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Antioxidant compounds from *Rosa laevigata* fruits

Xi Li [a,b], Wei Cao [c], Ying Shen [d], Ning Li [e], Xiao-Ping Dong [b], Kai-Jin Wang [e], Yong-Xian Cheng [a,*]

[a] *State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China*
[b] *College of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu 610075, China*
[c] *Department of Food Science and Technology, Northwest University, Xi'an 710069, China*
[d] *Department of Nephrology, The First People's Hospital of Yunnan Province, Kunming 650032, China*
[e] *School of Life Sciences, Anhui University, Anhui 230039, China*

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A B S T R A C T

Rosa laevigata fruit is a foodstuff used in China to brew wine, extract brown pigments, and make a healthy vinegar-based beverage. It is also widely used in traditional Chinese medicine to treat nephropathy. We isolated 20 compounds from the fruit, including two new lignans, rosalaevins A and B (**1** and **2**), and 18 phenolic compounds isolated for the first time. Their antioxidant potentials were evaluated with 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and ferric reducing antioxidant power (FRAP) assays, and a β-carotene linoleate model system. Several compounds exhibited powerful antioxidant capacities. No compound inhibited nitric oxide production in macrophage cells. These results provide new insights into the role of *R. laevigata* fruit in the prevention of nephropathy, and may lend support to the development of this fruit as a health-promoting food.

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1. Introduction

Diabetes mellitus has become one of the most common causes of diabetic nephropathy (DN), which ultimately develops into end-stage renal disease. It is estimated that approximately 40% of cases of end-stage renal disease are attributable to DN (Lasaridis & Sarafidis, 2003). Therefore, the search for compounds that are useful in the prevention of DN has become our priority. Previous studies have shown that two key mechanisms are involved in the development of DN: the formation of advanced glycation end products (AGEs) and oxidative stress. Therefore, new strategies that target AGEs or oxidative stress will be important for the improvement of DN (Adeline, Tan, Josephine, & Mark, 2007). The kidney is considered the congenital origin of the body in traditional Chinese medicine (TCM), and TCM practitioners have paid great attention to the protection of the kidney since ancient times. Therefore, TCM could make its own contribution to the prevention of kidney diseases. Shui-Lu-Er-Xian-Dan, which consists of *Euryale ferox* seeds and *Rosa laevigata* Michx. fruits, was created by Zun Hong (1120–1174 AD) in the South Song Dynasty, and is a representative traditional Chinese formula used for the treatment of kidney diseases. One of the authors found that this formula reduced proteinuria in clinical practice. This aroused our interest because several studies have indicated that advanced nephropathy leads to severe proteinuria, and the reduction of proteinuria is an important protective strategy against DN (Robert et al., 2005). Previously, we have studied *E. ferox* seeds and isolated several antioxidant compounds that were thought to be associated with the actions of this formula (Song et al., 2011). However, so far, little is known about the role of *R. laevigata* fruit in the prevention of proteinuria. *R. laevigata*, belonging to the family Rosaceae, is abundantly distributed in China. Its fruit is edible and is widely consumed in China, with effects on improving resistance to colds, sperm counts, kidney health, reducing inflammation, and the treatment of arteriosclerosis (Zou, Yang, & Chen, 2006). The Ministry of National Health of China has rated the fruit a new food resource, and has now developed it as a third-generation wild fruit food. In China, the fermentation product of the fruit is made into a healthy vinegar-based beverage (Lu, Li, Zeng, & Zhang, 2007). The fruit is also used as a foodstuff in the traditional brewing of wine and also for the extraction of brown pigments used as food additives. Previous pharmacological studies have indicated that this medicinal plant can inhibit arterial sclerosis, cure hyperpiesia, and has hepatoprotective activity (Liu, Lu, & Peng, 2011). Polysaccharides, flavonoids, and saponins from this herb have been characterized (Zhao, Guo, & Li, 2003). However, more intensive chemical and pharmacological investigations on the fruit are required. To continue our systematic study on the effects of Shui-Lu-Er-Xian-Dan on DN, we undertook an investigation of the *R. laevigata* fruit. Here, we report the isolation, structural characterization, and antioxidant potency of compounds from the *R. laevigata* fruit.

