PHYTOCHEMICAL STUDY OF *Retama sphaerocarpa*

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Retama sphaerocarpa Boissier is a species of the Fabaceae family [1]. This plant is used to cure rabies in the medicinal folk traditions of the east of Algeria. In the present paper we report the result obtained on the qualitative and quantitative analysis of secondary metabolites extracted from the medicinal plant *R. sphaerocarpa*, which has been the subject of several chemical and pharmacological investigations [2–6]. The aerial parts of this plant were collected during flowering in the Souk Naamane Region east of Algeria in May, June 2002.

The air-dried powdered aerial parts (950 g) of *R. sphaerocarpa* were extracted with 70% MeOH. The MeOH extract was evaporated to dryness. The residue was dissolved in boiling water and extracted with ethyl acetate and *n*-BuOH successively. The solvents was evaporated and the residue of the ethyl acetate and *n*-BuOH extracts was dissolved in small volumes of MeOH. Two-dimensional paper chromatography using 15% AcOH and BAW (*n*-BuOH–AcOH–H_2O, 4:1:5 upper phase) as solvents shows that the acetate and *n*-BuOH extract contains almost the same compounds representing flavonoids. The *n*-BuOH extract was applied to a column of polyamide MN SC6 and eluted with a gradient of toluene–MeOH with increasing polarity. Three flavonoids (**1–3**) contained in several fractions were isolated by preparative PC on Whatman 3MM paper using 15% AcOH, then by preparative TLC on polyamid DC6 eluted with H_2O–MeOH–methylethylketone-acetylacetone, 13:3:3:1.

The isoflavonoid glycoside (genistein 7-O-β-glucoside) was isolated from the intermediate fraction by crystallization. This compound has already been isolated from this plant [2]. Purification of each compound for spectral analysis was carried out using MeOH over Sephadex LH-20. These compounds were identified using UV and ^{1}H NMR spectra, chemical transformations, and comparison with authentic samples [7–9]. Flavonoids **1–2** are isolated for the first time from this genus. Compound **3** has been reported previously from another species of *Retama* [10] and from *R. sphaerocarpa* for the first time.

Compound (1), $C_{21}H_{20}O_{10}$, mp. 246–248°C; UV (λ_{max}, MeOH, nm): 332, 269; NaOH: 279, 326, 393; $AlCl_3$: 275, 303, 345, 381; $AlCl_3$/HCl: 276, 302, 343, 381.

^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 8.01 (2H, d, J = 8.3, H-2′, H-6′), 6.89 (2H, d, J = 8.3, H-3′, H-5′), 6.76 (1H, s, H-3), 6.25 (1H, s, H-6), 4.67 (1H, d, J = 9.8, H-1″); 3.05–4.0 (sugar protons). Characterized as apigenin 8-C-glucoside (vitexin).

Compound (2), $C_{27}H_{30}O_{17}$, mp. 196–198°C, UV (λ_{max}, MeOH, nm): 268, 347; NaOH: 275, 403; $AlCl_3$: 272, 327, 426; $AlCl_3$/HCl: 274, 296, 355, 385; NaOAc: 269, 350, 407.

^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.52 (1H, dd, J = 8.4–1.8, H-6′), 7.48 (1H, s, H-2′), 6.85 (1H, d, J = 8.4, H-5′), 6.82 (1H, s, H-8), 6.64 (3H, s, H-6), 4.82 (1H, d, J = 10.12, H-1″), 4.66 (1H, d, J = 9.9, H-1‴), 3.05–3.90 (sugar protons). Identified as quercetin 3,7-di-O-β-glucoside.

Acid hydrolysis of **2** produced quercetin and D-glucose.

Compound (3), $C_{27}H_{30}O_{15}$, mp. 250°C (decomp), UV (λ_{max}, MeOH, nm): 272, 330; NaOH: 282, 332, 396; $AlCl_3$: 277, 304, 348, 385; $AlCl_3$/HCl: 277, 302, 347, 385; NaOAc: 282, 376.

The ^{1}H NMR (250 MHz, Py-d_5, δ, J/Hz): proton signals at 8.26 (2H, d, J = 8.6, H-2′, H-6′); 7.31 (2H, d, J = 8.5, H-3′, H-5′); 6.61 (1H, s, H-3); 5.73 (1H, d, J = 10.0, H-1″), 5.84 (1H, d, J = 9.9, H-1‴), 3.05–3.90 (sugar protons). Identified as apigenin 6,8-di-C-glucoside (vicenin-2).

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