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Analytical Methods

Antioxidant activity of *Rhizoma Smilacis Glabrae* extracts and its key constituent-astilbin

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ABSTRACT

Rhizoma Smilacis Glabrae is widely consumed by Chinese as functional food and in folk medicine for its medicinal properties. In this study, methanol and water extracts of *Rhizoma Smilacis Glabrae* were prepared. The water extract was further divided into polysaccharide and supernatant fractions. Constituents in different extracts were analysed by capillary electrophoresis, and levels of total phenolics were also determined using the Folin-Ciocalteu method. Astilbin, the main constituent in the herb, was isolated and purified. Different antioxidant tests were employed to evaluate the antioxidant activities of the extracts and the isolated astilbin, and the results were compared with two commonly used synthetic antioxidants-butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Methanol, water extract and supernatant fraction showed concentration dependent antioxidant activity while polysaccharide didn't show any antioxidant activity. Purified astilbin showed the strongest antioxidant activity in comparison to any other extracts.

1. Introduction

Oxidative metabolism is essential for normal biological activities of cell. However, it also accompanies with the production of reactive oxygen species (ROS). When an excess of ROS is formed, it can result in cell death and tissue damage (Antolovich, Prenzler, Patsalides, McDonald, & Robards, 2002). The role of ROS has been implicated in many diseases, such as cardiovascular diseases, neurodegenerative disorders and cancer. Oxidation also affects food quality. It is a major cause for food deterioration through affecting colour, flavour, texture and nutritional value. Antioxidants are vital substances which possess the ability to reduce oxidative damage caused by ROS. As the possible carcinogenic effects of synthetic antioxidants, there is growing interest in replacing synthetic antioxidants with natural antioxidants from spices and herbs. Epidemiological studies have demonstrated a positive correlation between the intake of fruits and vegetables high in antioxidants (e.g. phenolic compounds) and prevention of diseases like atherosclerosis, cancer and also ageing (Kaur & Kapoor, 2001). Hence, the studies on natural antioxidant have gained increasing attention (Gulcin, Oktay, Kirecci, & Kufrevioglu, 2003; Kubola & Siriamornpun, 2008; Ozsoy, Can, Yanardag, & Akev, 2008; Silva, Malva, & Dias, 2008). In particular, antioxidants from Chinese herbal medicines (CHM) have become a hot topic as they are typically low in toxicity, rarely produce complications and have beneficial pharmacological activities (Guo, Weng, Wu, Li, & Bi, 2005; Han, Weng, & Bi, 2008). The various antioxidant properties of Chinese herbal medicines in the context of their biochemical mechanisms have been reviewed by Zhu et al. (2004).

Rhizoma Smilacis Glabrae, the dry rhizome of the *Smilax Glabra Roxb*., also called Tufuling in Chinese, is a well-known traditional Chinese medicine that widely used for detoxication, relieving dampness and diuretic. It is also the main ingredient of Guiling gao, an old traditional functional food popular in Southern China and Hong Kong. This food is said to help minimize the effects of damp-heat, nourish yin and clear toxins in the blood. It is specifically claimed to improve skin disorder. Pharmacological studies revealed that the methanol or water extract of this herb is anti-inflammatory (Jiang, Wu, Lu, Lu, & Xu, 1997), immunomodulatory (Jiang & Xu, 2003), protective against hepatocyte damage (Chen et al., 1999), and insulin sensitivity enhancement (Fukunaga, Miura, Furuta, & Kato, 1997). Chemical studies showed that flavonoids are the main bioactivity compounds in *Rhizoma Smilacis Glabrae* (Chen, Yin, Yi, Xu, & Chen, 2007), while some organic acids (e.g. syringic acid (Yi et al., 1995), shikimic acid (Zhang, 2001)) are also contained. Our study showed that astilbin is the main constituent in *Rhizoma Smilacis Glabrae*, and the content ranges from 1% to 4% (Zhang, Li, Lai, & Cheung, 2008). Due to the high content of this flavonoid, *Rhizoma Smilacis Glabrae* would show good antioxidant properties. However, to the best of our knowledge, no report about the antioxidant properties of *Rhizoma Smilacis Glabrae* has been published.

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The aim of this study was to evaluate the antioxidant properties of different extracts of *Rhizoma Smilacis Glabrae* and its main constituent-astilbin. The constituents in the extracts were also analyzed by capillary electrophoresis (CE). Their antioxidant properties were compared with two commonly used synthetic antioxidants-BHA and BHT.

2. Material and methods

2.1. Chemicals

BHT, BHA, taxifolin (>85%), shikimic acid (>99%), nitro blue tetrazolium (NBT), phenazine methosulphate (PMS), nicotinamide adenine dinucleotide (NADH), 1,1-diphenyl-2-picryl-hydrazil (DPPH), trichloroacetic acid, tween 20, linoleic acid, 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), ammonium thiocyanate, potassium persulfate, ferrous chloride and ferric chloride were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Standard astilbin (>99%) was gift from Prof. T. Chen (Nanjing University, China). Tested astilbin was isolated from *Rhizoma Smilacis Glabrae* in our lab, and was characterized by UV, FTIR, MS and NMR. The purity was >95.5% as determined by CE. Throughout the study Milli-Q deionised water was used. It was prepared by a Milli-Q water system (Millipore, MA, USA). All other chemicals were of analytical grade.

