Asian Journal of Chemistry; Vol. 23, No. 8 (2011), 3581-3586

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Phytochemical Profiles and Antioxidant Properties of *Sarcopyramis bodinieri* var. delicata

XI-GEN HUANG[1,*] and CHUN-PENG WAN[2]

[1]Department of Chemistry, Jiangxi Agricultural University, Nanchang 330045, P.R. China
[2]State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, P.R. China

*Corresponding author: Tel: +86 791 3813642; E-mail: hxg208@163.com

(*Received*: 27 October 2010; *Accepted*: 27 April 2011) AJC-9852

Sarcopyramis bodinieri var. delicata was extracted with 60 % aqueous ethanol and the extract was successively isolated by liquid-liquid partition to yield the extract. Total flavonol content and antioxidant properties of the three organic solvent extracts were conducted, the antioxidant activities were evaluated by measuring the ability of the extracts to scavenge the DPPH and $ABTS^{\bullet+}$ free radical. In addition, the reducing power, phosphomolybdenum reduction and Fe^{2+} chelation were also assessed. Among those organic fractions, the ethyl acetate fraction (EAF) exhibited the highest total flavonol content, which possessed significant free radical scavenging and antioxidant properties. Seven phenolic compounds were identified by LC-DAD-MS and NMR spectrum from ethyl acetate fraction. Additionally, four major aglycon compounds (quercetin, isorhamnetin, kaempferol and ellagic acid) were determined from hydrolyzed ethyl acetate fraction. The results obtained *in vitro* models clearly suggest that the ethyl acetate fraction from the extract of *Sarcopyramis bodinieri* var. delicata is a promising natural source for antioxidants.

Key Words: *Sarcopyramis bodinieri* var. delicata, Antioxidant properties, Flavonol constituents.

INTRODUCTION

In many biological systems, reactive oxygen species (ROS) are formed as natural byproducts of normal oxygen metabolism. The reactive oxygen species produced in cellular process include superoxide, hydroxyl radical and peroxyl radical, generally result in degradation of protein, lipid peroxidation and oxidation of DNA, which have been considered to be linked with many chronic diseases such as diabetes, cancers and atherosclerosis[1,2].

Flavonol are a large group of phenolic secondary metabolites of higher plants and exhibit powerful antioxidant capacities. Recently, the evaluation the antioxidant capacities of plants, vegetables and fruits extract arouse people extensive interests[3-6].

Sarcopyramis bodinieri var. delicata belonging to the family Melastomataceae, which is narrowly distributed in Fujian and Taiwan provinces in China. As a rare species, *Sarcopyramis bodinieri* var. delicata is widely used as hepatoprotective drugs in the local people[7]. The water extract of this dried herb could reduce aminotransferase and cure choleplania and hepatoma[8]. *Sarcopyramis bodinieri* var. delicata contains plentiful of flavonol compounds and we had isolated and identified many flavonol and phenolic acid compounds from this plant previously[9,10]. However, phytochemical profiles and antioxidant activity of the plant have not been reported previously. In continuation of our studies on the bioactive constituents from *Sarcopyramis bodinieri* var. delicata, in the present work, the free radical and antioxidant activity of the organic extracts of *Sarcopyramis bodinieri* var. delicata are evaluated *in vitro* and the phytochemical profiles also conducted by the LC-DAD-MS.

EXPERIMENTAL

Trolox, vitamin C, ferrozine and DPPH were all purchased from Sigma Chemical Company (St. Louis MO, USA); 2,2'-azino-*bis*-(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) was obtained from TCI-SU (Tokyo, Japan). Isoquercitrin was purified by our laboratory and identified by the NMR and ESI-MS spectrum. All of the organic solvents used were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. S (Shanghai, China). UV-spectra measurements were performed using UV-2450 spectrophotometer (Shimadzu, Japan). A Shimadzu LC-20 HPLC coupled with 3200 Q-trap ESI-MS spectrometer (ABI, American) were used to analyze and identify the chemical constituents, the NMR spectra were recorded on a Bruker Avance-600 FT NMR spectrometer.

Solvent extraction: *Sarcopyramis bodinieri* var. delicata were collected in April 2007 from the Yongchun country, Fujian province, China. A voucher specimen (RSC07) is deposited

at the Department of Pharmacy, School of Medicine, Xiamen University. The ground plant material (10 g) was extracted with 400 mL 60 % ethanol in a 1000 mL conical flask and refluxed at 80 ºC for 1 h. The extracts were vacuum filtered (Whatman No. 1) and concentrated *in vacuo* at 45 ºC, using a rotary evaporator (EYELA, N-1000, Tokyo, Japan). The dried extracts were suspended in 100 mL water and subjected to sequential liquid-liquid extraction with the equal volumn chloroform (CF), ethyl acetate (EAF) and *n*-butanol (BF). The three organic fractions were obtained after removal of the solvent and weighted to calculate the yield rate.