* Corresponding author. Tel./fax: +86 871 5223048.
E-mail address: yxcheng@mail.kib.ac.cn (Y.-X. Cheng).

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2. Materials and methods

2.1. General

Optical rotations were recorded on a Horiba SEPA-300 polarimeter. UV spectra were measured on a Shimadzu double-beam 210A spectrometer. IR spectra were obtained on a Tensor 27 spectrometer with KBr pellets. NMR spectra were collected on a Bruker AV-400 or a DRX-500 or a DRX 600 spectrometer. EIMS were measured on a Finnigan-4510 spectrometer. ESIMS and HRESIMS were recorded with an API QSTAR Pulsar 1 spectrometer. Column chromatography (CC) was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Inc., China), RP-18 (40–60 μm, Daiso Co., Japan), MCI gel CHP 20P (75–150 μm, Tokyo, Japan), and Sephadex LH-20 (Amersham Biosciences, Sweden). Semi-preparative HPLC was done on an Agilent 1200 liquid chromatograph, the column used was a 250 × 4.6 mm i.d., 5 μm, Zorbax SB-C_{18}.

2.2. Plant material

The *R. laevigata* fruits produced in Sichuan Province were obtained from Yunnan Corporation of Materia Medica (YCMM), Yunnan Province of China, in May 2009, and were identified by Mr. Hong-Yan Sun at YCMM. A voucher specimen (CHYX0315) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China of our institution.

2.3. Extraction and isolation

The air-dried and powdered fruits of *R. laevigata* (12 kg) were extracted with 80% EtOH (90 l × 3) under reflux. After evaporation under reduced pressure, the extract was suspended in H_2O and followed by successive partition with petroleum ether, EtOAc and *n*-BuOH. The EtOAc soluble extract (360 g) was fractionated by silica gel CC, eluted with gradient $CHCl_3$–MeOH. 11 fractions were collected on the basis of their TLC characteristics as follows: 1–5 (I), $CHCl_3$–MeOH (98:2, 5 l); 6–20 (II), $CHCl_3$–MeOH (96:4, 5 l; 94:6, 5 l; 92:8, 5 l); 21–35 (III), $CHCl_3$–MeOH (92:8, 5 l; 90:10, 10 l); 36–60 (IV), (88:18, 10 l; 85:15, 10 l; 80:20, 5 l). Fraction II (15.3 g) was submitted to MCI gel CHP 20P CC eluted with gradient aqueous MeOH to afford three portions: IIa, MeOH–H_2O (50:50, 500 ml; 55:45, 500 ml); IIb, MeOH–H_2O (55:45, 300 ml; 60:40, 500 ml); IIc, MeOH–H_2O (70:30, 500 ml; 90:10, 300 ml). Fraction IIa (3.3 g) was gel filtrated by Sephadex LH-20 (MeOH), and then applied to preparative TLC ($CHCl_3$–MeOH, 20:1) to produce compounds **6** (66.9 mg), **17** (5.6 mg), **18** (16.7 mg), and **19** (12.9 mg). Fraction IIb (1.2 g) was submitted to a Sephadex LH-20 column (MeOH) to yield a mixture containing compounds **5** and **12** which was further purified by semi-preparative HPLC (MeOH–H_2O, 65:35) to give compounds **5** (5.3 mg), and **12** (4.8 mg). Fraction III (24 g) was passed through MCI gel CHP 20P and washed with gradient aqueous MeOH to obtain four portions: IIIa, MeOH–H_2O (30:70, 300 ml; 40:60, 600 ml); IIIb, MeOH–H_2O (45:55, 500 ml; 50:50, 500 ml); IIIc, MeOH–H_2O (50:50, 600 ml; 55:45, 300 ml); IIId, MeOH–H_2O (55:45, 600 ml; 80:20, 500 ml). Fraction IIIa (845 mg) was subjected to Sephadex LH-20 column (MeOH), RP-18 (MeOH–H_2O, 20:80, 200 ml; 30:70, 200 ml; 40:60, 200 ml; 80:20, 200 ml) and finally purified by preparative TLC ($CHCl_3$–MeOH, 10:1) to give compounds **9** (17.7 mg), **16** (48 mg), and a mixture of **8** and **20**. This mixture was subjected to semi-preparative HPLC (MeOH–H_2O, 70:30) to afford compounds **8** (3.5 mg) and **20** (6.5 mg). Fraction IIIb (3 g) was separated by Sephadex LH-20 (MeOH) and RP-18 (MeOH–H_2O, 20:80, 30:70, 40:60, 45:55, 70:30, each 300 ml) to afford fractions IIIb-1 (MeOH–H_2O, 40:60, 300 ml) and IIIb-2 (MeOH–H_2O, 45:55, 250 ml). Fraction IIIb-1 (600 mg) was purified by silica gel CC ($CHCl_3$–Me_2CO, 4:1) to yield **14** (88.7 mg). Fraction IIIc (4.1 g) was passed through Sephadex LH-20 (MeOH), RP-18 (MeOH–H_2O, 30:70, 35:65, 40:60 45:55, 50:50, 80:20, each 300 ml), and purified by preparative TLC ($CHCl_3$–MeOH, 7:1) to give compounds **2** (11.7 mg), **3** (24.5 mg), and **11**(19.5 mg). Fraction IIId (6.4 g) was fractionated by RP-18 (MeOH–H_2O, 30:70, 40:60, 50:50, 55:45, 60:40, 80:20, each 300 ml) followed by preparative TLC ($CHCl_3$–Me_2CO, 2:1) to afford **1** (29.4 mg), **4** (69.6 mg), **7** (11.1 mg), **10** (8 mg), and **15** (16.5 mg). Fraction IV (22.8 g) was subjected to MCI gel CHP 20P CC (MeOH–H_2O, 40:60, 45:55, 50:50, 55:45, 60:40, 90:10, each 500 ml) to afford fractions IVa, IVb, and IVc (MeOH–H_2O, 50:50, 200 ml). Fraction IVc (2.3 g) was purified by silica gel CC ($CHCl_3$–MeOH, 5:1) to yield **13** (7 mg).

