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FLAVONOL GLYCOSIDES FROM *Convolvulus supinus* AND THEIR ANTIOXIDANT ACTIVITY

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Convolvulus is a fairly large genus with 250 species, almost cosmopolitan in distribution [1]. Various biological activities such as anticancer [2], antinociceptive [3], antidiarrhea [4], and antifungal [5] have been reported for *Convolvulus* species, which are characterized by the presence of phenolic compounds and alkaloids [6–9].

Algerian flora is represented by 17 species [10]. We have been interested in the phytochemical study and antioxidant activity of the endemic species *Convolvulus supinus* Coss. & Kralik (Convolvulaceae).

Aerial parts (1.5 kg) of *Convolvulus supinus* were macerated in a methanolic solution (80%) at room temperature. The extract was concentracted under low pressure, diluted with water, and filtered, then successively extracted with dichloromethane, ethyl acetate, and *n*-butanol.

The butanolic extract (12 g) was column chromatographed on polyamid SC_6, eluting with toluene–methanol with increasing polarity. A total of 103 fractions was obtained and combined into 19 main fractions (according to the number, color, and R_f of the sports). Fraction F-9 was separated by column chromatography on silica gel and eluted with an isocratic system of EtOAc–MeOH–H_2O (10:1:0.5) to afford compounds **1** and **2**. Fraction F-14 was subjected to column chromatography on silica gel and eluted with an isocratic system of EtOAc–MeOH–H_2O (10:1:1.5) to yield compound **3**. These compounds were identified by the use of spectral methods: UV, ^{1}H NMR, and ^{13}C NMR in addition to acid hydrolysis.

Acid Hydrolysis. The pure compounds were treated with 2M 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_2PO4, solvent Me_2CO–H_2O (9:1), revealed with aniline malonate.

Antioxidant Activity. The radical scavenging activity of the *n*-butanolic extract of *Convolvulus supinus* was measured by the slightly modified method of Hatano [11]. 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. The extract concentration providing 50% inhibition (IC_{50}) was calculated from the plot of inhibition percentage against extract concentration.

Compound 1. $C_{21}H_{20}O_{11}$, mp 272°C. UV (MeOH, λ_{max}, nm): 266, 350; + NaOH: 274, 325, 400; + $AlCl_3$: 275, 398; + $AlCl_3$/HCl: 275, 398; NaOAc: 275, 375; + H_3BO_3: 266, 357. ^{1}H NMR (400 MHz, CD_3OD, δ, ppm, J/Hz): 8.10 (2H, d, J = 9.0, H-6′, H-2′), 6.90 (2H, d, J = 9.0, H-5′, H-3′), 6.43 (1H, d, J = 2.5, H-8), 6.22 (1H, d, J = 2.5, H-6), 5.25 (1H, d, J = 7.5, Glc H-1″), 3.20–4.00 (sugar protons). ^{13}C NMR (100 MHz, CD_3OD, δ, ppm): 177.0 (C-4), 165 (C-7), 161.5 (C-5), 160 (C-4′), 157.5 (C-2), 157 (C-9), 134 (C-3), 130.5 (C-2′, 6′), 121.5 (C-1′), 115 (C-3′, 5′), 104 (C-10), 98.50 (C-6), 93.50 (C-8), 102.50 (C-1″), 76.7 (C-5″), 76.4 (C-3″), 74.5 (C-2″), 70.0 (C-4″), 61.50 (C-6″). Acid hydrolysis of compound **1** produced kaempferol and glucose. This compound was characterized as kaempferol 3-*O*-*β*-D-glucoside [12, 13].

