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A New Coumestan Glucoside from *Eclipta prostrata*

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Abstract – *Eclipta prostrata* is an annual herb, belonging to Asteraceae family, and has been traditionally used to improve immunity and treat hepatitis and bacterial disease in Korea. In this study, a new coumestan glucoside (**1**) along with ten known compounds (**2** – **11**) was isolated from *E. prostrata*. The chemical structures of isolates were elucidated to be wedelolactone-9-*O*-β-D-glucopyranoside (**1**), wedelolactone (**2**), demethylwedelolactone (**3**), apigenin (**4**), apigenin-7-sulfate (**5**), luteolin (**6**), luteolin-7-sulfate (**7**), luteolin-7-*O*-β-D-glucopyranoside (**8**), pratensein-7-*O*-β-D-glucopyranoside (**9**), 3,4-di-*O*-caffeoylquinic acid (**10**) and 3,5-di-*O*-caffeoylquinic acid (**11**) based on the spectroscopic evidence.
Keywords – *Eclipta prostrata,* Asteraceae, Phenolic compounds, Wedelolactone-9-*O*-β-D-glucopyranoside

Introduction

Eclipta prostrata is an annual herb, belonging to Asteracea family, and distributed in the tropical and subtropical areas, especially Asia and Africa.[1] *Eclipta prostrata* has been traditionally used to improve immunity and treat hepatitis and bacterial diseases in Korea.[2] In India, *E. prostrata* has been used to treat body pain, fever, hair loss, jaundice, liver enlargement and skin diseases.[3] Recent biological evidence revealed that the *E. prostrata* possessed anti-HIV,[4] anti-inflammation,[5] hepatoprotection,[6] hair growth,[7] periodontitis,[8] and osteoporotic effects.[9] The various phytochemical works have been performed to identify secondary metabolites, including flavonoids and coumastans,[10,11] triterpenoids[12,13] and thiopenes,[12] which are regarded to be biologically active components.

In this study, we focused on the chemical study of *E. prostrata*, which led to the isolation and determination of a new coumestan glucoside along with ten known compounds (Fig. 1). The chemical structures of isolates were established utilizing spectroscopic data, including one- (1D) and two-dimensional (2D) nuclear magnetic resonance (NMR), electrospray ionization-quadrupole-time of flight-mass spectrometry (ESI-Q-TOF-MS), polarimeter, ultraviolet (UV) and Fourier-transform infrared spectroscopy (FT-IR).

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Experimental

General experimental procedures – The preparative HPLC was performed using a Gilson HPLC system (Middleton, WI, USA) composed of a binary pump, a liquid handler, and a UV/Vis detector with a Luna C18(2) (21.2 × 250 mm I.D., 5 μm, Phenomenex, Torrance, CA, USA). 1D and 2D-NMR data were obtained using an Avance 500 spectrometer (Bruker, Karlsruhe, Germany). ESI-Q-TOF-MS spectra were recorded using an Agilent 6530 Q-TOF LC/MS system (Agilent Technologies, Santa Clara, CA, USA). Gas chromatography was performed using a GC353B-FSL (GL Sciences Inc., Tokyo, Japan) and a BPX50 capillary column (0.25 mm × 30 m, Trajan Scientific and Medical, Victoria, Australia). Silica gel 60 (40 - 63 μm, Merck, Germany), ZEOprep 90 C18 (40 - 63 μm, Zeochme, Uetikon, Switzerland) and Diaion HP-20 (Mistubishi chemical, Tokyo, Japan) were used to perform liquid column chromatography. Deionized water was produced using a Millipore Milli-Q water purification system. Organic solvents used for general experiments were purchased from Dae-Jung Chemical Co. Ltd. (Seoul, Korea).

