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Estrogenic and anti-estrogenic compounds from the Thai medicinal plant, *Smilax corbularia* (Smilacaceae)

Boonsong Wungsintaweekul, Kaoru Umehara *, Toshio Miyase, Hiroshi Noguchi

School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Shizuoka 422-8526, Japan

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ABSTRACT

From the rhizomes of *Smilax corbularia* Kunth. (Smilacaceae), 11 compounds, (2*R*,3*R*)-2″-acetyl astilbin, (2*R*,3*R*)-3″-acetyl astilbin, (2*R*,3*R*)-4″-acetyl astilbin, (2*R*,3*R*)-3″-acetyl engeletin, (2*R*,3*S*)-4″-acetyl isoastilbin, 2-(4-hydroxyphenyl)-3,4,9,10-tetrahydro-3,5-dihydroxy-10-(3,4-dihydroxyphenyl)-(2*R*,3*R*,10*R*)-2H,8H-benzo [1,2-b:3,4-b′] dipyran-8-one, 2-(4-hydroxyphenyl)-3,4,9,10-tetrahydro-3,5-dihydroxy-10-(3,4-dihydroxyphenyl)-(2*R*,3*R*,10*S*)-2H, 8H-benzo [1,2-b:3,4-b′] dipyran-8-one, 3,4-dihydro-7-hydroxy-4-(3,4-dihydroxyphenyl)-5-[(1*E*)-2-(4-hydroxyphenyl) ethenyl]-2H-1-benzopyran-2-one, 3,4-dihydro-7-hydroxy-4-(3,4-dihydroxy-phenyl)-5-[(1*E*)-2-(3,4-dihydroxyphenyl) ethenyl]-2H-1-benzopyran-2-one, 3,4-dihydro-7-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-5-[(1*E*)-2-(4-hydroxyphenyl) ethenyl]-2H-1-benzopyran-2-one, and 5,7,3′,4′-tetrahydroxy-3-phenylcoumarin along with 34 known compounds were isolated and characterized as 19 flavonoids, 14 catechin derivatives, 6 stilbene derivatives, and 6 miscellaneous substances. All isolates had their estrogenic and anti-estrogenic activities determined using the estrogen-responsive human breast cancer cell lines MCF-7 and T47D. The major constituents were recognized as flavanonol rhamnosides by the suppressive effect on estradiol induced cell proliferation at a concentration of 1 μM. Meanwhile, flavanonol rhamnoside acetates demonstrated estrogenic activity in both MCF-7 and T47D cells at a concentration of 100 μM, and they enhanced the effects of co-treated E2 on T47D cell proliferation at concentrations of more than 0.1 μM.

1. Introduction

As a part of our continued research on estrogenic compounds from Thai medicinal plants, a methanol extract of *Smilax corbularia* Kunth. (Smilacaceae) was investigated. *Smilax corbularia* is a climbing vine distributed in South-East Asia, and its rhizomes have mostly been used as a Thai folk medicine not only for the treatment of female diseases, such as breast and ovary cancers but for AIDS treatment (Tewtrakul et al., 2006). However, no scientific reports about the constituents of *S. corbularia* have been published.

This paper deals with the isolation of constituents from these plants, their structural characterization by spectroscopic methods, and the assessment of the estrogenic and anti-estrogenic activity of the isolates using the estrogen responsive MCF-7 and T47D cell lines.

2. Results and discussion

The methanol extract of rhizomes of *S. corbularia* (70 g) was partitioned with ethyl acetate and water. The ethyl acetate-soluble and water-soluble fractions had their estrogenic and anti-estrogenic activities tested in MCF-7 and T47D cells. As a result, it was found that the ethyl acetate-soluble fraction enhanced proliferation of T47D at 10 μg/mL and suppressed activity of co-treated estradiol in both cell lines, especially in MCF-7 cells, at 1 μg/mL. Due to these results, the ethyl acetate-soluble fraction was subjected to silica gel column chromatography and eluted with chloroform–methanol–water as a gradient solvent system to afford 15 combined fractions. Further purification of the active fractions was carried out using preparative HPLC to give 45 pure compounds including 11 new compounds (**1**–**11**). The following 34 known compounds were identified by comparisons of their spectroscopic data and optical rotations with the values reported in the literature: astilbin (**12**) (De Britto et al., 1995), neoastilbin (**13**) (De Britto et al., 1995), isoastilbin (**14**) (Gaffield et al., 1975), neoisoastilbin (**15**) (De Britto et al., 1995), engeletin (**16**) (Gaffield et al., 1975), isoengeletin (**17**) (Gaffield et al., 1975), (+) taxifolin (**18**) (Nonaka et al., 1987), (+) dihydrokaempferol (**19**) (Xu et al., 2005), naringenin (**20**) (Perry et al., 1999), eriodictyol (**21**) (Gaffield et al., 1975), homoeriodictyol (**22**) (Ibrahim et al., 2003), quercetin (**23**) (Lee et al., 2004), quercitrin (**24**) (Fukunaga et al., 1988), luteolin (**25**) (Sugamoto et al., 2008), (−) catechin (**26**) (Kumar and Rajapaksha, 2005), (−) epicatechin (**27**) (Ban et al., 2006), cinchonain Ia (**28**) (Chen et al., 1993), catechin-(7,8-b,c)-4b-(3,4-dihydroxyphenyl)-2(3H)-pyranone (**29**) (Chen et al., 1993), cinchonain Ib (**30**) (Chen

* Corresponding author. Tel./fax: +81 54 264 5661.
E-mail address: umehara@mail.u-shizuoka-ken.ac.jp (K. Umehara).

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et al., 1993), rhinchoin Ia (**31**) (Foo, 1987), cinchonain Id (**32**) (Chen et al., 1993), (4*S*,8*R*,9*S*)-4,8-bis(3,4-dihydroxyphenyl)-3,4,9,10-tetrahydro-5,9-dihydroxy-2H,8H-benzo[1,2-b:3,4-b′]dipyran-2-one (**33**) (Chen et al., 1993), cinchonain Ic (**34**) (Chen et al., 1993), (4*R*,8*R*,9*S*)-4,8-bis(3,4-dihydroxyphenyl)-3,4,9,10-tetrahydro-5,9-dihydroxy-2H,8H-benzo[1,2-b:3,4-b′]dipyran-2-one (**35**) (Chen et al., 1993), phyllocoumarin (**36**) (Foo, 1989), epiphyllocoumarin (**37**) (Foo, 1989), *trans*-resveratrol (**38**) (Nakajima et al., 1978), piceatannol (**39**) (Yao et al., 2005), isorhapontigenin (**40**) (Silayo et al., 1999), eucryphin (**41**) (Tschesche et al., 1979), (−) syringaresinol (**42**) (Deyama, 1983), 5-*O*-caffeoylshikimic acid (**43**) (Silayo et al., 1999), caffeic acid (**44**) (Flamini et al., 2001), and protocatechuic acid (**45**) (Wu et al., 2007) (Fig. 1).

