



This article is cited as: Özcan Ateş G. (2026). Evaluation of the In Vitro Antifungal Activity of Commercially Available Plant Extracts, Fixed and Essential Oils Against Aspergillus, Penicillium, and Trichoderma Species, Mantar Dergisi, 17(1) 43-52.

Geliş(Received) :01.01.2026

Kabul(Accepted) :24.02.2026

Research Article

Doi: 10.30708/mantar.1854027

Evaluation of the In Vitro Antifungal Activity of Commercially Available Plant Extracts, Fixed and Essential Oils Against *Aspergillus*, *Penicillium*, and *Trichoderma* Species

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Abstract: Plant extracts, fixed oils (FO), and essential oils (EO) are continually investigated for their biological activities. However, the antifungal activity of plant extracts and oils is less frequently studied. Therefore, in this study, the antifungal activities of commercially available 11 FOs, 24 EOs, 11 PEs, and one tar were primarily investigated against eight microfungi (*Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Penicillium chrysogenum*, *Penicillium oxalicum*, *Penicillium purpurogenum*, *Trichoderma* sp.-1, and *Trichoderma* sp.-2) species using the agar disc diffusion method. *Cuminum cyminum* seed FO, *Mentha piperita* EO, *Thymus* spp. EO, *Zingiber officinale* EO, and *Juniperus oxycedrus* tar exhibited inhibition zones ranging from 8.00 to 90.00 mm. Other plant extracts, FOs, and EOs were found to have no antifungal activity. Then, the inhibition of *M. piperita* EO, *Thymus* spp. EO and *J. oxycedrus* tar on radial growth, which showed the highest inhibition zone, were also examined. It was determined that they inhibited radial growth between 52% and 100%. Moreover, the effect of two *Zingiber officinale* EOs, which were determined to have both antifungal activity and not antifungal activity because of agar disc diffusion analysis, on radial growth was also investigated. *Zingiber officinale* EO, which has antifungal activity, inhibited radial growth by 66-100%. On the other hand, the *Z. officinale* EO promoted the growth of *Aspergillus fumigatus* and inhibited the radial growth of the different microfungi tested by up to 42.98%. As a result, the antifungal activity depends on the oil's composition. Therefore, the beneficial effects may not be seen in all commercial products as stated in the literature. For this reason, it is necessary to conduct more detailed studies of commercially available products.

Keywords: Antifungal activity, Fixed oil, Essential oil, Plant extracts.

Ticari Olarak Temin Edilen Bitki Ekstraktları, Sabit ve Esansiyel Yağların *Aspergillus*, *Penicillium* ve *Trichoderma* Türlerine Karşı İn Vitro Antifungal Aktivitesinin Değerlendirilmesi

Öz: Bitki ekstraktı (PE), sabit yağlar (FO) ve uçucu yağlar (EO) biyolojik aktiviteleri açısından sürekli olarak araştırılmaktadır. Bununla birlikte, ekstraktı ve yağlarının antifungal aktivitesi daha az sıklıkla incelenmektedir. Bu nedenle, bu çalışmada, agar disk difüzyon yöntemi kullanılarak, ticari olarak temin edilebilen 11 FOs, 24 EOs, 11 PEs ve bir katranın sekiz küf (*Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Penicillium chrysogenum*, *Penicillium oxalicum*, *Penicillium purpurogenum*, *Trichoderma* sp.-1, and *Trichoderma* sp.-2) türüne karşı antifungal aktiviteleri araştırılmıştır. *Cuminum cyminum* tohumu FO'su, *Mentha piperita* EO'su, *Thymus* spp. EO'su, *Zingiber officinale* EO'su ve *Juniperus oxycedrus* katranı, 8,00 ile 90,00 mm arasında inhibisyon bölgeleri göstermiştir. Diğer bitki ekstraktı, FO'lar ve EO'ların antifungal



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aktivitesi bulunmamıştır. Daha sonra, en yüksek inhibisyon bölgesini gösteren *M. piperita* EO'su, *Thymus* spp. EO'su ve *J. oxycedrus* katranının radyal büyüme üzerindeki inhibisyonu da incelenmiştir. Yapılan incelemelerde, bu yağların radyal büyümeyi %52 ile %100 arasında inhibe ettiği belirlendi. Ayrıca, agar disk difüzyon analizi nedeniyle hem antifungal aktiviteye sahip olduğu hem de antifungal aktiviteye sahip olmadığı belirlenen iki *Z. officinale* EO'sunun radyal büyüme üzerindeki etkisi de araştırıldı. Antifungal aktiviteye sahip olan *Z. officinale* EO radyal büyümeyi %66-100 oranında inhibe etti. Öte yandan antifungal aktiviteye sahip olmadığı belirlenen *Z. officinale* EO ise *Aspergillus fumigatus* büyümesini teşvik etti ve test edilen farklı küflerin radyal büyümesini %42,98'e kadar inhibe etti. Sonuç olarak, antifungal aktivite yağın bileşimine bağlıdır. Bu nedenle, literatürde belirtildiği gibi, faydalı etkiler tüm ticari ürünlerde görülmeyebilir. Bu nedenle, piyasada bulunan ürünler üzerinde daha detaylı çalışmalar yapılması gerekmektedir.

