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Full Length Research Paper

Comparative studies on the chemical composition and antioxidant activities of *Schisandra chinensis* and *Schisandra sphenanthera* fruits

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The chemical composition and antioxidant activity of the extracts and fractions from *Schisandra chinensis* and *Schisandra sphenanthera* fruits were comparatively studied here. Obvious differences were found in the yields and compositions of the essential oils, metal elements, polysaccharides, ethanol extracts and fractions of the two species. Total phenolic content and antioxidant capacity were different among the samples. Good correlation between antioxidant capacity and the content of phenolic and flavonols were found, which were R^2 = 0.8474 and R^2 = 0.7036, respectively. Various extracts and fractions provided different antioxidants, which demonstrated strong activities within the ethyl acetate fractions. These fractions might be valuable antioxidant natural sources and seemed to be applicable in both healthy medicine and food industry.

Key words: *Schisandra chinensis, Schisandra sphenanthera*, composition, antioxidant activity.

INTRODUCTION

Schisandra chinensis (*S. chinensis,* Bei Wu-wei-zi in Chinese) is the dried fruit of *Schisandra chinensis* (Turcz.) Baill. and *Schisandra sphenanthera* (*S. sphenanthera,* Nan Wu-wei-zi in Chinese) is the dried fruit of *Schisandra sphenanthera* Rehd. et Wils. Both plants belong to Schisandrae, Magnoliacene. *S. chinensis* (Turcz.) Baill. is distributed in north-eastern China and *S. sphenanthera* Rehd. et Wils grows in the most western and southern parts of China. *S. chinensis* and *S. sphenanthera* are used as superior traditional drugs and functional foods for thousands of years in China. These two fruits have some similar functions in treating chronic cough and dyspnea, nocturnal emission, spermatorrhea, enuresis, frequent urination, protracted diarrhea, spontaneous sweating, night sweating, impairment of body fluids with thirst, shortness of breath and feeble pulse, diabetes and wasting-thirst (Lu and Chen, 2009). Fruits, *S. chinensis* and *S. sphenanthera* mainly contain volatile oil, organic acid, vitamin, lignin, sesquiterpene, triterpenoid, amino acid, polysaccharide etc (Panossian and Wikman, 2008). The dried ripe fruits of both *S. chinensis* and *S. sphenanthera* have long been used as Wuweizi. Only in recent ten years, *S. chinensis* and *S. sphenanthera* had been distinguished in the Chinese Pharmacopoeia. Chemical constituents and contents of the bioactive components are quite different in *S. chinensis* and *S. sphenanthera* according to some studies (Lu and Chen, 2009). Much attention had been paid to the quality control and constituent analysis of Wuweizi in recent years. Gao (2003) established a TLC method to easily distinguish the fruits of *S. chinensis* and *S. sphenanthera*, using schizandrin, gomisin A, schisantherin A, deoxyschizandrin, schizandrin B and schizandrin C as references. Zhu et al. (2007) determined the contents of schizandrin, schisantherin A,

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deoxyschizandrin, and schizandrin in the fruits of *S. chinensis* and *S. sphenanthera* with HPLC. However, the qualitative and quantitative techniques were commonly established with the essential oils and lignans. There is no available information relating to the comparative analysis on the other bioactive constituents including polysaccharides, mineral elements, polyphenols and antioxidant activities between the two fruits of *S. chinensis* and *S. sphenanthera.* In this study, the composition of the two types of polysaccharides, the components of the two type of essential oils, the contents of the phenols and the antioxidant activities of the extracts, the mineral element composition of the two fruits of *S. chinensis* and *S. sphenanthera* were comparatively studied, which will be of benefit for the application of the two fruits of *S. chinensis* and *S. sphenanthera.*

The aim of the present study is to analyze the chemical components including the essential oils, mineral elements, polysaccharides and polyphenols of S. *chinensis* and *S. sphenanthera* with gas chromatography-mass spectrometry (GC-MS), inductive coupled plasma optical emission spectrometer (ICP-OES), and phytochemical methods. The antioxidant and radical-scavenging activities of the essential oil, ethanol extract, polysaccharide, petroleum fraction, ethyl acetate fraction, and *n*-BuOH fraction of ethanol extract of the two species were also evaluated.

