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Antimicrobial effect of *Prangos ferulacea* aqueous extract on some pathogenic fungal species and its interaction with nystatin antibiotic

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ABSTRACT

Prangos ferulacea is a plant that has been used in traditional medicine for its therapeutic properties. The present study was conducted to investigate the antifungal effect of *P. ferulacea* aqueous extract on *Alternaria alternata*, *Alternaria solani*, *Saccharomyces cerevisiae*, and *Fusarium solani*. The antifungal methods used were disc diffusion agar, well diffusion agar, minimum inhibitory concentration (MIC), and minimum fungicidal concentration (MFC). The extract concentrations used in the study were 20, 40, 60, and 80 mg/mL. The study found that the antifungal effect was concentration-dependent and increased with concentration. *S. cerevisiae* was the most sensitive species to the extract while *F. solani* was the most resistant. At 80 mg/mL, the inhibition zones for *S. cerevisiae* were found to be 11.90 mm and 13.00 mm in disc diffusion agar and well diffusion agar methods, respectively. The corresponding zones were 9.60 mm and 10.20 mm for *F. solani*, respectively. In the combined mode (interaction) of *P. ferulacea* aqueous extract with nystatin antibiotic, synergistic mode was observed for all fungal strains. The MIC and MFC values for *S. cerevisiae* were 32 and 128 mg/mL, respectively. The MIC and MFC results for *F. solani* were found to 256 and > 512 mg/mL, respectively. The results of this study showed that *P. ferulacea* aqueous extract could be used as a natural antifungal agent to inhibit the growth of pathogenic fungi on fresh fruits and vegetables.

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1- Introduction

Mushrooms play an important role in the food industry. They are used for various purposes such as creating the desired flavor, color and/or texture in food products, production of citric acid, enzymes and pigments used in food processing and use as poultry feed, agricultural silage, coffee extraction, functional foods. and so on are used. Saccharomyces cerevisiae is a yeast commonly used in the food industry to ferment bread, beer, and wine [1]. However, fungi can reduce the shelf life of food products.*Alternaria alternata* And *Alternaria solani* They can cause premature burn of potatoes and tomatoes, which leads to widespread damage to leaf tissues. Symptoms are seen in the form of expanding necrotic lesions [2, 3]. mushroom*Fusarium solani*It is a soil fungus that can cause root rot in plants such as potatoes [4]. In addition, mushrooms *Fusarium* ،*Alternaria* ،*Aspergillus* And*Penicillium* They are capable of producing mycotoxins, which can cause serious complications for humans and animals due to their carcinogenic, mutagenic, teratogenic, and immunosuppressive properties [5].

The control of plant pathogenic fungi in agricultural production is of global concern, because they cause significant economic losses to producers. About six million fungi are known; However, about 200 species have some pathogenicity associated with diseases that affect biodiversity, ecology, agriculture, and food security worldwide. Some estimates of the losses caused by plant pathogenic fungi in crops are 21.5% for wheat, 30% for rice, 22.5% for corn, 17.2% for potato and 21.4% for soybean, especially in the post-production stage. It is taken from [6]. Many strategies have been proposed to control fungal diseases, such as chemical fungicides, biological control methods, improving plant resistance to fungal diseases and obtaining new genotypes [7].

