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Antioxidant activities of *Salvia miltiorrhiza* and *Panax notoginseng*

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Abstract

Traditional Chinese medicinal herb *Salvia miltiorrhiza* (SM) and *Panax notoginseng* (PN) have been widely used for the prevention and treatment of vascular diseases in the clinics. To better understand their mechanisms of pharmacological actions, the in vitro antioxidant activities of extract of *Salvia miltiorrhiza* (ESM) and extract of *Panax notoginseng* (EPN) were evaluated with different antioxidant testing systems. Their activities of scavenging superoxide anion radicals, DPPH radicals, hydroxyl radicals, and hydrogen peroxide, chelating Ferrous ion, and ferric ion reducing power were assessed. The results showed that the mechanisms of their antioxidant effects were distinct and diverse. ESM possessed strong reducing power and high scavenging activities against free radicals including superoxide anion, hydroxyl and DPPH radicals, but a weaker scavenging activity for hydrogen peroxide. ferrous ion chelating activity of ESM was undetectable at the tested concentrations. In contrary, EPN exhibited strong ferrous ion chelating activity and high scavenging activities against hydrogen peroxide, hydroxyl radicals, and a weak activity against superoxide anion and DPPH free radicals. EPN did not show any ferric ion reducing power. Since their antioxidant mechanisms are complementary, the combined use of ESM and EPN might be even more beneficial. These antioxidant properties of SM and PN are likely part of the reasons that they are effective in the prevention and treatment of vascular diseases.
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Keywords: *Salvia miltiorrhiza*; *Panax notoginseng*; Antioxidant activity; Free radicals scavenging; Metal chelating; Reducing power

1. Introduction

Reactive oxygen species (ROS), such as superoxide anion, hydroxyl radicals and hydrogen peroxide, are chemically reactive molecules derived from oxygen. They are generated in living organisms as by-products through many metabolic pathways. ROS can readily react with and oxidize most biomolecules including carbohydrates, proteins, lipids and DNA. There is increasing evidence that accumulation of ROS in biological system causes oxidative damage to tissue that affects cellular integrity and functions. Oxidative damage caused by ROS has been frequently proposed to be associated with the pathogenesis of various diseases and health problems such as aging, arthritis, cancer, inflammation and heart diseases in human body (Abe & Berk, 1998; Busciglio & Yankner, 1995; Meerson, Kagan, Kozlov, Belkina, & Arkhipenko, 1982). Consequently, antioxidants that can neutralize direct ROS attacks and terminate free radical-mediated oxidative reaction would have beneficial activities in protecting the human body from such diseases (Havsteen, 2002; Hertog et al., 1995).

Herb medicines had traditionally played a major role in the management of human health and are still playing an active role in the health care in many countries. There is considerable interest in elucidating the mechanisms of their actions, so we can learn from them to develop better medicines. It has been suggested that for some herbs it is the natural antioxidants they contain conferred their biological activities (Loliger, 1991; Zhu et al., 2004). Most of the antioxidant potentials in herbs and spices are due to the

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redox properties of phenolic compounds that allow them to act as reducing agents, hydrogen donators and free radicals quenchers (Shahidi & Wanasundara, 1992). *Salvia miltiorrhiza* (SM) and *Panax notoginseng* (PN) are two of the most widely used medicinal plants in the clinics either by themselves or in combination with other herbs. The roots or rhizome of SM and PN are the main sources of Compound Danshen Dripping Pill, an oral herbal medicine that has been used in China, Korea and Russia for the treatment of cardiovascular diseases such as occlusive vasculitis, atherosclerosis, cerebral infarction and coronary artery diseases (Guo & Wang, 2003; Horie et al., 2005). Some biological activities of SM and PN are similar. They exhibited activities in modulating vascular tone such as promotion of blood circulation, removal of blood stasis and alleviation of pain (Lei & Chiou, 1986). They also showed activities in inhibiting platelet aggregation and exerting antioxidant effects (Ma & Xiao, 1998). In animal ischemia/reperfusion model, SM prevented myocardial infarction injury, decreased the lipid peroxidation (Kuang, Tao, & Tian, 1996; Zhou & Ruigrok, 1990), enhanced antioxidant enzyme activities (Ji, Tan, Zhu, Linz, & Zhu, 2003) and scavenged free radicals (Zhao, Jiang, Zhao, Hou, & Xin, 1996). PN decreased the plasma lipid level and fibrinogen in high-fat diet rats (Cicero, Vitale, Savino, & Arletti, 2003). Furthermore, PN also showed protective effects for myocardial injury by ischemia and stroke (Huang, Gu, Guan, & Wang, 1991; Li, Wang, Huang, Zhang, & Wei, 1991), for ethanol–induced hepatotoxicity (Lin, Wang, Wu, Huang, & Liu, 2003), and for liver fibrosis (Park, Lee, & Kim, 2005). These growing number of animal studies suggested that SM and PN exerted their biological activities likely through their potent antioxidant activities in vivo. However, their antioxidant activities on the molecule level remain to be determined.

