin (Apign), and chrysoeriol (Chrysrl), and seven flavonoid glycosides, Kaempf 3,7-di-*O*-glucoside (Kaempf3Glc7Glc), Kaempf 3-*O*-glucoside (Kaempf3Glc), Kaempf 7-*O*-glucoside (Kaempf7Glc), Lutln 7-*O*-glucoside (Lutln7Glc), Apign 7-*O*-glucoside (Apign7Glc), Apign 7-*O*-neohesperidoside (Apign7Neo), and chalcononaringenin 2′-glucoside (isosalipurposide), have been reported in tree peony flowers (*3*, *7*, *8*). Among those flavonoids, it is notable that Querc and Kaempf have high antioxidant activity; Apign and chalcononaringenin (Chalcon) have anticarcinogenic activity (*6*, *9*, *10*).

Tree peony flowers for edible and medicinal use in China mainly refer to traditional Chinese cultivars with purple, pink,

*Address correspondence to this author at the Institute of Botany, Chinese Academy of Sciences, Nanxincun 20, Xiangshan, Beijing 100093, China (telephone +86-10-62836654; fax +86-10-62590348; e-mail wanglsh@ibcas.ac.cn).

 Published on Web 08/27/2009

red, and white colors. Yellow flowers are rare: there are two species, *Paeonia delavayi* Franch. and *Paeonia ludlowii* (Stern et Taylor) D. Y. Hong, and a few cultivars in China as well as several cultivars from America, Japan, and France. To our knowledge, yellow flowers contain abundant flavonoids such as flavones, flavonols, and chalcones compared with other flowers. Nevertheless, their flavonoid composition and antioxidant activity still have not been evaluated systematically. It is of great importance to identify the flavonoids and evaluate the antioxidant activity to supply more scientific evidence for the development of yellow flowers.

High-performance liquid chromatography (HPLC) with a diode array detector (DAD) coupled with electrospray ionization (ESI) mass spectrometry (MS) has provided a reliable technique for the characterization of complex flavonoids according to UV and MS^n data (*11*). For the antioxidant activity evaluation, the 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^•$) and radical monocation of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{•+}$) are widely used for extracts or single compounds (*12*, *13*); besides, hydroxyl radical-scavenging activity (HRSA) and ferric reducing antioxidant power (FRAP) assays, etc., are commonly employed (*14*, *15*). The antioxidant reaction of plant extract is a complex process, and different antioxidant assays would be needed. For the further utilization of tree peony flowers, the objective of this study was to investigate the flavonoid composition and content, total polyphenol content, and antioxidant activities of yellow flowers to estimate their edible and medicinal value.

MATERIALS AND METHODS

Chemicals. Querc 3-*O*-rutinoside (rutin) and chlorogenic acid (CA) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Gallic acid was purchased from Shanghai Tauto Biotech (Shanghai, China). $DPPH^•$, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tripyridyl-*S*-triazine (TPTZ), 2-deoxyribose, ascorbic acid (Vc), butylated hydroxytoluene (BHT), and Folin−Ciocalteu's phenol reagent were purchased from Sigma-Aldrich (St. Louis, MO). Standards of Kaempf3Glc7Glc, Kaempf3Glc, Lutln7Glc, Apign7Glc, Apign7Neo, and Isorhmnt 3-*O*-glucoside (Isorhmnt3Glc) were generously provided by Dr. Xiao Wang (Shandong Analysis and Test Center, Shandong Academy of Sciences, Shandong, China). An authentic sample of Querc 3-*O*-glucoside (Querc3Glc) was obtained from blueberry cultivar 'Blue Crop' (*16*). HPLC grade methanol and acetonitrile were obtained from Promptar (ElK Grove, CA). Water from a Milli-Q System (Millipore, Billerica, MA) was used. All other regents used were of analytical grade.

Plant Materials. Petals of six tree peony cultivars, Chinese 'Yao Huang' (light yellow), American 'Golden Isles' (lemon-yellow petals with dark-red blotch at the base) and 'High Noon' (bright lemon-yellow with dark-red blotch), French 'Souvenir de Maxime Cornu' (bright lemon-yellow with red blotch and border) and 'Alice Harding' (lemon-yellow with red blotch), and Japanese 'Oukan' (bright lemon-yellow with dark-red blotch), were collected at Beijing Botanical Garden, Institute of Botany, Chinese Academy of Sciences (IBCAS), Beijing, China; and petals of two species, *P. delavayi* (variant I, pure bright yellow petals; variant II, bright yellow petals with slight pink at the base) and *P. ludlowii* (yellow) were collected at Yuzhong Peace Peony Garden, Gansu, China, in May 2007 (see the Supporting Information). The petals with blotches were divided into two parts, nonblotches and blotches, which were analyzed separately. All full-blown flower petals were air-dried and stored in a brown glass desiccator at room temperature until use (*17*).

Extraction and Preparation of the Flavonoids. The flavonoid extraction method was modified from that of Yang et al. (*18*). Approximately 0.15 g of each dry sample was extracted with 5 mL of 70% methanol aqueous at 4 °C with exclusion of light for 24 h. The suspension was filtered through a 0.22 μm membrane filter (Shanghai ANPEL Scientific Instrument, Shanghai, China) before the HPLC-DAD and HPLC-MS analyses.

Extraction and Preparation of the Polyphenolic Fractions. Almost 0.15 g of each dry nonblotch sample was crushed in 5 mL of 70% methanol aqueous at 4 °C with exclusion of light for 24 h and then filtered through qualitative filter paper (Hangzhou Special Paper Industry, Zhejiang, China). The filtrate was evaporated at 30 °C, and the residue was dissolved in 1 mL of water and then purified by solid-phase extraction (SPE) cartridge (500 mg, 3 mL) C_{18} Supelclean ENVI-18 cartridge (Supelco Park, Bellefonte, PA), which had been previously activated with methanol and water. The cartridge was successively rinsed with water (to remove sugars, acids, and other interfering substances) and methanol (to elute the polyphenolic fractions). The methanolic eluate was concentrated (at 30 °C), and the residue was redissolved in 1.5 mL of methanol for Folin−Ciocalteu test and antioxidant capacity assays.

HPLC-DAD Analysis. Analytical HPLC was performed with a system from Dionex (Sunnyvale, CA), including a P680 HPLC pump, an UltiMate 3000 autosampler, a TCC-100 thermostated column compartment, and a PDA100 photodiode array detector. The liquid chromatograph was equipped with a 5 μm ODS-80Ts QA C_{18} column (150 × 4.6 mm i.d., Tosoh, Tokyo, Japan), which was protected with a C_{18} guard cartridge (Shanghai ANPEL Scientific Instrument, Shanghai, China). The following solvent and gradient were used: A, 0.1% aqueous formic acid; B, 0.1% formic acid in acetonitrile (*19*); constant gradient from 10 to 22% B within 60 min; the flow rate was 0.8 mL min^{-1}; 10 μL of analytes was injected. Column temperature was maintained at 35 °C, DAD data were recorded from 200 to 800 nm, and chromatograms of flavonoids were recorded at 350 nm.

HPLC-ESI(±)-MS^2 Analysis. HPLC-ESI(±)-MS^2 analysis for flavonoids was carried out in an Agilent-1100 HPLC system equipped with a UV detector coupled to a LC-MSD Trap VL ion-trap mass spectrometer via an ESI source (Agilent Technologies, Palo Alto, CA). The HPLC separation condition was the same as described above. Both positive-ion (PI) and negative-ion (NI) ESI mass spectra were acquired. ESI was performed using capillary voltage of 3.5 kV, a nebulization pressure of 241.3 kPa, and a gas (N_2) temperature of 350 °C with flow rate of 6.0 L min^{-1}. Capillary offset voltage was 77.2 V for PI and −77.2 V for NI. MS and MS^2 spectra were recorded over the range from m/z 50 to 1000. The MS^2 scan of the target ions used the normalized collision energy of 30%.

