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Phenolic compounds and antioxidant properties of bulb extracts of *Lilium leucanthum* (Baker) Baker native to China

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RESEARCH ARTICLE

Abstract

Lilium leucanthum is popularly used as one of the ornamental lily plant and medicinal bulbs in its local distribution areas with a long history in China. The aim of this study was to provide sufficient experimental evidence for further utilisation of wild *L. leucanthum* as a source of natural antioxidants. Total phenolic content and antioxidant activity of three ecotypes of *L. leucanthum* from Zhenping, Zhouqu and Liuba County of China P.R. were determined using the method of Folin-Ciocalteau and 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity, cupric-reducing antioxidant capacity, hydroxyl radical scavenging activity, superoxide radical scavenging activity and lipid peroxidation inhibition. Ten antioxidant standards were used in high-performance liquid chromatography (HPLC) with diode array detection for phenolic compounds. The results showed that the tested samples from wild Zhenping ecotype had the highest phenolic content and the strongest antioxidant capacity among all three ecotypes, except for superoxide radical scavenging activity. HPLC analysis revealed that myricetin was the most abundant phenolic and the chlorogenic acid was the major phenolic acid in all bulb extracts tested. According to the linear correlation analysis, the total phenolic content was the major contributor to the total antioxidant capacity in lily bulb except for superoxide radical scavenging activity. The results suggest the wild Zhenping ecotype of the selected *L. leucanthum* would have a promising application as a potential source of food and medicinal herb by virtue of its valuable properties for human health.

Keywords: antioxidant activity, ecotype, *L. leucanthum*, phenolic compounds

1. Introduction

Reactive oxygen species (ROS), which includes the production of hydroxyl radicals, superoxide anions, and hydrogen peroxide, are continuously produced during normal physiological activities and may not have harmful effects on cell function at physiological concentrations (Zhang *et al.*, 2011). However, ROS are excessively generated in living organisms when exposed to ultraviolet rays, ozone, tobacco smoke, industrial exhausts and other exogenous stress factors. When the formation of ROS exceeds the capacity of cellular antioxidant defences to neutralise their effects, the delicate cellular balance will be disturbed (Reddy *et al.*, 2010). Several studies have demonstrated that the overproduction of ROS can contribute to DNA damage, protein oxidation and lipid peroxidation in living tissues and cells (Halliwell, 1996; Liang *et al.*, 2010; Muralikrishna and Hatcher, 2006). Hence, dietary supplements of antioxidants have become popular and effective in enhancing the body's antioxidant defences and balancing the ROS in the human body (Shukla *et al.*, 2009).

Antioxidants can be defined as any substance that, present in low concentrations compared to an oxidised substrate, effectively delays or inhibits oxidation of the substrate (Tiveron *et al.*, 2012). For the food industry, synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole are commonly used. Nevertheless, there is widespread agreement that synthetic antioxidants need to be replaced with natural antioxidants because

of their potential health risks and toxicity, most notably possible carcinogenic effects (Ito *et al.*, 1986; Safer and Al-Nughamish, 1999). In view of their safety, the use of naturally-occurring antioxidants has attracted considerable attention. As an important category of natural antioxidants, phenolic compounds universally exist in plants. They have attracted increasing attention as potential agents for preventing and treating many oxidative stress-related diseases (Fu *et al.*, 2011).

As an important source of natural antioxidants, many traditional Chinese medicinal herbs have proven to exhibit major antioxidant activity (Liao *et al.*, 2008; Wang *et al.*, 2005). Lily has been extensively used both as a food and a traditional Chinese medicine for many centuries in China, due to its health-promoting properties to treat bronchitis, pneumonia, chromic gastritis and provide nourishment and act as a tonic (You *et al.*, 2010). Recent reports have also disclosed antioxidant, antibacterial and anti-inflammatory properties of the methanol extract of lily bulbs (Luo *et al.*, 2012). *Lilium leucanthum* (Baker) Baker, a liliaceous species, is a native perennial bulbous plant which is restricted to the provinces of Shaanxi, Hubei, Chongqing, Gansu and Sichuan in China. This species is a high-value ornamental plant with potential for use both as a pot and cut-flower plant. Meanwhile, its bulb is also an important ingredient in some traditional Chinese medicines (Tang *et al.*, 2010b). It is possible that *L. leucanthum* contains bioactive components that contribute to good health. To the best of our knowledge, there is little literature on its chemical composition and bioactivity. Moreover, a systematic comparison of different ecotypes on the phytochemistry and antioxidant activities of *L. leucanthum* is lacking. Thus, detailed information about the health-promoting components and biological activities of *L. leucanthum* is helpful to give a better insight into its use as functional food and ingredient in pharmaceuticals, nutraceuticals, and medicines.

The aim of this study was to systematically evaluate the phenolic content and antioxidant capacity of three ecotypes of *L. leucanthum* native to China. Hopefully, this study will provide sufficient experimental evidence for the antioxidant activity and potential for further development and use of *L. leucanthum*.

2. Materials and methods

Chemicals

Folin-Ciocalteu's phenol regent, ferrozine, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,9-dimethyl-1,10-phenanthroline (neocuproine), 2-deoxyribose, nicotinamide adenine dinucleotide (NADH), nitroblue tetrazolium (NBT), phenazine methosulfate (PMS), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox), egg lecithin and all the phenolic compounds (purity >97%) were supplied by Sigma-Aldrich (Shanghai, China P.R.). All other chemicals were analytical grade and supplied by Xi'an Chemical Reagent Co. Ltd. (Xi'an, China P.R.).

