

## ***In vitro* antifungal activity of *Allium hirtifolium* in comparison with the miconazole**

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### **Abstract**

**Objective:** Shallots are important part of the diet for many people and there is long-held belief in their health enhancing properties. The aim of this study was to determine antifungal activity of shallot against reference fungal strains.

**Methods:** Alcoholic and aqueous extracts of shallot (*Allium hirtifolium*) were tested for *in vitro* antifungal activities against *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium gryseogenum*, *Alternaria*, *Microsporum canis* and *Trichophyton mentagrophytes*. The minimal inhibitory concentration (MIC) was determined using broth macrodilution method. The effects of shallot extracts were also compared with those of miconazole.

**Results:** *Allium hirtifolium* showed antifungal activity against all the fungi species tested with MIC values ranging from 0.058 to 0.8 mg/ml for alcoholic extract and 0.26 to 3.84 mg/ml for aqueous extract. The minimum fungicidal concentration (MFC) of alcoholic and aqueous extracts ranged from 0.1 to 12.8 mg/ml and 0.6 to 68.26mg/ml, respectively.

**Conclusions:** The results indicate that crude juice of shallot has antifungal activity and might be promising, at least, in treatment of fungal-associated diseases from mentioned fungi.

**Keywords:** *Allium hirtifolium*, miconazole, *aspergillus* spp, *alternaria*, *penicillium chrysogenum*, *microsporum canis*, *trichophyton mentagrophytes*

### **Introduction**

Fungal infections associated with significant morbidity are common in critically ill and immunocompromised patient [1-5].

The dermatophytes are a group of closely related fungi that have the capacity to invade the keratinized tissue of humans and other animals and produce dermatophytoses [6]. The incidence of dermatophytoses has increased over recent years, particularly in immunocompromised patients [7,8,9]. In addition, in patients with immune system depression such as cyto-

toxic therapy for cancer, leukemia, organ transplantation or those with AIDS, life-threatening deep mycoses can be caused by fungi which have been considered saprophytes and harmless healthy individuals [10].

Herbal medicines have been important sources of products in developing countries for treatment of common infections including fungal disease [11]. Since the fungal pathogens are eukaryotes, the treatment with common drugs may also affect the infected patients [12]. Hence as an alternative, cheap, affordable, ecofriendly, plant extracts may possibly be used for the treatment

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[13].

The use of Liliaceae bulbs as a food flavor is known world wide and, particularly, garlic and onion have been used in folk medicine since ancient time [14]. The oldest citation of shallot (a cultivated variety derived from *A. cepa* L) is found in the work of Fattorusso et al who described six different types of onions with their therapeutic uses, indicating shallot as the most important one [15].

Persian shallot (*Allium hirtifolium*) is a native plant in Iran [16] that belongs to genus Liliaceae. There are more than 500 species in this genus. Shallot produces a cluster of bulbs from a single planted bulb [17].

Recently, the antibacterial, antifungal and antiprotozoal properties of *Allium* species among shallot have been studied [16-23]. In this study, the antifungal effect of Iranian *Allium hirtifolium* has been studied against *Aspergillus* spp, *Penicillium gryseogenum*, *Alternaria* spp, *Trichophyton mentagrophytes* and *Microsporum canis*.

### Methods

**Plant material:** The bulbs of *Allium hirtifolium* (Persian shallot) were collected from Khansar, Iran in autumn and then prepared for the experiment.

**Extraction and isolation:** The bulbs of shallot were dried at room (20-22°C) and ground into a powder using a blender. The dried bulb powder was extracted by soxhlet's procedure for 14 hours (24). Two type of extraction were performed; alcoholic (170 mg powder in 500 ml methanol) and aqueous extraction (135 mg powder in 500 ml distilled water). The alcoholic and aqueous extracts were filtered and evaporated to dryness with a vacuum rotary evaporator. Afterwards, the extracts were freeze-dried and stored at 4°C.

**Microorganisms:** The reference fungal strains, included *Aspergillus fumigatus* PTCC 5009

(Persian Type Culture Collection), *Aspergillus flavus* PTCC 5006, *Aspergillus niger* PTCC 5011, *Penicillium chrysogenum* PTCC 5031, *Alternaria* PTCC 5224, and *Microsporum canis* PTCC 5069 were purchased from Iranian Scientific and Industrial Institute and the reference strain of *Trichophyton mentagrophytes* was obtained from Mycology department of Iran medical science university.

