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*Research article*

# Chemical Composition and Antimicrobial Efficacy of *Thymus fallax* Essential Oil against Foodborne Spoilage and Pathogenic Microorganisms[a]

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## ABSTRACT

The essential oil extracted from Thymus fallax Fisch. Fisch. & C.A.Mey., a plant traditionally employed in folk medicine, underwent chemical composition analysis through both gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS) techniques. A total of 28 components were identified, collectively constituting 79.0% of the total detected constituents. The predominant compounds were determined to be 1,8 cineol (9.9%), linalool (8.8%), and camphor (8.1%).

The antimicrobial potential of essential oils extracted from the aerial parts of the plant was assessed against various microorganisms, encompassing both foodborne spoilage and pathogenic bacteria and yeast. The essential oil demonstrated notable antibacterial and antifungal efficacy, with the level of activity increasing in proportion to the quantity of essential oil applied.

**Keywords:** *Thymus fallax*, essential oil, chemical composition, antimicrobial activity.



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*Araştırma makalesi*

# *Thymus fallax* Esansiyel Yağının Kimyasal Bileşimi ve Gıda Kaynaklı Bozulmaya ve Patojenik Mikroorganizmalara Karşı Antimikrobiyal Etkinliği


## ÖZ

Geleneksel olarak halk tıbbında kullanılan bir bitki olan *Thymus fallax* Fisch. & C.A.Mey.'den elde edilen uçucu yağ hem gaz kromatografisi (GC) hem de gaz kromatografisi-kütle spektrometrisi (GC/MS) teknikleri ile kimyasal bileşim analizine tabi tutuldu. Tespit edilen bileşenlerin %79,0'ını oluşturan toplam 28 bileşen tanımlandı. Baskın bileşiklerin 1,8 sineol (%9,9), linalool (%8,8) ve kafur (%8,1) olduğu belirlendi.

Bitkinin toprak üstü kısımlarından ekstrakte edilen uçucu yağın antimikrobiyal potansiyeli, hem gıda kaynaklı bozulmalara sebep olan hem de patojenik bakteri ve mayaları da kapsayan çeşitli mikroorganizmalara karşı değerlendirildi. Esansiyel uçucu yağ, uygulanan esansiyel yağın miktarıyla orantılı olarak artan aktivite seviyesi ile dikkate değer bir antibakteriyel ve antifungal etkinlik göstermiştir.


**Anahtar Kelimeler:** *Thymus fallax,* esansiyel yağ, kimyasal kompozisyon, antimikrobiyal aktivite.



## Introduction

Essential oils play a vital role in safeguarding plants by offering protection against bacterial, viral, fungal, and insect threats, as well as deterring herbivore attacks. Presently, there is a recognition of around 3,000 distinct essential oils, among which 300 hold significant commercial value within various industries, including pharmaceuticals, agriculture, food production, sanitation, cosmetics, and perfumery (Bakkali et al. 2008).

Foodborne diseases, arising from either foodborne infection or intoxication, stand as the predominant sources of global foodborne illnesses. These diseases stem from the infiltration of foodborne pathogens into consumable items, often facilitated by processes like cross-contamination, improper handling, and temperature mishandling. Notable among these pathogens are *Staphylococcus aureus*, *Salmonella* species, and *Escherichia coli*, recognized for their roles in foodborne infection and intoxication. In contrast, the quality of food products is compromised by food spoilage microorganisms, rendering them unsuitable for consumption. The limited shelf life of food items due to spoilage presents a major problem within the food industry (Jay 2000; Ray 2001; Tian et al. 2014; Dwivedi et al. 2017 ). Preventing the proliferation of pathogenic and spoilage microorganisms in food traditionally relies on the use of chemical preservatives, which function as antimicrobial agents to hinder the growth of