Plant materials

Bulbs of three wild ecotypes of *L. leucanthum* were collected from Zhouqu County (34°47' N and 104°22' E with an altitude of 1,400 m), Gansu province, Zhenping (31°54'N and 109°32' E with an altitude of 994 m) and Liuba County (33°38' N and 106°56' E with an altitude of 1,031 m), Shaanxi province, respectively on August 2009, and were authenticated by Professor Lixin Niu at the College of Horticulture, Northwest A&F University, where the voucher specimen of the plant materials was deposited. Fresh bulbs were washed with cold water, frozen in liquid nitrogen, freeze-dried (LGJ-10; Songyuan Huasheng Biotechnology Co. Ltd., Beijing, China P.R.), ground into powder using an electrical grinder (JP-250A-8; Jiugong Economy and Trade Co. Ltd., Shanghai, China P.R.), and stored at -20 °C until analysis.

Preparation of phenolic extracts

A fine dried lily bulb powder sample (10 g) was homogenised and extracted with ultrasonic assistance in 100 ml of acidified methanol solution (0.1% HCl, v/v) at 25 °C for 30 min in an external water bath. The homogenate was centrifuged at 12,000 rpm for 10 min at 4 °C (KDC-140HR; Zhongke Scientific Instruments Co. Ltd., Anhui, China P.R.). The residue of extraction was repeated three times under the same conditions. The supernatants obtained were combined and stored in a freezer at -20 °C for later analysis.

Prior to analysis, 50 ml of the extracts were pipetted into a 250 ml evaporation flask and concentrated to a volume of 10 ml on a rotary evaporator (SENCO-R series; Shensheng Biotech Co. Ltd., Shanghai, China P.R.) at 35 °C. The enriched extracts (aqueous phase) were then extracted three times with 10 ml of ethyl acetate. Then, the combined organic phases were evaporated to dryness under vacuum. Subsequently, the dried residuals were re-dissolved in 5 ml of methanol (HPLC grade). This methanol solution was filtered through a 0.45 μm filter and analysed by HPLC for individual phenolic compounds.

Determination of basic nutritional contents

Three wild ecotypes of *L. leucanthum* were evaluated for total soluble (%) and reducing sugars (%), starch (%), protein (%) and crude fibre (%) contents by the AACC approved method (AACC, 1999).

Determination of total phenolic, total flavonoid and total flavanol contents

The total phenolic content (TPC) in the bulb extract was determined using the Folin-Ciocalteu reagent according to the method of Halicia *et al.* (2005). Results were expressed as the equivalent of milligrams of gallic acid per kilogram of dry weight (mg GAE/kg).

Total flavonoid content (TFOC) was determined according to the method of Chang *et al.* (2002) with minor modification. In short, in a centrifuge tube, an aliquot of sample solution (300 µl) was mixed with methanol solution (2.7 ml), $NaNO_2$ (0.5 M, 200 µl), and $AlCl_3$ (0.3 M, 200 µl) in sequence. After 5 min, NaOH (1 M, 1 ml) was added to the reaction system, and the absorbance was measured against blank at 510 nm. The results were expressed as the equivalent in milligrams of rutin per kilogram of dry weight (mg RE/kg).

The determination of total flavanol content (TFAC) was performed colorimetrically by the vanillin method according to the method of Rockenbach *et al.* (2011). Results were expressed as the equivalent in milligrams of (+)-catechin per kilogram of dry weight (mg CE/kg).

Antioxidant activity evaluation

The ability of phenolics from the *Lilium* extracts to scavenge DPPH free radicals was determined. Scavenging activity was measured based on the method of Amarowicz *et al.* (2002) with slight modification. Briefly, an aliquot of sample (0.1 ml) was mixed with a 6.25×10^{-5} M solution of $DPPH^{\cdot}$ in methanol (3.9 ml). A control sample containing the equal volume of methanol in place of sample was used to measure the maximum $DPPH^{\cdot}$ absorbance. After a reaction for 20 min in the dark, the absorbance at 517 nm was recorded to determine the concentration of remaining $DPPH^{\cdot}$. The antioxidant capacity was expressed as micromoles trolox equivalents (TE)/kilogram sample of dry weight.

Cupric ion reducing antioxidant capacity (CUPRAC) was determined as described by Apak *et al.* (2004) with minor changes. Briefly, an aliquot of sample (0.1 ml) was mixed with 5 mM $CuSO_4$, 3.75 mM neocuproine (0.3 ml each), and distilled water (2.8 ml). After 30 min, absorbance was measured at 450 nm. Results were expressed in micromoles TE/kilogram sample of dry weight.