The stock solution of astilbin, taxifolin, shikimic acid, BHA and BHT was prepared at a concentration of 1.0 mg/ml in methanol and stored at −20 °C until use. To obtain the calibration curve of astilbin, taxifolin and shikimic acid by CE for quantitative analysis, the stock solutions were diluted with methanol. For antioxidant tests, the stock solutions were diluted with water (for superoxide radical-scavenging test, BHA and BHT were diluted with methanol).

2.2. Apparatus

P/ACE MDQ electrophoresis system equipped with a photodiode array detector (DAD) (Beckman Instruments, Fullerton, CA, USA) and 60.2 cm (50 cm from the inlet to detector)×50 μm I.D. fused-silica capillary tube (Beckman Instruments) was used. Agilent 8453 spectrophotometer UV-AG-2 with matched 10-mm quartz cells was used for absorbance measure.

2.3. Plant materials and solvent extraction

Dried samples of *Rhizoma Smilacis Glabrae* were purchased from the local herbal store in Hong Kong and were authenticated by Prof. Zhong-Zhen Zhao (School of Chinese Medicine, Hong Kong Baptist University, Hong Kong SAR). Before extraction, the sample was finely homogenized to powder. For water extraction (WE), 2.5 g sample was mixed with 100 ml water and refluxed for 1 h. The mixture was centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant was collected and concentrated to about 25 ml at 80 °C by rotary evaporation. The concentrated extract was lyophilized and labeled as WE. To obtain polysaccharide fraction, the concentrated extract was precipitated with four volumes of ethanol and stayed for 2 h. Then, the mixture was centrifuged at 5000 rpm for 10 min at 4 °C. The precipitate collected was lyophilized as polysaccharide. The removed supernatant was also lyophilized and labeled as SE fraction. For methanol extract (ME), 5.0 g sample was mixed with 100 ml methanol and sonicated for 30 min. The extract was filtered over Whatman No.1 paper. The methanol in the filtrate was removed by rotary evaporation at 50 °C. The residue was removed from the rockered flask by 15 ml water in five times and lyophilized. These extracts were stored at −20 °C until used. The stock solution of these extract were prepared weekly at a concentration of 1.0 mg/ml in water and stored at −4 °C. Less concentrated of these extract were prepared daily by diluting the stock solutions with water.

2.4. CE analysis

The capillary tube was conditioned prior to its daily use by flushing with 0.1 M NaOH for 5 min, followed by water for another 5 min and finally with the buffer for 5 min. The separation was carried out with running buffer of 20 mM borax with 3 mM β-cyclodextrin (buffer pH was 9.4 without modification), separation voltage of 25 kV and temperature of 25 °C. Samples were injected under the pressure of 0.5 psi for 10 s. Electropherograms were recorded at 214 nm. The ME and SE sample were prepared by mixing 400 μl stock solution (1 mg/ml), 400 μl methanol and 200 μl P-coumaric acid (internal standard, 100 μg/ml). The WE and polysaccharide sample were prepared by mixing 800 μl stock solution (1 mg/ml) with 200 μl P-coumaric acid.

2.5. Determination of total phenolic content

The total phenolic content in the extracts was determined spectrophotometrically, using the Folin-Ciocalteu method as described by Singleton, Orthofer, and Lamuela-Raventos (1999). For this purpose, an aliquot of 0.5 ml of the extract was added to 0.5 ml of Folin-Ciocalteu reagent, followed by addition of 0.5 ml of sodium carbonate solution (7.5%, w/v). The mixture was stirred and measured at 765 nm after staying 30 min. A blank sample consisting of water and reagents was used as a reference. The results were expressed as mg of gallic acid equivalents per g of dry matter, utilizing a calibration curve of gallic acid in the concentration ranged from 5 to 40 μg/ml.

2.6. Scavenging activity of DPPH radical

The scavenging activity of DPPH radical was assayed according to the method described by Chu, Chang, and Hus (2000) with some modifications. An aliquot of 1.0 ml of 0.1 mM DPPH radical solution dissolved in ethanol was mixed with 0.5 ml of extracts at various concentrations or water as negative control. The absorbance of the reaction mixture was measured at 517 nm at 10 min later. The DPPH radical-scavenging activity (%) was calculated by the following equation:

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

where A_{sample} is the absorbance in the presence of extracts and A_{control} is the absorbance of control (Gulcin, 2006a; Gulcin, 2006b).

2.7. Antioxidant activity by the $ABTS^{\cdot+}$ assay

ABTS radical cation ($ABTS^{\cdot+}$) scavenging of the extracts was determined according to the reference (Siddhuraju & Becker, 2007) with some minor modifications. Briefly, $ABTS^{\cdot+}$ was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate (final concentration). The mixture was stood in dark at room temperature for 12–16 h before use. Prior to assay, the solution was 70 times diluted in water to give an absorbance of 0.75 at 734 nm in a 1 cm cuvette. An aliquot of 1.0 ml of the diluted $ABTS^{\cdot+}$ solution was mixed with 0.1 ml of extracts at various concentrations or negative control (water). After staying 5 min at the room temperature, the mixture was monitor at 560 nm. The radical-scavenging activity was calculated using the following formula:

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

where A_{sample} is the absorbance in the presence of extracts and $A_{control}$ is the absorbance of control (Gulcin, 2007; Gulcin, Elias, Gepdiremen, & Boyer, 2006c).

