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Scientific Research

# Evaluation of the chemical characteristics and antifungal effect of *Zhumeria majdae* essential oil on molds causing orange fruit rot and spoilage during storage

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**ABSTRACT**

In this research, the chemical properties and antifungal effect of *Zhumeria majdae* essential oil on the molds that cause decay and spoilage of orange fruit during storage were investigated. The hydrodistillation method was used to extract *Z. majdae* essential oil. The amount of total phenol (according to Folin Ciocalteu method), the amount of total flavonoid (based on the colorimetric method of aluminum chloride), antioxidant effect (based on DPPH and ABTS free radical inhibition methods) and antifungal activity (based on disc diffusion agar, well diffusion agar, minimum inhibitory concentration, and minimum fungicidal concentration) of essential oil against *Penicillium italicum* and *Penicillium digitatum* were investigated. The content of total phenol and total flavonoid of the essential oil were equal to 51.38 mg GAE/g and 22.18 mg QE/g, respectively. *Z. majdae* essential oil was able to inhibit DPPH and ABTS free radicals (61.50% and 67.85%, respectively). The results of the antifungal effect of *Z. majdae* essential oil showed that the average diameter of the inhibition zone for the fungal strains of *P. italicum* and *P. digitatum* in the disc diffusion agar method was 11.10 and 13.70 mm, respectively, and in the well diffusion agar method it was 12.20 and 14.90 mm. The minimum inhibitory concentration for these strains was equal to 4 and 2 mg/ml and the minimum fungicidal concentration was equal to 64 and 16 mg/ml. According to the results, *Z. majdae* essential oil can be used as a natural antimicrobial agent in order to prevent the growth of fungal strains that cause rot and spoilage of orange fruit during storage.



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## 1- Introduction

Citrus fruits are among the most common fruit crops grown in subtropical and tropical regions around the world. Brazil has been leading the global citrus market for years, gravitating towards processing, followed by the United States and China. Significant economic losses in horticulture worldwide are generally a reflection of the occurrence of several diseases in the post-harvest stages. Diseases such as stem-end rot, anthracnose, black spot, and melanosis may be important under certain conditions, but Penicillium is considered the most important post-harvest disease in citrus production [1]. As reported, Penicillium accounts for up to 15% of all post-harvest diseases [2].

The primary fungal pathogen responsible for Penicillium in citrus fruits is *Penicillium digitatum*, commonly known as green mold [1]. Al-Sheikh and Yehia (2016) revealed that 39.5% of citrus fruit decay was attributed to *Penicillium digitatum* and *Penicillium italicum*, 25.5% to *Penicillium citrinum*, and 0.91% to *Fusarium solani* [3].

Post-harvest control of these pathogens is effectively achieved by synthetic chemical fungicides. However, the reduced number of permitted active ingredients, the increasing resistance of some post-harvest fungal pathogens to registered fungicides, and the growing consumer demand for high-quality and safe fruits and vegetables have intensified efforts to develop alternative control methods.

Extracts and essential oils derived from various plants have recently gained popularity and scientific interest due to their antibacterial and antifungal activities. Numerous findings have been reported on the antimicrobial properties of plant extracts containing diverse phenolic compounds [4-17]. Phenolic compounds, a rich source of biocides and preservatives, have long been explored as alternative post-harvest control tools.

*Zhumeria majdae* is an aromatic perennial shrub belonging to the Lamiaceae family, native to the southern regions of Iran. It typically grows on relatively bare rocky slopes. The essential oil has a strong pleasant aroma and the plant has been traditionally used as a remedy for stomachache, bloating, diarrhea, indigestion, colds, headaches, wound healing, antiseptic, and menstrual disorders. Phytochemically, the presence of certain compounds such as flavonoids, diterpenoids, and triterpenoids has been identified in Moringa concanensis. Additionally, its cytotoxic, anti-inflammatory, analgesic, antimicrobial, and antioxidant activities have been reported in various studies [18, 19]. Previous studies on the essential oil of Moringa concanensis have shown high levels of linalool (35.6–53%) and camphor (23.8–43%) [20, 21]. Therefore, the aim of this study was to extract the essential oil of Moringa concanensis, determine its total phenolic and flavonoid content, investigate its antioxidant activity, and evaluate its antifungal activity against the fungal strains Penicillium italicum and Penicillium digitatum in order to explore the potential of the essential oil as a natural food preservative.

