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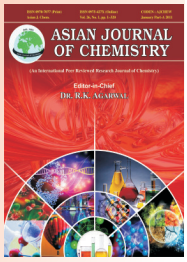

Phenolic Compounds and Antioxidant Property of Petal Extracts of Six *Lilium* Species Native to China

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Lily (*Lilium*) is used as an important edible and medicinal plant species with long history in China. In this study, petals of six *Lilium* species native to China were evaluated for their phenolic composition and dietary antioxidant potential. Eleven individual phenolic compounds were analyzed by high-performance liquid chromatography. The results showed that all petal extracts exhibited strong antioxidant activity, which generally correlated positively with the total phenolic contents, total flavonoid contents, total flavanol contents and total anthocyanin contents. Hierarchical cluster analysis showed that *L. davidii* and *L. lancifolium* belonged to the group with high phenolic content and high antioxidant activity. *L. davidii* var. *unicolor* and *L. sargentiae* were in the group with moderate phenolic content and antioxidant capacity, while *L. brownii* and *L. leucanthum* were clustered in the group with low phenolic content and weak antioxidant activity. It was suggested that lily petals could be used as a potential plant resource of antioxidants for functional food and pharmaceutical applications.

Keywords: *Lilium*, Petal, Phenolic compounds, Antioxidant activity.

INTRODUCTION

Lily of *Lilium*, a perennial ornamental crop belonging to the Liliaceae, has great ornamental, medicinal and edible value. The genus *Lilium*, which includes approximately 100 species, is native to Asia, Europe and North America in the Northern hemisphere. China with about 55 species is the diversity centre of wild *Lilium* in the World[1]. The lily petals, rich in polyphenol, have been extensively used as both a food and a traditional Chinese medicine in China[2]. They have been used to make various delicious folk foods, such as porridge, soup, teas and honey wine. Moreover, the lily petals have been used in traditional medicine as an antiinflammatory agent, a tonic and to treat bronchitis and stop bleeding from surgery[2]. In addition, extracts of petals are employed to make skin care products and essential oil. Although there exist different using ways of the lily in the pracitse, it still lack of enough constitute informations such as the antioxidant activity of the bioactive compounds of the lily petals.

It is well-known that degenerative or pathological processes such as atherosclerosis, aging and cancer in the human body are caused by oxidative damage[3]. Reactive oxygen species (ROS), including free radicals and nonradical species, are the main factor causing oxidative stress[4,5]. They can stimulate free radical chain reactions subsequently damaging the cellular biomolecules such as nucleic acids, lipids and proteins[6]. When ROS formation exceeds the capacity of cellular antioxidant defenses to neutralize their effects, the delicate cellular balance will be disturbed; furthermore, various pathophysiological disorders can ensue[7,8]. The human body has an antioxidant defence system, but for the dramatically increased ROS levels, the innate defense in the human body may not be enough for severe oxidative stress. It has been assumed that a diet rich in antioxidants strengthens this system. The network of antioxidants with different chemical properties may work in a synergistic way to protect the cells from damage[9,10].

The present epidemiological studies have revealed that the synthetic phenolic antioxidants such as buthylated hydroxytoluene (BHT), buthylated hydroxyanisole (BHA) and *tert*-butylhydroquinone (TBHQ) can effectively inhibit lipid oxidation[11]. However, they have limited use in many countries due to the possibility of causing adverse effects on human health[12,13]. For the food industry, it is highly interesting to find new and safe antioxidants from natural sources. Among the dietary antioxidants, phenolic compounds are the most abundant natural antioxidants[14].

Phenolic compounds are secondary metabolites commonly found in plant materials and products such as fruits, vegetables, herbs, beverages, wine and cocoa[15]. These compounds are useful in the defensive function against pathogens and radiation and are directly involved in the antioxidant activity, providing substantial protection against oxidative stress by inducing cellular endogenous enzymic protective mechanisms[16-19]. In view of the potential health benefits, there has been intensive research on natural antioxidants derived from plants.

Up to now, kaempferol, kaempferol glycosides, quercetin glycosides, regaloside, chalcone and cyanidin 3-O-β rutinoside have been characterized from the petals of *L. longiflorum* and the antioxidant activity of the petal extracts has been also disclosed[2,20]. Nevertheless, the antioxidant activity of the phenolic compounds obtained from *Lilium* species is still unclear. This is not conducive to the application of these valuable species. The objective of this study was to identify the main phenolic compounds from the petals of six *Lilium* species native to China and evaluate their antioxidant activities in order to provide valuable data for the further fully utilization of these *Lilium* species.

EXPERIMENTAL

L. brownii F. E. Brown ex Miellez, *L. leucanthum* Baker, *L. sargentiae* Wils., *L. davidii* Duch., *L. davidii* var. *unicolor* and *L. lancifolium* Thunb. were used in this study. All bulbs of six *Lilium* species were collected from its original distribution area in Shaanxi, Yunnan, Sichuan province on August 2011 (Fig. 1) and their botanical identification was performed by Professor Lixin Niu at the College of Horticulture, Northwest A&F University.

