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Characterization of Antioxidant Activity of Extracts from *Flos Lonicerae*

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Antioxidants are emerging as prophylactic and therapeutic agents for various diseases. However, little is known about the antioxidant property of the extract from *Flos Lonicerae*, a medically useful traditional Chinese medicine herb. Here the antioxidant capacity of water, methanolic and 70% ethanolic extracts prepared from *Flos Lonicerae* to scavenge DPPH radical and reduce Fe^{3+} to Fe^{2+} is evaluated. Chlorogenic acid, a major component of *Flos Lonicerae*, is identified and further purified from 70% ethanolic extract with HPLC and its antioxidant capacity is also characterized. The content of total phenolic compounds and chlorogenic acid in *Flos Lonicerae* is determined. The present results demonstrate that all *Flos Lonicerae* extracts examined here exhibit antioxidant activity and chlorogenic acid is a major contributor to this activity, which implicates that the *Flos Lonicerae* extract may serve as potential source of natural antioxidants for treatment of some diseases.

Keywords *Flos Lonicerae*; chlorogenic acid; antioxidant

INTRODUCTION

In recent years, antioxidants have gained a lot of importance due to their potential as prophylactic and therapeutic agents in many diseases (Venkat Ratnama et al., 2006). Free radicals are highly reactive molecules or chemical species containing unpaired electrons that can cause oxidative stresses that damage lipids, proteins, enzymes, carbohydrates, and DNA in cell and tissues, and have been known to be associated with pathogenesis of various disorders such as cancer, diabetes, cardiovascular diseases, neurodegenerative disorders, and other diseases. Antioxidants can scavenge and react with free radicals and prevent these damages caused by them (Ames et al., 1993; Aruoma et al., 1998; Block et al., 1992; Halliwel et al., 1999; Hertog et al., 1995; Venkat Ratnama et al., 2006). Therefore the discovery of antioxidants has lead to a medical revolution that is promising a new paradigm of healthcare.

Flos Lonicerae, also called Jinyinhua, is a commonly used TCM herb. It has latent-heat-clearing, antipyretic, detoxicant, and anti-inflammatory actions. It has been, therefore, prescribed to treat fever due to common cold, febrile disease, dysentery, carbuncles, and virulent swellings (Chang H. M et al., 1986; Ministry of Public Health of the People's Republic of China, 2000). Several kinds of major components, such as chlorogenic acid and its analogues, flavonoids, iridoid glucosides, and triterpenoid saponins have been investigated for their effective activities (Chai et al., 2005; Li et al., 2003; Song, et al., 2006). Although great progresses have been made in the studies and clinical applications about *Flos Lonicerae* in the last couples of years (Chang et al., 1986; Ministry of Public Health of the People's Republic of China, 2000), the antioxidant property of the *Flos Lonicerae* extract is still unclear. In this study, the antioxidant property of extracts differently prepared from *Flos Lonicerae* was tested with two in vitro assays, DPPH radical scavenging assay and ferric reducing assay, which have been widely used to evaluate the antioxidant capacity of plant extracts (Benzie & Strain, 1996; Sreejayan & Rao, 1996; Stratil et al., 2006; Villano et al., 2007). The present results demonstrate that all three extracts prepared here exhibit antioxidant activity, which provides us with new anticipation for application of the *Flos Lonicerae* extract as antioxidant in some diseases. To further explore the property of the *Flos Lonicerae* extract, we analyzed the contribution of chlorogenic acid, one major component of *Flos Lonicerae*, to the antioxidant activity of *Flos Lonicerae* extract. We also determined the content of chlorogenic acid and total phenolic compounds in *Flos Lonicerae* and its extract, and the relationship

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among total phenolic compounds content, chlorogenic acid content and the antioxidant activity of the extract was analyzed.

MATERIALS AND METHODS

Materials

The *Flos Lonicerae* sample was collected from HeNan Province of China. HPLC apparatus was purchased from American Lab Alliance Corporation. Beckman DU-640 spectrophotometer equipped with a thermostatized auto-cell-holder was purchased from Beckman Corporation. The chlorogenic acid reference, DPPH and Folin-Ciocalteu reagent were purchased from the Sigma corporation. All reagents used in HPLC analyses were HPLC grade. The other reagents were of analytical grade.

Methods

Preparation of Flos Lonicerae *Extracts*

Flos Lonicerae sample was dried at 50°C to constant weight. Approximately 10g of pulverized sample were added to a round-bottomed flask containing 250 mL of different solvents including distilled water, methanol and 70% ethanol solution respectively. The mixture was heated under reflux for 4 h. The extracts were filtered and the residue was re-extracted under the same conditions. Combine the extracts. The pooled extract was concentrated and evaporated to dryness with rotary evaporator under reduced pressure. The residue was dissolved with 30 mL of water into a 100 mL of flask and the product was stored at 4°C for 24 h followed by centrifugation with 10,000 rpm for 30 min. The supernatant fraction was designated as *Flos Lonicerae* crude extract and lyophilized to fine powder for long term storage and dissolved with methanol for identification and quantification analysis with HPLC.

