SUPPLEMENTARY MATERIAL

**Chemical constituents from the roots of *Leea thorelii* Gagnep**

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**Chemical constituents from the roots of *Leea thorelii* Gagnep**

Phytochemical investigation of the roots of *Leea thorelii* led to the isolation of nine compounds. Their structures were determined by spectroscopic evidence as bergenin (**1**), 11-*O*-acetyl bergenin (**2**), 11-*O*-(4'-*O*-methylgalloyl) bergenin(**3**), 3,5-dihydroxy-4-methoxybenzoic acid (**4**), (-)-epicatechin (**5**), 4"-*O*-methyl-(-)-epicatechin gallate (**6**), (-)-epicatechin gallate (**7**), microminutinin (**8**), and stigmasterol. Compounds **1**-**8** are the first reports from this plant and this is also the first report of the presence of **1**, **3**, **4**, **6** and **8** in the Vitaceae family.

**Keywords:** *Leea thorelii*, Vitaceae, flavan, bergenin, epicatechin

**Experimental**

***General experimental procedures***

Melting points were determined using Electrothermal IA9200 digital melting point apparatus (Bibby Scientific Limited, Staffordshire, UK). IR spectra were obtained using a Bruker Tenser 27 spectrophotometer (Agilent Technologies, USA). NMR spectra were recorded using CDCl3 and CD3OD as solvents on a Varian Mercury Plus 400 spectrometer (Varian, Inc., USA), the internal standards were referenced from the residue of those solvents. Column chromatography was carried out on Merck silica gel 60 (230–400 mesh) (Merck, Darmstadt, Germany). TLC was performed with precoated Merck silica gel 60 PF254 aluminum sheets (Merck, Darmstadt, Germany); the spots were visualized under UV light (254 and 365 nm) and further by spraying with anisaldehyde then heating until charred.

***Plant Material***

The roots of *L. thorelii* were collected from Ubolratana District, Khon Kaen Province, Thailand, in July 2011 and identified by Prof. Pranom Chantaranothai, Department of Biology, Khon Kaen University, Thailand where a voucher specimen (voucher number S. Kanokmedhakul-12) was deposited.

***Extraction and Isolation***

Air dried roots of *L. thorelii* (1.5 kg) were ground into powder and then extracted with EtOAc (3 x 10 L) to yield crude EtOAc extract (40 g, 2.66 %). The precipitate in the EtOAc extraction was filtered out and was recrystallised with MeOH to obtain colourless crystals of **1** (2.9 g). The filtrate of EtOAc was concentrated to yield a brown residue (38.1 g) and was subjected to column chromatography (CC) over silica gel, eluted with a gradient system of hexane:EtOAc (70:20), EtOAc, EtOAc:MeOH (90:10), and MeOH to give six fractions, FE1-FE6. Colourless needles ofstigmasterol (455.5 mg) were crystallized from FE2 in hexane. The filtrate was purified by flash column chromatography (FCC), eluted with a gradient system of CH2Cl2-EtOAc (90:10), EtOAc, EtOAc-MeOH (90:10), and MeOH to give six subfractions, FE2.1-FE2.6. Subfraction FE2.3 was purified by preparative TLC using hexane-EtOAc (85:15) as eluent to givecolourless needlesof **8** (2.0 mg). Fraction FE3 was purified by crystallisation from EtOAc, to give an orange solid **4** (76.5 mg). White solid of **5** (504.0 mg) was filtered out from the FE4 and its filtrate was purified by FCC, eluted with an isocratic system of hexane-EtOAc (40:60) to give three subfractions, FE4.1-FE4.3. Subfraction FE4.2 was purified by preparative TLC using CH2Cl2-EtOAc (60:40) as eluent to obtain a brown solid of **6** (21.1 mg). Subfraction FE4.3 was purified by preparative TLC using CH2Cl2-MeOH (85:15) as eluent to give a brown solid of **7** (71.0 mg) anda white solidof **2** (12.7 mg). Fraction FE5 was purified by FCC, eluted with a gradient system of CH2Cl2-EtOAc (70:30), EtOAc, EtOAc-MeOH (90:10), and MeOH to give five subfractions, FE5.1-FE5.5. Subfraction FE5.3 was purified by FCC, eluted with an isocratic system of hexane-EtOAc (50:50) to give colourless needles of **3** (143.7 mg).