The aim of this study was to investigate the in vitro antioxidant activities of extracts from *Salvia miltiorrhiza* (ESM) and *Panax notoginseng* (EPN). Studies included superoxide anion radical scavenging, DPPH free radical scavenging, hydroxyl radical scavenging and hydrogen peroxide scavenging activities, metal chelating activity, and reducing power. The differences of antioxidant properties of ESM and EPN were examined.

2. Materials and methods

2.1. Chemicals

Nitroblue tetrazolium (NBT), phenazine methosulfate (PMS), nicotinamide adenine dinucleotide (NADH), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), trichloracetic acid (TCA), 1,10-phenanthroline, potassium hexacyanoferrate [$K_3Fe(CN)_6$], and L-ascorbic acid were purchased from the Sigma Chemical Co. (St. Louis, USA). All other chemicals and solvents used were of analytical grade available commercially.

2.2. Preparation of extracts from Salvia miltiorrhiza and Panax notoginseng

The extract of *Salvia miltiorrhiza* (ESM) and the extract of *Panax notoginseng* (EPN) used in this study were provided by Research Institute of Tianjin Tasly Group Co. Ltd., Tianjin. These two extracts were prepared according to the methods described in Quality Standard for Compound Danshen Dripping Pills of Chinese Pharmacopoeia 2000, which is in accordance with the GMP guidelines. In brief, the fresh air-dried roots (30-mesh size) were extracted twice with seven volumes of boiled water under refluxing for 2 h. The combined aqueous extract was filtrated, condensed at 50–60 °C, and precipitated with ethanol (to a final ethanol concentration of 70%) over night. The supernatant was then collected and concentrated in vacuum (40 °C). Finally, the extract was lyophilized and kept in the dark at 4 °C. Extraction rates of ESM and EPN were 12% and 14%, respectively. Dried extract residue was weighed and re-dissolved in distilled water for further experiments.

2.3. Superoxide anion scavenging activity assay

The scavenging activity of the two extracts towards superoxide anion radicals was measured by the method of Liu, Ooi, and Chang (1997). Superoxide anions were generated in a non-enzymatic phenazine methosulfate–nicotinamide adenine dinucleotide (PMS–NADH) system through the reaction of PMS, NADH, and oxygen. It was assayed by the reduction of nitroblue tetrazolium (NBT). In these experiments the superoxide anion was generated in 3 ml of Tris–HCl buffer (100 mM, pH 7.4) containing 0.75 ml of NBT (300 μM) solution, 0.75 ml of NADH (936 μM) solution and 0.3 ml of different concentrations of the extracts. The reaction was started by adding 0.75 ml of PMS solution (120 μM) to the mixture. After 5 min of incubation at room temperature, the absorbance at 560 nm was measured with a Beckman (USA) spectrophotometer (Model DU-64). The superoxide anion scavenging activity was calculated according to the following equation:

$$\text{Scavenging rate} = [1 - (A_1 - A_2)/A_0] \times 100\% \quad (1)$$

where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract, A_2 was the absorbance without PMS.