undesirable microorganisms. Nevertheless, the growing demand for minimally-processed, longer-lasting food products and concerns about potential toxicities associated with chemical preservatives have prompted food manufacturers to explore alternative sources of antimicrobial compounds (Conner 1993; Nychas 1995; da Cruz Cabral et al. 2013; Burt 2004; Awad et al. 2022; Hou et al. 2022; Przybylska-Balcerek et al. 2022). Consequently, essential oils and plant extracts have emerged as promising natural antimicrobial agents, with potential applications in the food and pharmaceutical industries for the management of both pathogenic and spoilage microorganisms. In Türkiye, the genus *Thymus* is richly represented, comprising a total of 41 species, of which 24 are exclusive to the region. Referred to as "kekik" in Turkish, *Thymus* species find extensive use for their dried herbal parts, serving various purposes in herbal teas, culinary condiments, and traditional medicinal practices (Bagci and Baser 2005). Among these, Thyme (*T. fallax* Fisch & C.A.Mey), a member of the *Lamiaceae* family, stands out as a fragrant, perennial shrub that thrives in multiple regions worldwide, including the Western Mediterranean, Southern Italy, and Türkiye (Davis 1982; Baytop 1997; Gieto 2017, Maciel et al. 2022).

Prior research has examined the antimicrobial effects of the essential oil from *T. fallax* (Sokmen et al. 1999; Ozturk and Ercisli 2005; Unal et al. 2008; Goze et al. 2009; Kotan et al. 2010; Küçükbay 2014; Moshaverinia et al. 2022) and various other *Thymus* subspecies (Kim et al. 1995; Smıth-Palmer et al. 1998; Millezi et al. 2012; Sateriale et al. 2022) in previous studies. However, there is a notable absence of reports concerning the antimicrobial properties of *Thymus fallax* essential oils against foodborne spoilage and pathogenic microorganisms. The findings of the current study may serve as a valuable resource in the realm of food quality control. Therefore, the objective of this investigation was to assess the antimicrobial attributes of *T. fallax* essential oil, harvested in Türkiye, against a spectrum of foodborne and spoilage microorganisms, encompassing *Listeria, Enterococcus, Lactobacillus* and *Pedicoccus* spp., as well as the pathogenic microorganisms.

## Materıals and Methods

### *Plant Material*

Wild *T. fallax* plants, classified within the *Lamiaceae* family, were harvested during the flowering phase on Mount Ali (at an elevation of 1650 meters) in Talas, Kayseri, Türkiye. A voucher specimen (Collector number MO 1002) has been officially preserved in the Herbarium of the Department of Biology, Faculty of Science, University of Gazi. Post-collection, the plant was promptly identified and subsequently air-dried at room temperature in preparation for subsequent analysis.

### *Isolation of the Essential Oil*

The essential oils from the air-dried aerial sections of the plant samples were extracted through hydrodistillation employing a Clevenger-type apparatus. Following extraction, the oil was subjected to drying with anhydrous sodium sulfate and subsequently preserved at a temperature of +4°C until the time of analysis.

*Chemical Composition of the Essential Oil*

The chemical composition of the essential oil derived from T. fallax was analyzed at the Medicinal and Aromatic Plant and Drug Research Center (TBAM), situated within Anadolu University at Eskisehir, 26470, Türkiye, utilizing Hewlett-Packard GC/MS and GC instrumentation (Table 1).

