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Full Length Research Paper

Antioxidant activity of bioactive compounds extracted from *Ampelopsis grossedentata* leaves by optimized supercritical carbon dioxide

Le Ying, Ping Xu, Shuangru Huang and Yuefei Wang*

Department of Tea Science, Zhejiang University, Hangzhou 310058, P. R. China.

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Supercritical carbon dioxide (SC-CO_2) extraction of bioactive compounds including flavonoids and phenolics from *Ampelopsis grossedentata* leaves was carried out. Extraction parameters such as pressure, temperature, dynamic time and modifier concentration, were optimized using an orthogonal array design of L_9 (3^4), and antioxidant activities of the extracts were assessed by 2,2-diiphenly-1-picrylhydrazyl (DPPH) free radical scavenging assay and ferrous ion chelating (FIC) assay. Maximal yields of total flavonoids and phenolics were 5.99 and 3.26 mg GAE/g dry material, respectively, which were achieved under the optimal conditions of 200 bar, 50°C, 80 min and with a modifier of 95% ethanol. Moreover, results demonstrated that the obtained extract under optimized conditions showed the best DPPH radical scavenging ability, but an insignificant negative correlation ($p > 0.05$) was found between FIC and total flavonoid contents (TFC) or total phenolic contents (TPC) by Person correlation analysis. In addition, several unreported flavonoids have been detected in the extracts. These results indicate that SC-CO_2 could be a promising method for the extraction of bioactive compounds from *Ampelopsis grossedentata* leaves, and the extracts could act as different kinds of natural antioxidants and be applied in the food and pharmaceutical industry.

Key words: *Ampelopsis grossedentata*, supercritical carbon dioxide extraction, bioactive compounds, orthogonal array, optimization, antioxidant activity.

INTRODUCTION

Reactive oxygen species (ROS), such as superoxide anon radical, hydroxyl radical and peroxyl radical, are the major free radicals in human body that may induce damage to cell structures, DNA, lipids and proteins (Valko et al., 2007), leading to a variety of diseases such as cancers, cardiovascular disease, diabetes, neurological disorders and atherosclerosis (Dalle-Donne et al., 2006). Flavonoids and phenolics, as the ubiquitous phytochemicals that are universally present in many plants (Gan and Latiff, 2010), have been proved to possess remarkable antioxidant capacities *in vitro* and *in vivo* (Lee et al., 2010a; Mane et al., 2011), and been regarded as bioactive compounds which have the potential to protect human body against ROS and to retard the progress of many chronic diseases (Zhang et al. 2010). *Ampelopsis grossdentata* (Hand-Mazz) W. T. Wang as a kind of medicinal plant, which is widely distributed in southern China, and has been used as an herbal remedy for treating hepatitis, flu, hypertension, hyperglycemic and sore throat. In addition, its tender leaves and stems have been used as an herbal tea by local people for some centuries ago. These pharmacological functions of *A. grossdentata* may be due to its main bioactive constituents, such as dihydromyricetin and myricetin, and other bioactive substances (Gao et al., 2009), which have lots of biological functions, including antioxidant, hypoglycemic, antithrombotic, immunostimulating, anti-inflammatory and antibacterial activities (Zhang et al., 2007).

Percolation and soxhlet are the mainly conventional methods for the extraction of bioactive compounds from plants materials. But these methods have many disadvantages, including consumption of large amounts of organic solvent and long extraction time, resulting in low efficiency of extraction. Thus, a much safer and more

*Corresponding author. E-mail: zdxp@zju.edu.cn. Tel: +86 571 88982217. Fax: +86 571 88982217.

Table 1. The factors and levels of the orthogonal array design.

Factors	Levels		
	1	2	3
Pressure (bar)	150	200	250
Temperature (℃)	40	50	60
Dynamic time (min)	40	60	80
Modifier concentration (ethanol %)	95	85	75

