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# Essential oils as natural alternatives for controlling bacterial diseases in *Agaricus bisporus* cultivation

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**Abstract**

The increasing resistance of bacterial pathogens to chemical treatments in agriculture highlights the need for natural alternatives. This study evaluates the antibacterial properties of essential oils (EOs) from six medicinal plants: Eucalyptus camaldulensis, *Oliveria decumbens*, *Callistemon citrinus*, *Myrtus communis*, *Satureja khuzistanica*, and *Ocimum gratissimum*, against key bacterial pathogens of *Agaricus bisporus*, including *Pseudomonas tolaasii*, *Pseudomonas reactans*, *Ewingella americana*, and *Mycetocola* sp. EOs were extracted via hydrodistillation and analyzed using gas chromatography-mass spectrometry (GC-MS). Antibacterial activity was tested using the disk diffusion method, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays. Results showed significant antibacterial effects, especially for *S. khuzistanica* and *O. gratissimum*, which exhibited the highest inhibition zones (26.33 and 24.66 mm) and the lowest MIC (0.97 and 1.90 µL/mL) and MBC (1.95 and 3.50 µL/mL) values against *P. tolaasii*. GC-MS analysis identified bioactive compounds like thymol, carvacrol, and eugenol, known for their antimicrobial properties. Disease severity test on mushroom caps confirmed that EOs of *S. khuzistanica* and *O. gratissimum* significantly reduced bacterial infection symptoms. These findings suggest that EOs, particularly those from *S. khuzistanica* and *O. gratissimum*, could be sustainable, eco-friendly alternatives for managing bacterial diseases in mushroom cultivation. Further research is necessary to optimize application methods, assess long-term stability, and evaluate potential impacts on mushroom growth and quality.

***Keywords: Minimum Inhibitory Concentration, antibacterial activity, plant pathogens, GC-MS, sustainable agriculture***

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# اسانس های گیاهی به عنوان جایگزین طبیعی برای کنترل بیماری های باکتریایی در کشت قارچ خوراکی

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## چکیده

**در میان قارچ‌های کشت‌شده، قارچ تکمه‌ای سفید به‌طور گسترده در سراسر جهان کشت می‌شود. این محصول تحت تاثیر بیماری‌های باکتریایی مختلفی قرار می‌گیرد. در این پژوهش، تأثیر اسانس‌های استخراج‌شده از گیاهان دارویی بر رشد باکتری‌های بیماری‌زای قارچ خوراکی مورد بررسی قرار گرفت. نمونه‌های گیاهی شامل ریحان آفریقایی (*Ocimum gratissimum*)، مرزه خوزستانی (*Satureja khuzistanica*)، شیشه شور (*Callistemon citrinus*)، ریحان کلیمانجاروم (*Ocimum kilimandscharicum*)، مورد (*Myrtus communis*)، اُکالیپتوس (*Eucalyptus camaldulensis*) و لعل کوهستان (*Oliveria decumbens*)، از مناطق مختلف جمع‌آوری شد و ترکیبات شیمیایی اسانس‌ها با استفاده از دستگاه کروماتوگرافی گازی متصل به طیف‌سنج جرمی (GC-MS) شناسایی شد . آزمون‌های اثر ضدباکتریایی اسانس‌ها با روش نشت در دیسک بر روی باکتری‌های بیماری‌زا نظیر *Pseudomonas tolaasii*، *Ewingella americana*، *Pseudomonas reactans* و *Mycetocola* sp.، انجام شد. نتایج نشان داد که اسانس ریحان آفریقایی (*O. gratissimum*) بیشترین تأثیر را بر باکتری *P. tolaasii* با قطر هاله ۲۳/٦٦ میلی‌متر نشان داد. همچنین، اسانس مرزه خوزستانی (*S. khuzistanica*) با قطر هاله ۲٦/۳۳ میلی‌متر مؤثرترین اثر را بر سویه دیگری از این باکتری داشت. در آزمون بیماری‌زایی قارچ خوراکی، نتایج نشان داد که مرزه خوزستانی و ریحان کلیمانجاروم در کنترل علائم مؤثرتر بودند. علاوه بر این، آزمایش‌های حداقل غلظت بازدارندگی (MIC) و کشندگی (MBC) نشان داد که اسانس مرزه خوزستانی به‌ترتیب ۰/۹۷ و ۱/۹۵ میکرولیتر بر میلی‌لیتر، بیشترین تأثیر را بر باکتری *P. tolaasii* داشت. به‌طور کلی، نتایج این مطالعه نشان می دهد که اسانس ها می‌تواند به‌عنوان جایگزینی طبیعی و ایمن برای کنترل بیماری‌های باکتریایی در صنعت تولید قارچ خوراکی مورد استفاده قرار گیرد.**

***کلید واژه‌ها: اسانس گیاهی، قارچ خوراکی، فعالیت ضدباکتریایی، بیمارگر گیاهی***

دبیر تخصصی: دکتر رسول رضائی

## Introduction

Cultivating edible mushrooms, particularly *Agaricus bisporus*, is a vital agricultural industry due to its significant nutritional and economic value. However, bacterial pathogens such as *Pseudomonas tolaasii*, the causative agent of brown blotch disease, and *Ewingella americana* threaten crop yield and quality, causing both pre-harvest and post-harvest losses (Moallem et al., 2024; Hamidizade et al., 2023). These pathogens thrive in the humid environments essential for mushroom cultivation, making disease management a persistent challenge (Osdaghi et al., 2019). Additionally, bacterial contamination during storage and transport accelerates spoilage, reducing marketability (Azizpanah et al., 2023; Alegbeleye et al., 2022).