Anahtar kelimeler: Antifungal aktivite, Sabit yağ, Esansiyel yağ, Bitki ekstraktı.

Introduction

Fungi are various eukaryotic organisms ubiquitous in nature and mostly live in dead and decaying organic matter. Microfungi are an essential group of microorganisms for humans, as they produce antibiotics, enzymes, and food. In addition, they play crucial roles in ecosystem processes, such as the cycling of matter, biological transformations, and energy flows, due to their ability to decompose organic matter. In addition to its ecological and beneficial effects on human health, only a few types of fungi are pathogenic (Mikawlawng et al., 2014; Boddy, 2016).

Despite the development of new antifungal drugs, fungal infections are still a significant problem in medicine. In addition, the rapidly increasing resistance to existing antifungals is another critical issue. Moreover, foodborne diseases caused by contaminated food are also a significant public health problem (Ahmadi et al., 2020). Synthetic preservatives are used to prevent microbial spoilage and biosynthesis of toxic metabolites. However, today, consumers prefer safe, natural products with fewer synthetic additives (Kocić-Tanackov et al., 2015). Therefore, it is very important to search for new antifungal agents, given the use of natural preservatives and the increasing prevalence of antifungal resistance, to protect foods in line with consumer demands. Plants are the most significant source of research on both new antifungals and natural food preservatives.

Plant extracts (PE), fixed oils (FO), and essential oils (EO) such as *Lavandula angustifolia* L. (lavanta), *Origanum vulgare* L. (karakınık), *Rosmarinus officinalis* L. (biberiye), *Salvia officinalis* L. (adaçayı), or *Thymus vulgaris* L. (adi kekik), etc., are not only used as food preservatives and a source of new antifungal agents but are also frequently used in the cosmetic industry for antimicrobial preservatives, fragrances, or other beauty purposes. In addition, EOs are commonly used as natural preservatives, especially in cosmetic formulations (Dreger and Wielgus, 2013; Sarkic and Stappen, 2018; Karpiński, 2020; Sesli et al., 2020).

Plants and EO have been used for centuries for medicinal purposes. Interest in traditional medicine practices, especially herbal medicine, is increasing worldwide. Especially recently, due to the positive effects of various biological activities on health, research on EOs has increased in interest. The key driver of these studies is consumer demand for natural, safe, and effective health products. Therefore, studies on determining the pharmacological properties of plant extracts and EOs, and on their applications in various industries, draw attention (Ni et al., 2021). There are many studies in the literature on the antibacterial activities of thyme, mint, ginger, rosemary, cumin, clove, myrtle, black cumin, garlic, and various EOs (Sivropoulou et al., 1995; Norajit et al., 2007; Hussain et al., 2010; Çetin et al., 2011; Karagözlü et al., 2011; Mulyaningsih et al., 2011; Sienkiewicz et al., 2011; Sivasothy et al., 2011; Viuda-Martos et al., 2011; Wang et al., 2012; Bajalan et al., 2017). However, studies on antifungal studies against microfungi are few and have not been investigated in depth (Nazzaro et al., 2017; Hu et al., 2019; Mutlu-Ingok et al., 2020; Valková et al., 2021).

The documented biological activities of PE, FO, and EO have led to increased consumer interest in these products. As a result, many different manufacturers are marketing these products. However, the chemical composition of these products is unclear. To increase profits, manufacturers may reduce the concentration of pure substances in their products or mix them with other materials. Thus, this alteration of chemical composition also directly affects biological activity. Therefore, this study aims to determine the antifungal activity of commercial PEs, FOs, and EOs against eight microfungi species.

Material and Metod

Plant Extract, Fixed and Essential Oils

For the antifungal activity test, 11 FOs, 24 EOs, 11 PEs, and one tar (Table 1) were commercially obtained from herbalists in Çanakkale, Türkiye.

Table 1. Plant extracts (PE), fixed oils (FO), and essential oils (EO) were used in this study.

