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Phytoconstituents from the aerial parts of *Salvia dracocephaloides* Boiss. and their biological activities

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Abstract

MTT colorimetric cytotoxic bioassay, solvent fractionation and chromatographic purification of an 80% methanol extract of *Salvia dracocephaloides*, led to isolation and identification of eight known compounds, including 5-hydroxy-3,6,7,3′,4′-pentamethoxyflavone (**1**), cynaroside (**2**), salvigenin (**3**), eupatorin (**4**), cirsimaritin (**5**), *β*-sitosterol (**6**), oleanolic acid (**7**), and ursolic acid (**8**). The structures of the compounds were characterized using spectroscopic analyses including HRESI-MS, ^{1}H and ^{13}C-NMR and comparison with those previously reported in the literature. The biological activities of **1** and **2**, including their cytotoxicity against MCF-7 human breast cancer cell line and DPPH free radicals scavenging effect were studied *in vitro*. Compound **1** showed stronger cytotoxicity; IC_{50} of 7.2 µg/mL compared to that obtained for **2**; IC_{50} of 15.5 µg/mL. While, compound **2**, with 3′,4′ dihydroquinol functionality, displayed higher DPPH radical inhibitory activity with an IC_{50} of 10.7 µg/mL compared to that measured for **1**; 48.8 µg/mL.

Keywords: *Salvia dracocephaloides*, Lamiaceae, Cytotoxicity, DPPH radical scavenging activity, polymethoxyflavones

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Supplementary Spectral Data

5-Hydroxy-3,6,7,3′,4′-pentahydroxyflavone; ***1***, light yellowish powder; UV (MeOH) λ_{Max} nm (log ε) 223 (4.27), 271 (3.56), 363 sh (2.74) nm; ^{13}C NMR (100 MHz, in DMSO-d_6): δ 178.3 (C-4), 158.8 (C-7), 155.5 (C-2), 151.8 (C-5)*, 151.7 (C-9)*, 151.5 (C-4′)*, 148.5 (C-3′), 138.1 (C-3), 131.7 (C-6), 122.1 (C-1′), 122.1 (C-6′), 111.6 (C-5′), 111.2 (C-2′), 105.7 (C-10), 91.5 (C-8), 60.1 (6-OMe), 59.8 (3-OMe), 56.6 (7-OMe)*, 55.7 (3′-OMe)*, 55.7 (4′-OMe)*. ^{1}H NMR (400 MHz, in DMSO-d_6): δ 7.73 (1H, d, *J* = 8.2 Hz, H-6′), 7.66 (1H, s, H-2′), 7.16 (1H, d, *J* = 8.2 Hz, H-5′), 6.93 (1H, s, H-8); *Interchangeable values. Positive ion mode of HRESI-MS *m/z* 389.1220 $[M+H]^+$ (calcd for $C_{20}H_{21}O_8$, 389.1236) [1].

Cynaroside (Luteolin-7-O-β-D-glucopyranoside; 2), yellowish powder, $C_{21}H_{20}O_{11}$, ^{13}C NMR (75 MHz, in Pyridine-d_5): δ 183.55 (C-4), 166.04 (C-2), 164.62 (C-7), 163.12 (C-5), 158.51 (C-9), 152.59 (C-4′), 148.41 (C-3′), 123.25 (C-1′), 120.45 (C-6′), 117.61 (C-5′), 115.31 (C-2′), 107.21 (C-10), 104.67 (C-3), 102.37 (C-1″), 101.30 (C-6), 96.13 (C-8), 79.74 (C-5″), 79.02 (C-3″), 75.44 (C-2″), 71.89 (C-4″), 63.00 (C-6″). ^{1}H NMR (300 MHz, in Pyridine-d_5): δ 13.56 (1H, s, 5-OH), 7.97 (1H, d, *J* = 2.4 Hz, H-2′), 7.57 (1H, d, *J* = 8.4, 2.4 Hz, H-6′), 7.37 (1H, d, *J* = 8.4 Hz, H-5′), 7.04 (1H, d, *J* = 2.2 Hz, H-8), 6.95 (1H, s, H-3), 6.85 (1H, d, *J* = 2.2 Hz, H-6), 5.82 (1H, d, *J* = 7.6 Hz, H-1″), 4.62 (1H, m, H-6″a), 4.58 (1H, m, H-6″b), 4.48 (1H, dd, *J* = 8.7, 8.6 Hz, H-3″), 4.41 (1H, dd, *J* = 8.6, 7.3 Hz, H-4″), 4.36 (1H, dd, *J* = 8.6, 7.6 Hz, H-2″), 4.25 (1H, m, H-5″) [2].

