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Antioxidant phenylpropanoid glycosides from *Smilax bracteata*

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Abstract

From the ethanolic extract of *Smilax bracteata*, six phenylpropanoid glycosides, smilasides G–L (**1**–**6**), along with four known phenylpropanoid compounds, helonioside A, helonioside B, smilaside E, and (1-*p*-*O*-coumaroyl-6-*O*-feruroyl)-β-D-fructofuranosyl-α-D-glucopyranoside, and fourteen known phenolic compounds were isolated. Their structures were elucidated on the basis of spectroscopic analyses. Moreover, **1**–**6** exhibited moderate scavenging activities against DPPH radicals.

Keywords: *Smilax bracteata*; Smilacaceae; Smilaside G; Smilaside H; Smilaside I; Smilaside J; Smilaside K; Smilaside L; Phenylpropanoid glycosides

1. Introduction

Some of the *Smilax* plants distributed in Asia area including Taiwan, China, and Japan (Huang, 2000) are used as traditional medicine for treating syphilis, gout, and rheumatism in Taiwan (Kan, 1991). Recently, we have isolated six new phenylpropanoid glycosides (smilasides A–F) from *Smilax china* (Kuo et al., 2005). In a continuing search for bioactive constituents from the family Smilacaceae, we have found that the 95% EtOH extract of *Smilax bracteata* possessed antioxidative effects. Although several phenolic components including three phenylpropanoid glycosides have been reported previously (Li et al., 2002), there is still a lack of pharmacological reports on this plant or its isolates. We report herein that bioassay-directed fractionation led to isolation of six new phenylpropanoid glycosides, smilasides G–L (**1**–**6**), along with eighteen known phenolic compounds, helonioside A (Nakano et al., 1986), helonioside B (Nakano et al., 1986), smilaside E (Kuo et al., 2005), (1-*p*-*O*-coumaroyl-6-*O*-feruroyl)-β-D-fructofuranosyl-α-D-glucopyranoside (Li et al., 2002), tricin (Kuwabara et al., 2003), 5,7,4′-trihydroxy flavanone (Shirataki et al., 1985), 4,6,4′-trihydroxyaurone (Kesari et al., 2004), vitexin (Yin et al., 2004), isovitexin (Hosoya et al., 2005), quercetin (Chu et al., 2004), 3-*O*-α-L-rhamnopyranosyl quercetin (Wu and Chan, 1994), 3,7-*O*-α-L-dirhamnopyranosyl quercetin (Fico et al., 2003), resveratrol (Morikawa et al., 2002), peceatannol (Morikawa et al., 2002), veraphenol (Zhou et al., 1999), trans-scirpusin A (Morikawa et al., 2002), 2-β-D-glucopyranosyl-1,3,6,7-tetrahydroxy xanthone (Shahat et al., 2003), and 5-*O*-caffeoylshikimic acid (Fukuoka, 1982) from the EtOH extract. Structural elucidation of the new phenylpropanoid glycosides (**1**–**6**) were based on spectroscopic data analyses, including application of 1D and 2D NMR spectroscopic techniques (DEPT, ^{1}H–^{1}H COSY, HMQC, and HMBC).

2. Results and discussion

The 95% EtOH extract of the aerial part of *S. bracteata* was suspended in H_2O and partitioned with *n*-hexane, dichloromethane, and *n*-butanol, successively. The dichlo-

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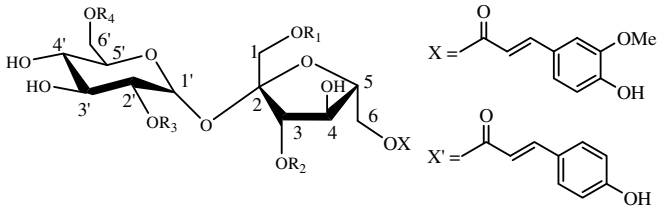
romethane layer was applied to on a silica gel column and then to a sephedex LH-20 column. The bioactive fractions so obtained were then subjected to preparative recycle HPLC, using a reversed-phase (ODS) column, to yield six new (**1**–**6**) (see Fig. 1) and four known phenylpropanoid glycosides. From the *n*-butanol layer, fourteen known phenolic compounds were obtained.

