SHORT REPORT

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Complete Isolation and Characterization of Polar Portion of *Mentha dumetorum* Water Extract

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Abstract: Four flavone glycosides, eriocitrin **(1)**, luteolin-7-O-rutinoside **(2)**, hesperidin **(3)**, apigenin-7-*O*-rutinoside **(4)** and rosmarinic acid **(5)** were isolated from the aerial parts of *Mentha dumetorum* (Lamiaceae) for the first time. The structural elucidations of the isolated compounds were achieved by spectral methods (1D- and 2D-NMR, and LC-TOF-HRMS).

Keywords: *Mentha dumetorum*; flavone glycosides; rosmarinic acid.

1. Plant Source

Mentha dumetorum was cultivated in application fields of Faculty of Agriculture, Gaziosmanpasa University by Prof. Dr. İsa Telci. Plant samples harvested at June 2012. We report on the structure elucidation of the four flavone glycosides and rosmarinic acid (**5**) (Figure 1).

2. Previous Studies

In literature, there is no any information about polyphenolic content of *Mentha dumetorum*.

3. Present Study

Grinded aerial parts of plant samples (500 g) were boiled in water (2 L) at open atmosphere for 1 h and cooled to room temperature. Plant residue was filtered off using filter paper. The filtrate was partitioned between ethyl acetate (1 L X 3) and water using separation funnel. Ethyl acetate phase was removed. The water phase partitioned between butanol (2 L X 3) and water. n-butanol phases were combined and dried over anhydrous Na_2SO_4 and evaporated to the dryness. n-butanol extract

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was obtained as yellowish slurry (12 g). The extract was chromatographed over slica gel column (5x120 cm, diameter x length).The column was eluted using 50% *n*-hexane-EtOAc (15 L), 25% *n*-hexane-EtOAc (15 L), 100% EtOAc (10 L), 95% EtOAc-MeOH (11.25 L), 90% EtOAc-MeOH (12.50 L), 85% EtOAc-MeOH (36.25 L) to give 400 fractions (250 mL each). According to TLC basis, similar fractions were combined as follows: fractions 1-125 (250 mg, fatty acid mixture, low polarity metabolites, not studied, Fr-0), fractions 132-151 (92 mg, Fr-1), fractions 165-192 (102 mg, Fr-2), fractions 210-236 (88 mg, Fr-3), fractions 265-400 (3450 mg, Fr-4).

Fr-1was purified using Flash chromatography on slica gel column (40 g, Redisep) eluting a linear gradient from 50 % *n*-Hexane-EtOAc to 100% EtOAc with 10 mL/min flow rate. The peak detected at 330 nm on flash chromatogram (15-21 fractions) was combined to give rosmarinic acid **(5)**. Fr-2 was solved in small amount of methanol and added EtOAc until a precipitate formed. Mixture was centrifuged for 5 min, 5000 rpm. Solvent was decanted and the precipitate was washed with acetone and evaporated to give apigenin-7-*O*-rutinoside **(4)** as pale yellow amorphous solid. Fr-3 gave hesperidin **(3)** as white amorphous solid. Fr-4 was chromatographed on slica gel column (250 g, 70-230 mesh). The column was eluted using 100% EtOAc (0.5L), 97% EtOAc-MeOH (1.25 L), 94% EtOAc-MeOH (1.75 L), 90% EtOAc-MeOH (1.5 L), %85 EtOAc-MeOH (1 L) to give 120 fractions (50 mL each). After TLC analysis of fractions; 18-64 (Fr-4a) and 72-87 (Fr-4b) were combined. Fr-4a (1250 mg) gave eriocitrin (**1**) and Fr-4b (612 mg) gave luteolin-7-*O*-rutinoside **(2)**.