Quantitive Analysis of Flavonoids. Flavonoids in each sample were measured semiquantitatively by linear regression of rutin and quantified at 350 nm as rutin equivalents. The flavonoids were expressed as milligrams of rutin per 100 mg of dry weight (DW). All of the samples were analyzed in triplicate.

Estimation of the Total Polyphenol Content. Total polyphenol (TP) concentrations in the extracts were determined according to the Folin−Ciocalteu method, using gallic acid as the standard (*20*). The TP concentration was calculated from the calibration curve, [TP (mg mL^{-1})] = $0.0749Abs_{760nm} + 0.0107$ ($r^2 = 0.9997$), and the TP content (TPC) was expressed as milligrams of gallic acid equivalents (mg of GAE) per 100 mg of DW of petals. All of the samples were analyzed in triplicate.

Free Radical Scavenging. *DPPH Assay*. The DPPH assay was used to evaluate the free radical scavenging activity on $DPPH^•$ of each sample, and Vc and BHT were used as the reference samples (*12*). All $DPPH^•$ concentrations were calculated from the calibration curve, [$DPPH^•$ (mg mL^{-1})] = $0.0356Abs_{515nm}$ ($r^2 = 0.9999$). The percentage of remaining $DPPH^•$ ($\%DPPH^•_{REM}$) in each sample tested was calculated according to the formula $\%DPPH^•_{REM}$ = [$DPPH^•$ (mg mL^{-1})]$_t$/[$DPPH^•$ (mg mL^{-1})]$_0$, where t is the time needed to reach the reaction steady state, 0 representing the initial. The ratio of [TP (mg)]/[$DPPH^•$ (mg)] was plotted against $\%DPPH^•_{REM}$ to calculate the efficient concentration (EC_{50}), the amount of sample necessary to decrease the initial $DPPH^•$ concentration by 50% (*20*). The antiradical power (ARP) is calculated as ARP = $1/EC_{50}$ (*12*). All of the samples were analyzed in triplicate.

ABTS Assay. The ABTS assay was based on the method of Re et al. (*13*) with slight modification. $ABTS^{•+}$ reagent was produced by reacting 10 mL of 7 mM ABTS solution with 178 μL of 140 mM potassium persulfate aqueous in the dark at room temperature for 13 h before use. The $ABTS^{•+}$ solution was diluted with ethanol to appropriate absorbance. One-tenth of a milliliter of diluted sample was added to 3.9 mL of diluted

$ABTS^{\bullet+}$ solution to react in the dark at room temperature for 6 min, and the absorbance at 732 nm was recorded. Trolox was used as standard with the final concentration ranging from 0 to 16.5 μM. Results were expressed as Trolox equivalent antioxidant capacity (TEAC, mmol of Trolox/g of DW). All of the samples were analyzed in triplicate.

HRSA Assay. The HRSA assay was performed according to the slightly modified method of Ghiselli et al. (*21*). Briefly, 100 μL of diluted sample, 690 μL of 10 mM phosphate buffer (PBS, pH 7.4) containing 2.5 mM 2-deoxyribose, 100 μL of 1.0 mM iron ammonium sulfate premixed with 1.04 mM EDTA, 100 μL of 1.0 mM Vc, and 10 μL of 0.1 M H_2O_2 were mixed and maintained at 37 °C for 1 h, and then 1.0 mL of cold 2.8% trichloroacetic acid was added followed by 0.5 mL of 1% thiobarbituric acid. The solutions were heated at 100 °C for 15 min and cooled, and the absorbance was measured at 532 nm. CA was used as standard with the final concentration ranging from 0.1 to 2.9 μM. Results were expressed as CA equivalent antioxidant capacity (CEAC, mmol of CA/10 g of DW) (*14*). All of the samples were analyzed in triplicate.

FRAP Assay. The FRAP assay was carried out according to the method of Fang et al. (*15*) with slight modification: the reaction was maintained for 1 h at room temperature. Trolox was used as standard with the final concentration ranging from 0 to 16.5 μM. Results were expressed as TEAC (mmol of Trolox/g of DW). All of the samples were analyzed in triplicate.

Statistical Analysis. One-way analysis of variance test (ANOVA) and bivariate correlate analysis were performed by SPSS 11.5 (SPSS Inc., Chicago, IL). Post hoc comparisons were accomplished with Duncan's test using the same statistical package. The differences were considered to be significant when $p < 0.05$.

RESULTS AND DISCUSSION

Identification of Flavonoids. In general, the common glycosylation of flavonoids occurs at the 7-hydroxyl in flavones and at the 3- and 7-hydroxyls in flavonols. The sugars usually refer to hexose (glucose, galactose, and rhamnose) and pentose (arabinose and xylose) (*22*). On this basis, the flavonoid from the yellow flowers of tree peony were identified according to HPLC retention times, UV−vis, and MS spectra (in both NI and PI modes), as well as reference data. In total, 26 flavonoids were detected, including 19 flavonols, 6 flavones, and 1 chalcone. **Figure 1** shows the flavonoid HPLC profiles in four samples (i.e., *P. delavayi* (variant I), cultivars 'Yao Huang', 'Souvenir de Maxime Cornu', and 'High Noon'). Compound data obtained in the HPLC-DAD analysis (including retention time and UV absorption maxima) and HPLC-ESI(±)-MS^2 analysis (such as molecular ion, aglycone ion, and main fragments observed in MS^2) and the list of samples in which each compound was detected, as well as references for identification, are summarized in **Table 1**.

The aglycone of each compound was identified according to previous research (*7*) by comparing its MS and UV spectra with published data (*23*). Seven aglycones were detected (**Figure 2**), including three flavonols [Querc (aglycone of compounds **1**, **2**, **9**, **10**, **11**, and **14**), Kaempf (aglycone of compounds **3**−**6**, **8**, **15**−**17**, and **22**), and Isorhmnt (aglycone of compounds **7**, **18**, **20**, and **23**)], three flavones [Lutln (aglycone of compounds **12** and **13**), Apign (aglycone of compounds **19** and **21**), and Chrysrl (aglycone of compounds **24** and **25**)], one chalcone (aglycone of compound **26**).

In chromatographic analysis, seven compounds were identified as Kaempf3Glc7Glc (compound **5**), Querc3Glc (compound **11**), Lutln7Glc (compound **12**), Kaempf3Glc (compound **16**), Apign7Glc (compound **19**), Isorhmnt3Glc (compound **20**), and Apign7Neo (compound **21**) by cochromatography and comparison of their UV spectra with corresponding standards. They were further confirmed by their mass spectra (details in **Table 1**). Among them, compounds **5**, **12**, **16**, **19**, and **21** were previously reported in tree peony (*3*, *24*).

