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ORIGINAL ARTICLE

Constituents from *Bupleurum montanum* (Coss. & Dur.) (Apiaceae)

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KEYWORDS

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Abstract A chemical investigation of the aerial parts of *Bupleurum montanum* (Coss. & Dur.) (Apiaceae) afforded five compounds, quercitin **1**, tamarexetin **2**, isorhamnetin-3-rutinoside **3**, kaempferol-3-*O*-β-rutinoside **4**, and 3,4-dihydroxybenzoic acid (Protocatechuic acid) **5**. The structural elucidation was performed mainly by MS, 1D and 2D NMR spectrum data.

1. Introduction

The genus *Bupleurum*, family Apiaceae, is widespread in the Mediterranean. The flora of Algeria contains 14 species of *Bupleurum* with 5 endemic species (*Bupleurum plantagineum* Desf., *Bupleurum atlanticum* Murb., *Bupleurum montanum* Coss., *Bupleurum balansae* Boiss. et Reut., *Bupleurum oligactis* Boiss.) (Quezel and Santa, 1963).

In previous phytochemical studies of *B. montanum*, we have reported the study of the chemical composition and antimicrobial activity of essential oil (Laouer et al., 2009). In the present and in continuation of our studies on Algerian Apiaceous plants (Bousetla et al., 2005; Benahmed et al., 2006, 2008) we describe the isolation and structural determination of five compounds from aerial parts of *B. montanum*. Compounds **1**–**5** were identified by GC–MS analysis and (UV, MS, ^{1}H NMR, and ^{13}C NMR spectroscopy) as: quercitin **1**, tamarexetin **2**, isorhamnetin-3-rutinoside **3**, kaempferol-3-*O*-β-rutinoside **4**, and 3,4-dihydroxybenzoic acid **5** (see Fig. 1).

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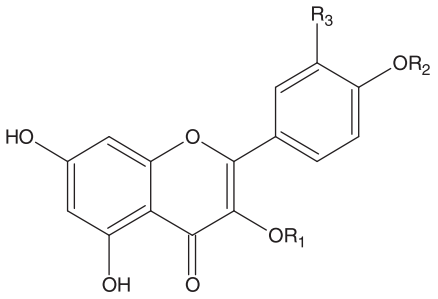
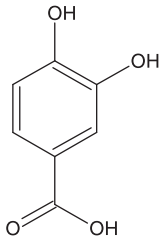


	R_1	R_2	R_3
1	H	H	OH
2	H	CH_3	OH
3	Rha(1→6) Glc	H	OCH_3
4	Rha(1→6) Glc	H	H



5

Fig. 1 Chemical structures of compounds **1**–**5**.

2. Experimental

2.1. General procedures

Ultra-violet absorption spectrum was recorded on Perkin–Elmer Lambda 2 UV spectrophotometer. NMR spectra were recorded on Bruker DMX 300 and chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as an internal reference. EI-MS data were obtained on a Hewlett Packard 5973 A quadripole mass spectrometer. Silica gel F_{254} was used for TLC. Spots were detected on TLC under UV light. Column chromatography was carried out on silica gel 60 Merck (6–35 μm, 20–45 μm, 70–200 μm).

2.2. Plant material

Aerial parts of *B. montanum* (Coss. & Dur.) (Apiaceae) were collected from Megress Mountain, Sétif, (Eastern Algeria) at 1500 m above sea level during the flowering period in Jun (2006), and identified by Pr. H. Laouer (Department of Biology, University Ferhat Abbas, Sétif, Algeria). A voucher specimen (B-6307) has been deposited in the Muséum d'Histoire naturelle de la ville de Nice, France.

2.3. Extraction and isolation

The air dried aerial parts of *B. montanum* (1000 g) were extracted three times with boiling 70% MeOH. The hydro-alcoholic solutions were concentrated under reduced pressure to dryness and the residue was dissolved in hot water and kept in cold overnight. After filtration, the aqueous solution was successively treated with ethylacetate and *n*-butanol, then the EtOAc and *n*-BuOH extracts were concentrated to dryness (Benahmed et al., 2006, Akkal et al., 2010).