2.4. DPPH free radicals scavenging activity assay

The scavenging activity for DPPH free radicals was measured according to the procedure described by Amarowicz, Naczk, and Shahidi (2000). An aliquot of 1.5 ml of 0.25 mM DPPH solution in ethanol and 1.5 ml of extract at various concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. Decolorization of DPPH

was determined by measuring the absorbance at 517 nm with a Beckman spectrophotometer. The DPPH radicals scavenging activity was calculated according to the following equation:

$$\text{Scavenging rate} = [1 - (A_1 - A_2)/A_0] \times 100\% \quad (2)$$

where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract, A_2 was the absorbance without DPPH.

2.5. Hydroxyl radicals scavenging activity assay

The scavenging activity for hydroxyl radicals was measured with Fenton reaction (Yu, Zhao, & Shu, 2004). Reaction mixture contained 60 μl of 1.0 mM $FeCl_2$, 90 μl of 1 mM 1,10-phenanthroline, 2.4 ml of 0.2 M phosphate buffer (pH7.8), 150 μl of 0.17 M H_2O_2, and 1.5 ml of extracts at various concentrations. The reaction was started by adding H_2O_2. After incubation at room temperature for 5 min, the absorbance of the mixture at 560 nm was measured with a Beckman spectrophotometer. The hydroxyl radicals scavenging activity was calculated according to the following equation:

$$\text{Scavenging rate} = [1 - (A_1 - A_2)/A_0] \times 100\%, \quad (3)$$

where A_0 was the absorbance of the control (blank, without extract), A_1 was the absorbance in the presence of the extract and A_2 was the absorbance without 1,10-phenanthroline.

2.6. Hydrogen peroxide scavenging activity assay

Hydrogen peroxide scavenging activity of the extract was estimated by replacement titration (Zhang, 2000). Aliquot of 1.0 ml of 0.1 mM H_2O_2 and 1.0 ml of various concentrations of extracts were mixed, followed by 2 drops of 3% ammonium molybdate, 10 ml of 2 M H_2SO_4 and 7.0 ml of 1.8 M KI. The mixed solution was titrated with 5.09 mM NaS_2O_3 until yellow color disappeared. Percentage of scavenging of hydrogen peroxide was calculated as

$$\text{Scavenging rate} = (V_0 - V_1)/V_0 \times 100\% \quad (4)$$

where V_0 was volume of NaS_2O_3 solution used to titrate the control sample in the presence of hydrogen peroxide (without extract), V_1 was the volume of NaS_2O_3 solution used in the presence of the extracts.

2.7. Fe^{2+} chelating activity assay

The chelating activity of the extracts for ferrous ions Fe^{2+} was measured according to the method of Dinis, Madeira, and Almeidam (1994). To 0.5 ml of extract, 1.6 ml of deionized water and 0.05 ml of $FeCl_2$ (2 mM) was added. After 30 s, 0.1 ml ferrozine (5 mM) was added. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. After 10 min at room temperature, the absorbance of the Fe^{2+}–Ferrozine complex was measured at 562 nm. The chelating activity of the extract for Fe^{2+} was calculated as

$$\text{Chelating rate} = (A_0 - A_1)/A_0 \times 100\%, \quad (5)$$

where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

2.8. Reducing power assay

The Fe^{3+} reducing power of the extracts was determined by the method of Oyaizu (1986) with slight modifications. The extract (0.75 ml) at various concentrations were mixed with 0.75 ml of phosphate buffer (0.2 M, pH 6.6) and 0.75 ml of potassium hexacyanoferrate [$K_3Fe(CN)_6$] (w/v 1%), followed by incubating at 50 °C in a water bath for 20 min. The reaction was stopped by adding 0.75 ml of trichloroacetic acid (TCA) solution (10%) and then centrifuged at 3000 r/min for 10 min. 1.5 ml of the supernatant was mixed with 1.5 ml of distilled water and 0.1 ml of ferric chloride ($FeCl_3$) solution (0.1%, w/v) for 10 min. The absorbance at 700 nm was measured as the reducing power. Higher absorbance of the reaction mixture indicated greater reducing power.