efficient technology is needed for the extraction of bioactive compounds. Supercritical carbon dioxide (SC-CO_2) extraction, as a non-toxic, high-efficiency and moderate method for the extraction of many kinds of components from plant matrices, has received considerable attention in recent years (Chiu et al., 2002; Cossuta et al., 2008). In order to improve the extraction efficiency of polar compounds, modifiers like methanol, ethanol, and water are commonly used in SC-CO_2 extraction of bioactive compounds like flavonoids and phenolics; but taking the safety of extraction processing and the resulting extracts into consideration, ethanol and water are more suitable for the extraction of bioactive compounds, which have the potential application in food and pharmaceutical industry (Iheozor-Ejiofor and Dey, 2009; Lee et al., 2010b). In previous reports, SC-CO_2, with a modifier of different concentrations of ethanol, has been successfully employed from a wide range of plants, such as pigeonpea (Kong et al., 2009), grape (Ghafoor, 2010), *Prunella vulgaris* (Zhang et al., 2010), and cherries (Serra et al., 2011). However, to the best of our knowledge, SC-CO_2 extraction of bioactive compounds from *A. grossedentata* leaves has not been reported in literature previously. Therefore, the purposes of this study were to use SC-CO_2 to extract bioactive compounds, including flavonoids and phenolics, from *A. grossedentata* leaves, to use an orthogonal array design (OAD) to determine the optimal conditions, to evaluate antioxidant activities of the extracts by 2,2-diiphenly-1-picrylhydrazyl (DPPH) free radical scavenging assay and ferrous ion chelating (FIC) assay, and to identify the main flavonoids using high performance liquid chromatography (HPLC).

MATERIALS AND METHODS

The leaves of *A. grossedentata* were obtained from Sanming in Fujian Province, China. They were dried at 40℃ for 24 h and then milled into powder by an herbal pulverizer (XB-02, Xiaobao Machinery Co. Ltd., China). The resulting flour passed through a 20 mesh sieve and stored in a refrigerator at 4℃ until needed.

Chemicals

Carbon dioxide (purity 99.9%) was supplied by Zhejiang Gas Company, China. Methanol and acetonitrile of HPLC grade were purchased from Tianjin Shield Company, China. Folin-Ciocalteu's phenol reagent, gallic acid, rutin, 3-(2-Pyridyl)-5,6-diphenrl-1,2,4-triazine-4',4"-disulfonic acid sodium salt (Ferrozine), 2,2-Diphenyl-1-pikryl-hydrazyl (DPPH), and all the flavonoid standards were purchased from Sigma-Aldrich, Germany. All other chemicals were analytical grade and purchased from Sinopharm Chemical Reagent Co. Ltd., China.

SC-CO_2 extraction

SC-CO_2 extraction was performed on a supercritical fluid extractor Spe-ed^{TM} SFE-2 (Applied Separation, USA). 20 g of *A. grossedentata* leaves was packed into a 50 ml extraction vessel filled with defatted cotton in both ends. The flow rate of CO_2 and modifier were maintained at 2 L/min and 0.5 ml/min, respectively. Liquid CO_2 and modifier were pumped into the extraction vessel after desired temperature was achieved.

In this study, a four-factor, three-level orthogonal array design (OAD) was chosen for optimization of SC-CO_2 extraction of *A. grossedentata* leaves. As shown in Table 1, extractions were performed at three different pressure (150, 200 and 250 bar), three different temperature (40, 50 and 60℃), three different dynamic time (40, 60 and 80 min) and three different concentration of ethanol as modifier (95, 85 and 75%).

The extracts were collected in a glass vial at room temperature and atmospheric pressure. The modifier was removed completely by a vacuum rotary evaporator (R205B, Shensheng Co. Ltd., China) at 40℃ (water-bath temperature). The dry extracts were adjusted to 50 ml with absolute ethanol as samples for further analysis.

Determination of total flavonoids

Total flavonoids were measured by a colorimetric assay of Kim et al. (2003). 0.5 ml of sample was added to a 10 ml volumetric flask containing 5 ml absolute ethanol. Then 0.3 ml of 5% (w/v) $NaNO_2$ was added to the flask. After 5 min, 0.3 ml of 10% (w/v) Al $(NO_3)_3$ was added.

At 6 min, 4 ml of NaOH (1 M) was added to the mixture and adjusted to 10 ml with absolute ethanol. The mixture was thoroughly mixed and the absorbance was measured at 510 nm (HP 8453 UV-Vis spectrophotometer, Hewlett Packard, USA). A calibration curve was obtained with rutin. The total flavonoid content (TFC) of extracts was expressed as rutin equivalents (mg RE/g dry material).

Determination of total phenolics

Total phenolics were determined using the Folin-Ciocalteu method by Ranilla et al. (2010) with a slight modification. Briefly, 1 ml of sample was transferred into a 10 ml volumetric flask and mixed with 6 ml of distilled water. To each sample, 0.5 ml of 50% (v/v)

Folin-Ciocalteu reagent was added and mixed. After 5 min, 1 ml of 5% (w/v) Na_2CO_3 was added to the mixture and adjusted to 10 ml with distilled water. After standing for 60 min at room temperature, the absorbance was measured at 760 nm. Gallic acid was used for constructing the standard curve. The total phenolic content (TPC) of extracts was expressed as gallic acid equivalents (mg GAE/g dry material).