The spectroscopic data of compounds **3-8** are extracted from the compounds simultaneously isolated from *S. russellii* and published elsewhere. Identification was deduced based on co-TLC with the authentic samples.

Salvigenin (3), light yellowish powder, $C_{18}H_{16}O_6$, ^{13}C NMR (125 MHz, in DMSO-d_6): δ 183.69 (C-4), 163.63 (C-2), 162.45 (C-4′), 158.71 (C-7), 152.68 (C-9), 152.07 (C-5), 135.48 (C-6), 128.38 (C-2′,6′), 122.75 (C-1′), 114.63 (C-3′,5′), 109.07 (C-10), 103.39 (C-3), 91.70 (C-8), 60.06 (6-OMe), 56.50 (7-OMe), 55.62 (4′-OMe). ^{1}H NMR (500 MHz, in DMSO-d_6): δ 12.89 (1H, s, 5-OH), 8.09 (2H, d, *J* = 8.9 Hz, H-2′,6′), 7.14 (2H, d, *J* = 8.9 Hz, H-3′,5′), 6.98 (1H, s, H-3), 6.96 (1H, s, H-8), 3.94 (3H, s, 7-OMe), 3.87 (3H, s, 4′-OMe), 3.74 (3H, s, 6-OMe). Positive ion mode of HRESI-MS *m/z* 351.0877 $[M+Na]^+$ (calcd. for $C_{18}H_{16}O_6Na$, 351.0845) [3].

Eupatorin (4), yellowish powder, $C_{18}H_{16}O_7$, ^{13}C NMR (125 MHz, in MeOD): δ 184.26 (C-4), 166.40 (C-2), 160.68 (C-7), 154.91 (C-9), 153.71 (C-5), 152.77 (C-4′), 148.33 (C-3′), 133.77 (C-6), 124.98 (C-1′), 120.14 (C-6′), 114.04 (C-2′), 112.70 (C-5′), 106.75 (C-10), 104.46 (C-3), 92.31 (C-8), 60.07 (6-OMe), 57.02 (7-OMe), 56.51 (4′-OMe). ^{1}H NMR (500 MHz, in MeOD): δ 12.74 (1H, s, 5-OH), 7.58 (1H, dd, *J* = 8.6, 2.1 Hz, H-6′), 7.47 (1H, d, *J* = 2.1 Hz, H-2′), 7.13 (1H, d, J = 8.6 Hz, H-5′), 6.86 (1H, s, H-8), 6.69 (1H, s, H-3), 4.02 (3H, s, 7-OMe), 3.99 (3H, s, 4′-OMe), 3.87 (3H, s, 6-OMe). Both positive and negative ion mode of HRESI-MS *m/z* 367.0821 $[M+Na]^+$ (calcd. for $C_{18}H_{16}O_7Na$, 367.0794), 343.0844 $[M-H]^+$ (calcd. for $C_{18}H_{15}O_7$, 343.0818) [4].

Cirsimaritin (5), yellowish powder, $C_{17}H_{14}O_6$, ^{13}C NMR (125 MHz, in DMSO-d_6): δ 182.67 (C-4), 164.61 (C-2), 161.08 (C-4′), 159.06 (C-7), 153.09 (C-9), 152.55 (C-5), 132.33 (C-6), 129.01 (C-2′,6′), 121.46 (C-1′), 116.58 (C-3′,5′), 105.52 (C-10), 102.96 (C-3), 92.05 (C-8), 60.51 (6-OMe), 56.92 (7-OMe). ^{1}H NMR (500 MHz, in DMSO-d_6): δ 12.96 (1H, s, 5-OH), 7.97 (2H, d, *J* = 8.8 Hz, H-2′,6′), 6.94 (1H, s, H-8), 6.92 (2H, d, *J* = 8.8 Hz, H-3′,5′), 6.85 (1H, s, H-3), 3.93 (3H, s, 7-OMe), 3.73 (3H, s,

6-OMe). Negative ion mode of HRESI-MS *m/z* 313.0694 $[M-H]^+$ (calcd. for $C_{17}H_{14}O_6$, 313.0712) [3].

