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Full Length Research Paper

Antioxidant activity and total phenolic content of medicinal plants associated with prevention and treatment of cardiovascular and cerebrovascular diseases

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In order to find new resources of natural antioxidants, antioxidant activities of 40 medicinal plants associated with prevention and treatment of cardiovascular and cerebrovascular diseases were evaluated using ferric reducing antioxidant power (FRAP) and Trolox equivalent antioxidant capacity (TEAC) assays, respectively, and their total phenolic contents were measured by the Folin-Ciocalteu method. Most of these plants were analyzed for the first time for their antioxidant activities. Generally, these plants had high antioxidant capacities and total phenolic contents. A significant correlation between the FRAP value and the TEAC value suggested that antioxidant components in these plants were capable of reducing oxidants and scavenging free radicals. A high correlation between antioxidant capacity and total phenolic content indicated that phenolic compounds could be the main contributor of the antioxidant activity of these plants. Several plants, such as *Sanguisorba offivinalis*, *Rosa chinensis*, *Millettia dielsiana*, *Polygonum cuspidatum*, *Caesalpinia sappan* and *Sophora japonica,* showed the highest antioxidant activities and total phenolic contents. These plants could be potential rich resources of natural antioxidants and could be developed into functional food or drug for prevention and treatment of diseases caused by oxidative stress.

Key words: Plant, antioxidant activity, phenolic content.

INTRODUCTION

There is abundant evidence that oxidative stress imposed by reactive oxygen species plays an important role in many chronic and degenerative diseases, such as atherosclerosis, ischemic heart disease, cancer, diabetes mellitus, neurodegenerative diseases and ageing (Azizova, 2002; Young and Woodside, 2001). The body's non-enzymatic antioxidant defense system is made up of some antioxidants, such as vitamin C, vitamin E, vitamin K and glutathione (Chae et al., 2004; Sies, 1993). The exogenous antioxidants are mainly comprised of synthetic and natural antioxidants. The synthetic antioxidants are widely used in food industry to protect food from oxidation and spoiling. However, some of synthetic antioxidants, such as butylated hydroxyanisole and butylated hydroxytoluene, have been found to be harmful for health due to their potential toxicity and carcinogenicity (Botterweck et al., 2000). On the other hand, it has been reported that natural antioxidants in fruits and vegetables were inversely related with the risk of many chronic diseases, such as cardiovascular diseases and cancer (Duthie et al., 2000; Leifert and

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Abeywardena, 2008). Because of potential health benefits of natural antioxidants (Eberhardt et al., 2000; Trichopoulou et al., 2003), they are expected to be an alternative to synthetic ones. Therefore, there is an increasing interest for researchers in seeking for new resources of natural antioxidants.

As an important category of phytochemicals, phenolic compounds are dietary constituents widely existing in plants and have been considered to have high antioxidant capacity and free radical scavenging capacity (Kahkonen et al., 2001; Robards et al., 1999). Phenolic compounds have attracted more and more attention as potential agents for preventing and treating many oxidative stress-related diseases. Several studies showed that phenolic compounds were the main antioxidant ingredients in several medicinal plants (Cai et al., 2004; Liu et al., 2008). In recent years, researches on antioxidant activities of medicinal plants have remarkably augmented by virtue of increased interest in their potential high antioxidant capacity and positive health benefits (Katalinic et al., 2006; Liu et al., 2008; Matkowski et al., 2008; Rafat et al., 2010; Veeru et al., 2009). The studies also showed that some medicinal plants possessed more potent antioxidant activity than common dietary plants (Cai et al., 2004). Therefore, evaluation of antioxidant activities of medicinal plants is very important because some plants possessing strong antioxidant activities can be screened out, which are potential resources of natural antioxidants, both for preparation of crude extracts and for further isolation and purification of antioxidant components. Furthermore, if the extract possessing strong antioxidant activity is nontoxic, further isolation and purification of antioxidant components is not necessary because health benefits of the extract might be from additive and synergistic effects of phytochemicals in the extract (Liu, 2003). At this condition, the extract can be directly used as either component of functional food or food additive.

