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Identification of antioxidants from rhizome of *Davallia solida*

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Abstract

Davallia solida rhizome has long been used as an herb tonic to treat osteoporosis, arthralgia, and arthritis. The aqueous extract of *D. solida* rhizome contains a high content of phenolic compounds [210.8 ± 4.6 mg catechin equivalents (CE)/g dry weight] and shows a strong 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity ($IC_{50} = 15.93 \pm 1.21$ μg dry weight/ml). Further solvent partition of the aqueous extract yielded chloroform, *n*-butanol, and water layers. Among them, *n*-butanol layer has the highest phenol content (806.3 ± 12.3 mg CE/g dry weight) and DPPH scavenging potential ($IC_{50} = 3.93 \pm 0.31$ μg dry weight/ml). Isolation and purification from the *n*-butanol layer identified 12 compounds. They included four new compounds: 3′-*O*-*p*-hydroxybenzoylmangiferin (**1**), 4′-*O*-*p*-hydroxybenzoylmangiferin (**2**), 6′-*O*-*p*-hydroxybenzoylmangiferin (**3**), and 3-*O*-*p*-hydroxybenzoylmangiferin (**4**); as well as eight known compounds: mangiferin (**5**), 2-*C*-β-D-xylopyranosyl-1,3,6,7-tetrahydroxyxanthone (**6**), 4β-carboxymethyl-(−)-epicatechin (**7**), 4β-carboxymethyl-(−)-epicatechin methyl ester (**8**), eriodictyol (**9**), eriodictyol-8-*C*-β-D-glucopyranoside (**10**), icariside E_5 (**11**), and icariside E_3 (**12**). DPPH scavenging and Trolox equivalent antioxidant capacity (TEAC) analyses revealed that the most potent antioxidants are **1**, **2**, and **3**, which exerted more than triple activity as compared with the positive controls, α-tocopherol and Trolox.
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Keywords: *Davallia solida*; DPPH; Trolox equivalent antioxidant capacity (TEAC)

1. Introduction

Davallia solida (Forst.) Sw. (Davalliaceae), which distributes widely in Malaya, Philippines, Taiwan, and Polynesia, is an epiphyte with creeping rhizome (Li, Liu, Huang, Koyama, & Devol, 1993). Traditionally, the dried rhizomes of *D. solida* are used as soup ingredient and can be found in nearly every healthy food store and herbal shop in Taiwan. Rhizome of this fern together with that of the same genus, *Davallia divaricata* and *Davallia mariesii*, have traditionally been used as substitutes for *Drynariae Rhizoma* [*Drynaria fortunei* (kunze) J. Sm; Gu-Sui-Bu in Chinese] for the treatment of osteoporosis, arthralgia, and arthritis.

It has been recognized that osteoporosis, arthralgia, and arthritis are inflammatory diseases and may be induced by oxidative stress (Geronikaki & Gavalas, 2006; Yalin et al., 2005). Consequently, the traditional function of *D. solida* may result from its anti-inflammatory or antioxidant activity. Previous studies on this genus plants have reported the isolation of xanthones, flavonoids, and triterpenes (Cui et al., 1990; Cui et al., 1992). 2-*C*-β-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone (mangiferin) and 2-*C*-β-D-xylopyranosyl-1,3,6,7-tetrahydroxyxanthone as well as their precursor, 4-*O*-β-D-glucopyranosyl-2,6,4′-trihydroxybenzophenone, have been isolated from *D. solida* (Rancon et al., 1999, 2001). These three compounds are potential

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modulators of *P*-glycoprotein that rejects chemotherapeutic drugs out of the cell (Rancon et al., 2001; Tchamo et al., 2000). It has also been demonstrated that 6,7-dihydroxyl xanthones are strong antioxidants and cause apoptosis in human cancer cell lines (HT-29, HL-60, SK-OV3, AGS, and A549) (Lee et al., 2005). Although there is a wide application of *D. solida*, no systematic report regarding to its antioxidant components could be found. The aim of this study is to search for the antioxidant principals using the total phenol content and the DPPH scavenging activity as index.

2. Materials and methods

2.1. General procedures

Optical rotations were measured on a JASCO DIP-370 digital polarimeter. UV spectra were obtained on a Hitachi 220-20 spectrophotometer. IR spectra were measured on a Hitachi 260-30 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Inova 500, Varian Unity Plus 400 MHz, or Varian Gemini 200 MHz spectrometer using TMS as an internal standard. Chemical shifts were reported in parts per million (δ) and coupling constants (J) were expressed in Hertz. LR-EI-MS were collected on a Bruker APEX II mass or a Quattro GCMS spectrometer having a direct inlet system. LR-ESI-MS and HR-ESI-MS were measured on a Bruker APEX II mass spectrometer. Purospher STAR RP-18e (Merck KGaA, Darmstadt, Germany), silica gel 60 (230–400 mesh, Merck), sephadex LH-20 (GE Healthcare UK Ltd, Buckinghamshire, England), and Diaion HP20SS (Mitsubishi Chemical Co., Japan) were used for column chromatography. Spots were detected by spraying TLC with 50% H_2SO_4 followed by heating on a hot plate.

