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Evaluation of the antifungal effect of *Badrashboo (Dracocephalum moldavica*) essential oil and its interaction with Nystatin on some fungal strains

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ABSTRACT

Although chemical antifungal preservatives are often used in various food products, the use of these substances has been limited due to their harmful effects on human health and the environment. Researchers have recently sought to replace these chemical compounds with natural and less dangerous substances. In this regard, using essential oils of medicinal plants can be considered a suitable alternative due to fewer side effects. Therefore, in the present research, after preparing the *Badrashboo* plant from the fields of Golmarz village located near Urmia city and drying it, extracting the essential oil from the *Badrashboo* was carried out using a Clevenger, and the antifungal effect of *Badrashboo* essential oil on some important fungal strains with disc diffusion agar and well diffusion agar, minimum inhibitory concentration, minimum fungicidal concentration and the interaction of *Badrashboo* essential oil with Nystatin were performed. The results of disk diffusion agar and well diffusion agar tests showed that *Badrashboo* essential oil had a significant antifungal effect on all studied fungal strains. The results of the minimum inhibitory concentration of essential oil for strains of *Saccharomyces cerevisiae*, *Candida albicans*, *Aspergillus niger*, *Fusarium solani*, and *Penicillium expansum* were 8, 16, 2, 8, and 4 mg/mL, respectively. The minimum fungicidal concentration for the mentioned strains was 32, 64, 8, 16, and 32 mg/mL respectively. Also, the results of the interaction of *Badrashboo* essential oil with Nystatin indicated the synergistic effect of *Badrashboo* essential oil with Nystatin. Considering the significant antifungal effect observed for *Badrashboo* essential oil in the present study, it can be used in the food and pharmaceutical industries.

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1- Introduction

Due to the growing consumer demand for reducing or eliminating the use of chemical additives in food, essential oils have emerged as a promising alternative for preventing microbial proliferation and the spread of pathogens [1]. In response to this demand, biopreservation technologies are gaining attention for their potential to enhance food safety, nutritional value, and sensory properties [2]. Many spices, medicinal plants, and their derivatives and essential oils, in addition to their traditional use as food ingredients and flavorings, also possess antibacterial and antifungal properties [3]. These plant products have few side effects on the functioning of different parts of the body due to their active medicinal compounds such as terpenes, alkaloids, flavonoids, and glycosides, and they also have good therapeutic potential [4]. The lipophilic nature of the hydrocarbon skeleton of the compounds present in essential oils and the hydrophilic nature of their functional groups are of primary importance in the antimicrobial properties of essential oils [5]. Fungal spoilage of food plays a key role in food spoilage and foodborne illnesses [6]. Therefore, the increasing resistance of fungi to classical drugs, their toxicity, and the associated costs are encouraging researchers to conduct further research in this area [7]. Hussaini et al. (2015) investigated the antimicrobial effect of clove essential oil on raw hamburger during frozen storage and found that the microbial load decreased more significantly with increasing clove essential oil concentration and frozen storage time [8]. The results of Fayazi et al. (2021), who studied the physical, mechanical, and antimicrobial properties of a carboxymethyl cellulose bioactive film containing *Trachysperrmum* essential oil and its effect on the shelf life of turkey meat, showed that the film containing 1% *Trachysperrmum* essential oil had a stronger antimicrobial effect on reducing the number of coliform bacteria and *Escherichia coli*, and the film containing the essential oil was able to increase the shelf life of turkey meat [9]. Tourahmad et al. (2023) also investigated the antimicrobial effect of laboratory-extracted and commercial cinnamon essential oils on a number of foodborne pathogens and reported that both essential oils showed promising antimicrobial activity against foodborne microorganisms [10]. Iran, due to its geographical location and climatic diversity, is considered one of the best regions in the world for growing various medicinal plants and has been a source of production and consumption of medicinal plants in the past. Of course, the medicinal and therapeutic properties of each plant depend on the type and amount of its effective compounds [11]. *Badrashboo*, with the scientific name *Dracocephalum moldavica*, is an aromatic herbaceous plant from the Lamiaceae family, also known as *Dragon head*. This plant is native to northwestern Iran and can be found in Tabriz, Urmia, Yazd, Mazandaran and the Alborz mountain range [12]. The height of this plant reaches 50 to 70 cm and the yield of extracted oil is between 0.1 and 0.8%. *Badrashboo* essential oil is mainly composed of citral, geranyl, neral, geranyl acetate and geraniol [13]. Citral, with the formula C10H16O (3,7-dimethyl-1,6-octadienal), is a pale yellow liquid with a citrusy lemon-like odor. It is a mixture of two geometric isomers, trans-geranial and cis-neral. These isomers have the ability to pass through cell membranes and have antifungal effects [14]. However, previous research has reported different major components for *Badrashboo* essential oil. These differences are due to factors such as the plant growth region, ecological and climatic conditions, and the storage time of the medicinal plants [15]. *Badrashboo* is a good source of protein, lipids, and fiber. Its oil is rich in unsaturated fatty acids (about 90%), mainly linolenic and linoleic acids (about 60% and 20%, respectively). *Badrashboo* leaves have been reported to contain various polyphenols, particularly hydroxycinnamic acids and flavonoids, luteolin and its glycosides, quercetin, diosmetin, kaempferol, apigenin, agastacin, and salvianoside. *Badrashboo* extract also exhibits antibacterial and antioxidant properties, along with cardioprotective effects. In some countries, an aqueous extract prepared from this plant by distillation is used as a beverage [16].

