Leaf and Flower Volatile Oil Components of Two Thyme Taxa
Origanum onites L. and Thymbra spicata var. spicata L. in Turkey

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Abstract

Medicinal and aromatic plants have a special importance with volatile oil components. Lamiaceae family members are important in pharmacology and perfumery industry because they contain volatile and aromatic oil. Origanum onites L. and Thymbra spicata var. spicata L. are the most widely used and most exported species. The volatile components of the leaves and flowers of Origanum onites L. and Thymbra spicata var. spicata taxa were determined by Headspace Solid Phase Microextraction (HS-SPME) technique combined with gas chromatography/mass spectrometry (GC / MS). 33 different components of Origanum onites were identified and the main components were p-cymene (11.45%), γ-terpinene (11.89%), linalool (14.35%), thymol (20.03%) and carvacrol (26.91%), respectively. For Thymbra spicata var. spicata L., 36 different compounds were identified and the main components were p-cymene (11.72%), γ-terpinene (10.96%), linalool (13.44%), thymol (18.92%) and carvacrol (27.34%), respectively. Oxygen containing monoterpenes have been found to be high.

Keywords: Origanum onites L., Thymbra spicata var. spicata L., SPME, carvacrol, thymol, Turkey

İki Kekik Taksonu Origanum onites L. ve Thymbra spicata var. spicata L.’nin Yaprak ve Çiçek Uçucu Bileşenleri

Öz

Tibbi ve aromatik bitkiler grubunda özellikle uçucu yağ içeriği açısından zengin olanların ayrı bir önemi bulunmaktadır. Lamiaceae familyasının ihracatını en çok yapılan ve uçucu yağ üretiminde kullanılan türler; Origanum onites L. ve Thymbra spicata var. spicata L. dir. Origanum onites L. ve Thymbra spicata var. spicata L. taksonlarını yaprak ve çiçeklerinin uçucu bileşenleri gaz kromatografisi/kütte spektrometresi (GC/MS) ile kombine edilmiş Tepe Boğluğu-Katı Faz Mikro Ekstraktisyon (HS-SPME) teknigi ile belirlenmiştir. Origanum onites’te 33 farklı bileşen tespit edilmiş olup ana bileşenler p-cyemne (%11.45), γ-terpinene (%11.89), linalool (%14.35), thymol (%20.03) ve carvacrol (%26.91)dur. Thymbra spicata var. spicata L.’da 36 farklı bileşen tespit edilmiş olup ana bileşenler p-cyemne (%11.72), γ-terpinene (%10.96), linalool (%13.44), thymol (%18.92) ve carvacrol (%27.34)dur. Oksijenli monoterpenlerin yükseğ oranda bulunduğu belirlenmiştir.

Anahtar Kelimeler: Origanum onites L., Thymbra spicata var. spicata L., SPME, carvacrol, thymol, Türkiye
Introduction

Volatile oils (essences, ethereal oils) and their aromatic extracts are widely used as a source of fragrance, food additives, cleaning products, in fragrance of cosmetics and medicines and also in taste industries, as a source of aroma-chemicals or as a starting material for synthesis of naturally identical and semi-synthetic beneficial aroma chemicals (Weiss, 1997). The Lamiaceae family members which are represented by 45 genera and 546 species in Turkey, are important in pharmacology and perfumery industry due to comprising volatile and aromatic oils (Secmen et al., 2000).

More than 15 plant species in Turkey are named and used as "thyme". The majority of these plants belong to Thymus of the Lamiaceae family, while others include the genus Origanum, Satureja, Majorana and Thymbra (Ozguven and Tansi, 1998; Kocabas and Karaman, 2001).

A well-known folk medicine and spice plant, thyme is mainly used for meat dishes, vegetable dishes, various sauces and salads, cheeses and sausage production, digestive system and upper respiratory tract disorders, indigestion, loss of appetite and cough. It has antiseptic, sedative, gas expectorant, expectorant, cramp solvent properties. Also, it is a highly sought-after spice plant in food storage in recent years due to its antibacterial effects on bacteria causing food spoilage and food poisoning (Bayram et al., 2010). Thyme is a good source of pollen for bees and a source of quality grass in milk-consuming animals (Ortiz and Fernandez, 1992).