Determination of antioxidant activities

DPPH assay

The DPPH free radical scavenging activity of the extracts was determined according to the method of Yang et al. (2009) and Thoo et al. (2010), with a slight modification. 200 µl of sample was added to 7.8 ml of ethanolic DPPH solution (60 µM). After vortexing the reaction mixture for 1 min, the tubes were kept in dark for 30 min and the absorbance (A_1) was measured at 517 nm. A control containing the same amount of absolute ethanol and DPPH radical was prepared and measured at the same wavelength (A_0). The DPPH radical scavenging effect (%) was calculated with the following equation:

DPPH scavenging effect (%) = $(1 – A_1 / A_0) \times 100$.

FIC assay

The chelating effect of the extracts on ferrous ion was assayed according to Yuan et al. (2008) with a few modifications. 1 ml of the sample was mixed with 1 ml of $FeSO_4$ (0.1 mM) for 30 s, then 1 ml ferrozine (0.25 mM) was added and the mixture was kept for 10 min at room temperature. The absorbance of the mixture was determined at 562 nm (A_1). A reagent control was measured by the same way (A_0) and the ability of sample for the ferrous ion was calculated with the following equation:

Chelating effect (%) = $(1 – A_1 / A_0) \times 100$.

High performance liquid chromatography (HPLC) analysis

The main flavonoid components of the extracts were analyzed using a high performance liquid chromatography (HPLC) method. Flavonoid standards including dihydromyricetin, myricetin, vitexin, quercitrin, luteolin, quercetin, apigenin, kaempferol and rutin, were prepared at 1 mg/ml in absolute ethanol. The HPLC analysis was performed with a Shimadzu SPD-20A ultraviolet detector, a SIL-20AC automatic sample injector and equipped with an Agilent TC-C18 reversed-phase column (4.6 × 150 mm × 5 µm). The temperature of the column during analysis was maintained at 35°C. The mobile phase consists of solvent A (3% acetonitrile + 0.5% acetic acid) and solvent B (50% acetonitrile + 0.5% acetic acid) with the elution profile as follows: 0 to 24 min, 27.5 to 45% B (linear gradient, v/v); 24 to 29 min, 40 to 80% B (linear gradient); 29 to 37 min, 80% B; 37 to 45 min, 27.5% B (equilibration). The flow rate was kept constant at 1.0 ml/min and the peaks were identified using UV absorbance at 360 nm. The injection volume was 10 µl each time.

Statistical analysis

All the experiments were carried out in triplicate. The results were expressed as means ± SD and evaluated by analysis of variance (ANOVA) followed by Turkey's studentized range test carried out on the SAS system for windows (Version 9.1, SAS Institute Inc., USA).

RESULTS AND DISCUSSION

Optimization of SC-CO_2 extraction

An orthogonal array design, L_9 (3^4), was performed to optimize pressure, temperature, dynamic time and modifier concentration at a CO_2 flow rate of 2 L/min and a modifier flow rate of 0.5 ml/min. Table 2 shows that the yields of total flavonoids and phenolics under experimental conditions were 1.46 to 5.99 mg RE/g dry material and 0.32 to 3.26 mg GAE/g dry material, respectively. Table 3 presents a further orthogonal analysis with the calculation of the K, k and R values. From the R values, it can be observed that modifier had a dominant effect on the yield of total flavonoids, followed by temperature, pressure and dynamic time. Meanwhile, the influence on the yield of total phenolics decreased in the following order: modifier concentration > temperature > dynamic time > pressure. As the ANOVA results showed in Table 4, all the parameters under experiments had significant effect on the yield of total phenolics ($p < 0.05$), and three of them, modifier concentration, temperature and pressure, showed significant effect on the yield of total flavonoids as well ($p < 0.05$). Moreover, the optimum conditions for SC-CO_2 extraction of flavonoids and phenolics by SC-CO_2 were both at 200 bar (A_2), 50°C (B_2), 80 min (C_3) and 95% modifier (D_1) based on the results of Table 3.

Effect of various pressures

Pressure had a significant (p <0.05) effect on the extraction of flavonoids and phenolics according to Table 4. The effect of various pressures, 150, 200 and 250 bar, on the yields of total flavonoids and phenolics is presented in Figure 1A. It can be observed that the yield of total flavonoids increased noticeably as pressure varied from 150 to 200 bar, while the yield of total phenolics increased slightly. However, the yields of total flavonoids and phenolics both decreased significantly with the increasing pressure when pressure was above 200 bar. A similar phenomenon was also observed on the SC-CO_2 extraction of bioactive flavonoid compounds from *Strobilanthes crispus* (Bimakr et al., 2010) and spearmint leaves (Liza et al., 2010). The increasing pressure will cause the increase of the density of SC-CO_2, leading to a shorter distance between the molecules. Therefore, the interaction between the analytes and CO_2 increased with a greater solubility of the analytes in CO_2 (Liza et al., 2010). In this study, the yields of total flavonoids and phenolics increased with increasing pressure in certain range. When the pressure was over the up-limit, decreasing yields of flavonoids and phenolics was observed. The volatility and polarity of extracted analytes might be responsible for the result (Wang et al., 2008). The different extents of enhancement on the yield of total flavonoids and phenolics from 150 to 200 bar can

Table 2. Results obtained at the experimental condition using L_9 (3^4) orthogonal array design.

