Comparative studies on antioxidant activities of extracts and fractions from the leaves and stem of *Epimedium koreanum* Nakai

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Abstract The antioxidant potency of various extracts and fractions from the leaves and stem of *Epimedium koreanum* Nakai was evaluated using three established methods, specifically the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity assay, the inhibitory effect on lipid peroxidation induced by Fe^{2+} /ascorbate (MDA) assay and the ferric reducing power (FRP) assay. The amounts of total phenolics and total flavonoids in the extracts and fractions were determined by spectrophotometric methods and the content of icariin was determined by HPLC. The results showed that all the extracts and fractions exhibited antioxidant activities at different magnitudes of potency. The leaf extract and fractions demonstrated superior antioxidant activity in most of the assays. The decreasing order of antioxidant activities among the extracts/fractions assayed through the three methods were found to be n-BuOH fraction>ethyl acetate fraction>ethanol extract>petroleum ether fraction>water fraction. A positive correlation was found between the amounts of total phenolics, total flavonoids and icariin and DPPH radical scavenging activity ($R^2=0.9935$, 0.9944 and 0.9997 , respectively) and inhibitory activity on lipid peroxidation ($R^2=0.9987$, 0.9830 and 0.9886 , respectively). The results suggested that Icariin was one of the main constituents contribute to the antioxidant activity of *Epimedium koreanum* Nakai and the n-BuOH fractions of leaf extract might be valuable antioxidant natural sources.

Keywords *Epimedium koreanum* Nakai · Flavonoids · Phenolic · Antioxidant activity · Fraction

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Introduction

Over the last few decades, interest in functional foods has been growing fast, leading to the discovery of new functional components or processes that can improve food processing, as well as products that may help to retard aging or avoid diseases. Free radicals and active oxygen can induce oxidant damage. Lipid peroxidation, that involves a series of free radical-mediated chain reaction processes, is also associated with several types of biological damage. The role of free radicals and active oxygen is becoming increasingly recognized in the pathogenesis of many human diseases, including cancer, aging, and atherosclerosis (Chen et al. 2005). So many studies have focused on the antioxidant activities by in vitro assays. Within the antioxidant compounds, flavonoids and phenolics, with a large distribution in nature, have been studied more comprehensively (Amico et al. 2008). Recently, traditional Chinese herbal medicines have been taken as the focus of investigation for natural antioxidants due to broad distribution and diversity as well as low toxicity (Lin et al. 2011). *Epimedium koreanum* Nakai has been used as a nutraceutical in functional foods or a phytopharmaceutical for preventing and curing some serious and fatal illnesses such as cardiovascular diseases, osteoporosis and to improve neurological and sexual function (Yu et al. 1999). There were over 130 constituents had been reported in *Epimedium*, including polyphenols, polysaccharide, steroids, alkaloid, lignin. Fifty one prenyl flavonoid glycosides and eighteen phenolic acids were identified or tentatively identified using the Q-TOF-MS, and an ethylene glycol (Click OEG) column by Wang (2010). Flavonoids was one of the most important contents of *Epimedium* and it had been shown to display a number of biological activities such as antioxidant (Giovannini et al. 2006; Sang et al.

2005) antiviral, antifungal (Friedman 2007), anticancerous (Li et al. 2007; Fresco et al. 2006) antiangiogenic (Oak et al. 2005) and anti-inflammatory activities (Dryden et al. 2006). Icariin ($C_{33}H_{40}O_{15}$; molecular weight: 676.67, ICA) was one of the main flavonoids of *Epimedium koreanum* Nakai and it was always used as the standard for the quality control of *Epimedium* related remedies. Icariin was reported to have multiple activities including vasodilatory effect on the coronary vessels by its inhibition on Ca^{2+} channels (Middleton 1998), cardioprotective effects during ischemia and reoxygenation (Behera et al. 2008), improving the spatial learning and memory abilities in aluminum-intoxicated rats and decreasing the level of $A\beta_{1-40}$ in the hippocampus of aluminum-intoxicated rats (Papetti et al. 2006).

