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Analysis of Essential Oil Composition of Endemic Marrubium parviflorum subsp. oligodon Grown in Turkey by Using SPME with GC/MS

Türkiye'de Yetiştirilen Endemik Marrubium parviflorum subsp. oligodon'un Uçucu Yağ Kompozisyonunun SPME Kullanılarak GC/MS ile Analizi

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Abstract

The aim of the study is to determine the essential oil profile and antioxidant activity of endemic *Marrubium parviflorum* subsp. *oligodon.* The essential oil contents were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) using Solid Phase Microextraction (SPME). In addition, total phenolic content and the capacity of antioxidant activity measured by DPPH• and FRAP assays were determined. Twenty-three (64.12%) essential oil components were determined. Pentadecanolide was found to be the major essential oil and palmitic acid was determined as main fatty acid in *Marrubium parviflorum subsp. oligodon*. Total phenolic content was found to be 39.9±0.31 mg GAE/g sample. FRAP value was found to be 48.91±0.33 µmol Fe/g sample and DPPH• scavenging activity was found to be 0.76±0.03 mg/mL. This study is the first report in which the essential oil content of *Marrubium parviflorum subsp. oligodon* was determined by SPME.

Keywords: *Marrubium parviflorum* subsp. *oligodon*, SPME, Lamiaceae, Essential oil

Özet

Bu çalışmanın amacı, endemik *Marrubium parviflorum* subsp. *oligodon*'un uçucu yağ profilini ve antioksidan aktivitesini belirlemektir. Esansiyel yağ içerikleri, Katı Faz Mikro Ekstraksiyon (SPME) kullanılarak Gaz Kromatografisi-Kütle Spektrometresi (GC-MS) ile analiz edildi. Ek olarak, DPPH• ve FRAP testleri ile ölçülen antioksidan aktivite kapasitesi ve toplam fenolik

madde miktarı belirlendi. Toplam 23 esansiyel yağ bileşeni (%64,12) belirlendi. *Marrubium parviflorum* subsp. *oligodon*'un toplam fenolik madde miktarı 39.9±0.31 mg GAE/g numune olarak bulundu. FRAP değeri 48.91±0.33 µmol Fe/g numune ve DPPH• süpürme aktivitesi 0.76±0.03 mg/mL olarak tespit edildi. Bu çalışma *Marrubium parviflorum* subsp. *oligodon*'un uçucu yağ içeriğinin SPME ile belirlendiği ilk rapordur.

Anahtar Kelimeler: *Marrubium parviflorum* subsp. *oligodon*, SPME, Lamiaceae, Uçucu yağ

Abbreviations: GAE, gallic acid equivalent; GC-MS, Gas Chromatography-Mass Spectrometry; SPME, Solid Phase Microextraction; FRAP, ferric reducing antioxidant power; DPPH•, 1,1-diphenyl2-picrylhydrazyl.

1. INTRODUCTION

Plants have been used for different purposes (fabric dyeing, treatment of diseases, essence, etc.) since ancient times. Today, plants constitute a large part of pharmaceutical raw materials. In recent years, the inadequacy of synthetic drugs produced against diseases and the presence of side effects have led people to use natural products. Therefore, the importance of medicinal and aromatic plants is increasing day by day (Ceylan, 2022). Turkey has a great potential in terms of having different climatic and ecological conditions and containing a large number of plant species and diversity (Şenkul & Kaya, 2017).

Essential oils are natural products obtained from the leaves, fruit, bark or root parts of plants. Although they are defined as oils because they do not mix with water, they are different from fixed oils (Ceylan, 1983). Essential oils have important properties such as antibacterial (Boyle, 1955), antiviral (Bishop, 1995), antioxidant (Sarıkurkcu et al., 2018), insecticidal (Karpouhtsis et al., 1998). Phenolic compounds are secondary metabolites produced by plants. These compounds have many properties such as anti-inflammatory, antimicrobial, antiviral, antimutagenic, anticarcinogenic, antiulcer, antioxidant effects (Balasundram et al., 2006; Moure et al., 2001).

