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Evaluation of the chemical characteristics and antifungal effect of *Lavandula sublepidota* essential oil on molds causing strawberry fruit rot and spoilage during storage

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ABSTRACT

Strawberries are highly prone to fungal decay. Therefore, utilizing naturally occurring compounds with potential antifungal properties, such as plant essential oils, could effectively control and prevent postharvest diseases in strawberries. This study evaluates the antifungal effectiveness of *Lavandula sublepidota* essential oil against fungal species responsible for strawberry rot, specifically *Aspergillus niger*, *Rhizopus stolonifer*, and *Botrytis cinerea*. The research also examined the essential oil's chemical composition, total phenolic and flavonoid content, and antioxidant activity. *L. sublepidota* essential oil was found to be rich in linalool (43.85%). The total phenolic content was recorded at 86.29 mg GAE/g, and the flavonoid content at 21.52 mg QE/g. This essential oil demonstrated significant scavenging activity against DPPH (61.53%) and ABTS (69.85%) free radicals. Antifungal testing revealed that *B. cinerea* was the most sensitive species, with inhibition zone diameters measuring 13.30 mm and 14.35 mm for disk and well diffusion agar methods, respectively. Furthermore, lower concentrations of the essential oil were necessary to inhibit or eliminate *B. cinerea*, with minimum inhibitory and fungicidal concentrations of 4 mg/ml and 128 mg/ml, respectively. These findings suggest that *L. sublepidota* essential oil could serve as a natural antifungal agent to inhibit the growth of harmful fungi on strawberries and extend their shelf life.

1- Introduction

Strawberries are a widely enjoyed fruit known for their numerous nutritional benefits. They are rich in vitamins, minerals, and antioxidants, which make them an essential part of a healthy diet. However, despite their popularity, strawberries are highly perishable and typically have a short shelf life of just a few days to a few weeks, depending on the environmental conditions. Their vulnerability to factors that diminish quality and quantity, such as physical damage, unfavorable temperatures, and microbial infections, often leads to substantial post-harvest losses. This perishability creates challenges for producers and retailers, underscoring the need for effective storage methods to preserve quality and extend the shelf life of strawberries [1-3].

Pathogenic fungi significantly contribute to the spoilage of strawberries, negatively impacting their marketability and safety. Fungi like *Botrytis cinerea* and *Rhizopus stolonifer* thrive in moist environments and can rapidly reproduce on the surface of strawberries. These pathogens not only result in considerable economic losses within the agricultural sector but also pose health risks to consumers [4, 5].

To address fungal spoilage, the food industry has developed and widely implemented synthetic preservatives and antifungal agents, which effectively inhibit fungal growth and prolong the shelf life of strawberries. However, concerns regarding potential health risks, environmental impacts, and the risk of fungal resistance have led to a search for safer alternatives. Growing consumer interest in natural products has intensified the exploration of plant-based preservatives as viable substitutes for synthetic agents in strawberry preservation [5, 6].

Particularly, plant preservatives, and essential oils in particular, have gained attention for their potential as natural antifungal agents. Essential oils are concentrated liquids derived from plants and are celebrated for their complex chemical compositions and powerful antimicrobial properties. Their capability to inhibit the growth of various fungal pathogens positions them as promising candidates for natural food preservation. Incorporating essential oils into food systems can provide a dual benefit: extending the shelf life of perishable items while aligning with consumer preferences for natural and minimally processed foods [7-14].

One plant that shows considerable promise as an antifungal agent is *Lavandula sublepidota*. This shrub is noted for its aromatic qualities and the bioactive compounds found in its essential oil [15-17]. Studies indicate that lavender essential oil exhibits significant antifungal activity [18-21], making it a strong candidate for preventing strawberry spoilage. The distinctive phytochemical profile of this essential oil supports its application in postharvest treatments and presents a natural alternative to synthetic fungicides.

While several studies have demonstrated the antifungal properties of essential oils against pathogens responsible for strawberry spoilage [22-24], the specific antifungal effects of lavender essential oil on strawberry fungal pathogens have yet to be explored. As scrutiny of synthetic preservatives increases, plant-based solutions—particularly those derived from

essential oils like lavender—offer a promising path toward ensuring food safety and maintaining quality. This paper aims to investigate the antifungal effects of *L. sublepidota* essential oil on fungal strains that compromise the post-harvest shelf life of strawberries, along with analyzing the essential oil's chemical composition, total phenolic and flavonoid content, and antioxidant activity.

