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RESEARCH ARTICLE

Isolation of Major Compounds of *Origanum micranthum* and *Origanum minutiflorum*

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Isolation of Major Compounds of Origanum micranthum and Origanum minutiflorum

SUMMARY

Origanum species are traditionally used frequently as spices and because of their medicinal effects. In this study, isolation studies were carried out by using column chromatography on Origanum micranthum Vogel and Origanum minutiflorum O. Schwarz & P.H. Davis. 4(3,4-dihydroxy benzoyl oxymethyl) phenyl-β-D-glucopyranoside (1), rosmarinic acid (2), 3-(3,4-dihydroxyphenyl)-2-hydroxypropionic acid (3), ursolic acid (4A), oleanolic acid (4B) were isolated from O. micranthum, rosmarinic acid (5), apigenin (6) and vicenin-2 (7) were isolated from O. minutiflorum at the end of the study.

Key Words: *Origanum micranthum, Origanum minutiflorum, Isolation, Secondary metabolite, Chromatography, Structure elucidations*

Origanum micranthum ve Origanum minutiflorum'un Başlıca Bileşenlerinin İzolasyonu

ÖZ

Origanum türlerinin baharat olarak ve tıbbi etkilerinden dolayı geleneksel kullanımı oldukça yaygındır. Bu çalışmada Origanum micranthum Vogel ve Origanum minutiflorum O. Schwarz & P.H. Davis üzerinde kolon kromatografisi yöntemiyle izolasyon çalışmaları gerçekleştirilmiştir. O. micranthum'dan 4(3,4-dihidroksi benzoil oksimetil)fenil-β-D-glukopiranozit (1), rozmarinik asit (2), 3-(3,4-dihidroksifenil)-2-hidroksipropionik asit (3), ursolik asit (4A), oleanolik asit (4B), O. minutiflorum'dan rosmarinik asit (5), apigenin (6) ve visenin-2 (7) izole edilmiştir.

Anahtar Kelimeler: *Origanum micranthum, Origanum minutiflorum, İzolasyon, Sekonder metabolit, Kromatografi, Yapı tayini*

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INTRODUCTION

Labiatae is a medically important family which is spread over a wide area over the earth (Atasu and Konuklugil, 1988). The *Origanum* genus belongs to Labiatae family and Turkey has large number of *Origanum* species. They are traditionally known as "kekik" which is used as spice and herbal tea. These species are also used in the production of essential oil and aromatic water (Baser, 2002). Some biological activities have been reported regarding to species of this genus such as antimicrobial, antioxidant, antimutagenic (Bostancıoglu et al, 2012; Chishti et al, 2013; Karaboduk et al, 2014; Sarikurkcu et al, 2015), antifungal, insecticidal, anticarcinogenic, antispasmodic (Bostancıoglu et al, 2012), antiviral, fungicide, nematocide, biocide, growth regulation effects (Karaboduk et al, 2014), antithrombin, angiogenic, antiparasetic and antihyperglycaemic activities (Chishti et al, 2013). *Origanum* species have been also used as expectorant, digestive, anti-diabetic, stimulant, tonic, menstrual regulator, sedatives, diuretic, analgesics, carminative, antiparasitic, antihelminthic, for gastrointestinal complaints and for colds, asthma (Chishti et al, 2013; Karaboduk et al, 2014; Sahin et al, 2004; Nakiboglu et al, 2007; Loizzo et al, 2009). *Origanum* species have been reported to contain essential oils, flavonoids, phenolic compounds, triterpenes. Essential oil of O*riganum* genus mostly contains thymol and carvacrol (Sezen Karaoglan, 2011).

In this study, isolation studies of *Origanum minutiflorum* Vogel and *Origanum minutiflorum* O. Schwarz & P.H. Davis were carried out by chromatographic methods. Their structures were identified by means of spectroscopic methods (1D- and 2D-NMR, EIMS).

MATERIALS AND METHODS

Plant Materials

O. micranthum was collected from Kozan district (Between Kozan Lakes Plateau and Feke Tapan Village, Mount Hopka, 1900 m) of Adana in August 2009. Plant samples are stored in Herbarium of Ankara University Faculty of Pharmacy (AEF 25873).

