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Control of Some Filamentous Fungi and Yeasts by Dehydrated *Allium* Extracts

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Abstract: The antifungal activities of dehydrated garlic (*Allium sativum* L.), onion (*Allium cepa* L.) and leek (*Allium porrum* L.) against *Aspergillus niger*, *Fusarium oxysporum*, *Candida albicans* ATCC 10231 and *Metschnikowia fructicola* were investigated. Inhibition activities of the ethyl alcohol or acetone extracts of dehydrated *Allium* species were studied by disc-diffusion and broth dilution methods. The Minimum Inhibitory Concentration (MIC) and the Minimum Fungicidal Concentration (MFC) were found in the range of 75 and 100 mg/mL (w/v). Ethyl alcohol extracts of dehydrated onion (*Allium cepa* L.) in the range of 75 and 175 mg/mL were determined as the most inhibitory MIC and MFC for *A. niger*, *F. oxysporum* and *C. albicans* respectively. The extracts possess antifungal activity against some of the tested filamentous fungi and yeasts at various concentrations.

Zusammenfassung: Es wurde die fungizide Wirkung dehydrierter Extrakte von Knoblauch (*Allium sativum* L.), Küchenzwiebel (*Allium cepa* L.) und Porree (*Allium porrum* L.) gegen *Aspergillus niger*, *Fusarium oxysporum*, *Candida albicans* ATCC 10231 und *Metschnikowia fructicola* untersucht. Die fungizide Wirkung der Äthanol- bzw. Aceton-Extrakte aus den genannten dehydrierten *Allium*-Spezies wurde mittels Disk-Diffusion-Test und spezieller Kultivierungsmethoden charakterisiert. Als "Minimum Inhibitory Concentration" (MIC) und "Minimum Fungicidal Concentration" (MFC) sind Werte im Bereich von 75 bis 100 mg/ml (w/v) zu betrachten. Äthanol-Extrakte aus dehydrierter *A. cepa* (75 bis 175 mg/mL) zeigten die höchsten MIC und MFC in Bezug auf *A. niger*, *F. oxysporum* bzw. *C. albicans.* Um die fungizide Wirkung der verwendeten Extrakte gegen die hier getesteten Pilze realisieren zu können, war es nötig, dem jeweiligen Einzelfall angespasste Konzentrationen einzusetzen.

1. Introduction

Plant extracts have been used for a wide variety of purposes for many thousands of years (Jones, 1996). A renewed interest in "natural preservatives" appears to be stimulated by present food safety concerns, growing problems with microbial resistance and a rise in production of minimal processed food. Many researches have documented the antifungal and antibacterial effects of plants (Irkin and Arslan, 2008; Hammer et al., 1999; Hughes and Lawson, 1991).

Large demands for fungicides exist in agriculture, food protection and medicine. Plant pathogens are estimated to cause yield reductions in crops of almost 20% worldwide. The presence and growth of fungi in food may cause spoilage result in a reduction in quality and quantity. Some filamentous fungi can attack some important crops such as tomatoes, bananas, sweet potatoes and pears (Phay et al., 1999). Food quality is guaranteed by controlling fungi that produce mycotoxins. Serious invasive fungal infections caused by yeasts, such as *Candida* spp. and filamentous fungi like *Aspergillus* spp. represent an increasing threat to human health. Incidence of infection by these microorganisms has risen dramatically in the last 20 years (Trakranrungsie et al., 2008; Pauw, 2000; Candlish et al., 2001; Juglal et al., 2002; Paster et al., 1999; Sahin and Korukluoglu, 2000).

During the last years, protection of food from spoilers and pathogens has been a great concern and was achieved by various physical and chemical methods. However, chemical additives and antibiotics used against fungi have many side effects; also a number of resistant microorganism strains have been determined at the clinical level (Elsom et al., 2003; Ruddock et al., 2005).