2.8. Reducing power

The reducing power of extracts was determined by the method of Gulcin (Gulcin et al., 2003). Briefly, an aliquot of 1.0 ml of extracts at various concentrations was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%, w/v, in water). The mixture was incubated at 50 °C for 20 min and the reaction was stopped by addition of 2.5 ml of trichloroacetic acid (10%, w/v, in water), followed by centrifugation at 1036 g for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%, w/v, in water), and the absorbance was measured at 700 nm against blanks that contained all reagents except the sample extracts.

2.9. Superoxide anion radical scavenging activity

The superoxide anion scavenging activity of extracts was measured by the method of Siddhuraju et al. (2007) and Gulcin et al. (2003) with some modifications. 150 μM nitroblue tetrazolium (NBT), 60 μM phenazine methosulphate (PMS) and 468 μM NADH were prepared in 16 mM Tris–HCl buffer at pH 8.0. The superoxide anion radical was generated by adding 1.0 ml of NBT, NADH, and sample extract (0.1–1 mg/ml) or control (water), respectively, and started by adding 1.0 ml of PMS. After staying 5 min at the room temperature, the mixture was monitor at 560 nm. The inhibition percentage was calculated using the following formula:

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

where A_{sample} is the absorbance in the presence of extracts and $A_{control}$ is the absorbance of control (Gulcin, 2007; Gulcin et al., 2006c).

2.10. Antioxidant activity in linoleic acid emulsion system

The inhibition of lipid peroxidation was assayed using the thiocyanate method as described by Siddhuraju et al. (2007). 0.5 ml of sample extracts or standards (500 μg/ml, BHA and BHT in ethanol, other in water) was mixed with 2.5 ml of linoleic acid emulsion and 2.0 ml of phosphate buffer (0.2 M, pH 7.0). The linoleic acid emulsion was prepared by homogenising 0.2804 g of linoleic acid, 0.2804 g of Tween 20 as emulsifier, and 50 ml phosphate buffer (0.02 M, pH 7.0). The reaction mixture was incubated at 37 C. Aliquots of 0.1 ml were taken at several intervals during incubation. The degree of oxidation was measured by sequentially adding ethanol (4.7 ml, 75%), ferrous chloride (0.1 ml, 0.02 M in 3.5% HCl), sample solution (0.1 ml), and ammonium thiocyanate (0.1 ml, 30%, w/v). After the mixture had rested for 3 min, the peroxide value was determined by monitoring absorbance at 500 nm. A control was performed with linoleic acid but without the samples. The degree of oxidation was measured for every 24 h until a day after the absorbance of the control reached its maximum. The lipid peroxidation inhibition (LPI)% was calculated as:

$$\text{LPI}(\%) = (1 - A_{sample,48h}/A_{control,48h}) \times 100$$

where $A_{sample,48h}$ is the absorbance in the presence of extracts after 48 h and $A_{control,48h}$ is the absorbance of control after 48 h (Gulcin, 2007; Gulcin et al., 2006c).

2.11. Statistical analysis

Data were expressed as means ± standard deviation (S.D.) of three parallel measurements. Statistical calculations were carried out by OriginPro Version 6.0 software (OriginLab Corporation, Northampton, USA). P values <0.05 were regarded as significant and P values <0.01 were regarded as very significant.

3. Results and discussion

3.1. Extraction yield and total phenolic content

The yield of different *Rhizoma Smilacis Glabrae* extracts was shown in Table 1. About 44.27% of the sample (w/w) can be extracted by water. By dividing the water extract into polysaccharide and supernatant fragments, the content of polysaccharide in *Rhizoma Smilacis Glabrae* was 32.05% (w/w). That may explain why ancient Chinese called *Rhizoma Smilacis Glabrae* as *yuliang*, meaning a superfluous foodstuff which can be eaten as foodstuff when the famine occurred. The solid substances extracted by methanol were around 7.66% (w/w). As polysaccharide cannot dissolve in methanol, main of the methanol extract should be organic small molecules.

It had been reported that the antioxidant activity of plant materials was well correlated with the content of their phenolic compounds (Velioglu, Mazza, Gao, & Oomah, 1998). So, it is important to determine the total phenolic content in the different extracts. The concentration of phenolics in the extracts, expressed as mg of gallic acid equivalents per g of extract, was shown in Table 1. As shown, phenolic content in different extracts was in the order: ME > SE > WE > polysaccharide. ME had the highest phenolic content, which reached 152.28 mg/g. For WE, the phenolic content in polysaccharide fraction was low to be detected by this spectrophotometric method, all phenolic compounds were presented in the supernatant fraction. ME has higher phenolic content than that of WE. However, the yield of WE was 5.7 times than that of ME, so the total amount of phenolic compound extracted between them was similar, which mean water and methanol have similar extraction efficiency of phenolic compound in *Rhizoma Smilacis Glabrae*.

3.2. Identification and quantification of constituents in the extracts

The characterization of constituents in different extract of *Rhizoma Smilacis Glabrae* was carried out using CE-DAD method. The

Table 1
Yield, content of total phenolics, astilbin, taxifolin and shikimic acid of different extracts (mg/g)[a].

