

Full Length Research Paper

Antifungal activity of *Allium obliquum*

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A hydroalcoholic extract of *Allium obliquum* was tested for *in vitro* germination and growth of *Aspergillus niger*, *Botrytis cinerea*, *Fusarium oxysporum* F. sp. *gladioli*, *Penicillium expansum* and *Sclerotinia sclerotiorum* on Czapek-agar nutritive medium. The minimum inhibitory concentration (MIC) of the plant extract varied between 50 and 80 μ l/ml, according to the fungal species.

Key words: Alliin, antifungal activity, *Allium obliquum*, HPLC, phytopathogenic fungi.

INTRODUCTION

Romania has 21 wild and 3 cultivated *Allium* species. *Allium obliquum* is an edible plant and a very rare perennial which is encountered in Romania in a single place (on limestone rocks in Turda Gorges). It is also encountered, as a wild species, in South-East Russia, Siberia and Central Asia (Ciocârlan, 2000). Traditionally, *Allium* plants are used for treating headaches, colds and stomach disorders (Keusgen et al., 2006).

Most of the research regarding the phytotherapeutic properties of *Allium* species is performed on *Allium cepa* and *Allium sativum* plants (Duke et al., 2002; Josling, 2003; Tămaș, 1999). *A. sativum* plant extract has antihypertensive (Al-Qattan et al., 1999; Ziyat et al., 1997), antidiabetic (Ziyat et al., 1997), hepatoprotective (Vimal and Devaki, 2004), immunostimulating, antioxidant and antitumor activities (Ayaz and Alpsoy, 2007), antiviral, antifungal (Motsei et al., 2003; Lemar et al., 2005; Thamburan et al., 2006), antihelminthic and antiparasitic (Guarrera, 1999) effects.

Allium plants and extracts contain different chemical

compounds. In *A. cepa* and *A. sativum* extracts different biologically active substances such as organosulphurous compounds like alliin and allicin, E/Z-ajoene, sterols, flavones and polyphenolcarboxylic acids (Duke et al., 2002; Huges and Lawson, 1991; Josling, 2003; Vimal and Devaki, 2004) were found.

Alliin is the precursor of allicin formed by the action of allinase enzyme. There is also a secondary substance resulting from alliin decomposition, called ajoene (Wang and Ng, 2003). Allicin has antibacterial, antiviral, antitumor, anticoagulation, antihypertensive, antiparasitic, hepatoprotective, etc., activity (Josling, 2003). It is also efficient against many fungal species, like *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans*, *Fusarium laceratum*, *Microsporum canis*, *Mucor racemosus*, *Penicillium* spp., *Rhizopus nigricans*, *Saccharomyces* spp., *Trichophyton granulosum*, etc. (Josling, 2003).

Medicinal plants remain a rich source of novel therapeutic agents. Many plant species have not been tested chemically or biologically. The aim of this study was to evaluate *A. obliquum* plant extract effect on the germination and growth of some phytopathogenic fungi. The quality of most *Allium* spp. relies upon organosulfur compounds mostly derived from alliin.

Therefore, a comprehensive documentation of alliin

concentration is required.

MATERIALS AND METHODS

Plant

A. obliquum L. (Liliaceae) plants were grown in the Agrobotanical Garden of the University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca, Romania, from seeds collected in Turda Gorges, Romania. The plant was identified by Dr. Gheorghe Groza. A voucher specimen (CL 659564) was deposited at the Herbarium of "A. Borza" Botanical Garden, "Babeş-Bolyai" University of Cluj-Napoca, Romania.