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Badrashboo is used as a tea and as an herbal medicine due to its renowned medicinal properties, such as treating stomach and liver ailments, headaches, and congestion [17]. There are also reports of its use in treating mucosal diseases, respiratory tract inflammation, and cardiovascular diseases with antioxidant, anti-inflammatory, anticoagulant, and vasodilatory effects [18]. In addition to the food industry, this plant has applications in the pharmaceutical, cosmetic, and fragrance industries [19]. Deziki et al. (2019), who investigated the effect of *Badrashboo* leaves on the quality of wheat flour bread, reported that breads enriched with the mentioned plant had a higher crumb firmness index than the control bread and the total phenolic content increased linearly with increasing plant percentage from 4.8 to 10.1 mg dry weight of the plant, resulting in an increase in the antioxidant activity of the bread as well. Sensory evaluation also indicated good consumer acceptability with the addition of 3 grams of dragonhead to 100 grams of wheat flour [16]. Wójtowicz et al. (2017), evaluated the application of *Badrashboo* leaves as a value-added functional ingredient in extruded corn snacks and concluded that corn snacks made with 5-20% dragonhead leaves had better nutritional value and were a good source of fiber. Additionally, due to the presence of phenolic compounds, particularly rosmarinic acid, they reported high antioxidant potential in this plant and found favorable sensory evaluation of the mentioned treatments [20]. Ashimovich et al. (2020), investigated the chemical composition and antioxidant and antibacterial activity of dragonhead essential oil and reported the main components of the mentioned oil to be geranyl acetate, geranial, and neral. They approved the use of dragonhead essential oil in the food and pharmaceutical industries due to its antioxidant and antimicrobial properties [19]. The results of Alizadeh et al. (2015), who investigated the antibacterial properties of borage and *Badrashboo* essential oils under laboratory conditions against four bacteria: Listeria monocytogenes, Staphylococcus aureus, Salmonella Typhimurium, and Escherichia coli using two methods, disc diffusion and broth dilution, indicated the inhibitory ability and antibacterial properties of both essential oils [21]. Given the growing interest of today's consumers in using foods containing natural preservatives and the many beneficial properties mentioned for the dragonhead plant, and considering that despite the cultivation of *Badrashboo* in different parts of Iran, there is still very little research on the antifungal effect of its essential oil, the aim of this study is to investigate the antifungal activity of *Badrashboo* essential oil alone and in combination with the antibiotic nystatin against the fungi: Saccharomyces cerevisiae, *Candida albicans*, *Aspergillus niger*, *Fusarium solani*, and *Penicillium expansum*.

2- Materials and Methods

The present study was conducted in 2024 with the participation of the Microbiology Laboratory of the Department of Health Food group of the Faculty of Veterinary Medicine of Shahid Chamran University of Ahvaz and the Food Industries Group of the University of Agricultural Sciences and Natural Resources of Khuzestan.

2-1- Preparation of Required Materials

For the present study, the following materials were used: Sabouraud Dextrose Agar (SDA) and Sabouraud Dextrose Broth (SDB) (Liofilchem Company), Dimethyl sulfoxide (DMSO) (Uni-Chem Company), Blank disks (Padtan Teb Company), Triphenyltetrazolium chloride (TTC) (Solarbio Company) and 0.22 µm syringe filters

2-2- preparing *Badrashboo* and extraction of its essential oil

The *Badrashboo* plant was purchased from the farms of Golmarz village in the central district of Urmia city, West Azerbaijan province, and was identified by the Faculty of Agriculture, Khuzestan University of Agricultural Sciences and Natural Resources with the scientific name *Dracocephalum moldavi L* and herbarium code KUAU653. The purchased plant was dried at room temperature and stored in the refrigerator until essential oil extraction. Essential oil extraction was performed using a Clevenger apparatus (MS.E- Iran). For this purpose, 100 grams of the plant was powdered each time with

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an electric grinder (Asan Toos Shargh- Iran) and poured into the Clevenger apparatus along with distilled water, and essential oil extraction was performed within 3 hours [22].

2-3- Preparation of microbial strains and standard of 0.5 McFarland

Standard microbial suspensions were first prepared from the following reference strains stored in the Food Microbiology Laboratory of the Department of Food Science and Engineering, Khuzestan University of Agricultural Sciences and Natural Resources: *Saccharomyces servisiae* PTCC 5052, *Candida albicans* PTCC 5027, *Aspergillus niger* PTCC 5010, *Fusarium solani* PTCC 5284 and *Penicilium expansum* ATCC 24692. The suspensions were prepared by inoculating the strains onto Sabouraud Dextrose Agar (SDA) plates and incubating them until growth was observed. The 0.5 McFarland standard was then prepared from the suspensions of the cultured microorganisms [23].

2-4- Evaluation of Antifungal Activity of *Badrashboo* Essential Oil by Disk Diffusion Agar (DDA)

The disc diffusion agar (DDA) test was performed according to the method of Aljafari et al. (2011), with slight modifications. Briefly, serial concentrations of 2, 4, 8, 16, 32, 64, and 128 mg/mL of *Badrashboo* essential oil were prepared in distilled water (9.5% w/v) and dimethyl sulfoxide emulsifier (0.5% w/v). The prepared concentrations were passed through a 0.22 µm syringe filter for sterilization and poured into sterile tubes. Then, 100 µl of the 0.5 McFarland suspension of each microorganism was spread on the surface of Sabouraud Dextrose Agar (SDA) medium. To assess the antifungal activity, 6 mm blank paper discs were placed at a fixed distance from each other and from the edge of the plate on the medium, and 20 µl of the prepared dragonhead essential oil concentrations were added to the discs. The fungal cultures were incubated at 27°C for 72 hours in an incubator (FG- Iran) and the diameter of the inhibition zones formed around the discs was measured and reported in millimeters [24].