2- Materials and Methods

2.1. Extraction of essential Oil

The aerial parts of the *L. sublepidota* plant were sourced from the National Botanical Garden of Iran. The scientific name of the plant was verified by the Herbarium Center of Khuzestan University of Agricultural Sciences and Natural Resources. The plants were cleaned, removing any damaged parts, and then crushed into a powder, which was stored in dry, dark containers for subsequent experiments. A total of 300 g of the powdered plant was measured and placed in a boiling flask, to which 1200 ml of distilled water was added. The essential oil was then extracted through water distillation using a Clevenger apparatus for three hours. The resulting essential oil was stored in a freezer at -4 °C until analysis [25].

2.2. Identification of chemical compounds

The chemical compounds in the essential oil were analyzed using gas chromatography (GC-FID) and gas chromatography/mass spectrometry (GC/MS) [15]. The GC analysis was performed on an Agilent 7890-A series gas chromatograph equipped with an FID detector, utilizing an HP-5 column (30 m × 0.32 mm ID; 0.25 µm film thickness). The injection and detector temperatures were set at 250 °C and 280 °C, respectively. The carrier gas flow (nitrogen) was maintained at 1 mL/min, and the oven temperature program was set from 60–210 °C at a rate of 4 °C/min, then increased to 240 °C at a rate of 20 °C/min, and held isothermally for 8.5 minutes. For GC/MS analysis, an Agilent gas chromatograph with an HP-5MS column was paired with a 5975-C mass spectrometer. Helium served as the carrier gas, with an ionization voltage of 70 eV. The ion source and interface temperatures were maintained at 230 °C and 280 °C, respectively. The mass range for detection was set from 45 to 550 amu, and the oven temperature program was aligned with the GC method. Identification of volatile compounds involved calculating their retention indices against n-alkanes (C8-C25) and essential oils on an HP-5 column under equivalent chromatographic conditions. Further identification was achieved by matching the recorded mass spectra with reference mass spectra. For quantitative assessments, the relative area percentages obtained by FID were utilized without correction factors [15].

2.3. Total phenol content

The total phenol content of the essential oils was determined using the Folin-Ciocalteau method. In this procedure, 125 µL of the sample was combined with 500 µL of distilled water and 125 µL of Folin-Ciocalteau reagent, and the mixture was allowed to stand for 6 minutes. Following this, 1.25 mL of 7% sodium carbonate and 1 mL of distilled water were added, and the solution was incubated for 90 minutes. The absorbance was measured at a wavelength of 760 nm, with gallic acid serving as the standard. Total phenol

content was expressed in milligrams of gallic acid per gram of essential oil (mg GA/g) [26].

2.4. Total flavonoid content

To measure the total flavonoid content, 0.5 mL of the essential oil was mixed with 2 mL of distilled water. Next, 0.15 mL of 5% sodium nitrate solution was added, followed by 0.15 mL of 10% aluminum chloride solution after 6 minutes. Then, 2 mL of 4% sodium hydroxide solution was included, and the final volume was adjusted to 5 mL with distilled water. This solution was incubated at room temperature for 15 minutes, and absorbance was measured at a wavelength of 510 nm. The results were reported in terms of milligrams of quercetin per gram of dry matter (mg QE/g) [25].

2.5. Antioxidant activity

The antioxidant activity of *L. sublepidota* essential oil was assessed using the DPPH and ABTS radical scavenging methods as outlined by Rahmati Junidabad et al. [26], with necessary modifications. For the DPPH assay, 2 mL of the essential oil was combined with 0.4 mL of a 0.5 mM methanolic DPPH radical solution, and the mixture was kept in the dark for 30 minutes before measuring absorbance at 517 nm. The percentage of DPPH radical scavenging was calculated using the following formula:

$$Scavenging\ \% = \left(\frac{A_{blank} - A_{sample}}{A_{blank}}\right) \times 100$$

In this equation, A_{blank} and A_{sample} are the blank and sample absorbances, respectively.