O. minutiflorum was kindly obtained from Inan Tarim in Antalya which was collected from Serik district (between Etler Village and Ovacik Highland road, 1800 m) of Antalya in September 2010. Plant samples are kept in Herbarium of Ankara University Faculty of Pharmacy (AEF 25949).

Extraction Studies

The aerial parts of *O. micranthum* (410 g) was gently dried and powdered. Extraction of powder were achieved in methanol at 40 °C (3×2 L). Extract was evaporated until dryness via rotary evaporator. Residue was firstly dissolved in H_2O:MeOH (9:1) and then subjected to liquid-liquid extraction with chloroform and ethyl acetate, respectively. Remaining phase was entitled as aqueous fraction. Afterwards, the solvents were evaporated in rotary evaporator. Thus, ethyl acetate, chloroform and aqueous fraction were obtained.

The aerial parts of *O. minutiflorum* (550 g) was dried and powdered. The powdered plant was extracted with methanol at 40 °C (3×2 L). The methanol extract was evaporated in the rotary evaporator. The dried extract was dissolved in H_2O:MeOH (9:1) and then subjected to liquid-liquid extraction with chloroform and ethyl acetate, respectively. Remaining phase was called aqueous fraction. Afterwards, all phases were evaporated and ethyl acetate, chloroform and aqueous fractions were obtained.

Isolation Studies

Isolation studies on *Origanum micranthum*

Ethyl acetate fraction

The ethyl acetate fraction was applied to the silica gel column. The elution was initiated with $CHCl_3$:MeOH:H_2O (80:20:2) solvent system and continued with $CHCl_3$:MeOH:H_2O (70:30:3, 50:50:5). Compound 1 was obtained from fractions 18-20. Fractions 27-28 were applied to the Sephadex LH-20 column with methanol as mobile phase. Compound 2 was obtained from fractions 7-9.

Aqueous fraction

The aqueous fraction was applied to the Sephadex LH-20 column in presence of methanol as mobile phase. Fractions 11-12 were applied to the

silica gel column. The elution was initiated with $CHCl_3$:MeOH:H_2O (80:20:2) solvent system, continued with $CHCl_3$:MeOH:H_2O (70:30:3, 50:50:5). Fractions 18-28 were applied to the silica gel column. Chromatographic separation were performed by $CHCl_3$:MeOH:H_2O (80:20:2) and continued with $CHCl_3$:MeOH:H_2O (70:30:3, 50:50:5). Fraction 40 applied to the Sephadex LH-20 column. Methanol was used as the mobile phase. Compound 3 was obtained from fractions 5-8.

Chloroform fraction

The chloroform fraction was applied to the silica gel column. The elution was initiated with n-hexane: EtOAc (100: 0) solvent system and continued in increasing proportions of EtOAc (90:10,……, 50:50). Compound 4A-4B (mixture) from fraction 17.

Isolation studies on *Origanum minutiflorum*

Ethyl acetate fraction

The ethyl acetate fraction was applied to the reversed-phase silica gel column. The elution was carried out with H_2O:MeOH (90:10, 80:20, ….., 0:100) solvent systems. Fractions 5-8 and 12-13 were studied.

Fractions 5-8 were applied to the Sephadex LH-20 column in presence of methanol as mobile phase. Fractions 6-12 were applied to the Sephadex LH-20 column again using methanol. Fractions 5-16 were applied to the silica gel column. Chromatographic separation were performed by $CHCl_3$:MeOH:H_2O (80:20:2) solvent system and continued with $CHCl_3$:MeOH:H_2O (70:30:3, 50:50:5). Compound 5 was obtained from fractions 25-27.

Fractions 12-13 were applied to the Sephadex LH-20 column. Methanol was used as the mobile phase. Compound 6 was obtained from fractions 10-11.

Aqueous fraction

The aqueous fraction was applied to the Sephadex LH-20 column in presence of H_2O:MeOH (1:1) as mobile phase. Fractions 3-10 were applied to the silica gel column. The elution was initiated with $CHCl_3$:MeOH:H_2O (80:20:2) solvent system, continued with $CHCl_3$:MeOH:H_2O (70:30:3, 50:50:5).

