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FLAVONOIDS, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF *Eryngium triquetrum*

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The genus *Eryngium* belongs to the Apiaceae family (Umbelliferae), subfamily Saniculoideae, which consists of about 300 species [1]. Plants of this genus have shown various activities such as anti-inflammatory [2], antimalaria and antifungal [3], antioxidant and antimicrobial [4], antidiabetic [5], and analgesic properties [6].

Air-dried and powdered aerial parts (1.5 kg) of *Eryngium triquetrum* Vahl. were macerated at room temperature with MeOH–H_2O (70:30, v/v) for 24 h three times. After filtration and concentration, the residue was dissolved in water (600 mL). The resulting solution was extracted successively with $CHCl_3$, EtOAc, and *n*-butanol. Concentration in *vacuo* led to the following extracts: $CHCl_3$ (0.8 g), EtOAc (1.3 g), and *n*-butanol (11 g).

The butanolic extract of *Eryngium triquetrum* (11 g) was column chromatographed on polyamide SC6 with a gradient of toluene–MeOH with increasing polarity; Fr. 4 (70 mg), obtained from 85% toluene, was subjected to silica gel column chromatography eluted with EtOAc–MeOH–H_2O (10:1:0.5), leading to two subfractions: Fr. 1 and Fr. 2. Subfraction 1 was separated by silica gel column chromatography eluted with CH_2Cl_2–MeOH (8:2) to afford compound **1** (10 mg). Subfraction 2 yielded compound **2** (60 mg), which was obtained as a yellow precipitate. Fraction 6 (70 mg), obtained from 80% toluene, was further subjected to silica gel column chromatography eluted with EtOAc–MeOH–H_2O (10:1:0.5) to give compound **3** (40 mg).

The ethyl acetate extract (1.3 g) was column chromatographed on silica gel with a gradient of $CHCl_3$–MeOH with increasing polarity. Fraction 2 (30 mg) and Fr. 3 (35 mg), obtained from 95% and 90% $CHCl_3$ successively, were separated by silica gel column chromatography eluted with EtOAc–MeOH–H_2O (10:1:0.5) to afford compounds **4** (9 mg) and **5** (8.5 mg). The structures of compounds **1–5** were established by chemical and spectral analysis, UV, NMR, acid hydrolysis, and HPLC-UV-DAD-MS, as well as by comparing their spectroscopic data with those reported in the literature.

Acid Hydrolysis. The pure compounds were treated with 2M HCl at 100°C for 1 h. The hydrolysates were extracted with EtOAc, and the aglycons 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), revealed with aniline malonate.

Compound 1. $C_{21}H_{20}O_{11}$. UV (MeOH, λ_{max}, nm): 267, 347; +NaOH: 275, 325, 401; +$AlCl_3$: 273, 398; +$AlCl_3$/HCl: 273, 397; +NaOAc: 274, 377; +H_3BO_3: 268, 355. ^{1}H NMR (250 MHz, CD_3OD, δ, ppm, J/Hz): 8.09 (2H, d, J = 8.9, H-6′, 2′), 6.91 (2H, d, J = 8.9, H-5′, 3′), 6.42 (1H, d, J = 1.9, H-8), 6.22 (1H, d, J = 1.9, H-6), 5.28 (1H, d, J = 7.3, Glc H-1″), 3.26–4.14 (sugar protons). This compound was characterized as kaempferol 3-*O*-*β*-D-glucoside [7].

Compound 2. $C_{30}H_{26}O_{13}$. UV (MeOH, λ_{max}, nm): 267, 315; +NaOH: 274, 368; +$AlCl_3$: 274, 403; +$AlCl_3$/HCl: 273, 403; +NaOAc: 274, 372; +H_3BO_3: 272, 371. ^{1}H NMR (400 MHz, CD_3OD, δ, ppm, J/Hz): 7.97 (2H, d, J = 8.9, H-6′, 2′), 7.39 (1H, d, J = 15.9, H-7‴), 7.28 (2H, d, J = 8.6, H-2‴, 6‴), 6.79 (2H, d, J = 8.9, H-5′, 3′), 6.77 (2H, d, J = 8.6, H-3‴, 5‴), 6.27 (1H, d, J = 1.7, H-8), 6.11 (1H, d, J = 1.7, H-6), 5.24 (1H, d, J = 7.2, Glc H-1″), 4.32 (1H, d, J = 11.5, H-6″a), 4.19 (1H, dd, J = 12.5, 5.6, H-6″b), 3.20–4.40 (sugar protons). In addition, HPLC-UV-DAD permitted the characterization of the compound as kaempferol 3-*O*-[6″-*O*-*E*-*p*-coumaroyl]-*β*-D-glucopyranoside [8].