In the literature, antioxidant activities of several groups of medicinal plants, such as those possessing anticancer activities, antiviral actions, heat-clearing properties and nutritious and tonic functions have been evaluated (Cai et al., 2004; Chen et al., 2005; Li et al., 2008; Liu et al., 2008). However, a special and important group of Chinese medicinal plants, which are traditionally used as herbal tea for prevention and treatment of cardiovascular and cerebrovascular diseases, have not been evaluated for their antioxidant activities systematically.

The cardiovascular and cerebrovascular diseases might be partly attributed to oxidative stress caused by reactive oxygen species and effects of these medicinal plants could be partly attributed to their antioxidant and free radical scavenging activities (Gong and Sucher, 1999; Ng et al., 2000). This prompted us to speculate that these medicinal plants could contain rich natural antioxidants. Therefore, the purpose of this study was to systematically evaluate the antioxidant activities of 40 medicinal plants and the correlation with their total phenolic contents. The results from this study are helpful for full utilization of these medicinal plants.

MATERIALS AND METHODS

Chemicals and plant materials

2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethylchromane-2- carboxylic acid (Trolox), 2,2'-azinobis(3-ethylbenothiazoline-6-sulfonic acid) diammonium salt (ABTS), Folin–Ciocalteu's phenol reagent and gallic acid were purchased from Sigma–Aldrich (St. Louis, MO). Iron (III) chloride 6-hydrate, iron (II) sulfate 7-hydrate, methanol, hydrochloric acid, acetic acid, sodium acetate, potassium persulphate and sodium carbonate were obtained from Tianjing Chemical Factory (Tianjing, China). All chemicals used in the experiments were of analytical grade. Forty selected medicinal plants were purchased from Beijing Tong-Ren-Tang drug retail outlet in Guangzhou of China.

Sample preparation

The plant sample was ground to fine powder with a special grinder for herbal medicine. A precisely weighed amount (about 0.5 g) of the powder was extracted with 10 mL of 80% methanol at 35°C for 24 h in a shaking bath according to the literature (Cai et al., 2004). The samples were then cooled down to the room temperature and centrifuged at 4000 rpm for 10 min. The supernatant was recovered for the evaluation of antioxidant capacity and total phenolic content.

Ferric-reducing antioxidant power (FRAP) assay.

The FRAP assay was carried out according to the procedure described by Benzie and Strain (1996) with minor modification. Briefly, the FRAP reagent was prepared from sodium acetate buffer (300 mM, pH 3.6), 10 mM TPTZ solution (40 mM HCl as solvent) and 20 mM iron (III) chloride solution in a volume ratio of 10:1:1, respectively. Especially, the FRAP reagent was prepared freshly daily and warmed to 37°C in a water bath before use. One hundred microliters of the diluted sample was added to 3 mL of the FRAP reagent. The absorbance of the reaction mixture was then detected at 593 nm after 4 min. The standard curve was constructed using $FeSO_4$ solution, and the results were expressed as µmol Fe (II)/g dry weight of plant material.

Trolox equivalent antioxidant capacity (TEAC) assay

The TEAC assay was carried out to determine the free radical scavenging capacity using the $ABTS^{\bullet+}$ radical cation, according to the method established in the literature (Re et al., 1999) with slight modification. Briefly, the $ABTS^{\bullet+}$ stock solution was prepared from 7 mM ABTS and 2.45 mM potassium persulfate in a volume ratio of 1:1 and then should be incubated in the dark for 16 h at room temperature and used within 2 days. The $ABTS^{\bullet+}$ working solution was prepared by dilution of the stock solution with ethanol to an absorbance of 0.70 ± 0.05 at 734 nm. All samples were diluted approximately to provide 20 to 80% inhibition of the blank absorbance. One hundred microliters of the diluted sample was mixed with 3.8 mL $ABTS^{\bullet+}$ working solution. The absorbance of the reaction mixture was then detected at 734 nm after 6 min of incubation at room temperature, and the percent of inhibition of absorbance at 734 nm was calculated. Trolox solution was used as a reference standard and the results were expressed as µmol Trolox/g dry weight of plant material.