*Eriocitrin **(1)**:* HRMS *m/z:*596.1844[M-H]$^-$.^{1}H NMR (DMSO-d_6), [aglycone moiety] δ_H: 2.60 (1H, dd, *J* = 17.10 and 2.92 Hz, H-3a), 3.20 (1H, dd, *J* = 17.10 and 12.48 Hz, H-3b),5.44 (1H, d, *J* = 12.48 and 2.92 Hz, H-2), 6.12 (1H, d, J=2,34 Hz, H-6), 6.14 (1H, d, *J* = 2.34 Hz, H-8), 6.77 (2H, brs, H-5' and H-6', overlapped), 6.78 (1H, brs, H-2'), 12.06 (1H, s, 5-OH), [sugar moiety] 1.08 (3H, d, *J* = 5.32 Hz, rhamnose H-6'"), 4.98 (1H, d, *J* = 7.02 Hz, H-1"), 4.54 (1H, brs, H-1'"). 3.10-3.70 (10 sugar protons), 5.40, 5.20, 5.02, 4.72, 4.65, 4.55 (6H, sugar hydroxyls).^{13}C NMR (DMSO-d_6), [aglycone moiety],δ_C: 42.6 (C-3), 79.1 (C-2) 95.9 (C-8), 96.7 (C-6), 103.8 (C-10), 114.9 (C-2'), 115.8 (C-5'), 118.6 (C-6'), 129.7 (C-1'), 145.6 (C-4'), 146.2 (C-3'), 163.0 (C-9), 163.5 (C-7), 165.5 (C-5), 197.6 (C-4); [sugar moiety]: glucose; 66.5 (C-6"), 70.0 (C-4"), 72.5 (C-2"), 76.7 (C-5"), 75.9 (C-3"), 99,8 (C-1"); rhamnose; 18.2 (C-6'"), 68.7 (C-5'"), 70.7 (C-3'"), 71.2 (C-2'"), 73.4 (C-4'"), 101.0 (C-1'").

*Luteolin-7-O-rutinoside **(2)**:* HRMS *m/z:* 593.1501[M-H]$^-$.^{1}H NMR (DMSO-d_6) [aglycone moiety]δ_H: 6.46 (1H, d, *J*=1.92 Hz, H-6), 6.74 (1H, d, *J*=1.92 Hz, H-8), 6.76 (1H, s, H-3), 6.92 (1H, d, *J*=8.42 Hz, H-4'), 7.42 (1H, dd, *J*= 8.42/2.08 Hz, H-6'), 7.45 (1H, d, *J*=2.08 Hz, H-2'), 13.05 (1H, s, 5-OH); [sugar moiety] 1.07 (3H, d, *J*= 5.80 Hz, H-6'"), 3.10-3.90 (10 sugar protons), 4.55 (1H, brs, H-1'"), 5.08 (1H, d, *J*= 7.24 Hz, H-1"), 5.53, 5.34, 5.23, 4.89, 4.62, 4.54 (6H, sugar hydroxyls); ^{13}C NMR (DMSO-d_6): [aglycone moiety] δ_C: 95.2 (C-8), 100.9 (C-6), 103.6 (C-3), 105.8 (C-10), 114.0 (C-6'), 116.5 (C-5') 119.7 (C-2'), 121.8 (C-1'), 146.2 (C-3'), 150.4 (C-4'), 158.3 (C-9), 161.6 (C-5), 163.3 (C-7), 165.0 (C-2), 182.3 (C-4); [sugar moiety]: glucose; 66.4 (C-6"), 70.0 (C-4"), 72.4 (C-2"), 76.7 (C-5"), 76.0 (C-3"), 100,9 (C-1"); rhamnose; 18.5 (C-6'"), 68.6 (C-5'"), 70.7 (C-3'"), 71.4 (C-2'"), 73.4 (C-4'"), 101.3 (C-1'").

*Hesperidin **(3)**:* HRMS *m/z:*609.1887 [M-H]$^-$.^{1}H NMR (DMSO-d_6), [aglycone moiety], δ_H:3.77 (3H, s, -OCH_3), 2.77 (1H, dd, *J* = 17.12 and 3.10 Hz, H-3a), 3.34 (1H, dd, *J* = 17.12 and 12.26 Hz, H-3b), 5.52 (1H, dd, *J* = 12.26 and 3.10 Hz, H-2), 6.14 (1H, d, J=2,32 Hz, H-6), 6.16 (1H, d, *J* = 2.32 Hz, H-8), 6.94 (2H, brs, H-5' and H-6',overlapped), 6.91 (1H, brs,H-2'), 12.02 (1H, s, 5-OH), [sugar moiety] 1.08 (3H, d, *J* = 6.38 Hz, rhamnose H-6'"), 4.97 (1H, d, *J* = 7.40 Hz, H-1"), 4.52 (1H, brs, H-1'"). 3.10-3.70 (10 sugar protons), 5.40, 5.19, 5.00, 4.70, 4.62, 4.48 (6H, sugar hydroxyls). ^{13}C NMR (DMSO-d_6), [aglycone moiety],δ_C=56.1 (-OCH_3), 78.8 (C-2), 42.5 (C-3), 96.0 (C-6), 96.8 (C-8), 103.7 (C-10), 112.6 (C-2'), 114.6 (C-5'), 118.4 (C-6') 131.3 (C-1'),146.9 (C-4'), 148.4 (C-3'), 162.9 (C-9), 163.5 (C-7), 165.6 (C-5), 197.4 (C-4) [sugar moiety]:glucose; 66.5 (C-6"), 70.0 (C-4"), 72.5 (C-2"), 76.7 (C-5"), 75.9 (C-3"), 99.9 (C-1"); rhamnose; 18.3 (C-6'"), 68.7 (C-5'"), 70.7 (C-3'"), 71.1 (C-2'"), 73.4 (C-4'"), 101.0 (C-1'").