The HRESIMS of smilaside G (**1**) suggested an elemental formula $C_{40}H_{42}O_{18}$ based on the quasi-molecular ion at *m/z* 833.2231 $[M + Na]^+$. The IR and UV spectra displayed absorption bands for the hydroxyl and α,β-unsaturated aromatic ester groups. In the ^1H NMR spectrum, signals for eight oxygenated methines [δ_H 5.60 (*d*, $J = 8.4$ Hz, H-3), 4.52 (*m*, H-4), 4.21 (*m*, H-5), 5.50 (*d*, $J = 3.6$ Hz, H-1′), 3.40 (*m*, H-2′), 3.66 (*t*, $J = 9.2$ Hz, H-3′), 3.40 (*m*, H-4′), 3.97 (*m*, H-5′)], three oxygenated methylenes [δ_H 4.33 (2H, *br s*, H-1), 4.54 (2H, *m*, H-6), 3.91 and 3.82 (each 1H, *m*, H-6′)], three pairs of olefinic protons [δ_H 7.71 (*d*, $J = 16.0$ Hz, H-α″) and 6.39 (*d*, $J = 16.0$ Hz, H-β″), 7.63 (*d*, $J = 16.0$ Hz, H-α‴) and 6.33 (*d*, $J = 16.0$ Hz, H-β‴), 7.62 (*d*, $J = 16.0$ Hz, H-α⁗), and 6.36 (*d*, $J = 16.0$ Hz, H-β⁗)], two benzylic moieties with A_2B_2 [δ_H 7.39 (2H, *d*, $J = 8.0$ Hz, H-2″, 6″) and 6.79 (2H, *d*, $J = 8.0$ Hz, H-3″, 5″), 7.47 (2H, *d*, $J = 8.8$ Hz, H-2‴, 6‴) and 6.73 (2H, *d*, $J = 8.8$ Hz, H-3‴, 5‴)], as well as AMX [δ_H 7.15 (1H, *br s*, H-2⁗), 7.05 (1H, *br d*, $J = 8.4$ Hz, H-6⁗), 6.79 (1H, *d*, $J = 8.0$ Hz, H-5⁗)] coupling patterns were observed. In addition, the ^{13}C NMR chemical shifts attributable to 27 sp^2 carbons were in good agreement with those of two *para*-coumaroyl and one feruloyl moieties. A characteristic anomeric signal appeared at δ_H 5.50 with a smaller coupling constant (*d*, $J = 3.6$ Hz, H-1′), together with the ^{13}C NMR spectrum showing 12 oxygenated carbon resonances containing two anomeric carbons, suggested that **1** possessed a disaccharide moiety. Detailed analysis of the ^1H and ^{13}C NMR spectroscopic data of **1** with the aid of DEPT, ^1H–^1H COSY, HMQC and HMBC analyses indicated that the two sugars were comprised of β-D-fructose and α-D-glucose moieties connected through a 2 → 1 linkage; thus, **1** should be a phenylpropanoid sucroside. Inspection of the HMBC spectrum of **1**, resulted in the following being noted: long range correlations between ester carbonyl carbons of two *para*-coumaroyl groups (δ_C 168.5 and δ_C 168.4) and H-1 (δ_H 4.33) and H-3 (δ_H 5.60) of fructose, respectively, and between an ester carbonyl carbon of feruloyl group (δ_C 169.0) and H-6 of fructose (δ_H 4.54). Accordingly, the structure of **1** was determined as (1,3-*O*-di-*p*-coumaroyl-6-*O*-feruloyl)-β-D-fructofuranosyl-(2 → 1)-α-D-glucopyranoside, and tentatively named as smilaside G.



	R_1	R_2	R_3	R_4
1	X'	X'	H	H
2	X'	X'	$COCH_3$	H
3	X'	X'	H	$COCH_3$
4	X	X'	H	H
5	X'	X	$COCH_3$	H
6	X	X	H	H

Fig. 1. Compounds **1**–**6** isolated from *Smilax bracteata*.

Smilaside H (**2**) was isolated as a yellow amorphous powder, and its HRESIMS gave a quasi-molecular ion at *m/z* 875.2432 $[M + Na]^+$ ($C_{42}H_{44}O_{19}Na$). The IR and UV spectra displayed absorption bands for the hydroxyl and α,β-unsaturated aromatic ester groups. The ^1H and ^{13}C NMR spectra of **2** were similar to those of **1** (Tables 1 and 2), along with the same substitution pattern, except for the presence of an acetyl group signal. Based on analysis of the HMBC spectrum, the long-range correlation noted between the proton at δ_H 4.62 (H-2′ of Glc) and the acetyl carbonyl carbon at δ_C 172.6 suggested assignment of the acetate group at C-2′ of Glc. Moreover, the changes for the proton chemical shifts of Glc produced from the acetate group may be found by comparing the NMR spectra of **1** and **2**. Thus, signals for H-2′ and C-2′ in **2** were shifted to lower fields (δ_H 3.40 for **1**, δ_H 4.62 for **2**; δ_C 73.0 for **1**, δ_C 74.5 for **2**), and C-1′ and C-3′ was shifted to higher field (δ_C 93.5 and 74.9 for **1**, δ_C 91.2 and 72.2 for **2**). From the above evidences, the structure of **2** was elucidated as (1,3-*O*-di-*p*-coumaroyl-6-*O*-feruloyl)-β-D-fructofuranosyl-(2 → 1)-(2-*O*-acetyl)-α-D-glucopyranoside.