Determination of total phenolic content

Total phenolic content were determined according to the literature (Li et al., 2007; Singleton and Rossi, 1965). Briefly, 0.50 mL of the diluted sample was added to 2.5 mL of 1:10 diluted Folin–Ciocalteu reagent. After 4 min, 2 mL of saturated sodium carbonate solution (about 75 g/L) was added. After 2 h of incubation at room temperature, the absorbance of the reaction mixture was measured at 760 nm. Gallic acid was used as a reference standard and the results were expressed as milligram gallic acid equivalent (mg GAE)/g dry weight of plant material.

Statistical analysis

All the experiments were performed in triplicate and the results were expressed as mean ± SD (standard deviation). Statistical analysis was performed using SPSS 13.0 and Excel 2003. The difference was considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Antioxidant capacities of 40 selected medicinal plants

The antioxidant capacity of the extract from plant is influenced by lots of factors, such as composition of the extract and test system and can not be fully described with one single method. A reliable antioxidant protocol requires the measurement of more than one property because most natural antioxidants are multifunctional. Therefore, it is essential to perform more than one type of antioxidant capacity measurement to take into account the various mechanisms of antioxidant action (Wong et al., 2006). In this study, ferric reducing antioxidant power (FRAP) and Trolox equivalent antioxidant capacity (TEAC) assays were used to evaluate the antioxidant capacities of 40 selected medicinal plants associated with prevention and treatment of cardiovascular and cerebrovascular diseases. The FRAP assay is on the basis of the capacity of antioxidant to reduce Ferric (III) ions to Ferrous (II) ions (Benzie et al., 1996). The TEAC assay is based on the ability of antioxidant to scavenge $ABTS^{\bullet+}$ radical and can measure antioxidant capacities of hydrophilic and lipophilic compounds in the same sample (Re et al., 1999). Generally, the FRAP and TEAC assays are simple, inexpensive, and usually employed methods for the evaluation of antioxidant capacity and could offer fast and reproducible results (Benzie et al., 1996; Cai et al., 2004; Katalinic et al., 2006).

The antioxidant capacities of 40 selected medicinal plants are shown in Table 1. As shown in Table 1, there was a large difference among the antioxidant capacities, from 0.24 to 2025.33 µmol Fe (II)/g dry weight for FRAP assay and from 6.15 to 1363.33 µmol Trolox/g dry weight for TEAC assay. These medicinal plants exhibited quite high antioxidant capacities when compared with some fruits, vegetables, seeds and other medicinal plants reported in the literature (Lako et al., 2007; Silva et al., 2007). For FRAP assay, 8 medicinal plants *Sanguisorba officinalis* (2025.33 ± 184.46 µmol Fe(II)/g), *Millettia dielsiana* (790.79 ± 52.51 µmol Fe(II)/g), *Salvia miltiorrhiza* (788.78 ± 102.81 µmol Fe(II)/g), *Rosa chinensis* (660.34 ± 58.27 µmol Fe(II)/g), *Sophora japonica* (577.88 ± 20.61 µmol Fe(II)/g), *Polygonum cuspidatum* (520.78 ± 39.17 µmol Fe(II)/g), *Sparganium stoloniferum* (338.73 ± 41.45 µmol Fe(II)/g) and *Caesalpinia sappan* (313.50 ± 44.66 µmol Fe(II)/g) had the highest antioxidant capacities, but *Cyathula officinalis* had the lowest antioxidant capacity with 0.24 µmol Fe(II)/g dry weight among the 40 medicinal plants. For TEAC assay, 7 medicinal plants *S. officinalis* (1363.33 ± 100.81 µmol Trolox/g), *R. chinensis* (758.65 ± 19.56 µmol Trolox/g), *M. dielsiana* (615.79 ± 27.90 µmol Trolox/g), *P. cuspidatum* (590.51 ± 24.23 µmol Trolox/g), *C. sappan* (417.48 ± 10.57 µmol Trolox/g), *S. japonica* (318.92 ± 16.22 µmol Trolox/g) and *Dalbergia odorifera* (307.39 ± 5.67 µmol Trolox/g) had the highest free radical scavenging capacities, but *Momordica cochinchinensis* showed the lowest free radical scavenging capacity with 6.15 µmol Trolox/g dry weight among the tested plants.