Acid hydrolysis of ethyl acetate fraction: The method of acid hydrolysis the extract of *S. bodinieri* var. delicata has described before[11]. Briefly, 10 mg ethyl acetate fraction was dissolved in 30 mL methanol mixed with 5 % hydrochloric acid (4:1, v/v). The solution was refluxed at 80 ºC for 1 h. The hydrolysis solution was filtered through a 0.45 μm filter before HPLC analysis.

Determination of total flavonol content (TFC): The total flavonol content were determined by the colorimetric method used isoquercitrin as standard reference. The absorbance of the standard reference and three organic fractions from extract of *S. bodinieri* var. delicata was measured at 355 nm using UV-2450 spectrophotometer (Shimadzu, Japan). Calibration curve was determined using a series of standard solutions in a range of concentration of 4.4-19.8 μg/mL. The calibration equation was for $Y = 0.0464X + 0.0039$ ($R^2 = 0.9958$). And the results were expressed as mg isoquercitrin/g dry extract (DE).

DPPH free radical-scavenging capacity: The DPPH free radical-scavenging activity of the extracts was determined according to the method described by Nadaroglu[12]. Briefly, DPPH solution (0.6 mM) was prepared in ethanol and to 0.5 mL of this solution was mixed with 0.5 mL of moderate concentration extract samples or Trolox (8-40 μg/mL) as a standard reference. The volume of the mixture solution was adjusted with ethanol to a final volume of 5 mL. After incubation in a dark place for 0.5 h at room temperature, the absorbance of the mixture was measured at 515 nm against ethanol as blank using UV-2450 spectrophotometer (Shimadzu, Japan). Each sample was measured in triplicate and averaged. The DPPH radical scavenging activity (RSA) was calculated according to the formula:

$$\text{DPPH RSA } (\%) = \left[\frac{(A_C - A_S)}{A_C}\right] \times 100$$

where A_C is the absorbance value of the control and A_S is the absorbance value of the added test samples solution.

$ABTS^{\bullet+}$ cation free radical-scavenging activity: For $ABTS^{\bullet+}$ assay, the procedure followed the method described by Roberta[13] with some modifications. ABTS was dissolved in water to make a concentration of 7 mM/L. $ABTS^{\bullet+}$ was produced by reacting the ABTS stock solution with 2.45 mM/L potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. For the test of samples, the $ABTS^{\bullet+}$ stock solution was diluted with 80 % methanol to give an absorbance of 0.70 ± 0.02 at 734 nm. After the addition of 4.5 mL of diluted $ABTS^{\bullet+}$ to 0.5 mL of moderate concentration extract samples or Trolox (2-24 μg/mL) as a standard reference. The absorbance reading was taken in 6 min after the initial mixing at 734 nm. Each sample was measured in triplicate and averaged. This activity is given as percentage $ABTS^{\bullet+}$ scavenging that is calculated by the same formula with DPPH.

Determination of the total antioxidant using phosphomolybdenum method: The total antioxidant capacity of the extract samples were evaluated according to the method described by Prieto[14]. An aliquot of 0.5 mL of moderate concentration extract samples solution (24-56 μg/mL) were combined with 4.5 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). In case of blank, 0.5 mL of ethanol was used in place of samples. The tubes were incubated in a boiling water bath at 95 ºC for 1.5 h. After the samples were cooled to room temperature, the absorbance of the aqueous solution of each samples were measured at 695 nm against blank in UV-2450 spectrophotometer. Vitamin C and Trolox were used as standard reference. Each sample was measured in triplicate and averaged. The total antioxidant activity was expressed as the absorbance of the sample at 695 nm. The higher absorbance value indicated higher antioxidant activity[15].

Reducing power assay: The reducing power of extract samples was determined according to the method of Nadaroglu[12]. 2.5 mL extract samples (8-40 μg/mL) were mixed with 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1 % potassium ferricyanide and the mixture was incubated at 50 ºC for 20 min. At the end of the incubation, 2.5 mL of 10 % trichloroacetic acid (TCA) were added to the mixture, which was centrifuged at 3000 rpm for 10 min. 2.5 mL of the upper layer of solution was mixed with 2.0 mL of distilled water and 0.5 mL of 0.1 % $FeCl_3$ and the absorbance was measured at 700 nm. Vitamin C and Trolox were used as standard references. Each sample was measured in triplicate and averaged. Increase in absorbance of the reaction mixture indicated the reducing power of the extract samples.