The spectroscopic features of the new compounds (**1**–**5**) were very similar to one another, suggesting they were flavonoid glycosides and 2″-acetyl astilbin (**1**) was assigned the molecular formula, $C_{23}H_{24}O_{12}$, as determined from its molecular ion $[M+H]^+$ peak at *m*/*z* 493.1355 in HRFABMS. The ^{1}H NMR spectrum of **1** showed a pair of doublet aromatic proton signals [δ 5.90 (d, J = 2 Hz) and 5.92 (d, J = 2 Hz)] and ABX-type aromatic proton signals [δ 6.76 (d, J = 8 Hz), 6.79 (dd, J = 8, 2 Hz) and 6.93 (d, J = 2 Hz)], and it was suggested that the aglycone moiety was a 2,3-*trans* flavanonol from the characteristic signals for H-2 and 3 observed at δ 5.11 (d, J = 10.5 Hz) and 4.53 (d, J = 10.5 Hz), respectively. The ^{1}H NMR spectrum also indicated the presence of a rhamnose moiety from the following signals: [δ 3.97 (d, J = 2 Hz), 1.18 (d, J = 6 Hz)], which are unique for the H-1″ and Me-6″ of the sugar, respectively. Acid hydrolysis of **1** gave (+)-taxifolin (**18**) as an aglycone and a sugar moiety, which was identified by GC analysis as L-rhamnose. The HMBC spectrum of **1** indicated the bonding position of L-rhamnose to be C-3 according to the H–C long-range connectivity of H-1″ [δ 3.97 (d, J = 2 Hz)] to C-3 (δ 78.3). The spectrum supported the NMR assignments since the aromatic proton signals at δ 6.79 (dd, J = 8, 2 Hz) and 6.93 (d, J = 2 Hz) showed correlations with C-2 (δ 83.7). Furthermore, a correlation of the proton resonance of rhamnose at δ 4.87 (H-2″) with the carbonyl carbon signal at δ 171.5 (Me<u>C</u>O-2″) suggested that the bonding position of the acetyl moiety was C-2″. The absolute configuration of the C-2 of **1** was determined as 2*R* due to the detection of a positive Cotton effect ($[\theta]_{328}$ + 6900, $[\theta]_{295}$ − 39,400) in the CD spectrum (Gaffield et al., 1975). On the basis of the above spectroscopic evidence, **1** was found to be a new compound with the structure (2*R*,3*R*)-5,7,3′,4′-tetrahydroxyflavanonol 2″-acetyl rhamnoside or (2*R*,3*R*)-2″-acetyl astilbin.

3″-Acetyl astilbin (**2**), 4″-acetyl astilbin (**3**), and 4″-acetyl isoastilbin (**5**) were assigned the molecular formula $C_{23}H_{24}O_{12}$, which is same as that of **1**, as determined from their molecular ion $[M+Na]^+$ peaks at *m*/*z* 515.1174, 515.1146, and 515.1188 in HRFABMS, respectively. Their ^{1}H NMR spectra indicated that they shared a pair of doublet aromatic proton signals around the δ 5.9, ABX-type aromatic proton signal at δ 6.8–7.0 and rhamnose sugar moieties. From their ^{1}H NMR spectra, **2** and **3** were deduced to be as 2,3-*trans* flavanonol rhamnosides from their H-2 coupling constants [**2**: δ 5.09 (d, J = 11 Hz), **3**: 5.06 (d, J = 11 Hz)], whereas **5** was found to be a 2,3-*cis* flavanonol rhamnoside [**5**: δ 5.42 (d, J = 2 Hz)]. Acid hydrolysis of these compounds (**2**,**3**, and **5**) gave (+)-taxifolin (**18**) as an aglycone with a common sugar moiety, which was identified by GC analysis as L-rhamnose. The HMBC spectra indicated that these compounds commonly bore an L-rhamnose moiety at their C-3 position, as determined by the H–C long range correlations of H-1″ [**2**: δ 4.01 (d, J = 1.5 Hz), **3**: 4.05 (d, J = 1.5 Hz), **5**: 4.96 (d, J = 1.5 Hz)] to C-3 (**2**: δ 75.7, **3**: 78.7, **5**: 74.7). Additionally, the bonding positions of their acetyl moieties were confirmed from the correlations between their rhamnose proton signals [**2**: δ 4.90 (dd, J = 10, 3, H-3″), **3**: 4.85 (t, J = 10, H-4″), **5**: 4.65 (t, J = 10, H-4″)] and carbonyl carbon signals [**2**: δ 172.7 (Me<u>C</u>O-3″), **3**: 172.6 (Me<u>C</u>O-4″), **5**: 172.6 (Me<u>C</u>O-4″)]. The absolute configurations of the C-2 of **2**, **3**, and **5** were determined to be 2*R* due to the detection of a positive Cotton effect at 330 nm and a negative Cotton effect at 295 nm in their CD spectra. From these results, the structures of 3″-acetyl astilbin (**2**), 4″-acetyl astilbin (**3**), and 4″-acetyl isoastilbin (**5**) were determined to be (2*R*,3*R*)-5,7,3′,4′-tetrahydroxyflavanonol 3″-acetyl rhamnoside or (2*R*,3*R*)-3″-acetyl astilbin (**2**), (2*R*,3*R*)-5,7,3′,4′-tetrahydroxyflavanonol 4″-acetyl rhamnoside or (2*R*,3*R*)-4″-acetyl astilbin (**3**), and (2*R*,3*S*)-5,7,3′,4′-tetrahydroxyflavanonol 4″-acetyl rhamnoside or (2*R*,3*S*)-4″-acetyl isoastilbin (**5**), respectively.

3″-Acetyl engeletin (**4**) was assigned the molecular formula $C_{23}H_{24}O_{11}$, as determined from its molecular ion $[M+Na]^+$ peak at 499.1237 in HRFABMS. The ^{1}H NMR spectrum of **4** indicated the structure to be a flavanonol rhamnoside like **2** from the similarity between the two spectra, and **4** was recognized to have a 1,4-disubstituted B-ring from its A_2B_2-type aromatic proton signals [δ 6.82 (d, J = 8 Hz), 7.36 (d, J = 8 Hz)] while ABX-type aromatic proton signals were observed in **2**. That is the only difference between these two compounds. Acid hydrolysis of **4** gave (+)-dihydrokaempferol (**19**) as an aglycone and a sugar moiety, which was identified as L-rhamnose by GC analysis. In the HMBC spectrum of **4**, correlations were observed (i) from the anomeric proton of rhamnose [δ 4.08 (d, J = 1.5 Hz) to C-3 (δ 79.2) and (ii) from H-3″ [δ 4.91 (dd, J = 10, 3 Hz)] to the carbonyl carbon (δ 172.6), which suggested its structure to be (2*R*,3*R*)-5,7,4′-trihydroxyflavanonol 3″-acetyl rhamnoside or (2*R*,3*R*)-3″-acetyl engeletin (**4**).

Corbulain Ia (**6**) and Ib (**7**) were assigned the molecular formula $C_{24}H_{20}O_8$, as determined from their molecular ion $[M+H]^+$ peak at *m*/*z* 437.1242 and the $[M]^+$ peak at *m*/*z* 436.1187 in HRFABMS, respectively. The ^{1}H and ^{13}C NMR spectroscopic data of **6** and **7** were similar to those of cinchonain Ia (**28**) and Ib (**30**), respectively. However, the ^{1}H NMR spectra showed the presence of a 1,4-disubstituted phenyl group [**6**: δ 7.09 (2H, d, J = 8.5 Hz), 6.68 (2H, d, J = 8.5 Hz); **7**: δ 7.28 (2H, d, J = 8.5 Hz), 6.77 (2H, d, J = 8.5 Hz)] and a 1,3,4-trisubstituted phenyl group [**6**: δ 6.60 (1H, d, J = 2 Hz), 6.69 (1H, d, J = 8 Hz), 6.51 (1H, dd, J = 8, 2 Hz); **7**: δ 6.52 (1H, d, J = 2 Hz), 6.60 (1H, d, J = 8 Hz), 6.42 (1H, dd, J = 8, 2 Hz)], instead of the two 1,3,4-trisubstituted phenyl groups found in the cinchonain I series. In the HMBC spectra of **6** and **7**, correlations were observed from the H-7″ [**6**: δ 4.44 (dd, J = 7,1.5); **7**: δ4.52 (dd, J = 7.5,1.5)] to C-2″ and 6″ [**6**: δ115.3 (C-2″), 119.4 (C-6″); **7**: δ115.0 (C-2″), 119.2 (C-6″)], which suggested the bonding position of the catechol group to be C-7″. The NOE spectrum of **7** indicated the configuration of C-7″ from enhancement of the H-2′ and H-6′ proton signals of its B-ring (δ 7.28) when H-7″ (δ 4.52) was irradiated, and no NOE enhancement was observed in the case of **6**. The CD spectra of **6** and **7** also showed good accordance to the corresponding compounds (**28** and **30**) (Chen et al., 1993). From these results, corbulain Ia (**6**) and Ib (**7**) were defined as 2-(4-hydroxyphenyl)-3,4,9,10-tetrahydro-3,5-dihydroxy-10-(3,4-dihydroxyphenyl)-(2*R*,3*R*,10*R*)-2H,8H-benzo [1,2-b:3,4-b′] dipyran-8-one (**6**) and 2-(4-hydroxyphenyl)-3,4,9,10-tetrahydro-3,5-dihydroxy-10-(3,4-dihydroxy phenyl)-(2*R*,3*R*,10*S*)-2H,8H-benzo [1,2-b:3,4-b′] dipyran-8-one (**7**).