**Table 1.** Antimicrobial activity of *T. fallax* essential oil against bacteria

| **Strains** | ***T. fallax* essential oil** | | **Standard antibiotic discs** | |
|---|---|---|---|---|
| | **Inhibiton zone (mm)** | | | |
| | 10µl | 20µl | Cefadroxil | Amikasin |
| *P. vulgaris* RSKK 96026 | 7.95±0.45 | 10.7±0.2 | 16.2 | 18.2 |
| *B. cereus* RSKK 863 | 23.5±1.2 | 27.2±0.8 | 24.0 | 17.5 |
| *E. coli* ATCC 11230 | 8.45±0.5 | 10.7±1.1 | 25.4 | 9.0 |
| *E. aerogenes* RSKK 720 | 12.3±3.3 | 14.0±0.0 | 12.7 | 10.0 |
| *S. aureus* ATCC 25923 | 13.0±1.0 | 15.0±0.2 | 24.6 | 16.3 |
| *S. epidermidis* MU 30 | 8.0±0.0 | 8.2±0.0 | 17.3 | 7.8 |
| *S. typhimurium* CCM 5445 | 7.2±0.0 | 9.5±0.0 | 21.8 | 9.0 |
| *P. fluorescens* RSKK 240 | 7.9±0.2 | 10.0±0.2 | 22.6 | 15.0 |
| *S. enteritidis* RSKK 171 | 7.3±0.4 | 10.0±0.0 | 20.0 | 17.3 |
| *P. aeruginosa* ATCC 29212 | 7.4±0.25 | 8.4±0.4 | 20.0 | 14.0 |
| *S. sonnei* RSKK 877 | 10.0±0.0 | 13±0,0 | 28.0 | - |
| *M. luteus* NRLL B-4379 | 10.4±1.15 | 15.3±0.7 | 37.3 | 19.0 |
| *Y. enterocolitica* ATCC 1501 | 10.7±0.2 | 12.7±1.6 | 21.0 | 11.0 |
| *B. subtilis* ATCC 6633 | 16.4±1.1 | 20.3±0.3 | 19.4 | 15.0 |
| *L. monocytogenes* | 14.5±0.0 | 15.7±0.0 | 19.0 | 13.0 |
| *A. hydrophila* | 8.1±0.1 | 10.8±0.2 | 16.7 | - |
| *E. faecalis* E. 27 | 9.6±0.5 | 10.3±0.2 | 15.0 | 10.6 |

*GC- MS*

The analysis was performed using a Hewlett-Packard GC/MS system with an Innowax FSC column (60 m x 0.25 mm diameter and a 0.25 µm film thickness). Helium was employed as the carrier gas. The initial GC oven temperature was maintained at 60°C for 10 minutes, then programmed to increase to 220°C at a rate of 4°C per minute, where it remained constant for an additional 10 minutes, and finally increased to 240°C at a rate of 1°C per minute. The split flow rate was adjusted to 50 mL/min, and the injector temperature was set at 250°C. Mass spectrometry was conducted at 70 eV with a mass range covering m/z 35 to 425. For library search purposes, the Wiley GC/MS Library and the BASER Library of Essential Oil Constituents were utilized.

*Gas Chromatography*

Gas chromatography analysis was conducted using a Hewlett Packard 6890 system equipped with an HP-Innowax FSC column (60 m x 0.25 mm □ , and a 0.25 µm film thickness). Nitrogen was employed as the carrier gas at a flow rate of 1 mL/min. The temperature within the GC oven was initially held at 60°C for 10 minutes, then ramped up to 220°C at a rate of 4°C/min, maintained at 220°C for an additional 10 minutes, and subsequently programmed to increase to

240°C at a rate of 1°C/min. The injector was set at a temperature of 250°C. To determine the percentage compositions, electronic integration measurements were performed using flame ionization detection (FID) at a temperature of 250°C.

*Microorganisms and Condition for Cultivation*

In this study, a diverse array of microorganisms were employed, including *Proteus vulgaris* RSKK 96026, *Bacillus cereus* RSKK 863, *Escherichia coli* ATCC 11230, *Enterobacter aerogenes* RSKK 720, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* MU 30, *Salmonella typhimurium* CCM 5445, *Pseudomonas fluorescens* RSKK 240, *Salmonella enteritidis* RSKK 171, *Pseudomonas aeruginosa* ATCC 29212, *Shigella sonnei* RSKK 877, *Micrococcus luteus* NRLL B-4379, *Yersinia enterocolitica* ATCC 1501, *Bacillus subtilis* ATCC 6633, *Listeria monocytogenes*, *Aeromonas hydrophila*, *Enterococcus faecalis* E. 27, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* ATCC 11842, *Lactobacillus casei*, *Lactobacillus plantarum* ATCC 2024, *Leuconostoc mesenteroides*, *Pediococcus pentosaceous*, *Streptococcus cremori*, *Streptococcus durans*, *Streptococcus lactis*, *Streptococcus thermophilus* 986, *Saccharomyces carlsbergenesis* 8090, *Candida tropicalis*, *Candida albicans* ATCC 10239, and *Candida krusei*.

