Chemistry of Natural Compounds, Vol. 46, No. 3, 2010

SECONDARY METABOLITES OF AN ALGERIAN *Phlomis bovei* AND THEIR ANTIOXIDANT ACTIVITIES

N. Zaabat,[1,3] S. Akkal,[1] N. Darboure,[3] H. Laouer,[2] M. G. Dijoux Franca,[3] and Helmut Duddeck[4]

UDC 547.972

The plants of the genus *Phlomis* are native to Turkey, North Africa, Europe, and Asia. *Phlomis bovei* De Noe, sy*n. Phlomis samia* Desfontaines (Lamiaceae), is a rare Algerian endemic plant, commonly known as Kayat El Adjarah [1] in the Algerian dialect or variously named Farseouan, Tarseouan, Iniji, R′ilef, and Azaref throughout the North of Africa [2]. It is one among the nine endemic plants recorded in the Rapport National sur la Diversite Biologique [1]. *P. bovei* is a herbaceous perennial plant, which grows up to 0.8 m and often develops a stout woody base.

Phlomis bovei was collected from the east of Algeria in june 2006 and was taxonomically identified by Dr. Hocine Laouer from the Department of Biology, University of Setif.

About 465 g of the air powdered leaves were extracted with *n*-hexane, CH_2Cl_2, and MeOH in succession at room temperature. The MeOH extract was fractionated over silica gel–VLC eluting with CH_2Cl_2 followed by increasing concentrations of MeOH. Fractions 3 and 4 (41 g), eluted with CH_2Cl_2–MeOH 50:50–25:75), were combined and further applied to column chromatography over silica gel using CH_2Cl_2–EtOAc and EtOAc–MeOH. Fraction L (1.73 g), eluted with 95:5 EtOAc–MeOH, was subjected to RP18 MPLC with H_2O–MeOH. Purification of fraction L6 (18 mg) by Sephadex LH20 (MeOH) allowed the isolation of compound **1.** Purification of fraction L17 (112 mg) by SPE RP18 MeOH–H_2O and TLC on silica gel (9–1) CH_2Cl_2–MeOH afforded compound **2**. Fraction L22 (62 mg) was subjected to SiO_2 TLC using (EtOAc–MeOH–H_2O 100:10:5), yielding **3** (9 mg).

The structures of these compounds were elucidated by UV, ^{1}H NMR, and ^{13}C NMR, and all these data were in good agreement with the respective literature data [3–8].

Compound 1, $C_{15}H_{10}O_5$, mp 347°C, UV (MeOH, λ_{max}, nm): 266, 330; +NaOH: 275, 324, 390; +$AlCl_3$: 277, 301, 341, 387; +$AlCl_3$/HCl: 277, 301, 341, 387; +NaOAc: 278, 300, 380; +NaOAc/H_3BO_3: 273, 279, 350. ^{1}H NMR (400 MHz, CD_3OD, δ, ppm, J/Hz): 7.78 (2H, d, J = 8.9, H-2′, H-6′), 6.90 (2H, d, J = 8.9, H-3′, H-5′), 6.51 (1H, s, H-3), 6.42 (1H, d, J = 2.1, H-8), 6.22 (1H, d, J = 2.1, H-6). This compound was characterized as 5,7,4′-trihydroxyflavone (apigenin) [6].

Compound 2, $C_{21}H_{20}O_{10}$, mp 226–228°C, MS–ES positive 433**,** UV (MeOH, λ_{max}, nm): 269, 334; + NaOH: 267, 381; + $AlCl_3$: 277, 349 sh, 383; + $AlCl_3$/HCl: 279, 346 sh, 382. ^{1}H NMR (400 MHz, CD_3OD, δ, ppm, J/Hz): 7.91 (2H, d, J = 8.8, H-2′, H-6′); 6.95 (2H, d, J = 8.8, H-3′, H-5′); 6.83 (1H, d, J = 2.5, H-8); 6.51 (1H, s, H-3), 6.54 (1H, d, J = 2.55, H-6); 5.20 (1H, d, J = 7.5, H-1″ Glu); 3.05–3.90 (sugar protons). Identified as apigenin 7-*O*-glucoside or apigetrin [7].

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

Compound 3, $C_{10}H_{10}O_2$, mp 110°C, ^{1}H NMR (400 MHz, CD_3OD, δ, ppm, J/Hz): 7.66 (2H, d, J = 8.85, H-2′, H-6′); 6.73 (2H, d, J = 7.56, H-3′, H-5′); 6.85 (1H, d, J = 12.6, H-4); 5.72 (1H, d, J = 12.6, H-3), ^{13}C NMR (CD_3OD, 75 MHz): 112.6 (C-3), 116.7 (C-3′,5′), 126.6 (C-1′), 133.9 (C-2′,6′), 145.6 (C-4), 170 (C-2). Identified as 4-(4′-hydroxyphenyl)-*trans*-but-3-en-2-one [8].

1) Laboratoire de Phytochimie et Analyses physicochimiques et Biologiques, Departement de Chimie, Faculte de Sciences exactes, 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, 19000 Setif, Algeria; 3) Universite de Lyon, UMR 5557 CNRS-UCBL, CESN, Dept de Botanique et Pharmacognosie, Faculte de Pharmacie, 8 avenue Rockefeller, 69373 Lyon Cedex 08, France; 4) Universitat Hannover, Institut fur Organische Chemie, Schneiderberg 1B, D-30167, Hannover, Germany. Published in Khimiya Prirodnykh Soedinenii, No. 3, p. 384, May–June, 2010. Original article submitted November 14, 2008.

 0009-3130/10/4603-0454

Compounds **1** and **2** are isolated for the first time from *P. bovei*. Compound **3** was isolated for the first time from a *Phlomis* species.

Free Radical Scavenging Activity. The CH_2Cl_2 and MeOH–H_2O extracts of the flowers of *P. bovei* were examined for *in vitro* antioxidant properties using the DPPH test.

The radical-scavenging activity was measured by the molar ratio of antioxidant to DPPH radical required for 50% reduction in DPPH radical concentration in 45 min. The results of our experiments demonstrated that the two compound-extracts tested possess radical-scavenging activity. It was also found that the free-radical-scavenging activity of MeOH–H_2O extract (EC_{50} 0.30) was stronger than that of CH_2Cl_2 extract (EC_{50} 0.50).

REFERENCES

1. P. Quezel and S. Santa, *Nouvelle Flore de l′Algerie et des Regions Desertiques Meridionales*, C.N.R.S, Paris, 1962, p. 805.
2. L. Trabut, in *Repertoire des Noms Indigenes des plantes spontanees, cultivees et utilisees dans le Nord de l′Afrique*. Collection du centenaire de l′Algerie, Imprimeries "La Typo-Litho" et Jules Carbonnel Reunies: Alger, 1935, p. 190.
3. T. J. Mabry, K. R. Markham, and M. B. Thomas, *The Systematic Identification of Flavonoids*, Springer, New York, 1970.
4. K. R. Markham, *Techniques of Flavonoid Identification*, Academic Press, London, 1982.
5. K. R. Markham and H. Geiger, *Advance in Research Since 1986*, J. B. Harborne ed., Chapman and Hall, London, 1993.
6. T. H. Simpson and L. Garden, *J. Chem. Soc.*, **46**, 38 (1952).
7. T. Wada and T. Hahori, *Nat. Resour.*, **32**, 67 (1953).
8. Das, Biswanath, G. Anjani, A. Kashinatham, B. Venkataiah, Rao, and S. Padma, *Nat. Prod. Sci.,* **4** (2), 78 (1998).