## 2- Materials and Methods

### 2.1. Essential Oil Extraction

The leaves were dried in the shade at ambient temperature and then ground into a powder. The powdered leaves were used for essential oil extraction. For each distillation cycle, 100 g of sample were used with water. The essential oil extraction process was carried out for 3 h, and then the essential oil was dried with anhydrous Na2SO4. The obtained essential oil was stored in dark containers at 4 °C [22].

### 2.2. Total Phenolic Content

Total phenolic content was determined using the method of Wang et al. (2017). The reaction mixture consisted of 0.5 mL of essential oil, 1 mL of Folin-Ciocalteu reagent, and 1 mL of Na2CO3 solution (7.5%). After incubation at room temperature for 2 h, the mixture was centrifuged at 3000 rpm for 10 minutes. The corresponding absorbance values were measured at 760 nm using a spectrophotometer. The total phenolic content of the essential oil





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was calculated based on the equation obtained from the gallic acid standard curve and the results were reported as milligrams of gallic acid equivalent per gram of essential oil (mg GAE/g) [23].

**2.3. Total Flavonoid Content**

Total flavonoid content was determined using the aluminum chloride method [24]. Briefly, the reaction mixture containing 0.5 mL of sample and 300 µL of NaNO2 solution (1:20 w/v) was vortexed for 10 seconds and incubated at 24 °C for 5 minutes. After that, 300 µL of AlCl3 (1:10 w/v), 2 mL of 1 M sodium hydroxide solution, and 1.9 ml of distilled water were added to the reaction mixture and mixed. The absorbance of the reaction mixture was determined at 510 nm. Quercetin was used to prepare the standard curve, and the results for total flavonoid content were expressed as milligrams of quercetin equivalent per gram of essential oil (mg QE/g).

**2.4. Antioxidant Activity**

**2.4.1. DPPH Radical Scavenging Activity**

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of the essential oil was evaluated according to the method of Saeidi et al. (2019) with necessary modifications [19]. Briefly, 2.5 ml of the essential oil solution was added to 1 mL of DPPH methanolic solution. After incubation at room temperature for 30 minutes, the absorbance of the solution was recorded at 518 nm. The radical scavenging activity of the essential oil was calculated using the following equation:

%inhibition = [(AC-AS)/AC)] *100

In this formula, AC the absorbance of the control sample, and AS represent the absorbance of the sample solution.

**2.4.2. ABTS Radical Scavenging Activity**

The ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging activity of the essential oil was measured using the method of Omidpanah et al. (2015) with necessary modifications. Briefly, a 7 mM solution of ABTS was prepared in water, and then the ABTS radical cation was generated by reacting the ABTS stock solution with a 2.45 mM potassium persulfate solution and allowing the mixture to stand in the dark at room temperature for 12-16 hours before use. The ABTS radical cation solution was diluted with ethanol to an absorbance of 0.7 at 734 nm. Then, 500 µL of distilled water, 500 µL of ethanol, 15 µL of ABTS radical cation solution, and 20 µL of essential oil were added to a test tube, and the absorbance of the solution was measured at 734 nm. Finally, the following equation was used to calculate the ABTS radical scavenging activity:

%inhibition = [(AC-AS)/AC)] *100

In this equation, AC and AS the absorbance of the control sample and the absorbance of the sample solution respectively [25].

**2.5. Antimicrobial Activity**

The antifungal activity of the essential oil of *Zhumeria majdae* against *Penicillium italicum* and *Penicillium digitatum* strains was evaluated using the methods of Rahmati Janabad et al. (2022) [26].

**2.5.1. Agar Disc Diffusion Assay**

In this assay, the *Zhumeria majdae* essential oil was first sterilized using a 0.22 µm sterile syringe filter. Then, blank disks were immersed in the essential oil for 15 min. Subsequently, the blank disks were placed on the surface of Sabouraud dextrose agar plates inoculated with the fungal strains. The plates were incubated at 27 °C for 72 h, and the diameter of the zones of inhibition (mm) around the disks was measured.

