

RESEARCH ARTICLE

Anti-Candidal Effect of Shallot against Chronic Candidiasis

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Received October 6, 2010; Revised February 29, 2011; Accepted April 5, 2011

This paper is available online at <http://ijpt.iums.ac.ir>**ABSTRACT**

Shallots are an important part of the diet of many populations and there is long-held belief in their health enhancing properties. The aim of this study was to determine anti-candidal activity of shallot against chronic candidiasis agents. Alcoholic and aqueous extracts of shallot (*Allium hirtifolium*) were tested for *in vitro* antifungal activities against 33 candida species isolated from patient with chronic candidiasis who were referred to Mirza-Kochak-Khan and Lolagar Hospitals. Minimal inhibitory concentration (MIC) was determined using broth microdilution method. *Allium hirtifolium* showed antifungal activity against all the candida species tested and anti-candidal activity of the alcoholic extract was very better than aqueous extract. The results indicate that crude juice of shallot has anti-candidal activity and might be promising in treatment of candidiasis.

Keywords: *Shallot, Candida, Allium hirtifolium, Minimal inhibitory concentration (MIC)*

Fungal infections associated with significant morbidity are increasing in critically ill and immunocompromised patient [1-5]. Candida vaginitis is a common problem attributable to overgrowth of Candida species. It is estimated that 75% of all women will experience an episode of candida vaginitis in their lifetime [6,7]. By the age of 25 years, nearly one-half of all college-age women will have had at least 1 episode of candida vaginitis [8]. Candida albicans accounts for 80-95% of all episodes of candida vaginitis worldwide [6,7]. A minority of women, however, suffer recurrent episodes of candidiasis which can significantly affect their quality of life and their sexual health.

While a number of factors are probably important in the pathogenesis of recurrent thrush, there are two main theories as to why some women experience frequent recurrences [9]: (1) re-infection either from a sexual partner or a reservoir of yeasts in the gut; (2) vaginal relapse due to incomplete eradication of the yeasts. In the 1990s, there was a significant increase in the prevalence of drug-resistant fungal infections due to candida species in patients hospitalized for mucosal or systemic diseases [10-12].

Herbal medicines have been important sources of products in developing countries for treatment of common infections including fungal disease [13]. Since the fungal pathogens are eukaryotes, the treatment may

also affect the infected patients [14]. Hence as an alternative, cheap and affordable eco-friendly plant extracts may possibly be used for the treatment [15].

In this study, the antifungal effect of *Allium hirtifolium* growing in Iran has been studied against candida species isolated from patient with chronic candidiasis for accessing to the new antifungal drug.

MATERIALS AND METHODS*Plant material*

The bulbs of *Allium hirtifolium* (Persian shallot) were collected from Khansar, Iran in autumn.

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. 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 vaccum rotary evaporator. Afterwards, the extracts were freeze-dried and stored at 4°C.

Table 1. Antifungal effects of alkaloid extract of *P. harmala* and miconazole on selected fungal strains

Strain	MIC (mg/mL)		
	Alcoholic extract	Aqueous extract	Miconazole
<i>Candida albicans</i> (23 spp)	0.05-3.2	12.8-25.6	0.0005-0.016
<i>Candida glabrata</i> (6 spp)	0.1-0.8	1.6-25.6	0.00025-0.256
<i>Candida parapsilosis</i> (2 spp)	0.2-0.4	9.6-25.6	0.002-0.016
<i>Candida krusei</i> (1 spp)	0.4	25.6	<0
<i>Candida guilliermondii</i> (1 spp)	0.2	6.4	0.0005
<i>Candida albicans</i> PTCC:5027	0.3	1.2	0.0005

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

Microorganisms

Candida species isolated from patient with chronic candidiasis who were referred to Mirza-Kochak-Khan and Lolagar Hospitals. The samples were collected by clinicians on site from the patient's vaginal pool using a sterile cotton swab. Swabs were directly inoculated onto a sterile slant containing Sabouraud dextrose agar. These samples were then cultured on petri dishes containing yeast peptone dextrose agar media at 37°C for 48 h. The colonies were identified by classical methods [16,17] using the following tests: formation of germinative tubes, study of micromorphology, assimilation of carbon and nitrogen sources, fermentation and urea hydrolysis. The differential medium CHROM agar *Candida* was used to confirm the result by colony morphology and pigmentation [18]. Culture-positive samples were plated on CHROM agar and grown at 37°C for 24 h. A germ tube test was performed for presumptive identification of *C. albicans*. Among 33 identified *Candida* species, the most common species were *Candida albicans* (23 spp), *Candida glabrata* (6 spp), *Candida parapsilosis* (2 spp), *Candida krusei* (1 spp) and *Candida guilliermondii* (1 spp).

Inoculum preparation

Stock inoculum suspensions of the *Candida* species were obtained from 24-h-old cultures on Sabouraud Dextrose Agar (SDA) at 37°C. The turbidity of the *Candida* suspensions was adjusted by the spectrophotometric method and the diluted (two times) *Candida* inoculum concentrations ranged from 0.8×10^3 to 4.2×10^3 CFU/mL.

Antifungal assay

The alcoholic extract of shallot was dissolved in DMSO (Dimethylsulphoxide), and aqueous extract was dissolved in sterile distilled water.

Microdilution test

Broth microdilution testing was performed in sterile, flat-bottomed 96-well microplates. Each micro-dilution well containing 100 µl of the diluted (two times) alcoholic and aqueous extract of shallot concentrations were inoculated with 100 µl of *Candida* species suspensions. Microdilution trays were incubated at 37°C

and were examined at 48 h. The result of *Candida* species MIC were compared with MIC of reference isolate of *Candida albicans* (PTCC: 5027).

RESULTS AND DISCUSSION

The MIC of alcoholic and aqueous extracts of shallot are presented in Table 1. 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 [19] which may be less toxic to human. Some studies have demonstrated the inhibitory effects of shallot extract against bacteria, protozoan and fungal strains [20-25]. Amin *et al.*, evaluated antifungal and antibacterial effects of shallot, garlic and onion extracts. In this study, three types of shallot extract were used; fresh, dried and autoclaved extracts, which showed that fungal species were more sensitive to shallot extract than bacteria [25]. In other study by Yin *et al.* on antifungal effects of *Allium* genus plants, growth inhibitory effects of each 7 *Allium* species strains on three *Aspergillus* species were indicated [22]. In a study, using chromatography, Wang *et al.* extracted a polypeptide from shallot, Ascalin (MW: 9.5 KDa), which not only had antifungal effects but blocked HIV reverse transcriptase enzyme [26]. 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 [23]. The inhibitory effects of alcoholic and aqueous extracts of shallot were studied against 33 *Candida* species separated from patient with chronic candidiasis. Broth microdilution method was used in this study and the results were compared with each other and miconazole and reference strain of *Candida albicans*. Comparison between alcoholic extract of shallot and aqueous extract shows that anti-candidal activity of alcoholic extract is more than that of aqueous extract. This indicates that the active ingredients of shallot against *Candida* strains, dissolve better in methanol than water, and for prospective supplementary researches

about antimicrobial properties of shallot, it seems that alcoholic extract is more useful than aqueous extract.

According to the fact that the shallot extracts were crude and contained the ineffective ingredients, the difference in the results of the extracts and miconazole seems reasonable. The most important result of this study is to show that shallot extract is effective against candida species and it looks promising that in future, we can obtain some effective antifungal agents with minimal side effects from shallot extract for chronic candidiasis treatment, but *in vivo* studies for evaluation of pharmacokinetic effects of shallot are required.

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