Many species of the genus *Allium* have traditionally been used for various purposes. Garlic (*Allium sativum* L.), onion (*Allium cepa* L.) and leek (*Allium porrum* L.) are commonly used

worldwide for food preparations and as seasoning agents (Ross et al., 2001; Yin and Cheng, 1998). Also, dehydrated *Allium* species have great commercial value because of their culinary and medicinal properties as nutraceutical. *Allium* vegetable powders have been sold as nutraceutical and dietary supplements all over the world. *Allium* is reported to possess antibacterial and antifungal activities, and contains powerful sulphur compounds and other numerous phenolic compounds which have been the subject of many researches (Benkeblia, 2004; Griffiths et al., 2002; Harris et al., 2001; Yin and Tsao, 1999). Dehydrated *Allium* species contains higher concentrations of sulphur compounds than the fresh forms according to Lanzotti (2006) and O'Gara et al. (2000).

In this study antifungal activities of these dehydrated plant extracts (garlic and onion bulbs, whole parts of leek) were investigated against some pathogen fungi (*Aspergillus niger*, *Fusarium oxysporum*) and some important yeasts (*Candida albicans, Metschnikowia fructicola*) by using disc-diffusion and the broth dilution methods.

2. Materials and Methods

2.1. Preparation of *Allium* Extracts

Dehydrated bulb of garlic (*Allium sativum* L.), onion (*Allium cepa* L.) and whole parts of leeks (*Allium porrum* L.) in flakes forms were purchased from Yenice Dehydrated Food Industry Factory, Yenice region Canakkale-Turkey were used as samples, soluble dry matter content all of the samples were determined according to method AOAC (1990). Samples (30 g) were extracted with 150 mL ethyl alcohol and acetone solvent for 4 h by using Soxhelet equipment. The extracts were filtered by using Whatman filter paper (no.1) and then concentrated in a vacuum rotary evaporator (Buchi, Switzerland) at 70 °C (Deba et al., 2008; Alanis-Garza et al., 2007). The residues were stored in a refrigerator at +4 °C until subsequent use. For the bioassay, the extracts were suspended in the same solvents (ethyl alcohol, acetone) at a concentration of 25–900 mg/mL. Then all the concentrated extracts were filterated through membrane filters (Cole-Parmer Instr., Comp., Illinosis, USA – 47 mm, 45 μm pore size) by the aid of nitrogen gas.

2.2. Test Fungi

Aspergillus niger and *Fusarium oxysporum* were isolated from foods in Uludag University Food Engineering Department in Bursa, Turkey and identified according to Raper and Fennel (1977), Pitt(1988), Frisvad and Filtenborg (1990). *Candida albicans* ATCC 10231 and *Metschnikowia fructicola* were obtained from Uludag University Medicine Faculty and Plant Protection Faculty in Bursa, Turkey, respectively. Stock cultures were maintained on Sabouraud Dextrose Agar (SDA) (Oxoid) slants at 4 °C. Ten milliliters of 1% (v/v) Tween 20 were added for conidia collection. Conidia were harvested by centrifugation at 1000 rpm for 10 min and washed with sterile distilled water three times. One ml of these conidia suspensions were added into the sterile Sabouraud Dextrose Broth (SDB) (Oxoid) and were incubated at 30 °C for 24 h according to Yin and Tsao (1999). SDB suspension concentrations of filamentous fungi were approximately 10^4 conidia/ml. Yeasts were grown in 50 ml sterile SDB for 18 h on a shaker incubator (Infors AGCH-4103, Bottmingen model) 50 rpm at 30 °C, producing yeast suspensions of approximately 10^6 cfu/ml. All of the filamentous fungi and yeasts in SD broth were enumerated on SD agar at 22–25 °C/4–5 days by using serial dilution methods (Tournas et al.,1998).

