Journal of Medicinal Plants Research Vol. 3(5), pp. 450-453, May, 2009
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875© 2009 Academic Journals

Full Length Research Paper

Anti fungal activity of shallot, *Allium ascalonicum* Linn. (Liliaceae), *in vitro*

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Accepted 12 May, 2009

The aim of the present study was to investigate anti fungal activities of the fresh extract of *Allium ascalonicum* Linn. (Liliaceae) against clinically important yeast, dermatophytes and some saprophytic fungi *in vitro*. The anti fungal effects of fresh crude juice of shallot were determined against 11 isolates of *Candida albicans*, three species of dermatophytes (*Microsporum gypseum*, *Trichophyton mentagrophytes* and *Epidermophyton floccosum*) and *Syncephalastrum*, *Aspergillus niger*, *Penicillium* sp., *Paecilomyces* sp., *Scopulariopsis* sp., *Cladosporium* sp., *Alternaria* sp., *Drechslera* sp.by agar well diffusion method. Minimal inhibitory concentration (MIC) of the fresh extract of *A. ascalonicum* was 0.25% for most tested fungi; however extract show remarkable activity against saprophytic fungi followed by Candida species and dermatophytes. It is concluded that fresh extract of *A. ascalonicum* has more anti-saprophytes effect at 0.25% with a mean diameter of inhibition zone 21.83 mm.

Key words: *Candida albicans*, saprophytic fungi, dermatophyte, *Allium ascalonicum*, anti fungal.

INTRODUCTION

Allium ascalonicum Linn. (Shallot) is an annual herbaceous plant of the family Liliaceae that grows in wide variety parts of the World. However shallot is probably of Asiatic origin. *A. ascalonicum*, mildly aromatic herb, which are used like onions to flavor food, particularly meats and sauces. Shallot has been used worldwide as a spice, food, and folk medicine and there is long held belief in their health enhancing properties. The bulb of shallot is of considerable importance in African cooking and in salads (Adeniyi and Anyiam, 2004). Many authors have studied the antimicrobial (Dankert et al., 1979; Adeniyi and Anyiam, 2004), anti viruses (Ashrafi et al., 2004) and anti parasite activities (Azadbakht et al., 2002) of *A. ascalonicum*. Antioxidant (Leelarungrayub et al., 2006), anti-diabetic (Adeniyi and Anyiam, 2004) and haematological effects (Owoyele et al., 2004) of *A. ascalonicum* were also reported.

Wang et al. reported ascalin, a new anti-fungal peptide from shallot bulbs that inhibit mycelial growth in *Botrytis cinerea*; however the reports about its activity on clinically important fungi are rare (Wang et al., 2002). At least four sulfur-containing compounds were detected in shallot (Ogra et al., 2005). Two new furostanol saponins (ascalonicoside A1/A2 and ascalonicoside B) were also isolated and described from bulbs of shallot (Fattorusso et al., 2002). Although the antimicrobial activity of plant extracts has been recognized for many years; few investigations have done on the anti fungal activities of *A. ascalonicum*. The aim of the present study was to investigate anti fungal activities of the fresh extract of *A. ascalonicum* against clinically important fungi *in vitro*.

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MATERIALS AND METHODS

Extraction

Fresh bulbs of *A. ascalonicum* were collected from Ilam, a province of South-West of Iran. The plant was identified in the department of Pharmacognosy, School of Pharmacy, Jundishapour University of Medical Sciences, Ahvaz, Iran where voucher specimens were deposited. Fresh shallot bulbs were washed, crushed and filtered by sterile cotton wool. Filtered crude juice was deposited in dark bottle at 4°C.

Fungal isolates

Eleven strains of *C. albicans* (isolated from different patients), eight Isolates of saprophytic fungi and five isolates of dermatophytes