Gnetumontanin E (**8**), F (**9**), and G (**10**) were assigned the molecular formula $C_{23}H_{18}O_6$, $C_{23}H_{18}O_7$, and $C_{24}H_{20}O_6$, respectively, as determined from their molecular ion peaks at *m*/*z* 390.1111 $[M]^+$, 406.1071 $[M]^+$, and 404.1288 $[M]^+$ in HRFABMS, respectively. The spectroscopic features of these compounds were very similar to one another and shared many features with those of gnetumontanin C, a stilbene analogue (Li et al., 2004). The ^{1}H NMR spectrum of **8** showed a pair of *meta*-coupled aromatic proton signals [δ 6.48 (d, J = 2.5 Hz) and 6.90 (d, J = 2.5 Hz)], aromatic proton resonances from a 1,3,4-trisubstituted phenyl group [δ 6.47 (dd, J = 8, 2 Hz), 6.53 (d, J = 2 Hz), and 6.70 (d, J = 8 Hz)], aromatic proton

responding from a 1,4-disubstituted phenyl group [δ 6.72 (d, J = 9 Hz), and 7.22 (d, J = 9 Hz)], *trans*-coupled olefinic proton resonances [δ 6.87 (d, J = 16 Hz), and 6.92 (d, J = 16 Hz)], and three aliphatic proton signals [δ 4.48 (dd, J = 7, 2 Hz), 3.06 (dd, J = 16, 7 Hz) and 2.89 (dd, J = 16, 2 Hz)]. Analysis of the ^{13}C NMR spectrum indicated the presence of a carbonyl carbon of a δ-lactone ring (δ 170.1), whereas that of the HMBC spectrum of **8** suggested the bonding position of dihydrocaffeic acid with *trans*-resveratrol from the H-C long range connectivity of H-7′ [δ 4.48 (dd, J = 7, 2 Hz)] to C-9 (δ 139.5), 11 (δ 154.5), 2′ (δ 115.2), 6′ (δ 119.5), and 9′ (δ 170.1). On the basis of the above spectroscopic evidence, gnetumontanin E (**8**) was determined to be a new compound with the structure 3,4-dihydro-7-hydroxy-4-(3,4-dihydroxyphenyl)-5-[(1*E*)-2-(4-hydroxyphenyl) ethenyl]-2H-1-benzopyran-2-one.

In the ^{1}H NMR spectrum of **9**, a second set of 1,3,4-trisubstituted phenyl proton signals [δ 6.71 (d, J = 8 Hz), 6.73 (dd, J = 8, 2 Hz), and 6.85 (d, J = 2 Hz)] was observed instead of the 1,4-disubstituted phenyl proton resonances recognized in the spectrum of **8**. The HMBC spectrum of **9** indicated the bonding position of dihydrocaffeic acid with piceatannol from the H-C long range connectivity of H-7′ [δ 4.47 (dd, J = 6.5, 1.5 Hz)] to C-9 (δ 139.5), 11 (δ 154.5), 2′ (δ 115.2), 6′ (δ 119.5), and 9′ (δ 170.1). On the basis of this spectroscopic evidence, gnetumontanin F (**9**) was determined to be 3,4-dihydro-7-hydroxy-4-(3,4-dihydroxyphenyl)-5-[(1*E*)-2-(3,4-dihydroxyphenyl) ethenyl]-2H-1-benzopyran-2-one.

The ^{1}H NMR spectrum of **10** showed the presence of an additional methoxy group when it was compared with that of **8**, and a nuclear Overhauser effect (NOE) was observed for one of the doublet signals [δ 6.73 (d, J = 2 Hz)], which was assigned to H-2′ by irradiation of the methoxy group signal [δ 3.75 (s)]. From these results, the structure of gnetumontanin G (**10**) was confirmed as 3,4-dihydro-7-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-5-[(1*E*)-2-(4-hydroxyphenyl) ethenyl]-2H-1-benzopyran-2-one.

5,7,3′,4′-Tetrahydroxy-3-phenylcoumarin (**11**) was assigned the molecular formula $C_{15}H_{10}O_6$, as determined from its molecular ion $[M]^+$ peak at *m/z* 286.0491 in the HRFABMS. The ^{1}H NMR spectrum of **11** showed ABX-type coupled aromatic proton signals [δ 7.84 (d, J = 2 Hz), 6.79 (d, J = 8 Hz), 7.40 (dd, J = 8,2 Hz)] and meta-coupled aromatic proton resonances [δ 6.11 (d, J = 2 Hz), 6.07 (d, J = 2 Hz)]. Analysis of the ^{13}C NMR spectrum showed the presence of a carbonyl carbon (δ 168.7) and a pair of olefinic carbons (δ 118.5, 141.3), which suggested that it has a coumarin analogue structure. Although the compound was reported by Baker as a synthetic product from an anhydrocatechin tetramethyl ether, this is the first report of its isolation from a natural source (Baker, 1929).

The estrogenic activity of the isolated compounds was tested by assessing their stimulatory effects on MCF-7 and T47D cell proliferation at concentrations of 0.1, 1, 10, and 100 μM, and their stimulatory activity was determined by comparing their effects with those of a positive control, estradiol (E2), at concentrations ranging from 1 to 100 pM (Table 6). Anti-estrogenic activity was also investigated using MCF-7 and T47D cell proliferation as an index. One hundred pM estradiol (E2) was initially used to enhance cell proliferation, and each compound was tested at 0.1, 1, 10, and 100 μM (Table 7).

The isolates from *S. corbularia* were classified into four groups from their structures, (i) flavonoids (**1**–**5**, **12**–**25**), (ii) catechin derivatives (**6**,**7**, **26**–**37**), (iii) stilbene derivatives (**8**–**10**, **38**–**40**), and (iv) others (**11**, **41**–**45**), and the major constituents in terms of their yield were flavanonol rhamnosides (**1**–**5**,**12**–**17**). Engeletin (**16**) showed anti-estrogenic activity, whereas its aglycone, dihydrokaempferol (**19**), exhibited estrogenic activity in both MCF-7 and T47D cells. Another flavanonol rhamnoside, astilbin (**12**), whose aglycone has an additional hydroxyl group at the C-3′ of **19**, moderately suppressed E2 activity in the cells. Meanwhile, acetates of these flavanonol rhamnosides (**1**–**4**) were recognized to have estrogenic activity in both MCF-7 and T47D cells at a concentration of 100 μM. Although these acetates enhanced the effects of co-treated E2 on T47D cell proliferation at concentrations ranging from 0.1 to 100 μM, they had a mild suppressive effect on E2-induced MCF-7 cell proliferation at concentrations lower than 10 μM.

Catechin (**26**) and epicatechin (**27**) exhibited inhibitory activity on E2-enhanced MCF-7 cell proliferation and attenuated 90% of E2 activity at a concentration of 10 μM. Their analogues (**6**, **7**, **28**–**37**) scarcely showed inhibitory activity against E2 induced MCF-7 cell proliferation at the tested concentrations, whereas they completely suppressed the effects of co-treated E2 on T47D cell proliferation at a concentration of 100 μM without any cytotoxicity, and some compounds whose catechol group was linearly aligned with their catechin skeleton (**32**, **34**) showed mild estrogenic activity against MCF-7 cells.