2-5- Evaluation of Antifungal Activity of *Badrashboo* Essential Oil by Well Diffusion Agar

The well diffusion assay was performed according to the method of Noshad et al. (2018), with slight modifications. Briefly, serial concentrations of 2, 4, 8, 16, 32, 64, and 128 mg/mL of *Badrashboo* essential oil were prepared in dimethyl sulfoxide emulsifier (0.5% w/v) and distilled water (9.5% w/v). The prepared concentrations were passed through a syringe filter for sterilization and poured into sterile tubes. Then, 100 µl of the 0.5 McFarland suspension of each microorganism was spread on the surface of Sabouraud Dextrose Agar (SDA) medium. To assess the antifungal activity, 6 mm diameter wells were created using the sterile tip of a Pasteur pipette under completely sterile conditions at a fixed distance from each other and from the edge of the plate on the medium. 20 µl of the prepared *Badrashboo* essential oil concentrations were added to the wells. The fungal cultures were incubated at 27°C for 72 hours and the diameter of the inhibition zones formed around the wells was measured and reported in millimeters [25].

2-6- Determination of Minimum Inhibitory Concentration (MIC) of *Badrashboo* Essential Oil

Serial concentrations of 0.25, 0.5, 1, 2, 4, 8, 16, 32, and 64 mg/mL of *Badrashboo* essential oil were prepared in dimethyl sulfoxide emulsifier (0.5% w/v) and distilled water (9.5% w/v). To determine the minimum inhibitory concentration (MIC), 100 µl of each essential oil concentration was added to each well of a 96-well plate containing 20 µl of the fungal suspension (0.5 McFarland concentration). Two wells at the end of each row were used as controls. In one well, 120 µl of the highest selected concentration (without microorganism) was added, and in another well, 100 µl of Mueller-Hinton broth was added along with 20 µl of the half-McFarland concentration of each microorganism (without essential oil). After incubation at 27°C for 72 hours, 20 µl of 5% Triphenyltetrazolium chloride solution was added to each well, and the plates were incubated again for 30 minutes. In this method, wells with fungal growth turned dark red or purple. The first well in which no fungal growth was observed and no color change was seen was recorded as the MIC [26].

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2-7- Determination of Minimum Fungicidal Concentration (MFC) of *Badrashboo* Essential Oil

The minimum fungicidal concentration (MFC) of *Badrashboo* essential oil was measured according to the method of Rahmati-Joneidabad and Alizadeh Behbahani (2020). Briefly, 100 µl aliquots were transferred from the wells identified as the minimum inhibitory concentration (MIC) to higher concentrations that had not shown any color change. These aliquots were inoculated onto Sabouraud Dextrose Agar (SDA) plates and incubated at 27°C for 72 hours. The lowest concentration of the plate that did not show any fungal growth was reported as the MFC of Badrashboo essential oil for each fungus studied [27].

2-8- Interaction of *Badrashboo* Essential Oil with Nystatin

This test was performed according to the method of Jalil Sarqaleh et al. (2023), to evaluate the interaction of *Badrashboo* essential oil with Nystatin. In this assay, the interaction of *Badrashboo* essential oil with Nystatin was evaluated against a number of fungal strains. Briefly, after determining the minimum inhibitory concentration (MIC) of *Badrashboo* essential oil for the fungal strains under study and determining the sub-inhibitory (1/2 MIC) concentration for each microorganism, it was added to Sabouraud Dextrose Agar (SDA) medium. Then, 100 µl of the 0.5 McFarland suspension of each microorganism was inoculated onto the medium. Nystatin discs were then placed at appropriate intervals from the edge of each plate, and the incubation and measurement of the inhibition zones of the discs were performed and reported in millimeters as in the disc diffusion method [28].

2-9- Statistical Analysis

The obtained data were analyzed using a completely randomized design and the SPSS software program (version 20). One-way analysis of variance (ANOVA) was used to investigate the antifungal effect of different concentrations of *Badrashboo* essential oil on various fungi. Duncan's multiple range test was used to compare the means. The antifungal activity of nystatin and its interaction with the essential oil were determined using the independent two-sample t-test.