Chemical fungicides have often been used to control these diseases, but this behavior is associated with negative environmental effects, possible human exposure to pesticides, and residue deposition on fruits. However, the effectiveness of synthetic fungicides has been reduced by the repeated development of resistance by pathogens. Hence there is a great demand for safe, alternative and effective agents. Currently, the search for natural products with new applications, especially related to pest management, is very active. Aromatic and medicinal plants in the field of controlling plant diseases, especially plant extracts with antimicrobial properties and containing a range of secondary metabolites such as alkaloids, quinones, flavonoids, glycosides, saponins, tannins and terpenoids, have been considered. are The concentration of these bioactive compounds in any plant species depends on the environmental conditions and the extraction system [8-16]. Jashir plant (*Prangos ferulacea*) has been used in traditional medicine due to its therapeutic properties. The functional and biological properties of jashir extract are due to its bioactive compounds such as saponin, anthraquinone, tannin, coumarins, furocoumarins, monoterpenes (especially alpha pinene), sesquiterpenes, phenolics and flavonoids. These compounds are responsible for the antimicrobial, antioxidant, antiviral, anticancer and antidiabetic activity of this plant [17-19]. Our previous study showed that Jashir's aqueous extract is rich in phenolic compounds such as coumaric acid, catechin, rutin, myrstein, caffeic acid, luteolin and kaempferol, which lead to inhibiting free radicals and preventing the growth of pathogenic bacteria such as*Listeria monocytogenes* ،*Bacillus cereus* ،*Salmonella Typhimurium* ،*Staphylococcus aureus* ، *Escherichia coli* ،*Shigella dysentery* And*Staphylococcus epidermidis* became [17].

According to the above, the aim of this study is to investigate its antifungal activity against fungi*Alternaria alternata*، *Alternaria solani*، *Saccharomyces cerevisiae*And *Fusarium solani*Was. The antifungal activity of the extract was investigated according to the antimicrobial methods of agar diffusion disc, agar well, minimum growth inhibitory concentration and minimum lethal concentration.

2- Materials and methods

2- 1- Materials

In this research, Saburod dextrose agar and broth (Merck, Germany) and triphenyltetrazolium

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chloride reagent (Sigma, USA) were used. Other chemicals were of laboratory grade.

2- 2- Extraction of the extract

Jashir plant was first identified by the herbarium of Khuzestan University of Agricultural Sciences and Natural Resources. The extract was extracted by soaking in solvent. For this purpose, the plant collected from the southern region of Iran was cleaned from mud and soil and then washed completely with distilled water. Then, the storage temperature of 37°C was used to dry the plant. Next, the dried plant was turned into powder using a laboratory mill. For extraction, the dry plant was mixed with distilled water at a ratio of 1 to 10 weight/volume and placed on a stirrer at room temperature for 24 hours. Then the sample was filtered using filter paper and immediately centrifuged. Finally, the evaporation operation was performed using a rotary device and the obtained extract was stored in the refrigerator [17].

2- 3- Investigating antifungal activity

Antifungal activity of jashir extract against fungi***Alternaria alternata*، *Alternaria solani*، *Saccharomyces cerevisiae*And *Fusarium solani*According to the antimicrobial methods of agar diffusion disk, agar well, the minimum growth inhibitory concentration and the minimum lethal concentration were investigated.**

2- 3- 1- Agar diffusion disk

Agar disk diffusion method is a common method to evaluate the antifungal activity of plant extracts. To perform this test, concentrations of 20, 40, 60, and 80 mg/ml were prepared from the aqueous extract of jashir plant, and then a syringe microfilter with a pore size of 0.22 microns was used for its sterilization. Next, the discs were soaked with the extract for 15 minutes. Microbial suspension (standard equal to 0.5 McFarland) was used in the inoculation stage. Then, the discs coated with the extract were placed on Sabourud dextrose agar culture medium and the culture medium was kept in a greenhouse at 27°C for 72 hours. Finally, the diameter of the growth halo around the disc was measured in millimeters and used as the antimicrobial effect of the extract [8, 9].

2- 3- 2- Agar well

In this test, Sabourud dextrose agar culture medium was prepared and poured into a petri dish. Next, the microbial suspension was spread on the environment. Then, wells with a diameter of 6 mm were created on the surface of the culture medium and filled with 20 microliters of extract with concentrations of 20, 40, 60, and 80 mg/ml. The culture medium was kept in an incubator at 27°C for 72 hours, and the diameter of the growth halo around the wells was measured (in millimeters) and expressed as the antifungal effect of the extract [14].

