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RESEARCH ARTICLE

SPME/GC-MS analysis of volatile organic compounds from *Origanum acutidens* (Hand.-Mazz.) Ietsw. - An endemic species in Turkey

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Abstract

In this present study, it was aimed to determine the composition of the essential oil obtained by water distillation from the aerial parts of *Origanum acutidens*. The oil was analysed by GC-FID and GC/MS. Carvacrol (69.3%), *p*-cymene (8.3%), γ-terpinene (6.3%), β-caryophyllene (2.7%) and borneol (2.2%) were found as main constituents. In addition, headspace-solid phase microextraction using a Polydimethylsiloxane-Divinylbenzene (PDMS/DVB – 65 µm) fiber of *O. acutidens* was performed and analysed by GC-FID and GC/MS. The main components were identified as: carvacrol (44.0%), *p*-cymene (25%), ϒ-terpinene (8%), β-caryophyllene (6.1%) and borneol (6.1%), respectively.

Keywords: *Origanum acutidens*, essential oil, carvacrol, Lamiaceae, HS-SPME

Introduction

The family Lamiaceae, which includes many plants with medicinal properties, is known for its richness of species, and most species are common in the Mediterranean. The family Lamiaceae is represented in Turkey by 782 taxa of which 346 taxa are endemic (44%). 24 of 28 hybrids discovered in the flora of Turkey are endemic (Başer et Kırımer, 2007).

The genus *Origanum* L. is an important member of the Lamiaceae family, represented by 31 taxa, 10 of which are hybrids in Turkey (Baytop, 1999; Davis, 1982, Celep & Dirmenci, 2017). Many studies were reported on the chemical composition and various biological activities of *Origanum* species, which are rich in essential oils.

Origanum species, traditionally are used as a sedative, antiseptic, against sore throat, diuretic, carminative and also in the treatment of gastrointestinal diseases and constipation (Baytop, 1999, Baser, 2008).

The endemic *Origanum acutidens* (Hand.-Mazz.) Ietsw. is a herbaceous perennial plant, flowering between June and August in Eastern Anatolia (Davis, 1982; Ietswaart , 1980) Growing on limestone and in non-calcareous soils between 1000-3000 m, sometimes in shady places, the plant needs very little moisture during the growing season In addition, *O. acutidens*, which can be widely used in urban landscape management, has showy, bright pink flowers and is an ornamental plant preferred due to its aromatic scent and its capacity to attract butterflies and bees (Karagoz and Karagoz, 2019). The species, which can grow up to 50 cm in length, is in hairless form, and its leaves bear intense odor (Ietswaart, 1980). *O. acutidens*, which is rich in carvacrol and thymol, is also rich in rosmarinic acid and therefore has antioxidant activity (Kordali et al., 2008). In addition, *O. acutidens* growing in the Eastern Anatolia region contains >20% thymol (Karagoz and Karagoz, 2019; Kordali et al., 2008). Essential oil yields of *O. acutidens* range from 0.73 to 1.7%

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(Cosge et al., 2009; Cakmakcı et al., 2009). Essential oil compositions of *O. acutidens* were determined by several studies (Table 1).

Table 1. Previous studies on essential oils of *O. acutidens*

Part distilled	**Main components (%)**	**Reference**
Aerial parts	Carvacrol (66.25%) *p*-Cymene (13.99%) Bomeol (1.96%) β-Caryophyllene (1.7%)	(Baser et al.,1997)
Aerial parts	Carvacrol (87.0%) *p*-Cymene (2.0%) Linalool acetate (1.7%) Borneol (1.6%) β-Caryophyllene (1.3%)	(Kordali et al., 2008)
Aerial parts	Carvacrol (67.5%) *p*-Cymene (14.0%) Borneol (1.5%) Terpinene 4-ol (1.4%)	(Figuérédo et al., 2006)
Aerial parts	Calvacrol (61.8 %) *p*-Cymene (15.5 %) Thymol (12.7 %)	(Gulec et al., 2014)
Aerial parts	Carvacrol (86.99 %) *p*-Cymene (1.95 %) Borneol (1.63 %) Linalool acetate (1.65 %) β-Caryophyllene (1.30 %)	(Tozlu et al., 2011)
Aerial parts	Carvacrol (76.2 %) *p*-Cymene (7.4 %) Borneol (3.2 %) γ-Terpinene (1.38%) Terpinene-4-ol (1.04%)	(Figuérédo et al., 2012)
Aerial parts	Carvacrol (61.8 %) *p*-Cymene (15.5 %) Thymol (12.7 %) γ-Terpinene (1.4%) Borneol (1.2%) Hexadecanoic acid (1.2%)	(Gulec et al., 2014)
Aerial parts and corollas	Carvacrol (67.51% and 52.33%) *p*-Cymene (9.43% and 17.51%) β-Caryophyllene (1.62 % and 2.04%) Tymoquinone (3.80% and 2.85%) Isoborneol (1.36% and 2.21%) β-Thujone (1.31% and 1.93%) β-Caryophyllene (1.62% and 2.04%)	(Cosge et al., 2009)
Aerial parts	Carvacrol (65%) *meta*-Cymene (9.15 %) *trans*-Caryophyllene (4.43 %) γ-Terpinene (3.54 %) α-Terpinene (1.86%) Thymol (1.10%)	(Goze et al., 2010)