2.9. Statistical analysis

Tests were carried out in triplicate for 3–5 separate experiments. L-ascorbic acid was used as a positive control. Values are presented as means ± SD. Statistical analysis was carried out with three or more groups using one-way analysis of variance (ANOVA) and Dunnetts' test. The values of $P < 0.05$ were considered statistically significant. The amount of extract needed to inhibit free radicals concentration by 50%, IC_{50}, was graphically estimated using a nonlinear regression algorithm.

3. Results

3.1. The scavenging activity for superoxide anion radicals

Superoxide anion radical, as the precursor of the more reactive oxygen species including hydroxyl and peroxynitrite radicals, is very harmful to the cellular components in a biological system. The superoxide anion radical scavenging activities of the extracts from *Salvia miltiorrhiza* and *Panax notoginseng* assayed by the PMS–NADH system were shown in Fig. 1. The superoxide scavenging activity of ESM was increased markedly with the increase of concentration, while the superoxide scavenging ability of EPN was not significantly different from 0.5 mg/ml to 3.0 mg/ml. At the concentration of 3 mg/ml, the scavenging rates of ESM and EPN reached 65% and 21%, respectively. The half inhibition concentration (IC_{50}) of ESM was 1.76 mg/ml. These results suggested that ESM had notably superior superoxide scavenging effects than EPN.

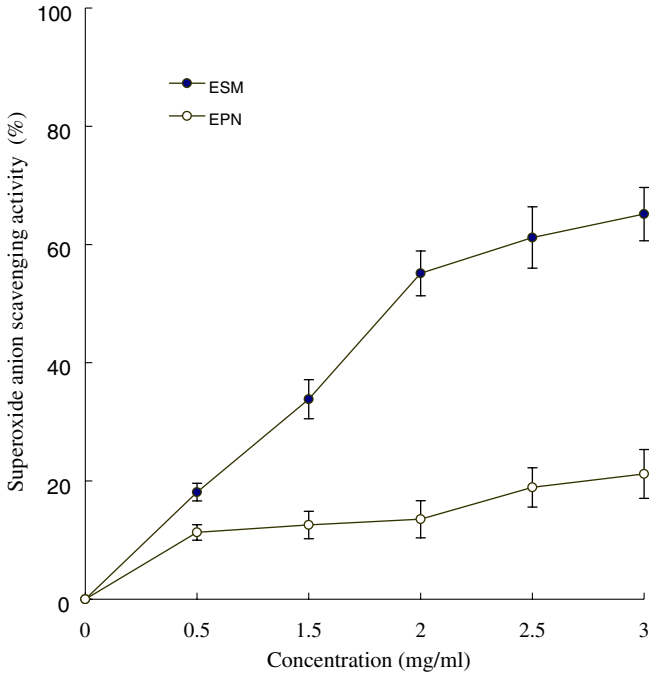

Fig. 1. Superoxide anion radical scavenging activities of extracts from *Salvia miltiorrhiza* (ESM) and from *Panax notoginseng* (EPN) at different concentrations by the non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS–NADH) method. Each value represents means ± SD ($n = 5$).

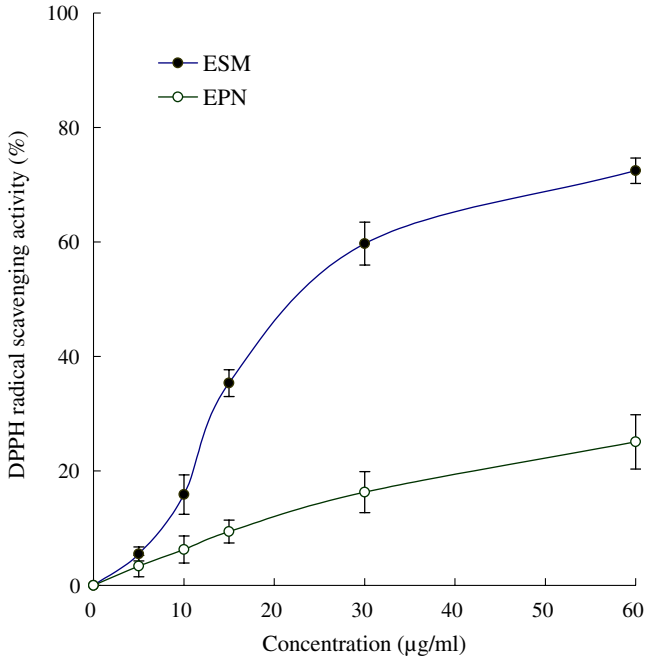

Fig. 2. Free radical (1,1-diphenyl-2-picrylhydrazyl (DPPH)) scavenging activities of extracts from *Salvia miltiorrhiza* (ESM) and from *Panax notoginseng* (EPN) at different concentrations. Each value represents means ± SD ($n = 5$).

