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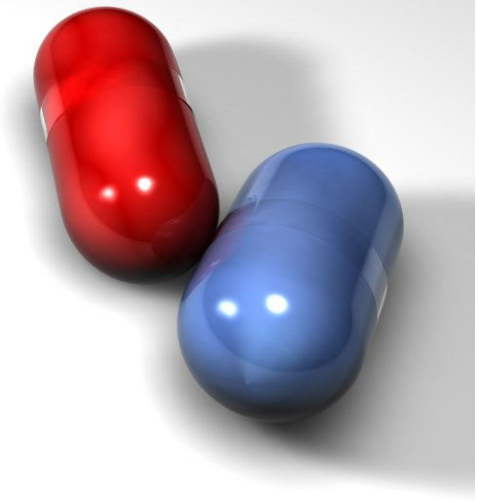

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Letter to the Editor

Phytochemical studies on *Origanum rotundifolium*

Sir,

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 (Aligiannis et al., 2001; Baser, 1978; Novak et al., 2000).

Origanum rotundifolium is a member of *Origanum* genus. This medicinal plant known in Anatolia as "Mercan kösk" isused as spices. Thymol, carvacrol, *p*-cymene and borneol were found to be as main constituents of the essential oil of *O. rotundifolium*. The essential oil of this plant shows various biological activities such as anti-oxidant, antibactial properties. These activities have been mainly attributed to constituents such as thymol, carvacrol, borneol, terpinenchemo type and spathulenol (Baser et al., 1993; Gormez et al., 2016; Goze et al., 2009; Kotan et al., 2010).

In the present study, we report on the isolation and structure elucidation of 3 metabolites of *O. rotundifolium*.

The aerial parts of *O. rotundifolium* were collected from Barhal (Yusufeli, Artvin Province, 560-620 m, Turkey). A voucher specimen was deposited at the Herbarium of Ankara University, Faculty of Pharmacy (AEF25873).

The air-dried and powdered aerial parts (400 g) of *O. rotundifolium* were extracted three times with MeOH at 40°C (3 × 2L). After filtration, the methanol extracts were evaporated under vacuum to dryness. Methanol extract (71.6 g) was dissolved in water-methanol (9:1) and partitioned with chloroform and then ethyl acetate, which were separately concentrated and dried under reduced pressure to give 18.3 g and 4.0 g residues, respectively. The remaining aqueous phase was 31.0 g.

Chloroform extract (2.7 g) was separated via silica gel column chromatography eluting with *n*-hexane-ethyl acetate (100:0, 90:10 ... 50:50). Fr. 6-7 gave the compound **1** (45 mg).

Ethyl acetate extract was subjected to reversed phase silica gel column chromatography using water: methanol (90:10, 80:20…..,100) solvent systems. The fractions 4-10 (1.79 g) were further purified by consecutive column chromatography on silica gel (chloroform-methanol-water, 80:20:2, 70:30:3,…, 50:50:5) and sephadex LH-20 (MeOH), respectively. Fr. 8 gave compound **2** (44 mg).

The remaining aqueous phase was subjected to reversed phase silica gel column chromatography using water: methanol (90:10, 80:20…..,100) solvent systems. Fr. 19-29 were separated via silica gel column chromatography eluting with chloroform-methanol-water (80:20:2, 70:30:3,…, 50:50:5). Fr. 84-121 gave compound **3** (217 mg).

Their structures were identified by means of spectroscopic methods (1D- and 2D-NMR, EIMS).

Compound **1**

EIMS *m/z*236 $[M+Na-H]^+$, $C_{14}H_{30}O$,^{1}H-NMR ($CDCl_3$, 400 MHz)*δ*: 3.66 (2H, t, *J*= 6.6 Hz, H-1), 1.28-1.60 (24H, H-2-13), 0.90 (3H, t, *J*= 6.8 Hz, H-14). ^{13}C-NMR ($CDCl_3$, 100 MHz) *δ*:22.7, 25.7, 29.4, 29.4, 29.6, 29.7, 29.7, 31.9, 32.82 (C-2-13), 14.1 (C-14).