Extraction

Fresh *A. obliquum* herba (leaves, stems and flowers fragments of 0.5 - 1 cm) was extracted with 50% ethanol (Merck, Bucuresti, Romania) in the Mycology Laboratory of Babes Bolyai University, Cluj-Napoca, Romania, by modified Squibb's repercolation method (Ionescu-Stoian and Savopol, 1977). Three successive applications of the same menstruum where repercolated to the plant material (150 g in the first, 90 g in the second, 60 g in the third percolator). In each percolator plant material was moistened with the menstruum, macerated for two days and than percolated at a rate of about 4 - 6 drops per minute for each 100 g of crude material. The first percolated fractions were reserved and the next fractions were poured upon the next percolator. Then reserved fractions (60 ml the first, 90 ml the second and 150 ml the third) were mixed and constitute the fluid extract 1:1 (w:v).

Preparation of fungal colonies

A. niger Tiegh. isolated from *A. cepa* L. bulbs, *Botrytis cinerea* Pers. isolated from *Rosa* L. flowers, *Fusarium oxysporum* f. sp. *gladioli* W.C. Snyder and H.N. Hansen isolated from *Gladiolus* × *hybridus* C. Morr. corms, *Penicillium expansum* Link isolated from *Malus domestica* Borkh. fruits and *Sclerotinia sclerotiorum* (Lib.) de Bary isolated from *Daucus carota* ssp. *sativus* (Hoffm.) Arcang. roots were included in this study. Colonies were grown in Petri dishes containing Czapek-agar medium (BD Difco, Budapest, Hungary), following inoculation into the central point and incubation at 22°C for 5 days.

Determination of antifungal activity

The antifungal activity of the *A. obliquum* extract, expressed as minimum inhibitory concentration (MIC), was determined by the agar-dilution assay (Bhandari et al., 2000), and was compared to the antimycotic drug fluconazole (2 mg ml⁻¹) (Krka, Novo Mesto, Slovenia) and a control (nutritive medium and 70% EtOH). The percentage of mycelial growth inhibition (P) at each concentration was calculated using the formula: $P = (C-T) \times 100/C$, where C is the diameter of the control colony and T is the diameter of the treated colony (Nidiry and Babu, 2005).

Alliin analysis

The analysis of alliin from *A. obliquum* extract was done using a newly developed liquid chromatography coupled with mass spectrometry detection (LC/MS). Briefly, an Agilent 1100 series HPLC system was used (Agilent Technologies, Darmstadt, Germany),

coupled with an Agilent Ion Trap SL mass spectrometer equipped with an electrospray ion source. The chromatographic separation of alliin was made using a Zorbax SB-C18 100 × 3.0mm i.d., 3.5 µm column (Agilent Technologies, Darmstadt, Germany). The mobile phase consisted in 100% ammonium acetate, 1 mM in water, isocratic elution, flow 1 ml/min. The mass spectrometer operated in positive multiple reaction monitoring mode, using nitrogen as nebulising and dry gas. The nebuliser was set at 70 psi, the dry gas flow was 12 L/min at 350 C temperature. The mass spectrometer was set to record the transition m/z 178 > m/z 88, which is specific to alliin (Sigma-Aldrich NV/SA, Bornem, Belgium). The retention time of alliin in above described conditions was 0.64 min.

RESULTS AND DISCUSSION

In the present study, the antifungal activities of the total *A. obliquum* plant extract were investigated. The extract had antifungal activity against the studied phytopathogenic fungi. As shown in Table 1, *A. obliquum* plant extract MIC was 50 µl/ml for *S. sclerotiorum*, 60 µl/ml for *B. cinerea*, 70 µl/ml for *F. oxysporum* f. sp. *gladioli*, and 80 µl/ml for *A. niger* and *P. expansum*.