	Yield of extract (%, w/w)	Total phenolics[b]	Astilbin content	Taxifolin content	Shikimic acid content
ME	7.66 ± 0.39	152.28 ± 10.57	245.65 ± 8.21	1.30 ± 0.86	13.22 ± 1.35
WE	44.27 ± 0.56	29.41 ± 3.14	38.11 ± 1.46	8.23 ± 0.78	5.74 ± 0.72
SE	10.98 ± 0.16	109.84 ± 6.76	142.18 ± 5.49	28.23 ± 2.32	19.93 ± 1.94
Polysaccharide	32.05 ± 0.23	ND[c]	1.16 + 0.78	ND[c]	ND[c]

[a] Values are expressed as means ± S.D. of triplicate measurements.
[b] As gallic acid equivalents.
[c] ND = not detected.

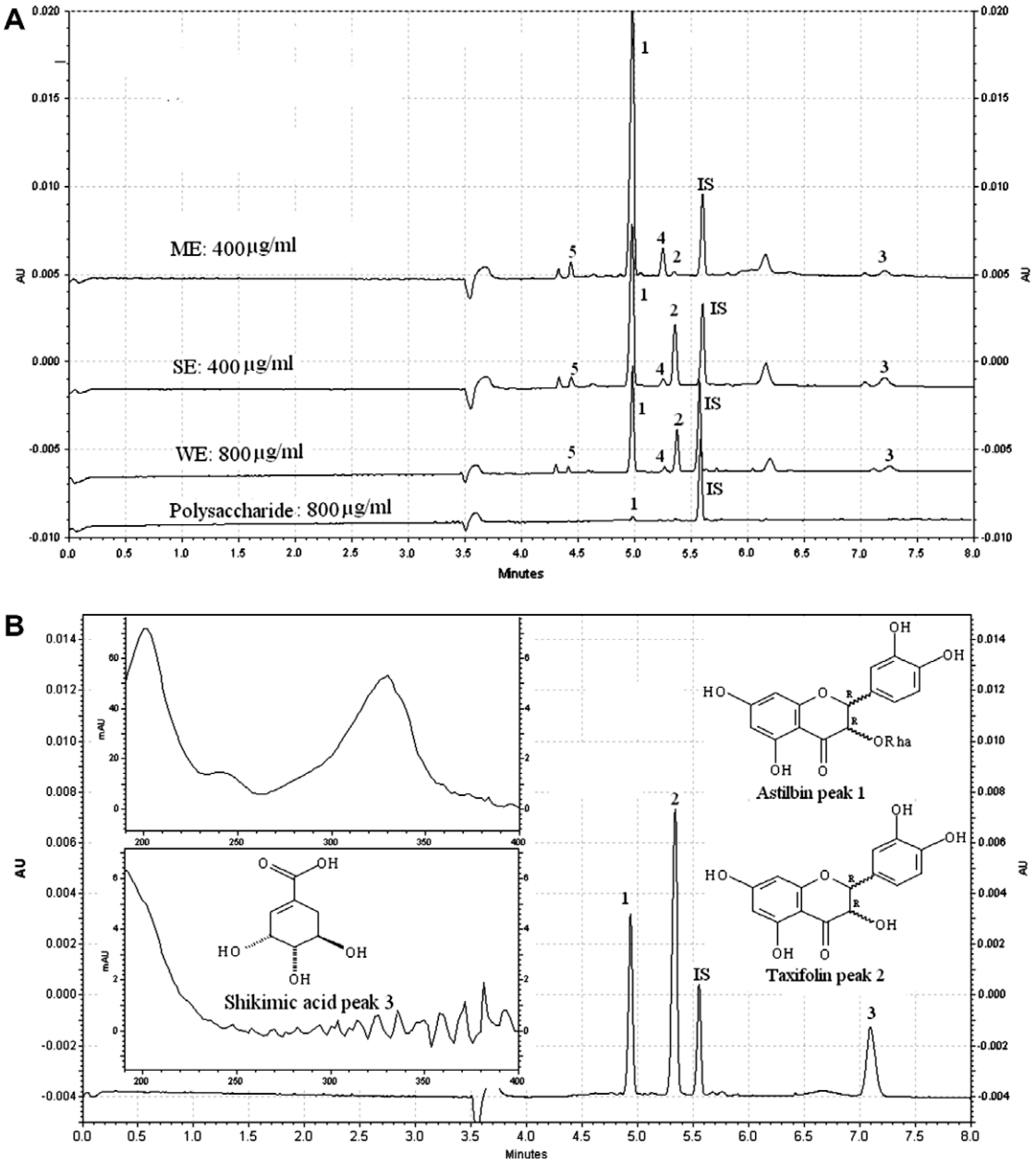

Fig. 1. (A) CE-DAD electropherograms of different extracts; (B) CE-DAD electropherogram, molecule structures and UV spectra of standards. Peaks: 1 = astilbin; 2 = taxifolin; 3 = shikimic acid.

running buffer was 20 mM borax with 3 mM β-cyclodextrin (buffer pH was 9.4 without modification), separation voltage was 25 kV and the capillary temperature was 25 °C. These separated conditions were optimized in our previous works (Zhang et al., 2008). Fig. 1A showed the typical electropherograms of different extracts. As shown, ME, WE and SE had similar electropherograms just with different peak area. However, only one small peak was found in the electropherogram of polysaccharide. The peaks in the electropherograms were identified by comparing the UV spectra and the migration times with that of standards, and by spiking the sample solution with standards. Thus, the most dominant peak (peak 1) was identified as astilbin, and peak 2 and peak 3 was identified as taxifolin and shikimic acid, respectively. The typical electropherogram, molecule structures and UV spectra of these standards were shown in Fig. 1B. Taxifolin was the flavonol glycoside of astilbin, and they have the same UV spectra. Peak 4 and 5 also have the same UV spectra with Peak 1, these compounds may be the isomers of astilbin as described by Chen et al. (2007).

