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NOTE

FLAVONOID AGLYCONES FROM *BALLOTA SAXATILIS* SUBSP. *SAXATILIS*

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ABSTRACT

From the aerial parts of Ballota saxatilis *subsp.* saxatilis, *the three flavone aglycones 6 hydroxy apigenol 7,4'-dimethyl ether (ladanein) (**1**), kaempferol 3,7,4'-trimethyl ether (**2**),and quercetin 3,7,3',4'-tetramethyl ether (retusin) (**3**), have been isolated and identified.*

INTRODUCTION

Ballota species have been known since the Dioscorides and infusions of the plant are used in Turkish folk medicine in cases of whooping cough and as an antiulcer, antispasmodic and sedative (Meriçli et al., 1988).

The genus *Ballota* (Lamiaceae) consists of about 33 species growing mainly in the Mediterranean region. In Turkey, the genus *Ballota* is represented by eleven species, and six subspecies, ten of which are endemic (Davis, 1972; Ferreres et al., 1986). *Ballota saxatilis* subsp. *saxatilis* Sieber ex J.& C. Presl. is distributed in Central Anatolia and has not been investigated before.

In this research, from the aerial parts of *Ballota saxatilis* subsp. *saxatilis*, one flavone and two flavonol were isolated. The flavonoids were identified as apigenol 7,4'-dimethyl ether (**1**), kaempferol 3,7,4'-trimethyl ether (**2**), and quercetin 3,7,3',4'- tetramethyl ether (**3**) by UV, MS and ^{1}H-NMR data.

Keywords: Ballota saxatilis subsp. *saxatilis*, Flavonoid aglycones, 6-Hydroxyapigenol 7,4'-dimethyl ether, Kaempferol 3,7,4'-trimethyl ether, Ladanein, Lamiaceae, Quercetin 3,7,3',4'- tetramethyl ether, Retusin.

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MATERIALS AND METHODS

General Experimental Procedures

UV spectra (in MeOH) were recorded using a Varian DMS 90 instrument. EIMS spectra were recorded using a VG ZAB spectrometer. ^{1}H-NMR spectra in $CDCl_3$ were recorded at 300 MHz on a Bruker 300 spectrometer. Melting points were recorded on a Dupont 910 DSC instrument.

Plant Material

Ballota saxatilis subsp. *saxatilis* Sieber ex J. & C. Presl. was collected in 1994 from flowering plants near Ermenek (Turkey) and identified by Prof. Dr. Mehmet Koyuncu. Voucher specimens are kept in the Herbarium of Ankara University, Faculty of Pharmacy (AEF. No. 18676, 18706).

Isolation of Flavonoids

Air-dried and powdered aerial parts of the plant (1.2 kg) were extracted with Me_2CO (10 L) at room temperature for 1 week. Solvent was evaporated, the residue extracted with EtOAc and the extract washed with H_2O and dried. The extract was concentrated to dryness *in vacuo* to afford a syrupy residue (60 g). From this residue, 25 g were subjected to CC on SiO_2 200 mesh (200 g) and eluted with light petroleum and with an increasing amount of EtOAc until 80:20 (v/v). The fractions collected were 50 ml each. Similar fractions were combined and they were chromatographed on a PTLC (Kieselgel 60 PF_{254}) using Me_2O: light

petroleum (98:2) to afford three flavonoid aglycones: ladanein **1** (500 mg), kaempferol-3,7,4'-trimethyl ether **2** (40 mg), and retusin **3** (30 mg).

Ladanein (6-hydroxy apigenol 7,4'-dimethyl ether) (1)

Yellow needles, m.p. 207°C (lit. 205°) (Barberan et al., 1985). UV λ_{max} (MeOH) 330, 284, 214; (+NaOMe) 398, 318, 214; (+$AlCl_3$) 358, 300, 210; (+$AlCl_3$/HCl) 355, 300, 214, 210; (+NaOAc) 330, 300, 210; (NaOAc/H_3BO_3) 334, 300, 210; EIMS *m/z* (% rel.int) 314.2 (M^+, 100), 296.1 [(M^+-CH_3)$^+$, 52], 285.1 [(M^+-CH_3)$^+$, 13], 268.1 (61), 240.1 (3), 182 (6), 152.0 (9).^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.89 (3H, s, OCH_3), 4.0 (3H, s, OCH_3), 6.59 (2H, s, H-3 and H-8), 7.15 [2H, dd (J = 2 and H-8 Hz) H-3' and H-5'], 8.0 [2H, dd (J = 2 and 8 Hz) H-2' and H-6'], 12.59 (1H, brs, 5-OH).