Ferrous ions chelating activity: Ferrous ions chelating activity of extract samples were estimated by the method of Soares[16]. In brief, 1 mL of the extract samples (240-1200 μg/mL) was added to 0.1 mL of $FeCl_2$ solution (2 mM/L) and 3.7 mL ethanol. The reaction was initiated by the addition of 0.2 mL ferrozine (5 mM/L) and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm, wherein the Fe^{2+} chelating ability of extract samples were monitored by measuring the ferrous ion-ferrozine complex at 562 nm. EDTA was used as standard reference. Each sample was measured in triplicate and averaged. The Fe^{2+} chelating activity was calculated according to the formula:

$$\text{Ferrous ions chelating } (\%) = \left[\frac{A_C - A_S}{A_C}\right] \times 100$$

where A_C is the absorbance value of the control and A_S is the absorbance value of the added test samples solution. The control solutions only contained $FeCl_2$ and ferrozine solutions.

HLPC-DAD-ESI-MS analysis: HPLC-DAD-ESI-MS system consist of an Shimadzu LC-20 HPLC coupled with

3200 Q-trap ESI-MS spectrometer (ABI, American), equipped with a vacuum degasser, a quaternary pump, an autosampler, a thermostatted column compartment, a diode array detector (DAD) and an ion-trap mass spectrometer with electrospray ionization interface. Shimadzu shimpack VP-ODS (150 mm × 4.6 mm i.d., 5 µm particle size) was used for separation. Solvents for the mobile phase were water-0.1 % formic acid (A) and methanol (B). The gradient elution was: 0-15 min, linear gradient 10-30 % B, 15-25 min, linear gradient 30-40 % B, 25-40 min, linear gradient 40-50 % B, 40-50 min, linear gradient 50-70 % B, 50-60 min, isocratic elution with 70 % B. The flow rate was 1.0 mL/min and the column was operated at 30 ºC. Peaks were detected with the DAD at 365 nm and the injection volume was 20 µL. The ESI negative TIC modes were used for MS detection. The m/z values of the monitored ions were from 100-800. The other parameters were as follows: capillary voltage, 3.5 kV; cone voltage, 30 V; extraction voltage, 5 V; RF voltage, 0.5 V; source temperature, 90 ºC; nitrogen gas flow for desolvation, 300 L/h and temperature of the nitrogen gas for desolvation, 350 ºC.

Statistical analysis: Results were given as mean ± standard deviation of three replicates. Experimental results were analyzed by SPSS version 16.0 (SPSS Inc. Chicago, IL). Differences between means were determined using one-way ANOVA and Duncan's test. The level of statistical significance was set at $p < 0.05$ and 0.01.

RESULTS AND DISCUSSION

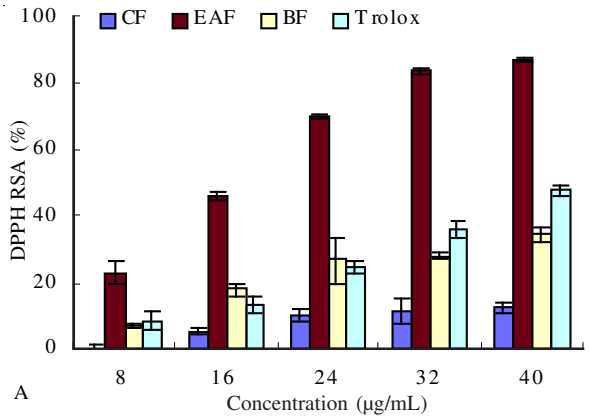
Percentage yield and total flavonol content (TFC): In the present study, the percentage yield and TFC of three organic fractions extracted from *S. bodinieri* var. delicata are shown in Table-1. Among the three fractions, the *n*-butanol fraction (BF) had the highest percentage yield value ($p < 0.01$), followed by the ethyl acetate fraction (EAF), while the chloroform fraction had the lowest value ($p < 0.01$). However, the maximum TFC were recorded in EAF (499.97 ± 5.84 mg isoquercitrin/g dry extracts) followed by chloroform fraction (298.57 ± 4.39 isoquercitrin/g dry extracts), while the *n*-butanol fraction contained the lowest TFC. Flavonol are a large group of second metabolite in plants, which had gained much attention, due to their multiple pharmacologic activities including antioxidant, anticancer, antiinflammatory and hypoglycemia activity. Many flavonol compounds had been isolated and identified from the genus of *Sarcopyramis* previously. The mono-glucoside derivatives of quercetin and isorhamnetin were the principal flavonol compounds of the plant, which were suitable extracted by ethyl acetate[10].