Trial	Pressure (A)	Temperature (B)	Dynamic time (C)	Modifier concentration (D)	Total flavonoid yield [a] (mg RE/g dry material)	Total phenolic yield [a] (mg GAE/g dry material)
1	1	1	1	1	2.17 ± 0.41^{cd}	2.01 ± 0.41^{b}
2	1	2	2	2	2.66 ± 0.57^{cd}	1.05 ± 0.04^{cd}
3	1	3	3	3	1.46 ± 0.44^{d}	0.34 ± 0.07^{e}
4	2	1	2	3	1.77 ± 0.15^{cd}	0.32 ± 0.01^{e}
5	2	2	3	1	5.99 ± 2.02^{a}	3.26 ± 0.61^{a}
6	2	3	1	2	2.12 ± 0.20^{cd}	0.36 ± 0.03^{e}
7	3	1	3	2	1.61 ± 0.02^{cd}	0.69 ± 0.25^{de}
8	3	2	1	3	2.76 ± 0.46^{c}	0.44 ± 0.04^{e}
9	3	3	2	1	4.63 ± 0.27^{b}	1.44 ± 0.43^{c}

[a] Values followed by different letters are different ($p < 0.05$) from one another.

Table 3. Analysis of L_9 (3^4) orthogonal array design results.

Parameter	Total flavonoids (mg RE/g dry material)				Total phenolics (mg GAE/g dry material)			
	Pressure (A)	Temperature (B)	Dynamic time (C)	Modifier concentration (D)	Pressure (A)	Temperature (B)	Dynamic time (C)	Modifier concentration (D)
K_1 [a]	6.30	5.53	7.06	12.80	3.40	3.02	2.82	6.71
K_2 [a]	9.86	11.42	9.04	6.39	3.94	4.76	2.81	2.10
K_3 [a]	9.00	8.20	9.06	5.97	2.57	2.15	4.29	1.11
k_1 [b]	2.10	1.84	2.35	4.27	1.13	1.01	0.94	2.24
k_2 [b]	3.29	3.81	3.01	2.13	1.31	1.59	0.94	0.70
k_3 [b]	3.00	2.74	3.02	1.99	0.86	0.72	1.43	0.37
R [c]	1.19	1.97	0.67	2.28	0.46	0.87	0.49	1.87
Optimal level	A_2	B_2	C_3	D_1	A_2	B_2	C_3	D_1

[a] K_i^A = Σ the yield of total flavonoids or total phenolics at A_i. [b] $k_i^A = K_i^A / 3$. [c] R_i^A = max { k_i^A } – min{ k_i^A }.

probably be explained by the different polarities of the main components of flavonoids and phenolics.

Effect of various temperature

Temperature was also appraised as a significant ($p < 0.01$) factor for the extraction of the flavonoids and phenolics, based on ANOVA results in Table 4. Figure 1B shows the effect of temperature on the yield of total flavonoids andphenolics. The yield of total flavonoids and phenolics increased as the temperature increased from 40 to 50°C. However, increasing temperature from 50 to 60°C caused decreasing yields of total flavonoids and phenolics. It is well-known that temperature has a dual effect on the extraction of total flavonoids and phenolics by SC-CO_2. Generally, higher temperature could accelerate mass transfer and improve the extraction yield (Wang et al., 2008). However, increasing

Table 4. ANOVA analysis of four parameters for SC-CO_2.

Source	Sum of squares	DF	Mean square	F-Value	*p*-Value
Total flavonoid yield					
Corrected model [a]	56.269	8	7.034	12.360	0.000
Pressure	6.900	2	3.450	6.063	0.010
Temperature	17.438	2	8.719	15.322	0.000
Dynamic time	2.639	2	1.320	2.319	0.127
Modifier concentration	29.291	2	14.645	25.736	0.000
Total phenolic yield					
Corrected model [b]	23.815	8	2.977	33.586	0.000
Pressure	0. 957	2	0.479	5.400	0.015
Temperature	3.538	2	1.769	19.961	0.000
Dynamic time	1.455	2	0.727	8.206	0.003
Modifier concentration	17.865	2	8.932	100.775	0.000

[a] R^2 = 0.846 (Adjusted R^2 = 0.778). [b] R^2 = 0.937 (Adjusted R^2 = 0.909).