Using P-coumaric acid (20 μg/ml) as the internal standard, the calibration curve of astilbin, taxifolin and shikimic acid were obtained and used for determining the content of these compounds in different extracts. The results were shown in Table 1. As shown, astilbin was the most dominant compound in the extracts, and the contents were 24.56%, 14.21% and 3.81% in ME, SE and WE, respectively. Comparing the yield and astilbin content of ME and WE, it confirmed the similar extraction efficiency of methanol and boil water for astilbin in *Rhizoma Smilacis Glabrae*. These extracts also contain some amount of taxifolin and shikimic acid. Besides small amount of astilbin, polysaccharide fraction contains no other polyphenolic compound. Astilbin was a dihydroflavonol with many bioactivities, such as antibacterial activity (Moulari et al., 2006), contact hypersensitivity inhibition activity (Fei, Wu, & Xu, 2005), etc. As astilbin was the most dominant bioactive compound in

Rhizoma Smilacis Glabrae extracts, we isolated and purified it. The antioxidant activity of purified astilbin was compared with the different extracts to test its role in the extracts.

3.3. DPPH radical-scavenging activity

DPPH radical is a stable free radical that commonly used as a substrate to evaluate antioxidant activity. The antioxidant can scavenge the radial by hydrogen donation, which caused the decrease of DPPH absorbance at 517 nm. Fig. 2A showed the scavenging effect of astilbin and different extracts on DPPH radical compared with that of BHA and BHT. A steady increase of scavenging effect with increasing concentration of extracts was found. Astilbin exhibited strong ability to quench DPPH radical, similar with that of BHA and BHT, the two commercial antioxidants used in the food industry. Both ME and SE factions also showed strong scavenging effect. In contrary, polysaccharide did not show any scavenging effect. Because less content of astilbin, the scavenging effect of WE was relatively low. The concentration of sample at which the inhibition percentage reaches 50% is defined as EC_{50} value. Thus, EC_{50} value is negatively related to the antioxidant activity, the lower EC_{50} value, the higher antioxidant activity of the tested sample. The EC_{50} of BHA, BHT, astilbin, ME, SE and WE were 15 ± 1, 20 ± 1and 24 ± 2, 43 ± 3, 58 ± 3 and 236 ± 17, respectively. According to the EC_{50} values, the order of DPPH radical scavenging ability of these substrates was: BHA > BHT > astilbin > ME > SE > WE > polysaccharide. Comparing with ME, SE and WE, purified astilbin exhibited the strongest scavenging activity, and the difference was very significant ($p < 0.01$).