HPLC Analysis

The dissolved extract was filtered through 0.22 μm membrane filter before loading it into the HPLC system (Lab Alliance Corporation; Pump, Series III; Detector, model 525). Chromatography was carried out on analytical column (Kromasil C18, 5 μm, 250 × 4.6 mm) and preparative column (Kromasil C18, 20μm, 250 × 22mm) at 25°C. The sample loops of 20 μL and 1 mL were used for sample injection. The mobile phase consisted of two solvent components: water (solvent A) and acetonitrile-citrate acid-water (20:40:40, V/V, solvent B). Gradient elution was that the solvent B was increased from 5–100% within 30 min. The flow rate was 0.2 mL/min for analytical and 0.8 mL/min for preparative purpose respectively. The wavelength used to detect chlorogenic acid was 324 nm. 20 μL of different concentrations (0.02–0.50 mg/mL) of chlorogenic acid reference was loaded into HPLC system for construction of the calibration curve by plotting the peak areas versus the concentrations. Each extract powder was dissolved with methanol to a concentration of 0.5 mg/mL and 20 μL of the crude extract was loaded into HPLC system for identification and quantification of chlorogenic acid in *Flos Lonicerae* respectively. The content of chlorogenic acid in *Flos Lonicerae* was determined from the corresponding calibration curve and the content was expressed as in mg of chlorogenic acid per gram of *Flos Lonicerae*. The peak area of chlorogenic acid in each extract was mean of three parallel measurements for chlorogenic acid quantification. Comparing the retention time with that of chlorogenic acid reference the chromatography peak was identified. The chlorogenic acid used for antioxidant activity assay was produced through preparative column separation and manually collected according to the retention time and chromatography peak of chlorogenic acid under the same chromatography conditions.

Determination of Total Phenolic Compounds Content

Total phenolic compound content in each extract was spectrophotometrically determined according to the Folin-Ciocalteu procedure by reading the absorbances at 760 nm against a methanol blank (Singleton & Rossi, 1965). Briefly, samples (150 μL, three replicates) were introduced into test tubes and then 750 μL of Folin-Ciocalteu reagent and 600 μL of sodium carbonate (7.5%) were added. The tubes were mixed and incubated at 50°C for 10 min. Absorption at 760 nm was measured. The total phenolic content was expressed as in mg of gallic acid equivalents (GAE) per gram of *Flos Lonicerae*.

Scavenging DPPH Radical Assay

The capacity of each sample to scavenge DPPH radical was spectrophotometrically measured according to Sreejayan-Rao procedure (Sreejayan & Rao, 1996). Briefly, the freshly prepared stock solution of each extract was diluted into different concentrations of solutions (50, 100, 250, 500, 1000, and 2000 μg/mL) with methanol. The preparative chlorogenic acid used in this assay was quantified and also dissolved with methanol. 100 μL of each solution was added into methanolic solution of DPPH radical (100 μM, 2.90 mL). The mixture was shaken vigorously for 30 sec and left to stand for 20 min at room temperature in dark area. Absorbance was recorded at 517 nm. The effect of DPPH radical scavenging of the sample was calculated according to the formula: Scavenging effect (%) = [(Ac-Aa) / Ac] × 100%. Where Ac is the absorbance of the control and Aa is the absorbance of the sample. DPPH radical scavenging reaction kinetics of the samples, including 1000 μg/mL of preparative chlorogenic acid, chlorogenic acid reference and 70% ethanolic extract, was observed. Absorbance was recorded at regular intervals of 15 sec for total 2 min after shaking the mixture vigorously for 30 sec. Ascorbic acid and methanol were served as positive and negative control respectively. The results are mean ± *SD* of three parallel measurements.

Ferric Reducing Antioxidant Power (FRAP) Assay

Ferric reducing antioxidant power (FRAP) assay is a simple and reliable colorimetric method and commonly used for measuring the antioxidant capacity (Benzie & Strain, 1996).

Briefly, 900 μL of FRAP reagent, prepared freshly and warmed at 37°C, was mixed with 90 μL of distilled water and 30 μL of test sample (100 μg). The FRAP reagent contained 2.5 mL of a 10 mM TPTZ solution in 40 mM HCl plus 2.5 mL of 20 mM $FeCl_3 \cdot 6H_2O$ and 25 mL of 0.3 M acetate buffer, pH 3.6. The mixture was shaken vigorously for 30 sec and left to stand for 30 min at room temperature and the readings at the absorption maximum (595 nm) were recorded. The ferric reducing reaction kinetics of the samples, including 100 μg of preparative chlorogenic acid, chlorogenic acid reference, and 70% ethanolic extract, was also observed. The readings were taken every 30 sec using a Backman DU-640 spectrophotometer at 37°C for up to 30 min. Ascorbic acid and methanol were served as positive and negative control, respectively. Results are mean ± *SD* of three parallel measurements.