Plant material – The 70% ethanol extract of *E. prostrata* was provided from Helixmith (Seoul, Korea). The whole part of *E. prostrata* was purchased from the Humanherb (Daegu, Korea) and extracted using 70% aqueous ethanol at room temperature for 48 hrs. The

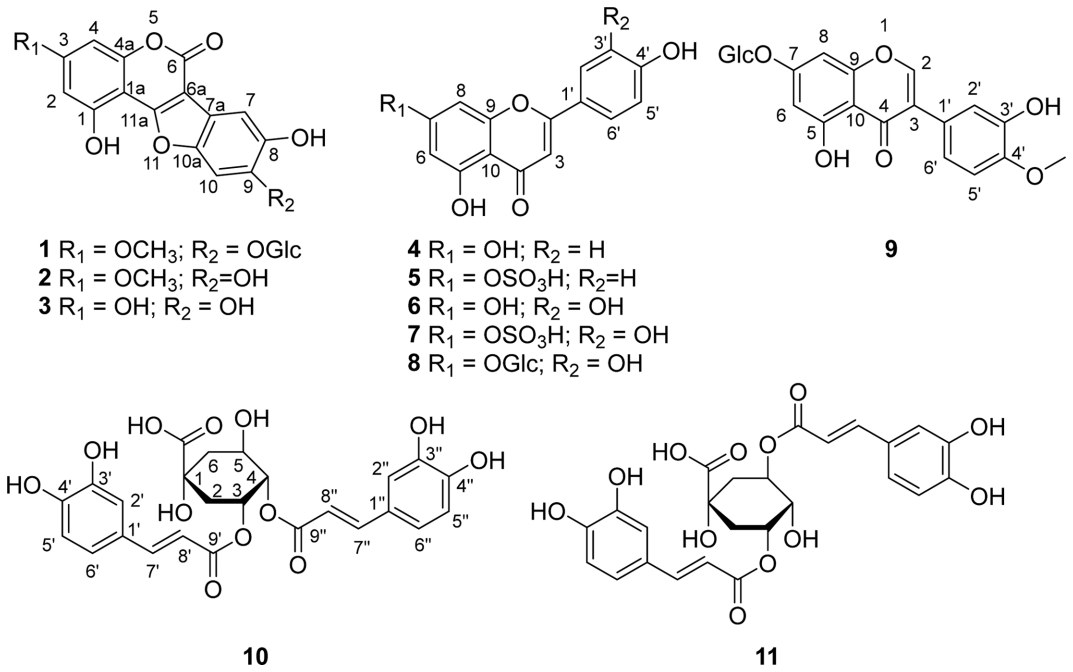

Fig. 1. Chemical structures of isolates from *Eclipta prostrata.*

extraction yield of 70% ethanol extract of *E. prostrata* was 9.62%.

