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Original article

Essential oil composition of two *Origanum* L. taxa from Bingol (Turkey)

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Summary. In this study aerial parts of the essential oils of *Origanum acutidens* L. and *Origanum vulgare* L. subsp. *gracile* (K. Koch) Ietswaart taxa were analyzed by HS-SPME/GC-MS. As a result forty one and thirty seven components were identified representing 89.7% and 90.4% of the oil, respectively. Carvacrol (37.5%), thymol (22.7%) and *p*-cymene (7.6%) were detected as main compounds of *O. acutidens*; carvacrol (30.8%), thymol (26.8%) and γ-terpinene (12.1%) were detected as the major constituents of *O. vulgare* subsp. *gracile*. With this study, chemotypes of studied taxa were identified as carvacrol and thymol. Additionly, the studied plant samples were found to be rich in essential oils. The results are discussed in respect to natural products, renewable resources and chemotaxonomy.

Key words: *Origanum*, essential oil, HS-SPME/GC-MS

Introduction

The Lamiaceae or Labiatae family (the mint family) occurs in more than 7200 species across approximately 240 genera which are classified in 7 subfamilies, which have a world-wide distribution (1). *Origanum* L. (oregano) is an important genus of the Lamiaceae family and comprises about 900 species, widespread throughout the world. In addition this genus contains some multipurpose medicinal plants and comprises 42 species and 18 hybrids widely distributed in Eurasia and North Africa (2). Members of *Origanum* genus are suffriticose or herbaceous perennials, hairy or glabrous, with several stems, ascending or erect, usually branched and comprises 8 sections, 43 species and 18 hybrids, most of them distributed in Anatolia, which means that nearly 50% of all *Origanum* taxa (23 species, 3 subspecies, 1 variety and 5 hybrids) are recorded to grow in Turkey. This means that 16 of 32 taxa are endemic (3, 4).

The taxa of *Origanum* are known in Turkey as "Yalancı kekik", "Kekik", "İstanbul kekiği" and "Keklik otu" Turkish characters must be written in English. *Origanum* taxa are traditionally used as sedative, diuretic, degasifier, sweater and antiseptic. They are also used for the treatment of gastrointestinal diseases and constipation. They are also used as spicy additives for food as an alternative to thyme. They are rich in essential oils and bitter substances. There are some reports on the chemical compositions and various biological activities of *Origanum* taxa (5). Medicinal and aromatic plants are valued for biological activities which can be justified from the fact that about 80% of the local population still depend on these plants for primary health care. The formation and accumulation of essential oil in plants has been reviewed by many workers (6). The compounds from the plant based essential oils are useful as an alternative therapy, either directly or as models for new synthetic products (7).

Some *Origanum* taxa are pungent, bitter, hot, stomachic, anthelmintic, alexipharmic, useful in diseases of the heart and blood, fevers, leucoderma and inflammation (8). An infusion of *Origanum* is used as a stimulant, sudorific, emmenagogue and galactagogue and is also useful in asthma, hysteria, paralysis and antibacterial activity (9). Tsimidou and Boskou (10) concluded that among the herbs and spices extensively studied, the plants obtained from the Labiatae family possess a significant antioxidant activity. Lagouri et al. (11) studied the antioxidant activity of essential oils and they found that oregano essential oil, rich in thymol and carvacrol, has a considerable antioxidant effect on the process of lard oxidation. In recent studies, Kilic and Bagci (5) examined the essential oil of *Origanum vulgare* subsp. *gracile* grown in Turkey, as well as the probability of using the plant as herbal tea. They detected thymol and carvacrol as the main compounds. Carvacrol and thymol are the main antimicrobial and antioxidant monoterpene phenolic compounds that constitute about 78–85% of *Origanum* essential oil. In addition to the antimicrobial and antioxidant properties, carvacrol and thymol provide the characteristic flavor and odor (12). The antimicrobial activity of these compounds is attributed to their lipophilic character that makes them more attractive to the cell membrane structures. Consequently, their presence cause membrane expansion, increases fluidity and permeability, disturbs embedded proteins, inhibits respiration, and alters ion transport processes (13). These compounds act as antioxidant agents quenching free radicals by donating hydrogen atoms or electrons, retarding lipid oxidation (14).

O. acutidens is an endemic species generally growing in northeastern Turkey; subshrub to 50 cm, branches to 10 pairs per stem, leaves subsessile, ovate, obtuse, glaucous; verticillaster 2-12 flowered; calyx 5-7.5 mm; corolla white or tinged pink; Fl.6-8; habitat generally calcerous and non-calcareous rocks, slopes and screes, 1000-3000 m. (4). *O. acutidens* has antitumor activity against breast cancer cell lines (15). *O. vulgare* is a perennial herb, to 100 cm, adpressed pilose, hirsute, or glabrous and often pruinose, corolla purple, pink or white and has four subspecies (subsp. *gracile* (K.Koch) Ietswaart, *hirtum* (Link) Ieswaart, *vulgare*, ve *viride* (Boiss.) ?Hayek) in Flora of Turkey (4).