3-Results and discussion

3-1- Disk Diffusion and Well Diffusion Assays

Table 1 presents the antifungal effect of *Badrashboo* essential oil using the disk diffusion method. The investigation into the effect of various *Badrashboo* essential oil concentrations on the growth inhibition of several standard fungi, including *Candida albicans*, *Saccharomyces cerevisiae*, Penicillium *expansum*, *Fusarium solani*, and *Aspergillus niger*, revealed a significant ($P < 0.05$) increasing trend in the diameter of the fungal growth inhibition zone for all microorganisms mentioned (except for the 2 mg/mL essential oil level, which had no effect on *Candida albicans* growth) as the essential oil level increased from 2 to 128 mg/mL. According to this table, the examination of the antifungal activity of different *Badrashboo* essential oil concentrations using the disc diffusion method revealed that at a concentration of 2 mg/mL, the diameter of the *Aspergillus niger* growth inhibition zone (10 mm) was significantly larger ($P < 0.05$) than that of *Saccharomyces cerevisiae* (7.67 mm). At a concentration of 4 mg/mL essential oil, *Aspergillus niger* exhibited the largest growth inhibition zone. As the *Badrashboo* essential oil concentration was gradually increased to 8, 16, 32, 64, and 128 mg/mL, the essential oil's ability to inhibit the growth of the studied fungi, as measured by the diameter of the growth inhibition zone, consistently improved. Starting from 16 mg/mL essential oil concentration, *Candida albicans* showed the smallest growth inhibition zone compared to the other fungi, with a statistically significant difference. At high essential oil concentrations of 64 and 128 mg/mL, the growth inhibition zones of *Saccharomyces cerevisiae*, *Penicillium expansum*, *Fusarium solani*, and *Aspergillus niger* were statistically similar ($P < 0.05$) and significantly larger ($P < 0.05$) than the growth inhibition zone of *Candida albicans*.

Table 1. The mean inhibition zone diameter (mm) of *Badrashboo* essential oil on some fungi species (disk diffusion agar)

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Microorganism	Badrashboo essential oil concentration (mg/mL)						
	2	4	8	16	32	64	128
C. albicans	-	9.67 ± 0.33^{dC}	11.00 ± 0.58^{dC}	12.00 ± 0.58^{cdD}	14.00 ± 0.58^{bcE}	16.33 ± 0.67^{bB}	22.00 ± 1.73^{aB}
S. cerevisiae	7.67 ± 0.33^{fB}	10.67 ± 0.33^{eBC}	13.00 ± 0.58^{deBC}	15.33 ± 0.33^{dC}	35.33 ± 0.88^{cC}	53.33 ± 2.03^{bA}	72.67 ± 0.88^{aA}
P. expansum	8.67 ± 0.33^{gAB}	11.33 ± 0.33^{fB}	13.67 ± 0.33^{eB}	24.00 ± 0.58^{dB}	38.00 ± 1.15^{cB}	52.67 ± 1.20^{bA}	70.67 ± 0.33^{aA}
F. solani	9.67 ± 0.33^{fAB}	11.67 ± 0.88^{fB}	21.67 ± 0.88^{eA}	26.33 ± 0.88^{dA}	32.00 ± 0.58^{cD}	55.67 ± 1.86^{bA}	71.33 ± 0.33^{aA}
A. niger	10.00 ± 1.15^{fA}	16.33 ± 0.33^{eA}	23.00 ± 1.00^{dA}	24.67 ± 0.88^{dAB}	45.33 ± 0.88^{cA}	57.67 ± 1.33^{bA}	73.33 ± 0.88^{aA}

* Means (±SE) with different lowercase letters in each row and uppercase letters in each column show a significant difference at $P \leq 0.05$

In Table 2, the antifungal activity of *Badrashboo* essential oil using the well dffusion method is presented. Similar to the disc diffusion method, the well diffusion method also revealed a significant ($P < 0.05$) increasing trend in the diameter of the growth inhibition zones for all five fungi (*Candida albicans*, *Saccharomyces cerevisiae*, *Penicillium expansum*, *Fusarium solani*, and *Aspergillus niger*) as the concentration of *Badrashboo* essential oil increased from 2 to 128 mg/mL. In contrast to the disc diffusion method, the well diffusion method demonstrated complete growth inhibition of *Candida albicans* even at a concentration of 2 mg/mL essential oil, with a statistically significant difference ($P < 0.05$). Evaluation of the growth inhibition effect of each *Badrashboo* essential oil concentration using the well diffusion method revealed no statistically significant difference in the diameters of the growth inhibition zones for all fungi at a concentration of 2 mg/mL essential oil. As the essential oil concentration increased to 4 mg/mL, the diameter of the growth inhibition zone for *Aspergillus niger* (14 mm) was significantly larger ($P < 0.05$) than that for *Candida albicans* (11.67 mm). This suggests that at this concentration, *Badrashboo* essential oil exhibited stronger antifungal activity against *Aspergillus niger* compared to *Candida albicans*. At 8 mg/mL essential oil, *Fusarium solani* exhibited the largest zone of inhibition (22.33 mm) with a statistically significant difference ($P < 0.05$). At 16 mg/mL *Badrashboo* essential oil, both *Fusarium solani* and *Aspergillus niger* had significantly larger inhibition zones (28 and 27 mm respectively) compared to other fungi ($P < 0.05$). The largest and smallest zones of inhibition at concentrations of 32 and 128 mg/mL essential oil belonged to *Aspergillus niger* and *Candida albicans*, respectively. At a concentration of 64 mg/mL essential oil, the diameters of the growth inhibition zones for *Saccharomyces cerevisiae* and *Fusarium solani* were significantly larger ($P < 0.05$) than those for the other fungi, while *Candida albicans* continued to exhibit the smallest growth inhibition zone.