3.2. The scavenging activity for DPPH radicals

DPPH molecule that contains a stable free radical has been widely used to evaluate the radical scavenging ability of antioxidants. The free radical scavenging activities of the two extracts, ESM and EPN, were also assayed by using DPPH. As shown in Fig. 2, both ESM and EPN reacted directly with and quenched DPPH radicals to different degrees with increased activities at higher concentrations. At all of the concentrations tested, ESM showed significantly stronger activities than EPN. At the concentration of 60 μg/ml, the scavenging activity of ESM reached a plateau of 73%. However, at same concentration, the scavenging effect of EPN was only 25%. The IC_{50} of ESM was 23.45 μg/ml. To obtain the same ESM IC_{50} scavenging activity, the concentration needed for EPN was 3.09 mg/ml, almost 14 times as that of ESM. Therefore, although both ESM and EPN showed DPPH scavenging activity, ESM was a significantly stronger scavenger for DPPH radicals.

3.3. The scavenging activity for hydroxyl radicals

Hydroxyl radical is very reactive and can be generated in biological cells through the Fenton reaction. Fig. 3 showed that both ESM and EPN exhibited concentration-dependent scavenging activities against hydroxyl radicals generated in a Fenton reaction system. The IC_{50} of ESM at 1.12 mg/ml was slightly lower than the IC_{50} of EPN at 1.74 mg/ml, suggesting that ESM was a slightly stronger hydroxyl radical scavenger. But at a higher concentration,

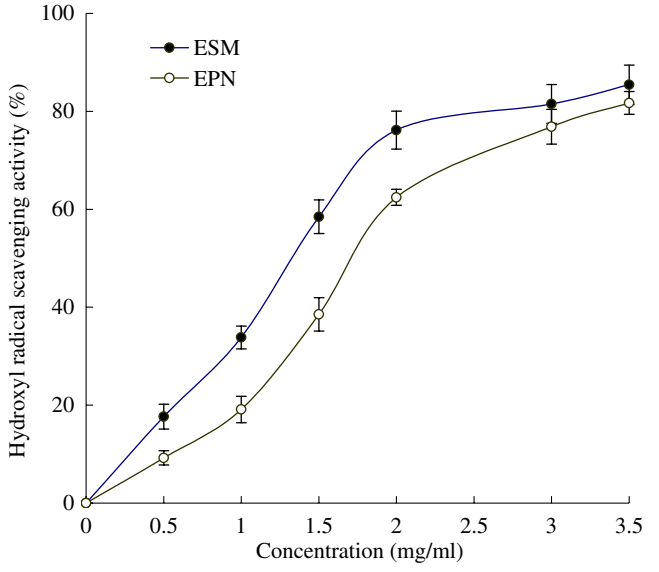

Fig. 3. Hydroxyl free radical scavenging activities of extracts from *Salvia miltiorrhiza* (ESM) and from *Panax notoginseng* (EPN) at different concentrations measured with the Fenton reaction. Each value represents means ± SD ($n = 5$).

like 3.5 mg/ml, ESM and EPN showed statistically the same scavenging activities.