Hydroxyl radical scavenging activity (HRSA) of *Lilium* bulb extracts was estimated using the method described by Sroka and Cisowski (2003) with a slight modification. Briefly, $FeSO_4$ (20 mM, 0.1 ml), H_2O_2 (45 µL, 0.15%), and salicylic acid (8 mM, 1 ml) were sequentially mixed with distilled water (4 ml). Subsequently, *Lilium* bulb extract (1 ml) was added to this mixture and left for 30 min at 37 °C. Readings of the coloured product were then taken at 593 nm, and the percentage of free radical scavenging activity was calculated as follows:

$$\text{scavenging activity (\%)} = (1 - A_{sample}/A_{control}) \times 100$$

The superoxide radical scavenging activity (SRSA) was measured by the method of Chen and Yen (2007) with a minor modification. Superoxide radicals were generated in PMS-NADH system by oxidation of NADH and assayed by reduction of NBT. The reaction mixture, which contained samples (0.1 ml) in methanol, NBT (1 ml, 156 µM) in phosphate buffer (100 µM, pH 7.4), NADH (1 ml, 468 µM) in phosphate buffer, and PMS (0.1 ml, 60 µM). After incubation at 25 °C for 8 min, the absorbance (A) was measured at 560 nm against methanol as control. The superoxide radical scavenging activity was calculated as follows:

$$\text{scavenging activity (\%)} = (1 - A_{sample}/A_{control}) \times 100$$

Lipid peroxidation inhibition (LPI) of tested sample was determined according to the method of Tsuda *et al.* (1994). Liposomes were prepared as follows: egg lecithin (5 g) was dispersed in a sodium phosphate buffer (500 ml, 20 mM, and pH 7.4) and sonicated for 30 min in an ice-cold water bath. A sample (0.5 ml) was mixed with liposomes (2 ml), 25 mM $FeCl_3$ (0.1 ml), 25 mM H_2O_2 (0.1 ml), 25 mM ascorbic acid (0.1 ml) and 0.2 M phosphate buffer (1.2 ml, pH 7.4). The reaction mixture was incubated at 37 °C for 4 h. At the end of the incubation, butylated hydroxyanisole (1 ml 20 mg/ml in methanol) was added to terminate the oxidation reaction. The extent of oxidation of liposomes was subsequently determined by measuring the thiobarbituric acid-reactive substances. The absorbance of the supernatant was measured spectrophotometrically at 532 nm. The lipid peroxidation inhibition was calculated as follows:

$$\text{inhibition (\%)} = (1 - A_{sample}/A_{control}) \times 100$$

Phenolic compounds analysis by HPLC

Lilium bulb extracts were analysed using 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-C_{18} column (250 mm × 4.0 mm, 5 µm). Chromatographic identification and confirmation of phenolic compounds was based on comparing their retention times with those of pure standards and quantification was made by using the external standard method. The standards, gallic acid, *p*-coumaric acid, chlorogenic acid, (+)-catechin, (–)-epicatechin, rutin, quercetin, phloridzin, kaempferol and myricetin were dissolved in methanol at a stock concentration of 1 mg/ml.

Calibration standard mixture was prepared by appropriate dilutions with methanol from the stock solution.

Elution was performed with a gradient mobile phase of solvent A being water-acetic acid (98:2, v/v) and solvent B being acetonitrile. The gradient elution conditions were as follows: 0-40 min, 5-40% B; 40-45 min, 40-100% B; 45-60 min, 100% B. The wavelength-switching program was employed. The column was held at 40 °C and then flushed at a flow rate of 0.5 ml/min. A volume of 10 µl was injected for each run in triplicate. The PDA detector scanned from 200 to 400 nm. Results were acquired and processed by the Shimadzu Workstation CLASS-VP 6.12 software and expressed as mg/kg sample of dry weight.

Statistical analysis

All analyses were carried out in triplicate and the results were presented as means ± standard deviation (SD). Data were analysed using one-way analysis of variance (ANOVA) followed by SPSS version 16.0 for Windows (IBM, Armonk, NY, USA). Significant differences were calculated according to Duncan's multiple range tests and differences at $P<0.05$ were considered statistically significant. A two-tailed Pearson's correlation test was processed to determine the correlations among different antioxidant methods and phenolic compounds.

3. Results and discussion

Basic nutritional composition

To our knowledge, there is a lack of available literature concerning the biochemical composition among different ecotypes of *L. leucanthum*. This species is naturally distributed in limited areas of the western part of China. Wild populations of the species can be found along the road side, on the borders of forests, and in forest clearings. It could be a potential source of valuable germplasm for further breeding of new commercial lily cultivars with high disease-resistance like traditional Chinese medicine herbs (Tang *et al.*, 2010b). The determination of the biochemical composition of the different ecotypes of *L. leucanthum* allowed selection of the best strain characterised by the highest content of basic nutritional composition and the results are shown in Table 1. The highest total soluble sugar and protein contents were found in wild Zhenping ecotype. The reducing sugar in the wild Zhouqu ecotype was higher than other two ecotypes. The wild Liuba ecotype had the highest amount of crude fibre (2.15%) content. No significant differences in the starch content were observed among the three ecotypes of *L. leucanthum*.

Comparison on phenolic compounds

It has been recognised that phenolic compound contents (such as flavonoids, stilbenes, and tannins) of botanical materials are associated with their antioxidant activities (Velioglu *et al.*, 1998). In this study, TPC, TFOC and TFAC of the three ecotypes of *L. leucanthum* were measured, as shown in Table 2, indicating a strong variation of phenolic concentrations in the tested samples.