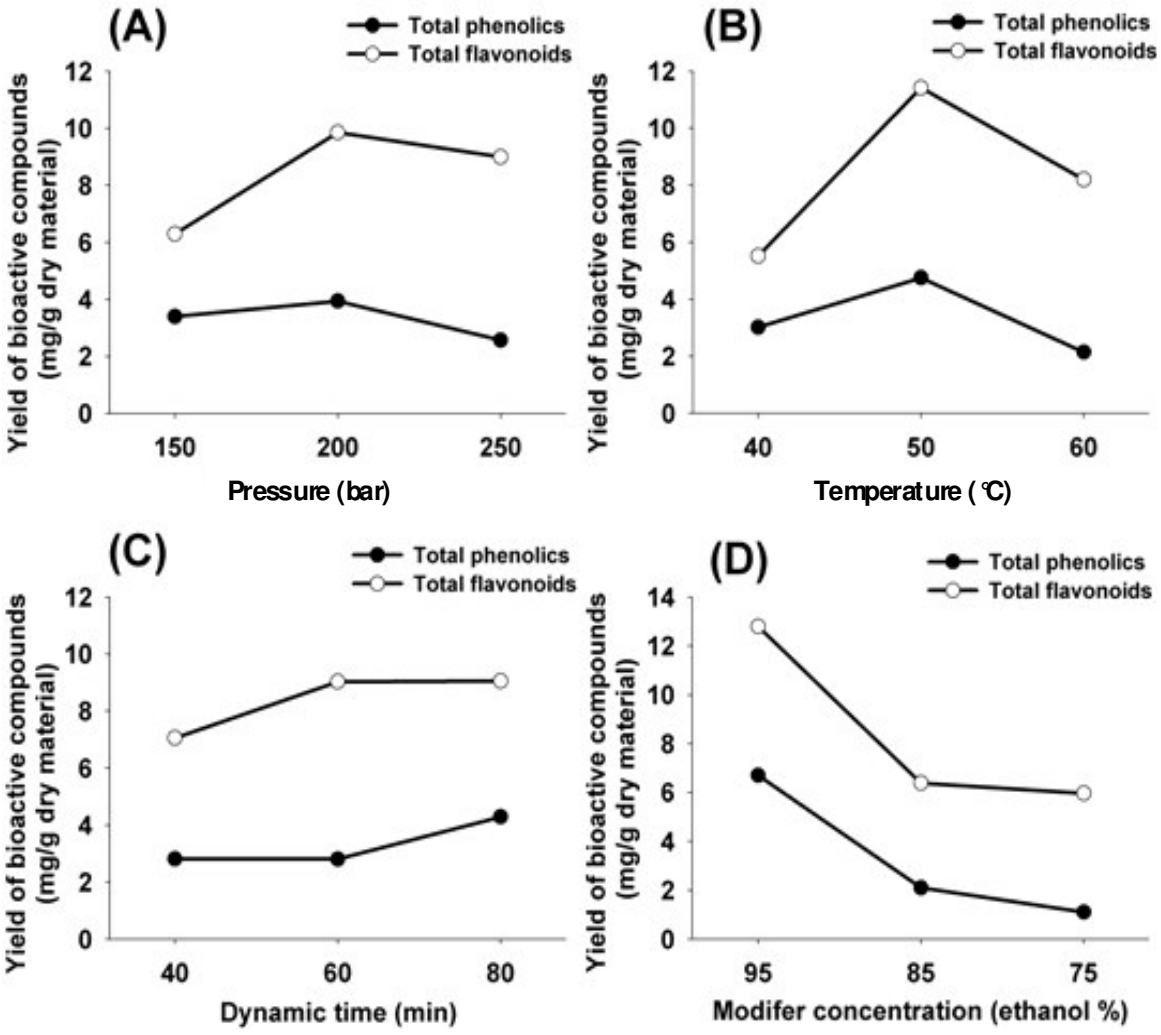

Figure 1. Effect of pressure, temperature, dynamic time and modifier on the yields of total flavonoids and phenolics.

temperature would reduce the solvent density and decrease the yield at pressures in the critical range.

Effect of various dynamic time

Figure 1C shows the effect of various dynamic time (40, 60 and 80 min) on the extraction yields of total flavonoids and phenolics. In this study, the yields of total flavonoids and phenolics were both enhanced by increasing dynamic time within a certain range. However, it was noteworthy that a further increase in the dynamic time (from 60 to 80 min) improved the yield of total flavonoids slightly, while a significant growth in the yield of total phenolics occurred as time increased from 60 to 80 min. Furthermore, dynamic time had no significant effect on the yield of total flavonoids with a *p*-value of 0.127 (Table 4), which is in agreement with the results of a previous research on SC-CO_2 extraction of rose geranium oil (Gomes et al., 2007).