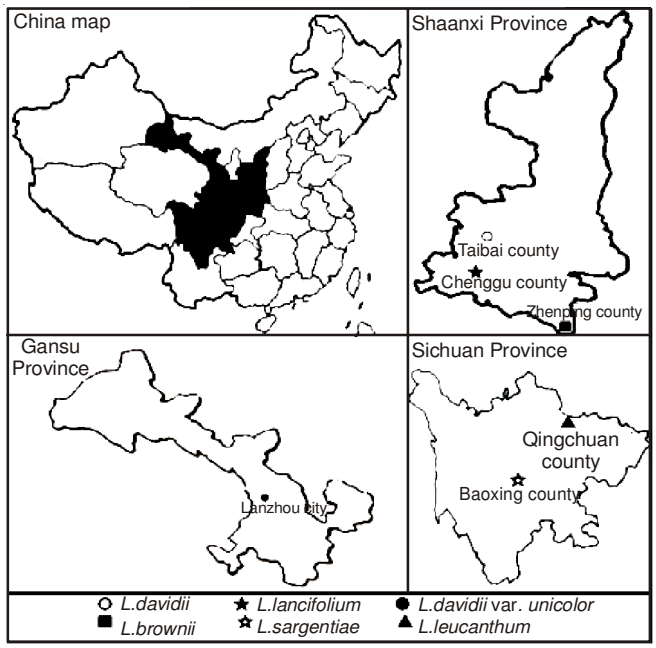

Fig. 1. Collection sites of six *Lilium* species native to china

Bulbs of *L. davidii* var. *unicolor* were collected from Lanzhou City (36° 24' N, 103° 40' E), Gansu and those of *L. leucanthum* and *L. sargentiae* were obtained from Qingchuan County (32° 12' N, 104° 36' E), Baoxing County (30° 22' N, 102° 48' E), Sichuan, respectively. Other lily bulb samples, including *L. brownii*, *L. davidii* and *L. lancifolium* were obtained from Zhenping County (31°42' N, 109°11' E), Taibai (34° 09' N, 107° 49' E) and Chenggu County (33° 15' N, 107° 20' E), Shaanxi, respectively.

The bulbs were planted into pots (21 cm in diameter, 1 bulb per pot) filled with mixed substrate of V(peat): V(perlite) = 1:1. The plants were grown in a heliogreenhouse of Northwest A&F University. Petals of six *Lilium* species were collected at the stage of 2 days post anthesis in May, June and July 2012. *L. brownii*, *L. sulphureum*, *L. leucanthum* and *L. sargentiae* have white petals. *L. davidii* var. *unicolor* has orange petals without spots. Others have orange petals with violet-black spots.

Samples were first frozen at -80 °C and then freeze-dried (LGJ-10, Songyuan Huasheng Biotechnology Co. Ltd., Beijing, China). The dry plant samples was ground to fine powder using an electrical grinder (JP-250A-8, Jiugong Economy and Trade Co. Ltd., Shanghai, China) and then kept in the refrigerator at -20 °C in labeled plastic bags under vacuum until extraction.

Extraction and determination of phenolics: 2.5 g of powder of each sample was homogenized and extracted by ultrasonic bath with 25 mL of acidified methanol solution (1 M HCl in 80 % methanol) at 25 °C for 1 h according to the method described by Jin *et al.*[21] with modifications. The homogenate was centrifuged at 12,000 rpm for 10 min at 4 °C and the residues was re-extracted in the same manner. All samples were extracted in triplicate. The supernatants were combined and concentrated under vacuum using a rotary evaporator at 40 °C. After lyophilizing, the sample powder was dissolved in 25 mL of acidified methanol. The prepared extract was stored at -20 °C for further analysis.

Total phenolic content assay was performed according to the Folin-ciocalteu colorimetric method of Singleton *et al.*[22] using gallic acid as standard. In short, extract of petals (0.10 mL) and distilled water (7.9 mL) were added into Folin-Ciocalteu reagent (0.5mL). After reaction for 5 min, 20 % Na_2CO_3 (1.5 mL) was added. Mixtures were left to stand for react at room temperature for 2 h in the dark before measuring absorbance at 765 nm against a blank (methanol) similarly prepared using a UV-visible spectrophotometer (UV-1700, Shimadzu Corp, Kyoto, Japan). The results were expressed as the equivalent to milligrams of gallic acid per 100 g of dry weight (mg RE/100 g).

Total flavonoid content (TFOC) was determined using the aluminum chloride ($AlCl_3$) colorimetric assay based on Chang *et al.*[23] using rutin as standard. Briefly, sample solution (1 mL), methanol solution (4 mL), $NaNO_2$ (0.5 M, 0.3 mL) and $AlCl_3$ (0.3 M, 0.3 mL) were added in a test tube successively. After 5 min, 4 mL of 1 M NaOH was added. The absorbance of the mixture was measured against blank at 510 nm. The results were expressed as the equivalent to milligrams of rutin per 100 g of dry weight (mg RE/100 g).

The quantitative determination of total flavanol content (TFAC) was estimated colorimetrically by the vanillin method described by Price *et al.*[24] using (+)-catechin as a standard. The determination carried out as follows, the vanillin reagent (5 mL, 0.5 g of reagent and 200 mL of 4 % HCl methanol) was added to sample solution (1 mL) and mixed well. Similarly, 4 % HCl in methanol (5 mL) and sample extracts (1 mL) were added in a test tube as a blank. After 20 min in the dark at room temperature the absorbances were measured at 500 nm. The absorbance of the blank was subtracted from that of the sample and the results were expressed as the equivalent to milligrams of (+)-catech in per 100 g of dry weight (mg CE/100 g).

Total anthocyanin content (TAC) of the samples was determined using the pH-differential method[25]. The potassium chloride buffer (0.2 M) and sodium acetate buffer (0.4 M) were prepared. The pH values of the buffer were 1 and 4.5, respectively. The obtained sample extracts were diluted with buffer to give an absorbance reading between 0.4 and 0.6 U. Absorbance was measured at 520 and 700 nm.

The absorbance values of the diluted samples (A) were calculated as follows:

$$A = (A_{\lambda 520} - A_{\lambda 700})_{pH1} - (A\lambda_{520} - A_{\lambda 700})_{pH4.5}$$

The total anthocyanin pigment (mg/100 g) was calculated (TAC) as follows:

$$TAC = (A \times MW \times DF \times Ve \times 100)/(\varepsilon \times 1 \times M)$$

The results were expressed as milligram of cyanidin-3-*O*-glucoside per 100 g of dry weight by using a molar absorptivity (ε) of 29,600 and a molecular weight (MW) of 499.38.

Where DF = Dilution multiples; Ve = total volume of each sample extract (25 mL); M = amount of powder of each sample for extracting (mg).

Phenolic compounds analysis by HPLC: Identification and quantification of phenolic compounds was performed on a Shimadzu liquid chromatography system (LC-2010AHT, Shimadzu Corp., Kyoto, Japan) equipped with a quaternary pump, a vacuum degasser, an autosampler, a photodiode array detector, a tunable UV-visible detector and a Hibar RT Lichrospher SB-C18 column (250 × 4 mm, 5 µm). The standard solutions of gallic acid, cyanidin-3-rutinoside, cyanidin, (+)-catechin, chlorogenic acid, (-)-epicatechin, dihydro-myricetin, rutin, *p*-coumaric acid, dihydroquercetin, kaem-pferol were prepared as serial dilutions to obtain a calibration curve.