**Inoculum preparation:** For saprophytes fungi, suspensions were prepared from mature cultures grown at 30°C on SDA slants. The fungal slants were covered with 1 ml sterile saline (0.9%) and gently probed with the tip of Pasteur pipette. Heavy particles were allowed to settle and the upper homogenous suspensions removed and then adjusted spectrophotometrically to 90% transmission at 530 nm. The adjusted suspension contained approximately  $1 \times 10^6$  to  $5 \times 10^6$  CFU/ml. For dermatophytes, suspensions were prepared from matured cultures grown at 30 (10-14 days) on SDA slants. The fungal colonies were covered with 2 ml sterile saline (0.9%) and the suspensions were made gently by probing the surface with the tip of Pasteur pipette. The resulted suspension was transferred to a sterile tube and allowed to settle. The upper homogenous suspensions were adjusted spectrophotometrically to 90% transmission at 530 nm.

Viable counts for all the fungi species were determined by quantitative plating onto SDA plates and plates incubated at 30°C until growth was established.

**Antifungal assay:** The freeze-dried extracts and miconazole powder were dissolved in DMSO (Dimethylsulphoxide), except for aqueous extract, which was dissolved in sterile distilled water.

**Broth Macrodilution method:** 1 ml of sterile liquid sabouraud medium was added to each 12 sterile capped tubes. 1 ml of alcoholic extract

suspension (51.2 mg/ml in medium) was added to tube 1. The contents were mixed and 1 ml was transferred to tube 2. This serial dilution was continued to tube 10. 50 µl of inoculum was added to tube 1 through 11 and the contents were mixed. The control medium (no inoculum and no drug) and inoculum control (no drug) tubes were prepared. The final concentrations of alcoholic extract ranged from 25.6 to 0.05 mg/ml. For aqueous extract and miconazole, the final concentrations ranged from 102.4 to 0.1 mg/ml and 0.256 to 0.0005 mg/ml, respectively. The tubes were incubated at 30 °C for 48 h (for saprophytes fungi) and 72 h (for dermatophytes) and were read visually. The lowest concentration of each tube that prevented visible growth was considered as the MIC. The MFC was determined by culturing 10 µl of the vortexed broth from tubes that lacked visible turbidity in the MIC assay on SDA plates at 30 °C for sufficient times. The MFC was defined as the lowest concentration at which the growth of fungal colony was completely inhibited.

*Statistical analysis:* Statistical analysis was conducted using the t-test.

### Results

The MIC of alcoholic and aqueous extracts of shallot and miconazole are presented in table 1. The MIC of alcoholic and aqueous extracts ranged from 0.058 to 0.8 mg/ml and 0.26 to

3.84 mg/ml, respectively. The least sensitive strain to the extracts was *Aspergillus niger* and the most sensitive strain *Alternaria*. The MIC of miconazole ranged from 0.00066 to 0.00533 mg/ml, that the least sensitive strain to miconazole was *Aspergillus fumigatus* and the most sensitive strain *Aspergillus flavus*.

The MFC of alcoholic and aqueous extracts and miconazole ranged from 0.1 to 12.8 mg/ml, 0.6 to 68.26 mg/ml and 0.008 to 0.1066 mg/ml, respectively (Table 2).

The MFC of shallot extracts of 3 fungal species, *P. chrysogenum*, *M. canis*, *T. mentagrophytes*, were equal to the MIC values.

### Discussion

The emergence of antifungal resistant strain of various fungi such as candida, dermatophyte and *Cryptococcus neoformans* has prompted into developing new strategies for fighting fungal infections [25] which may be less toxic to human. Some studies have demonstrated the inhibitory effects of shallot extract against bacteria, protozoan and fungal strains [16-20,26]. Amin et al, evaluated antifungal and antibacterial effects of shallot, garlic and onion extracts. In this study three types of shallot extract; fresh, dried and autoclaved extracts, were used and the results showed that fungal species were more sensitive to shallot extract than bacteria. The study by Amin et al, obtained MICs of the shallot extract against *Trichophyton mentagrophytes*