The TPC of all tested bulb extracts ranged from 6,686.40 to 21,360.00 mg gallic acid equivalent (GAE)/kg extract. The wild Zhenping ecotype of *L. leucanthum* had the highest amount of TPC, followed by that in wild Liuba ecotype, whereas wild Zhouqu ecotype of the tested samples had the lowest TPC. The significant difference in the TPC suggests that *L. leucanthum* collected from Zhenping County may be a better source of phenolic compounds than the other two ecotypes. Compared with some other traditional Chinese medicinal plants, the TPC measured in the *L. leucanthum* bulbs was higher than that in the *Angelica dahurica*, *Bupleurum chinense*, *Fritillaria cirrhosa* and *Gleditsia sinensis*, but was lower than the content reported recently in *Pueraria lobata* (Willd.) *Ohwi* (flower), *Tussilago farfara*, *Eriobotrya japonica*, *Ephedra sinica*, and *Dioscorea bulbifera* (Song *et al.*, 2010).

Flavonoids, the most common group of phytophenolics, are ubiquitously present in plants and have versatile functions. Flavonoids are compounds with strong antioxidant properties and can therefore effectively remove oxygen radicals from the body (Kang *et al.*, 2011). The wild Zhenping ecotype lily bulb contained the highest TFOC,

Table 1. The basic nutritional parameters in bulbs of three wild ecotypes of *Lilium leucanthum*.[1]

Ingredient	Wild Zhenping ecotype	Wild Zhouqu ecotype	Wild Liuba ecotype
Total soluble sugar (%)	6.33±0.41 a	4.88±0.22 b	4.15±0.12 c
Reducing sugar (%)	1.52±0.03 b	2.26±0.11 a	1.26±0.06 c
Starch (%)	56.41±3.39 a	61.43±2.78 a	53.72±1.15 a
Total protein (%)	14.90±1.17 a	6.29±0.47 c	11.18±0.50 b
Crude fibre (%)	1.49±0.03 b	2.01±0.23 a	2.15±0.20 a

[1] Values are means of 3 replicates ± standard deviation. Different letters within the same column indicate significant difference at $P<0.05$ by Duncan's test.

Table 2. Contents of total polyphenols (TPC), flavonoids (TFOC) and flavanols (TFAC) in bulb extracts of three wild ecotypes of *Lilium leucanthum*.[1]

Analytical index	Wild Zhenpin ecotype	Wild Zhouqu ecotype	Wild Liuba ecotype
TPC (GAE mg/kg)	21,360.00±292.80 a	6,686.40±304.88 c	17,081.60±789.94 b
TFOC (RE mg/kg)	5,078.54±62.64 a	1,780.52±61.69 c	4,081.30±68.59 b
TFAC (CE mg/kg)	2,150.78±195.30 a	719.42±64.54 c	1,156.94±47.16 b

[1] GAE mg/kg, RE mg/kg and CE mg/kg represent milligrams of gallic acid equivalents, milligrams of rutin equivalent and milligrams of (+)-catechin equivalent per kilogram of dry lily bulbs, respectively. Values are means of 3 replicates ± standard deviation. Different letters within the same column indicate significant difference at $P<0.05$ by Duncan's test.

with the mean of 5078.54 mg rutin equivalent (RE)/kg, followed by those in wild Liuba ecotype (4,081.30 mg/kg of RE) and wild Zhouqu ecotype (1,780.52 mg/kg of RE). Consumption of fruit and vegetable is well known as a way of obtaining a wide variety of flavonoids, which play a protective role by reducing the risk of cancer and cardiovascular diseases (Hollman *et al.*, 1996). In this study, the contribution of TFOC to the TPC value of the tested bulb extracts varied from 23.81% to 26.63%, indicating that the high concentration of flavonoids in lily bulbs somewhat substantiates its function in natural antioxidant products.

Quantitatively, flavanols represent a major group of flavonoids in the diet and have demonstrated positive effects on human health, including the recovery of endothelial function, improvements in insulin sensitivity, decreasing blood pressure, and reductions in platelet aggregation (Christian *et al.*, 2010). The TFAC was estimated by vanillin-HCl assay and the trend was similar to those observed for the TPC in the lily bulb extracts, where the wild Zhenping ecotype of *L. leucanthum* showed the highest TFAC with the amount of 2,150.78 mg (+)-catechin equivalent (CE)/kg, followed by that in wild Liuba ecotype (1,156.94 mg/kg of CE), whereas wild Zhouqu ecotype of the tested samples had the lowest TFAC (719.42 mg/kg of CE), which was only one third less than that in wild Zhenping ecotype.

Phenolic compounds by HPLC

In order to find out which phenolic constituents were present in the lily bulb extract, all the extracts were analysed by HPLC. Ten phenolic compounds were quantified from three ecotypes of *L. leucanthum* samples, including three phenolic acid (gallic acid, *p*-coumaric acid and chlorogenic acid), four flavonols (myricetin, rutin, quercetin, and kaempferol), two monomeric flavanols ((+)-catechin and (–)-epicatechin) and one chalcone (phloridzin). The representative chromatograms of the standard mixture solution and the wild Zhenping ecotype of *L. leucanthum* extract separation were depicted in Figure 1 and the phenolic contents of the tested samples were listed in Table 3. Significant differences in the concentrations of individual phenolic compounds from the three ecotypes of *L. leucanthum* were observed.