2.2. Plant

D. solida was obtained from the Taitung District Agricultural Research and Extension Station, Taitung, Taiwan in December 2004. A voucher specimen (DS001) was deposited in the Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung, Taiwan.

2.3. Extraction and fractionation

Rhizomes (8.0 kg) of *D. solida* were cut into small pieces and extracted with boiling water (3 × 20 l). A small portion (100 ml) of the combined aqueous extract was lyophilized to yield a dark-brown powder (32.1 mg), which was redissolved in deionized water to make stock solution (100 mg/ml) prior to use. It was denoted as the crude aqueous extract.

The rest of the aqueous extract was concentrated to a small volume (ca. 2 l) and partitioned with chloroform (1 l × 3) to yield the chloroform and the aqueous layers. The resulting aqueous layer was further partitioned with *n*-butanol (1 l × 3) to yield the *n*-butanol and the aqueous layers.

2.4. Total phenol content

Total phenol content was determined by the Folin–Denis method (Waterman & Mole, 1994). 850 μl of crude aqueous extract or subsequent layer, which was previously diluted with water, was mixed with 50 μl of Folin–Denis reagent (Sigma-Aldrich Co., St. Louis, MO, USA). 100 μl of saturated sodium carbonate was then added to the mixture. After exposing the mixture for 30 min at 50 °C and cooling down to room temperature, the amount of absorbance was measured at 760 nm. (+)-Catechol (Fluka Chemie, Bushs, Switzerland) was used as an analytical standard for total phenol quantification. Reference solutions were prepared in water from stock solution (100 μg/ml) to contain 0–50 μg/ml of catechol. The contents of phenolic compounds in crude extract and various layers were expressed as mg catechin equivalents (CE)/g dry weight.

2.5. Purification of compounds

The *n*-butanol layer (ca. 3 l) was evaporated under reduced pressure to yield a dried mixture (ca. 50.0 g). It was then passed through a Diaion HP20SS column (40 × 10 cm) and eluted with water–methanol mixtures (at ratios of 1:0, 1:3, 1:1, 3:1 and 0:1 (v/v), 4 l/each) to give five fractions, A1–A5, respectively. The obtained Fr. A4 (dry weight 9.6 g) was further passed through a silica gel (500 g) and eluted with a chloroform–methanol mixture [at a ratio 4:1 (v/v), 3 l] followed by preparative reverse-phase HPLC [Purospher, 20 × 250 mm, methanol:water = 1:3 (v/v), flow rate 3 ml/min] to yield compounds **1** (37.5 mg), **2** (27.1 mg), **3** (20.0 mg), and **4** (11.2 mg).

Compound **5** (ca. 300 mg) was obtained by re-crystallization of the Fr. A3 (dry weight 6.7 g) with methanol. The rest of Fr. A3 was then passed through preparative reverse-phase HPLC [Purospher, 20 × 250 mm, methanol:water = 1:1 (v/v), flow rate 3 ml/min] to yield compounds **6** (ca. 50 mg),**11** (11.8 mg), and **12** (10.0 mg).

Fr. A5 (dry weight 4.2 g) was chromatographed on Sephadex LH-20 (600 g) and eluted with 700 ml of methanol followed by preparative reverse-phase HPLC [Purospher, 20 × 250 mm, methanol:water = 1:1 (v/v), flow rate 3 ml/min] to yield compounds **7** (30.2 mg), **8** (21.2 mg), **9** (10.8 mg), and **10** (12.0 mg).

2.6. Acid hydrolysis of compounds 1–4

A solution of each compound (3.0 mg) in 6% aqueous HCl (3.5 ml) was refluxed for 2 h. The reaction mixture was diluted with water and then extracted with ethyl acetate. The resulting mangiferin (in water) and *p*-hydroxybenzoic acid (in ethyl acetate) were identified by their ^{1}H NMR spectra.