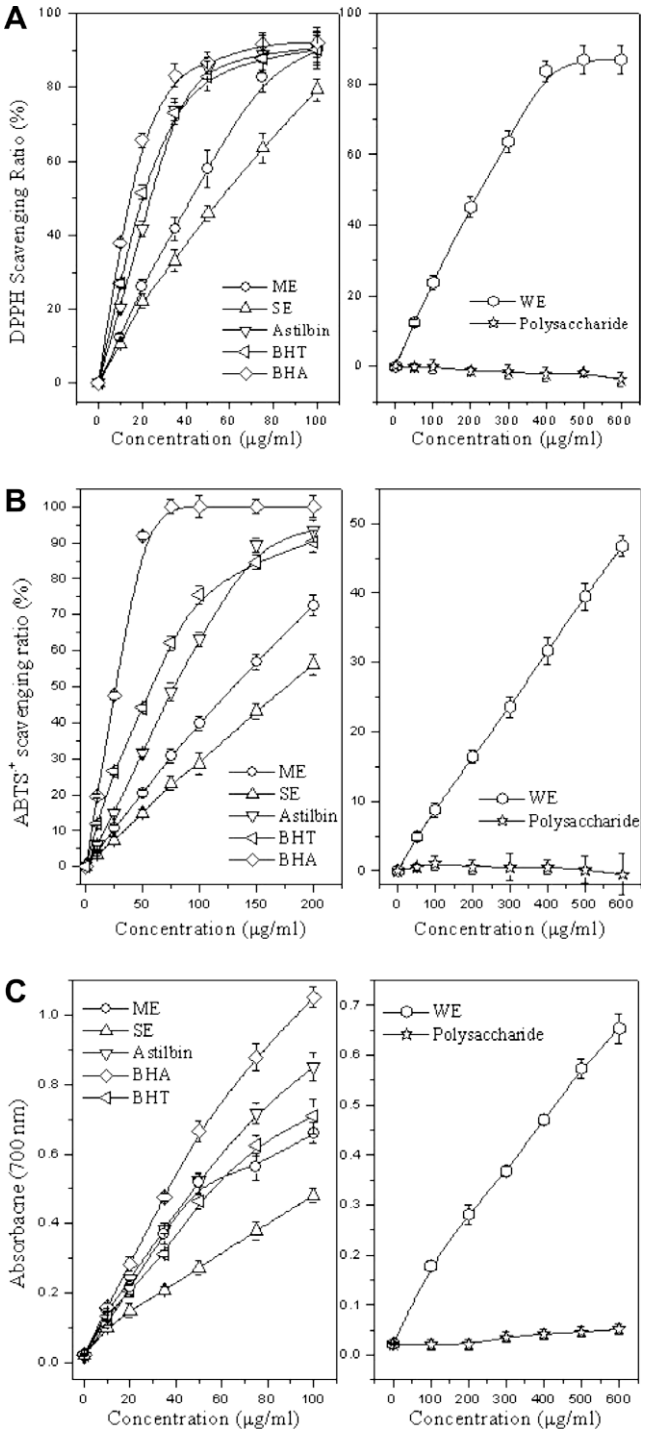

Fig. 2. Antioxidant activities of BHA, BHT, astilbin and different extracts of *Rhizoma Smilacis Glabrae*. (A) DPPH radical-scavenging assay; (B) ABTS radical-scavenging assay; (C) reducing power assay.

3.4. $ABTS^{\cdot+}$ radical-scavenging activity

The $ABTS^{\cdot+}$ radical assay is also one of the most commonly used methods to evaluate the antioxidant activity. As shown in Fig. 2B, a concentration response curve of extracts and standards to $ABTS^{\cdot+}$ assay was obtained, showed quite similar results compared with those obtained in DPPH assay. BHA showed the strongest scavenging effect, and polysaccharide did not show any effect. The scavenging efficiency of astilbin, ME and SE at 50 μg/ml was 31.52%, 20.40% and 14.83%, respectively. The EC_{50} of BHA, BHT, astilbin, ME, SE and WE were 26 ± 2, 60 ± 3, 78 ± 3, 130 ± 8, 177 ± 11 and 647 ± 31, respectively. Thus, the $ABTS^{\cdot+}$ scavenging ability of these substrates exhibited the following order: BHA > BHT > astilbin > ME > SE > WE > polysaccharide.

3.5. Reducing power

Fe^{3+} reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action, and can be strongly correlated with other antioxidant properties (Dorman, Peltoketo, Hiltunen, & Tikkanen, 2003). The reducing power of the extracts was measured by direct electron donation in the reduction of $[Fe(CN)_6]^{3-}$ to $[Fe(CN)_6]^{4-}$. The product was visualized by addition of free Fe^{3+} ions after the reduction reaction, by forming the intense Prussian blue colour complex, $(Fe^{3+})_4[Fe^{2+}(CN-)6]_3$, and quantified by absorbance measurement at 700 nm. Increased absorbance of the reaction mixture indicates increased reducing power.

Fig. 2C showed the dose-response curves for the reducing power of the extracts and standards. Except polysaccharide, all extracts showed the reducing power, astilbin even showed higher power than that of BHT. The EC_{50} value (the effective concentration at which the absorbance was 0.5) was very high for the WE (453 ± 16 μg/ml), compared with the astilbin, ME and SE, of which the EC_{50} values were 45 ± 2, 48 ± 2 and 110 ± 6 μg/ml, respectively. EC_{50} values of BHA and BHT were 40 ± 2 and 57 ± 3 respectively. Effectiveness in reducing power inversely correlated with EC_{50} values and was in descending order: BHA > astilbin > ME > BHT > SE > WE > polysaccharide. Astilbin and ME even showed better reducing power than that of BHT ($p < 0.05$).

3.6. Superoxide radical-scavenging activity

Superoxide radical is known to be very harmful to cellular components as a precursor of the more reactive oxygen species, contributing to tissue damage and various diseases. In the PMS-

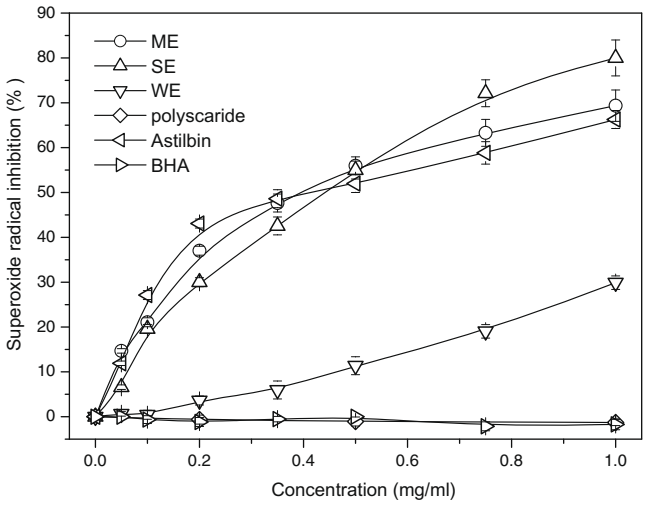

Fig. 3. Superoxide radical-scavenging of BHA, astilbin and different extracts of *Rhizoma Smilacis Glabrae.*

NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS-NADH coupling reaction, will reduce NBT (yellow dye) to blue coloured product called formazon. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. Two buffer systems for the reaction were found in the literatures. One is Tris–HCl buffer (16 mM, pH 8.0) (Gulcin et al., 2003), the other is phosphate buffer (0.1 M, pH 7.4) (Siddhuraju et al., 2007). We compared the two buffer system, and found Tris–HCl buffer was more sensitive and suitable for the reaction.