Table 2. The mean inhibition zone diameter (mm) of Badrashboo essential oil on some fungi species (well diffusion agar)

Microorganism	Badrashboo essential oil concentration (mg/mL)						
	2	4	8	16	32	64	128

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C. albicans	10.33±0.33^{f} A	11.67±0.33^{ef} B	13.00±0.58^{d} eD	14.00±0.58 dC	16.33±0.33 cB	20.00±0.58 bC	23.67±0.88^{a} D
S. cerevisiae	10.67±0.33^{f} A	12.67±0.88^{ef} AB	13.67±0.33^{e} CD	23.00±0.58 dB	40.00±0.58 cA	61.67±0.88 bA	74.33±0.88^{a} B
P. expansum	10.67±0.33 eA	12.00±0.58^{eA} B	14.67±0.33^{d} C	15.00±0.58 dC	41.67±0.33 cB	57.00±0.58 bB	72.00±0.58^{a} C
F. solani	10.67±0.33 gA	13.00±0.58^{fA} B	22.33±0.33^{e} A	28.00±0.58 dA	34.00±0.58 cD	60.67±0.66 bA	72.33±0.33^{a} BC
A. niger	10.67±0.33 gA	14.00±0.58^{fA} B	18.67±0.33^{e} B	27.00±0.88 dA	48.00±0.58 cA	56.00±1.53 bB	78.00±0.58^{a} A

* Means (±SE) with different lowercase letters in each row and uppercase letters in each column show a significant difference at $P \leq 0.05$

In a study by Sonboli et al. (2008), the antimicrobial activity of *Badrashboo* essential oil and its main components, citral, geranyl acetate, and geraniol, was evaluated using the disc diffusion method against a panel of pathogenic bacteria and fungi. The results demonstrated that all three compounds exhibited inhibitory zones against both bacterial and fungal strains. The researchers attributed the primary antimicrobial activity of *Badrashboo* essential oil to the presence of citral, which possesses potent biological activity. They also suggested that synergistic interactions between citral and other components of the essential oil, such as geraniol and geranyl acetate, contributed to the larger inhibition zones observed for the essential oil compared to the individual compounds alone [29]. Nazemi Salman et al. (2017), investigated antifungal activity of essential oils of *Prangos frulacea*, *Ziziphora tenuior*, *Ferula gummosa* and *Dracocephalum moldavica* against *candida albicans* . the results of the antimicrobial tests showed that the essential oil of *Badrashboo* and then *Ziziphora tenuior* had more antifungal effects among them. The tested extracts had and the diameter of the inhibitory halo of all essential oils studied (except *Ferula gummosa* essential oil) was greater than the standard drug chlorhexidine. The inhibition zone diameters of all tested essential oils (except *Ferula gummosa*) were larger than those of the standard chlorhexidine control [30]. In a study by El-Baky et al. (2008), the antimicrobial activity of Egyptian *Badrashboo* essential oil was evaluated against a panel of six bacterial strains and four fungal species, including Aspergillus niger, *Rhizopus stolonifer*, *Penicillium expansum*, and *Mucor hiemalis*. The results demonstrated that the essential oil exhibited potent antimicrobial activity against all tested microorganisms [31]. Hu et al. (2019), investigated the antimicrobial activity of *Badrashboo* extract against pathogenic *Staphylococcus aureus* strains. They attributed the antibacterial properties of the ethanolic extract to the presence of compounds such as rosmarinic acid and tannins [32]. While several studies have demonstrated the broad-spectrum antimicrobial activity of *Badrashboo* essential oil, including its efficacy against fungi, a study by Asimova et al. (2022), presented contrasting findings. The researchers evaluated the antimicrobial properties of *Badrashboo* essential oil against a panel of pathogenic bacteria and fungi. While the essential oil exhibited potent antibacterial activity, it only showed antifungal activity against *Saccharomyces cerevisiae* among the three fungi tested. The essential oil was ineffective against *Candida albicans* and *Aspergillus brasiliensis* [22]. Haghigi et al. (2003), compared the antimicrobial activity of ten plant extracts against three pathogenic oral microorganisms. They reported that *Badrashboo* extract had no antifungal activity against *Candida albicans*. This could be due to differences in climatic and environmental conditions where the plant grows, which can lead to variations in the essential oil

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composition [33]. Rahmati Jenadabad et al. (2021), investigated the antifungal activity of Khuzestani Savory essential oil against *Aspergillus niger*, *Botrytis cinerea*, and *Rhizopus stolonifer* using both disc diffusion and well diffusion methods. They found *Botrytis cinerea* to be the most resistant strain, while *Aspergillus niger* was the most susceptible strain to the essential oil [34]. In a study by Ghasemi et al. (2016), the chemical composition and antibacterial activity of native Ardabil mint essential oil were evaluated against *Streptococcus mutans*, *Lactobacillus rhamnosus*, and *Actinomyces viscosus* using the disc diffusion method. The researchers found that *Lactobacillus rhamnosus* was the most susceptible bacterial strain among those tested, while *Actinomyces viscosus* was the most resistant. Jafarpoor and Attai (2022), investigated the antifungal activity of *Trachyspermum ammi*, *Satureja hortensis*, and *Mentha piperita* essential oils against molds that contaminate Iranian white cheese. the most sensitive fungus, *Trichoderma harzianum*, with inhibition zones of 5.1, 5.3 and 2.9 cm for Satureja hortensis, *Trachyspermum ammi*, and *Mentha pipperita* essential oils, respectively. Also, among the studied molds, *Psilomyces varioti*, *Aspergillus niger* and *Aspergillus oryzae* showed the most resistance to essential oils [36]. Hussaini et al. (2011), evaluated the inhibitory effect of *carvacrol* essential oil on *Candida albicans* strains using the disc diffusion method to determine the zone of inhibition and the broth microdilution method to determine the minimum inhibitory concentration (MIC). The researchers reported that *carvacrol* essential oil exhibited significant antifungal activity against both fluconazole-resistant and -sensitive strains of *Candida albicans* [37]. Barzegar et al. (2021), investigated the antimicrobial activity of *Lawsonia inermis* aqueous extract against a panel of pathogenic bacteria. The researchers found that the minimum inhibitory concentration (MIC) was lowest for *Staphylococcus epidermidis* and highest for *Escherichia coli* and *Salmonella typhimurium*. The minimum bactericidal concentration (MBC) was also higher for *Escherichia coli* and *Salmonella typhimurium* compared to *Staphylococcus epidermidis* and *Listeria innocua* [38]. The present study's findings indicate that the well diffusion method yields larger inhibition zones for *Badrashboo* essential oil compared to the disc diffusion method. This observation aligns with the results of Nooshad and Alizadeh Behbahani (2023), who investigated the antimicrobial activity of *Thymus trautvetteri* extract and *Datura stramonium* essential oil against various foodborne pathogens. This difference in zone sizes may be attributed to the direct contact between the essential oil and microorganisms in the well diffusion method compared to the disc diffusion method. In the latter, the essential oil must diffuse from the disc surface into the medium to exert its antimicrobial effect [39&40].