Rosalaevin A (**1**). White solid. $[\alpha]_D^{27}$ −10.9 (*c* 0.20, $CHCl_3$); IR (KBr) ν_{max}: 3425, 2938, 2845, 1595, 1516, 1463, 1427, 1367, 1331, 1273, 1236, 1154, 1124, 1034, 910, 825, 731 cm^{-1}; UV ($CHCl_3$) λ_{max} (log ε): 280 (3.67), 240 (3.90), 210 (3.48) nm; ^1H and ^{13}C NMR data: see Table 1; ESIMS *m/z*: 609 $[M+Na]^+$; HRESIMS *m/z*: 609.2317 $[M+Na]^+$ (calculated for $C_{31}H_{38}O_{11}Na$, 609.2311).

Rosalaevin B (**2**). White solid. $[\alpha]_D^{27}$ −9.3 (*c* 0.20, MeOH); IR (KBr) ν_{max}: 3431, 2925, 2851, 1603, 1509, 1480, 1465, 1432, 1366, 1272, 1218, 1124, 1031, 994, 817 cm^{-1}; UV (MeOH) λ_{max} (log ε): 280 (3.88), 226 (4.28), 204 (4.84); ^1H and ^{13}C NMR data: see Table 1; ESIMS *m/z*: 389 $[M+Na]^+$; HRESIMS *m/z*: 389.1217 $[M+Na]^+$ (calculated for $C_{18}H_{22}O_8Na$, 389.1212).

2.4. DPPH radical-scavenging assay

A DPPH assay was performed as previously described (Blois, 1958; Yoshida et al., 1989). L-Ascorbic acid was used as the positive

Table 1
NMR data for compounds **1** and **2** (400 MHz for ^1H and 100 MHz for ^{13}C, in $CDCl_3$).