SPME is a method for combining sample preparation, extraction and condensation in a single step without solvent. Significant gains were obtained in the processing time and costs by this method, while the diagnosis was improved. In addition, it has been observed that there are positive developments in the sample preparation stage and the results. The effectiveness of the SPME method depends on the type and thickness of the material covering the fiber portion in the syringe. Besides, the fact that SPME method is as short as 1-30 minutes shows its advantage over other methods (Vas and Vekey, 2004; Araujo et al., 2007; Donmez and Salman, 2017).

The most widely exported and used in the production of essential oil are Origanum onites and Thymbra spicata var. spicata. Origanum onites and Thymbra spicata var. spicata has been the subject of more researches, mainly due to its wide range and volatile fat content. In other studies, it is seen that different methods are used to determine the essential oil components. The aim of this study was to determine the volatile components, quantities and classes of fresh leaves and flowers of Origanum onites and Thymbra spicata var. spicata that were collected from Sutlegen province of Kas county in Antalya, Turkey.

Materials and Methods

Origanum onites and Thymbra spicata var. spicata specimens that were collected from stands where these are grown naturally in Sutlegen province of Kas county in Antalya, Turkey during the vegetation period of 2017-2018, constitute the material of the study. Collecting site is situated in the southwestern of Turkey (36° 28' N; 29° 38' E'). The leaves and flowers of the plant samples collected from the research area were placed in paper packaging and transferred to the laboratory on the same day without ever being exposed to sunlight. The collected plant materials were dried at room temperature (25 °C). The volatile components of leaves and flowers were determined by the Head Space Solid Phase Micro Extraction (HS-SPME) technique combined with gas chromatography / mass spectrometry (GC/MS). On the basis of the solid phase micro extraction technique, 2 g of the leaves and flower samples taken from each sample were placed in a 10 mL vial and stored at 60 °C for 30 minutes after the mouth was sealed with a silicone cap.

The SPME apparatus was passed through the headspace with 75 µm thin Carboxen/ Polidimethylsiloxane (CAR / PDMS) coated fused silica fiber to adsorb volatiles then injected directly into the capillary column of the Shimadzu 2010 Plus GC-MS device (Restek Rx-5, MS 30 m x 0.25 mm, 0.25 µm). The device is connected to the same brand mass selector detector operated in El mode (70 eV). This procedure was repeated three times to compare the accuracy of the results and the results were given as average. Helium was used as carrier gas with a flow rate of 1.61 mL per minute. Injection and detection temperatures were set at 250°C. Retention Indices (RI) of volatile components are calculated according to the standard of C7-C30 alkane mixtures under the above chromatographic conditions. Identification of the compounds was performed by comparing the mass spectra and the compounds in the spectral library (Wiley, Nist, Tutor, FFNSC).

Results and Discussion

Volatile components, amounts and classes of leaves and flowers of Origanum onites L. and Thymbra spicata var. spicata were determined according to SPME (solid-based micro extraction) technique. 33 different components of Origanum onites were determined. Of them, p-cymene (11,45%), γ-terpinene (11,89%), linalool (14,35%), thymol (20,03%) and carvacrol (26,91%) were found as main components. Also, 36 components were determined for Thymbra spicata var. spicata. p-cymene (11,72%), γ-terpinene
were found to be high (Table 1). Oxygen containing monoterpenes were found to be high (Table 1).