Flavonoids are well known as powerful antioxidants. Therefore, it is valuable to evaluate the potential of antioxidant activity in *Epimedium koreanum* Nakai. Although the antioxidant activity of the flavonoids in *Epimedium koreanum* Nakai had been reported (Wang et al. 2007), only six flavonoids were compared by DPPH radical scavenging assay and no correlation between total phenolic and total flavonoids contents and antioxidant capacities was reported. In recent years, some investigations on the comparison of the different tissues of folk medicine have been conducted for the synthetic application (Zhang et al. 2010a). Though all plants have antioxidant activity, the most effective part (leaf or stem), the most effective fraction and constituents in *Epimedium koreanum* Nakai are still unknown till now. There was hardly any document reported the total phenolic and total flavonoids distribution in *Epimedium* leaves (EL) and *Epimedium* aerial stem (EAS). So a systematic study of the contents of the total phenolic, total flavonoids, icariin and antioxidant activities of extracts and fractions in different parts of *Epimedium koreanum* Nakai is necessary.

In this study, the objective was to evaluate the antioxidant activities and the contents of antioxidant compounds (flavonoid, icariin and phenolic contents) of different extracts and fractions in different parts (leaves and stem) of *Epimedium koreanum* Nakai. Moreover, correlations between the content of antioxidant compounds and antioxidant activities are also evaluated.

Materials and methods

Chemicals

2, 2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu's phenol reagent was obtained from Sigma-Aldrich (St. Louis, MO). Icariin was bought from National Institute for the Control of Pharmaceutical and Biological Products (Tianjin, China). HPLC solvents were obtained from Concord Tech-

nology Co., Ltd (Tianjin, P.R. China). All other chemicals and reagents were purchased locally and were of analytical grade.

Plant material

Leaves and stem of *Epimedium koreanum* Nakai (Berberidaceae), collected in August 2009 at Kuandian County, Liaoning Province, PR China, and were authenticated by associate Prof. Haixia Chen. Voucher specimens were deposited in the herbarium of this Key Laboratory. The leaves and stem were air dried and then pulverized into a homogeneous size by a disintegrator (FW100, Tianjin Taistite Instrument Co., LTD, Tianjin, China) and then sieved (30–40 mesh).

Extractions

The powder of leaves (20 g) and stem (20 g) were macerated separately at room temperature with ethanol 85% (v/v) for five days with daily shake-up. The leaves and stem powder repeatedly extracted for 3 times with ethanol (85%, v/v) at room temperature. After filtration, the two extracts were evaporated under reduced pressure to remove the ethanol. Each extract was suspended in water and partitioned successively with petroleum ether, ethyl acetate and n-butanol.

Determination of total phenolics content

Total phenolics were determined by the Folin-Ciocalteu method (Zhang et al. 2010b). Briefly, 0.5 ml of each sample was mixed with 0.5 ml of the Folin-Ciocalteu reagent and 0.5 ml of 100 mg/ml sodium carbonate (added 2 min after the Folin-Ciocalteu reagent). After initial mixing the opaque flasks were allowed to stand for 2 h. The optical density of the blue-colored samples was measured at 765 nm in a Shimadzu-UV-1201 (Shimadzu, Kyoto, Japan) spectrophotometer. Gallic acid was used as standard. The total phenolic content was expressed as mg gallic acid equivalent (GAE)/100 g DW.

Determination total flavonoids content

The amount of total flavonoids was measured by colorimetric assay (Jia et al. 1999). An aliquot (1 ml) of extracts or standard solution of quercetin was added to a 10 ml volumetric flask containing 4 ml of distilled H_2O . 0.3 ml of 5% $NaNO_2$ was added to flask. After 5 min, 0.3 ml of 10% $AlCl_3$ was added. At 6 min, 2 ml of 1 M NaOH was added to the mixture. Immediately, the reaction flask was diluted to 10 ml. Absorbance of the mixture was determined at 510 nm versus a blank. Total flavonoids of the extracts were expressed on an extracts weight basis as mg quercetin equivalents/g dry sample. The results were reported in quercetin equivalent (QE) per g of sample. All the measurements were taken in triplicate, means and standard deviation values were calculated.