Strain	MIC mg/ml		Miconazole
	Shallot alcoholic extract	Shallot aqueous extract	
<i>Aspergillus flavus</i>	0.13 ± 0.047	0.93 ± 0.5	0.00066 ± 0.00019
<i>A. fumigatus</i>	0.53 ± 0.19	0.88 ± 0.55	0.00533 ± 0.00189
<i>A. niger</i>	0.8 ± 0	3.84 ± 2.80	0.002 ± 0
<i>Penicillium chrysogenum</i>	0.2 ± 0	0.6 ± 0.28	0.00266 ± 0.00057
<i>Alternaria</i>	0.058 ± 0.031	0.26 ± 0.094	0.00133 ± 0.00047
<i>Microsporium canis</i>	0.1 ± 0	1.6 ± 0	0.00333 ± 0.00094
<i>Trichophyton mentagrophytes</i>	0.4 ± 0.28	1.6 ± 0	0.0035 ± 0.00324

The MIC was determined visually as the concentration that showed 100% inhibition. Assay was performed using broth dilution method. The MIC values represent the average of 3 independent experiments.

Table 1. Antifungal effects of shallot extracts and miconazole on selected fungal strains.

Strain	MFC mg/ml		
	Shallot		Miconazole
	alcoholic extract	aqueous extract	
<i>Aspergillus flavus</i>	0.8 ± 0.56	2.4 ± 1.13	0.01066 ± 0.00377
<i>A.fumigatus</i>	12.8 ± 0	51.2 ± 36.20	0.03733 ± 0.01995
<i>A.niger</i>	21.33 ± 6.027	68.26 ± 24.14	0.1066 ± 0.03017
<i>Penicillium chrysogenum</i>	0.2 ± 0	0.6 ± 0.28	0.00853 ± 0.00589
<i>Alternaria</i>	0.53 ± 0.19	12.8 ± 0	0.01293 ± 0.00434
<i>Microsporum canis</i>	0.1 ± 0	1.6 ± 0	0.00933 ± 0.00499
<i>Trichophyton mentagrophytes</i>	0.4 ± 0.28	1.6 ± 0	0.008 ± 0.00566

The MFC was determined as the lowest concentration of the agent at which no colonies were seen after sufficient times. The MFC values represent the average of 3 independent experiments.

Table 2. Fungicidal effects of shallot extracts and miconazole on selected fungal strains.

phytes, *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger* were 0.62, 20, 20 and 20 mg/ml respectively [26], which differ from our results in this study. In other study by Yin et al on antifungal effects of *Allium* genus plants, and growth inhibitory effects of each 7 *Allium* species strains on three *Aspergillus* species were indicated [18]. In a study, using chromatography, Wang et al extracted a polypeptide, Ascalin (MW: 9.5 KDa), from shallot which not only had antifungal effects but blocked HIV reverse transcriptase enzyme [27]. In other study by Dankert et al, antimicrobial effects of shallot, garlic and onion were assessed on 5 gram negative, 3 gram positive bacteria and 2 yeast species. In this study garlic extract showed growth inhibitory effect on all mentioned microorganisms, while shallot and onion extracts were not as effective [19].

In a study by Adeniyi and Anyiam the anti-helicobacter pylori potency of crude methanol extract of *Allium ascalonicum* (shallot) was investigated. The MICs of the extract against all of the tested strains ranged from 6.25 to 12.5 mg/ml [28]. The results of this study showed that the antifungal effects of shallot extract is better than anti-helicobacter pylori effects.

In this study, the inhibitory effects of alcoholic and aqueous extracts of shallot were studied against 7 fungal species including *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium chrysogenum*, *Al-*

*ternaria*, *Microsporum canis* and *Trichophyton mentagrophytes*. Broth dilution method was used in this study and the results compared with each other and the miconazole.

The results of this study differ from previous studies; which could be due to variation in extraction methods, fungal species, and incubation periods and [18,26].

Interestingly, comparison between alcoholic extract of shallot and aqueous extract, showed that antifungal activity of alcoholic extract was more than aqueous extract. This indicates that the active ingredients of shallot against fungal strains, dissolved better in methanol than water, and for prospective supplementary researches about antimicrobial properties of shallot, it seem that alcoholic extract is more useful than aqueous extract.

There were no significant differences between alcoholic and aqueous extracts of shallot in comparison with the miconazole. Since the shallot extracts were crude and contained the ineffective ingredients, the different in the results between the extracts and miconazole seemed reasonable.

There was not any limitation in performing this research and the most important result of this study was to show that shallot extract was effective against named fungal species and it looks promising that in future, we can obtain some effective antifungal agents with minimal side effects from shallot extract, but more invi-

vo studies for evaluation of pharmacokinetic effects of shallot are required.

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