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FLAVONOIDS OF *Campanula alata* AND THEIR ANTIOXIDANT ACTIVITY

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In addition to their use as ornamental plants, *Campanula* (Campanulaceae) species are used in traditional medicine to treat various diseases such as tonsillitis, laryngitis, bronchitis, and warts [1]. They possess stimulant properties and are used as emetics; they also possess refreshing, antiallergic, antiphlogistic, antioxidant, spasmolytic, antiviral, and antimicrobial properties [2, 3]. Flavonoids are the most important secondary metabolites of the *Campanula* genus [4–9].

Campanula alata Desf. (Campanulaceae) is an endemic species [10] collected from Constantine (Eastern Algeria) in April 2004 and authenticated by Prof. Gerard De Belair (Annaba, Algeria).

A voucher specimen was deposited in the Herbarium of the Laboratory of Therapeutic Substances (LOST) at Mentouri University (LOST/Ca/04/04).

Air-dried and powdered aerial parts (1 kg) of *Campanula alata* Desf. were macerated in a methanolic solution (70%). The extract was successively concentrated to dryness (under low pressure); the residue was dissolved in boiling water and extracted with petroleum ether, dichloromethane, ethyl acetate, and *n*-butanol, successively.

The butanolic extract was column chromatographed on Polyamid SC6 eluted with toluene–methanol with increasing polarity. Whatman 3MM paper chromatography using 15% AcOH and BAW [*n*-BuOH–AcOH–H_2O, 4:1:5 (upper phase)] and TLC on Polyamid DC6 eluted with H_2O–MeOH–metylethylketone–acetylacetone (13:3:3:1) followed by column flash chromatography over Sephadex LH-20 in MeOH led to four pure flavonoids **1–4** and a caffeic ester (**5**), which were identified by using UV, ^{1}H NMR, ^{13}C NMR, and MS analysis [11–13].

Acid Hydrolysis. The pure compounds were treated with 2 M HCl at 100°C for 1 h. The hydrolysates were extracted with EtOAc, and the aglycones were identified by their UV spectra in methanol and by comparison of their R_f with authentic samples.

Sugars were identified in the aqueous residue by comparison with authentic samples on silica gel TLC impregnated with 0.2 M NaH_2PO_4 [solvent Me_2CO–H_2O (9:1)] and revealed with aniline malonate.

Antioxidant Activity. The radical scavenging activity of the *n*-butanolic extract of *Campanula alata* (BECA) was measured by the slightly modified method of Hatano [14]. One milliliter of a 0.2 mM DPPH methanol solution was added to 4 mL of various concentrations of the extract in methanol. The mixture was shaken vigorously and left to stand at room temperature. After 30 min, the absorbance of the solution was measured at 517 nm and the antioxidant activity calculated using the following equation: Scavenging capacity % = 100 – [(Ab of sample – Ab of blank) × 100/Ab of control]. Methanol (1 mL) plus plant extract solution (4 mL) were used as a blank, while DPPH solution plus methanol was used as a negative control. The positive control was DPPH solution plus 1 mM rutin. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the plot of inhibition percentage against extract concentration.

Compound 1. $C_{15}H_{10}O_7$, yellow needles (acetone), mp >300°C. UV (MeOH, λ_{max}, nm): 256, 374; +$AlCl_3$: 271, 451; +$AlCl_3$/HCl: 266, 302sh, 361sh, 427. ^{1}H NMR spectrum (400 MHz, acetone-d_6, δ, ppm, J/Hz): 7.81 (1H, br.s, H-2′), 7.70 (1H, d, J = 8.0, H-6′), 6.99 (1H, d, J = 8.0, H-5′), 6.53 (1H, br.s, H-8), 6.27 (1H, br.s, H-6). This compound was characterized as quercetin [15].

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Compound 2. $C_{21}H_{20}O_{11}$, mp 239–242°C (MeOH). UV (MeOH, λ_{max}, nm): 267, 336; +NaOH: 275, 405; +$AlCl_3$: 275, 291, 330, 423; +$AlCl_3$/HCl: 275, 296, 355, 385; +NaOAc: 267, 400; +NaOAc/H_3BO_3: 260, 370. ^{1}H NMR (250 MHz, CD_3OD, δ, ppm, J/Hz): 7.42 (1H, dd, J = 9.4, J = 2.2, H-6′), 7.40 (1H, d, J = 2.2, H-2′), 6.93 (1H, d, J = 9.4, H-5′), 6.81 (1H, d, J = 2.2, H-8), 6.63 (1H, s, H-3), 6.52 (1H, d, J = 2.2, H-6), 5.10 (1H, d, J = 10, H-1′′), 3.25–4.25 (sugar protons). Acid hydrolysis of compound **2** produced luteolin and glucose. This compound was characterized as 5,3′,4′-trihydroxy-7-*O*-glucosylflavone (luteolin 7-glucoside) [16].