MATERIALS AND METHODS

Plant material

S. Chinensis and *S. sphenanthera* were purchased from Chinese medicinal herbs shop in Tianjin, and their producing areas were Liaoning and Guangxi. These two species were identified by Doctor Haixia Chen, who works in Pharmacognosy Department of School of Pharmaceutical Science and Technology, Tianjin University. Moisture content of the drugs was determined before the experiment and the result was 5.46% (w/w) in *S. chinensis* and 10.07% (w/w) in *S. sphenanthera.*

Essential oil extraction and GC-MS analysis

Volatile oil was extracted from *S. Chinensis* and *S. sphenanthera* with the method of steam distillation according to Oke et al. (2009). Volatile oil determination apparatus was used to isolate the sample for 5 h. The oils isolated were dried for 2 days with anhydrous sodium sulfate which were dehydrated in the condition of 105ºC for 2 days. The essential oils were kept at 4ºC until analysis.

The chemical composition of the essential oil was analyzed using GC-MS technique. The mass spectrometer was Agilent 6890 N GC/5975MSD-SCAN (Agilent Technologies, Palo Alto, CA, USA) in the electron impact (EI) ionisation mode (70 eV) and HP- 5 MS (bonded and cross-linked 5% phenyl-methylpolysiloxane, 30 m × 0.25 mm, coating thickness 0.25 µm) capillary column (Restek, Bellefonte, PA). Injector and detector temperatures were set at 230ºC. The oven temperature was held at 50ºC for 30 min, then programmed to 240ºC at rate of 5ºC /min. Helium (99.99%) was the carrier gas at a flow rate of 1 ml/min. Diluted samples (1/100 in hexane, v/v) of 1.0 µl were injected manually. The identification of the components was based on the comparison of their mass spectra with Nist 2005a libraries and as well as by comparison of their retention times.

Analysis of mineral elements

The wet-ashing method was employed for the digestion of the dried fruit of S. *chinensis* and S. *sphenanthera* according to our previous study (Chen et al., 2007). 0.5000 g of the samples was digested with 10 ml of concentrated HNO_3 and perchloric acid (1:1 v/v) and thereafter transferred to a 25 ml volumetric flask. It was diluted to volume with deionized water and stored in a clean polyethylene bottle. The mineral element content was determined by an inductively coupled plasma optical emission spectrometer (ICP-OES). Elements were determined using an ICP-OES **(**Varian, VISTA-MPX, USA) equipped with an axial torch, Scott-type spray chamber, and cross-flow nebulizer with gem tips. The plasma conditions are as follows. RF power 1000 W, Nebulizer flow 0.5 L/min, auxiliary flow 1.0 L/min, plasma flow 15 L/min, Sample flow 1.5 ml/min. An autosampler was used for the introduction of the solutions into the nebulizer. Standards, analytical blanks, and rinse blanks were matrix matched to the sample, so that all solutions contained 20% concentrated HNO_3 by volume.

Preparation of ethanol extracts and the fractions

One hundred of the dried and powered fruits of S. *chinensis* or *S. sphenanthera* were extracted by 1 L of ethanol at 80ºC for 3 h for 3 times. The extract was filtered and concentrated under vacuum at 50ºC by using a rotary evaporator (Heidolph, Laborota 4000, Schwabach, Germany), yielding a waxy material. The concentrated EtOH extract was suspended in H_2O and extracted by a series of solvents of increasing polarity including petroleum ether, ether ethyl acetate, *n*-butanol in sequence. Then four fractions of the two species were obtained and named petroleum ether fraction (PEF), ethyl acetate fraction (EAF), n-butanol fraction (BF) and water fraction (WF).

Extraction of polysaccharide and composition determination

Polysaccharides of S. *chinensis* and *S. sphenanthera* were extracted according to our previous reports (Chen et al., 2008)**.** Briefly, the residues after the ethanol extract of *S. chinensis* and *S. sphenanthera* were dried in air and then extracted with hot water (70°C) three times (1:20, w/v). The water extract was concentrated in a rotary evaporator under reduced pressure, precipitated by 95% (v/v) ethanol at 4°C for 24 h, and then centrifuged (3000 × *g*, 10 min). The precipitate was devolved in distilled water and the Sevag method was used to remove protein. Then the solution was precipitated by adding three times of volume of 95% (v/v) ethanol again. The precipitate was vacuum freeze-dried, and two kinds of polysaccharides were obtained from S. *chinensis* or *S. sphenanthera,* respectively.

The total sugar content of the polysaccharides was determined by phenol-sulfuric acid analysis using D-glucose as a standard (Dubois et al., 1956). The calibration curve was prepared using a standard solution of glucose from 5 to 200 mg/L (R^2 = 0.9949). Uronic acid content was determined by the carbazole-sulfuric acid method using galacturonic acid as a standard (Bradford, 1976). The standard solution of D-galacturonic acid from 8 to 80 µg/ml was used to prepare the calibration curve (R^2 = 0.999). Protein concentration was measured with the Bradford assay using bovine serum albumin as the standard (Bitter and Muir, 1962). The standard solution was bovine serum albumin from 0 to 100 µg/ml (R^2 = 0.9984).