RESULTS AND DISCUSSION

Identification and Quantification Analysis

It is well known that the pharmacological and biological activities of *Flos Lonicerae* are closely linked with its functional components (Chai et al., 2005; Chang et al., 1986; Li J et al., 2003; Song et al., 2006), of which chlorogenic acid has been extensively studied due to its high content and effective activities (Azuma et al., 2000; Delcy et al., 2002; Plumb et al., 1999; Yasuhisa et al., 2004). In this study, we prepared three *Flos Lonicerae* extracts with different solvents and the chlorogenic acid component was identified in all three extracts by comparing the retention time with that of chlorogenic acid reference [Figure 1A (a,b,c,e)] and quantified with HPLC (Table 1). The linear regression equation shows good linear regression within the test ranges (0.02–0.50 mg/mL) for chlorogenic acid standard.

The chlorogenic acid content in *Flos Lonicerae* extracts was calculated as shown in Table 1. The content (0.51–1.44%) of chlorogenic acid in *Flos Lonicerae* (HeNan) is lower than the content (3.36–3.83%) of chlorogenic acid in *Flos Lonicerae* (HanZhong) (Hai et al., 2006), but similar to the criterion (1.5%) provided in China Pharmacopoeia (2000 edition). Moreover, the total phenolic compounds content in *Flos Lonicerae* and its extracts were also quantified (Table 1).

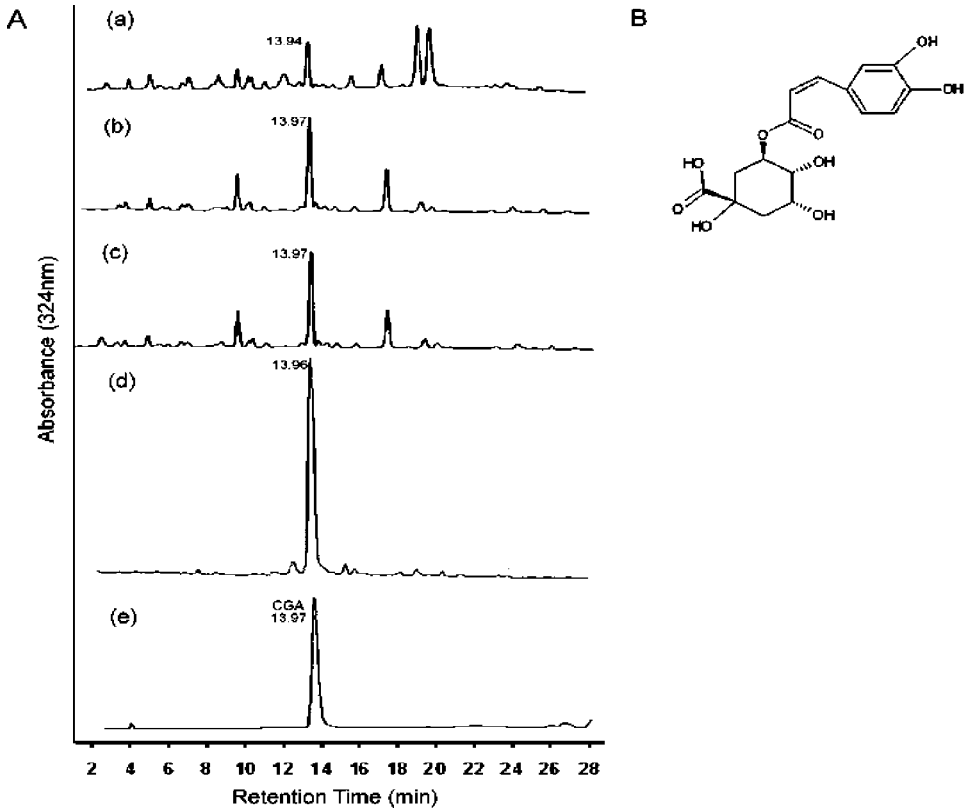

FIGURE 1. A, HPLC analyses. The HPLC profile a, b, and c represents the identification of chlorogenic acid in water, methanolic and 70% ethanolic extracts from *Flos Lonicerae* respectively. The HPLC profile d represents purified chlorogenic acid from 70% ethanolic extract with HPLC. There are several contaminant constituents in the prepared chlorogenic acid fraction around chlorogenic acid peak, which are caused by manual collection during purification. The peaks labeled with retention time are chlorogenic acid, because their retention time is identical with that of chlorogenic acid standard (e, 13.97 min). 10 μg the samples (a,b,c,d) were respectively loaded into the HPLC system except chlorogenic acid standard (5 μg). B, The structure of chlorogenic acid.

TABLE 1
Chlorogenic acid and total phenolic compounds contents in *Flos Lonicerae* and its extracts

Solvents	Chlorogenic Acid (mg/g)		Total Phenolic Compounds (mg GAE/g)	
H_2O	5.09 ± 0.64^a	134.54 ± 6.34^b	8.60 ± 0.83^a	205.43 ± 9.76^b
Methanol	13.89 ± 1.18^a	338.45 ± 13.36^b	2.32 ± 1.94^a	428.93 ± 16.39^b
70% Ethanol	14.38 ± 1.21^a	345.36 ± 13.45^b	2.54 ± 1.76^a	432.08 ± 16.54^b

Results are mean ± *SD* of three parallel measurements. a and b stand for chlorogenic acid or total phenolic compounds in *Flos Lonicerae*(a) and its extracts (b), respectively.