Compound **2**

$C_{18}H_{16}O_8$,^{1}H-NMR ($CDCl_3$, 400 MHz)*δ*: 7.02 (1H, d, *J*= 1.8 Hz, H-2), 6.76 (1H, d, *J*= 8.4 Hz, H-5), 6.92 (1H, dd, *J*= 8.1, *J*= 2.2 Hz, H-6), 7.50 (1H, d, *J*= 16.0 Hz, H-7), 6.27 (1H, d, *J*= 16.0 Hz, H-8), 6.75 (1H, d, *J*= 1.8 Hz, H-2'), 6.67 (1H, d, *J*= 8.1 Hz, H-5'), 6.62 (1H, dd, *J*= 8.1 Hz, *J*= 1.8 Hz, H-6'), 3.09 (1H, dd, *J*= 14.1 Hz, *J*= 3.1 Hz, Ha-7'), 2.92 (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'). ^{13}C-NMR ($CDCl_3$, 100 MHz) *δ*: 126.8 (C-1), 114.4 (C-2), 145.5 (C-3), 148.2 (C-4), 115.3 (C-5), 121.7 (C-6), 145.5 (C-7), 113.9 (C-8), 167.9 (C-9), 129.9 (C-1'), 116.3 (C-2'), 144.8 (C-3'), 143.6 (C-4'), 115.0 (C-5'), 120.6 (C-6'), 37.6 (C-7'), 76.3 (C-8'), 176.1 (C-9').

Compound **3**

EIMS *m/z* 561 $[M+Na]^+$,$C_{27}H_{22}O_{12}$, ^{1}H-NMR ($CDCl_3$, 400 MHz)*δ*: 7.10 (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), 5.11 (1H, dd, *J*= 6.2 Hz, H-10), 3.07(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), 6.63 (d, *J*= 8.0 Hz, H-16), 6.57 (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, signaloverlap). ^{13}C-NMR

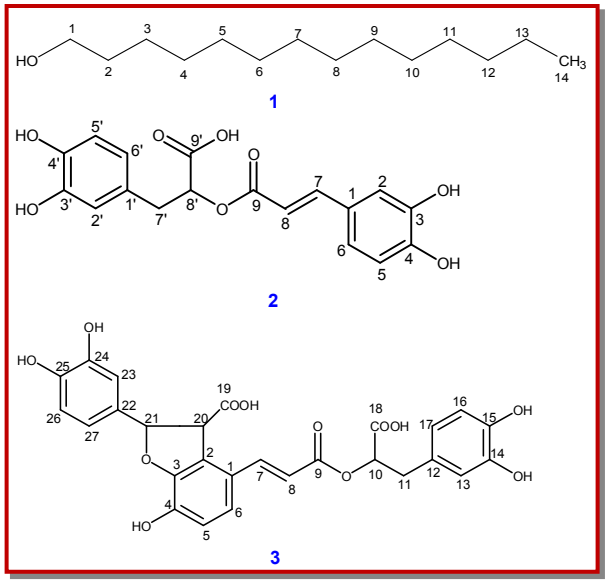

Figure 1: Compounds isolated from *O. rotundifolium*

($CDCl_3$, 100 MHz) δ:123.6 (C-1), 129.0 (C-2), 147.5 (C-3), 145.3 (C-4), 116.6 (C-5), 119.9 (C-6), 142.7 (C-7), 116.0 (C-8), 167.8 (C-9), 75.9 (C-10).

At the end of the extraction and isolation processes of aerial parts of *O. rotundifolium* 71.6 g methanol extract was obtained. Chloroform, ethyl acetate and remaining aqueous phase were 18.3 g, 4 g and 31 g, respectively after fractionation. 1-Tetradecanol (Compound **1**, 45 mg) was isolated from the chloroform phase; rosmarinic acid (Compound **2**, 44 mg) from ethyl acetate phase; lithospermic acid (Compound **3**, 217 mg) fromthe aqueous phase (Figure 1). ^{1}H-NMR and ^{13}C-NMR data comply with the data given in the literature (Junges et al., 2000; Kelley et al., 1975; Kelley et al., 1976).

Earlier studies have shown that *O. rotundifolium* has anti-oxidant, antibactial effects, including essential oils such as thymol, carvacrol, *p*-cymene and borneol (Gormez et al., 2016; Goze et al. 2009).

The isolation of 1-tetradecanol, rosmarinic acid and lithospermic acid from *O. rotundifolium* was recorded for the first time in this study.

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