2-3- MIC and MBC tests

Table 3 shows the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of *Badrashboo* essential oil against the tested fungi. *Badrashboo* essential oil exhibited inhibitory activity against *Aspergillus niger* and *Penicillium expansum* at concentrations of 2 and 4 mg/mL, respectively. *Badrashboo* essential oil demonstrated growth inhibition of *Saccharomyces cerevisiae* and *Fusarium solani* at a dose of 8 mg/mL. A higher concentration of 16 mg/mL was necessary for *Badrashboo* essential oil to inhibit the growth of *Candida albicans*. *Badrashboo* essential oil exhibited fungicidal activity against *Aspergillus niger* at 8 mg/mL and *Fusarium solani* at 16 mg/mL. A higher concentration of 32 mg/mL was required for the essential oil to kill *Saccharomyces cerevisiae* and *Penicillium expansum*. *Candida albicans*, the most resistant fungus, showed fungicidal activity only at the highest concentration tested (64 mg/mL).

Table 3. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the *Badrashboo* essential oil on some fungi strains

Microorganism	MIC (mg/mL)	MFC (mg/mL)
C. albicans	16	64
S. cerevisiae	8	32
P. expansum	4	32

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F. solani	8	16
A. niger	2	8

The cited study by Sonboli et al. (2008), investigated the antifungal activity of *Badrashboo* essential oil against three fungal strains: *Candida albicans*, *Saccharomyces cerevisiae*, and *Aspergillus niger*. They reported that the MIC of *Badrashboo* essential oil for all three fungi was 1.25 mg/mL [31]. In El-Baky et al. (2008), study, the minimum inhibitory concentration (MIC) of Egyptian *Badrashboo* essential oil against *Aspergillus niger*, *Rhizopus stolonifer*, *Penicillium expansum*, and *Mucor hiemalis* fungi was reported as 0.075, 0.085, 0.078 and 0.080 mg/mL, respectively. The researchers attributed the antifungal activity of *Badrashboo* essential oil to its high content of antimicrobial compounds with ketone, alcohol, and phenolic monoterpene structures, including significant amounts of geraniol, nerol, linalool geranial, and neral, which exert their antimicrobial effects through various mechanisms like disrupting cellular energy production, inhibiting biosynthesis, and damaging genetic material in the fungus [33]. Antimicrobial compounds present in essential oils also exert their effects on cytoplasmic membranes, causing alterations in their permeability and leading to the release of cellular contents. These compounds can also interfere with various cellular processes, including electron transport, nutrient uptake, fatty acid synthesis, adenosine triphosphatase (ATPase) activity, and other metabolic functions. This disruption is likely due to interactions with the enzymes responsible for these processes [41]. Researchers believe the antifungal properties of plant essential oils stem from the antimicrobial activity of phenolic compounds within them. This is likely due to the presence of an aromatic ring and a reactive phenolic hydroxyl group in their structure, allowing them to form hydrogen bonds with sulfhydryl groups in the active sites of fungal enzymes, thereby inactivating them. Additionally, plant essential oils, due to their hydrophobic nature, are attracted to and absorbed by the hydrophobic fungal mycelium, potentially hindering its growth [29]. Alizadeh Behbahani et al. (2013), evaluated the antifungal efficacy of aqueous and methanolic extracts derived from *Avicennia Marina* leaves against the fungal strains *Alternaria alternata* and *Penicillium citrinum*. Their findings demonstrated that the methanolic extract exhibited superior antifungal activity compared to the aqueous extract. According their findings the MIC values for the methanolic extract were 16 mg/mL for *A. alternata* and 8 mg/mL for *P. citrinum*, while the aqueous extract had higher MIC values of 32 mg/mL and 64 mg/mL, respectively. Similarly, the MFC values for the methanolic extract were 16 mg/mL for *A. alternata* and 32 mg/mL for *P. citrinum*, compared to the aqueous extract's MFC values of 64 mg/mL and 256 mg/mL, respectively [42]. In the study by Izady et al. (2014), investigating the antimicrobial activity of *purple coneflower*, Klebsiella pneumoniae was more sensitive compared to other Gram-negative bacteria, while the minimum fungicidal concentration for Bacillus cereus, Aspergillus niger, and Candida albicans was several times higher than for other microorganisms studied [43]. Alizadeh Amoli et al. (2020), compared the antimicrobial and antioxidant properties of *Mentha aquatica L*. They found the essential oil to be more effective against various bacteria than the ethanolic extract. The minimum inhibitory concentration (MIC) of the essential oil against *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhimurium* were 5.12, 5.12, 2.5 and 2.5 mg/mL, while the minimum fungicidal concentration (MFC) for them were 10, 10, 5.12 and 5.12 mg/mL. Interestingly, there wasn't a significant difference in effectiveness between Gram-positive and Gram-negative bacteria [44]. Figure 1 provides an illustration of the determination of the minimum inhibitory concentration of *Badrashboo* essential oil against the fungal species studied in this research.