### Table 1. SPME results for *Origanum onites* L. and *Thymbra spicata* var. *spicata* L.

| No | RI  | RT    | Constituents     | O. onites (%) | T. spicata (%) | Formula   | Category |
|----|-----|-------|------------------|---------------|----------------|-----------|----------|
| 1. | <700| 1.670 | 2-Methylpropenal  | -             | 0,17           | C₅H₈O     | AA       |
| 2. | <700| 1.873 | Acetic acid      | 0,25          | 0,17           | C₂H₄O₂     | FA       |
| 3. | <700| 2.178 | 2-Butenal        | 0,08          | 0,08           | C₅H₁₀O     | AAI      |
| 4. | <700| 2.220 | 3-Methylbutanal   | -             | 0,11           | C₆H₁₂O     | AA       |
| 5. | <700| 2.311 | 2-Methylbutanal   | -             | 0,12           | C₅H₁₀O     | AA       |
| 6. | <700| 2.531 | 1-Penten-3-one    | -             | 0,13           | C₅H₁₀O     | AAI      |
| 7. | <700| 2.680 | Pentanal         | 0,06          | -              | C₅H₁₀O     | AAI      |
| 8. | 703 | 2.695 | Furan            | -             | 0,17           | C₅H₁₀O     | AA       |
| 9. | 751 | 3.606 | (E)-2-Pentenal   | -             | 0,07           | C₅H₁₀O     | AAI      |
| 10.| 801 | 4.596 | Hexanal          | 0,07          | 0,08           | C₅H₁₀O     | AA       |
| 11.| 850 | 6.085 | (E)-2-Hexenal    | 0,13          | 0,83           | C₅H₁₀O     | AA       |
| 12.| 927 | 8.495 | α-Thujene        | 0,79          | 1,16           | C₁₀H₁₆O₂   | MH       |
| 13.| 933 | 8.734 | α-Piene          | 0,94          | 0,82           | C₁₀H₁₆O₂   | MH       |
| 14.| 957 | 9.365 | Camphene         | 0,40          | 0,11           | C₁₀H₁₆O₂   | MH       |
| 15.| 964 | 9.788 | Benzaldehyde     | 0,06          | 0,08           | C₅H₁₀O     | AAI      |
| 16.| 978 | 9.535 | β-Piene          | 0,18          | 0,18           | C₁₀H₁₆     | MH       |
| 17.| 979 | 9.805 | 3-Octenol        | 1,29          | 1,09           | C₅H₁₀O     | AAI      |
| 18.| 986 | 10.445| Vinyl amyl ketone| -             | 0,15           | C₅H₁₀O     | AAI      |
| 19.| 991 | 10.943| β-Myrcene        | 2,33          | 2,51           | C₁₀H₁₆O₂   | MH       |
| 20.| 1007| 10.647| α-Phellandrene   | 0,38          | 0,39           | C₁₀H₁₆O₂   | MH       |
| 21.| 1009| 10.742| α-3-Carene       | 0,14          | 0,10           | C₁₀H₁₆O₂   | MH       |
| 22.| 1013| 10.896| 2,4-Heptadienal  | 0,59          | 0,49           | C₅H₁₀O     | AA       |
| 23.| 1018| 11.102| α-Terpinene      | 1,84          | 1,57           | C₁₀H₁₆O₂   | MH       |
| 24.| 1025| 12.255| P-Cymene         | 11,45         | 11,72          | C₁₀H₁₆O₂   | MH       |
| 25.| 1030| 12.414| Limonene         | 1,37          | 1,32           | C₁₀H₁₆O₂   | MH       |
| 26.| 1031| 12.632| 1,8-Cineole      | 0,08          | 0,09           | C₅H₁₀O     | OM       |
| 27.| 1046| 13.163| β-Ocimene        | 0,37          | 1,34           | C₁₀H₁₆O₂   | MH       |
| 28.| 1058| 13.544| γ-Terpinene      | 11,89         | 10,96          | C₁₀H₁₆O₂   | MH       |
| 29.| 1067| 14.020| trans-Sabinene hydrate | 0,16 | 0,53 | C₁₂H₁₈O₂ | FA |
| 30.| 1101| 15.265| Linalool         | 14,35         | 13,44          | C₁₀H₁₆O₂   | OM       |
| 31.| 1180| 18.290| Terpineolene     | 0,41          | 0,44           | C₁₀H₁₆O₂   | MH       |
| 32.| 1198| 18.852| α-Terpineol      | 0,36          | 0,20           | C₅H₁₀O     | AAI      |
| 33.| 1300| 22.455| Thymol           | 20,03         | 18,92          | C₁₀H₁₆O₂   | OM       |
| 34.| 1317| 22.715| Carvacrol        | 26,91         | 27,34          | C₁₀H₁₆O₂   | OM       |
| 35.| 1418| 26.905| Caryophyllene    | 2,32          | 2,38           | C₁₅H₂₄     | SH       |
| 36.| 1454| 26.966| α-Humulene       | 0,17          | 0,12           | C₁₅H₂₄     | SH       |
| 37.| 1458| 27.453| Aromadendrene    | 0,21          | -              | C₁₅H₂₄     | SH       |
| 38.| 1508| 29.716| β-Bisabolene     | 0,12          | 0,62           | C₁₅H₂₄     | SH       |
| 39.| 1557| 29.735| Germacrone B     | 0,11          | -              | C₁₅H₂₄     | SH       |
| 40.| 1584| 32.035| Caryophyllene oxide | 0,16 | -     | C₁₅H₂₄O   | OS       |
| **TOTAL** | | | | **100** | **100** | | |
In previous studies, Erdemgil (1992) determined carvacrol as main essential oil component of *Origanum onites*. Also, carvacrol, γ-terpinene and β-bisabolene were found as main components by Ruberto et al. (1993). Results of other studies that were conducted the essential oil components of *Origanum onites*, are in parallel with our study. Unlike other studies, the main components were p-cymene, linalool and thymol in our study.