Phenolic acids are plant metabolites spread throughout the plant kingdom. The recent interest in phenolic acids comes from their potential protective role against diseases that cause oxidative damage such as coronary heart disease, stroke, and cancers through ingestion of fruits and vegetables (Elmastas *et al.*, 2006). The profile of phenolic acids in bulb extracts of wild ecotypes of *L. leucanthum* was analysed and it was clearly shown that chlorogenic acid was the most predominant phenolic compound in the tested phenolic acids, ranging from 10.91 to 25.47 mg/kg. The wild Zhouqu ecotype of *L. leucanthum* had the highest amount of chlorogenic acid, which was about 2.5-fold more than that in the wild Zhenping ecotype. For gallic acid, the wild Zhenping ecotype of the tested samples contained the highest concentration, with the value of 10.73 mg/kg, followed by the wild Liuba ecotype (9.62 mg/kg), while the wild Zhouqu ecotype had the lowest concentration (9.08 mg/kg). The amount of *p*-coumaric acid in tested bulb extracts, with a mean value (n=3) of 11.45 mg/kg, was approximately 1.6-fold less than that of chlorogenic acid.

Myricetin was the predominant phenolic compounds found in lily bulb extracts, ranging from 10.32 to 29.23 mg/kg. Myricetin is widely distributed in the human daily diet in foodstuffs such as fruits, beverages, tea and vegetables, and it has potent antioxidant properties, especially when it occurs in complex with iron; it also has a favourable effect on cognitive performance (Spencer *et al.*, 2009). Kaempferol was the second most abundant flavonol in the tested *L. leucanthum*. The wild Zhenping ecotype of the lily samples contained the highest concentration of kaempferol (23.57 mg/kg), followed by the wild Liuba ecotype and wild Zhouqu ecotype, with a value of 14.59 and 11.08 mg/kg, respectively. The levels of quercetin in all samples were much lower than that of kaempferol, ranging from 10.50 to 15.52 mg/kg. Kaempferol and quercetin flavonols have been reported to be capable of effectively protecting PC12 cells from the oxidative stress induced by H_2O_2 and reducing inflammation, tumorogenesis and cell damage caused by

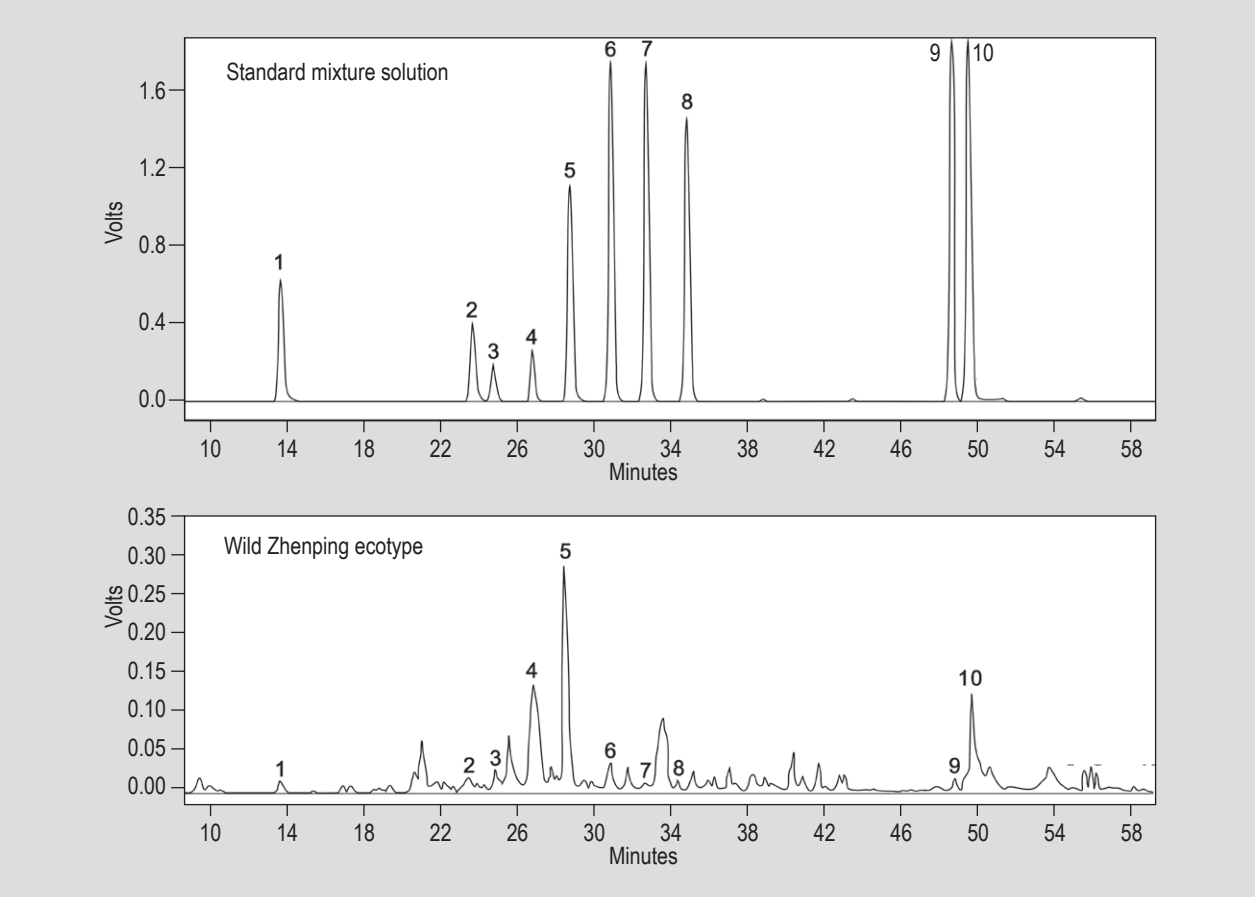

Figure 1. High-performance liquid chromatograms of individual polyphenolic constituents (1 = gallic acid; 2 = (+)-catechin; 3 = chlorogenic acid; 4 = (–)-epicatechin; 5 = myricetin; 6 = rutin; 7 = p-coumaric acid; 8 = quercetin; 9 = phloridzin; 10 = kaempferol) of the standard mixture solution and the wild Zhenping ecotype of *Lilium leucanthum*.

