

Phytochemical Studies on *Origanum acutidens*

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Abstract

Origanum species (Lamiaceae) are commonly used as herbal tea, flavoring agents and medicinal plants all over the world. Rosmarinic acid, lithospermic acid, vicenin-2, betulalbuside A, 8-OH-linaloyl glycoside, ursolic acid, oleanolic acid were isolated from the aerial parts of *Origanum acutidens* in this study. Their structures were identified by means of spectroscopic methods (1D- and 2D-NMR).

Keywords: *Origanum acutidens*, flavonoid, phenolic compound, triterpene, monoterpene glycosides

Introduction

The genus *Origanum* is one of the important genera of the Lamiaceae family. *Origanum* species have rich essential oil constituents and these plants are used as spices. They are also extensively used in the flavouring of food products and alcoholic drinks and perfumery for their spicy smell. *Origanum* species are used as antiseptic, stimulant, stomachic, expectorant, sudofiric and emmenagogic in the folk medicine (Aliğiannis et al.; 2001; Baser, 1978; Çeker et al., 2012; Novak et al., 2000; Ryman, 1992). *Origanum acutidens* (Hand.-Mazz.) is a member of *Origanum* genus. Carvacrol, *p*-cymene were found to be as main constituents of the essential oil of *O. acutidens* (Baser, 1997). The essential oil of this plant shows various biological activities such as antioxidant, antibacterial, insecticidal properties (Kordali, 2008; Sokmen et al., 2004). In the present study, we report on the isolation and structure elucidation of rosmarinic acid, lithospermic acid, vicenin-2, betulalbuside A, 8-OH-linaloyl glucoside, ursolic acid, oleanolic acid metabolites of *O. acutidens*.

Materials and Methods

Plant Material

The aerial parts of *O. acutidens* were collected from Esendal (Yusufeli, Artvin Province, 950 m, Turkey). A voucher specimen was deposited at the Herbarium of Ankara University, Faculty of Pharmacy (AEF23177).

Extraction and Isolation Studies

The air-dried and powdered aerial parts (200 g) of *O. acutidens* were extracted three times with MeOH at 40 °C (3 × 2 L). After filtration, the MeOH extracts were evaporated under vacuum to dryness. Methanol extract (50g) was dissolved in H₂O:MeOH (9:1) and partitioned with *n*-hexane, CHCl₃ and then EtOAc, which were separately concentrated and dried under reduced pressure to give 7 g, 5,8 g and 1,9 g residues, respectively. The remaining aqueous phase was 34.0 g. CHCl₃ extract was separated via silica gel column chromatography eluting with *n*-Hexane:EtOAc (100:0, 90:10 ... 50:50). Fraction 20-26 were further purified by silica gel column chromatography (Hexane:EtOAc, 6:4). Fraction 5-4 gave mixture triterpene compounds ursolic acid and oleanolic acid. EtOAc extract was subjected to reversed phase silica gel column chromatography using H₂O:MeOH (90:10, 80:20.....,100) solvent systems. Fractions 28-32 were further applied by column chromatography on silica gel (CHCl₃:MeOH:H₂O, 80:20:2, 70:30:3,..., 50:50:5). Fractions 26-37 was subjected to reversed phase silica gel column chromatography using H₂O:MeOH (70:30). Fraction 5-4 gave rosmarinic acid. The remaining aqueous phase was subjected to Sephadex LH-20 column chromatography using MeOH. Fr. 1-3 (OSA) and Fr. 4 (OSB) were studied separately. OSA were separated via reversed phase silica gel column chromatography using H₂O:MeOH (90:10, 80:20.....,100) solvent systems. Eight fractions were collected (OSA1-OSA8). OSA2 was subjected to reversed phase silica gel column chromatography using H₂O:MeOH (90:10, 80:20.....,100).