The fragmentation behaviors of six compounds (compounds **1**−**4**, **6**, and **7**) were similar to that of Kaempf3Glc7Glc in MS^2 analysis, which indicated that the six compounds were flavonol 3,7-diglycosides. Accordingly, the fragments in NI mode can give creditable information about the glycosylation position of different glycoses in flavonol 3,7-diglycosides (*25*). Ions generated from $[M - H]^-$ by losing the glycose substituent located at the 3-position ($[M - H - Gly_{(3)}]^-$) are labeled Y_0^{3-}, whereas $[M - H - Gly_{(7)}]^-$ is labeled Y_0^{7-}. In NI mode, the relative abundance of Y_0^{7-} is higher than that of Y_0^{3-} in MS^2 spectra of flavonol 3,7-diglycosides because $[M - H]^-$ more easily loses a glycose substituent located at the 7-position than at the 3-position (*25*). Thus, compound **2** was tentatively characterized as Querc 3-*O*-glucoside-7-*O*-arabinoside considering the pelargonidin arabinoside was reported in flowers of herbaceous peony (genus *Paeonia*, a close relative plant of tree peony) (*26*), and compounds **4** and **6**

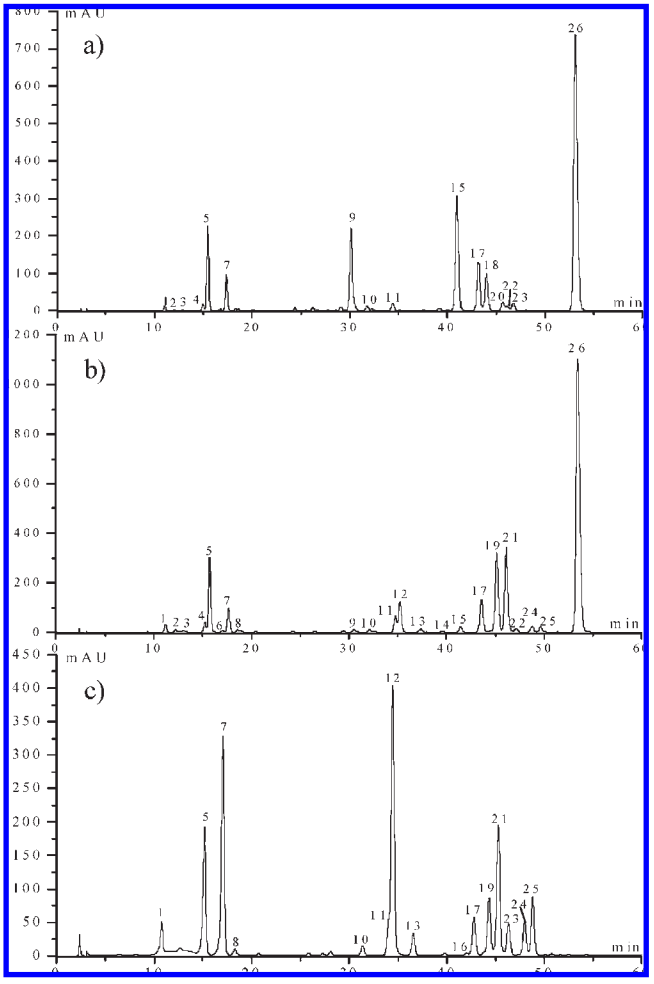

Figure 1. HPLC chromatograms at 350 nm of extracts from yellow flowers of tree peony: (**a**) species *P. delavayi* (variant I, pure bright yellow petals); (**b**) cultivar 'Yao Huang'; (**c**) flower mixture of cultivars 'Souvenir de Maxime Cornu' and 'High Noon'. Peaks: 1, quercetin 3,7-di-*O*-glucoside; 2, quercetin 3-*O*-glucoside-7-*O*-arabinoside; 3, kaempferol 3,7-di-*O*-hexoside; 4, kaempferol 3-*O*-arabinoside-7-*O*-glucoside; 5, kaempferol 3,7-di-*O*-glucoside; 6, kaempferol 3-*O*-glucoside-7-*O*-rhamnoside; 7, isorhamnetin 3,7-di-*O*-glucoside; 8, kaempferol derivative; 9, quercetin 3-*O*-galloylglucoside; 10, quercetin 7-*O*-glucoside; 11, quercetin 3-*O*-glucoside; 12, luteolin 7-*O*-glucoside; 13, luteolin 7-*O*-neohesperidoside; 14, quercetin 3-*O*-arabinoside; 15, kaempferol 3-*O*-galloylglucoside; 16, kaempferol 3-*O*-glucoside; 17, kaempferol 7-*O*-glucoside; 18, isorhamnetin 3-*O*-galloylglucoside; 19, apigenin 7-*O*-glucoside; 20, isorhamnetin 3-*O*-glucoside; 21, apigenin 7-*O*-neohesperidoside; 22, kaempferol 3-*O*-arabinoside; 23, isorhamnetin 7-*O*-glucoside; 24, chrysoeriol 7-*O*-glucoside; 25, chrysoeriol 7-*O*-neohesperidoside; 26, chalcononaringenin 2′-*O*-glucoside.

Table 1. Flavonoid Compounds Identified from the Extracts of Yellow Flowers of Tree Peony