Determination of total polyphenol content (TPC) and total flavonoid content (TFC)

Total polyphenol contents of the extracts and fractions were determined by the Folin-Ciocalteau method (Schwarz et al., 2009). The extract solution (0.25 ml) was mixed with 0.2 ml of 50% Folin-Ciocalteau reagent. The mixture was allowed to react for 5 min and 4.0 ml aqueous solution of 2% Na_2CO_3 was added. Then, the mixture was vortexed vigorously. At the end of incubation for 10 min at room temperature, absorbance of each mixture was measured at 750 nm. The same procedure was also applied to the standard solutions of gallic acid. The calibration curve was prepared with gallic acid solutions ranging from 0 to 72 mg/L (R^2 = 0.9992). Total phenol contents were expressed as 1 g gallic acid equivalents per mg of the extract.

Total flavonoid contents of the extracts and fractions were determined according to the colorimetric assay by the method which was developed by Alothman et al. (2009). The sample solution (6 ml) was mixed with 1 ml of 5% (w/v) $NaNO_2$. The mixture was allowed to react for 6 min and 1.0 ml of 10% (w/v) $AlCl_3$ was added. 6 min later, 10 ml of 4% (w/v) NaOH was added and distilled water was added to the final volume of 25 ml. The mixed solution was shaken vigorously and the absorbance of the mixture was measured at 510 nm. A calibration curve was prepared using a standard solution of catechin from 0 to 3.2 µg/ml (R^2 = 0.9986), and the results were also expressed on a fresh weight basis as mg catechin equivalents (CEQ)/1 g of sample.

DPPH free radical-scavenging assay

The radical scavenging activity of the extracts and fractions was determined following the method used by Bortolomeazzi et al. (2009). DPPH was dissolved in the ethanol at a final concentration of 1.2×10^{-4} M. Then add 2.9 ml DPPH solution to 0.1 ml sample solutions of different concentration. The mixed solution was allowed to stand for 30 min in the dark at 37°C for any reaction to take place. UV absorbencies of these solutions were recorded on a spectrometer (Rayleigh UV-9200, Beijing Rayleigh Analytical Instrument Corp.) at 517 nm using Vitamin C as control group and solution without extraction as blank group. Inhibition of free radical DPPH in percent (I%) was calculated as follow:

$$I\,\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

Reducing power assay

The ability of the extracts and fractions to reduce iron (III) was determined according to the method reported before (Ferreira et al., 2007). One hundred microlitre of each extracts or fractions at different concentrations were mixed with 0.7 ml of 0.2 M phosphate buffer (pH 6.6) and 2 ml potassium ferricyanide. The mixer was incubated at 50°C for 20 min, followed by the addition of 2 ml of 10% (w/v) trichloroacetic acid. The mixed solution was allowed to stand for 10 min and took 1 ml from the solution. Add 3.0 ml of 1.7×10^{-3} M ferric chloride to the prepared solution and stood for 10 min. After that reaction, the absorbance of the final solution was measured at 700 nm. With the increasing of the absorbance, the reducing power increases.

Inhibition of lipid peroxidation

Inhibition effects of the extracts and fractions on liposome peroxidation were performed as the method used by Zhang et al. (2009). Briefly, mice liver homogenate was suspended adequately in the 0.9 % (w/w) sodium chloride by the proportion of 1:30 (w/v). The mixed solution contained 0.1 ml of sample solution at different concentration, 0.25 ml of 1.0×10^{-4} M vitamin C, 0.25 ml of 1.0×10^{-5} M ferrous sulfate, 0.9 ml of 5.0×10^{-5} M phosphate buffer(pH 7.4), and 0.5 ml of liver homogenate. After shaking the mixed solution, Then the reaction was terminated by adding 1 ml of 20 % (w/v) trichloroacetic acid, and added 1.0 ml of 0.67% (w/v) TBA. Mix the solution and put it in the condition of 100°C for 15 min. After cooling the mixture was centrifuged at 3000 × g for 15 min. The supernatant absorbance was measured at 532 nm. The same procedure was repeated without the two drugs extractions as the control. Effect of inhibition of lipid peroxidation was calculated as follows:

$$I\,(\%) = (1-A_1/A_0)\times100$$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of extraction sample.

Statistical analysis

All the data were expressed as means ± standard deviation (SD) of three replications, and one factor ANOVA was used for the statistical analysis. The values were considered to be significantly different when $P<0.05$.