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Precipitate formed in the 43rd fraction was given vicenin-2. Fraction 44-45 were further applied by column chromatography on silica gel (CHCl₃:MeOH:H₂O, 80:20:2, 70:30:3, ..., 50:50:5). Fraction 2 was given mixture monoterpene glycosides compounds betulalbuside A and 8-OH-linaloyl glycoside. OSB was subjected to Sephadex LH-20 column chromatography using MeOH. Fraction 12-14 gave lithospermic acid.

Results and Discussion

At the end of the extraction and isolation processes of aerial parts of *O. acutidens* 50g MeOH extract was obtained. n-Hexane, CHCl₃, EtOAc and remaining aqueous phase were 7 g, 5,8 g and 1,9 g respectively after fractionation. Ursolic acid and oleanolic acid were isolated from CHCl₃ phase; rosmarinic acid from EtOAc phase; vicenin-2, betulalbuside A, 8-OH-linaloyl glycoside and lithospermic acid from the aqueous phase. ¹H-NMR and ¹³C-NMR data were compared with literature data.

Ursolic acid

C₃₀H₄₈O₃, ¹H-NMR (DMSO-*d*₆, 400 MHz) δ: 5.23 (1H, *m*, H-12), 3.14 (1H, *m*, H-3), 2.20 (1H, *d*, *j*= 11.7 Hz, H-18), 1.18 (CH₃), 0.96 (CH₃), 0.95 (CH₃), 0.94 (CH₃), 0.88 (CH₃), 0.81 (CH₃), 0.78 (CH₃), 2.08-1.28 (*m*, 20 H) ¹³C-NMR (DMSO-*d*₆, 100 MHz) δ: 38.3 (C-1), 26.7 (C-2), 78.5 (C-3), 38.7 (C-4), 55.6 (C-5), 18.3 (C-6), 33.1 (C-7), 39.6 (C-8), 47.7 (C-9), 36.9 (C-10), 23.2 (C-11), 125.7 (C-12), 138.5 (C-13), 42.1 (C-14), 28.0 (C-15), 24.1 (C-16), 47.7 (C-17), 53.2 (C-18), 39.2 (C-19), 39.2 (C-20), 30.6 (C-21), 36.9 (C-22), 27.6 (C-23), 14.8 (C-24), 15.2 (C-25), 16.5 (C-26), 22.9 (C-27), 180.5 (C-28), 16.6 (C-29), 20.4 (C-30) (Baykal et al., 1998; Jiang et al., 1995; Junges et al., 2000; Lin et al., 1987; Miyakshi et al., 1997; Tundis et al., 2002; Maillard et al., 1992).

Oleanolic acid

C₃₀H₄₈O₃, ¹H-NMR (DMSO-*d*₆, 400 MHz) δ: 5.23 (1H, *m*, H-12), 3.14 (1H, *m*, H-3), 2.84 (1H, *dd*, *j*= 13.8 Hz, *j*= 4.2 Hz, H-18), 1.16 (CH₃), 0.97 (CH₃), 0.94 (CH₃), 0.90 (CH₃), 0.84 (CH₃), 0.78 (CH₃), 2.08-1.28 (*m*, 24 H) ¹³C-NMR (DMSO-*d*₆, 100 MHz) δ: 38.7 (C-1), 26.7 (C-2), 78.5 (C-3), 38.8 (C-4), 55.6 (C-5), 18.3 (C-6), 32.6 (C-7), 39.4 (C-8), 48.2 (C-9), 37.0 (C-10), 22.8 (C-11), 122.5 (C-12), 144.0 (C-13), 41.7 (C-14), 27.5 (C-15), 22.9 (C-16), 46.5 (C-17), 41.6 (C-18), 46.1 (C-19), 30.4 (C-20), 33.7 (C-21), 32.4 (C-22), 27.7 (C-23), 14.7 (C-24), 15.1 (C-25), 16.5 (C-26), 25.2 (C-27), 180.5 (C-28), 32.8 (C-29), 23.3 (C-30) (Baykal et al., 1998; Jiang et al., 1995; Junges et al., 2000; Lin et al., 1987; Miyakshi et al., 1997; Tundis et al., 2002; Maillard et al., 1992).