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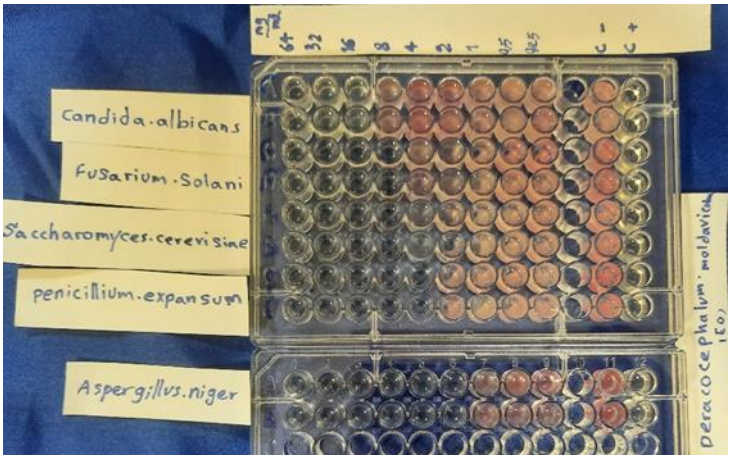

Fig. 1. The minimum inhibitory concentration (MIC) of *Badrashboo* essential oil on some fungi strain

As mentioned earlier, the antimicrobial property of *Badrashboo* essential oil is directly linked to its bioactive compounds. Research has shown that variations in factors like genetic origin, ecological factors, plant genetics, cultivation techniques, and even drying methods used for the plant before oil extraction can all lead to quantitative and qualitative differences in the essential oil composition of medicinal and aromatic plants [45]. Dumocos et al. (1994), studied the essential oil properties of *Badrashboo* seeds. Fresh plants contained about 0.4% volatile oils, with 43% being citral. This volatile oil content increased with cultivation altitude (e.g., 70% increase at 800 meters), while geraniol content decreased at higher altitudes. Additionally, volatile oil accumulation increased during the plant's reproductive stage, peaking at the end of flowering, which might explain variations observed in research results [46]. This observation could explain the discrepancies or similarities between the findings of the present study and previous investigations.

3-3- Interaction of *Badrashboo* essential oil with Nystatin

Antifungal activity of Nystatin and its interaction with *Badrashboo* essential oil were evaluated using the disc diffusion method and are presented in Figure 2. The results demonstrated that the combination of Nystatin and *Badrashboo* essential oil significantly increased the zone of inhibition for all five fungi tested, namely *Candida albicans*, *Saccharomyces cerevisiae*, *Penicillium expansum*, *Fusarium solani*, and *Aspergillus niger*, compared to Nystatin treatment alone ($P<0.05$).

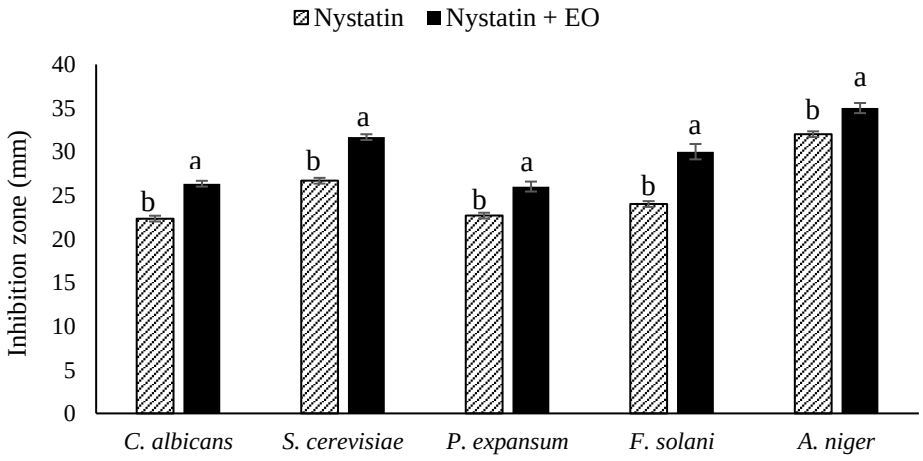

Fig. 2. The antifungal activity of Nystatin and its interaction with the *Badrashboo* essential oil by disk diffusion agar method. Means (±SE) with different letters in each fungus show a significant difference at $P \leq 0.05$

Ahmadnejad et al. (2023), investigated the antifungal activity of aqueous extract of *Calotropis procera* against *Alternaria alternata*, *Alternaria solani*, *Saccharomyces*