Effect of various modifiers

Modifier was identified as a significant factor ($p < 0.01$) on the yields of total flavonoids and phenolics based on ANOVA results in Table 4. Various concentrations of ethanol (95, 85 and 75%) were selected as modifiers to evaluate the effect on the yields of total flavonoids and phenolics. The modifier of 95% ethanol gave the highest yields of total flavonoids and phenolics, followed by 85 and 75% ethanol (Figure 1D), which suggested that higher concentration of ethanol would result in higher recovery of flavonoids and phenolics, whereas lower concentration of ethanol limited the solubility of flavonoids and phenolics in CO_2, leading to declined extraction yields (Kong et al., 2009).

Antioxidant activity

DPPH radical scavenging activity

DPPH is a useful reagent for investigating the free radical scavenging activities of compounds. Antioxidants transfer either electrons or hydrogen atoms to DPPH and thus neutralize free radical (Yuan et al., 2008). The DPPH radical scavenging activities of the SC-CO_2 extracts from *A. grossdentata* leaves under different experiment conditions are shown in Figure 2A. It can be observed that the DPPH radical scavenging effect of the extracts ranged from 29.66 to 95.95%. The extract 5 (according to OAD) exhibited the strongest DPPH radical scavenging ability, followed by the extract 1 (95.22%) and the extract 9 (88.88%). These results may be explained by these extracts enriched with flavonoid and phenolic compounds which always play an important role in the antioxidant capacity of the extracts (Pourmorad et al., 2006).

Ferrous ion chelating activity

Metal chelating activity is claimed as one of antioxidant mechanisms, since it reduces the concentration of the catalyzing transition metal in lipid peroxidation. Among the transition metals, iron is known as the most important lipid oxidation pro-oxidant due to its high reactivity. Hence, the high ferrous ion chelating ability of the extracts would be somewhat beneficial. As presented in Figure 2B, the extract 2 (75.11%) possessed the notable ferrous ion chelating abilities, followed by the extract 3 (47.72%) and the extract 8 (38.96%). However, it was unexpectable to observe that the extracts with relatively high ferrous ion chelating activity were not abundant either in flavonoids or phenolics. Generally, flavonoids and phenolics are known to act as antioxidants, both as radical scavengers and as metal chelators (Chu et al., 2000). By contrast, extracts 5, 9 and 1 enriched with bioactive compounds, showed the weakest ferrous ion chelating ability. These results may be explained by the presence of some other substance possessing remarkable ferrous ion chelating ability, which may be more easily extracted under the condition of lower concentrations of ethanol.

Person correlation analysis

Pearson correlation analysis was used to gain a better understanding of the relationship between different antioxidant responses and the contents of total flavonoids and phenolics of the extracts. As summarized in Table 5, a significant ($p < 0.01$) positive correlation (0.876) between TPC and DPPH indicated that TPC might be responsible for DPPH radical scavenging activity, which corresponded to the report by Thoo et al. (2010). Besides, TPC may mostly contribute to bioactive phenolic compounds of low-molecular weight, as the DPPH is known to react preferentially with low-molecular weight phenolic compounds (Paixão et al., 2007). Meanwhile, TFC was also highly related with DPPH, the coefficient between them was 0.626 with a significant difference ($p < 0.01$). However, weakly negative correlations with insignificant difference ($p > 0.05$) were observed between TFC and FIC, and TPC and FIC, which indicated that TPC and TFC did not have obvious influences on the ferrous ion chelating abilities of the extracts. A negatively significant ($p < 0.01$) correlation (-0.543) was found between DPPH and FIC, which may be explained by the different reaction mechanisms of the two antioxidant methods.