Fractions 90-149 were applied to the reversed-phase silica gel column. Chromatographic separation were performed by H_2O:MeOH (90:10, 80:20, ….., 0:100) solvent systems. A yellow precipitate formed in fractions 28-32. The precipitate was filtered on filter paper and washed with distilled water. Compound **7** was obtained.

Determination of chemical structure

The chemical structures of the isolated compounds were determined by 1D- and 2D-NMR, EIMS

RESULTS AND DISCUSSION

Isolation studies of *O. micranthum*

Compound 1: $C_{20}H_{22}O_{10}$, EIMS m/z 445 $[M+Na]^+$, ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 7.34 (1H, d, *J*= 2.2 Hz, H-2), 6.78 (1H, d, *J*= 8.4 Hz, H-5), 7.30 (1H, dd, *J*= 8.4 Hz, *J*= 2.2 Hz, H-6), 7.35 (2H, A_2B_2 system, d, *J*= 8.4 Hz, H-2', 6'), 7.02 (2H, A_2B_2 system, d, *J*= 8.4 Hz, H-3', 5'), 5.17 (2H, s, H-7'), 4.86 (1H, d, *J*= 7.3 Hz, H-1"), 3.11-3.33 (5H, m, H-2", 3", 4", 5", 6"b), 3.67 (1H, d, *J*= 11.0 Hz, H-6"a).

^{13}C-NMR (DMSO-d_6, 100 MHz) δ:121.2 (1), 116.9 (2), 145.7 (3) , 151.2 (4), 116.1 (5), 122.6 (6), 166.2 (7), 130.4 (1'), 130.3 (2'), 116.9 (3'), 157.9 (4'), 116.9 (5'), 130.3 (6'), 66.1 (7'), 101.0 (1"), 73.9 (2"), 77.7 (3"), 70.4 (4"), 77.3 (5"), 61.4 (6").

When the ^{1}H-NMR spectrum of the compound 1 was examined, protons of the A_2B_2 system were observed at δ_H= 7.02 and δ_H= 7.35 ppm. These signals supported the presence of the 1,4-disubstituted benzene structure in the molecule. The protons of the other aromatic ring were δ_H= 6.78, δ_H= 7.30 and δ_H= 7.34 ppm. The signal observed at δ_H= 5.17 ppm indicates a benzylic methylene containing acetoxy group. Anomeric proton signal of glucose was observed at δ_H = 4.86 ppm. Other proton signals of glucose were observed at δ_H= 3.67 ppm and δ_H= 3.11-3.33 ppm. In the ^{13}C-NMR spectrum, the characteristic signals of glucopyranosil were observed at δ_C= 61.4, 70.4, 73.9, 77.3, 77.7 and 101.0 ppm. A methylene (δ_C= 66.1 ppm), an ester (δ_C= 166.2 ppm), five substituted aromatic car-

bon (δ_C= 121.2, 130.4, 145.7, 151.2 and 157.9 ppm) and seven protonated carbon (δ_C= 116.1, 116.9 (3C), 122.6 and 130.3 (2C)) signals of the aglycone portion of the molecule were observed. When these findings were compared with the literature, it was observed that the compound 1 was 4(3,4-dihydroxy benzoyl oxymethyl)phenyl-β-D-glucopyranoside (Nakatani and Kikuzaki, 1987). The chemical structure of compound 1 is shown in Figure 1-A.

Compound 2: $C_{18}H_{16}O_8$, ^{1}H-NMR (CD_3OD, 400 MHz) δ: 7.02 (1H, d, *J*= 2.2 Hz, H-2), 6.76 (1H, d, *J*= 8.1 Hz, H-5), 6.92 (1H, dd, *J*= 8.3, *J*= 2.0 Hz, H-6), 7.50 (1H, d, *J*= 16.1 Hz, H-7), 6.26 (1H, d, *J*= 16.1 Hz, H-8), 6.74 (1H, d, *J*= 2.2 Hz, H-2'), 6.68 (1H, d, *J*= 8.1 Hz, H-5'), 6.62 (1H, dd, *J*= 8.1 Hz, *J*= 2.2 Hz, H-6'), 3.09 (1H, AB system (A), dd, *J*= 14.3 Hz, *J*= 3.5 Hz, Ha-7'), 2.94 (1H, AB system (B), dd, *J*= 14.3 Hz, *J*= 9.5 Hz, Hb-7'), 5.10 (1H, dd, *J*= 9.7, *J*= 3.4 Hz, H-8').