3.4. The scavenging activity of hydrogen peroxide

Although hydrogen peroxide is not very reactive, its high penetrability of cellular membrane leads to hydroxyl

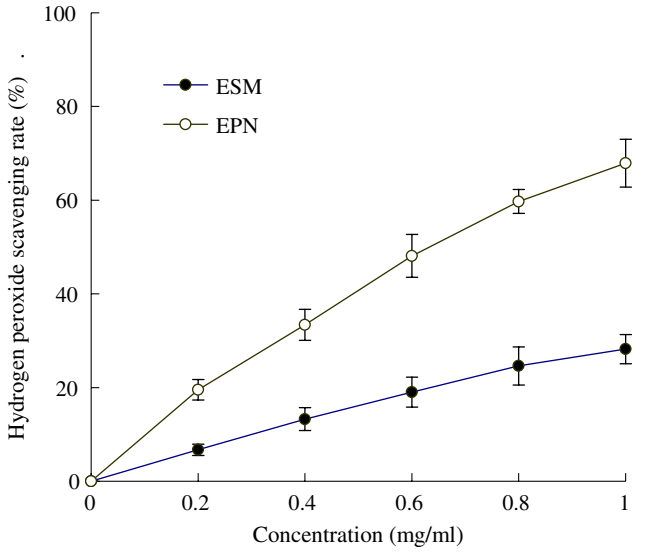

Fig. 4. Hydrogen peroxide scavenging activities of extracts from *Salvia miltiorrhiza* (ESM) and from *Panax notoginseng* (EPN) at different concentrations. Each value represents means ± SD ($n = 3$).

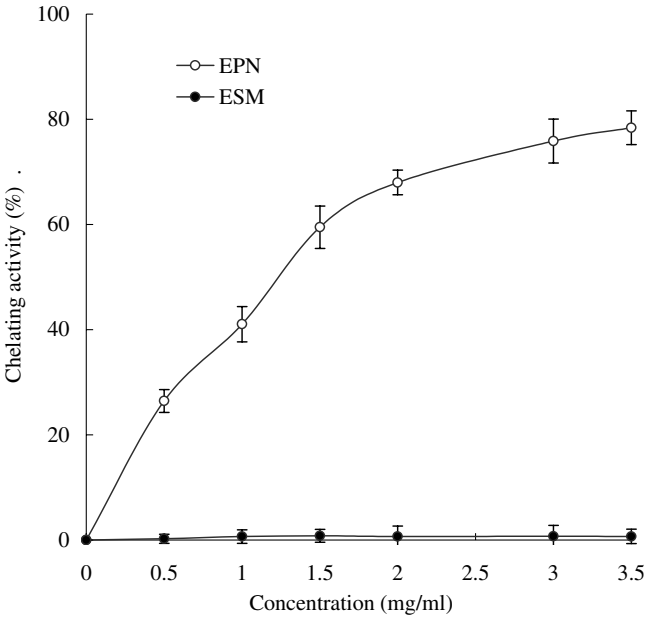

Fig. 5. Ferrous ion chelating activities of extracts from *Salvia miltiorrhiza* (ESM) and from *Panax notoginseng* (EPN) at different concentrations. Each value represents means ± SD ($n = 5$).

radical formation when it reacts with ferrous ion or superoxide anion radical in the cell. As shown in Fig. 4, EPN and ESM demonstrated hydrogen peroxide scavenging activity in a concentration dependent manner. Compared with ESM, EPN was more effective for scavenging hydrogen peroxide with an IC_{50} of 0.58 mg/ml. ESM scavenged 35% of hydrogen peroxide at the concentration of 1.0 mg/ml, while EPN exhibited a scavenging rate of 68% at same concentration.

3.5. The chelating activity for ferrous ion

Transition metals such as ion can stimulate lipid peroxidation by generating hydroxyl radicals through Fenton reaction and accelerate lipid peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals, therefore drive the chain reaction of lipid peroxidation. The chelating activities for ferrous ion of the extracts were assayed by the inhibition of formation of red-colored ferrozine and ferrous complex. As shown in Fig. 5, the formation of the red-colored complex was inhibited in the presence of EPN, indicating chelating activity. However, the color did not change much in the presence of ESM, suggesting weak chelating activity. The light absorbance of the red-colored complex was decreased linearly in an EPN concentration dependent manner with an IC_{50} of 1.2 mg/ml. At the concentration of 5 mg/ml, EPN chelated 81% of ferrous ions. While EPN have strong ferrous ion chelating activity, ESM did not show any chelating activity among the concentrations tested.

