ANTIIBACTERIAL ACTIVITY OF *THYMUS MİGRİCUS* ESSENTIAL OILS

Mehmet Çağlar Fırat*[1], Zeynep Akşit[2]

***ORCID: 0000-0003-4920-4920**
E-Posta: *mcfirat@erzincan.edu.tr
[1,2] Erzincan Binali Yıldırım Üniversitesi, Turizm ve Otelcilik MYO, Aşçılık Programı, Erzincan, Türkiye

Abstract

Thymus migricus is an endemic and highly biodiverse plant species belonging to the Lamiaceae family. This plant is often found in mountainous areas, especially on limestone grounds. The species is adapted to arid and semi-arid climates, especially in high mountain regions. Morphologically, *T. migricus* is generally recognized by its small-sized, densely hairy leaves and characteristic essential oil glands. Studies on the biochemical composition of *T. migricus* have highlighted the pharmacological potential of the plant. In particular, the leaves and flowers of *T.migricus* have been identified as a rich source of antioxidants, polyphenols and essential oils. These components may support the plant's antioxidant, anti-inflammatory, and antimicrobial properties. There have been some reports of *Thymus migricus* being used in Van herbed cheese, tea, and for traditional medicinal purposes in Eastern Turkey and Iran. In this study, *Thymus migricus* was collected from Erzincan Dumanlı Nature Park. The aerial parts of plant material were freshly used for essential oil isolation. The fresh aerial parts (400 g) were cut into small pieces and subjected to hydro distillation using a Neo-Clevenger-type apparatus in 2-liter deionized water and maintained for 2 hours. The hydro distillation process was carried out in triplicate. Decanted and dried over Na_2SO_4 essential oils were kept at +4 °C in sealed dark vials. The analysis was performed using the Thermo Scientific GC-MS instrument. Total 38 components, representing 99.25% of the total oils were identified. Main components of the oil were determined as carvacrol (46.91 %), thymol (18.06 %), p-cymene (9.17%) and γ-terpinene (6.14%). Antimicrobial susceptibility tests were applied to 7 pathogenic bacterial strains with these extracts. Imipenem was used as a positive control. Inhibition zones was measured as 35 mm for *Pseudomonas aeruginosa*, 18 mm for *E.coli*, 40 mm for *Bacillus cereus*, 41 mm for *Clostridium perfringens*, 39 mm for *Listeria monocytogenes*, 35 mm for *Staphylococcus aureus*, and 28 mm for *Salmonella enteritidis*. A strong antibacterial effect was observed in all 7 strains tested. Essentials oils of *Thymus migricus* appears to have potential applications in the pharmacology and food industries.