Resveratrol (**38**) is a phytoestrogen; however, it also showed anti-estrogenic activity against these two cell lines at and above concentrations of 1 μM in addition to suppressing E2 activity to less than 10% at a concentration of 10 μM, and its analogues (**8**, **10**) demonstrated mild anti-estrogenic activity against MCF-7 cells.

3. Conclusion

In our present investigation, 45 phenolic compounds including 11 new compounds were isolated from *S. corbularia* rhizomes and had their estrogenic and anti-estrogenic activity characterized. The extract did not display cytotoxicity against breast cancer cell lines in our study (data not shown) nor COR-L23 human non-small lung cancer in a previous study (Houghton et al., 2007). Some polyphenols isolated in this study, such as resveratrol, quercetin, and catechin, were used to determine their combined effect on MDA-MB-231 human breast cancer cells in nude mice, and they reduced primary tumor growth at dietary concentrations that did not exert any significant inhibitory effect on cell proliferation or cell cycle progression *in vitro* (Schlachterman et al., 2008). Although *S. corbularia* extracts have been used for treating breast and ovary cancers in Thailand, there is a disconnect between its usage and data supporting its use. The major constituents, flavanonol rhamnosides (**12**–**17**) were shown to occupy 15% of total amount of the methanol extract in this study, and were also recognized for their suppressive effect on estradiol at a concentration of 1 μM by monitoring human breast cancer cell proliferation. These data would help to bridge the gap between its common uses and the lack of studies on its effectiveness.

4. Experimental section

4.1. General experimental procedures

Optical rotations were measured on a JASCO DIP-360 digital polarimeter. CD spectra were recorded on a JASCO J-20A spectropolarimeter. UV spectra were recorded on a Hitachi U3410 spectrometer. ^{1}H and ^{13}C spectra were recorded on a JEOL JNM-α 400 instrument, and chemical shifts are given in δ (ppm) with tetramethylsilane (TMS) used as an internal standard at 35 °C. Inverse-detected heteronuclear correlations were measured using HMQC (optimized for $^1J_{C-H}$ = 145 Hz) and HMBC (optimized for $^nJ_{C-H}$ = 8 Hz) pulse sequences with a pulsed field gradient. HRFABMS data were obtained using a JEOL JMS 700 mass spectrometer and an *m*-nitrobenzyl alcohol matrix. HPLC was carried out with a JASCO model 887-PU pump and an 875-UV variable-wavelength detector for preparative separation with reversed-phase columns (Develosil-Lop-ODS column, 12–20 μm, 5 × 50 cm × 2, Nomura Chemical Co. Ltd., at 45 mL/min with detection at 205 nm; TSKgel ODS-80TS column, 5 μm,

6 × 60 cm × 2, Tosoh Chemicals Co. Ltd., at 45 mL/min with detection at 205 nm) and a JASCO model 880-PU pump and an 875-UV variable-wavelength detector for semi-preparative separation with reversed-phase column (TSKgel ODS-100 V column, 5 μm, 2 × 25 cm, Tosoh Chemicals Co. Ltd., at 9 mL/min with detection at 205 nm).

4.2. Chemicals

Eagle's MEM and RPMI-1640 media were obtained from Nissui Pharmaceutical Co. Ltd. (Tokyo, Japan). Fetal bovine serum (FBS) was purchased from Gibco (Grand Island, NY), antibiotics were obtained from Meiji Seika Kaisha Ltd. (Tokyo, Japan), L-glutamine was from Wako Pure Chemical Industries Ltd. (Osaka, Japan), and 17β-estradiol and dextran-coated charcoal (DCC) were from Sigma Chemicals (St. Louis, MO).

4.3. Plant material

Commercially available rhizomes of *Smilax corbularia* Kunth (Smilacaceae) were purchased from a traditional Thai herb store in Nakhon Sri Thammaraj, Thailand, in July 2007. The plant was identified by Prof. Akira Ueno, University of Shizuoka. A voucher specimen was deposited at the Laboratory of Pharmacognosy, University of Shizuoka, Japan (S007108).

4.4. Extraction and isolation

The powdered rhizomes of *S. corbularia* (1.2 kg) were extracted three times with MeOH (3 × 20 L) at room temperature, with the extracts combined and evaporated under reduced pressure to yield a viscous mass (203 g). A sample of the MeOH crude extract (70 g) was suspended in H_2O and partitioned with EtOAc to yield EtOAc (38 g) and H_2O-soluble fractions. The H_2O-soluble fraction was successively subjected to HP-20 column chromatography (cc) eluted with MeOH–H_2O (1:1, 3:1, v/v) and MeOH (5 L each) to yield MeOH–H_2O (1:1, v/v) (9.0 g), MeOH–H_2O (3:1, v/v) (0.5 g), and MeOH (0.1 g) v/v solubles, respectively.

The EtOAc-soluble fraction (38 g) was applied to a silica gel column and fractionated using a $CHCl_3$–MeOH–H_2O gradient solvent system. Fractions were collected and pooled by TLC analysis to afford 15 combined fractions.

From these combined fractions, fraction A [0.9 g, eluted with $CHCl_3$–MeOH (9:1)] was subjected to semipreparative HPLC with CH_3CN–H_2O (30:70) to yield **42** (8.2 mg; t_R 14.5 min). Fraction B [0.4 g, eluted with $CHCl_3$–MeOH (9:1)] was subjected to semipreparative HPLC with CH_3CN–H_2O (30:70) to yield **20** (3.5 mg; t_R 30 min) and **22** (1.0 mg; t_R 33 min). Fraction C [147.9 mg, eluted with $CHCl_3$–MeOH (9:1)] was subjected to semipreparative HPLC with MeOH–H_2O (35–50:65–50) to yield **19** (4.5 mg; t_R 21 min) **21** (1.9 mg; t_R 22 min), and **40** (0.5 mg; t_R 31 min). Fraction D [644.9 mg, eluted with $CHCl_3$–MeOH (9:1)] was subjected to preparative HPLC [TSK-Gel ODS-80TS column with MeOH–H_2O (45:55–65:35) linear gradient within 10 h] to afford **8** (9.4 mg; t_R 274 min), **10** (2.5 mg; t_R 324 min), **18** (65.5 mg; t_R 135 min), **23** (7.7 mg; t_R 300 min), **25** (2.7 mg; t_R 202 min), **36** (2.2 mg; t_R 154 min), **37** (3.1 mg; t_R 200 min), **38** (23.9 mg; t_R 178 min), **44** (5.7 mg; t_R 136 min), and **45** (10.4 mg; t_R 96 min). Fraction E [1.2 g, eluted with $CHCl_3$–MeOH (8:2)] was subjected to preparative HPLC [TSK-Gel ODS-80TS column with MeOH–H_2O (40:60–50:50) linear gradient within 10 h] to afford **1** (8.7 mg; t_R 225 min), **2** (30 mg; t_R 220 min), **3** (24.4 mg; t_R 280 min), **4** (1.7 mg; t_R 300 min), **5** (4.7 mg; t_R 240 min), **6** (4.5 mg; t_R 335 min), **7** (2.7 mg; t_R 154 min), **9** (1.3 mg; t_R 221 min), **11** (4.5 mg; t_R 336 min), **28** (25 mg; t_R 241 min), **33** (1.8 mg; t_R 158 min), **39** (14.2 mg; t_R 132 min), and **41** (23 mg; t_R 70 min). Fraction F of the EtOAc-soluble fraction [3.4 g, eluted with $CHCl_3$–MeOH (8:2)] was subjected to preparative HPLC [TSK-Gel ODS-80TS column with MeOH–H_2O (35:65–55:45) linear gradient within 10 h] to afford **2** (8.5 mg; t_R 255 min),**12** (10.5 mg; t_R 180 min), **15** (10.1 mg; t_R 240 min), **16** (614 mg; t_R 300 min), **17** (181 mg; t_R 348 min), **26** (50.3 mg; t_R 75 min), **27** (11 mg; t_R 81 min), **28** (60 mg; t_R 350 min), **29** (20.4 mg; t_R 168 min), **30** (46.5 mg; t_R 162 min), **31** (73.4 mg; t_R 237 min), **32** (12.5 mg; t_R 225 min), **33** (16.6 mg; t_R 150 min), **34** (3.1 mg; t_R 420 min), **35** (17.6 mg; t_R 144 min), and **43** (420 mg; t_R 42 min). Part (4 g) of fraction G [18.8 g, eluted with $CHCl_3$–MeOH (8:2)] was subjected to preparative HPLC [Develosil-Lop-ODS column with MeOH–H_2O (35:65), detector: UV 205 nm] to afford **12** (728.5 mg; t_R 190 min), **13** (95 mg; t_R 170 min), **14** (918 mg; t_R 225 min), **15** (300 mg; t_R 210 min), **24** (22 mg; t_R 240 min), **29** (22 mg; t_R 180 min), **30** (37 mg; t_R 110 min), and **31** (29 mg; t_R 210 min).