**2.5.2. Agar Well Diffusion Assay**

In this assay, five sterile wells were first created on the surface of Sabouraud dextrose agar plates using a sterile Pasteur pipette. Then, a microbial suspension (100 µL) was spread over the surface of the agar. After adding 20 µL of the essential oil to each well, the plates were incubated at 27 °C for 72 h. Finally, the diameter of the zones of inhibition around the wells was measured and reported as the antifungal activity of the essential oil.





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**2.5.3. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)**

The broth dilution method was employed to determine the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the essential oil. For this assay, a 256 mg/mL stock solution of the *Zhumeria majdae* essential oil was prepared and sterilized. Then, serial dilutions of the stock solution were prepared (0.05, 1, 2, 4, 8, 16, 32, 64, 128, and 256 mg/mL). Next, the essential oil concentrations were transferred to separate test tubes, and 100 µL of the microbial suspension was added to each concentration. The samples were incubated at 27 °C for 72 hours, and the turbidity in the test tubes was visually assessed. In this step, the first concentration of essential oil that prevented microbial growth (no turbidity in the medium) was reported as the minimum inhibitory concentration (MIC) of the *Zhumeria majdae* essential oil.

To determine the minimum fungicidal concentration (MFC), 10 µL aliquots from the tubes with no visible growth (MIC and lower concentrations) were transferred onto fresh Sabouraud dextrose agar plates and incubated at 27 °C for 72 h. The MFC was defined as the lowest concentration of essential oil that resulted in no visible fungal growth on the agar plates after incubation.

**2-6- Statistical Analysis**

All collected data were subjected to statistical analysis using one-way ANOVA. The mean difference between treatments was evaluated using Duncan's multiple range test at a 95% confidence level. The data were analyzed using SPSS software (version 22), and graphs were plotted using MS Excel 2019. All tests were repeated three times.

## 3- Results and Discussion

Phenolic compounds are secondary metabolites found in plant tissues such as flowers, seeds, roots, and edible parts. These compounds have been extensively studied due to their beneficial activities, including antioxidant, anti-inflammatory, anti-tumor, and antimicrobial properties. Over 8,000 phenolic compounds with diverse structures have been identified. Phenolic compounds possess a minimum of one aromatic ring with one or more hydroxyl groups in their chemical structure. Based on the number and arrangement of carbon atoms, phenolic compounds are classified into two main categories: flavonoids and non-flavonoids (i.e., phenolic acids, lignans, stilbenes, and other lower molecular weight compounds) [26]. The results for total phenol and total flavonoid content of *Zhumeria majdae* essential oil are shown in Figure 1. *Zhumeria majdae* essential oil contained 51.38 ±0.61 mg GAE/g total phenols and 22.18 ± 0.27 27 mg QE/g total flavonoids. Imani et al. (2015) reported that *Zhumeria majdae* essential oil contained 42.74 mg GAE/g total phenols [27]. Additionally, the total phenol and flavonoid content of *Zhumeria majdae* methanol extract were reported to be 50.1 µg GAE/mg and 48.4 µg QE/mg, respectively, while the values for the aqueous extract were 27.4 µg GAE/mg and 13.5 µg QE/mg, respectively [28]. The variations in the measured phenolic compound content in this study compared to the findings of other researchers could be attributed to the fact that the composition and quality of essential oils derived from plant sources are heavily influenced by factors such as the age and variety of the plant, geographical conditions, drying methods, and extraction methods employed for the isolation of the essential oils [16, 29].