Six plants, *S. officinalis*, *Rosa chinensis*, *M. dielsiana*, *P. cuspidatum*, *C. sappan* and *S. japonica,* had the highest antioxidant capacities among the 40 plants based on a combinative consideration of the results obtained by FRAP and TEAC assays. It has been reported that *C. sappan* has strong antioxidant activity, which was similar to those of ascorbic acid, rutin and vitamin E (Badami et al., 2003). This further provided that these six plants have strong antioxidant activities, which are comparable to famous antioxidants ascorbic acid and vitamin E. According to the literature, these 6 medicinal plants have many biological activities.

For example, *S. offivinalis* had neuroprotective property, anti-wrinkle activity and anti-allergic effect (Cai et al., 1999). *R. chinensis* is a well-known ornamental plant with its flowers, which usually used in traditional Chinese medicine, and its phenolic compounds had antibacterial activity. *M. dielsiana* showed significant anti-inflammatory activity, especially its components barbigerone and genistein. *P. cuspidatum* had antioxidant, antibacterial, antitumor and lipid-lowering activities. *C. sappan* showed antibacterial activity, and had the potential to be developed into an antibiotic. *S. japonica* had anti-platelet activity, anti-osteoporosis effect, and hepato and nephroprotective activities in normal and carcinogenic rat model. Actually, these plants are often used for the prevention and treatment of cardiovascular and cerebrovascular diseases because they could improve blood circulation or stop bleeding (Cai et al., 1999). Because of their high antioxidant activities, it could be speculated that these plants will be beneficial for cardiovascular and cerebrovascular diseases caused by oxidative stress, and might be developed into functional food or drug in the future.

The correlation between the data obtained from FRAP assay and those from TEAC assay are shown in Figure 1.

Table 1. Antioxidant capacities and total phenolic contents of 40 selected medicinal plants and herbs.