2.7. DPPH scavenging capacities

The crude aqueous extract, different layers and isolated pure compounds were evaluated for their activities to scavenge the stable DPPH radical (0.1 mM, Sigma) according to the method (Dinis, Maderia, & Almeida, 1994). The affinity of the test material to quench the DPPH free radical was evaluated according to the equation: scavenging $\% = (A_c - A_s)/A_c \times 100\%$. A_s and A_c are absorbance at 517 nm of the reaction mixture with sample and control, respectively. The IC_{50} values were obtained through extrapolation from linear regression analysis and denoted the concentration of sample required to scavenge 50% of DPPH radicals. All experiments were repeated at least three times.

2.8. Trolox equivalent antioxidant capacity (TEAC) analysis

The ABTS radical cation was prepared by mixing an ABTS stock solution (7 mM in water) with 2.45 mM potassium persulfate. This mixture has to remain for 12–24 h until the reaction is complete and the absorbance is stable. For measurements, the $ABTS^{2+}$ solution was diluted to an absorbance of 0.700 ± 0.020 at 734 nm. 1 ml of the $ABTS^{2+}$ solution and 100 μl antioxidant solution were mixed for 45 s and the absorbance at 734 nm was recorded after 1 min of incubation. TEAC is defined as the concentration (mM) of Trolox having the antioxidant equivalent to a 1.0 mM of the compound under investigation. To calculate the TEAC, the gradient of the plot of the percentage inhibition of absorbance vs. concentration plot for the antioxidant in question is divided by the gradient of the plot for Trolox (Re et al., 1999).

3. Results and discussion

3.1. The total phenol contents and DPPH scavenging potentials of the crude extract and different layers

The total phenol contents of the crude aqueous extract of *D. solida* rhizome (DS), as well as those of the chloroform, *n*-butanol, and water layers are 210.8 ± 4.6, 31.7 ± 1.0, 806.3 ± 12.3, and 34.7 ± 0.9 mg CE/g dry weight, respectively, as determined by the Folin–Denis reagent. The IC_{50} values for DPPH scavenging of DS, chloroform, *n*-butanol, and water layers are 15.93 ± 1.21, >200, 3.93 ± 0.31, and >200 μg dry weight/ml of reaction mixture, respectively. The total phenol content correlates well with DPPH scavenging activity indicating that the antioxidant compounds of DS are mainly polyphenolic compounds and exist in *n*-butanol layer.

*3.2. Identification of compounds **1–12** from the n-butanol layer*

Twelve phenolic compounds were further isolated from the *n*-butanol layer as described in Section 2.5. Their structures were determined as 3′-*O*-*p*-hydroxybenzoylmangiferin (**1**), 4′-*O*-*p*-hydroxybenzoylmangiferin (**2**), 6′-*O*-*p*-hydroxybenzoylmangiferin (**3**), 3-*O*-*p*-hydroxybenzoylmangiferin (**4**), mangiferin (**5**), 2-*C*-β-D-xylopyranosyl-1,3,6,7-tetrahydroxyxanthone (**6**), 4β-carboxymethyl-(−)-epicatechin (**7**), 4β-carboxymethyl-(−)-epicatechin methyl ester (**8**), eriodictyol (**9**), eriodictyol-8-*C*-β-D-glucopyranoside (**10**), icariside E_5 (**11**), and icariside E_3 (**12**). Compounds **1–4** are new compounds, while compounds **9–12** are identified for the first time from the genus *Davallia*. Their structures are shown in Fig. 1.

3′-*O*-*p*-Hydroxybenzoylmangiferin (**1**): $C_{26}H_{22}O_{13}$, pale yellow powder, LR-ESI-MS *m/z*: 565 $[M+Na]^+$, HR-ESI-MS *m/z*: 565.0957 (calcd for $C_{26}H_{22}O_{13}Na^+$, 565.0958), $[\alpha]_D^{25}$: −45° (c 0.36, MeOH), UV λ_{max}^{MeOH} (log ε): 256 (3.8), 338 (3.5), 402 (3.0) nm, IR ν_{max} (neat): 3410, 1647, 1700 cm^{-1}, ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) are given in Tables 1 and 2.