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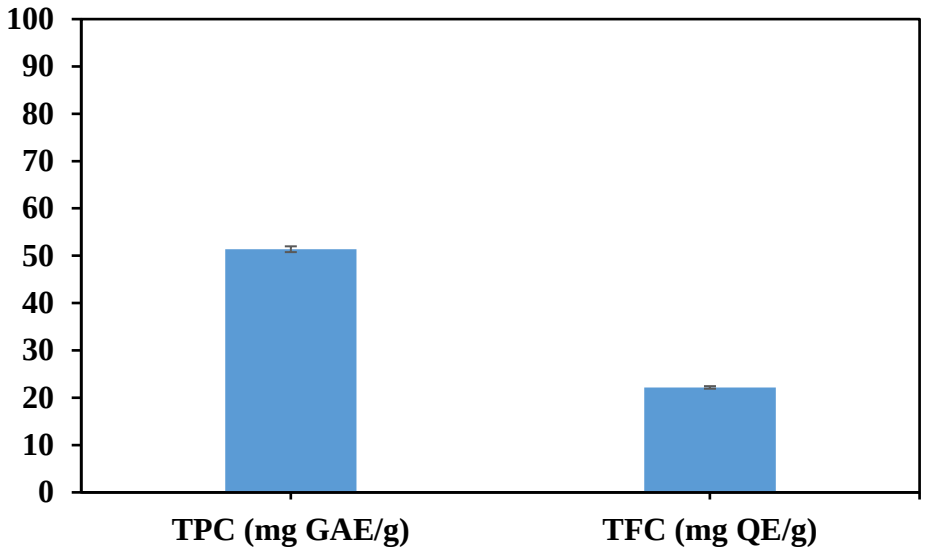


**Figure 1**. The total phenol content (TPC) and total flavonoid content (TFC) of *Zhumeria majdae* essential oil.

Free radicals have detrimental effects on the human body due to their oxidative nature. Antioxidants are compounds that counteract these oxidative effects and are beneficial in the treatment of certain chronic diseases. While lipid oxidation in food products can be prevented using antioxidants, synthetic antioxidants are not suitable options due to their toxicity. Researchers are exploring natural antioxidant sources as alternatives to synthetic antioxidants for better food preservation and reduced oxidative stress in living tissues [30-32]. Figure 2 illustrates the antioxidant activity results of *Zhumeria majdae* essential oil. *Zhumeria majdae* essential oil significantly inhibited both DPPH and ABTS free radicals, with antioxidant activities of 61.50 % and 67.85% against DPPH and ABTS radicals, respectively. The antioxidant properties of *Zhumeria majdae* are attributed to the presence of certain diterpenes, including labdanes [1], found in its roots. 12,16-di-deoxy-egyptinone B and 12-deoxy-salvipiquinone are diterpene quinones that have been isolated from *Zhumeria majdae* roots [20]. Antioxidant activity can also be found in aerial part extracts, which is attributed to their phenolic and flavonoid content. In a laboratory study, essential oils were extracted from the aerial parts of the *Zhumeria majdae* plant in five regions of Iran (Sirmand, Ghotabad, Sarchehan, Tangzagh, and Geno). Antioxidant properties were evaluated during the flowering stage using the DPPH radical scavenging assay. The antioxidant potency of the essential oils, expressed as IC50, ranged from 18.0 to 18.47 mg/mL [19]. Similar to the method employed in the previous study, the antioxidant activity of various *Zhumeria majdae* essential oils and extracts against the DPPH free radical was analyzed. The antioxidant activity of Zhumeria majdae essential oil and extracts (petroleum ether, chloroform, methanol, and aqueous) was evaluated using the DPPH radical scavenging assay. The IC50 values were found to be 20.5, 40.1, 45.8, 26.1, and 53.6 mg/mL, respectively [28]. Phenolic compounds play a major role in the antioxidant activity of essential oils and plant extracts.



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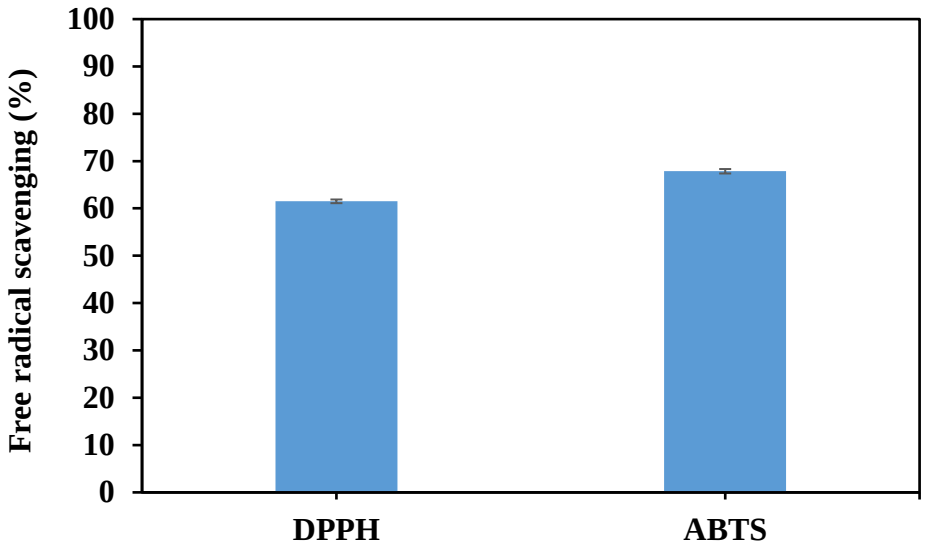