TABLE-1
YIELD AND TFC OF THREE ORGANIC FRACTIONS FROM THE EXTRACTS OF *S. bodinieri* var. delicata

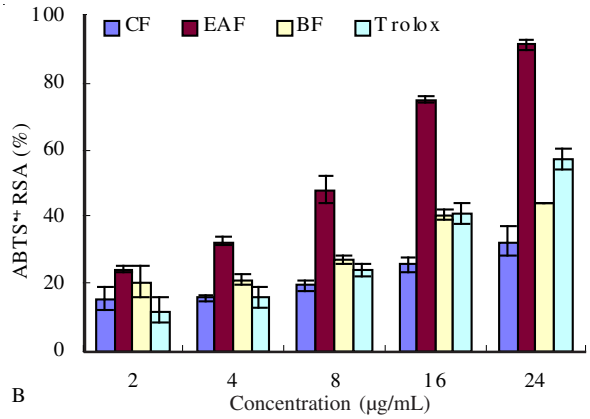
Sample	Yield (mg/g dry material)	Total flavonols content (mg isoquercitrin/g extracts)
Chloroform fraction	8.21 ± 0.98*	298.57 ± 4.39**
Ethyl acetate fraction	15.39 ± 1.12**	499.97 ± 5.84***
n-Buthanol fraction	36.31 ± 1.75***	87.31 ± 3.62*

Note: Values expressed are mean ± SD of triplicate experiments. Different letters within the same column indicate significant difference at $p < 0.05$ by Duncan's test.

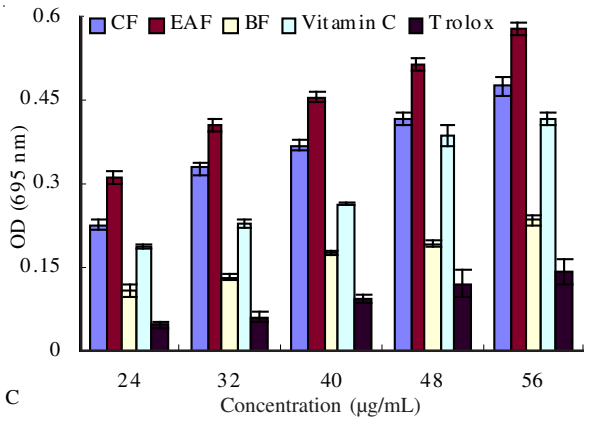
DPPH free radical-scavenging capacity: DPPH has been widely used in the evaluation of antioxidant activity of pure compounds and plant extracts. The efficacy of DPPH radical scavenging activity of the three fractions from *S. bodinieri* var. delicata and the standard material (Trolox) are shown in Fig. 1A. All of the four samples were able to inhibit the activity of DPPH radicals in a dose-dependent manner. The EAF had the strongest DPPH radical-scavenging activity compared with the standard material and other two fractions ($p < 0.01$), the *n*-butanol fraction showed equivalent activity to the standard material, while the chloroform fraction showed the lowest DPPH scavenging activity. An almost linear correlation between DPPH radical-scavenging capacity and total flavonol content in medicine plants and vegetables have been reported. Those results revealed that the EAF with the most TFC from *S. bodinieri* var. delicata has free radical scavengers, acting possibly as primary antioxidants.



