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Phenolics, acyl galactopyranosyl glycerol, and lignan amides from *Tetragonia tetragonioides* (Pall.) Kuntze

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Abstract Eleven antioxidative compounds, including five lignin amides, were isolated from the aerial part of *Tetragonia tetragonioides* (New Zealand spinach) using 1,1-diphenyl-2-picrylhydrazyl radical-scavenging assay-guided purification. The structures were determined by nuclear magnetic resonance and electrospray ionization-mass spectroscopy. These compounds were identified as methyl linoleate (**1**), methyl coumarate (**2**), methyl ferulate (**3**), 1-*O*-stearoyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol (**4**), 1-*O*-caffeoyl-β-D-glucopyranoside (**5**), *N*-*trans*-caffeoyltyramine (**6**), cannabisin B (**7**), cannabisin A (**8**), *N*-*trans*-feruloyltyramine (**9**), *N*-*cis*-feruloyltyramine (**10**), and *N*-*trans*-sinapoyltyramine (**11**). Compounds **1**, **2**, **4**, **5**, and **8**-**11** were isolated for the first time from this plant.

Keywords: *Tetragonia tetragonioides*, halophyte, lignan amide, phenolics, acyl galactopyranosyl glycerol

Introduction

Tetragonia tetragonioides (Pall.) Kuntze, New Zealand spinach, belongs to the family of Aizoaceae and is an edible halophyte (1). *T. tetragonioides* has been used as a leafy vegetable with similar flavor and texture as spinach (2). It is cultivated in Australia, New Zealand, and East Asia (2). Moreover, *T. tetragonioides* is widely distributed in the saline environment of coastal sand dunes in Korea and has been used as a traditional remedy for stomachache (3,4). Cerebrosides and steryl glucosides from *T. tetragonioides* have been identified as antiulcerogenic compounds (5-7). Recently, several *in vitro* biological effects, such as antibacterial, antioxidant, and α-glucosidase inhibitory activities, have been investigated (8-10). Several compounds such as diterpenes, flavonol glycosides, glycosyl imidates, and lignan amides have been isolated from *T. tetragonioides* (4,5,7,11,12). However, the biological activities and responsible chemical constituents of *T. tetragonioides* have not been evaluated to establish its value as a potential vegetable or food. During the investigation on the biological activities and chemical constituents of various edible halophytes, *T. tetragonioides* showed high *in vitro* free radical-scavenging activities with *Salicornia herbacea* and *Suaeda japonica* (data not shown). *T. tetragonioides* is considered as an antioxidant-rich vegetable that can reduce oxidative stress and prevent various diseases, including cardiovascular diseases, cancer, hypertension, and diabetes (13,14). Therefore, in this study, we investigated the antioxidative compounds present in the aerial parts of *T. tetragonioides*, and 11 antioxidative compounds, including a fatty acid, an acyl galactopyranosyl glycerol, three phenolics, and five lignin amides, were isolated from this plant using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay-guided purification.

Materials and Methods

Materials and chemicals The aerial part of *T. tetragonioides* was harvested in June 2012 in Jeonnam, Korea. This sample was immediately treated after harvesting. DPPH was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Deuterated methanol (CD_3OD) was obtained from Merck Co. (Darmstadt, Germany). All the solvents used for analyses were of high-performance-liquid-chromatography (HPLC) grade.

Extraction and solvent fractionation The fresh aerial part (20 kg) was homogenized with methanol (MeOH, 40 L) and extracted at room temperature for 24 h. The MeOH extract was filtered under vacuum through a No. 2 filter paper (Whatman, Maidstone, UK). The

residue was extracted with MeOH (32 L) and then filtered under vacuum through a No. 2 filter paper (Whatman). The MeOH extracts were combined and concentrated under vacuum at 38°C. The MeOH extracts (429 g) were suspended in distilled water (1.3 L) and successively partitioned with *n*-hexane (1.5 L, three times), ethyl acetate (EtOAc, 1.5 L, three times), and water-saturated *n*-butanol (BuOH, 1.5 L, three times). The fractions were concentrated under vacuum.

Assay for purification of antioxidants The fractions obtained after column chromatography were analyzed by thin-layer chromatography (TLC) (15). Each fraction was spotted on silica gel TLC plates (silica gel 60 F254, 0.25 mm thickness; Merck) and developed in a mixture of EtOAc/$CHCl_3$/acetic acid (3:1:2, v/v). The spots were detected by UV light (254 nm). Finally, the dried TLC plate was sprayed with a 200 μM DPPH EtOH solution. The antioxidative activity was measured by the visualization of the decolorized spots after spraying with the DPPH EtOH solution.

Isolation of antioxidative compounds from the EtOAc fraction The EtOAc fraction (8.36 g) was fractionated by chromatography of an silica gel (Kieselgel 60, 70-230 mesh, Merck) column (4.5×90 cm) and eluted with a step-wise system of *n*-hexane/EtOAc (10:0, 5:5, and 0:10, v/v, each 920 mL) followed by EtOAc/MeOH (5:5 and 0:10, v/v, each 920 mL). The 11 fractions (A-K) were grouped according to the DPPH radical assay based on the TLC profiles. Fraction A ($CHCl_3$/EtOAc=10:0, v/v, 149 mg) was purified by HPLC using a Porasil silica column (7.8×300 mm; 15-20 μm, Waters, Milford, MA, USA; flow rate, 5.0 mL/min; wavelength, 210 nm). Elution with a step-wise system of 100% *n*-hexane for 5 min and *n*-hexane/isopropanol/ethanol (98:1:1, v/v) afforded **1** (t_R 14.7 min, 5.0 mg) along with three unknown compounds. Fraction B ($CHCl_3$/EtOAc=5:5, v/v, 821 mg) was fractionated by the chromatography using an octadecylsilane (ODS, 70-230 mesh; YMC, Tokyo, Japan) column (35 g, 2.0×52.5 cm). Elution with a mixture of H_2O/MeOH (8:2 and 0:10, v/v) produced two subfractions (B1 and B2). Subfraction B10 (30.3 mg) was purified by ODS-HPLC (Shim-pack Prep-ODS (H) Kit, 5 μm; flow rate, 5.0 mL/min; wavelength, 254 nm) using a linear gradient system of 25% MeOH to 80% MeOH for 40 min, furnishing a mixture of **2** and **3** (t_R 12.1 min, 12.3 mg) along with 11 unknown compounds.

