

Heracleum platytaenium Boiss. Uçucu Yağının Elma Kara Leke ve Monilya Hastalığına Karşı Antifungal Aktivitesinin Belirlenmesi

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ÖZ

Bu çalışma; Türkiye'ye özgü endemik bir tür olan *Heracleum platytaenium* Boiss.'in uçucu yağının *Monilinia* spp. ve *Venturia inaequalis* gibi fitopatogenik funguslar üzerindeki antifungal aktivitesini incelemektedir. Uçucu yağ, bitkinin tam çiçeklenme döneminde toplanan meyvelerinden hidrodistilasyon yöntemiyle elde edilmiştir. Çalışmada; *Monilinia* spp. ve *V. inaequalis* izolatları üzerinde farklı konsantrasyonlarda (5 µL, 10 µL, 20 µL) uçucu yağın antifungal etkisinin vitro olarak değerlendirilmiştir. Antifungal etkinlik; miselyal büyüme inhibisyonu yüzdesi ve istatistiksel analizlerle belirlenmiştir. Sonuçlar, uçucu yağın her iki patojen üzerinde doza bağlı olarak değişen inhibisyon etkisi gösterdiğini ortaya koymuştur. *Monilinia* spp. için en yüksek inhibisyon oranı %48,8, *V. inaequalis* için ise %46,6 olarak kaydedilmiştir. Ekstraksiyon yılına bağlı olarak antifungal etkinlikte farklılıklar gözlemlenmiş, 2021 yılına ait uçucu yağ örneklerinin 2022 yılına göre daha yüksek inhibisyon oranına sahip olduğu belirlenmiştir. Çalışmanın bulguları, *H. platytaenium* uçucu yağının önemli bir biyopestisit potansiyeline sahip olduğunu ve fitopatojenlere karşı doğal bir mücadele yöntemi olarak değerlendirilebileceğini göstermektedir. Bu sonuçlar, bitkisel bazlı antifungal ajanların sürdürülebilir tarım uygulamalarındaki önemini vurgulamakta ve kimyasal fungusitlere alternatif olabilecek yeni yaklaşımlara ışık tutmaktadır.

Antifungal Activity of *Heracleum platytaenium* Essential Oil Against Fungi Causing Apple Scab and Monilia Disease

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ABSTRACT

This study investigates the antifungal activity of the essential oil of *Heracleum platytaenium* Boiss., an endemic species in Türkiye, against the phytopathogenic fungi *Monilinia* spp. and *Venturia inaequalis*. The essential oil was obtained by hydrodistillation from the fruits collected at the full flowering stage. The antifungal effects of different concentrations (5 µL, 10 µL, 20 µL) of the essential oil were evaluated *in vitro* on *Monilinia* spp. and *V. inaequalis* isolates. Antifungal activity was determined based on the percentage inhibition of mycelial growth and statistical analyses. The results showed that the essential oil exhibited a dose-dependent inhibitory effect on both pathogens. The highest inhibition rates were recorded as 48.8% for *Monilinia* spp. and 46.6% for *V. inaequalis*. Differences in antifungal activity were observed depending on the extraction year, with the 2021 essential oil samples showing higher inhibition rates compared to those from 2022. The findings suggest that *H. platytaenium* essential oil has significant potential as

a biopesticide and may serve as a natural control agent against phytopathogens. These results highlight the importance of plant-based antifungal agents in sustainable agricultural practices and offer new insights into alternatives to chemical fungicides.

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1. Introduction

Heracleum belongs to the Apiaceae family and consists of about 125 species globally. Of these, 23 species are found in Türkiye, nine of which are endemic (Pimenov and Leonov, 2004). The genus *Heracleum* includes various species that have been used in traditional medicine for centuries. They exhibit several pharmacological effects like anticonvulsant (Sayyah et al., 2005), antifungal and antioxidant (Liu et al., 2024), antihypertensive (Gao et al., 2014), anti-inflammatory (Yang et al., 2002), analgesic (Taniguchi et al., 2005) and antipyretic (Taniguchi et al., 2011). Moreover, some species are utilized in the food industry as a spice and flavoring agents (Souri et al., 2004).