Kilik (2006) were determined carvacrol, p-cymene, β-myrcene, γ-terpinene, α-terpinene and trans-caryophyllene as main essential oil components of *Thymbra spicata* var. *spicata* by hydrodistillation and GC / MS analysis. Tunen et al. (2011) found carvacrol as main component of *T. spicata* var. *spicata*. Carvacrol and thymol were found as main components in study of Al-Sheibany et al. (2005). Also, carvacrol, thymol, γ-terpinene and p-cymene were determined by Ravid and Putievsky (1985), carvacrol and γ-terpinene were found by Markovic et al. (2011) as main components of *Thymbra spicata*. In the study of Akgul et al. (1999), carvacrol, thymol, γ-terpinene and p-cymene were found as main components. Carvacrol, thymol, γ-terpinene and p-cymene were found by Fleisher and Fleisher (2005) and also carvacrol, α-thujene, myrcene, γ-terpinene and p-cymene were determined as main component of *T. spicata* by Barakat et al. (2013). All these results support our research. By the way, linalool was determined as different.

**Conclusions**

According to the SPME (solid-based micro extraction) technique, 33 components were determined for *Origanum onites* and also 36 for *Thymbra spicata* var. *spicata*. In both taxa, p-cymene, γ-terpinene, linalool, thymol and carvacrol were found to be main components. The results of the study were compared with leaf and flower samples obtained from different regions and analyzed by hydrodistillation method. They are collected from the nature in an inappropriate and dense manner due to the most widely used and used in the production of essential oil. This situation jeopardizes the extinction of species and narrows its natural distribution areas. Local people and traders should be made aware of the conscious collection and consumption. In addition to the therapeutic properties of the essential oils, it is necessary to take into consideration the harmful aspects and the place and dosage of the essential oils should be well adjusted. Thyme is also consumed as a well-known folk medicine, tea and spice plant. It is recommended that people with sugar and blood pressure should pay attention to their dose while drinking their tea. Detailed studies should be carried out in these areas in order to be used more extensively in the food, cosmetic and pharmaceutical industries.

**References**

Akgul, A., Ozcan, M., Chialva, F., Monguzzi, F. (1999). Essential Oils of Four Turkish Wild-Growing Labiatae Herbs: Salvia cryptantha Montbr. et Auch., Satureja cuneifolia Ten., Thymbra spicata L. and Thymus cilicicus Boiss. et Bal. Journal of Essential Oil Research, 11(2): 209-214.