For the ABTS assay, a cationic ABTS radical solution was prepared by mixing equal volumes of 7 mM ABTS solution and 2.4 mM potassium persulfate solution, and incubated in the dark at room temperature for 12-16 hours. Before use, this solution was diluted with absolute ethanol to achieve an absorbance of 0.7 at 734 nm. Subsequently, 3950 µL of the reaction mixture was combined with 50 µL of the essential oil. After incubating in the dark at room temperature for 30 minutes, the absorbance at 734 nm was measured against the control solution. The inhibitory effect was expressed as a percentage, calculated using the same formula as in the DPPH method.

2.6. Antifungal activity

The antifungal activity of lavender essential oil was evaluated against *Aspergillus niger*, *Rhizopus stolonifer*, and *Botrytis cinerea* using agar disk diffusion, agar well diffusion, and minimum inhibitory and lethal concentration methods.

2.6.1. Disk diffusion agar

Sterile discs were first immersed in sterile essential oil for 15 minutes, then placed on the surface of sterile Sabouraud dextrose agar, which had been inoculated with 100 µL of each fungal strain. The plates were incubated at 27 °C for 72 hours, after which the diameters of the inhibition zones were measured in millimeters [27].

2.6.2. Well diffusion agar

In this method, 20 µL of sterile essential oil was introduced into wells created on the surface of Sabouraud dextrose agar medium. Fungal strains were also added to each well. The culture medium was incubated at 27 °C for 72 hours, after which the diameter of the inhibition zone was measured in millimeters [27].

2.6.3. Minimum Inhibitory and fungicidal concentrations

Various concentrations of essential oil (0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, and 512 mg/mL) were prepared, and 5 mL of each concentration was added to 100 µL of fungal suspension in test tubes. After incubating the test tubes at 27 °C for 72 hours, the growth of the fungal strains was evaluated by assessing the turbidity of the medium. The tube that showed no turbidity was designated as the minimum inhibitory concentration (MIC). For the MFC test, 100 µL of the culture medium was transferred from the tubes without turbidity onto Sabouraud dextrose agar medium, which was then incubated at 27 °C for 72 hours. The concentration of essential oil that inhibited the growth of the fungal strains was identified as the minimum fungicidal concentration (MFC) of lavender essential oil [28].

2.7. Statistical analysis

All experiments were conducted in triplicate. The results were analyzed using Minitab software (version 16) and Tukey's test at a significance level of 5%.

3- Results and Discussion

3.1. Chemical composition

Gas chromatography coupled with mass spectrometry (GC/MS) analysis revealed 13 compounds in the essential oil of *L. sublepidota*, which collectively accounted for 98.42% of the oil's composition (Fig. 1). The compound with the highest concentration was linalool at 43.85%, followed by linalyl acetate (20.21%), beta-myrcene (9.85%), limonene (6.62%), 1,3,6-octatriene (5.24%), and terpinen-4-ol (4.75%). Consistent with these findings, Heidari et al. [16] identified 12 compounds in the essential oil of *L. sublepidota* through GC/MS, with linalool (43.3%), linalyl acetate (19.1%), beta-myrcene (11.61%), limonene (5.43%), and 1,3,1-octatriene (5.31%) being the most abundant.

In another study, the primary components of *L. sublepidota* essential oil were reported to include caryophyllene oxide (31.8%), spathulenol (10.4%), (E)-caryophyllene (6.9%), 14-hydroxy-9-epi-(E)-caryophyllene (5.3%), elemol (4.9%), and carvacrol (4.4%). Notably, linalyl acetate was not detected, and linalool was found at less than 0.3% [15]. For *Lavandula angustifolia*, the main constituents were identified as linalyl acetate (47.56%) and linalool (28.06%) [29]. The essential oil of *Lavandula bipinnata* was reported to contain trans-carveol, polygon, camphor, and menthol [30]. Variations in the reported percentages across different studies may be attributed to factors such as species differences, climatic conditions during growth, harvest timing, storage duration, and extraction methods used for the essential oil [31].