Traditional disease control measures rely heavily on synthetic antibiotics and chemical treatments. However, widespread and prolonged use of these methods has raised concerns regarding environmental pollution, the emergence of antibiotic-resistant strains, and regulatory restrictions on residue limits in agricultural products (Jamshidi et al., 2023; Rueda and Mogollón 2012). Consequently, there is a growing demand for safer and sustainable alternatives that effectively combat bacterial diseases while ensuring environmental safety and consumer health (Bahraminejad et al., 2016).

Essential oils (EOs) derived from medicinal plants have gained attention as natural antimicrobial agents. Their complex composition, rich in bioactive compounds such as terpenoids, phenols, and aldehydes, confers broad-spectrum antibacterial and antifungal properties (Aghraz et al., 2018). Studies have demonstrated the efficacy of EOs against various plant pathogens, with compounds like thymol, carvacrol, and eugenol exhibiting potent effects on both Gram-positive and Gram-negative bacteria (Mousavifar et al., 2023; Pandey et al., 2017).

Among these, *Satureja khuzistanica* and *Ocimum gratissimum* contain high phenolic compounds, making them promising candidates for agricultural applications. Thymol and carvacrol, the dominant constituents of *S. khuzistanica*, disrupt bacterial membranes and inhibit biofilm formation. Meanwhile, eugenol, abundant in *O. gratissimum*, interferes with bacterial enzymatic systems and disrupts energy metabolism (Memar et al., 2017; Zakaria Nabti et al., 2020). Previous studies have explored the antibacterial and antifungal effects of EOs from various medicinal plants, including *Matricaria chamomilla*, *Mentha piperita*, *M. spicata*, *Lavandula angustifolia*, *Ocimum basilicum*, *Thymus vulgaris*, *Origanum vulgare*, *Salvia officinalis*, *Citrus limon*, and *Citrus aurantium*. Their major components—linalyl acetate, linalool, limonene, α-pinene, β-pinene, 1,8-cineole, camphor, carvacrol, thymol, and menthol—have been evaluated for their inhibitory effects against *A. bisporus* pathogens such as *Verticillium fungicola*, *Trichoderma harzianum*, and *P. tolaasii*. *O. vulgare* demonstrated the most effective antimicrobial activity (Soković and van Griensven, 2006).

Despite extensive research on the antimicrobial properties of EOs, their application in managing mushroom diseases has been relatively underexplored. This study aims to evaluate the antibacterial effects of EOs from six medicinal plants (*Eucalyptus camaldulensis*, *Oliveria decumbens*, *Callistemon citrinus*, *Myrtus communis*, *S. khuzistanica*, and *O. gratissimum*) against key bacterial pathogens of *A. bisporus*, including *P. tolaasii*, *Pseudomonas reactans*, *E. americana*, and *Mycetocola* sp.

## Materials and Methods

### Plant Materials

The medicinal plants used in this study included *E. camaldulensis*, *O. decumbens*, *O.*

*kilimandscharicum*, *C. citrinus*, *M. communis*, *S. khuzistanica*, and *O. gratissimum*. The plant materials were collected from various regions of Iran and authenticated based on morphological characteristics (Table 1). Following collection, the plants were washed to remove impurities, dried under shade at room temperature, and ground into a fine powder using a laboratory mill for EO extraction.

**Table 1. Characteristics of the Plants Used in the Study.**

| Scientific Name | Collection Site | Plant Part Used |
|---|---|---|
| *Callistemon citrinus* | Shahid Chamran University, Ahvaz, Iran 31.303733491737635, 48.658651962418794 | Leaf |
| *Eucalyptus camaldulensis* | Shahid Chamran University, Ahvaz, Iran 31.303733491737635, 48.658651962418794 | Leaf |
| *Myrtus communis L.* | Shahid Chamran University, Ahvaz, Iran 31.303733491737635, 48.658651962418794 | Leaf |
| *Ocimum kilimandscharicum* | Horticultural Research Farm, Shahid Chamran University, Ahvaz, Iran 31.303293475406416, 48.659102573504626 | Aerial Parts |
| *Ocimum gratissimum* | Horticultural Research Farm, Shahid Chamran University, Ahvaz, Iran 31.303293475406416, 48.659102573504626 | Aerial Parts |
| *Oliveria decumbens* | Horticultural Research Farm, Shahid Chamran University, Ahvaz, Iran 31.303293475406416, 48.659102573504626 | Seeds & Flowers |
| *Satureja khuzistanica* | Dehloran, Ilam, Iran 32.693087517349305, 47.2695916168586 | Leaf |

## Essential Oil Extraction

EOs were extracted from dried plant materials using the hydro-distillation method with a Clevenger-type apparatus (SCI Finetech, Germany). For each plant, 100 g of powdered material was mixed with 1 L of distilled water in a 2 L distillation flask and heated for 3 hours. The extracted oils were collected, dried over anhydrous sodium sulfate to eliminate residual moisture, and filtered through Whatman No. 1 filter paper (Smith-Palmer et al., 2001). The purified oils were stored in sterile amber vials at 4°C until further analysis.