HPLC system for analysis of icarrin in EL extracts and EAS extracts

HPLC analysis was performed using Shimadzu LC-10AT HPLC (Shimadzu Company, Japan) and chromatographic separations were performed on a YMC ODS-C18 reversed-phase column (5 μ m, 250 mm $\times$ 4.6 mm i.d., YMC Co. Ltd, Kyoto, Japan) equipped with an Analytical KJ0-4282 C18 guard cartridge system (Phenomenex, Torrance, CA, USA).

The mobile phase consisted of methanol (A) and water (B). Baseline separation of icariin was achieved with an elution program as follows: 54% A held until 60 min. The UV detector was set at the wavelength of 270 nm. The flow rate was 1 ml/min, injection volume was 20 μ l. Samples and mobile phases B were filtered through 0.45 μ m Millipore filters. Mobile phases A was filtered through 0.22 μ m Millipore filters. Column temperature was maintained at 25 °C and the retention time for icariin were about 22 min. Flavonoids compounds were identified and quantified by comparing their retention time and absorption spectra of icariin peaks. The working calibration curves based on reference compounds of icariin showed good linearity over the range of 4.75–235 μ g/ml. The regression lines were $Y=40456X+22622$ ($R^2=0.9993$, $n=6$), where Y is the peak area of analysis, and X is the concentration of reference compound (μ g/ml).

Antioxidant capacity determined by DPPH radical scavenging activity

The DPPH radical scavenging activity of the extracts and fractions were determined according to our previous studies (Chen et al. 2009). Briefly, extracts of the leaves and stem of *Epimedium koreanum* Nakai was dissolved in 10 ml of absolute ethanol to give a final concentration of 1 mg/ml, then 2.9 ml of 120 μ M DPPH in ethanol were added to 0.1 ml of the extract solution. After shaking the mixture vigorously, the decrease of absorbance was measured at 517 nm until the reaction reached a plateau. Absolute ethanol was used as the control and ascorbic acid, a stable antioxidant, was used as a synthetic reference. The DPPH radical-scavenging activity in percentage of sample was calculated according to the following equation: DPPH scavenging, activity (%) = $(1 - A_{517 \text{ sample}}/A_{517 \text{ DPPH solution}}) \times 100$.

Inhibitory effect on lipid peroxidation induced by Fe^{2+} /ascorbate (MDA)

The inhibition of lipid peroxidation was assayed according to the method of Anup (Anup et al. 2006). Antioxidant of the different extracts could reduce the malondialdehyde (MDA) content. The mice livers were then cut into small pieces and homogenized in normal saline. The reaction

mixture was composed of tissue homogenate 0.5 ml, phosphate buffer (50 mM, pH 7.4) 0.9 ml, FeSO_4 (0.01 mM) 0.25 ml, ascorbic acid (0.1 mM) 0.25 ml, and 0.1 ml of different concentration (4000, 2000, 1000, 500 and 250 μ g/ml) of the extracts and the standard sample. The reaction mixture was incubated for 30 min at 37 °C and the reaction was terminated by adding 1 ml of Trichloroacetic acid (20% w/v) to the mixture. The mixture was incubated with 1 ml of thiobarbituric acid (0.67%) for 15 min at 100 °C, and use the freezing water to terminated the reaction. After centrifugation at 3000 $\times$ g for 15 min, the absorbance of the supernatant was detected at 532 nm. Tocopherol was used as the positive control.

Measurement of ferric reducing power (FRP)

The reducing power of the extracts and fractions were determined using the method of Cheng (Cheng et al. 2008). The solution (0.5 ml) at different doses were mixed with phosphate buffer (0.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$\text{K}_3\text{Fe}(\text{CN})_6$] (0.5 ml, 1%). The mixture was then incubated at 50 °C for 20 min. A portion of trichloroacetic acid (0.5 ml, 10%) was added to the mixture, which was then centrifuged for 10 min at 3000 $\times$ g. The upper layer of solution (0.5 ml) was mixed with distilled water (0.5 ml) and FeCl_3 (0.1 ml, 0.1%) for 10 min, and then the absorbance was measured at 700 nm in a spectrophotometer, with higher absorbance indicating greater reducing power. All the measurements were taken in triplicate and the mean values were calculated.