2.3 Antifungal Assays

2.3.1 Disc diffusion assay

Disc diffusion method was used to determine the antifungal activity of extracts (Benkeblia, 2004; Karaman et al., 2003). 0.2 ml of the yeast or fungi suspension was uniformly spread on sterile SDA Petri dishes. After inoculum absorption by agar, sterile filter discs (Schleicher & Schüll 2668, Germany, 6 mm diameter) were placed on the culture media and impregnated with 30 μl of each of the various dilutions (25–900 mg/ml) of the extracts. After 30 min, the plates were inverted and incubated at 30°C for 72 h for filamentous fungi and 48 h for yeasts. At the end of the incubation period, the inhibition halo diameters were measured using calipers and expressed in millimeters. When the inhibition halo observed was equal or higher than 10 mm, it was considered as positive antifungal activity (Gonzales et al., 2004). Each assay in this experiment was repeated three times.

2.3.2 Broth dilution method (Determination of MIC and MFC)

The minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) were determined according to Yin and Tsao (1999) and Rasooli and Abyaneh (2004). Plant extracts were diluted with the relevant solvents from 25 to 900 mg/mL. MFC was determined by broth dilution method: 0.5 ml from each dilution of the extracts was added to 4.5 ml fungi culture (10^4 cfu/ml for fungi and 10^6 cfu/ml for yeasts) in SB tubes. The tubes were incubated in a shaker incubator at 30° C (72 h for fungi and 48 h for yeasts). The lowest concentration of extracts that prevented visible growth in the tube was considered the MIC. The MFC was determined by culturing 0.2 ml from all tubes, which lacked the visible turbidity in the MIC assay, on SD agar plates at 30° for 72 h. The MFC was defined as the lowest concentration which completely inhibited the growth of the fungal colony on agar plates.

3. Results and Discussion

Ethyl alcohol and acetone extracts of dehydrated garlic, onion and leek showed antifungal activities *in vitro*. The antifungal activities of dehydrated garlic, onion and leek against filamentous fungi and yeasts are given as MIC (mg/mL) and MFC (mg/mL) (Tab. 1). Between 75->900 mg/mL MFC and 10.4–19 mm mean zone diameters against the tested fungi were obtained from all of the extracts[1].

The results showed that the extracts of dehydrated *Allium* vegetables mediated some degree of activity against fila-

[1] Soluble dry matter content of garlic, onion bulb flakes and whole dehydrated leek contain 5.5, 6 and 5.8% water, respectively.

Tab. 1 Minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) and diameter of inhibition zones/mm of the investigated garlic, onion and leek extracts against filamentous fungi and yeasts.

Fungi Treatment	Dehydrated Allium and Solvent		MIC[a]	MFC[a]	Diameter of inhibition zone/mm[b]
Aspergillus niger	Garlic	(A)	425±22.3	425± 22.3	13± 0.6
		(B)	300± 17.7	325± 11.2	19± 1.6
	Onion	(A)	75± 2.7	100± 2.7	13.6± 0.8
		(B)	175± 13.7	200±11.2	13± 1.1
	Leek	(A)	100± 4.5	125± 22.4	14.2± 0.8
		(B)	175±17.7	200±13.7	12.6± 0.8
Fusarium oxysporum	Garlic	(A)	225± 11.2	300± 11.2	11.8± 0.4
		(B)	150± 13.7	200± 13.7	12.2± 0.8
	Onion	(A)	100± 2.2	100± 4.2	12.6± 1.5
		(B)	175± 13.7	250± 11.2	12.4± 0.5
	Leek	(A)	125± 13.7	> 900	10.4± 1.5
		(B)	>900	>900	0
Metschnikowia fructicola	Garlic	(A)	175± 13.7	200± 11.2	14.2± 0.8
		(B)	300± 22.4	>900	12.2± 0.4
	Onion	(A)	150± 11.2	175± 11.2	13.2± 0.4
		(B)	150± 11.2	200± 13.7	14.2± 0.8
	Leek	(A)	100 ± 0	100±0	14.8± 0.4
		(B)	150± 11.2	>900	10.8±0.8
Candida albicans	Garlic	(A)	400± 11.2	425± 21	15.2±1.4
		(B)	350± 11.2	400± 11.2	13.4±0.8
	Onion	(A)	100± 11.2	175± 13.7	13.2±0.4
		(B)	150± 21	175± 13.7	13.2±1
	Leek	(A)	200± 11.2	225± 13.7	13.8±1
		(B)	300± 13.7	>900	11.2±0.4