Product By	Plant Species	Turkish Name of Plant Species (Güner et al., 2012)	Type	Origin	Commercial Name	Commercial Turkish Name
A	<i>Bryophyta</i> spp.	-	EO*	-	Balsam oil	Pelesenk Yağı
	<i>Rubus</i> spp.	Yapraklı Karayosunları	FO**	-	Moss oil	Yosun yağı
	<i>Cuminum cyminum</i> L.	-	FO	Berry	Blackberry oil	Böğürtlen yağı
B	<i>Daucus carota</i> L.	Acemkimyonu	FO	Seed	Cumin seed oil	Kimyon yağı
	<i>Myrtus communis</i> L.	Yabani havuç	FO	-	<i>Daucus carota</i> oil	Havuç yağı
	<i>Rosmarinus officinalis</i> L.	Mersin	EO	-	Myrti communi oil	Mersin yağı
C	<i>Triticum</i> spp.	Biberiye	EO	-	Rosmarini oil	Biberiye yağı
	<i>Allium sativum</i> L.	-	FO	-	<i>Triticum</i> spp. oil	Buğday yağı
	<i>Dianthus caryophyllus</i> L.	Sarımsak	EO	-	Garlic oil	Sarımsak yağı
D	<i>Elaeagnus</i> spp.	-	EO	-	Carnation oil	Karanfil yağı
	<i>Eucalyptus</i> spp.	-	EO	-	Russian olive oil	İğde yağı
	<i>Jasminum</i> spp.	-	EO	-	Eucalyptus aroma	Okalipütüs aroması
E	<i>Myrtus communis</i> L.	-	EO	-	Jasmin oil	Yasemin yağı
	<i>Rubus idaeus</i> L.	-	EO	-	Myrtle oil	Mersin yağı
	<i>Viola</i> spp.	Ahududu	PE***	-	Raspberry oil	Ahududu aroması
F	<i>Aloe vera</i> L.	-	EO	-	Violet oil	Menekşe yağı
	<i>Carthamus tinctorius</i> L.	Sarısabitir	FO	-	<i>Aloe vera</i> oil	Aloe vera yağı
	<i>Eucalyptus</i> spp.	Aspir	FO	-	<i>Carthamus tinctorius</i> oil	Aspir yağı
G	<i>Myrtus communis</i> L.	-	PE	-	Eucalyptus aroma	Okalipütüs aroması
	<i>Silybum marianum</i> (L.) Gaertn.	-	FO	-	Myrtle oil	Mersin yağı
	<i>Zingiber officinale</i> Roscoe	Deve diken	EO	-	<i>Silybum marianum</i> oil	Devediken yağı
H	<i>Zingiber officinale</i> Roscoe	-	FO	-	Ginger oil	Zencefil yağı
	<i>Mentha piperita</i> L.	-	EO	-	Ginger oil	Zencefil yağı
	<i>Thymus</i> spp.	-	EO	-	Mint oil	Nane yağı
I	<i>Aesculus hippocastanum</i> L.	-	EO	-	Thyme oil	Kekik yağı
	<i>Allium sativum</i> L.	At kestanesi	EO	-	Equus castanea oil	At kestanesi yağı
	<i>Aloe vera</i> L.	-	EO	-	Allium oil	Sarımsak yağı
J	<i>Centaurea minus</i> Rafn.	-	EO	-	<i>Aloe vera</i> oil	Aloe vera yağı
	<i>Juniperus oxycedrus</i> L.	Kırmızı kantron	EO	-	Centaurea minus oil	Kantaron yağı
	<i>Lavandula hybrida</i> Rev.	Katran ardıcı	EO	-	<i>Juniperus oxycedrus</i> tar	Ardıç katranı
K	<i>Melaleuca</i> spp.	-	EO	-	Lavandula hybrid oil	Karabaş otu yağı
	<i>Nigella sativa</i> L.	-	EO	-	Tea lignum oil	Çay ağacı yağı
	<i>Persea gratissima</i> L.	Çörekotu	FO	-	Nigella oil	Çörekotu yağı
L	<i>Sinapis</i> spp.	-	EO	-	Avocado oil	Avokado yağı
	<i>Urtica</i> spp.	-	EO	-	Sianpsis oil	Hardal yağı
	<i>Zingiber officinale</i> Roscoe	-	EO	-	Urticae oil	Isırgan tohumu yağı
M	<i>Viola odorata</i> Linn.	-	EO	-	Ginger oil	Zencefil yağı
	<i>Crataegus monogyna</i> Jacq.	Kokulu menekşe	EO	-	<i>Viola odorata</i> oil	Menekşe yağı
	<i>Cynara scolymus</i> L.	Yemişen	PE	Berry	Hawthorn berry extract	Alic Meyvesi Ekstraktı
N	<i>Nigella sativa</i> L.	Enginar	PE	Leaf	Artichoke leaf extract	Enginar Yaprağı Ekstraktı
	<i>Olea europaea</i> L.	-	PE	Seed	Black cumin seed extract	Çörekotu Tohumu Ekstraktı
	<i>Rosa canina</i> L.	Zeytin	PE	Leaf	Olive leaf extract	Zeytin Yaprağı Ekstraktı
O	<i>Silybum marianum</i> (L.) Gaertn.	Kuşburnu	PE	Berry	Rosehip berry extract	Kuşburnu Meyvesi Ekstraktı
	<i>Taraxacum officinale</i> Weber	-	PE	-	Virgin Mary thistle extract	Meryemana Diken Ekstraktı
	<i>Vitex agnus-castus</i> L.	-	PE	-	Dandelion extract	Karahindiba Ekstraktı
P	<i>Vitis vinifera</i> L.	Hayıt	PE	-	<i>Vitex agnus-castus</i> extract	Hayıt Ekstraktı
	-	Asma	PE	Seed	Grape seed extract	Üzüm Çekirdeği Ekstraktı