Acid hydrolysis of **1** yielded two hydrolysis products (mangiferin and *p*-hydroxybenzoic acid), which were identified by ^1H and ^{13}C NMR spectroscopy. The HR-ESI-MS spectrum of **1** showed a sodium-adduct molecular ion at *m/z*: 565.0957 $[M+Na]^+$ corresponding to the molecular formula, $C_{26}H_{22}O_{13}$. The IR spectrum indicated the presence of two carbonyl groups (1647 and 1700 cm^{-1}). The ^1H NMR shifts of **1** revealed typical signals of the AA′BB′ system of the *p*-hydroxybenzoyl group [δ 6.85 (2 H, d, $J = 8.6$ Hz) and 7.95 (2H, d, $J = 8.6$ Hz)], together with [δ 6.35 (1H, s), 6.79 (1H, s) and 7.42 (2H, s)] and the signals of the glucopyranose (Table 1). In addition, the structure of the glucose skeleton was assigned by a combination of COSY, HMQC, and HMBC experiments. Starting from the anomeric proton of the sugar unit (δ 5.26; H-3′), all proton signals could be identified (Table 1). The HMBC correlation of H-3″ and C-7″ (δc 168.3) indicated that mangiferin is benzoylated at C-3′. Furthermore, the HMBC spectrum of **1** showed obvious correlations between H-1′/C-1, H-1′/C-2, and H-1′/C-3. This evidence confirmed that the glucose is *C*-linked to the mangiferitin nucleus at C-2. This was also supported by the hydrolysis products mangiferin and *p*-hydroxybenzoic acid. Thus, the structure of **1** was unambiguously defined as 2-(3′-*O*-*p*-hydroxybenzoyl)-*C*-β-D-glucopyranosyl-1,3,6,7-tetrahydroxanthone, a new compound, which we named 3′-*O*-*p*-hydroxybenzoylmangiferin.

4′-*O*-*p*-Hydroxybenzoylmangiferin (**2**): $C_{26}H_{22}O_{13}$, pale yellow powder, LR-ESI-MS *m/z*: 565 $[M+Na]^+$, HR-ESI-MS *m/z*: 565.0961 (calcd for $C_{26}H_{22}O_{13}Na^+$, 565.0958), $[\alpha]_D^{25}$: −80°(c 0.30, MeOH), UV λ_{max}^{MeOH} (log ε): 255 (3.9), 339 (3.8), 401 (3.2) nm, IR_{max} (neat): 3410, 1651, 1701 cm^{-1}, ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) are given in Tables 1 and 2.

6′-*O*-*p*-Hydroxybenzoylmangiferin (**3**): $C_{26}H_{22}O_{13}$, pale yellow powder, LR-ESI-MS *m/z*: 565 $[M+Na]^+$, HR-ESI-MS *m/z*: 565.0959 (calcd for $C_{26}H_{22}O_{13}Na^+$, 565.0958), $[\alpha]_D^{25}$: −65° (c 0.55, MeOH), UV λ_{max}^{MeOH} (log ε): 256 (3.8), 338 (3.5), 402 (3.1) nm, IR ν_{max} (neat): 3410, 1652, 1708 cm^{-1}, ^1H NMR (CD_3OD, 400 MHz) and ^{13}C