## Bacterial Strains and Culture Conditions

The antibacterial activity of EOs was tested against four bacterial pathogens associated with *Agaricus bisporus*: *P. tolaasii*, *P. reactans*, *E. americana*, and *Mycetocola* sp. The bacterial isolates were obtained from the bacterial collection laboratory at the Shahid Chamran University of Ahvaz and Shiraz University. All isolates

represent previously identified or standard reference strains from international culture collections (French Collection of Phytopathogenic Bacteria, CFBP). They were used solely as reference strains for evaluating essential-oil activity, with no re-identification, taxonomic revision, or new characterization performed. The strains were maintained on nutrient agar (NA, Merck, Germany) slants at 4°C. For experimental assays, bacterial suspensions were prepared by culturing isolates in nutrient broth (NB, Merck, Germany) at 28°C for 24 hours. The bacterial concentration was adjusted to 0.5 McFarland standard (~1 × $10^8$ colony-forming units per milliliter (CFU/mL)) for antibacterial assays.

**Antibacterial Activity Assays**

**Disk Diffusion Method**

The antibacterial activity of EOs was assessed using the disk diffusion method. Sterile Whatman No. 1 filter paper disks (6 mm diameter) were impregnated with 10 µL of undiluted EO and placed on NA plates previously inoculated with bacterial suspensions (100 µL of $10^8$ CFU/mL). Two discs with sufficient distance were applied on the same Petri dish for each test. Plates were incubated at 28°C for 48 hours, and inhibition zone diameters (mm) were measured using a digital caliper. Ampicillin (10 µg/disc) was used as a positive control, while sterile water was a negative control. This experiment was conducted in triplicate (Bauer et al., 1996).

**Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)**

The MIC of bacterial strains sensitive to the essential oil, as determined by the disk diffusion method, was assessed using the macro dilution method. The bacterial inoculum was prepared from a 24-hour culture, and the suspension was adjusted to 0.5 McFarland standard. A mixture of the bacteria A mixture of the bacteria (optical density at 600 nm ($OD_{600}$) = 0.1, approximately $10^8$ CFU/mL) and diluted EO was incubated at 37°C for 24 hours in NA. After this period, the lowest concentration at which bacterial growth was inhibited was recorded as the MIC. In the next step, the contents of the tubes showing no bacterial growth were cultured on plates. The first plate showing no bacterial growth was considered the MBC (Parvin et al., 2010). This experiment was conducted in triplicate.

**Disease severity test on Mushroom Caps**

For this purpose, after surface sterilization of the edible button mushroom (*A. bisporus*), the cap was cut to 2–3 mm height using the Cap Cut method (Hamidizade et al., 2023). Then, two small wells were created on the mushroom cap using a sterile scalpel. One well was inoculated with 20 µL of a pure bacterial suspension, while the other well was filled with 20 µL of EO. Each treatment was tested in duplicate wells. Both wells were inoculated with the bacterial suspension without essential oil for the positive control. For the negative control, both wells contained sterile water without bacteria. All treatments, including controls, were performed in at least two independent replicates. The mushrooms were placed in sterile containers, with cotton moistened with water at the bottom to maintain humidity. After allowing the suspensions to diffuse, the containers were placed in sterile plastic bags and incubated at 27°C in complete darkness. After 24–48 hours, the experimental containers were removed from the plastic bags, and the results were recorded. Each treatment was performed with three biological replicates, each tested in duplicate (technical replicates), to ensure accuracy and reproducibility.

**Gas Chromatography-Mass Spectrometry (GC-MS) Analysis**

The chemical composition of the extracted EOs was analyzed using gas chromatography-mass spectrometry (GC-MS) with an Agilent 7890A GC coupled to an Agilent 5975C MS.

The analysis was conducted using an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm film thickness), with helium as the carrier gas at a constant flow rate of 1 mL/min. A 1 µL sample of EO, diluted in hexane, was injected with a split ratio of 1:50. The oven temperature program started at an initial temperature of 50°C, which was held for 2 minutes, followed by an increase at a rate of 10°C per minute until reaching 280°C, where it was maintained for 10 minutes. Ionization was performed using the electron impact (EI) mode at 70 eV. The major volatile compounds were identified by comparing their mass spectra with data available in the NIST and Wiley libraries. Retention indices were determined using a homologous series of *n*-alkanes ($C_8$–$C_{20}$) injected under the same conditions (Adams 2004, Davies 1990).

## Statistical Analysis

The experiments followed a completely randomized design. Data from EO-pathogenic bacteria interactions were analyzed using one-way ANOVA in SPSS 19.0 statistical software. Duncan's multiple range test assessed differences between mean values at a 95% significance level ($p < 0.05$).