A gradient solvent system was employed with solvent A containing water-acetic acid (99:1, v/v) and solvent B containing acetonitrile. The elution profile was as follows: 0-40 min, 95-0 % A, 5-100 % B; 40-60 min,100 % B. The column temperature was maintained at 40 °C and the flow rate was set at 0.5 mL/min. A volume of 10 µL was injected for each run in triplicate. The PDA detector scanned from 200 to 700 nm. The extracts were filtered through a 0.45 µm nylon filter before injection. Phenolic compounds were identified by their retention times after comparison with those of pure standards and quantified by the external standard method. Calibra tion was performed by injecting the standards three times at five different concentrations. Results were acquired, processed by the Shimadzu Workstation CLASS-VP 6.12 software and expressed as mg/100 g sample of dry weight.

Evaluation of antioxidant activity: Four methods were tested in this study for determining antioxidant activity: radical cation 2,2'-azino-*bis*-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picryl-hydrazyl (DPPH), cupric-reducing antioxidant capacity (CUPRAC) and hydroxyl radical scavenging activity (HRSA).

2,2'-Azino-*bis*-(3-ethylbenzothiazoline-6-sulphonic acid) free radical scavenging activity was determined as described by Re *et al*[26]. with slight changes. 2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) radical cation ($ABTS^{\bullet+}$) was produced by reacting 7 mM ABTS solution (5 mL) with 140 mM potassium persulfate aqueous solution (88 µL) and the mixture was left to stand in the dark at room temperature for 12 h. The $ABTS^{\bullet+}$ solution was diluted with methanol to give an absorbance at 732 nm of 0.70 ± 0.02. 0.1 mL of sample was added to 3.9 mL of diluted $ABTS^{\bullet+}$ solution. The absorbance of the solution was then measured at 732 nm after 8 min. Results were expressed as micromoles Trolox equivalents (TE)/100 g sample of dry weight.

2,2-Diphenyl-1-picryl-hydrazyl$^{\bullet}$ scavenging activity was analyzed according to the method of Brand williams, Cuvelier and Berset with some modifications[27]. Briefly, an aliquot of sample (0.1 mL) was mixed with a 6.25×10^{-5} M solution of $DPPH^{\bullet}$ in methanol (3.9 mL). Similarly, a blank was prepared by adding the equal volume of methanol in place of sample in order to measure the maximum $DPPH^{\bullet}$ absorbance. After a 0.5 h reaction in the dark, the decrease in absorbance of the resulting solution was measured at 517 nm. The antioxidant capacity was expressed as micromoles trolox equivalents (TE)/100 g sample of dry weight.

The cupric ion reducing capacity was performed according to the minor modified method of Apak *et al*.[28]. In short, 5 mM $CuSO_4$, 3.75 mM neocuproine, CH_3COONH_4 buffer (1 M, pH 7) solutions (1 mL each) and distilled water (0.6 mL) were added in a test tube successively, followed by addition of sample solution(1 mL) to make the final volume 4.1 mL. The absorbance was measured at 450 nm after 0.5 h. Results were expressed in micromoles Trolox equivalents (TE)/100 g of extract.

Finally, HRSA was estimated using the method described by Ghiselli *et al*.[29] with a slight modification. Briefly, an aliquot of sample (0.1 mL) was added to 690 µL of phosphate buffer (10 mM) at pH 7.4 containing 2.5 mM 2-deoxyribose. Simultaneously, a blank was prepared by adding the equal volume of methanol in place of sample. Subsequently, 0.1 mL of iron ammonium sulfate (1 mM) premixed with EDTA (1.04 mM) was added. The reaction was started by adding ascorbic acid (1 mM, 0.1 mL) and H_2O_2 (0.1 M, 10 µL). After 10 min in a water bath at 37 °C, cold trichloroacetic acid (2.8 %, 1 mL) and thiobarbituric acid (1 %, 0.5 mL) were added. The absorbance was measured at 532 nm after boiled for 8 min and cooled. The percentage of free radical scavenging activity was calculated as follows:

$$\text{Scavenging effect (\%)} = [1 - (A_{sample}/A_{control})] \times 100\ \%$$

RESULTS AND DISCUSSION

Total phenolic content (TPC), total flavonoid content (TFOC), total flavano content (TFAC) and total anthocyanin content (TAC).

Samples of the petals with different colours of six *Lilium* species were freeze-dried before extraction, since freezing samples may preserved higher levels of total phenolics because of enhanced plant cell rupture during ice crystal formation and melting[30,31]. And the methanol was used as extraction medium. The correlative phenols content (including total phenolic content, total flavonoid content, total flavanol content and total anthocyanin content) of the extracts from different *Lilium* samples were examined and shown in Table-1.

Phenolics, such as flavonoids, phenolic acids and tannins, are the most common and widely distributed group to the antioxidant capacity[32]. As shown in Table-1, the TPC varied from 5,637.88 to 11,265.87 mg gallic acid equivalent (GAE)/ 100 g dw and the mean value was 8268.03 mg GAE/100 g. The *L. davidii* had the highest amount of TPC followed by *L. lancifolium*, *L. sargentiae*, *L. davidii* var. *unicolor*, *L. brownii*, whereas *L. leucanthum* showed the lowest TPC among these species. Phenolic compounds are plant metabolites characterized by the presence of several phenol groups. Some of them are very reactive in neutralizing free radicals[33]. Compared with the report previously, the TPC of the petals extract of *Lilium* was higher than that of tree penoy cultivars and marigold flower which also used as traditional food and herbal medicine[3,34]. On the other hand, the TPC measured in the lily petals was greater than that in the onion, shallot, lily bulbs and other 30 Chinese medicinal plants[21,35].