3.6. The reducing power

In order to examine the reducing power of extracts, the Fe^{3+} to Fe^{2+} reduction in the presence of the extracts was investigated. As showed in Fig. 6, ESM and EPN showed very different reducing powers. ESM exhibited strong reducing power for Fe^{3+} in a dose dependent manner with an IC_{50} value of 0.35 mg/ml. However, EPN displayed little reducing power under the experimental concentrations.

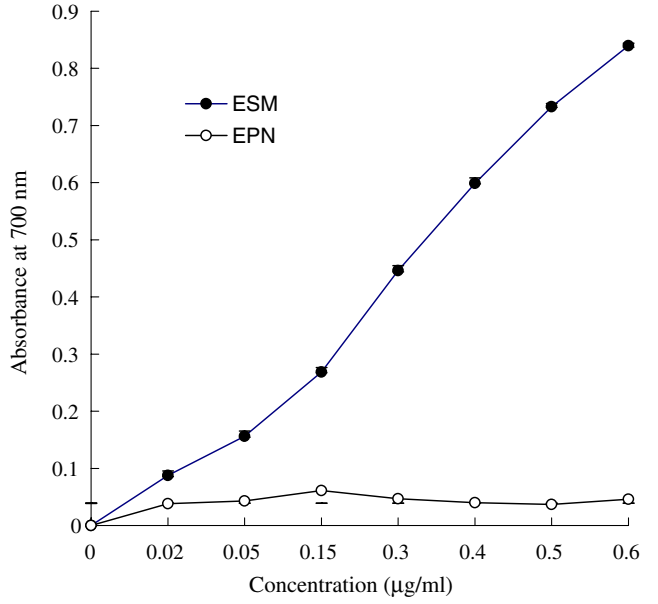

Fig. 6. Reducing power of extracts from *Salvia miltiorrhiza* (ESM) and from *Panax notoginseng* (EPN) at different concentrations by spectrophotometric detection of the Fe^{3+}–Fe^{2+} transformation. Each value represents means ± SD ($n = 5$). High absorbance at 700 nm indicates high reducing power.

4. Discussion

The mechanisms of actions of natural products from traditional Chinese herbal medicines have been focus on interest of their antioxidant activities. What learned from nature could help to overcome the toxicity issues of synthetic antioxidant compounds, such as butylated hydroxyanisole that is widely used in food procession (Chung, Chen, Hsu, Chang, & Chou, 2005), and to develop new chemical entities of antioxidants. *Salvia miltiorrhiza* and *Panax notoginseng* have been reported to be effective to treat myocardial infarction in animal model and to treat cardiovascular diseases in humans. Various studies have suggested that their biological functions are due to, at least partially, their protective effects against oxidation. Our previous study showed that *Salvia miltiorrhiza* protected endothelial cells from TNF-α induced damage (Ding, Zhao, Yuan, & Guo, 2005). In this study we demonstrated the antioxidant properties of extract of *Salvia miltiorrhiza* (ESM) and extract of *Panax notoginseng* (EPN) using various in vitro testing systems. Our data suggested that both ESM and EPN possess direct and potent radicals scavenging activities through multiple mechanisms.