Herbal parts	Carvacrol (72.0 %) *p*-Cymene (7.5 %) ϒ -Terpinene (5.3 %) β-Myrcene (1.7 %) β-Caryophyllene (1.0 %)	(Sokmen et al., 2004)
Aerial parts	Carvacrol (61.69%) *p*-Cymene (17.32%) ϒ -terpinene (4.05%) Borneol (3.96%)	(Altıntas and Demirtas, 2017)
Aerial parts	*m*-Cymene (39.2%) τ-Terpinene (3.4 %) ϒ- Terpinene (1.4 %) *allo*-aromadendrene (24.8%)	(Yilmaz et al., 2017)
Herbal parts	Carvacrol (47.46%) *p*-Cymene (22.22%) Borneol (3.41%) γ-Terpinene (2.91%) β-Caryophyllene (2.70%) Linalool (2.35%) 3-Octanone (1.84%)	(Cetin et al., 2011)

As seen in Table 1, carvacrol ratios of *O. acutidens* oils were reported as: 87.0% (Kordali et al., 2008; Tozlu et al., 2011); 76.2% (Figueredo et al., 2012); 67.5% (Cosge et al., 2009); 66.3% (Baser et al., 1997) and 65.0% (Goze et al.,2010). In addition, the essential oil obtained from *O. acutidens* was the main component of the oil, carvacrol followed by *p*-cymene, while other important components were borneol, thymol, β-caryophyllene, γ-terpinene, linalool, linalyl acetate, β-myrcene, *3*-octanone and thymol. Aerial parts of the plants were often subjected to distillation procedures by different researchers. In general, monoterpenes were the main groups in essential oils.

The SPME method has some advantages over traditional methods. SPME combines sampling, extraction, concentration and sample entry in a single solvent-free stage. Analytes in the sample are extracted directly on the extraction fiber. The method saves preparation time and disposal costs and can improve detection limits (Vas and Vekey, 2004).

The aim of this study is to reveal the essential oil content of *O. acutidens* by distillation as well as to determine the differences in volatile organic compound distribution using HS-SPME analysis.

Materials and Methods

Plant material and isolation of essential oil

O. acutidens was collected from Erzurum province (40° 9' 33 "K, 41° 2' 52" D, 2030 m.) of Turkey during flowering in July 2019. Voucher specimens are stored in the Herbarium of the Faculty of Pharmacy of Anadolu University, in Eskisehir, Turkey (ESSE 15529). Essential oil was obtained from the dried plant samples by hydrodistilation for 3 hours with the Clevenger-type apparatus and hydrodistillation of *O. acutidens* with a yield of 0.8 % (w/v) of essential oil.

Analysis of the essential oil

The oil was analyzed by capillary GC and GC/MS using a Agilent GC-MSD system.

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Headspace-solid phase microextraction (HS-SPME) analysis

30°C and 30 min sampling was carried out on plant materials using SPME fibre Polydimethylsiloxane-Divinylbenzene (PDMS/DVB - 65µm) - Blue (supplied by Supelco Bellefonte, USA). The fiber was directly desorbed at 250ºC for 10 min. in GC/MS.