The scavenging activities of the extracts on superoxide radicals were shown in Fig. 3. It was found that the superoxide-scavenging activities of the extracts increased with the increase of their concentrations. The results showed that BHA and polysaccharide did not show any superoxide-scavenging activity. The activity of BHT was not determined, because the buffer system was cloudy at tested concentration due to its poor water solubility. Astilbin, ME, SE and WE all showed superoxide-scavenging activity. The EC_{50} of Astilbin, ME and SE were 410 ± 18, 398 ± 27 and 432 ± 26, respectively. These data have no statistically significant difference ($p > 0.05$). The results showed that ME and SE might have some constituents which possess better superoxide-scavenging activity than astilbin. WE was comprised of large amount of polysaccharide and less content of polyphenols, so it exhibited weaker activity.

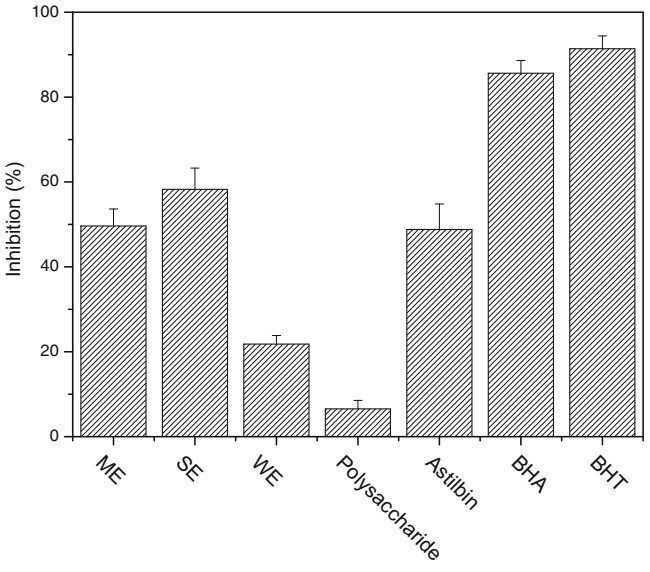

Fig. 4. Antioxidant activity determined in linoleic acid system.

3.7. Antioxidant activity determined in linoleic acid system

The antioxidation effects of the extracts and standards on the peroxidation of linoleic acid were investigated and the results were presented in Fig. 4. The results showed that astilbin and the extracts inhibited 6.5–58.3% peroxidation of linoleic acid after incubation for 48 h. The values were significantly ($P < 0.01$) lower than those of BHT (91.4%) and BHA (85.6%). In summary, the results showed that the inhibitory potential of different extracts and standards was in the order: BHT > BHA > SE > ME > astilbin > WE > polysaccharide.

4. Conclusion

The yield of methanol extract of *Rhizoma Smilacis Glabrae* was 7.66%. Because high content of polysaccharide (32.05%), the yield of water extract even reached 44.27%. According to the results from CE analysis, astilbin was the most dominant compound in all extracts, and in ME the content was high as 24.5%. Besides, taxifolin and shikimic acid were also contained. The studies of DPPH radical, ABTS radical and superoxide anion scavenging, reducing power and inhibition of linoleic acid peroxidation revealed that both methanol and water extract showed concentration dependent antioxidant activity. Purified astilbin showed the strongest antioxidant activity compared with that of ME, WE, SE and polysaccharide fractions. Because higher content of polyphenols, ME showed stronger antioxidant activity than that of WE and SE. Polysaccharide fraction did not show any antioxidant activity, all activity of WE resulted from SE fraction. Based on these results, we concluded that the antioxidant activity of *Rhizoma Smilacis Glabrae* extracts come from its polyphenolic compounds, and as the most dominant constituent, astilbin plays the central role. As boil water and methanol have similar extraction efficiency of astilbin, it can be concluded that, water extracts of *Rhizoma Smilacis Glabrae*, in the way which they are consumed as a foodstuff by Southern Chinese and Hong Kong people, can be used as an accessible source of natural antioxidants with consequent health benefits.

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References

Antolovich, M., Prenzler, P. D., Patsalides, E., McDonald, S., & Robards, K. (2002). Methods for testing antioxidant activity. *Analyst, 127*, 183–198.

Chen, T., Li, J. X., Cao, J. S., Xu, Q., Komatsu, K., & Namba, T. (1999). A new flavanone isolated from Rhizoma Smilacis Glabrae and the structural requirements of its derivatives for preventing immunological hepatocyte damage. *Planta Medica, 65*, 56–59.

Chen, L., Yin, Y., Yi, H. W., Xu, Q., & Chen, T. (2007). Simultaneous quantification of five major bioactive flavonoids in Rhizoma Smilacis Glabrae by high-performance liquid chromatography. *Journal of Pharmaceutical and Biomedical Analysis, 43*, 1715–1720.