2″-Acetyl astilbin (**1**): Pale brown amorphous powder; $[\alpha]_D^{25}$ 19.6° (*c* 0.3, MeOH); CD (*c* 0.1, MeOH) $[\theta]_{328}$ + 6900, $[\theta]_{295}$ − 39,400, $[\theta]_{224}$ + 19,700, $[\theta]_{208}$ − 26,570; UV (MeOH) λ_{max} (log ε) 220 (4.49), 230 (4.39), 290 (4.35), 330 (3.88) nm; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; HRFABMS *m/z* 493.1355 $[MH]^+$ (calcd for $C_{23}H_{25}O_{12}$, 493.1346).

3″-Acetyl astilbin (**2**): Brown amorphous powder; $[\alpha]_D^{25}$ 15.2° (*c* 0.2, MeOH); CD (*c* 0.1, MeOH) $[\theta]_{328}$ + 8900, $[\theta]_{294}$ − 34,400, $[\theta]_{223}$ + 32,500, $[\theta]_{209}$ − 36,410; UV (MeOH) λ_{max} (log ε) 220 (4.56), 230 (4.45), 290 (4.34), 330 (3.85) nm; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; HRFABMS *m/z* 515.1174 $[M + Na]^+$ (calcd for $C_{23}H_{24}NaO_{12}$, 515.1165).

4″-Acetyl astilbin (**3**): Brown amorphous powder; $[\alpha]_D^{25}$ − 20.4° (*c* 0.3, MeOH); CD (*c* 0.1, MeOH) $[\theta]_{332}$ + 4900, $[\theta]_{295}$ − 21,650, $[\theta]_{222}$ + 16,700, $[\theta]_{208}$ − 19,700; UV (MeOH) λ_{max} (log ε) 207 (4.67), 230 (4.46), 290 (4.30), 330 (4.07) nm; for ^1H and ^{13}C NMR data see Table 1; HRFABMS *m/z* 515.1146 $[M + Na]^+$ (calcd for $C_{23}H_{24}NaO_{12}$, 515.1165).

3″-Acetyl engeletin (**4**): Pale brown amorphous power; $[\alpha]_D^{25}$ 8.8° (*c* 0.1, MeOH); CD (*c* 0.1, MeOH) $[\theta]_{325}$ + 11,400, $[\theta]_{288}$ − 38,950, $[\theta]_{235}$ + 11,400, $[\theta]_{212}$ + 31,350; UV (MeOH) λ_{max} (log ε) 216 (4.59), 292 (4.36), 330 (3.98) nm; for ^1H and ^{13}C NMR spectroscopic data, see Table 2; HRFABMS *m/z* 499.1237 $[M + Na]^+$ (calcd for $C_{23}H_{24}NaO_{11}$, 499.1216).

4″-Acetyl isoastilbin (**5**): Reddish-brown amorphous powder; $[\alpha]_D^{25}$ − 111.2° (*c* 0.1, MeOH); CD (*c* 0.1, MeOH) $[\theta]_{343}$ + 3950, $[\theta]_{312}$ − 9850, $[\theta]_{283}$ − 7900, $[\theta]_{222}$ − 14,800; UV (MeOH) λ_{max} (log ε) 218 (4.46), 292 (4.31), 330 (3.96) nm; for ^1H and ^{13}C NMR spectroscopic data, see Table 2; HRFABMS *m/z* 515.1188 $[M + Na]^+$ (calcd for $C_{23}H_{24}NaO_{12}$, 515.1165).

Corbulain Ia (**6**): Dark red amorphous powder; $[\alpha]_D^{25}$ − 12.8° (*c* 0.1, MeOH); CD (*c* 1.0, MeOH) $[\theta]_{317}$ − 1300, $[\theta]_{283}$ − 1300, $[\theta]_{250}$ + 1150, $[\theta]_{227}$ − 10,460; UV (MeOH) λ_{max} (log ε) 214 (4.67), 283 (3.98), 335 (3.39) nm; for ^1H and ^{13}C NMR spectroscopic data, see Table 3; FABMS *m/z* 437.1242 $[MH]^+$ (calcd for $C_{24}H_{21}O_8$, 437.1236).

Corbulain Ib (**7**): Dark red amorphous powder; $[\alpha]_D^{25}$ 27.9° (*c* 0.3, MeOH); CD (*c* 1.0, MeOH) $[\theta]_{282}$ + 1300, $[\theta]_{230}$ + 14,800; UV (MeOH) λ_{max} (log ε) 211 (4.72), 281 (3.88), 315 (3.16) nm; for ^1H and ^{13}C NMR spectroscopic data, see Table 3; FABMS *m/z* 436.1187 $[M]^+$ (calcd for $C_{24}H_{20}O_8$, 436.1158).

Gnetumontanin E (**8**): Brown amorphous powder; $[\alpha]_D^{25}$ 7.4° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 209 (4.65), 296 (4.44), 309 (4.45), 318 (4.46) nm; for ^1H and ^{13}C NMR spectroscopic data, see Table 4. HRFABMS *m/z* 390.1111 $[M]^+$ (calcd for $C_{23}H_{18}O_6$, 390.1103).

Gnetumontanin F (**9**): Brown amorphous powder; $[\alpha]_D^{25}$ 0.2° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.65), 230 (4.44), 288 (4.15), 327 (4.13) nm; for ^1H and ^{13}C NMR spectroscopic data, see Table 4. HRFABMS *m/z* 406.1071 $[M]^+$ (calcd for $C_{23}H_{18}O_7$, 406.1053).

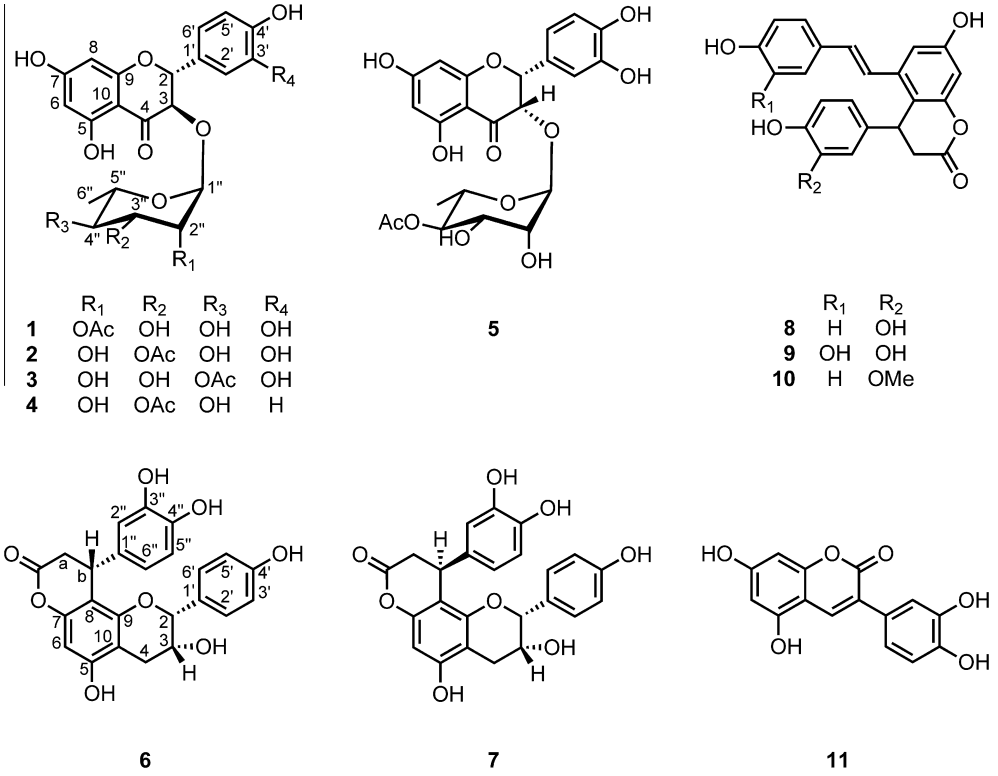

Fig. 1. New Compounds from Rhizomes of *S. corbularia*.