Kaempferol 3,7,4'-trimethyl ether (2)

Yellow needles, m.p. 143°C (lit 145–147°C) (Imre et al., 1988; Jaipetch et al., 1983). UV λ_{max} (MeOH) 345, 226, 210; (+NaOMe) 355, 280, 210; (+$AlCl_3$) 394, 344, 300, 274, 210; (+$AlCl_3$/HCl), 394, 344, 300, 274, 210; (+NaOAc) 345, 264, 210; (+NaOAc/H_3BO_3) 345, 264, 210; EIMS *m/z* (% rel.int): 328.1 (M^+, 100), 314,1 [(M-CH_3)$^+$, 7], 299.1 (23), 285.1(67), 242.1 (18), 227 (7), 201.1 (18), 150.1 (23), 135.1 (24).^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.85 (3H,s,OCH_3), 3.89 (3H,s, OCH_3), 3.91 (3H,s, OCH_3), 6.34 [1H, d (J = 2 Hz) H-6], 6.47 [1H, d (J = 2Hz) H-8], 7.03 [2H (dd (J = 2.5 and 8.5 Hz) H-3' and H-5'], 8.09 [2H, dd (J = 2.5 and 8.5 Hz) H-2' and H-6'], 11.57 (1H, brs, 5=OH).

Retusin (Quercetin 3,7,3',4'-tetramethyl ether) (3)

Yellow needles, m.p.153°C (lit. 160–161°C) (Jaipetch et al., 1983) UV_{max} (MeOH) 337, 210; (+NaOMe) 320, 210; (+$AlCl_3$) 384, 356, 300, 276; (+$AlCl_3$/HCl) 285, 356, 300, 276; (+NaOAc) 337, 270, 210; (+NaOAc/H_3BO_3) 337, 270, 210; EIMS *m/z* (% rel. int): 358 (M^+, 100), 343 [(M-CH_3)$^+$], 179 (10), 165 (24), 150 (15).^{1}H-NMR (300 MHz,$CDCl_3$) δ 3.81 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 6.43 (2H, s, H-8), 7.65 [1H, d (J = 8Hz) H-5'], 7.73 [1H, dd (J = 2.5 and 8.5 Hz) H-6'], 8.06 [1H, d (J = 2.5 Hz) H-2'], 12.59 (1H, s, 5-OH).

RESULTS AND DISCUSSION

Chromatography of the plant afforded one flavone and two flavonols, namely, ladanein **1**, kaempferol 3,7,4'-trimethyl ether **2** and retusin **3**. The identity of the isolated flavonoids was confirmed through interpretation of their physical and spectral characters, namely, m.p., UV, MS and ^{1}H-NMR and comparison with the reported data (Barberan et al., 1985; Collado et al., 1985; Imre et al., 1984; Jaipetch et al., 1983).

To our knowledge, this is the first report on the isolation of kaempferol 3,7,4'-trimethyl ether **2** and retusin **3** from the genus *Ballota*. Ladanein **1** was previously

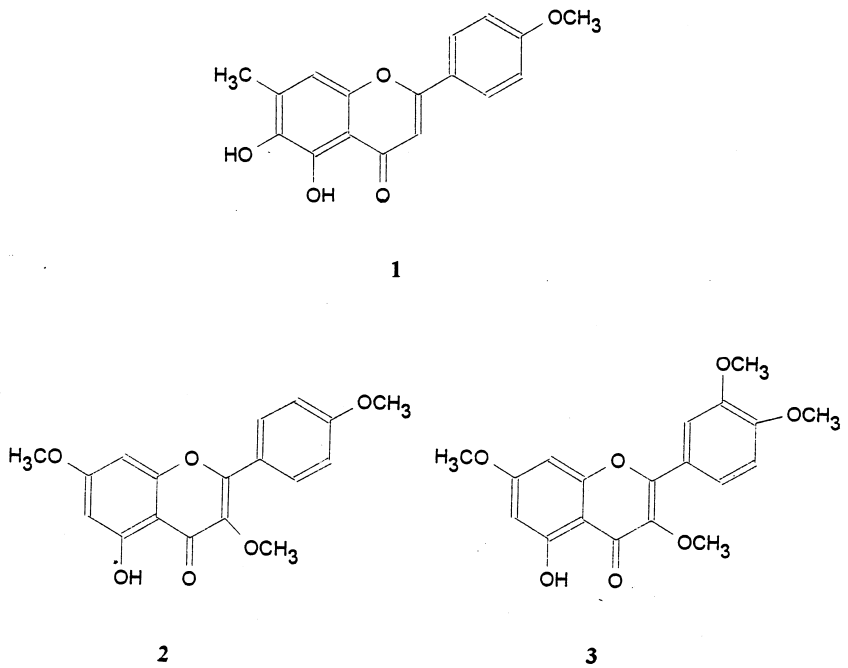

Fig. 1. Structures of the compounds **1**, **2** and **3**.

isolated and identified from *Ballota hirsuta* (Ferreres et al., 1986).

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