Original Article

UNVEILING THE CHEMICAL RICHES OF ORIGANUM ACUTIDENS: A QUEST FOR BIOACTIVE MOLECULES

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Abstract: Origanum species, such as Origanum acutidens, are valued for their rich essential oil constituents and find diverse applications in culinary and folk medicine. This study investigates the isolation and structural identification of key bioactive compounds from O. acutidens, revealing the presence of rosmarinic acid, lithospermic acid, vicenin-2, betulalbuside A, 8-OH-linaloyl glucoside, ursolic acid, and oleanolic acid metabolites. These compounds contribute to the plant's various biological properties, including antioxidant, antibacterial, and insecticidal activities. O. acutidens remains a promising source of phytochemicals with potential applications in the food and pharmaceutical industries.

Keywords: Origanum acutidens, essential oil, rosmarinic acid, lithospermic acid, vicenin-2, betulalbuside A, 8-OH-linaloyl glucoside, ursolic acid, oleanolic acid, bioactive compounds.

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 flavoring 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; 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, and 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 ºC (3 × 2 L). After filtration, the MeOH extracts were evaporated under vacuum to dryness. Methanol extract (50g) was dissolved in H_2O:MeOH (9:1) and partitioned with *n*-hexane, $CHCl_3$ 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. $CHCl_3$ 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 H_2O:MeOH (90:10, 80:20…..,100) solvent systems. Fractions 28-32 were further applied by column chromatography on silica gel ($CHCl_3$:MeOH:H_2O, 80:20:2, 70:30:3,…, 50:50:5). Fractions 26-37 was subjected to reversed phase silica gel column chromatography using H_2O: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 H_2O: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 H_2O:MeOH (90:10, 80:20…..,100).
Precipitate formed in the 43rd fraction was given vicenin-2. Fraction 44-45 were further applied by column chromatography on silica gel ($CHCl_3$:MeOH:H_2O, 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 $CHCl_3$ phase; rosmarinic acid from EtOAc phase; vicenin-2, betulalbuside A, 8-OH-linaloyl glycoside and lithospermic acid from the aqueous phase. ^{1}H-NMR and ^{13}C-NMR data were compared with literature data.

Ursolic acid

$C_{30}H_{48}O_3$, ^{1}H-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 (CH_3), 0.96 (CH_3), 0.95 (CH_3), 0.94 (CH_3), 0.88 (CH_3), 0.81 (CH_3), 0.78 (CH_3), 2.08-1.28 (m, 20 H) ^{13}C-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 (C7), 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

$C_{30}H_{48}O_3$, ^{1}H-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 (CH_3), 0.97 (CH_3), 0.94 (CH_3), 0.90 (CH_3), 0.84 (CH_3), 0.78 (CH_3), 2.08-1.28 (*m*, 24 H) ^{13}C-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

$C_{18}H_{16}O_8$, ^{1}H-NMR ($CDCl_3$, 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'). ^{13}C-NMR ($CDCl_3$, 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

$C_{27}H_{30}O_{30}$, ^{1}H-NMR ($CDCl_3$, 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), ^{13}C-NMR ($CDCl_3$, 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 (C7), 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 (C3‴), 71.5 (C-4‴), 82.5 (C-5‴), 61.8 (C-6‴) (Hussein et al.,1997; Xie et al., 2003).

Betulalbuside A

$C_{16}H_{28}O_7$, ^{1}H-NMR ($CDCl_3$, 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'). ^{13}C-NMR ($CDCl_3$, 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

$C_{16}H_{28}O_7$, ^{1}H-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 (C8), 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

$C_{27}H_{22}O_{12}$, ^{1}H-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, H20), 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.

References

Aligiannis, N., Kalpoutzakis, E., Mitaku, S., Chinou, I.B. (2001). Composition and antimicrobial activity of the essential oils of two *Origanum* species. Journal of Agricaltural and Food Chemistry, 49 (9), 4168-4170.

Baser, K.H.C. 1978. "The Turkish *Origanum* Species", in "Oregano; The Genera *Origanum* And *Lippia*" (S.E. Kintzios, ed.), Taylor & Francis, London and New York, pp. 109-126.

Original Article

Baser, K.H.C., Tumen, G., Duman, H. (1997). Essential Oil of *Origanum acutidens* (Hand.-Mazz.) letswaart, Journal of Essential Oil of Research, 9 (1), 91-92.

Baykal, T., Panayir, T., Taşdemir, D., Sticher, O., Calis, I. (1998). Triterpene saponins from *Scabiosa rotata*. Phytochemistry, 48 (5), 867-873.

Cai, X.F., Lee, I.S., Shen, G., Dat, N.T., Lee, J.J., Kim, Y.H. (2004). Triterpenoids from *Acanthopanax koreanum* Root and Their Inhibitory Activities on NFAT Transcrition. Archives of Pharmacal Research, 27, 825828.