2-3-3- minimum inhibitory concentration[1] (MIC)

To perform this test, successive concentrations of the extract were prepared and 125 microliters of each concentration was added to the wells of the 96-well plate. The microbial suspension was also added to the wells and the greenhouse was kept at 27°C for 72 hours. Next, 25 microliters of triphenyltetrazolium chloride reagent solution (5 mg/ml) was added to the wells. Microbial growth causes dark red color to appear in the wells. As a result, the lowest concentration in which no microbial growth was observed and no color change was observed was considered as the MIC of the extract [20].

2- 3- 4- Minimum concentration of fungicide[2] (MFC)

To determine the amount of MFC, 100 microliters of the culture medium that lacked microbial growth (absence of red color in the well) was cultured on Saburoud dextrose agar culture medium. The greenhouse was repeated according to the above conditions, and the minimum concentration that completely prevented the colony formation was considered as the MFC of the extract [21, 22].

[1]-Minimum inhibitory concentration

[2]- Minimum fungicidal concentration

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2-3-5- Investigating the interaction between Jashir aqueous extract and Nystatin antibiotic

To investigate the interaction of Jashir aqueous extract with nystatin antibiotic, the method of Alizadeh Behbahani et al. (2020) was used with the necessary changes. For this purpose, concentrations that were equivalent to half of the minimum inhibitory concentration were briefly used. Microbial culture was carried out on Sabourud dextrose agar culture medium containing Jashir extract. Then, the nystatin antibiotic disc was gently placed on the surface of the culture medium with the help of sterile forceps. In the following, the culture medium was carried out at 27 degrees Celsius for 72 hours and finally the diameter of the halo of non-growth around the disk was measured and reported in millimeters [23].

2-4- Statistical analysis

The tests were repeated three times and the data were analyzed using one-way analysis of variance with the help of Minitab software (version 17). The difference between the average results was checked using Tukey's test at the 95% confidence level ($p < 0.05$).

3. Results and Discussion

The antifungal activity of plant extracts is due to the presence of bioactive agents such as phenolic compounds, flavonoids, terpenoids and saponins. In the present study, the antifungal activity of Jashir aqueous extract against fungi*Alternaria alternata*، *Alternaria solani*، *Saccharomyces cerevisiae*And *Fusarium solani*It was checked. Antifungal effect results. The extract based on the agar disk diffusion method is presented in Figure 1.

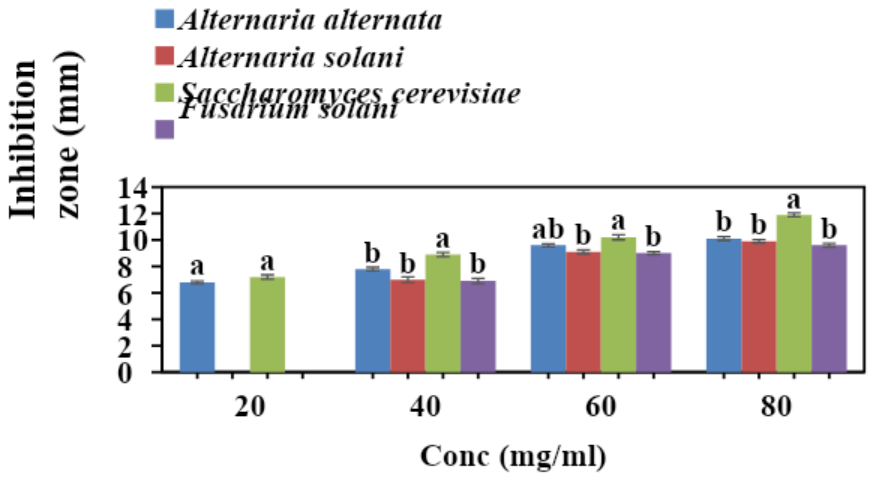

Figure 1. Antifungal activity of *Prangos ferulacea* extract based on disc diffusion agar method. Means with different superscripts at each concentration are significantly different ($p<0.05$).