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cerevisiae, and *Fusarium solani*. They reported a synergistic effect of the extract with Nystatin against all tested fungi and stated that the greatest synergistic effect was observed against *Saccharomyces cerevisiae* [47]. Zamanpour Broujeni et al. (2023), investigated the antimicrobial activity of *Nigella sativa* oil against some pathogenic bacteria and its interaction with the antibiotic Chloramphenicol. They reported that the essential oil had a synergistic effect with Chloramphenicol against all tested bacteria and increased the diameter of the inhibition zone [48]. Tanavar et al. (2019), evaluated the essential oil of *Mentha pulegium* against a number of foodborne pathogens and its interaction with the antibiotics Gentamicin and Chloramphenicol. They also confirmed the synergistic effect of *Mentha pulegium* essential oil with the two mentioned antibiotics [49]. However, in the study by Safavi et al. (2021), which investigated the interaction of *Shiraz thyme* essential oil with the antibiotics Gentamicin and Chloramphenicol against a number of bacteria, the interaction of *thyme* essential oil and the tested antibiotics only caused an increase in the diameter of the zone of inhibition compared to the single treatment against *Listeria innocua* [50]. Figure 3 illustrates the synergistic effect of *Badrashboo* essential oil with the antibiotic Nystatin against *Fusarium solani*.

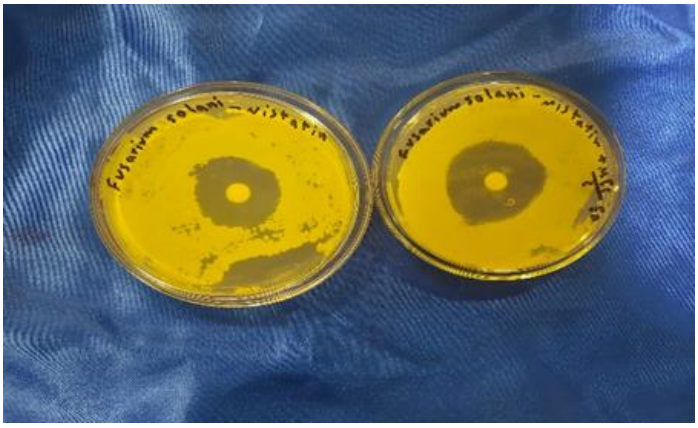

Fig. 3 The interaction of *Badrashboo* essential oil with Nystatin on *Fusarium solani.*

4- Conclusion

Based on the findings of the present study, *Badrashboo* essential oil, due to its remarkable antifungal properties and pleasant sensory characteristics and aroma, has the potential to serve as a valuable antifungal preservative in the food industry. Furthermore, it is recommended that clinical research be conducted on the therapeutic properties of *Badrashboo* essential oil to explore the possibility of utilizing this valuable plant as an antimicrobial drug. Undoubtedly, the discovery of cost-effective and less side-effect-prone treatment methods would be a significant step forward in promoting human health.

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مقاله علمی-پژوهشی

بررسی فعالیت ضدقارچی اسانس بادرشبو (*Dracocephalum moldavica*) و برهمکنش آن با آنتی‌بیوتیک نیستاتین علیه تعدادی از سویه‌های قارچی

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چکیده

اگرچه در حال حاضر اغلب از نگهدارنده‌های ضدقارچی شیمیای در فراورده‌های غذایی مختلف استفاده می شود، ولی استفاده از این مواد به دلیل اثرهای مضر آن‌ها بر سلامت انسان و محیط زیست محدود شده است. درسال‌های اخیر پژوهشگران به دنبال جایگزین کردن این ترکیب‌های شیمیایی با مواد طبیعی و کم خطر می‌باشند. در این راستا استفاده از اسانس گیاهان دارویی به دلیل عوارض جانبی کمتر می‌تواند جایگزین مناسبی محسوب شود. لذا در پژوهش حاضر پس از تهیه گیاه بادرشبو از مزارع روستای گلمرز واقع در نزدیکی شهرستان ارومیه و خشک کردن آن، اسانس‌گیری از گیاه بادرشبو به وسیله دستگاه کلونجر انجام شد و اثر ضدقارچی اسانس بادرشبو بر تعدادی از سویه‌های مهم قارچی با روش‌های انتشار در دیسک، انتشار در چاهک، حداقل غلظت بازدارندگی، حداقل غلظت کشندگی و برهمکنش اسانس بادرشبو با آنتی‌بیوتیک نیستاتین انجام شد. نتایج آزمایش‌های انتشار در دیسک و انتشار در چاهک نشان داد که اسانس بادرشبو تاثیر ضدقارچی قابل توجهی بر همه سویه‌های قارچی مورد مطالعه داشت. نتایج حداقل غلظت مهارکنندگی اسانس برای سویه‌های *ساکارومایسس سرویزیه*، *کاندیدا آلبیکانس*، *آسپرژیلوس نایجر*، *فوزاریوم سولانی* و *پنی‌سیلیوم اکسپنسوم* به ترتیب ۸، ۱۶، ۲، ۸ و ٤ میلی‌گرم بر میلی‌لیتر بود. حداقل غلظت کشندگی برای سویه‌های مذکور به ترتیب۳۲، ٦٤، ۸، ۱٦ و ۳۲ میلی‌گرم بر میلی‌لیتر بود. همچنین نتایج حاصل از برهمکنش اسانس بادرشبو با آنتی‌بیوتیک نیستاتین نیز حاکی از تاثیر هم‌افزایی اسانس مذکور با آنتی بیوتیک مورد آزمایش بود. با توجه به اثر قابل ملاحظه ضدقارچی مشاهده شده برای اسانس بادرشبو در پژوهش حاضر، می‌توان از آن در صنایع غذایی و دارویی استفاده کرد.

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