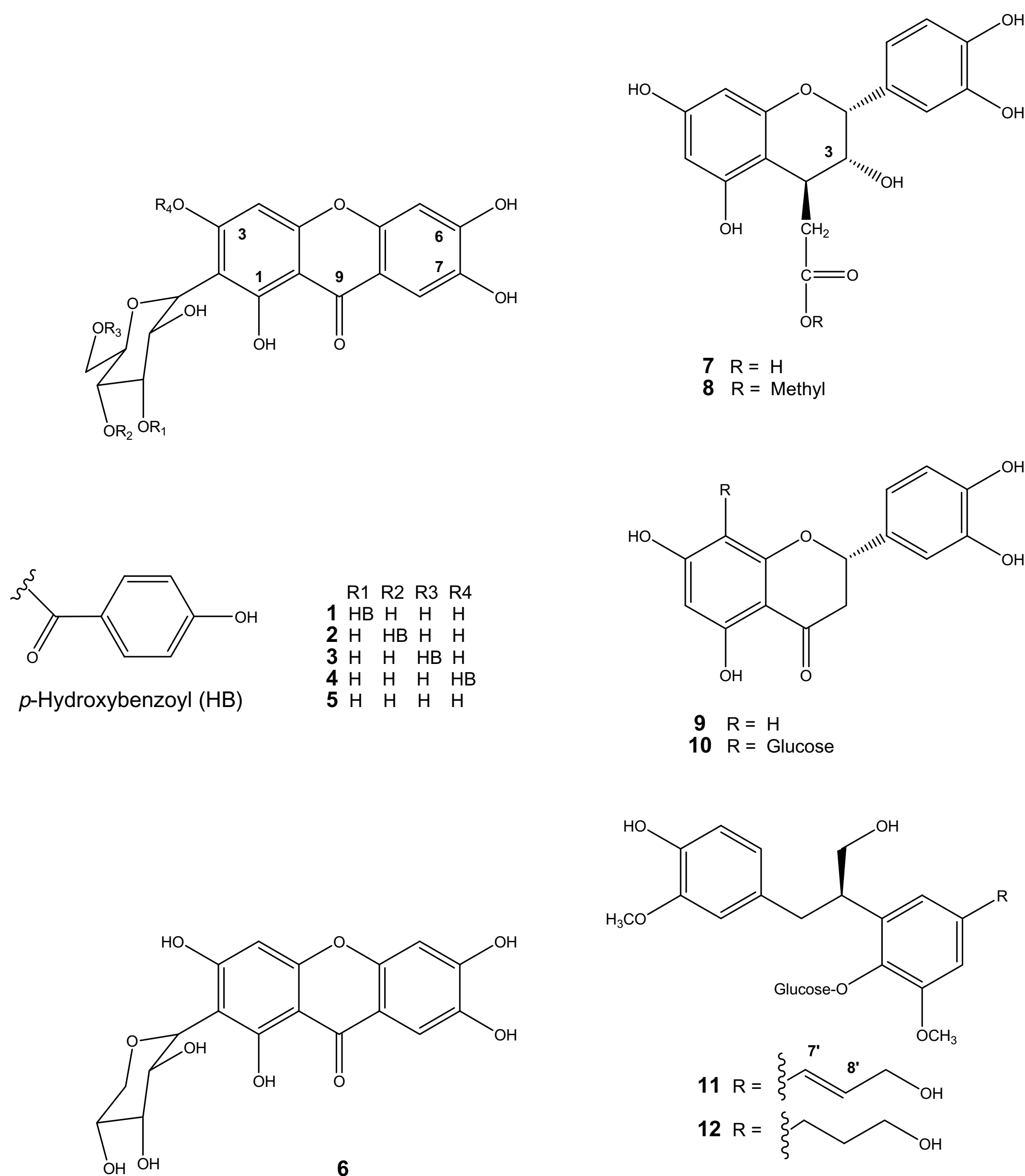

Fig. 1. Structures of compounds **1–12** isolated from rhizome of *D. solida*.

Table 1
^{1}H NMR (400 MHz) spectral data of compound **1–4**[a] in CD_3OD

Postition	1	2	3	4
4	6.35 (1H, s)	6.36 (1H, s)	6.33 (1H, s)	6.25 (1H, s)
5	6.79 (1H, s)	6.80 (1H, s)	6.76 (1H, s)	6.75 (1H, s)
8	7.42 (1H, s)	7.43 (1H, s)	7.39 (1H, s)	7.37 (1H, s)
1′	5.06 (1H, d, 9.6)	5.01 (1H, d, 9.6)	4.98 (1H, d, 9.6)	5.18 (1H, d, 9.6)
2′	4.53 (1H, t, 9.6)	4.42 (1H, m)	4.28 (1H, m)	4.60 (1H, m)
3′	5.26 (1H, t, 9.6)	3.79 (1H, t, 9.6)	3.72 (1H, m)	3.78 (1H, t, 9.2)
4′	3.74 (1H, m)	5.14 (1H, t, 9.6)	3.63 (1H, t, 9.2)	3.63 (1H, t, 9.2)
5′	3.56 (1H, m)	3.73 (1H, m)	3.54 (1H, t, 9.2)	3.50 (1H, m)
6a′	3.79 (1H, m)	3.58 (1H, m)	4.50 (1H, dd, 12.4, 4.4)	3.80 (1H, m)
6b′	3.91 (1H, m)	3.63 (1H, m)	4.57 (1H, dd, 12.4, 4.4)	3.93 (1H, m)
2″/6″	6.83 (1H, d, 8.8)	6.85 (1H, d, 8.8)	6.81 (1H, d, 8.8)	6.69 (1H, d, 8.8)
3″/5″	7.97 (1H, d, 8.8)	7.95 (1H, d, 8.8)	7.90 (1H, d, 8.8)	7.71 (1H, d, 8.8)

[a] Assignments confirmed by decoupling, ^{1}H-^{1}H COSY, NOESY, HMQC and HMBC.

Table 2
^{13}C NMR (100 MHz) spectral data of compound **1**–**4**[a] in CD_3OD

Position	1	2	3	4
1	163.4	163.5	163.3	163.4
2	107.4	107.5	107.3	106.4
3	165.2	165.3	165.2	153.2
4	94.8	94.7	94.8	102.9
4a	158.8	158.8	158.1	158.8
4b	155.5	155.9	155.6	155.6
5	103.2	103.4	103.4	103.4
6	153.1	153.2	153.1	153.1
7	144.9	145.1	144.9	144.9
8	109.1	108.9	109.0	109.0
8a	113.1	113.6	113.6	113.6
8b	103.2	103.2	103.2	103.4
9 (C=O)	181.2	181.3	181.2	181.1
1′	75.3	75.3	75.6	74.0
2′	70.7	72.5	72.5	73.2
3′	81.5	78.1	79.7	78.1
4′	70.2	73.0	71.8	71.8
5′	82.6	80.9	79.9	82.9
6′	62.7	62.8	64.8	62.8
1″	122.7	122.1	122.2	122.2
2″/6″	133.0	133.1	132.9	132.8
3″/5″	116.0	116.2	116.1	115.8
4″	163.4	163.7	163.5	163.3
7″ (O—C=O)	168.3	167.6	168.2	167.4
Key HMBC	C-7″/H-3′	C-7″/H-4′	C-7″/H-6′	C-7″/H-3

[a] Assignments confirmed by decoupling, ^1H-^1H COSY, NOESY, HMQC and HMBC.