A



B



C

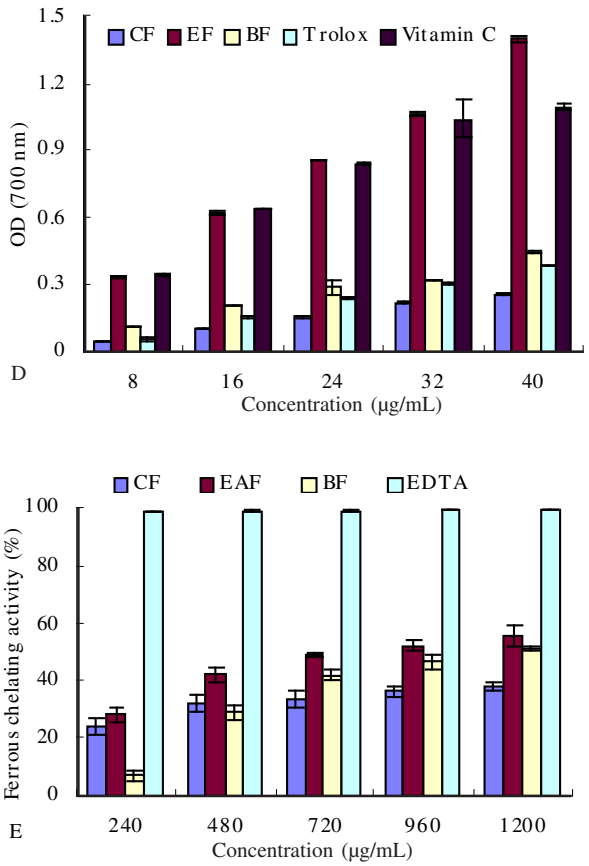


Fig. 1. Antioxidant activity of the three organic fractions from the ethanol crude extracts of *S. bodinieri* var. delicata by different solvents. Trolox, vitamin C and EDTA were used as standard materials. (A) DPPH radical scavenging activity, (B) $ABTS^{\bullet+}$ radical scavenging activity, (C) Total antioxidant activity determined by phosphomolybdenum method, (D) Reducing power, (E) Ferrous ion chelating activity. Values represent means ± SD from 3 replicate experiments

$ABTS^{\bullet+}$ cation free radical-scavenging activity: $ABTS^{\bullet+}$ assay is based on the antioxidant ability to react with $ABTS^{\bullet+}$ generated by potassium persulfate oxygenization the ABTS. This method is widely used to evaluate antioxidant activity in foods and medicines systems. $ABTS^{\bullet+}$ cation radical have a maximum of absorbance at 734 nm, extracts contain antioxidant components will decrease the absorption values of $ABTS^{\bullet+}$ at 734 nm by redox reaction directly scavenging the $ABTS^{\bullet+}$ cation radical. All of the three fractions from *S. bodinieri* var. delicata and Trolox exhibited $ABTS^{\bullet+}$ radical-scavenging activity in a concentration-dependent manner (Fig. 1B). The EAF from *S. bodinieri* var. delicata exhibited the highest radical scavenging activities when reacted with the $ABTS^{\bullet+}$ radical. In contrast, the chloroform fraction and *n*-butanol fraction showed equivalent activity to Trolox in the lower concentration (2-16 μg/mL), while them showed lower activity than Trolox increasing the concentration to 24 μg/mL.

Total antioxidant capacity determined by phosphomolybdenum method: The phosphomolybdate method has been routinely used to evaluate the total antioxidant capacity of plant, vegetable and fruit extracts. In the presence of antioxidant agent, Mo(VI) is reduced to Mo(V) and forms a green coloured phosphomolybdenum(V) complex with a maximum absorbance at 695 nm. The higher absorbance value at 695 nm indicated higher antioxidant activity. The total antioxidant activity of the three fractions from *S. bodinieri* var. delicata and the standard materials (Trolox and vitamin C) are shown in Fig. 1C. The chloroform fraction and EAF showed stronger total antioxidant activity than the two standard materials, while the *n*-butanol fraction only showed stronger activity than Trolox. The total antioxidant activities of the tested samples were in the order of EAF > CF > vitamin C > BF > Trolox within the concentration of 24-56 μg/mL. The results indicated that the EAF has powerful antioxidant activity than other fractions, which was coincidence with other *in vitro* antioxidant models.

Reducing power assay: In the reducing power assay, the presence of antioxidants in the samples would result in the reducing of Fe^{3+}-Fe^{2+} by donating an electron. The amount of Fe^{2+} complex can be determined by measuring the formation of Prussian blue at 700 nm. Increasing absorbance at 700 nm reveals an increase in reductive capacity[17]. Fig. 1D shows the dose-response manner for the reducing powers of the three fractions from the extract of *S. bodinieri* var. delicata and the standard materials (Trolox and vitamin C). All of the three fractions exhibited powerful reducing potential as compared with the standard materials. EAF showed comparable reductive capacity with vitamin C and higher activity than Trolox, while the chloroform fraction and *n*-butanol fraction showed comparable reductive capacity with Trolox. The highest reducing activity was for the EAF, compared to those of the other fractions. Similar to the results obtained from the DPPH, $ABTS^{\bullet+}$ to phosphomolybdenum assay.