Compound **3**, an amorphous powder, had a molecular formula of $C_{42}H_{44}O_{19}$ as determined from its pseudomolecular ion peak at *m/z* 875.2426 $[M + Na]^+$ in the HRESIMS. Like **2**, the ^1H and ^{13}C NMR spectra of **3** indicated that **3** possessed various moieties including sucrose, two coumaric acids, ferulic acid, and acetyl. The position of the acetyl group was determined at C-6′ instead of C-2′ in **2** of Glc, due to the long-range correlations between the proton at δ_H 4.14, 4.55 (H-6′ of Glc) and the carbonyl carbon at δ_C 173.0. Furthermore, by comparing the NMR spectra of **1** and **3**, the signals of H-6′ and C-6′ in **3** were shifted to lower field (δ_H 3.82, 3.91, δ_C 62.6 for **1**; δ_H 4.14, 4.55, δ_C 65.7 for **3**), while that of signal of C-5′ was shifted to higher field (δ_C 74.4 for **1**, δ_C 72.0 for **3**). This also supported the assignment of the acetyl group at C-6′ of Glc. Thus, the structure of smilaside I (**3**) was determined as (1,3-*O*-di-*p*-coumaroyl-6-*O*-feruloyl)-β-D-fructofuranosyl-(2 → 1)-(6-*O*-acetyl)-α-D-glucopyranoside.

Compound **4** was isolated as a yellow amorphous powder. Its molecular formula was established as $C_{41}H_{44}O_{19}$ from analysis of the HRESIMS pseudomolecular ion peak at *m/z* 863.2442 $[M + Na]^+$. In comparison to the NMR spectra of **4** and **3**, both have a sucrose and three phenylpropanoid moieties, whereas one of the coupling patterns of the benzyl protons in **4** was displayed by an AMX

Table 1
^{1}H NMR spectroscopic data (δ in methanol-d_4, J in Hz) for **1**–**6**[a,b]