Determination of Antioxidant Property of *Flos Lonicerae* Extracts

Increasing understanding of free radicals role in diseases is opening new area for the applications of antioxidants in prevention and therapy of healthcare system (Ames, 1993; Block, 1992; Halliwel & Gutteridge, 1999; Hertog, 1995; Venkat Ratnama et al., 2006). So far many antioxidants including superoxide dismutase, catalase, genistein, lycopene, quercetin, ellagic acid, coenzyme Q10, indole-3-carbinol, Vitamin C, and Vitamin E have been investigated to be pharmacologically active and therapeutic agents for treatment of various diseases. Although the delivery of some antioxidants through oral route can be improved through NDDS including chemical modifications, coupling agents, liposomes, microparticles, and nanoparticles, which are designed largely dependent on the physicochemical characteristics, biopharmaceutical properties and pharmacokinetic parameters of the antioxidant to be formulated, the investigation on antioxidants is still needed because some problems for its further application remain to be resolved, such as the bioavailability and the inability to reach the sites of action, etc. (Venkat Ratnama et al., 2006). Human antioxidant defense is equipped with many endogenous antioxidants to combat oxidative stresses. However, this defense system could be weakened or damaged under disease conditions (Anderson, 1996), which makes it necessary to rely on exogenous antioxidants including natural or synthesized antioxidants (Zhang et al., 2006). Herbal medicines with antioxidant properties have been widely examined and epidemiological studies also accepted these reagents to retard or inhibit the progression of some diseases (Schinella et al., 2003). Some extracts of TCM have been found to bear strong antioxidant activity to inhibit oxidative stresses and the in vivo studies of some plant extracts with antioxidant activity were performed to investigate their physicochemical and biopharmaceutical properties (German'o et al., 2006; Schinella et al., 2003; Venkat Ratnama et al., 2006). It was reported that blood lipid were markedly decreased by intravenous injection combined with concomitantly oral administration of *Flos Lonicerae* extract for cases of hyperlipidemia (Chang & Paul, 1986). Therefore, we suspect that the activity of *Flos Lonicerae* extract may be linked with its antioxidant components, because antioxidants have been thought to be involved in reducing production of free radicals and arterial deposition of cholesterol as well as blood lipid (Chang & Paul, 1986; Chenni et al., 2006; Ilker et al., 2004; José et al., 2003; Ramkumar et al., 2003; Ravi et al., 2001; Yang et al., 2006; Zhang et al., 2001). To determine whether the *Flos Lonicerae* extracts bear the antioxidant activity, the in vitro scavenging DPPH radical assay and ferric reducing assay were carried out. DPPH is one of the stable nitrogen centered free radicals. Although DPPH is artificial radical which can not be reproduced in vivo situation, they are widely adopted to evaluate the antioxidant activity of substances and can be an indicator of the antioxidant potential prior to their consumption (Villano, 2007). The scavenging DPPH radical assay results indicated that all *Flos Lonicerae* crude extracts examined here bear the capacity to scavenge DPPH free radical. 23–47% of DPPH radicals were scavenged within 20 min when the concentration of each extract is over 1000 μg/mL, which is, however, lower than that of ascorbic acid (81%), a potent antioxidant to scavenge free radicals (Figure 2A). The ferric reducing assay results also showed the similar results (Figure 2B).

Among three *Flos Lonicerae* extracts the antioxidant activity of water extract is the lowest and that of the other two extracts is relatively higher and similar, which is consistently linked with the total phenolic compounds content in the extracts (Table 1) and in agreement with previous reports about the correlation between antioxidant activity and total phenolic compounds content in the extracts (Katalinic et al., 2004; Sun & Ho, 2005).

According to the above analyses, the *Flos Lonicerae* extract is pharmacologically active and may function as free radical scavenger and metal chelator to finally prevent the generation of free radicals and inhibit the oxidative damages. Therefore, the *Flos Lonicerae* extract could serve as a novel potential source of natural antioxidants that could be applied in some diseases. In this study, however, we just characterized the in vitro antioxidant activity of the *Flos Lonicerae* extract, the studies and applications about the *Flos Lonicerae* extract as antioxidant are still in its infancy stage, in the future many factors should be considered for its further application such as its solubility, permeability, stability,