According to the results, the antimicrobial effect of the extract was dependent on the concentration and type of fungus. Increasing the concentration of the extract led to an increase in the diameter of the halo of no growth for the fungal strains. Fungal strains showed different behavior compared to the extract. At a concentration of 20 mg/ml of the extract, the antimicrobial effect for*Alternaria solani*And *Fusarium solani*It was not observed, but the diameter of the halo of non-growth for*Alternaria alternata* And *Saccharomyces cerevisiae*It was equal to 6.80 and 7.20 mm, respectively. Concentrations higher than 20 mg/ml of the extract resulted in preventing the growth of all fungal species. generally,*Saccharomyces cerevisiae*And*Fusarium solani* The most sensitive and resistant fungal strains with the highest and the lowest diameter of the non-growth halo, respectively, were Jashir's aqueous extract ($p<0.05$). So that the diameter of the non-

growth halo for these strains in the presence of 80 mg/ml extract concentration was equal to 11.90 and 9.90 mm, respectively.

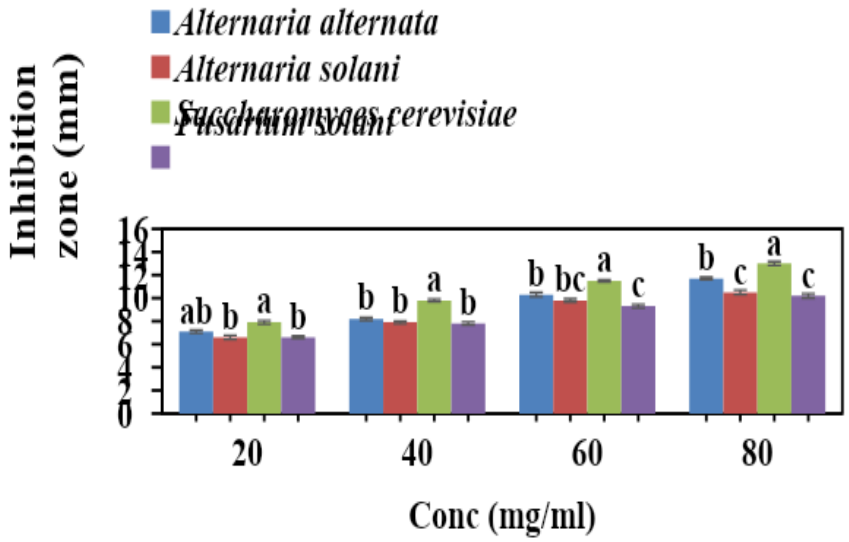

Figure 2. Antifungal activity of *Prangos ferulacea* extract based on well diffusion agar method. Means with different superscripts at each concentration are significantly different ($p<0.05$).

The results of the agar well antimicrobial test were in line with the findings of the agar disk diffusion test, and the antimicrobial effect of the extract was dependent on the type of fungus used and the concentration of the extract (Figure 2). The antifungal effect of the extract (no growth halo diameter) increased significantly with increasing its concentration. In addition, all concentrations of the extract were effective against the fungal strains. No growth halo diameter for fungi*Alternaria alternata*، *Alternaria solani*، *Saccharomyces cerevisiae*And *Fusarium solani*At the concentration of 80 mg/ml of the extract, it was equal to 11.70, 10.50, 13.00 and 10.20 mm respectively. In this regard, strains*Saccharomyces cerevisiae* With the highest diameter of the aura of lack of growth and strain*Fusarium solani* With the smallest diameter of the non-growth halo, they were respectively the most sensitive and resistant fungal species to the extract ($p<0.05$). It should be noted that the average diameter of the halo of non-growth in the agar well test was significantly higher than the antimicrobial disk diffusion agar method. In the diffusion method in the well, there is direct contact between the antimicrobial substance and the microorganism, but in the agar disk diffusion method, the antimicrobial substance must penetrate from the paper disk to the surface of the culture medium [20, 22].