# Results

## Antibacterial Activity of Essential Oils

The antibacterial activity of the tested EOs varied significantly among bacterial species. *O. gratissimum* and *S. khuzistanica* exhibited the highest inhibition zones against *P. tolaasii*, measuring 26.33 mm and 23.66 mm, respectively. Conversely, *M. communis* demonstrated the lowest antibacterial activity, with inhibition zones below 11 mm. EOs from *E. camaldulensis* and *C. citrinus* displayed moderate antibacterial effects. The effectiveness of each EO in inhibiting bacterial growth is presented as mean inhibition zone diameters (mm). Statistical analysis showed significant differences ($p < 0.05$) between the inhibition zones of different EOs (Table 2).

**Table 2. Mean inhibition zone diameters of essential oils against bacterial pathogens of edible mushrooms (Numbers with the same letters in each column do not show a significant difference at the 5% level.)**

| Essential Oil | *O.g* | *S.k* | *O.k* | *O.d* | *M.c* | *C.c* | *E.c* |
|---|---|---|---|---|---|---|---|
| *Pseudomonas tolaasii* 1127 | 24.66b | 26.33a | 19.66b | 20.00abc | 12.00a | 11.00b | 10.33c |
| *Pseudomonas tolaasii* 1037 | 23.66c | 26.00a | 17.00c | 19.00bc | 10.33c | 10.66bc | 11.66b |
| *Ewingella americana* 1034 | 17.66ef | 19.66b | 11.66d | 10.00d | 8.00de | 8.00d | 8.33d |
| *Ewingella americana* 1189 | 17.33f | 20.66b | 10.33d | 12.66cd | 10.66cb | 11.33b | 8.00d |
| *Mycetocola* 1169 | 18.33e | 26.00a | 19.66b | 24.00ab | 9.00d | 13.00a | 10.00c |
| *Mycetocola* 1162 | 22.00d | 25.33a | 16.00c | 21.66ab | 7.66e | 10.00c | 10.66bc |
| *Pseudomonas reactans* 1117 | 27.66a | 0.00c | 23.33a | 27.66a | 11.66ab | 11.66b | 13.00a |
| SEM | 1.09 | 1.5 | 1.7 | 1.8 | 0.8 | 1 | 1.01 |
| P treat | P < 0.05 | P < 0.05 | P < 0.05 | P < 0.05 | P < 0.05 | P < 0.05 | P < 0.05 |

*O.g- Ocimum gratissimum, C.k-Ocimum kilimandscharicum, S.k-Satureja khuzistani, E.c-Eucalyptus camaldulensis, O.d-Oliveria decumbens, C.c-Callistemon citrinus, M.c- Myrtus communis*

**Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)**

MIC and MBC assays confirmed the strong antibacterial properties of *S. khuzistanica* and *O. gratissimum*, which exhibited the lowest MIC (0.97 µL/mL) and MBC (1.95 µL/mL) against *P. tolaasii*. Higher MIC values were observed for *M. communis* and *O. decumbens*, indicating weaker antibacterial effects. The MIC and MBC values of each EO were compared across bacterial strains. *P. tolaasii* exhibited the highest susceptibility, while *Mycetocola* sp. demonstrated greater resistance to all tested oils (Figure 1; Table 3).

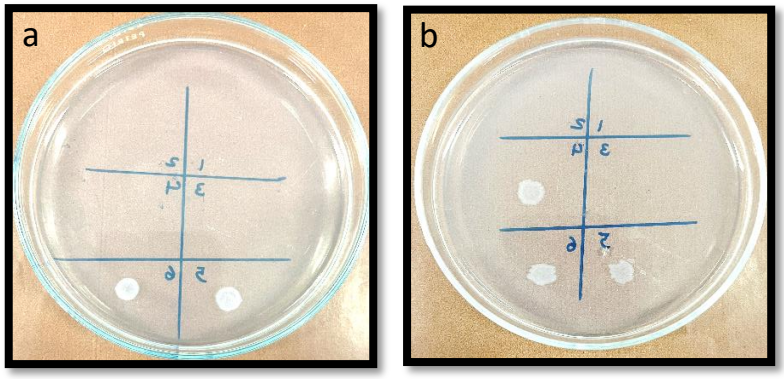



**Figure 1. The culture obtained from the ELISA plate after 24 hours was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). (a) Shows the growth of *Ewingella americana* 1034 in the presence of *Callistemon citrinus* essential oil (EO). (b) Depicts the growth of *Pseudomonas tolaasii* 1037 in the presence of *Myrtus communis* essential oil (EO).**

**Table 3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of essential oils against bacterial pathogens of *Agaricus bisporus*. Means in the same column followed by different letters are significantly different at $P < 0.05$ based on assumed statistical tests.**