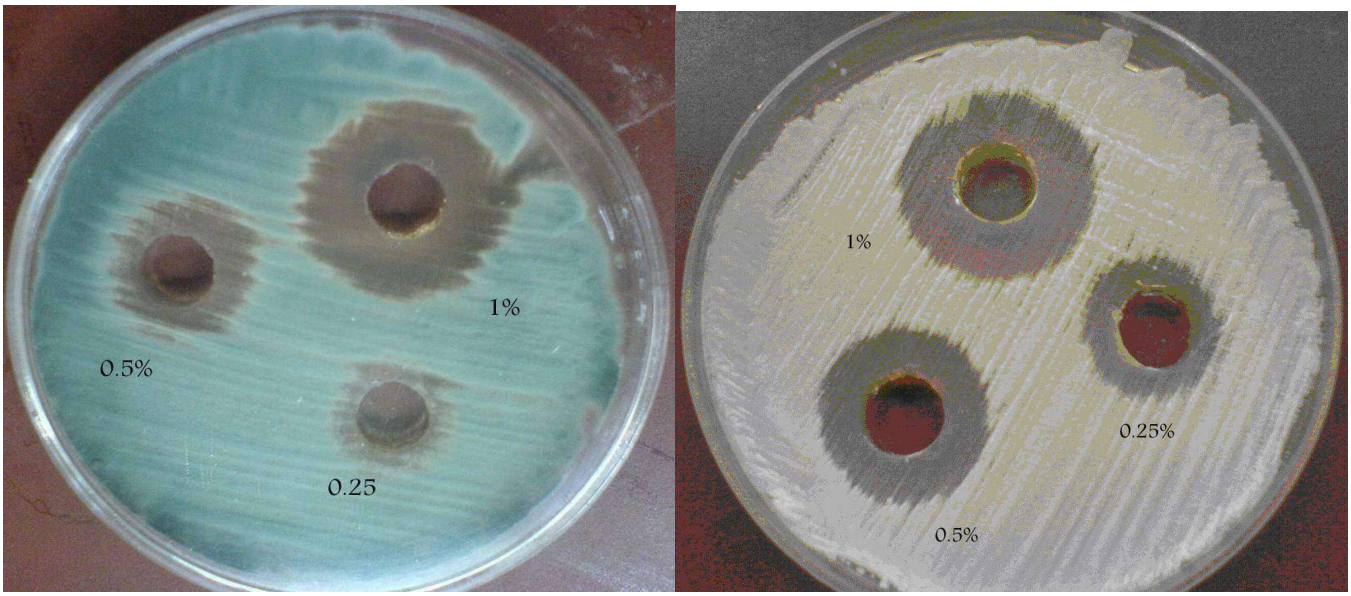

Figure 1. Diameter of inhibition zone in mm: a, (*Penicillium* sp.); b, *C. albicans.*

were studied. All *C. albicans* isolates were identified by standard methods, which included identification based on germ tube test, production chlamydoconidia on corn meal agar (Difco, UK), API 20 C AUX (bioMerieux SA, France) and CHROMagar Candida (CHROMagar Candida Company, Paris, France).

Saprophytic fungi contained, *Syncephalastrum* sp., *Scopulariopsis* sp., *Penicillium* sp., *Paecilomyces* sp., *A. niger, Cladosporium* sp., *Alternaria* sp. *and Drechslera* sp. were isolated from contaminated culture media and identified based on morphology and microscopic features. Dermatophytes consisting of *Microsporum gypseum* (1 isolate), *Trichophyton mentagrophytes* (2 isolates) and *Epidermophyton floccosum* (2 isolates) were identified on the basis of theirs macro and microscopic features, hair perforation test, urease and corn meal agar tests. Isolates were stored as suspendsions in sterile water at ambient temperature for future use.

Susceptibility tests

Anti fungal activity of A. ascalonicum was investigated by agar well diffusion method (Shahidi Bonjar et al., 2005). The yeasts and saprophytic fungi were subcultured onto Sabouraud's dextrose agar, SDA (Merck, Germany) and respectively incubated at 37°C for 24 h and 25°C for 2 - 5 days.

Dermatophytes were also subcultured on SDA and incubated at ambient temperature for 7-10 days. Suspensions of yeasts, saprophytic and dermatophytic fungi spore were prepared in sterile PBS and adjusted to a concentration of 10^6 cells/ml. Dipping a sterile swab into the fungal suspension and rolled on the surface of the agar medium. The plates were dried at room temperature for 15 min. Wells of 10 mm in diameter and about 7 mm apart were punctured in the culture media using sterile glass tube. 0.1 ml of several dilutions of fresh extracts was administered to fullness for each well. Plates were incubated at 37°C for yeasts and 25 - 29°C for saprophytic fungi and dermatophytes. After incubation, 24 h for yeasts, 2 - 4 days for saprophytic fungi and 7 - 10 days for dermatophytes, bioactivities were determined by measuring the diameter of inhibittion zone diameter in mm (Figure 1a, b). All experiments were made in triplicate and means were calculated.

RESULTS AND DISCUSSION

In the present study, the anti fungal activities of the fresh crude juice from shallot bulbs were investigated. Shallot is widely consumed components of the diet of many populations, particularly in Asian diets. It is widely believed to be beneficial to health and even curative potential against a range of debilitating conditions and diseases. Shallot bulbs are darker than garlic and have a stronger odour that correlates with its sulfide content (Azeez et al., 1990). Phytochemical analysis of shallot extracts has confirmed the presence of flavone, quercetins, ascalin and furostanol saponins (Wang et al., 2002; Fattorusso et al., 2002). Wang *et al*. reported ascalin as anti fungal from shallot bulbs that inhibit mycelial growth in fungi (Wang et al., 2002).