Other studies showed that crude extracts (water, ethanol, chloroform) of *A. sativum* had antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*, at minimum bacterial concentrations (MBC) depending on the species and on the type of plant extract (Abubakar, 2009). Another species with antimicrobial activity is *A. ascalonicum*. It has antifungal action against *C. albicans*, dermatophytes (*Microsporum gypseum*, *Trichophyton mentagrophytes* and *Epidermophyton floccosum*), *Syncephalastrum* spp., *A. niger*, *Penicillium* spp., *Paecilomyces* spp., *Scopulariopsis* spp., *Cladosporium* spp., *Alternaria* spp., *Drechslera* spp. at MIC of 0.25 % (Mahmoudabadi and Nasery, 2009). The aqueous extracts from *A. cepa* and *A. sativum* have antifungal action against *C. albicans* and other *Candida* isolates, against *Malassezia furfur* isolates and dermatophyte species (Ghahfarokhi et al., 2006). *A. cepa* plant extract also has antifungal action against *Trichophyton rubrum* and *T. mentagrophytes* species (Ghahfarokhi et al., 2004).

Antifungal activity of the total *A. obliquum* extract was species dependent. It was mostly active against *S. sclerotiorum* and *B. cinerea* but least active against *F. oxysporum* f. sp. *gladioli*, *A. niger* and *P. expansum*.

The inhibitory activity of the total *A. obliquum* plant extract on each fungus was more powerful than that of fluconazole. Fluconazole MIC for *S. sclerotiorum* was 80 µl/ml, for *F. oxysporum* f. sp. *gladioli* 100 µl/ml, for *B. cinerea* 120 µl/ml, and for *A. niger* and *P. expansum* 300 µl/ml (Table 1).

Alliin, allicin and E/Z-ajoene are biologically active compounds isolated from plant extracts of *A. sativum*, *A. cepa* and *A. ampeloprasum*, with antibacterial and antifungal activity (Huges and Lawson, 1991; Lemar et al., 2005; Thamburan et al., 2006). The analysis of alliin from *A. obliquum* extract by LC/MS determined 141.08 µg alliin/ml *A. obliquum* plant extract (Figure 1). Because

Table 1. *In vitro* radial growth inhibition of phytopathogenic fungi by the ethanolic *A. obliquum* extract and fluconazole.

Fungal species	<i>Allium obliquum</i> extract (µl/ml)	Colony ^a diameter (mm)	P ^a (%)	Fluconazole (µl/ml)	Colony ^b diameter (mm)	P ^b (%)
<i>Aspergillus niger</i>	C	22	0	C	22	0
	20	19.5	11.36±0.2 ^c	100	11.5	47.72±1.1 ^c
	40	15.5	29.54±0.1 ^c	150	9.5	56.81±1.1 ^c
	60	10.33	53.04±1.1 ^c	200	7.5	65.90±1.5 ^c
	70	4.5	79.54±2.1 ^c	250	4.5	79.54±1.8 ^c
	80	0	100	300	0	100
<i>Botrytis cinerea</i>	C	65	0	C	65	0
	10	59.5	8.46±0.1	20	40.33	37.95±0.8 ^c
	20	35.33	45.64±1.1 ^c	60	20.33	68.72±1.6 ^c
	40	15.66	75.90±1.8 ^c	100	5.5	91.53±2.2 ^c
	50	4.66	92.83±2.4 ^c	120	0	100
	60	0	100			
<i>Fusarium oxysporum</i> f.sp. <i>gladioli</i>	C	55	0	C	55	0
	20	45.66	16.98±1.1 ^c	20	6.33	88.49±1.4 ^c
	40	18.66	66.07±1.5 ^c	60	3.5	93.63±2.1 ^c
	60	4.66	91.52±1.8 ^c	80	2	96.36±1.6 ^c
	70	0	100	100	0	100
<i>Penicillium expansum</i>	C	13.5	0	C	13.5	0
	30	11.66	13.62±0.7 ^d	60	11.66	13.62±1.1 ^d
	50	7.83	42±0.9 ^c	120	11	18.51±1.1 ^c
	70	4.5	66.66±1.4 ^c	160	11	18.51±1.1 ^c
	80	0	100	200	10	25.92±1.3 ^c
				250	5.6	58.51±1.6 ^c
				300	0	100
<i>Sclerotinia sclerotiorum</i>	C	62	0	C	62	0
	10	54.5	12.09±0.8 ^c	20	30.33	51.08±1.1 ^c
	20	38.33	38.17±1.3 ^c	40	15.33	75.27±2.1 ^c
	30	18.66	69.90±2.1 ^c	60	5.16	91.67±1.8 ^c
	40	6.16	90.06±1.9 ^c	80	0	100
	50	0	100			

Legend: ^a = the effect of *A. obliquum* extract;

^b = the effect of fluconazole;

C = 70% aq. EtOH;

P = mycelial growth inhibition.