Compound 3. Mp 218–212°C. UV (MeOH, λ_{max}, nm): 257, 353; + NaOH: 272, 331, 404; + $AlCl_3$: 273, 300 sh, 434; +$AlCl_3$/HCl: 269, 359 sh; 396. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.60 (1H, d, J = 2.3, H-2′), 7.60 (1H, m, H-6′), 6.85 (1H, d, J = 8.9, H-5′), 6.40 (1H, d, J = 1.9, H-8), 6.20 (1H, d, J = 1.9, H-6), 5.40 (1H, d, J = 6.9, H-1′′Glc), 3.2–4.9 (sugar protons).

Acid hydrolysis of **3** produced quercetin and D-glucose. Compound **3** was identified as quercetin 3-*O*-glucoside [17].

Compound 4. $C_{27}H_{30}O_{16}$, mp 250–254°C. UV (MeOH, λ_{max}, nm): 257, 300sh, 356, +NaOH: 272, 325, 407; + $AlCl_3$: 275, 290, 350sh; +HCl: 268, 285sh, 350, 390; +NaOAc: 271, 385; +H_3BO_3: 263, 378. ^{1}H NMR (250 MHz, CD_3OD, δ, ppm, J/Hz): 7.80 (1H, d, J = 2, H-2′), 7.75 (1H, dd, J = 9, J = 2, H-6′), 6.85 (1H, d, J = 9, H-5′), 6.3 (1H, d, J = 2, H-8), 6.20 (1H, d, J = 2, H-6), 5.12 (1H, d, J = 7, H-1′′Glc), 4.55 (1H, d, H-1′′′Rha), 1.1 (3H, d, J = 6.2, H-6′′′Rha), 3.20–3.90 (10H, protons of rutinose). ^{13}C NMR (62.89 MHz, CD_3OD, δ): 177.9 (C-4), 164.6 (C-7), 161.5 (C-5), 157.9 (C-2), 157.06 (C-9), 148.4 (C-4′), 144.4 (C-3′), 134.2 (C-3), 122.1 (C-6′), 121.6 (C-1′), 116.2 (C-5′), 114.6 (C-2′), 104.17 (C-10), 103.3 (C-1′′), 100.9 (C-1′′′), 98.5 (C-6), 93.4 (C-8), 76.7 (C-5′′), 75.7 (C-3′′), 74.3 (C-2′′), 72.5 (C-4′′′), 70.7 (C-4′′), 70.6 (C-2′′), 69.9 (C-3′′′), 68.3 (C-5′′′), 67.1 (C-6′′), 16.4 (C-6′′′). Identified as quercetin-3-*O*-rutinoside [18].

Compound 5. $C_{10}H_{10}O_4$, yellowish crystals, mp 162–163°C. IR (KBr, ν, cm^{-1}): 3493 (OH), 3308 (C-H aromatic), 1681 (C=O), 1603 (C=C aromatic). UV (MeOH, λ_{max}, nm): 328, 300, 244, 219. ^{1}H NMR (400 MHz, CD_3OD, δ, ppm, J/Hz): 7.56 (d, J = 16.5, H-7), 7.05 (d, J = 2, H-2), 6.96 (dd, J = 8 and 2, H-6), 6.80 (d, J = 8, H-5), 6.27 (d, J = 16.5, H-8), 3.77 (s, OCH_3). ^{13}C NMR (100 MHz, CD_3OD, δ): 113.6 (C-8), 113.9 (C-2), 115.3 (C-5), 121.8 (C-6), 126.5 (C-1), 145.7 (C-3), 145.8 (C-7), 148.4 (C-4), 168.6 (C-9). Identified as methyl caffeate [19].

The butanolic extract of *Campanula alata* (BECA) exhibited good activity (IC_{50} 25.77 ± 0.2 μg/mL) compared with rutin (IC_{50} 3.01 ± 0.2 μg/mL).

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