In the present study the anti fungal activities of the fresh extract of *A. ascalonicum* were evaluated against 11 isolates of *C. albicans*, 8 isolates of saprophytic fungi and 5 isolates of dermatophytes by agar well diffusion method. Several concentrations of fresh extract (50, 40, 20, 10, 5, 4, 2, 1, 0.5, 0.25, 0.125 and 0.0625%) of *A. ascalonicum* were applied on isolates. The extract showed remarkable activities against tested isolates with the exception of *Scopulariopsis* sp. Table 1 shows details of mean MICs of fresh extract of *A. ascalonicum* against *C. albicans* isolates. As shown the lowest MIC for 11 isolates of *C. albicans* was 0.25%. The largest diameter of inhibition zone (20 mm) was shown by *C. albicans* no. 5 whereas *C. albicans* no. 12 was shown diameter of inhibition zone 13 mm. The mean diameter of inhibition zone for isolates of *C. albicans* was 16.81 mm for 0.25%, 21.28 mm for 0.5% and 25 mm for 1%.

Table 1. *In vitro* activities of *A. ascalonicum* against 11 isolates of *C. albicans*, indicated by diameter of inhibition zones (mm).

Isolates	0.25%	0.5%	1%
C. albicans (no 1)	16.3	20.7	24.7
C. albicans (no 2)	17.3	23	27
C. albicans (no 3)	17	21.7	25.3
C. albicans (no 5)	20	23.3	26.3
C. albicans (no 6)	14.7	19.7	23.3
C. albicans (no 7)	13.3	18.7	22.7
C. albicans (no 8)	19.7	23.7	26
C. albicans (no 9)	17	20.3	24.3
C. albicans (no 10)	18.3	23	25
C. albicans (no 11)	18.3	21	25.7
C. albicans (no 12)	13	19	24.7
Mean	16.81	21.28	25

Table 2. *In vitro* activities of *A. ascalonicum* against 8 isolates of saprophytic fungi, indicated by diameter of inhibition zones (mm).

Isolates	0.25%	0.5%	1%
Alternaria	29.3	32	39.3
Drechslera	29	33	38
Penicillium	16.3	20.3	28.7
Cladosporium	12.7	23.3	29
Paecilomyces	Grow	18.7	22.7
A. niger	Grow	18.7	22
Syncephalastrum	Grow	10.3	20
Scopulariopsis	Grow	Grow	Grow
Mean	21.83	22.33	28.53

Table 3. *In vitro* activities of *A. ascalonicum* against 5 isolates of dermatophytes, indicated by diameter of inhibition zones (mm).

Isolates	0.25%	0.5%	1%
M. gypseum (no 1)	11	19	29
T. mentagrophytes (no 1)	10.7	20.7	28.3
T. mentagrophytes (no 2)	16	20.3	25.7
E. floccosum (no 1)	15.3	20.6	26
E. floccosum (no 2)	17.6	24	30
Mean	14.12	20.92	27.8

Table 2 shows the details of mean MICs for saprophytic fungi. As shown isolates of *Alternaria* sp., *Drechslera* sp., *Penicillium* sp.and *Cladosporium* sp. (0.25%) are more susceptible to fresh extract of *A. ascalonicum*, followed by *Paecilomyces* sp., *A. niger* and *Syncephalastrum* sp. (0.5%). In the present study the MIC for *Scopulariopsis* sp. was more than 2%. The mean diameter of inhibition zone for 0.25% was 21.83 mm followed by 22.33 mm for 0.5% and 28.53 mm for 1%. The MICs of fresh extract of *A. ascalonicum* against three species of dermatophytes are presented in Table 3. The lowest diameter of inhibition zone was 10.7 mm for *T. mentagrophytes* (no 1) whereas the largest diameter of inhibition zone was 17.6 for *E. floccosum* (no 2). The mean diameter of inhibition zone for 5 isolates of dermatophytes was 14.12 mm for 0.25%, 20.92 mm for 0.5% and 27.8 mm for 1%. Amin et al. (2005) have shown that water extract of shallot has anti fungal activity. They tested *Aureobasidium pullulans*, *T. mentagrophytes* and *M. gypseum* against shallot extract. MIC was 0.15 mg/ml for *M. gypseum* and *T. mentagrophytes*.

Conclusion

It is concluded that the fresh crude juice of shallot bulbs has markedly anti fungal effect. Also shallot extract has more anti-saprophytes effect at 0.25% followed by *C. albicans* and dermatophytes.

ACKNOWLEDGMENTS

The authors are grateful to Miss Somaye Dalime and the department of Mycoparasitology, Ahvaz Jundishapur University of Medical Sciences for their help.

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