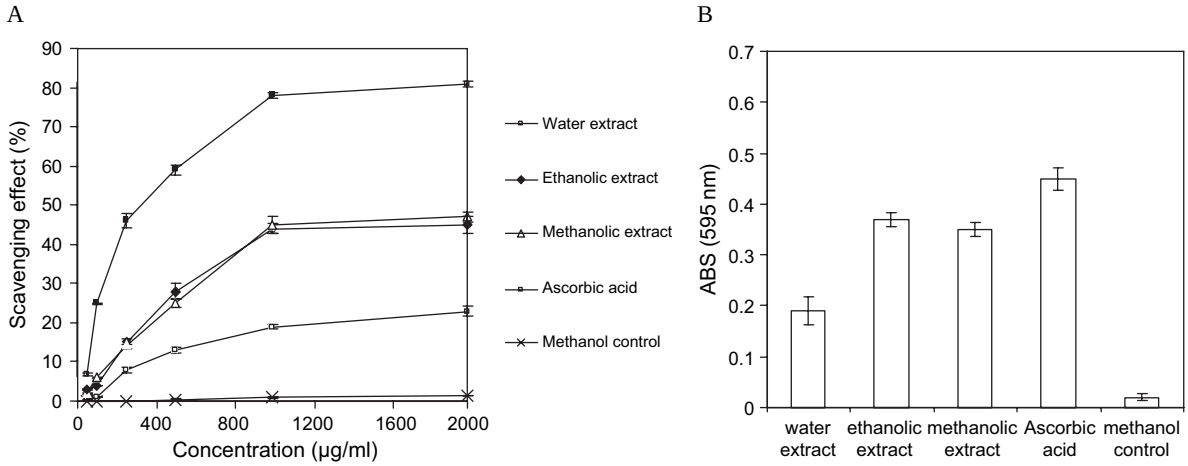

FIGURE 2. Determination of the antioxidant capacity of *Flos Lonicerae* crude extracts. A, Determination of the antioxidant capacity of *Flos Lonicerae* extracts to scavenge DPPH radical; B, Determination of the antioxidant capacity of *Flos Lonicerae* extracts to reduce Fe^{3+} to Fe^{2+}. Results are mean ± *SD* of three parallel measurements and calculated with MS Excel software.

metabolism, bioavailability, toxicological evaluation and clinical trials and so on.

Contribution of Chlorogenic Acid to Antioxidant Activity of *Flos Lonicerae* Extract

Chlorogenic acid, an ester formed between caffeic acid and quinic acid (Figure 1B), is one of the most naturally existed phenolic compounds found in plants and has been regarded as one of the most important chemical standards for quality evaluation of *Flos Lonicerae* (Chai et al., 2005). As a potent antioxidant and metal chelator, chlorogenic acid displays significant inhibitory effect on oxidative damage of liposome, deoxyribose and protein, and remarkable free radical scavenging activity (Chen et al., 2004; Dinis et al., 2002; Kono et al., 1998; Motoyo et al., 1994; Wang et al., 2006; Yasuhisa et al., 2004). Chlorogenic acid can modulate the activity of glucose-6-phosphatase involved in glucose metabolism (Hemmerle et al., 1997) and reduce the risk of cardiovascular diseases by decreasing oxidation of low density of lipoproteins (LDL), a major cholesterol-carrying lipoprotein in plasma (Heinecke et al., 1998; Nardini et al., 1995; Nardini et al., 1997; Steiberg et al., 1992). Chlorogenic acid can also reduce blood glucose, cholesterol and triacylglycerols, which partially result from the direct inhibition of glucose-6-phosphatase. As a well-known potential antioxidant, the investigation on the absorption and metabolism of chlorogenic acid was conducted. Choudhury et al. reported that intravenous or intraperitoneal administration of chlorogenic acid to rats results in very low concentrations of chlorogenic acid in urine (Choudhury et al., 1999), because chlorogenic acid could be picked up by liver in which the concentrations of cholesterol, triacylglycerol and minerals could be modified by chlorogenic acid (Azuma et al., 2000; Delcy et al., 2002). As for application, Shi, et al., reported that chlorogenic acid can prepared as a water-soluble antioxidant through microencapsulation, which is very helpful to promote its application in the future (Shi, 2006).

To further understand the antioxidant property of the *Flos Lonicerae* extracts, the major component of *Flos Lonicerae* chlorogenic acid was purified from 70% ethanolic extract with HPLC [Figure 1A(d,e)] and its antioxidant property was observed as shown in Figure 3 A, B. Preparative chlorogenic acid exhibits potent antioxidant activity, which is much higher than that of 70% ethanolic extract and even that of ascorbic acid. Moreover, the reaction kinetics of the test samples was observed respectively as shown in Figure 4 A, B. The present study indicates that higher content of chlorogenic acid exhibits higher efficiency to scavenge DPPH radical and reduce Fe^{3+} to Fe^{2+}. For example, preparative chlorogenic acid can scavenge 80% DPPH radicals within 20s and keep stable, while 70% ethanolic extract and ascorbic acid can only scavenge 15% and 45% DPPH radicals under the same conditions and finally keep stable after 65 and 35 sec, respectively. The antioxidant activity of preparative chlorogenic acid is slightly lower than that of chlorogenic acid standard, which is caused by the impurity of chlorogenic acid manually collected [(Figure 1A (d,e)]. Furthermore, the chlorogenic acid content in *Flos Lonicerae* extracts also reflects the antioxidant activity to some extent. The chlorogenic acid content in the water extract is lower than that of ethanolic and methanolic extracts by over 50%, which seems to be correlated with the antioxidant activity of the *Flos Lonicerae* extracts [Table 1, Figure 1A (a–c) and Figure 2]. According to the above analysis, it is evident that the antioxidant activity of *Flos Lonicerae* extract is mainly contributed by the presence of chlorogenic acid. Up to now, we have optimized the conditions for chlorogenic acid preparation from *Flos Lonicerae* extract and characterized its antioxidant property, which not only provides a new source for naturally, potential antioxidant but also helps to promote further investigation and