Fraction I (EtOAc/MeOH=5:5, v/v, 4,139 mg) was fractionated by Sephadex LH-20 column chromatography (4.0×70 cm; 70-230 mesh, Amersham Biosciences AB, Uppsala, Sweden). Elution with 100% MeOH afforded eight subfractions (I1-I8). Subfraction I1 [elution volume/total volume (Ve/Vt)=0.37-0.46, 1,299 mg] was fractionated by ODS column (3.0×77 cm) chromatography. Elution with a step-wise system of H_2O/MeOH (8:2, 6:4, 4:6, 2:8, and 0:10, v/v, each 1 L) provided 12 subfractions (I1a-I1l). Subfraction I1k [H_2O/MeOH=2:8-0:10, v/v, 714.3 mg] was subjected to semi-preparative ODS-HPLC [Shim-pack Prep-ODS (H) Kit; flow rate, 9.9 mL/min; wavelength, 254 nm] using a linear gradient of 20% MeOH (pH 2.65 adjusted with TFA)→100% MeOH for 40 min and with an isocratic of 100% MeOH for 60 min, afforded **4** (t_R 49.2 min, 10.0 mg) along with 15 unknown compounds. Moreover, compounds **5** (t_R 18.2 min, 10.0 mg), I6b (27.8 min, 55.3 mg), and a mixture of **9**-**11** (t_R 32.5 min, 5.0 mg) were purified from subfraction I6 (Ve/Vt=0.89-0.94, 283 mg) by semi-preparative ODS-HPLC using a linear gradient of 10% MeOH (pH 2.65 adjusted with TFA) 100% MeOH for 40 min. Subfraction I6b (27.8 min, 55.3 mg) was further purified by semi-preparative ODS-HPLC (40% MeOH, isocratic), affording **6** (t_R 9.8 min, 19.0 mg), **7** (t_R 12.5 min, 19.5 mg), and **8** (t_R 18.6 min, 5.0 mg).

Structural analysis The nuclear magnetic resonance (NMR) spectra were obtained using a ^{unit}INOVA 500 and 600 spectrometers (Varian, Walnut Creek, CA, USA). CD_3OD containing tetramethylsilane was used as the NMR solvent. All mass spectra were acquired using a hybrid ion-trap time-of-flight mass spectrometer (SYNAPT G2; Waters) equipped with an electrospray ionization source (ESI-MS).

Compound **1**: ^{1}H-NMR (500 MHz, CD_3OD) δ 2.81 (2H, m, H-2), 1.61 (2H, m, H-3), 1.29-1.33 (14H, m, H-4-7, 15-17), 2.06 (4H, m, H-8, 14), 5.40-5.49 (4H, m, H-9, 10, 12, 13), 2.31 (2H, t, *J*=7.5 Hz, H-11), 0.98 (3H, t, *J*=7.5 Hz, H-18), 3.67 (3H, s, OCH_3); ESI-MS (negative): *m/z* 293.1 $[M-H]^-$.

Compound **2**: ^{1}H-NMR (500 MHz, CD_3OD) δ 6.80 (2H, br. d, *J*=8.5 Hz, H-2, 6), 7.46 (2H, br. d, *J*=8.5 Hz, H-3, 5), 7.63 (1H, d, *J*=16.0 Hz, H-7), 6.36 (1H, d, *J*=16.0 Hz, H-8), 3.89 (3H, s, OCH_3); ESI-MS (negative): *m/z* 177.1 $[M-H]^-$.

Compound **3**: ^{1}H-NMR (500 MHz, CD_3OD) δ 7.19 (1H, d, *J*=1.8 Hz, H-2), 6.80 (1H, d, *J*=8.5 Hz, H-5), 7.07 (1H, dd, *J*=8.5, 1.8 Hz, H-6), 7.63 (1H, d, *J*=16.0 Hz, H-7), 6.33 (1H, d, *J*=16.0 Hz, H-8), 3.75 (3H, s, OCH_3), 3.76 (3H, s, OCH_3); ESI-MS (negative): *m/z* 207.1 $[M-H]^-$.

Compound **4**: ^{1}H-NMR (500 MHz, CD_3OD) δ 4.43 (1H, br. d, *J*=12.0 Hz, H-1a), 4.24 (1H, dd, *J*=12.0, 5.4 Hz, H-1b), 5.26 (1H, m, H-2), 3.99 (1H, dd, *J*=12.0, 5.4 Hz, H-3a), 3.75 (1H, dd, *J*=12.0, 6.0 Hz, H-3b), 2.33 (2H, m, H-2'), 1.61 (2H, m, H-3'), 1.26-1.37 (28H, m, H-4'-17'), 0.90 (3H, t, *J*=6.6 Hz, H-18'), 4.24 (1H, d, *J*=9.0 Hz, H-1''), 3.52 (1H, dd, *J*=9.0, 9.0 Hz, H-2''), 3.46 (1H, dd, *J*=9.0, 3.0 Hz, H-3''), 3.83 (1H, m, H-4''), 3.51 (1H, m, H-5''), 3.76 (1H, dd, *J*=12.0, 6.0 Hz, H-6''a), 3.73 (1H, dd, *J*=12.0, 5.4 Hz, H-6''b); ^{13}C-NMR (125 MHz, CD_3OD) δ 64.1 (C-1), 71.9 (C-2), 70.3 (C-3), 174.9 (C-1'), 35.2 (C-2'), 26.1 (C-3'), 30.2–30.9 (C-4'-17'), 14.6 (C-18'), 105.4 (C-1''), 76.9 (C-2''), 75.0 (C-3''), 70.3 (C-4''), 72.5 (C-5''), 62.5 (C-6''); ESI-MS (negative and positive): *m/z* 519.3 $[M-H]^-$ and 521.4 $[M+H]^+$.