Total flavonoid content of samples decreased as the following order: *L. davidii* > *L. lancifolium* > *L. davidii* var. *unicolor* > *L. brownii* > *L. sargentiae* > *L. leucanthum*. Regarding to TFC levels, the highest total flavonoid content level was also found in *L. davidii* with the mean of 5,836.52 mg rutin equivalent (RE)/100 g dw. *L. leucanthum* showed the lowest total flavonoid content of these species. Flavonoids found ubiquitously in plants are the most common group of phytophenolics[36]. Epidemiologic studies revealed that the consumption of flavonoids reduces the risk of cardiovascular disease, diabetes, arthritis and cancer due, at least in part, to their antioxidant and antiinflammatory activities[37]. Therefore, total flavonoid content in samples suggest that extracts of petals of *Lilium* may serve as a potential source of natural antioxidant for food and pharmaceutical application.

Flavanols, as a subgroup of the flavonoid family, represent a promising class of food components with respect to their ability to recover endothelial function, improve insulin sensitivity, decrease blood pressure and reduce platelet aggregation[38]. The results showed that total flavanol content varied from 181.19 mg (+)-catechin equivalent (CE) /100 g dw to 1,279.24 mg CE/ 100 g dw with the difference of 7-fold and decreased in the following order: *L. lancifolium* > *L. davidii* > *L. davidii* var. *unicolor* > *L. brownii* > *L. sargentiae* > *L. leucanthum*.

Colour is a psychological property of food products that affects the enjoyment of eating. Anthocyanins, as an important group of natural pigments belonging to the flavonoids, are largely responsible for the color of many plants which are widely present in fruits and vegetables and have been well characterized to possess bioactive properties[39,40]. Similar to the TPC, total flavonoid content and total flavanol content, the total anthocyanin content of *L. lancifolium* and *L. davidii*, species with orange petals and violet-black spots were significantly higher than that in the other tested *Lilium* species, with the mean of 479.51 mg Cyanidin-3-*O*-glucoside equivalent (CGE)/100 g dw and 357.62 mg CGE/100 g dw, respectively. And *L. leucanthum* still showed the lowest level of these species.

In this study, six species of different colours (white petals, orange petals, orange petals with violet-black spots) were used for further utilization of petals of *Lilium*. Levels of TPC,total flavonoid content, total flavanol content and total anthocyanin content in extracts of *Lilium* species with orange petals with/ without violet-black spots were significantly higher than those in the species with white petals except *L. sargentiae* and *L. davidii* var. *unicolor* , which showed a different tendency in TPC assay. Samples showed significant difference of antioxidant activity in these assays.

Antioxidant activities of lily petal extracts: Evaluation of the total antioxidant capacity of substances cannot be performed accurately by any single method due to the complex nature of phytochemicals[41]. Biological systems contain a multiplicity of antioxidant systems that may be involved in complex interactions, including synergistic or antagonistic reactions[13]. Many methods are available for analyzing antioxidant activity, with different concepts, mechanisms of action, ways of expressing results and applications. Of these, at least two different methods should be employed in order to evaluate the total antioxidant capacity of the plant products in vitro. In this study, four antioxidant assays, such as DPPH free radical scavenging activity, ABTS radical cation scavenging activity, cupric-reducing antioxidant capacity (CUPRAC) and hydroxyl radical scavenging activity (HRSA) were applied to evaluate the antioxidant properties of six *Lilium* species.

The DPPH radical scavenging capacity assay is one of the most simple and accurate methods in the determination of antioxidant activity *in vitro*[42]. $DPPH^{\bullet}$ is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule[43]. The antioxidants act as a hydrogen donor could terminate the oxidation process by converting free radicals to their stable forms[44]. The scavenging activity of radicals increased with increasing percentage of free radical inhibition[30]. The degrees of discoloration caused by the loss of the initial purple showed the potential for binding of the

TABLE-1
CONTENTS OF TOTAL PHENOLICS (TPC), FLAVONOIDS (TFOC), FLAVANOLS (TFAC) AND ANTHOCYANINS (TOTAL ANTHOCYANIN CONTENT) IN PETAL EXTRACTS FROM SIX *LILIUM* SPECIES

Species	TPC (GAE mg/100 g)	TFOC (RE mg/100 g)	TFAC (CE mg/100 g)	TAC (CGE mg/100 g)
L. brownii	6125.51 ± 58.30 e	2029.43 ± 39.80 d	428.54 ± 1.18 c	14.96 ± 1.06 c
L. leucanthum	5637.88 ± 30.72 e	1290.87 ± 30.28 f	244.59 ± 0.77 d	10.04 ± 0.31 c
L. sargentiae	8482.29 ± 31.29 c	1828.57 ± 47.71 e	181.19 ± 0.53 e	12.25 ± 0.29 c
L. davidii	11265.87 ± 139.41 a	5836.52 ± 29.71 a	1279.24 ± 34.56 a	357.62 ± 23.83 b
L. davidii var. *unicolor*	8060.78 ± 49.09 d	3102.01 ± 28.19 c	620.68 ± 20.73 b	20.75 ± 0.15 c
L. lancifolium	10035.83 ± 56.51 b	4514.83 ± 76.53 b	1302.59 ± 17.53 a	479.51 ± 4.68 a

GAE mg/100 g, RE mg/100 g, CE mg/100 g and CGE mg/100 g represent milligrams of gallic acid equivalent, milligrams of rutin equivalent, milligrams of (+)-catechin equivalent and milligrams of cyanidin-3-*O*-glucoside equivalent per 100 g of dry lily petals, respectively. Values are means of three replicates ± SD. Different letters (a–f) within the same column indicate significant difference at $p < 0.05$ by Duncan's test

free radicals[45]. In this study, samples showed significant difference of antioxidant activity in four assays ($p < 0.05$) and at the testing concentration. The antioxidant activity of all extracts determined as DPPH radical scavenging ability ranged from 287.59 to 2,505.31 μmol trolox equivalent (TE)/100 g dw (Table-2). The highest antioxidant activity was found for the petal extract of *L. davidii*, being about 8 times higher than the species with the lowest antioxidant activity, followed by *L. lancifolium*, *L. davidii* var. *unicolor*, *L. brownii* and *L. sargentiae*, while *L. leucanthum* yielded the lowest antioxidant capacity.