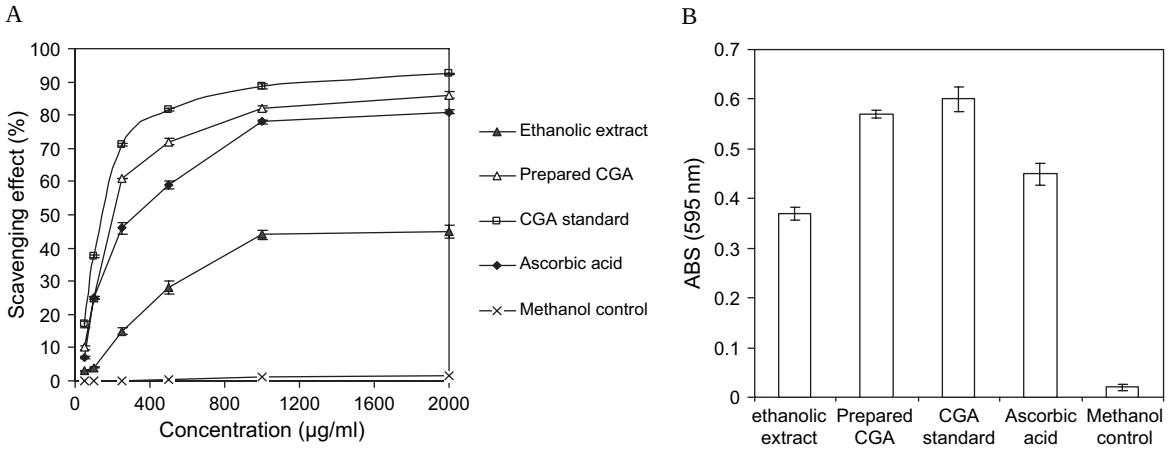

FIGURE 3. Determination of the antioxidant capacity of prepared chlorogenic acid from *Flos Lonicerae* extract. A, The antioxidant capacity of the samples to scavenge DPPH radical; B, The antioxidant capacity of the samples to reduce Fe^{3+} to Fe^{2+}. Results are mean ± *SD* of three parallel measurements and calculated with MS Excel software.

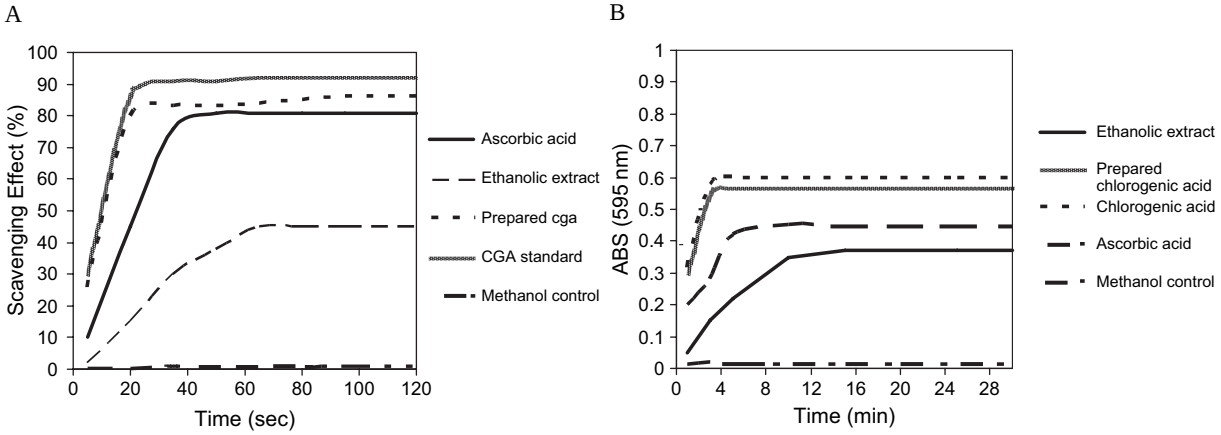

FIGURE 4. Reaction kinetics of DPPH radical scavenging (A) and ferric reducing (B). Results are mean of three parallel measurements and calculated with MS Excel software.

application of *Flos Lonicerae* extract as antioxidant, because the in vivo effects of chlorogenic acid as antioxidant have been extensively investigated with animal models (Azuma, 2000; Choudhury, 1999; Delcy, 2002; Plumb, 1999).

CONCLUSIONS

In this study, we characterized the antioxidant property of extracts from *Flos Lonicerae* with two different ways: DPPH radical scavenging assay and ferric reducing assay. The present results demonstrated that the extracts of *Flos Lonicerae* exhibit antioxidant activity and this activity is correlated with the total phenolic compounds content in the extract and mainly contributed by the presence of chlorogenic acid, which provides us with new pharmaceutical perspective for application of *Flos Lonicerae* extract as antioxidant in some diseases.