H	1	2	3	4	5	6
1	4.33 (*br s*, 2H)	4.32 (*br s*, 2H)	4.32 (*br s*, 2H)	4.33 (*br s*, 2H)	4.33 (*d*, 11.6	4.33 (*br s*, 2H)
	–	–	–	–	4.20 (*d*, 11.6)	–
3	5.60 (*d*, 8.4)	5.51 (*d*, 8.0)	5.61 (*d*, 8.4)	5.61 (*d*, 8.5)	5.61 (*d*, 8.4)	5.60 (*d*, 8.5)
4	4.52 (*m*)	4.50 (*m*)	4.55 (*m*)	4.51 (*m*)	4.50 (*m*)	4.50 (*m*)
5	4.21 (*m*)	4.20 (*m*)	4.19 (*m*)	4.33 (*m*)	4.21 (*m*)	4.18 (*br t*, 8.0)
6	4.54 (*m*, 2H)	4.54 (*m*, 2H)	4.55 (*m*, 2H)	4.55 (*m*, 2H)	4.54 (*m*, 2H)	4.54 (*m*, 2H)
1′	5.50 (*d*, 3.6)	5.64 (*d*, 3.0)	5.53 (*br s*)	5.51 (*d*, 3.0)	5.65 (*d*, 3.2)	5.51 (*d*, 3.5)
2′	3.40 (*m*)	4.62(*d d*, 8.0, 3.0)	3.44 (*br d*, 9.2)	3.42 (*d*, 10)	4.63 (*d*, 10.0, 3.6)	3.42 (*d*, 10)
3′	3.66 (*t*, 9.2)	3.89 (*m*)	3.63 (*t*, 8.8)	3.65 (*t*, 9.5)	3.89 (*m*)	3.66 (*t*, 9.0)
4′	3.40 (*m*)	3.50 (*t*, 8.8)	3.27 (*t*, 9.2)	3.42 (*t*, 9.5)	3.49 (*t*, 9.6)	3.42 (*t*, 9.5)
5′	3.97 (*m*)	3.94 (*m*)	4.19 (*m*)	3.97 (*m*)	3.95 (*m*)	4.00 (*br d*, 8.0)
6′	3.82 (*m*)	3.79 (*m*)	4.14 (*m*)	3.83 (*m*)	3.78 (*m*)	3.83 (*m*)
	3.91 (*m*)	3.92 (*m*)	4.55 (*m*)	3.92 (*m*)	3.93 (*m*)	3.92 (*m*)
OAc-2′	–	2.08 (*s*, 3H)	–	–	2.08 (*s*, 3H)	–
OAc-6′	–	–	2.09 (*s*, 3H)	–	–	–
X′ or X-1						
α″	7.71 (*d*, 16.0)	7.71 (*d*, 15.5)	7.72 (*d*, 15.6)	7.70 (*d*, 16.0)	7.63 (*d*, 16.0)	7.63 (*d*, 16.0)
β″	6.39 (*d*, 16.0)	6.45 (*d*, 16.0)	6.42 (*d*, 15.6)	6.42 (*d*, 16.0)	6.32 (*d*, 16.0)	6.35 (*d*, 16.0)
2″	7.39 (*d*, 8.0)	7.40 (*d*, 8.0)	7.40 (*d*, 8.0)	7.15 (*d*, 1.5)	7.39 (*d*, 8.0)	7.11 (*br s*)
3″	6.79 (*d*, 8.0)	6.80 (*d*, 8.0)	6.79 (*d*, 8.0)	–	6.72 (*d*, 8.4)	–
5″	6.79 (*d*, 8.0)	6.80 (*d*, 8.0)	6.79 (*d*, 8.0)	6.78 (*d*,7.5)	6.72 (*d*, 8.4)	6.73 (*d*, 8)
6″	7.39 (*d*, 8.0)	7.40 (*d*, 8.0)	7.39 (*d*, 8.0)	7.08 (*br d*, 8.0)	7.39 (*d*, 8.0)	7.01 (*br d*, 8.0)
OMe	–	–	–	3.86 (*s*)	–	3.87 (s)
X′ or X-3						
α‴	7.63 (*d*, 16.0)	7.64 (*d*, 16.0)	7.64 (*d*, 16.0)	7.62 (*d*, 16.5)	7.71 (*d*, 16.0)	7.69 (*d*, 16.0)
β‴	6.33 (*d*, 16.0)	6.33 (*d*, 16.0)	6.34 (*d*, 16.0)	6.32 (*d*, 16.0)	6.49 (*d*, 16.0)	6.42 (*d*, 16.5)
2‴	7.47 (*d*, 8.8)	7.48 (*d*,7.5)	7.48 (*d*, 8.0	7.37 (*d*, 8.5)	7.22 (*br* s)	7.15 (*br* s)
3‴	6.73 (*d*, 8.8)	6.73 (*d*, 7.0)	6.74 (*d*, 8.0)	6.71 (*d*, 8.5)	–	–
5‴	6.73 (*d*, 8.8)	6.73 (*d*, 7.0)	6.74 (*d*, 8.0)	6.71 (*d*, 8.5)	6.81 (*d*, 8.4)	6.78 (*d*, 7.5)
6‴	7.47 (*d*, 8.8)	7.48 (*d*, 7.5)	7.48 (*d*, 8.0)	7.37 (*d*, 8.5)	7.11 (*d*, 8.0)	7.08 (*d*, 8.0)
OMe	–	–	–	–	3.88 (*s*, 3H)	3.80 (*s*, 3H)
X′ or X-6						
α⁗	7.62 (*d*, 16.0)	7.64 (*d*, 16.0)	7.63 (*d*, 16.0)	7.62 (*d*, 16.5)	7.64 (*d*, 16.0)	7.63 (*d*, 16.0)
β⁗	6.36 (*d*, 16.0)	6.40 (*d*, 16.0)	6.38 (*d*, 16.0)	6.38 (*d*, 16.5)	6.40 (*d*, 16.0)	6.39 (*d*, 16.5)
2⁗	7.15 (*br* s)	7.18 (*br* s)	7.16 (*br s*)	7.15 (*d*, 1.5)	7.17 (*br s*)	7.16 (*br s*)
3⁗	–	–	–	–	–	–
5⁗	6.79 (*d*, 8.0)	6.79 (*d*, 8.0)	6.79 (*d*, 8.0)	6.78 (*d*, 7.5)	6.79 (*d*, 8.4)	6.77 (*d*, 8.0)
6⁗	7.05 (*br d*, 8.4)	7.06 (*br d*, 8.0)	7.06 (*br d*, 7.6)	7.05 (*br d*, 8.0)	7.06 (*br d*, 8.0)	7.06 (*br d*, 8.0)
OMe	3.85 (*s*, 3H)	3.87 (*s*, 3H)	3.86 (*s*, 3H)	3.86 (*s*, 3H)	3.86 (*s*, 3H)	3.86 (*s*, 3H)

[a] Assignments were confirmed by ^{1}H–^{1}H COSY, TOCSY, HMBC and HMQC.
[b] Compounds **1**,**3**,**5** were measured at 400 MHz and **2**,**4**,**6** at 500 MHz.

instead of an A_2B_2 pattern, along with the presence of signals for an additional methoxy group and the absence of an acetate group in **4**. These findings, together with the molecular formula of **4** which was 12 D[CH_2O (+) and CH_3CO(−)] less than that of **3**, indicated that not only a *para*-coumaroyl was replaced by a feruloyl moiety, but also an acetate unit was lost in **4**. Moreover, the HMBC spectrum showed cross-peaks for the carbonyl carbons of two feruloyl (δ_C 168.5, δ_C 169.1) and one *para*-coumaroyl groups (δ_C 168.4) with H-1 (δ_H 4.33), H-6 (δ_H 4.55) and H-3 (δ_H 5.61) of fructose, respectively, determining the position of these phenylpropanoid moieties. On the basis of the above corroborations, the structure of **4** was determined as (3-*O*-*p*-coumaroyl-1,6-*O*-diferuloyl)-β-D-fructofuranosyl-(2 → 1)-α-D-glucopyranoside, and named as smilaside J.