Compound **5**: ^{1}H-NMR (500 MHz, CD_3OD) δ 7.07 (1H, d, *J*=1.5 Hz, H-2), 6.79 (1H, d, *J*=8.0 Hz, H-5), 6.98 (1H, dd, *J*=8.0, 1.5 Hz, H-6), 7.66 (1H, d, *J*=16.0 Hz, H-7), 6.31 (1H, d, *J*=16.0 Hz, H-8), 5.58 (1H, d, *J*=8.0 Hz, H-1'), 3.39-3.47 (4H, m, H-2'-5'), 3.87 (1H, dd, *J*=12.0, 1.5 Hz, H-6'a), 3.70 (1H, dd, *J*=12.0, 4.5 Hz, H-6'b); ^{13}C-NMR (125 MHz, CD_3OD) δ 126.2 (C-1), 114.1 (C-2), 148.5 (C-3), 145.4 (C-4), 115.1 (C-5), 121.9 (C-6), 147.0 (C-7), 113.0 (C-8), 166.4 (C-9), 94.4 (C-1'), 74.9 (C-2'), 77.3 (C-3'), 69.7 (C-4'), 76.6 (C-5'), 60.9 (C-6'); ESI-MS (negative): *m/z* 341.0 $[M-H]^-$.

Compound **6**: ^{1}H-NMR (500 MHz, CD_3OD) δ 7.00 (1H, br. s, H-2), 6.77 (1H, d, *J*=8.0 Hz, H-5), 6.90 (1H, dd, *J*=8.0, 1.5 Hz, H-6), 7.38 (1H, d, *J*=15.5 Hz, H-7), 6.34 (1H, d, *J*=15.5 Hz, H-8), 7.06 (2H, br. d, *J*=8.5 Hz, H-2', 6'), 6.72 (2H, br. d, *J*=8.5 Hz, H-3', 5'), 2.75 (2H, t, *J*=7.5 Hz, H-7'), 3.46 (2H, t, *J*=7.5 Hz, H-8'); ^{13}C-NMR (125 MHz, CD_3OD) δ 126.8 (C-1), 113.6 (C-2), 147.4 (C-3), 145.3 (C-4), 115.0 (C-5), 120.7 (C-6), 140.7 (C-7), 117.0 (C-8), 167.9 (C-9), 129.9 (C-1'), 129.3 (C-2', 6'), 114.8 (C-3', 5'), 155.5 (C-4'), 34.4 (C-7'), 41.2 (C-8'); ESI-MS (negative): *m/z* 298.1 $[M-H]^-$.

Compound **7**: ^{1}H-NMR (500 MHz, CD_3OD) δ 4.29 (1H, d, *J*=4.0 Hz, H-1), 3.66 (1H, d, *J*=4.0 Hz, H-2), 7.16 (1H, s, H-4), 6.77 (1H, s, H-5), 6.51 (1H, s, H-8), 6.47 (1H, d, *J*=2.0 Hz, H-2'), 6.64 (1H, d, *J*=8.0 Hz, H-5'), 6.43 (1H, dd, *J*=8.0, 2.0 Hz, H-6'), 6.82 (2H, br. d, *J*=8.5 Hz, H-2'', 6''), 6.66 (2H, br. d, *J*=8.5 Hz, H-3'', 5''), 2.67 (2H, t, *J*=7.0 Hz, H-7''), 3.25 (1H, dt, *J*=14.0, 7.0 Hz, H-8''a), 3.20 (1H, dt, *J*=14.0, 7.0 Hz, 8''b), 6.98 (2H, br. d, *J*=8.5 Hz, H-2''', 6'''), 6.69 (2H, br. d, *J*=8.5 Hz, H-3''', 5'''), 2.69 (2H, t, *J*=7.0 Hz, H-7'''), 3.39 (1H, dt, *J*=14.0, 7.0 Hz, H-8'''a), 3.44 (1H, dt, *J*=14.0, 7.0 Hz, H-8'''b); ^{13}C-NMR (125 MHz, CD_3OD) δ 46.0 (C-1), 49.8 (C-2), 173.3 (C-2a), 125.9 (C-3), 169.1 (C-3a), 134.8 (C-4), 123.6 (C-4a), 115.3 (C-5), 144.0 (C-6), 147.1 (C-7), 115.7 (C-8), 133.4 (C-8a), 129.8 (C-1'), 114.8 (C-2'), 143.5 (C-3'), 144.6 (C-4'), 114.8 (C-5'), 118.8 (C-6'), 129.8 (C-1''), 129.3 (C-2'', 6''), 129.4 (C-3'', 5'')[a], 155.3 (C-4''), 34.1 (C-7''), 41.0 (C-8''), 130.0 (C-1'''), 129.4 (C-2''', 6''')[a], 114.8 (C-3''', 5'''), 155.4 (C-4'''), 34.3 (C-7'''), 41.3 (C-8'''). [a]The signals of C-3'', C-5'', C-3''', and C-5''' overlapped; ESI-MS (negative): *m/z* 595.3 $[M-H]^-$.