| Essential Oil | *P. tolaasii* 1037 | *P. tolaasii* 1127 | *P. reactans* 1117 | *E. americana* 1034 | *E. americana* 1189 | *Mycetocola* sp. 1162 | *Mycetocola* sp. 1169 |
|---|---|---|---|---|---|---|---|
| | MIC / MBC (µL/mL) | MIC / MBC (µL/mL) | MIC / MBC (µL/mL) | MIC / MBC (µL/mL) | MIC / MBC (µL/mL) | MIC / MBC (µL/mL) | MIC / MBC (µL/mL) |
| *C.c* | 31.25a/ 52.50a | 30.25a/ 48.50b | 25.00a / 45.00a | 35.00b / 70.00b | 32.50a/ 65.00a | 30.00a / 50.00a | 27.50a / 45.00a |
| *E.c* | 33.25a/ 62.70a | 32.50a/ 60.00a | 22.50a / 42.25a | 45.00a / 90.00a | 38.00a / 75.00a | 28.50a/ 48.00a | 28.50a/ 48.00a |
| *M.c* | 15.62b/ 28.25b | 12.50b/ 25.00c | 15.00b/ 25.00b | 18.50c / 32.00c | 17.00b / 28.00b | 20.00b/ 32.00b | 18.50b/ 30.00bc |
| *O.k* | 7.90c/ 12.62c | 7.00c/ 10.50d | 7.00c/ 12.00bc | 8.50d / 15.00d | 7.00c/ 12.50c | 8.00c/ 13.00c | 7.50c/12.00 |
| *O.g* | 2.10d/ 3.70d | 1.90d/ 3.50e | 5.00c/ 7.50c | 3.25e/4.50f | 3.00d/ 4.25d | 2.50d/4.00d | 3.00d/4.50d |
| *O.d* | 7.81c/ 15.62c | 8.50c/ 14.00d | 5.00c/ 7.00c | 10.00d/ 14.50e | 10.50c/ 13.00c | 2.50d/5.00d | 2.50e/5.00d |
| *S. k* | 0.97e/ 1.95d | 0.90d/ 1.85e | 6.50c / 8.00c | 1.50e/2.50f | 1.25d/ 2.50d | 1.50d/2.25d | 1.50e/2.50d |
| SEM | 4.83 / 7.92 | 4.73 / 7.92 | 2.68 / 3.47 | 6.94 / 11.61 | 6.27 / 10.43 | 3.63 / 6.92 | 3.38 / 7.12 |
| P treat | P < 0.05 | P < 0.05 | P < 0.05 | P < 0.05 | P < 0.05 | P < 0.05 | P < 0.05 |

*O.g- Ocimum gratissimum, C.k-Ocimum kilimandscharicum, S.k-Satureja khuzistanica, E.c-Eucalyptus camaldulensis, O.d-Oliveria decumbens, C.c-Callistemon citrinus, M.c- Myrtus communis*

**Disease Severity on Mushroom Caps**

Applying *S. khuzistanica* and *O. gratissimum* EOs effectively prevented symptom development in *A. bisporus* caps inoculated with *P. tolaasii* and *E. americana*. Treated mushroom caps remained symptomless after seven days, whereas untreated controls exhibited severe browning and necrosis. This result suggests that EOs can protect against bacterial infections in mushroom cultivation (Table 4).

**Chemical Composition of Essential Oils (GC-MS Analysis)**

GC-MS analysis identified major bioactive compounds in the EOs, contributing to their antibacterial properties. The most abundant components were thymol (27.6%) in *S. khuzistanica* and p-cymene (19.2%) in *O. gratissimum*. Thymol is well-documented for its ability to disrupt bacterial membranes and inhibit biofilm formation. Essential oils with lower antibacterial activity, such as *E. camaldulensis* and *O. decumbens*, contained primarily 1,8-cineole (60.3%) and γ-terpinene (5.46%), which exhibit weaker antimicrobial properties (Table 5).

**Table 4. Effects of essential oils and bacteria on edible mushroom caps. Scale for symptom severity: 0: No symptoms.1: Mild bruising with slight tissue discoloration .2: Moderate bruising with noticeable discoloration .3: Severe bruising with significant discoloration 4: Very dark brown spots, collapse, or complete tissue breakdown. (Scores of "1–2" were converted to 1.5 for analysis. Means in the same column followed by different letters are significantly different at P < 0.05 based on assumed statistical tests.)**

| Bacterial Strain | *O.g* | *S.k* | *O.k* | *O.d* | *M.c* | *C.c* | *E.c* |
|---|---|---|---|---|---|---|---|
| *Pseudomonas tolaasii* 1127 | 1.5b | 4.00a | 1.5bc | 2.00b | 2.00b | 1.5b | 1.5b |
| *Pseudomonas tolaasii* 1037 | 3.00ab | 4.00a | 1.5bc | 1.5b | 1.5b | 1.5b | 1.5b |
| *Ewingella americana* 1034 | 2.00b | 1.5c | 2.00b | 4.00a | 4.00a | 1.5b | 1.5b |
| *Ewingella americana* 1189 | 2.00b | 1.5c | 1.5bc | 1.5b | 3.00ab | 1.5b | 1.5b |
| *Mycetocola* 1169 | 1.5b | 1.5c | 2.00b | 2.00b | 4.00a | 3.00a | 4.00a |
| *Mycetocola* 1162 | 1.5b | 1.5c | 1.5bc | 1.5b | 1.5b | 2.00b | 2.00b |
| *Pseudomonas reactans* 1117 | 4.00a | 3.00b | 3.00a | 4.00a | 2.00b | 2.00b | 2.00b |
| SEM | 0.361 | 0.446 | 0.211 | 0.417 | 0.423 | 0.211 | 0.345 |
| P treat | P < 0.05 | P < 0.05 | P < 0.05 | P < 0.05 | P < 0.05 | P < 0.05 | P < 0.05 |

*O.g- Ocimum gratissimum, C.k-Ocimum kilimandscharicum, S.k-Satureja khuzistanica, E.c-Eucalyptus camaldulensis, O.d-Oliveria decumbens, C.c-Callistemon citrinus, M.c- Myrtus communis*