The ABTS radical is the one of the most widely used and stable chromogen compounds to measure the antioxidant activity of hydrogen-donating antioxidants and of chain breaking antioxidants[44,46]. It is more versatile as both the polar and non-polar samples can be evaluated for their scavenging activity[47]. All tested *Lilium* species had significant radical cation scavenging activities and individual species might significantly differ in their $ABTS^{\bullet+}$ scavenging capacities (Table-2). The greatest $ABTS^{\bullet+}$ scavenging capacity of 2,761.63 μmol TE/100 g dw was detected in *L. davidii* sample, while the *L. leucanthum* sample had the lowest $ABTS^{\bullet+}$ scavenging capacity of 975.63 μmol TE/100 g dw. Based on the mean value of each extract sample, the rank of radical cation scavenging activity was *L. davidii* > *L. lancifolium* > *L. brownii* > *L. davidii* var. *unicolor* > *L. sargentiae* > *L. leucanthum*. The radical scavenging of all species on $ABTS^{\bullet+}$ showed a similar trend with the results of the DPPH radical scavenging capacity assay except *L. brownii* and *L. davidii* var. *unicolor*, which showed an different tendency in these two assays. These differences could be due to the compositional differences in extracts and their solubility in different testing systems may also affect their capacities to act as antioxidants.

Determination of the redox potential of the substance focusing on detecting the reducing ability by reducing ability of iron and copper ions is a widely used method to estimate antioxidant capacity[21]. In CUPRAC assay, Cu(II) is reduced to Cu(I) by the action of electron-donating antioxidants. The CUPRAC assay treats the antioxidants in the samples as reductants and it is simple and easily standardized. This assay is superior to the generally used ferric ion reducing antioxidant power (FRAP) assay. First, the reaction conditions are more adaptable to real body systems; second, it can detect the reducing ability of thiol compounds[48]. For CUPRAC, there were significant difference amongst the samples ($p < 0.05$). The values of the samples varied from 944.56 μmol TE/100 g dw to 4,338.17 μmol TE/100 g dw. The CUPRAC of *L. davidii* with the highest value was nearly 4.5 times than that of *L. leucanthum* with the lowest value. The rank order based on the average TE values in accordance with the DPPH radical scavenging capacity assay was as follows: *L. davidii* > *L. lancifolium* > *L. davidii* var. *unicolor* > *L. brownii* > *L. sargentiae* > *L. leucanthum*.

Unlike DPPH and ABTS radicals, which are less relevant to the biological systems, hydroxyl radicals are commonly found in living tissues. Hydroxyl radicals can easily cross cell membranes, can readily react with most biomolecules including carbohydrates, proteins, lipids and DNA in cells and cause tissue damage or cell death.Thus, removing hydroxyl radicals is important for the protection of living systems[49]. In the present assays, deoxyribose assay was considered as a simple and low-cost alternative to pulse radiolysis for determination of rate constants for reaction of most biological molecules with hydroxyl radicals[29]. The HRSA of the samples decreased in the order: *L. davidii* > *L. davidii* var. *unicolor* > *L. lancifolium* > *L. sargentiae* > *L. brownii* > *L. leucanthum*. Similar to the other assays, the result suggested that petals extract of *L. davidii* was the primary hydroxyl radical scavengers while *L. leucanthum* showed the lowest antioxidant capacity. But other species showed a different trend with the results of the other assays. The results reveal lack of consistency among these methodologies. This fact could be explained by the different characteristics and mechanisms of action of the bioactive compounds present in the samples as well as the different principles used to detect antioxidant properties in each method.

It is now well accepted that in plants, phenolic compounds, belonging to the secondary metabolites, are useful in the defensive function against pathogens and radiation and are directly involved in the antioxidant activity[13]. Phenolics constitute the main bioactive phytochemicals that have been proven effective in the prevention of certain chronic diseases such as coronary heart diseases, cancers and diabetes, because of their free radical-scavenging activities[30]. The results of this study showed that the methanol petal extracts of six *Lilium* species had strong antioxidant activity, including DPPH radical, ABTS radical cation scavenging, hydroxyl radical scavenging activities and cupricion reducing capacity. Among the tested *Lilium* species, *L. davidii* had the greatest antioxidant activities, while *L. leucanthum* owned the lowest antioxidant capacities. Levels of those assays in extracts of *Lilium* species having orange petals with/without violet-black spots were significantly higher than those in the species with white petals except

TABLE-2
ANTIOXIDANT ACTIVITY DETERMINED BY THE 2,2-DIPHENYL-1-PICRYL-HYDRAZYL (DPPH), 2,2'-AZINO-*bis*-(3-ETHYLBENZOTHIAZOLINE-6-SULPHONIC ACID) (ABTS), CUPRIC-REDUCING ANTIOXIDANT CAPACITY (CUPRAC) AND HYDROXYL RADICAL SCAVENGING ACTIVITY (HRSA) ASSAYS OF THE BULB EXTRACTS FROM SIX *LILIUM* SPECIES

Species	DPPH (TE μmol/100 g)	ABTS (TE μmol/100 g)	CUPRAC (TE μmol/100 g)	HRSA (%)
L. brownii	1012.33 ± 18.09 d	1581.88 ± 31.25 c	1644.91 ± 10.47 d	53.77 ± 0.22 d
L. leucanthum	287.59 ± 3.66 f	975.63 ± 14.56 f	944.56 ± 7.43 f	49.07 ± 0.45 e
L. sargentiae	717.79 ± 3.03 e	1014.69 ± 5.36 e	1366.25 ± 7.08 e	54.59 ± 0.75 d
L. davidii	2505.31 ± 7.12 a	2761.63 ± 7.89 a	4338.17 ± 49.06 a	68.03 ± 1.34 a
L. davidii var. *unicolor*	1322.81 ± 4.16 c	1363.81 ± 11.26 d	2353.66 ± 18.51 c	59.67 ± 0.30 b
L. lancifolium	1839.69 ± 1.15 b	2011.46 ± 9.43 b	3453.61 ± 11.06 b	58.25 ± 0.37 c

TE μmol/100 g represents micromoles of trolox equivalents per 100 g of dry petals from six *Lilium* species for DPPH and ABTS free radical-scavenging capacity and CUPRAC. HRSA was expressed as the percentage of free radical-scavenging activity (%). Values are expressed as means ± SD (n = 3). Means in the same column followed by different letters (a–f) are significantly different ($p < 0.05$)

L. brownii and *L. davidii* var. *unicolor*, which showed different tendency in ABTS radical cation scavenging assay. Compared with some commonly consumed vegetables, six tested *Lilium* species had the higher antioxidant capacities than potato and carrot, but lower than some other vegetables, such as tomato, green bean, spinach, kale, broccoli and rhubarb, by measuring their radical cation scavenging activity[50]. In China, lily bulbs have been used as a vegetable and herb medicine for several centuries since Han dynasty (B.C. 202-A.D. 220). Moreover, the lily petals have also been used to make various delicious folk foods, such as porridge, soup, teas and honey wine,and regarded as traditional Chinese medicine. Thus the results in this study indicate that lily petals could be a potential source of natural antioxidants. In addition, it also provide data for using lily petals as natural antioxidants as well as, promoting the preservation of such *Lilium* species.