Extraction and isolation – The extract of *E. prostrata* (59.1 g) was suspended in H_2O and partitioned successively with *n*-hexane (*n*-Hex, 1.5 g), ethyl acetate (EtOAc, 3.5 g) and *n*-butanol (*n*-BuOH, 6.2 g). The EtOAc fraction (530.7 mg) was subjected to a silica gel column chromatography (C.C.) using a CH_2Cl_2-MeOH mixture (15:1, v/v) as an eluent to yield compound **7** (53.7 mg). The *n*-BuOH fraction (6.2 g) was subjected to a HP-20 column chromatography [MeOH-H_2O, 10:90 and 100:0 (v/v)] to divide two subfractions (Fr. 1 and 2). Fraction 2 (2.1 g) was chromatographed on a silica gel C.C. using a mixture of CH_2Cl_2-MeOH-H_2O (20:5:1, v/v/v) to afford seven subfractions (Fr. 2.1 – 2.7). Fraction 2.1 (176.3 mg) was separated by Sephadex LH-20 (MeOH as an eluent) to obtain two subfractions (Fr.2.1.1 - 2.1.2). Fraction 2.1.2 was subjected to a reversed-phase (RP) HPLC using a 25% aqueous MeCN as a mobile phase to isolate compound **6** (1.4 mg). Fraction 2.2 (140.3 mg) was loaded to a Sephadex LH-20 C.C. (MeOH) to yield two subfractions (Fr.2.2.1 - 2.2.2). Fraction 2.2.2 was separated by RP-HPLC using a MeCN-H_2O mixture [35:65 (v/v), 4.0 ml/min] to afford compound **2** (4.9 mg) and **9** (3.2 mg). Fraction 2.3 (255.0 mg) was subjected to RP-MPLC using a gradient elution of a MeOH-H_2O mixture [15:85 → 85:15 (v/v)] to give two subfractions (Fr.2.3.1-2.3.2). Compound **1** (8.0 mg) was isolated from Fr. 2.3.1 by RP-HPLC [MeCN-H_2O, 50:50 (v/v), 4.0 ml/min]. Compound **5** (7.1 mg) was isolated from Fr. 2.3.2 using RP-HPLC [MeOH-H_2O, 51:49 (v/v)]. Fraction 2.5 (339.6 mg) was separated using RP-MPLC with a gradient elution of a MeOH-H_2O mixture [15:85 → 85:15 (v/v), 4.0 ml/min] to give two subfractions (Fr. 2.5.1-2.5.2). Fraction 2.5.1 was further purified by RP-HPLC [MeCN-H_2O, 45:55 (v/v)] to give compound **3** (12.0 mg). Compound **4** (3.7 mg) was isolated from Fr.2.5.2 using RP-HPLC [MeCN-H_2O, 30:70 → 84:16 (v/v), 4.0 ml/min]. Fraction 2.6 (427.8 mg) was divided by RP-HPLC with a gradient elution of a MeOH-H_2O mixture [10:90 → 75:25 (v/v)] to afford two subfractions (Fr. 2.6.1 - 2.6.2). Compound **8** (6.2 mg) was isolated from Fr. 2.6.2 using RP-HPLC [MeCN-H_2O, 30:70 (v/v), 4.0 ml/min]. Fraction 2.7 (251.0 mg) was separated by Sephadex LH-20 using a MeOH to give two subfractions (Fr. 2.7.1 - 2.7.2). Compound **10** (2.9 mg) and **11** (3.6 mg) were isolated from Fr. 2.7.1 using RP-HPLC [MeCN-H_2O, 25:75 (v/v), 4.0 ml/min).

Sugar analysis – Sugar analysis of compound **1** was accomplished according to the previously reported method.[14]

Wedelolactone-9-*O*-β-D-glucopyranoside (1): white amorphous power; $C_{22}H_{20}O_{12}$; $[\alpha]_D^{25}$ -37.4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (Abs): 210.20 (2.11), 247.40 (1.11), 300.80 (0.46), 342.80 (1.34) nm; IR (neat) ν_{max}: 3324.40, 1710.85, 1632.34, 1201.19, 1072.71 cm^{-1}; ESI-Q-TOF-MS: *m/z* 475.0877 $[M-H]^-$; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.26 (1H, s, H-7), 7.59 (1H, s, H-10), 6.65

Table 1. ^{13}C-NMR data of compounds **1** – **3** (DMSO-d_6, 125 MHz)

Position	Compound		
	1	**2**	**3**
1	155.10	154.85	155.36
2	98.17	98.13	99.06
3	162.64	162.24	161.07
4	93.32	93.21	94.89
6	157.68	158.92	159.36
7	104.77	104.53	104.51
8	145.34	145.42	145.16
9	144.59	144.34	144.21
10	100.35	98.89	98.89
1a	96.55	96.71	95.41
4a	155.11	157.79	155.05
6a	101.39	101.69	100.90
7a	116.58	113.73	113.82
10a	148.32	148.86	148.67
11a	155.58	155.28	157.88
3-OCH_3	55.81	55.73	
1'	101.82		
2'	73.29		
3'	75.90		
4'	69.91		
5'	77.21		
6'	60.92		

(1H, d, J = 2.3 Hz, H-4), 6.47 (1H, d, J = 2.3 Hz, H-2), 4.89 (1H, d, J = 7.3 Hz, H-1'), 3.83 (3H, s, 3-OCH_3), 3.76 (1H, dd, J = 9.9, 5.3 Hz, H-6'a), 3.47 (2H, o, H-5', 6'b), 3.16-3.34 (3H, o, H-2', 3', 4'); ^{13}C-NMR (125 MHz, DMSO-d_6): Table 1.