Compound **5** has a quasi-molecular ion at m/z 905.2526 $[M + Na]^+$ ($C_{43}H_{46}O_{20}Na$) from analysis of its HRESIMS. The ^{1}H and ^{13}C NMR spectroscopic data of a sucrose and an acetyl residues in **5** were similar to those of **2** (Tables 1 and 2), except for two AMX and one A_2B_2 patterns of aromatic protons found in **5** rather than one AMX and two A_2B_2 patterns in **2**. These findings indicated that one *para*-coumaric acid was replaced in **5** by a ferulic acid, whose position was further established at C-1 of the sucrose unit on the basis of long range correlations observed between carbonyl carbon of feruloyl group (δ_C 168.3) and the H-1 of fructose (δ_H 4.33, 4.20). Taken together, this evidence indicated that, the structure of smilaside K was (1-*O*-*p*-coumaroyl-3,6-*O*-diferuloyl)-β-D-fructofuranosyl-(2 → 1)-(2-*O*-acetyl)-α-D-glucopyranoside (**5**).

Table 2
^{13}C NMR spectroscopic data (δ in methanol-d_4) for **1**–**6**[a,b]

C	1	2	3	4	5	6
1	66.0	66.2	66.3	66.1	66.3	66.1
2	103.5	103.7	103.4	103.5	103.7	103.5
3	79.1	79.5	79.0	79.1	79.5	79.1
4	74.1	74.2	73.6	74.1	73.6	74.1
5	81.0	81.0	81.1	81.0	81.0	81.1
6	66.0	65.7	65.5	65.9	65.7	65.9
1′	93.5	91.2	93.0	93.5	91.2	93.5
2′	73.0	74.5	72.8	73.0	74.4	73.0
3′	74.9	72.2	74.8	75.0	72.2	75.0
4′	71.5	71.3	72.1	71.6	71.4	71.6
5′	74.4	74.4	72.0	74.3	74.3	74.4
6′	62.6	62.3	65.7	62.7	62.4	62.7
OAc-2′	–	172.6	–	–	172.6	–
	–	21.1	–	–	21.1	–
OAc-6′	–	–	173.0	–	–	–
	–	–	20.9	–	–	–
X′ or X-1						
α″	147.9	148.0	147.9	148.2	147.3	147.4
β″	114.3	114.0	114.2	114.3	114.5	114.6
γ″	168.5	168.4	168.5	168.5	168.3	168.5
1″	127.0	126.8	127.0	127.2	126.9	127.3
2″	131.3	131.6	131.3	112.0	131.3	111.6
3″	116.8	117.1	116.8	149.5	116.8	149.6
4″	161.2	161.8	161.2	151.6	161.5	151.5
5″	116.8	116.8	116.8	116.6	116.8	116.6
6″	131.3	131.6	131.3	124.5	131.3	124.5
OMe	–	–	–	56.4	–	56.4
X′ or X-3						
α‴	147.2	147.4	147.2	147.3	148.2	148.3
β‴	114.7	114.3	114.6	114.5	114.6	114.2
γ‴	168.4	168.4	168.4	168.4	168.4	168.4
1‴	127.0	126.7	127.0	126.8	127.4	127.1
2‴	131.5	131.3	131.5	131.3	111.9	112.0
3‴	116.8	117.0	116.8	116.9	149.4	149.5
4‴	161.4	162.2	161.4	161.7	151.1	151.8
5‴	116.8	116.8	116.8	116.9	116.6	116.7
6‴	131.5	131.3	131.5	131.3	124.5	124.5
OMe	–	–	–	–	56.5	56.3
X′ or X-6						
α⁗	147.1	147.3	147.2	147.2	147.2	147.6
β⁗	115.1	114.9	115.1	114.8	115.0	114.8
γ⁗	169.0	169.0	168.9	169.1	169.0	169.1
1⁗	127.7	127.5	127.7	127.4	127.6	127.3
2⁗	111.7	111.6	111.6	111.6	111.6	111.6
3⁗	149.3	149.5	149.3	149.5	149.4	149.6
4⁗	150.6	151.5	150.7	151.2	150.8	151.5
5⁗	116.4	116.5	116.5	116.5	116.5	116.6
6⁗	124.3	124.4	124.3	124.4	124.4	124.5
OMe	56.5	56.5	56.5	56.4	56.5	56.4

[a] Assignments were confirmed by ^{1}H–^{1}H COSY, HMBC and HMQC.
[b] Compounds **1**,**3**,**5** were measured at 100 MHz and **2**,**4**,**6** at 125 MHz.