Scientific name	FRAP assay (μmol Fe(II)/g)	TEAC assay (μmol Trolox/g)	Total phenolic content (mg GAE/g)
Achyranthes bidentata Blume	12.54 ± 1.30	24.79 ± 3.55	1.34 ± 0.06
Achyranthes longifolia Mak.	18.66 ± 1.62	37.05 ± 2.11	3.15 ± 0.08
Agrimonia pilosa Ledeb.	255.39 ± 6.24	175.22 ± 2.56	14.10 ± 0.45
Artemisia anomala S. Moore	10.94 ± 1.18	32.77 ± 3.99	3.15 ± 0.02
Artemisia argyi Levl. et Vant.	241.19 ± 13.98	127.73 ± 4.63	12.87 ± 0.23
Biota orientalis (L.) Endl.	181.64 ± 11.84	135.95 ± 13.29	9.12 ± 0.59
Bletilla striata (Thunb.) Reichb. F.	27.24 ± 2.09	38.47 ± 2.33	2.75 ± 0.14
Boehmeria nivea (L.) Gaud.	139.10 ± 1.92	132.78 ± 9.35	10.07 ± 0.44
Caesalpinia sappan L.	313.50 ± 44.66	417.48 ± 10.57	40.97 ± 0.12
Campsis grandiflora Thunb.	96.68 ± 10.58	101.59 ± 1.97	8.93 ± 0.10
Carthamus tinctorius L.	26.05 ± 1.19	97.60 ± 6.58	7.26 ± 0.05
Celosia cristata L.	35.13 ± 1.79	34.83 ± 1.66	2.97 ± 0.16
Cephalanoplos segetum (Bge.) Kitam.	31.55 ± 0.90	32.52 ± 3.36	1.78 ± 0.13
Cirsium japonicum DC.	21.49 ± 1.79	24.86 ± 3.01	1.58 ± 0.18
Curcuma aromatica Salisb.	11.43 ± 1.27	7.66 ± 1.06	0.38 ± 0.05
Curcuma longa L.	122.03 ± 5.35	242.78 ± 21.93	15.33 ± 0.77
Curcuma wenyujin Y.H. Chen	12.15 ± 1.10	136.05 ± 9.50	3.67 ± 0.05
Curcuma zedoaria (Berg.) Rosc.	213.72 ± 26.72	245.03 ± 23.71	13.73 ± 0.66
Cyathula officinalis Kuan	0.24 ± 0.02	23.36 ± 4.33	3.73 ± 0.01
Dalbergia odorifera T. Chen	162.82 ± 11.48	307.39 ± 5.67	7.76 ± 0.30
Ilex pubescens Hook et Arn	44.41 ± 2.49	45.67 ± 0.15	4.70 ± 0.05
Impatiens balsamina L.	121.95 ± 13.30	107.60 ± 0.83	8.42 ± 0.16
Imperata cylindrica (L.) Beauv.	87.67 ± 3.27	43.23 ± 3.07	4.88 ± 0.31
Leonurus heterophyllus Sweet	14.26 ± 1.59	28.35 ± 3.50	3.03 ± 0.16
Ligusticum Chuanxiong Hort.	137.53 ± 13.22	72.20 ± 6.61	5.51 ± 0.08
Lycopus lucidus Turcz.	138.69 ± 10.83	120.98 ± 1.64	7.87 ± 0.17
Millettia dielsiana Harms	790.79 ± 52.51	615.79 ± 27.90	41.93 ± 1.04
Momordica cochinchinensis Lour.	6.16 ± 0.39	6.15 ± 0.44	0.87 ± 0.04
Panax notoginseng (Burk.) F.H. Chen	8.05 ± 0.92	6.80 ± 0.68	0.46 ± 0.04
Polygonum cuspidatum Sieb. et Zucc.	520.78 ± 39.17	590.51 ± 24.23	34.91 ± 0.22
Polygonum orientale L.	2.34 ± 0.37	9.43 ± 1.34	0.65 ± 0.06
Prunus persica (Linn) Batsch.	0.78 ± 0.07	7.81 ± 0.41	0.46 ± 0.02
Rosa chinensis Jacq.	660.34 ± 58.27	758.65 ± 19.56	38.06 ± 0.35
Rubia cordifolia L.	116.67 ± 10.97	89.49 ± 13.02	5.55 ± 0.12
Salvia miltiorrhiza Bge.	788.78 ± 102.81	185.67 ± 29.50	29.60 ± 1.68
Sanguisorba officinalis L.	2025.33 ± 184.46	1363.33 ± 100.81	75.71 ± 5.64
Scutellaria harbata D. Don	22.23 ± 2.10	26.35 ± 1.67	2.12 ± 0.08
Selaginella tamariscina Beauv.	150.43 ± 15.32	155.06 ± 1.06	11.18 ± 0.18
Sophora japonica L.	577.88 ± 20.61	318.92 ± 16.22	30.02 ± 2.71
Sparganium stoloniferum Buch.	338.73 ± 41.45	31.32 ± 2.45	2.58 ± 0.06

The results exhibited positive linear correlation (R^2 = 0.836) between them, which suggested that antioxidant components in these plants could reduce oxidants (such as ferric ions) and scavenge free radicals.

Total phenolic content of 40 selected medicinal plants

The total phenolic contents of 40 selected medicinal plants were estimated using the Folin–Ciocalteu method, which relied on the transfer of electrons from phenolic compounds to Folin–Ciocalteu reagent in alkaline medium, and is operationally simple, reproducible and used in many studies (Li et al., 2007; Singleton et al., 1965). As shown in Table 1, the difference among the total phenolic contents of these plants was very large, from 0.38 mg GAE/g dry weight to 75.71 mg GAE/g dry