The results of the antimicrobial effect of nystatin fungicide and its interaction with Jashir aqueous extract are reported in Figure 3. The results showed that nystatin is effective against all fungi and the most effect is on*Saccharomyces cerevisiae*was observed (Figure 4). The interaction of nystatin with jashir extract improved the antimicrobial activity of nystatin. So that the average diameter of the aura of nystatin growth is 15.40, 12.50, 20.00 and 17.50 mm for*Alternaria alternata*، *Alternaria solani*، *Saccharomyces cerevisiae*And *Fusarium solani* It increased to 17.70, 13.60, 21.60 and 17.90 mm in the presence of nystatin + extract, which shows the synergistic effect between the extract and nystatin.

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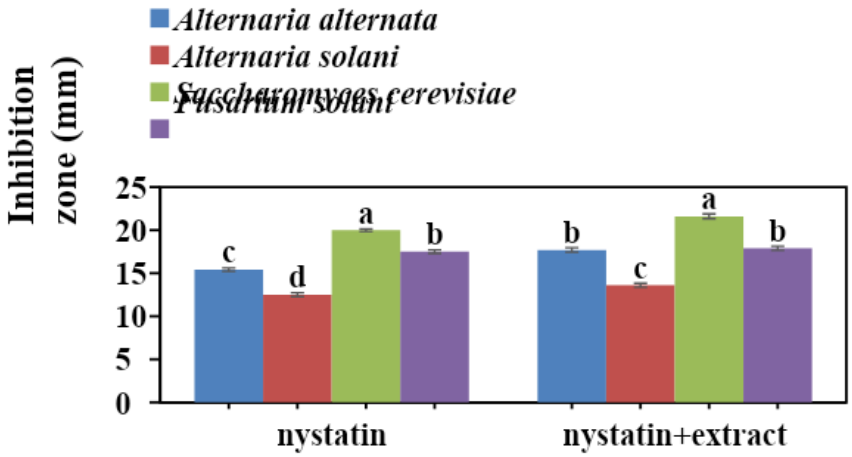

Figure 3. The mean inhibition zone diameter (mm) of nystatin and its interaction with *Prangos ferulacea* extract on some fungal species. Means with different superscripts are significantly different ($p<0.05$).

Figure 4. The antifungal effect of nystatin on *Saccharomyces cerevisiae*.

The results of the antimicrobial effect of the extract based on MIC and MFC methods are presented in Table 1. According to the results, the lowest MIC and MFC to*Saccharomyces cerevisiae*It was determined that the expression shows the highest sensitivity among the fungal strains to the extract. AmountsMIC To*Alternaria alternata*، *Alternaria solani*، *Saccharomyces cerevisiae*And *Fusarium solani* It was equivalent to 64, 128, 32 and 256 mg/ml, respectively. While the valuesMFC 256, >512, 128 and >512 mg/ml were obtained for these strains, respectively.

Table 1. Antifungal activity of *Prangos ferulacea* extract based on MIC and MFC methods.

Fungi species	MIC (mg/ml)	MFC (mg/ml)
Alternaria alternata	64	256
Alternaria solani	128	> 512
Saccharomyces cerevisiae	32	128
Fusarium solani	256	> 512

The antimicrobial activity of Jashir essential oil was evaluated based on broth microdilution method. The antimicrobial effect was concentration-dependent, and the MIC of Jashir essential oil varied between 100 and 200 µg/ml for gram-positive and gram-negative bacteria. was [24]. In another study, it was found that the antifungal activity of the methanol extract of Jashir against fungi*Aspergillus fumigatus* ، *Aspergillus acraseus* ،*Aspergillus niger* ، *Aspergillus versicolor* ،*Trichoderma was scared* ،*Penicillium funiculosum* ،*Penicillium acrochloron* And*Penicillium verrucosum* It is significant [25]. Antifungal effect of Jashir essential oil against*Candida albicans* has also been reported [26]. In addition, it has been shown that Jashir's aqueous extract is rich in phenolic compounds that prevent the growth of pathogenic bacteria such as*Listeria*