NMR (CD_3OD, 100 MHz) are given in Tables 1 and 2.

The molecular formulas of **2** and **3**, $C_{26}H_{22}O_{13}$, were established by HR-ESI-MS (**2**: $[M+Na]^+$ *m/z* 565.0961, **3**: $[M+Na]^+$ *m/z* 565.0959, calcd 565.0958). The ^1H NMR and ^{13}C NMR spectroscopic data of **2** and **3** were similar to those of **1** (Tables 1 and 2). Homologous to **1** the acid hydrolysis products of **2** and **3** were also mangiferin and *p*-hydroxybenzoic acid. The only difference between these three compounds is the substitution locus of the *p*-hydroxybenzoyl group at the glucose moiety. A combination of COSY and HMQC spectrum analysis identified the acylated glucopyranoses. The typical ^{13}C NMR acylation shift is about 2 ppm (Table 2), and the unusual high value of the chemical shift of H-4′ (5.14 ppm, **2**) and H-6′ (4.50 and 4.57 ppm, **3**), respectively, indicated the benzoylation is the *O*-linkage at C-4′ and C-6′, respectively. The above indications are further confirmed by a HMBC experiment showing the correlation of H-4′/C-7″ and H-6′/C-7″ of **2** and **3**, respectively. Thus, the proposed structures of 4′-*O*-*p*-hydroxybenzoylmangiferin (**2**) and 6′-*O*-*p*-hydroxybenzoylmangiferin (**3**) were determined unambiguously.

3-*O*-*p*-Hydroxybenzoylmangiferin (**4**): $C_{26}H_{22}O_{13}$, pale yellow powder, LR-ESI-MS *m/z*: 565 $[M+Na]^+$; HR-ESI-MS *m/z*: 565.0960 (calcd for $C_{26}H_{22}O_{13}Na^+$, 565.0958), $[\alpha]_D^{25}$: −70° (c 0.28, MeOH), UV λ_{max}^{MeOH} (log ε): 256 (3.7), 338 (3.5), 401 (3.0) nm, IR ν_{max} (neat): 3410, 1655, 1702 cm^{-1}, ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) are given in Tables 1 and 2.

Compound **4** showed a sodium-adduct molecular ion at *m/z* 565.0960 $[M+Na]^+$ by HR-ESI-MS, corresponding to the molecular formula, $C_{26}H_{22}O_{13}$. The ^1H NMR and ^{13}C NMR spectroscopic data of **4** was similar to those of **1** (Tables 1 and 2). The ^{13}C NMR shifts of **4** were confirmed by COSY, HMQC, and HMBC techniques (Table 2). The acidolysis products of **4** were mangiferin and *p*-hydroxybenzoic acid as well, but the chemical shifts of the glucose moiety did not show any acylation shift. Therefore, the benzoic acid should be bounded to the mangiferin skeleton. While an acylation shift at C-3 ($\delta_C = 153.2$) was observed, no acylation shift could be seen at C-1 ($\delta_C = 163.4$), C-6 ($\delta_C = 153.1$), or C-7 ($\delta_C = 114.9$) as shown in Table 2. A hydrogen bridge between O-1 and O-9 was observed in C_5D_5N (400 MHz). Therefore, the locus of benzoylation was C-3 and the proposed structure of **4** was unambiguously determined as 3-*O*-*p*-hydroxybenzoylmangiferin.

Mangiferin (**5**): $C_{19}H_{18}O_{11}$, yellow powder, LR-ESI-MS *m/z*: 445 $[M+Na]^+$, The ^1H NMR (400 MHz, CD_3OD) and ^{13}C NMR (CD_3OD, 100 MHz) data are consistent with the literature (Catalano et al., 1996).

2-*C*-β-D-Xylopyranosyl-1,3,6,7-tetrahydroxyxanthone (**6**): $C_{18}H_{16}O_{10}$, yellow powder, LR-ESI-MS *m/z*: 415 $[M+Na]^+$, The ^1H NMR (400 MHz, CD_3OD) and ^{13}C NMR (CD_3OD, 100 MHz) data are consistent with the literature (Rancon et al., 1999).