*Apigenin-7-O-rutinoside **(4)**:* HRMS *m/z:* 577.1549[M-H]$^-$.^{1}H NMR (DMSO-d_6), [aglycone moiety],δ_H: 6.18 (1H, d, *J*=1.98 Hz, H-6), 6.47 (1H, d, *J*=1.98 Hz, H-8), 6.78 (1H, s, H-3), 6.93(2H, d, *J*=8.76 Hz, H-3' and H-5'), 7.91 (2H, d, *J*= 8.76 Hz, H-2' and H-6'), 12.98 (1H, s, 5-OH); [sugar moiety] 1.10 (3H, d, *J*= 6.41 Hz, H-6'"), 3.10-3.90 (10 sugar protons), 4.54 (1H, brs, H-1'"), 5.04 (1H, d, *J*= 7.08 Hz, H-1"), 5.53, 5.34, 5.28, 4.78, 4.59, 4.48 (6H, sugar hydroxyls); ^{13}C NMR (DMSO-d_6): [aglycone moiety],δ_H: 94.4 (C-8), 99.3 (C-6), 103.2 (C-3), 104.0 (C-10), 116.4 (C-3' and C-5'), 121.6

(C-1'), 128.9 (C-2' and C-6'), 157.8 (C-9), 161.6 (C-5), 161.9 (C-4'), 164.1 (C-7), 164.8 (C-2), 182.1 (C-4);); [sugar moiety]: glucose; 66.3 (C-6''), 70.1 (C-4''), 72.4 (C-2''), 76.8 (C-5''), 75.9 (C-3''), 100.8 (C-1''); rhamnose; 18.3 (C-6'''), 68.7 (C-5'''), 70.7 (C-3'''), 71.4 (C-2'''), 73.4 (C-4'''), 101.3 (C-1''').

*Rosmarinic acid **(5)**:* HRMS *m/z:* 359.0836 $[M-H]^-$.^{1}H-NMR (400 MHz, DMSO-d_6), δ_H: 2.94 (*d*, 1H, H-7b', *J*= 10 Hz), 2.98 (*dd*, 1H, H- 7a', *J*= 10.1 Hz, *J*= 1.10 Hz), 5.08 (*dd*, 1H, H-8', *J*= 10 Hz, *J*= 2.8 Hz), 6.24 (*d*, 1H, H-8, *J*= 15.8 Hz), 6.54 (*d*, 1H, H-5', *J*= 7.92 Hz), 6.65 (*d*, 1H, H-6', *J*= 7.92 Hz), 6.69 (*d*, 1H, H-2', *J*= 1.5 Hz), 6.79 (*d*, 1H, H-5, *J*= 8.12 Hz), 7.02 (*d*, 1H, H-6, *J*= 8.12 Hz), 7.06 (*d*, 1H, H-2, *J*= 1.4 Hz), 7.46 (*d*, 1H, H-7, *J*= 15.8 Hz). ^{13}C-NMR (100 MHz, DMSO-d_6) δ_C:36.6 (C-7'), 73.2 (C-8'), 113.6 (C-8), 115.2 (C- 2), 120.5 (C-5'), 116.2 (C-5), 117.2 (C-2'), 115.8 (C-6'), 122.0 (C-6), 125.9 (C-1), 127.7 (C-1'), 145.4 (C-4'), 144.5 (C-3'), 146.4 (C-7), 149.1 (C-3), 146.0 (C-4), 166.4 (C-9), 171.3 (C-9').