Phytochemical studies have identified various bioactive compounds in *Heracleum* species. These include coumarins, furanocoumarin dimers, coumarin glycosides, anthraquinones, and stilbenes (Dincel et al., 2013). The essential oil of *Heracleum platytaenium* has shown strong biological potential. It exhibits antimicrobial (Akçin et al., 2013; Soyuçok, 2022; Buema et al., 2024) and anticandidal (Işcan et al., 2004) activities. However, the composition of essential oils can vary due to environmental conditions, genetic factors, abiotic stress, and the plant's growth stage (Bayır et al., 2014; Güneş et al., 2025). As a result, research has reported notable differences in the composition and quantity of essential oils from *Heracleum* species (Sajwaw et al., 2012; Kılıç et al., 2016).

Essential oils play a crucial role as natural antimicrobial agents. The frequent use of chemical fungicides in agriculture has caused serious concerns. These include environmental pollution, negative effects on human health, and the rise of resistant pathogens (Goswami et al., 2018). Because of this, plant-based biopesticides, especially essential oils, have gained attention as sustainable alternatives. Essential oils from various plant species have been tested for their inhibitory effects on phytopathogenic fungi. These studies highlight their potential as biocontrol agents (Deans and Svoboda, 1990; Demirel et al., 2024).

H. platytaenium is a monocarpic plant species endemic to Türkiye, characterized by its strong aromatic properties. It is primarily distributed in the Black Sea, Central Anatolia, and Aegean regions, typically growing in mixed forests, rocky slopes, and riverbanks (Bayan et al., 2016). While studies have investigated the antimicrobial properties of various *Heracleum* species (Wu et al., 2023; Liu et al., 2024; Segneanu et al., 2024), research specifically on *H. platytaenium* remains limited. Bayan et al. (2017) reported that the essential oil of *H. platytaenium* exhibited *in vitro* antifungal activity against *Alternaria solani* and *Monilia laxa*.

The genus *Monilinia*, particularly *Monilinia laxa*, is a significant fungal pathogen responsible for brown rot disease in stone fruit trees. This disease causes brown, soft rot and mummification of

infected fruits, leading to substantial crop losses (Vasić et al., 2016). *Venturia inaequalis*, on the other hand, is the causative agent of apple scab, a fungal disease that results in olive-green or brown lesions on leaves, fruits, and shoots, thereby reducing both yield and quality (Belete and Boyraz, 2018).

Traditionally, these diseases have been battled using chemical fungicides. However, their use is associated with environmental pollution, negative effects on human health, and the emergence of resistant pathogen strains (Goswami et al., 2018). Therefore, the application of natural compounds such as plant extracts and essential oils as antifungal agents offers sustainable and environmentally friendly alternatives. Research on the antibacterial qualities of *Heracleum* species point to their possibility as biological control agents against phytopathogens.

This study investigated the antifungal effects of *H. platytaenium* essential oil on *Monilinia* spp. and *V. inaequalis*, two phytopathogens that have not been widely studied in this context. By exploring its potential as a natural source for controlling these pathogens, the research aims to support the development of alternatives to chemical fungicides.

2. Materials and Methods

2.1. Collection of Plant and Fungal Material

The *H. platytaenium* species was collected at its full flowering stage and identified by Prof. Dr. Hasan Özçelik based on *Flora of Turkey Volume 4*. The type specimens of the species are preserved at the Süleyman Demirel University Gül Herbarium under the accession numbers 63.75.3.1-2. The study was conducted between 2021 and 2022 in the research and application fields of the Faculty of Agriculture, Isparta University of Applied Sciences. Seeds collected at full maturity in 2016 were stored at +4°C for one year. In the autumn of 2017, the stored seeds were sown in 32-cell seed viols containing a 3:1 perlite and peat mixture.

The experimental field was plowed deeply in the autumn of 2017, and in 2018, it was tilled again and prepared for sowing using a rotary tiller. In the spring of 2018, the obtained seedlings were transplanted under field conditions with a spacing of 60 cm between plants and 140 cm between rows. The planting was carried out in two rows, each 20 m in length. Weed control was carried out manually by hoeing, and irrigation was applied through a drip system when the soil surface was dry to a depth of 10 cm. General characteristics of the species were observed in 2019 and 2020, and seeds were harvested at full maturity in July 2021-2022.