Position	1		2	
	^{13}C	^1H	^{13}C	^1H
1	131.9		133.6	
2	111.1	6.69 (s)	110.7	7.01 (d, 1.8)
3	146.5		147.9	
4	145.3		146.3	
5	114.4	6.83 (d, 8.2)	115.1	6.77 (d, 8.0)
6	121.1	6.67 (overlap)	119.8	6.80 (dd, 8.0, 1.8)
7	33.2	2.89 (dd, 13.2, 5.2); 2.54 (m)	73.1	4.95 (d, 4.3)
8	42.3	2.68 (m)	87.9	4.01 (m)
9	73.0	4.04 (m); 3.75 (m)	60.7	3.80 (m); 3.38 (d, 9.4)
1′	139.9		129.3	
2′	102.5	6.60 (s)	154.6	
3′	152.9		93.9	6.20 (s)
4′	144.0		155.1	
5′	152.9		93.9	6.20 (s)
6′	102.5	6.60 (s)	154.6	
7′	82.8	4.86 (d, 6.0)		
8′	52.5	2.39 (m)		
9′	60.6	3.57 (m); 3.33 (m)		
1″	132.1			
2″	109.7	6.95 (brs)		
3″	146.3			
4″	145.3			
5″	114.2	6.85 (d, 8.0)		
6″	120.3	6.93 (overlap)		
7″	74.0	5.00 (d, 8.7)		
8″	88.9	3.89 (m)		
9″	60.8	3.93 (m); 3.77 (m)		
3-OMe	55.91	3.88 (s)	56.1	3.81 (s)
2′-OMe			56.1	3.78 (s)
3′-OMe	56.1	3.88 (s)		
5′-OMe	56.1	3.88 (s)		
6′-OMe			56.3	3.78 (s)
3″-OMe	55.88	3.88 (s)		

control. Reaction mixtures containing an EtOH solution of 200 μM DPPH (100 μl) and twofold serial dilutions of the sample (dissolved in 100 μl of EtOH, with sample concentrations in the range of 2–1000 μg/ml) were placed in a 96-well microplate and incubated at 37 °C for 30 min. After incubation, the absorbance was read at 517 nm with an Emax Precision Microplate Reader, and the means of three readings were calculated. Scavenging activity was calculated with the equation:

$$\%\,\text{scavenging activity} = [(\text{absorbance}_{\text{control}} - \text{absorbance}_{\text{sample}})/\text{absorbance}_{\text{control}}] \times 100$$

The SC_{50} value is the concentration of sample required to scavenge 50% of the DPPH radicals and was obtained by extrapolation from a linear regression analysis.

2.5. FRAP assay

The FRAP assay was performed according to a previously reported method (Pulido, Bravo, & Saura-Calixto, 2000), with slight modification. Briefly, the FRAP reagent was prepared from acetate buffer (pH 3.6), 10 mM TPTZ solution, and 20 mM iron (III) chloride solution in proportions of 10:1 (v:v), respectively. The FRAP reagent was prepared fresh daily and was warmed to 37 °C in a water bath before use. The samples (100 μl) were added to 4 ml of FRAP reagent. The absorbance of the reaction mixture was recorded after 10 min at 593 nm. A standard curve was constructed using 0.2, 0.4, 0.6, 0.8, and 1.0 ml of Trolox (1 mM). All the measurements were made in triplicate and the mean values were calculated.

2.6. Antioxidant assay using the β-carotene linoleate model system

The antioxidant activity of the compounds was evaluated with the β-carotene linoleate model system (Miller, 1971). A solution of β-carotene was prepared by dissolving 2 mg of β-carotene in 10 ml of chloroform. A 2 ml sample of this solution was pipetted into a 100 ml round-bottomed flask. After the chloroform was removed under vacuum, 40 mg of purified linoleic acid, 400 mg of Tween 40 emulsifier, and 100 ml of aerated distilled water were added to the flask with vigorous shaking. Aliquots (3.9 ml) of this emulsion were transferred into different test tubes containing the same concentrations of the phenolic compounds (0.025 mg/