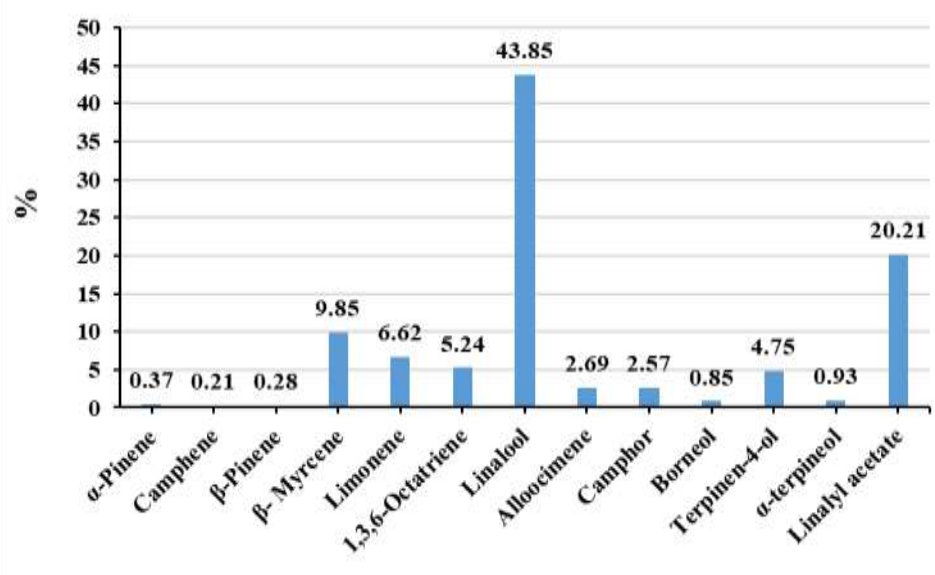

Figure 1. Chemical composition of *Lavandula sublepidota* essential oil identified by GC/MS. Compounds below 0.2% are not listed in the figure.

3.2. Total phenol and flavonoid contents

The essential oil of *L. sublepidota* was found to contain 86.29 mg GAE/g of total phenols and 21.52 mg QE/g of total flavonoids (Fig. 2). Keivanfar et al. [25] reported the total phenol content of *L. sublepidota* essential oil as 74.57 mg GAE/g and the total flavonoid content as 1831.44 mg QE/g. In another study, phenol content in five lavender flower samples from Romania ranged from 74.98 mg GAE/g to 88.89 mg GAE/g [32]. Bahmanzadegan et al. [15] identified and quantified a total of 11 phenolic compounds in the essential oil of *L. sublepidota* using high-performance liquid chromatography, including chlorogenic acid, quercetin, coumarin, hesperetin, carvacrol, caffeic acid, vanillin, trans-ferulic acid, sinapic acid, rosmarinic acid, and p-coumaric acid. The dominant phenolic compounds identified were sinapic acid (0.68 mg/g), trans-ferulic acid (0.58 mg/g), and rosmarinic acid (0.34 mg/g) [15]. Phenolic compounds in plant extracts are linked to their antioxidant properties, thanks to their redox characteristics, which enable them to function as reducing agents, hydrogen donors, and singlet oxygen quenchers [32–34].

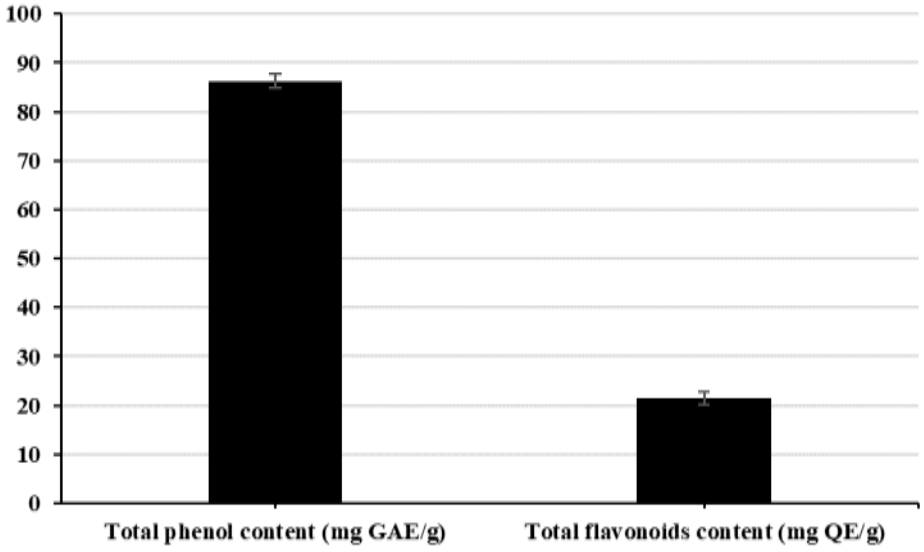

Figure 2. Total phenol content and total flavonoids content of *Lavandula sublepidota* essential oil.

