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FLAVONOIDS FROM *Chrysanthemum fuscatum*

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The genus *Chrysanthemum* (Asteraceae) is represented by 6 species in Algeria [1, 2]. As part of a systematic examination of these species, we investigated *C. fuscatum* Desf., which is endemic to the Algerian and Tunisian Sahara and has not been investigated previously. All parts of this plant are traditionally used as a blood purifier, a nutrient species, and to cure fever. This work concerned the phytochemical study of the *n*-BuOH soluble part of the aqueous–methanolic extract of the aerial parts.

Chrysanthemum fuscatum Desf., growing abundantly in Sahara [1, 2], was collected from the Dayas of Touggourt region in Southeast of Algeria in 2001 and authenticated by Pr. N. Khalfallah (Department of Nature and Life Sciences, Mentouri University, Constantine, Algeria). A voucher specimen was deposited in the Herbarium of the Department of Nature and Life Sciences, Mentouri University, Constantine under the code: CCF 05/01/01.

The dried aerial parts of *C. fuscatum* (4430 g) were macerated at room temperature with MeOH–H_2O (80:20 v/v). The MeOH extract was concentrated to dryness; the residue was dissolved in boiling water (400 mL for each kg of plant material). After filtration, the concentrate was taken up successively with EtOAc and *n*-BuOH. The organic layers were dried with Na_2SO_4 giving, after removal of solvents under reduced pressure, EtOAc (8.34 g) and *n*-BuOH (126 g) extracts, respectively.

Investigation of *n*-BuOH extract by two-dimensional paper chromatography using 15% AcOH and BAW (*n*-BuOH–AcOH–H_2O, 4:1:5 upper phase) as solvents and two-dimensionnal TLC chromatography on polyamid DC-6.6 plates using toluene–MeOH–methylethylketone (4:3:3) and water–EtOH–*n*-BuOH–AcOH (60:20:25:2) as systems of solvents showed that this extract contained several compounds representing flavonoids.

The *n*-BuOH extract was applied to a polyamid SC6 column eluted with a gradient of toluene–MeOH to yield 14 fractions from which two major fractions (F_8 and F_{11}) were obtained. These two fractions gave, after separation by preparative TLC, compounds **1**, **2**, **3**, **4**, and **5**. Purification of each compound for spectral analysis was carried out using MeOH over Sephadex LH-20 column.

The structures of the compounds were elucidated by UV, ^{1}H NMR, ^{13}C NMR, HMBC, and MS analysis. All these data were in good agreement with the literature [3–5].

Compound 1: $C_{16}H_{12}O_6$, mp 291°C; UV (λ_{max}, MeOH, nm): 271, 336; +NaOH: 279, 331, 399; +$AlCl_3$: 277, 302, 350, 387; +$AlCl_3$/HCl: 279, 301, 347, 382; +NaOAc: 278, 390; +NaOAc/H_3BO_3:276, 362.

^{1}H NMR (250 MHz, CD_3OD, δ, ppm, J/Hz): 7.85 (2H, d, J = 8.8, H-2′, H-6′), 6.93 (2H, d, J = 8.8, H-3′, H-5′), 6.65 (1H, s, H-3), 6.55 (1H, s, H-6), 3.68 (3H, s, 8-OCH_3).

This compound was identified as 5,7,4′-trihydroxy-8-methoxyflavone (isooscutellarein 8-Me-ether).

Compound 2: $C_{16}H_{12}O_6$, mp 285–290°C; UV (λ_{max}, MeOH, nm): 267, 345; +NaOH: 269, 401; +$AlCl_3$: 273, 423; +$AlCl_3$/HCl: 274, 390; +NaOAc: 267, 396; +NaOAc/H_3BO_3: 261, 374.

^{1}H NMR (CD_3OD, 250 MHz, δ, ppm, J/Hz): 7.45 (1H, dd, J = 8.6, J = 2.0, H-6′), 7.43 (1H, d, J = 2.0, H-2′), 6.93 (1H, d, J = 8.6, H-5′), 6.83 (1H, br.s, H-8), 6.63 (1H, s, H-3), 6.53 (1H, br.s, H-6), 3.68 (3H, s, 7-OCH_3). This compound was characterized as 5,3′,4′-trihydroxy-7-methoxyflavone (luteolin 7-Me-ether).