The pathogenic bacterial strains were cultivated overnight at 37 ± 0.1°C on Nutrient agar (NA) (Oxoid). Lactic acid bacteria (LAB) were cultured overnight at 37 ± 0.1°C on Lactic Agar (LA), while yeasts were grown overnight at 30 ± 0.1°C on Yeast Pepton Dextrose Agar (YEPD) (Oxoid). Detailed lists of these microorganisms can be found in Tables 1, 2, and 3. All microorganisms were sourced from the Biotechnology Laboratory, Department of Biology, Faculty of Science and Arts, Gazi University, Ankara, Türkiye.

*Antimicrobial Assay*

The antimicrobial activity of the plant's essential oils was assessed using the disc diffusion method, as outlined in previous studies (Collins et al. 1995; Murray et al. 1995). The inoculum for both bacterial and yeast cultures was prepared using No. 0.5 McFarland tubes, resulting in a concentration of 1 × 108 bacteria and 1 × 106 yeast per milliliter.

Sterilized Nutrient Agar (NA), Yeast Pepton Dextrose Agar (YEPD), and Lactic Agar (LA), maintained at a temperature of 45-50°C, were distributed into sterile petri dishes with a diameter of 9 cm (15 ml) after the addition of bacterial and yeast cultures (0.5 ml), ensuring even distribution across the medium. The plates were allowed to sit at room temperature for 15-20 minutes.

Subsequently, 10 µl and 20 µl of each essential oil were applied to sterile 6 mm discs (Schleicher and Schuell) using suction, and these discs were gently placed on the solid agar medium within the petri dishes. The plates containing yeast cultures were then incubated at 30°C for 48 hours, those with pathogenic bacterial strains were incubated at 37°C for 24 hours, and those with lactic acid bacteria were incubated at 30°C for 24 hours. Following the respective incubation periods, the diameters of the inhibition zones on the NA, YEPD, and LA were measured in millimeters.

To serve as positive reference standard antibiotics, amikacin (30 μg), cefadroxil (30 μg), and nystatin (30 μg) were employed. All experiments were conducted in triplicate, and the average and standard deviation (SD) were calculated for the inhibition zone diameters.

**Results and Discussion**

In this present study, the antimicrobial effectiveness of two distinct concentrations of *T. fallax* essential oil was investigated against foodborne spoilage and pathogenic microorganisms. The assessment of potency was quantitatively determined by observing the presence or absence of inhibition zones and measuring their respective diameters. For this reason 27 bacteria and 4 yeasts were used. The results are given in Table 1, 2 and 3.

**Table 2**. Antimicrobial activity of *T. fallax* essential oil against yeasts

| **Strains** | ***T. fallax* essential oil** | | **Standard Antibiotic disc** |
|---|---|---|---|
| | **10µl** | **20µl** | **Nystatin** |
| | **Inhibiton zone (mm)** | | |
| *S. carlsbergenesis* 8090 | 14.6±0.0 | 20.2±0.0 | 19.4 |
| *C. tropicalis* | 23.5±0.0 | 26.3±0.0 | 15.7 |
| *C. albicans* ATCC 10239 | 16.7±0.0 | 17.7±0.0 | 18.7 |
| *C. krusei* | 6.5±0.0 | 7.2±0.0 | 11.4 |