Results are the mean of 4 experiments ± SEM.

Results were analysed using the Students't-test.

^c = p < 0.001 versus C;

^d = p < 0.01 versus

The same doses of *A. obliquum* extract (10, 20, 30, 40, 50, 60, 70 and 80 µl/ml) and fluconazole (20, 60, 80, 100, 120, 150, 200, 250, 300 µl/ml) were tested against all fungal species. From the table were excluded the doses that related to the previous dose did not reduce significantly colony diameter

alliin content is involved in the antifungal effect, alliin *A. obliquum* extract had an important antifungal effect against *S. sclerotiorum*, *B. cinerea*, *F. oxysporum* f. sp. *gladioli*, *A. niger* and *P. expansum*, which makes it a candidate for the *in vivo* biological control of phytopatho-

genic fungi.

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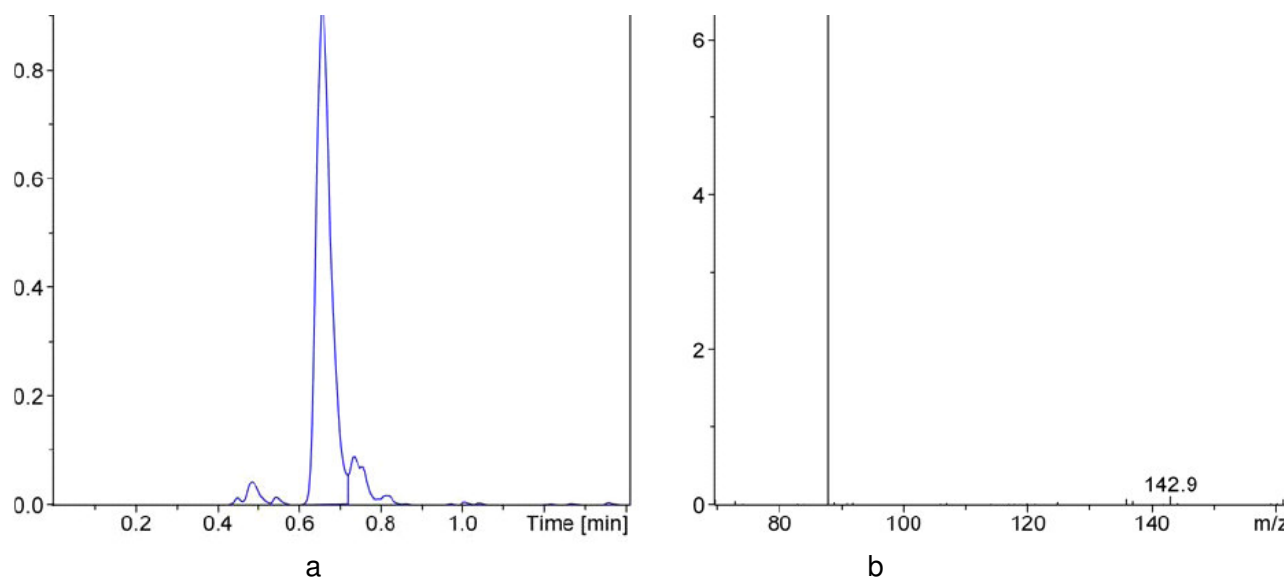


Figure 1. The chromatogram (a) and MS/MS (b) of alliin from *A. obliquum* extract

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