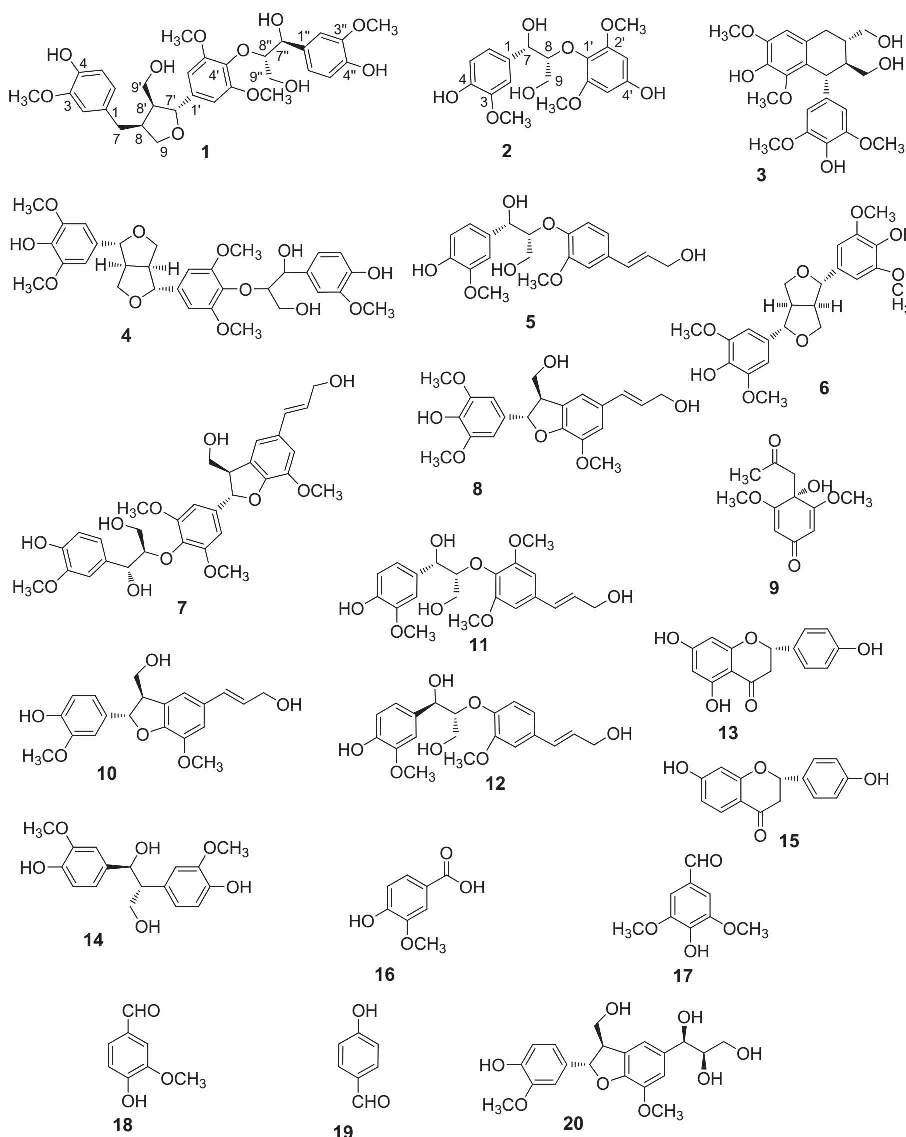

Fig. 1. Structures of compounds **1–20**.

ml, final concentration). Tertiary butylhydroquinone (TBHQ; 0.025 mg/ml) was used for comparative purposes. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm on a spectrophotometer (751-GW, China). The tubes were placed at 50 °C in a water bath. The measurement of absorbance was continued until the colour of the β-carotene had disappeared; a blank, devoid of β-carotene, was prepared for background subtraction. The antioxidant activity (AA) was calculated with the equation:

$$AA = (\beta\text{-carotene content after 2 h of assay}/\beta\text{-carotene content}) \times 100$$

2.7. Inhibition of nitric oxide production in lipopolysaccharide-stimulated RAW 264.7 macrophage cell line

The assay was performed according to a previously described method (Dirsch, Stuppner, & Vollmar, 1998). Each compound was dissolved in DMSO and further diluted in the medium to produce different concentrations, with a maximum concentration of 25 μM. The absorbance was measured at 570 nm with a 2104 Envision Multilabel Plate Reader (Perkin–Elmer Life Sciences, Inc., Boston, MA, USA). Cytotoxicity was determined with the MTT assay. MG-132 (Sigma–Aldrich, USA) was used as the positive control.