The present study sought to investigate the essential oil compounds of *Origanum acutidens* and *Origanum vulgare* subsp. *gracile*, to explain the chemotaxonomic significance and to determine chemotypes and to potential usefulness of studied samples.

Materials and Methods

Plant materials

Origanum acutidens was collected at the flowering stage in July 2016 in vicinity of Şaban village, Bingöl, Turkey. *Origanum vulgare* subsp. *gracile* was collected from Bingöl-Solhan, Hazarşah village, Aksakal Göl hamlet, stony and igneous slopes of stream 1600-1700 m, in June 2016. The taxonomic identification of plant materials was confirmed by plant taxonomist Dr. Ömer Kılıç, in Technical Vocational College, Bingöl University, Bingöl, Turkey. The voucher specimens have been deposited at the Department of Park and Garden Plants of Bingol University.

HS-SPME Procedure

"Five grams powder of aerial part of studied samples were carried out by a (HS-SPME) head space solid phase microextraction method using a divinyl benzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber, with 50/30 um film thickness; before the analysis the fiber was pre conditioned in the injection port of the gas chromatography (GC) as indicated by the manufacturer. For each sample, 5 g of plant samples, previously homogenized, were weighed in to a 40 ml vial; the vial was equipped with a "mininert" valve. The vial was kept at 35°C with continuous internal stirring and the sample was left to equilibratefor 30 min; then, the SPME fiber was exposed for 40 min to the headspace while maintaining the sample at 35°C. After sampling, the SPME fiber was introduced into the GC injector, and was left for 3 min to allow the analyzes thermal desorption. In order to optimize the technique, the effects of various parameters, such as sample volume, sample headspace volume, sample heating temperature and extraction time were studied on the extraction efficiency as previously reported by Verzera et al. (16).

GC-MS Analysis

"A Varian 3800 gas chromatograph directly inter faced with a Varian 2000 ion trap mass spectrometer (VarianSpa, Milan, Italy) was used with injector temperature, 260°C; injection mode, splitless; column, 60 m, CP-Wax 52 CB 0.25 mm i.d., 0.25 um film thickness. The oven temperature was programmed as follows: 45°C heldfor 5 min, then increased to 80°C at a rate of 10°C/min, and to 240°C at 2°C/min. The carrier gas was helium, used at a constant pressure of 10 psi; the transfer line temperature, 250°C; the ionisation mode, electron impact (EI); acquisit ion range, 40 to 200 m/z; scan rate, 1 us^{-1}. The compounds were identified using the NIST (National Institute of Standardsand Technology) library (NIST/WILEY/EPA/NIH), mass spectral library and verified by the retention indices which were calculated as described by Van den Dool and Kratz (17). The relative amounts were calculated on the basis of peak-area ratios. The identified constituents are listed in Table 1.

Table 1. Identified components of *Origanum* taxa (%).

Compounds	**RRI**	***O. acutidens***	***O. vulgare* subsp. *gracile***
Thujene	995	1.1	0.4
α-pinene	1021	0.9	0.7
Camphene	1034	0.5	0.1
Sabinene	1050	0.3	1.3
δ-3-carene	1052	-	0.1
1-octan-3-ol	1053	0.4	-
β-pinene	1055	0.3	0.1
3-oktanone	1060	0.5	-
β-mirsene	1064	1.0	1.4
α-phallendrene	1077	0.2	0.3
α-terpinene	1086	2.1	1.9
P-cymene	1093	7.6	2.3
Limonene	1095	0.4	-
1,8-cineole	1098	0.2	1.4
Cis-ocimene	1100	-	2.2
γ-terpinene	1119	5.0	12.1
Cis-sabinen-hydrate	1127	0.2	0.1
α-linalool	1148	0.7	0.2
Cis-anethol	1166	0.3	-
1-Borneol	1200	1.2	0.2
4-Terpineol	1285	0.8	0.1
Thymol	1297	22.7	26.8
Carvacrol	1302	37.5	30.8
Nerol acetate	1337	0.3	-
α-copaene	1360	-	0.1
β-caryophyllene	1366	0.1	-
Trans-caryophyllene	1394	0.5	2.4
β-gurjunene	1400	0.1	-
Aromadendrene	1406	0.1	0.2
α-Humulene	1410	-	0.1
Naphthalene	1415	0.2	0.3
Trans-verbenol	1420	0.1	-
Ledene	1425	0.2	0.1
γ-muurolene	1431	-	0.3
Germacrene-D	1435	0.3	0.4
Bicyclogermacrene	1436	0.2	0.2
Carvone	1440	0.1	1.6
Viridiflorene	1441	0.2	-
β-bisabolene	1452	0.1	0.3
α-amorphene	1455	-	0.2
δ-cadinene	1458	0.1	-
β-sesquiphellandrene	1462	0.1	0.2
Cis-α-bisabolene	1472	0.3	-
Spathulenol	1495	2.7	0.4
Caryophyllene oxide	1498	0.5	0.7
α-cadinol	1545	0.2	0.2
İzoaromadendrene epoksit	1547	-	0.1
2-Pentodecanone	1631	0.1	-
Ericosane	1699	-	0.1
Total		**89.7**	**90.4**

RRI*: Relative Retention Index

Results

In this study, carvacrol (37.5%), thymol (22.7%) and *p*-cymene (7.6%) were detected as the main compounds of *O. acutidens*; carvacrol (30.8%), thymol (26.8%) and γ-terpinene (12.1%) were detected as the major constituents of *O. vulgare* subsp. *gracile*.