"0" : Not detectable; [a] : mg/ml ± standard deviation; [b]: mm ± standard deviation; (A): ethylalcohol (B): acetone

mentous fungi and yeasts. *F. oxysporum*, *M. fructicola, C. albicans* were not inhibited by acetone extracts of dehydrated leek. *A. niger* and *F. oxysporum* were inhibited strongly (75 and 100 mg/mL MFC) by ethyl alcohol extract of dehydrated onion. Hughes and Lawson (1991) determined 200 mg/mL MIC for *C. albicans* from onion powder extracts but in this study inhibition effects of extracts of dehydrated onion flakes by ethyl alcohol and acetone were 100 and 150 mg/mL MIC for *C. albicans*.

However ethyl alcohol extract of dehydrated garlic showed less activity to *A. niger* and *C. albicans* with 425 mg/mL MFC. The inhibition zone diameters for *A. niger* and *C. albicans* were 19 mm for dehydrated garlic-acetone extract and 15.2 mm for dehydrated garlic-ethyl alcohol extract which were the most wide diameter zones in the study.

There are only a few publications about antifungal effects of leek; in relation to onion, cabbage, radish and garlic leek has shown the lowest inhibitory effect against yeasts (Kivanc and Kunduhoglu, 1997). In the present study extracts of leek by ethyl alcohol or acetone were also non-inhibitory against yeasts. We can demonstrate however, that *M. fructicola* and *A. niger* were sensitive to ethyl alcohol extracts of leek with 100 and 125 mg/ml MFC doses respectively. The anti-fungal activity of leek can be increased therefore by using the dehydrated form with a concentrated amount of sulfur compounds in relation to the dry weight.

Effective inhibition of fungi was related to the type of solvent used for extraction as well as to the *Allium* species. Ethyl alcohol extracts of *Allium* species seem to be more inhibitory than the acetone extracts. Ethyl alcohol extracts of *Allium* species contain phenolic and sulphur compounds (Benkeblia, 2004; Kyung and Lee, 2001) and have the lowest MIC among the solvents used for extraction. In studies by Naganawa et al. (1996) and Yoshida et al. (1987) an ajoene compound – a derivative of alliicin – was obtained from garlic after ethyl alcohol extraction. Ajoene was found to be very inhibitory against *A. niger*, *C. albicans* and *Paracoccidioides brasiliensis*.

For the growth-inhibition of *A. niger* Irkin and Korukluoglu (2007) found MFC of 450 mg/mL (fresh garlic), 275 mg/mL (fresh onion), and > 900 mg/mL (fresh leek) after ethyl alcohol extraction and MFC of 875, >900, and >900 mg/mL after acetone extraction. Again extracts of dehydrated *Allium* vegetables inhibit fungi growth much more than the fresh forms because of containing higher amounts of sulphur compounds related to the dry weight. Moreover, dehydrated *Allium* vegetables have more practically usage and storage conditions (Ross et al., 2001; Elsom et al., 2003; O'Gara et al., 2000).

The results suggested that similar studies about *Allium* plant extracts, as a natural preservative, could be an alternative to synthetic antimicrobial substances. Therefore, crude extracts obtained in future experiments should be chosen and evaluated according to their antifungial compounds. Dehydrated garlic, onion and leek extracts could safely be used as substitutes for fungicides to partially or completely inhibit the growth of fungi producing mycotoxins (Soliman and Badeaa, 2002).

4. Conclusions

Fungal diseases are a serious problem still all over the world. *Allium* species contain organosulphur compounds which have antimicrobial properties. *Allium* species are the main products in the dehydration industry. Dried garlic and onion are widely used in food industry in soups, ketchups, sauces and mayon-

naises. They are easy to use and have long shelf-life. Amounts of organosulphur compounds are concentrated in dehydrated *Allium* species and contain therefore higher amounts of antifungal compounds than the fresh forms. The potential for developing preparations of dehydrated *Allium* species for use as an alternative to synthetic fungicides seems to be important; the active compounds should be identified and characterized.

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