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ANTIOXIDANT ACTIVITY AND FLAVONOIDS OF *Stachys ocymastrum*

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Stachys genus (Lamiaceae) has shown various activities such as anti-inflammatory [1], antimicrobial [2], and antioxidant [3] activities. Because of the various biological interests in the secondary metabolites (flavonoids, diterpenes, phenylethanoid glycosides) of this genus, we have made a phytochemical study of the antioxidant activity of the species *Stachys ocymastrum* (L.) Briq. [4].

Aerial parts of *Stachys ocymastrum* (L.) Briq. were collected from Djebel El-Ouahch-Constantine (North Eastern Algeria) in June 2005 during the flowering stage. A voucher specimen has been deposited in the Herbarium of the Department of Chemistry, University Mentouri-Constantine, and authenticated by Prof. G. De Belair (University of Annaba, Algeria).

Air-dried and powdered aerial parts (890 g) of *Stachys ocymastrum* were macerated in a methanolic solution (70%) at room temperature. The extract was concentrated under low pressure, diluted, and filtered, then successively extracted with petroleum ether, dichloromethane, ethyl acetate, and *n*-butanol.

Compound **1** was isolated as a yellow solid that precipitated from the ethyl acetate extract; then, the butanolic and the ethyl acetate extracts were concentrated under reduced pressure. The two extracts were combined and column chromatographed on Polyamid SC6 with a gradient of toluene–MeOH with increasing polarity, affording compound **2** from fraction F-75. Successive separations using preparative TLC on silica gel eluted with CH_2Cl_2–MeOH (9:1) led to compound **3** from fraction F-57, while paper chromatography (Whatman No. 3MM) eluted with AcOH–H_2O (30:70) led to compound **4** from fraction F-70.

Compounds **1** and **2** were identified by ^{1}H NMR, ^{13}C NMR, Dept-135, ^{1}H–^{1}H COSY, HMBC, HMQC, and MS, while compounds **3** and **4** were identified by UV and ^{1}H NMR as well as by direct comparison with literature data [6–9].

Compound 1. $C_{30}H_{26}O_{12}$, mp 270°C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 12.97 (1H, s, 5-OH), 7.94 (2H, d, J = 8.7, H-2′, H-6′), 6.92 (2H, d, J = 8.7, H-3′, H-5′), 6.83 (1H, s, H-3), 6.82 (1H, d, J = 1.8, H-8), 6.47 (1H, d, J = 1.8, H-6), 5.17 (1H, d, J = 6.9, H-1″), 4.47 (1H, d, J = 11.6, H-6″$_b$), 4.16 (1H, dd, J = 11.9, 7.2, H-6″$_a$), 3.84 (1H, m, H-5″), 3.35 (1H, m, H-2″), 3.33 (1H, m, H-3″), 3.26 (1H, m, H-4″), 7.49 (1H, d, J = 15.9, H-*β*), 6.67 (2H, d, J = 8.5, H-3‴, H-5‴), 7.36 (2H, d, J = 8.5, H-2‴, H-6‴), 6.32 (1H, d, J = 15.9, H-*α*). ^{13}C NMR (125 MHz, DMSO-d_6, δ): 94.60 (C-8), 99.34 (C-6), 102.90 (C-3), 105.26 (C-10), 115.90 (C-3′, C-5′), 120.86 (C-1′), 128.45 (C-2′, C-6′), 156.80 (C-9), 161.02 (C-5), 161.28 (C-4′), 162.58 (C-7), 164.17 (C-2), 181.88 (C-4), 63.33 (C-6″), 69.87 (C-4″), 72.84 (C-2″), 73.70 (C-5″), 76.11 (C-3″), 99.37 (C-1″), 113.61 (C-*α*),115.57 (C-3‴, C-5‴), 124.78 (C-1‴), 130.00 (C-2‴, C-6‴), 144.85 (C-*β*), 159.68 (C-4‴), 166.37 (C=O). Mass spectrum, *m/z*: 601.1 $[M + Na]^+$, 623.1 $[M – H + 2Na]^+$, 639.1 $[M – H + Na + K]^+$. Characterized as apigenin 7-*O*-*β*-D-(6″-*O*-*p*-coumaroylglucopyranoside) [5].

Compound 2. $C_{27}H_{30}O_{16}$. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 12.04 (1H, s, 5-OH), 8.35 (1H, s, 8-OH), 7.99 (2H, d, J = 8.8, H-2′, H-6′), 6.96 (2H, d, J = 8.8, H-3′, H-5′), 6.85 (1H, s, H-3), 6.65 (1H, s, H-6), 5.11 (1H, d, J = 7.5, H-1″), 3.74 (1H, m, H-6″$_b$), 3.65 (1H, m, H-2″), 3.51 (1H, m, H-5″), 3.49 (1H, m, H-6″$_a$), 3.49 (1H, m, H-3″), 3.36 (1H, m, H-4″), 4.91 (1H, d, J = 8, H-1‴), 3.87 (1H, m, H-3‴), 3.64 (1H, H-5‴), 3.52 (1H, H-6‴$_b$), 3.42 (1H, H-6‴$_a$), 3.34 (1H, m, H-4‴), 3.21 (1H, m, H-2‴). ^{13}C NMR (125 MHz, DMSO-d_6, δ): 182.3 (C-4), 163.98 (C-2), 161.24 (C-4′), 152.35 (C-5), 151.13 (C-7), 144.24 (C-9), 128.56 (C-2′, C-6′), 127.02 (C-8), 121.17 (C-1′), 115.91 (C-3′, C-5′), 105.11 (C-10), 102.5 (C-3), 98.65 (C-6), 99.5 (C-1″), 81.17 (C-2″), 76.94 (C-3″), 75.59 (C-5″), 69.17 (C-4″), 60.4 (C-6″), 101.6 (C-1‴), 74.5 (C-5‴), 71.5 (C-2‴), 70.9 (C-3‴), 67.1 (C-4‴), 60.9 (C-6‴). Mass spectrum, *m/z*: 633.1 $[M + Na]^+$, 655.1 $[M – H + 2Na]^+$. Characterized as isoscutellarein 7-*O*-*β*-D-allopyranosyl-(1→2)-glucopyranoside [6, 7].

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Compound 3. $C_{15}H_{10}O_5$, mp 327°C, characterized as luteolin [8].

Compound 4. $C_{15}H_{10}O_6$, mp 347°C, characterized as apigenin [9].

Compound **1** was isolated for the first time from the genus.

Antioxidant Activity. The radical scavenging activity of the butanolic extract of *Stachys ocymastrum* (L.) Briq. was measured by the slightly modified method of Hatano [8]. The *n*-butanolic extract of *Stachys ocymastrum* (L.) Briq. exhibited good activity: IC_{50} 6.77 ± 0.2 μg/mL compared with the reference (rutin: IC_{50} 3.01 ± 0.2 μg/mL).

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