RESULTS AND DISCUSSION

GC-MS analysis of volatile oil

Steam by hydrodistillation, the dried fruits of *S. chinensis* yielded 0.68% (v/w) of essential oil and *S. sphenanthera* yielded 0.87% (v/w) of essential oil. Twenty-three compounds, representing 96.08% of the oil were identified for *S. chinensis* while twenty-five compounds, representing 94.14% of the oil were identified for *S. sphenanthera*. Quantitative and qualitative analytical results by GC-MS are shown in Table 1. There were great differences in the oil composition between *S. chinensis and S. sphenanthera*. Compounds 1,7-dimethyl-7-(4-methyl-3-pentenyl)-Tricyclo[2.2.1.0 (2,6)] heptanes (18.06%), 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-Naphthalene (15.58%), 2,4a,5,6,7,8-hexahydro-3,5,5,9-tetramethyl-1H-Benzocycloheptene (11.45%), 1-methyl-4-(1-methylethyl)-1,3-Cyclohexadiene (9.90%), (R)-1-methyl-4-(1,2,2-trimethylcyclopentyl)- Benzene (9.13%), were the major components of the oil of S. chinensis, while 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-Naphthalene (16.40%), 1,7-dimethyl-7-(4-methyl-3-pentenyl)-Tricyclo (2.2.1.0 (2,6)) heptanes (13.03%), 2,4a,5,6,7,8-hexahydro-3,5,5,9-tetramethyl--1H-Benzocycloheptene (11.42%), (R)- 1-methyl-4-(1,2,2-trimethylcyclopentyl)-Benzene (8.70%), (3R-trans)- 4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-Cyclohexene (8.48%) were the main components of the oil of *S. sphenanthera*. Though the composition of the major components was different, there were eleven compounds identified as the same between the components of the essential oils of *S. sphenanthera* and *S. chinensis*. Only three constituents (copaene, α-santalol and α-caryophyllene) of the thirty-nine compounds identified in *S. chinensis* previously were found in this

Table 1. Chemical composition of essential oil from the dried fruits of S. chinensis and S. sphenanthera.

No.	Retention time	Constituents	Percentage (%)	
			S. chinensis	*S. sphenanthera*
1	15.31	Copaene	1.34	0.95
2	15.99	Isoledene	0.77	0.62
3	16.44	(-)-1,7-dimethyl-7-(4-methyl-3-pentenyl)-tricyclo[2.2.1.0(2,6)]heptane	18.06	13.03
4	16.58	(1alpha, 4 alpha,8 alpha)-1,2,3,4,4α,5,6,8α-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-naphthalene	-	1.46
5	17.29	α-caryophyllene	0.83	0.68
6	17.44	2-isopropyl-5-methyl-9-methylene-bicyclo[4.4.0]dec-1-ene	2.84	2.32
7	17.78	1-methyl-4-(1-methylethyl)-1,3-cyclohexadiene	9.90	-
8	17.78	(3R-trans)-4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-cyclohexene	8.48	-
9	17.79	(-)-3,7,7-trimethyl-11-methylene-spiro[5.5]undec-2-ene	3.35	2.98
10	18.30	[s-(E,E)]-1-methyl-5-methylene-8-(1-methylethyl)-1,6-cyclodecadiene	2.31	-
11	18.45	2,4α,5,6,7,8-hexahydro-3,5,5,9-tetramethyl-1H-benzocycloheptene	11.45	11.42
12	18.53	7-(1-methylethylidene)-bicyclo[4.1.0]heptane	0.64	-
13	18.59	1-methyl-4-(1,2,2-trimethylcyclopentyl)-benzene	9.13	8.70
14	18.79	(1 alpha, 4 beta, 8 alpha)-1,2,3,4,4α,5,6,8α-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-naphthalene	15.58	-
15	18.80	1,2,4α,5,6,8α-hexahydro-4,7-dimethyl-1-(1-methylethyl)-naphthalene	-	18.33
16	18.98	(1S-cis)-1,2,3,5,6,8α-hexahydro-4,7-dimethyl-1-(1-methylethyl)-naphthalene	4.93	2.70
17	19.13	(E,E)-1,5-dimethyl-8-(1-methylethylidene)-1,5-cyclodecadiene	1.25	-
18	19.21	2,6,6,9-tetramethyl-tricyclo[5.4.0.0(2,8)]undec-9-ene	1.88	-
19	19.21	2,4α,5,6,7,8-hexahydro-3,5,5,9-tetramethyl-1H-benzocycloheptene	-	200
20	19.59	1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-cyclohexane	2.60	3.55
21	19.89	3,7,11-trimethyl-3-hydroxy-6,10-dodecadien-1-yl acetate	-	0.52
22	21.11	4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one	-	0.81
23	22.07	(1S-endo)-2-methyl-3-methylene-2-(4-methyl-3-pentenyl)-bicyclo[2.2.1]heptane	0.86	1.07
24	22.98	α-santalol	1.26	2.36

Table 1. Continued.