Free radicals can occur as a by-product of normal reactions in our body, such as energy production, lipid degradation, or inflammatory processes. These free radicals play an important role in the development of various pathological conditions such as lipid peroxidation, DNA damage and cellular degeneration (Losada-Barreiro & Bravo-Diaz, 2017). Phenolic compounds and essential oils in plants are natural antioxidants and antimicrobials. These components have protective properties against diseases caused by free radicals. Therefore, people prefer herbal antioxidants instead of synthetic antioxidants due to their side effects (Ceylan et al., 2021).

The genus *Marrubium* L. belongs to the Lamiaceae family and is represented by about 28 taxa in the flora of Turkey. Some *Marrubium* species are used for different purposes in the world and in our country due to their medical and ethnobotanical importance. It is also preferred in beekeeping due to its abundant nectar and abundant flowers (Akgül et al., 2008).

Marrubium parviflorum subsp. *oligodon*, is a perennial herbaceous plant endemic to Turkey and grows in the Central Anatolia Region. It is also known as "mountain tea" among the people and is used as an antipyretic in the treatment of colds (Altundag & Ozturk, 2011; Sarıkurkcu et al., 2018).

The most widely used method to obtain essential oil from plants is hydrodistillation. Recently, the use of SPME (solid phase microextraction) method, which does not require solvent and is a faster method, has been increasing (Xing et al., 2019). In previous studies, hydrodistillation was used for essential oil analysis in *Marrubium* species. Therefore, in our study, it was aimed to determine the antioxidant activity and essential oil content of *Marrubium parviflorum* subsp. *oligodon* by SPME/GC-MS. The results obtained in this study can be considered as the first report in which the essential oil content of *Marrubium parviflorum* subsp. *oligodon* was determined by SPME.

2. MATERIALS and METHODS

2.1. Plant Material

The plant was collected from Nevşehir (Turkey) and identified by Dr. Mustafa Karaköse. Plant was dried at room temperature. Air dried sample was powdered in an electric grinder (Waring Commercial, USA). The dry plant sample was divided into two. 5 g of the dry sample was weighed and methanolic extract was prepared for antioxidant activity assay. The remaining sample was used for essential oil analysis.

2.2. Total Phenolic Content

Total phenolic content (TPC) of plant was obtained by utilizing the Folin-Ciocalteu assessment (Slinkard & Singleton, 1997). Standard was Gallic acid (different concentration) in this work. Shortly, 0.5 N 400 µL Folin-Ciocalteu solution, 20 µL methanolic samples (1 mg/mL), 680 µL of distilled water were stirred and the solution was vortexed. After 3-minute waiting, 400 µL of sodium carbonate (10%) was added and the solution was vortexed again. Then, the solution was left about 2 h. Following time, absorbance of the solution was determined to be 760 nm.

2.3. Determination of Antioxidant Activity

FRAP and DPPH$^{\bullet}$ assays were utilized in the calculation antioxidant activity. The FRAP assay is reduction of Fe^{3+}-TPTZ compound to the Fe^{2+}-TPTZ compound with electron giving material this situation (Benzie & Szeto, 1999). The 100 µL of sample solution or blank and 3 mL of FRAP solution (including TPTZ, iron (III) chloride and acetate buffer) were added and the solution was vortexed. The absorbance rates on 593 nm were determined to be about 4 min for at 25 °C.

The cleaning capacity of DPPH$^{\bullet}$ radical (2,2-diphenyl-1-picrylhydrazyl) of metanolic extractions was defined utilizing the technique of Molyneux (2004). Various concentrations 0.75 mL of each sample extracts were vortexed together with 0.75 mL of a 0.1 mM of DPPH$^{\bullet}$ solution in methanol. After that, each extract was left at room temperature in the dark (50 min). Absorbance was monitored as 517 nm. Trolox was utilized as stock and amounts were explained as SC_{50} (mg sample per mL), the concentration of the antioxidant causing 50% DPPH$^{\bullet}$ scavenging.