Keywords: *Thymus migricus*; Essential oil; Antibacterial activity

Thymus migricus ESANSİYEL YAĞLARIN ANTİBAKTERİYEL ETKİNLİĞİ

Özet

Thymus migricus, Lamiaceae familyasına ait endemik ve biyolojik çeşitliliği oldukça yüksek bir bitki türüdür. Bu bitki genellikle dağlık bölgelerde, özellikle kireçtaşı zeminlerde bulunur. Tür, özellikle yüksek dağlık bölgelerdeki kurak ve yarı kurak iklimlere uyum sağlamıştır. Morfolojik olarak *T. migricus* genellikle küçük boyutlu, yoğun tüylü yaprakları ve karakteristik esansiyel yağ bezleri ile tanınır. *T. migricus*'un biyokimyasal bileşimi üzerine yapılan çalışmalar bitkinin farmakolojik potansiyelini ortaya çıkarmıştır. Özellikle *T.migricus*'un yaprak ve çiçeklerinin zengin bir antioksidan, polifenol ve uçucu yağ kaynağı olduğu tespit edilmiştir. Bu bileşenler bitkinin antioksidan, antiinflamatuar ve antimikrobiyal özelliklerini destekleyebilir. Türkiye'de Van otlu peynirinde, Azerbaycan ve İran'da *Thymus migricus*'un çayda ve geleneksel tıbbi amaçlarla kullanıldığına dair bazı bilgiler bulunmaktadır. Bu çalışmada *Thymus migricus* Erzincan Dumanlı Tabiat Parkı'ndan toplanmıştır. Bitki materyalinin toprak üstü kısımları taze olarak uçucu yağ izolasyonu için kullanıldı. Taze hava parçaları (400 gr) küçük parçalar halinde kesilerek Neo-Clevenger tipi aparat kullanılarak 2 litrelik deiyonize su içerisinde hidrodistilasyona tabi tutulmuş ve 2 saat bekletilmiştir. Hidro distilasyon işlemi üç tekrar halinde gerçekleştirilmiştir. Na_2SO_4 ile sudan arındırılan ve kurutulan esansiyel yağlar, kapalı koyu renkli şişelerde +4 °C'de saklandı. Analiz Thermo Scientific GC-MS cihazı kullanılarak yapılmıştır. Esansiyel yağların %99,25'ini temsil eden toplam 38 bileşen tespit edilmiştir. Yağın ana bileşenlerinin carvacrol (%46,91), timol (%18,06), p-simen (%9,17) ve γ-terpinen (%6,14) olduğu belirlendi. Bu ekstraktlarla 7 patojen bakteri suşuna antimikrobiyal duyarlılık testleri uygulanmıştır. Pozitif kontrol olarak imipenem kullanılmıştır. İnhibisyon zonları *Pseudomonas aeruginosa* için 35 mm, *E.coli* için 18 mm, *Bacillus cereus* için 40 mm, *Clostridium perfringens* için 41 mm, *Listeria monocytogenes* için 39 mm, *Staphylococcus aureus* için 35 mm ve *Salmonella enteritidis* için 28 mm olarak ölçülmüştür. Test edilen 7 suşun tamamında güçlü bir antibakteriyel etki gözlenmiştir. *Thymus migricus*'un esansiyel yağlarının farmakoloji ve gıda endüstrilerinde potansiyel uygulamaları olabileceği görülmektedir.

Anahtar Kelimeler: *Thymus migricus*; Esansiyel yağ, Antibakteriyel etki

I. INTRODUCTION

Thymus migricus is an endemic and extremely biodiverse plant species which is a member of the Lamiaceae family The aromatic plants in the Lamiaceae family are found almost everywhere in the world, but particularly in North-Western Asia and the Mediterranean region (Aras et al., 2021). This plant is frequently

located in hilly regions, particularly on limestone terrain (Mahdavi et al., 2014). The species, particularly in high mountain regions, is acclimated to arid and semi-arid climates. *T. migricus* is recognizable morphologically by its small, highly hairy leaves and distinctive essential oil glands. Research on *T. migricus*'s biochemical composition has brought attention to the plant's potential as a pharmacological agent . Particularly, it has been found that *T. migrus* leaves and flowers are a rich source of essential oils, polyphenols, and antioxidants (Takaloo et al., 2012; Rezaii et al., 2023; Yuca et al., 2022). The plant's antibacterial, anti-inflammatory, and antioxidant qualities might be strengthened by these constituents. Some reports have stated that *Thymus migricus* is used in tea, cheese, and traditional medicines in Eastern Turkiye, Azerbaijan and Iran (Afshar et al., 2016; Ocak & Köse, 2014; Tunçtürk et al., 2020).

II. MATERIAL AND METHODS

Plant Material:

Thymus migricus was collected from Erzincan Dumanlı Nature Park at an altitude of 2000-2100 meters on June 2023. Identification of spiece was implemented by Prof. Dr. Ali Kandemir at the Faculty of Biology, Erzincan Binali Yıldırım Üniversitesi.