*EO: essential oil, **FO: fixed oil, ***PE: plant extract, -: Unknown

Fungal Strains

In this study, microfungi isolates obtained from Prof. Dr. Özlem ABACI GÜNYAR of the Biology Department at Ege University were used. The isolates used to determine antifungal activity were *Aspergillus flavus* Link (cibil asper), *Aspergillus fumigatus* Fresen. (kiran asper), *Aspergillus niger* Tiegh. (kara asper), *Penicillium chrysogenum* Thom (penisilyum), *Penicillium oxalicum* Currie & Thom (gevrek penisilyum), *Penicillium purpurogenum* Stoll (species fungorum current name: *Talaromyces purpureogenus* Stoll) (bordo süpürge), *Trichoderma* sp.-1 (yeşilküf), and *Trichoderma* sp.-2 (yeşilküf) (Sesli et al., 2020; URL-1, 2026).

Preparation of Spore Suspension

The spore solution preparation method described by Kocić-Tanackov et al. (2015) was used with minor modification. Stock cultures were inoculated into Potato Dextrose Agar (PDA) (Merck, Germany) petri plates and incubated at 25°C for 7 days. After incubation, spores

were collected aseptically with 10 mL of physiological saline and transferred to sterile test tubes. Spore suspensions were adjusted to a 10⁶ spores/mL final concentration by hemocytometer. At the same time, serial dilutions were prepared from the spore solutions, and the spread plate method confirmed the final concentration.

Agar Disk Diffusion Method

The agar disc diffusion method was used, with minor modifications, as specified by Korukluoğlu et al. (2009). First, 100 µL of the prepared spore solution was inoculated onto the surface of PDA medium in 90 mm petri plates. Then, 6 mm-diameter sterilized paper discs were placed on the PDA surface, and 20 µL (the amount that the blank disk can absorb) of the PEs, FOs, or EOs were added. After 15 minutes, the petri dishes were incubated at 25 ± 2°C for 72 hours. Ketoconazole (50 µg) (Bioanalyze, Türkiye) was used as the positive control, and a sterile blank disc as the negative control. After

incubation, the inhibition zone diameters around the discs were measured in mm.

Inhibition of Radial Growth

Five EOs were selected to determine the inhibition of radial growth. F- *Mentha piperita* EO, F- *Thymus* spp. EO, and G- *Juniperus oxycedrus* tar, with the highest inhibition zone diameter, were selected for radial growth inhibition. In addition, E and G *Zingiber officinale* EOs were chosen to compare two different oils. PDA medium was prepared as 300 mL into a 500 mL Erlenmeyer flask and autoclaved at 121°C for 15 minutes. After autoclaving, the media were cooled to 45-50°C. 10 µL/mL of EOs was added to the cooled media under aseptic conditions. Next, 20 mL of medium containing EOs was poured into a 90 mm petri plate. Finally, 10 µL of spore solution (10⁴ spores/mL) was inoculated into the centre of the prepared medium. As a control, PDA media without EO were used. After inoculation, the petri plates were incubated at 25 ± 2°C for 3 days. Colony diameters were measured at the end of incubation, and inhibition of radial growth was calculated by the following equation (1):

$$\%I = (C - T) / C \times 100 \quad (1)$$

C: Colony diameter (mm) in the control plate

T: Colony diameter (mm) in the EOs containing plate

I: inhibition (%)

Statistical analysis

Table 2. FOs, EOs, and tar inhibition zones (in mm) tested against *Aspergillus* spp.