Both *Monilinia* spp. and *Venturia inaequalis* are known to cause serious damage and significant economic losses in fruit production. The *Monilinia* spp. and *V. inaequalis* isolates tested in this study were obtained from the fungal collection of the Plant Pathology Laboratory at the Faculty of Agriculture, Bingöl University, where they had been previously identified and preserved using standard phytopathological methods.

2.2. Essential Oil Extraction

The fruits of the species were harvested at full maturity in 2021-2022 and then ground. To extract the essential oil, 100 g of the material was distilled for three hours using a Clevenger apparatus.

2.3. Preparation of Culture Medium

To purify and propagate fungal isolates, Potato Dextrose Agar (PDA) medium was prepared and supplemented with distilled water. PDA was sterilized in an autoclave at 121.1°C for 15 minutes and then poured into sterile petri dishes under aseptic conditions. The prepared medium was used for both obtaining pre-cultures and forming experimental groups. In the experimental groups, different concentrations of essential oil (5 µL, 10 µL, 20 µL) were added to the medium and homogenized using a sterile pipette tip. The doses used in this study were selected to encompass and slightly extend beyond previously reported effective concentrations, in order to assess both moderate and high antifungal responses. Similar doses were reported in the aforementioned studies (Akçin et al., 2013; Bayan et al., 2016).

2.4. Preparation of Fungal Isolates

The fungal isolates were transferred onto PDA medium in 90 mm petri dishes and incubated under optimal temperature and humidity conditions for seven days.

2.5. Antifungal Activity Assay

Using a sterile cork borer, 8 mm diameter mycelial plugs were aseptically obtained from the actively growing margins of fungal colonies and inoculated at the center of Petri dishes containing potato dextrose agar (PDA). The plates were incubated under suitable temperature and humidity conditions until full colony development was achieved. Mycelial growth was monitored daily, and final colony diameters were measured at the end of the incubation period.

The experiments were designed in a completely randomized manner with three biological replicates per treatment. Measurements were taken independently for each replicate. A positive control (nystatin) and a negative control (medium without essential oil) were included to validate antifungal efficacy.

The inhibitory effect of the essential oil was quantified based on its impact on mycelial expansion, calculated using the following formula adapted from Deans and Svoboda (1990):

$$\text{Inhibition (\%)} = (\text{gc} - \text{gt}) / \text{gc} \times 100$$

Where:

- **gc** refers to the average colony diameter in the control group (mm),
- **gt** refers to the average colony diameter in the treatment group (mm).

2.6. Statistical Analysis

The data obtained from different years and treatment doses were analyzed using a factorial design with triplicate in a completely randomized design. The statistical analysis was performed using the GLM procedure of the SAS statistical software package (SAS, 2009) based on standard variance analysis (ANOVA). Differences between means were determined using the LSD multiple comparison test.

3. Results and Discussion

The essential oil of *H. platytenium*, applied at different doses, exhibited statistically significant antifungal activity ($p < 0.01$) against *Monilinia* spp., while the effects of year and the year \times dose interaction were found to be statistically insignificant. However, in the case of *Venturia inaequalis*, the antifungal effect of the essential oil was significantly influenced by year, dose, and their interaction ($p < 0.01$) (Table 1).

Table 1. Variance analysis table for the antifungal effect of *H. platytenium* essential oil against *Monilinia* spp. and *V. inaequalis* pathogens.

Sources of Variation	Degrees of Freedom	<i>Monilinia</i> spp. F Value	<i>V. inaequalis</i> F Value
Year	1	1.76	60.84**
Dose	4	1308.99**	6836.04**
Year x Dose	4	1.13	14.44**
Error	20		
General	29		
Cv		3.6	1.52

** : $p < 0.01$

The essential oil showed a dose-dependent antifungal effect against *Monilinia* spp. The inhibition zones for the different concentrations were measured as 71.3 mm at 5 μ L, 62.0 mm at 10 μ L, and 55.2 mm at 20 μ L. When compared with the negative control (90 mm), all tested doses demonstrated significant inhibition, though lower than the positive control (8 mm). Yearly comparison revealed that the highest antifungal activity was observed in 2021 at a dose of 20 μ L, where the inhibition zone was 53.3 mm. Conversely, the lowest antifungal effect was recorded in 2022 at a dose of 5 μ L, with an inhibition zone of 72.3 mm (Table 2).