2.4.SPME Procedure and GC/MS Analysis

GC analysis was performed on a Shimadzu QP2010 plus gas chromatography utilizing a TRB-5MS capillary column (30 m x 0.25 mm, film thickness, 0.25 µm). Shimadzu QP2010 Plus gas chromatography was connected to a Shimadzu QP2010 Ultra mass selector detector. One gram dry plant was added in a vial (10 mL), then the head space was placed to the solid-phase micro extraction apparatus (Supelco, USA). A polydimethylsiloxane/divinyl-benzene coating fiber was added in the head space. After at 50 °C with incubation time of 5 min, extraction was done at 10 min. Then fiber was inserted into the injection port of the GC-MS. The oven program was as follows: the first heat was 60 °C for 2 minutes, which was raised to 240 °C in 3 minutes, the last heat was kept at 250 °C for 4 minutes. The transporter gas was utilized as helium (99.999%) with a stable flux amount of 1 mL/minute. Detection was performed in electronic pulse range (EI); ionization tension adjusted to 70 eV and scanning range (40-400 m/z) was utilized to get mass. The volatile compounds were detected by comparison of their mass spectra of the two libraries (FFNSC1.2 and W9N11) (Renda et al., 2016).

3. RESULTS and DISCUSSION

Phenolic compounds are secondary metabolites produced by plants. They can be found not only in the fruit of the plant, but also in all parts of the plant such as leaves, roots and bark. These compounds protect the plant under different stress conditions such as drought, UV radiation,

pathogens and diseases. Phenolic compounds have many bioactive properties such as anti-inflammatory, antimicrobial, antiviral, antimutagenic, anticarcinogenic, antiulcer, antioxidant effects (Dietrich et al., 2004; Szajdek & Borowska, 2008).

Table 1 shows the results of total phenolic content, FRAP and DPPH analysis of *Marrubium parviflorum* subsp. *oligodon.* Total phenolic content was found to be 39.9±0.31 mg GAE/g sample. FRAP value was found to be 48.91±0.33 µmol Fe/g sample and DPPH• scavenging activity was found to be 0.76±0.03 mg/mL. Antioxidant activity analyzes performed in different *Marrubium* species in previous studies show diversity. Sarikurkcu et al. (2008) measured the antioxidant activity of *Marrubium parviflorum* subsp. *oligodon* using different solvents (hexane, water, methanol, ethyl acetate and dichloromethane). For the measurement of antioxidant activity, β-carotene-linoleic acid and DPPH• radical scavenging activity methods were used in the aerial part and essential oil of the plant. In addition, total phenolic and flavonoid were determined. While in total phenolic was high water extract (38.16 mg GAE/g), total flavonoid was high in methanolic extract (19.58 mg QE/g). Water extract was also found to be high in DPPH• scavenging activity. Our results are the second study examining the antioxidant activity of *Marrubium parviflorum* subsp. *oligodon*. There are also antioxidant activity studies with different *Marrubium* species in the literature. Total phenolic substance and DPPH radical scavenging activity were investigated in hexane and metanol extracts of *Marrubium parviflorum* and higher activity was found in methanolic extract (Yumrutaş & Saygıdeğer, 2010). In another study using different extraction solvents (petroleum ether, chloroform, ethyl acetate and n-butanol), it was reported that the DPPH• activity of *Marrubium parviflorum* Fisch. & C.A.Mey. was higher in ethyl acetate and n-butanol (Delnavazi et al., 2017).

Table 1. Result of total phenolic content and antioxidant activity

Sample	**Total phenolic content (mg GAE/g sample)**	**FRAP (µmol Fe/ g sample)**	**DPPH• (mg/mL)**
Marrubium parv.	39.9±0.31	48.91±0.33	0.76±0.03
Trolox	-	-	0.0025±0.11

Essential oils obtained from the leaves, fruit, bark or root parts of plants are of great interest in the pharmaceutical industry due to their biological potential (Kulaksız et al., 2018).