Gas chromatography (GC) and gas chromatography - mass spectrometry (GC/MS) analysis

GC-MS conditions

The oil was analyzed by capillary GC/MS using an Agilent GC-MSD system (Agilent Technologies Inc., Santa Clara, CA). HP-Innowax FSC column (Hewlett-Packard-HP, U.S.A.) (60 m × 0.25 mm i.d., with 0.25 µm film thickness) was used for separation of components in the oil and helium as a carrier gas (0.8 mL/min). The GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. The split flow was adjusted at 40:1 split ratio (at splitless mode for SPME). The injector temperature was set at 250 °C. Mass spectra were taken at 70 eV with the mass range *m/z* 35-450.

GC conditions

The GC analysis was done with Agilent 6890N GC system fitted with a FID detector set at a temperature of 300°C. To obtain the same elution order with GC–MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms by using Agilent ChemStation Plus® software with peak integration process.

Identification of compounds

Identification of essential oil components were performed by comparison of their mass spectra with those in the Baser Library of Essential Oil Constituents, Wiley GC/MS Library, Adams Library, MassFinder Library and confirmed by comparison of their retention indices. A homologous series of *n*-alkanes (C8–C24) were used as the reference points in calculation of relative retention indices (RRI). The relative percentages of the separated compounds were calculated from FID chromatograms. The analysis results are expressed as mean percentage as listed in Table 2 and Table 3.

Results and Discussion

O. acutidens oil yield was 0.8%. Twenty four compounds were identified in oil of the aerial parts representing 99.7 % of the *O. acutidens* essential oil. The main components of the oil were carvacrol (69.3%) and *p*-cymene (8.3%). As seen in Table 2, the terpenoids consist of the main portion of *O. acutidens* essential oil and oxygenated monoterpenes (73.5%) were the main group of constituents, followed by monoterpene hydrocarbons (19.6%), sesquiterpene hydrocarbons (5.0%), oxygenated sesquiterpenes (0.3%) and others (1.3%).

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Table 2. The composition of the essential oil of *O. acutidens*

No	RRI	Compounds	%	IM
1	1032	α-Pinene	0.6	t_R, MS
2	1035	α-Thujene	1.0	MS
3	1076	Camphene	0.5	t_R, MS
4	1174	Myrcene	1.7	t_R, MS
5	1188	α-Terpinene	1.2	t_R, MS
6	1255	γ-Terpinene	6.3	t_R, MS
7	1265	3-Octanone	0.8	t_R, MS
8	1280	*p*-Cymene	8.3	t_R, MS
9	1452	*1*-Octen-*3*-ol	0.5	t_R, MS
10	1474	*trans*-Sabinene hydrate	0.5	t_R, MS
11	1556	*cis-Sa*binene hydrate	0.3	t_R, MS
12	1611	Terpinen-4-ol	0.8	t_R, MS
13	1612	β-Caryophyllene	2.7	t_R, MS
14	1628	Aromadendrene	0.2	MS
15	1630	*trans*-Dihydrocarvone	0.1	t_R, MS
16	1634	*cis*-Dihydrocarvone	0.1	t_R, MS
17	1687	α-Humulene	0.1	t_R, MS
18	1708	Ledene	0.2	MS
19	1719	Borneol	2.2	t_R, MS
20	1726	Germacrene D	0.7	MS
21	1755	Bicyclogermacrene	1.1	t_R, MS
22	2144	Spathulenol	0.3	t_R, MS
23	2198	Thymol	0.2	t_R, MS
24	2239	Carvacrol	69.3	t_R, MS
		Monoterpene hydrocarbons	19.6	
		Oxygenated monoterpenes	73.5	
		Sesquiterpene hydrocarbons	5.0	
		Oxygenated sesquiterpenes	0.3	
		Others	1.3	
		Total (%)	99.7	

RRI: Relative retention indices experimentally calculated against n-alkanes; IM: Identification Method: t_R, Identification based on comparison with co-injected with standards on a HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the in-house Baser Library of Essential Oil Constituents, Adams, MassFinder and Wiley libraries (Adams, 2007; Hochmuth, 2008; McLafferty and Stauffer, 1989).

The volatile compounds were also obtained by the HS-SPME method and analyzed by GC-FID and GC/MS. The volatiles were trapped by SPME in a dynamic headspace set up. A blue - Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) fibre was used for 30 min. Seventeen volatile compounds were identified in the aerial parts representing 100.0% of the total headspace volatiles. Main components were identified as carvacrol (44%), *p*-cymene (25.0%), ϒ -terpinene (8.0 %), β-caryophyllene (6.1%) and borneol (6.1%) (Table 3).