Table 3. Phenolic composition of the bulb extracts from three wild ecotypes of *Lilium leucanthum*.[1]

Phenolic compounds	Retention time (min)	Wild Zhenping ecotype	Wild Zhouqu ecotype	Wild Liuba ecotype
Gallic acid	13.57	10.73±0.21 a	9.08±0.04 c	9.62±0.24 b
(+)-catechin	23.73	11.39±0.18 a	11.39±0.18 a	8.46±0.22 b
Chlorogenic acid	24.48	10.91±0.08 c	25.47±0.05 a	18.06±0.04 b
(–)-epicatechin	26.84	24.83±0.11 a	10.85±0.18 c	16.73±0.07 b
Myricetin	28.70	29.23±0.51 a	10.32±0.07 c	20.43±0.16 b
Rutin	31.00	11.44±0.20 b	13.57±0.26 a	9.85±0.15 c
p-coumaric acid	32.63	9.04±0.04 c	10.85±0.03 b	14.47±0.13 a
Quercetin	34.61	10.50±0.25 c	15.52±0.09 a	12.72±0.15 b
Phloridzin	48.88	8.35±0.33 a	8.44±0.33 a	6.52±0.440 b
Kaempferol	49.44	23.57±0.13 a	11.08±0.05 c	14.59±0.17 b

[1] Values, in mg/kg dry weight, are expressed as means ± standard deviation (n=3).
Means in the same line followed by different letters are significantly different ($P<0.05$).

oxidation (Hong *et al.*, 2009; Javanovic *et al.*, 1996). These two compounds have been found in Easter lily flowers and our results from *L. leucanthum* were in agreement with the previous report (Francis *et al.*, 2004). Highest level of rutin was found in the wild Zhouqu ecotype of *L. leucanthum* among the tested samples, followed by the wild Zhenping

ecotype, while the lowest amount was found in the wild Liuba ecotype. Phloridzin, as a chalcone, has high activity in the oxygen radical absorbance capacity assay and DPPH free radical scavenging activity (Bernonville *et al.*, 2010). Wild Zhouqu ecotype of the tested bulb extracts had the highest concentration, with a value of 8.44 mg/kg, a little higher than that in wild Zhenping ecotype, with a content of 8.35 mg/kg, while the wild Liuba ecotype had the lowest content (6.52 mg/kg).

With regard to the flavanols, (+)-catechin and (–)-epicatechin were the typical monomeric flavanol compounds identified in the bulbs of the *L. leucanthum* analysed. (+)-catechin and (–)-epicatechin have a positive correlation with free radical scavenging capacity and reducing power and are noted for their high antibacterial activity (Ksouri *et al.*, 2009). (+)-catechin was an abundant monomeric flavanol compound identified in the wild Zhenping ecotype and wild Zhouqu ecotype (with equal value of 11.39 mg/kg), whereas the value of the wild Liuba ecotype was only 8.46 mg/kg. There were significant differences amongst (–)-epicatechin of the tested samples (P<0.05), with values ranging from 10.85 to 24.83 mg/kg. Among the three ecotypes, the concentration of (–)-epicatechin in the wild Zhenping ecotype of *L. leucanthum* was more than 2.2-fold that of the wild Zhouqu ecotype.

The promising biological properties of lily bulb extracts, especially their strong antioxidant and antiradical activities *in vitro* and *in vivo*, are attributed to the phenolic compounds (Dai and Mumper, 2010). The different concentration of phenolic composition might explain the different antioxidant abilities of the lily bulb extracts observed above. It can also be speculated that phenolic compounds present in the extracts may exert their antioxidant capacity individually as well as synergistically.

Comparison of antioxidant activity

The antioxidant capacities of the samples may be influenced by several factors simultaneously, such as a test system, and cannot be fully described separately. Moreover, most natural antioxidants are multifunctional and may act through different mechanisms. Although many methods have been developed and tested in the literature, their advantages and limitations are still under discussion and no consensus has been reached to define a unique standard method capable of encompassing all the peculiarities exhibited by the different classes of antioxidants (Karadag *et al.*, 2009).

For this reason, it is necessary to use at least two complementary methods to evaluate the antioxidant capacity *in vitro*. In this study, six antioxidant assays, including DPPH free radical scavenging activity, CUPRAC, HRSA, SRSA and LPI were applied to accurately evaluate the antioxidant properties of the three ecotypes of *L. leucanthum* and the results are shown in Table 4.