Species belonging to Lamiaceae family are used in the treatment of various diseases in traditional medicine and the *Marrubium parviflorum* subsp. *oligodon*, we used in our study, is an endemic species belonging to this family. Composition of the essential oils of *Marrubium parviflorum* subsp. *oligodon* was identified with SPME with GC-MS (Table 2).

Tablo 2. Essential oil composition of *Marrubium parviflorum* subsp. *oligodon*

Compound	**RI Exp.**	***Marrubium parv.* (%)**
Aldehyde		
Hexanal	804	0.80
Alcohol		
Hexanol	1034	9.83
Lauryl alcohol	2307	0.53
Ketones		
Acetoin	712	4.27
Civetone	2155	2.78
Hydrocarbons		
Dodecane	1205	0.72
Pentadecane	1269	0.92
Octadecane	1286	1.98
Tridecane	1299	0.68
Tetradecane	1406	3.01
Hexadecane	1459	0.55
Lactones		
δ-Undecalactone	1642	1.50
Pentadecanolide	1841	14.61
Terpens		
İsopulegol	1150	4.22
Linalool	1065	0.67
Neomenthol acetate	1599	0.78
Carboxylic acids		
Valeric acid	986	1.25
Caproic acid	1125	0.64
Esters		
Dietthyl malonate	912	0.53
Tetrahydrofurfuryl butyrate	1606	4.47
Fatty acids		
Palmitic acid	1949	6.72
Heptadecanoic acid	2103	1.61
Other		
Anethole	1290	0.52
Oxybenzene	988	0.53
Unknown		35.88
Total	**-**	**100**

Twenty-three (64.12 %) essential oil components were determined in this study. Pentadecanolide (14.61 %) and hexanol (9.83%) were determined as the main components. It is seen that palmitic acid (6.72%) is the main fatty acid.

When the results obtained are examined, it is seen that ketone, aldehyde, terpene, ester, carboxylic acid and hydrocarbon classes components are also detected in our sample. In previous studies, essential oil determination was made by hydrodistillation method in *Marrubium* species. The results obtained in these studies differ from our study. Bal et al. (1999) made the analysis of essential oil in *Marrubium parviflorum subsp. oligodon* and identified 139 compounds. Hexadecanoic acid (15.4%), germacren D (11.1%) and beta-caryophyll (10.0%) were indicated as the major components. Sarikurkcu et al. (2018) were determined 31 compounds as essential oil in *Marrubium parviflorum* subsp. *oligodon* and the main components were reported as (Z,Z) -farnesyl acetone (19.28%), caryophyllene oxide (15.85%), palmitic acid (15.4%) and pulegon (7.15%). In the essential oil study with *Marrubium parviflorum subsp. parviflorum* collected from Bingöl, β-caryophyllene (20.3%), germakren D (18.8%), bicyclogermacrene (10.2%) and spathulenol (7.3%) were found as the main components of the essential oil of the plant. In addition, fatty acid analysis was performed and palmitic acid (46.89%) and stearic acid ester (28.43%) were found to be high (Kılıç, 2018). It is seen that there is germakren D as a common component in studies of Bal et al. (1999) and Kılıç (2018). Sarikurkcu et al. (2018) also detected germakren D (3.85%), but at a lower rate. But, germakren D could not be detected in our study. However, the main fatty acid of palmitic acid in our study is similar to these studies. In addition, while pentadecanolide could not be detected in these studies, pentadecnolide (1.7%) was detected in the study of Khanavi et al. (2005) with *Marrubium parviflorum* Fisch. & C. A. Mey.

4. CONCLUSION

It has been thought that the extraction method, the region where the plant was collected and the harvesting time were effective in the differences between the results obtained in our study and the results in the literature. As we emphasized before, hydrodistillation method was used to determine the essential oil content in previous studies. In this study, SPME method was preferred for essential oil analysis. It can be used in different studies and compared with the hydrodistillation method, more volatile components can be detected in plants. As a continuation of this study, it is planned to determine the essential oil component of the same plant by using both SPME and hydrodistillation methods at the same time.

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DECLARATIONS

The authors declare that they have no conflicts of interest.

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