	<i>Aspergillus flavus</i> (cibil asper)		<i>Aspergillus fumigatus</i> (kiran asper)		<i>Aspergillus niger</i> (kara asper)	
	Inhibition zone	%**	Inhibition zone	%**	Inhibition zone	%**
B- <i>Cuminum cyminum</i> seed FO	9.33 ± 0.78*	-56.26	6.00 ± 0.01***	-69.23	12.75 ± 0.97	-32.89
F- <i>Mentha piperita</i> EO	11.33 ± 0.49	-46.88	39.08 ± 5.18	200.41	15.83 ± 1.12	-16.68
F- <i>Thymus</i> spp. EO	43.67 ± 3.00	204.74	52.17 ± 2.89	267.54	45.5 ± 1.93	239.47
G- <i>Zingiber officinale</i> EO	8.00 ± 0.85	-62.49	8.50 ± 0.52	-56.41	6.00 ± 0.01	-68.42
G- <i>Juniperus oxycedrus</i> Tar	9.17 ± 0.72	-57.01	8.09 ± 0.67	-58.51	7.83 ± 0.72	-58.79
Ketoconazole (50 µg)	21.33 ± 0.88	-	19.5 ± 1.88	-	19.00 ± 1.04	-

*Data are given as mean ± standard deviation. **Percentage of antifungal efficacy compared to ketoconazole. ***Antifungal activity could not be determined.

Table 3. FOs, EOs, and tar inhibition zones (in mm) tested against *Penicillium* spp.

	<i>Penicillium chrysogenum</i> (penisilyum)		<i>Penicillium oxalicum</i> (gevrek penisilyum)		<i>Penicillium purpurogenum</i> (bordo süpürge)	
	Inhibition zone	%**	Inhibition zone	%**	Inhibition zone	%**
B- <i>Cuminum cyminum</i> seed FO	12.33 ± 1.23	-37.03	17.17 ± 1.64	-14.87	14.58 ± 0.79	-23.26
F- <i>Mentha piperita</i> EO	15.08 ± 1.98	-22.98	29.17 ± 1.85	144.62	15.17 ± 1.80	-20.16
F- <i>Thymus</i> spp. EO	55.67 ± 3.00	284.32	68.83 ± 6.15	341.25	90.00 ± 0.1	473.69
G- <i>Zingiber officinale</i> EO	11.50 ± 0.52	-41.27	19.00 ± 1.13	-5.80	12.00 ± 1.71	-36.84
G- <i>Juniperus oxycedrus</i> Tar	15.33 ± 2.27	-21.17	16.83 ± 2.13	-16.56	14.58 ± 1.08	-23.26
Ketoconazole (50 µg)	19.58 ± 1.62	-	20.17 ± 1.03	-	19.00 ± 0.85	-

*Data are given as mean ± standard deviation. **Percentage of antifungal efficacy compared to ketoconazole. ***Antifungal activity could not be determined.

All analyses were performed in triplicate to determine the antifungal activity of PEs, FOs, and EOs. The data were evaluated using Minitab 17.0. Results are given as mean ± standard deviation. A statistical comparison was not performed.

Results

The agar disc diffusion method was used to evaluate the antifungal activity of 11 FOs, 24 EOs, 11 PEs, and one tar against eight microfungi strains. Using the agar disc diffusion method, it was determined that commercially available 11 PEs, 9 FOs, and 21 EOs did not exhibit antifungal activity. Inhibition zone diameters are given in Tables 2, 3, and 4. It was determined that B- *Cuminum cyminum* seed FO, F- *Mentha piperita* EO, F- *Thymus* spp. EO, G- *Zingiber officinale* EO, and G- *Juniperus oxycedrus* tar had antifungal activity against the tested molds.

In the study, ketoconazole, used as a positive control, yielded inhibition zones ranging from 19.00 to 28.25 mm against the tested microfungi. F- *Thymus* spp. EO showed the largest inhibition zones (43.67 to 90.00 mm) against all tested microfungi and was determined to have 165.44% to 473.69% higher antifungal activity than ketoconazole. F- *M. piperita* EO showed vigorous antifungal activity against *A. fumigatus* (39.08 ± 5.18) and *P. oxalicum* (29.17 ± 1.85) species and was 200.41% and 144.62% more effective than ketoconazole, respectively.

Table 4. FOs, EO, and tar inhibition zones (in mm) tested against *Trichoderma* sp.

	<i>Trichoderma</i> sp.-1 (yeşil küf)		<i>Trichoderma</i> sp.-2 (yeşil küf)	
	Inhibition zone	%**	Inhibition zone	%**
B- <i>Cuminum cyminum</i> seed FO	6.00 ± 0.01	-78.76	6.00 ± 0.01	-77.28
F- <i>Mentha piperita</i> EO	15.25 ± 0.62	-46.02	18.50 ± 2.02	-31.48
F- <i>Thymus</i> spp. EO	64.50 ± 6.67	228.32	44.67 ± 3.39	165.44
G- <i>Zingiber officinale</i> EO	14.67 ± 0.49	-48.07	6.00 ± 0.01	-77.28
G- <i>Juniperus oxycedrus</i> Tar	15.33 ± 0.78	-45.73	6.00 ± 0.01	-77.28
Ketoconazole (50 µg)	28.25 ± 1.36	-	27.00 ± 1.95	-

*Data are given as mean ± standard deviation. **Percentage of antifungal efficacy compared to ketoconazole. ***Antifungal activity could not be determined.