25	23.68	(2-methylpropyl)-pyrazine	-	0.48
26	24.10	cis-α-santalol	2.98	-
27	24.10	3-methylenecyclohexene	-	4.36
28	24.61	2,2-dimethyl-cyclopropanecarboxanilide	2.1	-
29	24.61	[2R-(2 alpha, 4 alpha,8 beta)]-1,2,3,4,4α,5,6,8α-octahydro-4α,8-dimethyl-2-(1-methylethenyl)-naphthalene	-	3.79
30	24.98	8-methylene-3-oxatricyclo[5.2.0.0(2,4)]nonane	0.5	
31	24.98	4,11,11-trimethyl-8-methylene-,[1R-(1R,4Z,9S)]-bicyclo[7.2.0]undec-4-ene	-	0.48
32	25.15	oxide-alloaromadendrene	1.5	-
33	25.15	1-methyl-8-(1-methylethyl)-tricyclo[4.4.0.0(2,7)]dec-3-ene-3-methanol	-	3.06
Total			96.08	94.14

Table 2. Mineral analysis of *S. chinensis* and *S. sphenanthera* by ICP-OES (μg/g).

Mineral	*S. chinensis* (μg/g)	*S. sphenanthera* (μg/g)
B	209.0	256.0
Ca	12000.0	12800.0
Co	1.1	0.7
Cr	24.9	20.3
Cu	11.5	10.5
Fe	983.0	712.0
Mg	1220.0	1330.0
Mn	644.0	596.0
Ni	14.3	14.4
Pb	1.0	1.2
Se	0	119.0
Sr	99.3	13.3
Zn	175.0	146.0

study (Zhu et al., 2008). In Zhu's study, ylangene, 2,6-dimethyl-bicycle [3.1.1]hept-2 ene, 3-methanlo, 1-methyl-8(1-methylethy1) -tricyclo (4.4.0.02, 7)dec-3 ene were determined as major components of the essential oil obtained from *S. chinensis*. This may possibly be due to the fact that fruits were grown in different regions, which may have caused the differences in their chemical composition. Li et al. determined copaene and α-farnesene as the major components of the oil belonging to *S. chinensis* collected from Liaoning, China (Li et al., 2001). The differences in chemical composition of essential oils might depend on the climatic, seasonal, and geographic conditions.

Mineral analysis of *S. chinensis* and *S. sphenanthera*

Thirteen elements including B, Ca, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Se, Sr, Zn were determined by ICP-OES. The mineral elements contents of *S. chinensis* and *S. sphenanthera* are listed in Table 2. There were differences detected in the contents of elements in *S. chinensis* and *S. sphenanthera*. The results showed that *S. sphenanthera* had a high level of Ca (12800.0 μg/g), followed by Mg (1330.0 μg/g), Fe (712.0 μg/g), Mn (596.0 μg/g), B (256.0 μg/g), Zn (146.0 μg/g) and Se (119.0 μg/g), while *S. chinensis* had a high level of Ca (12000.0 μg/g), followed by Fe (9830.0 μg/g), Mg (1220.0 μg/g),

Table 3. Total flavonoid and polyphenol content of the extracts and fractions of *S. chinensis* and *S. sphenanthera*.

Component	Total polyphenol content (mg/g)		Total flavonoid content (mg/g)	
	S. chinensis	***S. sphenanthera***	***S. chinensis***	***S. sphenanthera***
Essential oil (EO)	-	-	-	-
Ethanol extract (EE)	26.79±17.06	26.94±7.21	9.52±1.53 [a]	15.84±2.52 [b]
Petroleum ether fraction (PEF)	6.34±1.38	2.39±0.85	47.42±2.37	59.08±2.95
Ethyl acetate fraction (EAF)	102.65±6.02 [a]	89.28±5.03 [b]	157.05±4.45 [a]	148.30±6.07 [b]
n-Butanol fraction (BF)	1.37±1.11	1.94±2.07	10.01±0.50	9.04±0.45
Water fraction (WF)	-	-	-	4.47±2.08

Values represent means ± standard deviations for triplicate experiments. Values followed by the different letter in the same row are significantly different ($p < 0.05$).