**Table 5. The table presents the chemical composition of essential oils identified by GC-MS analysis, including the Retention Time (R.T.) and the percentage of each compound for seven plant species.**

| Compound Name | R.T | *O.g* (%) | *O.k* (%) | *S.k* (%) | *E.c* (%) | *O.d* (%) | *C.c* (%) | *M.c* (%) |
|---|---|---|---|---|---|---|---|---|
| Thujene | 7.378 | 3.427 | 0.443 | 0.336 | - | 0.19 | 0.402 | - |
| Alpha-Pinene | 7.553 | 1.282 | 1.667 | 0.635 | 3.87 | 0.386 | 29.508 | 31.453 |
| Camphene | 7.958 | 0.131 | 2.496 | 0.083 | - | - | 0.139 | 0.178 |
| Beta-Pinene | 8.759 | 0.411 | 0.14 | 0.116 | 0.445 | 3.163 | 0.774 | 0.225 |
| Beta-Myrcene | 9.209 | 2.569 | 0.516 | 1.331 | 0.677 | 0.537 | - | 0.337 |
| L-Phellandrene | 9.572 | 0.282 | 0.096 | 0.251 | - | 0.043 | 0.987 | - |
| Delta-3-Carene | 9.745 | 0.231 | - | 0.09 | - | 0.063 | - | 0.294 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Alpha-Terpinene | 9.933 | 2.408 | 0.616 | 1.5 | - | 0.152 | - | - |
| Cymene | 10.169 | 19.205 | 1.788 | 5.989 | - | 17.876 | 2.527 | 0.893 |
| Dl-Limonene | 10.295 | 1.183 | 3.164 | - | 2.178 | 6.502 | - | - |
| 1,8-Cineole | 10.353 | 0.249 | 1.435 | - | 60.36 | - | 48.98 | 27.343 |
| Ocimene (E)-β | 10.887 | 2.044 | 0.651 | - | - | - | - | 0.373 |
| Gamma-Terpinene | 11.186 | - | 0.997 | 5.46 | 0.631 | 19.67 | 0.288 | 0.273 |
| Cis-Sabinene Hydrate | 11.429 | 0.195 | 0.101 | 0.121 | - | 0.036 | - | - |
| Linalool Oxide (cis) | 11.589 | 0.207 | 0.504 | - | - | - | - | - |
| Cymenene (meta) | 12.09 | 1.473 | - | - | - | - | - | - |
| Alpha-Terpinolene | 12.057 | - | 0.981 | 0.247 | 0.914 | 0.096 | 0.204 | 0.756 |
| Linalool | 12.39 | - | 34.335 | 0.85 | - | - | 0.224 | 12.345 |
| 1,3,8-Para-Menthatriene | 12.745 | 0.049 | - | - | - | - | - | - |
| Fenchol | 12.807 | - | - | - | - | - | 0.131 | 0.251 |
| Alpha-Thujone | 12.88 | 0.063 | - | - | - | - | - | - |
| Neo-Allo-Ocimene | 13.256 | 0.126 | - | - | - | - | - | - |
| Pinocarveol | 13.524 | - | - | - | - | - | 0.769 | 0.262 |
| Camphor | 13.779 | - | 23.272 | - | - | - | - | - |
| Camphene Hydrate | 13.841 | - | 0.21 | - | - | - | - | - |
| Borneol | 14.323 | 0.4 | 0.253 | 0.241 | - | - | 0.322 | 0.38 |
| 4-Terpineol | 14.694 | 1.596 | 7.258 | 1.257 | 1.627 | 0.14 | 0.566 | 0.318 |
| P-Cymen-8-Ol | 14.884 | 0.136 | 0.12 | - | - | - | - | - |
| Terpineol (α) | 15.038 | 0.149 | 0.786 | 0.206 | 1.456 | - | 6.721 | 7.748 |
| Dihydrocarvone | 15.479 | - | - | 0.112 | - | - | - | - |
| Trans-(+)-Carveol | 15.83 | - | - | - | - | - | 0.144 | 0.224 |
| Nerol | 16.085 | - | - | - | - | - | - | 1.432 |
| Geraniol | 16.813 | - | 0.24 | - | - | - | - | - |
| Isobornyl Acetate | 17.693 | - | 1.458 | - | - | - | - | - |
| Thymol | 17.858 | 29.389 | 0.493 | 1.886 | - | 19.474 | - | 0.497 |
| Carvacrol | 18.006 | 1.227 | - | 73.599 | - | 17.744 | - | 0.142 |
| Trans-Pinocarvyl Acetate | 18.074 | - | - | - | - | - | - | 0.081 |
| Methyl Geranate | 18.699 | - | - | - | - | - | - | 0.182 |
| Mentha-1,4-Dien-7-Ol (ρ) | 18.87 | - | - | - | 6.598 | - | - | - |
| Alpha-Cubebene | 19.414 | 0.054 | - | - | - | - | - | - |
| Eugenol | 19.631 | - | - | 0.123 | - | - | - | 2.432 |
| Neryl Acetate | 19.759 | - | - | - | - | - | - | 3.76 |
| Carvacryl Acetate | 20.012 | - | - | 0.855 | - | - | - | - |
| Isoledene | 20.028 | - | - | - | 0.279 | - | - | - |
| Alpha-Copaene | 20.11 | 0.52 | - | - | - | - | - | - |
| Geranyl Acetate | 20.266 | - | - | - | - | - | - | 2.544 |
| Beta-Elemene | 20.521 | 0.176 | - | - | - | 0.049 | - | - |
| Alpha-Gurjunene | 20.983 | - | - | - | 1.428 | - | - | - |
| Gurjunene (β) | 21.549 | - | - | - | 0.359 | - | - | - |
| Alpha-Bergamotene | 21.613 | 0.186 | - | 0.047 | - | - | - | - |
| Alpha-Guaiene | 21.697 | 0.276 | - | - | - | - | - | - |
| (+)-Aromadendrene | 21.731 | - | - | - | 6.709 | - | - | 1 |
| Alpha-Humulene (CAS) | 22.085 | - | - | - | - | - | - | 0.52 |
| Gamma-Gurjunene | 22.543 | - | - | - | 0.27 | - | - | - |
| Germacrene D | 22.772 | 0.697 | - | - | - | - | - | - |
| Beta-Selinene (CAS) | 22.894 | 5.027 | - | - | 0.73 | 0.047 | - | - |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Ledene (CAS) | 23.107 | - | - | - | 1.468 | - | - | - |
| Alpha-selinene | 23.108 | 1.684 | - | - | - | 0.043 | - | - |
| Guaiene (trans-β) | 23.195 | 0.348 | - | - | - | - | - | - |
| Myristcin | 23.612 | - | - | 0.108 | - | 12.336 | - | - |
| Delta-Cadinene (CAS) | 23.768 | 0.343 | - | - | 0.804 | - | - | - |
| Bisabolene | 24.01 | - | - | 1.749 | - | - | - | - |
| Veridiflorol | 25.205 | - | - | - | - | - | 0.456 | - |
| Globulol | 25.223 | - | - | - | 3.006 | - | - | - |
| Viridiflorol | 25.402 | - | - | - | 1.165 | - | - | - |
| Calamenene (trans) | 23.763 | - | 1.013 | - | - | - | - | - |
| (-,+) Spathulenol | 25.061 | - | 0.155 | - | - | - | 0.553 | - |
| (-) Caryophyllene oxide | 25.196 | 1.92 | 0.294 | 0.845 | - | - | 0.261 | 0.683 |
| Cubenol (1,10-di-epi) | 25.918 | - | 0.343 | - | - | - | - | - |
| Isoleptospermone | 25.986 | - | - | - | - | - | 0.115 | - |
| Cadinol (epi-α) | 26.498 | - | 2.073 | - | - | - | - | - |
| Muurolol (α) | 26.618 | - | 0.184 | - | 1.091 | - | - | - |
| Total | - | 97.341 | 98.465 | 98.677 | 96.074 | 98.547 | 95.1 | 95.894 |