Identification and determination of phenolic constituents in extracts: Typical phenolics that possess antioxidant activity are known to be mainly phenolic acids and flavonoids that widely occur in the plant kingdom[51]. In order to know the phenolic constituents present in extract of the petals of *Lilium* species from rural China, all the extracts were analyzed by HPLC. Three phenolic acids (gallic acid, *p*-coumaric acid and chorogenic acid), two flavonols (rutin and kaempferol), two monomeric flavanols [(+)-catechin and (-)-epicatechin], two dihydroflavonols (dihydromyricetin and dihydroquercetin) and two anthocyanins (cyanidin and cyanidin-3-rutinoside) were quantified. The representative chromatograms of the standard mixture solution, *L. davidii* and *L. brownii* extract separation are depicted in Fig. 2 and the phenolic contents of *Lilium* species are listed in Table-3. Statistically significant differences were found between the species analyzed for each compound assayed.

Regarding the phenolic acids, chlorogenic acid, *p*-coumaric acid and gallic acid were detected and quantified in the petal extracts analyzed. Natural phenolic acids are strong antioxidants and exhibit potential antifungal, antibacterial, antiinflammatory and anticancer activity[21]. Table-3 showed that chlorogenic acid was the major component of phenolic acids, with mean values (n=6) of 366.56 mg/100 g dw which was approximately 40-fold higher than that of gallic acid. Chlorogenic acid was detected in all the extracts of six *Lilium*

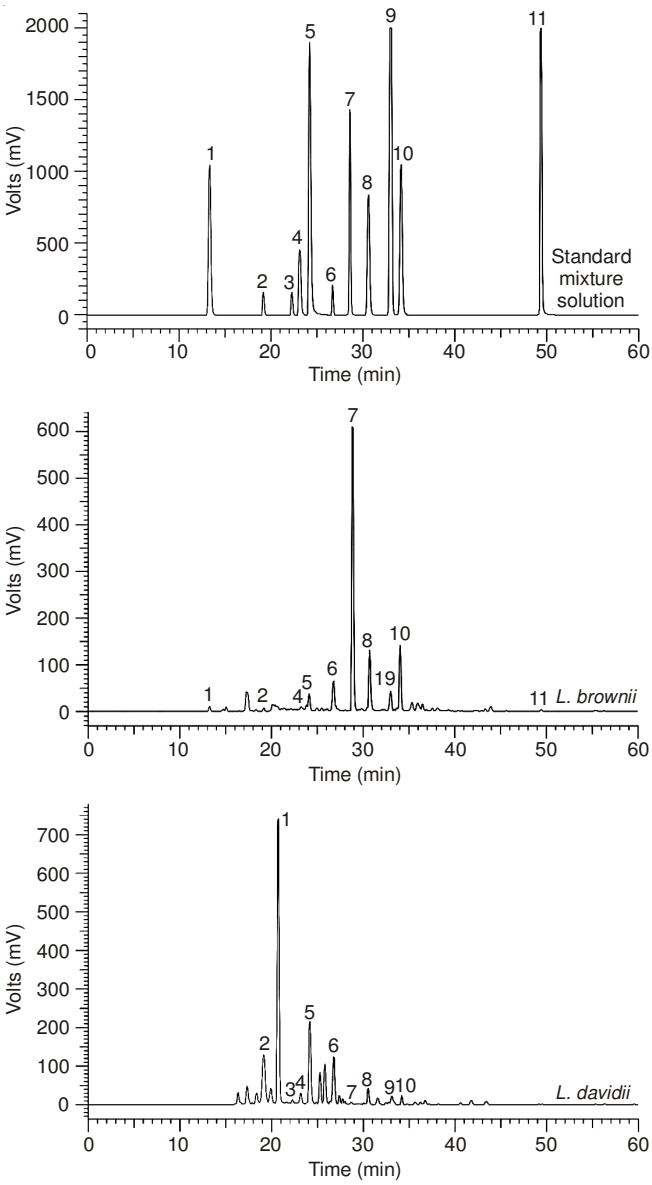

Fig. 2. HPLC trace of individual polyphenolic constituents (1, gallic acid; 2, cyanidin-3-rutinoside; 3, cyanidin; 4, (+)-catechin; 5, chlorogenic acid; 6, (-)-epicatechin; 7, dihydromyricetin; 8, rutin; 9, *p*-Coumaric acid; 10, dihydroquercetin; 11, kaempferol) of the standard mixture solution , L. *brownii* and L. *davidii*