Analysis of DPPH• scavenging activity is one method for detecting antioxidant activity and is universally used to evaluate the ability of free radicals to react with antioxidants (Tang *et al.*, 2010a). Trolox equivalent antioxidant capacity assay is one of the most commonly employed to measure the ability of a compound to scavenge DPPH radicals, and is widely used to screen antioxidant activity of fruits, vegetables, foods and plants; it is also applicable to lipophilic and hydrophilic antioxidants (Van den Berg *et al.*, 1999). For DPPH•, the values of tested samples varied from 4,194.30 to 4,956.75 μmol TE/kg. The DPPH• scavenging activity of the tested extracts decreased in the order: wild Zhenping ecotype > wild Liuba ecotype > wild Zhouqu ecotype. The results of the investigation showed that the higher the concentration of phenolic contents, the lower is the amount of remaining DPPH• and the higher is the free radical scavenging activity.

Table 4. Antioxidant activity determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH), cupric-reducing antioxidant capacity (CUPRAC), hydroxyl radical scavenging activity (HRSA), superoxide radical scavenging activity (SRSA) and lipid peroxidation inhibition (LPI) assays of the bulb extracts from three wild ecotypes of *Lilium leucanthum*.[1]

Antioxidant index	Wild Zhenping ecotype	Wild Zhouqu ecotype	Wild Liuba ecotype
DPPH (TE μmol/kg)	4,956.75±51.79 a	4,194.30±34.26 c	4,874.53±25.89 b
CUPRAC (TE μmol/kg)	7,793.35±58.07 a	6,362.31±46.90 c	7,085.13±85.03 b
HRSA (%)	44.43±1.18 a	30.33±0.30 c	38.31±0.52 b
SRSA (%)	26.10±0.65 b	23.30±1.13 c	26.95±1.33 a
LPI (%)	42.55±1.16 a	41.04±1.43 a	41.68±1.32 a

[1] TE μmol/kg represents micromoles of trolox equivalents per kilograms of dry bulbs from three wild ecotype forms of *L. leucanthum* for DPPH free radical-scavenging capacity, and cupric-reducing antioxidant capacity. Hydroxyl radical-scavenging activity, superoxide anion scavenging activity and lipid peroxidation inhibition were expressed as the percentage of antioxidant activity (%). Values are expressed as means ± standard deviation (n=3). Means in the same column followed by different letters are significantly different (P<0.05).

Determination of the redox potential of a substance is a widely used method to estimate antioxidant capacity. Electron donation is an important means by which antioxidants can promote the formation of less reactive species and may be assessed by the reducing power assay. The CUPRAC assay, treating the antioxidants in the samples as reductants in a redox-linked colorimetric reaction, is simple and could be easily standardised (Meng *et al.*, 2011). To measure the reductive ability of bulb extracts of three wild ecotypes of *L. leucanthum*, Cu^{2+}-Cu^{+} transformation was investigated in this study. As shown in Table 4, bulb extracts of different wild ecotypes of the selected samples demonstrated powerful Cu^{2+} reducing ability as TE. The reducing power of *L. leucanthum* was as follows: wild Zhenping ecotype > wild Liuba ecotype > wild Zhouqu ecotype. As reported in the previous study, regardless of the stage in the oxidative chain in which the antioxidant action is assessed, most non-enzymatic antioxidative activities such as scavenging of free radicals or inhibition of peroxidation are mediated by redox reaction (Zhu *et al.*, 2002). Our data implied that the reducing power of phenolic compounds probably played a role in the antioxidation of *L. leucanthum*.

Hydroxyl radicals can be synthesised in humans. They can easily cross cell membranes and react with most biomolecules such as carbohydrates, proteins, lipids, and DNA in cells, causing tissue damage or cell death. Thus, removing hydroxyl radicals is important for the protection of living systems (Jayaprakasha *et al.*, 2008). According to the investigation in this study, there were significant differences amongst the samples ($P<0.05$) which exhibited between 30.33 and 44.43% hydroxyl radical scavenging activity. The highest hydroxyl radical scavenging activity was found in wild Zhenping ecotype of the tested lily bulbs, followed by that in wild Liuba ecotype and wild Zhouqu ecotype. These results indicated that strong OH• scavenging activities of lily bulb extracts were closely related to their high levels of total phenolic contents and attributed to the scavenging of the radical by hydrogen donation.

The superoxide radical is one extremely reactive free radical formed in biological systems. Although superoxide was a weak oxidant, in most organisms, it could degrade continuously and form other active ROS, triggering peroxidation of lipids, and then induce pathological incidents such as arthritis and Alzheimer's disease. Thus, it is important to remove superoxide radicals (Tedesco *et al.*, 2000). The superoxide radical scavenging activities among the tested lily bulb samples were shown in Table 3 with the values ranging from 23.30 to 26.95%. The radical scavenging of all samples on $O_2^{\bullet-}$ showed a different order with the results of the HRSA assay. The inhibition of superoxide radical decreased in the order: wild Liuba ecotype > wild Zhenping ecotype > wild Zhouqu ecotype.

Lipid peroxidation may cause peroxidative tissue damage in inflammation, cancer, toxicity of xenobiotics and aging (Middleton *et al.*, 2000). In this study, the potential of lily bulb extracts was measured to inhibit lipid peroxidation in egg yolk phosphatidylcholine, induced by Fenton reaction. The result showed that all samples had strong inhibition of peroxidation but no significant difference was observed among the three ecotypes of *L. leucanthum*. The values varied from 41.04 to 42.55% and the inhibiting power of *L. leucanthum* was as follows: wild Zhenping ecotype > wild Liuba ecotype > wild Zhouqu ecotype.