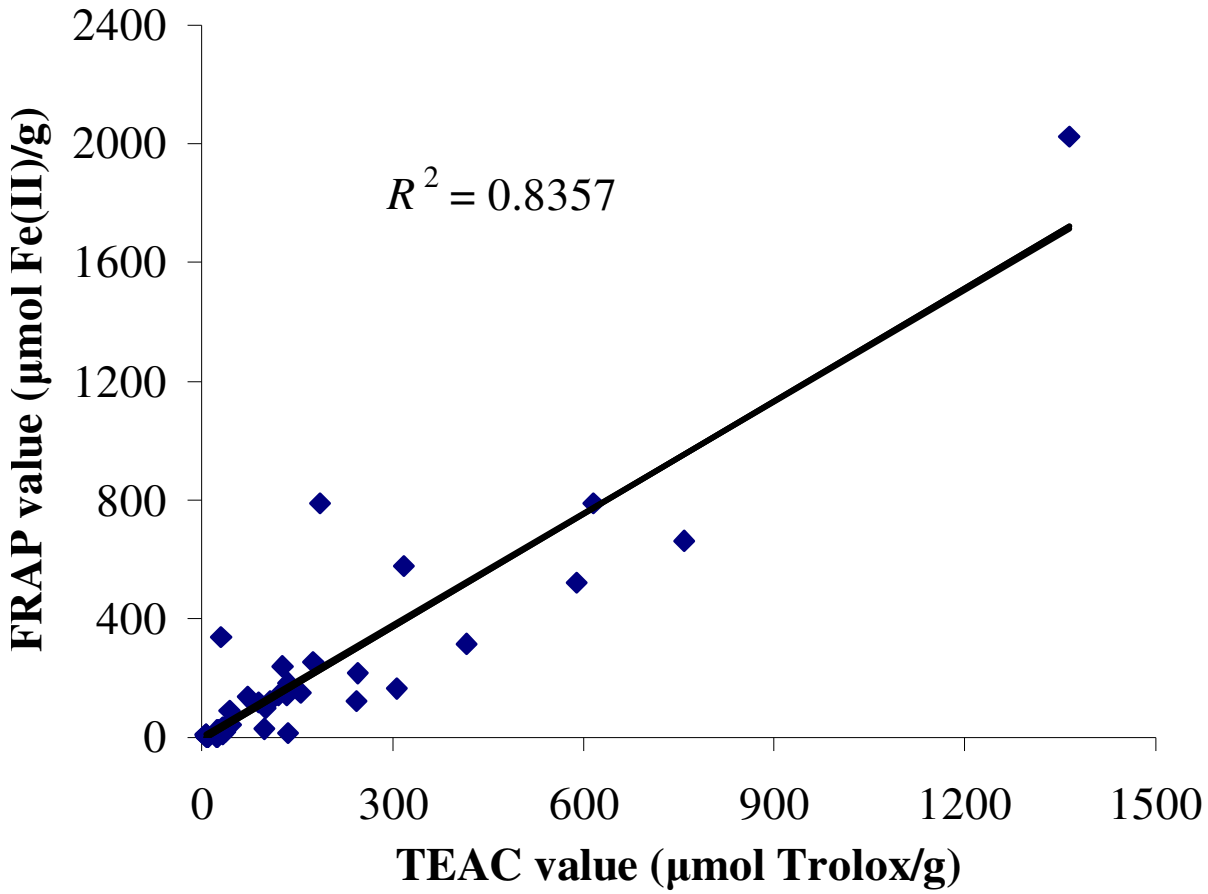

Figure 1. Correlation between the antioxidant capacities measured by the FRAP and TEAC assays.

weight. Seven plants *S. offivinalis* (75.71 ± 5.64 mg GAE/g), *M. dielsiana* (41.93 ± 1.04 mg GAE/g), *C. sappan* (40.97 ± 0.12 mg GAE/g), *R. chinensis* (38.06 ± 0.35 mg GAE/g), *P. cuspidatum* (34.91 ± 0.22 mg GAE/g), *S. japonica* (30.02 ± 2.71 mg GAE/g) and *S. miltiorrhiza* (29.60 ± 1.68 mg GAE/g) showed the highest total phenolic contents (> 20 mg GAE/g), but *Curcuma aromatica* had the lowest total phenolic content with 0.38 mg GAE/g among the tested plants. The total phenolic contents of these medicinal plants were generally high when compared with common fruits, vegetables and other medicinal plants reported in the literature (Lako et al., 2007; Silva et al., 2007).