Ferrous ions chelating activity: In this assay, the chelating agents inhibit the formation of ferrozine-Fe^{2+} complex with a maximum absorbance at 562 nm, thus decreasing the absorbance at 562 nm reveals the ferrous ions chelating activity. The lower absorbance indicates higher chelating capacity[16]. The chelating activity of the three fractions from *S. bodinieri* var. delicata slightly increased with their concentrations (Fig. 1E). However, all fractions presented much lower chelating power than EDTA (a positive control) ($p < 0.01$).

HPLC-DAD-ESI-MS analysis the constituents of EAF: Seven peaks of phenolic compounds were separated and identified by MS value coupled with the UV spectrum data, further confirmed by the NMR spectrum data (Table-2). Four major aglycon compounds were also identified from the hydrolyzed EAF. The HPLC-DAD chromatogram of EAF is shown in Fig. 2. Peak 1 yielded a quasimolecule ion at 463 $[M-H]^-$ and its MS/MS spectrum showed a prominent ion of $[M-H-Glu]^-$ at m/z 301. Moreover, UV spectrum of peak 1 showed λ_{max} at 258 and 356 nm, the structure of peak 1 was identified as isoquercitrin[10]. Peak 2 yielded a quasimolecule ion at 301 $[M-H]^-$, UV spectrum of peak 2 showed λ_{max} at 253 and 368 nm, based on those data coupled with the NMR spectrum data (Table-2), the structure of peak 2 was identified as ellagic acid[18]. Peak 3 yielded a quasimolecule ion at 623 $[M-H]^-$ and its MS/MS spectrum showed a fragment ion of $[M-H-Rha]^-$ at m/z 477 and $[M-H-Rha-Glu]^-$ at m/z 315. UV spectrum of peak 3 showed λ_{max} at 256 and 355 nm, the structure of peak 3 was identified as isorhamnetin-3-O-rutinoside[19]. Peak 4 yielded a quasimolecule ion at 477 $[M-H]^-$ and its MS/MS spectrum showed a prominent ion $[M-H-Glu]^-$ at m/z 315. UV spectrum

TABLE-2
RETENTION TIMES (RT), UV λ_{max} VALUES, ESI-MS AND NMR DATA FOR CONSTITUENTS FROM EAF

Peak No.	RT (min)	λ_{max} (nm)	Product ions (ESI-, m/z)	Identification compounds	NMR (DMSO-d_6, δ, ppm, 600 Mz for ^{1}H NMR and 150 Mz for ^{13}C NMR)
1	26.58	258, 356	463, 301	Isoquercitrin	7.58 (1H, dd, H-6'), 7.57 (1H, d, H-2'), 6.84 (1H, d, H-5'), 6.40 (1H, d, H-8), 6.19 (1H, d, H-6), 5.47 (1H, d, H-1'')
2	27.22	253, 368	301	Ellagic acid	7.47 (1H, s); ^{13}C-NMR 159.1 (C=O), 148.1 (C-3, 3'), 139.6 (C-5, 5'), 136.3 (C-4, 4'), 112.3 (C-6, 6'), 110.1 (C-1, 1'), 107.5 (C-2, 2')º
3	31.21	256, 355	623, 477, 315	Isorhamnetin-3-O-rutinoside	10.86 (1H, s, 4'-OH), 9.80 (1H, s, 7-OH), 7.86 (1H, d, H-6'), 7.52 (1H, dd, H-2'), 6.92 (1H, d, H-5'), 6.44 (1H, d, H-8), 6.21 (1H, d, H-6), 5.44 (1H, d, H-1''), 4.54 (1H, d, H-1'''), 4.01- 4.09 (2H, m, H-6''), 3.84 (3H, s, OCH_3), 0.97 (3H, d, CH_3)
4	31.59	256, 354	477, 315	Isorhamnetin-3-O-glucoside	7.95 (1H, d, H-2'), 7.50 (1H, dd, H-6'), 6.92 (1H, d, H-5'), 6.44 (1H, d, H-8), 6.21(1H, d, H-6), 5.57 (1H, d, H-1''), 3.84(3H, s, OCH_3)
5	36.03	256, 371	301	Quercetin	7.68 (1H, d, H-2'), 7.54 (1H, dd, H-6'), 6.88 (1H, d, H-5'), 6.40 (1H, d, H-8), 6.18 (1H, d, H-6)
6	42.71	265, 366	285	Kaempferol	12.47 (1H, s, OH-5), 8. 04 (2H, d, H-2', 6'), 6.93 (2H, d, H-3', 5'), 6.42 (1H, d, H-8), 6.17 (1H, d, H-6)
7	44.69	255, 371	315	Isorhamnetin	7.74 (1H, d, H-2'), 7.69 (1H, dd, H-6'), 6.94 (1H, d, H-5'), 6.48 (1H, d, H-8), 6.19 (1H, d, H-6), 3.84 (3H, s, OCH_3)