*β-Sitosterol (**6**)*, whitish crystal, $C_{29}H_{50}O$. ^{13}C NMR (75 MHz, in $CDCl_3$): δ 140.93 (C-6), 121.86 (C-5), 71.97 (C-3), 56.93 (C-14), 56.23 (C-17), 50.31 (C-9), 46.02 (C-22), 42.48 (C-13), 42.48 (C-4), 39.95 (C-12), 37.42 (C-1), 36.66 (C-10), 36.31 (C-18), 34.12 (C-20), 33.87 (C-2), 31.84 (C-7), 31.84 (C-8), 29.34 (C-25), 28.40 (C-16), 26.27 (C-15), 24.46 (C-21), 23.24 (C-23), 21.25 (C-11), 19.97 (C-26), 19.55 (C-27), 19.20 (C-19), 18.94 (C-28), 12.14 (C-24), 12.02 (C-29). ^1H NMR (300 MHz, in $CDCl_3$): δ 5.35 (1H, brs, H-6), 3.50 (1H, m, H-3), 1.01 (3H, s, H-19), 0.92 (3H, brs, H-21), 0.84 (3H, brs, H-26), 0.82 (3H, brs, H-27), 0.68 (3H, s, H-18). EI-MS *m/z* 414.71 $[M]^+$ [5].

*Oleanolic acid (**7**)*, whitish powder, $C_{30}H_{48}O_3$, ^{13}C NMR (75 MHz, in $CDCl_3$): δ 183.78 (C-28), 143.72 (C-13), 122.77 (C-12), 79.19 (C-3), 55.34 (C-5), 47.77 (C-9), 46.86 (C-17), 46.68 (C-19), 41.74 (C-18), 41.68 (C-14), 39.41 (C-8), 38.90 (C-4), 38.54 (C-1), 37.22 (C-10), 33.94 (C-21), 33.90 (C-22), 33.22 (C-29), 32.75 (C-7), 30.82 (C-20), 29.85 (C-2), 28.25 (C-23), 27.82 (C-15), 26.01 (C-27), 23.73 (C-30), 23.54 (C-16), 23.05 (C-11), 18.45 (C-6), 17.22 (C-26), 15.70 (C-24), 15.48 (C-25). ^1H NMR (300 MHz, in $CDCl_3$): δ 5.27 (1H, brs, H-12), 3.21 (1H, dd, *J* = 11.2, 5.1 Hz, H-3), 1.13 (3H, s, H-27), 0.98 (3H, s, H-23), 0.92 (3H, s, H-30), 0.90 (6H, brs, H-25,29), 0.73 (3H, s, H-24), 0.77 (3H, s, H-26). Positive ion mode of HRESI-MS *m/z* 479.3646 $[M+Na]^+$ (calcd. for $C_{30}H_{48}O_3Na$, 479.3501) [6].

*Ursolic acid (**8**)*, whitish powder, $C_{30}H_{48}O_3$, ^{13}C NMR (75 MHz, in $CDCl_3$): δ 180.93 (C-28), 138.14 (C-13), 125.76 (C-12), 79.00 (C-3), 55.55 (C-5), 55.14 (C-9), 53.11 (C-17), 47.83 (C-19), 42.39 (C-18), 39.72 (C-14), 39.24 (C-8), 38.94 (C-4), 37.18 (C-1), 37.09 (C-10), 33.31 (C-22), 32.53 (C-7), 30.92 (C-20), 30.57 (C-21), 29.88 (C-2), 28.27 (C-15), 28.22 (C-23), 26.99 (C-16), 24.47 (C-11), 23.51 (C-27), 21.29 (C-30), 18.56 (C-6), 17.08 (C-26), 16.29 (C-29), 15.79 (C-24), 15.60 (C-25). ^1H NMR (300 MHz, in $CDCl_3$): δ 5.26 (1H, brs, H-12), 3.20 (1H, m, H-3), 1.16 (3H, s, H-27), 1.10 (3H, s, H-23), 0.99 (3H, d, *J* = 6.7 Hz, H-30), 0.96 (3H, s, H-25), 0.94 (3H, *J* = 6.6 Hz, H-29), 0.82 (3H, s, H-26), 0.78 (3H, s, H-24). Positive ion mode of HRESI-MS *m/z* 479.3510 $[M+Na]^+$ (calcd. for $C_{30}H_{48}O_3Na$, 479.3501) [6].

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