Formation and accumulation of ROS is believed to be one of the mechanisms of myocardial damage by ischemia/reperfusion. During reperfusion, xanthine oxidase converts oxygen into superoxide anion, which in turn dismutates into H_2O_2, and generates hydroxyl radicals through the Fenton reaction, resulting in cell damages. Electron spin resonance (ESR) study showed that ROS, mainly the superoxide anion and semiquinone radicals, and partly H_2O_2, were involved in myocardial reperfusion. When treated with SM, ESR signal of the superoxide anion was disappeared (Zhao et al., 1996). Our in vitro assays showed that ESM have strong reducing power, high scavenging activities for superoxide anion radicals, hydroxyl radicals and DPPH radicals, and some weak activities to scavenge hydrogen peroxide. These activities are reminiscent of the activities of antioxidant enzyme superoxide dismutase and catalase. The major known active components of extract of *Salvia miltiorrhiza* are polyphenolic compounds, including Danshensu (3, 4-dihydroxyphenyllactic acid), salvianolic acids, protocatechualdehyde, tanshinone and its derivatives. Using the xanthine oxidase testing system, Danshensu was shown to scavenge superoxide anion, and tanshinone to scavenge the lipid free radicals, but not the superoxide anion (Zhao et al., 1996). Although in their study Danshensu and tanshinone did not show scavenging activity for hydroxyl radicals, phenolic caffeic acid and its derivatives from *Salvia officinalis*, a similar herb as *Salvia miltiorrhiza*, were shown to have high reaction rate towards hydroxyl radicals (Bors, Michel, Stettmaier, Lu, & Foo, 2004), suggesting that these compounds in *Salvia miltiorrhiza* might be responsible for this property. Flavanoids are another class of phenolic compounds that showed antioxidant activities. Their scavenging potential and metal chelating ability are dependent upon their unique phenolic structures and the number and position of the hydroxyl groups (Pazos, Gallardo, Torres, & Medina, 2005). Flavanoids such as Catechins from tea showed high chelating activity for ferrous iron (Sugihara, Ohnishi, Imamura, & Furuno, 2001). Surprisingly, the extract of *Salvia miltiorrhiza* did not show any chelating activity for ferrous iron. This could be due to the structural complexity of phenolic compounds in the extracts. The works to identify the chemical compounds that are responsible for the various antioxidant activities of ESM are underway.

The major active components of *Panax notoginseng* are saponin, including notogisenosides and gisenosides (Gan & Zhen, 1992; Dong et al., 2003). It has been reported that EPN and its active compounds exhibited inhibitive effects on lipid peroxidation (Lin et al., 2003; Park et al., 2005). Unsaturated lipids in cell membrane are susceptible to peroxidation. This chain reaction is initiated by hydroxyl radical attacking lipids and extended by the generated lipid hydroperoxide free radicals. It was reported that the extract of ginseng exhibited scavenging activity against DPPH radicals in vitro and scavenging activity for hydroxyl radicals to prevent lipid peroxide formation (Jung, Seog, Choi, & Cho, 2005; Zhang et al., 1996). It was also reported that EPN and ginsenosides significantly inhibited hemolysis induced by AAPH free radicals (Liu, Luo, Sun, Chen, & Wang, 2002; Ng, Liu, & Wang, 2004). In our experiments, EPN showed strong scavenging activity towards hydroxyl free radicals and hydrogen peroxide. Its scavenging ability towards DPPH is somewhat weaker. Because its strong protective effect against hydroxyl radicals, EPN might suppress the initiation of lipid oxidation by hydroxyl radicals and therefore have inhibitive effects on lipid peroxidation. This will result in cell membrane protection from oxidative injury.

Antioxidant properties of both ESM and EPN are thought to be part of the reasons that they are pharmacologically useful, but the mechanisms of their antioxidant activities are unclear. Our study showed that there are major differences in the mechanisms of actions of ESM and EPN. Generally, ESM showed stronger scavenging activities for superoxide anions, hydroxyl radicals, and DPPH radicals than EPN. However, EPN reacted with hydrogen peroxide more efficiently than ESM. Moreover, EPN exhibited no reducing power in a Fe^{3+}–Fe^{2+} system, but showed strong ferrous iron chelating activity, implying that EPN acted as Fe^{2+}-chelator, not as Fe^{3+}-reductant. In contrary, ESM showed strong Fe^{3+} reducing power, but no Fe^{2+} chelating activity. These findings expanded the possible mechanism of their antioxidant actions of ESM and EPN.

In conclusion, the antioxidant activities of extract of *Salvia miltiorrhiza* and extract of *Panax notoginseng* were evaluated in this study with five different in vitro testing systems. ESM was highly effective in scavenging free radicals such as superoxide, DPPH and hydroxyl radicals, while EPN was highly effective in chelating ferrous iron, and scavenging hydroxyl radicals and hydrogen peroxide.

Their antioxidant properties likely contributed to their cardiovascular protective effects and their other medicinal usages. It is clear that the antioxidant properties of ESM and EPN are distinct and complementary. Therefore, their combined use might be synergistic and more effective. The understanding of the antioxidant mechanisms of ESM and EPN might lead to the isolation and identification of new compounds with better antioxidant activities.

Acknowledgements

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