Correlation between antioxidant capacity and total phenolic content

The correlation between the antioxidant capacity and the total phenolic content of 40 selected plants are shown in Figure 2. The results showed a positive linear correlation between the antioxidant capacity and total phenolic content, which indicated that the phenolic compounds could be the main contributor of the antioxidant activity of these medicinal plants. This result was in agreement with many previous studies reported in the literature (Cai et al., 2004; Katalinic et al., 2006). But there were also some studies that did not find significant correlation between the antioxidant capacity and the total phenolic content (Marwah et al., 2007). Maybe, other components, such as polysaccharides, were the major antioxidant constituents of some medicinal plants (Kardosova and Machova, 2006).

In conclusion, antioxidant activities and total phenolic contents of 40 selected medicinal plants were evaluated and several plants *S. offivinalis*, *R. chinensis*, *M. dielsiana*, *P. cuspidatum*, *C. sappan* and *S. japonica* showed the highest antioxidant capacities and total phenolic contents. These medicinal plants could be potential rich resources of natural antioxidants and could be developed into functional food or drug for prevention and treatment of diseases caused by oxidative stress. In the future, the specific components with high antioxidant capacity in these medicinal plants should be isolated and identified and explored for their health effects with oxidative stress.

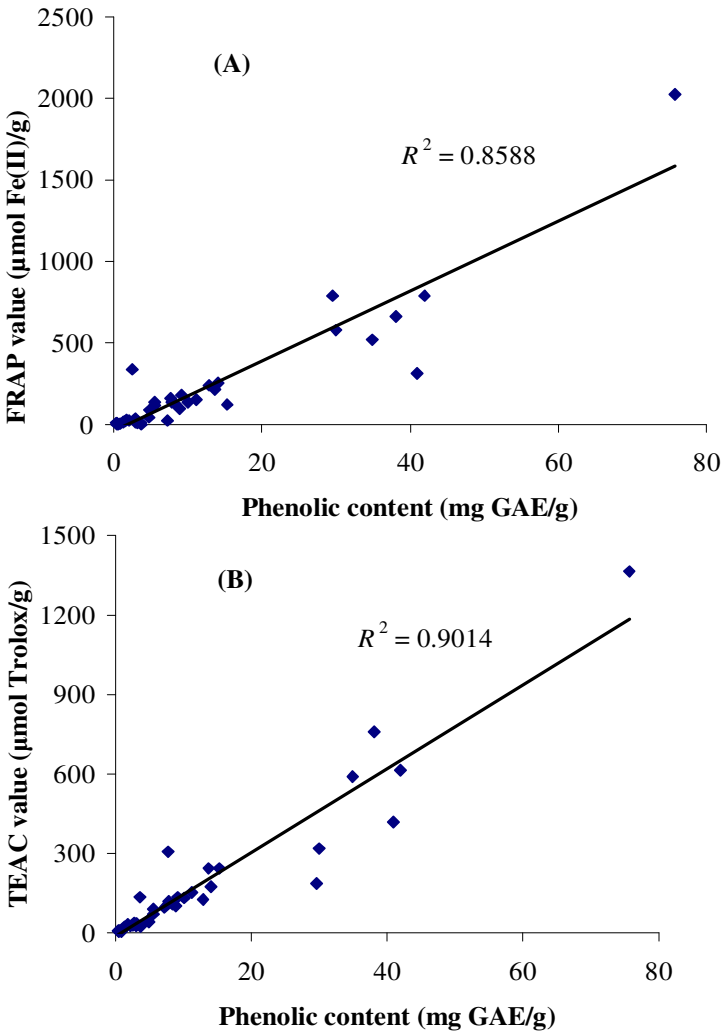

Figure 2. Correlation between the antioxidant capacity and total phenolic content. Antioxidant capacities were measured by the FRAP assay (A) and TEAC assay (B), respectively. GAE: gallic acid equivalents.

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