For *Venturia inaequalis*, the inhibition zones of essential oil varied slightly across years. The average inhibition was measured as 58.4 mm in 2021 and 61.0 mm in 2022. According to the dose, the inhibition zones were 71.0 mm at 5 μ L, 68.5 mm at 10 μ L, and 61.0 mm at 20 μ L. All values showed higher antifungal activity than the negative control (90 mm), but did not reach the efficacy of the positive control (8 mm) (Table 2). The strongest inhibitory effect was observed at the highest dose, suggesting a clear dose–response relationship.

Table 2. Mycelial measurements showing the antifungal activity of the essential oil based on the extraction year: (A) for *Monilinia* spp., (B) for *V. inaequalis*.

		Negative Control	Positive Control	5 μ L	10 μ L	20 μ L	Mean
<i>Monilinia</i> spp	2021	90	8	70.3	62.3	53.3	57.8
	2022	90	8	72.3	61.7	57.0	56.8
	Mean	90 ^a	8 ^e	71.3 ^b	62.0 ^c	55.2 ^d	
Lsd _{dose} :		2.48					
		Negative Control	Positive Control	5 μ L	10 μ L	20 μ L	Mean
<i>V.</i> <i>inaequalis</i>	2021	90 ^a	8 ^e	69.0 ^c	67.3 ^d	57.7 ^f	58.4 ^b
	2022	90 ^a	8 ^e	73.0 ^b	69.7 ^c	64.3 ^e	61.0 ^a
	Mean	90 ^a	8 ^e	71 ^b	68.5 ^c	61.0 ^d	

Lsd_{year}:1.38, Lsd_{year}:0.70, Lsd_{dose}:1.09, Mean: The "Mean" column presents the arithmetic average (in mm) of mycelial growth values from the years 2021 and 2022 for each treatment dose. These values are provided to illustrate the overall effect of each dose independently from the year.

The findings of our study revealed that the essential oil of *Heracleum platytaenium* exhibited a dose-dependent increase in antifungal activity against both phytopathogens. Notably, at a concentration of 20 μ L, inhibition rates reached up to 48.8% for *Monilinia* spp. and 46.6% for *Venturia inaequalis*. These results demonstrate that microbial activity can be effectively suppressed depending on the concentration of the essential oil. Similarly, Bayan et al. (2016) reported that at a 10 μ L dose, *H. platytaenium* essential oil caused near-complete inhibition of mycelial growth in certain phytopathogens.