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monocytogenes ،*Bacillus cereus* ،*Salmonella Typhimurium* ،*Staphylococcus aureus* ، *Escherichia coli* ،*Shigella dysentery* And*Staphylococcus epidermidis* becomes [17]. Antifungal activity can be achieved by killing the pathogenic fungal cell. Terpenes are the active antimicrobial compounds of plants. The mechanism of action of this group of compounds includes disruption of the membrane as well as destruction of fungal mitochondria. Another mechanism of antifungal effect of terpenes includes inhibition of electron transfer (inhibition of proton pump) and inhibition of ATPase in mitochondria. For phenolic compounds, the antifungal effect occurs through disruption of the fungal cell membrane [27, 28].

4- The final conclusion

This study showed that the aqueous extract of jashir has antifungal effect against*Alternaria alternata*، *Alternaria solani*، *Saccharomyces cerevisiae*And *Fusarium solani*Is. The antifungal effect was concentration-dependent and increased with the concentration of the extract. Therefore, Jashir aqueous extract can be used as a natural antifungal agent to inhibit the growth of pathogenic fungi in fresh fruits and vegetables. However, more studies are needed to determine the components of Jashir aqueous extract, its effectiveness in vivo and its potential use as a natural antifungal agent in food preservation.

5- Appreciation and thanks

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6- Resources

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اثر ضدمیکروبی عصاره آبی جاشیر بر برخی از گونه‌های قارچی بیماری‌زا و برهمکنش آن با آنتی‌بیوتیک نیستاتین

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چکیده

جاشیر گیاهی است که در طب سنتی به دلیل خواص درمانی آن مورد استفاده قرار می‌گرفته است. مطالعه حاضر به منظور بررسی اثر ضد قارچی عصاره آبی جاشیر بر روی *آلترناریا آلترناتا*، *آلترناریا سولانی*، *ساکارومایسس سرویزیه* و *فوزاریوم سولانی* انجام شد. روش‌های ضد قارچی مورد استفاده عبارت بودند از دیسک دیفیوژن آگار، چاهک آگار، حداقل غلظت مهارکنندگی (MIC) و حداقل غلظت قارچ‌کشی (MFC). غلظت عصاره مورد استفاده در این مطالعه ۲۰، ۴۰، ۶۰ و ۸۰ میلی‌گرم در میلی‌لیتر بود. این مطالعه نشان داد که اثر ضد قارچی وابسته به غلظت عصاره است و با غلظت افزایش می‌یابد. *ساکارومایسس سرویزیه* حساس‌ترین گونه به عصاره و *فوزاریوم سولانی* مقاوم‌ترین گونه بود. در ۸۰ میلی‌گرم بر میلی‌لیتر عصاره، قطر بازداری برای *ساکارومایسس سرویزیه* به ترتیب ۱۱/۹۰ میلی‌متر و ۱۳ میلی‌متر در روش دیسک دیفیوژن آگار و چاهک آگار بود. قطرهای بازداری مربوطه برای *فوزاریوم سولانی* به ترتیب ۹/۶۰ میلی‌متر و ۱۰/۲۰ میلی‌متر بود. در حالت ترکیبی (برهمکنش) عصاره آبی جاشیر با آنتی‌بیوتیک نیستاتین برای تمامی سویه‌های قارچی حالت هم‌افزایی مشاهده شد. مقادیر MIC و MFC برای *ساکارومایسس سرویزیه* به ترتیب ۳۲ و ۱۲۸ میلی‌گرم بر میلی‌لیتر بود. نتایج MIC و MFC برای *فوزاریوم سولانی* به ترتیب ۲۵۶ و > ۵۱۲ میلی‌گرم در میلی‌لیتر بود. نتایج این مطالعه نشان داد که عصاره آبی جاشیر می‌تواند به عنوان یک عامل ضد قارچ طبیعی برای مهار رشد قارچ‌های بیماری‌زا در میوه‌ها و سبزیجات تازه مورد استفاده قرار گیرد.