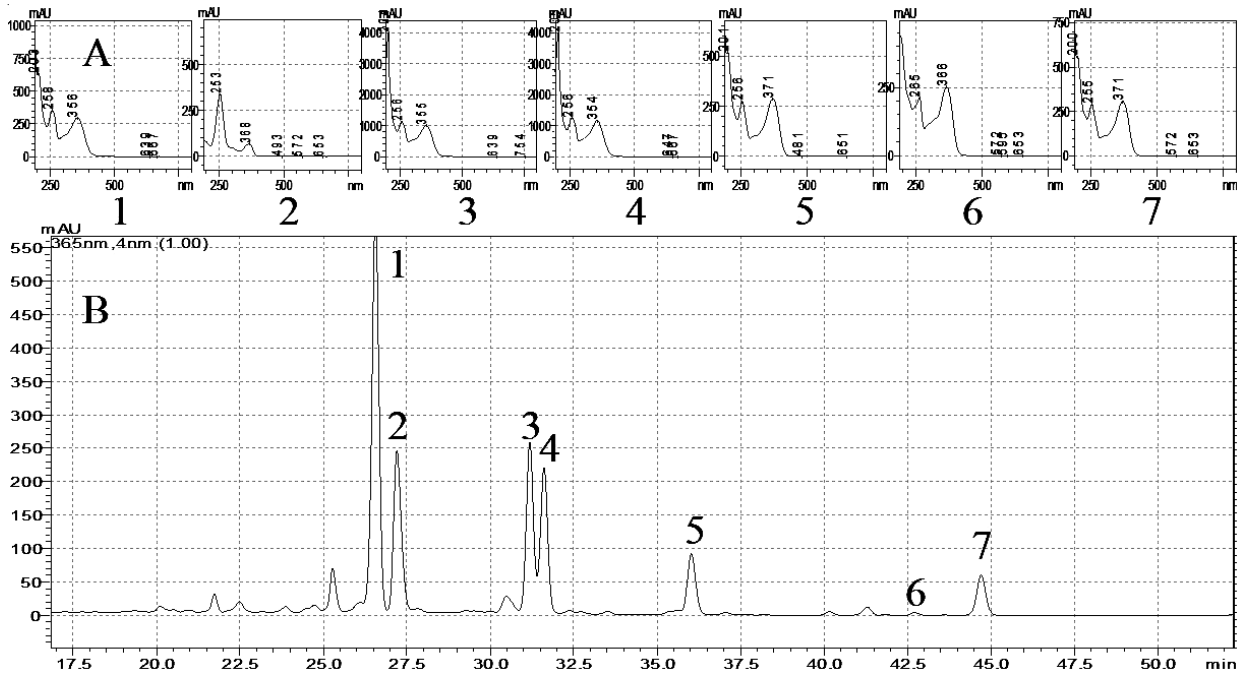


Fig. 2. HPLC-DAD chromatogram of EAF. (A) The UV spectrum of the seven peaks in methanol solution, (B) The HPLC chromatogram of EAF performed on Shimadzu LC-20 HPLC

of peak 4 showed λ_{max} at 256 and 354 nm, the structure of peak 4 was identified as isorhamnetin-3-O-glucoside[10]. Peak 5 yielded a quasimolecule ion at 301 $[M-H]^-$, UV spectrum of peak 5 showed λ_{max} at 256 and 371 nm, the structure of peak 5 was identified as quercetin[10]. Peak 6 yielded a quasimolecule ion at 285 $[M-H]^-$, UV spectrum of peak 6 showed λ_{max} at 263 and 366 nm, the structure of peak 6 was identified as kaempferol[20]. Peak 7 yielded a quasimolecule ion at 315 $[M-H]^-$, UV spectrum of peak 7 showed λ_{max} at 255 and 371 nm, the structure of peak 7 was identified as isorhamnetin[10]. Four aglycon components were detected in the hydrolysis ethyl acetate fraction (Fig. 3). The content of the two flavonols (quercetin and isorhamnetin) significantly increased after hydrolysis showed that the glucoside derivatives of quercetin and isorhamnetin were the principal constituents of the plant, which showed powerful antioxidant capacity described by many literatures.