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ABBREVIATIONS

HPLC	high performance liquid chromatography
DPPH	1,1-diphenyl-2-picrylhydrazyl
TPTZ	2,4,6-tripyridy-s-triazine
CGA	chlorogenic acid
GAE	gallic acid equivalents
TCM	traditional Chinese medicine
LDL	low-density lipoprotein
NDDS	novel drug delivery systems
SD	standard deviation

REFERENCES

Ames, B. N., Shigenaga, M. K., & Hagen, T. M. (1993). Oxidants and antioxidants and the degenerative diseases of aging. *Proc. Natl. Acad. Sci.*, 90, 7915–7922.

Anderson, D. (1996). Antioxidant defense against reactive oxygen species causing genetic and other damage. *Mutat. Res., 350*, 103–108.

Aruoma, O. I. (1998). Free radicals, oxidative stress, and antioxdants in human health and disease. *J. Am. Oil. Chem. Soc*, *75*, 199–212.

Azuma, K., Ippoushi, K., Nakayama, M., Ito, H., Higashio, H., & Terao, J. (2000). Absorption of chlorogenic acid and caffeic acid in rats after oral administration. *J. Agric. Food Chem*, *48*, 5496–5500.

Benzie, I. F., & Strain J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem*, *239*, 70–76.

Block, G., Patterson, B., & Subar, A. (1992). Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. *Nutr. Cancer*, *18*, 1–29.

Chai, X. Y., Li, S. L., & Li, P. (2005). Quality evaluation of *Flos Lonicerae* through a simultaneous determination of seven saponins by HPLC with ELSD. *J. Chromatogr. A., 1070*, 43–48.

Chang, H. M., & Paul, P. H. B. (1986). *Pharmacology and Applications of Chinese Material Medica, II*, 787–792.

Chen, Y. A., Wu, A. B., & Chen, C. Y. (2004). The influence of different treatment on the free radical scavenging activity of burdock and variations of its active components. *Food Chem., 86*, 479–484.

Chenni, A., Ait, Y. D., & Boukortt, F. O. (2006). Effect of aqueous extract of Ajuga iva supplementation on plasma lipid profile and tissue antioxidant status in rats fed a high cholesterol diet. *J. Ethnopharmacol., 109*, 207–213.

Choudhury, R., Srai, S. K., Debnam, E., & Rice-Evans, C. A. (1999). Urinary excretion of hydroxycinnamates and flavonoids after oral and intravenous administration. *Free Radic. Biol. Med, 27*, 278–286.

Delcy V., Rodriguez de Sotillo, & Hadley, M. (2002). Chlorogenic acid modifies plasma and liver concentrations of: cholesterol, triacylglycerol, and minerals in (fa/fa) Zucker rats. *J. Nutr. Biochem., 13*, 717–726.

Dinis, T. C., Santosa, C. L., & Almeida, L. M. (2002). The apoprotein is preferential target for peroxynitrite-induced LDL damage protection by dietary phenolic acids. *Free Radical Res., 36*, 531–543.

German'o, M. P., Angelo, V. D., Biasini, T., & Sanogo, R., et al. (2006). Evaluation of the antioxidant properties and bioavailability of free and bound phenolic acids from Trichilia emetica Vahl. *J. Ethnopharmacol., 105*, 368–373.

Hai, J., Wei, W., & Ding, R. (2006). Determination of chlorogenic acid in *Flos Lonicerae* of HanZhong by HPLC. *ShanXi Li Gong Xue Yuan Xue Bao* (in Chinese), *22*, 87–89.

Halliwel, B., & Gutteridge, J. M. C. (1999). *Free Radical in Biology and Medicine*, Oxford University Press, UK, 617.

Heinecke, J. W. (1998). Oxidants and antioxidants in the pathogenesis of atherosclerolis: Implication of the oxidized low density lipoprotein hypothesis. *Atherosclerosis, 141*, 1–15.

Hemmerle, H., Burger, H. J., & Below, P. et al. (1997). Chlorogenic acid and synthetic chlorogenic acid derivatives: novel inhibitors of hepatic glucose-6-translocase. *J. Med. Chem.*, 40, 137–145.

Hertog, M. G., Kromhout, D., Aravanis, C., Blackburn, H., & Buzina, R. et al. (1995). Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Arch. Intern. Med., 155*, 381–386.

Ilker, D., Mustafa, K., & Bilal, A. (2004). Effects of garlic extract consumption on blood lipid and oxidant/antioxidant parameters in humans with high blood cholesterol. *J. Nutr. Biochem., 15*, 373–377

José, C., & Fernández, C. (2003). Redox regulation and signaling lipids in mitochondrial apoptosis. *Biochem. Bioph. Res. Co., 304*, 471—479.

Katalinic, V., Milos, M., Mondum, D., & Music, I. et al. (2004). Antioxidant effectiveness of selected wines in comparison with (+) catechin. *Food Chem., 86*, 593–600.

Kono, Y., Kashine, S., Yoneyama, T., & Sakamoto, Y. (1998). Iron chelation by chlorogenic acid as a natural antioxidant. *Biosci. Biotech. Biochem., 62*, 22–27.