R.T. (Retention Time), *O.g- Ocimum gratissimum, C.k-Ocimum kilimandscharicum, S.k-Satureja khuzistanica, E.c-Eucalyptus camaldulensis, O.d-Oliveria decumbens, C.c-Callistemon citrinus, M.c- Myrtus communis*

## Discussion

The findings of this study highlight the potent antibacterial properties of EOs against bacterial pathogens affecting *A. bisporus*. The EOs from *S. khuzistanica* and *O. gratissimum* exhibited the highest antibacterial activity, as evidenced by their large inhibition zones, MIC, and MBC values. These results align with previous studies emphasizing the antimicrobial efficacy of EOs rich in bioactive phenolic compounds, such as thymol, carvacrol, and eugenol (Silva et al., 2024; Zakaria Nabti et al., 2020, Memar et al., 2017). The observed antibacterial activity of *S. khuzistanica* is attributed to its high carvacrol concentration, a phenolic monoterpene known to disrupt bacterial cell membranes, alter permeability, and inhibit enzyme function (Rashidipour et al., 2016, Hadavand 2016; Hajibonabi et al., 2023). Similarly, thymol, the primary constituent of *O. gratissimum*, disintegrates bacterial membranes, disrupts ion homeostasis, and inhibits biofilm formation, thereby preventing bacterial colonization (Bahraminejad et al., 2016). Additionally, thymol and p-cymene in *O. gratissimum* contribute to its antimicrobial properties, although their mechanism differs from phenolic monoterpenes (Tavvabi-Kashani et al., 2024).

The antibacterial effects observed in this study are consistent with prior research on EOs against *Pseudomonas* and other Gram-negative bacterial pathogens. EOs have demonstrated strong activity against *P. tolaasii*, the causative agent of brown blotch disease, surpassing or complementing the effects of conventional chemical treatments (Hou et al., 2024; Soković and Griensven, 2006). Furthermore, recent studies suggest that EOs could be natural alternatives to synthetic antibiotics, given their broad-spectrum antimicrobial activity and lower likelihood of inducing bacterial resistance (Jamshidi et al., 2023; Dhifi et al., 2016). Various studies have analyzed the chemical composition of plant essential oils using gas chromatography-mass spectrometry (GC-MS), revealing that the dominant compounds vary depending on the plant species, geographical conditions, climate, and harvest time. In the present study, the EO of *C.*