3. Results and discussion

3.1. Structure elucidation of compounds

The fruit of *R. laevigata* was refluxed with 80% EtOH. The EtOH extract was submitted to the consequent chromatographic separation on columns with silica gel, C_{18} reverse-phase silica gel, MCI gel CHP 20P, Sephadex LH-20 and semi-preparative HPLC to afford compounds **1–20** (Fig. 1).

Known compounds were each identified as polystachyol (**3**) (Samir, Panadda, Shahabuddin, Takashi, & Masami, 2006), buddlenol C (**4**) (Houghton, 1985), *erythro*-guaiacylglycerol-β-*O*-4′-coniferyl ether (**5**) (Samir et al., 2006), diasyringaresinol (**6**) (Guo, Wang, & Yao, 2006), buddlenol B (**7**) (Houghton, 1985), (−)-simulanol (**8**) (Hong et al., 2006), 4-acetonyl-3,5-dimethoxy-*p*-quinol (**9**) (Wu, Yang, Wu, & Liu, 1995), (+)-8-hydroxypinoresinol (**10**) (Yeo, Chin, Park, & Kim, 2004), *erythro*-guaiacylglycerol-β-*O*-4′-sinapyl ether (**11**) (Liao, Wu, & Yue, 2006), *threo*-guaiacylglycerol-β-*O*-4′-coniferyl ether (**12**) (Samir et al., 2006), dihydroapigenin (**13**) (Feng, Wang, & Zheng, 2008), 1,2-(1*S*,2*R*)-bis(4-hydroxy-3-methoxyphenyl)-1,3-propanediol (**14**) (Kazuko, Noriko, & Shigenobu, 1998), liquiritigenin (**15**) (Sun, Zhao, Liang, & Tu, 2010), and euscaphic acid (**20**) (Chakravarty, Mukhopadhyay, Saha, & Pakrashi, 1996; Deyama et al., 1987) by comparison of their ^{1}H, ^{13}C NMR, and MS data with those reported in the literatures. Simple compounds 4-hydroxy-3-methoxybenzoic acid (**16**), syringaldehyde (**17**), vanillin (**18**), and 4-hydroxybenzaldehyde (**19**) were directly identified by NMR and MS experiments, and by TLC comparison with authentic samples available in our laboratory. It was noted that all the known compounds were isolated from the fruits for the first time.

Compound **1** had the molecular composition of $C_{31}H_{38}O_{11}$ derived from its HRESIMS, ^{13}C NMR and DEPT spectra. The IR spectrum showed absorptions for hydroxy (3425 cm^{-1}) and aromatic (1595, 1516, 1463, 1427 cm^{-1}) groups. The UV spectrum displayed absorptions at 280 and 240 nm. The ^{1}H NMR spectrum showed four methoxy groups, two 1,3,4-trisubstituted benzene rings and one 1,3,4,5-substituted benzene. Four methoxy groups, 27 carbons were observed in the ^{13}C NMR spectrum. The above data implied that **1** is a sesquilignan derivative. Comparison of its NMR data with those of *erythro*-guaiacylglycerol-β-*O*-4′(5′)-methoxylariciresinol (**1a**) disclosed their extreme similarity (Han, Liu, Wang, & Yao, 2008). The only difference was that the coupling constant for H-7″ of **1** (8.7 Hz) is larger than that of **1a** (5.1 Hz), indicating the configuration difference. Comparison with literature data allowed the assignment of *threo*-relationship (Kazuko et al., 1998; Liao et al., 2006; Mei et al., 2009). ^{1}H–^{1}H COSY, HMQC, and HMBC correlations (Fig. 2) confirmed the structure and allowed the unambiguous assignment of its NMR data. Furthermore, the ROESY correlations of H-8/H-8′, H-7′/H-9b, and H-8/H-9a implied *cis*-orientation of H-8 and H-8′ and *trans*-configuration between H-7′ and H-8′, which also conformed to $J_{7',8'}$ value of 6.0 Hz. Therefore, the structure of **1** was determined to be *threo*-guaiacylglycerol-β-*O*-4′(5′)-methoxyllariciresinol, and named rosalaevin A.