Essential oil separation

The aerial parts of plant material were freshly used for essential oil isolation. The fresh aerial parts (400 g) were cut into small pieces and subjected to hydro distillation using a Neo-Clevenger-type apparatus in 2-liter deionized water and maintained for 2 hours. The hydro distillation process was carried out in triplicate. Decanted and dried over Na_2SO_4 essential oils were kept at +4 °C in sealed dark vials (Aksit et al., 2022).

Essential oil analysis

The analysis was performed using the Thermo Scientific GC-MS instrument equipped with a HP5-MS column with dimensions of 0.25 µm ID, 30 m x 250 µm. The oven program was set as follows: the initial temperature was set to 60 °C and held for 3 minutes. The temperature was then increased at a rate of 5°C per minute until it reached 200 °C. Finally, the temperature was held at this level for 5 minutes. Helium was used as the carrier gas at a flow rate of 1 ml per minute with the 50:1 split ratio. The injection volume was 1 µL, the solvent delay was 3 minutes, the GC transfer line temperature was set to 200 °C, and the ion source temperature was set to 250 °C. The ionization energy was set to 70 eV for EI source. The components were identified by comparing retention indices (RI) calculated by n-alkanes (C8-C30) analyzed under the same experimental

conditions, co-injection with standard compounds (for thymol, p-cymene, and -terpinene), and examining and comparing MS fragmentation patterns of each component with the corresponding MS libraries (Wiley 275 and NIST 08). The relative amounts of each component were calculated using the peak areas obtained from the flame ionization detector (FID) without using any correction factors (Aksit et al., 2022).

Disc-diffusion assay

Disc-diffusion test were applied to 7 pathogenic foodborne bacterial strains with these extracts according to (Adiguzel et al., 2007). *Pseudomonas aeruginosa, Staphylococcus aureus* (ATCC 6538), *Listeria monocytogenes, Bacillus cereus* (ATCC 12711, *Escherichia coli* (ATCC 25922), *Bacillus cereus, Clostridium perfringens,* and *Salmonella enteritis* were used as test microorganisms. The bacterial strains cultures were inoculated in Nutrient broth and the concentrations are adjusted to 1×10^6 cfu/mL. Imipenem was used as positive control.

III. RESULTS AND DISCUSSION

GC/MS Analysis of Essential Oil

Total 40 components are determined with GC/MS Analysis shown on Table 1. Carvacrol (46.91 %), thymol (18.06 %), p-cymene (9.17%) and γ-terpinene (6.14%) are the major components determined in essential oils of the extract. Genus Thymus, consisting 215 species, is known for its main compounds as thymol and carvacrol (Pirbalouti et al., 2015). Previous research implemented by Küçükbay et al., (Küçükbay et al., 2014) showed that main compounds of α-terpineol (30.6%), thymol (20.7%) and α-terpinyl acetate (14.9%). In another study, the main component was found to be thymol (36–44%). However, carvacrol (37%) was the primary component in one sample (C) for *T. migricus* collected from eastern province Van/Turkiye (Başer et al., 2002).

Table I. GS.MS data of the extract

Compound Number	**RT***	**Compound Name**	**Dumanlı Kekik (% area)**
1	4.13	Diacetone alcohol (C6/O)	0.24
2	5.93	α-Thujene (C10)	1.19
3	6.10	α-Pinene (C10)	2.59
4	6.47	Camphene (C10)	0.18
5	7.17	β-Pinene (C10)	0.30
6	7.25	Amyl vinyl carbinol (3-Octenol)(C8/O)	0.64
7	7.41	Amyl ethyl ketone (3-Octanone**)** (C8/O)	0.95
8	7.52	β-Myrcene (C10)	1.08
9	7.67	3-Octanol (C8/O)	0.29
10	7.88	α-Phellandrene (C10)	0.22
11	8.21	α-Terpinene (C10)	1.35
12	8.45	***p*-Cymene** (C10)	9.17
13	8.55	Limonene(C10)	0.68
14	8.63	Eucalyptol (C10/O)	2.42
15	9.38	**γ-Terpinene (C10)**	6.14
16	10.52	4-Thujanol (C10/O)	0.26
17	11.65	Pinocarveol (C10/O)	0.12
18	11.82	Verbenol(C10H16O)	0.16
19	12.41	Borneol (C10/O)	0.62
20	12.72	4-Terpineol (C10/O)	0.30
21	12.95	*p*-cymene-7-ol (C10/O)	0.11
22	13.11	2-norbornanol (fenchol) (c10/o)	0.27