no.	identification and tentative identification	t_R[a] (min)	UV λ_{max}[b] (nm)	ESI-NI MS/MS^2 (*m*/*z*)	ESI-PI MS/MS^2 (*m*/*z*)	tree peony samples[c]	ref
1	quercetin 3,7-di-*O*-glucoside	11.2	256, 353	625 $[M-H]^-$, 463 $[Y_0^{3-}$ and $Y_0^{7-}]$ (100), 301 $[Y_0^-]$ (32)	649 $[M+Na]^+$, 465 (69), 303 $[Y_0^+]$ (100)	1; 2; 4; blotch of 5; 6; 7; 8; 9	
2	quercetin 3-*O*-glucoside-7-*O*-arabinoside	12.2	256, 355	595 $[M-H]^-$, 463 [Y_0^{7-}] (100), 433 [Y_0^{3-}] (57), 301 $[Y_0^-]$ (73)	597 $[M+H]^+$, 465 (36), 435 (49), 303 $[Y_0^+]$ (50)	2; 6; blotches of 5, 7, 8, and 9	
3	kaempferol 3,7-di-*O*-hexoside	13.1	269, 343	609 $[M-H]^-$, 447 $[Y_0^{3-}$ and $Y_0^{7-}]$ (100), 285 $[Y_0^-]$ (10)	633 $[M+Na]^+$, 449 (18), 287 $[Y_0^+]$ (95)	blotches of 5 and 6	
4	kaempferol 3-*O*-arabinoside-7-*O*-glucoside	15.3	266, 347	579 $[M-H]^-$, 447 $[Y_0^{3-}]$ (1), 417 $[Y_0^{7-}]$ (100), 285 $[Y_0^-]$ (0.3)	603 $[M+Na]^+$, 449 (27), 287 $[Y_0^+]$ (100)	1; 2; 5; 6; 7; 8; 9	
5	kaempferol 3,7-di-*O*-glucoside	15.7	266, 346	609 $[M-H]^-$, 447 $[Y_0^{3-}$ and $Y_0^{7-}]$ (100), 285 $[Y_0^-]$ (13)	633 $[M+Na]^+$, 449 (70), 287 $[Y_0^+]$ (100)	1; 2; 3; 4; 5; 6; 7; 8; 9	standard
6	kaempferol 3-*O*-glucoside-7-*O*-rhamnoside	17.1	267, 345	593 $[M-H]^-$, 447 $[Y_0^{7-}]$ (74), 431 $[Y_0^{3-}]$ (44)	595 $[M+H]^+$, 449 (32), 433 (18), 287 $[Y_0^+]$ (100)	7; blotches of 6 and 8	
7	isorhamnetin 3,7-di-*O*-glucoside	17.7	255, 352	639 $[M-H]^-$, 477 $[Y_0^{3-}$ and $Y_0^{7-}]$ (100), 315 $[Y_0^-]$ (4)	663 $[M+Na]^+$, 479 (81), 317 $[Y_0^+]$ (100)	1; 2; 3; 4; 5; 6; 7; 8; 9	
8	kaempferol derivative	18.6	267, 347		287 $[Y_0^+]$ (41)	4; 5; blotch of 6; 7; 8; 9	
9	quercetin 3-*O*-galloylglucoside	30.5	266, 354	615 $[M-H]^-$, 463 (100), 300 $[Y_0-H]^{-\bullet}$ (18)	617 $[M+H]^+$, 303 $[Y_0^+]$ (42)	1; 2; blotches of 5 and 8; 6; 7; 9	
10	quercetin 7-*O*-glucoside	32.1	256, 365	463 $[M-H]^-$, 301 $[Y_0^-]$ (8)	487 $[M+Na]^+$, 303 $[Y_0^+]$ (100)	1; 2; 3; 4; blotches of 5, 6, 7, and 8; 9	
11	quercetin 3-*O*-glucoside	34.8	257, 353	463 $[M-H]^-$, 301 $[Y_0^-]$ (100), 300 $[Y_0-H]^{-\bullet}$ (16)	487 $[M+Na]^+$, 303 $[Y_0^+]$ (100)	1; 4; blotches of 5, 6, 7, 8, and 9	authentic sample
12	luteolin 7-*O*-glucoside	35.2	255, 348	447 $[M-H]^-$, 285 $[Y_0^-]$ (40)	449 $[M+H]^+$, 287 $[Y_0^+]$ (100)	2; 4; 5; 6; 7; 8; 9	standard
13	luteolin 7-*O*-neohesperidoside	37.4	253, 347	593 $[M-H]^-$, 447 (23), 285 $[Y_0^-]$ (2)	595 $[M+H]^+$, 449 (0.3), 287 $[Y_0^+]$ (100)	2; 4; 5; 6; 7; 8; 9	
14	quercetin 3-*O*-arabinoside	39.6	253, 353	433 $[M-H]^-$, 300 $[Y_0-H]^{-\bullet}$(50), 301 $[Y_0^-]$ (19)	435 $[M+H]^+$, 303 $[Y_0^+]$ (39)	1; blotches of 5, 6, and 8	
15	kaempferol 3-*O*-galloylglucoside	41.4	261, 348	599 $[M-H]^-$, 447 (10), 285 $[Y_0]^-$(5)	601 $[M+H]^+$, 449 (0.1), 287 $[Y_0^+]$ (23)	1; 2; 5; 6; 7; 8; 9	
16	kaempferol 3-*O*-glucoside	42.6	267, 351		449 $[M+H]^+$, 287 $[Y_0^+]$ (72)	4	standard
17	kaempferol 7-*O*-glucoside	43.6	266, 358	447 $[M-H]^-$, 285 $[Y_0^-]$ (32)	471 $[M+Na]^+$, 287 $[Y_0^+]$ (100)	1; 2; 4; 5; 6; 7; 8; 9	*3*
18	isorhamnetin 3-*O*-galloylglucoside	44.0	264, 354	629 $[M-H]^-$, 477 (7), 315 $[Y_0^-]$ (5)	653 $[M+Na]^+$, 317 $[Y_0^+]$ (46)	1	
19	apigenin 7-*O*-glucoside	45.1	267, 337	431 $[M-H]^-$, 269 $[Y_0^-]$ (9)	433 $[M+H]^+$, 271 $[Y_0^+]$ (100)	2; 4; 5; 6; 7; 8; 9	standard
20	isorhamnetin 3-*O*-glucoside	45.7	254, 354	477 $[M-H]^-$, 314 $[Y_0-H]^{-\bullet}$(5)	501 $[M+Na]^+$, 317 $[Y_0^+]$ (100)	1	standard
21	apigenin 7-*O*-neohesperidoside	46.1	267, 338	577 $[M-H]^-$, 269 $[Y_0^-]$ (17)	579 $[M+H]^+$, 271 $[Y_0^+]$ (100)	2; 4; 5; 6; 7; 8; 9	standard
22	kaempferol 3-*O*-arabinoside	46.2	266, 342	417 $[M-H]^-$, 284 $[Y_0-H]^{-\bullet}$(26)	441 $[M+Na]^+$, 287 $[Y_0^+]$ (100)	1; blotches of 5, 6, 7, and 8	
23	isorhamnetin 7-*O*-glucoside	47.0	254, 365	477 $[M-H]^-$, 315 $[Y_0^-]$ (57)	479 $[M+H]^+$, 317 $[Y_0^+]$ (100)	1; 4	
24	chrysoeriol 7-*O*-glucoside	48.8	252, 346	461 $[M-H]^-$, 299 $[Y_0^-]$ (25)	463 $[M+H]^+$, 301 $[Y_0^+]$ (100)	2; 4; 5; 6; 7; 8; 9	
25	chrysoeriol 7-*O*-neohesperidoside	49.6	251, 346	607 $[M-H]^-$, 461 (4), 299 $[Y_0^-]$ (40)	609 $[M+H]^+$, 301 $[Y_0^+]$ (100)	2; 4; 5; 6; 7; 8; 9	
26	chalcononaringenin 2′-*O*-glucoside	53.4	365	433 $[M-H]^-$, 271 $[Y_0^-]$ (100)	457 $[M+Na]^+$, 273 $[Y_0^+]$ (80)	1; 2; 5; 6; 7; 8; 9	*8*

[a] t_R, retention time on HPLC analysis. [b] Underlined data were the max UV absorption wavelength. [c] 1, *P. delavayi* (variant I, pure bright yellow petals); 2, *P. delavayi* (variant II, bright yellow petals with slight pink at the base); 3, *P. ludlowii*; 4, 'Yao Huang'; 5, 'Golden Isles'; 6, 'High Noon'; 7, 'Souvenir de Maxime Cornu'; 8, 'Oukan'; 9, 'Alice Harding'.

were tentatively identified as Kaempf 3-*O*-arabinoside-7-*O*-glucoside and Kaempf 3-*O*-glucoside-7-*O*-rhamnoside, respectively. Besides, compounds **1** and **7** were tentatively characterized as Querc 3,7-di-*O*-glucoside and Isorhmnt 3,7-di-*O*-glucoside, respectively. Compounds **3** and **5** showed similar MS^2 spectra; unfortunately, there was no more information provided for compound **3**, and it was tentatively identified as Kaempf 3,7-di-*O*-hexoside.

The MS data of compound **13** showed there were two substituents: one molecule of glucose and one rhamnose linked to the aglycone Lutln. The MS^2 fragment *m*/*z* 447 $[M-H-146u]^-$ was observed without *m*/*z* 431$[M-H-162\ u]^-$. That suggested

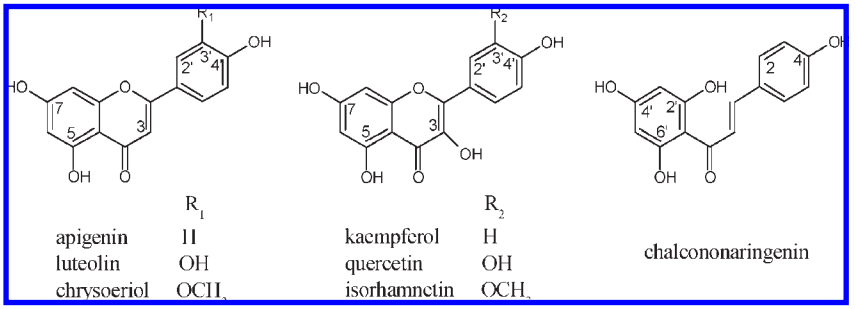

Figure 2. Chemical structures of the identified flavonoid aglycons.