Statistical analysis

The data were expressed as means $\pm$ standard error (SD) and analyzed using SPSS (version 13.0). One-way analysis of variance (ANOVA) and Tukey multiple comparisons were carried out to test any significant differences between the means. Differences between the means at the 5% confidence level were considered significant. IC_{50} values from the in vitro data were calculated by regression analysis. Each experiment was repeated three times. Correlation coefficients (r) to determine the relationship between variables were calculated using the Bivariate correlation statistical function.

Results and discussion

Extraction yield

As can be seen from Table 1, the yields of stem and leaf extracts and fractions were in the same order of: ethanol extract>n-BuOH fraction>ethyl acetate fraction>petroleum ether fraction>water fraction. Compared with those of stem,

Table 1 Extracts and fractions yields of aerial parts of *Epimedium koreanum* Nakai

Extracts and fractions ^a	Yield (g/100 g dry weight material)	
	Leaf	Aerial stem
EE	35.40	16.70
PEF	7.41	1.04
EAF	7.80	5.54
nBF	22.33	9.39
WF	0.54	0.70

^a EE ethanol extract; PEF petroleum ether fraction; EAF ethyl acetate fraction; nBF n-butanol fraction; WF water fraction.

the yields of leaf extracts and fractions were higher with the yields of ethanol extract and the n-BuOH fraction of 35.40 and 22.3 g/100 g dry weight material.

Total flavonoids content, total phenolic content

The content of the total phenolic compounds of ethanol extract (EE) and its different fractions (petroleum ether fraction, PEF; ethyl acetate fraction, EAF; n-butanol fraction, nBF and water fraction, WF) from the leaves (EL) and aerial stem (EAS) of *Epimedium koreanum* Nakai expressed as gallic acid equivalents is shown in Table 2. Results showed that nBF in the leaves of *Epimedium koreanum* Nakai samples contained the highest amount of total phenolic compounds followed by EAF, PEF, EE and WF, respectively. There was the similar tendency in phenolic content of the extract and fractions of the aerial

stem of *Epimedium koreanum* Nakai. Compared with the samples of EAS (phenol content were 75.81 mg GAE /g and 38.02 mg GAE /g for nBF and EAF, respectively), the samples of EL had the higher phenolic contents (phenol content were 182.99 mg GAE /g and 102.41 mg GAE /g for nBF and EAF, respectively). It was observed that the crude ethanol extract had a lower phenolic content as compared to the ethyl acetate fractions and n-butanol fractions.

Since *Epimedium* species are known to be rich in flavonoids, the contents of total flavonoids (TF) in the ethanol extracts and fractions from leaves and aerial stem of *Epimedium koreanum* Nakai were investigated (Table 2). In terms of different tissues, the total flavonoids content was higher in the leaves than that in the stems. The amounts of TF in different extracts and fractions varied widely, ranging from 5.44 to 67.41 mg QE/g. For the samples of EL, nBF had the highest TF content (67.41 mg QE/g) and followed by EAF, EF, PEF and WF. The tendency EAS samples were similar to those of EL, in which WF had the lowest TF content (5.44 mg QE/g). The positive correlation between phenolic content and antioxidant potential of various plant extracts had been well demonstrated in prior reports (Abdalbasit et al. 2009). Therefore, the high content of total phenols and total flavonoids in leaf fractions of *Epimedium koreanum* Nakai might indicate the strong antioxidant properties.

Identification of icariin using HPLC

Icariin is one of the main flavonoids of *Epimedium koreanum* Nakai with many bioactivities. Determination of

Table 2 Total phenolic contents and total flavonoids contents and antioxidant capacities of epimedium leaves and Epimedium aerial stem