^{13}C-NMR (CD_3OD, 100 MHz) δ: 126.7 (1), 114.3 (2), 145.6 (3), 148.2 (4), 115.3 (5), 121.8 (6), 145.5 (7), 113.9 (8), 167.9 (9), 129.9 (1'), 116.4 (2'), 144.7 (3'), 143.6 (4'), 115.1 (5'), 120.6 (6'), 37.5 (7'), 76.4 (8'), 176.5 (9').

In the ^{1}H-NMR spectrum of the compound 2, δ_H= 7.02 (1H, d, *J*= 2.2 Hz), δ_H= 6.92 (1H, dd, *J*= 8.3 Hz, *J*= 2.0 Hz), δ_H= 6.76 (1H, d, *J*= 8.1 Hz) and δ_H= 6.74 (1H, d, *J*= 2.2 Hz), δ_H= 6.68 (1H, d, *J*= 8.1 Hz), δ_H= 6.62 (1H, dd, *J*= 8.1, *J*= 2.2 Hz) aromatic ABX system protons (H-2,6,5 and H-2', 5', 6' protons, respectively) signals were observed. The chemical shift values at δ_H= 7.50 (1H, d, *J*= 16.1) and δ_H= 6.26 (1H, d, *J*= 16.1 Hz) ppm showed the presence of olefinic protons in the trans position relative to each other. It was determined by comparison with the literature that the proton (H-8') adjacent to the oxygen signal at δ_H= 5.10 (dd, *J*= 9.7, *J*= 3.5 Hz) ppm and the methylene protons at the C-7' position at δ_H= 3.09 (dd, *J*= 14.3, *J*= 3.6 Hz), δ_H = 2.94 (dd, *J*= 14.3, *J*= 9.5 Hz) ppm (Woo and Piao, 2004). When the ^{13}C-NMR spectrum was examined, four carbon atoms were substituted on the two phenyl rings with OH groups. These carbons signaled at δ_C= 148.2 (C-4), 145.6 (C-3), 144.7 (C-3') and 143.6 (C-4') ppm. Two carbonyl carbons δ_C= 176.5 (C-9'), 167.9 (C-9) ppm, olefinic carbons δ_C= 145.5 (C-7), 113.9 (C-8) ppm, one carbon atom adjacent to the ester δ_C= 76.4 (C-8 ') ppm. When these findings were compared with the literature, it was observed that the compound 2 was rosmarinic acid (Dapkevicius et al, 2002; Cai et al, 2004; Chiang et al; 2005, Junges et al, 2000). The chemical structure of compound 2 is shown in Figure 1-B.

Compound 3: $C_9H_{10}O_5$, EIMS m/z 221 $[M+Na]^+$, EIMS m/z 244 $[M+2Na]^+$, ^{1}H-NMR (CD_3OD, 400 MHz) δ: 6.65 (1H, d, *J*= 8.1 Hz, H-2), 6.73 (1H, d, *J*= 1.8 Hz, H-5), 6.59 (1H, dd, *J*= 8.1, *J*= 2.2 Hz, H-6), 2.96 (1H, dd, *J*= 14.1 Hz, *J*= 3.5 Hz, H-7a), 2.65 (1H, dd, *J*= 13.9 Hz, *J*= 8.1 Hz, H-7b), 4.08 (1H, dd, *J*= 8.3 Hz, *J*= 3.5 Hz, H-8)

^{13}C-NMR (CD_3OD, 100 MHz) δ: 130.6 (1), 116.6 (2), 144.6 (3), 143.4 (4), 114.9 (5), 120.8 (6), 40.6 (7), 73,4 (8), 179.2 (9).