Table 2. Relative Content of Flavonoid Glycoside and Total Flavonoid Content of Various Samples

	relative content of flavonoid glycoside[b,c] (%)													
	Querc			Kaempf			Isorhmnt			Lutln	Apign	Chrysrl	Chalcon	
sample[a]	3Gly7Gly	3Gly and 7Gly	3GalGlc	3Gly7Gly	3Gly and 7Gly	3GalGlc	3Gly7Gly	3Gly and 7Gly	3GalGlc	7Gly	7Gly	7Gly	2′Glc	TFC[d]
P. delavayi (variant I)	0.25	1.33	7.39	5.46	6.99	13.35	2.04	2.11	3.99	–	–	–	57.09	5.60 ± 0.26
P. delavayi (variant II)	0.74	0.35	0.67	1.49	0.67	+	3.13	–	–	14.11	15.90	1.02	61.92	12.26 ± 0.68
P. ludlowii	–	85.42	–	7.85	–	–	6.73	–	–	–	–	–	–	0.01 ± 4.0 × 10^{-3}
Yao Huang	3.80	4.21	–	11.84	2.53	–	22.14	2.66	–	26.36	17.88	8.03	–	6.08 ± 0.14
Golden Isles	–	–	–	2.87	2.14	0.34	0.52	–	–	4.83	47.84	3.08	38.38	9.44 ± 0.15
Golden Isles (blotch)	1.38	2.75	0.96	4.34	3.17	0.64	2.58	–	–	8.13	4.97	1.48	69.41	4.83 ± 0.26
High Noon	0.27	–	+	8.82	4.50	0.70	1.21	–	–	5.15	41.36	1.80	36.19	9.66 ± 0.45
High Noon (blotch)	2.15	3.87	0.49	10.05	7.53	0.87	2.87	–	–	3.19	7.94	0.49	60.42	5.89 ± 0.18
Souvenir de Maxime Cornu	0.20	–	0.17	4.04	1.96	0.41	1.62	–	–	3.49	27.29	4.05	56.51	7.49 ± 0.06
Souvenir de Maxime Cornu (blotch)	0.79	1.58	0.46	3.74	5.56	0.52	2.55	–	–	3.02	4.81	2.22	74.43	3.76 ± 0.20
Oukan	0.35	–	–	11.66	5.50	0.61	1.27	–	–	5.00	53.11	2.34	20.15	7.83 ± 0.29
Oukan (blotch)	3.43	4.59	0.25	15.04	9.15	0.46	3.93	–	–	3.77	10.51	0.51	48.10	3.19
Alice Harding	0.54	0.09	0.21	5.49	0.64	0.21	1.74	–	–	2.16	25.87	0.43	62.50	13.20 ± 0.14
Alice Harding (blotch)	1.39	1.48	1.01	4.75	3.28	0.77	3.10	–	–	3.25	8.60	1.09	71.05	7.10 ± 0.26

[a] variant I, pure bright yellow petals; variant II, bright yellow petals with slight pink at the base. [b] Average of the three values of each sample. "+" indicates <0.005 mg of rutin per 100 mg of dried petals; "–" indicates not detected. [c] Querc, quercetin; Kaempf, kaempferol; Isorhmnt, isorhamnetin; Lutln, luteolin; Apign, apigenin; Chrysrl, chrysoeriol; Chalcon, chalcononaringenin; 3Gly7Gly, 3,7-diglycoside; 3Gly and 7Gly, 3-glycoside and 7-glycoside; 3GalGlc, 3-galloylglucoside; 7Gly, 7-glycoside; 2′Glc, 2′-glucoside. [d] Average of three values of each sample ± SD. TFC, total flavonoid content, mg of rutin/100 mg of DW.

Lutln was glycosylated by a disaccharide. In addition, the relative abundance of m/z 449 $[M + H - 146\ u]^+$ and m/z 287 $[Y_0^+, M + H - (146 + 162)\ u]$ indicated the 1→2 linkage of interglycosidic linking in the glycoside (*27*). By comparison of the UV spectra with that of standard Lutln7Glc, finally, compound **13** was tentatively identified as Lutln 7-*O*-neohesperidoside. In this case, compound **25** was tentatively characterized as Chrysrl 7-*O*-neohesperidoside.

All $[M - H]^-$ ions of three compounds (compounds **9**, **15**, **18**) lost m/z 152 u fragments in MS^2 analysis. With regard to the presence of galloyl compounds in the root cortex of tree peony (*28*), these compounds were recognized as flavonol galloylglucosides. By comparing UV spectra with those of standards Querc3Glc, Kaempf3Glc, and Isorhmnt3Glc, the three compounds were tentatively identified as Querc 3-*O*-galloylglucoside (compound **9**), Kaempf 3-*O*-galloylglucoside (compound **15**), and Isorhmnt 3-*O*-galloylglucoside (Isorhmnt3GalGlc, compound **18**).

In NI mode of MS^2, the relative abundances of the radical aglycone ($[Y_0 - H]^{\bullet -}$) and the aglycone ion (Y_0^-) are usually different in flavonoid-3-glycoside and flavonoid-7-glycoside. When the glycosylation occurs at the 3-position of the aglycone, the relative abundance of $[Y_0 - H]^{\bullet -}$ is higher than that of Y_0^-, and the contrary phenomenon appears in flavonoid-7-glycoside (*25*). On the basis of the MS and UV spectra of five compounds (details in **Table 1**), they were tentatively characterized as Querc 7-*O*-glucoside (compound **10**), Querc 3-*O*-arabinoside (compound **14**), Kaempf 7-*O*-glucoside [compound **17**, which was previously reported in tree peony flower (*3*)], Kaempf 3-*O*-arabinoside (compound **22**), Isorhmnt 7-*O*-glucoside (compound **23**), and Chrysrl 7-*O*-glucoside (compound **24**).

Compound **26** was tentatively identified as Chalcon 2′-glucoside (4,4′,6′-trihydroxychalcone 2′-glucoside, isosalipurposide) according to the yellow pigment in flowers of *P. delavayi* var. *trollioides* (*8*).

In addition to the aglycone ion information of compound **8**, no other available data were obtained in MS spectra, and it was tentatively identified as kaempferol derivative.

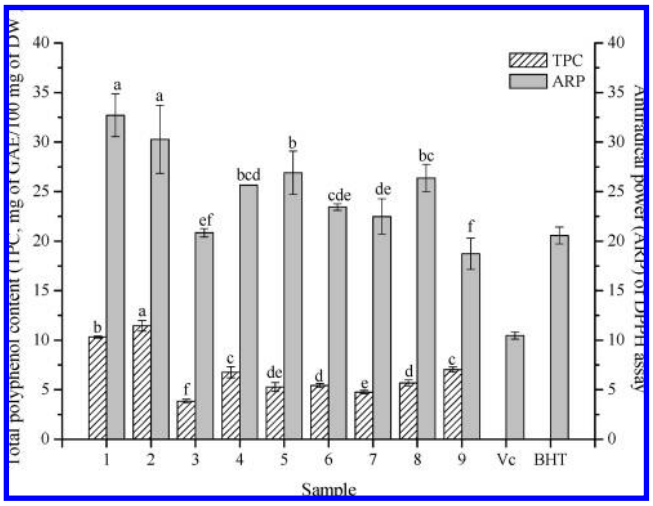

Figure 3. Total polyphenol content (TPC) and antiradical power (ARP) of each tree peony flower sample (mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($p < 0.05$). Samples are identified in the figure by numbers: 1, *P. delavayi* (variant I, pure bright yellow petals); 2, *P. delavayi* (variant II, bright yellow petals with slight pink at the base); 3, *P. ludlowii*; 4, 'Yao Huang'; 5, 'Golden Isles'; 6, 'High Noon'; 7, 'Souvenir de Maxime Cornu'; 8, 'Oukan'; 9, 'Alice Harding'.