3.3. Antioxidant effect

The antioxidant activity results of *Lavandula sublepidota* essential oil are illustrated in Fig. 3. The findings indicate that the essential oil exhibited inhibitory activities of 61.53% against DPPH radicals and 69.85% against ABTS radicals. Research on the antioxidant properties of *L. sublepidota* essential oil is limited. However, one study reported that the antioxidant activity of *L. sublepidota* essential oil was approximately 89%, which was notably higher than that of the synthetic antioxidant TBHQ, which showed an activity of 87% [25]. In another study, the antioxidant activity of the methanol extract of *L. sublepidota*, based on DPPH radical inhibition, was reported to be 2146 µg/mL [15]. The high antioxidant activity of essential oils is likely due to the elevated levels of phenolic and flavonoid compounds present in the extracted oil, which enhance the transport of free radicals and exhibit greater inhibitory capacity. It has been noted that increasing the concentrations of phenolic and flavonoid compounds directly enhances the ability of essential oils to inhibit free radicals. Higher concentrations of phenolic compounds lead to an increase in hydroxyl groups (-OH), which enhances the potential for hydrogen donation to free radicals [35, 36]. The results suggest that the antioxidant capacity of essential oils is influenced by their active components, which can vary due to factors such as climatic conditions, harvest timing, extraction methods, and others [14, 31, 37, 38].

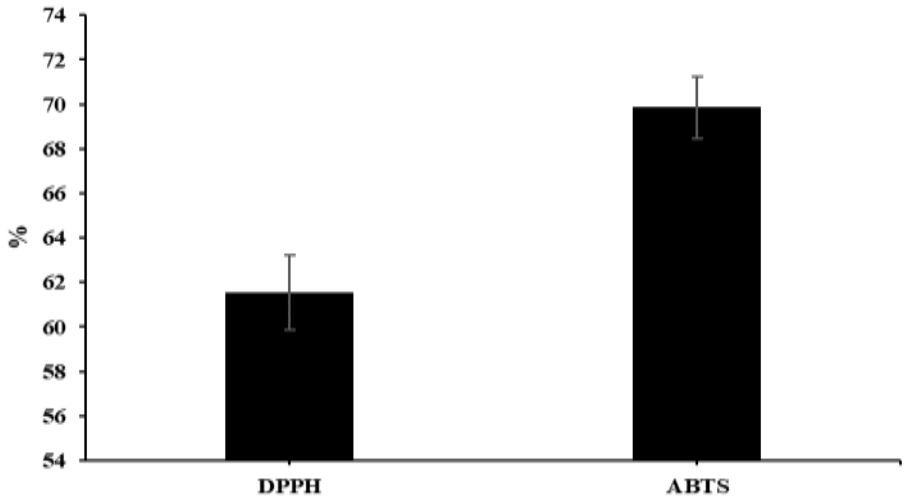

Figure 3. The antioxidant activity of *Lavandula sublepidota* essential oil based on DPPH and ABTS radical scavenging methods.

3.4. Antifungal effect

The results of the antifungal activity of the essential oil, assessed using the disk diffusion agar method, revealed that the largest growth inhibition zone (13.30 mm) was observed for the *B. cinerea* strain ($p < 0.05$) (Fig. 4). In contrast, the *R. stolonifer* strain exhibited the smallest inhibition zone diameter at 9.60 mm. The well diffusion agar test showed similar findings, with *R. stolonifer* showing a growth inhibition zone of 10.20 mm, while *B. cinerea* demonstrated a larger zone of 14.35 mm, indicating that *R. stolonifer* was the most resistant strain and *B. cinerea* the most sensitive to *L. sublepidota* essential oil ($p < 0.05$).