The molecular formula of compound **2** was determined to be $C_{18}H_{22}O_8$ on the basis of its HRESIMS, ^{13}C NMR and DEPT spectra. The IR spectrum absorptions at 3431, 1603, 1509, 1480, 1465 and 1432 cm^{-1} indicated the presence of hydroxy and aromatic functionalities. The UV spectrum showed absorptions at 280 nm. The ^{1}H and ^{13}C NMR spectroscopic data revealed two benzene rings. The coupling patterns of the aromatic protons suggested a 1,3,4-trisubstituted benzene and a 1,2,4,6-tetrasubstituted or a 1,3,4,5-tetrasubstituted benzene ring. Three methoxyl, one oxymethylene and two oxymethine were observed. The ^{1}H–^{1}H COSY spectrum showed correlations of H-5/H-6, and H-7/H-8/H-9. HMBC correlations of H-7/C-1, H-2, 3-CH_3O/C-3, and ROESY correlation of CH_3O/OH-2 (δ 8.79 in DMSO-d_6)/H-3 suggested the linkage of C-7 to C-1 and the substitution pattern of this benzene ring. ROESY correlations of OH-4′ (δ 9.27) measured in DMSO-d_6 with H-3′, and HMBC correlation of OH-4′ with C-3′ clarified the substituted pattern in the symmetric benzene ring. The linkage of C-8-*O*-C-1′, which is typical for neolignans, was supported by a weak HMBC correlation of H-9 (δ 3.91)/C-1′ (δ 129.3) when detected in DMSO-d_6 with a parameter of $J_{H,C}$ value of 4 Hz. The above HMBC correlation of OH-4′ with C-3′ and the down field shift of C-8 (δ 87.9) also supported this conclusion. The $J_{7,8}$ value of 5.1 Hz indicated the *erythro*-configuration of **2** (Kazuko et al., 1998; Liao et al., 2006; Mei et al., 2009). As a result, compound **2** was assigned as *erythro*-2-(4-hydroxy-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-1,3-propanediol, with the trivial name rosalaevin B.

3.2. Evaluation of the antioxidant properties of the compounds

Shui-Lu-Er-Xian-Dan, consisting of *E. ferox* seeds and *R. laevigata* fruits, is commonly used to treat kidney diseases, including proteinuria. Oxidative stress is one of the general pathological processes in kidney disease, thus we assessed the antioxidant properties of the compounds in the fruit of *R. laevigata*. Three methods were used, DPPH and FRAP assays, and a β-carotene linoleate model system. Our results show that compounds **1–3**, **6–8**, and **14** exhibited strong effects against the DPPH radical, with SC_{50} values of 20.8, 17.8, 7.9, 10.8, 14.9, 23.2, and 10.9 μM, respectively (Table 2), which were stronger than or similar to that of the positive control (L-ascorbic acid). The stronger activity of compound **11** compared to compounds **5** and **12** implies the importance of the OMe-2′ moiety in enhancing the compound's radical-scavenging capacity. Although compounds **8** and **10** are

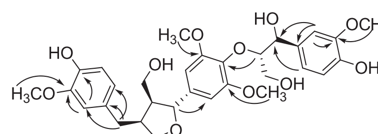

Fig. 2. Key HMBC correlations for compound **1**.

structurally similar to one another, the difference in their activities may result from the contribution of the OMe-5 moiety in the molecule of **8**. In the FRAP assay, the compounds that showed better antioxidant capacities were **6** (150.7 μM Trolox/g), 3 (145.3 μM Trolox/g), and **14** (108.6 μM Trolox/g; Table 2), in accordance with their antioxidant potencies established in the DPPH assay. As shown in Table 2, compounds **3** and **6** were most active in the β-carotene linoleate model system, and notably, the antioxidant potency of compound **6** was equivalent to that of TBHQ, the positive control used in this study. The data from these three antioxidant assays clearly show that compound **6** has good antioxidant activity, suggesting that plants with high compound **6** contents can be considered potential sources of natural antioxidants.