When the ^{1}H-NMR spectrum was examined, the signals of the protons of the aromatic ring were δ_H= 6.73 (1H, d, *J*= 1.8 Hz) ppm, δ_H= 6.65 (1H, d, *J*= 8.1 Hz) ppm and δ_H= 6.59 (1H, dd, *J*= 2.2 Hz, *J*= 8.1 Hz) ppm. These signals supported the 3,4-disubstituted benzene structure in the molecule. The metin proton signaled at δ_H= 4.08 (1H, dd, *J*= 3.5, *J*= 8.3 Hz) ppm. The signals observed at δ_H= 2.96 (1H, dd, *J*= 3.5, *J*= 14.1 Hz) ppm and δ_H= 2.65 (1H, dd, *J*= 8.1, *J*= 13.9 Hz) ppm showed the presence of methylene protons. In the ^{13}C-NMR spectra were examined, the carbonyl carbon was observed at δ_C= 179.2 ppm, and the characteristic signals of the substituted aromatic ring were observed at δ_C= 144.6 and 143.4 ppm. Three protonated carbons were observed at δ_C= 120.8 ppm, δ_C= 116.6 ppm and δ_C= 114.9 ppm. The signal observed at δ_C= 73.4 ppm is attributed to the metin carbon, the signal observed at δ_C= 40.6 ppm is attributed to the methylene carbon. These spectral findings showed that the compound 3 was 3-(3,4-dihydroxyphenyl)-2-hydroxypropionic acid when compared with the literature (Dai et al, 2010; Kelly et al, 1976). The chemical structure of compound 3 is shown in Figure 1-C.

Compound 4A: $C_{30}H_{48}O_3$, ^{1}H NMR (CD_3OD, 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 (3H, CH_3), 0.96 (3H, CH_3), 0.95 (3H, CH_3), 0.94 (3H, CH_3), 0.88 (3H, CH_3), 0.81 (3H, CH_3), 0.78 (3H, CH_3), 2.08-1.28 (24H, m). ^{13}C NMR (CD_3OD, 100 MHz): δ 38.3 (1), 26.7 (2), 78.5 (3), 38.7 (4), 55.6 (5), 18.3 (6), 33.1 (7), 39.6 (8), overlopped signal (9), 36.9 (10), 23.2 (11), 125.7 (12), 138.4 (13), 42.1 (14), 28.0 (15), 24.1 (16), overlopped signal (17), 53.2 (18), 39.2 (19), 39.2 (20), 30.6 (21), 36.9 (22), 27.6 (23), 14.8 (24), 15.2 (25), 16.5 (26), 22.9 (27), 180.5 (28), 16.6 (29), 20.4 (30).

Compound 4B: $C_{30}H_{48}O_3$, ^{1}H NMR (CD_3OD, 400 MHz): δ 5.23 (1H, m, H-12), 3.14 (1H, m, H-3), 2.84 (1H, dd, J = 13.8, J = 4.2 Hz, H-18), 1.16 (3H, s, CH_3), 0.97 (3H, s, CH_3), 0.94 (3H, s, CH_3), 0.90 (3H, s, CH_3), 0.84 (3H, s, CH_3), 0.78 (3H, s, CH_3), 0.78 (3H, s, CH_3). 2.08-1.28 (24H,m) ^{13}C NMR (CD_3OD, 100 MHz): δ 38.7 (1), 26.7 (2), 78.5 (3), 38.8 (4), 55.6 (5), 18.3 (6), 32.6 (7), 39.4 (8), overlopped signal (9), 37.0 (10), 22.8 (11), 122.5 (12), 144.0 (13), 41.7 (14), 27.5 (15), 22.9 (16), 46.5 (17), 41.6 (18), 46.1 (19), 30.4 (20), 33.7 (21), 32.4 (22), 27.7 (23), 14.7 (24), 15.1 (25), 16.5 (26), 25.2 (27), 180.5 (28), 32.8 (29), 23.3 (30).