*Bergenin (1):* colourless crystals; m.p. = 144-146 °C; [α]D27 -44.8 (*c* 0.16, EtOH);  *R*f: 0.19 (CH2Cl2-MeOH, 9:1); IR (neat) νmax (cm-1): 3390, 2959, 1703, 1613, 1463, 1375, 1349, 1235, 1127, 1093, 1071. 1H NMR (400 MHz, CD3OD): *δ* 3.66 (1H, ddd, *J* = 1.6, 7.6, 9.2 Hz, H-2), 3.43 (1H, t, *J* = 8.8, 9.2 Hz, H-3), 3.81 (1H, t, *J* = 8.8, 9.2 Hz, H-4), 4.05 (1H, d, *J* = 10.2 Hz, H-4a), 7.08 (1H, s, H-7), 4.94 (1H, d, *J* = 10.2 Hz, H-10b), 4.02 (1H, d, *J* = 11.2 Hz, H-11a), 3.69 (1H, d, *J* = 11.2 Hz, H-11b), 3.89 (3H, s, OCH3-9); 13C NMR (100 MHz, CD3OD) *δ* 81.5 (C-2), 70.4 (C-3), 74.1 (C-4), 79.9 (C-4a), 164.3 (C-6), 117.9 (C-6a), 109.6 (C-7), 150.8 (C-8), 140.8 (C-9), 147.9 (C-10), 115.8 (C-10a), 72.8 (C-10b), 61.2 (C-11), 59.5 (OCH3-9).

*11-O-Acetyl bergenin (2):*white solid; m.p. = 158-160 °C; [α]D27 -34.2 (*c* 0.1, MeOH); *R*f: 0.19 (CH2Cl2-MeOH, 9:1); IR (neat) νmax (cm-1): 3356, 2929, 2852, 1715, 1613, 1512, 1352, 1238, 1170, 1092, 1024. 1H NMR (400 MHz, CD3OD): *δ* 3.81 (1H, m, H-2), 3.46 (1H, m, H-3), 3.85 (1H, m, H-4), 4.07 (1H, dd, *J* = 9.6, 10.4 Hz, H-4a), 7.09 (1H, s, H-7), 4.99 (1H, d, *J* = 10.4 Hz, H-10b), 4.65 (1H, m, H-11a), 4.23 (1H, m, H-11b), 3.90 (3H, s, OCH3-9), 2.11 (2H, s, H-2′, 6′); 13C NMR (100 MHz, CD3OD) *δ* 80.3 (C-2), 71.8 (C-3), 75.6 (C-4), 81.3 (C-4a), 165.8 (C-6), 119.4 (C-6a), 111.3 (C-7), 152.7 (C-8), 142.4 (C-9), 149.3 (C-10), 117.2 (C-10a), 74.3 (C-10b), 64.6 (C-11), 60.9 (OCH­3-9), 172.6 (C-1′), 20.6 (C-2′).