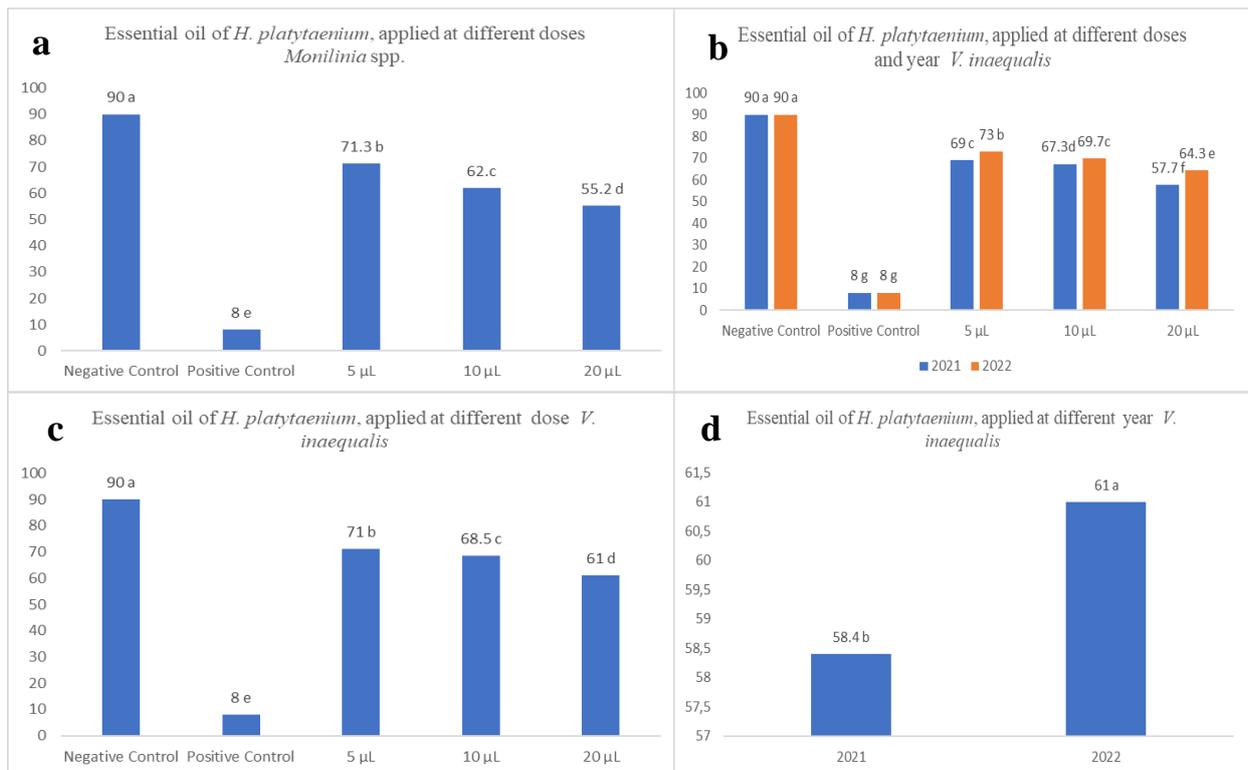


Figure 1. Antifungal activity of *Heracleum platytaenium* essential oil against *Monilinia* spp. and *Venturia inaequalis* at different doses (5, 10, and 20 μ L) and extraction years (2021 and 2022). (a) Effect on *Monilinia* spp. at different doses, (b) Effect on *V. inaequalis* at different doses and years, (c) Effect on *V. inaequalis* at different doses, (d) Effect on *V. inaequalis* from different extraction years (Other graphs were not included as the results were not statistically significant)

In addition, significant differences were observed in the antifungal activity of the essential oil between the two years of the study. The samples obtained in 2021 exhibited higher inhibition rates compared to those collected in 2022. This variation may be attributed to year-dependent changes in the chemical composition of the essential oil. A similar finding was reported by Mechergui et al. (2016), who emphasized that the biological activity of essential oils is closely related to the environmental conditions (e.g. temperature, precipitation, soil moisture) of the growing year. In this context, variations between years may affect the major components of essential oils and thereby influence their bioactivity. Therefore, it is crucial to consider both the year and environmental factors during the standardization process of plant-derived products intended for use as biopesticides.

The essential oils of *H. crenatifolium* Boiss., *H. sphondylium* L. subsp. *ternatum* (Velen.) Brummitt, and *H. platytaenium* Boiss. (Umbelliferae) were extracted using hydrodistillation. GC and GC/MS analyses identified octyl acetate as the main compound in all three species. The concentrations were 93.7%, 87.6%, and 31.6%, respectively. In *H. platytaenium*, octyl butyrate was also found as a major component. The antifungal effects of these essential oils were tested against *Candida glabrata* using the microdilution method. The results showed strong inhibitory effects in all samples (Akçin et al., 2013).