When the ^{13}C-NMR spectrum is examined, the signal observed at δ_C= 180.5 ppm belongs to a carboxyl carbon. The presence of $\Delta^{12(13)}$ function in triterpenic structure was determined by carbon resonances at δ_C= 125.7 (CH; C-12), δ_C= 138.5 (C; C-13) and δ_C= 122.5 (CH; C-12), δ_C= 144.0 (C; C-13). Oleophinic proton (H-12) was observed at 5.23 ppm. These properties are characteristic for the triterpenic skeleton of the urs-12-ene and olean-12-ene, respectively (Baykal et al, 1998; Miyakoshi et al, 1997). The carbon 5 of both compounds was observed at 55.6 ppm. The signals belonging to the 9th carbons of compounds 4A and 4B and 17th carbon of 4A overlap with the solvent signals. Carbon 18 of the compound 4A resonated at 53.2 ppm and carbon 18 of the compound 4B resonated at 41.6 ppm. When the ^{1}H-NMR spectrum was examined, δ_H= 2.20 (d) signal belonged to proton C (18) of compound 4A, and δ_H= 2.84 (dd) signal belonged to proton C (18) of compound 4B. It was determined that the signals observed at δ_H= 0.78-1.18 ppm belong to the methyl protons of 4A and 4B based on the literature. Resonances at δ_H= 3.14 ppm and δ_C= 78.5 ppm showed the presence of a secondary hydroxyl group at the carbon 3 atom of both compounds (Jiang et al, 1995). Spectroscopic findings and literature records show that these components are a mixture of ursolic acid and oleanolic acid, respectively (Lin et al, 1987; Junges et al, 2000; Tundis et al, 2002; Maillard et al, 1992). The chemical structures of compounds 4A and 4B are shown in Figure 1-D and 1-E.

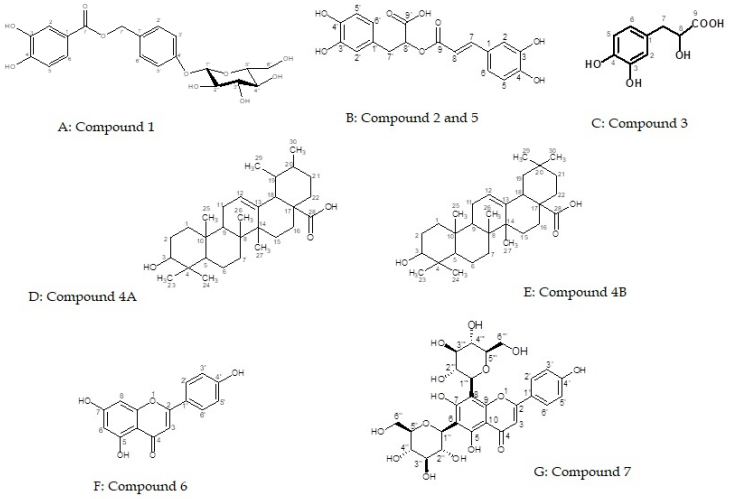

Figure 1. Chemical formulas of isolated compounds

Isolation studies of *O. minutiflorum*

Compound 5:

Similar to *O. micranthum*, rosmarinic acid was obtained from *O. minutiflorum*. Since the spectral values and interpretations of rosmarinic acid are described in the previous section, this compound is not disclosed in this section. The chemical structure of compound 5 is shown in Figure 1B.

Compound 6: $C_{15}H_{10}O_5$, ^{1}H-NMR (CD_3OD, 400 MHz) δ: 6.59 (1H, s, H-3), 6.20 (1H, d, *J*= 2.0 Hz, H-6), 6.45 (1H, d, *J*= 2.0, H-8), 7.86-7.84 (2H, AA'XX' system, AA', quasi d, *J*=9.0 Hz, H-2'ye H-6'), 6.94-6.91 (2H, AA'XX' system, XX', quasi d, *J*= 9.0 Hz, H-3' ve H-5').

^{13}C-NMR (CD_3OD, 100 MHz) δ: 165.1 (C-2), 102.6 (C-3), 182.6 (C-4), 161.6 (C-5), 99.0 (C-6), 164.9 (C-7), 93.9 (C-8), 158.3 (C-9), 102.6 (C-10), 122.1 (C-1'), 128.3 (C-2'), 115.8 (C-3'), 162.0 (C-4'), 115.8 (C-5') , 128.3 (C-6').

According to the ^{1}H-NMR spectrum of compound 6, the C-2' and C-6' proton signals were observed at δ_H= 7.86-7.84 (2H, AA'XX' system, AA', quasi d, *J*=9.0 Hz) ppm, the C-3' and C-5' proton signals were at 6.94-6.91 (2H, AA'XX' system, XX', quasi d, *J*=9.0 Hz) ppm, C-3, C-8 and C-6 protons were at δ_H = 6.59 (1H, s), 6.45 (1H, d, *J*= 2.0 Hz) and δ_H = 6.20 (1H, d, *J*= 2.0 Hz) ppm. This signal, seen as singlet at δ_H= 6.59 ppm, indicated that the molecule was flavone.