**Figure 2**. The antioxidant activity of *Zhumeria majdae* essential oil based on DPPH and ABTS radical scavenging methods.

The results of the antifungal activity of *Zhumeria majdae* essential oil against fungal strains are presented in Figure 3 using the agar disc diffusion method. Significant antifungal activity was observed against the strains *Penicillium italicum* and *Penicillium digitatum*. The mean diameter of the inhibition zones for *P. digitatum* was significantly larger than that for *P. italicum* (13.70 mm vs 11.10 mm, respectively) ($p < 0.05$).

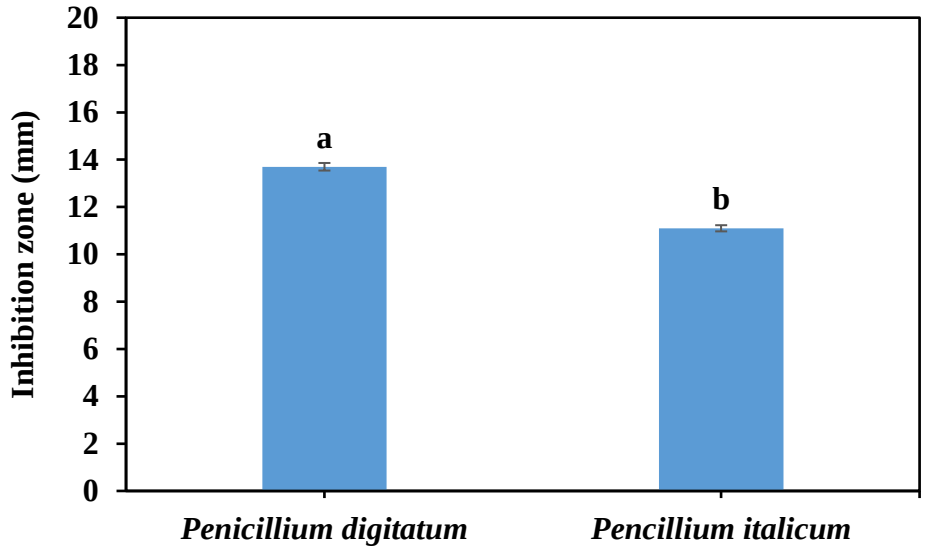


**Figure 3**. The antibacterial activity of *Zhumeria majdae* essential oil based on disc diffusion agar method

Similar results were observed using the agar well diffusion method (Figure 4). The *Penicillium digitatum* strain exhibited greater sensitivity to Persian mullein essential oil compared to the *Penicillium italicum* strain, with an inhibition zone diameter of 14.90 mm versus 12.20 mm, respectively ($p < 0.05$). Additionally, the mean inhibition zone diameter was larger in the agar well diffusion method compared to the agar disc diffusion method. This is likely because, in the agar well diffusion method, the fungal strains are in direct contact with the essential oil, whereas in the agar disc diffusion method, the rate of diffusion of the antimicrobial agent from the disc surface into the medium determines its inhibitory effect [9].