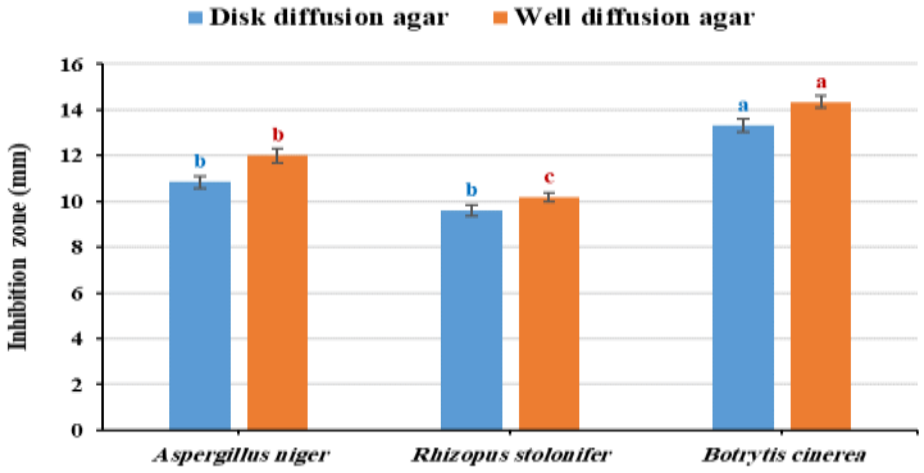

Figure 4. The antifungal activity of *Lavandula sublepidota* essential oil based on disk diffusion agar and well diffusion agar methods. Treatments labeled with different letters show significant differences at $p < 0.05$.

The results of the minimum inhibitory concentration (MIC) test for the essential oil are shown in Table 1. Overall, higher concentrations of the essential oil resulted in decreased growth of the fungal strains. The MIC values were determined to be 16 mg/mL for *A. niger*, 8 mg/mL for *R. stolonifer*, and 4 mg/mL for *B. cinerea*. The minimum fungicidal concentration (MFC) test produced similar findings, with MFC values of 256 mg/mL for both *A. niger* and *R. stolonifer*, and 128 mg/mL for *B. cinerea*. These results are consistent with those obtained from the disk diffusion agar and well diffusion agar tests, indicating that *R. stolonifer* was the most resistant strain and *B. cinerea* was the most sensitive to *L. sublepidota* essential oil.

Table 1. The antifungal activity of *Lavandula sublepidota* essential oil based on minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) methods.

		Essential oil concentration (mg/mL)										**Controls**	
Method	**Strain**	0.5	1	2	4	8	16	32	64	128	256	Positive control	Negative control
MIC	*A. niger*	+	+	+	+	+	-	-	-	-	-	+	-
	R. stolonifer	+	+	+	+	-	-	-	-	-	-	+	-
	B. cinerea	+	+	+	-	-	-	-	-	-	-	+	-
MFC	*A. niger*	+	+	+	+	+	+	+	+	+	-	+	-
	R. stolonifer	+	+	+	+	+	+	+	+	+	-	+	-
	B. cinerea	+	+	+	+	+	+	+	+	-	-	+	-

+ Growth; - No growth

Heidari et al. (2019) evaluated the microbial growth inhibition zones of *L. sublepidota* essential oil against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus*, and *Escherichia coli* across four concentrations (12.5, 25, 50, and 100 mg/mL) using disk diffusion agar and well diffusion agar methods. They also determined the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for these microorganisms. Their findings indicated that *S. aureus* was the most sensitive, while *P. aeruginosa* was the most resistant, with inhibition diameters of 30.70 mm and 10.10 mm, respectively, at a concentration of 100 mg/mL. The MIC values for *P. aeruginosa*, *S. aureus*, *B. cereus*, and *E. coli* were found to be 32, 8, 16, and 16 mg/mL, respectively [16].

Mazraeh et al. (2024) demonstrated that foliar spraying of copper nanoparticles significantly enhanced the antibacterial and antifungal efficacy of *L. sublepidota* essential oil compared to control conditions. Following the application of 25 mg/L of copper nanoparticles, the MIC of the essential oil for *Salmonella typhimurium*, *E. coli*, and *Candida albicans* decreased, with *C. albicans* showing the highest sensitivity to lavender essential oil among the tested microbial isolates at various copper nanoparticle doses [39].