**Table 3.** Antimicrobial activity of T. fallax essential oil against LAB.

| **Strains** | ***T. fallax* essential oil** | |
|---|---|---|
| | **10µl** | **20µl** |
| | **Inhibiton zone (mm)** | |
| *L. acidophilus* | - | 7.3±0.2 |
| *L. bulgaricus* ATCC 11842 | 7.7±0.5 | 13.4±0.2 |
| *L. casei* | 8.0±0.1 | 8.8±0.8 |
| *L. plantarum* ATCC 2024 | 10.2±0.5 | 11.3±0.1 |
| *Leu. mesenteroides* | 9.0±0.0 | 13.0±1.2 |
| *P. pentosaceous* | 9.0±0.9 | 10.0±0.2 |
| *S. cremoris* | 9.0±0.0 | 9.2±0.4 |
| *S. durans* | - | 7.4±0.5 |
| *S. lactis* | 8.6±0.6 | 10.6±0.1 |
| *S. thermophilus* 986 | 9.3±0.7 | 9.7±0.1 |

Both doses of essential oil displayed antibacterial activities on all the bacteria. In addition, different doses of essential oil showed different antibacterial effects on these strains, but the differences were not excessive. However, no effect was observed for Lactobacillus acidophilus and *Streptococcus durans*.

*Pseudomonas fluorescens* RSKK 240 and *Salmonella typhimurium* CCM 5445 have been the most resistant bacteria against thyme essential oil. However, the highest activity was obtained from the essential oil on *Escherichia coli* ATCC 11230. As shown in Table 1, cefadroxil had similar effect on the growth of *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes*. Furthermore, the inhibition effect of amikacin were much lower than the essential oil on *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Listeria monocytogenes* and *Aeromonas hydrophila.*

The overwhelming majority of documented cases of foodborne illnesses with identified causes can be attributed to bacterial agents. Among these bacterial pathogens are *Campylobacter jejuni*, *Salmonella*, *Staphylococcus aureus*, *Escherichia coli* O157:H7, *Shigella*, *Bacillus cereus*, *Clostridium perfringens*, *Listeria monocytogenes*, *Yersinia enterocolitica*, and *Clostridium botulinum*. These bacterial pathogens, which are linked to human food poisoning, may originate in production animals, making them potential sources of contamination. The sequence of events spanning from the stages of slaughter, processing, storage, and food preparation can facilitate the proliferation of these contaminating microorganisms. According to assessments by experts in public health and food safety, millions of cases of illness, both domestically and globally, can be directly attributed to foodborne pathogens each year (Oliver et al. 2005).

The worldwide impact of foodborne illnesses is challenging to quantify accurately. Nevertheless, it is estimated that approximately 2.1 million children in developing countries succumb to diarrheal-related diseases annually, with suspicions that food or water serves as the transmission vehicle for a significant portion of these illnesses (WHO 2002).

Ten µl and 20µl of the essential oil inhibited the growth of all LAB tested and the inhibition zones ranged between 7.7-10.2 mm and 7.3-13.4 mm, respectively. However, the essential oil (10µl) showed no inhibition effect on *Lactobacillus acidophilus* and *Streptococcus durans*. The most sensitive LAB to the essential oil in the present study were *Lactobacillus bulgaricus* and *Leu. mesenteroides.* The diameters of maximum inhibitory zones at the highest essential oil dosage (20µl) were as large as 13.4 mm for *Lactobacillus bulgaricus* and 13.0 mm for *Leu. mesenteroides.* The least sensitive LAB to the essential oil was *Lactobacillus acidophilus*, and the diameter of inhibitory zones at the highest essential oil was 7.3 mm.

Moreover, all the fungi were inhibited by the essential oil. The inhibition zones of its essential oil (10µl and 20µl) on *Candida albicans* were 16.7 and 17.7 mm, respectively. The essential oil (10µl and 20µl) inhibited the growth of *Saccharomyces carlsbergenesis* and the inhibition zones were 14.6 and 20.2 mm, respectively. The most effect of its essential oil was on *Candida tropicalis* and the inhibition zones ranged between 23.5 and 26.3 mm. However, it is interesting to note that the strains of *Candida albicans, Candida tropicalis, Saccharomyces carlsbergenesis* and *Candida krusei*, which are present in food as spoilage and pathogen bacteria, were found to be affected at test concentrations in our study.