Compound 3. $C_{27}H_{30}O_{16}$. UV (MeOH, λ_{max}, nm): 269, 314; +NaOH: 276, 321, 365; +$AlCl_3$: 276, 399; +$AlCl_3$/HCl: 277, 399; +NaOAc: 275, 374; +H_3BO_3: 275, 372. ^{1}H NMR (400 MHz, CD_3OD, δ, ppm, J/Hz): 7.95 (2H, d, J = 8.9, H-6′, 2′),

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7.72 (1H, d, J = 15.9, H-7‴), 7.48 (2H, d, J = 8.6, H-2‴, 6‴), 7.40 (2H, d, J = 15.9, H-7⁗), 7.30 (2H, d, J = 8.6, H-2⁗, 6⁗), 6.86 (2H, d, J = 8.9, H-5′, 3′), 6.81 (2H, d, J = 8.6, H-5‴, 3‴), 6.80 (2H, d, J = 8.6, H-5⁗, 3⁗), 6.45 (1H, d, J = 15.9, H-8⁗), 6.25 (1H, d, J = 2.0, H-8), 6.07 (1H, d, J = 15.9, H-8‴), 6.05 (1H, d, J = 2.0, H-6), 5.65 (1H, d, J = 8.2, Glc H-1″), 4.37 (1H, dd, J = 11.9, 1.9, H-6″a), 4.22 (1H, dd, J = 11.9, 6.6, H-6″b), 3.40–5.20 (sugar protons). In addition, HPLC-UV-DAD permitted the characterization of compound **3** as kaempferol 3-*O*-[2″,6″-di-*O*-*E*-*p*-coumaroyl]-*β*-D-glucoside [7, 9, 10].

Compound 4. $C_{27}H_{30}O_{15}$. UV (MeOH, λ_{max}, nm): 267, 352; +NaOH: 275, 325, 403; +$AlCl_3$: 274, 398; +$AlCl_3$/HCl: 275, 396; +NaOAc: 274, 376; +H_3BO_3: 269, 357. ^{1}H NMR (250 MHz, CD_3OD, δ, ppm, J/Hz): 8.09 (2H, d, J = 9.0, H-6′, 2′), 6.92 (2H, d, J = 9.0, H-5′, 3′), 6.44 (1H, d, J = 2.1, H-8), 6.24 (1H, d, J = 2.1, H-6), 5.16 (1H, d, J = 7.7, Glc H-1″), 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). In addition, HPLC-UV-DAD permitted the characterization of compound **4** as kaempferol 3-*O*-[*α*-L-rhamnosyl-(6→1)-*O*-*β*-D-glucoside [11].

Acid hydrolysis of compound **4** produced kaempferol and glucose + rhamnose, confirming the nature of the two sugars.

Compound 5. $C_{27}H_{30}O_{16}$. UV (MeOH, λ_{max}, nm): 257, 358; +NaOH: 274, 325, 409; +$AlCl_3$: 274, 432; +$AlCl_3$/HCl: 275, 425; +NaOAc: 273, 388; +H_3BO_3: 264, 381. ^{1}H NMR (250 MHz, CD_3OD, δ, ppm, J/Hz): 7.55 (1H, dd, J = 8.2, 2.1, H-6′), 7.53 (1H, d, J = 2.1, H-2′), 6.83 (1H, d, J = 8.2, H-5′), 6.36 (1H, d, J = 2.0, H-8), 6.17 (1H, d, J = 2.0, H-6), 5.33 (1H, d, J = 7.3, Glc H-1″), 4.38 (1H, sl, Rha H-1‴), 0.99 (3H, d, J = 6.2, Rha CH_3), 3.00–3.90 (sugar protons). In addition, HPLC-UV-DAD permitted the characterization of compound **5** as quercetin 3-*O*-[*α*-*L*-rhamnosyl-(6→1)-*O*-*β*-D-glucoside] [7, 9].

Acid hydrolysis of compound **5** produced quercetin and glucose + rhamnose, confirming the nature of the two sugars.

Phenols Quantification. Total phenolics was quantified according to the Folin–Ciocalteu method using pyrogallol as a standard [12].

Antioxidant Activity. The free radical scavenging activity of the *n*-butanolic extract of *Eryngium triquetrum* Vahl. (BEET) was measured by a slightly modified method of Hatano [13, 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.

Antibacterial Activity. Susceptibility of the bacterial strains to the chloroform extract of *Eryngium triquetrum* Vahl. (CEET) was investigated using the disk diffusion method and by comparing their antibiogram inhibition zones to those reported by the National Committee for Clinical Laboratory Standards (NCCLS) [15]. A range of microorganisms, namely *Escherichia coli* ATCC 25922, *Escherichia coli*, *Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus*, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Morganella morganii* were used. The reference strains were obtained from the Pasteur Institute (Algiers). The other strains were obtained from the laboratory of bacteriology, Benbadis Hospital, Constantine, using conventional methods (clinical isolation).

The five flavonol glycosides were reported for the first time from the species *E. triquetrum* Vahl., and two were isolated for the first time from the genus, which are kaempferol-3-*O*-*β*-(6″-*O*-*E*-*p*-coumaroyl)-*β*-D-glucopyranoside and kaempferol-3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside.

A significant phenolic content (>16 g/100 g of dry extract) and good radical scavenging activity were found for the BEET (IC_{50} 136 μg/mL), compared with the reference (quercetin IC_{50} 12 μg/mL).

The CEET inhibited the growth of the tested miroorganisms. The best antibacterial activity was observed against *Staphylococcus aureus*, *Staphylococcus aureus* ATCC 43300, *Escherichia coli* ATCC 25922, *Escherichia coli*, and *Morganella morganii* with 30, 24, 22, 20, and 20 mm inhibition zone diameters, respectively, with 80 μg/mL MIC value.

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