Chemistry of Natural Compounds, Vol. 43, No. 5, 2007

FLAVONOIDS OF *Retama sphaerocarpa* LEAVES AND THEIR ANTIMICROBIAL ACTIVITIES

S. Louaar,[1] S. Akkal,[1] H. Laouer,[2] and D. Guilet[3]

UDC 547.972

Retama sphaerocarpa (Fabaceae), known in Algeria as "Retam", was collected during flowering in the Souk Naamane Region east of Algeria in June 2002, and the species was identified by Dr. H. Laouer from the Department of Biology (University of Setif, Algeria) on the basis of Quezel and Santa (1963) [1]. A voucher specimen (P00383267) has been deposited in the Herbarium of Museum National d'Histoire Naturelle, Paris, France

This research is a contribution to the chemical study of some plants growing in Algeria, with the objective of discovering natural products with potential biological activity. As part of our continuing study of *Retama sphaerocarpa* [2] we have undertaken a reexamination of the chemical constituents of the aerial parts and roots of *R. sphaerocarpa*. We report here the isolation and identification of two flavonoids; these compounds are new for this genus. The leaves of *Retama sphaerocarpa* were investigated for antimicrobial properties. All fractions showed activity against *Staphylococcus aureus*.

Air-dried powder from the aerial parts (950 g) of *R. sphaerocarpa* was extracted three time with boiling 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. Analysis by two-dimensional paper chromatography using 15% AcOH and BAW (*n*-BuOH–AcOH–H_2O 4:1:5 upper phase) as solvents revealed identical separation of the flavonoids in the acetate and *n*-BuOH extracts. The *n*-BuOH extract was applied to a column of polyamide MN SC6 and eluted with a gradient of toluene–MeOH with increasing polarity. Two flavonoids (**1**, **2**), contained in several fractions, were isolated by preparative PC on whatman 3MM paper using AcOH 15%, then by preparative TLC on polyamide DC6 eluted with (H_2O–MeOH–methylethylacetone:acetylacetone) 13:3:3:1.

Purification of each compound for spectral analysis was carried out using MeOH over Sephadex LH-20. The chemical structures of the compounds were determined using UV, 1D, and 2D NMR spectrometry.

The structures of the pure compounds were improved using UV, ^{1}H NMR, and ^{13}C NMR methods. Multiple-pulse 2D NMR experiments (^{1}H–^{1}H COSY, ^{13}C–^{1}H HETCOR, and ^{13}C–^{1}H COLOC) were used for the structure elucidation of compound **2**.

Compound 1, $C_{17}H_{12}O_6$, mp 298–302°C. ^{1}H NMR (DMSO-d_6, δ, ppm, J/Hz): 8.13 (s, H-2), 7.92 (d, J = 8.7, H-5), 6.93 (dd, J = 8.7, 2.2, H-6), 6.87 (s, H-2′), 6.86 (d, J = 2.2, H-8), 6.81 (s, H-5), 6.01 (s, OCH_2O). Identified as 7-hydroxy-6′-mehoxy-3′,4′-methylenedioxyisoflavone(6′-methoxypseudobaptegenin) [3].

Compound 2, $C_{21}H_{20}O_{10}$, mp 194°C (dec.). UV (MeOH, λ_{max}, nm): 262, 331 sh; (MeOH+NaOAc): 273, 325 sh; (MeOH+$AlCl_3$): 272, 311 sh; (MeOH+AlCl+HCl): 275, 311 sh; ^{1}H NMR (CD_3OD, δ, ppm, J/Hz): 8.13 (1H, s, H-2), 7.37 (2H, d, J = 9, H-6′, H-2′), 6.84 (2H, d, J = 9, H-5′, H-3′), 6.29 (H, s, H-6), 4.92 (1H, d, J = 9.8, H-1″glu); 3.3–4.7 (sugar protons). Identified as 5,7,4′-trihydroxyisoflavone-8-*C*-glucoside (Genistein 8-*C*-glucoside) [4, 5].

Thus, to our knowledge compounds **1** and **2** are new for this genus.

Antibacterial Assay. Antibacterial activity was assessed using the disk diffusion method [6, 7]. The *n*-BuOH crude extract was the most active against *Staphylococcus aureus* ATCC 43300. Table 1 presents the results.

1) Departement de Chimie, Faculte de Sciences, Universite Mentouri Constantine, Route d'Ain el Bey, 25000 Constantine, Algerie, Fax: 213 31 62 49 12, e-mail: salah4dz@yahoo.fr; 2) Department of Biology, University Ferhat Abbas, Setif, Algeria; 3) Departement de Botanique Homeopathie, Pharmacognosie et Biologie Cellulaire, Universite Claude Bernard Lyon I. Published in Khimiya Prirodnykh Soedinenii, No. 5, pp. 508-509, September-October, 2007. Original article submitted May 29, 2006.

 0009-3130/07/4305-0616

TABLE 1. Antibacterial Activity of the Aerial Parts of *Retama sphaerocarpa*

Strains of bacteria	Extracts	Inhibition zone, mm							
		1/5v/v			1/10v/v			ethanol	gentamycin
		R1	R2	R3	R1	R2	R3		
Staphylococcus aureus									
ATCC 43300	A	12S	11S	12S	8S	7S	8S	-	16
	B	20C	20C	20C	15C	15C	15C	-	16
ATCC 29213	A	11S	11S	13S	8S	7S	8S	-	35
	B	11S	10S	11S	9S	9S	8S	-	35

_____

A: AcOEt extract; B: *n*-BuOH extract; R1: repetition n = 1, R2: repetition n = 2, R3: repetition n = 3.

REFERENCES

1. P. Quezel and S. Santa, *Nouvelle flore de l'Algerie et des regions desertiques et meridionales*, Tome II, Edition CNRS, Paris, 1963.
2. S. Louaar, S. Akkal, A. Bousetla, K. Medjroubi, L. Djarri, and E. Seguin, *Chem. Nat. Comp.*, **41**, 107 (2005).
3. E. Venkata Rao, M. Sree Rama Murthy, and R. S. Ward, *Phytochemistry*, **23**, 1493 (1984).
4. A. P. Rauter, A. Martins, C. Borges, J. Ferreira, J. Justino, M. R. Bronze, Ana V. Coelho, Young H. Choi, and R. Verpoorte, *J. Chromatogr. A*, **1089**, 59 (2005).
5. R. Mekkiou, H. Touahar, M. G. Dijoux-franca, A. M. Mariotte, S. Benayache, and F. Benayache, *Biochem. Syst. Ecol.*, **33**, 635 (2005).
6. A. W. Bauer, W. M. M. Kirby, J. C. Sherries, and P. Truck, *Am. J. Clin. Pathol*, **45**, 493 (1966)
7. NCCLS, Performance standards for antimicrobial disk susceptibilities tests, Villanova, PA, USA, Approved Standard NCCLS Publication, 1993.