The results indicate that the essential oil of *T. fallax* has a capacity to inhibit the growth of foodborne spoilage and pathogen microorganisms. For this reason, the chemical composition of its essential oil was determined. A total of 28 compounds were detected by using GC and

GC-MS method (Table 4). The major compounds of the essential oil of this plant were 1,8-cineole (9.9%), linalool (8.8%) and camphor (8.1%) detected, respectively (Table 4). 1,8-cineole (Bosnić et al. 2006; Vuuren and Viljoen, 2007; Hendry et al. 2009), linalool (Bagamboula et al. 2004; Krist et al. 2008) and camphor (Santoyo et al. 2005; Safaei-Ghomi and Batooli 2010) are also known to exhibit antibacterial activity.

**Table 4.** The chemical composition of the essential oil from *T. fallax*.

| | Compound | Percentage (%) |
|---|---|---|
| 1 | 1,8-cineole | 9.9 |
| 2 | linalool | 8.8 |
| 3 | camphor | 8.1 |
| 4 | β-caryophyllene | 6.2 |
| 5 | spathulenol | 4.5 |
| 6 | (E)-β-ocimene | 4.3 |
| 7 | α-terpineol | 3.7 |
| 8 | borneol | 3.4 |
| 9 | bicyclogermacrene | 3.2 |
| 10 | germacrene D | 3.1 |
| 11 | (E)-nerolidol | 3.1 |
| 12 | elemol | 3.0 |
| 13 | α-cadinol | 2.2 |
| 14 | trans-cadinol | 1.7 |
| 15 | bornyl acetate | 1.6 |
| 16 | camphene | 1.4 |
| 17 | alloaromadendrene | 1.3 |
| 18 | myrcene | 1.2 |
| 19 | (Z)-β-farnesene | 1.2 |
| 20 | Germacrene D-4-ol | 1.2 |
| 21 | δ-cadinene | 1.1 |
| 22 | caryophyllene oxide | 1.1 |
| 23 | *p*-cymene | 0.9 |
| 24 | *trans*-α-bergamotol | 0.9 |
| 25 | α-pinene | 0.8 |
| 26 | β-bisabolene | 0.8 |
| 27 | carvacrol | 0.2 |
| 28 | thymol | 0.1 |
| | **Total** | **79** |

There are some studies in the literature examining the chemical composition and antimicrobial effectiveness of the *T. fallax* species found in Türkiye (Öztürk and Ercişli et al. 2005, Göze et al. 2009, Moshaverinia et al. 2022, Bozhüyük and Kordalı, 2022). From a broader perspective, these studies not only focus on different aspects of *T. fallax* but also use different methodologies and produce different results. Although their common denominator is the discovery of the medical use of *T. fallax*, the research goals, approaches, and findings of each differ significantly. While this study placed great emphasis on examining the antibacterial and antifungal effects of the plant, Göze et al. (2009) took a more comprehensive approach and tested the antimicrobial activity of a wider range of microorganisms. Similarly, while Öztürk and Ercişli's (2005) study focused on the antibacterial effect of the methanol extract of the plant, while this study examined the chemical composition of the plant's essential oil and its antimicrobial and antifungal effects in detail. In conclusion, the essential oil of *T. fallax* showed

strong activities against *Candida tropicalis, Escherichia coli, Yersinia enterocolitica, Listeria monocytogenes* and *Aeromonas hydrophila* in this study. Moreover, it's worth noting that the dosages of essential oil we applied were notably lower than those of conventional antibiotics. This in vitro investigation furnishes substantial evidence suggesting that this plant is a promising reservoir of antimicrobial compounds with efficacy against the foodborne spoilage and pathogenic microorganisms under examination. Consequently, the essential oil extracted from *T. fallax* presents the potential to serve as a source of preservatives that may find utility in the realms of both the food and pharmaceutical industries.

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**Conflict of Interest**

No known or potential conflict of interest exist for any author.

**Kaynaklar**

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