4β-Carboxymethyl-(−)-epicatechin (**7**): $C_{17}H_{16}O_8$, amorphous powder, ESI-MS: *m/z* 371 $[M+Na]^+$, ^1H NMR (400 MHz, CD_3OD) and ^{13}C NMR (CD_3OD, 100 MHz) data are identical to those in the literature (Hwang, Kashiwada, Nonaka, & Nishioka, 1990).

4β-Carboxymethyl-(−)-epicatechin methyl ester (**8**): $C_{18}H_{18}O_8$, amorphous powder, ESI-MS: *m/z* 385 $[M+Na]^+$, ^1H NMR (400 MHz, CD_3OD) and ^{13}C NMR (CD_3OD, 100 MHz) data are identical to those in the literature (Hwang et al., 1990).

Eriodictyol (**9**): $C_{15}H_{12}O_6$, amorphous powder, ESI-MS: *m/z* 311 $[M+Na]^+$, ^1H NMR (400 MHz, CD_3OD) and ^{13}C NMR (CD_3OD, 100 MHz) data are identical to those in the literature (Hsu & Chen, 1993).

Eriodictyol-8-*C*-β-D-glucopyranoside (**10**): $C_{21}H_{22}O_{11}$, amorphous powder, ESI-MS: *m/z* 473 $[M+Na]^+$, ^1H NMR (400 MHz, CD_3OD) and ^{13}C NMR (CD_3OD, 100 MHz) data are identical to those in the literature (Hsu & Chen, 1993).

Icariside E_5 (**11**): $C_{26}H_{34}O_{11}$, amorphous powder, ESI-MS: *m/z* 545 $[M+Na]^+$, ^1H NMR (400 MHz, CD_3OD) and ^{13}C NMR (CD_3OD, 100 MHz) data are consistent with the literature (Iorizzi et al., 2001).

Icariside E_3 (**12**): $C_{26}H_{36}O_{11}$, amorphous powder, ESI-MS: *m/z* 547 $[M+Na]^+$, ^1H NMR (400 MHz, CD_3OD) and ^{13}C NMR (CD_3OD, 100 MHz) data are consistent with the literature (Miyase, Ueno, Takizawa, Kobayashi, & Oguchi, 1988).

3.3. DPPH and ABTS radical scavenging potentials of identified compounds

Table 3 shows the DPPH scavenging activities of compounds **1**–**12** isolated from the *n*-butanol layer of *D. solida*. All of these compounds, except icariside E_3 (**12**), displayed stronger or equal DPPH scavenging activity, compared with the common antioxidant supplement, α-tocopherol. All of these compounds, except the icarisides (**11, 12**), are catecholic polyphenols. Catecholic polyphenols have been extensively exploited both because of their wide-ranging pharmacological properties and ability for donating electrons (Boots, Haenen, den Hartog, & Bast, 2002). For example, quercetin, luteolin, catechin, and anthocyanidin, which possess catechol moiety have been investigated intensively (Doss, Potta, Hescheler, & Sachinidis, 2005; Rice-Evans, Miller, & Paganga, 1996).

Table 3 also shows the Trolox equivalent antioxidant capacity (TEAC) values of compounds **1**–**12**. Among them, the strongest ABTS radical scavengers are 3′-*O*-*p*-hydroxybenzoylmangiferin (1), 4′-*O*-*p*-hydroxybenzoylmangiferin (2) and 6′-*O*-*p*-hydroxybenzoylmangiferin (3) with TEAC values about 3.5 mM. It has been demonstrated that mangiferin is widely distributed in higher plants such as the Anacardiaceae and Gentianaceae families, especially in the leaves and the bark (Catalano et al., 1996). In addition to its well-documented antioxidant properties (Dar et al., 2005; Sanchez et al., 2000; Sato, Kawamoto, Tamura, Tatsumi, & Fujii, 1992), mangiferin has a wide range of pharmacological activities including anti-diabetic (Ichiki et al., 1998), anti-HIV (Guha, Ghosal, & Chattopadhyay, 1996), anti-cancer (Yoshimi et al., 2001), and anti-inflammatory (Garrido et al., 2004). Recently, it has been found that mangiferin can increase the mitochondrial permeability transition-mediated apoptosis in cancer cells (Andreu, Delgado, Velho, Curti, & Vercesi, 2005). In our research it was found that mangiferin (**5**), its derivatives (benzoyl compounds **1**–**3**) and 2-*C*-β-D-xylopyranosyl-1,3,6,7-tetrahydroxyxanthone (**6**) were the major antioxidants in DS. They have more than double potencies of DPPH and ABTS radical scavenging activities as compared with α-tocopherol and Trolox. However, 3-*O*-*p*-hydroxybenzoylmangiferin (**4**) has a significantly lower antioxidant activity compared with other xanthone derivatives (**1**–**3** and **5**–**6**). This indicates that in addition to the catechol moiety, the free 3-hydroxyl group may also play a significant role in the free radical scavenging activity of mangiferin. The benzyol mangiferins (**1**–**3**) although exhibit compatible DPPH bleaching effects but have significantly higher ABTS scavenging activities compared with mangiferin (**5**) and 2-*C*-β-D-xylopyranosyl-1,3,6,7-tetrahydroxyxanthone (**6**). This result suggests that the hydroxybenzyol moiety may play a more important role in reducing ABTS radical than DPPH radical.