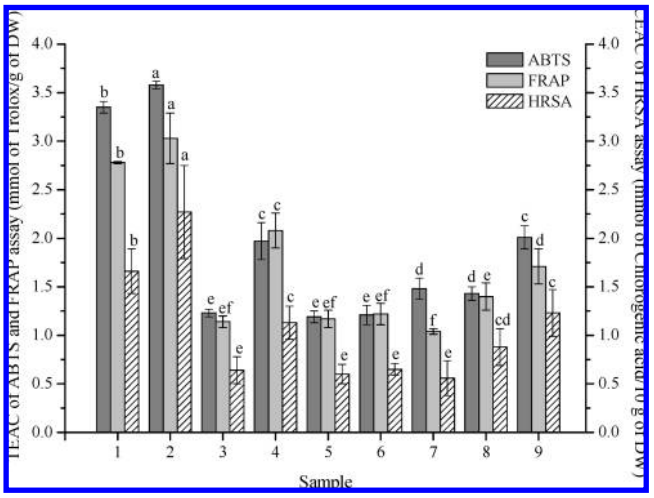

Figure 4. Antioxidant capacities (TEAC, for ABTS and FRAP assay; CEAC, for HRSA assay) of each tree peony flower sample (mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($p < 0.05$). Samples are identified in the figure by numbers: 1, *P. delavayi* (variant I, pure bright yellow petals); 2, *P. delavayi* (variant II, bright yellow petals with slight pink at the base); 3, *P. ludlowii*; 4, 'Yao Huang'; 5, 'Golden Isles'; 6, 'High Noon'; 7, 'Souvenir de Maxime Cornu'; 8, 'Oukan'; 9, 'Alice Harding'.

Composition and Content of Flavonoids. The composition of flavonoids and the results of quantitative analysis are presented in **Table 2**. Different kinds of flavonoid glycosides such as flavonol 3,7-diglycoside, 3- or 7-monoglycoside, and 3-acylglucoside, flavone 7-glycoside, and chalcone 2′-glucoside were detected. The Querc, Isorhmnt, and Chrysrl glycosides as well as flavonol acylglucosides had not been reported in tree peony before. The flavonoid compositions were not identical in each sample. The kinds of flavonoid glycosides in species *P. delavayi* were more than that in other samples, whereas *P. ludlowii* had the least. In most cultivars with blotches, the types of flavonoid glycosides in the blotch parts were more than that in the corresponding nonblotches. Acylated flavonol glycosides and chalcone glucoside were detected in *P. delavayi* as well as in American, French, and Japanese cultivars, which had close syngenetic relationships with *P. delavayi* (*29*). It was notable that Isorhmnt3GalGlc and Isorhmnt3Glc were unique in *P. delavayi* (variant I), and isosalipurposide was common in species *P. delavayi* and related cultivars. These compounds would be considered as characteristics in the flavonoid fingerprints of these tree peonies. Besides, isosalipurposide was found as the main flavonoid in tree peony petals bearing yellow flowers, and the cultivar 'Yao Huang' with light yellow flowers did not contain that compound. There were only trace flavonol glycosides tested in the flowers of *P. ludlowii*, which were scarcely studied before. The yellow pigment components in *P. ludlowii* flowers need to be further investigated. In general, the relative contents of Kaempf, Lutln, and Apign derivatives were higher than that of other flavonol and flavone derivatives.

The total flavonoid content (TFC) varied in different samples (**Table 2**). The cultivar 'Alice Harding' gave the highest value followed by *P. delavayi* (variant II), whereas *P. ludlowii* showed the lowest. TFCs of the nonblotch parts of the other five cultivars ('Golden Isles', 'High Noon', 'Souvenir de Maxime Cornu', 'Oukan', and 'Alice Harding') were higher than that of 'Yao Huang', because of the high content of isosalipurposide (varied from 1.58 ± 0.05 to 8.25 ± 0.16 mg of rutin/100 mg of DW, data not shown). TFCs in all blotch parts were lower than that of the corresponding nonblotches. Because the blotch areas in petals were quite small, so the nonblotches were taken to do TP quantitative analysis and antioxidant activity evaluation.

Total Polyphenol Content. The TPC displayed significant differences in various samples under ANOVA analysis ($p < 0.01$) (**Figure 3**). TPCs for each sample ranged from 3.85 ± 0.20 (*P. ludlowii*) to 11.45 ± 0.54 mg of GAE/100 mg of DW (*P. delavayi*, variant II). Species *P. delavayi* showed much higher TPC than the cultivars. The TPC of cultivars decreased in the order 'Alice Harding' > 'Yao Huang' > other cultivars tested. The difference of TPC in cultivars 'Golden Isles', 'High Noon', and 'Oukan' was not significant.

Antioxidant Activity. Individual tree peony samples showed significant difference of antioxidant activity in four assays ($p < 0.01$). The DPPH$^{\bullet}$ scavenging activity was evaluated by the parameter ARP: the larger the ARP, the more efficient the antioxidant (*12*). The ARP of species *P. delavayi* (variant II) extract was the highest (32.71 ± 2.16), and that of cultivar 'Alice Harding' was the lowest (18.72 ± 1.59) (**Figure 3**). There was no significant difference of ARP for cultivars 'Yao Huang', 'Golden Isles', and 'Oukan' extracts. **Figure 3** also shows the ARP of two reference standards, Vc and BHT. The results suggested the extracts of most tree peony flowers gave higher ARP than Vc and BHT, but the time needed to reach the reaction steady stage in the DPPH$^{\bullet}$ assay of tree peony samples was much longer than that of Vc and as long as that of BHT (about 8−10 h). The results of ABTS and FRAP assays were similar, and most TEAC values of the ABTS assay were slightly higher than that of the FRAP assay (**Figure 4**). A strongly significant correlation between the two methods was observed ($r = 0.967$, $p < 0.0001$), which suggested the two methods were comparable in the case of tree peony flowers. For the ABTS assay, TEAC values varied from 1.19 ± 0.06 ('Golden Isles') to 3.58 ± 0.04 mmol of Trolox/g of DW (*P. delavayi*, variant II); for the FRAP assay, the values ranged from 1.04 ± 0.03 ('Souvenir de Maxime Cornu') to 3.03 ± 0.26 mmol of Trolox/g of DW (*P. delavayi*, variant II). The hydroxyl radical is an extremely reactive free radical formed in biological systems with a lot of damage. The CEAC values of HRSA ranged from 0.56 ± 0.18 ('Souvenir de Maxime Cornu') to 2.27 ± 0.48 mmol of CA/10 g of DW (*P. delavayi*, variant II) (**Figure 4**). There was no