Mn (644.0 µg/g), B (209.0 µg/g) and Zn (175.0 µg/g).

There was no Se element detected in *S. chinensis*. The results agreed with Li's study, in which trace elements including Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Ag, Cd, Pb in fruit, leaf and stem in *S. Chinensis* were determined (Li et al., 2010). In Li's study, it was found that the mayor elements were K, Ca, Mg, Fe, Na and the contents of the elements were deferent in different parts and different producing areas of *S. Chinensis*. The differences in mineral composition might be due to the different species, climatic, seasonal, and geographic conditions of *S. chinensis* and *S. sphenanthera*.

Compositional analysis

There were differences on the yields of essential oil (EO), ethanol extract (EE) and polysaccharide in dried fruits between *S. chinensis* and *S. sphenanthera,* which were 6.8 ml/kg, 198.2 mg/g, 36.2 mg/g for *S. chinensis* and 8.7 ml/kg, 271.8 mg/g, 46.7 mg/g for *S. sphenanthera*, respectively. The yields of the four fractions, petroleum ether fraction (PEF), ethyl acetate fraction (EAF), n-butanol fraction (BF) and water fraction (WF) were 159.5, 90.6, 200.5, 549.4 mg/kg for *S. chinensis* and 283.9, 70.0, 210.7, 435.4 mg/kg for *S. sphenanthera*, respectively. Both in the four fractions of *S. chinensis* and *S. sphenanthera,* WF was the most and EAF was the smallest fraction. The contents of uronic acid, neutral sugar and protein in the polysaccharides of *S. chinensis* and *S. sphenanthera* were 49.60, 37.52, 4.83 and 49.77, 40.69, 1.71%, respectively. The results were similar to Zhao's study, in which it was showed the content of neutral sugar in polysaccharide of *S. chinensis* was 44.46% (Zhao et al., 2008). In Gao's study, the content of uronic acid in the polysaccharide of *S. chinensis* was 2.59 to 2.96% (Gao, 2009). The distinctive difference of the the content of uronic acid may be due to the different methods of extraction and purification. There was no difference in the contents of uronic acid while there were obvious differences in the contents of neutral sugar and protein in the two kinds of polysaccharide of *S. chinensis* and *S. sphenanthera*.

The contents of total polyphenol and total flavonoid in the extracts and fractions of *S. chinensis* and *S. sphenanthera* are presented in Table 3. There was no polyphenol and flavonoid detected in EO extract and WF fraction of *S. chinensis* and *S. sphenanthera* except the total flavonoid content in WF fraction of *S. sphenanthera was* 4.47 mg/g. The PEF fraction and BF fraction were in low contents of total polyphenol and total flavonoid. The EAF fractions of *S. chinensis* and *S. sphenanthera* were rich of polyphenol and flavonoid, which were 102.65 and 89.28 and 157.05, 148.30 mg/g, respectively. There were distinctive differences in the contents of total polyphenol and total flavonoid in EAF fractions of *S. chinensis* and *S. sphenanthera*. The contents of flavonoid EAF fraction of *S. chinensis* was much higher than that of Han's study, which was 7.92 mg/g in *S. chinensis* ethanol extract (Han et al., 2007), it was suggested that EAF fraction of *S. chinensis* might be good source of polyphenols and flavonoids.

DPPH radical-scavenging activity

DPPH is a stable free radical which shows maximum absorbance at 517 nm. The absorbance reduces when DPPH radicals encounter a proton-donating substrate such as an antioxidant. The decrease in absorbance is taken as a measure for radical-scavenging (Bougatef et al., 2009). The IC_{50} of the extracts and fractions of *S. chinensis* and *S. sphenanthera* on DPPH radical-scavenging activity are summarized in Table 4. The IC_{50} values of the extracts and fractions showed various scavenging efficacy against DPPH free radicals, ranged from 0.032 to 0.703 mg/ml, In *S. chinensis* and *S. sphenanthera* fruits, the ethanol extract and the ethyl acetate fraction demonstrated the highest inhibition potency with IC_{50} values of 0.135 and 0.032 mg/ml for *S. chinensis* and 0.142 and 0.033 mg/ml for *S. sphenanthera*. In contrast, a general tendency was also observed that there were no scavenging activities

Table 4. DPPH radical-scavenging of extracts/fractions of *S. chinensis* and *S. sphenanthera.*