Table 1
NMR spectroscopic data (400 MHz, MeOH-d_4) for Compounds **1**–**3**.

Position	**1**		**2**		**3**	
	δ_H (J in Hz)	δ_C[a]	δ_H (J in Hz)	δ_C[a]	δ_H (J in Hz)	δ_C[a]
2	5.11, d (10.5)	83.7	5.09, d (11)	83.9	5.06, d, (11)	84.0
3	4.53, d (10.5)	78.3	4.57, d (11)	75.7	4.59, d (11)	78.7
4		195.3		195.7		196.0
5		165.6		165.6		165.5
6	5.90, d (2)	96.4	5.89, d (2)	96.4	5.90, d (2)	96.3
7		168.8		168.9		168.7
8	5.92, d (2)	97.5	5.92, d (2)	97.5	5.93, d (2)	97.4
9		164.1		164.2		164.2
10		102.5		102.5		102.5
1′		128.9		129.3		129.2
2′	6.93, d (2)	115.1	6.96, d (2)	115.5	6.96, d (2)	115.6
3′		146.7		146.6		146.6
4′		147.4		147.4		147.5
5′	6.76, d (8)	116.5	6.79, d (8)	116.3	6.81, d (8)	116.4
6′	6.79, dd (8, 2)	120.4	6.84, dd (8, 2)	120.5	6.84, dd (8, 2)	120.5
1″	3.97, d (1.5)	99.3	4.01, d (1.5)	102.1	4.05, d (1.5)	102.0
2″	4.87, dd (3, 1.5)	73.2	3.70, dd (3, 1.5)	69.5	3.56, dd (3.5, 1.5)	71.8
3″	3.82, dd (10, 3.5)	70.5	4.90, dd (10, 3)	75.7	3.80, dd (10, 3.5)	70.3
4″	3.25, t (10)	74.1	3.50, t (10)	71.1	4.85, t (10)	75.5
5″	4.17, m	70.6	4.35, m	70.6	4.41, m	68.3
6″	1.18, d (6)	17.8	1.20, d (6)	17.8	1.05, d (6.5)	17.6
–CO		171.5		172.7		172.6
$-CH_3$	1.97, s	21.7	2.07, s	21.0	2.08, s	21.0

[a] Assignments were based on HMQC and HMBC experiments.

Table 2
NMR spectroscopic data (400 MHz, MeOH-d_4) for Compounds **4** and **5**.

Position	**4**		**5**	
	δ_H (J in Hz)	δ_C[a]	δ_H (J in Hz)	δ_C[a]
2	5.15, d (11.5)	83.8	5.42, d (2)	82.1
3	4.60, d (11.5)	79.2	4.07, d (2)	74.7
4		195.8		193.6
5		165.9		166.3
6	5.91, d (2)	97.6	5.91, d (2)	97.7
7		168.7		169.8
8	5.89, d (2)	96.4	5.97, d (2)	96.5
9		164.2		164.6
10		102.2		101.6
1′		128.7		128.8
2′	7.36, d (9)	130.1	6.95, br.s	114.9
3′	6.82, d (9)	116.4		146.7
4′		159.5		146.8
5′	6.82, d (9)	116.4	6.82, d (8)	116.2
6′	7.36, d (9)	130.1	6.79, dd (8, 2)	118.9
1″	4.08, d (1.5)	102.2	4.96, d (1.5)	99.2
2″	3.64, dd (3, 1.5)	69.5	3.67, dd (3, 1.5)	71.8
3″	4.91, dd, (10, 3)	75.7	3.58, dd (10, 3)	70.0
4″	3.50, t (10)	71.1	4.65, t (10)	75.0
5″	4.39, m	70.7	2.12, m	67.1
6″	1.20, d (6.5)	17.8	0.76, d (6.5)	17.8
–CO		172.6		172.9
$-CH_3$	2.07, s	21.0	2.06, s	21.1

[a] Assignments were based on HMQC and HMBC experiments.

Gnetumontanin G (**10**): Pale brown amorphous powder; $[\alpha]_D^{25}$ 11.0° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.64), 230 (4.45), 290 (4.35), 305 (4.39), 319 (4.40) nm; for ^1H and ^{13}C NMR spectroscopic data, see Table 4. HRFABMS *m/z* 404.1288 $[M]^+$ (calcd for $C_{24}H_{20}O_6$, 404.1260).

5,7,3′,4′-Tetrahydroxy-3-phenylcoumarin (**11**): Dark brown amorphous powder; $[\alpha]_D^{25}$ 13.0° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.48), 264 (4.02), 290 (4.00), 404 (4.13) nm; for ^1H and ^{13}C NMR spectroscopic data, see Table 5; HRFABMS *m/z* 286.0491 $[M]^+$ (calcd for $C_{15}H_{10}O_6$, 286.0477).

Table 3
NMR spectroscopic data (400 MHz, MeOH-d_4) for **6** and **7**.

Position	**6**		**7**	
	δ_H (J in Hz)	δ_C[a]	δ_H (J in Hz)	δ_C[a]
2	4.98, br.s	80.2	4.86, br.s	79.8
3	4.20, m	67.0	4.27, m	66.4
4	2.93, dd (17, 4.5)	29.0	2.92, dd (17.5, 4)	29.7
	2.82, dd (17, 3)		2.85, dd (17.5, 3)	
5		157.3		157.4
6	6.20, s	96.4	6.20, s	96.3
7		152.1		152.1
8		106.0		106.1
9		153.4		153.5
10		105.1		105.3
1′		131.0		131.2
2′	7.09, d (8.5)	128.9	7.28, d (8.5)	128.9
3′	6.68, d (8.5)	115.8	6.77, d (8.5)	115.9
4′		157.8		157.9
5′	6.68, d (8.5)	115.8	6.77, d (8.5)	115.9
6′	7.09, d (8.5)	128.9	7.28, d (8.5)	128.9
1″		135.5		135.5
2″	6.60, d (2)	115.3	6.52, d (2)	115.0
3″		146.5		146.3
4″		145.2		145.1
5″	6.69, d (8)	116.5	6.60, d (8)	116.5
6″	6.51, dd (8, 2)	119.4	6.42, dd (8, 2)	119.2
α	3.01, dd, (16, 7)	38.4	3.01, dd (16.5, 7.5)	38.6
	2.85, dd (16, 1.5)		2.85, dd (16.5, 1.5)	
β	4.44, dd (7, 1.5)	35.4	4.52, dd (7.5, 1.5)	35.5
–COO–		170.6		170.7

[a] Assignments were based on HMQC and HMBC experiments.

Table 4
NMR spectroscopic data (400 MHz, MeOH-d_4) for Compounds **8**–**10**.