Wedelolactone (2): white amorphous power; $C_{16}H_{10}O_7$; ESI-Q-TOF-MS: *m/z* 315.0511 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.24 (1H, s, H-7), 7.16 (1H, s, H-10), 6.62 (1H, d, J = 2.3 Hz, H-4), 6.45 (1H, d, J = 2.3 Hz, H-2), 3.81 (3H, s, 3-OCH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): Table 1.

Demethylwedelolactone (3): white amorphous power; $C_{15}H_8O_7$; ESI-Q-TOF-MS: *m/z* 299.0191 $[M-H]^-$; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.21 (1H, s, H-7), 7.14 (1H, s, H-10), 6.39 (1H, d, J = 2.1 Hz, H-4), 6.35 (1H, d, J = 2.1 Hz, H-2); ^{13}C-NMR (125 MHz, DMSO-d_6): Table 1.

Apigenin (4): pale yellow powder; $C_{15}H_{10}O_5$; ESI-Q-TOF-MS: *m/z* 271.0606 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.93 (2H, d, J = 8.8 Hz, H-2', 6'), 6.92 (2H, d, J = 8.8 Hz, H-3', 5'), 6.79 (1H, s, H-3), 6.48 (1H, d, J = 2.1 Hz, H-8), 6.19 (1H, d, J = 2.1 Hz, H-6); ^{13}C-NMR (125 MHz, DMSO-d_6): Table 2.

Apigenin-7-sulfate (5): pale yellow powder; $C_{15}H_{10}O_8S$; ESI-Q-TOF-MS: *m/z* 349.0018 $[M-H]^-$; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.97 (2H, d, J = 8.5 Hz, H-2', 6'), 7.02 (1H, d, J = 2.1 Hz, H-8), 6.93 (2H, d, J = 8.5 Hz, H-3', 5'), 6.87 (1H, s, H-3), 6.56 (1H, d, J = 2.1 Hz, H-6); ^{13}C-NMR (125 MHz, DMSO-d_6): Table 2.

Luteolin (6): pale yellow powder; $C_{15}H_{10}O_6$; ESI-Q-TOF-MS: *m/z* 285.0399 $[M-H]^-$; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.41 (1H, o, H-6'), 7.40 (1H, d, J = 2.0 Hz, H-2'), 6.88 (1H, d, J = 8.2 Hz, H-5'), 6.67 (1H, s, H-3), 6.44 (1H, d, J = 2.1 Hz, H-8), 6.18 (1H, d, J = 2.1 Hz, H-6); ^{13}C-NMR (125 MHz, DMSO-d_6): Table 2.

Luteolin-7-sulfate (7): pale yellow powder; $C_{15}H_{10}O_9S$; ESI-Q-TOF-MS: *m/z* 364.9966 $[M-H]^-$; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.46 (2H, m, H-2', 6'), 7.03 (1H, d, J = 2.1 Hz, H-8), 6.89 (1H, d, J = 8.5 Hz, H-5'), 6.77 (1H, s, H-3), 6.51 (1H, d, J = 2.1 Hz, H-6); ^{13}C-NMR (125 MHz, DMSO-d_6): Table 2.