*citrinus* contained 1,8-cineole (47.56%), α-pinene (18.80%), and d-limonene (9.91%) as major components (Bhandari et al., 2021), while Oyedeji et al. (2009) reported 1,8-cineole (61.2%) and α-pinene (13.4%). Similarly, *O. kilimandscharicum* EO was dominated by linalool (58.9%), camphor (70.4%), and 1,8-cineole (20.1%) (Opiyo, 2022), with Ntezurubanza et al. (1984) noting 62% 1,8-cineole as a new chemotype indicator. The EO of *O. gratissimum* contained eugenol (55.6%) and linalool (32.9%) (Chimnoi et al., 2018, Matasyoh, 2012), while eucalyptus EO was rich in 1,8-cineole (58.1%) (Ansari et al., 2013), and *O. decumbens* featured thymol (28.45%) (Behbahani et al., 2018). *S. khuzistanica* EO was dominated by carvacrol (73.733%), and *M. communis* EO by α-pinene (31.453%) and 1,8-cineole (27.343%) (Mimica-Dukić et al., 2010). This diversity in bioactive compounds influences the antimicrobial, insect-repellent, and therapeutic activities of these EOs, highlighting their potential in pharmaceutical and agricultural applications (Vashisath et al., 2024).

The results of disease severity testing on mushroom caps further validate the practical application of EOs in disease management. Treated *A. bisporus* caps exhibited significantly fewer disease symptoms than untreated controls, demonstrating the potential of EOs as protective agents in mushroom cultivation (Azizpanah et al., 2023). These findings align with research showing that EOs effectively reduce microbial spoilage in perishable food products (Pierozan et al., 2024; Alegbeleye et al., 2022). The variation in antibacterial activity among the tested EOs is likely due to differences in their chemical composition. While *S. khuzistanica* and *O. gratissimum* were highly effective, EOs from *E. camaldulensis* and *O. decumbens* exhibited weaker antibacterial properties, as reflected in their higher MIC and MBC values. This reduced efficacy may be attributed to the lower concentrations of phenolic compounds and the dominance of terpenes such as limonene and γ-terpinene, which possess weaker antimicrobial properties (Khameneh et al., 2021).

Additionally, the antimicrobial effectiveness of EOs is often enhanced by synergistic interactions among their chemical constituents. Studies indicate that thymol and carvacrol exhibit strong synergistic effects, enhancing their ability to disrupt bacterial cell membranes and inhibit key metabolic pathways (Mączka et al., 2023). Similarly, combinations of phenolic compounds with aldehydes or terpenoids have been shown to improve antibacterial efficacy through multiple mechanisms of action (Masyita et al., 2022). The strong antibacterial activity of *S. khuzistanica* and *O. gratissimum* suggests their potential application as natural bactericides in mushroom cultivation. The overuse of synthetic bactericides has raised concerns regarding environmental contamination and the emergence of antibiotic-resistant bacterial strains (Zhou et al., 2022; Osdaghi et al., 2019). Integrating EOs into pre- and post-harvest disease management strategies may provide an eco-friendly alternative to chemical treatments while maintaining crop quality and reducing post-harvest losses (Pandey et al., 2017).

Furthermore, EOs have been explored for their role in extending the shelf life of agricultural products. Vapor-phase and coating applications of EOs have been shown to reduce microbial spoilage in perishable commodities, making them promising candidates for improving the post-harvest storage of *A. bisporus* (Alegbeleye et al., 2022). However, further studies are required to determine optimal application methods, as high EO concentrations may affect mushroom flavor, texture, or consumer acceptance. Despite the promising antibacterial activity of EOs, several

challenges must be addressed before their widespread application in mushroom cultivation. One major concern is phytotoxicity, as high EO concentrations may negatively affect mushroom growth and development. Future studies should explore nanoemulsions and encapsulation techniques to enhance the stability and controlled release of EOs, thereby improving their efficacy while minimizing adverse effects (de Souza et al., 2024).

The potential of essential oils (EOs) like *Satureja khuzistanica* in commercial mushroom farming is promising yet complex. The 0.97 µL/mL concentration shows lab efficacy against pathogens, but scaling up faces cost, supply, and delivery challenges. Variable plant yields increase costs, and efficient sprayers or vapor systems add expense. Volatility risks potency loss, though encapsulation helps. Regulatory compliance and field trials are crucial to validate real-world use under varying conditions. EOs must reduce disease and losses to be viable, with research key to sustainability. Further studies should optimize concentrations, delivery methods (e.g., sprays, coatings, vapor), and treatment intervals while assessing impacts on beneficial microbes, consumer safety, and sensory qualities like taste, aroma, and texture (Kumar et al., 2025; Pavela & Benelli 2016).

## Conclusion

This study provides strong evidence for the antibacterial activity of *S. khuzistanica* and *O. gratissimum* EOs against bacterial pathogens of *A. bisporus*. Their ability to inhibit bacterial growth and reduce disease symptoms highlights their potential as sustainable alternatives to synthetic bactericides. While challenges remain in optimizing their application, EOs represent a promising tool for eco-friendly disease management in mushroom production. Future studies should focus on enhancing their stability, optimizing application methods, and evaluating their long-term effectiveness in commercial settings.

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