Quantification of the main flavonoids

Dihydromyricetin, myricetin and several other common

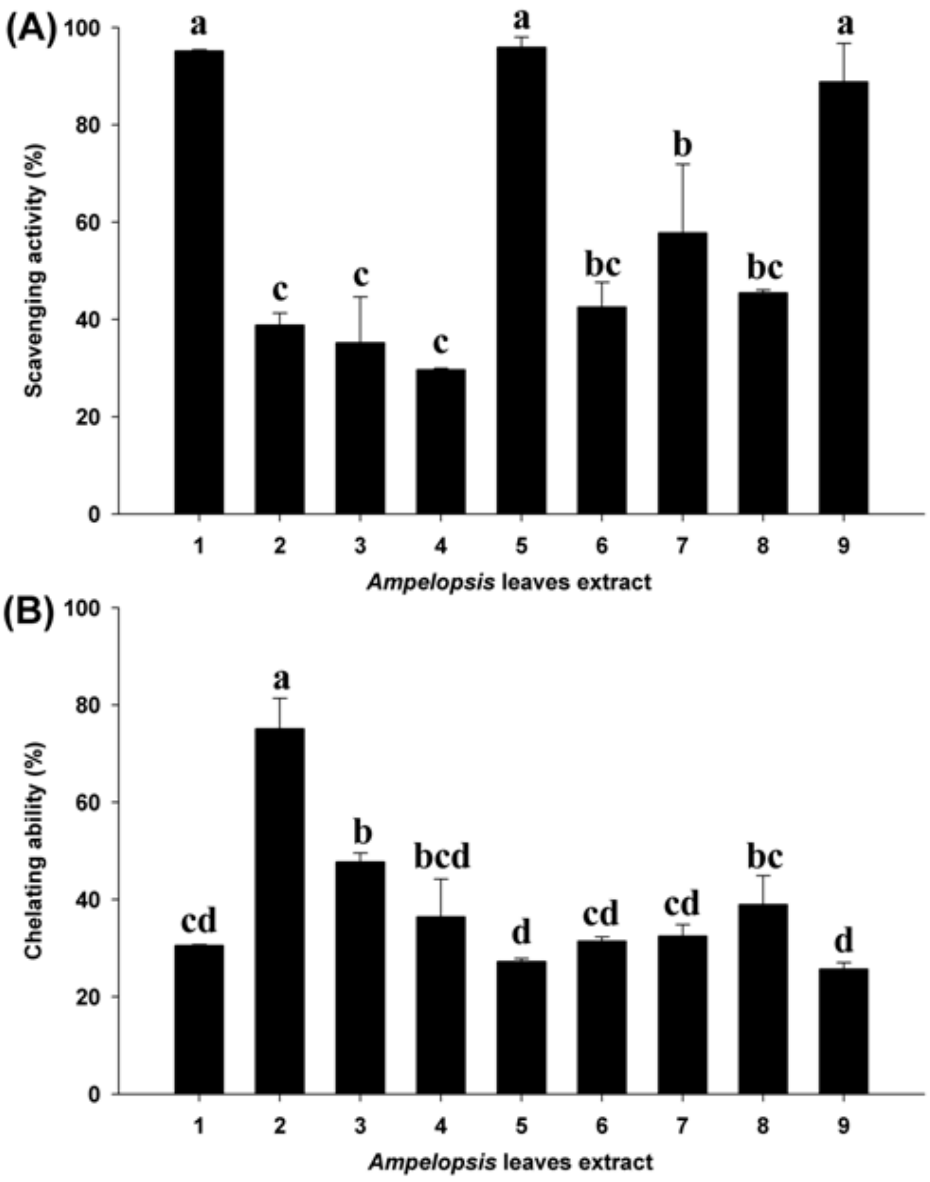

Figure 2. Antioxidant activities of *Ampelopsis grossedentata* leaves extracts (1-9 according to OAD) by the DPPH radical scavenging assay (A) and ferrous ion chelating assay (B).

flavonoids, including vitexin, quercitrin, luteolin, quercetin, apigenin, kaempferol and rutin, were analyzed by HPLC in order to determine the yields of main flavonoids by SC-CO_2 extraction from *A. grossdentata.* The yields of the individual flavonoid are shown in Table 6. As seen from the results, all the flavonoids, including dihydromyricetin, myricetin,vitexin, quercitrin, luteolin, quercetin, apigenin, kaempferol and rutin, were detected under the optimum conditions (the extract 5), and the extract 5 also possessed the highest yield of dihydromyricetin (1948.13 ± 2.98 µg/g dry material), which was considered as the characteristic flavonoid in *A. grossdentata*. Although, dihydromyricetin, myricetin and two flavonoid glycosides have been reported in *A. grossdentata* previously (Du et al., 2004; Zhang et al., 2007), its flavonoid profile has not been illustrated clearly yet. Our results demonstrated that vitexin, quercitrin, luteolin, quercetin, apigenin, kaempferol and rutin also existed in *A. grossdentata* leaves. However, the total amount of individual flavonoid was much lower than that of total flavonoids measured by colorimetric assay. This could be explained, at least partly, by that performance of colorimetric assay that tend to be more easily interfered with than that of HPLC, but it still strongly suggest that some other unidentified

Table 5. Correlation values between total flavonoid content (TFC), total phenolic content (TPC), DPPH radical scavenging activity (DPPH) and ferrous ion chelating effect (FIC).