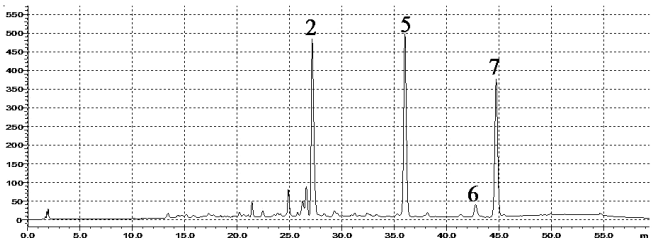


Fig. 3. HPLC-DAD chromatogram of hydrolyzed EAF

Conclusion

This paper described the antioxidant and radical-scavenging capacities of three organic fractions from the extract of *S. bodinieri* var. delicata. The TFC in the three fractions were also determined by the colorimetric method using isoquercitrin as standard material. The highest TFC was found in the EAF, which showed the powerful antioxidant activities in all test

models *in vitro*. Seven phenolic compounds were identified by LC-DAD-MS and NMR spectrum from the EAF. Additionally, four major aglycon compounds (quercetin, isorhamnetin, kaempferol and ellagic acid) were determined from hydrolyzed EAF. The derivatives of quercetin and isorhamnetin are the principal constituents of the plant. The results clearly suggest that the EAF from the extract of *S. bodinieri* var. delicata is a promising natural source for antioxidants.

ACKNOWLEDGEMENTS

The authors gratefully acknowledged the financial support by the Jiangxi Province Natural Science Foundation (JXNSF[2010]).

REFERENCES

1. J.K. Willcox, S.L. Ash and G.L. Catignani, *Crit. Rev. Food Sci. Nutr.*, **44**, 275 (2004).
2. P.A. Riley, *Int. J. Radiat. Biol*., **65**, 27 (1994).
3. M.D.M. Rufino, R.E. Alves, E.S. deBrito, J. Perez-Jimenez, F. Saura-Calixto and J. Mancini-Filho, *Food Chem*., **121**, 996 (2010).
4. N. Andarwulan, R. Batari, D.A. Sandrasari, B. Bolling and N. Wijaya, *Food Chem*., **121**, 1231 (2010).
5. P.C. Shieh, G. Agoramoorthy and D.H. Kuo, *Asian J. Chem*., **21**, 5556 (2009).
6. G. Agoramoorthy, F.A. Chen, V. Venkatesalu, D.H. Kuo and P.C. Shea, *Asian J. Chem*., **20**, 1311 (2008).
7. X.M. Wang, C.P. Wan, S.R. Zhou and Y. Qiu, *Molecules*, **13**, 1399 (2008).
8. M. Liao, J. Zhang and Y. Zhang, *Herald Med.*, **5**, 571 (2010).
9. J.Y. Yang, C.P. Wan and Y. Qiu, *J. Chin. Med. Mater*., **33**, 542 (2010).
10. C.P. Wan, X. Zheng, H.F. Chen, X. Zou, Z.R. Song, S.R. Zhou and Y. Qiu, *China J. Chin. Mater. Med*., **34**, 172 (2009).
11. A.R. Zuo, C.P. Wan and S.R. Zhou, *West China J. Pharm. Sci*., **24**, 300 (2009).
12. H. Nadaroglu, N. Demir and Y. Demir, *Asian J. Chem*., **21**, 5123 (2009).
13. R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans, *Free Radic. Biol. Med*., **26**, 1231 (1999).
14. P. Prieto, M. Pineda and M. Aguilar, *Anal. Biochem*., **269**, 337 (1999).
15. K.N. Prasad, B. Yang, S.Y. Yang, Y.L. Chen, M.M. Zhao, M. Ashraf and Y.M. Jiang, *Food Chem*., **116**, 1 (2009).
16. A.A. Soares, C.G.M. de Souza, F.M. Daniel, G.P. Ferrari, S.M.G. da Costa and R.M. Peralta, *Food Chem*., **112**, 775 (2009).
17. I. Gülçin, E. Bursal, M.H. Sehitoglu, M. Bilsel and A.C. Gören, *Food Chem. Toxicol.*, **48**, 2227 (2010).
18. H. Ueda, Y. Tachibana, M. Moriyasu, K. Kawanishi and S.M. Alves, *Phytomedicine*, **8**, 377 (2001).
19. S.A. El Batran, N.S. Abdel-Azim, K.A. Abdel-Shafeek, A.E.A. Shahat and M.M. El-Missiry, *Nat. Prod. Sci*., **11**, 233 (2005).
20. H.Q. Lan, *J. Chin. Med. Mater*., **33**, 547 (2010).