Selected native *L. leucanthum* bulbs investigated in this study were shown to be a novel rich source of antioxidant compounds. This study demonstrated that the bulbs of *L. leucanthum* had high potential value for fruit and vegetable growers as well as food and nutraceuticals manufacturers because of their high phenolic contents. The results also clearly showed that the methanol bulb extracts of three wild ecotypes of *L. leucanthum* had strong antioxidant activity, including DPPH, CUPRAC, HRSA, SRSA and LPI. Among the tested samples, the wild Zhenping ecotype of *L. leucanthum* had the greatest antioxidant activities, followed by the wild Liuba ecotype, while the wild Zhouqu ecotype possessed the lowest antioxidant capacities, except for superoxide radical scavenging activity, of which the highest was in the wild Liuba ecotype. We could also deduce that the strong antioxidant capacities of these bulb extracts were due to their high phenolic contents. This assertion was confirmed by the correlation studies between the total phenolic and antioxidant activities. Our study provided valuable information on the antioxidant capacity of *L. leucanthum* and highlighted the crucial influence of ecotype on elemental content, phenolic compounds and antioxidant capacity of the lily bulb extracts. Furthermore, it also revealed the importance of selecting lily cultivars for specific nutritional purposes or assigning parental lines in functional breeding programs.

Correlation amongst different antioxidant variables

Correlation analysis was conducted to explore the relationships amongst the different antioxidant variables measured for the selected *L. leucanthum* samples. Despite the presence of a wide range of total antioxidant capacities and total phenolic contents among the tested samples, linear positive relationships could be found between CUPRAC and total phenolic contents (TPC, TFOC and TFAC) at 0.05 level, as well as between the HRSA value and the total phenolic contents, as shown in Table 5. The DPPH free radical scavenging activity was significantly correlated with TPC and TFOAC at the 0.98 and 0.05 level, respectively. The linear correlation coefficients between LPI and TFOC, and between LPI and TFAC were 0.95 ($P<0.05$) and 0.99 ($P<0.01$), respectively. However, no significant correlation was found between the phenolic components of tested

Table 5. Linear correlation coefficients between phenolic composition and antioxidant capacity (panel A), and among the different methods for quantifying antioxidant capacity (panel B)[1].

	DPPH	CUPRAC	HRSA	SRSA	LPI
Panel A					
TPC	0.98*	0.97*	0.99*	0.87	0.95
TFOC	0.98*	0.98*	0.99*	0.87	0.95*
TFAC	0.80	0.97*	0.96*	0.57	0.99**
Panel B					
DPPH	1.00				
CUPRAC	0.91	1.00			
HRSA	0.94	1.00**	1.00		
SRSA	0.95	0.74	0.78	1.00	
LPI	0.87	1.00**	0.99*	0.67	1.00

[1] * = correlation is significant at P=0.05; ** = correlation is significant at P=0.01.
TPC = total polyphenols content; TFOC = total flavonoids content; TFAC = total flavanols content; DPPH = 2,2-diphenyl-1-picrylhydrazyl; CUPRAC = cupric-reducing antioxidant capacity; HRSA = hydroxyl radical scavenging activity; SRSA = superoxide radical scavenging activity; LPI = lipid peroxidation inhibition.

L. leucanthum samples and SRSA. The strong correlations between the results using different methods of measuring antioxidant capacity and the total phenolic content showed that phenolic compounds considerably contributed to the antioxidant activities of the tested samples, and therefore could play an important role in the beneficial effects of *L. leucanthum*. These results correspond to other reports in the literature (Jiang and Zhang, 2012).

Amongst the methods used for quantifying antioxidant activities, the correlation between LPI and CUPRAC, HRSA was 1.00 (P<0.01) and 0.99 (P<0.05), respectively. The correlation between CUPRAC and HRSA was 1.00 (P<0.01). This result suggested that CUPRAC, HRSA and LPI assays were almost comparable and interchangeable in characterising the *L. leucanthum* antioxidant activities.

4. Conclusions

Our results indicate that the phenolic contents and antioxidant properties showed statistically significant differences among the different ecotypes of *L. leucanthum*. The wild Zhenping ecotype of the tested samples had the highest phenolic contents (TPC, TFOC and TFAC) and the strongest antioxidant capacity, except for superoxide radical scavenging activity which was highest in the wild Liuba ecotype, while the wild Zhouqu ecotype of *L. leucanthum* had the lowest phenolic content and the weakest antioxidant activity. With regard to the individual phenolic compounds, the wild Zhenping ecotype of *L. leucanthum* contained the highest contents of gallic acid, (−)-epicatechin, myricetin, and kaempferol, and those of chlorogenic acid, rutin, quercetin and phloridzin were the highest in the bulb extract of the wild Zhouqu ecotype, while the wild Liuba ecotype had the highest concentration of *p*-coumaric. There was an equal content of (+)-catechin between the wild Zhenping ecotype and wild Zhouqu ecotype, which was higher than that in the wild Liuba ecotype. According to the linear correlation analysis, the total phenolic contents were the major contributors to the total antioxidant capacity in lily bulb except for superoxide radical scavenging activity. Overall, these assays have been useful for their applications in the food industry and the results of this study showed that the extract of the wild ecotypes of *L. leucanthum* can be used as an easily accessible source of natural antioxidants and as a possible food supplement or in the pharmaceutical industry.

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