Compound **8**: ^{1}H-NMR (500 MHz, CD_3OD) δ 7.73 (1H, s, H-4), 7.19 (1H, s, H-5), 6.98 (1H, s, H-8), 6.81 (1H, d, *J*=2.0 Hz, H-2'), 6.89 (1H, d, *J*=8.0 Hz, H-5'), 6.69 (1H, dd, *J*=8.0, 2.0 Hz, H-6'), 7.12 (2H, br. d, *J*=8.5 Hz, H-2'', 6''), 6.75 (2H, br. d, *J*=8.5 Hz, H-3'', 5''), 2.30 (2H, t, *J*=7.0 Hz, H-7''), 3.18 (2H, t, *J*=7.0 Hz, H-8''), 6.93 (2H, br. d, *J*=8.5 Hz, H-2''', 6'''), 6.67 (2H, br. d, *J*=8.5 Hz, H-3''', 5'''), 2.83 (2H, t, *J*=7.0 Hz, H-7'''), 3.52 (2H, t, *J*=7.0 Hz, H-8'''); ^{13}C-NMR (125 MHz, CD_3OD) δ 131.1 (C-1), 136.6 (C-2), 171.0 (C-2a), 129.4 (C-3), 170.1 (C-3a), 125.1 (C-4), 128.3 (C-4a), 109.9 (C-5), 147.7 (C-6), 148.1 (C-7), 109.0 (C-8), 129.3 (C-8a), 129.8 (C-1'), 117.3 (C-2'), 143.7 (C-3'), 144.8 (C-4'), 114.7 (C-5'), 121.7 (C-6'), 130.0 (C-1''), 129.5 (C-2'', 6''), 114.9 (C-3'', 5''), 155.3 (C-4''), 33.8 (C-7''), 41.4 (C-8''), 130.2 (C-1'''), 129.1 (C-2''', 6'''), 114.8 (C-3''', 5'''), 155.5 (C-4'''), 34.3 (C-7'''), 41.5 (C-8'''); ESI-MS (negative): *m/z* 593.3 $[M-H]^-$.

Compound **9**: ^{1}H-NMR (500 MHz, CD_3OD) δ 7.16 (1H, br. s, H-2), 6.72 (1H, d, *J*=8.5 Hz, H-5), 6.99 (1H, dd, *J*=8.5, 2.0 Hz, H-6), 7.43 (1H, d, *J*=15.5 Hz, H-7), 6.43 (1H, d, *J*=15.5 Hz, H-8), 3.87 (3H, s, OCH_3), 7.06 (2H, br. d, *J*=8.5 Hz, H-2', 6'), 6.71 (2H, br. d, *J*=8.5 Hz, H-3', 5'), 2.75 (2H, t, *J*=7.5 Hz, H-7'), 3.46 (2H, t, *J*=7.5 Hz, H-8'); ^{13}C-NMR (125 MHz, CD_3OD) δ 128.4 (C-1), 115.5 (C-2), 148.0 (C-3), 145.3 (C-4), 114.8 (C-5), 126.9 (C-6), 140.0 (C-7), 118.5 (C-8), 167.4 (C-9), 55.4 (OCH_3), 129.8 (C-1'), 129.3 (C-2', 6'), 114.8 (C-3', 5'), 155.5 (C-4'), 34.4 (C-7'), 41.2 (C-8'); ESI-MS (negative and positive): *m/z* 312.1 $[M-H]^-$ and 314.3 $[M+H]^+$.

Compound **10**: ^{1}H-NMR (500 MHz, CD_3OD) δ 7.16 (1H, br. s, H-2), 6.72 (1H, d, *J*=8.5 Hz, H-5), 6.99 (1H, dd, *J*=8.5, 2.0 Hz, H-6), 6.63 (1H, d, *J*=12.5 Hz, H-7), 5.88 (1H, d, *J*=12.5 Hz, H-8), 3.86 (3H, s, OCH_3), 7.06 (2H, br. d, *J*=8.5 Hz, H-2', 6'), 6.71 (2H, br. d, *J*=8.5 Hz, H-3', 5'), 2.70 (2H, t, *J*=7.5 Hz, H-7'), 3.42–3.34 (2H, m, H-8'); ^{13}C-NMR (125 MHz, CD_3OD) δ 128.4 (C-1), 115.5 (C-2), 148.0 (C-3), 145.3 (C-4), 114.8 (C-5), 126.9 (C-6), 135.7 (C-7), 121.6 (C-8), 167.4 (C-9), 55.3 (OCH_3), 129.8 (C-1'), 129.3 (C-2', 6'), 114.8 (C-3', 5'), 155.5 (C-4'), 34.0 (C-7'), 41.2 (C-8'); ESI-MS (negative and positive): *m/z* 312.1 $[M-H]^-$ and 314.3 $[M+H]^+$.

Compound **11**: ^{1}H-NMR (500 MHz, CD_3OD) δ 7.16 (2H, br. s, H-2, 6), 7.43 (1H, d, *J*=15.5 Hz, H-7), 6.43 (1H, d, *J*=15.5 Hz, H-8), 3.87 (6H, s, OCH_3), 7.06 (2H, br. d, *J*=8.5 Hz, H-2', 6'), 6.71 (2H, br. d, *J*=8.5 Hz, H-3', 5'), 2.75 (2H, t, *J*=7.5 Hz, H-7'), 3.46 (2H, t, *J*=7.5 Hz, H-8'); ^{13}C-NMR (125 MHz, CD_3OD) δ 126.9 (C-1), 104.5 (C-2, 6), 148.0 (C-3, 5), 135.8 (C-4), 140.0 (C-7), 118.5 (C-8), 167.4 (C-9), 55.4 (OCH_3, ×2), 129.8 (C-1'), 129.3 (C-2', 6'), 114.8 (C-3', 5'), 155.5 (C-4'), 34.4 (C-7'), 41.2 (C-8'); ESI-MS (negative and positive): *m/z* 342.0 $[M-H]^-$ and 344.2 $[M+H]^+$.