Chiang, Y.M., Chang, J.Y., Kuo, C.C., Chang, C.Y., Kuo, Y.H. (2005). Cytotoxic triterpenes from the aerial roots of *Ficus microcarpa*. Phytochemistry, 66, 495-501.

Ceker, S., Nardemir, G., Alpsoy, L., Agar, G., Mete, E. (2012). Anti-Genotoxic and Anti-Oxidant Effects of *Origanum rotundifolium* on Human Lymphocytes In vitro. Jeobp., 15 (3), 415-423.

Dapkevicius, A., Van Beek, T.A., Lelyveld, G.P., Van Veldhuizen, A., Groot, A.D., Linssen, J.P.H., Venskutonis, R. (2002).Isolation and structure elucidation of radical scavengers from *Thymus vulgaris* leaves. Journal of Natural Products, 65, 892-896.

Hussein, S.A.M., Barakat, H.H., Nawwar, M.A.M, Willuhn, G. (1997). Flavonoids from *Ephedra aphylla*. Phytochemistry, 45, 1529-1532.

Jiang, Z.H., Zhou, R.H., Masuda, K., Ageta, H. (1995). A rearranged ursane triterpenoid from *Rhoiptelea chiliantha*. Phytochemistry, 40 (1), 219-224.

Junges, M.J., Fernandes, J.B., Vieria, P.C., Fernandes, M.F., Filho, E.R., Frühauf, M., Baranano, A.G. (2000). Triterpenous ursanicos e oleanicos do caule de *Eugenia florida* DC. Revista de Pesquisa E Pos-Graduaçao, 1, 13-20.

Kelley, C.J., Mahajan, J.R., Brooks, L.C, Neubert, L.A., Breneman, W.R., Carmack, M. (1975). Polyphenolics Acids of *Lithospermum ruderale* Dougl. Ex Lehm. (Boraginaceae). 1. Isolation and Structure Determination of Lithospermic acid. Journal of Organic Chemistry. 40(12), 1804-1815.

Kelley, C.J., Harruff, R.C., Carmack, M. (1976). Polyphenolics Acids of *Lithospermum ruderale* II. Carbon-13 Nuclear Magnetic Resonance of Lithospermic and Rosmarinic Acids. Journal of Organic Chemistry, 41(3), 449-455.

Kordali, S., Cakir, A., Ozer, H., Cakmakci, R., Kesdek, M., Mete, E. (2008). Antifungal, phytotoxic and insecticidal properties of essential oil isolated from Turkish *Origanum acutidens* and its three components, carvacrol, thymol and p-cymene. Bioresource Technology, 99, 8788-8795.

Lin, C.N., Chung, M.I., Gan, K.H., Chiang, J.R. (1987). Xanthones from formosan *Gentianaceous* plants. Phytochemistry, 26 (8), 2381-2384.

Maillard, M., Adewunmi, C.O., Hostettmann, K. (1992). A triterpene glycoside from the fruits of *Tetrapleura tetraptera*. Phytochemistry, 31(4), 1321-1323.

Original Article

Miyakoshi, M., Isoda, S., Sato, H., Hirai, Y., Shoji, J., Ida, Y. (1997). 3-alpha-hydroxy-oleanene type triterpene glycosyl esters from leaves of *Acanthopanax spinosus*. Phytochemistry, 46 (7), 1255-1259.

Novak, J., Bitsch, C., Langbehn, J., Pank, F., Skoula, M., Gotsiou, Y., Franz, C.M. (2000). Ratios of cis- and transSabinene Hydrate in *Origanum majorana* L. and *Origanum microphyllum* (Bentham) Vogel. Biochemical Systematics and Ecolgy, 28 (7), 697-704.

Ryman, D. (1992)."Aromatherapy, The encyclopaedia of plants and oils and how they help you″, Piatkus, UK, pp. 163-165.

Sokmen, M., Serkedjleva, J., Dalerera, D., Gulluce, M., Pollsslou, M., Tape, B., Akpulat, H.A., Sahin, F., Sokmen, A.(2004). In vitro antioxidant, antimicrobial and antiviral activities of the essential oil and various extracts from herbal parts and callus cultures of *Origanum acutidens*. Journal of Agricaltural and Food Chemistry, 52, 3309-3312.

Tundis, R., Deguin, B., Menichini, F., Tillequin, F. (2002). Iridoids from *Putoria calabrica*. Biochemical Systematics and Ecology, 30, 689-691.

Woo, E.R., Piao, M.S. (2004). Antioxidative Constituents from *Lycopus lucidus*. Archives of Pharmacal Research, 27, 173-176.

Xie,, C., Veitch, N.C., Houghton, P.J., Simmonds, M.S.J. (2003). Flavone C-glycosides from *Viola yedoensis*. Chemical and Pharmaceutical Bulletin, 51(10), 1204-1207.

Yalçın, F.N., Ersoz, T., Akbay, P., Çalış, I. 2003. Phenolic, Megastigmane, Nucleotide, Acetophenon and Monoterpene Glycosides from *Phlomis samia* and *P. carica*. Turkish Journal of Chemistry, 27, 703-711.