The very similar ^{1}H and ^{13}C NMR spectroscopic signals of **1** and **6** indicated that the latter also possessed a sucrose moiety as well as three phenylpropanoid groups. The aromatic resonances displayed by the three AMX patterns in the ^{1}H NMR spectrum of **6**, together with the quasi-molecular ion {*m*/*z* 893.2532 $[M + Na]^+$ ($C_{42}H_{46}O_{20}Na$)} of **6**, which showed 60 D more than that of **1**, permitted deduction that two *para*-coumaroyl groups in **1** were replaced by two feruloyl groups in **6**. In the HMBC spectrum of **6**, the locations of the feruloyl units were confirmed at C-1, C-3 and C-6, due to the long-range correlations between three carbonyl carbons of esters and H-1, H-3 and H-6 of fructose, respectively. Based on the above evidence, the structure of **6** (smilaside L) was elaborated as (1,3,6-*O*-triferuloyl)-β-D-fructofuranosyl-(2 → 1)-α-D-glucopyranoside.The antioxidant activity of compounds **1-6** was evaluated by using the stable 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH) method, and their ED_{50} values are listed in Table 3.

Table 3
Antioxidant activity of compounds **1**–**6**

Compounds	ED_{50} (10^{-5} M)
1	7.193
2	7.935
3	6.847
4	2.667
5	3.021
6	3.270
(±)-α-Tocopherol[a]	2.820

[a] Positive control.

3. Conclusion

As shown in Table 3, compounds **4**–**6** showed higher antioxidant activity and very close data to that of the positive control, compared with **1**–**3**. The major structural differences between **1**–**3** and **4**–**6** were that the former compounds had one feruloyl unit, whereas the latter had two or three feruloyl units. These findings, together with free formed ferulic acid exhibiting better antioxidant activity than coumaric acid (Foti et al., 2004), indicated that the substituted feruloyl group has a crucial role for the antioxidant activity in the phenylpropanoid glycosides.

Although *Smilax sp*. are known for their steroidal contents (Ju and Jia, 1992, 1993; Ju et al., 1994; Sashida et al., 1992; Kubo et al., 1992), characteristic natural products having a sucrose core along with one to three phenylpropanoid moieties were also isolated by us and the other laboratories (Kuo et al., 2005; Li et al., 2002; Chen et al., 2000; Cheng et al., 2004). Due to our promising antioxidant activity results mentioned as above as well as by Fabre et al. (2000), together with the other reported bioactive effects including cytotoxicity (Kuo et al., 2005), chemopreventive activity (Takasaki et al., 2001), histamine release inhibition (Wang et al., 2003), PGE2 production inhibition (Wang et al., 2003), phenylpropanoid glycosides can generally be noted for their broad-spectrum pharmacological usage. In addition, these natural products being comprised of phenylpropanoid glycosides would provide the other proof of chemotaxonomy in the genus *Smilax*.

4. Experimental

4.1. General experimental procedures

Melting points were determined using a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were obtained on a JASCO P-1020 polarimeter. NMR spectra were recorded using a Bruker Unity Plus 400 MHz spectrometer. HRESIMS were determined using a Finingan MAT 95S mass spectrometer. UV spectra were measured with a GBC 918 spectrophotometer. IR spectra were recorded as KBr disks, using an IR-FT Mattson Genesis II spectrometer. For CC, silica gel 60 (70–230, 230–400 mesh, Merck), Diaion HP 20 (Mitsubishi Chemicals) and Sephadex LH-20 (Phramacia) were employed, whether precoated silica gel (Merck 60 F-254) plates were used for TLC. TLC spots were detected by spraying with 5% H_2SO_4 and then heating at 110 °C. MPLC manipulations were performed on a system equipped with a Buchi pump B-688, Buchi B-684 fraction collector and Buchi columns. HPLC separations were performed on a Shimadzu LC-8A series apparatus with a SPD-20A UV detector, equipped with a 250 × 20 mm i.d. preparative Cosmosil $5C_{18}$ AR-II column (Nacalai Tesque, Inc.).

4.2. Plant material

The aerial parts of *S. bracteata* (6.0 kg) were collected in the middle mountains of Taiwan, in November 2005 and identified by professor Muh-Tsuen Kao, National Research Institute of Chinese Medicine, Taipei. A voucher specimen (No. NRICM20051110A) has been deposited in the National Research Institute of Chinese Medicine, Taipei, Taiwan.