Extracts/fractions/standard antioxidants	IC_{50} (mg/ml)	
	S. chinensis	***S. sphenanthera***
Essential oil (EO)	-	-
Ethanol extract (EE)	0.135±0.003	0.142±0.002
Crude polysaccharide (CP)	0.361±0.023 [a]	0.324±0.013 [b]
Petroleum ether fraction (PEF)	0.254±0.011	0.251±0.008
Ethyl acetate fraction (EAF)	0.033±0.003	0.033±0.002
n-Butanol fraction (BF)	0.155±0.004 [a]	0.270±0.009 [b]
Water fraction (WF)	0.703±0.036	0.490±0.051
Vitamin C	0.005±0.003	

The IC_{50} value is expressed as mg/ml and represents the concentration of extracts or fractions that is required for 50% of free radicals inhibition (DPPH method). Lower IC_{50} value indicates the higher antioxidant activity. Values represent means ± standard deviations for triplicate experiments. Values followed by the different letter in the same row are significantly different ($p < 0.05$).

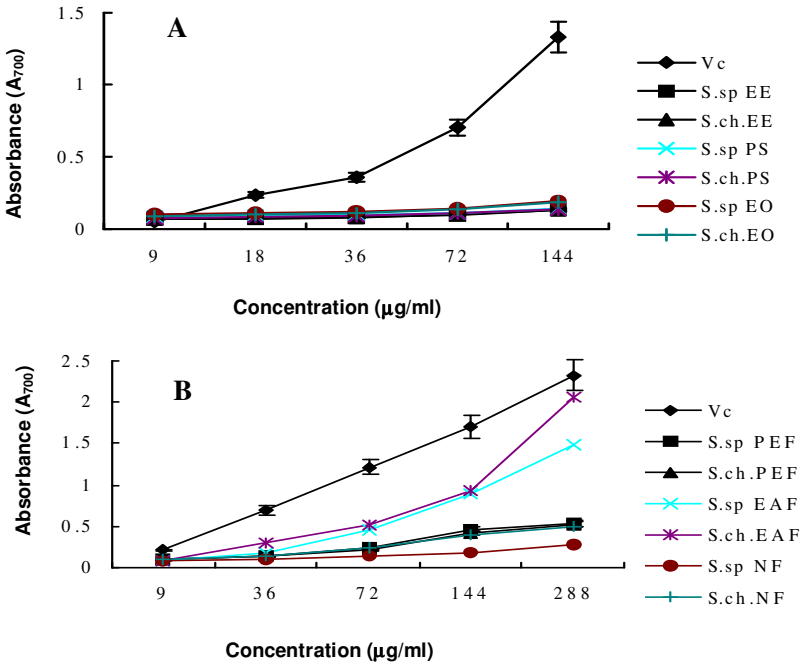

Figure 1. Reducing power of extracts (A) and fractions (B) from *S. chinensis* and *S.sphenanthera* at different concentrations. EO: Essential oil; EE: Ethanol extract; PS: Polysaccharide; PEF Petroleum ether fraction; EAF: Ethyl acetate fraction; BF: n-Butanol fraction; WF: Water fraction.

detected in the essential oils and the petroleum ether and water fractions had the lowest scavenging ability on the DPPH free radicals. These results indicated that the ethanol extract and ethyl acetate fraction of studied fruits contain antioxidant compounds with high potency in scavenging DPPH radicals and could be used as antioxidants in preservation of foods. The results were in agreement with Ma et al.'s study, in which it was showed that the ethanol extract of *S. Sphenanthera* had higher DPPH radical-scavenging activity than the water extract (Ma et al., 2010).

Ferric reducing/antioxidant power

The reducing power assay is often used to evaluate the ability of antioxidant to donate electron. Some studies reported that there was a direct correlation between reducing power and antioxidant activity (Bougatef et al., 2009). Figure 1 A and B shows the reducing power of

Table 5. Lipid peroxidation inhibition of extracts/fractions from *S. chinensis and S. sphenanthera* (mg/ml).

Extracts/fractions/standard antioxidants	IC_{50} (mg/ml)	
	S. chinensis	***S. sphenanthera***
Essential oil (EO)	0.041±0.001	0.036±0.002
Ethanol extract (EE)	0.033±0.004 [a]	0.025±0.004 [b]
Crude polysaccharide (CP)	0.173±0.003	0.150±0.006
Petroleum ether fraction (PEF)	0.028±0.001	0.027±0.001
Ethyl acetate fraction (EAF)	0.026±0.002	0.028±0.002
n-Butanol fraction (BF)	0.060±0.033 [a]	0.099±0.007 [b]
Water fraction (WF)	0.228±0.014	0.223±0.017
α-tocopherol	0.082±0.028	