Rosmarinic acid

C₁₈H₁₆O₈, ¹H-NMR (CDCl₃, 400 MHz) δ: 7.03 (1H, *d*, *j*= 1.8 Hz, H-2), 6.75 (1H, *d*, *j*= 8.4 Hz, H-5), 6.90 (1H, *dd*, *j*= 8.1, *j*= 2.2 Hz, H-6), 7.49 (1H, *d*, *j*= 16.0 Hz, H-7), 6.25 (1H, *d*, *j*= 16.0 Hz, H-8), 6.75 (1H, *d*, *j*= 1.8 Hz, H-2'), 6.68 (1H, *d*, *j*= 8.1 Hz, H-5'), 6.63 (1H, *dd*, *j*= 8.1 Hz, *j*= 1.8 Hz, H-6'), 3.08 (1H, *dd*, *j*= 14.1 Hz, *j*= 3.1 Hz, Ha-7'), 2.91 (1H, *dd*, *j*= 14.1 Hz, *j*= 9.7 Hz, Hb-7'), 5.08 (1H, *dd*, *j*= 9.7, *j*= 3.5 Hz, H-8'). ¹³C-NMR (CDCl₃, 100 MHz) δ: 126.7 (C-1), 114.4 (C-2), 145.6 (C-3), 148.1 (C-4), 115.2 (C-5), 121.6 (C-6), 145.3 (C-7), 113.8 (C-8), 167.7 (C-9), 129.9 (C-1'), 116.2 (C-2'), 144.8 (C-3'), 143.6 (C-4'), 115.1 (C-5'), 120.4 (C-6'), 37.7 (C-7'), 76.1 (C-8'), 176.2 (C-9') (Cai et al., 2004; Chiang et al., 2005; Dapkevicius et al., 2002; Woo and Piao, 2004).

Vicenin-2

C₂₇H₃₀O₃₀, ¹H-NMR (CDCl₃, 400 MHz) δ: 8.00 (2H, *d*, *j*= 8.4 Hz, H-2', H-6'), 6.88 (2H, *d*, *j*= 8.1 Hz, H-3', H-5'), 6.78 (1H, *s*, H-3), 4.78 (1H, *d*, *j*= 9.9 Hz, H-1''), 4.74 (1H, *d*, *j*= 9.9 Hz, H-1'''), 3.88-3.14 (10 H, glucose protons), ¹³C-NMR (CDCl₃, 100 MHz) δ: 164.7 (C-2), 103.3 (C-3), 182.9 (C-4), 159.0 (C-5), 108.1 (C-6), 161.8 (C-7), 105.9 (C-8), 155.8 (C-9), 104.5 (C-10), 122.2 (C-1'), 129.7 (C-2'), 116.5 (C-3'), 159.3 (C-4'), 116.5 (C-5'), 129.7 (C-6'), 74.0 (C-1''), 71.1 (C-2''), 78.4 (C-3''), 69.7 (C-4''), 81.5 (C-5''), 60.4 (C-6''), 74.8 (C-1'''), 72.5 (C-2'''), 79.5 (C-3'''), 71.5 (C-4'''), 82.5 (C-5'''), 61.8 (C-6''') (Hussein et al., 1997; Xie et al., 2003).

Betulalbuside A

C₁₆H₂₈O₇, ¹H-NMR (CDCl₃, 400 MHz) δ: 5.03 (1H, *dd*, *j*= 10.9, *j*= 1.5 Hz, Ha-1), 5.19 (1H, *dd*, *j*= 17.4, *j*= 1.5 Hz, Hb-1), 5.90 (1H, *dd*, *j*= 17.4, *j*= 10.9 Hz, H-2), 2.10 (2H, *m*, H-4), 1.53 (2H, *m*, H-5), 5.47 (1H, *bt*, *j*= 7.0, H-6), 4.19 (1H, *d*, *j*= 11.4 Hz, part A of the AB system, Ha'-8), 4.03 (1H, *d*, *j*= 11.4 Hz, part A of the AB system, Ha'-8), 5.47 (1H, *bt*, *j*= 7.0, H-6), 4.19 (1H, *d*, *j*= 11.4 Hz, part B of the AB system, Hb'-8), 1.68 (3H, *bs*, H-9), 1.26 (3H, *s*, H-10), 4.23 (1H, *d*, *j*= 7.7 Hz, H-1'). ¹³C-NMR (CDCl₃, 100 MHz) δ: 111.0 (C-1), 145.0 (C-2), 74.0 (C-3), 41.7 (C-4), 22.3 (C-5), 129.0 (C-6), 131.7 (C-7), 74.7 (C-8), 12.9 (C-9), 26.5 (C-10), 101.4 (C-1'), 73.9 (C-2'), 76.4 (C-3'), 70.5 (C-4'), 76.9 (C-5'), 61.6 (C-6') (Yalçın et al., 2003).