B- *C. cyminum* seed FO produced inhibition zones ranging from 9.33 to 17.17 mm against *A. flavus*, *A. niger*, *P. chrysogenum*, *P. oxalicum*, and *P. purpurogenum*, and showed lower antifungal activity compared to ketoconazole. G- *Z. officinale* EO was effective against all tested molds except *A. niger* and *Trichoderma* spp.-2, yielding inhibition zones between 8.00 and 19.00 mm. G- *J. oxycedrus* tar was effective against all tested microfungi except *Trichoderma* spp.-2, producing inhibition zones between 7.83 and 16.83 mm. This demonstrates the species-specific antifungal potential of the oils.

The radial growth inhibition of F- *M. piperita* EO, F- *Thymus* sp. EO, and G- *J. oxycedrus* tar, which gave the highest inhibition zone diameter in agar disc diffusion results, were also investigated. In addition, radial growth inhibition was also determined due to the different antifungal activity of *Z. officinale* EO from companies E

and G on the tested microfungi. The effect of inhibition of radial growth is given in Figure 1.

F- *M. piperita* EO at a concentration of 10 µL/mL inhibited all tested microfungi by 100.00%, while F- *Thymus* sp. EO inhibited them by 52.00% to 77.83%. G- *J. oxycedrus* tar and G-*Z. officinale* EO inhibited the tested *Penicillium* species and *Trichoderma* spp.-1 by 100%, while *Aspergillus* species and *Trichoderma* spp.-2 were inhibited by 66.04% to 85.77%. When comparing the effect of *Z. officinale* EO from two different companies on radial growth, G-*Z. officinale* EO provided 66.04% to 100% inhibition. In contrast, E-*Z. officinale* EO provided 9.87% to 42.98% inhibition against all tested molds except *A. fumigatus* and *Trichoderma* spp.-1. Moreover, E-*Z. officinale* EO even increased the radial growth of *A. fumigatus* by 7.45%. Also, it neither increased nor inhibited radial growth against *Trichoderma* spp.-1.

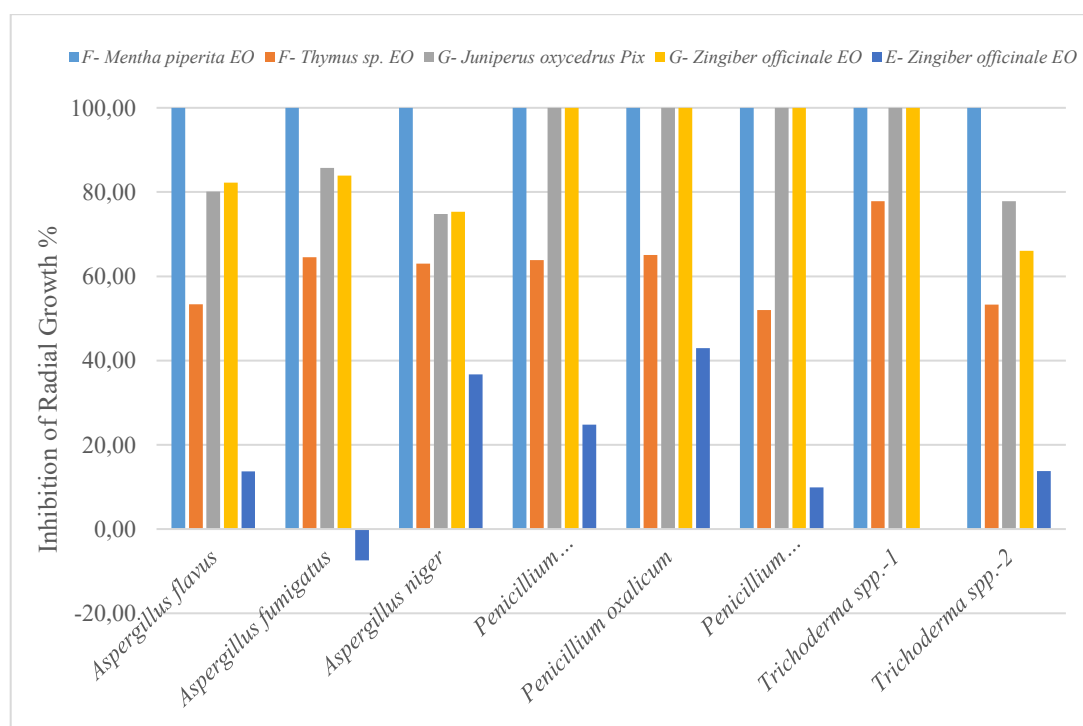


Figure 1. Radial growth inhibition (%) in PDA containing EO compared with PDA without EO.