Table 3. Correlation Coefficients between the Contents of Total Polyphenol, Total Floavonoid, and Flavonoid Glycosides and Each Antioxidant Activity Assay

antioxidant activity assay[a]	TPC[b]	TFC[b]	Querc[c] glycosides	Kaempf[c] glycosides	Lutln[c] glycosides	Apign[c] glycosides	Isorhmnt[c] glycosides	Chrysrl[c] glycosides	isosalipurposide
DPPH	0.664**	0.019	0.530**	0.268	0.266	−0.207	0.222	−0.014	−0.029
ABTS	0.969**	0.264	0.686**	0.069	0.398*	−0.450*	0.321	−0.298	0.444*
HRSA	0.854**	0.334	0.522**	−0.033	0.445*	−0.331	0.274	−0.217	0.489**
FRAP	0.961**	0.247	0.757**	0.124	0.518**	−0.420*	0.463*	−0.191	0.332

[a] DPPH, anti-DPPH$^{\bullet}$ assay; ABTS, anti-ABTS$^{\bullet+}$ assay; HRSA, hydroxyl radical-scavenging activity assay; FRAP, ferric reducing antioxidant power assay. [b] TPC, total polyphenol content; TFC, total flavonoid content. **, correlation is significant at the 0.01 level (two-tailed). [c] Querc, quercetin; Kaempf, kaempferol; Isorhmnt, isorhamnetin; Lutln, luteolin; Apign, apigenin; Chrysrl, chrysoeriol. *, correlation is significant at the 0.05 level (two-tailed); **, correlation is significant at the 0.01 level (two-tailed).

significant difference of CEAC values for species *P. ludlowii* and cultivars 'Golden Isles', 'High Noon', and 'Souvenir de Maxime Cornu'. All four assays showed that the antioxidant activity of species *P. delavayi* was higher than that of other samples, whereas *P. ludlowii* was the least effective antioxidant. On the whole, 'Yao Huang' displayed relatively high antioxidant activity among the cultivars.

Significant positive correlations were found between TPC and antioxidant capacity value in each assay (**Table 3**). Abundant phenolic compounds in tree peony flower can remove free radicals in the antioxidant reaction and enhance the reducing power to contribute significantly to antioxidant activity. This indicated the TP might also contribute to antisenescence and antiinflammation activity.

Relationships of Antioxidant Activity and Flavonoid Composition. A positive correlation between TPC and TFC was observed ($r = 0.408$). This was reasonable because the flavonoid is a subtype of polyphenols. Although flavonoids play an important role in the antioxidant activity of plant extracts (*30*), no significant correlation between antioxidant capacity value and TFC was observed (**Table 3**), which indicated that different types of flavonoid acted diversely in the antioxidant reaction. The antioxidant activities of flavonoids depend on their chemical structures. The number and position of hydroxyl or methoxyl groups on the phenolic ring are the main factors. The *o*-dihydroxy structure in the B ring contributes to the high radical scavenging activities (*6*). Both Lutln and Querc have 5,7- and 3′,4′-dihydroxyl groups in the A and B ring, respectively, and the 3-hydroxyl group of flavonol enhances the antioxidant activity (*31*). Therefore, among common aglycones, the antioxidant activity decreases in the order Querc > Kaempf > Lutln > Apign, and the antioxidant activity of their glycosides decreases slightly (*6*). Correlation coefficients between the content of flavonoid glycosides and each antioxidant activity assay are listed in **Table 3**. Although the contents of Kaempf, Lutln, and Apign type glycosides were relatively high in the extracts of tree peony, the content of Querc glycosides significantly correlated with the antioxidant activity (**Table 3**), which indicated that the increase of the Querc derivative content was helpful to enhance the antioxidant activity. In addition, a significant correlation ($p < 0.05$) between antioxidant activity and the total content of Kaempf, Lutln, and Querc type glycosides was observed in this study for the DPPH assay ($r = 0.472$), the ABTS assay ($r = 0.486$), the FRAP assay ($r = 0.622$), and the HRSA assay ($r = 0.422$). This suggested the samples with higher antioxidant activity had higher contents of Kaempf, Lutln, and Querc derivatives, which agreed with the findings of Wojdyło et al. (*31*). Apign and Chrysrl were least effective in free radical scavenging activity (**Table 3**), but it is not suitable to evaluate the bioactivity on cells of Apign by free radical scavenging test only (*6*). Apign is not only a cancer chemopreventive agent, having the ability to inhibit cell proliferation in cancer cell types, but also binds to DNA and forms ligand−DNA involved in vital DNA functions (*9*). In flowers of cultivar 'Yao Huang', the total content of Kaempf, Lutln, Querc, and Apign glycosides amounted to nearly 70% of TFC, and the contents of flavonol glycosides and flavone glycosides were the highest among the samples tested (**Table 2**). This indicated that 'Yao Huang' had valuable potential bioactive ability.

The chalcone derivatives have very high antioxidant activity, compared with BHT, because of the hydroxyl group on the 4-position of the B ring (*32*). According to Yagura et al. (*10*), isosalipurposide (compound **26**) is an active anticarcinogen, presenting stronger inhibitory effects than Querc to SENCAR mouse skin transformed cells. In our research, isosalipurposide was found to be the main flavonoid detected in flowers of species *P. delavayi* as well as American, French, and Japanese cultivars. There was a positive correlation between the isosalipurposide content and the antioxidant capacity in ABTS and HRSA assays (**Table 3**). Moreover, it is notable that cultivars 'Golden Isles', 'High Noon', 'Souvenir de Maxime Cornu', 'Oukan', and 'Alice Harding' contained high quantities of Apign glycosides. These cultivars were considered to be potential resources for the development of new drugs because the abundant isosalipurposide and Apign derivatives in them may contribute to the anticarcinogenic activity.

The present study provided the chemical basis for setting up a flavonoid fingerprint of tree peony flowers and for clarifying them as functional foods and traditional medicines. Cultivars 'Yao Huang', 'Golden Isles', 'High Noon', 'Souvenir de Maxime Cornu', 'Oukan', and 'Alice Harding' should be considered as natural resources for the development of new drugs or functional foods. This study is valuable for further studies and full development of tree peony flowers. Further studies on the determination of other phenolic profiles and physiological and pharmacological properties of flower extracts need to be carried out. Furthermore, tree peony species or cultivars rich in bioactive components would be selected for intense use.

ABBREVIATIONS USED

HPLC, high-performance liquid chromatography; DAD, diode array detector; ESI, electrospray ionization; MS, mass spectrometry; DPPH, 1,1-diphenyl-2-picrylhydrazyl; BHT, butylated hydroxytoluene; ABTS, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; TPTZ, 2,4,6-tripyridyl-*S*-triazine; HRSA, hydroxyl radical-scavenging activity; FRAP, ferric reducing antioxidant power; TEAC, Trolox equivalent antioxidant capacity; CEAC, chlorogenic acid equivalent antioxidant capacity; TPC, total polyphenol content; TFC, total flavonoid content; ARP, antiradical power.

ACKNOWLEDGMENT

We thank Dr. Xiao Wang (Shandong Analysis and Test Center, Shandong Academy of Sciences, Shandong, China) for his generous supply of standards.

Supporting Information Available: Flowers of the tree peony surveyed in this paper. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Li, J. J. Chapter I: The cultivated history of Chinese tree peony and herbaceous peony. In *Chinese Tree Peony and Herbaceous Peony*; Li, J. J., Ed.; China Forestry Publishing House: Beijing, China, 1999; pp 16−19 (in Chinese).

(2) Zhou, L. Tree peony flower and diet therapy. *World Cuisine* **2001**, *3*, 39 (in Chinese).