O. acutidens and *O. vulgare* subsp. *gracile* included high concentrations of thymol (22.7% - 26.8%, respectively) and carvacrol (37.5% - 30.8%, respectively) (Table 1). These compounds are also major constituents of *O. vulgare* subsp. *gracile* from different vegetation periods (unflowered, flowered and seeded) in Elazığ vicinity (5). The main constituents of the essential oils of this subspecies were found as thymol, γ-terpinene, α-terpinolene, carvacrol, p-cymene. It is also determined that some components show differences in different vegetation periods qualitatively and quantitatively.

Discussion

While *p*-cymene, α–terpinene and thymol amount has increased in seeded vegetation periods, α–terpinolene and carvacrol amounts has found to be more in flowered vegetation periods (5). Sivropoulou et al. reported that three *Origanum* essential oils, *Origanum vulgare* subsp. *hirtum*, *Origanum dictamnus*, and a commercially available *Origanum* oil were analyzed by gas chromatography-mass spectrometry (GC-MS) and showed a high content of carvacrol, thymol, γ-terpinene, and *p*-cymene representing 73.7%, 92.8%, and 87.8% of the total oil, respectively (18). Similarly in this research carvacrol, thymol, γ-terpinene, and *p*-cymene constituted a high content of studied samples (Table 1). Significant quantitative differences between the two oils were apparent only between the two isomeric phenols, carvacrol and thymol, and their biosynthetic precursors γ-terpinene and *p*-cymene. The concentration of other components varied greatly among the two oils but particularly that of carvacrol (37.5- 30.8%) and thymol (22.7-26.8%) (Table 1). Due to its low content of carvacrol, the commercial *Origanum* oil cannot be characterized as a typical "oregano" oil (19). The high amount of carvacrol found in the *O. vulgare* subsp. *gracile* and *O. acutidens* oils (Table 1) have also been observed in several other Greek wild populations of *Origanum* taxon. It should be noted that in some cases thymol, instead of carvacrol, is the major component of the Greek (20) and Turkey oregano essential oils (Table 1).

In another study, forty-one constituents were determined in the essential oil of *O. microphyllum*, representing 98.66% of the oil. The oil was characterized by the presence of terpin-4-ol (24.86%), γ-terpinene (13.83%), linalool (10.81%) (21). On the other hand, sabinene (14.24-24.23%), cis-sabinene hydrate (22.45-31.09%), trans-sabinene hydrate (12.42-26.34%), and linalool (9.37- 14.16%) were found as the main volatile constituents of *O. microphyllum*, from CH_2Cl_2 leaf extract and from the leaves-flowers (separately) using the headspace method, as reported by (22). Whereas in the present study the essential oils of studied *Origanum* taxa were shown to contain mainly carvacrol, thymol, *p*-cymene, γ-terpinene and other compounds (Table 1), these differences probably depend on the different analytical method, different environmental factors as well as on the different plant material investigated. Baydar et al., (23) reported that the major constituent of the oils determined by GC was cavracrol (86.9% in *O. onites*, 84.6% in *O. minutiflorum*, 75.5% in *T. spicata* and 53.3% in *S. cuneifolia*). Similarly, in this research carvacrol was the major compound of studied samples (Table 1). Among the monoterpenes, *p*-cymene was found in high percentage of *O. acutidens* (7.6%) and in low percentage of *O. vulgare* subsp. *gracile* (2.3%) (Table 1).

In conclusion, *Origanum acutidens* and *Origanum vulgare* subsp. *gracile* evidenced a similarity, with reference to the presence of the main constituents; carvacrol and thymol were among the principal one in both species. Also the percentages of *p*-cymene, γ-terpinene, spathulenol and other compounds were comparable. This study demonstrates the occurrence of carvacrol and thymol chemotypes of *Origanum acutidens* and *Origanum vulgare* subsp. *gracile* in Eastern Anatolian region of Turkey. In addition, the essential oil results have given some clues on the chemotaxonomy of the genus patterns and usability of these species as natural product. According to these results, studied plants were found to be rich in respect to essential oils. So these plants can be used different purposes in industry, ethnobotany and can be cultivated to richened natural products. In addition, many plant species are threatened due to overharvesting for medicinal or other use, so there is great need to protect plant diversity. There is also a need to develop more sustainable ways of obtaining industrial products from renewable resources. The cultivation of medicinal and aromatic plants for industrial products can address these issues. Futhermore the essential oils were natural products preventing the growth of foodborne pathogens or spoilage organisms in the test systems. Further work is necessary to explore the efficacy, and palatability, of suitable concentrations of these essential oils in foods.

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