Position	**8**		**9**		**10**	
	δ_H (J in Hz)	δ_C[a]	δ_H (J in Hz)	δ_C[a]	δ_H (J in Hz)	δ_C[a]
1		130.1		130.3		130.1
2	7.22, d (9)	129.4	6.85, d (2)	113.9	7.24, d (9)	129.0
3	6.72, d (9)	116.6		146.7	6.72, d (9)	116.6
4		158.8		147.0		158.9
5	6.72, d (9)	116.6	6.71, d (8)	116.9	6.72, d (9)	116.6
6	7.22, d (9)	129.4	6.73, dd (8, 2)	120.6	7.24, d (9)	129.0
7	6.87, d (16)	132.9	6.81, d (16)	133.3	6.88, d (16)	133.0
8	6.92, d (16)	122.7	6.85, d (16)	122.6	6.95, d (16)	122.7
9		139.5		139.5		139.5
10		115.5		115.4		115.5
11		154.5		154.5		154.5
12	6.48, d (2.5)	103.9	6.48, d (2)	103.8	6.49, d (2.5)	103.9
13		158.8		158.8		158.9
14	6.90, d (2.5)	109.7	6.89, d (2)	109.7	6.90, d (2.5)	109.8
1′		134.7		134.6		134.7
2′	6.53, d (2)	115.2	6.53, d (2)	115.2	6.73, d (2)	111.9
3′		146.7		146.5		149.4
4′		145.5		145.5		146.8
5′	6.70, d (8)	116.9	6.69, d (8)	116.4	6.71, d (8)	116.7
6′	6.47, dd (8, 2)	119.5	6.47, dd (8, 2)	119.5	6.52, dd (8, 2)	120.5
7′	4.48, dd (7, 2)	38.1	4.47, dd (6.5, 1.5)	38.1	4.57, dd (7, 1.5)	38.3
8′a	2.89, dd (16, 2)	39.0	2.90, dd (16, 1.5)	39.0	2.93, dd (16, 1.5)	38.8
8′b	3.06, dd (16, 7)		3.07, dd (16, 6.5)		3.09, dd (16, 7)	
9′		170.1		170.1		170.1
3′-OCH_3					3.75, s	56.4

[a] Assignments were based on HMQC and HMBC experiments.

4.5. Acid hydrolysis of glycosides (*1–5*, *12–17* and *24*)

Sugar identification was conducted according to a previously reported procedure (Luecha et al., 2009). Compound **1** (2 mg) was heated to 100 °C with dioxane (0.05 mL) and 5% H_2SO_4 (0.05 mL) for 1 h. After dilution with H_2O, the reaction mixture was extracted twice with EtOAc. The EtOAc layer was evaporated to give (+)-taxifolin (**18**) as an amorphous solid, and **18** was identified by direct comparison with an authentic sample. The aqueous layer was passed through an Amberlite IRA-60E column. The H_2O eluate was then concentrated, and the residue was treated with D-cysteine (0.05 mg) in H_2O (0.03 mL) and pyridine (0.015 mL) at 60 °C for 1 h with stirring. After the solution had been concentrated and the reaction mixture dried, pyridine (0.015 mL), hexamethyldisilazane (0.015 mL), and trimethylsilyl chloride (0.015 mL) were added to the residue. The reaction mixture was then heated at 60 °C for 30 min, and the supernatant was analyzed by GC. GC conditions: column GL Sciences TC-l, 0.25 mm x 30 m; column temp: 235 °C; carrier gas: N_2; t_R (min): D-rhamnose (11.4), L-rhamnose (11.6). The t_R of L-rhamnose was detected from **1**. Acid hydrolysis of other compounds was performed in the same manner as used for **1**, and the t_R of L-rhamnose was detected in these compounds.

Table 5
NMR spectroscopic data (400 MHz, MeOH-d_4) for Compound **11**.

Position	**11**	
	δ_H (J in Hz)	δ_C[a]
2		168.7
3		118.5
4	7.88, s	141.3
5		155.5
6	6.11, d (2)	99.2
7		160.6
8	6.07, d (2)	91.1
9		155.7
10		106.2
1′		128.6
2′	7.84, d (2)	119.4
3′		145.9
4′		149.3
5′	6.79, d (8)	116.0
6′	7.40, dd (8, 2)	127.0

[a] Assignments were based on HMQC and HMBC experiments.

Table 6
Cell proliferation stimulatory activities of isolates against MCF-7 and T47D cells.

Compound	Estrogenic activity (EqE in mM)			
	MCF-7		T47D	
	EqE_{10}[a]	EqE_{100}[a]	EqE_{10}[a]	EqE_{100}[a]
1	39	–	1	20
2	55	–	12	93
3	15	–	6	27
4	23	–	28	
5	22	–	11	73
6	51	–	>100	–
14	>100	–	40	–
15	53	–	73	–
19	6	10	8	29
20	3	–	5	55
22	28	–	64	–
23	88	–	81	–
27	>100	–	82	–
32	32	–	>100	–
33	70	–	>100	–
37	97	–	>100	–
38	21	–	1	–
39	>100	–	18	–
40	>100	–	6	–
Daidzein	0.2	6	0.4	8

[a] EqE_{10} and EqE_{100} represent the concentration of the compound that stimulated cell proliferation equivalent to that induced by 10 pM and 100 pM estradiol, respectively. These values were determined by linear regression analysis using at least five different concentrations in quadruplicate. Compounds **7**–**13**, **16**–**18**, **21**, **24**–**26**, **28**–**31**, **34**–**36**, **41**–**45** were inactive.

Table 7
Inhibitory activities of isolates against E2-Enhanced MCF-7 and T47D cell proliferation.

Compound	Anti-estrogenic activity (μM)							
	MCF-7				T47D			
	$iEqE_{50}$[a]	$iEqE_{10}$[a]	$iEqE_1$[a]	IL[b]	$iEqE_{50}$[a]	$iEqE_{10}$[a]	$iEqE_1$[a]	IL[b]
1	<0.1	–	–		–	–	–	
2	<0.1	–	–		–	–	–	
3	<0.1	–	–		–	–	–	
4	<0.1	–	–		–	–	–	
5	<0.1	–	–		–	–	–	
6	–	–	–		3	33	76	
7	>100	–	–		<0.1	10	95	
8	4	10	41		<0.1	–	–	M[c]
9	>100	–	–		<0.1	–	–	M[c]
10	<0.1	–	–	M[c]	<0.1	–	–	M[c]
11	<0.1	0.2	>100		<0.1	–	–	M[c]
12	<0.1	–	–	M[c]	<0.1	–	–	M[c]
13	<0.1	–	–	M[c]	<0.1	–	–	
14	<0.1	–	–	M[c]	–	–	–	
15	<0.1	–	–	M[c]	–	–	–	
16	<0.1	0.7	>100		<0.1	–	–	M[c]
17	0.8	9	>100		<0.1	<0.1	–	S[d]
18	<0.1	0.2	5		<0.1	<0.1	–	S[d]
19	–	–	–		<0.1	<0.1	–	
20	–	–	–		<0.1	<0.1	–	
21	9	14	28		<0.1	0.8	10	
22	<0.1	–	–		0.3	–	–	
23	<0.1	–	–		1	–	–	
24	1.3	>100	–		0.8	–	–	
25	<0.1	–	–	M[c]	<0.1	–	–	M[c]
26	3	38	>100		<0.1	–	–	M[c]
27	0.4	10	>100		<0.1	–	–	M[c]
28	<0.1	–	–	M[c]	1	–	–	
29	>100	–	–		13	36	68	
30	>100	–	–		2	28	64	
31	<0.1	–	–	M[c]	<0.1	19	59	
32	<0.1	–	–		<0.1	19	60	
33	<0.1	–	–		<0.1	20	60	
34	>100	–	–		17	37	67	
35	>100	–	–		5	30	65	
36	>100	–	–		<0.1	<0.1	>100	
37	–	–	–		33	70	>100	
38	0.8	3	14		0.7	8	–	
39	>100	–	–		<0.1	–	–	
40	<0.1	–		M[c]	20	41	70	M[c]
41	<0.1	–	–	M[c]	<0.1	–		M[c]
42	1	4	7		3	11	24	
43	<0.1	–	–	M[c]	<0.1	–	–	M[c]
44	<0.1	–	–	M[c]	<0.1	–	–	M[c]
45	<0.1	–	–	M[c]	<0.1	–	–	M[c]
Tam[e]	0.1	0.5	5		0.1	0.8	15	

[a] $iEqE_{50}$, $iEqE_{10}$, and $iEqE_1$ represent the concentrations of the compound that inhibited the cell proliferation enhanced by 100 pM of E_2 to levels equivalent to those induced by 50 pM, 10 pM, and 1 pM of E_2 treatment, respectively. These values were determined by linear regression analysis using four different concentrations.
[b] IL Inhibitory level of the compound.
[c] Mild inhibition (M), more than 50% inhibition through the concentrations tested.
[d] Strong inhibition (S), more than 90% inhibition through the concentrations tested.
[e] Tamoxifen.