Results and Discussion

The MeOH extracts of *T. tetragonioides* aerial parts were solvent-fractionated to obtain *n*-hexane (23.62 g), EtOAc (8.36 g), and BuOH (28.05 g) fractions. The free radical-scavenging activities of these fractions were determined by DPPH radical assay on a TLC plate (15). The EtOAc fraction showed various spots with free radical-scavenging activity. Eleven compounds (**1**-**11**) were purified and isolated from the EtOAc fraction of *T. tetragonioides* aerial parts using various column chromatography techniques such as silica gel, ODS, Sephadex LH-20, and normal- and reverse-phase HPLC. The purification procedure for 11 compounds obtained from the aerial parts of *T. tetragonioides* is indicated in Fig. 1. The structure of the purified compounds was determined by NMR and MS analyses. Out of the 11 isolated compounds, three compounds, which have been isolated from this plant previously (4), were identified as methyl ferulate (**3**) (16), *N-trans*-caffeoyltyramine (**6**) (17), and cannabisin B (**7**) (18) based on a comparison of their NMR and MS data with those reported in the previous study.

The molecular weight of **1** was 294, as shown by a pseudomolecular ion peak at *m/z* 293.1 $[M-H]^-$ in the ESI-MS (negative) spectrum. The ^{1}H-NMR spectrum (CD_3OD) suggested that **1** was a typical unsaturated fatty acid. The characteristic proton signals including two olefinic double bonds at δ 5.40-5.49 (4H, m, H-9, 10, 12, 13) and a methoxy group at δ 3.67 (3H, s, OCH_3) were observed in the ^{1}H-NMR spectrum. Thus, compound **1** was identified as methyl linoleate from a comparison of its MS and ^{1}H-NMR spectra with those reported previously (19) (Fig. 2).

Compound **2** showed a pseudomolecular ion peak at *m/z* 177.1 $[M-H]^-$ in the ESI-MS (negative) spectrum, indicating that its molecular weight was 178. The ^{1}H-NMR spectrum of **2** showed the two proton

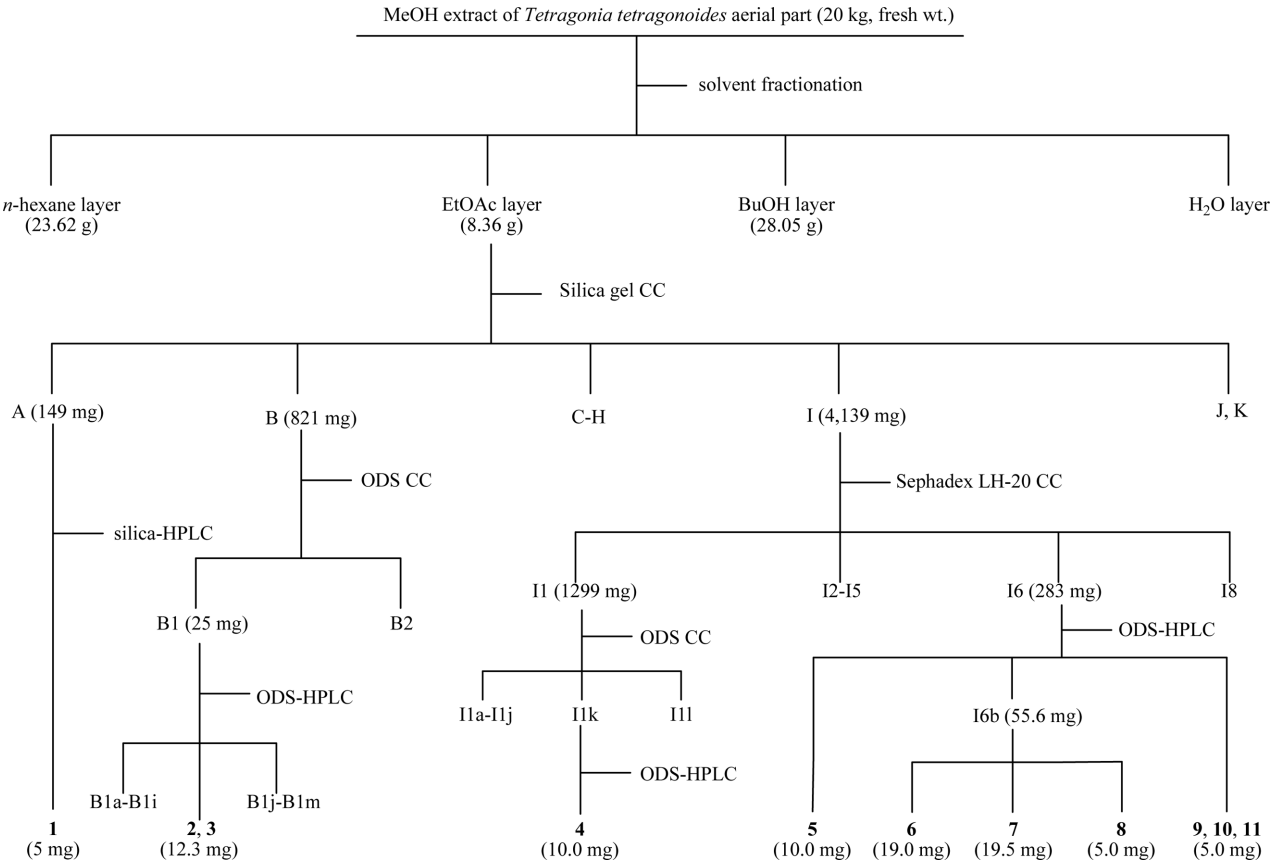

Fig. 1. Procedure used to purify and isolate **1**-**11** from the aerial parts of *Tetragonia tetragonioides*. CC, column chromatography.

signals of a *para*-substituted benzene ring at δ 6.80 (2H, br. d, *J*=8.5 Hz, H-2, 6) and 7.46 (2H, br. d, *J*=8.5 Hz, H-3, 5) and two proton signals of the *trans* olefinic double bond at δ 7.63 (1H, d, *J*=16.0 Hz, H-7) and 6.36 (1H, d, *J*=16.0 Hz, H-8). Moreover, a methoxy proton signal at δ 3.89 (3H, s, OCH_3) was observed in the ^{1}H-NMR spectrum. The MS and ^{1}H-NMR results indicated that **2** has a 4-hydroxycinnamic acid moiety and a methoxy group. The ^{1}H-NMR spectrum of **2** was matched with that of methyl coumarate, previously isolated from pear fruits (20). Thus, compound **2** was unambiguously identified as methyl coumarate (Fig. 2).