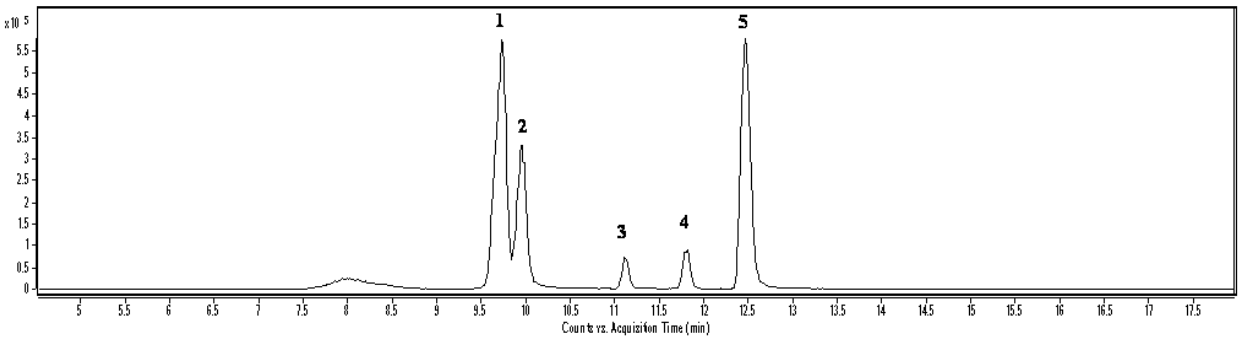

Figure 1. HPLC-TOF-MS chromatogram of water extract of *Mentha dumetorum.*

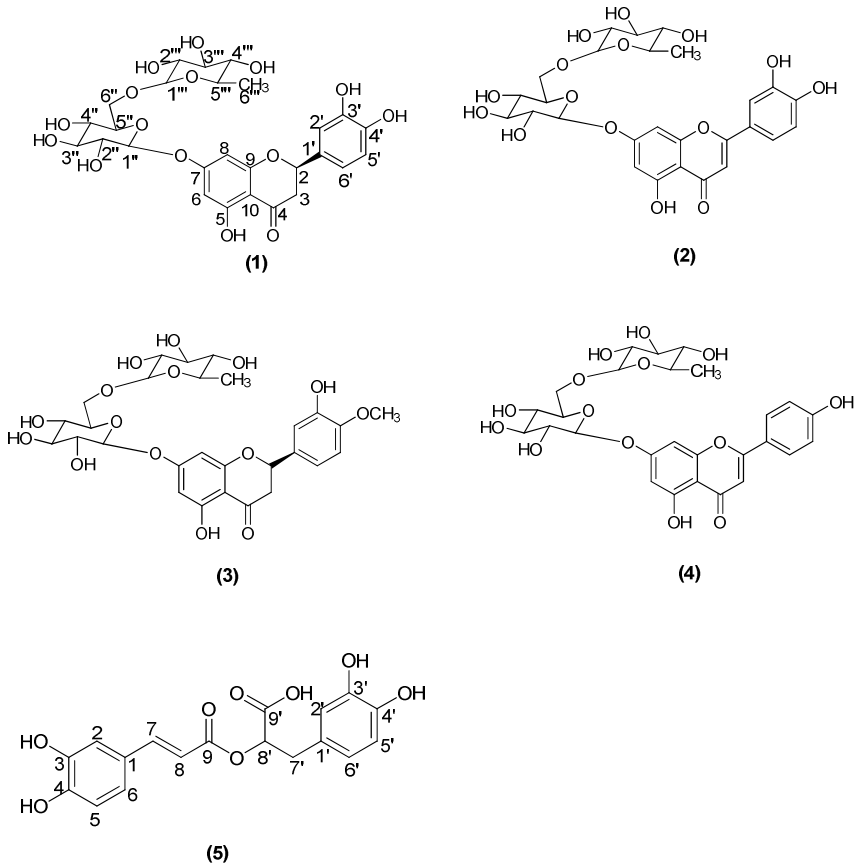

Figure 1. Chemical structure of isolated molecules **(1-5)**.

In this study, from the aerial parts of *Mentha dumetorum*, four flavone glycosides, eriocitrin **(1)**, luteolin-7-O-rutinoside **(2)**, hesperidin **(3)** apigenin-7-O-rutinoside **(4)** as well as rosmarinic acid **(5)** were isolated from hot water extract by fractionation of the butanol phase through an open column

chromatography on silica gel. In this study we achieved the isolation and characterization of all compounds revealed on LC-TOF-MS chromatogram (Figure 1) of polar portion of *Mentha dumetorum* hot water extract.

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