Luteolin-7-*O*-β-D-glucopyranoside (8): pale yellow powder; $C_{21}H_{20}O_{11}$; ESI-Q-TOF-MS: *m/z* 447.0926 $[M-H]^-$; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.45 (1H, dd, J = 8.3, 2.1 Hz, H-6'), 7.42 (1H, d, J = 2.1 Hz, H-2'), 6.90 (1H, d, J = 8.3 Hz, H-5'), 6.79 (1H, d, J = 2.3 Hz, H-8), 6.76 (1H, s, H-3), 6.44 (1H, d, J = 2.3 Hz, H-6), 5.08 (1H, d, J = 7.5 Hz, H-1"), 3.70 (1H, d, J = 10.4 Hz, H-6"a), 3.14-3.50 (4H, o, H-2", 3", 4", 5"), 3.47 (1H, m, H-6"b); ^{13}C-NMR (125 MHz, DMSO-d_6): Table 2.

Pratensein-7-*O*-β-D-glucopyranoside (9): pale yellow powder; $C_{22}H_{22}O_{11}$; ESI-Q-TOF-MS: *m/z* 463.1243 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.47 (1H, s, H-2), 7.16 (1H, d, J = 2.1 Hz, H-2'), 7.01 (1H, dd, J = 8.2, 2.1 Hz, H-6'), 6.83 (1H, d, J = 8.2 Hz, H-5'), 6.73 (1H, d, J = 2.2 Hz, H-8), 6.47 (1H, d, J = 2.2 Hz, H-6), 5.07 (1H, d, J = 7.4 Hz, H-1"), 3.80 (3H, s, 4'-OCH_3), 3.70 (1H, dd, J = 9.6, 5.2 Hz, H-6"a), 3.45 (1H, dd, J = 9.6, 4.5 Hz, H-6"b), 3.21-3.50 (4H, o, H- 2", 3", 4", 5"); ^{13}C-NMR (125 MHz, DMSO-d_6): Table 2.

3,4-Di-*O*-caffeoylquinic acid (**10**): white amorphous power; $C_{25}H_{24}O_{12}$; ESI-Q-TOF-MS: *m/z* 515.1190 $[M-H]^-$; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.47 (1H, d, J = 15.9 Hz, H-7'), 7.41 (1H, d, J = 15.9 Hz, H-7"), 7.05 (1H, d, J = 2.1 Hz, H-2'), 7.00 (1H, d, J = 2.1 Hz, H-2"), 6.99 (1H, o, H-6'), 6.93 (1H, dd, J = 8.2, 2.1 Hz, H-6"), 6.74 (1H, d, J = 8.2 Hz, H-5'), 6.72 (1H, d, J = 8.2 Hz, H-5"), 6.27 (1H, d, J = 15.9 Hz, H-8'), 6.16 (1H, d, J = 15.9 Hz, H-8"), 5.47 (1H, brs, H-3), 4.99 (1H, brs, H-4), 3.92 (1H, brs, H-5), 1.80-2.08 (4H, o, H-2a, 2b, 6a, 6b); ^{13}C-NMR (125 MHz, DMSO-d_6): Table 3.

3,5-Di-*O*-caffeoylquinic acid (11): white amorphous power; $C_{25}H_{24}O_{12}$; ESI-Q-TOF-MS: *m/z* 515.1191 $[M-H]^-$; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.48 (1H, d, J = 15.9

Table 2. ^{13}C-NMR data of compounds **4** – **9** (DMSO-d_6, 125 MHz)

Position	Compound					
	4	**5**	**6**	**7**	**8**	**9**
1						
2	163.73	164.35	164.14	164.35	164.48	154.89
3	102.85	102.23	102.87	103.00	103.20	121.72
4	181.75	182.93	181.68	182.02	181.92	180.51
5	161.16	160.53	157.29	160.52	161.15	161.67
6	98.82	103.06	98.84	102.02	99.54	99.59
7	164.12	159.56	163.91	159.53	162.96	163.02
8	93.97	97.84	93.87	97.54	94.72	94.52
9	157.31	156.39	161.48	156.32	156.96	157.19
10	103.69	105.72	103.70	105.65	105.34	106.09
1'	121.17	121.05	121.50	121.30	121.39	122.62
2'	128.49	128.68	113.37	113.32	113.58	115.29
3'	115.95	116.04	145.75	145.76	145.80	146.81
4'	161.41	161.36	149.71	149.86	149.94	147.28
5'	115.95	116.06	116.03	116.13	115.98	113.23
6'	128.49	128.68	119.00	119.15	119.19	121.43
1"					99.86	99.81
2"					73.12	73.08
3"					76.35	76.41
4"					69.52	69.58
5"					77.15	77.19
6"					60.60	60.61
4'-OCH_3						55.72