Values represent means ± standard deviations for triplicate experiments. Values followed by the different letter in the same row are significantly different ($p < 0.05$).

extracts and fractions of *S. chinensis* and *S. sphenanthera*. The reducing power of the extracts and fractions increased with increased concentrations. Both the ethyl acetate fractions from *S. chinensis* and *S. sphenanthera* showed the strongest ferric reducing ability, the reducing power was 0.207, for *S. chinensis* and 0.194, for *S. sphenanthera* when they were at the concentration of 144 µg/ml. The reducing power of the ethyl acetate fractions from *S. chinensis* was higher than that from *S. sphenanthera*. These data showed that the ethyl acetate fraction might contain either the higher concentration or the most potent compounds having the most potent efficacy in reducing metal ions, which act as catalysts of oxidative reactions. These results were according with the previous reports, in which both the ethyl acetate and butanol fractions of *Chenopodium quinoa* and *Amaranthus spp.* seeds showed the strongest ferric reducing ability (Nsimba et al., 2008)

Lipid peroxidation inhibitory activity

Lipid peroxidation was an oxidative deterioration process of polyunsaturated fatty acids which was damaged by radical. The content of malondialdehyde (MDA) can indicate the degree of lipid peroxidation (Su et al., 2009). Table 5 shows the inhibitory activity of extracts and fractions on lipid peroxidation. The inhibitory activity of extracts and fractions, expressed in the term of IC_{50} was in the range of 0.026 ± 0.002 to 0.228 ± 0.014 mg/ml. The strongest antioxidant potency for the ethyl acetate fractions of both *S. chinensis* and *S. sphenanthera* were detected with the IC_{50} of 0.026 and 0.028 mg/ml and the weaker for the water fractions with the IC_{50} of 0.228 and 0.223 mg/ml for *S. chinensis* and *S. sphenanthera,* respectively. This activity tendency was accorded with that of the scavenging activity on DPPH radicals. From the results on lipid peroxidative inhibitory activities, the ethyl acetate and petroleum ether fractions were even better than the positive control, α-tocopherol, the IC_{50} of which was 0.082 mg/ml. These results suggested that the ethyl acetate fractions might contain the strongest lipid peroxidate inhibitory compounds. There were different trends in antioxidant capacity between the DPPH, reducing power and lipid peroxidation inhibitory assays. This phenomenon was also found by the previous studies on the antioxidant activity of various extracts and fractions of *C. quinoa* and *Amaranthus spp.* Seeds (Nsimba et al., 2008).

Total phenols content and total flavonols content versus the radical scavenging activity

The antioxidant capacity was related to the compounds including polyphenols and flavonols. The correlation between antioxidant capacity and total phenols content and total flavonols content were investigated in many studies (Nsimba et al., 2008). In this work, the correlation between the DPPH radical scavenging activity and total phenols content and total flavonols content of the extracts and fractions were studied respectively using a linear regression analysis. Values of IC_{50} in DPPH free radical scavenging assay were used as antioxidant capacity in this study.

The correlation coefficient between total phenolics' content and DPPH scavenging activity was R^2=0.8474 (y = -0.0021x + 0.2378). The correlation coefficient between total flavonols' content and DPPH scavenging activity was R^2=0.7036 (y = -0.0014x + 0.2674). Though the correlation coefficients calculated for the antioxidant assays versus total phenols content and total flavonols content revealed differences in correlation of the antioxidant power to the phenolic compounds. The high correlation values between total phenols and total flavonols and the antioxidative activity suggested that the major antioxidant compounds in studied *S. chinensis* and *S. sphenanthera* might be phenolics and flavonols. The results were in good accordance with Du's study, in which it was showed the good relationship between antioxidant

capacity and total phenols content and total flavonols content (Du et al., 2009).

Conclusions

S. chinensis and *S. sphenanthera* have been used as traditional Chinese medicine for thousands of years. The chemical composition and antioxidant activity of the extracts and fractions from these two fruits were firstly comparatively studied here. There were obvious difference in the yields and composition of the essential oils, metal elements, polysaccharides, ethanol extracts and fractions between *S. chinensis* and *S. sphenanthera.* Both the two species *S. chinensis* and *S. sphenanthera* might be rich in several phytonutrients that could act as powerful dietary antioxidants. Total phenolic content and antioxidant capacity had different values among the samples and there was observed a good correlation between antioxidant capacity and total phenols content and total flavonols content. Various extracts and fractions might provide different antioxidants, which in general demonstrated strong activities within the ethyl acetate fractions. The detailed information on the structure of the most active compounds and the differences in both fractions need further investigation.

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