It has been suggested that flavonols with a free 3-hydroxy group are the strongest antioxidants and radical scavengers among the flavonoids (Burda & Oleszek, 2001; Hollman & Katan, 1997). In our study we showed that epicatechin derivatives (**7**, **8**) with a free 3-hydroxy group have compatible free radical scavenging compared with eriodictyols (**9**, **10**) without 3-hydroxy group (Table 3). This supports the notion that the combination C4 carbonyl moiety at A ring and catechol in B ring is one of the typical antioxidant structures for flavonoids (Furusawa, Tanaka, & Ito, 2005). In addition, eriodictyol (**9**) and eriodictyol-8-*C*-β-D-glucopyranoside (**10**) although have similar DPPH reducing potency, the presence of glucoside moiety significantly attenuates the ABTS radical scavenging activity (1.67 mM vs. 1.16 mM).

It has been found that icariside E_5 (**11**) is an antioxidant and can protect Jurkat cells from oxidative stress-induced apoptosis (Iorizzi et al., 2001). Here we found that there was a dramatic difference in antioxidant potency between icariside E_3 and E_5 (Table 3). It suggests that the double bond between 7′ and 8′ of icariside E_5 is involved in the free radical scavenging.

In this report, we have shown for the first time the antioxidant compounds of DS, which exhibited strong DPPH and ABTS radical scavenging activity *in vitro*. The total phenol contents correlated well with the DPPH scavenging potencies during purification. It has been proposed that the additive and synergistic effects of phytochemicals in fruits and vegetables are responsible for their antioxidant and anti-cancer activities (Liu, 2003). The traditional function

Table 3
The antioxidant activities of phenolic compounds isolated from the *n*-butanol layer of *D. solida*

Samples	IC_{50} for DPPH scavenging (μM)[a]	TEAC (mM)[b]
3′-*O*-*p*-Hydroxybenzoylmangiferin (**1**)	8.94 ± 1.49	3.47 ± 0.03
4′-*O*-*p*-Hydroxybenzoylmangiferin (**2**)	8.93 ± 1.24	3.56 ± 0.06
6′-*O*-*p*-Hydroxybenzoylmangiferin (**3**)	8.46 ± 1.26	3.54 ± 0.02
3-*O*-*p*-Hydroxybenzoylmangiferin (**4**)	21.52 ± 1.30	0.74 ± 0.06
Mangiferin (**5**)	9.95 ± 1.25	2.19 ± 0.02
2-*C*-β-D-Xylopyranosyl-1,3,6,7-tetrahydroxyxanthone (**6**)	10.43 ± 1.01	2.12 ± 0.06
4β-Carboxyl-(−)-epicatechin (**7**)	15.19 ± 1.20	1.35 ± 0.03
4β-Carboxymethyl-(−)-epicatechin (**8**)	12.65 ± 1.28	1.90 ± 0.05
Eriodictyol (**9**)	17.43 ± 0.66	1.67 ± 0.02
Eriodictyol-8-*C*-β-D-glucopyranoside (**10**)	16.58 ± 0.48	1.15 ± 0.02
Icariside E_5 (**11**)	15.64 ± 1.41	0.96 ± 0.08
Icariside E_3 (**12**)	32.96 ± 1.54	0.42 ± 0.03
α-Tocopherol (reference)	26.56 ± 2.14	
Trolox (reference)		1.00 ± 0.08

[a] IC_{50} for DPPH scavenging: concentration (in μM) necessary for reduction 50% DPPH radical. Values represent means ± SEM ($n = 3$).

[b] TEAC: concentration (in mM) of Trolox having the ABTS radical scavenging potential equivalent to 1.0 mM of the tested compound. Values represent means ± SEM ($n = 3$).

of DS may be therefore attributed in part to its antioxidant polyphenolic constitutes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <doi:10.1016/j.foodchem.2007.08.066>.

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