Discussions

In this study, the antifungal activity of fixed oil, essential oil, and tar extracts from plants against various microfungi was evaluated, and the findings revealed that antifungal activity varied significantly with mold species and the chemical composition of the extracts.

It was determined that the inhibition zone diameter of *F. Thymus* spp. EO showed a larger inhibition zone than the positive control, ketoconazole, an antifungal. Furthermore, it was determined that *F. M. piperita* EO gave the second-highest inhibition zone diameter and even had a higher inhibition zone diameter than the positive control against *A. fumigatus* and *P. oxalicum*. As a result, *M. piperita* and *Thymus* spp. EOs had highly antifungal activity. Our results regarding the antifungal activity of *Mentha piperita* and *Thymus* sp. EOs are consistent with the work of other researchers (Gonçalves et al., 2007; de Sousa Barros et al., 2015; Plavšić et al., 2020; Yan et al., 2021). Yan et al. (2021) determined that mint and thyme EOs at 150 µL/L concentration inhibited radial growth of *Rhizopus stolonifer* (Ehrenb.) Vuill. by 92-98%, and in our study, it was determined that *Thymus* spp. EOs completely inhibited the radial growth of all tested fungi.

B-C. cyminum seed FO was determined to give an inhibition zone diameter between 9.33 and 17.17 mm against *Aspergillus* and *Penicillium* strains. However, this FO was found to be ineffective against *A. fumigatus* and two *Trichoderma* strains. El-Said and Goder (2014) found that *C. cyminum* EO was effective against 90 different fungal isolates, including *A. fumigatus* and *Trichoderma* strains, while Kedia et al. (2014) found the antifungal activity of *C. cyminum* seed EOs against aflatoxin-producing *A. flavus*. Although previous studies have determined that *C. cyminum* essential oils possess antifungal activity, this study showed low or even no antifungal activity. This may be due to differences in the chemical composition and type of oils, as well as variations in the susceptibility of the tested microorganisms. Furthermore, variations in commercial products also affect antifungal activity. This situation demonstrates that the antifungal activity of *C. cyminum* essential oils cannot be generalized and that individual assessment is necessary, especially for commercial products, and that determining their chemical composition is critical.

When the antifungal activity of *Z. officinale* EOs from two different companies (E and G) was evaluated, the EO from company G exhibited antifungal activity. In contrast, the EO of company E lacked antifungal activity. It was determined that G- *Z. officinale* EO gave an 8-19 mm inhibition zone diameter against eight tested mold species. Sasidharan and Menon (2010) determined the

antifungal activity of *Z. officinale* EOs obtained from fresh and dried ginger rhizomes against *Trichoderma* spp., *Penicillium* spp., and *A. niger*. Hence, the study results are consistent with the results of Sasidharan and Menon (2010). Likewise, Sharma and Sharma (2011) and López et al. (2017) determined the antifungal activity of *Z. officinale* EOs against dermatophytes. Abdullahi et al. (2020) determined that *Z. officinale* EOs completely inhibited radial growth of 27% to 50% of *Fusarium oxysporum* Schldt., *Colletotrichum falcatum* Went, *Ganoderma boninense* Pat., *Pyricularia oryzae* Cavara, and *Rigidoporus microporus* (Sw.) Overeem phytopathogens at a concentration of 1 µL/mL. They reported that it completely inhibited the radial growth of phytopathogens at a concentration of 6 µL/mL. The researchers' results are similar to those of this G-*Z. officinale* EO.

Furthermore, this study revealed that even at a higher concentration (10 µL/mL), E-*Z. officinale* EO promotes the growth of *A. fumigatus*, and its antifungal efficacy may vary between products. This result may be due to differences in chemical composition among commercial essential oils and to the hormetic effect, in which lower concentrations of active components stimulate radial growth by reducing volatile components over time, through oxidation or degradation during storage. The fact that the maximum inhibition rate of E-*Z. officinale* EO on *P. oxalicum* was determined to be only 42.98% showing that the difference in antifungal activity in oils from two different companies does not indicate that antifungal activity is generalizable. These results demonstrate that the antifungal activity of *Z. officinale* EO depends on multiple factors, including plant source, chemical composition of the resulting product, and production method. This once again emphasizes the necessity of product-specific biological and chemical evaluations before applying commercial products as antifungal agents.