Compound 2. $C_{27}H_{30}O_{15}$, mp 272°C. UV (MeOH, λ_{max}, nm): 267, 351; + NaOH: 275, 325, 401; + $AlCl_3$: 273, 398; + $AlCl_3$/HCl: 274, 398; NaOAc: 273, 371; + H_3BO_3: 267, 351. ^{1}H NMR (400 MHz, CD_3OD, δ, ppm, J/Hz): 8.10 (2H, d, J = 8.9, H-6′, 2′), 6.90 (2H, d, J = 8.9, H-5′, 3′), 6.43 (1H, d, J = 2.0, H-8), 6.22 (1H, d, J = 2.0, H-6), 5.20 (1H, d, J = 7.5, Glc H-1″),

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4.50 (1H, d, J = 1.5, Rha H-1‴), 1.15 (3H, d, J = 6.2, Rha H-6‴), 3.20–4.00 (sugar protons). ^{13}C NMR (100 MHz, CD_3OD, δ, ppm): 176.5 (C-4), 164.5 (C-7), 161.5 (C-5), 160.0 (C-4′), 158.0 (C-2), 157.0 (C-9), 134.0 (C-3), 131.5 (C-2′, 6′), 121.5 (C-1′), 114.5 (C-3′, 5′), 104.5 (C-10), 99.0 (C-6), 93.5 (C-8), 103.5 (C-1″), 76.6 (C-3″), 75.8 (C-5″), 74.5 (C-2″), 70.1 (C-4″), 67.25 (C-6″), 101.0 (C-1‴), 72.5 (C-4‴), 71.0 (C-2‴), 70.7 (C-3‴), 68.25 (C-5‴), 16.0 (C-6‴). HMBC experiment established a correlation between C-6″ at δ 67.25 with H-1‴ at δ 4.50 which permitted the characterization of compound **2** as kaempferol 3-*O*-[α-L-rhamnosyl(1→6)-*O*-β-D-glucoside] [12, 13].

Acid hydrolysis of compound **2** produced kaempferol and glucose + rhamnose, confirming the nature of the two sugars.

Compound 3. $C_{27}H_{30}O_{16}$, mp 272°C. UV (MeOH, λ_{max}, nm): 266, 352; + NaOH: 275, 325, 400; + $AlCl_3$: 273, 398; + $AlCl_3$/HCl: 274, 398; NaOAc: 274, 377; + H_3BO_3: 269, 357. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 8.10 (2H, d, J = 8.4, H-6′, 2′), 6.75 (2H, d, J = 8.4, H-5′, 3′), 6.35 (1H, d, J = 1.1, H-8), 6.15 (1H, d, J = 1.1, H-6), 5.20 (1H, d, J = 6.1, Glc H-1″), 4.50 (1H, d, J = 7.5, Glc H-1‴), 3.20–4.00 (sugar protons). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 177.5 (C-4), 164.0 (C-7), 161.2 (C-5), 160.5 (C-4′), 159.0 (C-2), 157.5 (C-9), 132.0 (C-3), 131.7 (C-2′, 6′), 122.5 (C-1′), 114.2 (C-3′, 5′), 105.0 (C-10), 99.0 (C-6), 93.5 (C-8), 101.5 (C-1″), 77.15 (C-3″), 77.15 (C-5″), 72.5 (C-2″), 72.23 (C-4″), 63.0 (C-6″), 106.90 (C-1‴), 77.2 (C-3‴), 77.1 (C-5‴), 74.4 (C-2‴), 67.0 (C-4‴), 60.6 (C-6‴). An HMBC experiment established a correlation between C-6″ at δ 63.0 with H-1‴ at δ 7.50, which permitted the characterization of compound **3** as kaempferol 3-*O*-[β-D-glucosyl(1→6)-*O*-β-D-glucoside] [14].

Acid hydrolysis of compound **3** produced kaempferol and glucose, confirming the nature of the sugar.

The three flavonol glycosides are reported for the first time from the species *C. supinus.* Compound **1** has been reported from the single species *C. arvensis* [15], while compounds **2** and **3** are new for the genus *Convolvulus.*

The butanolic extract of *Convolvulus supinus* Coss. & Kralik exhibited good antioxidant activity (IC_{50} = 3.3 ± 0.2 μg/mL) compared with the reference (rutin IC_{50} = 3.01 ± 0.2 μg/mL).

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