*11-O-(4'-O-Methylgalloyl)-bergenin (3):*colorless needles; m.p. = 144-146 °C; [α]D26 +43.8 (*c* 0.5, EtOH); *R*f: 0.30 (CH2Cl2-MeOH, 9:1); IR (neat) νmax (cm-1): 3377, 2946, 2851, 1710, 1595, 1522, 1352, 1238, 1170, 1092, 1024. 1H NMR (400 MHz, CD3OD): *δ* 3.96 (1H, t, *J* = 2.0, 9.4 Hz, H-2), 3.56 (1H, t *J* = 8.8, 9.5 Hz, H-3), 3.88 (1H, t *J* = 8.8, 9.5 Hz, H-4), 4.10 (1H, t, *J* = 9.6, 10.4 Hz, H-4a), 7.07 (1H, s, H-7), 5.01 (1H, d, *J* = 10.4 Hz, H-10b), 4.85 (1H, dd, *J* = 2.0 Hz, H-11a), 4.41 (1H, dd, *J* = 6.8, 12.0 Hz, H-11b), 3.89 (3H, s, OCH3-9), 3.86 (3H, s, OCH3-4'), 7.09 (1H, s, H-2', 6'); 13C NMR (100 MHz, CD3OD) *δ* 80.5 (C-2), 71.8 (C-3), 75.4 (C-4), 81.2 (C-4a), 165.7 (C-6), 119.3 (C-6a), 111.2 (C-7), 152.3 (C-8), 142.2 (C-9), 149.2 (C-10), 116.9 (C-10a), 74.3 (C-10b), 64.9 (C-11), 61.0 (OCH3-9), 60.8 (OCH3-4'), 126.1 (C-1'), 110.3 (C-2', 6'), 151.7 (C-3', 5'), 141.4 (C-4'), 167.7 (C-7').

*3,5-Dihydroxy-4-methoxybenzoic acid (4):* orange solid; m.p. = 238-242 °C; *R*f: 0.26 (CH2Cl2-MeOH, 9:1); IR (neat) νmax (cm-1): 3475, 3316, 3197, 2954, 2849, 1710, 1594, 1512, 1452, 1520, 1360, 1090, 1054. 1H NMR (400 MHz, CD3OD): *δ* 7.04 (2H, s, H-2, 6), 3.86 (3H, s, OCH3-4); 13C NMR (100 MHz, CD3OD) *δ* 60.7 (OCH3-4), 110.4 (C-2, 6), 127.2 (C-1), 141.0 (C-4), 151.6 (C-3, 5), 169.9 (C-7).

*(-)-Epicatechin (5):* white solid; m.p. = 244-246 °C ((241-245 °C);[α]D25 -69.9 (*c* 1.0, acetone); *R*f = 0.24 (CH2Cl2-MeOH, 9:1); IR (neat) νmax (cm-1): 3359, 2916, 2851, 1629, 1607, 1518, 1468, 1357, 1280, 1195, 1142, 1091, 1042. 1H NMR (400 MHz, CD3OD): *δ* 4.81(1H, brs, H-2), 4.17(1H, brs, H-3), 2.87 (1H, dd, *J* = 16.8, 4.8 Hz, H-4), 2.74 (1H, dd, *J* = 16.8, 2.8 Hz, H-4), 5.94 (1H, d, *J* = 2.4 Hz, H-6), 5.92 (1H, d, *J* = 2.4 Hz, H-8), 6.97 (1H, d, *J* = 2.0 Hz, H-2′), 6.76 (1H, d, *J* = 8.0 Hz, H-5′), 6.80 (1H, dd, *J* = 2.0, 8.0 Hz, H-6′); 13C NMR (100 MHz, CD3OD) *δ* 79.9 (C-2), 67.5 (C-3), 29.3 (C-4), 100.1 (C-4a), 157.7 (C-5), 96.4 (C-6), 158.0 (C-7), 95.9 (C-8), 157.4 (C-8a), 132.3 (C-1′), 115.3 (C-2′), 145.8 (C-3′), 146.0 (C-4′), 115.9 (C-5′), 119.4 (C-6′).