The molecular weight of **4** was 520, as indicated by the pseudo-molecular ion peaks at *m/z* 519.3 $[M-H]^-$ and 521.4 $[M+H]^+$ in the ESI-MS (negative and positive) spectra. The ^{1}H-NMR spectrum showed the presence of a glycerol moiety, including two oxygenated methylene proton signals at δ 4.43 (H-1a), 4.24 (H-1b), 5.26 (1H, m, H-2), 3.99 (H-3a), and 3.75 (H-3b) and an oxygenated methine proton signal at δ 5.26 (H-2), and the proton signals of a stearic acid moiety, including the 32H methylene proton signals at δ 2.33 (H-2'), 1.61 (H-3'), 1.26-1.37 (H-4'~H-17'), and a methyl proton signal at δ 0.90 (H-18'). Moreover, the presence of a β-D-galactopyranose moiety was indicated by the coupling constant (*J*=9.0 Hz) of the anomeric proton signal at δ 4.24 (H-1''), other proton signals at δ 3.83-3.46 (6H, H-2''~H-6''), and the coupling constant (*J*=3.0 Hz) of H-3'' at δ 3.46. The proposed structure of the β-D-galactopyranose moiety was confirmed by the proton-proton correlations in the ^{1}H-^{1}H correlation spectroscopy (COSY) experiment. The ^{13}C-NMR spectrum of **4** revealed 27 carbon signals, including one carbonyl carbon at δ 174.9 (C-1') and 6 sugar and 3 glycerol carbons at δ 105.4-62.5. Based on the MS and 1D-NMR spectra, compound **4** was suggested to be composed of glycerol, stearic acid, and β-D-galactopyranose moieties. Their moieties were also assigned by 2D-NMR experiments, such as ^{1}H-^{1}H COSY, heteronuclear single-quantum correlation (HSQC), and heteronuclear multiple-bond correlation (HMBC) experiments. In particular, the HMBC correlations (Fig. 2, arrows) of δ 4.43 (H-1a) to δ 174.9 (C-1'), δ 3.99 (H-3a) to δ 105.4 (C-1''), and δ 4.25 (H-1'') to δ 70.3 (C-3) indicated that stearic acid and β-D-galactopyranose moieties were attached to the C-1 and C-3 of the glycerol moiety by ester and ether bonds, respectively. As a result, compound **4** was identified as (2*S*)-1-*O*-stearoyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol by comparing the ^{1}H- and ^{13}C-NMR spectra with those reported previously (21) (Fig. 2).

Compound **5** showed a pseudomolecular ion peak at *m/z* 341.0 $[M-H]^-$ in the ESI-MS (negative) spectrum, indicating that its molecular weight was 342. The ^{1}H-NMR spectrum showed the presence of a caffeic acid moiety, including three trisubstituted benzene ring proton signals at δ 7.07 (1H, d, *J*=1.5 Hz, H-2), 6.79 (1H, d, *J*=8.0 Hz, H-5), and 6.98 (1H, dd, *J*=8.0, 1.5 Hz, H-6), and *trans*