Chu, Y. H., Chang, C. L., & Hus, H. F. (2000). Flavonoid content of several vegetables and their antioxidant activity. *Journal of the Science of Food and Agriculture, 80*, 561–566.

Dorman, H. J. D., Peltoketo, A., Hiltunen, R., & Tikkanen, M. J. (2003). Characterization of the antioxidant properties of de-odourised aqueous extracts from selected Lamiaceae herbs. *Food Chemistry, 83*, 255–262.

Fei, M. J., Wu, X. F., & Xu, Q. (2005). Astilbin inhibits contact hypersensitivity through negative cytokine regulation distinct from cyclosporin A. *Journal of Allergy and Clinical Immunology, 116*, 1350–1356.

Fukunaga, T., Miura, T., Furuta, K., & Kato, A. (1997). Hypoglycemic effect of the rhizomes of *Smilax glabra* in normal and diabetic mice. *Biological & Pharmaceutical Bulletin, 20*, 44–46.

Gulcin, I., Oktay, M., Kirecci, E., & Kufrevioglu, O. I. (2003). Screening of antioxidant and antimicrobial activities of anise (Pimpinella anisum L.) seed extracts. *Food Chemistry, 83*, 371–382.

Gulcin, I. (2006a). Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid). *Toxicology, 217*, 213–220.

Gulcin, I. (2006b). Antioxidant and antiradical activities of L-carnitine. *Life Sciences, 78*, 803–811.

Gulcin, I., Elias, R., Gepdiremen, A., & Boyer, L. (2006c). Antioxidant activity of lignans from fringe tree (Chionanthus virginicus L.). *European Food Research and Technology, 223*, 759–767.

Gulcin, I. (2007). Comparison of in vitro antioxidant and antiradical activities of L-tyrosine and L-Dopa. *Amino Acids, 32*, 431–438.

Guo, J. M., Weng, X. C., Wu, H., Li, Q. H., & Bi, K. S. (2005). Antioxidants from a Chinese medicinal herb - Psoralea corylifolia L. *Food Chemistry, 91*, 287–292.

Han, H., Weng, X. C., & Bi, K. S. (2008). Antioxidants from a Chinese medicinal herb – Lithospermum erythrorhizon. *Food Chemistry, 106*, 2–10.

Jiang, J. Y., Wu, F. H., Lu, J. F., Lu, Z. H., & Xu, Q. (1997). Anti-inflammatory activity of the aqueous extract from Rhizoma Smilacis Glabrae. *Pharmacological Research, 36*, 309–314.

Jiang, J. Y., & Xu, Q. (2003). Immunomodulatory activity of the aqueous extract from rhizome of Smilax glabra in the later phase of adjuvant-induced arthritis in rats. *Journal of Ethnopharmacology, 85*, 53–59.

Kaur, C., & Kapoor, H. C. (2001). Antioxidants in fruits and vegetables-the millennium's health. *International Journal of Food Science and Technology, 36*, 703–725.

Kubola, J., & Siriamornpun, S. (2008). Phenolic contents and antioxidant activities of bitter gourd (Momordica charantia L.) leaf, stem and fruit fraction extracts in vitro. *Food Chemistry, 110*, 881–890.

Moulari, B., Pellequer, Y., Lboutounne, H., Girard, C., Chaumont, J. P., Millet, J., & Muyard, F. (2006). Isolation and in vitro antibacterial activity of astilbin, the bioactive flavanone from the leaves of Harungana madagascariensis Lam. ex Poir. (Hypericaceae). *Journal of Ethnopharmacology, 106*, 272–278.

Ozsoy, N., Can, A., Yanardag, R., & Akev, N. (2008). Antioxidant activity of leaf extracts Smilax excelsa L.. *Food Chemistry, 110*, 571–583.

Siddhuraju, P., & Becker, K. (2007). The antioxidant and free radical scavenging activities of processed cowpea (Vigna unguiculata (L.) Walp.) seed extracts. *Food Chemistry, 101*, 10–19.

Silva, B. A., Malva, J. O., & Dias, A. C. P. (2008). St. John's Wort (Hypericum perforatum) extracts and isolated phenolic compounds are effective antioxidants in several in vitro models of oxidative stress. *Food Chemistry, 110*, 611–619.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in enzymology, 299*, 152–178.

Velioglu, Y. S., Mazza, G., Gao, L., & Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry, 46*, 4113–4117.

Yi, Y. J., Cao, Z. Z., Yang, W. H., Hong, W. Q., Cao, Y., & Leng, Z. K. (1995). Chemical studies of smilax glabra(III). Isolation and identification of smiglanin from smilax glabla. *Acta Pharmaceutica Sinica, 30*, 718–720.

Zhang, Y. T. (2001). *Traditional chinese medicine*. Beijing: High education press.

Zhu, Y. Z., Huang, S. H., Tan, B. K. H., Sun, J., Whiteman, M., & Zhu, Y. C. (2004). Antioxidants in Chinese herbal medicines: A biochemical Perspective. *Natural Product Reports, 21*, 478–489.

Zhang, Q. F., Li, S. C., Lai, W. P., & Cheung, H. Y. (2008). β-Cyclodextrin facilitates simultaneous analysis of six bioactive components in *Rhizoma Smilacis Glabrae* by capillary zone electrophoresis. *Food Chemistry*. doi:10.1016/j.foodchem.2008.07.071.