The lipophilicity of the essential oil components allows them to penetrate the lipid layers of bacterial cell membranes and mitochondria, disrupting their structure and integrity, which may contribute to their antibacterial activity [40]. Due to their hydrophobic nature, essential oils can infiltrate fungal

mycelium and inhibit its growth. However, the precise mechanism behind their antifungal effects is not well understood, likely because of the numerous compounds present in essential oils. These compounds appear to enhance membrane permeability, cause damage, and disrupt fungal morphology [26, 41].

Ciocarlan et al. (2018) noted that the antifungal activity of *Lavandula angustifolia* essential oil against *Candida utilis* could be attributed to the presence of compounds such as beta-phlandrene, alpha-terpenyl acetate, and ligustilide. These compounds potentially lead to cell wall and cytoplasmic membrane damage, reduce cytoplasm surrounding the nucleus, disrupt plasma membrane lipid function, and ultimately alter cell permeability, causing intracellular compounds to leak out [42]. Variations in the antimicrobial properties of essential oil across different treatments may result from changes in the quantities or types of polyphenolic and flavonoid compounds present [31].

4- Conclusion

The findings of this study revealed that linalool, linalyl acetate, limonene, and beta-myrcene are the primary components of *L. sublepidota* essential oil. This essential oil exhibited high levels of phenols and flavonoids, demonstrating significant activity in inhibiting the free radicals DPPH and ABTS. Additionally, the antifungal assessments indicated that the *B. cinerea* strain was the most sensitive to this essential oil among strawberry pathogenic fungi. Consequently, *L. sublepidota* essential oil has the potential to be utilized as an herbal remedy to extend the shelf life of strawberries and other foods.

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ارزیابی ویژگی‌های شیمیایی و اثر ضدقارچی اسانس اسطوخودوس فلس‌دار (*Lavandula sublepidota*) بر کپک‌های عامل پوسیدگی و فساد میوه توت فرنگی طی انبارمانی

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چکیده

میوه توت‌فرنگی نسبت به فساد قارچی بسیار حساس است. در این راستا، استفاده از ترکیبات با منشأ طبیعی که دارای فعالیت ضد قارچی بالقوه هستند (مانند اسانس‌های گیاهی)، می‌تواند راه‌حل مؤثری برای کنترل و پیشگیری از بیماری‌های پس از برداشت میوه توت‌فرنگی باشد. در این مطالعه، فعالیت ضد قارچی اسانس اسطوخودوس فلس‌دار (*Lavandula sublepidota*) بر روی گونه‌های قارچی که باعث پوسیدگی توت‌فرنگی می‌شوند، یعنی *آسپرژیلوس نایجر*، *رایزوپوس استولونیفر* و *بوتریتیس سینه‌را*، مورد ارزیابی قرار گرفت. ترکیبات شیمیایی، محتوای فنول کل و فلاونوئیدها و فعالیت آنتی‌اکسیدانی اسانس نیز تعیین شد. اسانس اسطوخودوس فلس‌دار غنی از لینالول (۴۳/۸۵ درصد) بود. میزان فنول و فلاونوئید کل اسانس به ترتیب ۸۶/۲۹ mg GAE/g و ۲۱/۵۲ mg QE/g بود. اسانس اسطوخودوس فلس‌دار فعالیت قابل‌توجهی در مهار رادیکال آزاد DPPH (۶۱/۵۳ درصد) و ABTS (۶۹/۸۵ درصد) داشت. نتایج ضد قارچی نشان داد که *بوتریتیس سینه‌را* حساس‌ترین گونه قارچی به اسانس بود. قطر هاله عدم رشد در روش دیسک و دیفیوژن آگار برای این سویه به ترتیب ۱۳/۳۰ میلی‌متر و ۱۴/۳۵ میلی‌متر بود. علاوه بر این، غلظت کمتری از اسانس برای مهار رشد یا کشتن *بوتریتیس سینه‌را* مورد نیاز بود؛ بطوریکه، حداقل غلظت مهارکنندگی و قارچ‌کشی برای این سویه به ترتیب ۴ و ۱۲۸ میلی‌گرم در میلی‌لیتر به دست آمد. بر اساس نتایج، اسانس اسطوخودوس فلس‌دار می‌تواند به عنوان یک عامل ضد قارچ طبیعی برای جلوگیری از رشد قارچ‌های بیماری‌زا بر روی میوه توت‌فرنگی و افزایش ماندگاری آن استفاده شود.