It was determined that G- *J. oxycedrus* tar gave an inhibition zone diameter between 8 and 16 mm. Ertürk (2006) reported that *J. oxycedrus* tar bought from the market created 20 mm inhibition against *A. niger*. On the other hand, Karaman et al. (2003) determined that *J. oxycedrus* methanol and water extract did not have antifungal activity against *Alternaria alternata*, *A. flavus*, *F. oxysporum*, and *Penicillium* spp. strains. Furthermore, Diğrak et al. (1999) determined that *J. oxycedrus* leaf, bark, and fruit of chloroform, acetone, and methanol extracts did not have antifungal activity against *Penicillium italicum*. These findings demonstrate that antifungal activity can vary considerably depending on the plant part used, the product obtained from the plant, the extraction method, and the final product.

Tar is a complex, viscous substance formed by the pyrolytic decomposition of plant tissues, considered more versatile than the aqueous fraction because it contains less water and is less acidic. Tars contain compounds with high biological activity, such as monophenols, including guaiacol, syringol, and their derivatives, and oligomeric phenols, including stilbene, phenylcoumarin, and resorcinol (Saires et al., 2024). In contrast, plant extracts obtained with solvents such as water, methanol, and ethanol may be devoid of these components, depending on solvent properties and extraction temperature, thereby reducing antifungal activity. However, factors such as raw materials, production methods, and storage conditions of commercial tars affect their chemical composition and, consequently, their biological activity. Therefore, to evaluate the antifungal potential of *J. oxycedrus*, not only the plant species but also the product type and extraction method should be considered.

On the other hand, while some plant extracts, fixed and essential oils were reported to have antifungal activity in the literature, 11 PEs, 9 FOs, and 21 EOs were found to lack antifungal activity. In the literature, it has been stated that *Rosmarinus officinalis* EO (Özcan and Chalchat, 2008), *Myrtus communis* EO (Özcan and Erkemn, 2001; Gumus et al., 2010), *Syzygium aromaticum* (L.) Merr. & LM Perry EO (Chee and Lee, 2007; Pinto et al., 2009), *Eucalyptus* spp. EO (Ramezani et al., 2002; Gakuubi et al., 2017), *Daucus carota* EO (Tavares et al., 2008), *Allium sativum* oil (Li et al., 2014), *Jasminum officinale* L. EO (Kurkina et al., 2020), *Viola odorata* EO (Stević et al., 2014), seaweed extract (Pérez et al., 2012), and *Aloe vera* (De Rodríguez et al., 2005) have antifungal activity. This difference is due to the different chemical compositions of EOs.

EO has antimicrobial and other biological activities due to its bioactive volatile components. Compounds found in EOs that are critical for biological activity are terpenes, alcohols, acids, esters, epoxides, aldehydes, ketones, amines, and sulfides. These components are significant, and minor components should not be ignored for biological activities. These major and minor components of EOs are affected by various factors (Burt, 2004; Özcan and Zorba, 2016). The chemical composition of EOs, ecological and geographical

characteristics (soil properties, climatic conditions, collection place), different chemotype variations associated with the age of the plant and harvest time, postharvest processing and extraction method of EO, and plant part used in extraction (leaf, stem, flower, etc.). In addition to these factors, using different types of microorganisms, different concentration ranges, performing analyses in different media, and using different methods are other factors that cause different results in antimicrobial activity (Petretto et al., 2018; Fidan et al., 2019; Mutlu-Ingok et al., 2020; Ni et al., 2021). In addition to all these, EOs are volatile at room temperature and can be affected by oxygen and temperature changes. Therefore, methods to increase the stability and activity of EOs are required to preserve biological activity. (Mutlu-Ingok et al., 2020; Koçak et al., 2021).

In conclusion, it is a promising natural source for new antifungal agents that are less harmful to the environment and human health. However, as this study shows, the beneficial effects of each commercially available essential oil depend on its composition. Indeed, *E-Z. officinale* EO promoted the growth of *A. fumigatus* rather than inhibiting its radial growth. Therefore, detailed studies are needed on products used for medicinal purposes and commercially available to the public.

Conflicts of interest

The author declares no competing interests.

Ethical Statement: It is declared that scientific and ethical principles have been followed while carrying out and writing this study and that all the sources used have been properly cited (Gülçin ÖZCAN ATEŞ)

Acknowledgement

I sincerely wish to thank Prof. Dr. Özlem ABACI GÜNYAR for the microfungus isolates. The study was carried out at the Food Engineering Microbiology Laboratory of Çanakkale Onsekiz Mart University. I am grateful to Prof. Dr. Nükhet Nilüfer ZORBA for allowing me to use the laboratory. Part of the results were presented on the 12th. Antimicrobial Chemotherapy Days (İstanbul, Türkiye), 2016.

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