4.6. Cell culture

MCF-7 and T47D human breast cancer cells were purchased and cultured as described in a previous report. Estrogenic and anti-estrogenic activity assays were performed according to the previously reported method (Umehara et al., 2009).

4.7. Data and statistical analysis

Statistical differences were determined by analysis of variance followed by Dunnett's multiple comparison test. Statistical significance was established at the $p < 0.05$ level.

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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.phytochem.2010.12.018.

References

Baker, W., 1929. CCIX. Some substances derived from the anhydrocatechin tetramethyl ethers. J. Chem. Soc., 1593–1604.

Ban, J.Y., Jeon, S.Y., Bae, K., Song, K.S., Seong, Y.H., 2006. Catechin and epicatechin from *Smilacis chinae* rhizome protect cultured rat cortical neurons against amyloid beta protein (25–35)-induced neurotoxicity through inhibition of cytosolic calcium elevation. Life Sci. 79, 2251–2259.

Chen, H.F., Tanaka, T., Nonaka, G., Fujioka, T., Mihashi, K., 1993. Phenylpropanoid-substituted catechins from *Castanopsis hystrix* and structure revision of cinchonains. Phytochemistry 33, 183–187.

De Britto, J., Manickam, V.S., Gopalakrishnan, S., Ushioda, T., Tanaka, N., 1995. Determination of aglycone chirality in dihydroflavonol 3-*O*-α-L-rhamnosides by ^{1}H NMR spectroscopy. Chem. Pharm. Bull. 43, 338–339.

Deyama, T., 1983. The constituents of *Eucommia ulmoides* OLIV. I. Isolation of (+)-medioresinol di-*O*-β-D-glucopyranoside. Chem. Pharm. Bull. 31, 2993–2997.

Flamini, G., Antognoli, E., Morelli, I., 2001. Two flavonoids and other compounds from the aerial parts of *Centaurea bracteata* from Italy. Phytochemistry 57, 559–564.

Foo, L.Y., 1987. Phenylpropanoid derivatives of catechin, epicatechin and phylloflavan from *Phyllocladus trichomanoides*. Phytochemistry 26, 2825–2830.

Foo, L.Y., 1989. Flavanocoumarins and flavanophenylpropanoids from *Phyllocladus trichomanoides*. Phytochemistry 28, 2477–2481.

Fukunaga, T., Nishiya, K., Kajikawa, I., Watanabe, Y., Suzuki, N., Takeya, K., Itokawa, H., 1988. Chemical studies on the constituents of *Hyphear Tanakae* HOSOKAWA from different host trees. Chem. Pharm. Bull. 36, 1180–1184.

Gaffield, W., Waiss, A.C., Tominaga, T., 1975. Structural relations and interconversions of isomeric astilbins. J. Org. Chem. 40, 1057–1061.

Houghton, P., Fang, R., Techatanawat, I., Steventon, G., Hylands, P.J., Lee, C.C., 2007. The sulphorhodamine (SRB) assay and other approaches to testing plant extracts and derived compounds for activities related to reputed anticancer activity. Methods 42, 377–387.

Ibrahim, A.S., Galal, A.M., Ahmed, M.S., Mossa, G.S., 2003. *O*-Demethylation and sulfation of 7-methoxylated flavanones by *Cunninghamella elegans*. Chem. Pharm. Bull. 51, 203–206.

Kumar, N.S., Rajapaksha, M., 2005. Separation of catechin constituents from five tea cultivars using high-speed counter-current chromatography. J. Chromatogr. A 1083, 223–228.

Lee, J.H., Ku, C.H., Baek, N., Kim, S., Park, H.W., Kim, D.K., 2004. Phytochemical constituents from *Diodia teres*. Arch. Pharm. Res. 27, 40–43.

Li, X.M., Lin, M., Wang, Y.H., Liu, X., 2004. Four new stilbenoids from the lianas of *Gnetum montanum f Megalocarpum*. Planta Med. 70, 160–165.

Luecha, P., Umehara, K., Miyase, T., Noguchi, H., 2009. Antiestrogenic constituents of the Thai medicinal plants *Capparis flavicans* and *Vitex glabrata*. J. Nat. Prod. 72, 1954–1959.

Nakajima, K., Taguchi, H., Endo, T., Yosioka, I., 1978. The constituents of *Scirpus fluviatilis* (TORR.) A. GRAY. I.: The structures of two new hydroxystilbene dimers, Scirpusin A and B. Chem. Pharm. Bull. 26, 3050–3057.

Nonaka, G., Goto, Y., Kinjo, J., Nohara, T., Nishioka, I., 1987. Tannins and related compounds LII. Studies on the constituents of the leaves of *Thujopsis dolabrata* SIEB. *et* ZUCC. Chem. Pharm. Bull. 35, 1105–1108.

Perry, K.S.P., Nagem, T.J., De Almeida, W.B., 1999. Conformational study of naringenin in the isolated and solvated states by semiempirical and *ab initio* methods. Struct. Chem. 10, 277–284.

Schlachterman, A., Valle, F., Wall, K.M., Azios, N.G., Castillo, L., Morell, L., Washington, A.V., Cubano, L.A., Dharmawardhane, S.F., 2008. Combined resveratrol, quercetin, and catechin treatment reduces breast tumor growth in a nude mouse model. Trans. Oncol. 1, 19–27.

Silayo, A., Ngadjui, B.T., Abegaz, B.M., 1999. Homoisoflavonoids and stilbenes from the bulbs of *Scilla nervosa subsp Rigidifolia*. Phytochemistry 52, 947–955.

Sugamoto, K., Matsushita, Y., Matsui, T., Kurogi, C., Park, J.C., 2008. Isolation and analysis components of Ashitaba (*Angelica keiskei* Koidzumi) leaves growing in Miyazaki and Korea. Memoirs of the Faculty of Engineering, Miyazaki University 37, 75–80.

Tewtrakul, S., Itharat, A., Rattanasuwan, P., 2006. Anti-HIV-1 protease- and HIV-1 integrase activities of Thai medicinal plants known as Hua-Khao-Yen. J. Ethnopharmacol. 105, 312–315.

Tschesche, R., Delhvi, S., Sepulveda, S., Breitmaier, E., 1979. Eucryphin, a new chromone rhamnoside from the bark of *Eucryphia cordifolia*. Phytochemistry 18, 867–869.

Umehara, K., Nemoto, K., Matsushita, A., Terada, E., Monthakantirat, O., De-Eknamkul, W., Miyase, T., Warashina, T., Degawa, M., Noguchi, H., 2009. Flavonoids from the heartwood of the Thai medicinal plant *Dalbergia parviflora* and their effects on estrogenic-responsive human breast cancer cells. J. Nat. Prod. 72, 2163–2168.

Wu, H.C., Toit, E.S., Reinhardt, C.F., Rimando, A.M., Kooy, F., Meyer, J.M., 2007. The phenolic, 3, 4-dihydroxybenzoic acid, is an endogenous regulator of rooting in *Protea cynaroides*. Plant Growth Regul. 52, 207–215.

Xu, J., Li, X., Zhang, P., Li, Z., Wang, Y., 2005. Antiinflammatory constituents from the roots of *Smilax bockii* WARB. Arch. Pharm. Res. 28, 395–399.

Yao, C., Lin, M., Liu, X., Wang, Y., 2005. Stilbene derivatives from *Gnetum cleistostachyum*. J. Asian Nat. Prod. Res. 7, 131–137.