Parameter	TFC	TPC	FIC	DPPH
TFC	1.000	0.756 **	-0.264	0.626**
TPC		1.000	-0.285	0.876**
FIC			1.000	-0.543**

**Correlation is significant at $p < 0.01$.

Table 6. Quantification of the main flavonoids from *Ampelopsis grossdentata* leaves (µg/g dry material)[a].

	Dihydromyricetin	Myricetin	Vitexin	Quercitrin	Luteolin	Quercetin	Apigenin	Kaempferol	Rutin	Total yield
1[b]	1165.28 ± 20.21^{c}	25.09 ± 2.44^{b}	19.88 ± 2.74^{b}	6.75 ± 1.63^{b}	1.05 ± 0.12^{bc}	0.14 ± 0.01^{bcd}	0.66 ± 0.06^{de}	1.58 ± 0.01^{d}	4.23 ± 0.29^{b}	1224.66 ± 13.50^{b}
2	14.41 ± 0.61^{gf}	-	-	-	0.43 ± 0.01^{de}	0.09 ± 0.02^{d}	0.60 ± 0.06^{de}	1.83 ± 0.12^{d}	2.19 ± 0.17^{c}	19.55 ± 0.62^{d}
3	-	0.76 ± 0.10^{c}	-	-	0.48 ± 0.01^{cde}	0.20 ± 0.02^{ab}	0.45 ± 0.05^{e}	2.76 ± 0.04^{a}	-	4.64 ± 0.08^{e}
4	8.65 ± 0.42^{g}	0.66 ± 0.11^{c}	-	-	0.78 ± 0.01^{cde}	0.16 ± 0.01^{abc}	0.86 ± 0.08^{cd}	2.37 ± 0.01^{bc}	2.56 ± 0.13^{c}	16.04 ± 0.52^{c}
5	1948.13 ± 2.98^{a}	72.91 ± 12.45^{a}	38.33 ± 4.20^{a}	23.90 ± 5.51^{a}	4.53 ± 0.42^{a}	0.22 ± 0.01^{a}	1.46 ± 0.04^{ab}	2.41 ± 0.01^{bc}	10.53 ± 0.03^{a}	2102.42 ± 5.29^{a}
6	94.40 ± 1.18^{e}	0.75 ± 0.08^{c}	0.80 ± 0.12^{c}	-	0.84 ± 0.03^{cd}	0.21 ± 0.04^{a}	1.14 ± 0.03^{bc}	2.37 ± 0.11^{bc}	2.63 ± 0.16^{c}	103.13 ± 0.85^{bc}
7	144.95 ± 2.19^{d}	0.38 ± 0.05^{c}	4.36 ± 0.82^{c}	-	0.38 ± 0.02^{de}	0.09 ± 0.00^{cd}	1.52 ± 0.02^{a}	2.45 ± 0.06^{b}	-	154.14 ± 3.12^{d}
8	39.64 ± 1.90^{f}	-	2.30 ± 0.26^{c}	-	0.21 ± 0.01^{e}	-	0.83 ± 0.18^{cd}	1.70 ± 0.03^{d}	-	44.68 ± 1.43^{f}
9	1349.50 ± 6.36^{b}	6.35 ± 1.88^{c}	6.55 ± 0.23^{c}	3.85 ± 1.06^{b}	1.61 ± 0.17^{b}	0.21 ± 0.01^{ab}	0.78 ± 0.09^{d}	2.18 ± 0.08^{c}	-	1371.03 ± 5.14^{ef}

[a] Values in the same column followed by different letters are significantly different ($p < 0.05$). [b] *Ampelopsis* leaves extracts (1-9 according to OAD).

flavonoids have been extracted by SC-CO_2, and probably played an important role in the antioxidant activity of the extracts. Thus,isolation and characterization of these unidentified flavonoids should be carried out in further study.

Conclusions

Based on the yields of total flavonoids and phenolics, the optimal condition for extraction of bioactive compounds from *A. grossedentata* leaves was found to be 200 bar, 50°C, 80 min and with a modifier of 95% ethanol. An insignificant negative correlation ($p > 0.05$) was observed between FIC and total flavonoid contents (TFC) or total phenolic contents (TPC) by Person correlation analysis. Moreover, several unreported flavonoids such as: Luteolin and vitexin, have been detected in the extracts. Based on the results, we conclude that SC-CO_2 could be a promising alternative for efficient extraction of bioactive compounds from *A. grossdentata*. Meantime, the extracts obtained by SC-CO_2 have effective antioxidant capacity and could act as different kinds of natural antioxidant agents and a wait a major breakthrough for the applications in the food and pharmaceutical industry.

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