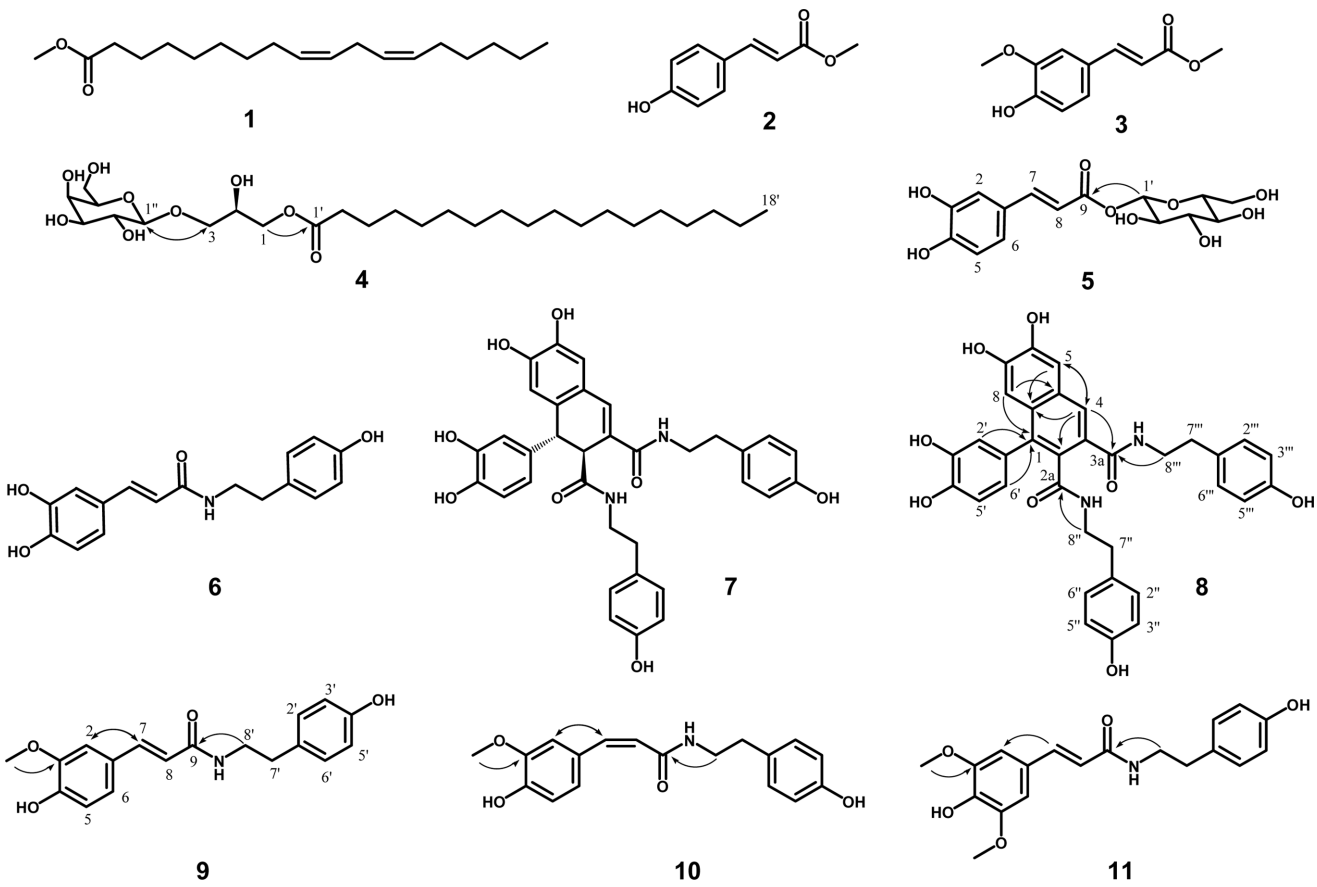

Fig. 2. Structures of the compounds isolated from the aerial parts of *Tetragonia tetragonioides* and the important HMBC correlations (arrows) for **4**, **5**, and **8**-**11**.

double bond proton signals at δ 7.66 (1H, d, *J*=16.0 Hz, H-7) and 6.31 (1H, d, *J*=16.0 Hz, H-8). Moreover, a β-glucopyranose moiety was indicated by the coupling constant (*J*=8.0 Hz) of the anomeric proton signal at δ 5.58 (1H, d, *J*=8.0 Hz, H-1') and other proton signals at δ 3.39-3.87 (6H, m, H-2'-H-6') in the ^{1}H NMR spectrum. The ^{1}H-NMR result of **5** was supported by the ^{13}C-NMR spectrum, with 15 carbon signals, including one carbonyl carbon at δ 166.4 (C-9), eight *sp*^{2} carbons at δ 148.5-114.1, and six sugar carbons at δ 94.4-60.9. Compound **5** was suggested to be caffeic acid attached to a β-glucopyranose moiety. In particular, the β-glucopyranose moiety was esterified with caffeic acid, which was confirmed by the correlation of δ 5.58 (1H, d, *J*=8.0 Hz, H-1') to δ 166.4 (C-9) in the HMBC experiment. The ^{1}H- and ^{13}C-NMR spectra of **5** were consistent with those of 1-*O*-caffeoyl-β-D-glucopyranoside, which was isolated previously from *Spathodea campanulata* (22). Therefore, compound **5** was unambiguously identified as 1-*O*-caffeoyl-β-D-glucopyranoside (Fig. 2).

Compound **8** showed pseudomolecular ion peaks at *m/z* 593.3 [M-H]$^{-}$ and 595.10 [M+H]$^{+}$ in the ESI-MS (negative and positive) spectra, respectively, indicating that the molecular weight of **8** was 594. The ^{1}H-NMR spectrum of **8** showed the presence of trisubstituted benzene proton signals at δ 6.81 (1H, d, *J*=2.0 Hz, H-2'), 6.89 (1H, d, *J*=8.0 Hz, H-5'), and 6.69 (1H, dd, *J*=8.0, 2.0 Hz, H-6') and three singlet proton signals of the *sp*^{2} methine at δ 7.73 (1H, s, H-4), 7.19 (1H, s, H-5), and 6.98 (1H, s, H-8). Moreover, the four proton signals of two *para*-substituted benzene rings at δ 7.12 (2H, br. d, *J*=8.5 Hz, H-2'', 5''), 6.75 (2H, br. d, *J*=8.5 Hz, H-3'', 5''), 6.93 (2H, br. d, *J*=8.5 Hz, H-3''', 5'''), and 6.67 (2H, br. d, *J*=8.5 Hz, H-2''', 6''') and four *sp*^{3} methylene proton signals at δ 2.30 (2H, t, 7.0), 3.18 (2H, t, 7.0), 2.83 (2H, t, 7.0), and 3.52 (2H, t, 7.0) were observed in the ^{1}H-NMR spectrum. These results were also supported by the ^{13}C-NMR spectrum, with 34 carbon signals, including two carbonyls at δ 170.1 (C-2a) and 170.0 (C-3a), 28 *sp*^{2} carbons at δ 155.3-109.0, and four *sp*^{3} carbons at δ 41.5-33.8. The MS and 1D-NMR data indicated that the two carbonyl groups at δ 170.1 (C-2a) and 170.0 (C-3a) were nitrogen-bearing carbons. Moreover, the ^{1}H- and ^{13}C-NMR spectra of **8** were very similar to those of cannabisin B (**7**) except for one olefinic double bond. Therefore, compound **8** was suggested to be a lignin amide, comprising two caffeoyltyramine moieties. The connectivities of **8** were further determined by ^{1}H-^{1}H-COSY, HSQC, and HMBC experiments. The 6,7-dihydroxy-1-(3,4-dihydroxyphenyl)naphthalene and two tyramine moieties were confirmed from the spectra. In particular, the HMBC correlations (Fig. 2, arrows) of δ 3.18 (H-8) to δ 170.0 (C-2a) and δ 3.52 (H-8) to δ 170.1 (C-3a) indicated that the two tyramine

moieties were attached to the two carbonyl groups, respectively. A correlation (Fig. 2, arrows) of δ 7.73 (H-4) to δ 170.1 (C-3a) was observed in the HMBC spectrum, indicating that one amide carbonyl group was coupled to the C-3 of 6,7-dihydroxy-1-(3,4-dihydroxyphenyl)naphthalene. Another amide carbonyl group may be attached to the C-2 position remaining in the 6,7-dihydroxy-1-(3,4-dihydroxyphenyl)naphthalene structure. The ^{1}H- and ^{13}C-NMR spectra of **8** were consistent with those of cannabisin A, previously isolated from *Cannabis sativa* (23). Thus, the structure of **8** was unambiguously determined as cannabisin A (Fig. 2).