Hz, H-7'), 7.45 (1H, d, J = 15.9 Hz, H-7"), 7.05 (1H, brs, H-2'), 7.03 (1H, brs, H-2"), 6.99 (2H, o, H-6', 6"), 6.77 (1H, d, J = 8.2 Hz, H-5'), 6.76 (1H, d, J = 8.2 Hz, H-5"), 6.24 (1H, d, J = 15.9 Hz, H-8'), 6.17 (1H, d, J = 15.9 Hz, H-8"), 5.30 (1H, m, H-5), 5.18 (1H, m, H-3), 3.81 (1H, m, H-4), 1.90-2.18 (4H, o, H-2a, 2b, 6a, 6b); ^{13}C-NMR (125 MHz, DMSO-d_6): Table 3.

Result and Discussion

The molecular formula of compound **1** was determined to be $C_{22}H_{20}O_{12}$ according to a positive ion peak at *m/z* 475.0877 $[M-H]^-$ in the ESI-Q-TOF-MS spectrum. The IR spectrum displayed absorption bands at 3324 (OH, phenolic), 1711 (δ-lactone carbonyl), 1632 (C=C, conjugated), 1201(C-O, furan) and 1072.71 cm^{-1} (C-O, phenolic). The ^{1}H- NMR spectrum of compound **1** showed the characteristic resonances of wedelolactone structure,[10] showing a set of *meta*-coupled aromatic proton signals [δ_H 6.65 (1H, d, J = 2.3 Hz, H-2) and 6.47 (1H, d, J = 2.3 Hz, H-4)], two singlet resonances [δ_H 7.59 (1H, s, H-7) and 7.26 (1H, s, H-10)] and one methoxy group at δ_H 3.83(3H, s, 3-OC$\underline{H}_3$). Additionally, an anomeric proton signal of sugar moiety was observed at δ_H 4.89 (1H, d, J = 7.3 Hz, H-1"). The ^{13}C-NMR spectrum of **1** showed 22 signals, including a wedelolactone[10] and a β-glucopyranoside resonances [δ_C 101.82 (C-1'), 77.21 (C-5'), 75.90 (C-3'), 73.29 (C-2'), 69.91 (C-4') and 60.92 (C-6')]. The acid hydrolysis and GC result revealed the presence of D-glucose. The position of glucose at C-9 was determined from the HMBC correlation peak at δ_H 4.89 (H-1") / δ_C 144.59 (C-9) (Fig. 2). Based on the spectroscopic evidence (Table 1), the structure of compound **1** was determined to be wedelolactone-9-*O*-β-D-glucopyranoside which was identified for the first time in nature.

The molecular formula of compound **2** was determined to be $C_{16}H_{10}O_7$ using ESI-Q-TOF-MS spectrum. The ^{1}H- and ^{13}C-NMR data of compound **2** was close to those of **1** except the absence of a glucosyl moiety. From the comparison of ^{1}H and ^{13}C-NMR data with those of reported values, the structure of **2** was elucidated to be wedelolactone.[10]

The molecular formula of compound **3** was determined as $C_{15}H_8O_7$ from the negative ion peak at *m/z* 299.0191

Table 3. ^{13}C-NMR data compounds **10** and **11** (DMSO-d_6, 125 MHz)