Samples ^a	Total phenol (mg GAE/g dry sample)	Total flavonoids (mg QE/g dry sample)	IC ₅₀ (DPPH) (mg/ ml)	IC ₅₀ (MDA) (mg/ ml)
Leaves				
EE	71.75±4.97	30.85±3.42	1.59±0.14	1.19±0.38
PEF	78.76±0.24	29.42±1.64	2.06±0.12	1.08±0.41
EAF	102.41±0.46	59.19±0.05	1.77±0.16	1.69±0.05
nBF	182.99±0.60	67.41±0.55	1.10±0.16	0.82±0.20
WF	64.19±2.54	7.22±1.78	12.69±1.59	2.52±0.00
Aerial stem				
EE	22.75±0.16	21.67±1.03	8.42±0.09	3.02±0.49
PEF	19.51±0.00	29.76±1.13	4.66±0.08	1.25±0.98
EAF	38.02±1.08	49.03±0.12	2.64±0.13	2.26±0.09
nBF	75.81±2.19	31.93±1.79	1.97±0.28	0.90±0.45
WF	15.23±3.40	5.44±0.17	93.26±19.31	5.03±0.00
Vitamin C	—	—	1.42	—
tocopherol	—	—	—	2.50

Values are presented as mean±SD (n=3).

^a EE ethanol extract; PEF petroleum ether fraction; EAF ethyl acetate fraction; nBF n-butanol fraction; WF water fraction.

icariin in the different extracts and fractions of leaves and stems of *Epimedium koreanum* Nakai was carried out by HPLC analysis. The substance was identified by a comparison of the retention time and ultraviolet spectra with icariin. The retention time of icariin was about 23.3 min (Fig. 1) and the icariin content were shown in Fig. 2. Icariin was found to be one of the major components in the EAF and nBF fractions. The EAF fractions had the higher contents of icariin with 4.42 and 4.16 mg/100 g DW for EL and EAS, respectively. While the WF fractions of EL and EAS had the lowest icariin contents with 0.061 and 0.038 mg/100 g DW, respectively.

DPPH radical scavenging activity

The DPPH assay is used as tool for the in vitro evaluation of extracts and fractions, and its results can indicate the presence of phenolic and flavonoids compounds in plant extracts (Rice-Evans et al. 1996). Brand-William et al. established the DPPH method (Brand-Williams et al. 1995) and it has been widely used to test free radical-scavenging ability of various samples in present studies. The DPPH

radical scavenging activities of the extracts and fractions were dose-dependent (Fig. 3a and b). Both nBFs from leaves and stem of *Epimedium koreanum* Nakai exhibited high scavenging activity toward DPPH with the IC_{50} values (the amount of antioxidant material required to scavenge 50% of free radical in the assay system) of 1.10 and 1.97 mg/ml, respectively (Table 2). While the IC_{50} values were found to be the least in WF fractions of leaves and stem (12.69 and 93.26 mg/ml). It seemed that nBFs were superior to all extracts and fractions tested with regard to scavenging abilities. The IC_{50} values of extracts and fractions of leaves were significantly lower than stem in DPPH free radicals assay. The results were not fully agreed with Boligon's study (Boligon et al. 2009), in which very good antioxidant activities were found for the butanolic and ethyl acetate fractions from both stem bark and leaves, with similar antioxidant profiles. In addition, the considerable differences might depend on the distinct distribution of the antioxidant constituents in which the plant has biosynthesized. Leaves antioxidant activity was found to be similar to the well known antioxidant ascorbic acid used as a reference. The results suggested that our extraction proce-

Fig. 1 HPLC analysis of icarrin. **a** Typical chromatogram for icarrin at the concentration of 117 μ g/ml; retention time= 23.3 min; **b** Typical chromatogram for fraction sample at the concentration of 500 μ g/ml; retention time=23.6 min

Fig. 2 The icariin contents of different extracts and fractions from leaves (EL) and stem (EAS) of *Epimedium koreanum* Nakai. *EE* ethanol extract; *PEF* petroleum ether fraction; *EAF* ethyl acetate fraction; *nBF* *n*-butanol fraction; *WF* water fraction

dures could concentrate more active compounds in nBF fractions by successively extracted with petroleum ether, acetic ether, and *n*-butyl alcohol. As active compounds present in medicinal plants are in low concentrations, applying selective solvents for the extraction procedure is recommended.