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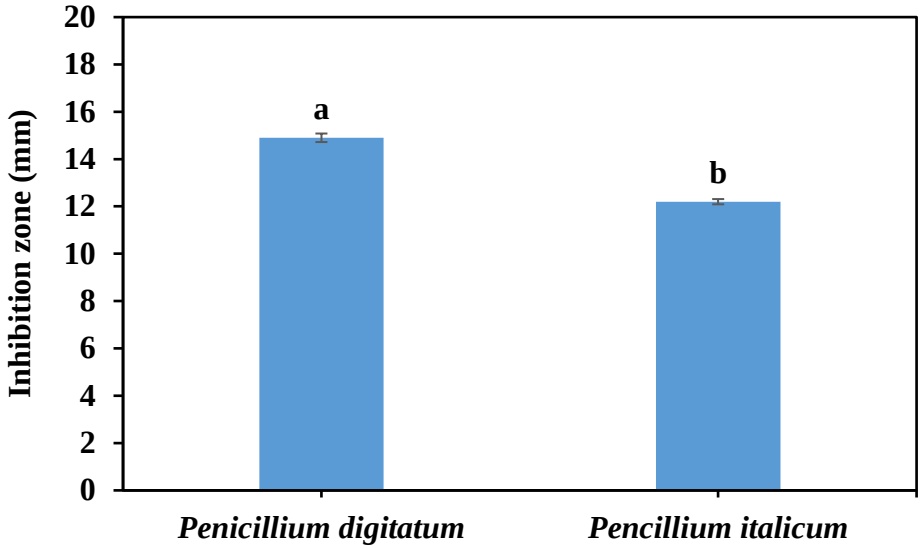


**Figure 4**. The antibacterial activity of *Zhumeria majdae* essential oil based on well diffusion agar method

The results of the minimum inhibitory concentration (MIC) of Persian mullein essential oil against the fungal strains are presented in Table 1. According to the results, the antifungal activity of Persian mullein essential oil was dependent on both its concentration and the type of fungal strain. Essential oil concentrations of 0.5 and 1 mg/mL did not exhibit antifungal activity against any of the fungal strains. The antifungal activity of the essential oil was significant at higher concentrations. The *Penicillium digitatum* strain was unable to grow in the presence of 2 mg/mL of the essential oil, demonstrating the highest sensitivity to Persian mullein essential oil. According to the results, the MIC values for the *Penicillium italicum* and *Penicillium digitatum* strains were 4 mg/mL and 2 mg/mL, respectively.

**Table 1**. Minimum inhibitory concentration of *Zhumeria majdae* essential oil

| Microorganism | Essential oil concentration (mg/mL) | | | | | | | | | | Negative control | Positive control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | | |
| *Penicillium digitatum* | + | + | - | - | - | - | - | - | - | - | - | + |
| *Penicillium italicum* | + | + | + | - | - | - | - | - | - | - | - | + |

+ grown; - not grown

The results of the minimum fungicidal concentration (MFC) were consistent with the MIC findings; however, higher concentrations of the essential oil were required to eliminate the fungal strains (Table 2). *Penicillium italicum* and *Penicillium digitatum* were identified as the most resistant and most sensitive strains to Persian mullein essential oil, with MFC values of 64 mg/mL and 16 mg/mL, respectively.

**Table 2**. Minimum fungicidal concentration of *Zhumeria majdae* essential oil

| Microorganism | Essential oil concentration (mg/mL) | | | | | | | | | | Negative control | Positive control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | | |
| *Penicillium digitatum* | + | + | + | + | + | - | - | - | - | - | - | + |
| *Penicillium italicum* | + | + | + | + | + | + | + | - | - | - | - | + |

+ grown; - not grown



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In line with the findings of this study, the antifungal activity of *Zhumeria majdae* essential oil was investigated against pathogenic fungi such as *Candida albicans*, *Trichophyton mentagrophytes*, *Aspergillus flavus*, *Trichophyton rubrum*, *Microsporum canis*, *Microsporum gypseum*, and *Epidermophyton floccosum*. The results demonstrated that Candida albicans (with an inhibition zone diameter of 29.05 mm and an MIC of 0.031 µg/mL) and Aspergillus flavus (with an inhibition zone diameter of 7.84 mm and an MIC of 0.25 µg/mL) were the most susceptible and most resistant fungal strains to the essential oil, respectively [27]. Furthermore, the antifungal activity of *Zhumeria majdae* essential oil has been demonstrated against *Candida albicans*, *Aspergillus niger*, *Aspergillus flavus*, *Saccharomyces cerevisiae*, *Fusarium* strains, and *Botrytis cinerea* in various studies [33-36]. Terpenes and flavonoids are the main components in the essential oil that contribute to its antifungal properties. These properties may also be due to the presence of linalool and camphor in the essential oil [27]. Additionally, the antimicrobial activity of phenolic compounds in plant extracts and essential oils has been shown to be due to the presence of an aromatic nucleus and a phenolic OH group in their structure, which can inactivate fungal enzymes by forming hydrogen bonds with thiol groups in the active sites of target enzymes. Moreover, the hydrophobic nature of plant extract and essential oil compounds allows them to be easily absorbed by fungal mycelia and inhibit their growth [5, 37].