T. migricus collected from East Azarbaijan, In the Mishoo habitat, the main compounds were linalool (65.57%) and citronellol (15.63%), while in the Espiran habitat, the main compounds were linalool (46.36%) and geraniol (26.74%) (Dizajeyekan et al., 2016). Our findings indicate carvacrol is the main compound which is parallel to Başer et al., 2002 however differ from Dizajeyekan et al., 2016) and Küçükbay et al., 2014. It is common knowledge that the composition of plants is influenced by environmental factors and different locations. Yavari et al. 2010, reported that *T.migricus*, which grows at lower altitudes than others, has a higher essential oil content.

Disc-diffusion assay:

Disc diffusion tests are first step for determinin antimicrobial acticity of any compound. Table 2 shows antibacterial activity of *T.migricus* essential oil. Essential oils of *T. migricus* showed strong antibacterial effect against test microorganisms. Inhibition zones varied 18-41mm and *Listeria monocytogenes and B. cereus* are observed as the most susceptible bacteria against essential oils among others.

Table 2. Disc-diffusion assay

Test microorganism	***Essential oil****	***Imipenem***
Pseudomonas aeruginosa	35	44
E.coli	18	22
Bacillus cereus	40	37
Clostridium perfringens	41	43
Listeria monocytogenes	39	36
Staphylococcus aureus	35	41
Salmonella enteritidis	28	39

* Diameter of disc diffusion (mm);

From a general point of view essential oils of *T. migricus* showed strong antibacterial effect against test microorganisms. Prior research on the antimicrobial properties of some Thymus species' essential oils revealed activity against fungi (Wild, 1994) and bacteria (Kotan et al., 2010). In our research main compound carvacrol is known for antibacterial, antiviral and antifungal activities (Imran et al., 2022). Recent approaches are using

carvacrol as a coating material for foods (Imran et al., 2022; Tao et al., 2022). Thymol is another antimicrobial agent *T. migricus* has (Falcone et al., 2005). Synergic antimicrobial effect of thymol and carvacrol is also suggested (Memar et al., 2017). These components have been previously reported for their antimicrobial properties and are known to disrupt bacterial cell membranes, inhibit enzymatic processes, and interfere with essential cellular functions. *p*-Cymene with a 9,17 %, seems to be the main antimicrobial agent is thyme (Bagamboula et al., 2004; Marchese et al., 2017). Bacteria, both causing infection and intoxication as well as Gram (-) and Gram (-) bacteria negatively affected from essential oil. On account of this antibacterial effect of Dumanlı *Thymus migricus* may be attributed to high carvacrol; thymol and *p*-Cymene content.

CONCLUSION

The investigation into the antibacterial effect of *Thymus migricus* essential oil has yielded valuable insights into its potential therapeutic applications. The experimental results consistently demonstrated a significant antibacterial activity against seven bacterial strains, suggesting that *Thymus migricus* essential oil possesses notable antimicrobial properties. It is essential to acknowledge the limitations of this study, including the need for further research to elucidate the specific mechanisms underlying the antibacterial activity of *Thymus migricus* essential oil. Additionally, in vivo studies and clinical trials are needed to evaluate the safety, efficacy, and potential food industry and therapeutic applications of this essential oil in real-world settings. Some factors such as location, altitute, season, temperature and soil composition affecting the essential oil constent. Therefore antibacterial effect may vary independently.

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