Determination antioxidant capacity by MDA

Lipid peroxidation is an oxidative alteration of polyunsaturated fatty acids in the cell membranes that generates a number of degradation products. MDA (malondialdehyde), one of the products of lipid peroxidation, has been studied widely as an index of lipid peroxidation and as a marker of oxidative stress (Ardestani and Yazdanparast 2007). The addition of

different extracts and fractions from leaves and stem of *Epimedium koreanum* Nakai to the liver lipid peroxide induced by FeSO_4 -ascorbic acid significantly reduced TBARS formation. Very good antioxidant activities were found for the EtOAc and *n*-BuOH fractions from both leaves and aerial stem with the IC_{50} of 1.69 and 2.26 mg/ml, 0.82 and 0.90 mg/ml, respectively (Table 2). The water fractions had the lowest inhibition of lipid peroxidation with the IC_{50} of 2.52 mg/ml for leaves and 5.30 mg/ml for stem. Even in the leaves, the IC_{50} values of nBF fraction were as four times better than that of water fraction. This fact could be explained on the basis of the similarity between compounds with high antioxidant activity extracted by these organic solvents. Many other studies have demonstrated that butanolic and ethyl acetate fractions are good sources of antioxidant com-

Fig. 3 Scavenging activity against DPPH free radical and reducing power of different extracts and fractions from leaves (EL) and stem (EAS) of *Epimedium koreanum* Nakai. *EE* ethanol extract; *PEF* petroleum ether fraction; *EAF* ethyl acetate fraction; *nBF* *n*-butanol fraction; *WF* water fraction. **a** scavenging activity against DPPH free radical of EL. **b** scavenging activity against DPPH free radical of EAS. **c** reducing power of EL. **d** reducing power of EAS

pounds (Schubert et al. 2007; Tseng et al. 1997; Turkmen et al. 2006; Tung et al. 2007). Compared with the extract and fractions from stem, the extracts and fractions from leaves were found to own higher antioxidant activity. The IC₅₀ value for nBF fractions was 0.82 mg/ml, which was significantly smaller than that for the positive control tocopherol (2.50 mg/ml). The inhibition of lipid peroxidation of the fractions might be caused by the absence of ferryl-perferryl complex or by scavenging hydroxyl radicals or by chelating the iron ions. The results suggested that the nBF fraction of *Epimedium koreanum* Nakai might be a good source of antioxidant.

Measurement of ferric reducing power (FRP)

Transition metals are known to play key roles in lipid peroxidation in both biological and food system. Particularly, reaction of ferrous iron with hydrogen peroxide generates the hydroxyl radicals, which are the most reactive and detrimental reactive oxygen. The ferric reducing power (FRP) assay is a simple assay that gives fast, reproducible results (Benzie and Strain 1996). The FRP is versatile and can be readily applied to both aqueous and alcohol extracts of different plants. In this assay, the antioxidant activity is determined on the basis of the ability to reduce ferric (III) iron to ferrous (II) iron (Wong et al. 2006), the value reflects the reducing power. As evident in Fig. 3 (c and d), a dose-dependent ferric reducing power was observed for these samples. The greater reducing power of EAF and nBF fractions from leaves and stem of *Epimedium koreanum* Nakai correlated with their marked antioxidative action, indicating the possible contribution of reducing power to this activity. When the concentration was at 1.0 mg/ml, the reducing power of the extract and fractions from leaves decreased in the order, nBF>EAF>PEF>EE>WF, while the decreasing order was nBF>EAF>PEF>EE>WF for the extract and fractions from stem. These results were also correlated with the flavonoids and phenolic content, the major active substance with the higher antioxidant activity.

Correlation of phytochemical contents with antioxidant activities

The beneficial effects of plant phenols and flavonoids have been attributed to their antioxidant properties, including their ability to scavenge superoxide anions, and hydroxyl and nitric oxide radicals, and to inhibit lipid peroxidation in a variety of experimental systems. Recent reports have established a highly positive rela-

tionship between total phenols or flavonoids and antioxidant activity in many plant species (Zhang et al. 2010a, b). To explore the influence of antioxidant compounds on the antioxidant capacity in *Epimedium koreanum* Nakai, correlations coefficients of the total phenolic, total flavonoid and icariin content using MDA and DPPH activities are shown in Table 3. For the leaves of *Epimedium koreanum* Nakai, the results showed that the total phenolic content and total flavonoid content correlated highly with MDA and DPPH activities ($R^2=0.9935$, 0.9944 and 0.9987, 0.9880, respectively). Furthermore, the correlation coefficient of the icariin content with MDA and DPPH activities was the highest ($R^2=0.9997$ and 0.9836, respectively) in the present study. However, for the stem of *Epimedium koreanum* Nakai, the correlations of total phenolic, total flavonoids and icariin with DPPH and MDA activity were from 0.6140 to 0.9958. The coefficient of icariin was not fully accord with that of total phenolic and total flavonoid, which suggested that icariin was one of the main antioxidant constituents of *Epimedium koreanum* Nakai. Full identification and characterization of candidate compounds that may account for the antioxidant activity of *Epimedium koreanum* Nakai requires further study.