Li, H. J., Li, P, & Ye, W. C. (2003). Determination of five major iridoid glucosides in *Flos Lonicerae* by high-performance liquid chromatography coupled with evaporative light scattering detection. *J. Chromatogr. A., 1008*, 167–172.

Ministry of Public Health of the People's Republic of China. (2000). Pharmacopoeia of the People's Republic of China, Chemical Industry Press, 177.

Motoyo, O., Hideko, M., & Hideo, I. et al. (1994). Inhibitory effects of chlorogenic acids on linoleic acid peroxidation and haemolysis. *Phytochemistry, 36*, 579–583.

Nardini, M., D'Aquino, M., & Tomassi,G. et al. (1995). Inhibition of human low-density lipoprotein oxidation by caffeic acid and other hydroxycinnamic acid derivatives. *Free Radic. Biol. Med., 19*, 541–552.

Nardini, M., Natella, F., Gentili, V., Di-Felice, M., & Scaccini, C. (1997). Effect of caffeic acid dietary supplementation on the antioxidant defense system in rat: An in vivo study. *Arch. Biochem. Biophys., 342*, 157–160.

Plumb, G., Garcia-Conesa, M., & Kroon, P. et al. (1999). Metabolism of chlorogenic acid by human plasma liver intestine and gut microflora. *J. Sci. Food Agric, 79*, 390–392.

Ramkumar, K. M., Rajesh, R., & Anuradha, C. V. (2003). Food restriction attenuates blood lipid peroxidation in carbon tetrachloride–intoxicated rats. *Nutrition, 19*, 358–362.

Ravi, J., Adhikari, S., & Patro, B. S. (2001). Free radical scavenging behavior of folic acid: evidence for possible antioxidant activity. *Free Radical Biol. Med., 30*, 1390–1399.

Schinella, G. R., & Tournier, H. A. et al. (2003). Antioxidant activity of anti-inflammatory plant extracts. *Life Sci., 70*, 1023–1033.

Shi, G. R., Rao, L. Q., Yu, H. Z., Xiang, H., Pen, G. P., Long, S., & Yang, C. (2006). Yeast-cell-based microencapsulation of chlorogenic acid as a water-soluble antioxidant. *J. Food Eng., 80*, 1060–1067.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *Am. J. Enol. Viticult., 16*, 144–158.

Song, Y., Li, S. L., Wu, M. H., Li, H. J., & Li, P. (2006). Qualitative and quantitative analysis of iridoid glycosides in the flower buds of Lonicera species by capillary high performance liquid chromatography coupled with mass spectrometric detector. *Anal. Chim. Acta*, *564*, 211–218.

Sreejayan, N., & Rao, M. N. A. (1996). Free radical scavenging activity of curcuminoids. *Drug Res., 46*, 169–171.

Steiberg, D. (1992). Antioxidants in the prevention of human atherosclerosis. *Circulation, 85*, 2337–2344.

Stratil, P., & Klejdeus, B. et al. (2007). Determination of phenolic compounds and their antioxidant activity in fruits and cereals. *Talanta, 71*, 1741–1751.

Sudip, C., & Anwessha, B. et al. (2007). Interaction of flavonoids with red blood cell membrane lipids and proteins: antioxidant and antihemolytic effects. *Int. J. Biol. Macromol., 41*, 42–48.

Sun, T., & Ho, C. T. (2005). Antioxidant activities of buckwheat extracts. *Food Chem., 90*, 743–749.

Venkat Ratnama, D., Ankolaa, D., Bhardwaja, V., Sahanaa, D. K., & Ravi Kumar, M. N. V (2006). Role of antioxidants in prophylaxis and therapy: A pharmaceutical perspective. *J. Control. Rel., 113*, 189–207.

Villano, D., & Fernandez-Pachon, M. S. et al. (2007). Scavenging ability of polyphenolic compounds towards DPPH free radical. *Talanta, 71*, 230–235.

Wang, B. S., & Yen, G. C. et al. (2006). Protective effects of burdock (Arctium lappa lone) on oxidation of low-density lipoprotein and oxidative stress in RAW 267.7 macrophages. *Food Chem., 101*, 729–738.

Yang, R. L., Le, G. W., & Li, A.l. (2006). Effect of antioxidant capacity on blood lipid metabolism and lipoprotein lipase activity of rats fed a high-fat diet. *Nutrition, 22*, 1185–1191.

Yasuhisa, K., Kazuo, K., & Seiichi, T. et al. (2004). Antioxidant activity of polyphenolics in diets: Rate constants of reactions of chlorogenic acid and caffeic acid with reactive species of oxygen and nitrogen. *BBA, 1335*, 335–342.

Zhang, H. Y., Yang, D. P., & Tang, G. Y. (2006). Multipotent antioxidants: from screening to design. *Drug Discov. Today, 11*, 749–754.

Zhang, Z. S., Chang, Q., Zhu, M., & Huang, Yu. et al. (2001). Characterization of antioxidants present in hawthorn fruits. *J. Nutr. Biochem., 12*, 144–152.