TABLE-3
PHENOLIC COMPOSITION OF THE PETAL EXTRACTS FROM SIX *LILIUM* SPECIES

Phenolic compounds	Retention time (min)	*L. brownii*	*L. leucanthum*	*L. sargentiae*	*L. davidii*	*L. davidii* var. *unicolor*	*L. lancifolium*
Gallic acid	13.29	3.48 ± 0.31 b	2.94 ± 0.02 b	20.62 ± 2.54 a	ND	ND	ND
Cyanidin-3-rutinoside	19.21	9.46 ± 0.59 d	8.91 ± 0.24 d	5.038 ± 0.76 d	324.83 ± 6.77 b	17.47 ± 1.18 c	429.54 ± 6.77 a
Cyanidin	22.53	ND	ND	ND	12.359 ± 2.44 b	4.17 ± 0.87 c	24.39 ± 2.60 a
(+)-Catechin	23.39	26.75 ± 1.24 e	89.33 ± 2.55 b	104.25 ± 4.23 a	65.41 ± 5.67 c	17.46 ± 4.28 f	37.33 ± 3.51 d
Chlorogenic acid	24.33	98.25 ± 3.32 f	158.57 ± 2.78 e	396.54 ± 5.77 c	545.38 ± 7.83 b	306.18 ± 5.77 d	694.45 ± 7.62 a
(–)-Epicatechin	26.82	168.07 ± 4.79 b	56.23 ± 0.36 e	75.01 ± 2.24 d	302.85 ± 6.92 a	149.24 ± 4.27 c	151.87 ± 3.71 c
Dihydromyricetin	28.71	164.64 ± 7.20 a	20.53 ± 1.23 c	6.08 ± 3.39 d	3.48 ± 0.59 d	7.7933 ± 2.30 d	27.16 ± 1.27 b
Rutin	30.69	1077.07 ± 9.55 b	784.06 ± 8.69 c	1392.33 ± 8.66 a	314.37 ± 7.92 e	333.68 ± 8.41 d	49.76 ± 2.58 f
p-Coumaric acid	33.13	56.42 ± 3.62 a	ND	53.89 ± 3.76 a	41.50 ± 4.29 b	43.91 ± 3.99 b	29.02 ± 1.83 c
Dihydroquercetin	34.12	80.81 ± 4.28 c	101.32 ± 3.54 b	172.15 ± 6.23 a	23.49 ± 5.72 d	17.53 ± 2.39 d	5.20 ± 0.57 e
Kaempferol	49.49	6.32 ± 0.32 b	ND	12.957 ± 1.89 a	ND	3.62 ± 0.74 b	ND

Values, in mg/100 g dw, are expressed as means ± SD (n = 3). Means in the same line followed by different letters (a–e) are significantly different ($p < 0.05$). ND = Not detected

six *Lilium* species and mainly abundant in *L. lancifolium* and *L. davidii* (694.45 and 545.38 mg/100 g dw, respectively), with the mean value of 619.92 mg/100 g dw,which was 5 times higher than the lowest level found in *L. brownii*, followed by *L. sargentiae*, *L. davidii* var. *unicolor*, *L. leucanthum*, *L. brownii*. It is reported that by scavenging peroxyradical chlorogenic acid inhibited the initiation of chain lipid peroxidations by organic free radical[52]. Chlorogenic acid can limit low-density lipid oxidation, the major determinant of the initial events in atherosclerosis[53]. Furthermore, it removes particularly toxic reactive species by scavenging alkylperoxyl radicals and may prevent carcinogenesis by reducing the DNA damage they cause[54,55]. *L. brownii* contained the highest concentration of *p*-coumaric acid (56.42 mg/100 g dw) which was found in all the samples of six *Lilium* species except *L. leucanthum* with insignificant difference and *L. lancifolium* yieded the lowest content, with the value of 29.02 mg/100 g dw. Minor quantities of gallic acid (2.94-20.62 mg/100 g dw) were found in *Lilium* species with white petals (*L. brownii*, *L. leucanthum* and *L. sargentiae*) with the mean value of 9.01 mg/100 g dw.

Rutin and dihydroquercetin were the dominant flavonol and dihydroflavonol compounds identified in the extracts. Furthermore, dihydromyricetin also exhibited a comparable level. These compounds were detected in all the extracts. *L. sargentiae* and *L. brownii* contained abundant rutin with mean values of 1,392.33 and 1,077.07 mg/100 g dw, respectively. For the species having orange petals with/without violet-black spots, the highest value was found in *L. davidii* var. *unicolor* with 3-folds lower than *L. sargentiae*. Rutin is a type of flavonol with various biological activities that may protect against spatial memory impairment accompanying hippocampal pyramidal neuron loss[56]. The levels of dihydroquercetin in *L. leucanthum* and *L. sargentiae* (172.15 and 101.32 mg/100 g dw, respectively) were higher than those in other species. *L. brownii* had the the highest level of dihydromyricetin which were significantly higher than that in the other tested *Lilium* species, with the mean of 164.64 mg/100 g dw, reaching 11 times higher than the amounts detected in the other species analyzed. As previously indicated, dihydromyricetin has been shown to be effective in inhibiting hypertension, relieving cough, protecting the liver and decreasing blood sugar[57]. And dihydroquercetin have been reported to effectively exhibit neuroprotective actions against the oxidative injuries induced in cortical cell cultures. The levels of kaempferol found in part of the petal extracts were significantly lower than other compounds.

Consistent with previous study on bulbs of *Lilium*, (+)-Catechin and (-)-epicatechin were the typical monomeric flavanol compounds identified in the petals of the *Lilium* species analyzed[21]. (-)-Epicatechin was the dominant monomeric flavanol compound identified in *L. davidii*, followed by *L. brownii*, *L. davidii* var. *unicolor*, *L. lancifolium*, *L. sargentiae*, whereas the value of *L. leucanthum* was only 56.23 mg/100 g dw which was approximately 5-folds lower than that of *L. davidii*. Statistically significant differences of the levels of (+)-catechin in extracts were found between the varieties. *L. sargentiae*, *L. leucanthum* and *L. davidii* were identified to be the species which contained abundant (+)-catechin (104.25, 89.33 and 65.41 mg/100 g dw, respectively). (+)-Catechin and (-)-epicatechin have a positive correlation with $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging capacity and attenuate the copper-mediated and endothelial cell-mediated oxidation of LDL. The antioxidant activity is attributed to the presence of a catechol group on the B ring, which can trap free radicals and chelate redox-active metals[56,58]. In recent years, this kind of compounds have attracted interest regarding cardiovascular health[59]. (-)-Epicatechin and other flavanols proved to be inhibitory at the enzyme level[58].