Conclusions

In conclusion, it was demonstrated that *Epimedium koreanum* Nakai possesses effective antioxidant activity, which included free radical and ferric reducing power and lipid peroxidation inhibition, especially the nBF and EAF fractions from leaves of *Epimedium koreanum* Nakai which due to the high contents of total phenolic, total flavonoid and icariin. These parts might possibly be valuable antioxidant natural sources and seemed to be applicable in both healthy medicine and food industry.

Table 3 Correlation coefficients of the content of total phenolic, total flavonoid and icariin with the MDA and DPPH assays

	Total phenolic	Total flavonoids	icariin	Ascorbic acid	tocopherol
Leaves					
DPPH	0.9935	0.9944	0.9997	0.9959	–
MDA	0.9987	0.9880	0.9836	–	0.9846
Aerial stem					
DPPH	0.6140	0.6492	0.7149	0.9959	–
MDA	0.7587	0.9958	0.8661	–	0.9846

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References

- Abdalbasit AM, Ramlah MI, Maznah I, Norsharina I (2009) Antioxidant activity and phenolic content of phenolic rich fractions obtained from black cumin (*Nigella sativa*) seedcake. *Food Chem* 116:306–312
- Amico V, Chillemi R, Mangiafico S, Spatafora C, Tringali C (2008) Polyphenol enriched fractions from Sicilian grape pomace. HPLC-DAD analysis and antioxidant activity. *Bioresour Technol* 99:5960–5966
- Anup S, Shereen RH, Shivanandappa T (2006) Antioxidant activity of the roots of *Decalepis hamiltonii* (Wight & Arn). *LWT- Food Science and Technology* 36:1059–1065
- Ardestani A, Yazdanparast R (2007) Antioxidant and free radical scavenging potential of *Achillea santolina* extracts. *Food Chem* 104:21–29
- Behera BC, Verma N, Sonone A, Makhija U (2008) Antioxidant and antibacterial properties of some cultured lichens. *Bioresour Technol* 99:776–784
- Benzie IFF, Strain JJ (1996) The ferric reducing ability of plasma as a measure of “antioxidant power” the FRAP assay. *Anal Biochem* 239:70–76
- Boligon AA, Pereira RP, Feltrin AC, Machado MM, Janovik V, Rocha BT, Athayde ML (2009) Antioxidant activities of flavonol derivatives from the leaves and stem bark of *Scutia buxifolia* Reiss. *Bioresour Technol* 100:6592–6598
- Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. *Food Sci Tech* 28(1):25–30
- Chen HX, Zhang M, Xie BJ (2005) Components and antioxidant activity of polysaccharide conjugate from green tea. *Food Chem* 90:17–21
- Chen HX, Qu ZS, Fu LL, Dong P, Zhang X (2009) Physicochemical properties and antioxidant capacity of 3 polysaccharides from green tea, oolong tea, and black tea. *J Food Sci* 74:C469–C474
- Cheng H, Chang WH, Chen CS, Liao JW, Huang CJ, Lu FJ, Chia YC, Hsu HK, Wu JJ, Yang HL (2008) Antioxidant activities of *Toona sinensis* leaves extracts using different antioxidant models. *Food Chem Toxicol* 46:105–114
- Dryden GW, Song M, McClain C (2006) Polyphenols and gastrointestinal diseases. *Curr Opin Gastroenterol* 22:165–170
- Fresco P, Borges F, Diniz C, Marques MP (2006) New insights on the anticancer properties of dietary polyphenols. *Med Care Res Rev* 26:747–766
- Friedman M (2007) Overview of antibacterial, antitoxin, antiviral, and antifungal activities of tea flavonoids and teas. *Mol Nutr Food Res* 51:116–134