(3) Wang, X.; Cheng, C. G.; Sun, Q. L.; Li, F. W.; Liu, J. H.; Zheng, C. C. Isolation and purification of four flavonoid constituents from the flowers of *Paeonia suffruticosa* by high-speed counter-current chromatography. *J. Chromatogr., A* **2005**, *1075*, 127–131.

(4) Shi, J. Y.; Gong, J. Y.; Liu, J. E.; Wu, X. Q.; Zhang, Y. Antioxidant capacity of extract from edible flowers of *Prunus mume* in China and its active components. *LWT−Food Sci. Technol.* **2009**, *42*, 477–482.

(5) Wang, X.; Shi, X. G.; Zheng, C. C.; Liu, J. H. Effects of extract from peony flowers on removal of reactive oxygen species and preventing DNA damage caused by hydroxyl radical. *Food Ferment. Ind.* **2004**, *30*, 55−58 (in Chinese).

(6) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Antioxidant properties of phenolic compounas. *Trends Plant Sci.* **1997**, *2*, 152–159.

(7) Wang, L. S.; Shiraishi, A.; Hashimoto, F.; Aoki, N.; Shimizu, K.; Sakata, Y. Phenetics in tree peony species from china by flower pigment cluster analysis. *J. Plant Res.* **2001**, *114*, 33–43.

(8) Harborne, J. B. Comparative biochemistry of flavonoids—I. Distribution of chalcone and aurone pigments in plants. *Phytochemistry* **1966**, *5*, 111–115.

(9) Nafisi, S.; Hashemi, M.; Rajabi, M.; Tajmir-Riahi, H. A. DNA adducts with antioxidant flavonoids: morin, apigenin, and naringin. *DNA Cell Biol.* **2008**, *27*, 433–442.

(10) Yagura, T.; Motomiya, T.; Ito, M.; Honda, G.; Iida, A.; Kiuchi, F.; Tokuda, H.; Nishino, H. Anticarcinogenic compounds in the Uzbek medicinal plant *Helichrysum maracandicum*. *J. Nat. Med.* **2008**, *62*, 174–178.

(11) Vallejo, F.; Tomás-Barberán, F. A.; Ferreres, F. Characterisation of flavonols in broccoli (*Brassica oleracea* L. var. *italica*) by liquid chromatography−UV diode-array detection−electrospray ionization mass spectrometry. *J. Chromatogr., A* **2004**, *1054*, 181–193.

(12) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of free radical method to evaluate antioxidant activity. *Lebensm.-Wiss. -Technol.* **1995**, *28*, 25–30.

(13) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* **1999**, *26*, 1231–1237.

(14) Wang, S. Y.; Jiao, H. J. Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. *J. Agric. Food Chem.* **2000**, *48*, 5677–5684.

(15) Fang, Z. X.; Zhang, Y. H.; Lü, Y.; Ma, G. P.; Chen, J. C.; Liu, D. H.; Ye, X. Q. Phenolic compounds and antioxidant capacities of bayberry juices. *Food Chem.* **2009**, *113*, 884–888.

(16) Cho, M. J.; Howard, L. R.; Prior, R. L.; Clark, J. R. Flavonoid glycosides and antioxidant capacity of various blackberry, blueberry and red grape genotypes determined by high-performance liquid chromatography/mass spectrometry. *J. Agric. Food Chem.* **2004**, *84*, 1771–1782.

(17) Zhang, J. J.; Wang, L. S.; Shu, Q. Y.; Liu, Z. A.; Li, C. H.; Zhang, J.; Wei, X. L.; Tian, D. K. Comparison of anthocyanins in non-blotches and blotches of the petals of Xibei tree peony. *Sci. Hortic.* **2007**, *114*, 104–111.

(18) Yang, R. Z.; Wei, X. L.; Gao, F. F.; Wang, L. S.; Zhang, H. J.; Xu, Y. J.; Li, C. H.; Ge, Y. X.; Zhang, J. J.; Zhang, J. Simultaneous analysis of anthocyanins and flavonols in petals of lotus (*Nelumbo*) cultivars by high-performance liquid chromatography-photodiode array detection/electrospray ionization mass spectrometry. *J. Chromatogr., A* **2009**, *1216*, 106–112.

(19) Lin, L. Z.; Harnly, J. M. A screening method for the identification of glycosylated flavonoids and other phenolic compounds using a standard analytical approach for all plant materials. *J. Agric. Food Chem.* **2007**, *55*, 1084–1096.

(20) Mansouri, A.; Embarek, G.; Kokkalou, E.; Kefalas, P. Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (*Phoenix dactylifera*). *Food Chem.* **2005**, *89*, 411–420.

(21) Ghiselli, A.; Nardini, M.; Baldi, A.; Scaccini, C. Antioxidant activity of different phenolic fractions separated from an Italian red wine. *J. Agric. Food Chem.* **1998**, *46*, 361–367.

(22) Markham, K. R. Introduction to the flavonoids. In *Techniques of Flavonoid Identification*; Markham, K. R., Ed.; Academic Press: London, U.K., 1982; pp 5−6.

(23) Harborne, J. B. Plant polyphenols 6. The flavonol glycosides of wild and cultivated potatoes. *Biochem. J.* **1962**, *84*, 100–106.

(24) Egger, K.; Keil, M. Flavonglykoside in blüen von *Paeonia arborea* und *Paeonia suffruticosa*. *Planta* **1969**, *88*, 154−156 (in German).

(25) Ablajan, K. *Study on the Mass Spectrometric Analytical Method of Flavonoid Glycosides*. Ph.D. Dissertation, Peking Union Medical College, Beijing, China, 2006 (in Chinese).

(26) Jia, N.; Shu, Q. Y.; Wang, D. H.; Wang, L. S.; Liu, Z. A.; Ren, H. X.; Xu, Y. J.; Tian, D. K; Tilt, K. M. Identification and characterization of anthocyanins by high-performance liquid chromatography−electrospray ionization−mass spectrometry in herbaceous peony species. *J. Am. Soc. Hortic. Sci.* **2008**, *133*, 418–426.

(27) Ma, Y.; Cuyckens, F.; Van den Heuvel, H.; Claeys, M. Mass spectrometric methods for the characterisation and differentiation of isomeric *O*-diglycosyl flavonoids. *Phytochem. Anal.* **2001**, *12*, 159–165.

(28) Xu, S. J.; Yang, L.; Zeng, X.; Zhang, M.; Wang, Z. T. Characterization of compounds in the Chinese herbal drug Mu-Dan-Pi by liquid chromatography coupled to electrospray ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 3275–3288.

(29) Li, J. J. Chapter III: The origin, evolution, and classification of cultivars. In *Chinese Tree Peony and Herbaceous Peony*; Li, J. J., Ed.; China Forestry Publishing House: Beijing, China, 1999; pp 67 (in Chinese).

(30) Maisuthisakul, P.; Pasuk, S.; Ritthiruangdej, P. Relationship between antioxidant properties and chemical composition of some Thai plants. *J. Food Compos. Anal.* **2008**, *21*, 229–240.

(31) Wojdyło, A.; Oszmiański, J.; Czemerys, R. Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chem.* **2007**, *105*, 940–949.

(32) Ohkatsu, Y.; Satoh, T. Antioxidant and photo-antioxidant activities of chalcone derivatives. *J. Jpn. Pet. Inst.* **2008**, *51*, 298–308.

Received June 19, 2009. Revised manuscript received August 8, 2009. Accepted August 09, 2009. We appreciate the support of the National Natural Science Foundation of China, No. 30571306, as well as the Knowledge Innovation Program of the Chinese Academy of Sciences, No. KSCX2-YW-N-043.