The role of nitric oxide in DN is complex. Most studies have concluded that increased nitric oxide release contributes to the hyperfiltration and microalbuminuria that characterizes early DN. Conversely, several factors, including hyperglycemia, AGEs, oxidative stress, and the activation of protein kinase C, may reduce nitric oxide production in advanced nephropathy, causing severe proteinuria. In this sense, the negative effects of the compounds (IC_{50} values > 25 μM, data not shown) in the nitric oxide production assay may be beneficial for advanced nephropathy (Prabhakar, 2004).

The results discussed above partly explain the effects of Shui-Lu-Er-Xian-Dan on reducing proteinuria, which are probably related to the antioxidant potential of many components of the *R. laevigata* fruit. This suggests that this edible fruit is a good source of natural antioxidants, which can protect human health, especially against DN.

Acknowledgments

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Table 2
Antioxidant activities of the selected isolates[a].

Sample	DPPH (SC_{50} μM)[c]	μM Trolox/g[d]	AA (%)[e]
1	20.8 ± 1.26	81.4 ± 3.2	12.6 ± 0.41
2	17.8 ± 0.94	76.8 ± 3.6	1.82 ± 0.17
3	7.9 ± 0.27	145.3 ± 7.1	14.4 ± 0.62
4	n.a.[b]	91.2 ± 2.9	7.6 ± 0.17
5	33.8 ± 1.42	55.1 ± 2.2	4.3 ± 0.13
6	10.8 ± 0.68	150.7 ± 4.9	18.4 ± 0.72
7	14.9 ± 0.82	70.5 ± 1.8	9.8 ± 0.45
8	23.2 ± 1.90	89.4 ± 3.2	9.0 ± 0.38
9	n.a.[b]	30.5 ± 1.3	1.8 ± 0.25
10	38.0 ± 2.59	39.0 ± 2.4	4.3 ± 0.18
11	28.1 ± 1.1.2	49.5 ± 1.7	4.3 ± 0.27
12	30.1 ± 1.95	63.3 ± 2.1	6.1 ± 0.12
13	n.a.	34.4 ± 2.0	4.0 ± 0.12
14	10.9 ± 0.70	108.6 ± 5.4	9.8 ± 0.23
15	n.a.[b]	28.6 ± 1.3	5.4 ± 0.13
16	53.6 ± 4.10		
17	n.a.[b]		
18	250.4 ± 16.35		
19	n.a.[b]		
20	80.1 ± 3.26	31.5 ± 1.2	5.4 ± 0.16
Ascorbic acid	22.2 ± 0.87		
TBHQ			19.9 ± 0.82

[a] Means ± SD ($n = 3$).
[b] n.a. - no activity.
[c] DPPH radical scavenging activity.
[d] Ferric reducing antioxidant power.
[e] Antioxidant activity (AA) in a β-carotene linoleate model system.

Appendix A. Compound name

Polystachyol (**3**)
Buddlenol C (**4**)
Erythro-guaiacylglycerol-β-*O*-4′-coniferyl ether (**5**)
Diasyringaresinol (**6**)
Buddlenol B (**7**)
(−)-Simulanol (**8**)
4-Acetonyl-3,5-dimethoxy-*p*-quinol (**9**)
(+)-8-Hydroxypinoresinol (**10**)
Erythro-guaiacylglycerol-β-*O*-4′-sinapyl ether (**11**)
Threo-guaiacylglycerol-β-*O*-4′-coniferyl ether (**12**)
Dihydroapigenin (**13**)
1,2-(1*S*,2*R*)-Bis(4-hydroxy-3-methoxyphenyl)-1,3-propanediol (**14**)
Liquiritigenin (**15**)
4-Hydroxy-3-methoxybenzoic acid (**16**)
Syringaldehyde (**17**)
Vanillin (**18**)
4-Hydroxybenzaldehyde (**19**)
Euscaphic acid (**20**)

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foodchem.2011.07.076.

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