In another study, Bayan et al. (2016) investigated the effects of *H. platytaenium* essential oil on phytopathogenic fungi, including *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL), *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Botrytis cinerea*, and *Verticillium dahliae*. The dominant components of the essential oil were reported as myristicin (27.47%), octyl acetate (25.10%), 1-octanol (16.90%), and octyl 2-methylbutyrate (9.88%). The antifungal activity of the essential oil at various concentrations (0, 0.5, 1, 1.5, 2, 5, and 10 μ L) was assessed using the agar well diffusion method. The results demonstrated that at 10 μ L, the essential oil completely inhibited the mycelial growth of FORL, *R. solani*, and *S. sclerotiorum* (100% inhibition). Significant growth reductions were also observed in the other tested pathogens. The LC90 values were calculated as 17.53 μ L for FORL, 15.52 μ L for *R. solani*, 8.83 μ L for *S. sclerotiorum*, 15.58 μ L for *B. cinerea*, and 149.54 μ L for *V. dahliae*. These findings indicate that *H. platytaenium* essential oil exhibits significant antifungal activity against certain phytopathogenic fungi and holds potential for use in biological control applications.

In this study, the antifungal activity of *H. platytaenium* essential oil against *Monilinia* spp. and *V. inaequalis*, two significant phytopathogenic fungi, was investigated. The results demonstrated that the essential oil caused dose-dependent mycelial inhibition in both fungal species (Table 1).

The percentage inhibition rates of *Monilinia* spp. and *Venturia inaequalis* at different doses of *H. platytaenium* essential oil are shown in Figure 1. The graph illustrates a dose-dependent inhibition pattern for both pathogens, with the highest activity observed at 20 μ L. The antifungal activity of *H. platytaenium* essential oil differed based on the extraction year. Overall, the 2022 samples showed lower inhibition rates than those from 2021 (Tables 1 and 2, Figure 1). At a concentration of 20 μ L, the inhibition rate for *Monilinia* spp. was 47.7% in 2021 and 42.2% in 2022, while for *V. inaequalis*,

the rates were 46.6% and 37.7%, respectively (Table 2). Similarly, mycelial growth measurements indicated that the 2021 essential oil samples provided stronger inhibition than those from 2022 (Table 1).

These results show that *H. platytaenium* essential oil possesses considerable fungicidal activity, indicating its usefulness as a biopesticide for plant pathogen control. Further, this study aids in filling the gap of research that exists on the antifungal effects of this oil. For effective control of important agricultural pathogens such as *Monilinia* spp. and *V. inaequalis*, sustainable agriculture must seek alternatives like these (Goswami et al., 2018). Phytopathogenic antifungal activity often varies from year to year and this may depend on shifts in the growing conditions, or some degree of environmental stress, something as simple as the essential oil's chemical composition. Phytopathogens are effective only if host resistance fails, so manipulating essential oil composition may give positive results (Bahadori et al., 2016; Mechergui et al., 2016). Achieving these variations is critical to ensure that their use with certain essential oils and herbs will be effective in biological control without negative consequences.

The dose-dependent antifungal activity observed in our study highlights the strong bioactive potential of *H. platytaenium* essential oil, particularly at 20 μ L concentrations. These findings not only align with previous reports on *Heracleum* species but also expand the understanding of their use against *Monilinia* spp. and *Venturia inaequalis*. For instance, Akçin et al. (2013) and Bayan et al. (2016) demonstrated significant antifungal effects of *Heracleum* essential oils against other phytopathogens, supporting our results.

In addition to essential oils, studies have also explored the antifungal effects of alternative compounds such as boron-based formulations. Boric acid and other boron compounds have shown promising inhibitory effects against fungal pathogens by disrupting membrane function and metabolic activity. However, unlike essential oils, boron compounds may pose phytotoxic risks depending on concentration and application method. Thus, essential oils offer a more sustainable and eco-friendly approach, especially for integrated pest management strategies.

Considering the rise of fungicide-resistant strains and environmental concerns associated with synthetic chemicals, our findings reinforce the value of essential oils as potential biopesticides and encourage further exploration of both plant-based and inorganic alternatives under field conditions.

In conclusion, these results illustrate that *H. platytaenium* possesses qualities that deem it effective and a viable contender for further research in essential oil antifungal properties, making it an effective solution for agricultural diseases. Finally, further work is essential to corroborate these findings against various ecological and environmental conditions. In addition, formulation and field studies, as well as toxicity evaluations need to be done to investigate the bio-fungicide efficacy.

Conflict of Interest Statement

The authors declare that there is no conflict of interest.

Authors Contribution Statement

The authors declare that they have contributed equally to the manuscript.

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