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Table 3. Headspace volatiles of *O. acutidens*

No	RRI[a]	Compounds	%	IM
1	1174	Myrcene	2.0	t_R, MS
2	1188	α-Terpinene	2.3	t_R, MS
3	1255	y-Terpinene	8.0	t_R, MS
4	1265	*3*-Octanone	0.8	t_R, MS
5	1280	*p*-Cymene	25.0	t_R, MS
6	1393	*3*-Octanol	0.4	MS
7	1452	*1*-Octen-3-ol	1.3	t_R, MS
8	1474	*trans*-Sabinene hydrate	0.5	t_R, MS
9	1556	*cis*-Sabinene hydrate	0.3	t_R, MS
10	1611	Terpinen-4-ol	1.1	t_R, MS
11	1612	β-Caryophyllene	6.1	t_R, MS
12	1628	Aromadendrene	1.1	MS
13	1630	*trans*-Dihydrocarvone	0.5	t_R, MS
14	1634	*cis*-Dihydrocarvone	0.2	t_R, MS
15	1719	Borneol	6.1	t_R, MS
16	2198	Thymol	0.3	t_R, MS
17	2239	Carvacrol	44.0	t_R, MS
		Total (%)	100.0	

RRI: Relative retention indices experimentally calculated against n-alkanes; IM: Identification Method: t_R, Identification based on comparison with co-injected with standards on a HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the in-house Baser Library of Essential Oil Constituents, Adams, MassFinder and Wiley libraries (Adams, 2007; Hochmuth, 2008; McLafferty and Stauffer, 1989).

Figure 1. General view of *O. acutidens*

In this study, the essential oil content of the hydrodistilled herbal parts of *O. acutidens* was revealed and the differences in the volatile organic compound distribution were determined by HS-SPME analysis. Twenty-four components constituting 99.1% of the total components detected were characterized while seventeen volatile components were identified representing 100.0% of the total headspace volatiles. Our results indicated that carvacrol was the main constituent (44.0-SPME; %-69.3%-EO) in the oil of *O. acutidens*.

The essential oil composition of *O. acutidens* was previously reported (Baser et al., 1997; Caglar et al., 2007; Figuérédo et al., 2006; Kordali et al., 2008; Sokmen et al., 2004; Yildirim et al., 2005), where it was shown that carvacrol (66.0–72.0%) and *p*-cymene (7.5–14.0%) were the major components of the oil. In another study, Gulec *et al.,* (2014) reported that the *O. acutidens* essential oil and its aromatic monoterpene components showed potent antibacterial activity against pathogenic bacterial strains. In addition, carvacrol (61.8 %), *p*-cymene (15.5 %) and thymol (12.7 %) that possessed antimicrobial activity were found as the major components. Findings were almost in agreement with the previous studies.

Although it was stated in previous studies on *Origanum* species that their essential oils are rich in carvacrol, thymol, *y*-terpinene and *p*-cymene and carvacrol is the main component (Bouchra et al., 2003; Daouk et al., 1995; Esen et al., 2007; Mueller-Riebau et al., 1995; Soković et al., 2002; Soylu et al., 2006), in some *Origanum* oils belonging to different genotypes, thymol had a higher percentage than carvacrol (Bendahou et al., 2008; Daferera et al., 2003; Esen et al., 2007). When our findings compared with previous data, carvacrol content in *O. acutidens* (69.3%-44.0%) was much higher than thymol (0.2%-0.3%). Other volatile components were similar to studies in the literature. Some slight differences among the volatile components in our study and previous studies were observed (Table 1).

Since the study is aimed to determine the natural scent of *O. acutidens*; HS-SPME was made at room temperature, the ratio of natural fragrance compounds given by the plant to the environment were determined and the amount of carvacrol was found to be lower than in essential oil.

Recent researches have revealed that carvacrol is very effective against cancer cells. It has been reported to be effective against prostate cancer cells, lung cancer cells, oral cancer cells and brain tumors (Junk et al., 2018; Liang & Lu, 2012; Ozkan & Erdogan, 2012; Baser, 2008). Therefore, the species with high carvacrol content like *O. acutidens* are important not only commercially but also for biological activity.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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