*4"-O-Methyl-(-)-epicatechin gallate (6):*brown solid; m.p. = 247-249 °C; [α]D25 -11.7 (*c* 0.2, acetone); *R*f = 0.22 (CH2Cl2-MeOH, 9:1); IR (neat) νmax (cm-1): 3358, 2946, 2851, 1696, 1604, 1520, 1438, 1373, 1237, 1144, 1097, 1053. 1H NMR (400 MHz, CD3OD): *δ* 5.03 (1H, brs, H-2), 5.54 (1H, brs, H-3), 3.01 (1H, dd, *J* = 4.4, 17.6 Hz, H-4), 2.87 (1H, d, *J* = 17.6 Hz, H-4), 5.98 (1H, s, H-6), 5.98 (1H, s, H-8), 6.94 (1H, d, *J* = 2.0 Hz, H-2′), 6.71 (1H, d, *J* = 8.4 Hz, H-5′), 6.81 (1H, dd, *J* = 2.0, 8.4 Hz, H-6′), 6.93 (2H, s, H-2′′, 6′′), 3.83 (3H, s, OCH3-4′′); 13C NMR (100 MHz, CD3OD) *δ* 78.5 (C-2), 70.3 (C-3), 26.8 (C-4), 99.3 (C-4a), 157.8 (C-5), 96.6 (C-6), 157.8 (C-7), 95.9 (C-8), 157.2 (C-8a), 131.4 (C-1′), 115.0 (C-2′), 145.9 (C-3′), 145.9 (C-4′), 116.0 (C-5′), 119.3 (C-6′), 126.5 (C-1′′), 110.3 (C-2′′, 6′′), 151.5 (C-3′′, 5′′), 141.2 (C-4′′), 60.7 (OCH3-4′′), 167.1 (C-7′′).

*(-)-Epicatechin-3-O-gallate (7):*brown solid; m.p. = 254-257 °C;[α]D25.5 -124.4 (*c* 0.2, acetone); *R*f: 0.06 (CH2Cl2-MeOH, 9:1); IR (neat) νmax (cm-1): 3358, 2916, 2852, 1692, 1610, 1518, 1448, 1338, 1235, 1144, 1098, 1039. 1H NMR (400 MHz, CD3OD): *δ* 5.03 (1H, brs, H-2), 5.52 (1H, brs, H-3), 3.00 (1H, dd, *J* = 4.8, 17.6 Hz, H-4), 2.85 (1H, dd, *J* = 2.0, 17.6 Hz, H-4), 5.97 (1H, s, H-6), 5.97 (1H, s, H-8), 6.93 (1H, d, *J* = 2.0 Hz, H-2′), 6.70 (1H, d, *J* = 8.4 Hz, H-5′), 6.81 (1H, dd, *J* = 2.0, 8.4 Hz, H-6′), 6.95 (2H, s, H-2′′, 6′′); 13C NMR (100 MHz, CD3OD) *δ* 78.6 (C-2), 70.0 (C-3), 26.8 (C-4), 99.4 (C-4a), 157.8 (C-5), 96.6 (C-6), 157.8 (C-7), 95.9 (C-8), 157.2 (C-8a), 131.4 (C-1′), 115.1 (C-2′), 145.9 (C-3′), 145.9 (C-4′), 116.0 (C-5′), 119.4 (C-6′), 121.4 (C-1′′), 110.2 (C-2′′, 6′′), 146.2 (C-3′′, 5′′), 139.7 (C-4′′), 167.6 (C-7′′).

*Microminutinin (8):* colourless needles; m.p. = 114-116 °C; *R*f = 0.85 (CH2Cl2-MeOH, 9:1); IR (neat) νmax (cm-1): 2926, 2854, 1732, 1615. 1H NMR (400 MHz, CDCl3): *δ* 6.24 (1H, d, *J* = 9.6 Hz, H-3), 7.64 (1H, d, *J* = 9.6 Hz, H-4), 7.31 (1H, d, *J* = 8.4 Hz, H-5), 6.78 (1H, d, *J* = 8.0 Hz, H-6), 6.55 (1H, d, *J* = 5.6 Hz, H-2′), 4.67 (1H, *br*d, *J* = 5.2 Hz, H-3′), 4.50 (1H, dd, *J* = 1.2, 12.6 Hz, H-5′), 4.41 1H, d, *J* = 12.6 Hz, H-5′), 5.73 (1H, d, *J* = 1.6 Hz, H-7′), 5.20 (1H, d, *J* = 1.6 Hz, H-7′); 13C NMR (100 MHz, CDCl3) *δ* 160.3 (C-2), 112.5 (C-3), 143.9 (C-4), 129.5 (C-5), 106.8 (C-6), 162.1 (C-7), 113.3 (C-8), 151.4 (C-9), 113.5 (C-10), 113.6 (C-2′), 48.5 (C-3′), 144.3 (C-4′), 70.6 (C-5′), 109.4 (C-7′).