Anthocyanins, present in fruits and vegetables as natural colorants, have been well characterized to possess bioactive properties[40]. They are the aglycone units of anthocyanins and there are six major types found widely in plants[39]. In this study, cyanidin and cyanidin-3-rutinoside were detected in petals of six *Lilium* species. Cyanidin-3-rutinoside was identified in all the samples of six *Lilium* species. *L. lancifolium* and *L. davidii*, having orange petals with violet-black spots, contained abundant cyanidin-3-rutinoside, with average value of 377.18 mg/100 g dw, reaching values 36 times higher than the amounts detected in the other species analyzed. Cyanidin was found in *L. davidii*, *L. davidii* var. *unicolor* and *L. lancifolium*, species having orange petals with/without violet-black spots, ranging from 4.17 to 24.39 mg/100 g dw. As previously indicated, anthocyanins contribute a major part of the antioxidant ability to suppress both peroxyl radical-induced chemical and intracellular oxidation. This kind of compound have been shown to use in the functional food and nutraceutical industry with recent reports on the various potential health benefits, that include a reduced risk of coronary heart disease, visual improvement and anticarcinogenic, antimutagenic and anti-inflammatory effects. Many of these health benefits have been attributed to the antioxidant property of anthocyanins[60].

Table-3 showed that there were differences in the major component of phenolic compounds between the various species likely due to genotypic differences. The difference of phenolic composition might explain the different antioxidant abilities of extracts of petals of *Lilium* species observed above. Also, it can be speculated that phenolic compounds present in the extracts may exert their antioxidant capacity individually as well as synergistically. It is possible that some phenolic compounds identified from the extracts might create a synergistic effect that increases the bioactivity. Some compounds may contribute more to the bioactivity. It is also possible that some compounds with a minor content and strong activity may have not been identified.

Relationships amongst different antioxidant variables: Correlation analysis was used to explore the relationships amongst the different antioxidant variables measured for all petal extracts of six *Lilium* species (Table-4). All the antioxidant assays, except ABTS, were significantly correlated with TPC at the 0.01 level. This indicated that abundant phenolic compounds was helpful to enhance the antioxidant activity. Significant positive correlations at the 0.01 level were found between total flavonoid content and antioxidant capacity values in each assay. CUPRAC was significantly correlated with total flavanol content at the 0.01 level. The results suggest that flavonoid compounds, a major class of phenolic compounds in *Lilium* petals, can remove free radicals in the antioxidant reaction and enhance the reducing power to contribute to antioxidant activity. Total flavonoid content were significantly

TABLE-4
LINEAR CORRELATION COEFFICIENTS BETWEEN PHENOLIC COMPOSITION AND ANTIOXIDANT CAPACITY (PANEL A), AND AMONG THE DIFFERENT METHODS FOR QUANTIFYING ANTIOXIDANT CAPACITY (PANEL B)

	DPPH	ABTS	CUPRAC	HRSA
Panel A				
Total phenolics content (TPC)	0.89**	0.79*	0.90**	0.88**
Total flavonoid content (TFOC)	0.99**	0.95**	1.00**	0.93**
Total flavano content (TFAC)	0.93**	0.90**	0.96**	0.78*
Total anthocyanin content (TAC)	0.81*	0.80*	0.86*	0.62
Panel B				
2,2-Diphenyl-1-picryl-hydrazyl (DPPH)	1.00			
2,2'-Azino-*bis*-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS)	0.96**	1.00		
Cupric-reducing antioxidant capacity (CUPRAC)	0.99**	0.95**	1.00	
Hydroxyl radical scavenging activity (HRSA)	0.95**	0.87*	0.92**	1.00

*Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level

correlated with DPPH, ABTS and CUPRAC at 0.01 level and the linear correlation coefficients between total anthocyanin content and DPPH, total anthocyanin content and ABTS, total anthocyanin content and CUPRAC were significant at the 0.05 level. However, no significant correlation was found between total anthocyanin content and HRSA.

Regarding the different methods, the significant correlation between methods was confirmed with four methods (DPPH, ABTS, CUPRAC and HRSA). There were significant positive correlations between DPPH and other three assays at 0.01 level. ABTS and CUPRAC were significantly correlated with CUPRAC and HRSA at the 0.01 level, respectively. ABTS was only significantly correlated with HRSA with the correlation of 0.87 at 0.05 level. This showed that using of more than one method is suitable for the analysis of *in vitro* antioxidant activity of these samples.

Cluster analysis: Data on phenolic contents and antioxidant capacity were used to carry out a cluster analysis of the *Lilium* species. The dendrogram that was generated by cluster analysis (Fig. 3) shows a certain correlation within species belonging to the *Lilium* genus. As the results show, *L. davidii* var. *unicolor* and *L. sargentiae* are arranged in one group characterized by moderate phenolic content and antioxidant capacity. *L. davidii* and *L. lancifolium* belong to the group with high phenolic content and strong antioxidant power, while *L. brownii* and *L. leucanthum* belong to the third group with low phenolic content and weak antioxidant activity.

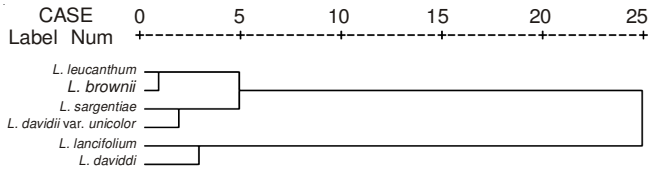

Fig. 3. Dendrogram plot visualizing the clustering of the petal extracts from six *Lilium* species in this study based on their phenolic composition and antioxidant properties

Conclusion

The total and individual phenolic contents of petal extracts of six *Lilium* species native to China were evaluated and the antioxidant activities of petals of these species were assessed. Significant difference exists in the antioxidant capacity as well as in the phenolic content of petal extracts of *Lilium* assayed. Chlorogenic acid was the major component of phenolic acids and cyanidin-3-rutinoside, the major kind of anthocyanin in lily petals, was identified in all the samples. (-)-Epicatechin was the typical monomeric compounds of flavanol and rutin was the dominant flavonol. According to the linear correlation analysis, the amount of phenolic compounds of the petals of *Lilium* analyzed showed significant correlation with the antioxidant activity of the samples. *L. davidii* was markedly superior to the other species in the four antioxidant assays, as well as in the total phenolic content, total flavonoid content and total flavanol content assays. *L. lancifolium* was arranged in the same group with *L. davidii* for the high levels in phenolic content and antioxidant capacity. Overall, the results of this research support the extensive use of the petals of *Lilium*. They are important for selecting *Lilium* species with strong antioxidant activity and high phenolic content for use as a plant resource of valuable antioxidants, especially for natural antioxidants and pharmaceutical applications.

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