4.3. Extraction and isolation

The aerial parts of *S. bracteata* (6.0 kg) were extracted with 95% EtOH 50 L for 12 h, three times at room temperature. The dried EtOH extract was concentrated under reduced pressure. The EtOH extract (403 g) was next suspended in H_2O, and this suspension was successively extracted with *n*-hexane, CH_2Cl_2, and *n*-BuOH. The CH_2Cl_2 layer provided 50 g of an extract which was separated by MPLC silica gel CC eluted with $CHCl_3$/MeOH (100:0, 20:1, 10:1, 5:1, 2:1, 0:100), to yield 6 fractions (Fr D1 to Fr D6). Fraction D2 ($CHCl_3$:MeOH, 20:1, 6.3 g) was then submitted to Sephadex LH-20 CC eluted with $CHCl_3$:MeOH (1:1), to afford three fractions (Fr D2.1 to Fr D2.3). Fr D2.2 (2.5 g) was subjected to silica gel MPLC CC eluted with $CHCl_3$–MeOH from 100:0 to 80:20, to give 8 fractions (Fr D2.2.1 to Fr D2.2.8). In turn, preparative TLC ($CHCl_3$:MeOH, 6:1, runs for two times) of Fr D2.2.4 afforded 3 parts, with part 2 purified by recycle semi-preparative HPLC (250 × 20 mm i.d., Cosmosil $5C_{18}$ AR-II column, CH_3CN:H_2O, 30:70, recycle for four times), to afford compounds **3** (5.1 mg) and **5** (7.0 mg). Fr D2.2.6 was purified by preparative TLC ($CHCl_3$:MeOH, 5.5:1, runs for two times) to afford helonioside A (12 mg), helonioside B (22 mg), and smilaside E (21 mg). Fraction D3 ($CHCl_3$:MeOH, 10:1, 3.8 g), was then submitted to Sephadex LH-20 CC eluted with $CHCl_3$:MeOH (1:2), to afford five fractions (Fr D3.1 to Fr D3.5). Fr D3.2 (700 mg) subjected to Sephadex LH-20 CC eluting with MeOH:H_2O (3:1), to afford three fractions (Fr D3.2.1 to Fr D3.2.3). By low bar ODS CC eluted with MeOH–H_2O from 30:70 to 100:0, three parts (Fr D3.2.3.1 to Fr D3.2.3.3) were furnished. Fr D3.2.3.3 was further purified by preparative TLC ($CHCl_3$:MeOH, 5.5:1, runs for 2 times) to afford compounds **2** (11 mg), **4** (25 mg), **6** (12.1 mg), and (1-*O*-*p*-coumaroyl-6-*O*-feruroyl)-β-D-fructofuranosyl-α-D-glucopyranoside (12 mg). Fraction D4 ($CHCl_3$:MeOH, 10:1, 7.1 g) was subjected to Sephadex LH-20 CC eluting with $CHCl_3$:MeOH (1:2) to give 7 parts (Fr D4.1 to Fr D4.7). Fr D4.5 were purified by Sephadex LH-20 CC eluting with MeOH:H_2O (2:1) to afford compound **1** (75 mg). The *n*-BuOH extract (100 g) was partitioned with Diaion HP 20 column eluting with pure H_2O, H_2O:MeOH (3:7, 6:4, v/v) and MeOH to give 4 parts (Fr B1 to Fr B4). Fr B4 (35 g) was subjected to MPLC (silica gel 60) eluted with $CHCl_3$/MeOH (100:0–0:100 gradient) to afford 13 fractions (Fr B4.1 to Fr B4.13). Further chromatography of Fr B4.3 (2.91 g) on a Sephadex LH-20 column with $CHCl_3$:MeOH (1:1) as eluent generated 9 fractions (Fr B4.3.1 to Fr B4.3.9). Fr B4.3.5 (22 mg) was purified with Sephadex LH-20 (100% MeOH) to afford tricin (5.1 mg). Fr B4.3.9 was recrystallized by MeOH to give 5,7,4′-trihydroxy flavanone (6.8 mg). Fr B4.5 was submitted to Sephadex LH-20 CC eluted with $CHCl_3$:MeOH (1:1), to give resveratrol (10 mg). Fr B4.6 was subjected to Sephadex LH-20 CC eluting with $CHCl_3$–MeOH (1:1), to afford 11 parts; part 7 was further purified by semi-preparative HPLC (250 × 10 mm i.d., Cosmosil 5 C_{18} AR-II column, CH_3CN:H_2O, 29:71) to give 4,6,4′-trihydroxyaurone (8.2 mg) and part 11 was purified by preparative TLC ($CHCl_3$:MeOH, 6:1) to give veraphenol (3.5 mg). Fr B4.11 was subjected to Sephadex LH-20 CC eluting with pure MeOH, to afford 7 parts (Fr B4.11.1 to Fr B4.11.7). Fr B4.11.2 was then purified by semi-preparative HPLC (250 × 20 mm i.d., Cosmosil $5C_{18}$ AR-II column, MeOH:H_2O, 60:40) to afford vitexin (30 mg) and isovitexin (24 mg). Fr B4.11.7 was purified by preparative TLC ($CHCl_3$:MeOH, 5.5:1) to give trans-scirpusin A (3.1 mg) and quercetin (1.5 mg). Fr B3 (22 g) was applied to an MPLC (silica gel 60) eluted column with $CHCl_3$/MeOH (100:0–0:100 gradient) to afford 14 fractions (Fr B3.1 to Fr B3.14). 3-*O*-α-L-rhamnopyranosyl quercetin (1.2 g), 3,7-*O*-α-L-dirhamnopyranosyl quercetin (1.0 g), and 2-β-D-glucopyranosyl-1,3,6,7-tetrahydroxy xanthone (1.0 g) were purified by recrystallization from Fr B3.5, Fr B3.10, and Fr B 3.11, respectively. Peceatannol (1.7 mg) and 5-*O*-caffeoylshikimic acid (2.3 mg) were purified by Sephadex LH-20 CC eluting with pure MeOH from Fr B3.3 and Fr B3.6, respectively.