In the ^{13}C-NMR spectrum, 13 carbon signals were seen. It was decided that the signal seen at δ_C= 128.3 belongs to the C-2' and C-6' carbons and that the signal seen at δ_C= 115.8 belongs to the C-3' and C-5' carbons. These spectral values were consistent with apigenin when compared with the literature (Wawer and Zielinska, 2001; Li et al, 1997). The chemical structure of compound 6 is shown in Figure 1-F.

Compound 7: $C_{27}H_{30}O_{15}$ EIMS m/z 617 $[M+Na]^+$, ^{1}H-NMR (CD_3OD, 400 MHz) δ: 8.00 (2H, d, *J*= 8.4, 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 (12H, glucose protons)

^{13}C-NMR (CD_3OD, 100 MHz) δ: 164.7 (2), 103.3 (3), 182.9 (4), 159.0 (5), 108.1 (6), 161.8 (7), 105.9 (8), 155.8 (9), 104.5 (10), 122.2 (1'), 129.7 (2'), 116.5 (3'), 159.3 (4'), 116.5 (5') , 129.7 (6'), 74.0 (1"), 71.1 (2"), 78.4 (3"), 69.7 (4"), 81.5 (5"), 60.4 (6"), 74.8 (1'"), 72.5 (2'"), 79.5 (3'"), 71.5 (4'"), 82.5 (5'"), 61.8 (6'").

According to the ^{1}H-NMR spectrum of compound 7, the C-2' and C-6' proton signals were observed at δ_H= 8.00 (2H, d, *J*= 8.4 Hz) ppm, the C-3' and C-5' proton signals were at 6.88 (2H, d, *J*= 8.1 Hz) ppm, C-3 proton was at δ_H= 6.78 (1H, s) ppm. This signal, seen as singlet at δ_H= 6.78 ppm, indicated that the molecule was flavone. The signals observed at δ δ_H= 4.78 and 4.74 ppm belong to the anomeric protons of glucose molecules. The signals of the anomeric protons were doubled and the interaction constant *J*= 9.9 Hz showed that the glucose was in β configuration.

In the ^{13}C-NMR spectrum, 25 carbon signals were seen. It is supported by the literature that the signals seen at δ_C= 129.7 and δ_C= 116.5 ppm belong to C-2', C-6' and C-3', C-5', respectively. The signals seen between δ_C= 60.4-82.5 ppm belong to 2 glucose molecules. It was decided that the signals seen at δ_C= 74.0 and 74.8 ppm belong to the anomeric carbon atom by comparing with the literature.

The H-6 and H-8 proton signals, which were not observed in the ^{1}H-NMR spectrum, supported the binding of glucose molecules to the C-6 and C-8 carbons. These spectral values were consistent with vicenin-2 when compared with the literature (Xie et al, 2003; Hussein et al, 1997). The chemical structure of compound 7 is shown in Figure 1-G.

CONCLUSION

4(3,4-dihydroxy benzoyl oxymethyl)phenyl-β-D-glucopyranoside, rosmarinic acid, 3-(3,4-dihydroxyphenyl)-2-hydroxypropionic acid, ursolic acid, oleanolic acid were isolated from *O. micranthum*, rosmarinic acid, apigenin and vicenin-2 from *O. minutiflorum* by column chromatographic methods. Rosmarinic acid, ursolic acid, oleanolic acid, apigenin and vicenin-2 are frequently quantified in the

genus *Origanum* (Bellakhdar et al, 1988; Kaukaulitsa et al, 2006; Kosar et al, 2003; Sezen Karaoglan et al, 2017). However, 4(3,4-dihydroxy benzoyl oxymethyl) phenyl-β-D-glucopyranoside and 3-(3,4-dihydroxyphenyl)-2-hydroxypropionic acid are rarely encountered in this genus (Lin et al, 2008; González et al, 2017). Our findings are consistent with the literature records.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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