- Giovannini C, Filesi C, D’Archivio M, Scaccocchio B, Santangelo C, Masella R (2006) Polyphenols and endogenous antioxidant defences: effects on glutathione and glutathione related enzymes. *Ann Ist Super Sanita* 42:336–347
- Jia ZS, Tang MC, Wu JM (1999) The determination of flavonoid content in mulberry and their scavenging effects on superoxide radicals. *Food Chem* 64:555–559
- Li Y, Fang H, Xu W (2007) Recent advance in the research of flavonoids as anticancer agents. *Mini Rev Med Chem* 7:663–678
- Lin LZ, Cui C, Wen LR, Yand B, Luo W, Zhao MM (2011) Assessment of in vitro antioxidant capacity of stem and leaf extracts of *Rabdosia serra* (MAXIM.) HARA and identification of the major compound. *Food Chem* 126(1):54–59
- Middleton E (1998) Effect of plant flavonoids on immune and inflammatory cell function. *Adv Exp Med Biol* 439:175–182
- Oak MH, Bedoui J, Schini-Kerth VB (2005) Antiangiogenic properties of natural polyphenols from red wine and green tea. *J Nutr Biochem* 16:1–8
- Papetti A, Daglia M, Aceti C, Quaglia M, Gregotti C, Gazzani G (2006) Isolation of an in vitro and ex vivo antiradical melanoidin from roasted barley. *J Agric Food Chem* 54:1209–1216
- Rice-Evans CA, Miller NJ, Paganga G (1996) Structure–antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med* 20:933–956
- Sang S, Hou Z, Lambert JD, Yang CS (2005) Redox properties of tea polyphenols and related biological activities. *Antioxid Redox Signaling* 7:1704–1714
- Schubert A, Pereira DF, Zanin FF, Alves SH, Beck RCR, Athayde ML (2007) Comparison of antioxidant activities and total polyphenolic and methylxanthine contents between the unripe fruit and leaves of *Ilex paraguariensis* A. St Hil *Pharmazie* 62:876–880
- Tseng TH, Kao ES, Chu CY, Chou FP, Lin HW, Wang CJ (1997) Protective effects of dried flower extracts of *Hibiscus sabdariffa* L. against oxidative stress in rat primary hepatocytes. *Food Chem Toxicol* 35:1159–1164
- Tung YT, Wu JH, Kuo YH, Chang ST (2007) Antioxidant activities of natural phenolic compounds from *Acacia confusa* bark. *Bioresour Technol* 98:1120–1123
- Turkmen N, Sari F, Velioglu YS (2006) Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin–Ciocalteu methods. *Food Chem* 99:835–841
- Wang T, Zhang JC, Chen Y, Huang F, Yang MS, Xiao P (2007) Comparison of antioxidative and antitumor activities of six flavonoids from *Epimedium koreanum*. *China Journal of Chinese Materia Medica* 32(8):715–718
- Wang YQ, Guo ZM, Jin Y, Zhang XL, Wang L, Xue XY, Liang XM (2010) Identification of prenyl flavonoid glycosides and phenolic acids in *Epimedium koreanum* Nakai by Q-TOF-MS combined with selective enrichment on “click oligo (ethylene glycol)” column. *J Pharm Biomed Anal* 51:606–616
- Wong CC, Li HB, Cheng KW, Chen F (2006) A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. *Food Chem* 97:705–711
- Yu S, Chen K, Li S, Zhang K (1999) In vitro and in vivo studies of the effects of a Chinese herb medicine on osteoclastic bone resorption. *Chin J Dent Res* 2:7–11
- Zhang H, Jiang L, Ye S, Ye YB, Ren FZ (2010a) Systematic evaluation of antioxidant capacities of the ethanolic extract of different tissues of jujube (*Ziziphus jujuba* Mill.) from China. *Food Chem Toxicol* 48:1461–1465
- Zhang Y, Li X, Wang ZZ (2010b) Antioxidant activities of leaf extract of *Salvia miltiorrhiza* Bunge and related phenolic constituents. *Food Chem Toxicol* 48:2656–2662