Compounds **9**-**11**, which were purified as a mixture from *T. tetragonioides*, were suggested to be feruloyltyramine derivatives by their ^{1}H- and ^{13}C-NMR spectra, i.e., the skeletal structure was similar, but the methoxy group and configuration of olefinic double bond in ferulic acid differed. The ^{1}H-NMR spectrum showed an unequal proton signal intensity of the olefinic double bond at a ratio of **9**/**10**/**11**=5:5:4. The structures of **9**-**11** could be assigned by ^{1}H-^{1}H COSY, HSQC, and HMBC experiments. The ^{1}H-NMR spectrum of **9** showed the signals for ferulic acid moiety [trisubstituted benzene ring protons at δ 7.16 (1H, br. s, H-2), 6.72 (1H, d, *J*=8.5 Hz, H-5), and 6.99 (1H, dd, *J*=8.5, 2.0 Hz, H-6), a methoxy proton at δ 3.87 (3H, s, OCH_3), and *trans* olefinic double bond protons at δ 7.43 (1H, d, *J*=15.5 Hz, H-7) and 6.43 (1H, d, *J*=15.5 Hz, H-8)]. Moreover, the signals for tyramine moiety [two *para*-substituted benzene ring protons at δ 7.06 (2H, br. d, *J*=8.5 Hz, H-2', 6') and 6.71 (2H, br. d, *J*=8.5 Hz, H-3', 5') and two methylene protons at δ 2.75 (2H, t, *J*=7.5 Hz, H-7') and 3.46 (2H, t, *J*=7.5 Hz, H-8') were observed in the ^{1}H-NMR spectrum. This result was supported by the ^{13}C-NMR spectrum, with 18 carbon signals of ferulic acid and tyramine moieties at δ 167.4-34.4. In particular, the HMBC correlations (Fig. 2, arrows) of δ 3.46 (H-8') and δ 3.46 (H-8') to δ 167.4 (C-9) indicated that the amine group of tyramine was coupled to the carbonyl group of ferulic acid as an amide bond. Therefore, the structure of **9** was determined as *N-trans*-feruloyltyramine (Fig. 2). Moreover, the ^{1}H-NMR spectrum of **10** was very similar to that of **9**, except for the chemical shifts and coupling patterns of the *cis* olefinic double bond proton signals at δ 6.63 (1H, d, *J*=12.5 Hz, H-7) and 5.88 (1H, d, *J*=12.5 Hz, H-8). The structure of **10** was confirmed as *N-cis*-feruloyltyramine based on 1D- and 2D-NMR experiments (Fig. 2). The molecular weights (MW. 313) of **9** and **10** were confirmed from the pseudomolecular ion peaks at *m/z* 312.1 $[M-H]^-$ and 314.3 $[M+H]^+$ in the ESI-MS (negative and positive) spectra. Therefore, compounds **9** and **10** were identified as *N-trans*-feruloyltyramine and *N-cis*-feruloyltyramine by comparing their ^{1}H- and ^{13}C-NMR spectra with those reported previously (24) (Fig. 2).

The molecular weight of **11** was 343, as shown by the pseudo-molecular ion peaks detected at *m/z* 342.1 $[M-H]^-$ and 344.3 $[M+H]^+$ in ESI-MS (negative and positive) spectra, respectively. The ^{1}H-NMR spectra of **11** showed the characteristic proton signals of sinapic acid moiety including one 2H benzene ring methine at δ 7.16 (2H, br. s, H-2, 6) and a 6H methoxy groups at δ 3.87 (6H, s, OCH_3) instead of ferulic acid moiety in the structure of **9**. The MS and ^{1}H-NMR spectra suggested that **11** was *N-trans*-sinapoyltyramine, as confirmed by the ^{13}C- and 2D-NMR experiments. As a result, the structure of **11** was identified as *N-trans*-sinapoyltyramine by comparing the ^{1}H- and ^{13}C-NMR spectra with those reported previously (25) (Fig. 2).

In this study, 11 antioxidative compounds, including a fatty acid derivative, an acyl galactopyranosyl glycerol, three coumaric acids, and six lignan amides, were isolated from the aerial part of *T. tetragonioides*. Compounds **3**, **6**, and **7** have been previously isolated from this plant (4,11). Compounds **1**, **2**, **4**, **5**, and **8**-**11** were identified for the first time from *T. tetragonioides*. Compounds **1** and **2** showed very weak DPPH radical-scavenging activities on a TLC plate (data not shown). However, coumaric acid derivatives (**2**, **3**, and **5**) and *N*-coumaroyltyramine derivatives (**6** and **9**-**11**) showed significant free radical-scavenging activities (25-27). Moreover, cannabisin B (**7**) has been reported to exhibit a high DPPH radical-scavenging activity and inhibitory activity against the *in vitro* oxidation of human low-density lipoprotein (28). Recently, it was reported that **7** exhibited antiproliferative activity in human hepatocarcinoma HepG2 cells by inhibiting the activation of AKT and mammalian target of rapamycin (29). Cannabisin A (**8**), containing a double bond between the C-1 and C-2 positions of cannabisin B (**7**), may exhibit high antioxidative activity, despite the activity not being evaluated in this study; i.e., the catechol structure is well known as an active site for the free radical-scavenging activity of phenolic compounds (27). Therefore, compounds **7** and **8** bearing two catechol moieties are expected to exhibit high antioxidative activities. Moreover, the phenolics (**2**, **3**, and **5**) and their amides (**6**-**11**) identified in this study may be partially responsible for the antioxidative activity of *T. tetragonioides*. These results provide useful information to better understand the chemical constituents and biological activities of *T. tetragonioides*. Further studies are needed to isolate and identify antioxidative compounds of *T. tetragonioides* and evaluate their antioxidative activities along with the 11 compounds isolated and characterized in this study.

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