8-OH-linaloyl glycoside

$C_{16}H_{28}O_7$, 1H -NMR ($CDCl_3$, 400 MHz) δ : 5.16 (1H, *dd*, $j = 11.0$, $j = 1.1$ Hz, Ha-1), 5.24 (1H, *dd*, $j = 18.0$, $j = 1.5$ Hz, Hb-1), 6.09 (1H, *dd*, $j = 18.0$, $j = 11.0$ Hz, H-2), 1.65 (2H, *m*, H-4), 2.10 (2H, *m*, H-5), 5.39 (1H, *bt*, $j = 6.6$, H-6), 3.90 (2H, *bs*, H-8), 1.63 (3H, *bs*, H-9), 1.34 (3H, *s*, H-10), 4.31 (1H, *d*, $j = 7.7$ Hz, H-1'). ^{13}C -NMR ($CDCl_3$, 100 MHz) δ : 113.9 (C-1), 143.3 (C-2), 80.1 (C-3), 40.0 (C-4), 22.1 (C-5), 125.8 (C-6), 134.6 (C-7), 67.8 (C-8), 12.6 (C-9), 23.3 (C-10), 98.1 (C-1'), 73.9 (C-2'), 76.7 (C-3'), 70.5 (C-4'), 77.0 (C-5'), 61.6 (C-6') (Yalçın et al., 2003).

Lithospermic acid

$C_{27}H_{42}O_{12}$, 1H -NMR ($CDCl_3$, 400 MHz) δ : 7.12 (1H, *d*, $j = 8.4$ Hz, H-6), 7.95 (1H, *d*, $j = 16.0$ Hz, H-7), 6.24 (1H, *d*, $j = 16.0$ Hz, H-8), 5.12 (1H, *dd*, $j = 6.2$ Hz, H-10), 3.08 (*bd*, $j = 13.0$ Hz, Ha-11), 2.94 (*dd*, $j = 13.0$ Hz, $j = 9.0$ Hz, Hb-11), 6.87 (1H, *s*, H-13), 6.62 (*d*, $j = 8.0$ Hz, H-16), 6.60 (*bd*, $j = 8.0$ Hz, H-17), 4.26 (1H, *d*, $j = 6.2$ Hz, H-20), 5.87 (1H, *d*, $j = 6.2$ Hz, H-21), 6.85 (1H, *s*, H-23), 6.71-6.75 (3H, *m*, signal overlap). ^{13}C -NMR ($CDCl_3$, 100 MHz) δ : 123.5 (C-1), 129.0 (C-2), 147.5 (C-3), 145.3 (C-4), 116.4 (C-5), 119.9 (C-6), 142.7 (C-7), 116.0 (C-8), 167.8 (C-9), 75.9 (C-10) (Kelley et al., 1975; Kelley et al., 1976).

Earlier studies have shown that *O. acutidens* has antioxidant, antibacterial, insecticidal, including essential oils mainly carvacrol, *p*-cymen (Baser, 1997; Kordali, 2008; Sokmen et al., 2004). The isolation of rosmarinic acid, lithospermic acid, vicenin-2, betulalbuside A, 8-OH-linaloyl glycoside, ursolic acid, oleanolic acid from *O. acutidens* was recorded for the first time in this study.

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