Al-Sheibany IS (2005). Isolation and Identification of Volatile oils from Iraqi Thyme (*Thymbra Spicata*) and study the antimicrobial activity. Irq. Nat. J. Chem. 18: 289-298.

Araujo HC, Lacerda MEG, Lopes D, Bizzo HR, Kaplan MAC (2007). Studies On The Aroma Of Mate (*Ilex paraguayensis* St.Hil.) Using Headspace SolidPhase Microextraction. Phytochem. Anal. 18: 469-474.

Barakat A, Wakim LH, Apostolides NA, Sroug G, Beyrouthy ME (2013). Variation in the essential oils of *Thymbra spicata* L. growing wild in Lebanon according to the date of harvest. J. Essent Oil Res. 25: 506-511.

Bayram E, Kirci S, Tans S, Yilmaz G (2010). “Tibbi Ve Aromatik Bitkiler Üretiminin Arttırılması Olanakları”. Türkiye Ziraat Mühendisliği VII. Teknik Kongresi Bildiriler Kitabi-I,437–456, 11–15 Ocak 2010, Ankara.

Donmez IE, Salman H (2017). Yaban Mersini (*Myrtus communis* L.) Yaprak ve Meyvelerinin Uçucu Bileşenleri. Turk. J. For. 18(4): 328-332.

Erdemgil FZ (1992). *Origanum onites* L.Uçucu Yağının Bileşimi. Anadolu Üniversitesi, Sağlık Bilimleri Enstitüsü, Yüksek Lisans Tezi, 53s.

Fleisher, Z, Fleisher A (2005). Extract Analyses of Satureja thymbra L. and Thymbra spicata L. Aromatic Plants of the Holy Land and the Sinai. Part XVII. J. Essent Oil Res. 17: 32-35.

Kılıç T (2005). Analysis of Essential Oil Composition of Thymbra spicata var. spicata: Antifungal, Antibacterial and Antimycobacterial Activities. Z. Naturforsch. 61: 324-328.

Kocabas YZ, Karahan S (2001). Essential oils of Lamiaceae family from South East Mediterranean Region (Turkey). Pak J Biol Sci. 4(10):1221-1223.
Markovic T, Chatzopoulou P, Siljegovic J, Nikolic, M, Glamoclija J, Ciric A, Sokovic M, 2011. Chemical Analysis and antimicrobial activities of the essential oils of Satureja thymbra L. and Thymbra spicata L. and their main components. Arch. Biol Sci. 63 (2): 457-464.

Ortiz PL, Fernandez I (1992). Microscopic Study of Honey and Apiary Pollci From the Province of Seville. Departamento de Biologia Vegetaly, Ecologia facultad de Biologia, Apdo, 1095.41080, Sevilla Spain, Abstract.

Ozgüven, M., Tansi, S (1998). In Situ Conservation of Aromatic Plant in Southeastern Turkey Wild Species. The Proceedings of International Symposium on in Situ Conservation of Plant Genetic Diversity, 177-183 S.

Ravid U, Putievsky E (1985). Composition of Essential Oils of Thymbra spicata and Satureja thymbra Chemotypes. Planta Med. 51(4):337-8

Ruberto G, Biondi D, Meli R, Piattelli M (1993). Volatile Flavour Components of Sicilian Origanum onites L.. Flavour Fragr J. 8(4):197 - 200.

Secmen O, Gemici Y, Görk G, Bekat L, Leblebici E (2000). Tohumlu Bitkiler Sistematiği. Ege Üniversitesi, Fen Fakültesi Yayınları Serisi No: 116, İzmir.

Tumen G, Ermin N, Ozek T, Kurcuoglu M, Başer KHC (2011). Composition of Essential Oils from Two Varieties of Thymbra spicata L. J Essent Oil Res. 6(5):463-468.

Vas G, Vekey K (2004). Solid-Phase microextraction: apowerful sample preparation tool prior to mass spectrometric analysis. J. Mass Spectrom. 39:233- 254.

Weiss EA (1997). Essential Oil Crops. J Agric Sci. 129, 121-123.