The EtOAc extract (5 g) was chromatographed on a silica gel column by gradient elution with CH_2Cl_2/MeOH with increasing polarity. Four main fractions (A1–A4) were collected. Each fraction was then subjected to repeated chromatography on silica gel by column chromatography and thin layer chromatography to yield compounds **1** (8 mg), **2** (9.3 mg) and **3** (8.2 mg). The *n*-BuOH extract was subjected to a silica gel column chromatography by elution with a gradient of CH_2Cl_2/MeOH with increasing polarity to give five fractions (B1–B5). Fraction B2 (3.5 g) was further separated by silica gel column chromatography eluting with EtOAc/MeOH/H_2O (25:3:1) to obtain **4** (40 mg). Compound **5** was obtained from fractions B4 and B5 which were combined and subjected to a silica gel column chromatography then to TLC on silica gel using EtOAc/MeOH/H_2O (25:3:1) as eluant.

2.3.1. Quercitin (*1*)

^{1}H NMR (300 MHz, in ppm, DMSO-d_6): δ 7.5 (^{1}H, d, J = 2.1 Hz, H-2′), 7.3 (1H, dd, J = 8.5 Hz, J = 2.1 Hz, H-6′), 6.7 (1H, d, J = 8.5 Hz, H-5′), 6.3 (1H,d, J = 1.9 Hz, H-8), 6.1 (1H, d, J = 1.9 Hz, H-6). ^{13}C NMR (100 MHz, in ppm, DMSO-d_6): δ 176.2 (C-4), 164.2 (C-7), 161.0 (C-5), 156.5 (C-8a), 148.0 (C-4′), 147.1 (C-2), 145.4 (C-3′), 136.1 (C-3), 122.3 (C-1′), 120.3 (C-6′), 115.9 (C-5′), 115.4 (C-2′), 103.3 (C-4a), 98.5 (C-6), 93,7 (C-8). EI-MS m/z $[M]^{+\cdot}$ at 302 (100%).

2.3.2. Tamarixetin (*2*)

UV (λ_{max} in MeOH): gives bands at 371 and 254 nm for band I and II, addition of NaOH; 411, 322 and 274, $AlCl_3$; 428 and 264 while HCl; 427 and 263. ^{1}H NMR (300 MHz, in ppm, DMSO-d_6): δ 7.8 (1H, d, J = 1.54 Hz, H-2′), 7.8 (1H, dd, J = 7.5 Hz, J = 1.5 Hz, H-6′), 7.01 (1H, d, J = 7.5 Hz, H-5′), 6.54 (1H,d, J = 1.8 Hz, H-8), 6.25 (1H, d, J = 1.8 Hz, H-6), and 3.9 (3H, S, OMe). ^{13}C NMR (100 MHz, in ppm, DMSO-d_6): δ 175.8 (C-4), 163.8 (C-7), 160.6 (C-5), 156.1 (C-8a), 148.7 (C-4′), 146.6 (C-2), 146.6 (C-3′), 135.7 (C-3), 121.9 (C-1′), 121.6 (C-6′), 115.5 (C-5′), 111.5 (C-2′), 102.9 (C-4a), 98.2 (C-6), 93,6 (C-8). EI-MS m/z $[M]^{+\cdot}$ at 316 (100%).

2.3.3. Isorhamnetin-3-rutinoside (*3*)

UV (λ_{max} in MeOH): gives bands at 356 and 254 nm for band I and II, addition of NaOH; 412, 326 and 273, $AlCl_3$; 403 and 268, as like as HCl; 403 and 268. ^{1}H NMR (300 MHz, in ppm, DMSO-d_6): δ 7.86 (1H, d, J = 1.7 Hz, H-2′), 7.53 (1H, dd, J = 8.5 Hz, J = 1.7 Hz, H-6′), 6.5 (1H, d, J = 8.5 Hz, H-5′), 6.43 (1H,d, J = 1.7 Hz, H-8), 6.2 (1H, d, J = 1.7 Hz, H-6), 5.45 (1H, d, J = 7.2, H-1″), 4.41 (1H, H-1‴), 3.83 (3H, S, OMe) and 0.98 (3H, d, J = 6.0, H-6‴). ^{13}C NMR (100 MHz, in ppm, DMSO-d_6): δ 177.2 (C-4), 164.3 (C-7), 161.1 (C-5), 156.4 (C-8a), 149.3 (C-4′), 156.4 (C-2), 146.2 (C-3′), 132.9 (C-3), 120.9 (C-1′), 122.2 (C-6′), 115.2 (C-5′), 113.2