## 4- Conclusion

The results of this research demonstrate that the essential oil obtained from the Mullein plant is rich in bioactive phenolic compounds. The essential oil exhibited high antioxidant activity against DPPH and ABTS radicals, suggesting its potential as a substitute for synthetic antioxidants. Additionally, its antifungal activity against *Penicillium italicum* and *Penicillium digitatum* was significant, with the strongest antifungal effect observed against *Penicillium digitatum*. Therefore, Mullein essential oil can be considered a valuable medicinal compound with potential applications in the fields of medicine, pharmaceuticals, food, and agriculture. However, further research is needed to elucidate the precise mechanisms underlying the antimicrobial and antioxidant effects of Mullein essential oil.

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# ارزیابی ویژگی‌های شیمیایی و اثر ضد قارچی اسانس مورخوش (*Zhumeria majdae*) بر کپک‌های عامل پوسیدگی و فساد میوه پرتقال طی انبارمانی

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**چکیده**

در این پژوهش، ویژگی‌های شیمیایی و اثر ضد قارچی اسانس مورخوش (*Zhumeria majdae*) بر کپک‌های عامل پوسیدگی و فساد میوه پرتقال طی انبارمانی بررسی گردید. روش تقطیر با آب برای استخراج اسانس مورخوش مورد استفاده قرار گرفت. مقدار فنول کل (مطابق روش فولین سیوکالتو)، میزان فلاونوئید کل (بر اساس روش رنگ سنجی کلرید آلومینیوم) و اثر آنتی‌اکسیدانی (بر پایه روش‌های مهار رادیکال آزاد DPPH و ABTS) و فعالیت ضد قارچی (بر پایه روش‌های دیسک دیفیوژن آگار، چاهک آگار، حداقل غلظت مهارکنندگی و حداقل غلظت کشندگی) اسانس در برابر *پنی‌سیلیوم ایتالیکوم* و *پنی‌سیلیوم دیجیتاتوم* بررسی گردید. محتوای فنول کل و فلاونوئید کل اسانس به ترتیب برابر با ۵۱/۳۸ mg GAE/g و ۲۲/۱۸ mg QE/g به دست آمد. اسانس مورخوش قادر به مهار رادیکال‌های آزاد DPPH و ABTS بود (به ترتیب ۶۱/۵۰ و ۶۷/۸۵ درصد). نتایج اثر ضد قارچی اسانس مورخوش نشان داد که میانگین قطر هاله عدم رشد برای سویه‌های قارچی *پنی‌سیلیوم ایتالیکوم* و *پنی‌سیلیوم دیجیتاتوم* در روش دیسک دیفیوژن آگار به ترتیب برابر با ۱۱/۱۰ و ۱۳/۷۰ میلی‌متر و در روش چاهک آگار برابر با ۱۲/۲۰ و ۱۴/۹۰ میلی‌متر می‌باشد. حداقل غلظت مهارکنندگی رشد برای این سویه‌ها معادل ۴ و ۲ میلی‌گرم در میلی‌لیتر و حداقل غلظت کشندگی برابر با ۶۴ و ۱۶ میلی‌گرم در میلی‌لیتر بود. مطابق نتایج، اسانس مورخوش را می‌توان بصورت ماده ضد میکروب طبیعی به‌منظور جلوگیری از رشد سویه‌های قارچی عامل پوسیدگی و فساد میوه پرتقال طی انبارمانی استفاده نمود.



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