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Compound 3: $C_{21}H_{20}O_{11}$, mp. 240°C; UV (λ_{max}, MeOH, 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 **3** produced luteolin and glucose. This compound was characterized as 5,3′,4′-trihydroxy-7-*O*-glucosylflavone (luteolin 7-glucoside).

Compound 4: $C_{21}H_{20}O_{10}$, mp 246–248°C; UV (λ_{max}, MeOH, nm): 272, 328; +NaOH: 279, 330, 399; +$AlCl_3$: 279, 398; +$AlCl_3$/HCl: 279, 384, 398; +NaOAc: 278, 390; +NaOAc/H_3BO_3: 279, 398.

Mass spectrum (EI, 70 eV), *m/z* (I_{rel} %): 283 [M-149 (from sugar)]$^+$ (9.30), 270 [apigenin]$^+$ (100), 121 $(C_7H_5O_2)^+$ (47.26), 118 $(C_8H_6O)^+$ (13.01).

^{1}H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 7.83 (2H, d, J = 8.8, H-2′, H-6′), 6.91 (2H, d, J = 8.8, H-3′, H-5′), 6.59 (1H, s, H-3), 6. 51 (1H, s, H-6), 4.85 (1H, d, J = 9.0, H-1″), 4.14 (1H, t, J = 9.2, H-2″), 3.85 (1H, dd, J = 12.2, J = 2.8, H-6″a), 3.72 (1H, dd, J = 12.2, J = 5.3, H-6″b), 3.38–3.47 (3H, m, H-3″, H-4″, H-5″).

^{13}C NMR (125 MHz, CD_3OD): 182.49 (C-4), 164.78 (C-2), 163.41 (C-7), 161.28 (C-4′), 160.53 (C-9), 157.25 (C-5), 127.98 (C-2′, C-6′), 121.64 (C-1′), 115.57 (C-3′, C-5′), 107.70 (C-8), 103.60 (C-10), 102.38 (C-3), 93.75 (C-6), sugar carbons: 81.1 (C-5″), 78.61 (C-3″), 73.75 (C-1″), 71.04 (C-2″), 70.24 (C-4″), 61.32 (CH_2OH). All these data were confirmed by 2D NMR experiments.

This compound was identified as apigenin 8-*C*-glucoside (vitexin).

Compound 5: $C_{22}H_{22}O_{12}$, mp. 268°C; UV (λ_{max}, MeOH, nm): 268, 351; +NaOH: 274, 319, 410; +$AlCl_3$: 269, 299, 405; +$AlCl_3$/HCl: 269, 299, 403; +NaOAc: 274, 309, 387; +NaOAc/H_3BO_3: 270, 312, 366.

^{1}H NMR (250 MHz, CD_3OD, δ, ppm, J/Hz): 7.96 (1H, d, J = 2.1, H-2′), 7.61 (1H, dd, J = 8.4, J = 2.1, H-6′), 6.94 (1H, d, J = 8.4, H-5′), 6.42 (1H, d, J = 2.1, H-8), 6.23 (1H, d, J = 2.1, H-6), 5.43 (1H, d, J = 8.3, H-1″), 3.97 (3H, s, 3′-OCH_3), 3.38–3.95 (sugar protons). Acid hydrolysis of compound **5** produced 3′-methylquercetin and glucose. This compound was characterized as 5,7,4′-trihydroxy-3′-methoxy-3-*O*-glucosylflavone (cacticin).

All these compounds were isolated for the first time from *Chrysanthemum fuscatum*.

The *n*-butanol extract of *C. fuscatum* was evaluated for hepatoprotective activity in rats with acute hepatitis induced by isoniazid and rifampicin. The extract in an oral dose (200 mg/kg body weight) exhibited a significant protective effect by lowering lipid peroxidation and by enhancing the glutathione system.

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