4.3.1. Smilaside G (**1**)

Yellowish glass, mp 110–112 °C. $[\alpha]_D^{25} + 73.75$ ($c = 1.6$, MeOH). UV λ_{max} (MeOH) nm: 316.6, 299.7 (sh), 231.5, 198.8; IR υ_{max} (KBr) 3376, 2944, 2836, 1693, 1631, 1514, 1447 cm^{-1}; HRESIMS m/z 833.2231 $[M + Na]^+$ (Calc. for $C_{40}H_{42}O_{18}Na$: 833.2269), for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.2. Smilaside H (**2**)

Yellowish glass, mp 131–133 °C. $[\alpha]_D^{25} + 45.35$ ($c = 0.86$, MeOH). UV λ_{max} (MeOH) nm: 316.8, 299.5 (sh), 231.2, 198.8; IR υ_{max} (KBr) 3376, 2944, 2837, 1697, 1632, 1604, 1515, 1448 cm^{-1}; HRESIMS m/z 875.2432 $[M + Na]^+$ (Calc. for $C_{42}H_{44}O_{19}Na$: 875.2374), for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.3. Smilaside I (**3**)

Yellowish glass, mp 139–142 °C. $[\alpha]_D^{25} + 70.00$ ($c = 0.7$, MeOH). UV λ_{max} (MeOH) nm: 316.8, 299.8 (sh), 231.0, 199.6; IR υ_{max} (KBr) 3396, 2946, 2837, 1704, 1632, 1604, 1515, 1448 cm^{-1}; HRESIMS m/z 875.2426 $[M + Na]^+$ (Calc. for $C_{42}H_{44}O_{19}Na$: 875.2374), for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.4. Smilaside J (**4**)

Yellowish glass, mp 148-151 °C. $[\alpha]_D^{25} + 61.05$ ($c = 1.9$, MeOH). UV λ_{max} (MeOH) nm: 322.0, 300.0 (sh), 234.8, 219.0; IR υ_{max} (KBr) 3365, 2943, 2837, 1697, 1631, 1603, 1515, 1449 cm^{-1}; HRESIMS m/z 863.2442 $[M + Na]^+$ (Calc. for $C_{41}H_{44}O_{19}Na$: 863.2374), for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.5. Smilaside K (**5**)

Yellowish glass, mp 145–148 °C. $[\alpha]_D^{25} + 107.84$ ($c = 0.51$, MeOH). UV λ_{max} (MeOH) nm: 321.4, 299.8 (sh), 233.6, 219.0; IR υ_{max} (KBr) 3404, 1700, 1630, 1603, 1515 cm^{-1}; HRESIMS m/z 905.2526 $[M + Na]^+$ (Calc. for $C_{43}H_{46}O_{20}Na$: 905.2480), for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.6. Smilaside G (**6**)

Yellowish glass, mp 232–233 °C. $[\alpha]_D^{25} + 39.42$ ($c = 1.04$, MeOH). UV λ_{max} (MeOH) nm: 327.2, 299.8 (sh), 235.8, 218.0; IR υ_{max} (KBr) 3365, 2931, 2850, 1699, 1631, 1597, 1516, 1455 cm^{-1}; HRESIMS m/z 893.2532 $[M + Na]^+$ (Calc. for $C_{42}H_{46}O_{20}Na$: 893.2480), for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.4. Scavenging activity of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical

The radical scavenging activity of Smilasides G to L (**1**–**6**) on DPPH free radicals was measured using the method of Rangkadilok et al. (2007) and Chung et al. (2002) with minor modifications. An aliquot of each sample (120 μL, 100–10 μg/mL), or (±)-α-tocopherol (40–10 μg/mL), was mixed with of 0.75 mM DPPH (30 μL in MeOH) in a 96-well microplate. The mixture was shaken vigorously using an orbital shaker in the dark at room temperature for 30 min with the absorbance measured at 517 nm using an ELISA reader. The negative control was the measurement using MeOH to replace the sample in the react solution. The DPPH radical scavenging activity of compounds **1**–**6** were compared to the negative control and (±)-α-tocopherol as positive control. The final results were performed as the concentrations of ED_{50}, which is the concentration of sample required to cause 50% inhibition against DPPH radicals in react solution.

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