Position	Compound	
	10	**11**
1	73.78	73.05
2	36.23	36.94
3	67.87	70.76
4	72.20	69..19
5	65.23	70.46
6	38.04	36.94
1'	125.46	125.63
2'	114.90	114.82
3'	145.58	145.60
4'	148.45	148.39
5'	115.73	115.82
6'	121.41	121.38
7'	145.35	144.74
8'	114.10	114.18
9'	165.92	166.19
1"	125.44	125.55
2"	114.75	114.82
3"	145.56	145.60
4"	148.36	148.26
5"	115.73	115.74
6"	121.37	121.14
7"	145.17	144.74
8"	113.99	113.95
9"	165.74	166.19
COOH	175.82	176.78

[M-H]$^-$. The ^{1}H and ^{13}C-NMR spectra of **3** were close to those of **2** except that a methoxy group was replaced by a hydroxy group. Therefore, compound **2** was identified to be demethylwedelolactone.[15]

The ^{1}H-NMR spectrum of **4** showed an AX spin system of benzene ring [δ_H 7.93 (2H, d, J = 8.8 Hz, H-2', 6') and 6.92 (2H, d, J = 8.8 Hz, H-3',5')], a singlet at δ_H 6.79 (1H, s, H-3) and two *meta*-coupled aromatic proton signals [δ_H 6.48 (1H, d, J = 2.1 Hz, H-8) and 6.19 (1H, d, J = 2.1 Hz, H-6)], corresponding to an apigenin skeleton. In the ^{13}C-NMR spectrum, 13 carbon resonances were observed, which were consistent with those of apigenin resonances. Therefore, compound **4** was determined to be apigenin.[16]

The molecular formula of compound **5** was determined to be $C_{15}H_{10}O_8S$ from an ion peak at *m/z* 349.0018 [M-H]$^-$ from the negative ion ESI-Q-TOF-MS spectrum. The ^{1}H-NMR spectrum of **5** was close to that of apigenin but the H-6 and H-8 resonances showed downfield shifts to

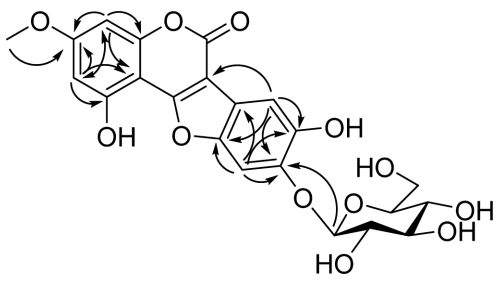

Fig. 2. HMBC correlations of compound **1**.

6.56 (1H, d, J = 2.1 Hz, H-6) and 7.02 (1H, d, J = 2.1 Hz, H-8), respectively. The ^{13}C-NMR of **5** showed the characteristic chemical shift change compared to that of apigenin (**4**) due to the presence of a sulfate group which was linked to the C-7 position. When the carbon resonances of compound **5** (Table 2) were compared to those of apigenin (**4**), an upfield shift for *ipso* carbon (-4.56 ppm for the C-7), downfield shifts for two *ortho* carbons (+4.24 and +3.87 ppm for C-6 and C-8, respectively), and downfield shift for *para* carbon (-2.03 ppm for C-10) were observed. Therefore, compound **5** was identified to be apigenin-7-sulfate.[17]

The ESI-Q-TOF-MS of compound **6** showed a molecular ion peak at *m/z* 285.0399 [M-H]$^-$, corresponding to the molecular formula of $C_{15}H_{10}O_6$. The ^{1}H-NMR spectrum of **3** showed resonances characteristic for a luteolin skeleton including an 1,3,4-trisubstitued benzene ring [δ_H 7.41(1H, o, H-6'), 7.40 (1H, d, J = 2.0 Hz, H-2'), 6.88 (1H, d, J = 8.2 Hz, H-5')], a singlet proton at δ_H 6.67 (1H, s, H-3), and two *meta*-coupled aromatic proton signals [δ_H 6.44 (1H, d, J = 2.1 Hz, H-8), 6.18 (1H, d, J = 2.1 Hz, H-6)]. The ^{13}C-NMR spectrum displayed 15 carbon signals, which was consistent with the luteolin structure. Therefore, compound **6** was identified as luteolin.[18]

The molecular formula of compound **7** was determined to be $C_{15}H_{10}O_9S$ from the negative ion peak at *m/z* 364.9966 [M-H]$^-$ from the ESI-Q-TOF-MS. The patterns of ^{1}H-, and ^{13}C-NMR spectra were similar to those of luteolin (**6**) but showed chemical shift differences at C-6, 7, 8 and 10 positions due to the sulfation at C-7 position as in the case of compound **5**. Therefore, compound **7** was established to be luteolin-7-sulfate.[19]

The molecular formula of compound **8** was established to be $C_{21}H_{20}O_{11}$ based on the ESI-Q-TOF-MS spectrum (*m/z* 447.0926 [M-H]$^-$). The ^{1}H-NMR spectrum indicated that compound **8** possessed a luteolin skeleton with a sugar moiety, which was deduced from an anomeric proton signal at δ_H 5.08 (1H, d, J = 7.5 Hz, H-1"). The ^{13}C-NMR spectrum of **8** showed resonances for a luteolin

and a glucose moieties [δ_C 99.86 (C-1"), 77.15 (C-5"), 76.35 (C-3"), 73.12 (C-2"), 69.52 (C-4") and 60.60 (C-6")]. The location of glucose at C-7 was determined from an HMBC correlation peak at δ_H 5.08 (H-1")/δ_C 162.96 (C-7). Base on the spectroscopic data, the chemical structure of compound **8** was established to be luteolin-7-*O*-β-D-glucopyranoside.[20]

The molecular formula of compound **9** was established to be $C_{22}H_{22}O_{11}$ from the positive ion peak at *m/z* 463.1243 $[M+H]^+$. The ^{1}H-NMR spectrum displayed signals for the 1,3,4-trisubstitued benzene ring [δ_H 7.16 (1H, d, *J* = 2.1 Hz, H-2'), 7.01 (1H, dd, *J* = 8.2, 2.1 Hz, H-6'), 6.83 (1H, d, *J* = 8.2 Hz, H-5')], a characteristic signal of isoflavone at δ_H 8.47 (1H, s, H-2), one methoxy resonance at δ_H 3.80 (3H, s, 4'-OCH_3) and an anomeric proton resonance at δ_H 5.07 (1H, d, *J* = 7.4 Hz, H-1"). The position of methoxy signal at δ_H 3.80 was correlated to a carbon signal at δ_C 147.28 (C-4') from the HMBC experiment which revealed that the aglycone of **9** was pratensein. The ^{13}C-NMR spectrum indicated the presence of 22 carbon signals including a pratensein and a glucose moieties [δ_C 99.81 (C-1"), 77.19 (C-5"), 76.41 (C-3"), 73.08 (C-2"), 69.58 (C-4") and 60.61 (C-6")]. The position of glucose was confirmed by the HMBC experiment, in which a correlation was observed between the H-1' (δ_H 5.07) and C-7 (δ_C 163.02). Thus, the structure of **9** was determined to be pratensein-7-*O*-β-D-glucopyranoside.[21]

Compound **10** and **11** were identified to be 3,4-di-*O*-caffeoylquinic acid and 3,5-di-*O*-caffeoylquinic acid, respectively, whose spectroscopic data were compared to a previous literature (Table 3).[22]

Lee at. al. reported a paper on the chemical analysis of *E. prostrata* using a HPLC-PDA-MS method which identified caffeoylqunic acid derivatives, sulfated flavonoids, wedelolactone derivatives and triterpenoids.[23] The current study is consistent with the previous report and could be a good chemical reference of *E. prostrata*.

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